GROUP A ELEMENTS: PROJECT MANAGEMENT

1. TITLE AND APPROVAL SHEETS

Monitoring and Reporting Project Plan (MRPP) and Quality Assurance Project Plan (QAPP)

for Conditional Waiver No. 4 for Discharges from Agricultural and Nursery Operations in the Santa Margarita River Watershed within Riverside County, California

Submitted to
California Environmental Protection Agency
Regional Water Quality Control Board, Region 9, San Diego
9174 Sky Park Court, Suite 100
San Diego, CA 92123

Submitted by
Upper Santa Margarita Irrigated Lands Group
P.O. Box 892411
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Submittal Date
January 1, 2012
Rev. 1 - October 23, 2012
Rev. 2 – July 29, 2013

Prepared by
AEI-CASC Consulting
1470 Cooley Drive
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APPROVAL SIGNATURES

MONITORING GROUP

Title:	Name:	Signature:	Date:
President of the Board, Upper Santa Margarita Irrigated Lands Group	Ben R. Drake		
Administrator, Upper Santa Margarita Irrigated Lands Group	Kourtney Drake		

CALIFORNIA REGIONAL WATER QUALTY CONTROL BOARD, REGION 9, SAN DIEGO

Title:	Name:	Signature:	Date:
Executive Officer	David W. Gibson		
Engineering Geologist	Roger N. Mitchell, P.G.		

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3. DISTRIBUTION LIST

This Monitoring and Reporting Project Plan (MRPP) and Quality Assurance Project Plan (QAPP) is available to all members in good standing of the Upper Santa Margarita Irrigated Lands Group (USMILG). In addition, copies of the MRPP/QAPP are being provided to the following individuals.

Name(s):	Affiliation:	Address:
Board Members: Steve Corona Andy Domenigoni Ben R. Drake Dan Hollingsworth Leo McGuire	Upper Santa Margarita Irrigated Lands Group	P.O. Box 892411 Temecula, CA 92589
Administrator: Kourtney Drake	Upper Santa Margarita Irrigated Lands Group	P.O. Box 892411 Temecula, CA 92589
Executive Officer and Staff: David W. Gibson Roger N. Mitchell, P.G.	California Regional Water Quality Control Board, Region 9, San Diego	Board, 9174 Sky Park Court, Suite 100, San Diego, CA 92123

4. PROJECT/TASK ORGANIZATION

Involved Parties and Roles

The Upper Santa Margarita Irrigated Lands Group (USMILG) is a runoff monitoring group organized to assist owners of irrigated farmland in the Santa Margarita River Watershed within Riverside County in complying with water quality regulations being mandated by the California Regional Water Quality Control Board, Region 9, San Diego Region (RWQCB). The regulations affect property owners that produce or expect to produce $1,000 or more gross income from agriculture or nursery products during any given year.

The USMILG will serve as the lead agency for complying with the RWQCB's Conditional Waiver No. 4 monitoring program requirements. As the lead agency, USMILG will oversee the development, submittal, and implementation of a Monitoring and Reporting Project Plan (MRPP) and Quality Assurance Project Plan (QAPP) to meet the Group Monitoring requirements of Conditional Waiver No. 4. The beneficiaries of USMILG's efforts will be the property owners of record that have formally joined the USMILG.

The USMILG is a California non-profit 501(c)(3) corporation directed by a five member Board of Directors including Steve Corona, Andy Domenigoni, Ben Drake, Dan Hollingsworth, and Leo McGuire. The Board of Directors is responsible for overall operation of the USMILG. Day-to-day operations of USMILG are managed by the group's Administrator, Kourtney Drake.

The USMILG has contracted with AEI-CASC Consulting to prepare the MRPP and QAPP and to implement the monitoring program. AEI-CASC Consulting's assistance to USMILG is managed by the firm's Engineering Director, Jeff Endicott, P.E., BCEE, CPESC, QSD.

The USMILG will contract for laboratory analyses and bioassessment field services when needed once the MRPP and QAPP are approved. By contracting these services closer to the time of need, USMILG will have the benefit of being able to have several qualified laboratories and bioassessment firms propose on the work.

Table 1. (Element 4) Personnel responsibilities.

Name	Organizational Affiliation	Title	Contact Information (Telephone number, fax number, email address.)
Steve Corona Andy Domenigoni Ben Drake Dan Hollingsworth Leo McGuire	Upper Santa Margarita Irrigated Lands Group	Board Member	P.O. Box 892411 Temecula, CA 92589
Kourtney Drake	Upper Santa Margarita Irrigated Lands Group	Administrator	*P.O. Box 892411 Temecula, CA 92589* Email: kdrake@usmilg.org
Jeff Endicott, P.E. BCEE, CPESC	AEI-CASC Consulting	Engineering Director	1470 E. Cooley Drive Colton, CA 92324 Phone: (909) 835-7537 Fax: (909) 783-0108 Email: jendicott@aei-casc.com
Analytical Laboratory	TBD	TBD	TBD
Bioassessment	TBD	TBD	TBD

Quality Assurance Officer Role

Kourtney Drake, USMILG Administrator, will serve as the Project Director and Quality Assurance Officer for development, submittal, and implementation of the MRPP and QAPP. Ms. Drake will monitor the work of the consultant preparing and implementing the MRPP and QAPP, including review of progress reports and recommending approval of progress reports and applications for payment to the USMILG Board of Directors. Ms. Drake will review and comment on consultant submittals, including reports and data summaries, and recommend approval of the same to the USMILG Board of Directors.

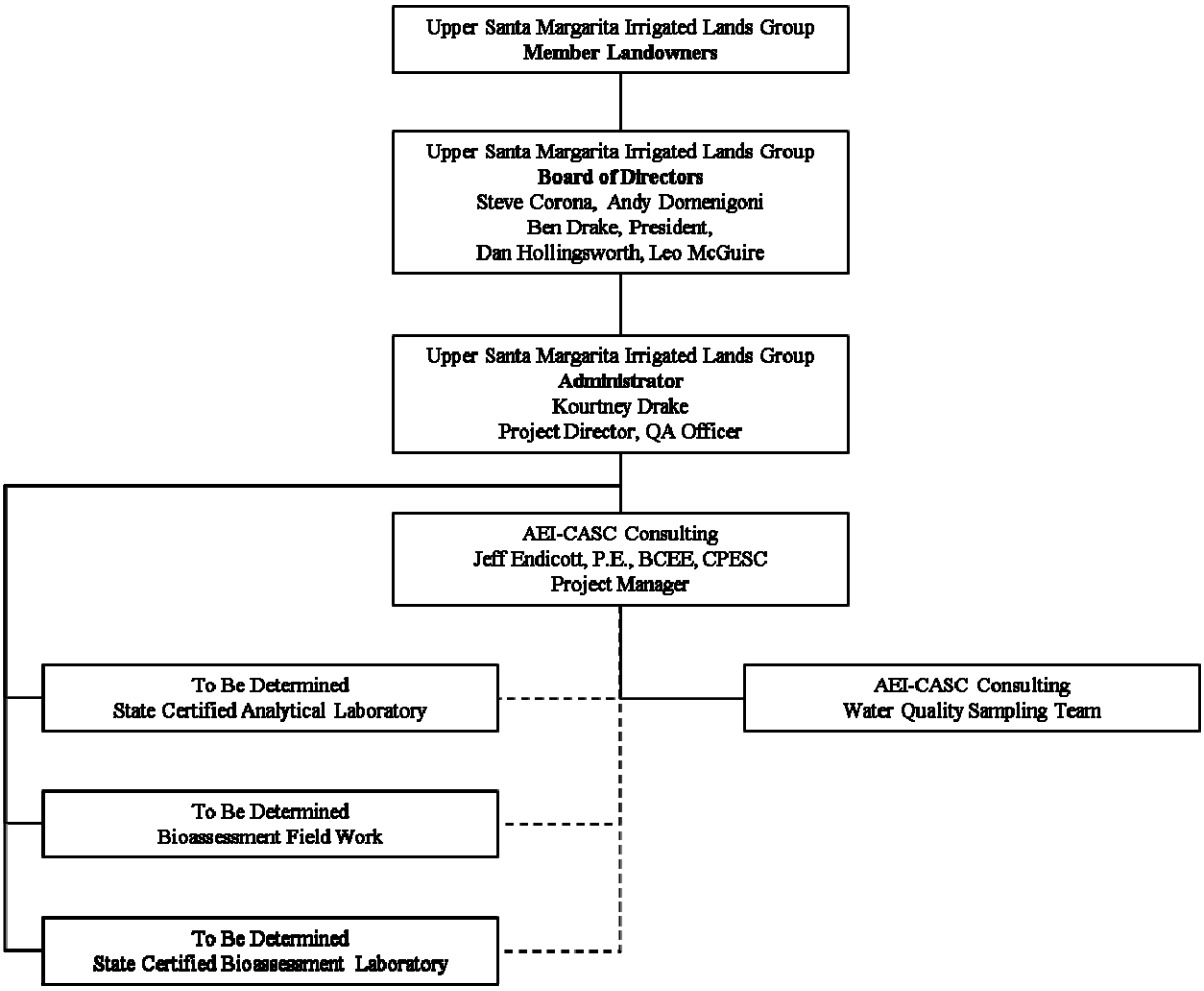
Persons Responsible for QAPP Update and Maintenance

Jeff Endicott, P.E., BCEE, CPESC, QSD, AEI-CASC Consulting Engineering Director, will be responsible for maintaining and updating the MRPP and QAPP. When significant updates to the MRPP and QAPP are identified, an update recommendation will be presented to Kourtney Drake, USMILG Administrator and project Quality Assurance Officer: Ms. Drake will review the updates and submit updates to the USMILG Board of Directors for approval. Ministerial updates to the MRPP and QAPP can be approved by the Quality Assurance Officer.

Organizational Chart and Responsibilities

Figure 1 shows the relationship between the USMILG members, the USMILG Board of Directors, the USMILG Administrator, and the consultant and contract laboratories that will be contracted to perform the monitoring described in this QAPP.

Figure 1. Organizational chart.



5. PROBLEM DEFINITION/BACKGROUND

Problem Statement

The California Regional Water Quality Control Board, Region 9, San Diego Region (RWQCB) has determined that discharges from lands used for agricultural or nursery operations can be a source of sediment, dissolved solids, nutrients, pesticides, hydrocarbons, pathogens, and other pollutants which can adversely affect the quality of waters of the state if growing operations, irrigation return flows, and storm water runoff are not properly managed. While the federal Clean Water Act (CWA) exempts agricultural storm water runoff and irrigation return flows from regulation under the National Pollutant Discharges Elimination System (NPDES) program, the California Water Code provides for regulation of these discharges through issuance of Waste Discharge Requirements (WDRs).[1]

The RWQCB examined options for regulation of agricultural storm water runoff and irrigation return flows using WDRs. Due to the limited RWQCB resources available to issue WDRs to each agricultural and nursery operation in the RWQB's jurisdiction, and due to the costs to the regulated entities associated with annual permit fees and monitoring requirements that go along with WDRs, the RWQCB has adopted a waiver program. The waiver program allows agricultural and nursery operations to discharge storm water runoff and irrigation return flows without WDRs in place on the condition that the discharger complies with the requirements of the waiver. In general, to qualify for the waiver dischargers must:

- Enroll, as an individual or as part of a group, in the waiver program.
- Implement management measures and/or best management practices to minimize or eliminate the discharge of pollutants that may adversely impact the quality or beneficial uses of waters of the State.
- Implement a monitoring and reporting program.

Agricultural and nursery operations that comply with the waiver conditions are not expected to pose a threat to the quality of waters of the State and may continue to operate and discharge without WDRs.

Conditional Waiver No. 4 – Discharges from Agricultural and Nursery Operations, was adopted by the RWQCB 2007.[2] Among other things, Conditional Waiver No. 4 requires that all property owners with irrigated farm or nursery land within the Santa Margarita Watershed of Riverside County, either individually or as part of a group, develop and implement a monitoring and reporting program. Agricultural and nursery operations subject to the waiver include operations that produce $1,000 or more gross income from agriculture or nursery products during any given year. This Monitoring and Reporting Project Plan (MRPP) / Quality Assurance Project Plan (QAPP) describes the group monitoring and reporting program proposed by the Upper Santa Margarita Irrigated Lands Group (USMILG).

USMILG is a California non-profit 501(c)(3) corporation organized to assist owners of irrigated farmland in the Santa Margarita River Watershed within Riverside County in complying with requirements of Conditional Waiver No. 4. Membership in USMILG is voluntary and is comprised of owners of irrigated farmland that recognize the benefits of joining a monitoring group in order to share in the cost of developing and implementing the monitoring and reporting requirements of Conditional Waiver No. 4. USMILG is not an enforcement agent or otherwise associated with the RWQCB. USMILG is directed by a five member Board of Directors including Steve Corona, Andy Domenigoni, Ben Drake, Dan Hollingsworth, and Leo McGuire. Day-to-day operations of USMILG are managed by the group's Administrator, Kourtney Drake.

USMILG will serve as the lead agency for complying with the RWQCB's Conditional Waiver No. 4 monitoring program requirements. As the lead agency, USMILG will oversee the development, submittal, and implementation of a MRPP/QAPP to meet the group monitoring requirements of Conditional Waiver No. 4. The beneficiaries of USMILG's efforts will be the property owners of record that have formally joined the USMILG.

[1]California Regional Water Quality Control Board, Region 9, San Diego Region, *Conditional Waiver No. 4 – Discharges from Agricultural and Nursery Operations*, Resolution R9-2007-0104, October 10, 2007.
[2]Ibid.

USMILG has contracted with AEI-CASC Consulting to prepare the MRPP and QAPP and to implement the monitoring program. AEI-CASC Consulting's assistance to USMILG is managed by the firm's Engineering Director, Jeff Endicott, P.E., BCEE, CPESC, QSD.

The USMILG will contract out laboratory analyses and bioassessment field services when needed once the MRPP/QAPP are approved. By contracting these services closer to the time of need, USMILG will have the benefit of being able to have several qualified laboratories and bioassessment firms propose on the work.

Decisions or Outcomes

This MRPP/QAPP will provide water quality data and biological data to contribute to the characterization and assessment of surface water quality in the Santa Margarita River watershed within Riverside County, California. USMILG's focus is on data and reporting contributions geared towards the assessment of irrigated farmlands' contribution to water quality. Similar monitoring and reporting efforts are being conducted by others, including efforts by cities, Riverside County, and Riverside County Flood Control and Water Conservation District: their efforts are generally focused on the assessment of urban areas' contribution to water quality.

The water quality data will be used to assess the quality of receiving waters compared to water quality limits contained in the document, *Water Quality Control Plan for the San Diego Basin (9)*[3] (Basin Plan). For irrigated agriculture, the water quality data will help in the assessment of Conditional Waiver No. 4's ability to protect surface water quality through the waiver program in lieu of issuing Waste Discharge Requirements (WDRs) to agricultural groups or to individual agricultural discharges. The water quality data may also help in the assessment of potential pollutant sources and help lead to identification of pollutant sources and the ultimate minimization or elimination of these sources. The biological data will be used to contribute data to the future development, by others, of a biological integrity index for receiving waters. Until a biological integrity index is developed and adopted, a process anticipated to take many years, the biological data collected as part of this project will not be suitable for use in assessing receiving waters in accordance with Basin Plan standards since the Basin Plan does not include biological metrics.

Water Quality or Regulatory Criteria

The water quality and biological data collected as part of this project may be used in the future for listing and/or delisting water bodies from the state's 303(d) list of impaired water bodies, for setting or implementing Total Maximum Daily Loads (TMDLs), and the determination of the effectiveness of waiver programs, among other purposes. To provide data of sufficient quality and comparability to data being collected in other efforts, the USMILG's MRPP/QAPP generally utilizes Standard Operating Procedures (SOPs) and analytical methods consistent with the requirements of the California Surface Water Ambient Monitoring Program (SWAMP).[4]

[3]California Regional Water Quality Control Board, Region 9, San Diego Region, *Water Quality Control Plan for the San Diego Basin (9,)* adopted September 8, 1994 and as subsequently amended.
[4]California State Water Resources Control Board, *California Surface Water Ambient Monitoring Program*, http://www.waterboards.ca.gov/water_issues/programs/swamp/.

6. PROJECT/TASK DESCRIPTION

Work Statement and Produced Products

The Upper Santa Margarita Irrigated Lands Group's (USMILG) Monitoring and Reporting Project Plan (MRPP) and Quality Assurance Project Plan (QAPP) provides for water quality and biological monitoring at sampling locations in the Santa Margarita River watershed area of Riverside County. Primary work products will be water quality data, biological data, and an annual monitoring report summarizing the results of the prior year's monitoring program.

Constituents to be Monitored and Measurement Techniques

The California Regional Water Quality Control Board, Region 9, San Diego Region (RWQCB) has provided the minimum testing requirements applicable to the USMILG for qualification under Conditional Waiver No. 4. The list of water quality parameters and biological parameters to be monitored along with the corresponding sample collection and analytical procedures are summarized in Tables 8, 9, and 10.

Project Schedule

The project schedule is shown below.

Table 2. (Element 6) Project schedule timeline.

Activity	Anticipated Date of Initiation	Anticipated Date of Completion	Deliverable	Deliverable Due Date
Develop MRPP/QAPP	Completed	Completed	MRPP/QAPP	Completed
RWQCB Reviews MRPP/QAPP	Completed	Completed	Comments on MRPP/QAPP	Completed
Revise and Resubmit MRPP/QAPP	Completed	Completed	Revised MRPP/QAPP	Completed
Year 1 Monitoring Water Quality (Wet)	Completed	Completed	Water Quality Analytical Data	Completed
Year 1 Monitoring Water Quality (Dry)	5/1/2013	9/1/2013	Water Quality Analytical Data	10/31/2013
Year 1 Monitoring Bioassessment	5/1/2013	9/1/2013	Bioassessment Data	10/31/2013
Year 1 Annual Report and Schedule for Year 2	9/1/2013	10/31/2013	Annual Report and Year 2 Schedule	12/31/2013
Year 2 Monitoring Water Quality (Wet and Bed Sediment)	11/1/2013*	4/30/2014*	Water Quality Analytical Data	6/30/2014*
Year 2 Monitoring Water Quality (Dry)	5/1/2014*	9/1/2014*	Water Quality Analytical Data	10/31/2014*
Year 2 Monitoring Bioassessment	5/1/2014*	9/1/2014*	Bioassessment Data	10/31/2014*
Year 2 Annual Report and Schedule for Year 3	9/1/2014*	10/31/2014*	Annual Report and Year 3 Schedule	12/31/2014*
Year 3 Monitoring Water Quality (Wet and Bed Sediment)	11/1/2014*	4/30/2015*	Water Quality Analytical Data	6/30/2015*
Year 3 Monitoring Water Quality (Dry)	5/1/2015*	9/1/2015*	Water Quality Analytical Data	10/31/2015*
Year 3 Monitoring Bioassessment	5/1/2015*	9/1/2015*	Bioassessment Data	10/31/2015*
Year 3Annual Report and Schedule for Year 4	9/1/2015*	10/31/2015*	Annual Report and Year 4 Schedule	12/31/2015*

Activity	Anticipated Date of Initiation	Anticipated Date of Completion	Deliverable	Deliverable Due Date
Year 4 Monitoring Water Quality (Wet and Bed Sediment)	11/1/2015*	4/30/2016*	Water Quality Analytical Data	6/30/2016*
Year 4 Monitoring Water Quality (Dry)	5/1/2016*	9/1/2016*	Water Quality Analytical Data	10/31/2016*
Year 4 Monitoring Bioassessment	5/1/2016*	9/1/2016*	Bioassessment Data	10/31/2016*
Year 4Annual Report and Schedule for Year 5	9/1/2016*	10/31/2016*	Annual Report and Year 5 Schedule	12/31/2016*
Year 5 Monitoring Water Quality (Wet and Bed Sediment)	11/1/2016*	4/30/2017*	Water Quality Analytical Data	6/30/2017*
Year 4 Monitoring Water Quality (Dry)	5/1/2017*	9/1/2017*	Water Quality Analytical Data	10/31/2017*
Year 5 Monitoring Bioassessment	5/1/2017*	9/1/2017*	Bioassessment Data	10/31/2017*
Year 5 Annual Report	9/1/2017*	10/31/2017*	Annual Report	12/31/2017*

*The Annual Report will confirm the schedule for the subsequent year. Extent of monitoring pending funding, to be confirmed in Annual Report for the subsequent year.

Geographical setting

The MRPP/QAPP describes monitoring to be conducted in the Santa Margarita River watershed of Riverside County, California. At the request of the RWQCB, the sampling locations will be located on receiving waters or tributaries thereto that are located downstream of the dams and reservoirs located in the watershed. The sampling locations will be located downstream of irrigated farmlands. Appendix A includes a map of the watershed that is the subject of this MRPP/QAPP.

Constraints

Key constraints of the proposed monitoring program include financial constraints and monitoring constraints.

Owners of irrigated farmland, like most Americans, are facing severe economic challenges and the funds available to develop and implement a monitoring program are limited. The RWQCB has indicated that the expected financial contribution towards monitoring under Conditional Waiver No. 4 is $5 per acre per year for group monitoring.[5] USMILG has a group enrollment of approximately 400 members covering approximately 4,500 acres of irrigated farmland. This acreage will generate approximately $32,000 per year to conduct the monitoring program including sample collection field work, laboratory analysis of samples, bioassessment field work, taxonomic analysis of biological samples, and report development.

The Santa Margarita River watershed area of Riverside County is relatively arid with few year round perennial streams and creeks. Most streams and creeks in the area are ephemeral, flowing only during intense or extended rain events sufficient to produce runoff and for a short duration thereafter. Water quality sampling of ephemeral streams is not particularly difficult, but establishing the rate of flow can be a challenge due to lack of a control section where a depth vs. flow relationship can be established with sufficient accuracy to be of value. For bioassessment work, the index period based on the watershed's eco-region is May 1 to September 1 which corresponds to a period where streams may be completely dry, thereby, reducing the applicability of bioassessment techniques.

[5]Gorham, Cynthia, Senior Environmental Scientist, California Regional Water Quality Control Board, Region 9, San Diego Region, in a June 10, 2011 meeting with the Upper Santa Margarita Irrigated Lands Group in Winchester, California.

7. Quality Objectives and Criteria for Measurement Data

Data Quality Indicators for this project will consist of the following:

Measurement or Analyses Type	Applicable Data Quality Indicators
Field Testing	Accuracy, Precision, and Completeness
Laboratory Testing, Conventionals	Accuracy, Precision, and Completeness
Laboratory Analysis, Non-Conventionals	Accuracy, Precision, and Completeness
Laboratory Analysis, Non-Conventional Biological	Accuracy, Precision, and Completeness

Accuracy will be determined by measuring one or more selected performance testing samples or standard solutions from sources other than those used for calibration.

Precision will be determined on both field and laboratory replicates. The number of replicates for field measurements will be 3 and the number of laboratory replicates will be 2.

Recovery will not be utilized as a data quality indicator. See accuracy. Recovery involves spiking samples with a known quantity of a constituent and determining the percentage recovery of the spike. This process adds additional analysis costs. In lieu of recovery, the project will utilize field blanks and field splits (duplicates) to "test" the analytical procedures.

Completeness is the number of analyses generating useable data for each analysis divided by the number of samples collected for that analysis.

Method sensitivity is managed by the inclusion of the required California Surface Water Ambient Monitoring Program (SWAMP) target reporting limits where they exist. Where SWAMP target reporting limits do not exist, standard method appropriate reporting limits are proposed.

Table 3 presents measurement quality objectives for data obtained through measurements in the field. Table 4 presents measurement quality objectives for data obtained through laboratory analyses.

Table 3. (Element 7) Measurement quality objectives for field data.

Group	Parameter	Accuracy	Precision	Recovery	Target Reporting Limit	Completeness
Field Testing	Algae Diatoms	DFG Procedure	DFG Procedure		--	90%
Field Testing	Algae Soft	DFG Method	DFG Method		--	90%
Field Testing	Depth	Propose 0.05 m	0.01 m		0.02 m	90%
Field Testing	Dissolved Oxygen	± 0.2mg/L	0.1 mg/L		0.2 mg/L	90%
Field Testing	pH	± 0.2 pH units	± 0.1 pH units		N/A	90%
Field Testing*	Specific Conductance	± 2 µS/cm	± 1 µS/cm		2.5 µS/cm	90%
Field Testing	Temperature	± 0.1 ºC	± 0.1 ºC		N/A	90%
Field Testing*	Turbidity	± 1 NTU	± 1 NTU		5 NTU	90%
Field Testing	Flow velocity and Discharge	Standard Operating Procedures (SOPs) for conducting field measurements for water quality and bioassessment purposes are provided in Appendix B.				
Field Testing	Unshaded solar radiation	--*	--*		$Cal/cm^2/day$*	--*

*Laboratory analysis is optional.
*Refer to the manufacturer's specifications for measuring device.

Table 4. (Element 7) Measurement quality objectives for laboratory data.

Group	Parameter	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Conventional	Alkalinity as $CaCO_3$	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		1 mg/L	90%
Conventional	Ammonia as N (NH_3)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.1 mg/L	90%
Conventional	Ammonium as N (NH_4^+)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0. 1 mg/L	90%
Conventional	$CaCO_3$ (Total Hardness)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		1 mg/L	90%
Conventional	Carbon (Particulate)*	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		2,600 mg/kg in 10mg sample	90%
Conventional	Chloride	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.25 mg/L	90%
Conventional	Nitrate as N (NO_3)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.01 mg/L	90%
Conventional	Nitrite as N (NO_2)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.01 mg/L	90%
Conventional	Nitrogen Total (Direct Measurement)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.1 mg/L	90%
Conventional	Nitrogen (Particulate)*	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		165 mg/kg in 10mg sample	90%
Conventional	Organic Carbon (Dissolved)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.6 mg/L	90%
Conventional	Orthophosphate as P (Dissolved; SRP)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.01 mg/L	90%
Conventional	Orthophosphate as P (Total)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.01 mg/L	90%
Conventional	Phosphorus (Dissolved)*	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.014 mg/L (Not Listed) (Proposed)	90%
Conventional	Phosphorus (Particulate)*	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		160 mg/kg in 10mg sample	90%
Conventional	Phosphorus (Total)	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.014 mg/L (Not Listed) (Proposed)	90%

Group	Parameter	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Conventional	Sulfate	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		1.0 mg/L	90%
Conventional	TDS	N/A	RPD<25% (N/A if native concentration of either sample<RL)		10 mg/L	90%
Conventional	TKN, Dissolved (as N)*	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.1 mg/L	90%
Conventional	TKN, Total (as N)*	80-120%	RPD<25% (N/A if native concentration of either sample<RL)		0.1 mg/L	90%
Conventional	TSS	N/A	RPD<25% (N/A if native concentration of either sample<RL)		0.5 mg/L	90%
Non-Conventional	Glyphosates (Water)	Reference Materials: 70-130% recovery if certified, otherwise 50-150% recovery. Matrix Spikes: 50-150% recovery	RPD<25% (N/A if native concentration of either sample<RL)			90%
Non-Conventional	Organochlorine Pesticides (Sediment)	Reference Materials: 70-130% recovery if certified, otherwise 50-150% recovery. Matrix Spikes: 50-150% recovery	Per Method		N/A	90%
Non-Conventional	Organochlorine Pesticides (Water)	Reference Materials: 70-130% recovery if certified, otherwise 50-150% recovery. Matrix Spikes: 50-150% recovery	RPD<25% (N/A if native concentration of either sample<RL)		0.050 µg/L	90%
Non-Conventional	Organophosphate (Water)	Reference Materials: 70-130% recovery if certified, otherwise 50-150% recovery. Matrix Spikes: 50-150% recovery	RPD<25% (N/A if native concentration of either sample<RL)			90%
Non-Conventional	Pyrethroids (Sediment)	Reference Materials: 70-130% recovery if certified, otherwise 50-150% recovery. Matrix Spikes: 50-150% recovery	Per Method			90%
Non-Conventional Biological	Algae Diatoms	Standard Methods (Proposed)	Standard Methods (Proposed)			90%
Non-Conventional Biological	Algae Soft	Standard Methods (Proposed)	Standard Methods (Proposed)			90%
Non-Conventional Biological	Amphipod 10-d Hyalella Chronic	Standard Methods (Proposed)	Standard Methods (Proposed)			90%
Non-Conventional Biological	Ash Free Dry Mass	Standard Methods (Proposed)	Standard Methods (Proposed)			90%

Group	Parameter	Accuracy	Precision	Recovery	Target Reporting Limits	Completeness
Non-Conventional Biological	Chlorophyll-a	Standard Methods (Proposed)	Standard Methods (Proposed)		0.002 mg/L	90%
Note*: If approved SWAMP procedures are unavailable for analysis of constituents in particulate phase, then alternatively sample and analyze for TKN (dissolved and total) and P (dissolved and total). If SWAMP testing procedures become available for the particulate constituents listed above, then particulates will be tested for utilizing those measurement quality objectives.						

8. Special Training Needs/Certification

Specialized Training or Certifications

Field Personnel

No specialized training or certifications are required for personnel collecting water quality samples or making field measurements. Staff acting as the "lead" for water quality sampling events shall have experience not less than having obtained samples of a similar nature for at least 5 events. Staff acting as the "lead" for bioassessment field work shall have experience not less than having participated in bioassessment field work of a similar nature for at least 5 sites.

Prior to sampling or field work, staff designated as "lead" shall familiarize themselves with the objectives of the sampling and field work and the applicable Standard Operating Procedures (SOPs). "Lead" staff shall then brief support staff on appropriate procedures before beginning the work and during the work as needed.

All staff conducting field work shall familiarize themselves with requirements to conduct their work in a safe manner. All staff conducing field work shall be familiar with their employer's health and safety plans and shall conduct their work in accordance with those plans. When work cannot be conducted safely and in accordance with health and safety plans, the work must be stopped and not resumed until it can be conducted in a safe manner.

Laboratory Personnel

Laboratories utilized on this project for water quality analyses shall be certified by the California Department of Health Environmental Laboratory Accreditation Program (ELAP).[6] Laboratory personnel conducting laboratory analyses of project water quality samples shall have sufficient training and/or certifications for compliance with ELAP requirements.

Laboratories utilized on this project for bioassessment laboratory work shall be acceptable to the California State Water Resources Control Board (SWRCB). Laboratory personnel conducting laboratory analyses of project bioassessment collections shall have sufficient training and/or certifications for compliance with SWRCB requirements.

Training and Certification Documentation

All consultants, laboratories, agencies, and others participating in field work or laboratory work associated with this project shall maintain records of their staffs' training. These records shall be made available upon request of the Project QA Officer. Table 5 provides an example of how staff training may be documented.

Training Personnel

All consultants, laboratories, agencies, and others with staff participating in the field work or laboratory work shall ensure that their staff receive or have received training appropriate to the duties assigned. Training shall include both initial training and periodic refresher training.

[6]California Department of Health, Environmental Laboratory Accreditation Program. http://www.cdph.ca.gov/certlic/labs/Pages/ELAP.aspx.

Table 5. (Element 8) Example training documentation format: specialized personnel training or certification.

Specialized Training Course Title or Description	Training Provider	Personnel Receiving Training/ Organizational Affiliation	Location of Records & Certificates *

*If training records and/or certificates are on file elsewhere, then document their location in this column. If these training records and/or certificates do not exist or are not available, note this.

9. DOCUMENTS AND RECORDS

The official repository of documents for this project will be at the offices of the Upper Santa Margarita Irrigated Lands Group (USMILG): USMILG Administrator Kourtney Drake will be the responsible individual. In addition, AEI-CASC Consulting, who has been retained by USMILG to assist with development and implementation of the monitoring program, will maintain a duplicate set of documents in its files located at the AEI-CASC Consulting office in Colton, California: Project Manager Jeff Endicott will be the responsible individual.

The Monitoring and Report Project Plan (MRPP) and Quality Assurance Project Plan (QAPP) will be distributed to the individuals and entities identified previously in Group A, Element 3. In addition, the MRPP/QAPP will be distributed to staff and laboratories as needed to support the effective conduct of field work and laboratory work required by the MRPP/QAPP.

Table 6. (Element 9) Document and record retention, archival, and disposition information.

Record Type	Identify Type Needed	Retention	Archival	Disposition
Plan	MRPP/QAPP and Amendments	Original, E-Document	Bound Report, Project E-Files	5 Years from Date Generated*
Field Records	Field Log, Chain of Custody	Original, Scanned Original	Notebook, Project E-Files	5 Years from Date Generated*
Analytical Records	Laboratory Data Report, QA Sample Data Report	Original, Scanned Original	Notebook, Project E-Files	5 Years from Date Received*
Reports	Annual Report	Original, E-Document	Bound Report, Project E-Files	5 Years from Date Submitted*
General	Project Correspondence	Original or E-Document	Notebook, Project E-Files	5 Years from Date Sent or Received*

*Disposition is for Scanned Originals and Electronic Documents (E-Documents) stored as Project Electronic Files (E-Files). Physical copies may be destroyed sooner than the disposition date shown for E-Files.

GROUP B: DATA GENERATION AND ACQUISITION

10. SAMPLING PROCESS DESIGN

This Monitoring and Reporting Project Plan (MRPP) and Quality Assurance Project Plan (QAPP) are part of the Upper Santa Margarita Irrigated Lands Group's (USMILG) program to comply with the requirements of *Conditional Waiver No. 4 – Discharges from Agricultural and Nursery Operations* adopted by the California Regional Water Quality Control Board, Region 9, San Diego Region (RWQCB). The MRPP/QAPP is designed to support the decisions and outcomes described in Group A, Element 5 of the MRPP/QAPP:

- To provide water quality data and biological data to contribute to the characterization and assessment of surface water quality in the Santa Margarita River watershed within Riverside County, California.
- To provide data and reporting contributions geared towards the assessment of irrigated farmlands' contribution to water quality.

The following experimental design is based on the objectives of the SWMP and QAPP:

Monitoring Location Identification

Three monitoring locations within the Santa Margarita River Watershed (HUB Number: 902.00) have been identified for this project:

Description	Location	Site ID
Unknown Creek at Via De Los Robles	33° 27' 22.65" N, 117° 11' 50.24" W	UK01
Santa Gertrudis Creek at Rancho California Road	33° 32' 49.48" N, 117° 02' 38.28" W	SG02
Unknown Creek at El Prado Drive	33° 27' 52.18" N, 117° 14' 26.60" W	UK03

The monitoring locations were selected to support the decisions and outcomes described in Group A, Element 5 of the MRPP/QAPP. Key criteria for selection of the monitoring locations are summarized below:

- **Drainage Area.** The drainage area above the monitoring location includes a significant percentage of irrigated farmland. The drainage area is located in the Santa Margarita River watershed of Riverside County. The drainage area is not located above the major dams in the Santa Margarita River watershed of Riverside County**.**
- **Monitoring Suitability.** The monitoring location is suitable for collecting water quality data and biological data.
- **Safety.** The monitoring locations can be accessed safely during rain events. The ability to obtain runoff water quality samples without wading is desirable.
- **Stability.** The monitoring location is stable. Sites upstream of a bridge or permanent culvert are likely to provide a monitoring location that does not change from year to year.
- **Access.** Permission to access the monitoring locations can be obtained from the landowner. Sites located on public property are preferred, followed by sites on land owned by members of the USMILG, followed by sites where written permission can be obtained.

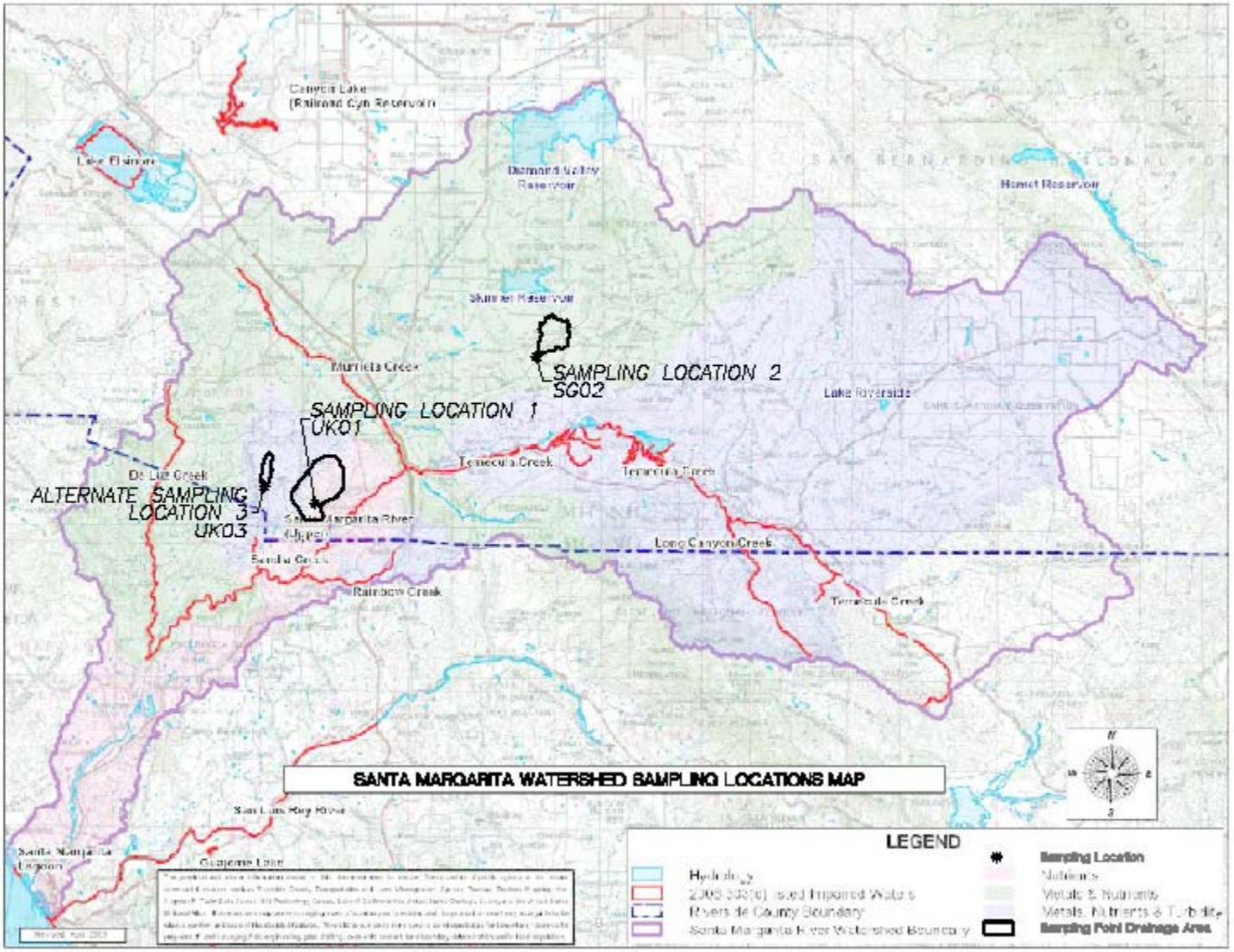


Figure 2. Monitoring locations shown within the Santa Margarita Watershed.

Site ID UK01

The first site is located at an existing unnamed creek running parallel to Via De Los Robles Road, southwest of Via Vaquero Road, within the City of Temecula and southwest Riverside County (see Figure 3). The sampling site can be easily accessed from the existing paved roadway. The existing creek flows southerly, passing under an existing dirt road way, which provides access to an existing drainage culvert that will be used to obtain grab samples (upstream side) (see Figure 4). The estimated drainage catchment area for this site is approximately 1,200 acres, based on review of U.S. Geological Survey topographic maps and has not been field verified to account for changes resulting from grading, development, road improvements, and drainage improvements (see Figure 5). Land use in the drainage area includes irrigated citrus and avocado land, open space, roadways, and rural residential (preliminary assessment).

Figure 3. Site ID UK01 Location

Figure 4. Site ID UK01 Grab Sample Location

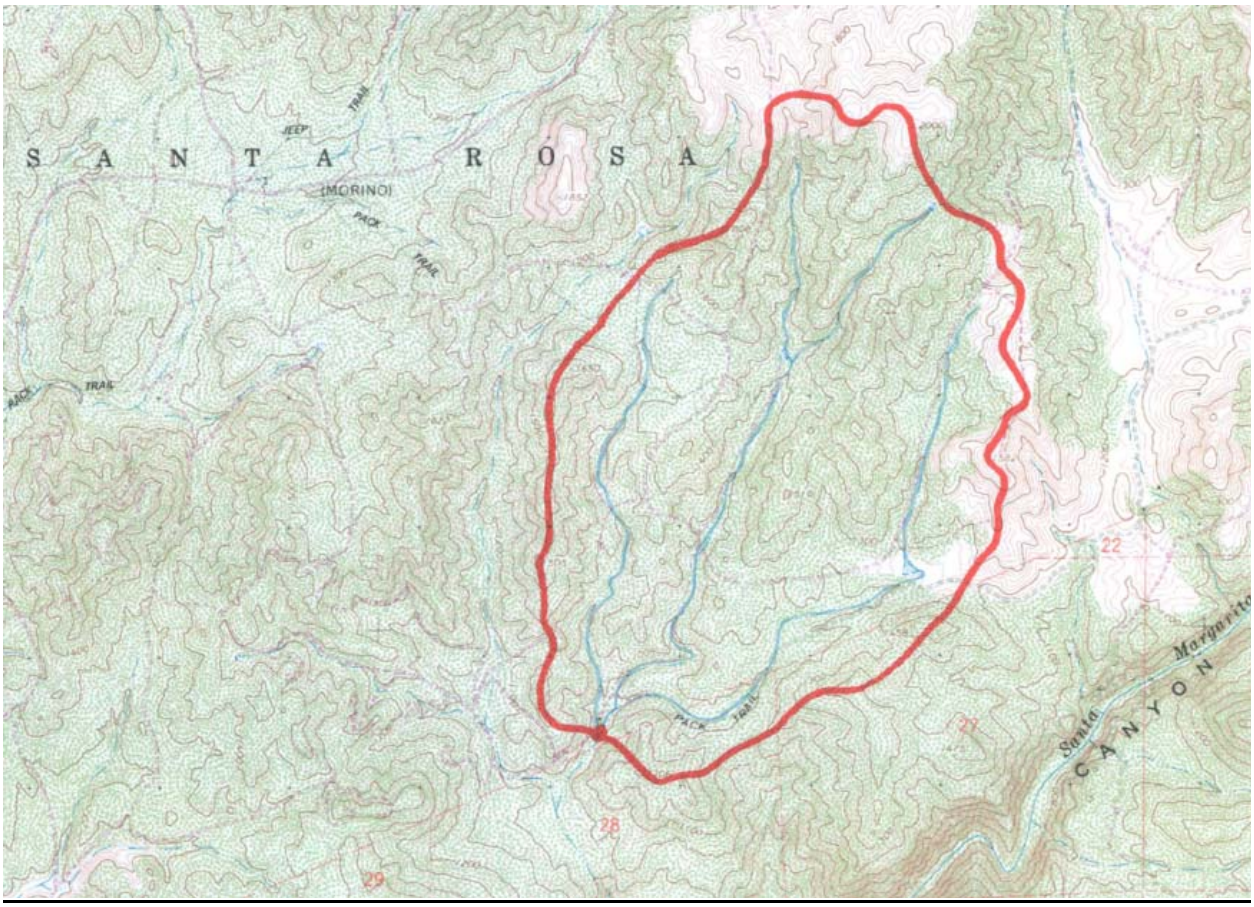


Figure 5. Site ID UK01 Watershed Delineation

Site ID SG02

The second site is located in the Santa Gertrudis Creek along Rancho California Road, approximately 300' north of Loma Ventosa Lane, in the City of Temecula and southwest Riverside County (see Figure 6). The sampling site is easily accessed from the existing paved roadway. The creek flows southerly, passing under Rancho California Road, which provides access to the existing drainage culverts that will be used to obtain grab samples (upstream side) (see Figure 7). The estimated drainage catchment area for this site is approximately 1,000 acres based on review of U.S. Geological Survey topographic maps and has not been field verified to account for changes resulting from grading, development, road improvements, and drainage improvements (see Figure 8). Land uses in the drainage area includes irrigated vineyards and olives, open space, roadways, commercial, and rural residential (preliminary assessment).

Figure 6. Site ID SG02 Location

Figure 7. Site ID SG02 Grab Sample Location

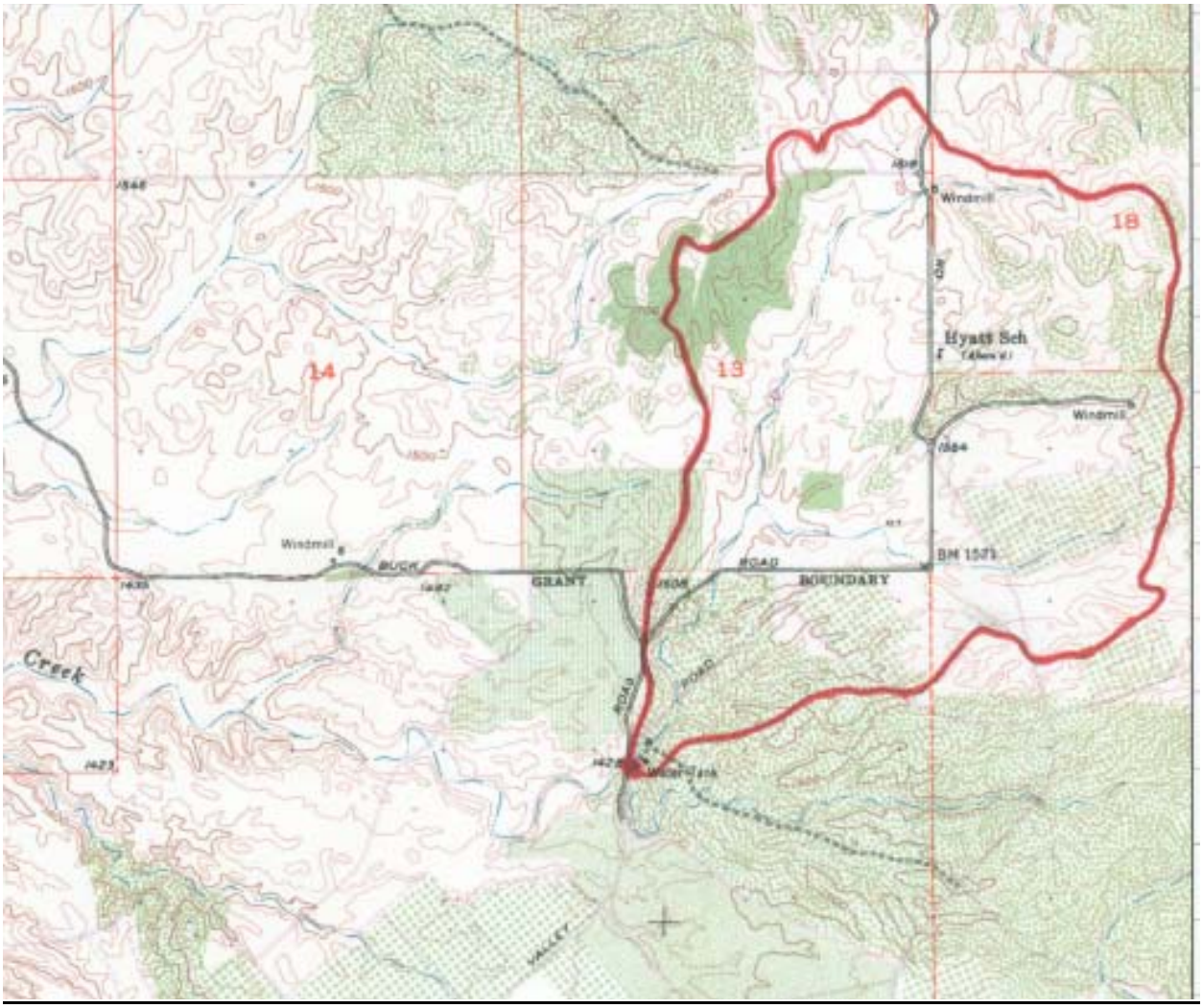


Figure 8. Site ID SG02 Watershed Delineation

Site ID UK03

The third site is located at an existing unnamed creek which intersects El Prado Road, north of Sandia Creek Drive, within the City of Temecula and southwest Riverside County (see Figure 9). The sampling site can be easily accessed from the existing paved roadway. The existing creek flows westerly, passing under El Prado Road, which provides access to existing drainage culverts that will be used to obtain grab samples (upstream side) (see Figure 10). The estimated drainage catchment area for this site is approximately 3,200 acres based on review of U.S. Geological Survey topographic maps and has not been field verified to account for changes resulting from grading, development, road improvements, and drainage improvements (see Figure 11). Land uses in the drainage area include irrigated avocado and citrus, open space, roadways, and rural residential.

Figure 9. Site ID UK03 Location

Figure 10. Site ID UK03 Grab Sample Location

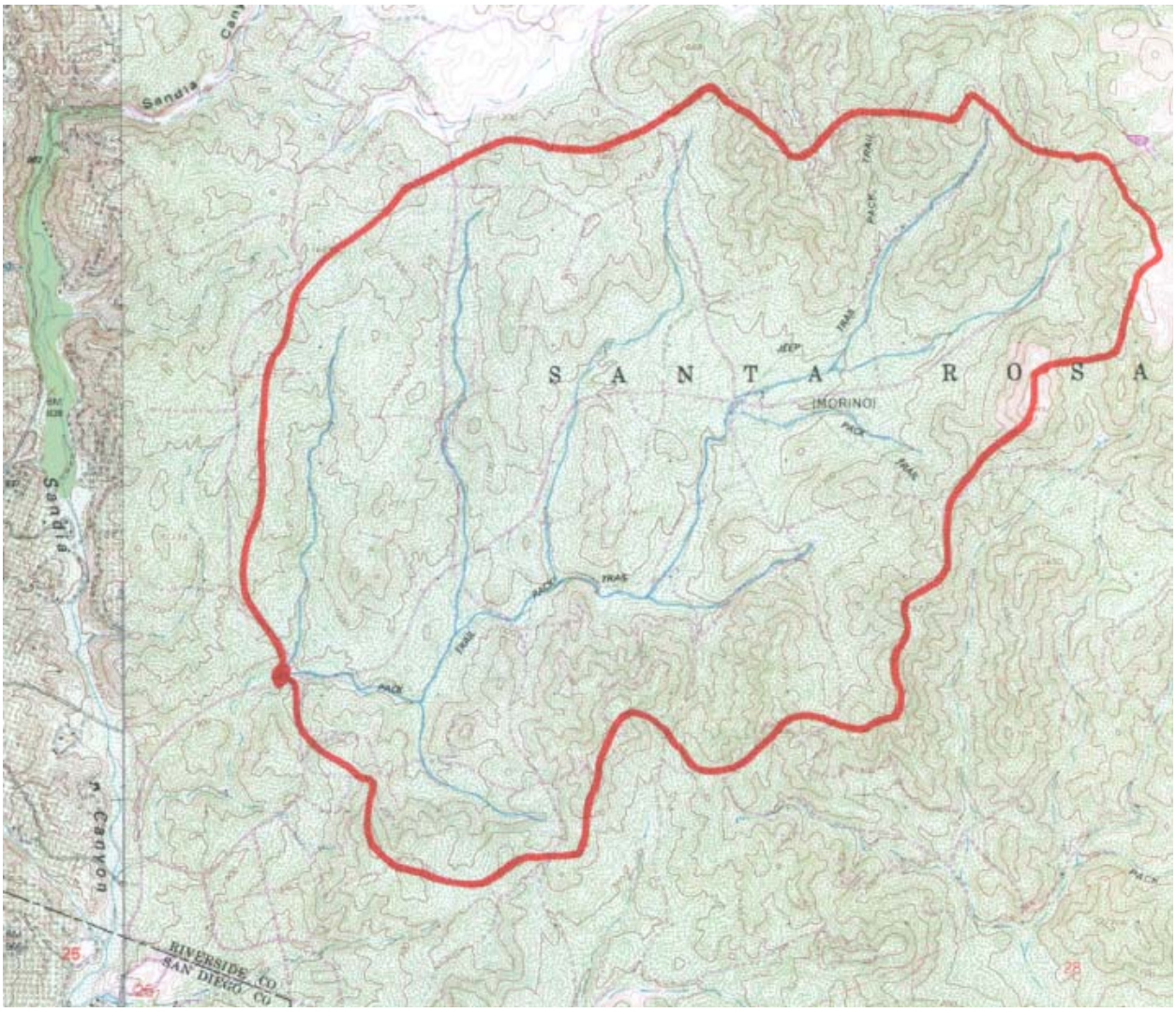


Figure 11. Site ID UK03 Watershed Delineation

Monitoring Schedule

Monitoring activities conducted under this MRPP/QAPP will be conducted at two of the three sites identified for monitoring above. The priority of monitoring at the sites shall be as follows:

- First - Primary Site: UK01
- Second - Primary Site SG02
- Third - Alternate Site: UK03

Site UK03 will be used as an alternate site in the event that either of the two primary sites become permanently inaccessible due to access or safety reasons. In the event that a sampling location becomes permanently inaccessible, the sampling team will reassess the sampling locations as defined herein the MRPP/QAPP accordingly.

Monitoring at these sites will be classified into three categories: water quality sampling, bed sediment sampling, and bioassessment sampling and field procedures. A breakdown of the parameters that will be monitored under each category is provided in Group B, Element 11 of this MRPP/QAPP.

Water Quality Sampling

Wet Season Sampling

Beginning in the 2012-2013 wet season (Nov 1 to April 30), sampling conducted under this category will be collected twice per wet season at two site locations. This sampling will occur within 48 hours following a storm event forecasted to produce at least 0.75 inches of rain within a 24-hour period. Throughout the season, weather will be continuously monitored using information obtained from the National Weather Service. This sampling category will provide a total number of two sampling events at two sites per season, for a total of four sets of sample data.

Sampling mobilization triggered by the 0.75 inches in a 24-hour period will only be conducted if the "lead" sampler concludes that there is sufficient flow at the individual sites to obtain satisfactory samples. If the "lead" sampler determines that there is not sufficient flow present at any site, no sampling will occur for this event and field documentation shall indicate why no sampling occurred.

Dry Season Sampling

Beginning in the 2013 dry weather season (May 1st to September 1st), sampling conducted under this category will be collected once per dry weather season at two site locations. Dry season sampling will be coordinated with the Bioassessment monitoring in order to meet the required water chemistry analysis. Specific timing of when sampling and field measurements are to occur will be determined by the Project Manager and coordinated with the sampling team. In the event that ephemeral and intermittent streams are dry during the season, the sampling team will document the reason that no sampling occurred for each proposed sampling event. This sampling category will provide a total number of one sampling event at two sites per season, for a total of two sets of sample data.

Bed Sediment Sampling

Beginning in the 2013-2014 wet season (Nov 1 to April 30), sampling conducted under this category will be collected once per wet season at two site locations. This sampling will only occur during the first storm event that is actually sampled for water quality based on that criteria. Sampling teams will ensure to complete water quality sampling collection prior to obtaining sediment bed samples. This sampling category will provide a total number of one sampling event at two sites per season, for a total of two sets of sample data.

Bioassessment Sampling and Field Procedures

Beginning in 2013, sampling conducted under this category will be collected during the watershed's ecoregion index period (May 1 to September 1) and includes one sampling and field measurement event per index period. Specific timing of when sampling and field measurements are to occur will be determined by the Project Manager and coordinated with the sampling team. The goal of the timing of specific sampling and field measurement timing will be to conduct the activities when waterbody levels are at base flows, as higher flows may affect the local biological

community. This sampling and field measurement category will provide a total of one bioassessment data set per index period and will be reported with the following wet season's data. For example, the bioassessment data collected in 2013 will be reported with the 2012-2013 wet season data.

Table 7 summarizes the overall sampling effort to be conducted per this MRPP/QAPP.

Table 7. (Element 10) Compliance sampling schedule.

Reporting Year	Water Quality Sampling					Bioassessment[2]
	Wet Season		Dry Season	Stream Bed Sediment Samples[1]		
	Storm Event 1	Storm Event 2	Dry Season Event 1	Storm Event 1	Storm Event 2	Index Period
2012-2013[3]	L_1, L_2	L_1, L_2	L_1	--	--	L_1
2013-2014	L_1, L_2	L_1, L_2	L_1, L_2	L_1, L_2	--	L_2
2014-2015	L_1, L_2	L_1, L_2	L_1, L_2	L_1, L_2	--	L_1
2015-2016	L_1, L_2	L_1, L_2	L_1, L_2	L_1, L_2	--	L_2
2016-2017	L_1, L_2	L_1, L_2	L_1, L_2	L_1, L_2	--	L_1
X[4]	L_1, L_2	L_1, L_2	L_1, L_2	L_1, L_2	--	L_2

Note 1: Includes monitoring for toxicity (Amphipod Hyalella) and pesticides/herbicides
Note 2: Includes Algae and Benthic Macroinvertebrate Bioassessments
Note 3: Water quality samples obtained in the 2012-2013 sampling season will not be analyzed for organochlorine pesticides, organophosphate, or glyphosates.
Note 4: Subsequent years of monitoring will be conducted in the pattern demonstrated above.
L_1: Sampling Location 1 (UK01)
L_2: Sampling Location 2 (SG02)
L_3: Alternate Sampling Location (UK03) may be used in substitute, if necessary.

Safety

Care must be exercised in selecting representative sampling locations and in sample collection to ensure the safety of the sampling team. Avoid entering a water body for sampling if possible. Sampling personnel will utilize the following safety precautions to avoid potential safety hazards:

- Be alert for the presence of snakes, rodents, insects, and poisonous plants and avoid contact. Long pants are recommended for all field crews
- Be aware of trip and slip hazards.
- Obey all caution and danger signage.
- Use proper lifting techniques as necessary for lifting heavy objects.
- Be prepared for potential rough terrain. Wear boots with non-slip soles. Use appropriate caution when walking in rough terrain. Two person-sampling crews are recommended when working from or descending steep banks where the water is greater than knee deep.
- Be prepared for potential weather hazards. Observe weather conditions, avoid lightning strike prone features, wear appropriate clothing, and be aware of flash flood dangers.
- Reagents, preservatives - Wear appropriate personal protective equipment (PPE) when using reagents or preservatives.

11. SAMPLING METHODS

Sampling methods for water, sediment, algae and BMI samples are provided Appendix B.

All Sample Types Except Algal and BMI Samples: *Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment in the Surface Water Ambient Monitoring Program (SWAMP).*[7]

Algae Samples: *Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California.*[8]

BMI Samples: *Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California.*[9]

Water Quality Sampling (Wet Season and Dry Season)

Water Quality samples collected for analyzing the constituents listed below will be sub-surface grab samples collected using techniques that minimize sample contamination and will be conducted in conformance with the procedures specified within this QAPP. Samples will be collected at the sampling site locations specified in the Sampling Process Design and shall be obtained from the centroid of the stream flow, whenever feasible, based on depth of the sampled stream. Water quality samples collected for analysis of the constituents listed below will be collected into appropriate pre-cleaned containers. If at any time the sampling team suspects that the sample container has possibly been contaminated, a fresh sample shall be collected into a new sample container.

- Alkalinity (as $CaCO_3$)
- Ammonia (as N)
- Ammonium (as N)
- $CaCO_3$ (Hardness)
- Carbon (Particulate)**
- Chloride
- Dissolved Organic Carbon
- Glyphosate*
- Nitrate as Nitrogen (NO_3)
- Nitrite as Nitrogen (NO_2)
- Nitrogen Total
- Nitrogen (Particulate)**
- Organochlorine pesticides*
- Organophosphate*
- Orthophosphate (Total, as P)
- Phosphorus (Particulate)**
- Phosphorus (Dissolved)**
- Phosphorus (Total) **
- pH
- Specific conductivity (EC)
- Sulfate
- Temperature
- TDS
- TKN (Dissolved)**
- TKN (Total)**
- TSS
- Turbidity
- Flow velocity and Discharge

**Constituent not slated for analysis in 2012-2013*

***If SWAMP testing protocols become available for the particulate constituents listed above, the particulates will be tested for utilizing those protocols. However, until such time, alternatively sample*

[7] California Department of Fish and Game, *Marine Pollution Studies Laboratory, Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program(SWAMP)*, October 15, 2007.

[8] Fetscher, A. Elizabeth, Southern California Coastal Water Research Project; Busse, Lilian, San Diego Regional Water Quality Control Board; and Ode, Peter R., California Department of Fish and Game: *Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California*, June 2009 with updates May 2010.

[9] Ode, Peter R., California Department of Fish and Game, *Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California*, February 2007.

and analyze for TKN (dissolved and total) and P (dissolved and total).

Sediment Bed Sampling

Monitoring of pesticides within sediment and sediment toxicity to the amphipod *Hyalella Azteca (10-day chronic)* will be conducted by obtaining bed sediment samples per the SOPs. For sediment toxicity testing, analysis will be conducted per the SOP provided in Appendix B for *Methods for Measuring Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates* (EPA 600\R-99/064 March 2000)[10], or another SWAMP comparable SOP utilized by the actual laboratory performing the test. All bed sediment samples shall be collected after any required water chemistry samples are collected, in areas where the sediment remains undisturbed by sampling activities. Sediment testing parameters per this QAPP are listed below:

- Organochlorine pesticides
- Pyrethroids
- Amphipod Hyalella (10 day)

Bioassessment Sampling and Field Procedures

Bioassessment monitoring conducted under this Plan includes sampling of stream algae and benthic invertebrates utilizing the Reachwide Benthos Method (RWB) only, and will be conducted in conformance with the procedures specified within this QAPP. The targeted riffle method specified in the SOPs will not be utilized per this QAPP. Bioassessment monitoring and sampling will be conducted during the index period of May 1 to September 1, and will only occur when the streams are at base flow, as higher flows can affect the local biological community.

Bioassessment monitoring includes the measurement of the ambient water chemistry and measurements of the surrounding physical habitat. To ensure measurements are taken prior to any sampling disturbance that may affect the sample result, all bioassessment monitoring activities will be conducted in the order below, as discussed in the SOP:

1. Sampling of ambient water chemistry (performed under Dry Season Sampling)
2. BMI sampling
3. Algae Sampling
4. Physical habitat data collection

If a sample collected is determined by the sampling team to be unusable due to contamination or sampling collection error, another sample may be collected only if it may be done without comprising the quality due to prior sampling activity disturbances. Samples and/or measurements collected during the bioassessment monitoring are as follows:

- Alkalinity (as $CaCO_3$)*
- Ammonia (as N)*
- Ammonium (as N)*
- Chloride*
- Dissolved Organic Compound*
- Dissolved Oxygen*
- Nitrate as Nitrogen (NO_3)*
- Nitrite as Nitrogen (NO_2)*
- Nitrogen Total*
- Orthophosphate (Total, as P)*
- pH
- Algal sampling for Taxonomic ID
- Algal sampling for Biomass Assessment
- BMI Sampling for Taxonomic ID
- Wetted width
- Bankfull dimensions
- Depth and pebble county + CPOM
- Percent Algal cover
- Cobble embeddedness
- Canopy cover over stream
- Human Influence
- Bank stability

[10] United States Environmental Protection Agency, Methods for Measuring Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates, Second Edition, March 2000.

- Phosphorus (Total)*
- Specific conductivity (EC)*
- Temperature
- Days of algal accrual
- Turbidity*
- Flow Habitat Delineation
- Flow velocity and discharge
- Unshaded solar radiation
- Photo documentation

Note - Constituents to be collected and analyzed under Dry Season Sampling*

Sample Integrity Procedures

For all sampling and monitoring performed under this QAPP, the sampling team shall maintain sample integrity and prevent cross-contamination by ensuring the following:

- Wear a clean pair of powder-free nitrile gloves prior to the collection and handling of each sample at each location (1-pair recommended, 2-pair preferred);
- Not contaminate the inside of the sample bottle or lids by allowing it to come into contact with any material other than the water sample;
- Discard sample bottles or sample lids that have been dropped onto the ground prior to sample collection;
- Not leave the cooler lid open for an extended period of time once samples are placed inside;
- Not sample near a running vehicle where exhaust fumes may impact the sample;
- Not touch the exposed end of a sampling tube, if applicable;
- Avoid allowing rainwater to drip from rain gear or other surfaces into sample bottles;
- Not eat, smoke, or drink during sample collection, nor sneeze or cough in the direction of an open sample bottle;
- Minimize the exposure of the samples to direct sunlight, as sunlight may cause biochemical transformation of the sample;
- Decontaminate sampling equipment prior to sample collection using a tri-sodium phosphate (TSP) solution water wash and triple rinse with distilled or de-ionized water; and
- Dispose of decontamination water/soaps appropriately (i.e., do not discharge to the storm drain system or receiving water).

Corrective Actions

The sampling team has the primary responsibility of responding to failures in the sampling or measurement activities. If monitoring equipment fails, the sampling team will document the failure on the sampling field logs and will not record data values for any variables in question.

Failure to collect a sample due to safety concerns will be documented by the sampling team on the sampling field logs. The sampling team will then notify the Project Manager, who will determine the actions to be taken in order to obtain a replacement sample, if feasible. All corrective actions taken will be documented on the sampling field logs.

12. SAMPLE HANDLING AND CUSTODY

Sample Handling

All samples will be handled, prepared, transported and stored in a manner so as to minimize bulk loss, analyte loss, contamination or biological degradation. Proper sample handling requirements for water, sediment, and biological samples are listed in Table 8 on the following page. If holding times cannot be met, supporting scientific literature must be provided as justification. For more information refer to the SWAMP QAPrP Element B3 and consult with State Board QA Officer, Bill Ray, SWRCB QA Officer, (916) 341-5583, bray@waterboards.ca.gov.

The contracted laboratory will be responsible for providing appropriate sample containers and, where necessary, preservatives for each analysis. As additional volumes are necessary for laboratory QA/QC, sample containers preferably will provide for at least twice the volume necessary to perform the analysis. Based on some stringent holding times, close coordination will be required between the laboratory and sampling team to make sure analyses are completed within holding times.

Sample Bottle Identification

Sample bottles should be pre-labeled to the extent possible before each stormwater monitoring event. Pre-labeling bottles simplifies field activities and leaves only date, time, sample number, and sampling personnel names to be filled out in the field. Each sample collected will be labeled with the following information:

- Project Name
- Site ID (refer to Element 10)
- Sample ID Number
- Matrix/Sample Type (water/sediment/habitat)
- Constituent(s)
- Analysis Type
- Collected by
- Preservative

Field samples, field blanks, and field duplicate samples will be labeled as described below. These samples will be labeled, recorded on the chain-of-custody form, and then transported to the analytical laboratory. Each stormwater sample collected will receive a unique alphanumeric code (sample I.D. number) for tracking. This code will be standard for all samples and contain information as it relates to the site, date of sample collection, and type of sample. The required sample identification numbers are listed below along with an example for the first sampling event at a site.

- Site ID (refer to Element 10)
- Sample ID: 0612051440 = Year, month, day, and military time (YYMMDDTTTT)
- #00 = Sample type
- 000 = Primary sample
- 500 = Field duplicate sample
- 600 = Field blank

Field Sample: XX0X-0612051440-000
Field Duplicate: XX0X-0612051440-500 (or as otherwise specified by the Contracted Laboratory)
Sample Field Blank: XX0X-0612051440-600 (or as otherwise specified by the Contracted Laboratory)

Matrix spike/matrix spike duplicate (MS/MSD) samples and samples identified for laboratory replicate analysis will be clearly noted on the chain-of-custody form. No special sample identification numbers are required. Descriptions and required frequencies of these QA/QC samples are presented in Table 12.

Laboratory Chain of Custody

All samples to be analyzed by a laboratory will be accompanied by a Chain of Custody (COC) form provided by the contracted laboratory. Proper transferring of responsibility must be recorded on the COC signature fields, the final signature will be of the receiving lab personnel. COC procedures will be strictly adhered to for QA/QC purposes. Laboratories shall maintain custody logs sufficient to track each sample submitted and to analyze or preserve each sample within specified holding times. An example COC is provided in Appendix D.

A sample is considered under custody if:

- it is in actual possession;
- it is in view after in physical possession;
- it is placed in a secure area (accessible by or under the scrutiny of authorized personnel only after in possession)

Field Log

Field crews shall be required to keep a field log for each sampling event. The following items should be recorded in the field log for each sampling event:

- time of sample collection;
- sample ID numbers, including etched bottle ID numbers for Teflon™ mercury sample containers and unique IDs for any replicate or blank samples;
- the results of any field measurements (temperature, D.O., pH, conductivity, turbidity) and the time that measurements were made;
- qualitative descriptions of relevant water conditions (e.g. color, flow level, clarity) or weather (e.g. wind, rain) at the time of sample collection;
- a description of any unusual occurrences associated with the sampling event, particularly those that may affect sample or data quality.

The field crews shall have custody of samples during field sampling. Chain of custody forms will accompany all samples during shipment to the Contracted Laboratory. All water quality samples will be transported to the analytical laboratory directly by the field crew or by overnight courier.

Table 8. (Element 12) Sample handling and custody.

Parameter	Container	Volume	Initial Preservation	Holding Time
Conventional Water Quality Constituents				
Alkalinity (as $CaCO_3$)	Polyethylene Bottles	300 mL	Cool to 6 °C and store in the dark	14 days
Ammonia (as N)	Polyethylene Bottles	500 mL	Cool to 6 °C and store in the dark. Samples may be preserved with 2 mL of $H2SO_4$ per L	48 hours; 28 days if acidified
Ammonium (as N)	Polyethylene Bottles	500 mL	Cool to 6 °C and store in the dark. Samples may be preserved with 2 mL of $H2SO_4$ per L	48 hours; 28 days if acidified
$CaCO_3$ (Hardness)	Polyethylene Bottles	300 mL	Cool to 6 °C and store in the dark. Acidify with HNO_3 to pH<2	6 months
Carbon (Particulate)[5]	PENDING[5]	PENDING[5]	PENDING[5]	PENDING[5]
Chloride	Polyethylene Bottles	300 mL	Cool to 6 °C and store in the dark	28 days
Dissolved Organic Carbon	40-mL glass vial	40 mL	Cool to 6 °C and store in the dark	28 days
Nitrate as Nitrogen (NO_3)	Polyethylene Bottles	150 mL	Cool to 6 °C and store in the dark. Acidify with H_2SO_4 to pH<2	48 hours or 28 days if acidified
Nitrite as Nitrogen (NO_2)	Polyethylene Bottles	150 mL	Cool to 6 °C and store in the dark	48 hours
Nitrogen Total	Polyethylene Bottles	300 mL	Cool to 6 °C and store in the dark	48 hours unless calculated from nitrate + nitrite (as N) and nitrite (as N) analyses
Nitrogen (Particulate) [5]	PENDING[5]	PENDING[5]	PENDING[5]	PENDING[5]

Parameter	Container	Volume	Initial Preservation	Holding Time
Orthophosphate (Total, as P)	Polyethylene Bottles	150 mL	Cool to 6 ˚C and store in the dark	48 hours
Orthophosphate (Dissolved, as P) *Soluble Reactive Phosphorus*	Polyethylene Bottles	150 mL	Filter within 15 minutes of collection; Cool to 6 ˚C and store in the dark	48 hours
pH	Field Measurement	Field Measurement	Field Measurement	15 mins Field Measurement
Phosphorus (Dissolved as P)[5]	Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	28 days
Phosphorus (Particulate)[5]	PENDING[5]	PENDING[5]	PENDING[5]	PENDING[5]
Phosphorus (Total as P)[5]	Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	28 days
Specific conductivity (EC)[4]	Polyethylene Bottles	500 mL	Cool to 6 ˚C and store in the dark If analysis is not completed within 24 hours of sample collection, sample should be filtered through a 0.45 micron filter and stored in the dark at 6 °C.	28 days
Sulfate	Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	28 days
Temperature	Field Measurement	Field Measurement	Field Measurement	Field Measurement
TDS	Polyethylene Bottles*	1000 mL	Cool to 6 ˚C and store in the dark	7 days
TKN Dissolved (as N)[5]	Polyethylene Bottles	500 mL	Cool to 6 ˚C and store in the dark. Acidify with H_2SO_4 to pH<2	28 days
TKN Total (as N)[5]	Polyethylene Bottles	500 mL	Cool to 6 ˚C and store in the dark. Acidify with H_2SO_4 to pH<2	28 days
TSS	500-mL amber glass jar or Polyethylene Bottles*	1000 mL	Refrigeration or icing to 6°C, to minimize microbiological decomposition of solids, is recommended.	7 days
Turbidity[4]	Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	48 hours
Non-Conventional Water Quality Constituents (Pesticides and Herbicides)				
Glyphosate[1] (water)	1000-mL I-Chem 200-Series amber glass bottle, with Teflon lid-liner	1000 mL/per individual analyses (QC samples or other analytes require additional sample bottles)	Cool to 6 °C in the dark.	6 months at -20 °C; Samples must be analyzed within 7 days of collection or thawing
Organochlorine[1, 2] Pesticides (sediment)	Pre-cleaned 250-mL I-Chem 300 Series amber glass jar with Teflon lid liner	500 g (two jars)	Cool to 6 °C in the dark	1 year at -20 °C; Samples must be extracted within 14 days of collection or thawing and analyzed within 40 days of extraction.
Organochlorine[1, 2] Pesticides (water)	1000-mL I-Chem 200-Series amber glass bottle, with Teflon lid-liner	1000 mL/per individual analyses (QC samples or other analytes require additional sample bottles)	Cool to ≤6 °C in the dark; pH 5-9.	Samples must be extracted within 7 days of collection and analyzed within 40 days of extraction.
Organophosphate[1, 2] (water):	1000-mL I-Chem 200-Series amber glass bottle, with Teflon lid-liner	1000 mL/per individual analyses (QC samples or other analytes require additional sample bottles)	Cool to ≤6 °C in the dark; pH 5-9.	Samples must be extracted within 7 days of collection and analyzed within 40 days of extraction.
Pyrethroids[1, 2] (sediment)	1000-mL I-Chem 200-Series amber glass bottle, with Teflon lid-liner	1000 mL/per individual analyses (QC samples or other analytes require additional sample bottles)	Cool to 6 °C in the dark.	Samples must be extracted within 7 days of collection and analyzed within 40 days of extraction.
Biological and Bioassessment Parameters				
Algae Diatoms	Refer to Appendix B for SOPs for Collecting Stream Algae Samples			
Algae Soft	Refer to Appendix B for SOPs for Collecting Stream Algae Samples			

Parameter	Container	Volume	Initial Preservation	Holding Time
Alkalinity (as $CaCO_3$)	Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	14 days
Ammonia (as N)	Polyethylene Bottles	500 mL	Cool to 6 ˚C and store in the dark. Samples may be preserved with 2 mL of $H2SO_4$ per L	48 hours; 28 days if acidified
Ammonium (as N)	Polyethylene Bottles	500 mL	Cool to 6 ˚C and store in the dark. Samples may be preserved with 2 mL of $H2SO_4$ per L	48 hours; 28 days if acidified
Amphipod 10-d Hyalella Chronic1	Refer to Appendix B for Sample SOPs for Freshwater Sediment Toxicity Tests using Hyalella[3]			
Ash Free Dry Mass	Refer to Appendix B for SOPs for Collecting Stream Algae Samples			
Benthic Substrate	Plastic or Glass Bottles	Variable (Refer to SOPs Collecting Benthic Macroinvertebrate Samples)	70% ethyl alcohol, OR 70% isopropyl alcohol, OR Add formalin to produce a 5-10% formalin solution. Store in dark and away from extremes of hot and cold.	5 years
Chloride	Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	28 days
Chlorophyll-a	Please refer to method requirements	500 mL	Centrifuge or filter as soon as possible after collection. If processing must be delayed, hold samples on ice or at 6 ˚C and store in the dark.	Samples must be frozen or analyzed within 4 hours of collection. Filters can be stored frozen for 28 days.
Dissolved Organic Carbon	40-mL glass vial	40 mL	Cool to 6 ˚C and store in the dark	28 days
Dissolved Oxygen[4]	Amber glass bottle or Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	8 hours
Nitrate as Nitrogen (NO_3)	Polyethylene Bottles	150 mL	Cool to 6 ˚C and store in the dark. Acidify with H_2SO_4 to pH<2	48 hours or 28 days if acidified
Nitrite as Nitrogen (NO_2)	Polyethylene Bottles	150 mL	Cool to 6 ˚C and store in the dark	48 hours
Orthophosphate (Dissolved, as P) *Soluble Reactive Phosphorus*	Polyethylene Bottles	150 mL	Filter within 15 minutes of collection; Cool to 6 ˚C and store in the dark	48 hours
pH	Field Measurement	Field Measurement	Field Measurement	15 mins Field Measurement
Phosphorus (Total as P)	Polyethylene Bottles	300 mL	Cool to 6 ˚C and store in the dark	28 days
Specific Conductance[4]	Polyethylene Bottles	500 mL	Cool to 6 ˚C and store in the dark If analysis is not completed within 24 hours of sample collection, sample should be filtered through a 0.45 micron filter and stored in the dark at 6 °C.	28 days
Temperature	Field Measurement	Field Measurement	Field Measurement	Field Measurement

Note 1: Constituent will not to be monitored during first monitoring year.
Note 2: Appendix E includes a comprehensive list of this family of pesticides/herbicides that may be detected in samples.
Note 3: Standard Operating Procedures will be pursuant to the Contracted Laboratory.
Note 4: Constituent may be alternatively analyzed in laboratory.
Note 5: If approved SWAMP procedures are unavailable for analysis of constituents in particulate phase, then alternatively sample and analyze for TKN (dissolved and total) and P (dissolved and total). If SWAMP sampling and testing procedures become available for the particulate constituents listed above, then particulates will be handled according to those protocols.

13. ANALYTICAL METHODS

Methods

Analytical methods used for the USMILG monitoring program and SJRIPP Monitoring Program will follow the State of California and the U.S. EPA approved standard laboratory and field methods for all applicable water quality analyses as summarized in the following tables.

Instrumentation

Refer to Element 15 - Instrument/Equipment Testing, Inspection, and Maintenance for a summary of field and laboratory equipment to be used.

Sample Disposal

Sampled analyzed in the field will not require disposal. Disposal of water, sediment, and specimen samples analyzed by the Contracted Laboratory will be disposed of in accordance with their SOPs. The Contracted Laboratory's SOPs will be provided in Appendix B.

Laboratory Turnaround

Typical laboratory turnaround time is general two to three weeks from the date samples are submitted for analysis.

Corrective Actions

Refer to Element 14 - Quality Control for a summary of QC activities and corrective actions.

Table 9. (Element 13) Field analytical methods.

Analyte	Laboratory / Organization	Project Action Limit (units, wet or dry weight)[1]	Reporting Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits[2]	
				Standard Operating Procedure	Modified for Method yes/no	MDLs	Method[2]
Conventional Water Quality Constituents							
pH	Contracted Storm Sampler	--	NA	SOP[4]	No	--	--
Specific conductivity[3]	Contracted Storm Sampler	--	2.5 µS/cm	SOP[4]	No	--	--
Temperature	Contracted Storm Sampler	--	N/A	SOP[4]	No	--	--
Turbidity[3]	Contracted Storm Sampler	--	0.5 NTU	SOP[4]	No	--	--
Non-Conventional Water Quality Constituents (Pesticides and Herbicides)							
None	--	--	--	--	--	--	--
Biological and Bioassessment Parameters							
Algae Diatoms	Contracted Biological Sampler	--	N/A	SOP[4]	No	--	--
Algae Soft	Contracted Biological Sampler	--	N/A	SOP[4]	No	--	--
Benthic Substrate	Contracted Biological Sampler	--	N/A	SOP[4]	No	--	--
Dissolved Oxygen[3]	Contracted Biological Sampler	--	N/A	SOP[4]	No	--	--
pH	Contracted Biological Sampler	--	N/A	SOP[4]	No	--	--
Specific conductivity[3]	Contracted Biological Sampler	--	2.5 µS/cm	SOP[4]	No	--	--
Temperature	Contracted Biological Sampler	--	N/A	SOP[4]	No	--	--

Analyte	Laboratory / Organization	Project Action Limit (units, wet or dry weight)[1]	Reporting Limit (units, wet or dry weight)	Analytical Method		Achievable Laboratory Limits[2]	
				Standard Operating Procedure	Modified for Method yes/no	MDLs	Method[2]
Unshaded solar radiation	Contracted Biological Sampler	--	cal/cm^2/day	--	No	--	--

Note 1: Not applicable to this Watershed Characterization and Monitoring Project.
Note 2: Not applicable to field measurement.
Note 3: Constituent may be alternatively analyzed in laboratory.
Note 4: Refer to Appendix B for the Standard Operating Procedures.
Note: N/A = Not applicable

Table 10. (Element 13) Laboratory analytical methods.

Analyte	Laboratory / Organization[1]	Project Action Limit (units, wet or dry weight)[2]	Reporting Limit (units, wet or dry weight)	Analytical Method[3]		Achievable Laboratory Limits[4]	
				Analytical Method	Modified for Method yes/no	MDLs	Method
Conventional Water Quality Constituents							
Alkalinity (as $CaCO_3$)	Contracted Laboratory	--	1 mg/L	EPA 310.1, QC 10303311A, SM 2320 B, or SM 2340 B	No	--	--
Ammonia (as N)	Contracted Laboratory	--	0.1 mg/L	EPA 350.1, EPA 350.2, EPA 350.3, QC 10107061G, SM 4500-NH3 C v18, or SM 4500-NH3 D v20,21	No	--	--
Ammonium (as N)	Contracted Laboratory	--	0.1 mg/L	EPA 350.1, EPA 350.2, EPA 350.3, QC 10107061G, SM 4500-NH3 C v18, or SM 4500-NH3 D v20,21	No	--	--
$CaCO_3$ (Hardness)	Contracted Laboratory	--	1 mg/L	EPA 200.7, QC 10301311B, SM 2340 B, or SM 2340 C	No	--	--
Carbon (Particulate)[8]	Contracted Laboratory	--	2600 mg/kg in 10 mg sample	EPA 4	No	1300 mg/kg in 10 mg sample	EPA 440
Chloride	Contracted Laboratory	--	0.25 mg/L	EPA 300.1	No	--	--
Dissolved Organic Carbon	Contracted Laboratory	--	0.6 mg/L	EPA 415.1, EPA 415.1M, SM 5310 B, or SM 5310 C	Yes	--	--
Nitrate as Nitrogen (NO_3)	Contracted Laboratory	--	0.01 mg/L	EPA 300.0, EPA 300.1, EPA 353.2, EPA 353.3, MBARI TRNo90-2, QC 10107041B, or Whitledge, et al., 1981	No	--	--

Analyte	Laboratory / Organization[1]	Project Action Limit (units, wet or dry weight)[2]	Reporting Limit (units, wet or dry weight)	Analytical Method[3]		Achievable Laboratory Limits[4]	
				Analytical Method	Modified for Method yes/no	MDLs	Method
Nitrite as Nitrogen (NO_2)	Contracted Laboratory	--	0.01 mg/L	EPA 300.0, EPA 300.1, EPA 354.1, QC 10107041B, SM 4500-NO2 B, SM 4500-NO2 BM, or Whitledge, et al., 1981	No	--	--
Nitrogen Total	Contracted Laboratory	--	0.1 mg/L	QC 10107044B,	No	--	--
Nitrogen (Particulate)[8]	Contracted Laboratory	--	165 mg/kg in 10 mg sample	EPA 4	No	80 mg/kg in 10 mg sample	EPA 440
Orthophosphate (Total, as P)	Contracted Laboratory	--	0.01 mg/L	EPA 365.2, MBARI TRNo90-2, or SM 4500-P E	No	--	--
Orthophosphate (Dissolved, as P) *Soluble Reactive Phosphorus*	Contracted Laboratory	--	0.01 mg/L	EPA 300.0, EPA 300.1, EPA 365.1M, EPA 365.2, EPA 365.3, QC 10115011M, SFSU-RTC BLAA MNo G-175-96, SM 4500-P E, or SM 4500-P EM	Yes	--	--
Phosphorus (Dissolved as P)[8]	Contracted Laboratory	--	0.03 mg/L (proposed)	EPA 365.1M, or SM 4500-P F	No	--	--
Phosphorus (Particulate)[8]	Contracted Laboratory	--	160 mg/kg in 10 mg sample	Aspila et al. 1976, EPA 365.5	No	80 mg/kg in 10 mg sample	Aspila et al. 1976, EPA 365.5
Phosphorus (Total as P)[8]	Contracted Laboratory	--	0.03 mg/L (proposed)	EPA 365.1M, or SM 4500-P F	No	--	--
Specific Conductivity[5]	Contracted Laboratory	--	2.5 µS/cm	EPA 120.1, or SM 2510 B	No	--	--
Sulfate	Contracted Laboratory	--	1.0 mg/L	EPA 300.0	No	--	--
TDS	Contracted Laboratory	--	10 mg/L	SM 2540 C	No	--	--
TKN (dissolved)[8]	Contracted Laboratory	--	0.1 mg/L	EPA 351.3	No	--	--
TKN (total)[8]	Contracted Laboratory	--	0.1 mg/L	EPA 351.3	No	--	--
TSS	Contracted Laboratory	--	0.5 mg/L	EPA 160.2, EPA 160.2M, or SM 2540 D	Yes	--	--
Turbidity[5]	Contracted Laboratory	--	0.5 ntu	EPA 180.1, or SM 2130 B	No	--	--
Non-Conventional Water Quality Constituents (Pesticides and Herbicides)							
Glyphosate[6] (water)	Contracted Laboratory	--	--[7]	EPA 547, or EPA 547M	Yes	--	--
Organochlori-ne[6] Pesticides (sediment)	Contracted Laboratory	--	--[7]	EPA SW846 Method 8081	No	--	--
Organochlori-ne[6] Pesticides (water)	Contracted Laboratory	--	--[7]	EPA SW846 Method 8081	No	--	--

Analyte	Laboratory / Organization[1]	Project Action Limit (units, wet or dry weight)[2]	Reporting Limit (units, wet or dry weight)	Analytical Method[3]		Achievable Laboratory Limits[4]	
				Analytical Method	Modified for Method yes/no	MDLs	Method
Organophosp-hate[6] (water)	Contracted Laboratory	--	--[7]	EPA SW846 Method 8141	No	--	--
Pyrethroids[6] (sediment)	Contracted Laboratory	--	--[7]	EPA 8081 P, or EPA 1660M	Yes	--	--
Biological and Bioassessment Parameters							
Alkalinity (as $CaCO_3$)	Contracted Laboratory	--	1 mg/L	EPA 310.1, QC 10303311A, SM 2320 B, or SM 2340 B	No	--	--
Ammonia (as N)	Contracted Laboratory	--	0.1 mg/L	EPA 350.1, EPA 350.2, EPA 350.3, QC 10107061G, SM 4500-NH3 C v18, or SM 4500-NH3 D v20,21	No	--	--
Ammonium (as N)	Contracted Laboratory	--	0.1 mg/L	EPA 350.1, EPA 350.2, EPA 350.3, QC 10107061G, SM 4500-NH3 C v18, or SM 4500-NH3 D v20,21	No	--	--
Amphipod 10-d Hyalella Chronic[1]	Contracted Laboratory	--	--[7]	EPA 600/R-99-064, or EPA 600/R-99-064M	Yes	--	--
Ash Free Dry Mass	Contracted Laboratory	--	N/A	WRS 73A.3	No	--	--
Chloride	Contracted Laboratory	--	0.25 mg/L	EPA 300.1	No	--	--
Chlorophyll-a	Contracted Laboratory	--	0.002 mg/L	EPA 445.0, EPA 445.0M, or SM 10200 H-2b	Yes	--	--
Dissolved Organic Carbon	Contracted Laboratory	--	0.6 mg/L	EPA 415.1, EPA 415.1M, SM 5310 B, or SM 5310 C	Yes	--	--
Dissolved Oxygen[5]	Contracted Laboratory	--	0.2 mg/L	EPA 360.2	No	--	--
Nitrate as Nitrogen (NO_3)	Contracted Laboratory	--	0.01 mg/L	EPA 300.0, EPA 300.1, EPA 353.2, EPA 353.3, MBARI TRNo90-2, QC 10107041B, or Whitledge, et al., 1981	No	--	--
Nitrite as Nitrogen (NO_2)	Contracted Laboratory	--	0.01 mg/L	EPA 300.0, EPA 300.1, EPA 354.1, QC 10107041B, SM 4500-NO2 B, SM 4500-NO2 BM, or Whitledge, et al., 1981	No	--	--

Analyte	Laboratory / Organization[1]	Project Action Limit (units, wet or dry weight)[2]	Reporting Limit (units, wet or dry weight)	Analytical Method[3]		Achievable Laboratory Limits[4]	
				Analytical Method	Modified for Method yes/no	MDLs	Method
Orthophospha-te (Dissolved, as P) *Soluble Reactive Phosphorus*	Contracted Laboratory	--	0.01 mg/L	EPA 300.0, EPA 300.1, EPA 365.1M, EPA 365.2, EPA 365.3, QC 10115011M, SFSU-RTC BLAA MNo G-175-96, SM 4500-P E, or SM 4500-P EM	Yes	--	--
Phosphorus (Total as P)	Contracted Laboratory	--	0.03 mg/L (proposed)	EPA 365.1M, or SM 4500-P F	No	--	--
Specific Conductance[5]	Contracted Laboratory	--	2.5 µS/cm	EPA 120.1, or SM 2510 B	No	--	--

Note 1: Laboratory contracted for sample analysis will be selected in accordance with the QAPP and SWMP requirements.
Note 2: Not applicable to this Watershed Characterization and Monitoring Project.
Note 3: Analytical Methods will be selected from those acceptable pursuant to the SWMP and in correlation with the Contracted laboratory.
Note 4: Achievable Laboratory Limits will be based on the Analytical Methods selected in correlation with the Contracted Laboratory.
Note 5: Constituent may be alternatively measured in field.
Note 6: Constituent will not to be monitored during first monitoring year.
Note 7: Reporting Limit to be determined by Contracted Laboratory.
Note 8: If approved SWAMP procedures are unavailable for analysis of constituents in particulate phase, then alternatively sample and analyze for TKN (dissolved and total) and P (dissolved and total). If SWAMP procedures become available for the particulate constituents listed above, then particulates will be tested for utilizing those protocols.
EPA: Environmental Protection Agency
M: Modified
MBARI: Monterey Bay Aquarium Research Institute
QC: Lachat QuikChem Flow Injection Analyzer Method
SM: Standard Methods for the Examination of Water and Wastewater, *20th edition*.
WRS: Willamette Research Station Analytical Laboratory, Corvallis, OR
Aspila, K.I., H. Agemian, and A.S.Y. Chau. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. Analyst. 101: 187-197.

14. QUALITY CONTROL

All laboratories contracted by USMILG will follow quality assurance and quality control programs in accordance with guidelines established by the State of California SWMP and the U.S. EPA. Laboratories are required to submit a copy of their SOPs for laboratory quality control to the USMILG's Administrator and Project Manager for review and approval (see Appendices to this QAPP for the SOPs of laboratories being used by this project). Table 11 summarizes Sampling (Field) QC activities. Table 12 summarizes Analytical QC activities.

All laboratory data will be entered into the database pursuant to Element 19 herein, and will be filed in the project archives maintained by USMILG along with related materials such as field forms, chain of custody forms, photographs, correspondence, etc. The Project Manager and USMILG's Administrator will review all laboratory data and will request additional re-analysis as warranted (refer to Group C – Assessment and Oversight).

Table 11. (Element 14) Sampling (Field) QC.

Matrix: **Samplewater**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Conventional Constituents (Field)**		
Analytical Method/SOP Reference: See Group B, Table 9		
# Sample locations: 2		
Field QC[1, 2]	**Frequency/Number per sampling event**	**Acceptance Limits**
Field Blanks	--[3]	<MDL (for analyte of interest)
Cooler Temperature	4° C	4° C
Field Duplicates[4]	5% of total number of samples per sample event	RPD <25%
Note 1: Equipment Blanks will not be used, as they are not applicable to grab samples. Note 2: Per SOPs, Trip Blanks are not required since not sampling for volatile organic compounds (VOCs) via SM 8260. Note 3: Water DOC must have Field Blanks analyzed at 5% rate. For other analytes of interest conduct random performance evaluation during field audit. If acceptable performance, no field blanks required until next field audit. If non-acceptable, 5% field blanks must be conducted until next field audit. Note 4: Field Duplicates will be used in lieu of Collocated Samples.		
Matrix: **Samplewater**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Non-Conventional Constituents – Pesticides and Herbicides (Field)**		
Analytical Method/SOP Reference: See Group B, Table 9		
# Sample locations: 2		
Field QC[1, 2]	**Frequency/Number per sampling event**	**Acceptance Limits**
Field Blanks	--[3]	<MDL (for analyte of interest)
Cooler Temperature	4° C	4° C
Field Duplicates[4]	5% of total number of samples per sample event	RPD <25%
Note 1: Equipment Blanks will not be used, as they are not applicable to grab samples. Note 2: Per SOPs, Trip Blanks are not required since not sampling for volatile organic compounds (VOCs) via SM 8260. Note 3: For other analytes of interest conduct random performance evaluation during field audit. If acceptable performance, no field blanks required until next field audit. If non-acceptable, 5% field blanks must be conducted until next field audit. Note 4: Field Duplicates will be used in lieu of Collocated Samples.		
Matrix: **Sediment**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Non-Conventional Constituents – Pesticides and Herbicides (Field)**		
Analytical Method/SOP Reference: See Group B, Table 9		
# Sample locations: 2		
Field QC[1, 2, 3]	**Frequency/Number per sampling event**	**Acceptance Limits**
Cooler Temperature	4° C	4° C
Field Duplicates[4]	1/20 samples or 1/batch (whichever is more frequent)	RPD <25%

Note 1: Equipment Blanks will not be used, as they are not applicable to grab samples.
Note 2: Per SOPs, Trip Blanks are not required since not sampling for volatile organic compounds (VOCs) via SM 8260.
Note 3: No Trip or Field Blanks required.
Note 4: Field Duplicates will be used in lieu of Collocated Samples.

Matrix: **Sediment**

Sampling SOP: See Appendix B

Analytical Parameter(s): **Biological Toxicity Testing (Hyalella azteca) Field**

Analytical Method/SOP Reference: See Group B, Table 9

Sample locations: 2

Field QC	Frequency/Number per sampling event	Acceptance Limits
Assess percent of data successfully collected	1 per sampling event	a) Survival in the controls must be ≥80%. b) Measurable growth in controls. c) All performance criteria outlined in SOP are met.
Field Duplicates[1]	Per SOPs (Appendix B)	a) Survival in the controls must be ≥80%. b) Measurable growth in controls. c) All performance criteria outlined in SOP are met.

Note 1: Field Duplicates will be used in lieu of Collocated Samples. For collection of Field Duplicates, duplicate sample locations shall be selected pursuant to the SOPs in Appendix B.

Matrix: **Habitat/ Benthic**

Sampling SOP: See Appendix B

Analytical Parameter(s): **Bioassessment Constituents (Field)**

Analytical Method/SOP Reference: See Group B, Table 9

Sample locations: 2

Field QC	Frequency/Number per sampling event	Acceptance Limits
Re-examination (sorting, counting, identification)	1 per 10 benthic invertebrate samples	≤5% difference
Assess percent of data successfully collected	1 per sampling event	100%
Field Duplicates[1]	10% of the study sites	

Note 1: Field Duplicates will be used in lieu of Collocated Samples. For collection of Field Duplicates, duplicate sample locations shall be selected pursuant to the SOPs in Appendix B.

Corrective Actions for Field Sampling

Determine cause of problem (e.g., equipment contamination, improper cleaning, exposure to airborne contaminants, etc.), remove sources of contamination, & reanalyze all suspect samples or flag all suspect data. Further corrective action will be determined by USMILG's Administrator and Project Manager (refer to Group C – Assessment and Oversight).

Corrective Actions for Field Sampling (Biological and Bioassessment)

Resolve differences in identification and enumeration for both precision and accuracy. Reschedule sample events as necessary or appropriate. Further corrective action will be determined by USMILG's Administrator and Project Manager (refer to Group C – Assessment and Oversight).

Table 12. (Element 14) Analytical QC.

Matrix: **Laboratory Blank**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **All Constituents**		
Analytical Method/SOP Reference: See Group B, Table 10		
# Sample locations: Not Applicable		
Laboratory QC	**Frequency/Number**	**Acceptance Limits**
Method Blank	One per 20 Samples or per Analytical Batch, whichever is more frequent.	Blanks <MDL for target analyte
Instrument Blank	Per Laboratory SOP Method	Per Laboratory SOP Limits
Matrix: **Samplewater**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Conventional Constituent**		
Analytical Method/SOP Reference: See Group B, Table 10		
# Sample locations: 2		
Laboratory QC	**Frequency/Number**	**Acceptance Limits**
Lab. Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates
Lab. Matrix Spike	One per 20 Samples or per Analytical Batch, whichever is more frequent.	%Recovery = 80-120% or Control Limits based on 3x the standard deviation of laboratory's actual method recoveries
Matrix Spike Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates
Matrix: **Laboratory Blank**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Non-Conventional Constituents – Pesticides and Herbicides**		
Analytical Method/SOP Reference: See Group B, Table 10		
# Sample locations: 2		
Laboratory QC	**Frequency/Number**	**Acceptance Limits**
Lab. Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates
Lab. Matrix Spike	One per 20 Samples or per Analytical Batch, whichever is more frequent.	%Recovery = 50-150% or Control Limits based on 3x the standard deviation of laboratory's actual method recoveries
Matrix Spike Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates
Surrogates	In every calibration standard, sample, and blank analyzed for organics by GC or isotope dilution GC-MS; added to samples prior to extraction	Determined by Contracted Laboratory

Matrix: **Sediment**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Non-Conventional Constituents – Pesticides and Herbicides**		
Analytical Method/SOP Reference: See Group B, Table 10		
# Sample locations:		
Laboratory QC	**Frequency/Number**	**Acceptance Limits**
Lab. Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates
Lab. Matrix Spike	One per 20 Samples or per Analytical Batch, whichever is more frequent.	%Recovery = 50-150% or Control Limits based on 3x the standard deviation of laboratory's actual method recoveries
Matrix Spike Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates
Surrogates	In every calibration standard, sample, and blank analyzed for organics by GC or isotope dilution GC-MS; added to samples prior to extraction	Determined by Contracted Laboratory

Matrix: **Sediment**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Biological Toxicity Testing (Hyalella azteca) Field**		
Analytical Method/SOP Reference: See Group B, Table 10		
# Sample locations: 2		
Laboratory QC	**Frequency/Number**	**Acceptance Limits**
Assess percent of data successfully collected	1 per sampling event	a) Survival in the controls must be ≥80%. b) Measurable growth in controls. c) All performance criteria outlined in SOP are met.

Matrix: **Laboratory Blank**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Bioassessment Constituents**		
Analytical Method/SOP Reference: See Group B, Table 10		
# Sample locations: 2		
Laboratory QC	**Frequency/Number**	**Acceptance Limits**
Lab. Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates
Lab. Matrix Spike	One per 20 Samples or per Analytical Batch, whichever is more frequent.	%Recovery = 80-120% or Control Limits based on 3x the standard deviation of laboratory's actual method recoveries
Matrix Spike Duplicate	One per 20 Samples or per Analytical Batch, whichever is more frequent.	RPD <25% for duplicates

Matrix: **Habitat/ Benthic**		
Sampling SOP: See Appendix B		
Analytical Parameter(s): **Bioassessment Constituents**		
Analytical Method/SOP Reference: See Group B, Table 10		
# Sample locations: 2		
Field QC	**Frequency/Number per sampling event**	**Acceptance Limits**
Re-examination (sorting, counting, identification)	1 per 10 benthic invertebrate samples	≤5% difference
Assess percent of data successfully collected	1 per sampling event	100%

Corrective Actions for Laboratory Analysis

Determine cause of problem (e.g., contaminated reagents, equipment), remove sources of contamination, recalibrate (as applicable), and reanalyze all suspect samples or flag all suspect data. Further corrective action will be determined by USMILG's Administrator and Project Manager. Zero percent recovery requires rejection of all suspect data (refer to Group C – Assessment and Oversight).

15. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Field measurement equipment will be properly maintained and calibrated per the manufacturer's requirements. This includes battery checks, routine replacement of membranes, and cleaning of conductivity electrodes. All equipment will be inspected and tested prior to the start of sampling to verify that the instrument is operating appropriately. If the instrument fails to operate within appropriate parameters, the Project Manager will take the appropriate steps to ensure replacement equipment is available.

All laboratories contracted for this MRPP/QAPP will operate using quality assurance and quality control programs to maintain their equipment in accordance with their SOPs, which include those specified by the manufacturer and those specified by the analytical method. The Project Manager, or designated personnel, will review the laboratory's SOP for laboratory equipment maintenance for compliance with the SWAMP.

Table 13. (Element 15) Testing, inspection, maintenance of sampling equipment and analytical instruments.

Equipment / Instrument	Maintenance Activity, Testing Activity or Inspection Activity	Responsible Person	Frequency	SOP Reference
ExTech Multiparameter Monitoring Or equal	Maintenance and calibrations	Project Manager, or designated personnel	Maintenance: Conducted per manufacturer's specifications Calibrations: prior to sampling collection	Per manufacturer's specifications
MicroTpw Turbidity Monitoring Or equal	Maintenance and calibrations	Project Manager, or designated personnel	Maintenance: Conducted per manufacture's specifications Calibrations: prior to sampling collection	Per manufacturer's specifications
Laboratory analytical instruments for Conventional constituents	Maintenance and calibration	Contracted Laboratory's Director	Maintenance: Conducted per manufacturer's specifications Calibrations: verification every 20 samples after initial calibration using a different source from initial calibration.	Per contracted Lab SOP and equipment manufacturer's specifications

*Note: These constituent tests may be alternatively analyzed in laboratory.

16. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All laboratories contracted by USMILG will operate using quality assurance and quality control programs to maintain their equipment in accordance with their SOPs, including those specified by manufacturer and analytical method. All laboratories are required to submit a copy of the SOPs for laboratory equipment maintenance for review by the Project Manager for compliance with the SWAMP.

An ExTech Multiparameter probes, or equivalent, will be used to make field measurements for conductivity, dissolved oxygen, pH, temperature, and turbidity.

A MicroTpw Turbidity meter, or equivalent, will be used to make field measurements for turbidity.

All field instruments will be inspected and properly calibrated prior to sampling activities per the manufacturer's specification.

17. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

The Contracted Laboratory will supply the sample containers necessary for the monitoring program. Sampling materials such as storage coolers, ice, latex gloves, plastic storage bags, field measurement equipment/tools, and waterproof pens will be provided by the contracted water quality sampler and contracted biological sampler, as applicable. All field sampling crews will implement quality assurance and quality control programs in accordance with the SOPs, which include those specified by the manufacturer and those specified by the method. Field crews will examine the sampling supplies for damage as they are received/purchased the supplies, and prior to use in the field. Field measurement/analytical devices will be managed in accordance with Group B, Element 15 of this MRPP/QAPP.

Table 14. (Element 17) Inspection/acceptance testing requirements for consumables and supplies.

Project-Related Supplies / Consumables	Inspection / Testing Specifications	Acceptance Criteria	Frequency	Responsible Individual
Nitrile Gloves	Visual inspection	Brand new/ Uncontaminated	Prior to each sampling event	Assigned Sampler
Sampling Bottles	Visual inspection	Brand new/ Decontaminated	Prior to each sampling event	Assigned Sampler
Grab Sample Cup	Visual Inspection	Uncontaminated	Prior to each sampling event	Assigned Sampler

18. NON-DIRECT MEASUREMENTS (EXISTING DATA)

There are no existing water quality data and biological data available to provide added benefit to this QAPP/MRPP for the characterization and assessment of surface water quality in the Santa Margarita River watershed.

19. DATA MANAGEMENT

Data will be maintained as described in Group A, Element 9 of this MRPP/QAPP. The Project Manager, or designated personnel, will maintain an inventory of data and forms, and will periodically check the inventory against the records in their possession. Data samples will be collected according to the procedures outlined in Group B, Element 10 of this MRPP/QAPP. Field measurements will be recorded on standard field log forms included in Appendix C. Analytical samples will be transferred to the laboratory under required COC procedures using a standard COC obtained from the contracted laboratories prior to sampling activities. A sample COC is provided in Appendix D.

All laboratory and field measurement data submitted to USMILG for inclusion in the project database will follow the guidelines and formats established by SWAMP (http://www.waterboards.ca.gov/swamp/qapp.html) and will be uploaded to the database through batch set electronic means.

All contract laboratories will maintain a record of transferred data records. Prior to upload, a QA/QC review will be conducted to check data for completeness, validity of analytical methods, validity of sample locations, and validity of sample dates. The QA/QC will involve using automated data checking tools, which assess that new data to be uploaded follow specified rules, including issues such as alpha-numeric formatting, units of measurement, missing information, and others. The sample location information will be checked to ensure that sites are correctly referenced and that identifiers and descriptions match any corresponding records within the existing database. Data not passing this QA/QC review will be returned to the Project Manager or originating laboratory for clarification and or correction. When all data within a batch set passes QA/QC requirements, the data will be uploaded to the database. A unique batch number, date loaded, originating laboratory, and the person who loaded the data will be recorded in the database, so that data can be identified and removed in the future if necessary.

Data will be stored in such that data is backed up daily with the possibility of rollback, if necessary. All computer software and equipment is routinely tested, maintained and scanned for viruses to ensure proper working equipment and integrity of the digital database file. Project data will also be formatted for upload into the SWAMP Information Management System (IMS) database.

Upload to the IMS will be via the California Environmental Data Exchange Network (CEDEN) at http://www.ceden.org/. The USMILG will be assigned to an appropriate Regional Data Center, likely the Southern California Coastal Water Research Project (SCCWRP), which collates ambient water quality data for the southern California region. Data will be uploaded using the specified CEDEN or SWAMP template (Excel format). Should either of these database formats not be ready or available at the time data is ready for upload, data will be reported in the format specified by USMILG. The USMILG QAPP and MRPP data will used, in part, for the RWCQB's integrated reports.

GROUP C: ASSESSMENT AND OVERSIGHT

20. ASSESSMENTS & RESPONSE ACTIONS

Data reviews will be made on an annual basis/during preparation of season reports. Reviews will be conducted by the Project Manager and where appropriate may include the USMILG's Administrator. These periodic reviews will include sampling equipment and calibration, sampling procedures during sampling, auditing of (sampling) data and the database to evaluate data accuracy and completeness.

If an audit discovers any discrepancy or data quality concern, the Project Manager will discuss the observed discrepancy and concerns with the appropriate person responsible for the task. The discussion will begin with whether the information collected is accurate, what were the cause(s) leading to the deviation, how the deviation might impact data quality, and what corrective actions might be considered. The Project Manager can require that certain corrective actions be made within a defined time schedule.

The USMILG's Administrator and Project Manager have the authority to halt all sampling and analytical work by both the sampling team and contract laboratory if the deviations noted are considered detrimental to data quality.

21. REPORTS TO MANAGEMENT

The Project Manager will share data and preliminary analyses with USMILG's Administrator in the form of oral quarterly progress reports and when appropriate. All contracted laboratories will provide QA/QC report, which summarize the Project's overall adherence to established analytical standard operating practices (SOPs), and responds to information from the results of the audit and on-site observations.

Quarterly progress reports will discuss project status, any significant field or laboratory issues, timeliness of scheduled field and analytical activities, any Quality Assurance problems, or other issues with recommended solutions, if applicable.

The Annual Report, submitted by September 1 of each year, will summarize the results of QA/QC assessments and evaluations, completeness of the monitoring data, the annual calibration exercise findings, and any lab and/or field performance audits that were conducted throughout the life of the project.

GROUP D: DATA VALIDATION AND USABILITY

22. DATA REVIEW, VERIFICATION, AND VALIDATION REQUIREMENTS

Data generated by sampling and monitoring activities specified within this MRPP/QAPP will be reviewed by the Project Manager, or designated personnel, against the data quality objectives cited in Element 7, and the quality assurance/control practices cited in Group B, Elements 14 through17 of this MRPP/QAPP. Data will be separated into three categories:

1. Data meeting all data quality objectives
2. Data meeting failing precision or recovery criteria
3. Data failing to meet accuracy criteria

Data meeting all quality objectives, but with failures of QA/QC practices shall be reviewed to determine the impact of the failure on the quality of the data. Once the failure impacts are determined, the data will be moved into either the first or last category.

Data falling into the first category are considered usable by the Project. Data falling into the last category will be considered not usable. For data falling into the second category, all aspects of the data's quality will be assessed to determine the data's usability for this Project. If sufficient evidence is found supporting use of this data, the data will be moved into the first category and flagged with a "J" per EPA specifications.

23. VERIFICATION AND VALIDATION METHODS

All data recorded in the field, including field measurements, observations and chain of custody forms, will be checked visually by the Project Manager, or specified personnel, and recorded as checked by initials and dates. Field data will be checked to ensure that all necessary data and activities were completed, including:

- Collection of all water samples, field blanks and field replicates
- Reporting correct units of measurement
- Measured values fall within expected ranges

Data validation will also include a check to ensure that samples were delivered to laboratories within required holding times and that all sample handling and custody protocols were followed.

In addition to field data validation, there will be a validation of water quality analysis results. This will involve a review of 10% of all laboratory water quality analysis reports by the Project Manager, or designated personnel. This review will involve verifying that all required parameters were measured, reported in the correct units, and that results fall within expected ranges.

The Project Manager will be responsible for all field data validation reviews. Each of the contract Laboratory QA Officers will also perform checks of all of its records and each of the contract Laboratory Directors will 10%. All checks by the contract laboratories will be reviewed by appropriate Project personnel.

Issues, including missing data, incomplete site visits, reporting errors (such as incorrect units of measure or incorrect date/time information, etc.), or data management errors will be communicated to responsible party immediately and documented in the QA/QC Reports for either field sampling, laboratory activities, or database management. Reconciliation and correction will be done by a committee composed of the Project Manager, Lead sampler from the sampling team, Analyst, the Contract Laboratory QA Officers, and Laboratory Director. Any corrections require a unanimous agreement that the correction is appropriate.

24. RECONCILIATION WITH USER REQUIREMENTS

The intent of the monitoring conducted under this plan is to provide water quality data and biological data to contribute to the characterization and assessment of surface water quality in the Santa Margarita River watershed. USMILG's focus is on data and reporting contributions geared towards the assessment of irrigated farmlands' contribution to water quality.

The reports produced in the Project will describe some of the limitation of the data. This may include constraints specified in Group B, Element 19 and 20 of this MRPP/QAPPP, and the ability for data to meet Data Quality Objectives (DQO). For data that do not meet DQOs, the Project Manager has to options:

1. Retain the data for analytical purposes, but flag data for QA deviations.
2. Do not retain the data and exclude them from all calculations and interpretations.

The decision of the two options above will be based on MQOs listed in Group A, Element 7 of this MRPP/QAPP. If qualified data are to be used then it must be made clear in the annual report that these deviations do not alter conclusions within the report. Validated project data collected will be compatible with SWAMP database requirements. The USMILG's Administrator will submit these data to the SWAMP database.

The water quality data will be used to assess the quality of receiving waters compared to water quality limits contained in the Basin Plan. For irrigated agriculture, the water quality data will help in the assessment of Conditional Waiver No. 4's ability to protect surface water quality through the waiver program in lieu of issuing Waste Discharge Requirements (WDRs) to agricultural groups or to individual agricultural discharges. The water quality data may also help in the assessment of potential pollutant sources and help lead to identification of pollutant sources and the ultimate minimization or elimination of these sources. The biological data will be used to contribute data to the future development, by others, of a biological integrity index for receiving waters. Until a biological integrity index is developed and adopted, a process anticipated to take many years, the biological data collected as part of this project will not be suitable for use in assessing receiving waters in accordance with Basin Plan standards since the Basin Plan does not include biological metrics

The water quality and biological data collected as part of this project may also be used in the future for listing and/or delisting water bodies from the state's 303(d) list of impaired water bodies, for setting or implementing Total Maximum Daily Loads (TMDLs), and the determination of the effectiveness of waiver programs, among other purposes.

Appendix A

Watershed Maps

RIVERSIDE COUNTY FLOOD CONTROL AND WATER CONSERVATION DISTRICT

RIVERSIDE, CALIFORNIA

CANYON LAKE

CITY OF MENIFEE

LAKE ELSINORE

DIAMOND VALLEY RESERVOIR

SKINNER RESERVOIR

CITY OF WILDOMAR

CITY OF MURRIETA

CITY OF TEMECULA

Cole Creek

Adobe Creek

Warm Springs Creek

Murrieta Creek

Temecula Creek

Santa Margarita River

RIVERSIDE COUNTY
SAN DIEGO COUNTY

- LEGEND -

- Wet/Dry Weather Monitoring Stations
- MS4 Facility Lines
- US Freeways
- State Highways
- Primary Arterial Roads
- Secondary Collector Roads
- Area Controlled by Vail Lake & Lake Skinner
- Watercourses
- Waterbodies
- HUC Subwatersheds
- Incorporated Areas
- Riverside County Boundary
- Santa Margarita River Watershed Boundary

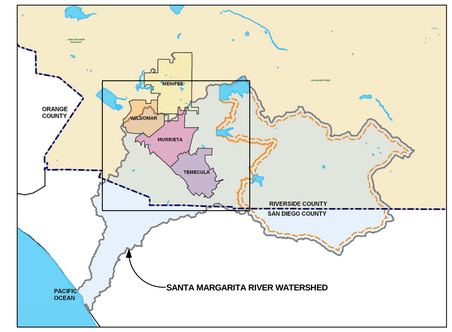
>

URBAN LAND USE:

- Parks/Recreation
- Industrial
- Commercial
- Urban Residential (less than 1 acre)
- Streets

NON-URBAN LAND USE:

- Agriculture
- Exempt/Public
- Preserves/Open Space
- Rural Residential (1 acre or more)



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0 1 2 3 4 Miles

The graphical and tabular information shown on this document may be derived from a variety of public agency and/or private commercial sources such as Riverside County Transportation and Land Management Agency, Thomas Brothers Mapping, the Stephen P. Teale Data Center, GIS Technology Center, State of California, the United States Geologic Survey and the United States National Atlas. These sources may possess varying levels of accuracy and precision and this product is meant only as a guide to the relative position and scale of the depicted features. This GIS document is in no case to be interpreted as fundamental or decisive for purposes of land surveying, field engineering, plan drafting, code enforcement, land boundary determination and/or land acquisition.

RIVERSIDE COUNTY FLOOD CONTROL AND WATER CONSERVATION DISTRICT
GEOGRAPHIC INFORMATION SYSTEM

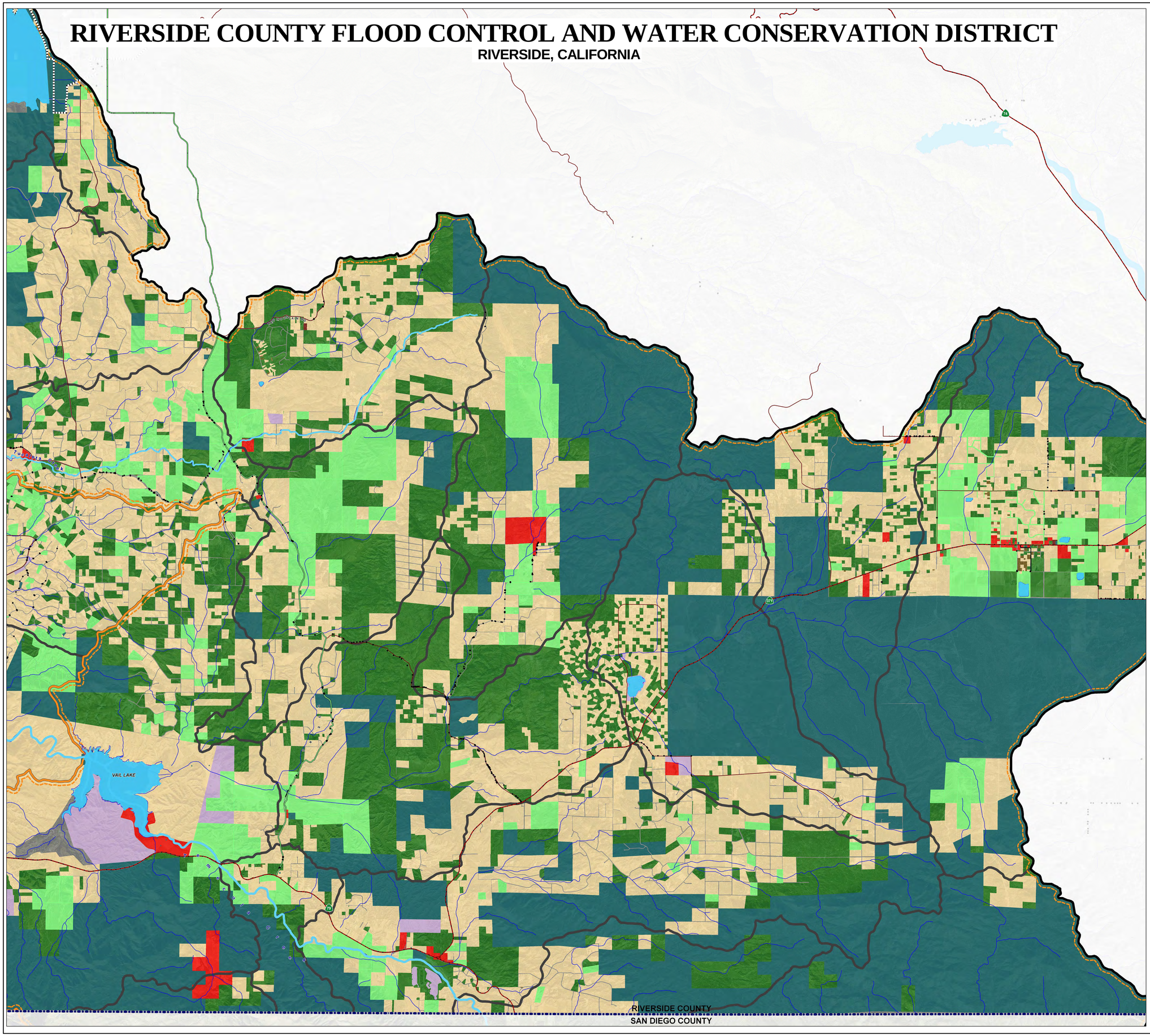
SANTA MARGARITA RIVER WATERSHED LANDUSE MAP with Current MS4 Facilities Delineated

Map 1 of 2

REVISED OCT2009

RIVERSIDE COUNTY FLOOD CONTROL AND WATER CONSERVATION DISTRICT

RIVERSIDE, CALIFORNIA



- LEGEND -

- Wet/Dry Weather Monitoring Stations
- MS4 Facilities
- US Freeways
- State Highways
- Primary Arterial Roads
- Secondary Collector Roads
- Area Controlled by Vail Lake & Lake Skinner
- Watercourses
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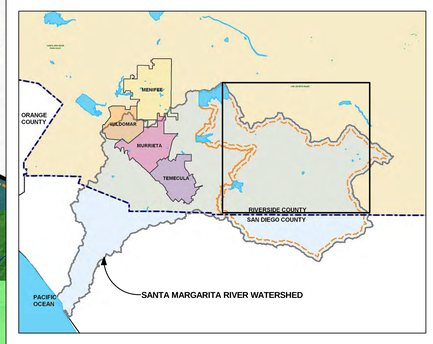
- Preserves/Open Space
- Rural Residential (1 acre or more)



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RIVERSIDE COUNTY FLOOD CONTROL AND WATER CONSERVATION DISTRICT GEOGRAPHIC INFORMATION SYSTEM

SANTA MARGARITA RIVER WATERSHED LANDUSE MAP with Current MS4 Facilities Delineated

Map 2 of 2

REVISED OCT2009

Appendix B

Standard Operating Procedures

Marine Pollution Studies Laboratory – Department of Fish and Game (MPSL-DFG) Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (SWAMP)

Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California

Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California

Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT)
List of Freshwater Macroinvertebrate Taxa from California and Adjacent States including Standard Taxonomic Effort Levels

Methods for Measuring Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates

Marine Pollution Studies Laboratory – Department of Fish and Game (MPSL-DFG) Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (SWAMP)

The SOPs below are for reference and information purposes only, the documents are not required by the Surface Water Ambient Monitoring Program (SWAMP). Please see the SWAMP Quality Assurance Management Plan (http://www.swrcb.ca.gov/swamp/qamp.html) for more information regarding SWAMP QA/QC requirements.

Table of Contents

Field Measurements

Field Data Sheets

Field data sheets are used to record field observations, probe measurements, and water and sediment chemistry sampling. Field data sheets are provided through the Marine Pollution Studies Laboratory website at:
http://mpsl.mlml.calstate.edu/swdwnlds.htm
Click on the *Field Data Sheets* for the most recent versions. There are guidelines provided below to standardize what is recorded on all data sheets and that should be helpful in completing each form. The Beaufort Scale (see at the end of this document) is also used for specifications and equivalent wind speeds for water conditions. The entries discussed below and on the field data sheets are recorded at each sampling site.

Notes to Standardize SWAMP Field Data Sheets (For in the field use)

Upon arrival at a sampling site, record visual observations on the appearance of the water and other information related to water quality and water use.

Key Reminders to identify samples:

1. **Sample Time** is the SAME for all samples (Water, Sediment, & Probe) taken at the sampling event. Use time of FIRST sample as it is important for the chain of custody (COC).
2. **Left Bank/Right Bank**
   Left bank is defined as the bank to the left of the observer when facing downstream, and the *right bank* is to the right of the observer when facing downstream

FIELD OBSERVATIONS: (each one of these observations has a *Comment* field in the database so use comment space on data sheet to add information about an observation if necessary)

1. **DOMINANT SUBSTRATE**: if possible; describe DOMINANT substrate type; use UNK if you cannot see the dominant substrate type
2. **WADEABILITY**: in general, is the water body being sampled wadeable to the average person AT the POINT of SAMPLE
3. **BEAUFORT SCALE**: use scale 0-12; refer to scales listed at the end of this document.
4. **WIND DIRECTION**: records the direction from which the wind is blowing
5. **PICTURES:** Digital photos are taken to help document the actual sampling site. The convention is to take photos facing DOWNSTREAM, overlooking the site. Right bank and left bank are thus defined in this downstream-facing direction. Document any discrepancies from this convention. Only one photo is necessary, if both, left and right

bank, fit into one frame. Record all photos in the field data sheet space to record picture numbers given by camera; be sure to rename accordingly back in the office. All photos should be renamed and saved with the StationCode_yyyy_mm_dd_uniquecode (e.g. 123ABC123_2007_07_01_BBDS).

6. **SITE ODOR**: Note if hydrogen sulfide odor, musty odor, sewage odor, etc. is in the sampling reach
7. **SKY CODE**: Note recent meteorological events that may have impacted water quality
8. **OTHER PRESENCE**: VASCULAR refers to terrestrial plants or submerged aquatic vegetation (SAV) and NONVASCULAR refers to plankton, periphyton etc.
9. **PRECIPITATION**: Note if any precipitation is occurring during sampling
10. **PRECIPITATION LAST 24 HOURS**: Note how much precipitation has occurred within the last 24-h of sampling
11. **WATER ODOR**: Note if the sample water being collected has odor
12. **WATER CLARITY**: this describes the clarity of the water while standing creek side; clear represents water that is clear to the bottom, cloudy may not be clear to bottom but greater than 4" can be seen through the water column.
13. **WATER COLOR**: This is the color of the water from standing creek side
14. **OBSERVED FLOW**: Visual estimates in cubic ft/s.

SAMPLE DETAILS:

1. **EVENT TYPE**: Note the event type based which type of media is being collected
2. **SAMPLE TYPE**: GRAB samples are when bottles are filled from a single depth; INTEGRATED sample are taken from MULTIPLE depths and combined.
   a. GRAB: use 0.1 for subsurface samples; if too shallow to submerge bottle; depth =0
   b. INTEGRATED: -88 in depth sampled, record depths combined in sample comments
3. **SAMPLING CREW**: J. Smith, S. Ride (first person listed is crew leader)
4. **STARTING BANK**: Which side of the stream was accessed first. Bearings are always recorded looking downstream
5. **OCCUPATION METHOD**: What media was used to access the site
6. **TARGET LAT/LONG**: Refers to the existing station location that the sampling crew is trying to achieve; can be filled out prior to sampling
7. **ACTUAL LAT/ LONG**: is the location of the current sample event.
8. **SAMPLE LOCATION**: describes from where IN water body sample was taken: Can be combined; ex: bank/thalweg or midchannel /thalweg
9. **HYDROMODIFICATION:** Describe existing hydromodifications such as a grade control, drainage pipes, bridge, culvert
10. **HYDROMOD LOC**: if there was an IMMEDIATE (with in range potentially effecting sample) hydromodification; was sample taken upstream or downstream of modification; if there is no hydromodification, NA is appropriate
11. **STREAM DEPTH, WIDTH & DISTANCE FROM BANK**: describe in meters at point of sample. Distance from bank should be recorded from the starting bank

Field Data Logbook

A Field Data Logbook or a Field Folder is taken into the field on each sampling trip. The use of bound or loose-leaf notebooks is left up to the entity conducting the monitoring. A good safety precaution against the loss of a bound field data logbook is to photocopy the current pages upon returning from the field. These pages are kept on file at the specific sample collection entity's office. If a loose-leaf notebook is used, take care to remove original field data log sheets from the notebook and file in the office. Copies of the field data log sheets may be left in the notebook for future reference.

Field Data Logbooks (bound or loose leaf sheets) are maintained on file indefinitely in each regional office or contract laboratory office. They are never discarded, since the logbook may be the only written record of field measurements. Field Data Logbooks are reviewed periodically during SWAMP QA site visits. At this point, these field notes are not inclusive of the information that would be collected for biological assessment work, and several other data measurement types.

Flow

Sampling crews should be notified on reconnaissance forms if it is known that there is an operational United States Geological Survey (USGS) gage is located at or nearby a sampling site. If there is a USGS gage nearby, a gage height in feet is recorded and later converted to an instantaneous flow value and recorded in the logbook. The gage height is always to be reported to the USGS for conversion to flow. If a USGS gage is not available, a flow measurement should be taken, if requested. See Instantaneous Flow Measurement information starting on page 13 in this document. In addition, it is recommended that a flow severity value is recorded at each stream or river station that is not tidally influenced. See the Flow Severity section starting on page 13 of this document. Centroid velocity measurements may also be taken as a minimum acceptable rough characterization of the stream flow as requested, although this measurement is not to be recorded as a flow, since it is only a velocity measurement.

Record of Samples Collected for Purposes of Chemical Analysis

The general types of chemical samples to be collected are listed for each site, since this may vary from site-to-site (e.g., metals-in-water, pesticides-in-sediments, routine water quality). Analyses authorization forms are recommended since different authorized laboratories perform different chemical analyses. The method of preservation for each chemical sample is recorded, as appropriate.

Record of Data Submission

The *Logbook* field must indicate in some manner whether data recorded in the logbook has been transcribed onto data forms and submitted to the SWAMP data management staff.

Other Observations

Water Appearance Note general appearance (e.g., color, unusual amount of suspended matter, debris or foam)

Sediment Appearance

Color, Odor and sediment composition should be noted.

Weather Note recent meteorological events that may have impacted water quality; (e.g., heavy rains, cold front, very dry, very wet)

Biological Activity Note excessive macrophyte, phytoplankton or periphyton growth. The observation of water color and excessive algal growth is very important in explaining high chlorophyll a values. Other observations such as presence of fish, birds and spawning fish are noted.

Watershed or Instream Activities Note instream or drainage basin activities or events that are impacting water quality (e.g., bridge construction, shoreline mowing, livestock watering upstream).

Record of Pertinent Observations Related to Water Quality and Stream Uses If the water quality conditions are exceptionally poor, note that standards are not met in the observations, (e.g., dissolved oxygen is below minimum criteria). Note uses (e.g., swimming, wading, boating, fishing, irrigation pumps, navigation). Eventually, for setting water quality standards, the level of use will be based on comments related to the level of fishing and swimming activities observed at a station.

Specific Sample Information Note specific comments about the sample itself that may be useful in interpreting the results of the analysis (e.g., number of sediment grabs, or type and number of fish in a tissue sample). If the sample was collected for a complaint or fish kill, make a note of this in the observation section.

Missing Parameters If a scheduled parameter or group of parameters is not collected, make some note of this in the comments.

Field Data Measurements

While collecting water samples (see Field Collection Procedures for Water Samples section), record appropriate field measurements. When field measurements are made with a multiparameter instrument, it is preferable to place the sonde in the body of water to be sampled and allow it to equilibrate in the dissolved oxygen (D.O.) mode while water samples are collected. Field measurements are made at the centroid of flow, if the stream visually appears to be completely mixed from shore to shore. *Centroid* is defined as the midpoint of that portion of the stream width which contains 50% of the total flow. For routine field measurements, the date, time and depth are reported as a grab. Measure Quality Objectives (MQO's) for field measurements are listed in appendix C of the SWAMP QAMP.

Recommended Depths for Conducting Field Data Measurements

Water Depth Less than 5 ft (<1.5 m) If the water depth is less than 5 ft (1.5 m), grab samples for water are taken at approximately 0.1 m (4 in.), and multi-probe measurements are taken at approximately 0.2 m (8 in.). This is because all sensors have to be submerged, so 0.1 m would not be deep enough. But taking a grab sample at 0.2 m is not always feasible, as it is difficult to submerge bottles to that depth, and in many cases the bottle will hit the stream bottom.

Water Depth Greater than 5 ft (>1.5 m) If the water depth at the sampling point exceeds 5 ft (1.5 m) in depth, a vertical profile of dissolved oxygen, temperature, pH and specific conductance are made using the multiparameter probe equipment. The depth of the sonde at the time of measurement is most accurately determined from the depth sensor on the multiparameter sonde rather than depth labels on the cable.

Vertical Depth Profiles and Depth-Integrated Sample Collection If depth integration sampling is being conducted, or if vertical profile measurements are requested, multi-probe measurements are made starting at a depth of 0.2 m, and are then conducted at 1.0, 2.0, 3.0, 4.0, and 5.0 m depths after that until 5.0 m depth is reached. Beginning at 5.0 m, measurements are made every 5.0 m through depth profile.

Field data for multiparameter vertical depth profiles are recorded in final form on the SWAMP Field Data Sheets and submitted to the SWAMP data management staff.
Go to http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting.

Water Temperature (^{O}C)

Water temperature data are recorded for each SWAMP visit in final form in a Field Data Logbook and submitted to the SWAMP data management staff.
See http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting.

Temperature Sampling Procedures

Temperature is measured in-stream at the depth(s) specified above. Measuring temperature directly from the stream by immersing a multiprobe instrument or thermometer is preferred.

Hand Held Centigrade Thermometer

If an electronic meter is not available, the temperature is measured with a hand-held, centigrade thermometer (Rawson, 1982).

- In wadeable streams, stand so that a shadow is cast upon the site for temperature measurement.

- Hold the thermometer by its top and immerse it in the water. Position the thermometer so that the scale can be read.
- Allow the thermometer to stabilize for at least one minute, then without removing the thermometer from the water, read the temperature to the nearest 0.1^{o} C and record.
- Do not read temperature with the thermometer out of the water. Temperature readings made with modern digital instruments are accurate to within $\pm$ 0.1^{o} C.

Temperature Measurement from a Bucket

When temperature cannot be measured in-stream, it can be measured in a bucket-Nalgene or plastic. Care must be taken to insure a measurement representative of in-stream conditions.

The following conditions must be met when measuring temperature from a bucket:

- The bucket must be large enough to allow full immersion of the probe or thermometer.
- The bucket must be brought to the same temperature as the water before it is filled.
- The probe must be placed in the bucket immediately, before the temperature changes.
- The bucket must be shaded from direct sunlight and strong breezes prior to and during temperature measurement.
- The probe is allowed to equilibrate for at least one minute before temperature is recorded.
- After these measurements are made, this water is discarded and another sample is drawn for water samples which are sent to the laboratory.

pH (standard units)

pH data is recorded for each SWAMP visit in final form on the Field Data Sheets and submitted to the SWAMP data management staff. See http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting.

pH Sampling Equipment

The pH meter should be calibrated according to the recommended procedures for calibration and maintenance of SWAMP field equipment. Calibration directions are listed in the manufactures field equipment operations manual. The pH function is pre and post calibrated every 24 h of use for multiparameter instruments.

pH Sampling Procedures

In-stream Method

Preferably, pH is measured directly in-stream at the depth(s) specified earlier in this document. Allow the pH probe to equilibrate for at least one minute before pH is recorded to the nearest 0.1 pH unit.

pH Measurement from a Bucket

When pH cannot be measured in-stream, it can be measured in a bucket-Nalgene or plastic. The following precautions are outlined above; “Temperature Measurement from a Bucket”.

Potential Problems

- If the pH meter value does not stabilize in several minutes, out gassing of carbon dioxide or hydrogen sulfide, or the settling of charged clay particles may be occurring (Rawson, 1982).
- If out gassing is suspected as the cause of meter drift, collect a fresh sample, immerse the pH probe and read pH at one minute.
- If suspended clay particles are the suspected cause of meter drift, allow the sample to settle for 10 min, then read the pH in the upper layer of sample without agitating the sample.
- With care, pH measurements can be accurately measured to the nearest 0.1 pH unit.

Dissolved Oxygen (mg/L)

Dissolved oxygen (D.O.) data is recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff.
See http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting.

Dissolved Oxygen Sampling Equipment

The dissolved oxygen meter should be calibrated according to the recommended procedures for calibration and maintenance of SWAMP field equipment. Calibration directions are listed in the manufactures field equipment operations manual.

Multiprobe Instrument

Pre and post calibrate the D.O. sensor every 24 h and for elevations greater than 500 ft on the multiprobe instrument. Preferably, D.O. is measured directly in-stream at the depth(s) specified in the Field Measurements section above. The D.O. probe must equilibrate for at least 90 s before D.O. is recorded to the nearest 0.1 % saturation or mg/L. Care must be taken at profile stations to insure that the reading is stable for each depth. Since dissolved oxygen takes the longest to stabilize, record this parameter after temperature, conductivity and pH. If the D.O. probe has an operable, automatic stirrer attached, the D.O. probe does not have to be manually stirred. However, if the probe is not equipped with an automatic stirrer, manual stirring must be provided by raising and lowering the probe at a rate of 1 ft/s (0.3m/s) without agitating the water surface. If the stream velocity at the sampling point exceeds 1 ft/s, the probe membrane can be pointed upstream into the flow and manual stirring can be avoided (Rawson, 1982).

D.O. Measurement from a Bucket

When D.O. cannot be measured in-stream, it can be measured in a bucket-Nalgene or plastic, following precautions outlined in the Temperature Measurement from a Bucket listed above. During equilibration and reading, water should be moved past the membrane surface at a velocity of 1 ft/s (0.3 m/sec), either by automatic stirrer or manual stirring. If stirred manually in a bucket, the water surface is not agitated (Rawson, 1982).

24-Hour Average D.O. (if requested in special study)

Unattended 24-Hour D.O. Data Collection

Why Collect 24-Hour Data

Dissolved oxygen sampling for standards compliance is targeted to water bodies where low instantaneous D.O. levels indicate partial or nonsupport of designated aquatic life uses. Intensive monitoring is conducted with automated equipment that is preset to record and store field measurements hourly over one 24-h period. Four or more dissolved oxygen measurements may also be made manually at 4-6-h intervals over one 24-h period, as long as one is made near sunrise (0500-0900 h) to approximate the daily minimum. However, data collected with automated equipment is preferred.

When to Take Measurements

All 24-h D.O. monitoring events must be spaced over an index period representing warm-weather seasons of the year (approx March 15-October 15), with between one-half to two-thirds of the measurements occurring during the critical period (July 1-September 30). The ***critical period*** of the year is when minimum stream flows, maximum temperatures, and minimum dissolved oxygen concentrations typically occur in area streams. **A flow measurement must be taken at the time of deployment.** In a perennial stream, a 24-h data for standards compliance can not be used if the flow is less than the 7Q2. In perennial streams, the D.O. criterion to do not apply for flows under the 7Q2. A period of about one month must separate each 24-h sampling event. Additional samples may be collected outside the index period to further characterize a water body, but that information is generally not used for assessing standards compliance.

Frequency of Measurements

The measurement interval should be no more than once per 15 min and no less than once per hour.

Where to Take Measurements

For purposes of determining standards compliance with the 24-h average criteria, samples collected near the surface will be considered representative of the mixed surface layer. In deep streams, reservoirs, and tidally influenced water bodies, automated equipment is positioned between 1 foot (from the surface) to one-half the depth of the mixed surface layer. At least 10 24-h monitoring events (using the 24-h criteria and/or absolute minimum criteria) at each site within a 5-year period are recommended to provide adequate data for assessment.

When to Collect Other Routine Samples, if doing 24-hour D.O. measurements

Other routine field measurements and water samples should be collect at either the time of deployment, at the reference check, or when the multiprobe recording 24-h data is retrieved. When ever possible, flow must be measured at the 24-h site.

Priority for Scheduling 24-Hour Sampling Events

- < 303d listed waterbodies
- < Waterbodies with Concerns for DO problems (too few samples available for full use assessment).

- Occurrence of low D.O. concentrations observed during the day
- Waterbodies with trends indicating declining D.O. concentrations
- Waterbodies which would contribute to an Ecoregion data set

Data Reporting for 24-hour D.O. measurements
Dissolved oxygen values recorded over the 24-h period are summed and divided by the number of measurements to determine the average concentration, which is compared to the 24-h criterion. The lowest D.O. value from each 24-h set is compared to the minimum criterion. There will be occasions when a complete 24-h data set won't be possible. For example, if there are 18 measurements instead of 24, a time weighted diurnal average needs to be calculated. This can be easily done using GW Basic.

Support of assigned aquatic life use is based on 24-h D.O. average and minimum criteria for each monitoring event. Report the 24-h average D.O. value, number of measurements over a 24-h period, and the minimum, and maximum values. Report data as a time composite sample with a beginning and ending date and time, covering the 24-h period measured.

Specific Conductance (µS/cm)

Specific conductance should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff.
See http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting.

Specific Conductance Sampling Equipment
The conductivity meter should be calibrated according to the recommended procedures for calibration and maintenance of SWAMP field equipment. Calibration directions are listed in the manufactures field equipment operations manual.

Specific Conductance Sampling Procedure
Preferably, conductivity is measured directly in-stream at the depth(s) specified earlier in this document. Allow the conductivity probe to equilibrate for at least one minute before specific conductance is recorded to three significant figures (if the value exceeds 100). The primary physical problem in using a specific conductance meter is entrapment of air in the conductivity probe chambers. The presence of air in the probe is indicated by unstable specific conductance values fluctuating up to $\pm$100 µS/cm. The entrainment of air can be minimized by slowly, carefully placing the probe into the water; and when the probe is completely submerged, quickly move it through the water to release any air bubbles.

If specific conductance cannot be measured in-stream, it should be measured in the container it can be measured in a bucket-Nalgene or plastic. The following precautions are outlined above; "Temperature Measurement from a Bucket".

Salinity (parts per thousand--ppt, or ‰)
The value for salinity is computed from chloride concentration or specific conductance. The calculation assumes a nearly constant ratio for major ions in an estuary when seawater is diluted

by river water. This assumption does not hold for cases where salinity is less than about three parts per thousand. Salinity determinations at such low values are only approximate. In estuarine waters, salinity is a relevant and meaningful parameter. Often the salinity may be low, approaching that of freshwater. Nevertheless, this is useful information. Determine if a station is estuarine from historical records (i.e., experiences cases where salinity is >2.0 ppt) and always report salinity at this station, regardless of the salinity during periods of high flow.

Salinity is measured directly in-stream at the depth(s) specified earlier in this document. Salinity data should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff. See http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting.

Values between 2.0 ppt and 1.0 ppt should be reported as <2.0 ppt rather than the actual value and values <1.0 ppt should be reported as <1.0 ppt. The field instruments compute salinity from specific conductance and temperature, and display the value in parts per thousand. Report salinity values above 2.0 ppt to the nearest 0.1 ppt.

Secchi Disc Transparency (meters)--if requested in special study

Secchi disk transparency should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff. See http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting.

Secchi Disk Sampling Equipment

- Secchi disk, 20 cm in diameter
- Measuring tape

Secchi Disk Transparency Sampling Procedures

Preferably, Secchi disk transparency is measured directly in-stream wherever conditions allow. The Secchi disk should be clean, weighted and suspended with chain, wire, or Dacron line (the line used to suspend the Secchi disk should not be nylon or cotton; stretching may cause erroneous readings). Another option is to attach the Secchi disk to a metal rod calibrated in metric units.

Average Turbidity

The Secchi disk should be lowered vertically in a location shielded from direct sunlight. Glare from the water's surface will affect the accuracy of the measurement. Don't wear sunglasses.

Slowly lower the disk until it disappears from view. The person viewing the disk should maintain an eye level of less than two meters above the water's surface. Note the depth at which the disk disappears from view.

Slowly raise the disk until it becomes visible. Note the depth at which the disk reappears.

Compute the mathematical average of the two depths noted and record the average value to two significant figures in the field logbook. The recorded average value is the Secchi disk transparency.

High Turbidity (Muddy Water)

In streams with very high turbidity, high velocity, and/or poor access, it may be necessary to measure Secchi disk transparency in a bucket. Fill the bucket from the centroid of flow being careful not to disturb the substrate.

Follow steps above for measuring the Secchi disk depth within 30 s after raising the filled bucket from the water's surface. Or, re-suspend the solids by stirring, then quickly make the measurement.

Record Secchi disk transparency to two significant figures.

Low Turbidity (Clear Water)

Some bodies of water will be so clear and shallow that it will not be possible to lower the Secchi disk until it disappears from view.

Measure and record the depth at the deepest point accessible. Report Secchi disk transparency as greater than the deepest depth measured.

Example (Low Turbidity): South Fork Rocky Creek is a small (<1 ft^3/s) clear stream. The stream in the vicinity of the sampling site was less than 1 m deep and the bottom was clearly visible everywhere. However, a pool was located in the stream next to a bridge. The maximum depth of the pool was 2.6 m at which depth the Secchi disk was still visible. Therefore, Secchi disk transparency for South Fork Rocky Creek was recorded as > 2.6 m.

Importance of Secchi Disk Data

Eutrophication, the natural aging process in reservoirs and lakes is accelerated by human activities which add nutrients to lakes, reservoirs, and the surrounding watersheds. Section 314 of the Clean Water Act (CWA) of 1987 requires all states to classify lakes and reservoirs according to trophic state. Although chlorophyll a is the most direct measure of algal biomass, other indices and programs utilize Secchi disk depth as the primary factor.

Turbidity Measurement with Turbidity Meter

Nephelometric Turbidity can be determined by measuring the amount of scatter when light is passed through a sample using a turbidity meter. The LaMotte 2020 Turbidity meter is a suitable instrument for example.

Meters should be calibrated using a standard close to the expected sample value.

For instructions on how to operate the instruments refer to the manufacturer's manual. Turbidity measurements can be executed together with water sampling. The turbidity sample has to be representative for the sampled water mass. Make sure that no gas bubbles are trapped in the vial for the reading and that the outside of the vial is wiped completely clean (i.e., meaning free of moisture, lint and fingerprints). Take several measurements to assure an accurate reading. Do not record values that vary greatly. If variations are small, record an average. If settling particles are present, record a reading before and one after settling. The meter might have to be recalibrated with a different standard, if the sample water readings are outside of the calibration standard limits.

Days Since Last Significant Precipitation

Significant precipitation is defined as any amount that visibly influences water quality. Water quality in small to medium streams and in the headwaters of many reservoirs is influenced by runoff during and immediately after rainfall events. This influence is site specific and poorly studied. As part of a new initiative to understand and regulate the adverse effects of runoff, SWAMP would like to associate recent rains or melted snow with ambient water quality, using a parameter defined as "days since last significant precipitation". Record the number of days, rounded to the nearest whole number, since a rain has occurred that, in the best professional judgment of monitoring personnel, may have influenced water quality. If it is raining when the sample is collected, or has rained within the last 24-h, report a value of <1. If it has been a long time since a significant rain, record this as greater than that particular value, for example >7 days. If confidence about the recent history of precipitation is low, draw a line through the space on the data form.

Flow Severity -- recommended new parameter

Flow severity should be noted for each SWAMP visit to non-tidally influenced flowing streams and submitted in the comments on the SWAMP Field Data Sheet. It should be recorded even if flow is visible but not measurable on that sampling visit. There are no numerical flow guidelines associated with flow severity. This is an observational measurement that is highly dependent on the knowledge of monitoring personnel. It is a simple but useful piece of information when assessing water quality data. For example, a bacteria value of 10,000 with a flow severity of 1 would represent something entirely different than the same value with a flow severity of 5. The six flow severity values are; 1=No Flow, 2= Low Flow, 3 = Normal Flow, 4 = Flood, 5 = High Flow, and 6 = Dry. The following are detailed descriptions of severity values:

1 **No Flow** When a flow severity of one (1 = no flow) is recorded for a sampling visit, then a flow value of zero ft^3/s should also be recorded for that sampling visit. **A flow severity of one (1) (no flow) describes situations where the stream has water visible in isolated pools.** There should be no obvious shallow subsurface flow in sand or gravel beds between isolated pools. Low flow does not only apply

to streams with pools. It also applies to long reaches of bayous and streams that have no detectable flow but may have water from bank to bank.

2 **Low Flow** When stream flow is considered low a flow severity value of two (2) is recorded for the visit and the corresponding flow measurement is also recorded for that visit. In streams too shallow for a flow measurement but with detected water movement, record a value of < 0.10 cfs. Note: Use a stick or other light object to verified the direction of water movement (i.e., movement is downstream and not the affect of wind.) What is low for one stream could be high for another.

3 **Normal Flow** When stream flow is considered normal, a flow severity value of three (3) is recorded for the visit and the corresponding flow measurement is also be recorded for that visit. Normal is highly dependent on the stream. Like low flow, what is normal for one could be high or low for another stream.

4 and 5 **Flood and High Flow** Flow severity values for high and flood flows have long been established by EPA and are not sequential. Flood flow is reported as a flow severity of four (4) and high flows are reported as a flow severity of five (5). High flows would be characterized by flows that leave the normal stream channel but stay within the stream banks. Flood flows are those which leave the confines of the normal stream channel and move out on to the flood plain.

6 **Dry** When the stream is dry a flow severity value of six (6 = dry) is recorded for the sampling visit. In this case the flow is not reported. This will indicate that the stream is completely dry with no visible pools.

Flow information for over 200 USGS sites is available on the Internet. The address is **http://water.usgs.gov/index.html**. This is useful information in determining flow conditions prior to sampling. This information may be included in general observations.

Flow Measurement Method (Reporting)

The method (or instrument) used to measure flow is noted by reporting a method number. The method numbers are:

1- Flow Gage Station (USGS/IBWC)	3- Electric (ex. Marsh-McBirney)
2- Mechanical (ex. Pigmy meter)	4- Weir/Flume
5- Other (orange peel, etc.)	

Flow (ft^3/s)

If requested, flow data should be recorded for each monitoring visit to non-tidal, flowing streams. Flow data should be recorded in final form on a Field Data Sheet and submitted to the SWAMP data management staff. See http://mpsl.mlml.calstate.edu/swdwnlds.htm for detailed information on data reporting. The following are two exceptions to the flow reporting requirement:

No Flow/ Pools — If there is no flow at a stream site and accessible, isolated pools remain in the stream bed, collect and report the required field data and laboratory samples from the pools and report instantaneous flow. Under these conditions, flow (ft^3/s) should be reported as zero. The reported flow severity value should be one. Pools may represent natural low-flow conditions in some streams and the chemistry of these pools will reveal natural background conditions.

Dry — If the stream bed holds no water, the sampling visit is finished. Report that the stream was "dry" in the observations and record a value of six (meaning "dry") for flow severity. No value is reported for flow since there is no water.

Flow Measurement

If a flow measurement is required at a site, measure and record flow after recording visual observations. The intent of measuring flow first is to delay collection of chemical and biological water samples with limited holding times. Care must be taken not to collect water samples in the area disturbed during flow measurement. There are several acceptable flow measurement methods that can be used.

U.S. Geological Survey (USGS) Gaging Station

Some SWAMP Stations are sampled at sites where the USGS maintains flow gaging equipment. On any type of sampling visit to a site that has a USGS flow gage, observe and record the gage height to the nearest hundredth of a foot in the field logbook. Upon return to the office, contact the USGS office responsible for maintaining the gage. USGS personnel can provide the flow value in cubic feet per second (ft^3/s) that corresponds to the gage height. Although SWAMP personnel may have a rating curve available to them, shifts associated with changes in the stream bed may occur over time. Always call the USGS to determine the shift. At some sites the shift changes frequently. At others, the relation between stream flow and gage height is almost unchanging. If a gage is no longer maintained by USGS, cross out the recorded gage height and be prepared to measure flow by another method on the return visit to that site.

Several factors may influence the accuracy of the USGS rating curves that are used to convert gage height to flow. If there is any doubt about the accuracy of a USGS gage height reading or flow rating curve, sampling personnel should measure the flow if possible.

Gage height may be indicated at a USGS gage by one of three methods:

Staff Gage

Staff gages are enameled steel plates (with the appearance of large measuring tapes) bolted to some stable structure. For example, staff gages may be bolted to concrete bridge abutments, pillars, or docks. The staff gage face is white with black lettering and gradations. The gradations shown are feet, tenths of a foot, and 0.02 of a foot. The point at which the water level crosses the staff gage should be recorded to the nearest hundredth of a foot.

Wire Weight Gage

Wire weight gages are locked, metal boxes with approximate dimensions of 15 in. long x 12 in. tall x 12 in. deep. Wire weight gages are usually affixed to bridge rails near mid-stream. They must be unlocked with a USGS key. The wire weight gages house a weight attached by wire cable to a graduated reel (gradations are tenths and hundredths of feet) with a counter at one end.

When the reel is released the weight can be gradually lowered until the bottom of the weight contacts the water surface. At the point of contact, the weight causes the water surface to ripple slightly. Maintaining the weight in that position, record the counter value to the nearest whole number and the point indicated by the stylus on the graduated reel to the nearest hundredth of a foot. Determine if the gage is the movable type that can be moved to multiple locations on the bridge. This type is common on braided streams. A correction value is stamped on the bridge near each point that the gage can be attached. Record the corrected value as the gage height in feet.

Bubble Gage

Bubble gages are locked in metal sheds that are approximately 4 ft wide x 4 ft deep x 6.5 ft tall. The gage houses are most frequently located on the shore near a bridge but sometimes are attached to bridge pillars near mid-stream or established on the stream bank far from any bridge. The gage house must be unlocked with a USGS key. Bubble gages in gage houses usually indicate the gage height in two or three locations. A counter attached to the manometer system indicates gage height in feet. Some gage houses have stilling wells that can be entered. Often there is a staff gage on the inside wall.

Most bubble gages are also equipped with digital recorders. Digital recorders consist of two white, coded discs, approximately 4 in. in diameter with a punch tape overlapping a portion of each disc. The discs are marked with 100 gradations. As the front of the digital recorder is viewed, the stylus at the disc on the left indicates height in feet. The stylus at the disc on the right indicates gage height in hundredths of feet. The gage height from both discs should be added and the number recorded in the field logbook as gage height to the nearest hundredth of a foot.

Many USGS metal sheds also contain a surface level recorder. This devise can be opened to determine how stable stream flow has been prior to the sampling event. Record observations concerning the flow hydrograph.

Instantaneous Flow Measurement

Water quality monitoring visits to sites where there are no nearby USGS flow gauges will require water quality monitoring personnel to measure flow, when requested by Regional Water Quality Control Boards (Regional Boards).

Flow Measurement Equipment

Flow meter

One of the following or an equivalent:

- Marsh-McBirney Electronic meter
- Montedoro-Whitney Electronic meter
- Price Pigmy meter (with timer and beeper)
- Price meter, Type AA (with Columbus weight)

Additional Equipment

- Top-setting wading rod (preferably measured in tenths of feet)(see Figure 1).
- Tape measure (with gradations every tenth of a foot).

Flow Measurement Procedure (USGS, 1969)

Select a stream reach with the following characteristics:

- Straight reach with laminar flow (threads of velocity parallel to each other) and bank to bank. These conditions are typically found immediately upstream of riffle areas or places where the stream channel is constricted.
- The site should have an even streambed free of large rocks, weeds, and protruding obstructions that create turbulence. The site should not have dead water areas near the banks, and a minimum amount of turbulence or back eddies.

Flat Streambed Profile (cross section)

Stretch the measuring tape across the stream at right angles to the direction of flow. When using an electronic flow meter, the tape does not have to be exactly perpendicular to the bank (direction of flow). When using a propeller or pigmy type meter, however, corrections for deviation from perpendicular must be made.

If necessary and possible, modify the measuring cross section to provide acceptable conditions by building dikes to cut off dead water and shallow flows, remove rocks, weeds, and debris in the reach of stream one or two meters upstream from the measurement cross section. After modifying a streambed, allow the flow to stabilize before starting the flow measurement.

Record the following information on the flow measurement form (see example Flow Measurement Forms at end of this document):

- Station Location and Station ID
- Date
- Time measurement is initiated and ended
- Name of person(s) measuring flow
- Note if measurements are in feet or meters
- Total stream width and width of each measurement section
- For each cross section, record the mid-point, section depth and flow velocity

Measuring the Stream Width

Measure and record the stream width between the points where the tape is stretched (waters edge to waters edge).

Determining the Number of Flow Cross Sections

Determine the spacing and location of flow measurement sections. Some judgment is required depending on the shape of the stream bed. Measurements must be representative of the velocity within the cross-section. If the stream banks are straight and the depth is nearly constant and the bottom is free of large obstructions, fewer measurements are needed, because the flow is homogeneous over a large section. Flow measurement sections do not have to be equal width. However, they should be unless an obstacle or other obstruction prevents an accurate velocity measurement at that point. ***No flow measurement section should have greater than 10% of the total flow.***

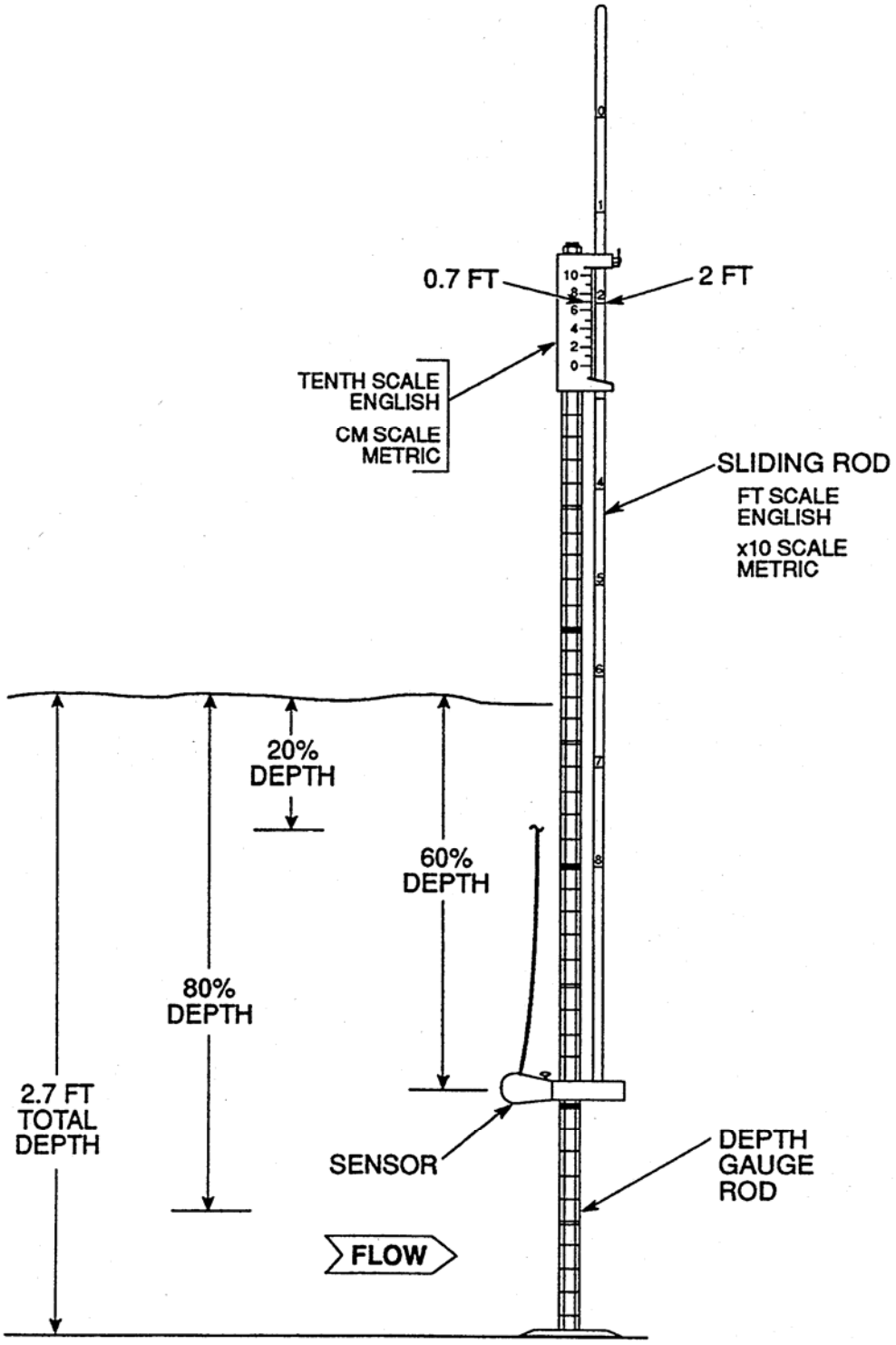
If the *stream width is less than 5 ft*, use flow sections with a width of 0.5 ft (See example 1 on page 23 of this document). If the *stream width is greater than 5 ft*, the minimum number of flow measurements is 10. The preferred number of flow measurement cross sections is 20-30 (See Example 2 on page 24 on this document). The total stream width is 26 ft with 20 measurements, section widths will be 1.3 ft (26/20 = 1.3).

Determining the Mid-Point of the Cross Section

To find the mid-point of a cross section, divide the cross section width in half. Using Example 2 (see forms at end of document);

- The total stream width is 26 ft with 20 cross sections and each cross section width is equal to 1.3 ft.
- Divide 1.3 ft in half and the mid-point of the first section is 0.65 ft. In this example the tape at waters edge is set at zero (0) ft.
- By adding 0.65 to zero the mid-point of the first section is 0.65 ft.
- Each subsequent mid-point is found by adding the section width (1.3 ft) to the previous mid-point. For example; MIDPOINT #1 is 0.65 + 0.0 = 0.65; MIDPOINT #2 is 0.65 + 1.3= 1.95 ft; MIDPOINT #3 is 1.95 + 1.3 = 3.25 ft and ....MIDPOINT # 20 is 24.05 +1.3.
- Place the top setting wading rod at 0.65 ft for the first measurement.
- Using a top setting wading rod, measure the depth at the mid-point of the first flow measurement section and record to the nearest 0.01 ft.

Figure 1. Top-Setting Wading Rod (Marsh-McBirney)



Adjusting the Sensor Depth at a Cross Section

Adjust the position of the sensor to the correct depth at each mid-point. The purpose of the top setting wading rod is to allow the user to easily set the sensor at 20%, 60%, and 80% of the total depth. The total depth can be measured with the *depth gage rod*. Each single mark represents 0.10 foot, each double mark represents 0.50 foot, and each triple mark represents 1.00 foot (see Figure 2).

For Depths < 2.5 Ft	If the depth is less than 2.5 ft, only one measurement is required at each measurement section. To set the sensor at 60% of the depth, line up the foot scale on the *sliding rod* with the *tenth scale*, located on top of the depth gage rod. If, for example, the total depth is 2.7 ft (as shown on Figure 2), then line up the 2 on the foot scale with the 7 on the tenth scale (Marsh-McBirney 1990).
For Depths > 2.5 Ft	If the depth is greater than 2.5 ft, two measurements should be taken at 20% and 80% of the total depth. To set the sensor at 20% of the depth, multiply the total depth by two. For example, if the total depth is 2.7 ft, the rod would be set at 5.4 ft (2.7 x 2). Line up the 5 on the sliding rod with the 4 on the tenth scale.
For Depths > 2.5 Ft (cont)	To set the sensor at 80% of the depth, divide the total depth by two. For example, the total depth is 2.7 ft the rod would be set at 1.35 ft (2.7/2). Line up the 1 on the sliding rod with the 0.35 on the tenth scale. The average of the two velocity measurements is used in the flow calculation. See page 2-36 for an example of a flow form recording measurements for depths greater than 2.5 ft. NOTE: The point where the rod is set for 20 and 80% of the depth will not equal values derived by calculating 20 and 80% of the total depth.

Measuring Velocity (this has typically been measured at 6/10 of the total depth, for velocity-only measurements)

- Position the meter at the correct depth and place at the mid-point of the flow measurement section. Measure and record the velocity and depth. The wading rod is kept vertical and the flow sensor kept perpendicular to the tape rather than perpendicular to the flow while measuring velocity with an electronic flow meter. When using a propeller or pigmy-type meter, however, the instrument should be perpendicular to the flow.
- Permit the meter to adjust to the current for a few seconds. Measure the velocity for a minimum of 20 s with the Marsh-McBirney and Montedoro-Whitney meters. Measure velocity for a minimum of 40 s (preferably 2 min with the Price and pigmy meters).
- When measuring the flow by wading, stand in the position that least affects the velocity of the water passing the current meter. The person wading stands a minimum of 1.5 ft downstream and off to the side of the flow sensor.

- A flow sensor, equipped with cable and weight may be used to measure flows where the water is too deep to wade. Follow the procedure involving meters attached to wading rods.
- Report flow values less than 10 ft^2/s to two significant figures. Report flow values greater than 10 ft^3/s to the nearest whole number, but no more than three significant figures.
- In cases where the flow is low and falling over an obstruction, it may be possible to measure the flow by timing how long it takes to fill a bucket of known volume.

Avoid measuring flow in areas with back eddies. The first choice would be to select a site with no back eddy development. However, this can not be avoided in certain situations. Measure the negative flows in the areas with back eddies. These negative values will be included in the final flow calculation.

Calculating Flow

To calculate flow, multiply the width x depth (ft^2) to derive the area of the flow measurement section. The area of the section is then multiplied by the velocity (ft/s) to calculate the flow in cubic feet per second (cfs or ft^3/sec) for that flow measurement section. When flow is calculated for all of the measurement sections, they are added together for the total stream flow (see Figure 2).

Q=Total Flow (or discharge), W=Width, D=Depth, V=Velocity.

$$Q = (W_1 * D_1 * V_1) + (W_2 * D_2 * V_2) + ... (W_n * D_n * V_n)$$

What to Do with Negative Values

Do not treat cross sections with negative flow values as zero. Negative values obtained from areas with back eddies should be subtracted during the summation of the flow for a site.

Flow Estimate (ft^3/s)

Flow estimate data may be recorded for a non-tidally influenced stream when it is not possible to measure flows by one of the methods described above. Flow estimates are subjective measures based on field personnel's experience and ability to estimate distances, depths, and velocities. If flow can not be measured at a routine non-tidal station, a new site should be selected where flow can be measured.

Flow Estimate Procedure

- Observe the stream and choose a reach of the stream where it is possible to estimate the stream cross section and velocity.
- Estimate stream width (ft) at that reach and record.
- Estimate average stream depth (ft) at that reach and record. Estimate stream velocity (ft/s) at that reach and record. A good way to do this is to time the travel of a piece of floating debris. If doing this method from a bridge, measure the width of the bridge. Have one person drop a floating object (something that can be distinguished from other

floating material) at the upstream side of the bridge and say start. The person on the downstream side of the bridge will stop the clock when the floating object reaches the downstream side of the bridge. Divide the bridge width by the number of seconds to calculate the velocity. The velocity can be measured at multiple locations along the bridge. These velocities are averaged. If this is done alone, watch for road traffic.

< Multiply stream width (ft) times average stream depth (ft) to determine the cross sectional area (in ft^2) which when multiplied by the stream velocity (in ft/s) and a correction constant, gives an estimated flow (ft^3/s).

Example: A stream sampler conducted a sampling visit to a stream while the flow meter was being repaired. The sampler looked at the creek downstream from the bridge and saw a good place to estimate flow. The stream width was around 15 ft. It appeared the average depth on this reach was about 0.75 ft. The sampler timed a piece of floating debris as it moved a distance of 10 ft in 25 s downstream over the reach. An estimated flow with a smooth bottom was calculated using the following formula.

Width x Depth x Velocity x A (correction factor)= estimated flow
15 ft (width) x 0.75 ft (depth) x 2.5 ft/s (velocity) x A =25 ft^3/s (cfs)

A is a correction constant: 0.8 for rough bottom and 0.9 for smooth bottom

Estimated flow should be reported to one or two significant figures.

Experienced field personnel are able to estimate flow to within 20% of actual flow for total flows less than 50 ft^3/s. The best way to develop this skill is to practice estimating flow before making measurements at all monitoring visits to non-tidally influenced flowing streams and then compare estimated flows with those obtained from USGS gages or from instantaneous flow measurements

Figure 2. Stream Flow (Discharge) Measurement

Example 1.
Stream Flow (Discharge) Measurement
Small Stream < 5 Ft Wide and #2.5 Ft Deep

Stream:___**_OAK CREEK**_____Date:__**5/29/91**___
Station Description:_____**at US Hwy 90A**_____
Time Begin:__**1545**_____Time End:__**1630**_____Meter Type:_**_Marsh-McBirney**_____
Observers:_____**BK/MK**_____Stream Width*:____**5 ft**_____ Section Width:____**0.5 ft**_____
Observations:_____

Section Midpoint (ft)	Section Depth (ft)	Observational Depth** Ft	Velocity		Area W x D (ft^2)	Discharge (Q) V x A (ft^3/s)
			At Point (ft/s)	Average (ft/s)		
0.25	**0.55**			**0.05**		**0.01375**
0.75	**0.80**			**0.11**		**0.044**
1.25	**0.85**			**0.27**		**0.42635**
1.75	**0.90**			**0.49**		**0.2205**
2.25	**1.10**			**0.58**		**0.275**
2.75	**1.50**			**0.72**		**0.540**
3.25	**1.20**			**0.76**		**0.456**
3.75	**0.90**			**0.76**		**0.342**
4.25	**0.75**			**0.44**		**0.165**
4.75	**0.30**			**0.00**		**0.00**

m^3/s x 35.3 = ft^3/s

Total Discharge (3Q) (ft^3/s)	**2.4826**

Example 2.

Stream Discharge Measurement Example (Larger Stream > 5 Ft and #2.5 Ft Deep)

Stream:___**_RED RIVER**_____Date:__**5/28/91**___

Station Description:_____**Post Oak Creek 40 m Below Sherman WWTP Outfall**_____

Time Begin:__**1542**_____Time End:__**1601**_____Meter Type:_**Marsh-McBirney**_____

Observers:_____**CM, EW, DO**_____Stream Width*:____**26 ft**___ Section Width:___**1.3 ft**_____

Observations:_____

Section Midpoint (ft)	Section Depth (ft)	Observational Depth** ft	Velocity		Area W x D (ft^2)	Discharge (Q) V x A (ft^3/s)
			At Point (ft/s)	Average (ft/s)		
0.65	0.55			2.03	0.715	1.451
1.95	0.40			2.04	0.520	1.061
3.25	0.42			2.02	0.546	1.103
4.55	0.38			1.77	0.494	0.874
5.25	0.40			1.75	0.520	0.910
7.15	0.42			1.93	0.546	1.054
8.45	0.40			1.99	0.52	1.035
9.75	0.37			1.92	0.481	0.924
11.05	0.37			1.56	0.481	0.750
12.35	0.43			1.32	0.559	0.738
13.65	0.40			1.36	0.520	0.707
14.95	0.42			1.33	0.546	0.726
16.25	0.40			1.35	0.520	0.702
17.55	0.45			1.64	0.585	0.959
18.85	0.48			1.70	0.624	1.061
20.15	0.48			2.00	0.624	1.248
21.45	0.50			1.95	0.650	1.268
22.75	0.40			2.18	0.520	1.134
24.05	0.48			1.71	0.624	1.067
25.35	0.50			0.60	0.650	0.390
				Total Discharge (3Q) (ft^3/s)		**19.162**

m^3/s x 35.3 = ft^3/s

Example 3.

Stream Flow (Discharge) Measurement (Larger Stream > 5 Ft and >2.5 Ft Deep)

Stream:____ARROYO COLORADO_____Date:__**6/16/98**__

Station Description:_____Downstream of Harlingen WWTP_____

Time Begin:__**1400**_____Time End:__**1445**_____Meter Type:__**Marsh-McBirney**_____

Observers:_____**JD, CK**_____Stream Width*:___**47.5 ft**___ Section Width:___**2.375 ft**_____

Observations: *Note that the starting point is at 4.7 ft on the measuring tape and not zero.

Section Midpoint (ft)	Section Depth (ft)	Observational Depth** ft	Velocity		Area W x D (ft^2)	Discharge (Q) V x A (ft^3/s)
			At Point (ft/sec)	Average (ft/sec)		
4.70	0.73			0.65	1.73	1.127
7.08	1.10			1.08	2.61	2.822
9.45	1.85			0.90	4.39	3.954
11.83	2.20			1.05	5.23	5.486
14.20	2.20			1.44	5.23	7.531
16.58	2.45			1.09	5.82	6.342
18.95	2.55	0.20	1.75	1.76	6.06	10.659
		0.80	1.76			
21.33	2.60	0.20	1.79	1.56	6.18	9.633
		0.80	1.32			
23.70	2.70	0.20	1.63	1.45	6.41	9.298
		0.80	1.26			
26.10	3.05	0.20	1.68	1.42	7.24	10.286
		0.80	1.15			
28.48	3.10	0.20	1.23	0.96	7.36	7.068
		0.80	0.69			
30.85	2.90	0.20	1.22	1.06	6.89	7.301
		0.80	0.89			
33.23	2.84	0.20	0.60	0.49	6.75	3.305
		0.80	0.37			
35.60	2.65	0.20	0.80	0.51	6.29	3.210
		0.80	0.21			
37.98	2.65	0.20	0.85	0.91	6.29	5.727
		0.80	0.96			
40.35	2.20			0.28	5.23	1.464
42.73	2.30			0.16	5.46	0.874
45.10	2.05			0.51	4.87	2.483
47.48	1.10			0.49	2.61	1.280
49.86	0.65			0.62	1.54	0.957

m^3/s x 35.3 = ft^3/s | **Total Discharge (3Q) (ft^3/s)**

Stream Flow (Discharge) Measurement Form

Stream:____Date:___________
Station Description:____
Time Begin:________ Time End:__________ Meter Type:_________________
Observers:_______________Stream Width*:__________ Section Width:________
Observations:____

Section Midpoint (ft) (m)	Section Depth (ft) (m) (cm)	Observational Depth** ft-m-cm	Velocity		Area W x D (ft^2) (m^2)	Flow (Q) V x A (m^3/s) (ft^3/s)
			At Point (ft/s) (m/s)	Average (ft/s)(m/s)		

$m^3/s \times 35.3 = ft^3/s$

Total Flow (Discharge) (3Q) (ft^3/s): ________

* Make a minimum of 10 measurements when the total width is > 5.0 ft, 20 measurements preferred.

** When water is < 2.5 ft deep take one measurement at each cross section. When water is > 2.5 ft deep, take two measurements at each cross section; one at 2 the total depth and the other at 2 x the total depth. Average the two velocity measurements. See SWAMP Procedures Manual for a detailed flow measurement method.

Summary of Significant Figures for Reporting Field Parameters

Parameter	Field Data Reporting Requirements
Water Temperature (^oC)	Report temperature to the nearest tenth of a degree. Round insignificant figures 0 through 4 down and 5 thru 9 up.
pH (s.u.)	Report pH to the nearest tenth of a pH standard unit.
D.O. mg/L **D.O.** (% saturation)	Report dissolved oxygen to the nearest tenth of a mg/L. Report % saturation to the nearest tenth of a percent
Specific Conductance (micro siemens/cm)	Report specific conductance to only three significant figures if the value exceeds 100. Do not report ORP which is displayed by some multiprobes.
Salinity (ppt)	Report salinity values above 2.0 ppt to the nearest tenth of a part per thousand. In estuarine waters report the actual values displayed by the multiprobe above 2.0 ppt and values less than 2.0 as <2.0 or <1.0 only. Determine if a station is estuarine (i.e., experiences cases where salinity is >2.0 ppt) and always report salinity at this station, regardless of the salinity during periods of high flow.
Secchi Disk (meters)	Report Secchi depth transparency in meters to two significant figures.
Days Since Last Significant Precipitation(days)	Report whole numbers. If it is raining when the sample is collected or has rained within the last 24 h, report a value of <1. If it has been over a week since a rainfall event, report a value of > 7.
Flow (ft^3/s)	Report instantaneous flow values less than 10 ft^3/s to two significant figures. Report flow values greater than 10 ft^3/s to the nearest whole number, but no more than three significant figures. When there is no flow (pools), report as 0.0. When there is no water, don't report a value, but report as "dry" in the observations.
Flow Severity (1-no flow, 2-low, 3-normal, 4-flood, 5-high, 6-dry)	When there is no flow (pools), report the severity as 1, and the instantaneous flow as 0.0 ft^3/s. If the stream is dry, record only flow severity, as a value of 6.

BEAUFORT SCALE: Specifications and equivalent speeds for use at sea

FORCE	EQUIVALEN SPEED 10 m above ground		DESCRIPTION	SPECIFICATIONS FOR USE AT SEA
	Miles/hour	knots		
0	0-1	0-1	Calm	Sea like a mirror
1	1-3	1-3	Light air	Ripples with the appearance of scales are formed, but without foam crests.
2	4-7	4-6	Light Breeze	Small wavelets, still short, but more pronounced. Crests have a glassy appearance and do not break.
3	8-12	7-10	Gentle Breeze	Large wavelets. Crests begin to break. Foam of glassy appearance. Perhaps scattered white horses.
4	13-18	11-16	Moderate Breeze	Small waves, becoming larger; fairly frequent white horses.
5	19-24	17-21	Fresh Breeze	Moderate waves, taking a more pronounced long form; many white horses are formed. Chance of some spray.
6	25-31	22-27	Strong Breeze	Large waves begin to form; the white foam crestsare more extensive everywhere. Probably some spray.
7	32-38	28-33	Near Gale	Sea heaps up and white foam from breaking waves begins to be blown in streaks along the direction of the wind.
8	39-46	34-40	Gale	Moderately high waves of greater length; edges of crests begin to break into spindrift. The foam is blown in well-marked streaks along the direction of the wind.
9	47-54	41-47	Severe Gale	High waves. Dense streaks of foam along the direction of the wind. Crests of waves begin to topple, tumble, and roll over. Spray may affect visibility.
10	55-63	48-55	Storm	Very high waves with long over- hanging crests. he resulting foam, in great patches, is blown in dense white streaks along the direction of the wind. On he whole the surface of the sea takes on a white appearance. The 'tumbling' of the sea becomes heavy and shock-like. Visibility affected.

Last edited on 09 January, 1999 Dave Wheeler weatherman@zetnet.co.uk
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http://www.zetnet.co.uk/sigs/weather/Met_Codes/beaufort.htm

Field Collection Procedures for Water Samples

Scope and Application

This protocol describes the techniques used to collect water samples in the field in a way that neither contaminates, loses, or changes the chemical form of the analytes of interest. The samples are collected in the field into previously cleaned and tested (if necessary) sample bottles of a material appropriate to the analysis to be conducted. Pre-cleaned sampling equipment is used for each site, whenever possible and/or when necessary. Appropriate sampling technique and measuring equipment may vary depending on the location, sample type, sampling objective, and weather. Trade names used in connection with equipment or supplies do not constitute an endorsement of the product.

Summary of Method

Appropriate sample containers and field measurement gear as well as sampling gear are transported to the site where samples are collected according to each sample's protocol. Water velocity, turbidity, temperature, pH, conductivity, dissolved oxygen as well as other field data are measured and recorded using the appropriate equipment. These field data measurement protocols are provided in the SWAMP Field Measurement SOP. Samples are put on ice and appropriately shipped to the processing laboratories. This procedure has been modified from the Texas Natural Resources Conservation Commission's Procedure Manual for Surface Water Quality Monitoring, with major input from the United State's Geological Survey's (USGS's) National Water Quality Assessment (NAWQA) Protocol for Collection of Stream Water Samples, for which due credit is herewith given.

WATER SAMPLE COLLECTION

Water chemistry and bacteriological samples, as requested, are collected at the same location. *Water samples are best collected before any other work is done at the site*. If other work (e.g., sediment sample collection, flow measurement or biological/habitat sample collection or assessment) is done after or downstream of the collection of water samples, it might be difficult to collect representative samples for water chemistry and bacteriology from the disturbed stream. Care must be taken, though, to not disturb sediment collection sites when taking water samples.

The following general information applies to all types of water samples, unless noted otherwise:

Sample Collection Depth

Sub-Surface Grab Sample Samples are collected at 0.1 m below the water surface. Containers should be opened and re-capped under water in most cases.

Depth-integrated Sample If a depth-integrated sample is

taken, the sample is pumped from discrete intervals within the entire water column.

Surface Grab Sample Samples are collected at the surface when water depth is <0.1 m. Since there is a difference in water chemistry on the surface, compared to subsurface, surface water should be noted on the field data sheet as 0 m.

Where to Collect Samples

Water samples are collected from a location in the stream where the stream visually appears to be completely mixed. Ideally this would be at the centroid of the flow (*Centroid* is defined as the midpoint of that portion of the stream width, which contains 50% of the total flow), but depth and flow do not always allow centroid collection. For stream samples, the sampling spot must be accessible for sampling physicochemical parameters, either by bridge, boat or wading. Sampling from the shoreline of any water body (meaning standing on shore and sampling from there) is the least acceptable method, but in some cases is necessary.

In reservoirs, lakes, rivers, and coastal bays, samples are collected from boats at designated locations provided by Regional Water Quality Control Boards (Regional Boards).

Sampling Order if Multiple Media are Requested to be Collected

The order of events at every site has to be carefully planned. For example, if sediment is to be collected, the substrate can not be disturbed by stepping over or on it; water samples can not be taken where disturbed sediment would lead to a higher content of suspended matter in the sample. *For the most part, water samples are best collected before any other work is done at the site*. This information pertains to walk-in sampling.

Sample Container Labels

Label each container with the station ID, sample code, matrix type, analysis type, project ID, and date and time of collection (in most cases, containers will be pre-labeled). After sampling, secure the label by taping around the bottle with clear packaging tape.

Procedural Notes

For inorganic and organic water samples, bottles do not have to be rinsed if they are I-Chem 200 series or higher or ESS PC grade or higher. This means that the sample bottles are analyzed for contamination, and a certification of analysis is included with the bottles. Other sample containers are usually rinsed at least three times if the bottles do not meet these

requirements. See filling instruction for each type of analyses if there is uncertainty. If applicable to the sample and analysis type, the sample container should be opened and re-capped under water.

Sample Short-term Storage and Preservation

Properly store and preserve samples as soon as possible. Usually this is done immediately after returning from the collection by placing the containers on bagged, crushed or cube ice in an ice chest. Sufficient ice will be needed to lower the sample temperature to at least 4 °C within 45 min after time of collection. Sample temperature will be maintained at 4 °C until delivered to the laboratory. Care is taken at all times during sample collection, handling and transport to prevent exposure of the sample to direct sunlight. Samples are preserved in the laboratory, if necessary, according to protocol for specific analysis (acidification in most cases).

Field Safety Issues

Proper gloves must be worn to prevent contamination of the sample and to protect the sampler from environmental hazards (disposable polyethylene, nitrile, or non-talc latex gloves are recommended, **however, metals and mercury sample containers can only be sampled and handled using polyethylene gloves as the outer layer**). Wear at least one layer of gloves, but two layers help protect against leaks. One layer of shoulder high gloves worn as a first (inside) layer is recommended to have the best protection for the sampler. Safety precautions are needed when collecting samples, especially samples that are suspected to contain hazardous substances, bacteria, or viruses.

Sample Handling and Shipping

Due to increased shipping restrictions, samples being sent via a freight carrier require additional packing. Although care is taken in sealing the ice chest, leaks can and do occur. Samples and ice should be bagged placed inside a large trash bag inside the ice chest for shipping. Ice should be double bagged to prevent melted ice water from leaking into the sample. The large trash bag can be sealed by simply twisting the bag closed (while removing excess air) and taping the tail down. Prior to shipping the drain plug of the ice chests have to be taped shut. Leaking ice chests can cause samples to be returned or arrive at the lab beyond the holding time.

Although glass containers are acceptable for sample collection, bubble wrap must be used when shipping glass.

Chain of Custody (COC) Forms

Every shipment must contain a complete Chain of Custody (COC) Form that lists all samples collected and the analyses to be performed on these samples.

Make sure a COC is included for every laboratory, every time you send a shipment of samples. Electronic COCs can also be emailed to the various laboratories but must be sent before the samples arrive at their destinations.
Include region and trip information as well as any special instructions to the laboratory on the COC.

The original COC sheet (not the copies) is included with the shipment (insert into ziplock bag) One copy goes to the sampling coordinator, and the sampling crew keeps one copy.

Samples collected should have the salinity (in ppt), depth of collection, and date/time collected for each station on every COC.

Write a comment on this form, if you want to warn the laboratory personnel about possibly hazardous samples that contain high bacteria, chlorine or organic levels.

Field QC Samples for Water Analyses

Field duplicates are currently submitted at an annual rate of 5%. Field travel blanks are required for volatile organic compounds at a rate of one per cooler shipped. Field blanks are required for trace metals (including mercury and methyl mercury), DOC, and volatile organic compounds in water at a rate of 5%. See Appendix C of the SWAMP QAMP for detailed Field QC requirements.

Field Site Data Sheets

Each visited field site requires a field observation completed SWAMP Field Data Sheet, even if no samples are collected (i.e. at a site which is found to be dry). If water and/or sediment samples are collected, all elements of the SWAMP Field Data Sheet must be completely filled out.

General Pre-Sampling Procedures

Instruments. All instruments must be in proper working condition. Make sure all calibrations are current. Multi-probe sondes should be pre-calibrated every morning prior to sampling and post-calibrated within 24 h of the original calibration. Conductivity should also be calibrated between stations if there is a significant change in salinity. Dissolved oxygen sensors should be re-calibrated if there is a 500 ft

change in elevation.

Calibration Standards. Pack all needed calibration standards.

Sample Storage Preparations. A sufficient amount of cube ice, blue ice and dry ice as well as enough coolers of the appropriate type/size must be brought into the field, or sources for purchasing these supplies identified in advance.

Sample Container Preparation. After arriving at the sample station, pack all needed sample containers for carriage to the actual collection site, and label them with a pre-printed label containing Station ID, Sample Code, Matrix info, Analysis Type info, Project ID and blank fields for date and time (if not already pre-labeled).
Safety Gear. Pack all necessary safety gear like waders, protective gloves and safety vests.

Walk to the site. For longer hikes to reach a sample collection site, large hiking backpacks are recommended for transport of gear, instruments and containers. Tote bins can be used, if the sampling site can be accessed reasonably close to the vehicle.

GPS. At the sampling site, compare/record reconnaissance GPS reading with current site reading and note differences. GPS coordinates should be in Decimal Degrees (e.g. 38.12345 -117.12345).

COLLECTION OF WATER SAMPLES FOR ANALYSIS OF CONVENTIONAL CONSTITUENTS

In most streams, sub-surface (0.1 m below surface) water is representative of the water mass. A water sample for analysis of conventional constituents is collected by the grab method in most cases, immersing the container beneath the water surface to a depth of 0.1 m. Sites accessed by bridge can be sampled with a sample container-suspending device. Extreme care must be taken to avoid contaminating the sample with debris from the rope and bridge. Care must also be taken to rinse the device between stations. If the centroid of the stream cannot be sampled by wading, sampling devices can be attached to an extendable sampling pole.

In some cases, depth-integrated sampling is required, as requested by Regional Boards. This is useful when lakes or rivers are stratified and a sample is wanted that represents the entire water column. Depth-integrated sample collection is explained later in this document.

Conventional Water Constituents, Routinely Requested in SWAMP

Chloride, sulfate, nitrite, nitrate (or nitrate+nitrate), ortho-phosphate, fluoride, total phosphorus, ammonia, TKN, alkalinity, chlorophyll a.

Conventional Water Constituents, Occasionally Requested in SWAMP

Total Suspended Solids (TSS) or Suspended Sediment Concentration (SSC), Total Dissolved Solids (TDS--especially if total metals requested), Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), hardness (if trace metals analysis is requested).

Conventional Water Constituents Sample Volume

Due to the potential for vastly different arrays of requested analyses for conventional constituents, please refer to table at the end of this document, as well as the Sample Handling Requirements Tables in Appendix C of the QAMP, for information on the proper volume to collect for the various types of analyses.

Conventional Water Constituents Sample Container Type

Due to the potential for vastly different arrays of requested analyses for conventional constituents, please refer to table at the end of this document, as well as the Sample Handling Requirements Tables in Appendix C of the QAMP, for information on the proper type of sample containers.

Chlorophyll a Syringe Sample Method

Chlorophyll a syringe method: Chlorophyll a is sampled by forcing water with a 60-mL syringe through a filter holder containing a 25-mm glass microfiber filter. The 60-mL syringe and an in-line filter holder are rinsed three times with the ambient water before filtration. The syringe is then filled with 60 mL of ambient water. The filter holder is then removed and a 25-mm glass microfiber filter is placed inside. The filter holder is then screwed onto the syringe and the ambient water is then flushed through the filter. The filter holder is removed every time more water needs to be drawn into the syringe. The process is then repeated until the desired amount of Chlorophyll a is present (usually 60 to 360 mL depending on the water clarity). When filtering is complete the filter holder is opened and the filter is removed with tweezers without

touching the Chlorophyll a. The filter is then folded in half, then again, in half with the Chlorophyll a inside the folds. The folded filter is then wrapped in aluminum foil and placed in an envelope labeled with the site information and the volume filtered. The envelope is then immediately placed on dry ice until transferred to the lab.

Collection of Water Samples for Analysis of Trace Metals (Including Mercury)

When deciding to measure total and dissolved metals in water the purpose of the sampling must be considered. Water quality standards for the protection of aquatic life are determined for the dissolved form of heavy metals in most cases, although this, too, can vary within different Basin Plans for different regions. The exception to routinely conducting dissolved metals analyses is usually mercury (and often selenium). Water quality standards usually apply to the total form of mercury (and often selenium), and not the dissolved form of these elements. Several regions are interested in conducting total metals analyses, in order to address specific issues. In order to budget inputs, transport, and accumulation of metals, it is necessary to know the concentration of total metals in the water column, sediments, effluent, etc. Sample collection for trace metals and mercury in water requires "Clean Hands/Dirty Hands" methodology.

Metals-in-water: General Information

Unless otherwise requested to collect for total metals analysis, dissolved metals are collected for all elements with the exception of mercury. Metals-in-water samples should **not** be collected during periods of abnormally high turbidity if at all possible. Samples with high turbidity are unstable in terms of soluble metals, and it is difficult to collect a representative grab sample. Special study sampling, however, may be an exception. For example, wet weather sampling is likely to include some samples with high turbidity.

Metals-in-water: Sample Collection Depth

Collect a metals sample from a depth of 0.1 m using a sub - surface grab method, or at discrete depths using a depth-integrated sampling method with a peristaltic pump (described further down). In most streams, sub-surface water is representative of the water mass. For the purpose of determining compliance with numerical toxic substance standards, a sample taken at the surface is adequate.

Metals-in-water: Sample Volume

Refer to table at end of this document, as well as Sample Handling Requirements Tables in Appendix C of the QAMP, for specific information on the proper volume to collect for trace metals analyses. Generally, for procedures most commonly used for analysis of metals in water (total or dissolved metals); one 60-mL polyethylene container is filled with the salinity recorded on the field data sheet and COC. Generally, for the procedures most commonly used for analysis of mercury in water (whether total or dissolved), one 250-mL glass or teflon container is filled, regardless of the salinity. All containers are pre-cleaned in the lab using HNO_3.

Metals-in-water:

Sampling Equipment

The method of choice for the collection of water samples for trace metals analysis in small, wadeable streams is the grab method, where the sampler submerges the sample bottle or syringe beneath the surface of the water until filled. The procedure for filtration of water samples for trace metals (including mercury) analysis must be performed within the 48-h maximum holding time (as well as acid preservation), and with extreme care to avoid contamination of the water sample. Considering these factors, it is best to use a **field** filtration system, such as a set-up with peristaltic pump with in-line filter, or a set-up with a syringe filter, if filtered water is required. Samples are pumped and/or filtered directly into the sample container. This minimizes contamination by using no intermediate sampling device. Samples can also be filtered in lab if need be Un-powdered (no-talc) polyethylene gloves are always worn during sampling for metals-in-water.

Depth-integrated sampling is useful when lakes or rivers are stratified and a representative sample is wanted which represents the entire water column. The method involves a peristaltic pump system with enough Teflon tubing to pump at the desired depth with an inline filter. Alternatively, mercury and metal samples can be filtered in the laboratory as long as they are filtered within the 48-hr maximum holding time and filter equipment blanks are analyzed for five percent of all cleaned equipment.

Equipment Preparation

It is best if the metals-in-water sampling materials are prepared by a laboratory that can guarantee contamination-free sampling supplies. If a laboratory assembles a Metals-in-Water Sample Collection Kit, it should contain the following items packaged together **for each sample**:

- Tubing with an in-line filter (disposable, 0.45 µm) attached for dissolved metals-in-water sampling. This same tubing is used for total metals-in-water samples without filter. If an in-line pumping system is not used, an acid cleaned syringe and filter are packed.
- Sample containers- polyethylene for total and dissolved samples and blanks; Glass or Teflon for total and dissolved mercury.
- Acid preservation is performed in the laboratory.
- Metals-free DI water (for blanks).

- Powder-free polyethylene gloves

If a laboratory is not assembling collection kits, individuals should take care to keep containers in the original packaging. When removed from the box, sample containers are placed in plastic bags (ziplock bags). Although filters come individually wrapped, they should also be stored in new ziplock bags to avoid possible contamination.

The filtering equipment is pre-cleaned according to laboratory protocol. Clean tubing is put into clean containers, such as large ziplock bags. Metals-free filter cartridges with the capacity to filter several liters are commercially available. Equipment blanks are run at the laboratory on batches of metals-in-water sampling equipment prior to their distribution to field staff. One to two liter containers with metals-free deionized water are taken into the field for travel blanks. Metals-free deionized water is supplied by the laboratory performing metals analysis. The deionized water containers are kept clean and dust-free on the outside by wrapping in two plastic bags.

Dissolved and Total Metals-in-Water: Detailed Collection Techniques

- ***Sub-Surface Grab Method***
- ***Syringe Filtration Method (for sub- surface collection)***
- ***Peristaltic Pumping Method (Using Tubing/In-line Cartridge Filters)for sub- surface collection or for depth-integrated collection***

Metals-in-water Sample Collection:

Sub-Surface Grab Method

Unfiltered Samples (for total metals analysis, if requested, and for mercury almost always, unless otherwise requested): Some samples can be sampled directly from the ambient water either by wading into the stream and dipping bottles under the surface of the water until filled, or by sampling from a boat and dipping the bottle under the surface of the water until it is filled. The bottles are cleaned according to laboratory protocol. It is very critical that all the acid is rinsed out of the bottles before the samples are taken. Personnel involved in field sample collection/processing wear polyethylene gloves. The laboratory pre-cleaned glass or Teflon™ 250 mL (for mercury) or polyethylene 60 mL (for

metals) sample bottles are taken from the double-wrapped plastic bags using "Clean Hands/Dirty Hands" techniques. The dirty hands person opens the first bag, and the clean hands person opens the inner bag around the bottle. The clean hands person then removes the bottle from the inner bag. The clean hands person dips the bottle into the ambient water, with the cap on, to approximately 0.1 m (avoiding disturbing surface scums), placing the cap back on the bottle before being removed from the water, rinses the bottle five times with ambient water, making sure the threads of the bottle get rinsed as well, and fills the bottle to the top. The lid is secured and the bottle is put back into the inner clean bag and sealed by the clean collector. The dirty hands collector then seals the outer bag.

Metals-in-water Sample Collection:

Syringe Filtration Method (for sub-surface collection)

Filtered Samples (for dissolved metals analyses): Sub-surface water samples are filtered for dissolved trace metals analysis (not for mercury, however, in almost all cases) using the following syringe filtration method.

The syringe (60 cc size, pre-cleaned in the laboratory) and in-line filter are pre-packed in two ziplock bags. The syringe and filter are taken out of the bags using "Clean Hands/Dirty Hands" technique, as previously described. The sub-surface water sample is collected by 1) wading out into the centroid portion of the stream, or by leaning over the edge of the boat, and aspirating water into the syringe, filling and rinsing the syringe five times with ambient water; 2) attaching the filter onto the syringe and filling the syringe body; 3) rinsing the filter with a few milliliters of the sample; 4) rinsing the sample bottle five times with the filtered ambient water; and 5) extruding the sample through the syringe filter and completely filling each bottle. The bottles are taken out of and put back into their bags using "Clean Hands/Dirty Hands".

Metals-in-water Sample Collection--

Peristaltic Pump

The basic "Clean Hands/Dirty Hands" technique is also applied in the use of a peristaltic pump with an in-line filter cartridge for metals-in-water sample collection. Dirty Hands removes the plastic cover from the end of the pump tubing and inserts the tubing into the sampling container. Dirty Hands holds the tubing in place. The in-line cartridge filter is attached to the outlet end of the tubing.

Clean Hands takes the plastic cover off the other end of the tubing. Dirty Hands turns on the pump and flushes l L of ambient water through the tubing to purge it for dissolved

metals.

Clean Hands removes the cap from the sample bottle and uses the pump to fill it with ambient water. Clean Hands puts the cap back on the bottle and places it in the plastic bag.

Metals-in-water Sample Collection:

Depth-Integrated Sampling, using In-line Cartridge Filter and Peristaltic Pump

Preparation for Depth-integrated sample collection:
Depth-integrated sampling is useful when lakes or rivers are stratified, and a representative sample is wanted that represents the entire water column to the extent possible. The method utilized to date for SWAMP involves a peristaltic pump system with enough Teflon tubing to pump from the desired depth. Regional Boards must request depth-integrated sampling.

The tubing set consists of a small length of CFLEX tubing that fits in the peristaltic pump, with an appropriate length of Teflon tubing on the suction side of the pump and a 3-ft section of Teflon tubing on the discharge side of the pump.

The tubing set is pre-cleaned in 10% reagent grade HCL at the laboratory, and to date in SWAMP, a new pre-cleaned tubing set is used for each site. However, the same peristaltic tubing set can be used at multiple sites, as long as it has been cleaned in the field between stations, according to protocol as outlined below. If this is to be done, however, and Dissolved or Total Organic Carbon samples are collected, equipment blanks should be collected at each site until it is determined that the blanks are acceptably low.

The field cleaning procedure for tubing that is to be re-used is:

- Pump phosphate free detergent through tubing.
- Pump 10% HCL through tubing.
- Pump methanol through tubing.
- Pump 1 l of blank water (Milli-Q) through.

All reagents must be collected in appropriate hazardous waste containers (separated by chemical), and transport, as well as disposal, must follow appropriate local, state, and federal regulations.

If a field blank is needed, collect it after the 1 L of blank water is pumped through. Pump the amount of ambient water

equivalent to 3 times the volume of the tubing before sampling the next site.

Filtered and Unfiltered Samples, Depth-integrated:
It is recommended to attach the tubing to a line with depth measurement markers (preferably in meters). At the end of this line should be a trace metal-safe weight, which hangs about one meter below the tubing end, avoiding any sediment intake from the bottom of the water column with the pump tubing.

At the site, Dirty Hands sets up the pump, while Clean Hands takes a bottle from the plastic bag and places it in a container holder or on a clean surface. A container holder can be anything trace metal clean that supports the bottle, freeing up the collector's hands. Clean Hands takes the outlet-end of the tubing (with the in-line filter cartridge attached) out of the bag, and places it in the peristaltic pump head. The outlet end is long enough to allow easy bottle filling; the other end is long enough to easily reach beneath the water surface and to the desired depth. Dirty Hands closes the pump head, locking the tubing in place.

Make sure that all bottles are filled with a depth-integrated water sample. This can be accomplished by dividing the total vertical length of the water column into 2 to 10 equal intervals, and sampling each interval equally, filling the bottles at each depth proportional to the number of intervals sampled. For example, if 10 intervals are sampled, every bottle is filled $1/10^{th}$ full at each depth sampled. A very common method of dividing the water column is by first determining the depth of the thermocline. Samples are taken at the midpoint between the surface and the thermocline, at the midpoint between the top of the thermocline and the bottom of thermocline, and at the midpoint between the bottom of the thermocline and just above the bottom of the water column. For these methods, all containers have to be filled at the same time. Note the number of intervals sampled on the data sheet.

When filling bottles, Clean Hands immerses the intake tube directly into the water at the appropriate depth, and Dirty Hands operates the pump to flush the tubing with a minimum of 1L of ambient water through the tubing and filter.

Clean Hands removes the cap from the sample bottle, holds

the tubing outlet with the in-line filter cartridge over the container opening (without touching the container), and allows the container to fill. The container is filled and rinsed five times with ambient water, and is then filled to the top for the actual sample. Clean Hands puts the cap back on the bottle, and places the bottle back it in the plastic bag. Whenever Clean Hands touches the boat or equipment, which may be contaminated, gloves should be changed immediately.

(Note for Unfiltered samples: *If an unfiltered sample is required for total metals, total mercury, conventional constituents, toxicity, or synthetic organics, the same procedure is used as described above, except the filter is detached from the end of the tubing before filling the bottles.)*

When sampling is finished, the tubing is brought to the surface, clean water (Milli-Q or deionized) is pumped through system, and the tubing is stored in a polyethylene bag.

The tubing set can be used at multiple sites, as long as it has been cleaned in the field between stations (see field cleaning procedure above). However, if Dissolved or Total Organic Carbon samples (in water) are collected, equipment blanks should be collected at enough sites until it is determined the blanks are appropriate.

Metals-in-water Sample Collection:

Collecting the Sample:

Composite Bottle

The sample collection methodologies are identical to those described above except the sample is collected first into a composite bottle(s). The sample is collected in an amber glass 4-L bottle for mercury and methyl mercury, and a 4-L polyethylene bottle for other trace metals. The compositing bottle is cleaned according to SWAMP SOP.SC.G.1. It is very critical that all the acid is rinsed out of the bottle and that the bottle is rinsed with sample water (five times) before the sample is taken. The sample is collected by the grab or pumping method after being rinsed five times with ambient water and is brought inside the water quality vehicle or sampling box for processing. Personnel involved in sample processing don polyethylene gloves. During sampling the dirty hands person opens the bag holding the composite bottle and opens the outer plastic bag. The clean hands person opens the inner plastic bag, removes the bottle and holds the bottle while the Dirty Hands sampler controls the flow of water

through the pump into the bottle.

Preparing sample aliquots from a composite bottle into smaller sample bottles using an inline pump and filter:

The dirty hands person opens the first bag, and the clean hands person opens the inner bag around the composite bottle. The clean hands person then removes the bottle from the inner bag and places the bags and the bottle in a designated clean place.

This process is repeated until all sample bottles are lined up on the clean bench with their tops still on.

The top of the bottles are loosened so that they fit very loosely on top of the bottles so the clean hands person can remove the caps and pour or pump water into the bottles easier.

The clean hands person shakes the 4-L sample in a steady and slow up and down motion for two full minutes.

Samples that are not to be filtered (including TSS/SSC) are subsampled out of the bottle by pouring out of the large compositing bottle into the sample bottles. The compositing bottle is shaken for 15 s between these subsamples.

Each sample bottle is rinsed five times with ambient water before filling.

For the clean pumping system setup procedure, see above.

(The equipment or field blank is processed exactly like a sample following the same steps.)

The clean end of the tubing used for suction is placed into 1 L bottle. Approximately 750 mL of Milli-Q are then pumped through the system to purge any residual contamination.

The 250-mL sample bottles are then filled to the neck and capped as soon as possible.

Note: if volatile organics are to be collected they should be pumped directly into the sample containers before the compositing procedure.

Metals-in-water: Short-term Sample Preservation

After collecting the sample, the double-bagged container is placed in another plastic bag for shipping, and placed on ice in the ice chest, cooled to 4 °C. This is to prevent possible contamination from other samples in the ice chest. Metals-in-water samples are acid-preserved in the lab.

Metals-in-water: Sample Container Label

Label each outer sample-bag with the station ID, sample code, matrix type, analysis type, project ID, and date and time of collection.

Metals-in-water: Field Equipment Blank

Pumping Method. If required, field blanks are collected at the last site of a sampling trip, with the same tube and filter used to collect the last dissolved metals-in-water sample of the day (before the ambient sample is collected); and with the tube used for the last total metals-in-water sample of the day. If each sample is taken using a new set of tubing, a separate tubing-set should be used for the blank.

The same Clean Hands/Dirty Hands collection techniques are followed for the field blank as the samples, pumping trace metal-free water from a clean container supplied by the laboratory.

Syringe Method. If required, field blanks are collected in much the same way as in the pumping method. "Clean Hands/ Dirty Hands" techniques are used. The syringe is taken out of the double bags, deionized water is aspirated into the syringe, syringe is rinsed five times with ambient water, the filter is attached, and the blank water is extruded into a sample bottle. A minimum of one blank per trip is taken, if required.

Grab Method. Bottles full of deionized water or Milli-Q are opened at the site for the same length of time the sample bottles are open.

COMPANION SAMPLES FOR METALS-IN-WATER

A hardness analysis should be requested by the Regional Water Control Board whenever metals-in-water are to be analyzed from an inland (freshwater) site. Estuarine/marine sites do not require hardness analysis.
If a total metals sample is collected, it is recommended to submit a sample for total suspended solids/suspended sediment concentration (TSS/SSC) in a companion sample for "conventionals in water".

Hexavalent Chromium

Very rarely, a request may be made for conducting hexavalent chromium analysis in water samples. Acidification alters the hexavalent form of chromium. A separate (un-acidified) sample must be submitted if hexavalent chromium is to be analyzed. Filter and submit a minimum of 500 mL water. The sample is collected in a DI-water-rinsed plastic or glass container, placed on ice, and shipped to the lab in time for analysis to begin within 24 h of collection. The lab must be notified when a hexavalent chromium sample will arrive. Hexavalent chromium is not usually analyzed on unfiltered samples.

FIELD QC SAMPLE COLLECTION REQUIREMENTS FOR METALS-IN-WATER

In order to assess contamination, "blanks" are submitted for analysis. Special projects may have other requirements for blanks. The same group of metals requested for the ambient samples are requested for the blank(s). Run a blank for each type of metal sample collected. Blanks results are evaluated (as soon as available) along with the ambient sample results to determine if there was contamination or not. See Appendix C of the QAMP for MQO's regarding frequency and types of field QC samples.

Field Equipment Blank (Ambient Blank)

Submit an equal volume (equal to the ambient sample) of metals-free deionized water that has been treated exactly as the sample at the same location and during the same time period. Use the same methods as described above (Grab sample, pumping method, syringe method). At least one ambient blank per field trip is required each for trace metal and Mercury samples in water. *If contamination is detected in field equipment blanks, blanks are required for every metals-in-water sample until the problem is resolved*.

Laboratory Equipment Blank

Laboratory Equipment Blanks for pumping and sampling equipment (Metals-in-Water Sample Collection Kits and Syringe Filtration Kits) are run by the laboratory that cleans and distributes the collection materials. It documents that the materials provided by the laboratory are free of contamination. When each batch of tubes, filters, bottles, acid and deionized water are prepared for a sampling trip, about five percent of the Mercury sampling materials are chosen for QC checks. Trace metal equipment needs to be subjected to an initial blank testing series. If these blanks are acceptable only occasional re-testing is required for TM equipment. The QC checks are accomplished by analyzing metals-free water which has been pumped through the filter and tube; collected

in a sample container; and preserved.

Field Duplicates

Five percent Field Duplicates are submitted every year. (If less than 20 samples are collected during an event, submit one set of duplicates per event.)

Collection of Water Samples for Analysis of Synthetic Organic Compounds

Collect organic samples at a depth of 0.1 m by submerging the sample container by hand. If depth-integrated sampling is required, use the in-line peristaltic pump methodology described previously. Since organic compounds tend to concentrate on the surface of the sampling device or container, the sampling device and sample container are ***not*** to be rinsed with ambient water before being filled.

Sample Containers and Collection
Also refer to Appendix C of the SWAMP QAMP for a list of sample volumes and containers.

Pesticides/ Herbicides

The sample container for pesticides and herbicides is a new, clean, unused amber glass jar with a Teflon-liner inside the cap. Collect one liter of water for each of the three sample types (Organophosphorus Pesticides, Organochlorine Pesticides and Chlorinated Herbicides). **EACH ANALYSIS TYPE REQUIRES A SEPARATE JAR**. Minimize the air space in the top of the jar. Preserve immediately after collection by placing on ice out of the sunlight.

Semi-volatile Organics

The sample container for semi-volatile organics must also be new, clean, unused amber glass bottles with a Teflon-liner inside the cap, and pre-rinsed with pesticide-grade hexane, acetone, or methylene chloride. Fill jars to the top and place on ice in the dark. In addition to other sample information, label the jar Semi-volatiles.

Volatile Organics:

Volatile Organic Carbon (VOC), Methyl-Tert Butyl Ether (MTBE) and (BTEX)

The sample containers for volatiles are VOA vials. Fill the 40-mL VOA vials to the top and cap without trapping any air bubbles. If possible, collect directly from the water, keeping the vial under water during the entire collection process. To keep the vial full while reducing the chance for air bubbles, cap the vials under the water surface. Fill one vial at a time and preserve on ice. The vials are submitted as a set.

If the vial has been pre-acidified for preservation, fill the vial quickly, without shaking using a separate clean glass jar. Fill the vial till the surface tension builds a meniscus, which extends over the top end of the vial, then cap tightly and check for bubbles by turning the vial on its head.

Ensure that the pH is less than 2. If the water may be alkaline or have a significant buffering capacity, or if there is concern that pre-acidified samples may have the acid wash out, take a

few practice vials to test with pH paper. It may take more than two drops, and it will then be known how to preserve the other samples that are being submitted to the lab. If an alternative method has proven successful, continue with that method.

Note: If vigorous foaming is observed following acidification, discard that sample and collect another set. Do not acidify the second set. Mark the sample clearly "not acidified" and the lab will run them immediately. Holding time is 14 days with acid, 24 h without acid.

Collect three VOA vials, if VOC, MTBE and BTEX are required, two vials, if only VOC is required and two vials, if only MTBE and BTEX are require. The vials may be taped together to keep them together.

Perchlorate

Surface water samples for perchlorate should be collected in a new unused polyethylene or glass container. Perchlorate samples should be placed immediately on ice to maintain temperature at 4 ^{o}C. The sample holding time is 28 days, under refrigeration.

Sample Treatment in Presence of Chlorine

(NOTE: This treatment has not been performed in SWAMP, but may be in the future, or if a known or suspected chlorine residual is suspected and this information is made known by a Regional Board SWAMP contact beforehand.)

If in stream chlorine residual is suspected, measure the chlorine residual using a separate water subsample. Free chlorine will oxidize organic compounds in the water sample even after it is collected. If chlorine residual is above a detectable level, (i.e., the pink color is observed upon adding the reagents) immediately add 100 mg of sodium thiosulfate to the pesticides, herbicides, semivolatiles and VOA samples; invert until sodium thiosulfate is dissolved. Record the chlorine residual concentration in field logbook. If chlorine residual is below detectable levels, no further sample treatment necessary.

VOA Trip Blank

Submit one Trip Blank for VOA samples (2- 40 mL VOA vials) for each sampling event. Trip Blanks are prepared in advance just before the sampling trip and transported to the field. Ask the laboratory for DI water and specify that it is for a VOA trip blank. VOA blanks require special purged water. Trip blanks demonstrate that the containers and sample handling did not introduce contamination. The trip blank vials

are never opened during the trip.

Field QC Samples If required, field Duplicates and field blanks are submitted at a rate subject to the discretion of the project manager. Refer to Appendix C of the SWAMP QAMP for details on required blanks and duplicates.

BACTERIA AND PATHOGENS IN WATER SAMPLES

Summary of Collection Procedure (Based on EPA water quality monitoring procedures)

Make sure the containers are sterilized; either factory-sealed or labeled.

Whirl-pak® bags

- Label the bottle as previously described for SWAMP.
- Tear off the top of the bag along the perforation above the wire tab just prior to sampling. Avoid touching the inside of the bag. If you accidentally touch the inside of the bag, use another one.
- If wading into the stream, try to disturb as little bottom sediment as possible. Be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you. You may also attach your bottle to an extension pole to sample from deeper water.
- If taking sample from a boat, carefully reach over the side and collect the water sample on the upstream side of the boat.
- Hold the two white pull-tabs in each hand and lower the bag into the water on your upstream side with the opening facing upstream. Open the bag midway between the surface and the bottom by pulling the white pull-tabs. The bag should begin to fill with water. You may need to "scoop" water into the bag by drawing it through the water upstream and away from you. Fill the bag no more than 3/4 full.
- Lift the bag out of the water. Pour out excess water. Pull on the wire tabs to close the bag. Continue holding the wire tabs and flip the bag over at least 4-5 times quickly to seal the bag. Don't try to squeeze the air out of the top of the bag. Fold the ends of the wire tabs together at the top of the bag, being careful not to puncture the bag. Twist them together, forming a loop.
- If the samples are to be analyzed in the lab, place them in a cooler with ice or cold packs for transport to the lab.

Screw cap containers

- Label the bottle as previously described for SWAMP.
- Remove the cap from the bottle just before sampling. Avoid touching the inside of the bottle or cap. If you accidentally touch the inside, use another bottle.
- If wading into the stream, try to disturb as little bottom sediment as possible. Be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you. You may also attach your bottle to an extension pole to sample from deeper

water.

- If taking sample from a boat, carefully reach over the side and collect the water sample on the upstream side of the boat.
- Hold the bottle near its base and plunge it (opening downward) below the water surface. If you are using an extension pole, remove the cap, turn the bottle upside down, and plunge it into the water, facing upstream. Collect a water sample 2" beneath the surface. You can only use this method if the sample bottles do not contain sodium thiosulfate.
- Turn the bottle underwater into the current and away from you. In slow moving stream reaches, push the bottle underneath the surface and away from you in an upstream direction.
- Alternative sampling method: In case the sample bottle contains preservatives/chlorine removers (i.e. Sodium-Thiosulfate), it cannot be plunged opening down. In this case hold the bottle upright under the surface while it is still capped. Open the lid carefully just a little to let water run in. Fill the bottle to the fill mark and screw the lid tight while the bottle is still underneath the surface.
- Leave a 1-in. air space so that the sample can be shaken just before analysis. Recap the bottle carefully, remembering not to touch the inside.
- If the samples are to be analyzed in the lab, place them in a cooler with ice or cold packs for transport to the lab.

Pouring from another clean bottle

- Due to different sampling conditions (high turbidity, rough water etc.) it is sometimes easy to pour water from another clean bottle into the bacteria bottle. This helps to make sure that the sample water is only being filled to the desired line and no overfilling occurs.

TOXICITY IN WATER

Sample Collection

Using the standard grab sample collection method described previously for water samples, fill (for typical suite of water toxicity tests conducted) the required amount of 2.25-L amber glass bottles with water, put on ice, and cool to 4 °C. Label the containers as described above and notify the laboratory of the impending sample delivery, since there is a 48-hr maximum sample hold time. Sample collection must be coordinated with the laboratory to guarantee appropriate scheduling.

Summary of Sample Container, Volume, Initial Preservation, and Holding Time Recommendations for Water Samples

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
Conventional Constituents in Water				
Alkalinity	Polyethylene bottles (see **NOTE(1)** below)	100 mL	Cool to 4°C, dark	14 days at 4°C, dark
Chloride (Cl), Sulfate (SO_4) and Fluoride (F)	Polyethylene bottles (see **NOTE(1)** below)	300 mL	Cool to 4°C, dark	28 days at 4°C, dark
Ortho-phosphate (OPO_4)	Polyethylene bottles (see **NOTE(1)** below)	150 mL	Cool to 4°C, dark	48 h at 4°C, dark
Nitrate + Nitrite (00630) ($NO_3 + NO_2$)	Polyethylene bottles (see **NOTE(1)** below)	150 mL	Cool to 4°C, dark	48 h at 4°C, dark
Total Keldjahl Nitrogen (TKN)	Polyethylene bottles (see **NOTE(1)** below)	600 mL	Cool to 4°C, dark	Recommend: 7 days Maximum: 28 days Either one at 4°C, dark
Total Dissolved Solids (TDS)	Polyethylene bottles (see **NOTE(1)** below)	1000 mL	Cool to 4°C, dark	7 days at 4°C, dark
Ammonia (NH_3)	Polyethylene bottles (see **NOTE(1)** below)	500 mL	Cool to 4°C, dark	48 h at 4C dark; if acidify, 28 days at 4°C, dark
Total Phosphorus (TPO_4)	Polyethylene bottles (see **NOTE(1)** below)	300 mL	Cool to 4°C, dark	28 days at 4°C, dark
(1)NOTE: **The volume of water necessary to collect in order to analyze for the above constituents is typically combined in four 1-L polyethylene bottles, which also allows enough volume for possible re-analysis and for conducting lab spike duplicates. This is possible since the same laboratory is conducting all of the above analyses; otherwise, individual volumes apply.**				
Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC)	125 mL amber glass vial 250 ml amber for TOC/DOC	125 mL for TOC only 250 mL for TOC/DOC	Cool to 4°C, dark Cool to 4°C, dark	28 days at 4°C, dark 28 days at 4°C, dark
Total Suspended Solids (TSS)	250 mL plastic bottle	250 mL	Cool to 4°C, dark	7 days at 4°C, dark
Suspended Sediment Concentration (SSC)	125 mL polyethylene bottle	Up to 125ml depending on turbidity of water	Cool to 4°C, dark	7 days at 4°C, dark
Chlorophyll *a* **Pheophytin *a***	1-L amber polyethylene bottle Aluminum Foil, GFC Filters	1000 mL (one bottle)	Cool to 4°C, dark	Keep at 4°C, dark, but must filter within 48 h.

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
Chlorophyll a **Pheophytin *a***		20-420 mL	Freeze, Dry ice	Filters may be stored frozen up to 30 days.
Non-Routine Compounds in Water Samples				
OIL AND GREASE	1-L glass jar with Teflon lid-liner, rinsed with hexane or methylene chloride	1000 mL (one jar)	Add 2 mL conc. H_2SO_4 to pH <2; cool to 4°C, dark.	28 days at 4°C, dark
PHENOLS	1-L glass jar with Teflon lid-liner	1000 mL (one jar)	Add 2 mL conc. H_2SO_4 to pH <2; cool to 4°C, dark.	28 days at 4°C, dark
CYANIDE	1-L cubitainer	1000 mL (one cubitainer)	Add 2 mL 1:1 NaOH to make pH > 12; Add 0.6 g ascorbic acid if residual Cl present. Cool to 4°C, dark.	14 days at 4°C, dark
BIOCHEMICAL OXYGEN DEMAND (BOD)	4-L cubitainer	4000 mL (one cubitainer)	Cool to 4°C, dark. Add 1g FAS crystals per liter, if residual Cl present.	48 h at 4°C, dark
CHEMICAL OXYGEN DEMAND (COD)	1-L cubitainer	110 mL (one cubitainer)	Add 2 mL conc. H_2SO_4 to make pH <2. Cool to 4°C, dark.	28 days at 4°C, dark
Trace Metals in Water Samples				
DISSOLVED METALS (except Dissolved Mercury)	60 mL polyethylene bottle, pre-cleaned in lab using HNO_3	60 mL (one bottle)	Filter at sample site using 0.45 micron in-line filter, or syringe filter. Cool to 4°C, dark. Acidify in lab, within 48 hrs, using pre-acidified container (ultra-pure HNO_3) for pH<2.	Once sample is filtered and acidified, can store up to 6 months at room temperature
DISSOLVED MERCURY	250 mL glass or Teflon bottle, pre-cleaned in lab using HNO_3	250 mL (one bottle)	Cool to 4°C, dark. Filter in lab within 48 h, using bench top Hg filtration apparatus. Acidify in lab within 48 hrs, with pre-tested HCL to	Once sample is filtered and acidified, can store up to 6 months at room temperature

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
			0.5%.	
TOTAL METALS (except Total Mercury)	60 mL polyethylene bottle, pre-cleaned in lab using HNO_3	60 mL (one bottle)	Cool to 4°C, dark. Acidify in lab within 48 hrs, with pre-acidified container (ultra-pure HNO_3), for pH<2.	Once sample is acidified, can store up to 6 months at room temperature
TOTAL MERCURY	250 mL glass or Teflon bottle, pre-cleaned in lab using HNO_3	250 mL (one bottle)	Cool to 4°C, dark. Acidify in lab within 48 hrs, with pre-tested HCL to 0.5%.	Once sample is acidified, can store up to 6 months at room temperature.
HEXAVALENT CHROMIUM (filtered)	600 mL plastic or glass bottle	600 mL (one bottle)	Cool to 4°C, dark No acid	Keep at 4°C, dark for up to 24 h; must notify lab in advance.
HARDNESS	200 mL polyethylene or glass bottle	200 mL (one bottle)	Cool to 4°C, dark OR Filter and add 2 mL conc. H_2SO_4 or HNO_3 to pH < 2; Cool to 4°C, dark.	48 h at 4°C, dark 6 months at 4°C, dark
Synthetic Organic Compounds in Water Samples				
VOLATILE ORGANIC ANALYTES (VOA's) including VOC, MTBE and BTEX	40 mL VOA vials	120 mL (three VOA vials)	All vials are pre-acidified (50% HCl or H_2SO_4) at lab before sampling. Cool to 4°C, dark	14 days at 4°C, dark
PESTICIDES & HERBICIDES* □Organophosphate Pesticides □Organochlorine Pesticides □Chlorinated Herbicides **SEMI-VOLATILE ORGANICS*** **POLYCHLORINATED* BIPHEYNYL AND AROCHLOR COMPOUNDS** **TPH, PAH, PCP/TCP***	1-L I-Chem 200-series amber glass bottle, with Teflon lid-liner (per each sample type)	1000 mL (one container) ***Each sample type requires 1000 mL in a separate container**	Cool to 4°C, dark If chlorine is present, add 0.1g sodium thiosulfate	Keep at 4°C, dark, up to 7 days. Extraction must be performed within the 7 days; analysis must be conducted within 40 days.
Toxicity Testing Water Samples				
TOXICITY IN WATER	Four 2.25 L amber glass bottles	9000 mL	Cool to 4°C, dark	48 hrs at 4°C, dark

Parameters for Analysis in WATER Samples	Recommended Containers (all containers pre-cleaned)	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time (analysis must start by end of max)
Bacteria and Pathogens in Water Samples				
E. Coli	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both E. coli and Enterococcus analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 h at 4°C, dark if data for regulatory purposes; otherwise, 24 hrs at 4C, dark if non-regulatory purpose.
Enterococcus	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both E. coli and Enterococcus analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 h at 4°C, dark if data for regulatory purposes; otherwise, 24 hrs at 4C, dark if non-regulatory purpose.
FECAL COLIFORM	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both fecal and total coliform analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 h at 4°C, dark if data for regulatory purposes; otherwise, 24 hrs at 4C, dark if non-regulatory purpose.
TOTAL COLIFORM	Factory-sealed, pre-sterilized, disposable Whirl-pak® bags or 125 mL sterile plastic (high density polyethylene or polypropylene) container	100 mL volume sufficient for both fecal and total coliform analyses	Sodium thiosulfate is pre-added to the containers in the laboratory (chlorine elimination). Cool to 4°C; dark.	STAT: 6 h at 4°C, dark if data for regulatory purposes; otherwise, 24 hrs at 4C, dark if non-regulatory purpose.

Field Collection Procedures for Bed Sediment Samples

Bed sediment (hereafter termed "sediment") samples are collected after any water samples are collected where water and sediment are taken in the same reach. Care must be taken not to sample sediments that have been walked on or disturbed in any manner by field personnel collecting water samples. Sediment samples are collected into a composite jar, where they are thoroughly homogenized in the field, and then aliquoted into separate jars for chemical or toxicological analysis. Sediment samples for metals and organics are submitted to the respective analytical laboratories in separate glass jars, which have been pre-cleaned according to laboratory protocol.

Sediment chemistry samples give information regarding both trends in contaminant loading and the potential for adverse effects on sediment and aquatic biota. In order to compare samples over time and from site to site, they must be collected in a consistent manner. If a suitable site for collecting sediments cannot be found at a station, sampling personnel should not collect the sediment sample, and should instead attempt to reschedule the sample collection. If this is not possible, make a note so that the missing sample is accounted for in the reconciliation of monitoring events during preparation of sample collection "cruise reports". Sites that are routinely difficult to collect should be considered for elimination or relocation from the sample schedule, if appropriate.

Characteristics of Ideal Sediment Material to be Collected

Many of the chemical constituents of concern are adsorbed onto fine particles. One of the major objectives in selecting a sample site, and in actually collecting the sample while on site, is to obtain recently deposited fine sediment, to the extent possible. Avoid hard clay, bank deposits, gravel, disturbed and/or filled areas. Any sediment that resists being scooped by a dredge is probably not recently deposited fine sediment material. In following this guidance, the collection of sediment is purposefully being biased for fine materials, which must be discussed thoroughly in any subsequent interpretive reporting of the data, in regards to representation of the collected sample to the environment from which it was collected.

Characteristics of an Ideal Site

Quiescent areas are conducive to the settling of finer materials (EPA/USACOE, 1981).

Choose a sampling site with lower hydrologic energy, such as the inner (depositional) side of bends or eddies where the water movement may be slower. Reservoirs and estuaries are generally depositional environments, also.

Selecting the Appropriate Sediment Type for Analysis

Sediment will vary from site to site and can vary between sample events at a particular site.

Streams and Rivers: Sediment collection in flowing streams is often a challenge. In areas of frequent scouring there may not be sufficient sediment for collection during or following periods of high flow. Sediment collection during these times may prove unsuccessful and may have to be rescheduled or cancelled.

When the suspended load in rivers and streams precipitates due to reduction of velocity, most of the resulting sediment will be fine-grained. More often than not, a dredge or mechanical grab device does not function well for collection of sediment in smaller streams. In many cases, sediment will have to be collected using a pre-cleaned polyethylene scoop. Collect the top 2 cm for analysis. Five or more (depending on the volume of sediment needed for conducting analyses) fine-sediment sub-sites within a 100-m reach are sampled into the composite jar.

Reservoirs and Estuaries: Collect the top 2 cm for analysis. Five or more grabs are composited for the sediment sample, depending on the volume of sediment needed for conducting analyses.

GENERAL PROCEDURE FOR COLLECTION OF BED SEDIMENT

After choosing an appropriate site, and identifying appropriate fine-grained sediment areas within the general reach, collect the sample using one or more of the following procedures, depending on the setting:

A. Sediment Scoop Method—Primary Method for Wadeable, Shallow Streams

- The goal is to collect the top 2 cm of recently-deposited fine sediment only.
- Wear gloves and protective gear, in areas of potential exposure hazards, per appropriate protocol (make sure gloves are long enough to prevent water from overflowing gloves while submerging scoop).
- Survey the sampling area for appropriate fine-sediment depositional areas before stepping into the stream, to avoid disturbing possible sediment collection sub-sites.
- Carefully enter the stream and start sampling at the closest appropriate reach, then continue sampling UPSTREAM. Never advance downstream, as this could lead to sampling disturbed sediment.
- Stir, do not shake, collected sediment with a polyethylene scoop for at least 5 min making

sure all sediment is completely homogenized.

- Quickly scoop sediment out of the homogenizing jar into desired sampling jars making sure to stir the sediment in the homogenizing jar in between each aliquot.
- Inspect each individual sediment jar making sure of consistent grain size throughout the entire sample collection.
- Single bag all sediment containers to prevent cross contamination.
- Make sure all containers are capped tightly and stored in a cooler on cube ice at 4 °C.
- Check cooler temperature and record in log book every 8-12 h or whenever sampler suspects that the temperature has not been maintained at 4 °C.

B. Hand Core Method-Alternate method for wadeable shallow streams with fine sediment

- A hand core is used in wadeable streams where there is very fine sediment.
- The hand core sampler consists of a 3-in. diameter polycarbonate core that is 8 inches long. Samplers push the core into the sediment to the desired depth, pull the core out of the sediment, and cap the bottom with a polyethylene core cap or by placing their hand underneath the cap to hold the sediment in place.
- Hand cores are usually measured and marked at 2 cm length so the sampler knows how far to deploy the core into the sediment.
- Sediment is then emptied into a homogenizing jug and aliquoted accordingly.

C. Sediment Grab Method—Primarily for Lake, River, Bridge, and Estuarine Settings (or deeper streams)

Description of sediment grab equipment:

- A mechanical sediment grab is used for the SWAMP bed sediment collection field effort for lake, river, bridge, and estuarine/coastal settings (or deeper, non-wadeable streams).
- The mechanical grab is a stainless steel “Young-modified Van Veen Grab", and is 0.5 m^2 in size.
- The mechanical grab is deployed primarily from a boat, and is used in deeper, non-wadeable waters, such as lakes, rivers, estuaries, and coastal areas.
- It is also deployed by field personnel from land in settings which allow its use: primarily from bridges; from smaller vessels in streams or drainage channels too deep or steep to wade into, but too shallow for a larger boat.

Deploying and retrieving the grab:

- Slowly lower the grab to the bottom with a minimum of substrate disturbance.
- Retrieve the closed dredge at a moderate speed (e.g., less than two feet per second).
- Upon retrieval, open the lids of the sediment grab, examine the sample to ensure that the sediment surface is undisturbed and that the grab sample should not be rejected.

Rejection Criteria—reject the sample if the following are not met:

- Mud surface must not be pressing out of the top of the sampler. If it is, lower the grab more slowly.
- Overlying water must not be leaking out along the sides of the sediment in the grab. This ensures the surficial sediment is not washed out.
- Sediment surface is flat and level in the sampler. If it is not level, the grab has tilted over before closing.

Processing the sediment sample from the grab equipment:

- The water overlying the sediment in the grab is very gently decanted by slightly tipping the grab with the lid closed until the water runs out the top.
- The decanting process should remove all of the overlying water but not remove the surficial sediments. The laboratory reports percent water for the sample, so overlying water is not included in the sample container.
- The sediment is examined for depth of penetration, color and thickness of top aerobic zone, and texture. These observations are recorded in the logbook.
- Collect the top 2 cm from at least five sub samples, and otherwise, exclude the bottom-most layer and composite.
- In streams or other settings with excessive bottom debris (e.g., rocks, sticks, leaves) where the use of a grab is determined to be ineffective (e.g., dredge does not close, causing loss of sediment), samples may be collected by hand using a clean plastic scoop, or by a variety of coring methods, if appropriate for the situation.
- Sediment is handled as described below in the metals and organic sections.

Cleaning the Grab Equipment and Protection from Potential Contaminating Sources:

- The sediment sampler will be cleaned prior to sampling EACH site by: rinsing all surfaces with ambient water, scrubbing all sediment sample contact surfaces with Micro™ or equivalent detergent, rinsing all surfaces with ambient water, rinsing sediment sample contact surfaces with 5% HCl, and rinsing all sediment sample contact surfaces with methanol.
- The sediment grab will be scrubbed with ambient water between successive deployments at ONE site, in order to remove adhering sediments from contact surfaces possibly originating below the sampled layer, thus preventing contamination from areas beyond target sampling area.
- Sampling procedures will attempt to avoid exhaust from any engine aboard any vessel involved in sample collection. An engine will be turned off when possible during portions of the sampling process where contamination from engine exhaust may occur. It is critical that sample contamination be avoided during sample collection. All sampling equipment (e.g., siphon hoses, scoops, containers) will be made of non-contaminating material and will be appropriately cleaned before use. Samples will not be touched with un-gloved fingers. In addition, potential airborne contamination (e.g., from engine exhaust, cigarette smoke) will be avoided.

D. Core Method--alternative for fast-moving, wadeable streams

The core method is used in soft sediments when it is difficult to use the other methodologies. The cores can be used in depths of water from 0 to 10 ft by using a pole deployment device or in deeper water using SCUBA divers. The pole deployment device consists of a pole that attaches to the top of the core. The top of the core is fitted with a one-way valve, which allows the core to be filled with sediment, but when pulled from the sediment catches the sediment within the core. The core is then brought to the surface and the sediments within the core are extruded out the top of the core so that 2 cm of sediment is above the top of the plastic core. The 2 cm of sediment is then sliced off and placed in the homogenizing jar. A new core, homogenizing jar, and device used to slice off the top two cm. are used at each station unless the equipment is cleaned using laboratory protocols.

E. Sediment Grab Method – Primarily used from bridges or for streams with restricted bank access.

Description and sampling procedure for the Eckman sediment grab

- The Eckman grab is 0.2 m^2 in size with a lead "messenger" that triggers the spring loaded doors.
- The primary use is for sampling from bridges or from small vessels in streams or drainage channels too deep or steep to wade into, but too shallow for a larger boat.
- The grab must be cleaned with a Micro™ and tap water rinse before sampling and in-between sample stations.
- To deploy the grab, pull the spring loaded doors open and hook the cables on the actuator plate.
- With a rope, lower the grab to the desired sample reach making sure that the grab has penetrated the sediment. Clip the "messenger" on the rope and release it while maintaining tension on the rope. Pull up the grab once the "messenger" has activated the doors.
- While wearing clean poly gloves, open the top hatch and remove the top 2 cm of sediment with a clean polyethylene scoop. Place the sediment into the homogenizing jug and repeat the sampling process until there is enough desired sediment. See general procedures for processing of bed sediment samples, once they are collected for sediment homogenization and aliquoting into sample jars.

GENERAL PROCEDURE FOR PROCESSING OF BED SEDIMENT SAMPLES, ONCE THEY ARE COLLECTED

Sediment Homogenization, Aliquoting and Transport

For the collection of bed sediment samples, the top 2 cm is removed from the scoop, or the grab, or the core, and placed in the 4-L glass compositing/homogenizing container. The composited sediment in the container is homogenized and aliquoted on-site in the field. The sample is stirred

with a polyethylene scoop for at least 5 min, but longer if necessary, until sediment/mud appears homogeneous. All sample identification information (station numbers, etc.) will be recorded prior to homogenizing and aliquoting.

The sediment sample is then aliquoted, using a clean plastic scoop, into appropriate containers for trace metal chemistry, organic chemistry, and toxicity testing. All sample containers will be packed surrounded by enough ice to keep them cool for 48 h. Each container will be sealed in one large plastic bag to prevent contact with other samples or ice or water.

Metals and Semi-volatile Organics in Sediment	For trace metals and semi-volatile organics, a minimum of three grabs is distributed to the composite bottle and/or sample containers. Mixing is generally done with a polyethylene scoop. Make sure the sample volume is adequate, but the containers do not need to be filled to the top. Seal the jars with the Teflon liner in the lids.
Sediment Conventionals	Sediment conventionals are sometimes requested when sediment organics, sediment metals, and/or sediment toxicity tests are requested for analysis of samples. The collection method is the same as that for metals, semi-volatile organics, and pesticides. Sediment conventionals include: grain size analysis and total organic carbon. These are used in the interpretation of metals and organics in sediment data.
Sample Containers	See "Sediment Sample Handling Requirements" table at end of this document.
Sediment Sample Size	Must collect sufficient volume of sediment to allow for proper analysis, including possible repeats, as well as any requested archiving of samples for possible later analysis. See "Sediment Sample Handling Requirements" Table at end of this document.
Labeling	Label the jars with the station ID, sample code, matrix type, project ID, time, and date of collection, as well as the type of analysis requested (e.g., metals, conventionals, organics, or archives).
Short-term Field Preservation	Immediately place the labeled jar on ice, cool to 4 °C, and keep in the dark at 4 °C until delivery to the laboratory.
Field Notes	Fill out the SWAMP Station Occupation Data Sheet and the Sediment Data Sheet. Make sure to record any field notes that are not listed on the provided data sheets. This information can be reported as comments with the sediment analytical results.

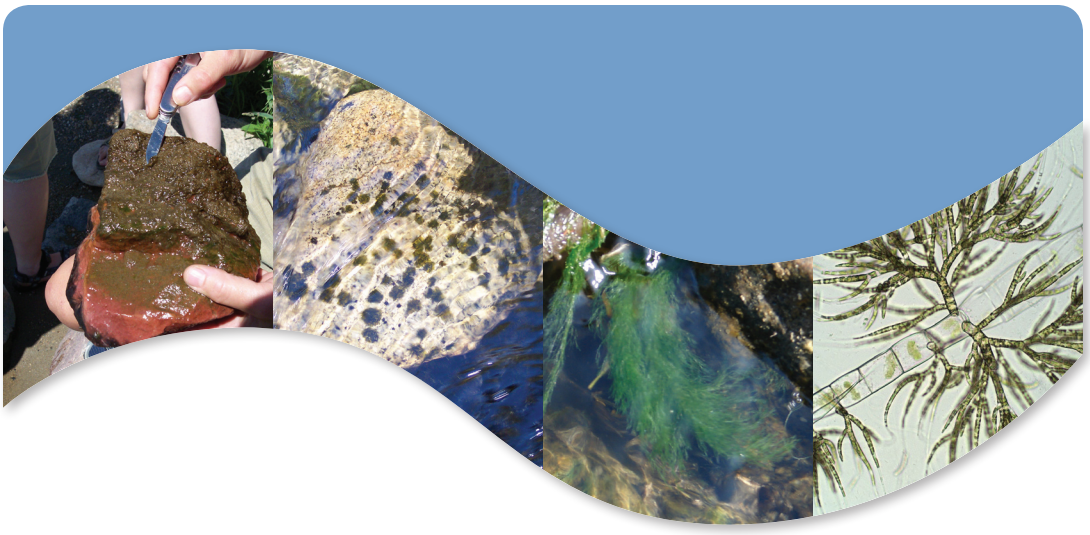
Summary of Sample Container, Volume, Preservation, and Storage Requirements for SWAMP Bed Sediment, Biota, and Tissue Samples (for contaminant analysis)

Parameters for Analysis	Recommended Containers	Typical Sample Volume (mL)	Initial Field Preservation	Maximum Holding Time
Bed Sediment Samples				
Trace Metals, including Hg and As (except for Se--see below)	60-mL I-Chem 300-series clear glass jar with Teflon lid-liner; Pre-cleaned	60 mL (one jar)	Cool to 4 °C, dark, up to 14 days	12 months(1) (-20 °C)
Selenium (separate container required)	60-mL I-Chem 300-series clear glass jar with Teflon lid-liner; Pre-cleaned	60 mL (one jar)	Cool to 4 °C, dark, up to 14 days	12 months(1) (-20 °C)
Synthetic Organic Compounds	250-mL I-Chem 300-series amber glass jar with Teflon lid-liner; Pre-cleaned	500 mL (two jars)	Cool to 4 °C, dark, up to 14 days	12 months(1) (-20 °C)
Sediment TOC	250-mL(3) clear glass jar; Pre-cleaned	125 mL (one jar)	Cool to 4 °C, dark, up to 28 days	12 months(2) (-20 °C)
Sediment Grain Size	250-mL(3) clear glass jar; Pre-cleaned	125 mL (one jar)	Cool to 4 °C, dark, up to 28 days	28 days (4 °C) ***Do not freeze***
Sediment Toxicity Testing	1-L I-Chem wide-mouth polyethylene jar with Teflon lid-liner; Pre-cleaned	2 (two jars filled completely)	Cool to 4 °C, dark, up to 14 days	14 days (4 °C) ***Do not freeze***

(1) Sediment samples for parameters noted with one asterisk (*) may be refrigerated at 4 °C for up to 14 days maximum, but analysis must start within the 14-day period, or the sediment sample must be stored frozen at minus (-) 20 °C for up to 12 months.

(2) Sediment samples for sediment TOC analysis can be held at 4°C for up to 28 days, and should be analyzed within this 28-day period, but can be frozen at any time during the initial 28 days, for up to 12 months at minus (-) 20 °C.

(3) Sediment samples for TOC AND grain size analysis can be combined in one 250 mL clear glass jar, and sub-sampled at the laboratory in order to utilize holding time differences for the two analyses. If this is done, the 250 mL combined sediment sample must be refrigerated only (not frozen) at 4 °C for up to 28 days, during which time the sub-samples must be aliquoted in order to comply with separate storage requirements (as shown above).



SWAMP
Surface Water Ambient Monitoring Program

SWAMP Bioassessment Procedures 2010

Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California

June 2009, updated May 2010

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Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California

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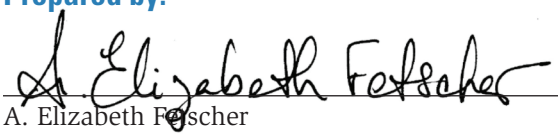

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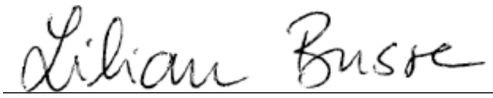

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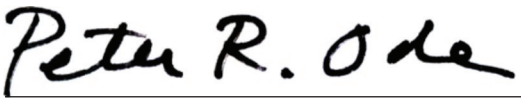

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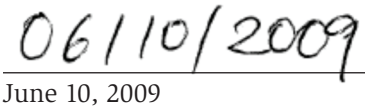
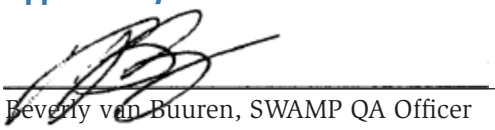


June 10, 2009

Approved by:



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Approval Date:

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ACKNOWLEDGEMENTS

This Standard Operating Procedures (SOP) manual represents the contributions of a wide range of researchers and field crews. The algal specimen collection methodology presented represents a modification of the U.S. Environmental Protection Agency's (EPA's) Environmental Monitoring and Assessment Program (EMAP) multihabitat sampling protocol (Peck et al. 2006). Point-intercept estimation of macroalgal cover has been adapted from the U.S. Geological Survey's (USGS's) National Water Quality Assessment (NAWQA) pilot procedures (J. Berkman, pers comm.), and assessment of microalgal thickness has been adapted from Stevenson and Rollins (2006). The physical habitat (PHab) methods are identical to those presented in the SWAMP Bioassessment protocol of Ode (2007), with the exception of the point-intercept method for determining algal cover, which is an add-on to the PHab pebble count procedure. The PHab procedures are, in turn, minor modifications of those used in EMAP and developed by EPA's Office of Research and Development (ORD, Peck et al. 2006).

The current version of the protocol was established with contributions from the following people:

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Erick Burres (State Water Resources Control Board, Clean Water Team)
Will Hagan (SWAMP QA Team)
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LIST OF ACRONYMS & ABBREVIATIONS L

Acronyms & Abbreviations	Definitions
AFDM	Ash-Free Dry Mass
BMI	Benthic Macroinvertebrate
chl a	Chlorophyll *a*
CPOM	Coarse Particulate Organic Matter
DO	Dissolved Oxygen
EMAP	Environmental Monitoring and Assessment Program (of the U.S. EPA)
GPS	Global Positioning System
IBI	Index of Biotic Integrity
MCM	Margin-Center-Margin
NAD	North American Datum
NAWQA	National Water Quality Assessment (of the U.S. Geological Survey)
NBO	Neutrally Buoyant Object
NNE	Nutrient Numeric Endpoints
OSPR	Office of Spill Prevention and Response (of the California Department of Fish and Game)
PHab	Physical Habitat
QAPrP	Quality Assurance Program Plan (of SWAMP)
ORD	Office of Research and Development (of the U.S. EPA)
RBP	Rapid Bioassessment Procedures
RWB	Reachwide Benthos
SOP	Standard Operating Procedures
SWAMP	Surface Water Ambient Monitoring Program
TRC	Targeted Riffle Composite

SECTION 1 INTRODUCTION

This document is the Standard Operating Procedure (SOP) for collecting and field-processing stream algae for the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP). Instructions are provided for the following:

- collection of samples for taxonomic identification of diatoms and soft-bodied algae
- collection of samples for determination of biomass based on chlorophyll *a* and ash-free dry mass (AFDM)
- estimation of percent algal cover

The document is designed to serve as a stand-alone SOP if algae are the only bioindicators being assessed at a given site. However, it can also serve as an add-on module to the existing SWAMP SOP for bioassessment using benthic macroinvertebrates (BMIs). Much of the procedure for collecting physical habitat (PHab) data is identical for these two assemblages. However, some PHab elements assessed in conjunction with BMI bioassessment are not included for algal bioassessment, because they are more specific to BMI habitat needs than to algae. Conversely, one PHab element for algal bioassessment (i.e., point-intercept estimation of algal cover) is not part of the BMI SOP. It should also be noted that, while the standard PHab protocol associated with BMI sampling includes both a "Full" and a "Basic" (simplified) version, a distinction between basic and full protocols for algae has not been established.

This SOP requires the reachwide benthos (RWB) sampling method to be used whenever algae bioassessment is conducted under the SWAMP program. Other appropriate sampling methods will be allowed if specific monitoring objectives require the use of alternative methods or if consistent data comparability in long-term monitoring projects is desired. For SWAMP funded projects, the project proponent must have the approval of the SWAMP bioassessment coordinator and the SWAMP Quality Assurance Officer before the use of alternative methods. For other projects and/or programs working towards SWAMP comparability, deviations should be approved by their project manager and project Quality Assurance (QA) officer.

For quick reference, Table 1 provides a list of elements common and distinct to the two SWAMP bioassessment assemblages. In general, if both BMIs and algae are being collected at a given site, the PHab procedure as described in Ode (2007) should be followed, with the exception of the pebble count, which should be conducted according to this SOP, because it incorporates instructions for algal cover point-intercept data collection. More specifically, if bioassessment involving the Full BMI protocol plus algae is to be implemented at a given site, practitioners should follow the Full protocol of Ode (2007), and add only Section 3.4 (re: water chemistry), Sections 4 and 5, and Sections 6.9-6.11 from this SOP.

Table 1
Sample and data collection elements included in algal and BMI bioassessment (Ode 2007).
X indicates elements included in algal bioassessment. F indicates elements that are part of the "Full" protocol for conducting BMI bioassessment, B corresponds to elements of the "Basic" BMI protocol, and O indicates elements that are "Optional".

Element	Algae[1]	BMIs
Layout of reach, marking transects, recording GPS coordinates	X	B, F
Notable field conditions	X	B, F
Temperature, pH, specific conductance, DO, alkalinity	X	B, F
Turbidity, Silica	O	O
Water chemistry for lab analysis (see list in Section 3.4)	X	
Algal Sampling for Taxonomic IDs	X	
Algal Sampling for Biomass Assessment	X	O
BMI Sampling for Taxonomic IDs		B, F
Wetted Width	X	B, F
Bankfull Dimensions	X	F
Depth and Pebble Count + CPOM	X	F
Percent Algal Cover (point-intercept with Pebble Count)	X	
Cobble Embeddedness	X	F
Canopy Cover	X	B, F
Gradient	X	B[2], F
Sinuosity		F
Human Influence	X	F
Riparian Vegetation		F
Instream Habitat		F
Bank Stability	X	B, F
Flow Habitat Delineation	X	B, F
Discharge	X	F
Photo documentation	X	B,F
Selected Rapid Bioassessment Procedure (RBP) visuals		F

1. A distinction between Basic and Full protocols for algae has not been established.
2. For BMIs, a single, reachwide measurement of gradient is required for Basic, but gradient is measured between all adjacent transect pairs for Full.

Depending upon the requirements of the monitoring effort, different components of this SOP might be incorporated or omitted. For instance, if stream productivity in terms of algae is the primary concern of the assessment, one may wish to collect only biomass samples and algal cover point-intercept data. Alternatively, one will need to collect algal assemblages (for quantification of diatom and/or soft-bodied algal taxa) in order to make more refined inferences about water quality and stream condition (e.g., by applying an algal Index of Biotic Integrity (IBI)).

This SOP is organized in such a way as to facilitate the inclusion or omission of certain elements based on the goals of the monitoring effort. A list of field supplies is provided in Appendix A. It is organized according to the materials needed for each type of sampling and data collection. In order to facilitate decisions about algal indicators to assess for program-specific needs, the introduction to Section 4 discusses what algal indicators serve which monitoring purposes.

SECTION 2 GETTING STARTED

Several considerations come into play when planning an algae-sampling effort. For instance, time of year can be an important determinant of stream algae abundance as well as the type of community likely to be encountered. Likewise, a minimum amount of surface water is a prerequisite to conducting bioassessment. The following section provides guidelines to help practitioners determine when sampling is appropriate for a given reach and also some pointers to help prepare for field work.

2.1 WHEN TO SAMPLE

It is recommended that sampling for stream algae be carried out during the same period as BMI sampling, generally from May through September, depending on the region. This time frame may eventually be modified (e.g., expanded) based on the results of ongoing index period studies.

It should be noted that high-velocity storm flows can remove macroalgae and biofilms from the stream bottom. Sampling must be done at least a month after any storm event that has generated enough stream power to mobilize cobbles and sand/silt capable of scouring stream substrates, in order to allow ample time for recolonization of scoured surfaces (Round 1991; Kelly et al. 1998; Stevenson and Bahls in Barbour et al. 1999).

2.2 BEFORE SETTING OUT FOR THE FIELD

- Proper precautions should be taken at all times in order to avoid transferring invasive organisms and pathogens between sites. This includes the implementation of effective equipment decontamination procedures. Refer to Appendix B for additional information.
- Use the equipment checklist provided in Appendix A to make sure all necessary supplies are brought along.
- Check with contract lab on sampling containers, and shipping and storage of samples.
- Have in mind at least three sites to visit per day (target two, but plan for at least one additional site as a back up if one of the first two sites is not useable.)
- Prepare, and double check, site dossiers to make sure they are complete with maps/directions to sites and scaled aerial photo(s). Bring along county maps, atlases, and Thomas Guides to further aid location of sites. Also bring along any site access permits, passes, and/or keys, as needed (and be aware that some landowners require notice prior to each site visit).

2.3 BEFORE LEAVING VEHICLE FOR SITE

Make sure the vehicle is parked in a safe spot and there are no "No Parking" signs. Stick a business card with cell phone number in the driver's window. Be sure to display the brown administrative pass placard if you are on National Forest land (or a letter of permission, if applicable).

2.4 DETERMINING WHETHER SITE IS APPROPRIATE FOR SAMPLING

Make an initial survey of the potential monitoring reach from the stream banks (being sure to not disturb the instream habitat). Ensure that there is sufficient water in the stream reach to facilitate collection of algae and water samples. In order for a reach to be in appropriate condition for sampling, at least half of the reach should have a wetted width of at least 1m, and there should be no more than 3 transects that are completely dry. If there is some flexibility in terms of where to place the sampling reach, strive for as few dry transects as possible (and preferably none).

Sites should be safe to sample and legally accessible. The time required to access the sampling sites should also be a consideration in planning which sites to visit, in order to ensure that sample holding times can be met (see Table 2 on page 11 for holding-time information).

3 REACH DELINEATION AND WATER CHEMISTRY SAMPLING

Before sample and data collection can begin, the monitoring reach must be identified and delineated. This requires setting up sampling transects along the stream reach of interest. Once the reach is delineated, information about reach location and condition will need to be documented. Water chemistry parameters must also be recorded, and certain samples collected.

A set of field forms for recording information about monitoring sites, algal samples, and associated water chemistry and PHab data is provided on the SWAMP website (see below). The field forms are also available in electronic version on an portable computer. It is imperative that you confirm throughout the data collection effort at each site that all necessary data have been recorded on the field forms correctly, by double-checking values, and confirming spoken values with your field partner(s). As a general practice, you should conduct a final check across all datasheets to confirm that there are no missing values before you leave the site, and rectify any blanks. *Note: Field forms may be updated periodically. It is imperative that field crews ensure that they are always using the most current field forms. Updated forms can be accessed from the SWAMP website at: http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads*

3.1 DELINEATING AND DOCUMENTING THE MONITORING REACH

To delineate the monitoring reach, you will need to scout it in its entirety in order to make sure that it is of adequate length for sampling algae. During this process, try to stay out of the channel as much as possible, to avoid disturbing the stream bottom, which could compromise the samples and data that will be collected.

SWAMP's standard algae (and BMI) sampling layout consists of a 150 m reach or a 250 m reach, depending upon the average wetted width of the channel. In some circumstances (see below), reach length can be < 150 m, but this should be avoided whenever possible. If the actual reach length is other than 150 m or 250 m, this should be noted and explained on the field forms. Under these circumstances, you will need to determine the useable length of the reach, and how to space your transects so that you can fit them into the reach at equal distances from one to the next.

The wetted channel is the zone that is inundated with water and the wetted width is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Estimate the average wetted width of the reach. If this value is ≤ 10 m, you will end up using 150 m for your monitoring reach length. If the average wetted width is > 10 m, you will use a 250 m long reach.

To set up the monitoring reach, begin a little outside of what you anticipate will be the outer boundary (based on aerials and maps) and count 150 large steps, or 250 large steps (for most adults, a large step is roughly equal to a meter), by walking along the bank. This will give a rough idea about the location of the ends of the study reach. However, keep in mind that once this is determined, the actual distances between transects and intertransects (and consequently, the reach length) will need to be more accurately measured.

As you go, identify where hydrologic inputs that could potentially modify the water chemistry environment occur along the length of the reach. If possible, there should be no tributaries or "end-of-pipe" outfalls feeding into the channel within the monitoring reach. Other features that should not be present within a monitoring reach are: bridge crossings (which shade the stream bottom and can artificially reduce or prevent algal growth), changes between natural and man-made (i.e., concrete) channel bottoms, waterfalls, and impoundments (dams and weirs). If any of such features occur within the reach, and there is not enough room to accommodate a 150-m reach or 250-m reach entirely upstream or downstream of such a feature, then the reach can be somewhat < 150 m. Whatever the reach length turns out to be (150 m, 250 m, or other), record it on the datasheet under "Reach Length".

3.2 MARKING THE TRANSECTS

The monitoring reach will be divided into 11 equidistant main transects that are arranged perpendicularly to the direction of flow. There will also be 10 additional transects (designated "inter-transects"), one between each pair of adjacent main transects, to give a total of 21 transects per monitoring reach. Main transects are designated "A" through "K", while inter-transects are designated by their nearest upstream and downstream main transects ("AB", "BC", etc.).

Once you have identified the upper and lower limits of the monitoring reach, determine the coordinates of the downstream end using a Global Positioning System (GPS) set to the North American Datum 1983 (NAD 83)[3], and record this information in decimal degrees (to five decimal places) on the datasheet under "Reach Documentation". Install a colored flag at water's edge on one of the banks at this location to indicate the first "main transect", or "A". Establish the positions of the remaining transects and inter-transects by heading along the entire length of the monitoring reach (again, staying out of the water/channel as much as possible) and using the transect tape or a segment of rope of appropriate length to measure off successive segments of 7.5 m (for streams of wetted width ≤ 10 m), or 12.5 m (for streams > 10 m wetted width). For monitoring reaches of non-standard length, you will divide the total, targeted length of the reach by 20 to derive the distance between the adjacent main, and inter-, transects. As you measure off the distances, always follow the virtual, mid-channel line, and not the water's edge (which may be irregular, and not reflective of the true stream curvilinear distance).

3. Be aware that some GPS units re-set themselves to factory default settings when the batteries are changed. This can include the datum. Therefore, anytime you remove batteries from your unit, double check that the unit is still set to the NAD83 datum after the batteries have been replaced.

At the end of each measured segment as you head along the stream, mark the transect location on the bank with a flag. We recommend alternating between two different flag colors (e.g., orange could correspond to main transects, and yellow to inter-transects). Determine transect orientations, and where on the banks to place the flags, by visually projecting perpendicularly from the mid-channel to the banks. Refer to Figure 1 for a visual representation of proper transect alignment relative to the stream's direction of flow. When you have finished, the downstream-most flag will correspond to main transect "A", and the upstream-most flag (the 21st in the entire series of main and inter- transects) will correspond to main transect "K".

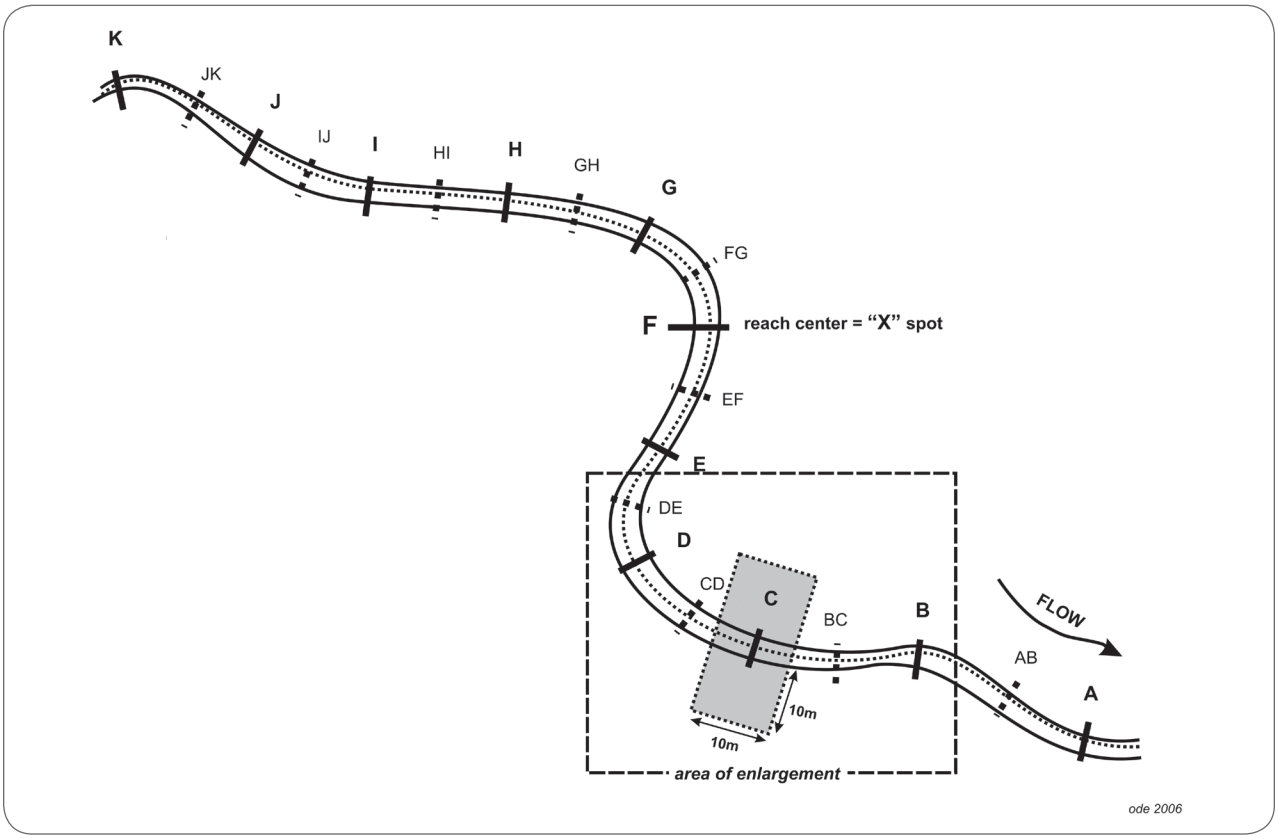

Figure 1. Reach layout geometry for physical habitat (PHab) and biological sampling showing positions of 11 main transects (A-K) and the 10 supplemental inter-transects (AB-JK). The area highlighted in the figure is expanded in Figure 11. Note: reach length = 150 m for streams ≤ 10 m average wetted width, and reach length = 250 m for streams > 10 m average wetted width (reprinted from Ode 2007).

3.3 NOTABLE FIELD CONDITIONS

Record under "Notable Field Conditions" any evidence of recent flooding, fire, or other disturbances that might influence algae samples. Especially note if flow conditions have been affected by recent rainfall, which can cause significant under-sampling of algal biomass and diversity. If you are unaware of recent fire or rainfall events, select the "no" option on the forms. Record the dominant land use and land cover in the area surrounding the reach by evaluating land cover within 50 m of either side of the stream reach. You can

use a scaled aerial photograph of the site and vicinity to guide you. *Note: Before heading out to the field, it is convenient to add a 150 m (or 250 m) line adjacent the stream to be sampled in order to get an idea about the anticipated approximate upstream and downstream boundaries of the monitoring reach.*

3.4 WATER CHEMISTRY

Measure and record common ambient water chemistry measurements (pH, dissolved oxygen (DO), specific conductance, alkalinity, and water temperature) just outside of the reach, at the downstream end, near the same location that the GPS coordinates were taken. This should be done in such a way that it does not interfere with biotic sampling and PHab data collection, but also in such a way that water samples are not compromised by other sampling activities upstream (e.g., by suspension of matter from the stream bottom into the water column, and consequently the introduction of this matter into the water chemistry samples).

Water chemistry measurements are typically taken with a handheld, water-quality meter (e.g., YSI, Hydrolab), but field test kits (e.g., Hach) can provide acceptable information if they are properly calibrated. For appropriate calibration methods, calibration frequency, and accuracy checks, consult the current SWAMP Quality Assurance Program Plan (QAPrP)[4], or follow manufacturer's guidelines. *Note 1: If characteristics of the site prohibit downstream entry, measurements may be taken at other points in the reach. In all cases, ambient chemistry measurements should be taken at the start of the survey (i.e., before algae sampling and PHab data collection). Note 2: A digital titrator (e.g., Hach) using low-concentration acid (such as 0.16N H_2SO_4) as the titrant is recommended for determining alkalinity in low-alkalinity streams (i.e., approximately 100 mg/L $CaCO_3$ or less).*

A suite of analytes must also be evaluated to aid in interpretation of the algal data. These are listed below. Consult the SWAMP QAPrP for specific instructions on the proper techniques for collecting, preserving, and storing these water samples until analysis.

- Nitrate as N (NO_3)
- Nitrite as N (NO_2)
- Ammonia as N (NH_3)
- Nitrogen, Total (TN)[5]
- Orthophosphate as P (dissolved; SRP)
- Phosphorous, Total (TPHOS)
- Dissolved Organic Carbon (DOC)
- Chloride (Cl)

- Silica as SiO_2, dissolved (Note: this analyte is recommended for research purposes, but is not part of the standard algae protocol)

4. This document is available online from the SWAMP website: http://www.swrcb.ca.gov/water_issues/programs/swamp/docs/qapp/swamp_qapp_master090108a.pdf
5. Total Nitrogen can be calculated from Total Kjeldahl Nitrogen (TKN), Nitrate (NO_3) and Nitrite (NO_2)

SECTION 4
REACHWIDE BENTHOS SAMPLING OF ALGAE

The following is a short introduction of several types of algal indicators that can be monitored as part of a bioassessment effort. For a more detailed discussion, see Fetscher and McLaughlin (2008). The most appropriate indicators to include in a given program will ultimately depend upon that program's goals, because the various indicators provide information at varying levels of resolution and applicability to different uses. Likewise, the various indicators require different levels of investment in terms of field work and lab work. Percent algal cover, for instance, is a rapid means of estimating algal primary productivity that can be carried out entirely in the field and is conducted in tandem with the PHab pebble count. Therefore, percent algal cover is an appropriate, fast, and inexpensive parameter for citizen monitoring groups if they are concerned about increased algal biomass. Other estimators of algal biomass include chlorophyll *a* and AFDM, which involve quantitative collection of algae, preservation, and subsequent laboratory analysis. Algal biomass is a key component of the California Nutrient Numeric Endpoints (NNE) framework. Higher resolution information about algal assemblages can be used in algal IBIs, and offers more in-depth insight into water quality. For this type of data, algal specimens must be collected quantitatively (and qualitatively, in the case of soft-bodied algae). The quantitative samples are then fixed/preserved carefully and subjected to taxonomic analysis.

While the percent algal cover data are recorded in conjunction with standard PHab procedures, and do not require the collection of samples, all the other types of data described in this protocol require reachwide benthos (RWB) sampling of algal specimens in a manner analogous to that which is carried out for BMIs.

All four of the algal samples described in this SOP: chlorophyll *a*, AFDM, diatom assemblage, and soft-bodied algal assemblage, can be obtained from a single composite sample generated by the RWB method. Which combination of these samples to prepare and submit for laboratory processing will depend on the needs of the monitoring program. To aid in the selection of algal indicators, Table 2 provides a summary of their attributes.

Table 2
Types of algal indicators and considerations for their assessment.

	Algal indicator for	Collection method	Collection vessel	Preservation/fixation methods and holding times	Qualitative live sample required?
Percent algal cover	Stream productivity measured as algal abundance	Point-intercept add-on to the PHab pebble count	N/A	N/A	N/A
Chlorophyll a[6]	Stream productivity measured as algal biomass; key indicator for the Nutrient Numeric Endpoints (NNE) framework	RWB sample collection	Glass-fiber filter	Wet ice, dark (foil-wrapped); freezing within 4h, and analysis within 28d	N/A
AFDM	Stream productivity measured as biomass of organic matter (including algae); indicator for the NNE framework	RWB sample collection	Glass-fiber filter (pre-combusted[7])	Wet ice, dark (foil-wrapped); freezing within 4h, and analysis within 28d	N/A
Diatoms	Used in IBIs. Indicative of factors such as trophic status; organic enrichment; low DO; siltation; pH; metals	RWB sample collection	50 mL centrifuge tube	Add 10% buffered formalin for a 2% final concentration immediately after collection; keep dark and away from heat	Optional
Soft-bodied algae[8]	Used in IBIs. Indicative of factors such as nitrogen limitation/ trophic status; siltation; pH; temperature, light availability, nuisance/ toxic algal blooms	RWB sample collection	50 mL centrifuge tube	Keep unfixed samples in dark on wet (NOT DRY) ice; add glutaraldehyde (to a 2.5% final concentration) as soon as possible, but no later than 4 days after sampling; after fixing, keep dark and away from heat	Required

6. It is valuable to assess both chlorophyll *a* and phaeophytin *a* (the degradation product of the former) content of algal samples, as this may provide a more robust assessment of algal biomass.
7. Precombustion is recommended in order to remove any possible residual organic matter from the filter.
8. For the purposes of this SOP, the soft-bodied assemblage includes cyanobacteria (an explanation of the rationale for this is provided in Fetscher and McLaughlin 2008)

4.1 GENERAL CONSIDERATIONS FOR SAMPLING ALGAE

This SOP describes the RWB method for collecting stream algae. It employs an objective approach for selecting sub-sampling locations that is built upon the 11 main transects described in the previous section. This approach is analogous to the SWAMP procedure for BMI sampling (Ode 2007), and is ultimately based on EPA's Environmental Monitoring and Assessment Program (EMAP; Peck et al. 2006). After collection, the 11 sub-samples are composited into a single sample per site (sampling reach).

The RWB method can be used to sample any wadeable stream reach since it does not target specific habitats. Because sampling locations are defined by the transect layout, the position of individual sub-sampling spots may fall within a variety of "erosional[9]" or "depositional[10]" habitats, each of which has implications for the type of substrate likely to be encountered and therefore the type of sampling device to use.

For the RWB method, the sub-sampling position alternates between left, center, and right portions of the transects, as one proceeds upstream from one transect to the next. These sampling locations are defined as the points at 25% ("left[11]"), 50% ("center") and 75% ("right") of the wetted width in high-gradient systems, and at "margin-center-margin" (MCM) positions in low-gradient systems. The RWB-MCM method should be only used in low-gradient streams where channel substrates are nearly uniform, resulting in low diversity within the channel. The interim cut-off between "low" and "high" gradient is 1%. Best professional judgment can be used to estimate whether the stream reach should be treated as low- or high-gradient. However, if there is uncertainty about the gradient, it should be measured prior to collecting the biotic assemblage samples. See Section 4.2 for specific instructions about where algae sampling locations should be positioned at the margins of low-gradient sampling reaches.

Algae should be sampled prior to PHab data collection (described in Sections 6-8), so as not to disturb the algae by trampling the transects, as occurs during the PHab process. Furthermore, to avoid disturbing the transects for eventual collection of PHab data, as with BMIs, algae should be collected at a location that is systematically offset from each transect (see Section 4.2).

4.2 COLLECTION OF ALGAE IN CONJUNCTION WITH BENTHIC MACROINVERTEBRATES

If only algae (or only BMIs) are being collected for bioassessment, then the specimens should be collected 1m downstream of the transects. If both assemblages are being sampled, then the algae should be collected upstream of the spot where the BMIs are collected, according to the schematic in Figure 2. BMIs must be collected BEFORE algae at each of the transects, in order to minimize the chances disturbing BMIs during algal collection. After the BMIs are collected at each spot, the algae sample should be taken ¼ m upstream

9. Erosional – habitats in the stream that are dominated by fast-moving water, such as riffles, where stream power is more likely to facilitate erosion (suspension) of loose benthic material than deposition; examples of "erosional" substrates include cobbles and boulders.
10. Depositional – habitats in the stream that are dominated by slow-moving water, such as pools, where deposition of materials from the water column is more likely to occur than erosion (or (re)suspension) of bed materials; examples of "depositional" substrates include silt and sand.
11. For our purposes, "left" is defined as the left bank when facing downstream.

from the center of the upper edge of the "scar" in the stream bottom left from the BMI sampling. It is important to make sure that the surface from which algae will be collected has not been disturbed (by the BMI sampling, or otherwise) prior to sampling the algae.

Note: If only algae (and not BMIs) are being collected in a low-gradient reach, the collection location should be 1 m downstream of the transect and, for each of the "margin" positions, at a distance of 15 cm from the wetted margin of the bank. Fifteen centimeters is chosen because it is approximately ½ the width of a D-frame net.

If duplicates are to be sampled (of either or both assemblages), locations for sampling them should be arranged as depicted in Figure 2 (the duplicates are shown in light grey). *Note: For convenience, only Transects A through C are shown, but the same pattern of placement should be rotated across all 11 transects.*

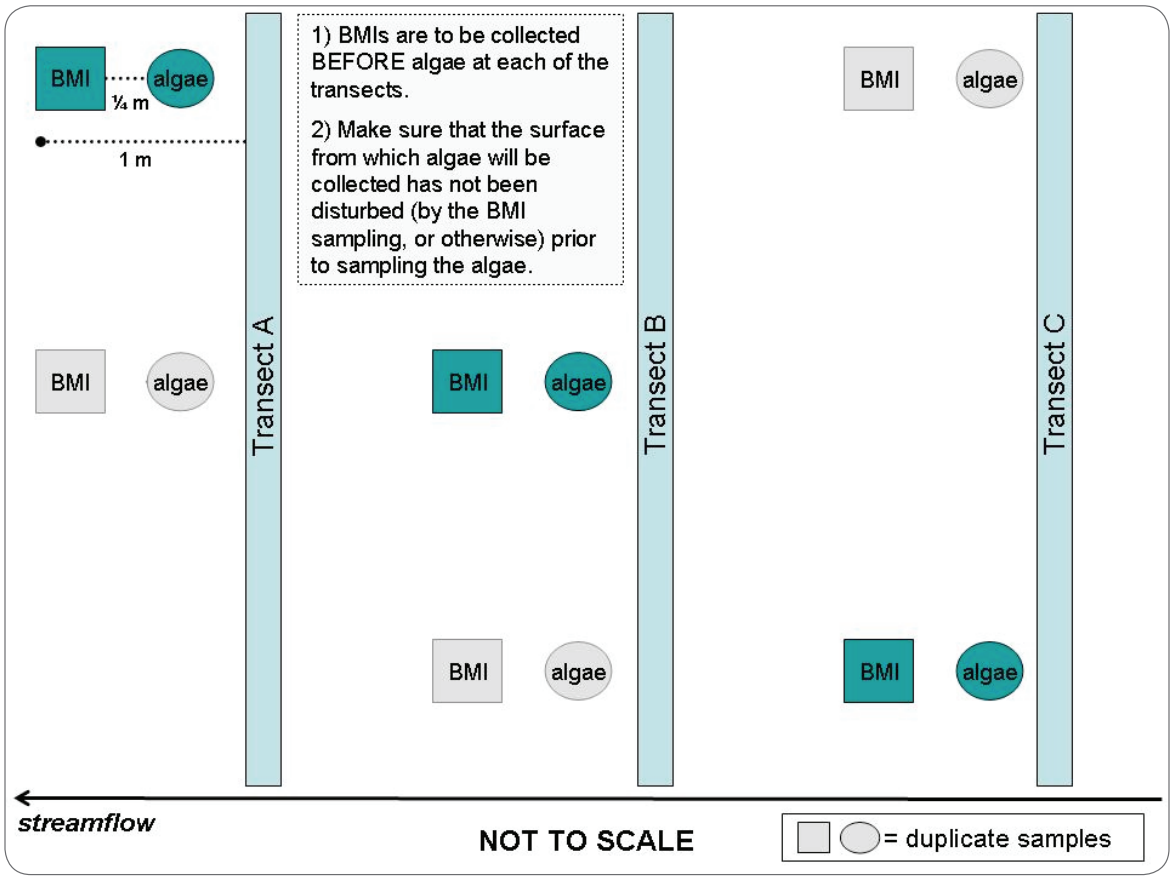

Figure 2. Sampling array for collection of algae, BMIs, and duplicates of each assemblage. For convenience, only Transects A through C are shown, but the same pattern of placement should be rotated across all 11 transects.

4.3 PROCEDURE FOR COLLECTION OF QUANTITATIVE ALGAL SAMPLES

During all phases of algae sampling and processing, in order to preserve specimen integrity, every attempt should be made to keep the sample material out of the sun, and in general, to protect the algae from heat and desiccation, as much as possible. This is necessary in order to reduce the risk of chlorophyll *a* degradation, limit cell division post-collection, and curb senescence/decay of live soft-bodied algae (especially for the qualitative samples; see Section 5.4). The need to maintain the integrity of the algal samples during collection and processing should always be borne in mind when planning the sampling scheme for a given site.

In addition, before sampling at any given site, the dish tub that will contain sample material must be scrubbed with a stiff-bristled brush or scouring pad and thoroughly rinsed with stream water, so that no algal material is carried over from the previous site to contaminate the current sample. The same applies to all other algae sampling apparati (toothbrushes for scrubbing, graduated cylinders, turkey basters, PVC and rubber delimiters, spatulas, syringe scrubbers, etc.).

4.3.1 Identifying the Sampling Locations

As with BMIs, algae sample collection should begin at Transect A and proceed upstream to Transect K. Except in circumstances in which the substrate to be sampled cannot be removed from the stream, a single sample of substrate material that corresponds to the objectively determined sampling point is gathered at each transect and placed in the plastic dish tub. The sample should always be collected from the substrate that is "uppermost" within the stream, and therefore has the highest probability of exposure to sun. For example, if at a given sampling point there is a thick layer of macroalgae above the stream bottom, the substrate collected at that point would be macroalgae itself, not the cobble, sand, or whatever other substrate lies beneath it. Proceeding from transect to transect with the dish tub, the sample collector rotates through the three collection positions in the following order: left at the first transect ("A"), center at the next transect ("B"), right at the next transect ("C"), then back to the left side ("D"), and so on through Transect K.

As substrates are gathered, a tally is taken of the number of samples that correspond to each of the classes of sampling device based on the surface area they sample: 1) 12.6 cm^2 for the PVC or rubber delimiters, and 2) 5.3 cm^2 for the syringe scrubber. The tallies are recorded in the Algae Samples field form under Collection Device. This information will ultimately be used to determine total stream surface area sampled at each site, which in turn will be used to calculate the soft-bodied algal biovolume and the biomass values. It may be helpful to use a tally meter in order to avoid having to carry a datasheet during substrate collection.

4.3.2 Collecting Erosional Substrates

If the substrate type that falls under the sampling spot is in erosional habitat and can be removed from the stream (e.g., a cobble, a piece of wood, or a piece of coarse gravel with an exposed surface area of at least 12.6 cm^2), carefully lift the substrate, moving slowly in an effort to disturb its top surface as minimally as

possible, and remove it from the stream. Then wipe any excess sand, silt, or BMIs, if present, off the bottom of the piece of substrate, and place it in the dish tub. It is helpful to place the substrate in such a way that makes it obvious what surface was facing upward when it was removed from the stream. Eventually, when you isolate a sample of algae from this substrate, you will want to obtain your sample from the portion of the substrate that had been exposed to the surface of the stream (and not buried) during the period leading up to the sampling event. For pieces of substrate with an exposed surface area that is < 12.6 cm^2, the PVC delimiter should be used (Section 4.3.3).

Be sure to place the substrate (e.g., cobble) in the dish tub in such a way that surfaces covered with non-target algae are not rubbing against anything, which could cause non-target algae to slough off into the tub, thus artificially inflating the amount of algae collected. To avoid this problem, and especially if a large number of cobbles are likely to be sampled across a given stream reach, one may choose to isolate the algal specimen from each cobble as it is selected, rather than collecting all the cobbles into the dish tub and then isolating the algal specimens from them after all transects have been sampled. See Section 4.3.9 for further elaboration on this alternative approach.

Figure 3. PVC Delimiter

4.3.3 Collecting Depositional Substrates

If the substrate type that falls under the sampling spot is removable and is in depositional habitat (e.g., silt, sand, fine gravel), and/or has an exposed surface area per particle that is < 12.6 cm^2, you will use a PVC delimiter. This is a plastic coring device with an internal diameter of 4 cm (Figure 3). Instructions for making a PVC delimiter are provided in Appendix C.

Isolate a specific quantity of sand/silt/gravel, centered on the sampling spot, by pressing into the top 1 cm of sediment with a PVC delimiter. Gently slide a masonry or kitchen spatula beneath the delimiter, being careful to keep the collected sediment contained within. Pull the PVC delimiter out of the water (with the spatula still in place) and remove any extra sediment from the spatula around the outside of the delimiter. Transfer the contents held in the delimiter by the spatula to the dish tub. Be sure not to pour the sediment sample on top of any cobbles that may be in the dish tub, as this could result in the sloughing of non-target algae from the cobbles into the dish tub, thus artificially inflating the amount of algae collected.

4.3.4 Collecting Sections of Macroalgae

If the substrate you hit on a given transect is a mass of macroalgae (e.g., a mass of attached filamentous algae underwater, or an unattached, floating mat that is believed to be native to the reach being sampled, and not imported from upstream), position the spatula directly under the macroalgae and press the PVC delimiter into the algae to define a 12.6 cm^2 area. Use a sharp razor blade or knife to cut away and discard any extra material from around the edges of the delimiter (do not simply pull it away, as this will distort the specimen and remove biomass from the targeted material). Add the macroalgal specimen that was isolated by the PVC delimiter to the dish tub.

When collecting a mass/mat of macroalgae, it is important to capture the full thickness of the macroalgae within the delimiter. To do this, from the side of the sampling area, slide your hand under the mat to feel where the bottom is, slide the spatula down to that spot, and then press the PVC delimiter downward slowly to "sandwich" the targeted section of macroalgae between the delimiter and the spatula. It is important to try not to bunch the macroalgae up nor stretch it out unnaturally, as the goal is to collect a representative sample of the algae as it occurs in the stream.

4.3.5 Collecting Sections of Macrophytes

If the substrate to be sampled is part of an immersed macrophyte, or old, dead leaves settled at the bottom of a pool, use the PVC delimiter/spatula combination to isolate a 12.6 cm^2 section of substrate that has been exposed to the surface of the stream. As with the macroalgae (Section 4.3.4), cut away and discard the extra material that falls outside the delimiter using a razor blade.

Figure 4. Syringe Scrubber

4.3.6 Collecting from Concrete, Bedrock, and Boulders

If the substrate falling under a sampling spot cannot be removed from the water (as in the case of bedrock, a boulder, or a concrete channel bottom), use a "syringe scrubber" device (Davies and Gee 1993; Figure 4) to collect an algae sample underwater. Instructions for making a syringe scrubber are provided in Appendix C.

To use this device, affix a fresh, white scrubbing pad circle onto the bottom of the syringe plunger using the Velcro® hooks on the end of the plunger. Press the plunger down so that the bottom of the scrubbing pad is flush with the bottom of the barrel. Then submerge the instrument, press the syringe firmly against the substrate, and rotate the syringe scrubber 3 times in order to collect the biofilm from the substrate surface onto the scrubbing pad. If the surface of the substrate where your sampling point fell is not flat enough to allow for a tight seal with the syringe barrel, objectively choose whatever sufficiently flat area on the exposed face of the substrate is closest to where the original point fell, and sample there.

After sampling, and before removing the syringe scrubber from the substrate, gently retract the plunger just slightly, so it is not up against the substrate anymore, but not so much that it pulls a lot of water into the barrel. Carefully slide the spatula under syringe barrel (which should be pulled just slightly away from the substrate on one side to allow the spatula to slide under), trying not to allow too much water to rush into the barrel. Then pull the instrument back up out of the water with the spatula still firmly sealed against the syringe-barrel bottom.

Hold the syringe scrubber over the dish tub and then remove the spatula, allowing any water to fall into the tub. Carefully detach the pad from the plunger and hold the pad over the tub. Using rinse water sparingly, remove as much algal material from the pad as possible by rinsing it off with the wash bottle, or a turkey baster, filled with stream water (from the current site—never carried over from a previous site), and

wringing it into the dish tub before discarding the used pad. Start this process by rinsing from the backside of the pad (the side that had been affixed to the plunger) to "push" the collected algae forward out of the front surface of the pad.

It is recommended that a fresh (new) pad be used each time a sample is collected, even within the same stream reach. Under no circumstances should the same pad be used at more than one site.

4.3.7 Collecting from Other Substrate Types

If other substrate types are encountered, they can be sampled from as long as there is good reason to believe that they were not recently introduced into the stream (e.g., by flowing from the upstream regions, or by recently falling into the stream), as they would then not be representative of the local instream environment. Use the collection instrument you deem to be most appropriate to sample the substrate and, as with any substrate, be sure to account for the surface area sampled (in this case, using the "Other" box on the Collection Device portion of the field forms).

4.3.8 Removal of Algae from Collected Substrates

After having sub-sampled substrates across the monitoring reach, there should be 11 transects' worth of material in the dish tub. Depending on the types of habitats in the stream and substrates encountered, the tub may contain cobbles, and/or sand, and/or gravel, and/or small pieces of wood, macroalgae, or macrophyte. Now a measured quantity of the algae clinging to these substrates must be removed and suspended in water to form a "composite sample" according to the instructions in the following sections.

For erosional substrate types that were removed from the stream (e.g., cobbles and small pieces of wood), use a rubber delimiter to isolate a 12.6 cm^2 area from which algae will be removed. A rubber delimiter can be made from a mountain bike tube with a hole cut out and reinforced with an appropriately sized rubber washer (Figure 5). Appendix C describes the procedure for making a rubber delimiter.

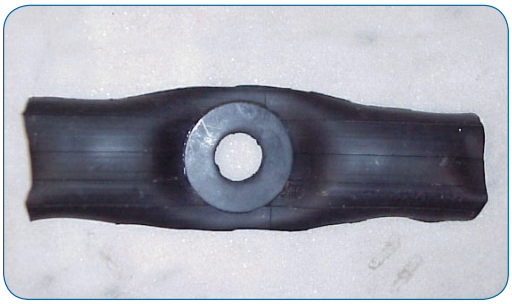

Figure 5. Rubber Delimiter

Wrap the rubber delimiter around the substrate to expose the desired sampling surface through the hole. Take care to ensure that the surface that will be scrubbed is truly the upper (generally at least somewhat "slimy") surface of the substrate as it had been oriented in the stream. Dislodge attached algae from this area by brushing it with a firm-bristled toothbrush (remember that this toothbrush must first have been thoroughly rinsed since the previous site to avoid contamination with algal specimens from other streams). If there is a thick mat of algae, or the algae is firmly encrusted on the surface of the substrate, use forceps or a razor blade first to dislodge the larger matter and put this in the dish tub. Then scrub the area with the brush.

Make sure that the entire surface within the delimiter has been scrubbed well in order to remove all the algae in that area. Fill a wash bottle or turkey baster with stream water from the current site (never carried over from a previous stream). Using as minimal a volume of water as possible, rinse the scrubbed algae from the sample area into the dish tub. Take care to squirt water only on the surface that is showing through the hole in the delimiter, and not anywhere else on the substrate's surface. It is helpful to invert the rock when rinsing so that the target surface is facing down toward the dish tub, and the rinsate drips off the sampling spot directly into the tub rather than flowing along the (non-target) sides of the substrate. Use water sparingly for each piece of substrate, because you should attempt to use no more than 400-500 mL total for the full suite of 11 samples collected along the transects (this includes any water used for rinsing algae off of sampling devices into the dish tub). After scrubbing is complete, rinse the delimiter and the brush into the dish tub, also. The scrubbed part of the substrate should feel relatively rough when you have finished, meaning that essentially all of the algae have been removed. After the sampling area on the piece of substrate has been thoroughly scrubbed and rinsed, the piece of substrate can be returned to the stream.

For depositional samples (e.g., silt, sand, or gravel), there is no need to isolate a specific area of the substrate within the dish tub, because the sample area was pre-isolated by using the PVC delimiter during collection. Simply massage all the sand and/or silt in the dish tub thoroughly between the fingers to dislodge any clinging algae. For pieces of gravel, use a toothbrush to remove algal material from surfaces.

Rinse the sediment thoroughly (but as sparingly as possible) with stream water so as to create a suspension of the dislodged microalgae (i.e., the sample). The final volume of the sample liquid in the dish tub will be measured before the algal taxonomic and biomass samples are prepared (described below). To do this, the liquid in the tub will be separated from the rinsed sediment such that the volume measured does not include sediment. After the liquid sample has been retrieved and measured, the rinsed sediment will be discarded back into the stream.

Other types of substrate, like pieces of macrophyte or dead leaves that had been collected with the PVC delimiter, should also be massaged between the fingers and rinsed into the tub in order to remove the algae coating them.

For macroalgal clumps there is a special step required for processing the samples. This procedure is described in detail in Section 5.3.

4.3.9 Alternative Approach: Processing Samples at Each Transect

It is also acceptable to isolate the algal specimens from each "piece" of substrate collected before moving on to the next transect. This approach has the disadvantage of requiring that all algae sampling/scraping tools be carried along with the collector as s/he proceeds up the stream, and that s/he pause to isolate the algae several times across the stream reach rather than one time at the end of all the transects. However, it limits the amount of substrate material that needs to be carried in the dish tub, thus making it lighter. This could be particularly important if a large number of cobbles are encountered across sampling points, such that it

could be difficult or impossible to carry them all to Transect K, or to carry them in such a way that non-target algae can easily be prevented from sloughing off into the tub via abrasion. For convenience, one may elect to wear a fisherman's vest to facilitate carrying all the algae sampling/scraping tools that will need to be brought along on the substrate sampling trip if employing this alternative approach.

SECTION 5
ALGAL SAMPLE PROCESSING

Four different types of laboratory samples may be prepared from the composite sample:

- Identification/Enumeration Samples
  1. Diatoms
  2. Soft-bodied algae
- Biomass Samples
  3. Chlorophyll *a* ("chl *a*")
  4. Ash-free dry mass ("AFDM")

5.1 GENERAL CONSIDERATIONS FOR PROCESSING ALGAL SAMPLES

The general process for sample preparation is as follows. The Identification/enumeration samples are each aliquoted into 50-mL centrifuge tubes and chemically fixed (preserved). Diatom samples are fixed in the field with formalin immediately following collection, and soft-bodied algae samples are fixed in a laboratory with glutaraldehyde within four days of collection. The chlorophyll *a* and AFDM samples are collected on filters in the field and stored on wet ice, and then frozen as soon as possible after returning from the field (and within four hours of collection). The filters are kept frozen until analysis, which should occur within 28 days of collection. If the field crew is spending the night in a hotel, it is necessary to buy dry ice to freeze the biomass filters upon finishing the day's fieldwork, and to keep them on dry ice until the samples can be transferred to the freezer back at the lab.

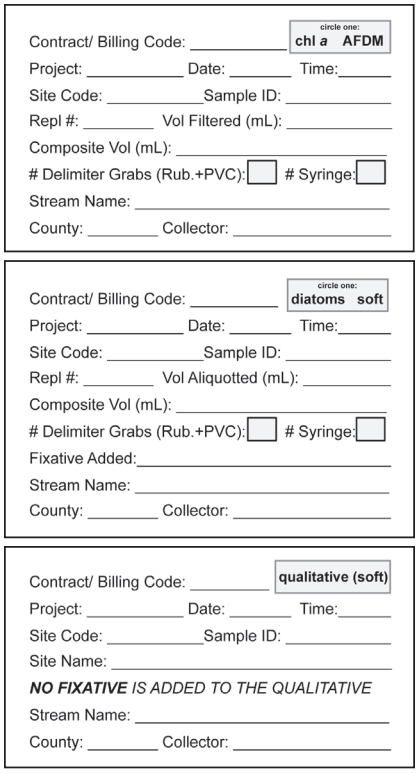

Figure 6. Labels for biomass and taxonomic identification samples.

Algae sample labels are shown in Figure 6. Recorded on each sample label are the volume of the composite sample (described in Sections 5.2.1 and 5.3.2), and the TOTAL area of stream bottom sampled (based on which sampling devices were used; described in Sections 4.3.2 - 4.3.7), as well as the volume of sample aliquoted (for the taxonomic ID samples) or filtered (for the chlorophyll *a* and ADFM samples). All of these values should be recorded on the field forms, as well, under the Algae Samples section. On the sample labels, the sample type: "chl *a*", "AFDM", "diatoms", or "soft" is circled, and all the remaining information on each label, like Site Code, Date, and site coordinates is filled out.

Before preparing the algae samples it is necessary to determine two things:

- Are there any clumps of macroalgae in the composite sample (as opposed to just microalgae suspended in liquid)?

AND

- Is a soft-bodied algal taxonomic sample going to be prepared?

The answers to these questions will determine the course of action for preparing the algae samples for a given site:

- If there is no macroalgal clump, liquid composite sample will simply be added to each taxonomic ID sample tube (40 mL for diatoms and 45 mL for soft-bodied algae). Biomass samples will also be prepared using the liquid composite sample, as is.
- If there is a macroalgal clump present, but no soft-bodied sample will be prepared, the entire clump will be chopped into fine bits (resulting in strands that are eyelash-length or shorter) and incorporated directly into the liquid portion of the composite sample, and the mixture will be shaken to homogenize it before preparing the diatom and/or biomass samples.
- If there is a macroalgal clump AND a soft-bodied algal taxonomic ID sample is to be prepared, then a more complex procedure must be employed in order to properly process the macroalgae before preparing the various samples.

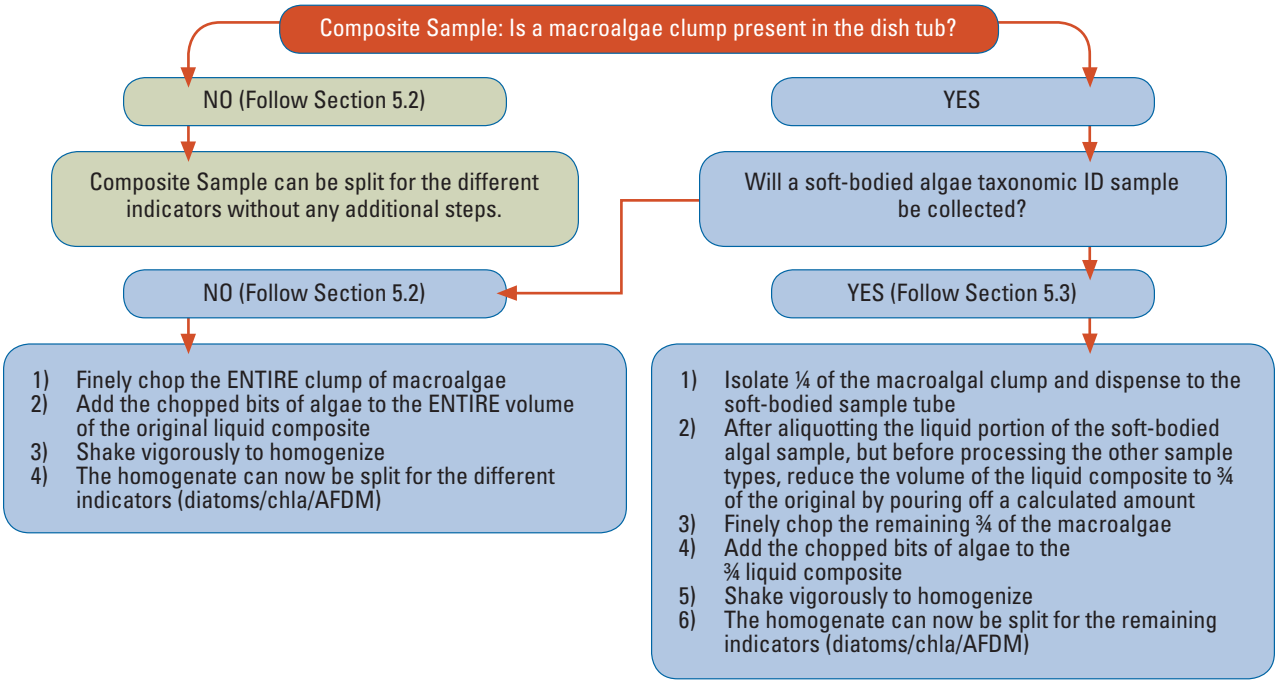

Figure 7. Summary of major sample-processing decision points based on presence of macroalgal clump(s) and need to prepare soft-bodied algal samples.

Figure 7 provides a summary of the various sample-processing steps that are involved, and the following sections describe the procedures in detail. Use Section 5.2. if there is NO macroalgal clump present in the dish tub OR a soft-bodied algal sample will be NOT be prepared. Use Section 5.3 if there IS a macroalgal clump present AND a soft-bodied algal sample will be prepared.

5.2 SAMPLE PROCESSING WHEN THERE IS NO MACROALGAL CLUMP OR WHEN NO SOFT-BODIED SAMPLE IS BEING PREPARED

This section describes the sample-processing procedure for the situation in which there was either 1) no macroalgal clump in the dish tub containing the composite sample material, or 2) no soft-bodied algal sample will be prepared. If there was no macroalgal clump but both soft bodied algae AND other sample types are to be prepared, follow all the instruction in this section with the exception of the final portion of Section 5.2.1 that is italicized and in orange font, and discusses how to process macroalgae when preparing only diatom and/or biomass samples.

If there is a macroalgal clump, but no soft-bodied algae sample is to be collected, follow the instructions in this section, including the final, italicized portion of Section 5.2.1, and skip Section 5.2.2, which deals with soft-bodied algal sample processing.

5.2.1 Measuring the Composite Liquid Volume

Once algal specimens have been removed from all the substrates (e.g., sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Section 4, thoroughly agitate the liquid to get as much as possible of the microalgae into suspension, and then immediately pour the liquid into a CLEAN graduated cylinder to measure its volume. Try to leave all substrate material (e.g., silt, sand) behind. Transfer the measured liquid into a CLEAN 1L plastic bottle. Using a minimal amount of stream water, rinse the substrate once or twice with stream water until it appears that little to no additional suspended material (microalgae) is coming off. Add this rinsate to the graduated cylinder to measure it also. If necessary, repeat this process (regularly agitating the dish tub) until all the liquid has been measured and transferred to the sample bottle. Use water sparingly, because the total sample volume (plus rinsate) should be no more than about 400-500 mL.

Because you are leaving as much as possible of the silt, sand, and any large substrate material behind, the final volume should reflect only the liquid component of the sample plus rinse water. Record the total volume of all the liquid that had been in the dish tub, including any that was used for rinsing the substrates and sampling devices, on the field sheet under the Algae Samples section. This is the COMPOSITE VOLUME. This value will also be recorded on all algae sample labels (i.e., for the diatom and soft-bodied algae taxonomic ID samples, the chlorophyll *a*, and the AFDM).

Note: If no soft-bodied algae sample is to be prepared, but there is a macroalgal clump in the sample, separate the clump from the liquid portion of the sample, measure and record the composite volume of the liquid (as described above), then cut the macroalgal clump into very fine pieces (resulting in strands that are eyelash-length or shorter) with CLEAN scissors and add these pieces to the composite liquid. The pieces should be chopped small enough so that they practically "blend" into the liquid (i.e., distinct fragments of macroalgae are not easily discernible), because the goal is to "homogenize" the macroalgae into the liquid as much as possible. Shake vigorously to homogenize the macroalgal fragments into the liquid. Then proceed to Section 5.2.3 and beyond to prepare the diatom and/or biomass samples.

5.2.2 Preparing the Soft-Bodied Algae Taxonomic ID Sample

Pour freshly-agitated liquid composite sample into the soft-bodied algae sample tube to the 45 mL mark. Midway through pouring, the composite sample should be swirled some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. Completely fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Place the tube in the wet ice chest to keep it in the dark and as cold as possible, but make sure it is never allowed to freeze.

Note: As soon as possible, and no longer than four days after collection of the sample, glutaraldehyde must be added to the tube (to a final concentration of 2.5%, by adding 5 mL of 25% glutaraldehyde to 45 mL of sample) and distributed throughout the sample by agitation and turning the tube upside down repeatedly. Glutaraldehyde is necessary for fixing soft-bodied algal samples in order to preserve fine morphological features and the color of pigments, as both can be crucial characters for taxonomic determination. Glutaraldehyde is a hazardous substance that poses a number of safety risks. As such, it should be handled in a fume hood by trained personnel wearing appropriate gear. Refer to Appendix D for an SOP for the use of glutaraldehyde.

Members of the field crew can either have the glutaraldehyde added to the samples back at their own lab, or arrange for the glutaraldehyde to be added to the samples by the taxonomy lab. In either case, the unfixed samples must be kept in the dark and on wet ice (but not allowed to freeze), and must be fixed within four days of collection (and preferably sooner). If the taxonomy lab will be adding the fixative, it is imperative to plan ahead to arrange for this to be done, and also to clearly mark which tubes will need to have fixative added to them. Once the samples are fixed, it is no longer imperative to store them on wet ice. Following fixation, they can simply be stored in a cool, dark place.

5.2.3 Preparing the Diatom Taxonomic ID Sample

Diatom samples should be fixed as soon as possible after collection to reduce the possibility of cell division post-sampling. A 10% solution of buffered formalin is used to fix diatoms, and instructions for preparing this solution are provided in Appendix C.

To prepare the diatom sample, aliquot 40 mL of freshly-agitated composite liquid into the diatom ID sample tube, swirling the composite sample bottle again midway through pouring to keep the microalgae suspended. Add 10 mL of the 10% buffered formalin to the sample. This can be done using a small syringe or bulb pipette. Alternatively, if preferred, the centrifuge tubes for the diatom samples can be pre-loaded with 10 mL of the 10% buffered formalin and 40 mL of sample can be added carefully to the fixative, to avoid having to dispense the fixative in the field.

Notes: Fixatives such as formalin must be used with great care. Be sure to wear formalin-safe gloves and safety goggles when using the fixative, as it should never be touched with bare hands or allowed to splash onto skin or into eyes. Also make sure it is used only in a very well-ventilated place and avoid breathing in any fumes. Minimize the amount of time that vessels containing formalin are open. Fixative added to the sample must not be allowed to ooze outside the vessel that contains it, including the sample tubes. Refer to Appendix E for an SOP for the use of formalin.

Cap the tube tightly and shake it to mix the formalin into the sample. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Keep the fixed diatom samples in the dark and away from extreme heat.

5.2.4 Preparing the Biomass Samples

The remaining composite sample liquid can be used to prepare the chlorophyll *a* and AFDM filters as described below.

Chlorophyll *a* samples:

The procedure to filter chlorophyll *a* samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light, and minimize chlorophyll *a* degradation thereby. For the chlorophyll *a* samples, use CLEAN filter forceps (rinsed with DI water three times) to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of a CLEAN filtering tower apparatus (rinsed with DI water three times), and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter reservoir on top. Never touch the filters with hands or anything other than clean forceps.

Agitate the composite sample to resuspend all the microalgal material. Carefully measure 25 mL using a small, CLEAN graduated cylinder (rinsed with DI water three times). Midway through pouring the 25 mL, swirl the composite sample again to ensure that the material is still fully suspended. Pour the remainder of the 25 mL, and then pour the measured sample into the filter reservoir. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the reservoir.

To filter the sample, create a gentle vacuum with the hand pump. Be sure to proceed very slowly, and pump only one stroke at a time until all of the liquid in the sample is passed through the filter. Pressure on the sample should never exceed 7 psi, as this could cause cells to burst and release contents, including chlorophyll *a*, into the filtrate and be lost. If it becomes impossible to filter a whole 25 mL of the sample and

remove the water efficiently, discard the filter and try again with a smaller volume (e.g., 10 mL). It is not necessary to collect on multiple filters to try to achieve a total volume of 25 mL. Simply filter as much as possible on a single filter, up to 25 mL, and then use that filter as the sample. Be sure to record the volume of the composite sample that was actually filtered, both on the datasheet, and on the sample label.

Rinse the sides of the filter reservoir with a few mL of DI water, and continue filtering until the water is drawn down. The filter should not be sucked dry, but rather left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst. After the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (like tiny seedlings or bits of leaves). If so, remove them with clean forceps, being careful not to remove any algae in the process. If possible, rinse the removed items with DI water onto the filter before discarding them. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Being careful not to remove any of the collected material from the filter, fold the filter in half (with the sample material on the inside) using the forceps, and place it inside a clean, snap-top Petri dish[13]. Envelop the Petri dish completely within a small sheet of aluminum foil in order to prevent any light from reaching the filter. Place the covered Petri dish and its corresponding, completely filled-out sample label (face outward) into a 100 mL Whirl-pak bag[14], purge as much of the air out of the bag as possible, "whirl" it shut, and seal it tightly with its wire tabs, so that water in the cooler will not be able to enter the bag. *Note 1: If the Whirl-pak bags contain a lot of air, they will float on top of the ice water in the cooler, and they then run the risk of not being kept cold enough. Shove the sample packet down into the ice in the cooler. Note 2: A clean, clear plastic centrifuge tube is also an acceptable container in which to store the filter. It must also be properly labeled, wrapped in aluminum foil, and kept submerged in wet ice.*

Keep chlorophyll *a* filters as cold as possible and place in the freezer or dry ice as soon as possible (and within four hours of collection); the holding time for the chlorophyll *a* filters is 28 days from collection, when kept frozen.

Ash-free dry mass (AFDM) samples:

For the AFDM samples, you should use glass-fiber filters (47 mm, 0.7 µm pore size) that have been precombusted. Never touch the filters with hands or anything other than a CLEAN forceps (rinsed with DI water three times). The filters to use should be labeled "for AFDM", and stored in aluminum sleeves. Follow the same process as that used for chlorophyll *a* sample filtering. After all the liquid has passed through, check the filter to see if there are any pieces of non-algal plant matter (such bits of leaves or wood). If so, remove them with a clean forceps (rinsed with DI water three times), being careful not to remove any algae in the process. The goal with AFDM, for the purposes of this SOP, is to target the ALGAL portion of the organic matter in the sample, and therefore field crews should do their best to remove non-algal contributors

13. It may be beneficial to write the Site Code or sample ID code on the Petri dish itself, in addition to filling out the full sample label.
14. Other bag types are acceptable only if they are water-tight (note that Ziploc bags often leak when submerged).

of organic matter from the sample. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Use the forceps to fold the AFDM filter in half (with the sample material on the inside) and wrap it loosely in a small sleeve of clean aluminum foil. Be careful not to squeeze the filter, which could cause the sample to ooze from the filter onto the aluminum sleeve. Store the filter in a sealed 100 mL Whirl-pak bag containing a completely filled-out sample label, including the volume that was filtered (i.e., 25 mL or otherwise). As with the chlorophyll *a*, purge as much of the air out of each bag as possible, "whirl" it shut, and seal tightly with the wire tabs. Shove the sample packet down into the ice in the cooler. *Note: A clean, clear plastic centrifuge tube is also an acceptable container in which to store the filter. It must also be properly labeled and kept submerged in wet ice.*

Keep AFDM filters as cold as possible until the samples can be frozen back at the lab that evening, or place on dry ice until they can be stored in the lab freezer. The holding time for the AFDM samples is 28 days from collection, when kept frozen.

5.3 PROCESSING SOFT-BODIED AND OTHER SAMPLE TYPES WHEN A MACROALGAL CLUMP IS PRESENT

The following is a description of how to proceed when a soft-bodied algal taxonomic ID sample is to be prepared AND macroalgal clump(s) are present in the sample in the dish tub. A flowchart of this procedure is provided in Appendix F. It is recommended that this flowchart be printed out in color, laminated (if possible) or printed out on water-proof paper, and brought along to the field for quick reference on handling macroalgal clumps in the composite sample. The reason for the extra step in the processing of the macroalgae for the purposes of the soft-bodied algae sample is that it maintains larger, more intact macroalgal specimens for examination in the laboratory, rather than chopping up all of the macroalgal specimens before sending them to the lab. This is important, because availability of intact specimens greatly improves the chances that the taxonomist will be able to identify the soft-bodied algae to low taxonomic levels.

5.3.1 Isolating and Dividing the Macroalgal Clump

For this procedure, the macroalgal clump is first removed from the dish tub, wrung out gently, and rolled into a cylinder shape that is relatively even in thickness along its length. If there is more than one type of macroalgae in the sample, the various types should be layered on top of one another lengthwise so that they are represented in roughly constant proportions across the length of the "cylinder". The cylinder is measured with a ruler and a quarter of its length is cut off with scissors and put into the (still empty) soft-bodied algae ID centrifuge tube[15]. The clump is pushed down into the tube, and the top is flattened, so that the volume

15. It is unlikely that the ¼ macroalgal clump will occupy all the space in the sample tube, but if it does, a second tube will be needed in order to accommodate all the sample material plus liquid. If such an action is taken, it should be noted in the Comments section of the field sheets and the tubes should be clearly identified as belonging to the same sample, for record keeping purposes.

of the clump can be estimated using the graduations on the tube. The estimated volume of this clump will be used in a calculation (see Equation 1 and Figure 8). The remaining three-quarters length of cylinder is set aside in the shade/cool. It is recommended that this section be placed in a Ziploc bag, sealed, and put in the wet ice cooler.

5.3.2 Measuring the Composite Liquid Volume

Once algal specimens have been removed from all the substrates (e.g., sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Section 4, gently agitate the dish tub to suspend the microalgae in the liquid, and then start pouring this suspension into a CLEAN graduated cylinder to measure the volume of the liquid. Try to leave all substrate material (e.g., silt, sand) behind. Transfer the measured liquid into a CLEAN 1L plastic bottle. Using a minimal amount of stream water, rinse the substrate once or twice with stream water until it appears that little to no additional suspended material (microalgae) is coming off. Add this rinsate to the graduated cylinder to measure it also. If necessary, repeat this process (regularly agitating the dish tub) until all the liquid has been measured and transferred to the sample bottle. Use water sparingly, because the total sample volume plus rinsate should be no more than about 400-500 mL.

Because you are leaving as much of the silt, sand, and any large substrate material behind as possible, the final volume should reflect only the liquid component of the sample plus rinse water. Record the total volume of all the liquid that had been in the dish tub, including any that was used for rinsing the substrates and sampling devices, on the field sheet under the Algae Samples section. This is the COMPOSITE VOLUME. This value will also be recorded on all algae sample labels (i.e., for the diatom and soft-bodied algae taxonomic ID samples, the chlorophyll *a*, and the AFDM).

5.3.3 Preparing the Soft-Bodied Algae Taxonomic ID Sample

Pour freshly-agitated liquid composite sample from the 1-L bottle into the soft-bodied algae sample tube (on top of the clump of macroalgae) up to the 45 mL mark. Midway through pouring, the composite sample should be swirled some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. Completely fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Place the tube in the wet ice chest to keep it in the dark and as cold as possible, but make sure it is never allowed to freeze.

Note: As soon as possible, and no longer than four days after collection of the sample, glutaraldehyde must be added to the tube (to a final concentration of 2.5%, by adding 5 mL of 25% glutaraldehyde to 45 mL of sample) and distributed throughout the sample by agitation and turning the tube upside down repeatedly. Glutaraldehyde is necessary for soft-bodied algal samples in order to preserve fine morphological features and the color of pigments, as both can be crucial characters for taxonomic determination. Glutaraldehyde is a hazardous substance that poses a number of safety risks. As such, it should be handled in a fume hood by trained personnel wearing appropriate gear. Refer to Appendix D for an SOP for the use of glutaraldehyde.

Members of the field crew can either have the glutaraldehyde added to the samples back at their own lab, or arrange for the glutaraldehyde to be added to the samples by the taxonomy lab. In either case, the unfixed samples must be kept in the dark and on wet ice (but not allowed to freeze), and must be fixed within four days of collection (and preferably sooner). If the taxonomy lab will be adding the fixative, it is imperative to plan ahead to arrange for this to be done, and also to clearly mark which tubes will need to have fixative added to them. Once the samples are fixed, it is no longer imperative to store them on wet ice. Following fixation, they can simply be stored in a cool, dark place.

5.3.4 Preparing the Diatom Taxonomic ID Sample

After the soft-bodied algal sample has been prepared, and before preparing the diatom sample (and biomass samples, which will be discussed in the next section), the volume of the remaining composite liquid must be reduced to equal ¾ of the original volume[16]. This is necessary because ¼ of the macroalgae clump was taken out of the composite sample but a full ¼ was not removed from the water portion. As such, the original ratio between water and macroalgae must be restored before further sample preparation.

The following procedure is used to reduce the volume of liquid composite to ¾ of the original. For convenience, you can use this formula (Figure 8) to calculate how many mL to pour off and discard from the composite:

Equation 1. Adjusting the volume of composite sample

volume (mL) of composite to pour off = (0.25 * C) – 45 + A

where "C" is the original composite volume and "A" is the approximate volume of the clump of macroalgae that was placed in the soft-bodied algae sample tube (tamped down and flattened). You may wish to fill out a copy of the Ratio Restoration worksheet shown in Figure 8 to calculate the amount of composite to pour off.

Liquid Portion of Composite Sample: _____ mL = C

Volume of 1/4 macroalgal chunk: _____ mL = A

Volume of Liquid Composite to Pour Off: (0.25 * _____ C) – 45 + _____ A = _____ mL

Figure 8. Ratio restoration worksheet.

16. For example, if the original composite volume was 480mL, you will be discarding enough composite liquid to get down to 360 mL.

As always, be sure to agitate the composite liquid adequately in order to resuspend any settled microalgae before pouring off the calculated volume.

Once the required amount of composite liquid has been discarded, the remaining ¾ of the macroalgal clump ("cylinder") is cut into very fine pieces with a scissors (resulting in strands that are eyelash-length or shorter), and these are added to the reduced-volume composite liquid. The pieces should be chopped small enough so that they practically "blend" into the liquid (i.e., distinct fragments of macroalgae are not easily discernible), because the goal is to "homogenize" the macroalgae into the liquid as much as possible. Now the ratio of macroalgae to liquid from the original sample in the dish tub is restored. Cap the composite bottle and shake vigorously to homogenize the bits into the liquid as much as possible, while not agitating so hard as to risk busting cells and releasing chlorophyll.

Diatom samples should be fixed as soon as possible after collection to reduce the possibility of cell division post-sampling. A 10% solution of buffered formalin is used to fix diatoms, and instructions for preparing this solution are provided in Appendix C.

To prepare the diatom sample, aliquot 40 mL of freshly-agitated sample homogenate into the diatom ID sample tube, swirling the composite sample bottle again midway through pouring to keep the algal material suspended. Add 10mL of the 10% buffered formalin to the sample. This can be done using a small syringe or bulb pipette. Alternatively, if preferred, the centrifuge tubes for the diatom samples can be pre-loaded with 10 mL of the 10% buffered formalin and 40 mL of sample can be added carefully to the fixative, to avoid having to dispense the fixative in the field.

Note: Fixatives such as formalin must be used with great care. Be sure to wear formalin-safe gloves and safety goggles when using the fixative, as it should never be touched with bare hands or allowed to splash onto skin or into eyes. Also make sure it is used only in a very well-ventilated place and avoid breathing in any fumes. Minimize the amount of time that vessels containing formalin are open. Fixative added to the sample must not be allowed to ooze outside the vessel that contains it, including the sample tubes. Refer to Appendix E for an SOP for the use of formalin.

Cap the tube tightly and shake it to mix the formalin into the sample. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Keep the fixed diatom samples in the dark and away from extreme heat.

5.3.5 Preparing the Biomass Samples

The remaining composite sample homogenate can be used to prepare the chlorophyll *a* and AFDM filters according to the following procedure.

Chlorophyll *a* samples:

The procedure to filter chlorophyll *a* samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light, and minimize chlorophyll *a* degradation thereby. For the chlorophyll *a* samples, use CLEAN filter forceps (rinsed with DI water three times) to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of CLEAN filtering tower apparatus (rinsed with DI water three times), and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter reservoir on top. Never touch the filters with hands or anything other than clean forceps.

Agitate the composite sample homogenate to resuspend all the macroalgal fragments and microalgal material. Carefully measure 25 mL using a small, CLEAN graduated cylinder (rinsed with DI water three times). Midway through pouring the 25 mL, swirl the homogenate again to ensure that the material is still fully suspended. Pour the remainder of the 25 mL, and then pour the measured homogenate into the filter reservoir. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the reservoir.

To filter the sample, create a gentle vacuum with the hand pump. Be sure to proceed very slowly, and pump only one stroke at a time until all of the liquid in the sample is passed through the filter. Pressure on the sample should never exceed 7 psi, as this could cause cells to burst and release contents, including chlorophyll *a*, into the filtrate and be lost. If it becomes impossible to filter a whole 25 mL of the sample and remove the water efficiently, discard the filter and try again with a smaller volume (e.g., 10 mL). It is not necessary to collect on multiple filters to try to achieve a total volume of 25 mL. Simply filter as much as possible on a single filter, up to 25 mL, and then use that filter as the sample. Be sure to record the volume of the composite sample that was actually filtered, both on the datasheet, and on the sample label.

Rinse the sides of the filter reservoir with a few mL of DI water, and continue filtering until the water is drawn down. The filter should not be sucked dry, but rather left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst. After all the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (like tiny seedlings or bits of leaves). If so, remove them with clean forceps, being careful not to remove any algae in the process. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Fold the filter in half (with the sample material on the inside) using the forceps, and place it inside a clean, snap-top Petri dish. Envelope the Petri dish completely within a small sheet of aluminum foil in order to prevent any light from reaching the filter. Place the covered Petri dish and its corresponding, completely filled-out sample label (face outward) into a 100 mL Whirl-pak bag, purge as much of the air out of the bag as possible, "whirl" it shut, and seal it tightly with its wire tabs, so that water in the cooler will not be able to enter the bag. *Note: If the Whirl-pak bags contain a lot of air, they will float on top of the ice water in the cooler, and they then run the risk of not being kept cold enough. Shove the sample packet down into the ice in the cooler.*

Keep chlorophyll *a* filters as cold as possible and place in the freezer or dry ice as soon as possible (and within four hours of collection); the holding time for the chlorophyll *a* filters is 28 days from collection, when kept frozen.

Ash-free dry mass (AFDM) samples:

For the AFDM samples, you should use glass-fiber filters (47 mm, 0.7 µm pore size) that have been precombusted. Never touch the filters with hands or anything other than a clean forceps (rinsed with DI water three times). The filters to use should be labeled "for AFDM", and stored in aluminum sleeves. Follow the same process as that used for chlorophyll *a* sample filtering. After all the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (such as bits of leaves or wood). If so, remove them with clean forceps (rinsed with DI water three times), being careful not to remove any algae in the process. Remove the filter from the filtering device. *Note: Always thoroughly rinse the sides of the filter reservoir and the interface between the mesh filter seating and the screw-on part of the reservoir with DI water between samples.*

Use the forceps to fold the AFDM filter in half (with the sample material on the inside) and wrap it loosely in a small sleeve of clean aluminum foil. Be careful not to squeeze the filter, which could cause the sample to ooze from the filter onto the aluminum sleeve. Store the filter in a sealed 100 mL Whirl-pak bag containing a completely filled-out sample label, including the volume that was filtered (i.e., 25 mL or otherwise). As with the chlorophyll *a*, purge as much of the air out of each bag as possible, "whirl" it shut, and seal tightly with the wire tabs. Shove the sample packet down into the ice in the cooler.

Keep AFDM filters as cold as possible until the samples can be frozen back at the lab that evening, or place on dry ice until they can be stored in the lab freezer. The holding time for the AFDM samples is 28 days from collection, when kept frozen.

5.4 PROCEDURE FOR COLLECTING QUALITATIVE ALGAL SAMPLES

If your program calls for the collection of soft-bodied taxonomic ID samples, then you will also need to collect a "qualitative" sample at every monitoring reach. The qualitative samples consist of a composite of all types of soft-bodied algae visible within the reach. This is of value because it can provide a fairly exhaustive list of soft-bodied algal taxa present at the site and can also aid identification of taxa captured in the RWB sampling, since it allows larger, more intact specimens to be collected than those that may end up in the more heavily processed quantitative sample (described above). In addition, if the qualitative sample is kept cool and in the dark, and is delivered to the lab in a timely manner (i.e., as quickly as possible, and within two weeks of collection), the live specimens can be cultured, which can also aid in identification. For example, some taxa in the Zygnematales cannot be identified to species level unless they are in a sexual phase during examination. If asexual at the time of collection (which is the typical situation), live specimens could be induced to a sexual phase in the lab. Collection of a qualitative diatom sample is optional, and is typically not needed for general bioassessment purposes.

For qualitative soft-bodied algal samples, collect specimens of all obviously different types of macroalgal filaments and mats, as well as microalgae (in the forms of scrapings using a razor blade or knife), and depositional samples (suctioned from along the surface of sediments using a clean turkey baster). Note that some algae (e.g., species of *Chara*, *Paralemanea*, and *Vaucheria*) have morphologies similar to submerged macrophytes or mosses. In addition, algae are not always green, and may instead be dark-brownish, golden, reddish, or bluish-green. Some cyanobacteria (which should also be collected for the qualitative sample), such as members of the genus *Nostoc*, look like gelatinous globules, or sacs, ranging in size anywhere from smaller than a pea to larger than a lime. Online image searches of these taxa and others will help the collector develop an eye for the variety of types of algae that may be encountered in streams. If you suspect something may be algae, but are not sure, it is always preferable to collect some of it for the qualitative sample. The laboratory will determine whether it qualifies for inclusion in the species list. Collect from as many distinct locations as possible throughout the reach so as to capture as much of the apparent diversity in the reach as you can. Also, when possible, try to grab part of the holdfast structures that attached the macroalgae to the substrate, as these structures can be useful for taxonomic identification.

Since these samples are merely qualitative, and not quantitative, you need not worry about collecting them in a manner that is representative of their relative abundances within the reach. *Note: If there is only a small amount of macroalgae in the stream, it should be allocated preferentially to the soft-bodied algae laboratory sample, as opposed to the diatoms (if a diatom qualitative sample is being collected), because it is primarily needed for the soft-bodied algal identification work (although diatoms can live as epiphytes on macroalgae, so macroalgal samples are also of values for the diatom work).*

Using a thick, waterproof marker, label a Whirl-pak bag with the Site Code, Date, Sample ID, and "soft" (or "diatom", if also collecting a diatom sample). Fill the bag with a total volume of up to 100 mL of qualitative algae sample + water. Purge any extra air from the bag, seal with the wire tabs by twisting them together (not just folding them, as this can result in leakage), tuck the ends of the wire tabs inward so that they cannot poke other bags, and store in the cooler on wet ice in the field. Be careful not to place the bags right up against ice or frozen blue-ice bags, because this could cause the algae to freeze and thus destroy the sample. Unlike with the quantitative samples, **do not add glutaraldehyde or formalin** (or any other fixative) to these qualitative samples. Keep the qualitative samples on wet ice and refrigerate immediately upon return to the lab. Because they are not preserved, these samples should be examined by a taxonomist as soon as possible (and within two weeks, at most), as they can decompose fairly rapidly. Decomposition is of particular concern for the soft-bodied algae sample.

If it is impossible to get the soft-bodied qualitative samples to a taxonomist within two weeks of sample collection, then split the qualitative samples in half, transfer one half to a 50 mL centrifuge tube and preserve it with glutaraldehyde (to a 2.5% final concentration) and leave the other half un-fixed (but continue to store in the cold/dark until examination by a taxonomist). This should be done in order to preserve part of the sample for morphological identification, but still maintain some possibility of keeping some specimens alive, in case culturing is necessary. *Note: Glutaraldehyde is a hazardous substance that can*

pose health and safety risks. Add glutaraldehyde in a fume hood, wearing safety goggles and glutaraldehyde-safe gloves. Refer to Appendix D for more detailed instructions on the safe handling of glutaraldehyde.

5.5 ALGAL SAMPLING QUALITY ASSURANCE / QUALITY CONTROL

The SWAMP bioassessment group is currently developing guidelines for quality assurance and quality control for bioassessment procedures. Future revisions to this document will include more specific information covering personnel qualifications, training and field audit procedures, procedures for field calibration, procedures for chain of custody documentation, requirements for measurement precision, health and safety warnings, cautions (actions that would result in instrument damage or compromised samples), and interferences (consequences of not following the SOP).

It is recommended that duplicate sampling of algae occur at 10% of study sites. The recommended method for collecting duplicates is at adjacent positions along the sampling transect according to the scheme depicted in Figure 2. Both samples should be collected at each transect before moving on to the next transect. When duplicate samples are collected at a site, the full suite of information about the algae samples (composite volume, numbers of each sampling device used, amount filtered, etc.) will need to be recorded for each replicate. This information can be recorded on a duplicate copy of the "Algae Samples" field sheet. Alternatively, the data cells on this sheet can be divided in half to accommodate information for each replicate. If the latter, it is important to keep track of which values go with which replicate.

In addition to including composite volume, area sampled (total, for all sampling devices used), and amount filtered (for the biomass samples) on the sample labels and field sheets, this information should also be included on the chain-of-custody sheets that are submitted to the algae analytical and taxonomy laboratories. This will facilitate efficient calculation of several types of data output, because this information is needed both for the biomass results and for the soft-bodied algae biovolume results.

6 PHYSICAL HABITAT TRANSECT-BASED MEASUREMENTS TO ACCOMPANY ALGAL BIOASSESSMENT

Once all algae samples have been collected at a given transect, PHab data collection can begin there. PHab data are designed to assess the physical habitat conditions of the stream reach being sampled. Knowledge about the PHab parameters can aid interpretation of the biotic assemblage data collected. Data for the following PHab parameters will be entered on transect-specific datasheets (corresponding to each of the 11 main transects along the monitoring reach). These datasheets are provided on the SWAMP website.

It should be noted that the data collection procedures for the parameters below reflect those that are described in the SWAMP BMI Bioassessment SOP (Ode 2007). With respect to PHab assessment, the only deviation between this SOP and that of Ode (2007) is in terms of omission of certain parameters. However, where there is overlap in parameters between the two SOPs, they are assessed in exactly the same manner. The one exception to this is the addition, in this SOP, of percent algal cover determination to the pebble count as described in Ode (2007). Also, note that because the datasheets are multi-purpose datasheets, developed for both BMIs and algae, they include some PHab parameters that are not a part of this SOP. Specifically, the following PHab data that appear on the datasheets are not collected when only algae are being sampled: 1) Riparian vegetation, and 2) Instream habitat complexity. As such, these sections are not filled out on the datasheets when only algae samples are being collected.

6.1 WETTED WIDTH

The wetted channel is the zone that is inundated with water and the wetted width is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Measure the wetted stream width and record this in the box at the top of the Transect data form.

6.2 BANKFULL WIDTH

The bankfull channel is the zone of maximum water inundation in a normal flow year (one-to-two year flood events). Since most channel formation processes are believed to act when flows are within this zone, bankfull dimensions provide a valuable indication of relative size of the waterbody.

Scout along the stream margins to identify the location of the bankfull margins on either bank by looking for evidence of annual or semi-annual flood events. Examples of useful evidence include topographic, vegetative, or geologic cues (changes in bank slope, changes from annual to perennial vegetation, changes in the size distribution of surface sediments). While the position of drift material caught in vegetation may be

a helpful aid, this can lead to very misleading measurements. *Note: The exact nature of this evidence varies widely across a range of stream types and geomorphic characteristics. It is helpful to investigate the entire reach when attempting to interpret this evidence because the true bankfull margin may be obscured at various points along the reach. Often the bankfull position is easier to interpret from one bank than the other; in these cases, it is easiest to infer the opposite bank position by projecting across the channel. Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (these heights should be equal).*

Stretch a tape from bank to bank at the bankfull position. Measure the width of the bankfull channel from bank to bank at bankfull height and perpendicular to the direction of stream flow.

6.3 BANKFULL HEIGHT

Measure bankfull height (the vertical distance between the **water surface** and the height of the bank, Figure 9) and record in the boxes at the top of the Transect data form under "Bankfull Width" and "Bankfull Height".

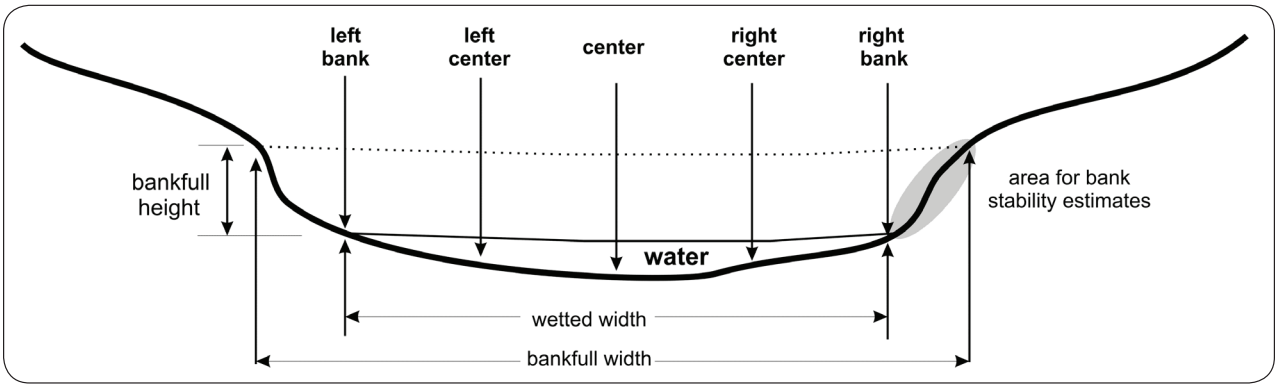

Figure 9. Cross sectional diagram of a typical stream channel showing locations of substrate measurements, wetted and bankfull width measurements, and bank stability visual estimates (reprinted from Ode 2007).

6.4 "PEBBLE COUNT": TRANSECT SUBSTRATES

Particle size frequency distributions often provide valuable information about instream habitat conditions that affect benthic communities. The Wolman pebble count technique is a widely used and cost-effective method for estimating the particle size distribution and produces data that correlate with costly, but more quantitative bulk sediment samples. Coarse particulate organic matter (CPOM, particles of organic material such as leaves that are greater than 1.0 mm in diameter) is a general indicator of the amount of

allochthonous organic matter available at a site, and its measurement can provide valuable information about the basis of the food web in a stream reach. The presence of CPOM associated with each particle is quantified at the same time that particles are measured for the pebble counts.

Transect substrate measurements are taken at five equidistant points along each transect (Figure 9). Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (e.g., a stadia rod or measuring tape). Once the positions are identified, lower a folding meter stick through the water column perpendicular to both the flow and the transect to identify the particle located at the tip of the meter stick. *Note: It is important that you are not subjective about selecting a particle, as this will result in failing to generate an accurate assessment of the size class distribution of particles present in that stream reach.*

6.5 DEPTH

With the folding meter stick, measure the depth from the water surface to the top of the particle to the nearest cm and record on the datasheet.

Table 3
Particle size class codes, descriptions, and measurements (adapted from Ode 2007)

Size Class	Code Size Class Description	Common Size Reference	Size Class Range
RS	bedrock, smooth	larger than a car	> 4 m
RR	bedrock, rough	larger than a car	> 4 m
XB	boulder, large	meter stick to car	1 - 4 m
SB	boulder, small	basketball to meter stick	25 cm - 1.0 m
CB	cobble	tennis ball to basketball	64 - 250 mm
GC	gravel, coarse	marble to tennis ball	16 - 64 mm
GF	gravel, fine	ladybug to marble	2 – 16 mm
SA	sand	gritty to ladybug	0.06 – 2 mm
FN	fines	not gritty	< 0.06 mm
HP	hardpan (consolidated fines)		< 0.06 mm
WD	wood		
RC	concrete/ asphalt		
OT	other		

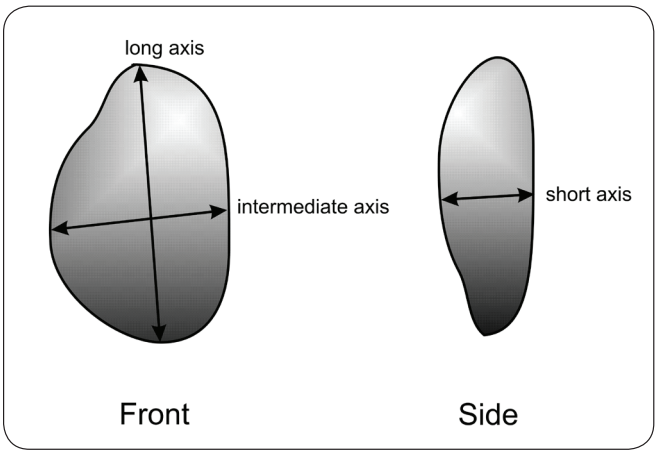

Figure 10. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts (reprinted from Ode 2007).

6.6 PARTICLE SIZE CLASS

Remove the particle from the streambed. Assign the particle to one of the size classes listed in Table 3 (these are also provided in a box on the transect form), based on its intermediate axis length (Figure 10). Record this information under Substrate size class.

Be sure to use measurements or the established codes for particle size class. If the latter, confirm the 2-letter codes for the particles as you call them out to your partner recording the data to ensure you are using the correct codes.

6.7 COBBLE EMBEDDEDNESS

It is generally agreed that the degree to which fine particles fill interstitial spaces has a significant impact on the ecology of benthic organisms and fish, but techniques for measuring this impact vary greatly. Here we define embeddedness as the volume of cobble-sized particles (64-250 mm) that is buried by fine and sand particles (< 2.0 mm diameter).

When a cobble-sized particle is encountered during the pebble count, visually estimate the percentage of the cobble's volume that has been buried by fine/sand particles (this will likely require removing the cobble from the streambed). Record, to the nearest 5%, the embeddedness of up to 25 cobble-sized particles within the sampling reach in the corresponding "% Cobble Embed" field for each cobble.

If 25 cobbles are not encountered during the pebble count, supplement the cobbles by conducting a "random walk" through the reach. Starting at a random point in the reach, follow a line from one bank to the other at a randomly chosen angle. Once at the other bank reverse the process with a new randomly chosen angle. Enter any of these additional embeddedness values at the bottom of the first page of the set of field forms, under "ADDITIONAL COBBLE EMBEDDEDNESS MEASURES".

If 25 cobble sized particles are not present in the entire reach, then record the values for however many cobbles are present.

6.8 CPOM

Record the presence or absence of Coarse Particulate Organic Matter (CPOM) that is > 1 mm diameter, and within 1 cm of the particle.

6.9 ALGAL COVER

Algal cover refers to the amount of algae in the stream reach, both in terms of 1) microalgal coatings ("slimy-ness") on stream substrates and 2) macroalgae (e.g., filaments, mats, globules). Algal cover is estimated by a point-intercept approach that entails collecting information about the presence/absence of both types of algae (as well as thickness, for the microalgae) at each of the points along the transects associated with the pebble count. If the imaginary point corresponding to each pebble in the pebble count intercepts algae, then algae is recorded as "present" at that point. The percentage of the points across the sampling reach that have algae present yields an estimate of the percent algal cover.

For each point along the pebble count, record information about algae as follows. For any film-like coating of algae (referred to as "Microalgae" on the datasheet) present on the surface of the substrate at that point, estimate the presence/thickness category according to the scheme in Table 4. For thicker microalgal layers, a small metal or plastic rod with demarcations at 1, 5, and 20 mm can be used for measurement. For layers too thin to measure, use the diagnostic criteria listed in the last column of Table 4. Note that these thickness codes refer only to microalgal coatings/films on substrate. They do not refer to thickness of macroalgal filaments/mats; macroalgal thickness is not assessed in this protocol. Be sure to collect microalgal thickness data from whatever substrate is topmost within the stream and therefore is most likely to be exposed to sunlight. Sometimes this substrate is not the actual pebble used in the pebble count, but rather a substrate type that occurs above the pebble, such as a thick mat of macroalgae that is above (and covering) the stream bottom. Microalgal species (which can include diatoms and unicellular soft-bodied algae) can grow as "epiphytes" upon macroalgal filaments and mats, coating them with a slimy, tinted film.

Table 4
Microalgal thickness codes and descriptions (adapted from Stevenson and Rollins 2006).

Code	Thickness	Diagnostics
0	No microalgae present	The surface of the substrate feels rough, not slimy.
1	Present, but not visible	The surface of the substrate feels slimy, but the microalgal layer is too thin to be visible.
2	<1mm	Rubbing fingers on the substrate surface produces a brownish tint on them, and scraping the substrate leaves a visible trail, but the microalgal layer is too thin to measure.
3	1-5mm	
4	5-20mm	
5	> 20mm	
UD	Cannot determine if a microalgal layer is present	
D	Dry point	

Note: Sometimes, due to the nature of the substrate, it can be difficult to discern whether a microalgal layer is present (particularly if it is very thin). For example, in the case of very fine sediments, the dark color of the silt can obscure the diagnostic color of a microalgal layer, and the inherent "sliminess" of very fine silt may make tactile determination of microalgae impossible. Therefore, when silt is the substrate, only relatively thick layers of microalgae might be easily discernible. If presence/absence of a microalgal layer cannot be determined with certainty, score microalgal thickness as "UD".

In addition to recording the presence and thickness of microalgae on the surfaces of substrates, record the presence/absence of attached macroalgae in the water column, as well as unattached, floating macroalgal mats on the water's surface, corresponding to each pebble count sampling point. Do this by envisioning an imaginary "line" extending from the water's surface down to the stream bottom where the target "pebble" lies (particularly in turbulent water, it may be helpful to use a viewing bucket (Appendix C) in order to see below the water's surface; the use of the viewing bucket is optional). If this line intercepts macroalgae, either floating on the water's surface, or somewhere within the water column, the appropriate algal class(es) should be recorded as "present". Attached macroalgal filaments have an obvious physical connection to something (like a cobble, boulder, or a gravel bed) lying on the bottom of the stream, whereas for unattached macroalgae, there is no obvious physical connection with the streambed, and the algae is just freely floating at or near the water's surface. For each class of macroalgae (Attached and Unattached), mark "P" (for "present") if intercepted by the sampling point and "A" (for "absent") if not intercepted.

Bear in mind that, because pebble counts span the "wetted width" of each transect, the expectation is that even the pebbles at the bank positions will generally be at least moist, and sometimes even submerged. As such, it is important to realize that algal cover can occur at the bank positions of the pebble count as well as intermediate positions across the stream. An exception to this is when the pebble surface is completely dry. Section 6.11 provides instructions for data collection in this situation.

6.10 MACROPHYTES

If a vascular plant (i.e., a macrophyte) is intercepted by the imaginary line associated with the pebble count point, mark "P" for "present" under Macrophytes. Otherwise, mark "A" for absent. Include only herbaceous plants that are rooted underwater. Examples of macrophytes include cattails, tules, rushes, sedges, monkeyflowers, speedwells, knotweeds, and watercress.

6.11 DRY SUBSTRATES

To determine how to collect data at dry sampling points, it is necessary to first establish whether the dry area in question lies within the stream's active channel (i.e., therefore regularly inundated during storms), or whether the point is on a stable island (i.e., therefore rarely, if ever, inundated). Stable islands are typically

vegetated, often with woody shrubs or trees, and have heights near or exceeding bankfull height. Pebble counts should not be conducted on stable islands. If the transect spans a portion of the study reach in which the channel is bifurcated such that there are two channels with an intervening island, the entire transect should be placed across the dominant channel, and all five pebble count points should be located on that side.

If the point falls on a dry surface that is within the usual active channel (i.e., subject to regular disturbance by flows), then pebble count/algal cover data from the dry point should be recorded as follows:

- score Depth as 0
- score particle Size Class and Embeddedness as described above for wet particles
- score all the algae variables (Microalgae, Macroalgae Attached, and Macroalgae Unattached) as "D" for "dry"
- leave CPOM and Macrophytes "blank" (i.e., do not circle anything). These parameters will register as NR (Not Recorded) in the database.

Ordinarily, the sampling transect would span the wetted width of the channel, but when no water is present at a given transect, evidence of the typical wetted extent of the active channel will need to be used to infer appropriate transect boundaries. Such indicators can include the transition from vegetated to unvegetated area (i.e., moving from banks to active channel), as well as the presence of dried algae, water stains, microtopographic transitions, changes in substrate composition, and others.

6.12 BANK STABILITY

The vulnerability of stream banks to erosion is often of interest in bioassessments because of its direct relationship with sedimentation.

For each transect, record a visual assessment of bank vulnerability in the region between the wetted width and bankfull width of the stream margins and between the upstream and downstream inter-transects. Choose one of three vulnerability states: eroded (evidence of mass wasting), vulnerable (obvious signs of bank erosion or unprotected banks), or stable.

6.13 HUMAN INFLUENCE

For the left and right banks, estimate a 10 x 10 m riparian area centered on the edges of the transect (see Figure 11). In the "Human Influence" section of the Transect data sheet, record the presence of 14 human influence categories in three spatial zones relative to this 10 x 10 m square (between the wetted edge and bankfull margin, between the bankfull margin and 10 m from the stream, and between 10 m and 50 m beyond the stream margins): 1) walls/rip-rap/dams, 2) buildings, 3) pavement/cleared lots, 4) roads/

railroads, 5) pipes (inlets or outlets), 6) landfills or trash, 7) parks or lawns (e.g., golf courses), 8) row crops, 9) pasture/ rangelands, 10) logging/ timber harvest activities, 11) mining activities, 12) vegetative management (herbicides, brush removal, mowing), 13) bridges/ abutments, 14) orchards or vineyards. Circle all combinations of impacts and locations that apply, but be careful to not double-count any human influence observations.

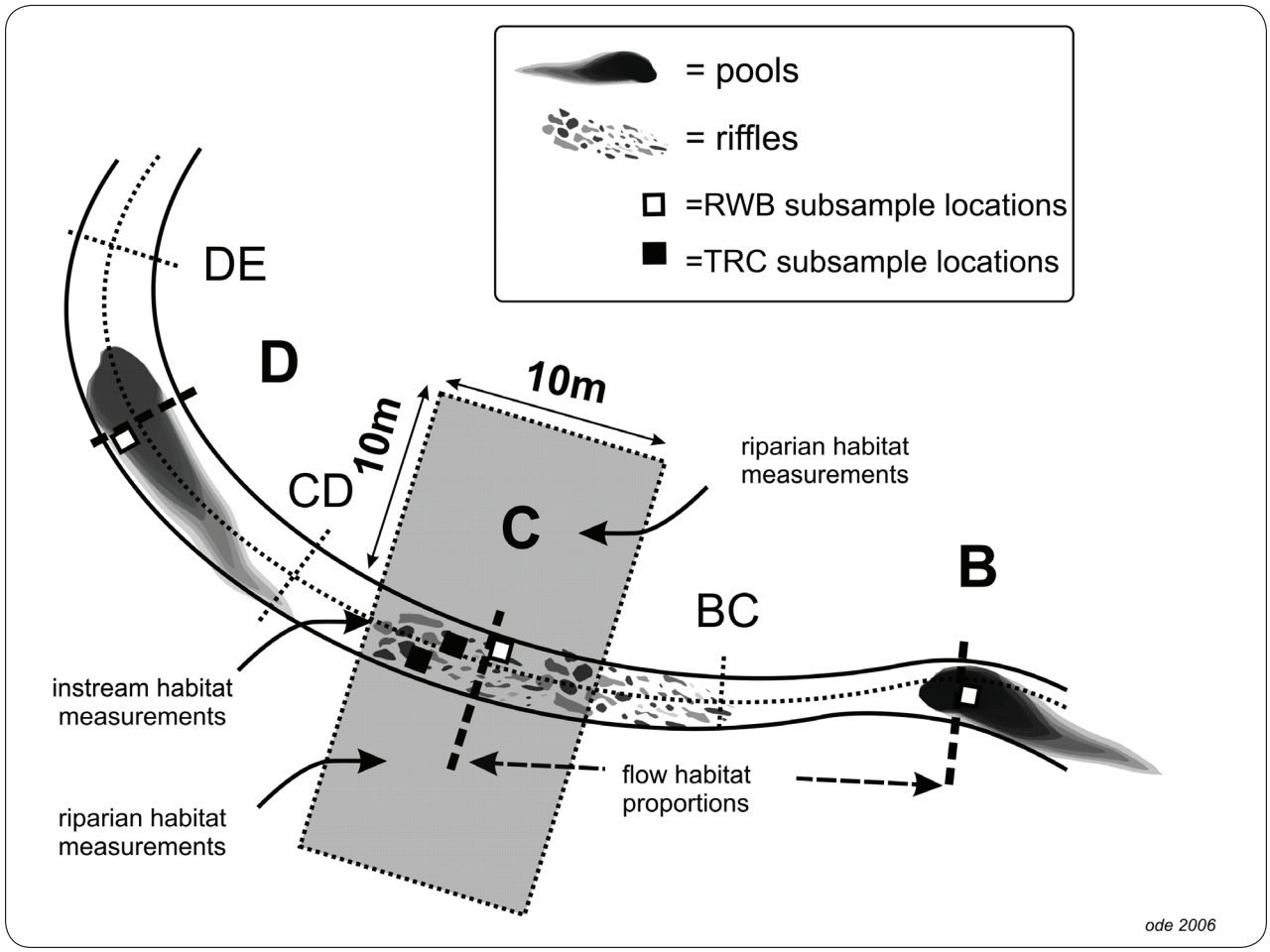

Figure 11. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting algae samples (the white square, labeled "RWB" in the legend box) and flow habitat proportion measurements (reprinted from Ode 2007).

Record the presence of any of the 14 human influence categories in the stream channel within a zone 5 m upstream and 5 m downstream of the transect.

6.14 DENSIOMETER READINGS (CANOPY COVER)

The densiometer is read by counting the number of line intersections that are obscured by overhanging vegetation. Before using, the densiometer should be modified by taping off the lower left and right portions of the mirror in order to emphasize overhead vegetation over foreground vegetation (the main source of bias in canopy density measurements; see Figure 12.)

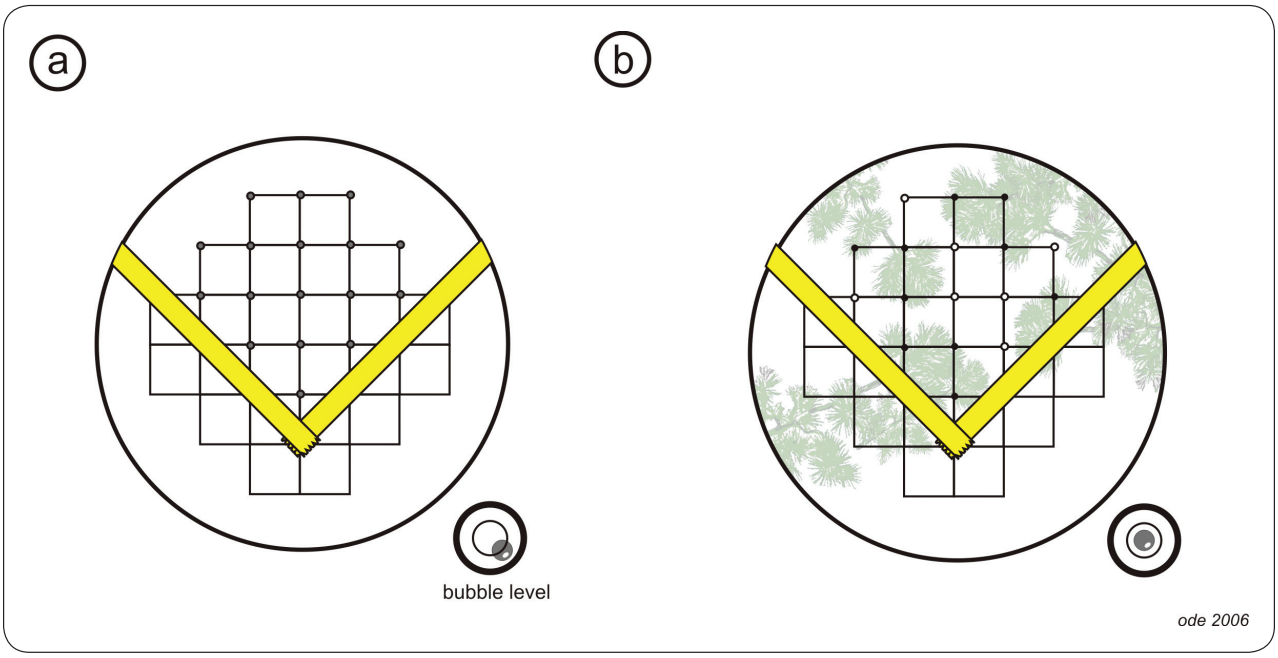

Figure 12. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 10 covered intersection points out of 17 possible. Note the position of the bubble level in (b) when the densiometer is leveled (reprinted from Ode 2007).

All densiometer readings should be taken with the bubble leveled, and 0.3 m (1 ft) above the water surface. The densiometer should be held just far enough from the squatting observer's body so that his/her forehead is just barely obscured by the intersection of the two pieces of tape.

Take and record four 17-point readings from the center of each transect: a) facing upstream, b) facing downstream, c) facing the left bank, d) facing the right bank. Optional readings can also be taken at the left and right banks (facing away from the stream, for these positions).

7 PHYSICAL HABITAT INTER-TRANSECT-BASED MEASUREMENTS

While most measures are taken at or relative to the main transects, a few measures are recorded at transects located at the midpoint between main transects. These are called "Inter-transects". The following measurements are taken relative to the Inter-transects: 1) Wetted Width, 2) Flow Habitats, and 3) "Pebble Count": Transect Substrates (including algal cover, as for the main transects).

7.1 INTER-TRANSECT WETTED WIDTH

Measure the same way that Transect wetted width was measured.

7.2 INTER-TRANSECT SUBSTRATES AND PERCENT ALGAL COVER

Collect these data the same way that Transect substrates and percent algal cover data were collected.

7.3 FLOW HABITATS

Because many benthic organisms prefer specific flow and substrate microhabitats, the proportional representation of these habitats in a reach is often of interest in bioassessments. There are many different ways to quantify the proportions of different flow habitats. This procedure produces a semi-quantitative measure consisting of 10 transect-based visual estimates.

At each Inter-transect, identify the percentage of six different habitat types in the region between the upstream Transect and downstream Transect: 1) cascades, 2) falls, 3) rapids, 4) riffles, 5) runs, 6) glides, 7) pools, and 8) dry areas. Record percentages to the nearest 5% — the total percentage of surface area for each section must equal 100%.

A description of each of these flow habitat types is provided below:

- cascades: short, high-gradient drops in stream bed elevation often accompanied by boulders and considerable turbulence
- falls: high-gradient drops in elevation of the stream bed associated with an abrupt change in the bedrock
- rapids: sections of stream with swiftly flowing water and considerable surface turbulence (rapids tend to have larger substrate sizes than riffles)
- riffles: "shallow/fast"; riffles are shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence (< 0.5 m deep, > 0.3 m/s)
- runs: "deep/fast"; long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool (> 0.5 m deep, > 0.3 m/s)
- glides: "shallow/slow"; sections of stream with little or no turbulence, but faster velocity than pools (< 0.5 m deep, < 0.3 m/s)
- pools: "deep/slow"; a reach of stream that is characterized by deep, low-velocity water and a smooth surface (> 0.5 m deep, < 0.3 m/s)
- dry: any surface area within the channel's wetted width that is above water

After you have collected all the above Transect-, and Inter-transect-, based measurements, collect data on Gradient. Also, if you have not already done so, take photographs at specific Transects, as indicated below. After you have collected Gradient data at each Transect, and have taken photographs where indicated, remove the corresponding flag from the stream bank.

7.4 PHOTOGRAPHS

Take a minimum of four (4) photographs of the reach at the following locations: a) Transect A facing upstream, b) Transect F facing upstream, c) Transect F facing downstream, and d) Transect K facing downstream. It is also desirable to take a photograph at Transect A facing downstream and Transect K facing upstream to document conditions immediately adjacent to the reach. Digital photographs should be used. Record the image numbers on the front page of the field form under "Photographs". *Note: An easy way to keep track of which site each series of photographs belongs to is to take a close-up of the front data sheet (containing legible site code and date) for that site prior to taking the series of photos.*

SECTION 8
REACHWIDE MEASUREMENTS

This last section describes PHab measurements of attributes specific to the stream reach as a whole. These include gradient of the reach and stream discharge.

8.1 GRADIENT

The gradient of a stream reach is one of the major stream classification variables, giving an indication of potential water velocities and stream power, which are in turn important controls on aquatic habitat and sediment transport within the reach. The data collected for gradient are recorded on the "Slope and Bearing" form.

Note: An autolevel should be used for reaches with a percent slope of less than or equal to 1%. Either a clinometer or an autolevel may be used for reaches with a percent slope of greater than 1%, and sometimes a clinometer is preferable in really steep areas that are also heavily vegetated. The following description is for clinometer-based slope measurements. In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff. In these cases, default to use of an autolevel, which is described further below.

Clinometer method:

Transect to transect measurements taken with a clinometer are used to calculate the average slope through a reach. This measurement works best with two people, one taking the readings at the upstream transect ("backsighting") and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section). Otherwise, leave these blank.

Beginning with the upper transect (Transect K), one person (the measurer) should stand at the water margin with a clinometer held at eye level. A second person should stand at the margin of the next downstream transect (Transect J) with a stadia rod flagged at the eye level of the person taking the clinometer readings. Be sure you mark your eye level while standing on level ground! Adjust for water depth by measuring from the same height above the water surface at both transects. This is most easily accomplished by holding the base of the stadia rod at water level. *Note: An alternative technique is to use two stadia rods pre-flagged at the eye-height of the person taking the readings.*

Use a clinometer to measure the percent slope of the water surface (not the streambed) between the upstream transect and the downstream transect by sighting to the flagged position on the stadia rod. The clinometer reads both percent slope and degree of the slope. Be careful to read and record percent slope

rather than degrees slope (the measurements differ by a factor of ~2.2). Percent slope is the scale on the right hand side as you look through most clinometers. *Note: If an autolevel or hand level is used, record the elevation difference (rise) between transects and the segment length (run) instead of the percent slope (autolevel instructions are provided in the following section).*

If the stream reach geometry makes it difficult to sight a line between transects, divide the distance into two sections and record the slope and the proportion of the total segment length between transects for each of these sections in the appropriate boxes on the slope form (supplemental segments). Do not measure slope across dry land (e.g., across a meander bend).

Proceed downstream to the next transect pair (I-J) and continue to record slope between each pair of transects until measurements have been recorded for all transects. If you have finished all the other transect and inter-transect based measurements for PHab, you may remove the transect flags as you go.

Autolevel method (preferred):

To measure gradient using an autolevel, identify a good spot to set up the autolevel, preferably somewhere around the center of the reach (if there is good visibility from this location to both the upstream and downstream ends of the reach.) Set up the autolevel on very stable, and preferably fairly flat, ground. Set the height of the autolevel to comfortable eye level for the operator. Level the plane of view of the autolevel by balancing it using the bubble. Start by adjusting the legs, and then fine-tune the adjustment using the knobs. Once balanced, begin "shooting" the change in the height of the water level of the stream from transect to transect. Try to start with one of the outer transects (like A). Have a field partner at Transect A hold the Stadia rod at water's edge and perpendicular to the ground. Viewing through the autolevel (and focusing as necessary), look at the Stadia rod and note to the smallest demarcation on the stadia rod the height at which the autolevel line of view (i.e., the middle line in the viewfinder) hits. Record this information, and then have the Stadia rod holder proceed to the next transect (e.g., Transect B), again holding the base of the Stadia rod at water's edge. Very carefully, rotate the head of the autolevel so that it points to the new Stadia rod location. *Note: Take care not to bump the autolevel out of its position, because if this happens, you will not be able to take a height measurement of Transect B's water surface relative to that of Transect A, to determine the slope between the two transects.*

If the autolevel is bumped out of position before all the measurements are done, or if there is a point along the reach at which there is no longer a clear line of sight from the autolevel to the Stadia rod positioned at the transect, at water's edge, a new location must be set up for the autolevel. In order to maintain a relationship between water heights of the various transects already measured, it will be necessary to "re-shoot" the height of the water at the last transect for which a valid measurement was attained. From there, assuming there is no more disturbance to the position of the autolevel, you can continue cycling through the remaining transects from the new position. On the Slope and Bearing Form corresponding to autolevel use, indicate when the autolevel's position has been changed. If it is necessary to move the autolevel at some point, the transect that was measured from the original and the new position will be listed twice on

the datasheet: once for the original position, and once for the new. Also indicate the distance between main transects (i.e., 15 m, 25 m or other). These pieces of information will later be used to determine the slopes between transects and for the reach as a whole.

8.2 STREAM DISCHARGE

Stream discharge is the volume of water that moves past a point in a given amount of time and is generally reported as cubic feet per second (cfs) or cubic meters per second (cms). Because discharge is directly related to water volume, discharge affects the concentration of nutrients, fine sediments and pollutants; and discharge measurements are critical for understanding impacts of disturbances such as impoundments, water withdrawals and water augmentation. Discharge is also closely related to many habitat characteristics including temperature regimes, physical habitat diversity, and habitat connectivity. As a direct result of these relationships, stream discharge is often also a strong predictor of biotic community composition. Since stream volume can vary significantly on many different temporal scales (diurnal, seasonal, inter-annually), it can also be very useful for understanding variation in stream condition.

It is preferable to take discharge measurements in sections where flow velocities are greater than 0.5 ft/s and most depths are greater than 15 cm, but slower velocities and shallower depths can be used. If flow volume is sufficient for a transect-based "velocity-area" discharge calculation, this is by far the preferred method. If flow volume is too low to permit this procedure or if your flow meter fails, use the "neutrally buoyant object/ timed flow" method.

8.2.1 Discharge: Velocity Area Method

The layout for discharge measurements under the velocity-area (VA) method is illustrated in Figure 13. Flow velocity should be measured with either a Swoffer Instruments propeller-type flow meter or a Marsh-McBirney inductive probe flow meter.

Select the best location in the reach for measuring discharge. To maximize the repeatability of the discharge measurement, choose a transect with the most uniform flow (select hydraulically smooth flow whenever possible) and simplest cross-sectional geometry. It is acceptable to move substrates or other obstacles to create a more uniform cross-section before beginning the discharge measurements.

Data for this parameter will be entered in the "Discharge Measurements" section of the datasheet with the basic site information at the top ("Reach Documentation"). Measure the wetted width of the discharge transect and divide this into 10 to 20 equal segments. The use of more segments gives a better discharge calculation, but is impractical in small channels. A minimum of 10 intervals should be used when stream width permits, but interval width should not be less than 15 cm.

At each interval, record the distance from the bank to the end of the interval. Using the top-setting rod that comes with the flow velocity meter, measure the median depth of the interval. Standing downstream of the

transect to avoid interfering with the flow, use the top-setting rod to set the probe of the flow meter at the midpoint of the interval, at 0.6 of the interval depth (this position generally approximates average velocity in the water column), and at right angles to the transect (facing upstream). See Figure 13 for positioning detail.

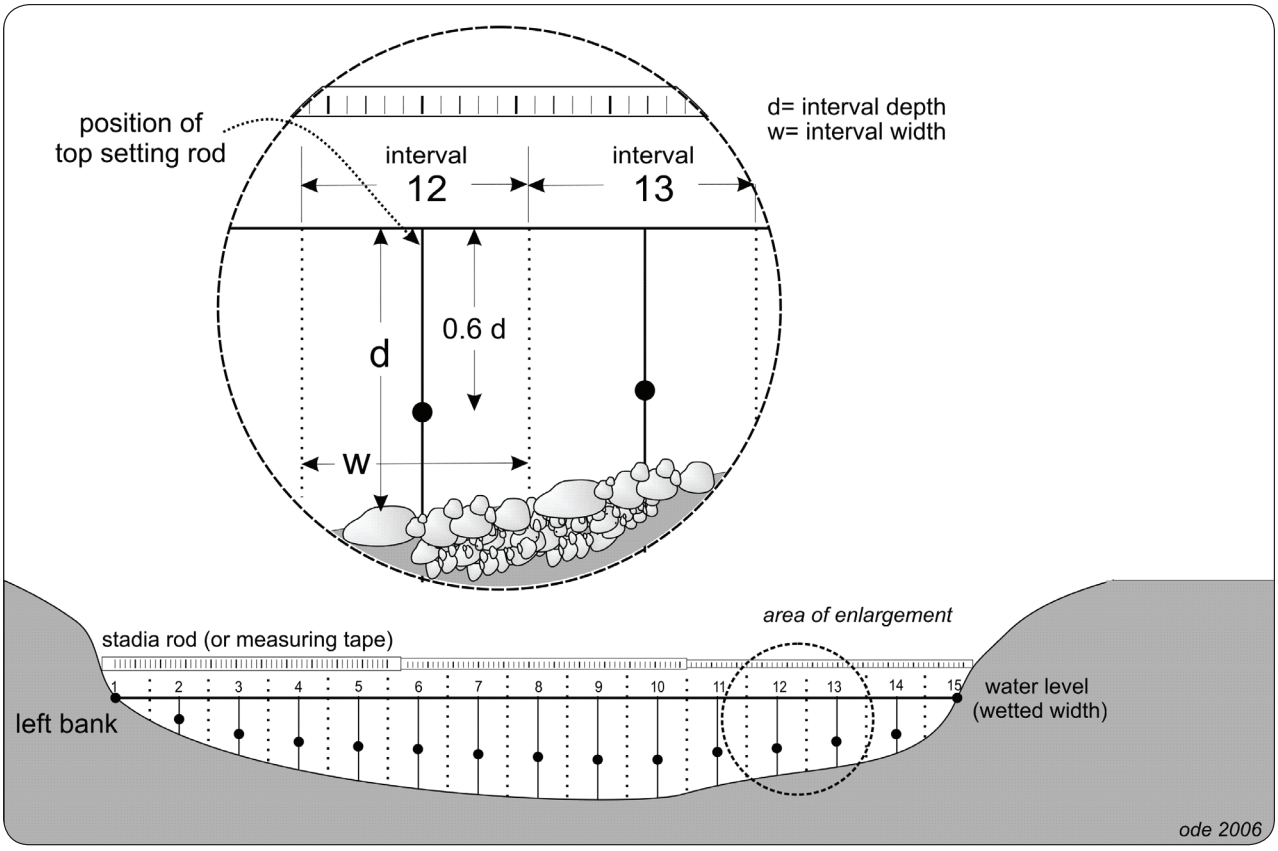

Figure 13. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots; reprinted from Ode 2007).

Allow the flow velocity meter to equilibrate for 10-20 seconds, then record velocity to the nearest ft/s. If the option is available, use the flow averaging setting on the flow meter. *Note 1: Under very low flow conditions, flow velocity meters may register readings of zero even when there is noticeable flow. In these situations, record a velocity of 0.5x the minimum flow detection capabilities of the instrument. Complete these steps on each of the intervals across the stream. Note 2: The first and last intervals usually have depths and velocities of zero.*

8.2.2 Discharge: Neutrally Buoyant Object Method

If streams are too shallow to use a flow velocity meter, the neutrally buoyant object (NBO) method should be used to measure flow velocity. However, since this method is less precise than the flow velocity meter

it should only be used if absolutely necessary. A neutrally buoyant object (one whose density allows it to just balance between sinking and floating) will act as if it were nearly weightless, thus its movement will approximate that of the water it floats in better than a light object. A piece of orange peel works well. To estimate the flow velocity through a reach, three transects are used to measure the cross-sectional areas within the test section sub-reach and three flow velocity estimates are used to measure average velocity through the test reach. To improve precision in velocity measurements, the reach segment should be long enough for the float time to last at least 10-15 seconds.

The position of the discharge sub-reach is not as critical as it is for the velocity-area method, but the same criteria for selection of a discharge reach apply to the neutrally buoyant object method. Identify a section that has relatively uniform flow and a uniform cross sectional shape.

The cross sectional area is estimated in a manner that is similar to, but less precise than, that used in the velocity area method. Measure the cross sectional area in one to three places in the section designated for the discharge measurement (three evenly-spaced cross sections are preferred, but one may be used if the cross section through the reach is very uniform). Record the width once for each cross section and measure depth at five equally-spaced positions along each transect.

Record the length of the discharge reach.

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GLOSSARY OF TERMS USED IN SOP G

Terms & Definitions	
TERM	**DEFINITION**
Aliquot	a measured portion of a sample, or subsample, or to measure a portion of a sample or subsample
Ash-free dry mass (AFDM)	the portion, by mass, of a dried sample that is represented by organic matter; the concentration of AFDM per stream surface area sampled can be used as a surrogate for algal biomass
Benthic algae	algae that are anchored to, or have at one point been anchored to, the stream bottom, in contrast to planktonic algae which are free-floating in the water column
Biofilm	a matrix/film adhering to stream substrata and consisting of microorganisms (e.g., algae, fungi, bacteria, protozoans) and detritus
Chlorophyll *a*	primary light receptor/photosynthetic pigment in algae and higher plants; the concentration of this pigment per stream surface area sampled provides an estimate of algal biomass
Composite sample	volume of all the liquid material amassed during sampling, including water used for rinsing substrate and sampling devices. Final composite volume should not exceed 400-500 ml.
Cyanobacteria	historically referred to as "blue-green" algae, but actually bacteria that are capable of photosynthesis and co-occur with true benthic algae in streams; useful as a bioindicator, and field-sampled and laboratory-processed concurrently with soft-bodied algae
Depositional	habitats in the stream that are dominated by slow-moving water, such as pools, where deposition of materials from the water column is more likely to occur than erosion (or (re)suspension) of loose bed materials; examples of "depositional" substrates include silt and sand
Diatom	a unicellular alga that possesses a rigid, silicified (silica-based) cell wall in the form of a "pill box"
Erosional	habitats in the stream that are dominated by fast-moving water, such as riffles, where stream power is more likely to facilitate erosion (suspension) of loose benthic material than deposition; examples of "erosional" substrates include cobbles and boulders
Homogenate	mixture of liquid composite sample and finely chopped fragments of macroalgae
Index of Biotic Integrity (IBI)	a quantitative assessment tool that uses information about the composition of one or more assemblages of organisms to make inferences about condition the environment they occupy (e.g., the assemblage of interest could be diatoms or benthic macroinvertebrates living in a stream)
Macroalgae	soft bodied algae that form macroscopically discernible filaments, mats, or globose structures
Microalgae	diatoms and microscopic soft-bodied algae, including unicellular forms; can co-occur with other microorganisms in a biofilm
Reachwide benthos (RWB)	method for biotic assemblage sample collection that does not target a specific substrate type, but rather objectively selects sampling locations across the reach, allowing for any of a number of substrate types to be represented in the resulting composite sample
Soft-bodied algae	non-diatom algal taxa; for the purposes of this SOP, cyanobacteria are subsumed under this assemblage
Wetted width	the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water

List of Supplies for Stream Algae Sampling and Associated Data Collection A

Table 5.
General Supplies and Ambient Water Chemistry Collection

Item	Quantity / Site	Specifications
Sampling SOP (this document)	1/person	
Equipment decontamination supplies		See Appendix B
Hip or chest waders, or wading boots/shoes (not felt-soled)	at least 1 pair/ person	
Digital camera	1	
Full set of datasheets printed on waterproof paper (e.g., Rite-in-the-Rain™)	1 full set (and spare set recommended)	
Fine-tipped and thick-tipped waterproof/alcohol-proof pens and markers; pencils	2-3 each	
Clipboard	2-3	
Site dossier containing site maps, aerials, etc.	1	Add a 150 m scale line to aerials adjacent to stream
Thomas Guide and regional maps	as needed	
Centigrade thermometer	1	
pH meter	1	
DO meter and spare membrane	1	
Conductivity meter	1	
Turbidimeter and vial(s) (optional)	1	
Field alkalinity meter or test kit (e.g., Hach)	1	
Water chemistry containers	as needed	
Calibration standards	1	
Spare batteries for meters	as needed	
First aid kit	1	

Table 6.
Algal Taxonomic and Biomass Sample Collection

Needed for[1]:	Item	Quantity / Site	Specifications
D, S, C, A	White dish tub, rectangular, plastic, 11.5 qt	1	Use white, not colored
D, S, C, A	Scrubbing brush or scouring pad to clean dish tub, etc.	1	
D, S, C, A	Composite sample receiving bottle with cap, 1 L, plastic	1	Fisher 02-912-038
D, S, C, A	Graduated cylinder, 500 mL and 25 mL, plastic	1 each	Fisher 03-007-42 & 03-007-39
D, S, C, A	Bottle brush to clean graduated cylinders, etc.	1 sm, 1 lg	
D, S, C, A	PVC delimiter, 12.6 cm^2 area	1	See Appendix C
D, S, C, A	Spatula (> 12.6 cm^2 surface area)	1	
D, S, C, A	Rubber delimiter, 12.6 cm^2 area	1	See Appendix C
D, S, C, A	Toothbrush, firm-bristled	1	
D, S, C, A	Syringe scrubber, 60 mL syringe, 5.3 cm^2 area	1	See Appendix C
D, S, C, A	White (non-pigmented) scrubbing-pad circles	11 per replicate	See Appendix C
D, S, C, A	Tally meter (optional)	1	Ben Meadows 9JB-102385
D, S, C, A	Scissors	1	
D, S, C, A	Wash bottles	2	Label bottles with "stream water", and "DI water"
D, S, C, A	Razor blades or Swiss army knife	1	
D, S, C, A	Sample labels (printed on waterproof paper)	4 per replicate	See Figure 6
D, S, C, A	Clear plastic tape, 5 cm wide	Length of ~20cm per replicate	
D, S, C, A	Ice chest with wet ice	1 (2 preferred if multiple sites to be sampled)	
D, S, C, A	Fisherman's vest (optional)	1	
D, S	Centrifuge tubes, 50 mL, plastic	2 per replicate	Cole Parmer 06344-27
D, S	Rack for 50 mL centrifuge tubes	1	
D	10% formalin solution buffered with borax	10 mL per replicate	See Appendix C
D	Formalin-resistant gloves	1 pair	
D	Safety goggles or face shield	1	
D	Small syringe or bulb pipette	1	

1. "D" = diatom sample, "S" = soft-bodied algal sample, "C" = chlorophyll a sample, "A" = ash-free dry mass sample

Needed for[1]:	Item	Quantity / Site	Specifications
D	Vermiculite packing material	as needed	
S	Turkey baster	1	
S (see note)	25% glutaraldehyde solution (to be dispensed in a laboratory fume hood, wearing appropriate safety gear)	5 mL per replicate	*Note: could be added by taxonomy lab, with prior notification*
S	Calculator	1	
S	Small metric ruler (waterproof)	1	
S	Small Ziploc bag	1	
S, C, A	Whirl-pak bag, 100 mL	3 per replicate	Cole Parmer 06498-00
C, A	Filter forceps	1	Fisher 0975350
C, A	Filtering chamber/tower, 47 mm, plastic	1	Hach 2254400
C, A	Hand vacuum pump	1	Fisher 13-874-612B
C, A	Aluminum foil	~100 cm^2 per replicate	
C, A	Deionized water	500 mL	
C, A	Dry ice (if not returning to lab immediately following the day's fieldwork)	10 lbs	
C	Glass fiber filter, 47 mm, 0.7 µm pore size	1 per replicate	Fisher 09804142H
C	Snapping Petri dish, 47 µm	1 per replicate	Fisher 08-757-105
A	Glass fiber filter, 47 mm, 0.7 µm pore size; foil-wrapped and pre-combusted for ash-free dry mass (AFDM)	1 per replicate	

1. "D" = diatom sample, "S" = soft-bodied algal sample, "C" = chlorophyll a sample, "A" = ash-free dry mass sample

Table 7.
Physical Habitat Data Collection

Item	Quantity / Site	Specifications
GPS receiver	1	
Transect tape; 150 m	1	
Lengths of rope (7.5 m and 12.5 m)	1 each	
Small metric folding ruler (waterproof)	1	
Digital watch	1	
Stadia rod	1	
Clinometer	1	
Autolevel and tripod	1	
Current velocity meter and top-setting rod	1	
Convex spherical densiometer	1	Taped to expose only 17 intersections of the grid (see Figure 12)
Transect flags	21 total	Two colors; label with main transect (11 ct.) and inter-transect (10 ct.) names
Algae viewing bucket (optional)	1	See Appendix C
Small/slender rod with 1, 5, and 20 mm marks	1	For measuring microalgal thickness
Rangefinder (optional)	1	
Fresh orange peel	1	

B Information Resources for Avoiding Introduction of Invasive Species and Pathogens into Streams

The following is an adaptation of an excerpt taken from an EMAP-based Quality Assurance Project Plan developed by the California Department of Fish and Game Aquatic Bioassessment Laboratory (2008).

Organisms of concern in the U.S. include, but may not be limited to, Eurasian watermilfoil (*Myriophyllum spicatum*), New Zealand mud snail (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*), *Myxobolus cerebralis* (the sporozoan parasite that causes salmonid whirling disease), and *Batrachochytrium dendrobatidis* (a chytrid fungus that threatens amphibian populations).

Field crews must be aware of regional species of concern, and take appropriate precautions to avoid transfer of these species. Crews should make every attempt to be apprised of the most up-to-date information regarding the emergence of new species of concern, as well as new advances in approaches to hygiene and decontamination to prevent the spread of all such organisms (e.g., Hosea and Finlayson, 2005; Schisler et al., 2008).

There are several online sources of information regarding invasive species, including information on cleaning and disinfecting gear:

Whirling Disease Foundation
www.whirling-disease.org

USDA Forest Service - Preventing Accidental Introductions of Freshwater Invasive Species
www.fs.fed.us/invasivespecies/documents/Aquatic_is_prevention.pdf

California Department of Fish and Game
www.dfg.ca.gov

U.S. Geological Survey Nonindigenous Aquatic Species: general information about freshwater invasive species
http://nas.er.usgs.gov

Protect Your Waters - Co-sponsored by the U.S. Fish and Wildlife Service
www.protectyourwaters.net/hitchhikers

The California State Water Resources Control Board Aquatic Invasive Species website

www.swrcb.ca.gov/water_issues/programs/swamp/ais

REFERENCES

Hosea, R.C. and B. Finlayson. 2005. Controlling the spread of New Zealand mudsnails of wading gear. California Department of Fish and Game, Office of Spill Prevention and Response, Administrative Report 2005-02, Sacramento.

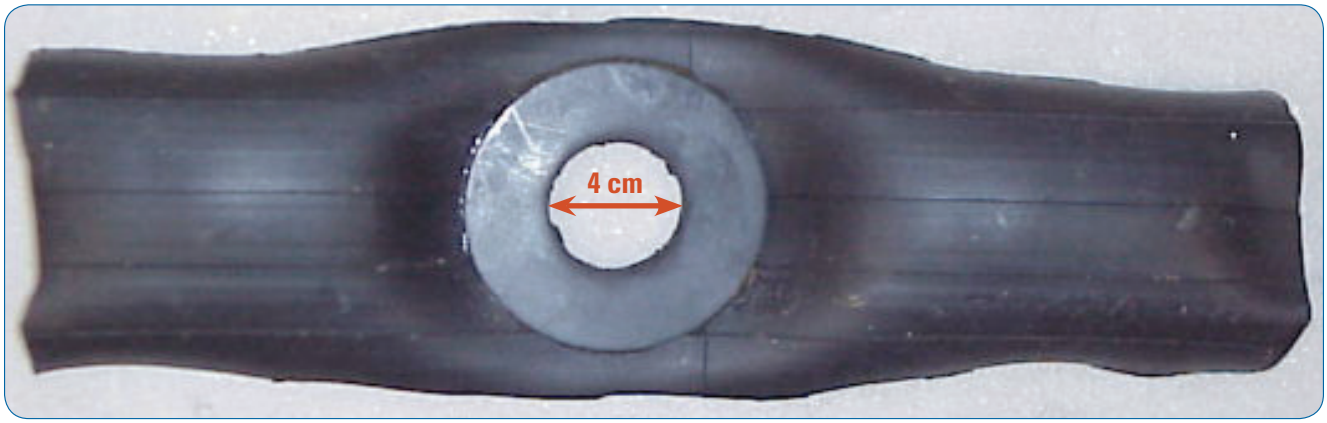
Schisler, G.J., N.K.M. Vieira, and P.G. Walker. 2008. Application of Household Disinfectants to Control New Zealand Mudsnails. North American Journal of Fisheries Management 28:1171-1176.

CONSTRUCTION OF ALGAE SAMPLING TOOLS C

This appendix provides step-by-step instructions for constructing the devices used for sampling algae. It also provides a recipe for the formalin fixative for diatoms.

1. RUBBER DELIMITER

The rubber delimiter for use on "erosional"/hard substrates like cobbles and wood is made from a sliced-open mountain bike inner tube that has a 4-cm diameter hole cut in the middle. The hole should be reinforced with a rubber gasket affixed to the tube with rubber cement.

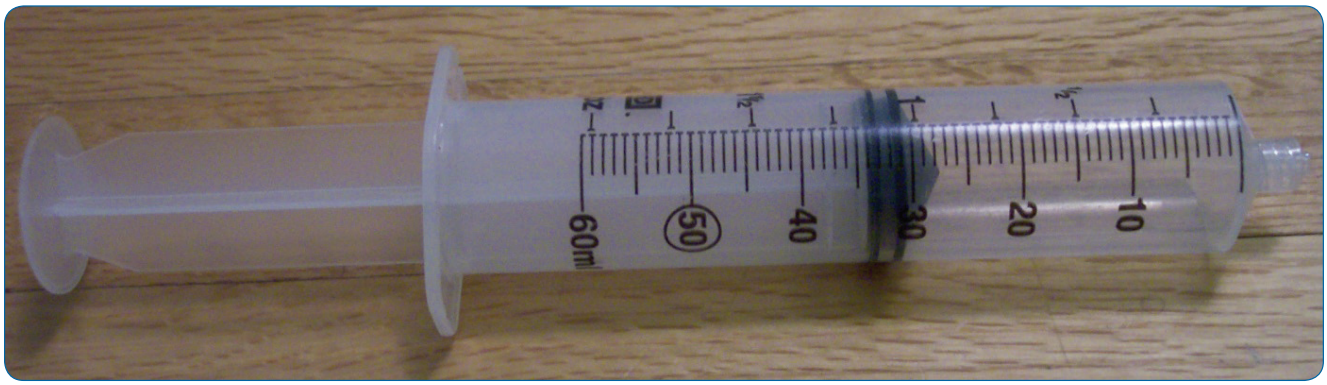


2. PVC DELIMITER

The PVC delimiter for "depositional"/soft substrates like sand, small gravel, and silt is made from a 1½" sewer cleanout, which can be found at a home-improvement or plumbing supply store. The hole in the bottom of the cleanout is 4 cm in diameter. The bottom edge of the cleanout is filed to make it sharp, to ease insertion into silt/sand. To facilitate consistent sampling, it is useful to paint a bright line indicating a depth of 1 cm around the outer surface of the bottom of the sampling device. This indicates the depth to which to insert the delimiter when sampling.

3. SYRINGE SCRUBBER

The syringe scrubber is for use on hard substrates that cannot be picked up out of the stream, like submerged bedrock and concrete channel bottoms. It is made from a 60 mL syringe barrel with the end cut off and its plunger fitted with Velcro-type material. Disposable, white (non-pigmented) scrubbing pads circles are then affixed to the end of the plunger and used to scrub the algae from the substrate.



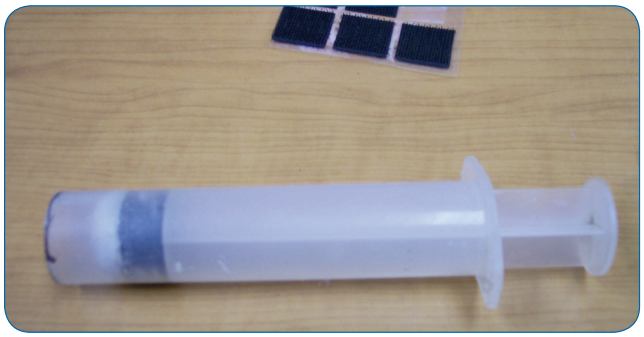
You will need a 60-mL plastic syringe for each sampler you want to make. Remove the plunger and saw the conical end off the plastic syringe, then sand the bottom so it is flat all the way around and fits tightly against a flat surface.

Firmly affix the rubber end to the plastic plunger by removing the rubber tip, applying glue to the "naked" end of the plunger, and replacing the rubber cap. Allow glue to cure. Then cut the conical part off the plunger tip so that only a flat surface of rubber remains.

Cut a circle of Velcro®-style hook material to fit the size of the plunger. Use a waterproof adhesive to affix the "Velcro®" circle to the end of the plunger.

Obtain some white scrubbing pad material (make sure it is not pigmented so it will not end up interfering with eventual chlorophyll *a* analysis of the samples collected.) Cut a supply of circles to fit the size of the plunger.

Before each sampling event, attach a fresh circular scrubbing pad to the end of the plunger. This is a head-on view of the plunger, with the scrubbing pad circle attached.



This is what the syringe sampler looks like when it is ready to be used.

4. VIEWING BUCKET (OPTIONAL)

A viewing bucket can be useful for visualizing submerged algae, particularly in instances of a turbulent stream surface that obscures the stream bottom. A viewing bucket can be constructed from a narrow cylinder of clear Plexiglas (approximately 8 inches in diameter) whose bottom is fitted with a circle of thick glass, and secured in place with a silicone seal. If desired, one or two handles can also be fashioned out of Plexiglas and attached to the side(s) of the cylinder. The use of the viewing bucket is optional.

5. PREPARING A 1-L SOLUTION OF 10-PERCENT BUFFERED FORMALIN (MOULTON ET AL. 2002)

1. Add 100 mL of formaldehyde (37-40%) to 900 mL of water in a chemically resistant, non-breakable bottle.
2. Add about 3 g of borax to 10 mL of water and mix.
3. Add dissolved borax solute, to buffer formalin solution.
4. Tightly seal the bottle and mix by carefully inverting the bottle several times.
5. Label the outside of the bottle with "10-percent buffered formalin," the date of preparation, and related hazardous chemical stickers.

REFERENCE

Moulton II, S.R., J.G. Kennen, R.M. Goldstein, and J.A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program, Open-File Report 02-150.

STANDARD OPERATING PROCEDURES (SOP) FOR USING GLUTARALDEHYDE FOR THE PRESERVATION OF SOFT ALGAE

D

(adapted from the Aquatic Bioassessment Laboratory, California Department of Fish and Game)

Note: Glutaraldehyde must only be handled by trained individuals who understand the safe handling and use of this chemical

1. SCOPE AND APPLICATION

Glutaraldehyde is a colorless liquid with a pungent odor used as a preservative and sterilant. This SOP covers the use of Glutaraldehyde by Department of Fish and Game OSPR laboratories as a preservative for soft bodied algae.

2. PHYSICAL HAZARDS

The physical hazards associated with the use of Glutaraldehyde include:

- Incompatibility with strong oxidizing substances and bases
- Corrosive to metals
- Production of Carbon Monoxide and Carbon Dioxide during decomposition
- Discolors on exposure to air

3. HEALTH HAZARDS

The health hazards associated with the use of Glutaraldehyde include;

Inhalation

- Regulatory limit of 0.05 ppm as a ceiling level
- Chemical burns to the respiratory tract
- Asthma and shortness of breath
- Headache, dizziness, and nausea

Skin

- Sensitization or allergic reactions, hives
- Irritations and burns
- Staining of the hands (brownish or tan)

Eyes

- Irritation and burns. Eye contact causes moderate to severe irritation, experienced as discomfort or pain, excessive blinking and tear production
- May cause permanent visual impairment
- Conjunctivitis and corneal damage

Ingestion

- Gastrointestinal tract burns; Central nervous system depression, excitement
- Nausea, vomiting
- Unconsciousness, coma, respiratory failure, death

Note: Oral toxicity of Glutaraldehyde ***increases*** *with dilution*

4. ENGINEERING CONTROLS

Strict engineering controls will be followed when using Glutaraldehyde. This chemical and processes using this chemical will only be used under a laboratory fume hood meeting the requirements of Title 8, CCR Section 5154.1. At no time will containers of Glutaraldehyde be opened outside of an operating fume hood. Personnel using Glutaraldehyde will designate an area of the lab for its use. The area where it is used will be noticed with a sign reading:

CAUTION GLUTARALDEHYDE IN USE

Only trained personnel will be allowed to enter the designated area when using Glutaraldehyde.

5. PERSONAL PROTECTIVE EQUIPMENT

Personal Protective Equipment (PPE) is required to be worn at all times when working with Glutaraldehyde. This includes:

Eye Protection

- Chemical splash goggles; or
- Safety glasses with face shield

Hand Protection

- Nitrile or Polyvinyl Chloride (vinyl) gloves

Body Protection

- Lab coat with polypropylene splash apron that cover the arms

Any PPE with noticeable contamination will be immediately removed and the affected area washed with water. Gloves and apron will be removed before leaving the designated area. Disposable PPE (gloves and aprons) will not be re-worn. Disposable PPE will be disposed of in a sealed waste receptacle approved for hazardous waste. Any non-disposable PPE (lab coats, chemical goggles) with noticeable contamination will be rinsed or cleaned as soon as practical, and secured in a manner that does not allow contamination of laboratory personnel. Respiratory protection will not be required as long as strict engineering controls are followed.

6. SAFETY SHOWER AND EYEWASH

All employees using Glutaraldehyde must be aware of the location and use of the laboratory safety shower and eyewash, and must be able to reach it within 10 seconds from the time of contamination. At no time will processes using Glutaraldehyde be allowed that do not provide access to a safety shower and eyewash. Employees who have skin or eye contact with Glutaraldehyde will immediately stop all processes and proceed to the safety shower and eyewash station. The employee will rinse the affected area for a minimum of 15 minutes. If eye contact has occurred, the upper and lower eyelids must be lifted to allow adequate flushing of the eyes.

7. SPECIAL HANDLING PROCEDURES AND STORAGE REQUIREMENTS

Procedures will be followed that reduce exposure to Glutaraldehyde vapor to the lowest reasonable level. This includes:

- Ensure Glutaraldehyde is only used under a fume hood
- Use only enough Glutaraldehyde to perform the required procedure
- Every effort must be made to minimize splashing, spilling, and personnel exposure
- Once specimens are preserved, they will be capped or secured in a way that does not allow Glutaraldehyde vapor to escape into the lab
- At no time will open containers be removed from the fume hood
- All containers of Glutaraldehyde or solutions containing Glutaraldehyde will be appropriately marked with the chemical name, and hazard warning label at the end of the work day or whenever there is a personnel change
- Glutaraldehyde will be stored in tightly closed containers in a cool, secure, and properly marked location

8. WASTE DISPOSAL

Excess Glutaraldehyde and all waste material containing Glutaraldehyde must be placed in an unbreakable secondary container labeled with the following “HAZARDOUS WASTE GLUTARALDEHYDE.” Wastes will be disposed of through the laboratory hazardous waste contract.

9. SPILL AND ACCIDENT PROCEDURES

Drips and splashes will be wiped up immediately with a sponge, towel, or mop. Any material used to clean spills will be disposed of as hazardous waste. Large spills (Greater than 300 CC) require response by a local Hazmat team. The Hazmat team will be called by the laboratory supervisor. In the event of a large spill personnel will immediately leave the laboratory, and not re-enter until cleared by the laboratory supervisor.

10. TRAINING

All personnel engaged in the use of Glutaraldehyde will be trained on the hazards associated with this chemical, before use. The training will include;

- OSPR's Hazard Communication Program and information contained in the chemical's Material Safety Data Sheet (MSDS)
- Health hazards and routes of exposure
- Specific procedures and techniques for use and handling
- Use of PPE and engineering controls
- The contents and requirements of this Standard Operating Procedure.

STANDARD OPERATING PROCEDURES (SOP) FOR USING FORMALIN FOR THE PRESERVATION OF DIATOMS

E

(adapted from the US EPA EMAP program; Peck et al. 2006)

Note: Formalin must only be handled by trained individuals who understand the safe handling and use of this chemical. All personnel engaged in the use of formalin will be trained on the hazards associated with this chemical before use. The training will include the information contained in the chemical's Material Safety Data Sheet (MSDS).

Formaldehyde (or formalin) is highly allergenic, toxic, and dangerous to human health (potentially carcinogenic) if utilized improperly. Formalin vapors and solution are extremely caustic and may cause severe irritation on contact with skin, eyes, or mucous membranes. Formaldehyde is a potential carcinogen, and contact with it should be avoided. Wear gloves and safety glasses and always work in a well-ventilated area. In case of contact with skin or eyes, rinse immediate with large quantities of water. Store stock solution in sealed containers in a safety cabinet or cooler lined with vermiculite or other absorbent material. If possible, transport outside the passenger compartment of a vehicle.

During the course of field activities, a team may observe or be involved with an accidental spill or release of hazardous materials. In such cases, take the proper action and do not become exposed to something harmful. The following guidelines should be applied:

- First and foremost during any environmental incident, it is extremely important to protect the health and safety of all personnel. Take any necessary steps to avoid injury or exposure to hazardous materials. You should always err on the side of personal safety for yourself and your fellow field crew members.
- Never disturb, or even worse, retrieve improperly disposed hazardous materials from the field and bring them back to a facility for disposal. To do so may worsen the impact to the area of the incident, incur personal or organizational liability, cause personal injury, or cause unbudgeted expenditures of time and money for proper treatment and disposal of material. However, it is important not to ignore environmental incidents. You are required to notify the proper authorities of any incident of this type so they can take the necessary actions to respond properly to the incident.

Follow Department of Transportation (DOT) and the Occupational Safety and Health Administration (OSHA) regulations for handling, transporting, and shipping hazardous material such as formalin and ethanol. Regulations pertaining to formalin are in the Code of Federal Regulations (CFR, specifically 29 CFR 1910.1048).

These requirements should be summarized for all hazardous materials being used for the project and provided to field personnel. Transport formalin and ethanol in appropriate containers with absorbent material. Dispose of all wastes in accordance with approved procedures (e.g., National Institute for Occupational Safety and Health 1981, US EPA 1986).

To dispense formalin in the field, wear formalin-safe gloves and safety goggles. Use a small syringe or bulb pipette to add 10 mL of 10% buffered formalin solution to 40 mL of the diatom sample in a 50 mL centrifuge tube. Alternatively, in order to avoid dispensing formalin solution in the field, clean 50 mL centrifuge tubes that will hold the diatom samples can also be pre-loaded with 10 mL of 10% buffered formalin in a laboratory fume hood prior to going into the field.

The preparation of the 10% buffered formalin stock solution should always be done by trained personnel under a laboratory fume hood while wearing protective gloves, clothing, and goggles.

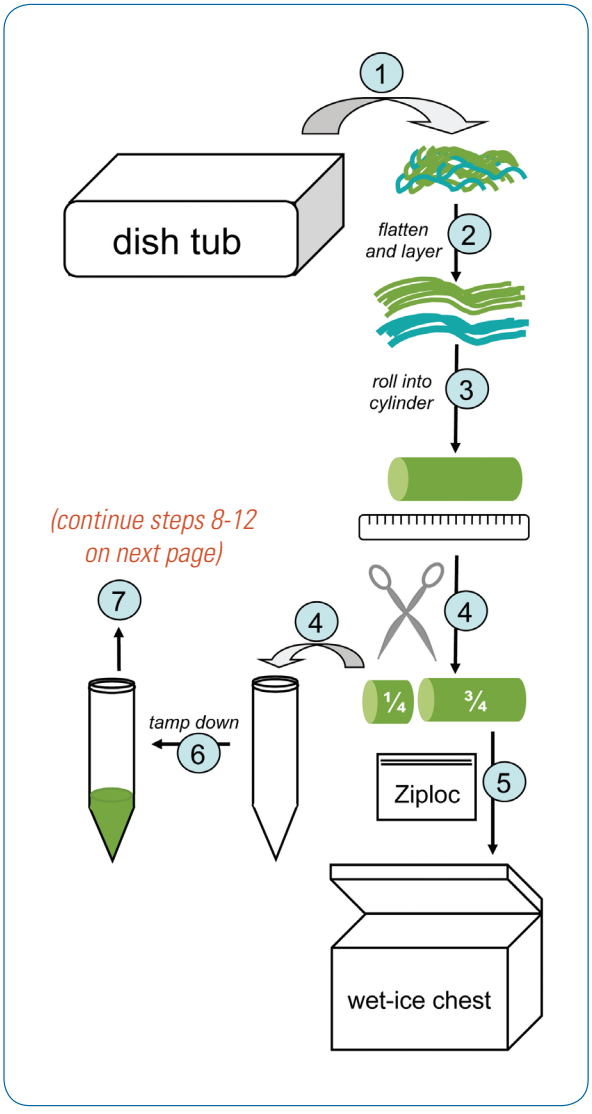
REFERENCE

Peck, D. V., A. T. Herlihy, B. H. Hill, R. M. Hughes, P. R. Kaufmann, D. Klemm, J. M. Lazorchak, F. H. McCormick, S. A. Peterson, P. L. Ringold, T. Magee, and M. Cappaert. 2006. Environmental Monitoring and Assessment Program-Surface Waters Western Pilot Study: Field operations manual for wadeable streams. U.S. Environmental Protection Agency, Washington, D.C. EPA/620/R-06/003.

PROCESSING SOFT-BODIED ALGAL AND DIATOM SAMPLES WHEN MACROALGAL CLUMPS ARE IN THE SAMPLE

F

The first step involves delivering a known quantity of macroalgae to the soft-bodied algae sample tube.



1) Gently wring excess water out of macroalgae and remove from dish tub.

2) Flatten each distinct taxon of macroalgae into even "sheets" and lay atop one another to distribute the volume of each as equally as possible.

3) Once the various layers of macroalgae are evenly spread upon one another, gently roll the stack into a cylinder shape that is roughly straight and even in thickness along its length.

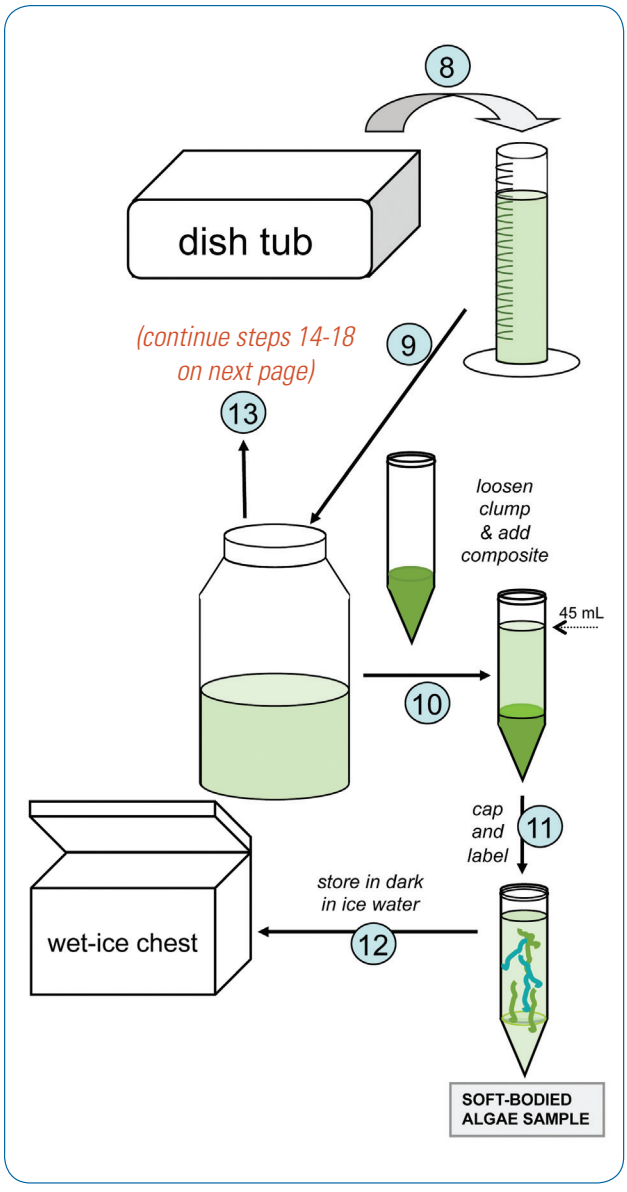
4) Measure and cut off ¼ of the cylinder and place that piece into the empty soft-bodied algae taxonomic ID sample tube.

5) Seal the remaining ¾ of the macroalgae in a clean plastic bag and place inside a cool, dark place such as the wet-ice chest.

6) Using a clean, blunt-ended object, tamp down the ¼ clump of macroalgae in the tube to make it dense and flatten the top surface. Estimate the volume of the macroalgal clump using the graduations on the tube and record this value on the Ratio Restoration worksheet (Figure 8).

7) Add composite sample solution to the tube according to directions on next page.

Some of the liquid composite sample is now added to the tube containing the macroalgae, but first the volume of the entire liquid composite collected must be measured.



8) Agitate the composite sample in the dish tub in order to suspend and mix the microalgae. Wait a few seconds to let the sand/silt settle. Quickly pour only the liquid (leaving silt/sand behind) into a graduated cylinder to measure its volume. Rinse substrate as necessary. Record TOTAL volume of the composite liquid (+ rinsate) on the datasheet, sample labels, and Ratio Restoration worksheet.

9) Pour the liquid composite sample into a clean, 1 L sample bottle

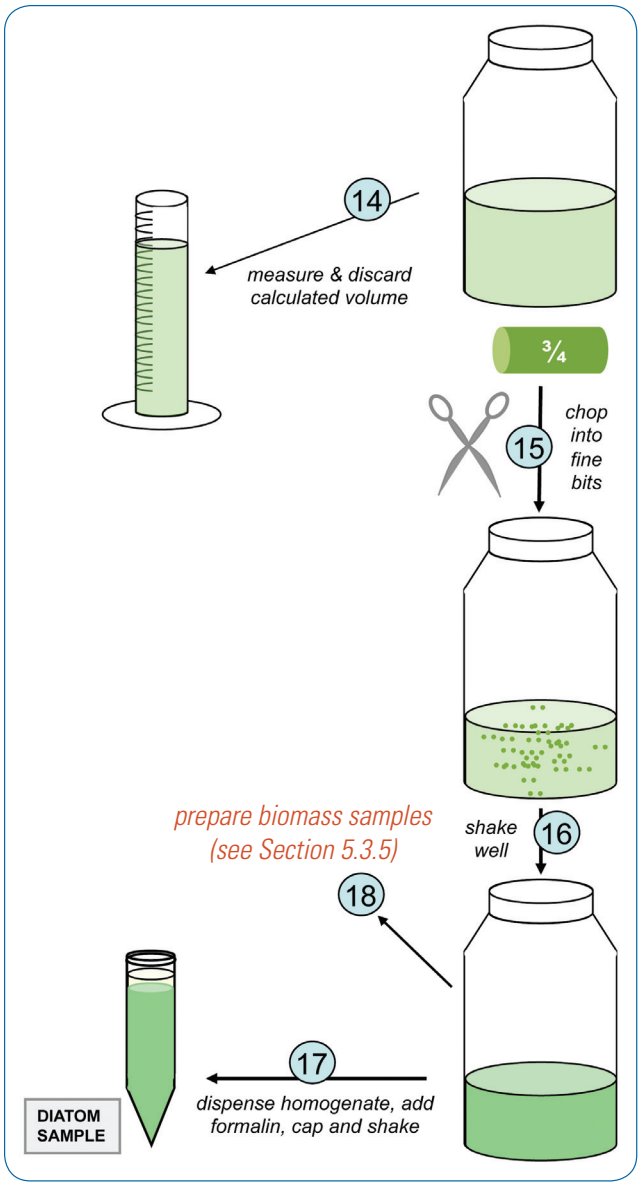
10) Loosen the macroalgae in the sample tube a little so it is no longer a dense clump lodged in the bottom and then pour freshly-agitated composite liquid into sample tube up to the 45 mL mark.

11) Cap the sample tube tightly. Affix a filled-out label to sample tube and cover with clear tape.

12) Place the tube in the dark in a wet-ice chest (not dry ice). Do not allow the algae to freeze. **Glutaraldehyde will need to be added to the tube within 4 days of sample collection, and preferably as soon as possible.**

13) According to directions on the next page, restore the original ratio of macroalgae to liquid composite in order to prepare the remainder of the samples.

The remainder of the macroalgae is now cut into tiny bits, which are added back to the liquid composite. **But the original ratio of macroalgae:liquid must first be restored.** The diatom and biomass samples are then prepared.



14) Use your Ratio Restoration worksheet to determine how much of the liquid composite to pour off. First shake the bottle vigorously, then measure and discard the appropriate volume.

15) Remove the macroalgal clump from the wet-ice chest. Chop the algae into **very fine** (eyelash length or smaller) pieces and add these to the liquid composite.

16) Cap and shake the bottle vigorously in order to homogenize the chopped algae into the liquid as thoroughly as possible.

17) Pour 40 mL of the freshly-agitated homogenate into the diatom sample tube. Add 10 mL 10% buffered formalin solution, observing all formalin safety precautions. Cap the tube, shake, and affix a sample label.

18) After both taxonomic ID samples have been prepared, the remainder of the homogenate is used for the biomass samples (chlorophyll *a* and ash-free dry mass). 25 mL of freshly shaken homogenate is filtered for each biomass sample. See Section 5.3.5.

SWAMP
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SWAMP
Surface Water Ambient Monitoring Program

SWAMP Bioassessment Procedures 2007

Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California

February 2007

California Water Boards

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Standard Operating Procedures for Collecting Benthic Macroinvertebrate Samples and Associated Physical and Chemical Data for Ambient Bioassessments in California

Prepared by: Peter R. Ode Peter Ode, SWAMP Bioassessment Coordinator, California Department of Fish and Game, Aquatic Bioassessment Laboratory

Preparation Date: January 23rd 2007

Approved by: Beverly van Buuren, SWAMP QA Officer

Approval Date: 01/17/07

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ACKNOWLEDGEMENTS

The protocols described here represent the contributions of a wide range of researchers and field crews. Most of the physical habitat methods are close modifications of those used in the U.S. Environmental Protection Agency's (EPA's) Environmental Monitoring and Assessment Program (EMAP) and developed by EPA's Office of Research and Development (ORD, Peck et al. 2004). The benthic macroinvertebrate collection methods are based on EMAP methods (EPA's targeted riffle methods were derived in turn from methods developed at Utah State University; Hawkins et al. 2003).

The current version of these protocols was established by Peter Ode (Department of Fish and Game's (DFG's) Aquatic Bioassessment Laboratory (ABL)) and David Herbst (UC Santa Barbara's Sierra Nevada Aquatic Research Laboratory) with significant contributions from staff at the ABL (Jim Harrington, Shawn McBride, Doug Post, Andy Rehn, and Jennifer York), the Surface Water Ambient Monitoring Program (SWAMP) Quality Assurance (QA) Team, Thomas Suk and other members of the SWAMP bioassessment committee (Mary Adams, Lilian Busse, Matt Cover, Robert Holmes, Sean Mundell, and Jay Rowan) and three external reviewers: Chuck Hawkins, Dave Peck, and Phil Kaufmann.

Ode, P.R.. 2007. Standard operating procedures for collecting macroinvertebrate samples and associated physical and chemical data for ambient bioassessments in California. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP 001.

SWAMP GUIDANCE SG

SWAMP GUIDANCE FOR MACROINVERTEBRATE FIELD PROTOCOLS FOR WADEABLE STREAMS

Background: The SWAMP Bioassessment Committee met in December, 2004, and agreed that the SWAMP Quality Assurance Management Plan (QAMP) should be amended to provide greater consistency in bioassessment sampling protocols for wadeable streams. The Committee's recommendations were reviewed and accepted by the full SWAMP Roundtable[1] in February, 2005 (some of the key considerations are contained in Appendix A).

The current guidance for macroinvertebrate sampling under the SWAMP program is as follows:

1. For ambient bioassessment monitoring of wadeable streams in California, two methods are to be used at sites with riffle habitats (i.e., one "multihabitat" sample, and one sample that targets the "richest" habitat):
   - **For sites with sufficient riffle habitat,** the two samples shall be: (1) the reachwide benthos (RWB) method (also known as "multihabitat" sampling.); and (2) the targeted-riffle composite (TRC) method.
   - **For low-gradient sites that do not have sufficient riffle habitat**, the RWB method is the standard method, but we also recommend the option of collecting a sample with (2) the "Margin-Center-Margin" (MCM) method until ongoing methods comparisons are completed (see Appendix A).
   - **Notes:** (1) The protocols for each method are provided in this document; (2) Other appropriate method(s) will be allowed if the specific monitoring objectives require use of alternative method(s). (See Item #2, below.); (3) The protocol recommendations specified above will be reevaluated as results become available from ongoing methods comparison studies. (See Appendix A for more information.)

2. The SWAMP QAMP allows flexibility in sampling methods so that the most appropriate method(s) may be used to address hypothesis tests and project-specific objectives that differ from program objectives. Such situations may include, but are not necessarily limited to, special studies (e.g., evaluation of point source discharges, above/below comparisons where statistical replication is needed), stressor identification investigations, and long-term monitoring projects where consistent data comparability is desired and an alternative method is needed to achieve that comparability. In addition, in some rare cases where funding limitations would make it cost-prohibitive to complete a project in compliance with the protocols listed in #1, above, the project proponent may request to complete laboratory analysis of only one sample, and "archive" one of the macroinvertebrate samples (i.e., the RWB sample in streams with riffles) to reduce lab costs. Deviations from the protocols specified in #1 above may be granted by the SWAMP Bioassessment Coordinator or the full SWAMP Roundtable.

1. The SWAMP Roundtable is the coordinating entity for the program. Participants include staff from the State and Regional Water Boards, USEPA, the Department of Fish and Game, the Marine Pollution Studies Laboratory, Moss Landing Marine Laboratories, contractors, and other interested entities.

SECTION 1 INTRODUCTION

This document describes two standard procedures (TRC and RWB) for sampling benthic macroinvertebrate (BMI) assemblages for ambient bioassessments. This document also contains procedures for measuring instream and riparian habitats and ambient water chemistry associated with BMI samples. These sampling methods replace previous bioassessment protocols referred to as the California Stream Bioassessment Procedure (CSBP, Harrington 1995, 1999, 2002).

These procedures can produce quantitative and repeatable measures of a stream's physical/habitat condition and benthic invertebrate assemblages, but they require field training and implementation of QA measures throughout the field season.

The sampling layout described here provides a framework for systematically collecting a variety of physical, chemical, and biological data. The biological sampling methods are designed to nest within the overall framework for assessing the biotic, physical, and chemical condition of a reach. The layout used in these procedures and most of the physical habitat methods are close modifications of those used in EPA's EMAP and developed by EPA's ORD (Peck et al. 2004). Data collected using this methodology are generally directly comparable to equivalent EMAP data, except for the difference in reach length. Other exceptions are noted in the text.

The following steps are presented in an order suggested for efficient data collection. The specific order of collection for the physical parameters may be modified according to preferences of field crews, with the caveat that care must always be taken to not disturb the substrates within the streambed before BMI samples are collected.

PHYSICAL HABITAT METHODS

The physical habitat scoring methods described here can be used as a stand-alone evaluation or used in conjunction with a bioassessment sampling event. However, measurements of instream and riparian habitat and ambient water chemistry are essential to interpretation of bioassessment data and should always accompany bioassessment samples. This information can be used to classify stream reaches, associate physical and chemical condition with biotic condition, and explain patterns in the biological data.

Because bioassessment samples can be collected to answer a variety of questions, this document describes the component measures of instream and riparian habitat as independent modules. Although individual modules can be added or subtracted from the procedure to reflect specific project objectives, a standard set of modules will normally accompany bioassessment samples. This document describes two standard groupings of modules that represent two different levels of intensity for characterizing the chemical and physical habitat data (Table 1). The BASIC physical habitat characterization represents a minimum amount of physical and chemical data that should be taken along with any ambient BMI sample, the FULL physical habitat characterization represents the suite of data that should be collected with most professional level bioassessment samples (e.g., SWAMP regional monitoring programs). In addition to these data, we also briefly introduce additional data modules (e.g., excess sediment, periphyton) that can be collected as supplements to the full set (OPTIONAL). Table 1 lists the physical and chemical variables that should be measured under the different levels.

Note: *SWAMP intends to develop guidance for selecting appropriate physical habitat modules to the intended uses of data. Until this guidance is available, users of these protocols should consult with representatives of the Regional Water Quality Control Boards (Regional Boards) or the SWAMP Bioassessment Coordinator when selecting modules.*

FIELD CREW SIZE AND TIME ESTIMATES

These methods are designed to be completed by either two or three (or more) person field crews. A very experienced field crew can expect to complete the full suite of physical habitat measurements and the two BMI sampling protocols in approximately two hours. Less experienced crews will probably take closer to three or four hours to complete the work depending on the complexity of the reach. Note that this estimate includes only time at the site, not travel time between sites.

Equipment and Supplies

Recommended equipment and supplies are listed in Table 2.

Table 1. Summary of physical habitat and water chemistry and proposal for basic, full, and optional levels of effort.

Survey Task	Parameter(s)	Basic	Full	Option	Comments
REACH DELINEATION and WATER QUALITY [Conducted before entering stream to sample BMIs or conduct any habitat surveys]	Layout reach and mark transects, record GPS coordinates	X	X		Use 150-m reach length if wetted width ≤10 m; Use 250-m reach length if wetted width > 10 m
	Temperature, pH, specific conductance, DO, alkalinity	X	X		Multi-meter (e.g., YSI, Hydrolab, VWR Symphony)
	Turbidity, Silica			X	Use test kit or meter
	Notable field conditions	X	X		Recent rainfall, fire events, dominant local landuse
CROSS-SECTIONAL TRANSECTS BASIC Measurements at main 11 transects only FULL Measurements at 11 main transects (A, B, C, D, E, F, G, H, I, J, K) or 21 transects (11 main plus 10 inter-transects) for substrate size classes only	Wetted width	X	X		Stadia rod is useful here
	Flow habitat delineation	X	X		Record proportion of habitat classes in each inter-transect zone
	Depth and Pebble Count + CPOM		X		5 -point substrate size, depth and CPOM records at all 21 transects
	Cobble embeddedness		X		All cobble-sized particles in pebble count. Supplement with "random walk" if needed for 25
	Slope (%)	See reach scale	X		Average slope calculated from 10 transect to transect slope measurements. Use autolevel for slopes ≤ 1%; clinometer is OK for steeper gradients
	Sinuosity		X		Record compass readings between transect centers
	Canopy cover	X	X		Four densiometer readings at center of channel (facing L bank R bank, Upstream +Downstream)
	Riparian Vegetation		X		Record % or categories
	Instream Habitat		X		
	Human Influence		X		
	Bank Stability	X	X		Eroding / Vulnerable / Stable
	Bankfull Dimensions		X		
	Excess Sediment Transect Measures (optional)				
	Bankfull width and height, bank angles			X	
	Large woody debris counts			X	Tallies of woody debris in several size classes
	Thalweg profile			X	100 equidistant points along thalweg

Survey Task	Parameter(s)	Basic	Full	Option	Comments
DISCHARGE TRANSECT	Discharge measurements		X		Velocity-Area Method or Neutrally Buoyant Object Method
REACH SCALE MEASUREMENTS:	EPA-RBP visual scoring of habitat features	*		X	*Used for citizen monitoring and comparison with legacy data
	Selected RBP visuals:		X		Channel alteration, sediment deposition, epifaunal substrate (redundant if doing EPA-RBP scoring)
	Slope (%, not degrees)	X	See transect scale		Single measurement for entire reach only for BASIC. Use autolevel for slopes ≤ 1%, clinometer is OK for higher gradients
	Photo documentation	X	X		Upstream (A, F, K) Downstream (F)
OTHER OPTIONAL COMPONENTS					
FOOD RESOURCE QUANTIFICATION	Periphyton (3 replicates)			X	Qualitative characterization of diatom growth and filamentous algal growth, quantification of biomass (AFDM, chl-a)
	CPOM & FPOM (3 replicates)			X	CPOM field measure of wet mass >1 mm particles, FPOM as 0.25 – 1 mm fraction (AFDM in lab)

Table 2. Field equipment and supplies

Physical Habitat	BMI Collection	General/ Ambient Chemistry
• GPS receiver • topographic maps • measuring tape (150-m) • small metric ruler or gravelometer for substrate measurements • digital watch, random number table or ten-sided die • stadia rod • clinometer • autolevel (for slopes < 1%) • handlevel (optional) • current velocity meter • stopwatch for velocity measurements • convex spherical densitometer • flags/ flagging tape • rangefinder	• D-frame kick net (fitted with 500-µ mesh bag) • standard # 35 sieve (500-µ mesh) • wide-mouth 500-mL or 1000 mL plastic jars • white sorting pan (enamel or plastic) • 95% EtOH • fine tipped forceps or soft forceps • waterproof paper and tape for attaching labels • 10-20-L plastic bucket for sample elutriation • preprinted waterproof labels (e.g., Rite-in-the-Rain™) • disposable gloves/ elbow length insulated gloves	• sampling SOP (this document) • hip or chest waders, or wading boots/shoes • field forms printed on waterproof paper (e.g., Rite-in-the-Rain™) • clip board and pencils • digital camera • centigrade thermometer • pH meter • DO meter • conductivity meter • field alkalinity meter • water chemistry containers • calibration standards • spare batteries for meters • first aid kit

SECTION 2
REACH DELINEATION AND WATER QUALITY

REACH LAYOUT AND GENERAL DOCUMENTATION

The systematic positioning of transects is essential to collecting representative samples and to the objective quantification of physical habitat measures. The standard sampling layout consists of a 150-m reach (length measured along the bank) divided into 11 equidistant transects that are arranged perpendicular to the direction of flow (Figure 1, Figure 2). Ten additional transects (designated "inter-transects") located between the main transects give a total of 21 transects per reach. Main transects are designated A through K while inter-transects are designated by their nearest upstream and downstream transects (e.g., AB, BC, etc.). In extreme circumstances, reach length can be shorter than 150 m (e.g., if upstream and downstream barriers preclude a 150-m reach), but this should be avoided whenever possible. If the actual reach length is other than 150 m or 250 m this should be noted and explained on the field forms.

Note 1: *The standard reach length differs from that used in the EMAP design, in which reach length was defined as 40x stream width, with a minimum reach length of 150 m. The EMAP reach length approach is used to ensure that enough habitat is sampled to support accurate fish assemblage estimates and relatively precise characterization of channel characteristics (e.g., residual pool volumes and woody debris estimates, which that are critical for relative bed stability estimates). Programs wishing to sample fish assemblages or produce relative bed stability estimates should strongly consider adopting the EMAP guidance for setting reach length.*

Note 2: *Streams > 10 m wetted width should use a reach length of 250 m. Some very large streams (i.e., > 20-m wetted width) may not be adequately represented even by a 250-m reach. In these cases, field crews should define a reach length that is representative of the larger stream segment being studied (i.e., attempt to include two to three meander cycles, or four to six riffle-pool sequences when possible).*

Note 3: *When the exact reach location is not restricted by the sampling design, attempt to position reaches upstream of bridges to avoid this influence.*

Step 1. Upon arrival at the sampling site, fill out the reach documentation section of the field forms (site and project identification, stream and watershed name, crew members, and date/time). If known at the time of sampling, record the Site Code following SWAMP site code formats. Determine the geographic coordinates of the downstream end of the reach (preferably in decimal degrees to at least four decimal places) with a GPS receiver and record the datum setting of the unit (preferably NAD83/ WGS84).

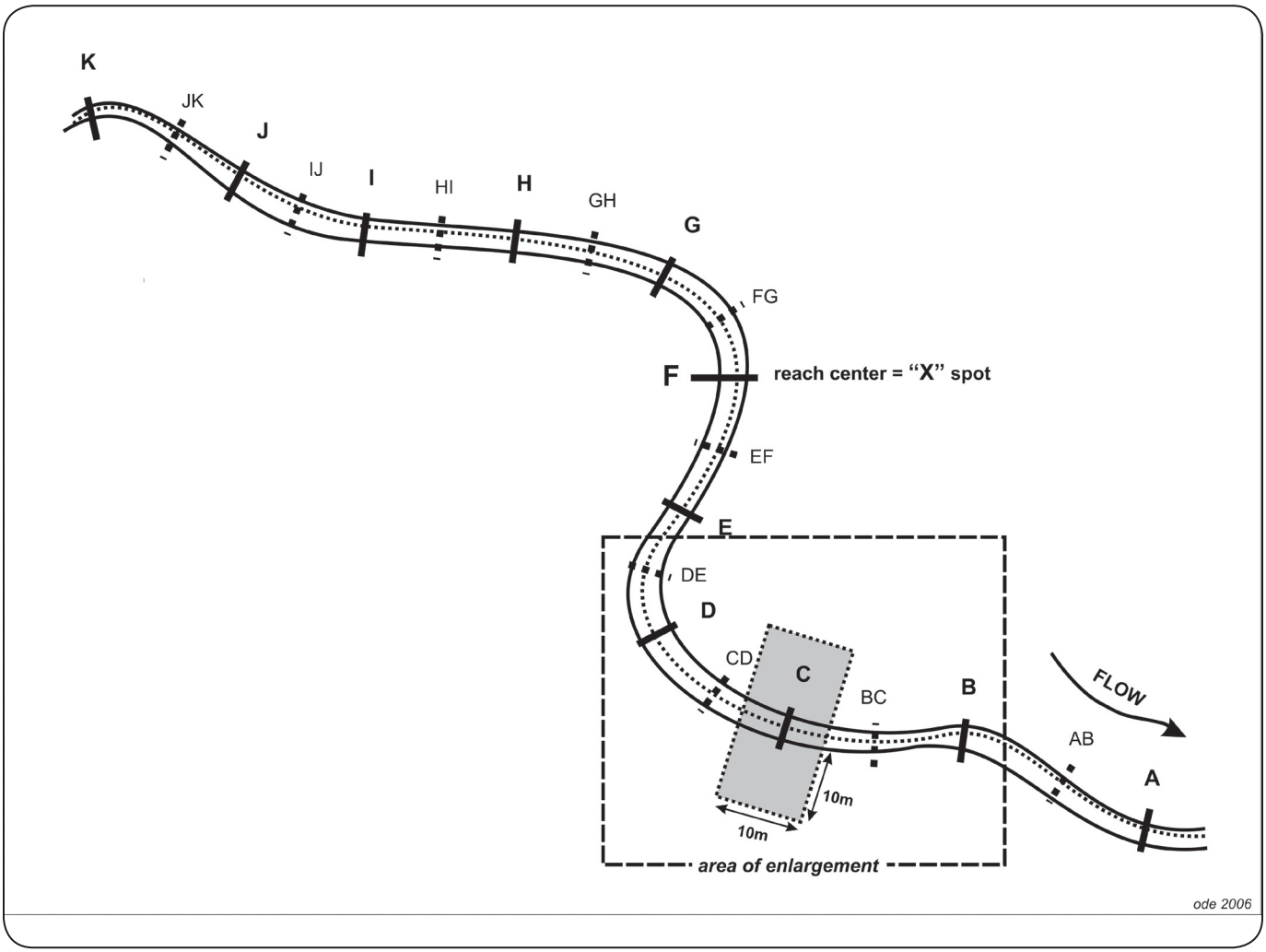

Figure 1. Reach layout geometry for physical habitat and biological sampling showing positions of 11 main transects (A – K) and the 10 supplemental inter-transects (AB- JK). The area highlighted in the figure is expanded in Figure 2. Note: reach length = 150 m for streams ≤ 10-m average wetted width, and reach length = 250 m for streams > 10-m average wetted width.

Step 2. Once a site has been identified, make an initial survey of the reach from the stream banks (being sure to not disturb the instream habitat). If TRC samples will be collected, identify all riffle habitats suitable for sampling (see Section IIIa for suitable habitat types) and note their positions so that a subset can be identified for sampling.

Step 3. Determine if the average wetted width is greater or less than 10 m. If the average wetted width ≤ 10 m, use a 150-m reach length. If the average wetted width > 10 m, use a 250-m reach length.

Step 4. Starting at one end of the reach, establish the position of the 11 main transects (labeled A-K from downstream to upstream) by measuring 15 m (25 m for streams > 10 m wetted width) along the bank from the previous transect. The 10 inter-transects should be established equidistant from the adjacent main transects (i.e., 7.5 m from main transects for 150-m reaches, 12.5 m for 250-m reaches). Since the data collection will start at the downstream end, is often easiest to establish transects starting from the upstream end. For easy setup and breakdown, mark the main transects with easily removable markers (e.g., large washers tied with strips of flagging, surveyor's flags).

Note 1: *While it is usually easiest to establish transect positions from the banks (this also reduces disturbance to the stream channel), this can result in uneven spacing of transects in complex stream reaches. To avoid this, estimate transect positions by projecting from the mid-channel to the banks.*

Note 2: *Flagging of a single bank is recommended to reduce mistakes caused by missed markers.*

Step 5. Measure and record common ambient water chemistry measurements (pH, DO, specific conductance, alkalinity, water temperature) at the downstream end of the reach (near same location as the GPS coordinates were taken). These are typically taken with a handheld water quality meter (e.g., YSI, Hydrolab), but field test kits (e.g., Hach) can provide acceptable information if they are properly calibrated. For appropriate calibration methods and calibration frequency, consult the current SWAMP QAMP (Appendix F), or follow manufacturer's guidelines.

Note 1: *If characteristics of the site prohibit downstream entry, measurements may be taken at other points in the reach. In all cases, ambient chemistry measurements should be taken at the beginning of the reach survey.*

Note 2: *Alkalinity test kits may not perform well in low ionic strength waters. Programs should consider collecting lab samples for these sites (see SWAMP QAMP for guidance on collecting water chemistry samples).*

Step 6. Take a minimum of four (4) photographs of the reach at the following locations: a) Transect A facing upstream, b) Transect F facing upstream, c) Transect F facing downstream, and d) Transect K facing downstream. It may also be desirable to take a photograph at Transect A facing downstream and Transect K facing upstream to document conditions immediately adjacent to the reach. Digital photographs should be used when possible. Record the image numbers on the front page of the field form.

Note 1: *When possible, photograph names should follow SWAMP coding conventions ("StationCode_yyyy_mm_dd_uniquecode"). The unique code should include one of the following codes to indicate direction: RB (right bank), LB (left bank), BB (both banks), US (upstream), DS (downstream). SWAMP suggests using unique codes created by the camera to facilitate file organization. Example: 603WQLB02_2004_03_20_RBDS1253.*

Step 7. Record the dominant land use and land cover in the area surrounding the reach (evaluate land cover within 50 m of either side of the stream reach).

Step 8. At the bottom of the form, record evidence of recent flooding, fire, or other disturbances that might influence bioassessment samples. Especially note if flow conditions have been affected by recent rainfall, which can cause significant under-sampling of BMI diversity (see note in the following section). If you are unaware of recent fire or rainfall events, select the "no" option on the forms.

SECTION 3
COLLECT BENTHIC MACROINVERTEBRATES

MULTIPLE HABITAT AND TARGETED RIFFLE PROTOCOLS

Note 1: *BMI samples intended for ambient bioassessments are generally collected when streams are at or near base flow (i.e., not influenced by surface runoff) as sudden flow increases can dramatically alter local community composition.*

Note 2: *Guidance for choosing among TRC sampling, RWB sampling or both will be provided in a separate document (see Appendix A for current guidance for sampling under SWAMP).*

Once the reach transects have been laid out, the biological samples (BMIs and algae if included) should be collected before any other physical habitat measures so that substrates are not disturbed prior to sampling. Both TRC and RWB methods use 500-µ mesh D-frame nets (see list of BMI sampling equipment in Table 2). The two samples can be collected at the same time by carrying two D-nets and compositing the material from the two samples in their respective nets. If a two person field crew is responsible for both the physical habitat data and benthic invertebrate samples, it is generally best to collect the benthos at each transect, then immediately record the physical habitat data before moving to the next transect. Obviously, this requires especially careful handling of the D-nets during the course of sampling to avoid loss or contamination of the samples. It can be helpful to clearly label the two D nets as RWB and TRC. Larger field crews may choose to split the sampling between biological team and a physical habitat team and have the biological team go through the reach first. The positions of the TRC and RWB subsampling locations are illustrated in Figure 2.

SECTION III A. TARGETED RIFFLE COMPOSITE PROCEDURE

The TRC method is designed for sampling BMIs in wadeable streams that contain fast-water (riffle/run) habitats and is not appropriate for waterbodies without fastwater habitats. The RWB protocol should be used in these situations. Riffles are often used for collecting biological samples (e.g., the old CSBP methods) because they often have the highest BMI diversity in wadeable streams. This method expands the definition to include other fast water habitats, however care should be taken when attempting to apply this method in low gradient streams.

Note: *Since all streams (even low gradient streams) have variation in flow habitats within the channel, this guidance should not be interpreted as including areas within low gradient streams that are only marginally faster than the surrounding habitats. The RWB protocol should be applied in these situations.*

The TRC was developed by the Western Center for Monitoring and Assessment of Freshwater Ecosystems (www.cnr.usu.edu/wmc) in Logan, Utah (Hawkins et al. 2003) and slightly modified by the EPA program (Peck et al. 2004). The TRC has been widely used in California (US Forest Service (USFS), the EMAP Western Pilot, and the California Monitoring and Assessment Program (CMAP)), and in the interest of methodological consistency between state and federal water resource agencies, has been adopted as the standard riffle protocol for bioassessment in California. The version described here is the EMAP modification, which distributes the sampling effort throughout the reach.

Sampling Locations – Acceptable Habitat Types

Riffles are the preferred habitat for TRC sampling, but other fast water habitats are acceptable for sampling if riffles are sparse. Common flow-defined habitat types are listed in Table 3 in decreasing order of energy. Most streams contain some or all of the following fast water habitat types: 1) cascades/falls, 2) rapids, 3) riffles, 4) runs. All of these are acceptable for TRC sampling if riffles are not available.

Note: *Because the common habitat types are arranged on a continuum between high to low energy environments, the categories grade into each other continuously and are not discrete. Thus, determination of habitat types requires somewhat subjective decision-making.*

Table 3. Common habitat types in stream channels, arranged in decreasing order of energy

Flow Habitat Type	Description
Cascades	Short, high gradient drop in stream bed elevation often accompanied by boulders and considerable turbulence
Falls	High gradient drop in elevation of the stream bed associated with an abrupt change in the bedrock
Rapids	Sections of stream with swiftly flowing water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles
Riffles	Shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence; (< 0.5 m deep, > 0.3 m/s)
Step-Runs	A series of runs that are separated by short riffles or flow obstructions that cause discontinuous breaks in slope
Runs	Long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool; (> 0.5 m deep, > 0.3 m/s)
Glides	A section of stream with little or no turbulence, but faster velocity than pools; (< 0.5 m deep, < 0.3 m/s)
Pools	A reach of stream that is characterized by deep, low-velocity water and a smooth surface ; (> 0.5 m deep, < 0.3 m/s)

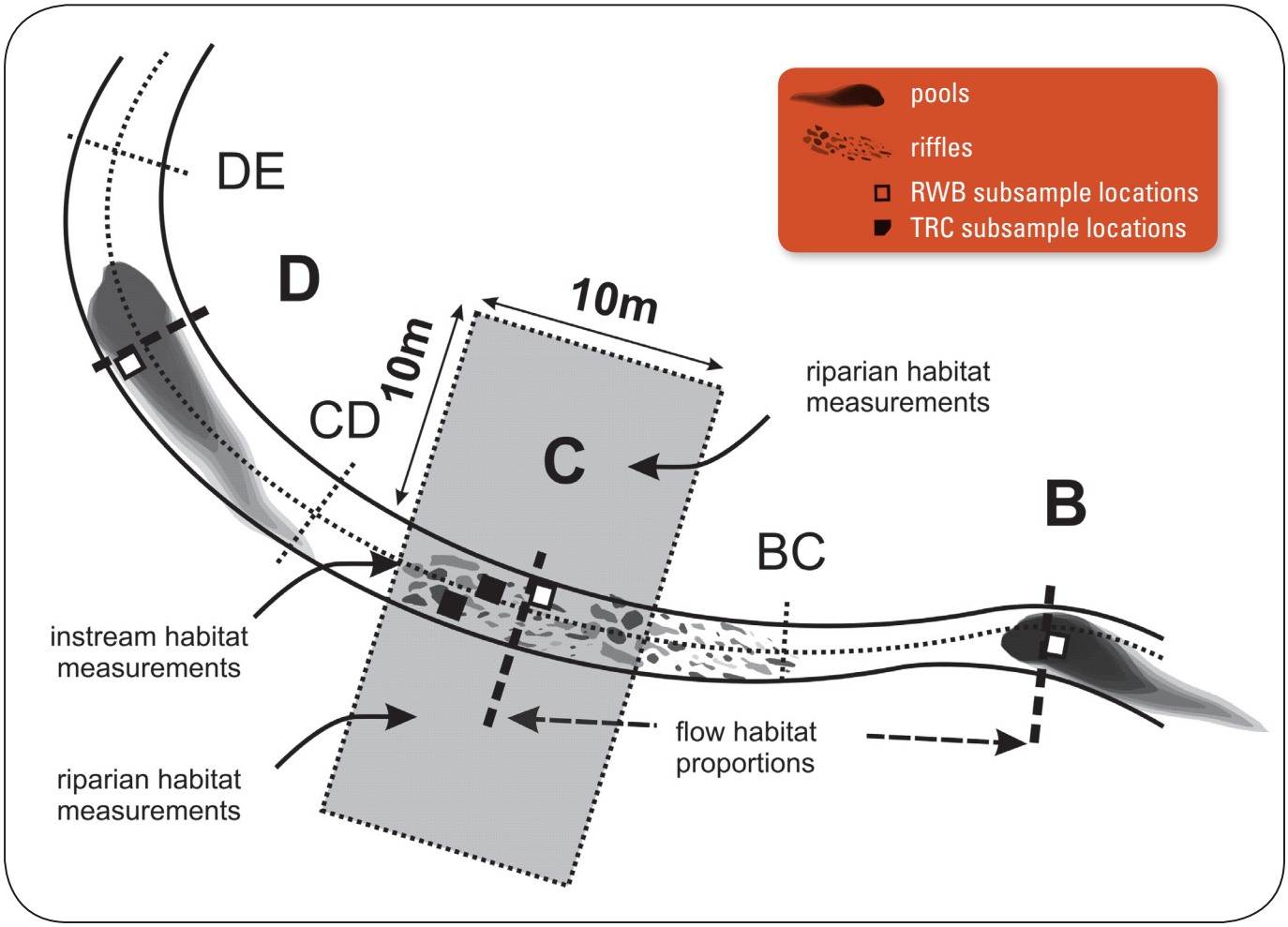

Figure 2. Section of the standard reach expanded from Figure 1 showing the appropriate positions for collecting benthic macroinvertebrate samples, instream and riparian habitat measurements and flow habitat proportion measurements.

Sampling Locations – Selecting Habitat Units

A TRC sample is a composite of eight individual kick samples of 1 ft^2 (0.09 m^2) of substrate each. During your initial layout of the reach, take a mental note of the number and position of the main riffles in a reach (and other fast water habitats if needed). Randomly distribute the eight sub-samples among the fast water habitats in the reach, giving preference to riffles where possible. Unless you are sampling in small streams, try to avoid very small riffle units (i.e., < 5 ft^2). If fewer than eight riffles are present in a reach, more than one sample may be taken from a single riffle, especially if the riffles are large.

Sampling Procedure

Begin sampling at the downstream end of the reach at the first randomly selected riffle and work your way upstream.

TRC-Step 1. Determine net placement within each habitat unit by generating a pair of random numbers between 0 and 9. Examples of convenient random number generators include the hundredths place on the stopwatch feature of a digital watch, a 10 sided die and a random number chart. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the riffle width from right bank. For example, if the two generated random numbers are 4 and 7, you will walk upstream 40% of the distance of the riffle and then go 70% of the distance across the riffle (see Figure 3). This position is the center of the 1 ft^2 (0.09 m^2) sampling quadrat for that riffle. If you are unable to sample this location because it is too deep or it is occupied by a large boulder, select a new pair of random numbers and pick a new spot.

TRC-Step 2. Position a 500-µ D-net (with the net opening perpendicular to the flow and facing upstream) quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid, and if necessary remove, large rocks that prevent the sampler from seating properly on the stream bottom.

TRC-Step 3. Holding the net in position on the substrate, visually define a square quadrat that is one net width wide and one net width long upstream of the net opening. Since D-nets are 12 inches wide, the area within this quadrat is 1ft^2 (0.09 m^2). Restrict your sampling to within that area. If desired, a wire frame of the correct dimensions can be placed in front of the net to help delineate the quadrat to be sampled, but it is often sufficient to use the net dimensions to keep the sampling area consistent.

TRC-Step 4. Working backward from the upstream edge of the sampling plot, check the quadrat for heavy organisms such as mussels, snails, and stone-cased caddisflies. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Remove and clean all of the rocks larger than a golf ball ($\sim$3 cm) within your sampling quadrat such that all the organisms attached to them are washed downstream into your net. Set these rocks outside your sampling quadrat after you have cleaned them. If the substrate is consolidated or comprised of large, heavy rocks, use your feet to kick and dislodge the substrate to displace BMIs into the net. If you cannot remove a rock from the stream bottom, rub it (concentrating on cracks or indentations) thereby loosening any attached insects. As you are disturbing the plot, let the water current carry all loosened material into the net.

Note 1: *Brushes are sometimes used in other bioassessment protocols to help loosen organisms, but in the interest of standardizing collections, do not use a brush when following this protocol.*

Note 2: *In sandy-bottomed streams, kicking within run habitats can quickly fill the sampling net with sand. In these situations, follow the standard procedures but use care to disturb the substrate gently and avoid kicking.*

TRC-Step 5. Once the coarser substrates have been removed from the quadrat, dig your fingers through the remaining underlying material to a depth of about 10 cm (this material is often comprised of gravels and finer particles). Thoroughly manipulate the substrates in the quadrat.

Note: *The sampler may spend as much time as necessary to inspect and clean larger substrates, but should take a standard time of 30 seconds to perform Step 5.*

TRC-Step 6. Let the water run clear of any insects or organic material before carefully lifting the net. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net, but be careful to avoid having any water or foreign material enter the mouth of the net during this operation.

TRC-Step 7. Move upstream to the next randomly selected habitat unit and repeat steps one through six, taking care to keep the net wet but uncontaminated by foreign material when moving the net from riffle to riffle. Sometimes, the net will become so full of material from the streambed that it is no longer effective at capturing BMIs. In these cases, the net should be emptied into sample jars as frequently as necessary, following guidelines described below in the "Preparation of BMI Sample Jars" section. Continue until you have sampled eight $1ft^2$ ($0.09\ m^2$) of benthos.

TRC-Step 8. PROCEED to Section IIIc. Filling and Labeling BMI Sample Jars.

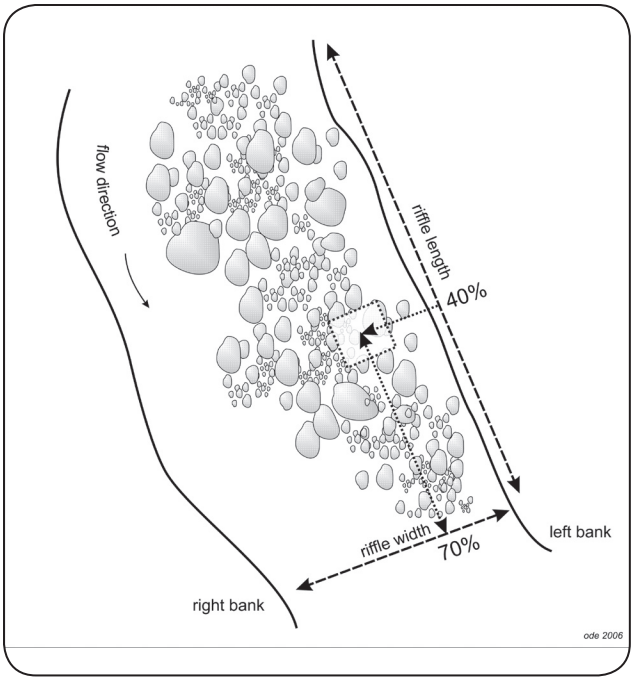

Figure 3. Example showing the method for selecting a subsampling position within a selected riffle under the TRC method. In this example, the random numbers 4 and 7 were selected

SECTION III B. REACHWIDE BENTHOS (MULTIHABITAT) PROCEDURE

The RWB procedure employs an objective method for selecting subsampling locations that is built upon the 11 transects used for physical habitat measurements. The RWB procedure can be used to sample any wadeable stream reach since it does not target specific habitats. Because sampling locations are defined by the transect layout, the position of individual sub-samples may fall in a variety of erosional or depositional habitats.

Note: *Sampling locations should be displaced one meter downstream of the transects to avoid disturbing substrates for subsequent physical habitat assessments.*

RWB -Step 1. The sampling position within each transect is alternated between the left, center and right positions along a transect (25%, 50% and 75% of wetted width, respectively) as you move upstream from transect to transect. Starting with the downstream transect (Transect

A), identify a point that is 25% of the stream width from the right bank (note that the right bank will be on your left as you face upstream). If you cannot collect a sample at the designated point because of deep water obstacles or unsafe conditions, relocate the point as close as possible to the designated position.

Note: *A modification to this procedure is currently being investigated by SWAMP. This "margin-center-margin" (MCM) modification replaces the samples at 25% and 75% of wetted width with samples of the marginal habitats (including emergent and submergent vegetation).*

RWB -Step 2. Place a 500-µ D-net in the water so the mouth of the net is perpendicular to and facing into the flow of the water. If there is sufficient current in the area at the sampling point to fully extend the net, use the normal D-net collection technique to collect the sub-sample (TRC-Step 3 through TRC-Step 6 above). If flow volume and velocity is not sufficient to use the normal collection technique, use the sampling procedure for "slack water" habitats (RWB-Step 3 through RWB-Step 7 below).

RWB -Step 3. Visually define a 1 ft^2 (0.09 m^2) quadrat that is one net-width wide and one net-width long at the sampling point.

RWB -Step 4. Working backward from the upstream edge of the sampling plot, check the quadrat for heavy organisms such as mussels and snails. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Remove and clean all of the rocks larger than a golf ball within your sampling quadrat such that all the organisms attached to them are washed downstream into your net. Set these rocks outside your sampling quadrat after you have cleaned them. Large rocks that are less than halfway into the sampling area should be pushed aside. If the substrate is consolidated or comprised of large, heavy rocks, use your feet to kick and dislodge the substrate to displace BMIs into the net. If you cannot remove a rock from the stream bottom, rub it (concentrating on cracks or indentations) thereby loosening any attached insects.

RWB -Step 5. Vigorously kick the remaining finer substrate within the quadrat with your feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net all the time so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds. For vegetation-choked sampling points, sweep the net through the vegetation within a 1ft^2 (0.09 m^2) quadrat for 30 seconds.

Note: *If flow volume is insufficient to use a D- net, spend 30 seconds hand picking a sample from 1ft^2 of substrate at the sampling point, then stir up the substrate with your gloved hands and use a sieve with 500-µ mesh size to collect the organisms from the water in the same way the net is used in larger pools.*

RWB -Step 6. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

RWB -Step 7. PROCEED to Section IIIc: Filling and Labeling BMI Sample Jars

SECTION III C. FILLING AND LABELING BENTHIC MACROINVERTEBRATE SAMPLE JARS

Step 1. Once all sub-samples (eight for TRC, 11 for RWB) have been collected, transfer benthos to a 500-mL or 1000-mL wide-mouth plastic sample jar using one of the following methods.

Note: *Field elutriation should only be used by well-trained field crews who are proficient at removing all benthic organisms from the discarded inorganic material. Training in the recognition of aquatic invertebrates is highly recommended.*

Step 1a. Complete Transfer of all Sampled Material – Invert the contents of the kick net into the sample jar. Perform this operation over a white enameled tray to avoid loss of any sampled material and make recovery of spilled organisms easier. If possible, remove the larger twigs and rocks by hand after carefully inspecting for clinging organisms, but be sure not to lose any organisms. Use forceps to remove any organisms clinging to the net and place these in the sample jar.

Step 1b. Field Elutriation of Samples – Empty the contents of the net into a large plastic bucket (10-20 L is sufficient). Use forceps to remove any organisms clinging to the net and place these in the bucket. Add stream water to the bucket and gently swirl the contents of the bucket in order to suspend the organic material (being certain to not introduce entrained organisms from the source water). Pour the organic matter from the bucket through a 500-µ sieve (or use the 500-µ net). Repeat this process until no additional material can be elutriated (i.e., only inorganic material is left in the bucket). If possible, remove the larger twigs and rocks by hand after carefully inspecting for clinging organisms, but be sure not to lose any organisms. Transfer all of the material in the sieve (invertebrates and organic matter) into the sample jar. Carefully inspect the gravel and debris remaining in the bottom of the bucket for any cased caddisflies, clams, snails, or other dense animals that might remain. Remove any remaining animals by hand and place them in the sample jar.

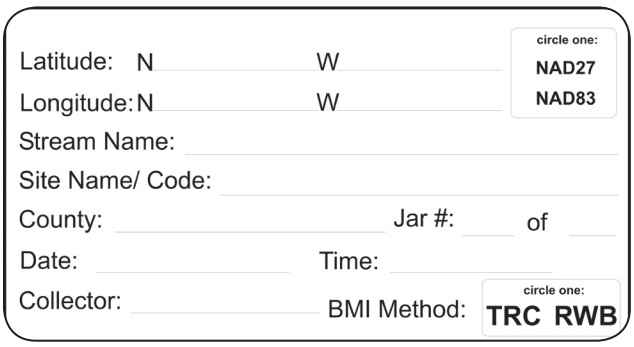

Figure 4. Example date - locality label for all BMI samples.

Step 2. Place a completed date/locality label (see Figure 4) on the inside of the jar (use pencil only as most "permanent" inks dissolve in ethanol) and completely fill with 95% ethanol. Place a second label on the outside of the jar. Note that the target concentration of ethanol is 70%, but 95% ethanol is used in the field to account for dilution from water in the sample. If organic and inorganic material does not accumulate in the net quickly, it may be possible to transfer all the material in the net into one jar. Otherwise, divide the material evenly among several jars

(being careful to clearly label them as part of a set). To ensure proper preservation of benthic macroinvertebrates it is critical that the ethanol is in contact with the BMIs in the sample jar. Never fill a jar more than 2/3 full with sampled material, and gently rotate jars that contain mostly mud or sand to ensure that the ethanol is well distributed. If jars will be stored for longer than a month prior to processing, jars should not contain more than 50% sample material.

SECTION 4 MAIN CROSS-SECTIONAL TRANSECT MEASURES

SECTION IVA. PHYSICAL MEASURES

The majority of physical habitat measurements in this protocol are made relative to the main cross-sectional transects (Figure 5). All the measures taken relative to each transect are recorded on forms specific to that transect. Start with the downstream transect (Transect A) and repeat steps 6-15 for all 11 main transects.

Module A. Transect Dimensions: Wetted Width and Bankfull Dimensions

Wetted Width – The wetted channel is the zone that is inundated with water and the wetted width is the distance between the sides of the channel at the point where substrates are no longer surrounded by surface water. Measure the wetted stream width and record this in the box at the top of the transect form.

Bankfull Width and Depth – The bankfull channel is the zone of maximum water inundation in a normal flow year (one to two year flood events). Since most channel formation processes are believed to act when flows are within this zone (Mount 1995), bankfull dimensions provide a valuable indication of relative size of the waterbody.

Note: *Bankfull dimensions are notoriously difficult to assess, even by experienced field crews (see Heil and Johnson 1995). It is often useful to discuss the interpretation of bankfull locations among the field crew members to reach a consensus. The USFS Stream Team provides a good set of instructional videos for improving consistency in accurate bankfull measurements (http://www.stream.fs.fed.us/publications/videos.html).*

Step 1. Scout along the stream margins to identify the location of the bankfull margins on either bank by looking for evidence of annual or semi-annual flood events. Examples of useful evidence includes topographic, vegetative, or geologic cues (changes in bank slope, changes from annual to perennial vegetation, changes in the size distribution of surface sediments). While the position of drift material caught in vegetation may be a helpful aid, this can lead to very misleading measurements.

Note: *The exact nature of this evidence varies widely across a range of stream types and geomorphic characteristics. It is helpful to investigate the entire reach when attempting to interpret this evidence because the true bankfull margin may be obscured at various points along the reach. Often the bankfull position is easier to interpret from one bank than the other; in these cases, it is easiest to infer the opposite bank position by projecting across the channel. Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (these heights should be equal).*

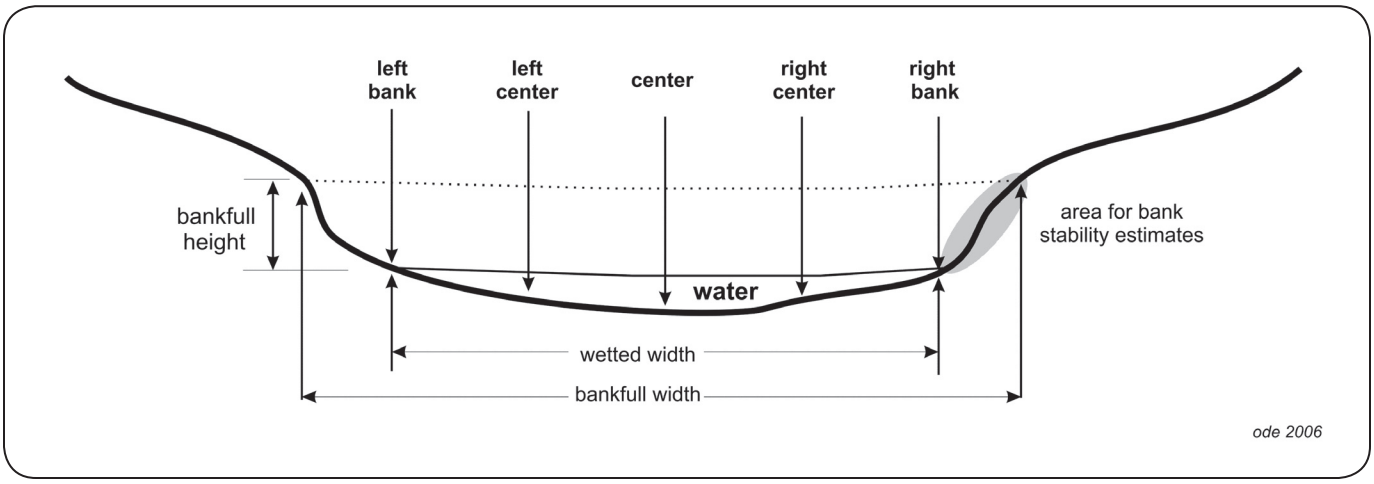

Figure 5. Cross sectional diagram of a typical stream channel showing locations of substrate measurements, wetted and bankfull width measurements, and bank stability visual estimates.

Step 2. Stretch a tape from bank to bank at the bankfull position. Measure the width of the bankfull channel from bank to bank at bankfull height and perpendicular to the direction of stream flow.

Step 3. Measure bankfull height (the vertical distance between the water height of the water and the height of the bank, Figure 5) and record.

Module B. Transect Substrate Measurements

Particle size frequency distributions often provide valuable information about instream habitat conditions that affect BMI distributions. The Wolman pebble count technique (Wolman 1954) is a widely used and cost-effective method for estimating the particle size distribution and produces data that correlates with costly, but more quantitative bulk sediment samples. The method described here follows the EMAP protocol, which records sizes of 105 particles in a reach (five particles from each of 11 main transects and 10 inter-transects).

Note: *The size cutoff for the finest particle sizes in the EMAP protocol (< 0.06 mm) differs from that used by the Sierra Nevada Aquatic Research Laboratory (SNARL) program (0.25 mm), although the narrative description for this cutoff is the same (the point at which fine particles rubbed between one's fingers no longer feel gritty).*

Coarse particulate organic matter (CPOM, particles of decaying organic material such as leaves that are greater than 1.0 mm in diameter) is a general indicator of the amount of allochthonous organic matter available at a site, and its measurement can provide valuable information about the basis of the food web in a stream reach. The presence of CPOM associated with each particle is quantified at the same time that particles are measured for the pebble counts.

Step 1. Transect substrate measurements are taken at five equidistant points along each transect (Figure 5). Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (a stadia rod is especially helpful here). Once the positions are identified, lower a graduated rod (e.g., a marked ski pole) though the water column perpendicular to both the flow and the transect to objectively select the particle located at the tip of the rod.

Step 2. Measure the depth from the water surface to the top of the particle with the graduated rod and record to the nearest cm.

Step 3. Record the presence or absence of CPOM >1mm within 1 cm of the particle.

Step 4. If the particle is cobble-sized (64-250 mm), record the percent of the cobble that is embedded by fine particles (<2 mm) to the nearest 5% (see cobble embeddedness text below).

Step 5. Remove the particle from the streambed, then measure and record the length of its intermediate axis to the nearest mm (see Figure 6). Alternatively, assign the particle to one of the size classes listed in the bottom of the transect form. Particle sizes classes can be estimated visually or with a quantitative measuring device (e.g., pass/ no-pass template, "gravelometer"). Regardless of the method, all particles less than 0.06 mm should be recorded as fines, all particles between 0.06mm and 2.0 mm recorded as sand. Field crews may want to carry vials containing sediment particles with these size ranges until they are familiar with these particles.

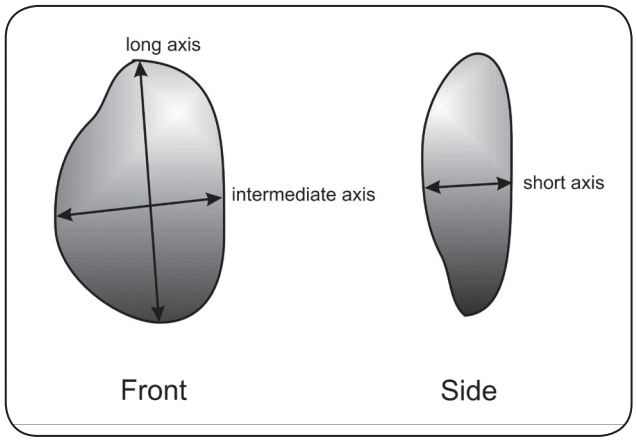

Figure 6. Diagram of three major perpendicular axes of substrate particles. The intermediate axis is recorded for pebble counts.

Module C. Cobble Embeddedness

The quantification of substrate embeddedness has long been a challenge to stream geomorphologists and ecologists (Klamt 1976, Kelley and Dettman 1980). It is generally agreed that the degree to which fine particles fill interstitial spaces has a significant impact on the ecology of benthic organisms and fish, but techniques for measuring this impact vary greatly (this is summarized well by Sylte and Fischenich 2002, http://stream.fs.fed.us/news/streamnt/pdf/StreamOCT4.pdf). Here we define embeddedness as the volume of cobble-sized particles (64-250 mm) that is buried by fine particles (<2.0 mm diameter).

Note: *This method differs from the EMAP method for measuring embeddedness, which measures embeddedness of all particles larger than 2 mm.*

Table 4. Size class codes and definitions for particle size measurements

Size Class Code	Size Class Description	Common Size Reference	Size Class Range
RS	bedrock, smooth	larger than a car	> 4 m
RR	bedrock, rough	larger than a car	> 4 m
XB	boulder, large	meter stick to car	1 - 4 m
SB	boulder, small	basketball to meter stick	25 cm - 1.0 m
CB	cobble	tennis ball to basketball	64 - 250 mm
GC	gravel, coarse	marble to tennis ball	16 - 64 mm
GF	gravel, fine	ladybug to marble	2 – 16 mm
SA	sand	gritty to ladybug	0.06 – 2 mm
FN	fines	not gritty	< 0.06 mm
HP	hardpan (consolidated fines)		< 0.06 mm
WD	wood		
RC	concrete/ asphalt		
OT	other		

Step 1. Every time a cobble-sized particle is encountered during the pebble count, remove the cobble from the stream bed and visually estimate the percentage of the cobble's volume that has been buried by fine particles. Since visual estimates of volume and surface area are subject to large amounts of observer error, field crews should routinely calibrate their estimates with each other and with other field crews.

Step 2. In the spaces to the right of the pebble count data, record the embeddedness of all cobble-sized particles encountered during the pebble count.

Note: *The cobble embeddedness scores do not correspond with the specific particles in the pebble count cells to the left, but are merely a convenient place to record the data.*

Step 3. If 25 cobbles are not encountered during the pebble count, supplement the cobbles by conducting a "random walk" through the reach. Starting at a random point in the reach, follow a transect from one bank to the other at a randomly chosen angle. Once at the other bank reverse the process with a new randomly chosen angle. Record embeddedness of cobble-sized particles in the cobble embeddedness boxes on the transect forms until you reach 25 cobbles. If 25 cobble-sized particles are not present in the entire reach, then record the values for cobbles that are present.

Module D. Canopy Cover

This method uses the Strickler (1959) modification of a convex spherical densiometer to correct for over-estimation of canopy density that occurs with unmodified readings. Read the densiometer by counting the number of line intersections that are obscured by overhanging vegetation (see Figure 7). Taping off the lower left and right portions of the mirror emphasizes overhead vegetation over foreground vegetation (the main source of bias in canopy density measurements). All densiometer readings should be taken with the bubble leveled and 0.3 m (1 ft) above the water surface.

Step 1. Using a modified convex spherical densitometer, take and record four 17-point readings all taken from the center of each transect: a) facing upstream, b) facing downstream, c) facing the left bank, d) facing the right bank.

Note: *This method deviates slightly from that of EMAP (in which two additional readings are taken at the left and right wetted edges to increase representation of bank vegetation).*

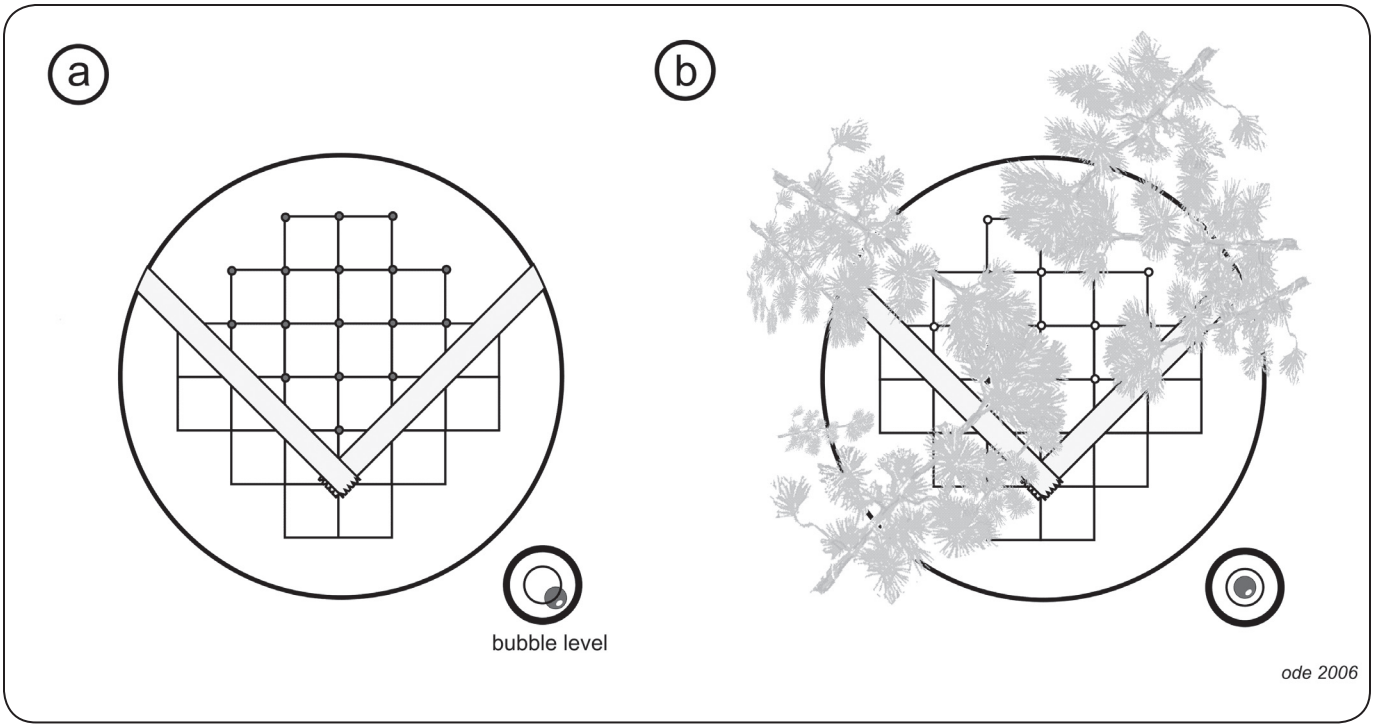

Figure 7. Representation of the mirrored surface of a convex spherical densiometer showing the position for taping the mirror and the intersection points used for the densiometer reading. The score for the hypothetical condition in (b) is 10 covered intersection points out of 17 possible. Note the position of the bubble level in (b) when the densiometer is leveled.

Module E. Gradient and Sinuosity

The gradient of a stream reach is one of the major stream classification variables, giving an indication of potential water velocities and stream power, which are in turn important controls on aquatic habitat and sediment transport within the reach. The gradient (slope) of a stream reach is often strongly correlated with many BMI metrics and other physical habitat measures and is therefore very useful when interpreting BMI data.

The "full" physical habitat method uses 10 transect to transect measurements to calculate the average slope through a reach. Although this is a little more time intensive than the reach-scale transect measures used in the "basic" protocol, it results in more precise slope determination and the ability to quantify slope variability within a reach. Sinuosity (calculated as the ratio of the length of the flow path between the ends of the reach and the straight line distance between the ends of the reach, Kaufmann et al. 1999) is measured at the same time as slope. These two measurements work best with two people, one taking the readings at the upstream transect ("backsighting") and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section). Otherwise, leave these blank.

Note 1: *An auto level should be used for reaches with a percent slope of less than or equal to 1%. All methods (clinometer, hand level, or auto level) may be used for reaches with a percent slope of greater than 1%. The following description is for clinometer-based slope measurements, but the same principles apply to use of an auto or hand level.*

Note 2: *In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff before taking readings. In these cases, default to use of an autolevel.*

Step 1. Beginning with the upper transect (Transect K), one person (the measurer) should stand at the water margin with a clinometer held at eye level. A second person should stand at the margin of the next downstream transect (Transect J) with a stadia rod flagged at the eye level of the person taking the clinometer readings. Be sure you mark your eye level while standing on level ground! Adjust for water depth by measuring from the same height above the water surface at both transects. This is most easily accomplished by holding the base of the pole at water level.

Note: *An alternative technique is to use two stadia rods pre-flagged at the eye-height of the person taking the readings.*

Step 2. Use a clinometer to measure the percent slope of the water surface (not the streambed) between the upstream transect and the downstream transect by sighting to the flagged position on the stadia rod. The clinometer reads both percent slope and degree of the slope. Be careful to read and record percent slope rather than degrees slope (these measurements differ by a factor of ~ 2.2). Percent slope is the scale on the right hand side as you look through most clinometers (e.g., Suunto models).

Note: *If an auto level or hand level is used, record the elevation difference (rise) between transects and the segment length (run) instead of the percent slope.*

Step 3. If the stream reach geometry makes it difficult to sight a line between transects, divide the distance into two or three sections and record the slope and the proportion of the total segment length between transects for each of these sections in the appropriate boxes on the slope form (supplemental segments).

Note: *Never measure slope across dry land (e.g., across a meander bend).*

Step 4. Take a compass reading from the center of each main transect to the center of the next main transect downstream and record this bearing to the nearest degree on the slope and bearing section of the form. Bearing measurements should always be taken from the upstream to downstream transect.

Step 5. Proceed downstream to the next transect pair (I-J) and continue to record slope and bearing between each pair of transects until measurements have been recorded for all transects.

SECTION IVB. VISUAL ESTIMATES OF HUMAN INFLUENCE, INSTREAM HABITAT, AND RIPARIAN VEGETATION

The transect-based approach used here permits semi-quantitative calculations from visual estimates even though most are categorical data (i.e., either presence/ absence or size classes) because we can calculate the percentage of transects that fall into different categories. These modules are adapted directly from EMAP protocols with some modifications as noted.

Module F. Human Influence

The influence of human activities on stream biota is of critical concern in bioassessment analyses. Quantification of human activities for these analyses is often performed with GIS techniques, which are very useful but are not capable of accounting for human activities occurring at the reach scale. Reach scale observations are often critical for explaining results that might seem anomalous on the basis of only remote mapping tools.

Step 1. For the left and right banks, estimate a 10 x 10 m riparian area centered on the edges of the transect (see Figure 2). Record the presence of 11 human influence categories in three spatial zones relative to this 10 x 10 m square (between the wetted edge and bankfull margin, between the bankfull margin and 10 m from the stream, and between 10 m and 50 m beyond the stream margins): 1) walls/rip-rap/dams, 2) buildings, 3) pavement/cleared lots, 4) roads/railroads, 5) pipes (inlets or outlets), 6) landfills or trash, 7) parks or lawns (e.g., golf courses), 8) row crops, 9) pasture/ rangelands, 10) logging/ timber harvest activities, 11) mining activities, 12) vegetative management (herbicides, brush removal, mowing), 13) bridges/ abutments, 14) orchards or vineyards. Circle all combinations of impacts and locations that apply, but be careful to not double-count any human influence observations.

Step 2. Record the presence of any of the 11 human influence categories in the stream channel within a zone 5 m upstream and 5 m downstream of the transect.

Module G. Riparian Vegetation

Riparian vegetation (vegetation in the region beyond the bankfull margins) has a strong influence on the composition of stream communities through its direct and indirect roles in controlling the food base, moderating sediment inputs and acting as a buffer between the stream channel and the surrounding environment. These methods provide a cursory survey of the condition of the riparian corridor. Observations are made in the same 10 x 10 m riparian area used for assessing human influence (see Figure 2).

Note: *Riparian vegetation measurements should only include living or recently dead vegetation.*

The riparian vegetation categories used here were condensed from the EMAP version, which further breaks the canopy classes into different components. However, because we have consolidated EMAP categories into fewer categories rather than creating new categories, existing EMAP data can be easily converted to this format simply by combining the appropriate categories.

Step 1. Divide the riparian zone into three elevation zones: 1) ground cover (< 0.5 m), 2) lower canopy (0.5 m - 5 m), and 3) upper canopy (> 5 m). Record the density of the following riparian classes: 1) Upper Canopy–Trees and Saplings, 2) Lower Canopy–Woody Shrubs and Saplings, 3) Woody Ground Cover–Shrubs, Saplings, 4) Herbaceous Ground Cover–Herbs and Grasses, and 5) Ground Cover–Barren, Bare Soil and Duff. Artificial banks (e.g., rip-rap, concrete, asphalt) should be recorded as barren.

Step 2. Indicate the areal cover (i.e., shading) by each riparian vegetative class as either: 1) absent, 2) sparse (< 10%), 3) moderate (10-40%), 4) heavy (40-75%), or 5) very heavy (> 75%).

Module H. Instream Habitat Complexity

Instream habitat complexity was developed by the EMAP program to quantify fish concealment features in the stream channel, but it also provides good information about the general condition and complexity of the stream channel. Estimates should include features within the banks and outside the wetted margins of the stream.

Step 1. Record the amount of nine different channel features within a zone 5m upstream and 5m downstream of the transect (see Figure 2): 1) filamentous algae (long-stranded algal forms that are large enough to see with the naked eye), 2) aquatic macrophytes (include mosses and vascular plants), 3) boulders (> 25 cm), 4 and 5) woody debris (break into two classes- larger and smaller than 30 cm diameter), 6) undercut banks, 7) overhanging vegetation, 8) live tree roots and 9) artificial structures (includes any anthropogenic objects including large trash objects like tires and shopping carts). Indicate the areal cover of each feature as either: 1) absent, 2) sparse (< 10%), 3) moderate (10-40%), 4) heavy (40-75%), or 5) very heavy (> 75%).

SECTION 5
INTER-TRANSECT MEASURES

While most measures are taken at or relative to the main transects, a few measures are recorded at transects located at the midpoint between main transects. These are called "inter-transects".

Module B (Part 2) Pebble Counts (same as for transects, but no cobble embeddedness measures)

Step 1. Divide the wetted stream width by four to get the distance between the five points (Left Bank, Left Center, Center, Right Center and Right Bank) and use a measuring device to locate the positions of these points (a stadia rod is especially helpful here, see Figure 5). Once the positions are identified, lower a graduated rod through the water column perpendicular to both the flow and the transect to objectively select the particle located at its tip.

Step 2. With the graduated rod, measure the depth from the water surface to the top of the particle and record to the nearest cm.

Step 3. Remove the particle from the streambed, then measure and record the length of its intermediate axis to the nearest mm (see Figure 6). Alternatively, assign the particle to one of the size classes listed in the bottom of the transect form (see Table 3 for a list of size classes). Particle size classes may be estimated visually or with a quantitative measuring device (e.g., pass/ no-pass template, gravelometer). Regardless of the method, all particles less than 0.06 mm should be recorded as fines, while all particles between 0.06 mm and 2.0 mm should be recorded as sand. Field crews may want to carry vials containing sediment particles with these size ranges until they are familiar with these particle size classes.

Step 4. Record the presence (P) or absence (A) of any CPOM within 1 cm of each particle.

Module J. Flow Habitats

Because many benthic macroinvertebrates prefer specific flow and substrate microhabitats, the proportional representation of these habitats in a reach is often of interest in bioassessments. There are many different ways to quantify the proportions of different flow habitats (for example, see text on EMAP's "thalweg profile" below). Like the riparian and instream measures listed above, this procedure produces a semi-quantitative measure consisting of 10 transect-based visual estimates.

Note: *The categories used here are based on those used in the EMAP protocol, with pools combined into one class and cascades and falls combined into another class.*

Step 1. At each inter-transect, identify the proportion of six different habitat types in the region between the upstream transect and downstream transect: 1) cascades/falls, 2) rapids, 3) riffles, 4) runs, 5) glides, 6) pools, 7) dry areas. Record percentages to the nearest 5% — the total percentage of surface area for each section must total 100%.

SECTION 6 DISCHARGE

Stream discharge is the volume of water that moves past a point in a given amount of time and is generally reported as either cubic meters per second (cms) or cubic feet per second (cfs). Because discharge is directly related to water volume, discharge affects the concentration of nutrients, fine sediments and pollutants; and discharge measurements are critical for understanding impacts of disturbances such as impoundments, water withdrawals and water augmentation. Discharge is also closely related to many habitat characteristics including temperature regimes, physical habitat diversity, and habitat connectivity. As a direct result of these relationships, stream discharge is often also a strong predictor of biotic community composition. Since stream volume can vary significantly on many different temporal scales (diurnal, seasonal, inter-annually), it can also be very useful for understanding variation in stream condition.

This procedure (modified from the EMAP protocol) provides for two different methods for calculating discharge. It is preferable to take discharge measurements in sections where flow velocities are greater than 0.15 m/s and most depths are greater than 15 cm, but slower velocities and shallower depths can be used. If flow volume is sufficient for a transect-based "velocity-area" discharge calculation, this is by far the preferred method. If flow volume is too low to permit this procedure or if your flow meter fails, use the "neutrally buoyant object/ timed flow" method.

Note: *Programs that sample fixed sites repeatedly may want to consider installing permanent discharge estimation structures (e.g., stage gauges, wiers).*

Module K. Discharge: Velocity Area Method

The layout for discharge measurements under the velocity-area (VA) method is illustrated in Figure 8. Flow velocity should be measured with either a Swoffer Instruments propeller-type flow meter or a Marsh-McBirney inductive probe flow meter. Refer to the manufacturers' instrument manuals for calibration procedures.

VA-Step 1. Select the best location in the reach for measuring discharge. To maximize the repeatability of the discharge measurement, choose a transect with the most uniform flow (select hydraulically smooth flow whenever possible) and simplest cross-sectional geometry. It is acceptable to move substrates or other obstacles to create a more uniform cross-section before beginning the discharge measurements.

VA-Step 2. Measure the wetted width of the discharge transect and divide this into 10 to 20 equal segments. The use of more segments gives a better discharge calculation, but is impractical in small channels. A minimum of 10 intervals should be used when stream width permits, but interval width should not be less than 15 cm.

VA-Step 3. Record the distance from the bank to the end of the first interval. Using the top-setting rod that comes with the flow velocity meter, measure the median depth of the first interval.

VA-Step 4. Standing downstream of the transect to avoid interfering with the flow, use the top-setting rod to set the probe of the flow meter (either the propeller or the electromagnetic probe) at the midpoint of each interval, at 0.6 of the interval depth (this position generally approximates average velocity in the water column), and at right angles to the transect (facing upstream). See Figure 8 for positioning detail.

VA-Step 5. Allow the flow velocity meter to equilibrate for 10-20 seconds then record velocity to the nearest m/s. If the option is available, use the flow averaging setting on the flow meter.

Note: *Under very low flow conditions, flow velocity meters may register readings of zero even when there is noticeable flow. In these situations, record a velocity of 0.5x the minimum flow detection capabilities of the instrument.*

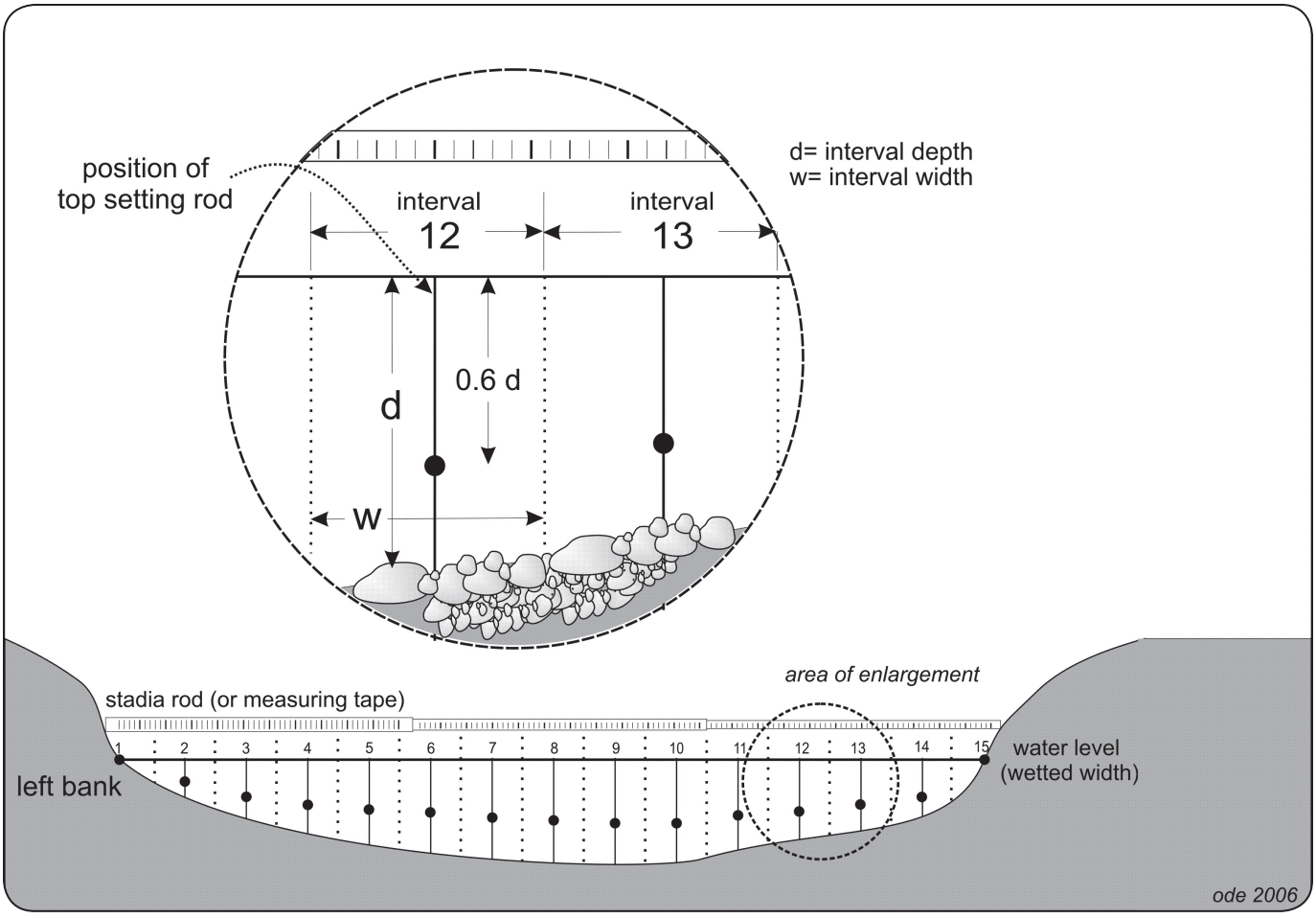

Figure 8. Diagram of layout for discharge measurements under the velocity-area method showing proper positions for velocity probe (black dots).

VA-Step 6. Complete Steps 3 through 5 on the remaining intervals.

Note: *The first and last intervals usually have depths and velocities of zero.*

Module L. Discharge: Neutrally Buoyant Object Method

If streams are too shallow to use a flow velocity meter, the neutrally buoyant object (NBO) method should be used to measure flow velocity. However, since this method is less precise than the flow velocity meter it should only be used if absolutely necessary. A neutrally buoyant object (one whose density allows it to just balance between sinking and floating) will act as if it were nearly weightless, thus it's movement will approximate that of the water it floats in better than a light object. To estimate the flow velocity through a reach, three transects are used to measure the cross-sectional areas within the test section sub-reach and three flow velocity estimates are used to measure average velocity through the test reach. To improve precision in velocity measurements, the reach segment should be long enough for the float time to last at least 10-15 seconds.

NBO-Step 1. The position of the discharge sub-reach is not as critical as it is for the velocity-area method, but the same criteria for selection of a discharge reach apply to the neutrally buoyant object method. Identify a section that has relatively uniform flow and a uniform cross sectional shape.

NBO-Step 2. The cross sectional area is estimated in a manner that is similar but less precise than that used in the velocity area method. Measure the cross sectional area in one to three places in the section designated for the discharge measurement (three evenly-spaced cross sections are preferred, but one may be used if the cross section through the reach is very uniform). Record the width once for each cross section and measure depth at five equally-spaced positions along each transect.

NBO-Step 3. Record the length of the discharge reach.

NBO-Step 4. Place a neutrally buoyant object (e.g., orange, rubber ball, heavy piece of wood, etc.) in the water upstream of the discharge reach and record the length of time in seconds that it takes for the object to pass between the upstream and downstream boundaries of the reach. Repeat this timed float three times.

SECTION 7
POST-SAMPLING OBSERVATIONS

Module M. Rapid Bioassessment Procedures Visual Assessment Scores (for Basic Physical Habitat, or optional supplement)

EPA's Rapid Bioassessment Procedures (RBPs, Barbour et al. 1999) include a set of 10 visual criteria for assessing instream and riparian habitat. The RBP has been used in the CSBP since its first edition (1995) and thus, this information is often valuable for comparison to legacy datasets. The criteria also have a useful didactic role since they help force the user to quantify key features of the physical environment where bioassessment samples are collected.

Module N. Additional Habitat Characterization (Full Physical Habitat only)

The RBP stream habitat visual estimates described in Step 1 are not included in the Full Physical Habitat version because they are generally replaced by more quantitative measurements of similar variables. However, we have found that three of the RBP measures are reasonably repeatable and include them in the reachwide assessment portion of the Full Physical Habitat version.

Note: *This is the only case in which a measurement included in the basic procedure is not included in the full.*

Module O. Reach Slope (for Basic Physical Habitat only)

Reach slope should be recorded as percent slope as opposed to degrees slope to avoid confusion. Slope measurements work best with two people, one taking the readings at the upstream transect and the other holding a stadia rod at the downstream transect. If you cannot see the mid point of the next transect from the starting point, use the supplemental sections (indicating the proportion of the total length represented by each section).

An auto level (with a tripod) should be used for reaches with a percent slope of less than or equal to 1%. All methods (clinometer, hand level, or auto level) may be used for reaches with a percent slope of greater than 1%. In reaches that are close to 1%, you will not know whether you are above or below the 1% slope cutoff. In these cases, default to use of an autolevel.

Step 1. Divide the reach into multiple segments such that stadia rod markings can be easily read with the measuring device to be employed (this is especially a factor for clinometer and hand level readings).

Step 2. Use a clinometer, hand level, or auto level to measure the percent slope of the water surface (not the streambed) between the top and bottom of each segment. Be sure to adjust for water depth by measuring from the same height above the water surface at both transects. Also be sure to record percent slope, not degrees slope. Record the segment length for each of these sections in the appropriate boxes on the BASIC slope form.

SECTION 8
OPTIONAL EXCESS SEDIMENT MEASURES

Future editions of these protocols will include supplemental modules, including a full discussion of the measurements used for calculating the excess sediment index (sometimes referred to as log relative bed stability, LRBS). However, since several of the measurements in EMAP's physical habitat protocols are interwoven into the layout of this protocol, a brief overview of the additional measurements collected for the LRBS calculations is included here for information purposes only. For detailed explanations of these measurements, consult Peck et al. 2004.

Woody Debris Tallies

Large woody debris (logs, snags, branches, etc.) that is capable of obstructing flow when the channel is at bankfull condition (just short of flood stage) contributes to the "roughness" of a channel. The effect of this variable is to reduce water velocity and thereby reduce the stream's competence to move substrate particles. The EMAP protocol tallies all woody debris with a diameter greater than 10 cm (~4") into one of 12 size classes based on the length and width of each object. Tallies are conducted in the zone between the main transects.

Thalweg Measurements

A stream's thalweg is a longitudinal profile that connects the deepest points of successive cross-sections of the stream. The thalweg defines the primary path of water flow through the reach. Thalweg measurements perform many functions in the EMAP protocols, producing measurements for the excess sediment calculations (residual pool volume, stream size, channel complexity) and flow habitat variability.

SECTION 9
OPTIONAL PERIPHYTON QUANTIFICATION

Periphyton Quantification

Characterization of periphyton has a dual role in bioassessments, as periphyton is both a food and habitat resource for benthic macroinvertebrates and fish and an effective bioindicator on its own. Quantification of periphytic resources will be covered under a separate SWAMP bioassessment protocol, but will include procedures for qualitative characterization of diatom assemblages, documentation of filamentous algal growth, and biomass quantification (e.g., ash-free dry mass and chlorophyll a).

SECTION 10
QUALITY ASSURANCE & CONTROL PROCEDURES

The SWAMP bioassessment group is currently developing guidelines for quality assurance and quality control for bioassessment procedures. Future revisions to this document will include guidance covering personnel qualifications, training and field audit procedures, procedures for field calibration, procedures for chain of custody documentation, requirements for measurement precision, health and safety warnings, cautions (actions that would result in instrument damage or compromised samples), and interferences (consequences of not following the standard operating procedure, SOP).

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DEFINITIONS OF TERMS USED IN SOP D

Terms & Definitions	
TERM	**DEFINITION**
ABL	California Department of Fish and Game's Aquatic Bioassessment Laboratory
Allocthonous	Derived from a source external to the stream channel (e.g., riparian vegetation) as opposed to autochthonous, which indicates a source inside the stream channel (e.g., periphyton).
Ambient Bioassessment	Biological monitoring that is intended to describe general biotic condition as opposed to a diagnosis of sources of impairment
Bankfull	The bankfull channel is the zone of maximum water inundation in a normal flow year (one to two year flood events)
BMI	Benthic macroinvertebrates: bottom-dwelling invertebrates large enough to be seen with the unaided eye
Cobble Embeddedness	The volume of cobble-sized particles (64-250 mm) that is buried by fine particles (<2.0 mm diameter)
CPOM	Coarse particulate organic matter (CPOM, particles of decaying organic material such as leaves that are greater than 1.0 mm in diameter)
CSBP	California State Bioassessment Procedures
DFG	California Department of Fish and Game
EMAP	The U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program
EPA	The U.S. Environmental Protection Agency
Fines	Substrate particles less than 0.06 mm diameter (not gritty to touch)
Inter-transects	Transects established at points equidistant between the main transects
MCM	Margin-Center-Margin alternative procedure for sampling low gradient habitats
ORD	EPA's Office of Research and Development
QAMP	Quality assurance management plan
RBP	EPA's Rapid Bioassessment Procedures
Reach	A segment of the stream channel
Riparian	An area of land and vegetation adjacent to a stream that has a direct effect on the stream.
RWB	Reach-wide benthos composite sampling method for benthic macroinvertebrates, also referred to as multi-habitat method
SCCWRP	Southern Coastal California Water Research Project
SNARL	Sierra Nevada Aquatic Research Laboratory
Substrate	The composition of a streambed, including both inorganic and organic particles
SWAMP	The State Water Resources Control Board's Surface Water Ambient Monitoring Program
Thalweg	A longitudinal profile that connects the deepest points at successive cross-sections of the stream. The thalweg defines the primary path of water flow through the reach

TERM	DEFINITION
Transects	Lines drawn perpendicular to the path of flow used for standardizing sampling locations
TRC	Targeted riffle composite sampling method for benthic macroinvertebrates
USFS	The United States Forest Service
Wadeable Streams	Streams that can be sampled by field crews wearing chest waders (generally less that 0.5 m - 1.0 meters deep)

APPENDIX A

FACTORS TO CONSIDER WHEN RECOMMENDING/ CHANGING BIOASSESSMENT METHODS

Beyond the primary considerations of precision and accuracy, there are at least five other key issues that SWAMP has considered and should consider in the future, when recommending or changing its official methods for bioassessment. These issues include:

1. Costs of Collecting Samples via Multiple Protocols – Collecting, processing, and interpreting samples using more than one method for each indicator (e.g., algae, macroinvertebrates, fish) per site adds significant costs to bioassessment monitoring programs. SWAMP should strive to identify the minimum set of protocols necessary for each indicator. However, this should not come at the expense of sound monitoring. If more than one method is needed to interpret the biological response, then this decision should be based on a cost-benefit assessment.

2. Costs of Maintaining Multiple SWAMP Protocols – While multiple methods for monitoring a given indicator may provide additional accuracy in specific habitats, there are significant costs to maintaining multiple protocols:

a. Need to maintain method-specific infrastructure (e.g., separate reference samples, separate indices of biotic integrity (IBIs), separate O/E models, etc.).
b. May lose or impair ability to compare across sites if different methods are used (see Issue 5 below).
c. Guidance on when to use methods becomes more complex. For example, we need to define very specifically which methods to use at each water body type; and thus, which tools can be used to interpret them.

Recommendation: *SWAMP should maintain as few protocols as necessary. If we elect to add new or modified protocols it should be because we have determined that the added value is worth all of the costs listed above.*

3. Separating Physical Impairment from Water Quality Impairment – One of the original reasons for adding a multihabitat component to SWAMP bioassessment programs was the potential for distinguishing physical and water quality impairment sources (see recommendations in Barbour and Hill 2002). In regards to macroinvertebrate indicators, the conventional wisdom has been that reachwide (RW, sometimes referred to as multihabitat or MH) samples should be relatively more responsive to physical habitat alteration (i.e., fine sediment inputs) than targeted-riffle (TR) samples because it is believed that erosional habitats take longer

to respond to sediment stresses, and because pockets of riffle habitat are thought to act as refugia from habitat loss. To the extent that this is true, RW and TR samples may offer complementary information that allows us to separate these sources of impairment.

While very few studies have addressed this conventional wisdom directly, recent studies suggest that this may not be as much a factor as previously believed. In a recent comparison of TR and RW samples at nearly 200 sites statewide, the ABL found at most weak evidence to support this notion (Rehn et al. 2007). Gerth and Herlihy (2006) came to the same conclusion in their analysis of ~500 sites in the eastern and western United States. However, this issue is far from resolved and SWAMP scientists currently are not in agreement regarding this issue. Since the majority of bioassessment programs in California have emphasized targeted riffle sampling, SWAMP will undoubtedly want to evaluate this question further before making any policy decision to discontinue TR sampling.

Recommendation: *Until this issue can be evaluated further and resolved to SWAMP's satisfaction, ambient macroinvertebrate sampling should include collection of both RW samples and richest targeted habitat (TR or MCM) samples at every site. (The TR method should be used where sufficient riffles are present, and the MCM method should be used at low-gradient sites where sufficient riffle habitat is not available.)*

4. Compatibility with Previous Data – To address this issue, at least three sets of macroinvertebrate sampling method comparisons have been conducted in California.

a. **Targeted Riffle Methods –** Comparisons are complete. Samples collected under the current TR protocols are considered interchangeable with both CSBP and SNARL samples (Ode et al. 2005, Herbst and Silldorff 2006).
b. **Low Gradient Sand-Dominated Streams –** Collaborative studies are currently underway between Water Board Regions 3 and 5, the Southern California Coastal Water Research Project (SCCWRP), and ABL to compare the performance of: (1) the "low-gradient" CSBP; (2) RW samples; and (3) a modification of the RW method designed to emphasize habitats along stream margins (MCM). The results of these low-gradient methods comparisons are not yet available.
c. **Targeted Riffle vs. Reachwide Methods –** A recent comparison of RW and TR samples collected from nearly 200 EMAP/ CMAP sites is in peer review press (Rehn et al. 2007). Results demonstrate remarkably similar performance of the methods across a wide range of habitats. Gerth and Herlihy (2006) recently published a similar analysis with the same conclusions. However, the bioassessment committee has yet to carefully review and discuss these analyses and their implications for SWAMP biomonitoring.

5. Comparability Among Sites – The ability to compare biological condition across sites is a common requirement of most ambient bioassessment programs. This type of analysis is confounded if different methods are used at these sites. One of the big advantages of reachwide (i.e., multihabitat) methods is that they can be applied anywhere because they don't require a specific habitat for sampling. Statewide

bioassessments and most regional programs will require the ability to compare their bioassessment results among multiple sites (e.g., within a watershed, within a region, statewide).

INTERIM RECOMMENDATIONS FOR MACROINVERTEBRATE SAMPLING (UPDATED DECEMBER 2006):

1. Until we can reach consensus on the outstanding issues (i.e., whether a single method for macroinvertebrate sampling will meet our needs, and the outcome of RW vs. MCM comparison studies for low-gradient wadeable streams/rivers), SWAMP recommends collecting both a reachwide (i.e., multihabitat) and a targeted habitat sample at each site. In high gradient streams, this means using both the RW and TR methods. In low-gradient streams, we recommend collecting both RW and MCM samples until the results are available from the low-gradient ("non-riffle") comparison. In rare cases where monitoring objectives cannot be met following these recommendations, the SWAMP Bioassessment Coordinator may authorize deviations. For example, where project-specific objectives differ from ambient monitoring, the SWAMP Bioassessment Coordinator may authorize alternate methods. In rare cases where funding is extremely limited and the cost of following the above recommendations would be prohibitive, the SWAMP Bioassessment Coordinator may authorize cost-saving options such as collecting both samples, but archiving one of the samples for later lab analysis.

2. SWAMP should develop guidance specifying when and where different methods should be used. For example, at "low gradient" sites, what is the slope cut-off (or other channel feature criteria to use) when deciding whether to apply TR or MCM? In addition, while SWAMP may eventually choose to adopt a single method (such as RW) at most sites, some regions may determine that the value of targeted habitat sampling merits continued sampling with supplemental protocols. In the latter case, or if SWAMP determines that distinct methods are needed for different habitat types, the guidance should specify the types of waterbodies or classes of waterbodies that require different methods.

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Surface Water
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Program

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Water Boards

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Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT)

List of Freshwater Macroinvertebrate Taxa from California and Adjacent States including Standard Taxonomic Effort Levels

1 March 2011

Austin Brady Richards and D. Christopher Rogers

Table of Contents 2

1.0 Introduction

The Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT) is charged through its charter to develop standardized levels for the taxonomic identification of aquatic macroinvertebrates in support of bioassessment. This document defines the standard levels of taxonomic effort (STE) for bioassessment data compatible with the Surface Water Ambient Monitoring Program (SWAMP) bioassessment protocols (Ode, 2007) or similar procedures. The STE is based on our current understanding of macroinvertebrate taxonomy, and this document was prepared following the STE Rules (Rogers & Richards, 2006). This list includes aquatic taxa found in streams and lakes primarily in California as well as surrounding states and ecoregions. It must be emphasized that this document is grey literature, and does not supercede any peer-reviewed literature. This document is a compliation and distillation of data gleaned from the peer-reviewed literature, museum records, the input of various taxonomic experts, and the SWAMP database. Specialized references are suggested for some taxa, however this document is not a procedural guideline, but rather a list of defined, reproducible endpoints. For the latest version of the STE document,visit the link on the SAFIT website (http://safit.org/ste.html). Although tolerance values and functional feeding group information was included in earlier versions of the STE, this information has been left out of the 2006 and present versions but are available on the SAFIT website (http://safit.org/TVFFG.html).

This STE list should not be interpreted as a comprehensive list of the aquatic macroinvertebrate fauna of the southwestern United States, although such a list is being developed by SAFIT. This STE list includes the macroinvertebrate taxa encountered in bioassessment samples as of the date of this revision, together with literature records from published taxonomic literature. The higher level taxa in this list (Phylum through Order) are organized hierarchically to reflect phylogenetic relationships, while Family through Species are listed alphabetically. The higher taxonomy of the Arthropoda is currently a subject of debate. Therefore, for this version of the STE List we have maintained a more traditional presentation of the superordinal tiers of the Arthropoda.

This information will be integrated into the SWAMP database and the California Environmental Data Exchange Network (CEDEN). Any suggestions for modifications of this list should comply with the STE Rules, and be sent to the attention of Austin Brady Richards, CDFG Aquatic Bioassessment Laboratory (arichards@csuchico.edu) or D. Christopher Rogers, Kansas Biological Survey (branchiopod@gmail.com) or any member of SAFIT's Standard Taxonomic Effort committee (see STE Rules, sections 2.2 and 2.6).

For definitions of the terms used in this document, please refer to Appendix II of the Rules document.

1.1 Acknowledgments

We want to thank the SAFIT membership for their cooperation and their role with SSCWRP in formalizing SAFIT. We gratefully acknowledge SWAMP for support and funding in this endeavor. Larry Serpa, Doug Post and Jon Lee each provided detailed reviews of the master source file and STE. Joseph Furnish provided comments on the Mollusca. We especially would like to thank the following persons for reviewing sections of the STE list from the 2006 version: Allison Brigham (Lepidoptera), Eric Chapman (Haliplidae), Doug Post (Dytiscidae), John Sandberg (Plecoptera), Joe Slusark (Ephemeroptera), Cheryl B. Barr (Byrrhoidea), William D. Shepard (Byrrhoidea), John T. Polhemus (Hemiptera), David E. Ruiter (Trichoptera), Andrew Rehn (Odonata), Rosser W. Garrison (Odonata), Norman Penny (Megaloptera and Neuroptera), Brian J. Krestian (Chironomidae); Raphael Mazor, Jonathan Lee, Tom King, Robert W. Wisseman, Dave Herbst and Michael Bogan of SNARL, and Peter Ode each provided detailed general reviews. We would like to thank Chris Rosamond, John Epler, Martin Spies and Peter S. Cranston for their input on the California and Nevada chironomid fauna. We would also like to thank Teresa Richards for her assistance with the formatting of this document.

2.0 Standard Taxonomic Effort

The goal of this document is to standardize levels of taxonomic effort among labs conducting the SWAMP bioassessment protocols or similar protocols. For benthic macroinvertebrate (BMI) datasets to be compatible, taxa need to be identified to a common, reproducible level, thus SAFIT defines levels of taxonomic resolution for all labs performing the SWAMP; i.e. the standard taxonomic effort or STE).

2.1 Rules for Developing a Standard Taxonomic Effort

Earlier versions of this document were developed by the predecesor of SAFIT, the California Aquatic Macroinvertebrate Laboratory Network (CAMLnet). During the recent reorganization of CAMLnet into SAFIT, SAFIT membership identified a need to formalize the rules for standardizing the reporting of taxonomic data used in bioassessment. This discussion led to the drafting of the first version of a rules document to accompany the STE (Rogers and Richards, 2006, herein after referred to as the STE Rules). This document defines SAFIT's rules for the validity of taxonomic names and provisional taxa, their use and reporting format in bioassessment datasets. The STE Rules document also outlines the procedures and criteria for subsequent revisions of the STE list with the proposed formation of an oversight committee for the STE.

2.2 Changes from the Previous Version

This STE is revised from the 2006 version (Richards and Rogers, 2006). Since 2006, we have added newly described taxa, made necessary changes in nomenclature including synonyms, we've deleted erroneous distribution records, and added new distribution records as well as new and additional taxonomic literature pertaining to the SAFIT region. The most visible change from the previous version is the addition of Utah to the list. The list of taxa and distribution records for Utah is still incomplete and will be a focus of the next revision of the STE. Also new for the list is the addition of authorities for all taxonomic names in the list, not just species. This ties each name to the peer-reviewed literature. Excluded taxa (as listed in the STE Rules section 3.4.3) have been added to the list with specific notes that they are excluded. Although all users of this STE and the STE Rules are ultimately responsible for their own data, we have tried to make this list easier to use and the information easier to find.

2.3 The SAFIT Standard Taxonomic List

A practical level of standard effort is determined by cost-effectiveness of identification relative to effort. Obviously, cost-effectiveness is highly dependent on taxonomic skills, but it is also determined by the availability of accurate keys and peer reviewed literature, and the degree of special methodology (e.g., slide mounting) needed to identify taxa.

Some bioassessment programs use the availability of species keys to establish standard levels of effort, and for some taxonomic groups we do provide references to species keys where they exist and if they meet the requirements of the STE Rules. However, under the SWAMP, the objective is to identify all taxa to a relatively even level of taxonomic effort. At the time of the previous revision of this list, two levels of standard effort were defined. Level I roughly corresponds to genus level identifications for all groups (where possible) except for the Chironomidae which are taken only to family and monotypic taxa which may be taken to species. Level II roughly corresponds to species level identifications for most taxonomic groups and genus/species group level identification for the Chironomidae. Taxonomic levels of effort (and exceptions) are listed for each taxonomic group.

3.0 Methods and Materials

We prepared a list of the benthic macroinvertebrates relevant to the SWAMP bioassessment protocols or similar procedures. All data was compiled based upon the standards presented in the Rules document. All accessible pertinent peer-reviewed literature was reviewed for relevant taxa and distributional records.

This document is grey literature, and does not supercede any peer-reviewed literature. It is a compliation and distillation of the peer-reviewed literature, museum records, the input of various taxonomic experts, and the ABL database, as follows the standards in the Rules document.

3.1 Habitat Information

The primary focus of this list is benthic macroinvertebrates. A few non-benthic taxa have been included and are marked as such. In future versions of this list, it is hoped that guidance on the taxonomy of all aquatic and semiaquatic invertebrates can be included. Basic habitat association (lotic, lentic and estuarine) has been included for the various taxa. This section is still under construction and will be further developed in subsequent revisions.

3.2 Geographic Scope

The STE began as a guidance document for California only. As the California Aquatic Macroinvertebrate Laboratory Network (CAMLNet) evolved into SAFIT, the area of coverage increased to include the Southwest in general. The STE has been expanded to include California and adjacent states. Washington was also included since many aquatic invertebrates have distributions ranging from California to Washington in the Cascade and Coastal Ranges. Some information has been given for distributions in Baja California as well. Utah is included in this revision of the STE and work is underway to add the western sections of Colorado and New Mexico. It is hoped that future revisions of this list will flesh out these distributions and add other sections of the southwest. All distribution information has been gathered from the peer-reviewed literature, museum records and the ABL database. Thus, this list is not meant to be a checklist for any of the groups therein, but simply a summary of available distributional information. Future revisions of the STE may include distributional updates based on bioassessment surveys and should not be taken as peer-reviewed published data by itself. This document is grey literature. We also stress that identifications should not be based solely on distribution.

3.3 Abbreviations in the STE list

CA=California, OR=Oregon, WA=Washington, NV=Nevada, AZ=Arizona, UT=Utah, Baja=Baja California (at present this term doesn't distinguish between Baja California Norte and Baja California Sur); "X"=published distributional or habitat records, "?" unpublished, but known distributional or habitat records, including those validated by taxonomic experts.

3.4 Life Stage Terminology

The information in the STE list primarily deals with those life stages of invertebrates that are aquatic. Some additional information is given for the terrestrial life stages. The term "larva" (plural: larvae) has historically been applied only to the immature, pre-pupal stage of holometabolous insects. However, in recent years, the term larva has also been applied to the immature or "nymph" stage of hemimetabolous insects. Both terms may appear in this document, although the compilers of this present edition prefer to reserve the name nymph for the immatures of hemimetabolous insect orders (Ephemeroptera, Odonata, Plecoptera, Hemiptera) and use the name larva in association with the holometabolous orders (Megaloptera, Trichoptera, Lepidoptera, Coleoptera, Diptera).

4.0 Rare, Threatened and Endangered Species

Rare, threatened and endangered species are defined to include aquatic macroinvertebrate species listed as threatened or endangered under the federal Endangered Species Act (ESA) (50 CFR 17.11 for listed animals and various Federal Register notices for proposed species), the California Endangered Species Act (CESA), and the California Environmental Quality Act (CEQA). This does not cover aquatic macroinvertebrate species listed under state law in adjacent states. Rare, threatened and endangered species are afforded various levels of protection under the aforementioned laws. Any individual, private company or agency that violates these laws may be subject to substantial fines, imprisonment, or both. Inclusion of names of rare, threatened and endangered aquatic macroinvertebrates in this document and the STE list is meant to be strictly informative and in no way authorizes collecting or harming these taxa without proper permits.

Rare species are species that may be given some protection under CEQA depending upon the action being reviewed under a specific CEQA document. These species typically are not protected, however they may at any time become listed under CESA or ESA.

Threatened species are species that are partially protected under CESA and ESA. While it is illegal to collect, harm, harass, or kill threatened species, some activities may still be legal (varying depending on the species) without the requirement of permits.

Endangered species are fully protected under CESA, CEQA and ESA. It is illegal to collect, harm, harass, or kill endangered species without the appropriate state Memorandum of Understanding and/or federal 10(A) 1(a) permits.

5.0 Literature Cited

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APPENDIX I

THE SAFIT STANDARD TAXONOMIC EFFORT LIST

Phylum: Silicea
Standard Effort Level I: Phylum
Standard Effort Level II: Phylum
Standard Taxonomic Reference: Reiswig, Frost and Ricciardi (2010)
Reviewed by:

The freshwater sponges are generally identified using Reiswig, Frost and Ricciardi (2010). They are not typically enumerated as a quantitative part of benthic samples, as they are colonial and sessile. However, their presence in samples should be noted, as most species are indicators of clean, well oxygenated water. Eernisse and Peterson (2004) showed that Porifera is paraphyletic and suggested the use of Silicea to avoid the paraphyly.

Taxonomic Hierarchy	Habitat				Distribution							Literature Cited	Comments
Phylum	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Silicea Gray, 1867	X	X	X	X	X	X	X	X	X	X	X	Reiswig, Frost and Ricciardi (2010); Eernisse and Peterson (2004)	Eernisse and Peterson showed that Porifera was paraphyletic; suggested use of Silicea to avoid the paraphyly.

Literature Cited

Eernisse, D. J., and K. J. Peterson. 2004. The history of animals. Chapter 13. [pp. 197-208]. In: J. Cracraft and M. J. Donoghue (editors), Assembling the Tree of Life. First ed. Oxford University Press, New York, New York, U.S.A.

Reiswig, H. M., T. M. Frost, and A. Ricciardi. 2010. Porifera. [pp. 91-123]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.

Phylum: Cnidaria
Standard Effort Level I: Genus
Standard Effort Level II: Genus
Standard Taxonomic Reference: Slobodkin and Bossert (2010)
Reviewed by:

Cnidarians are generally identified using Slobodkin and Bossert (2010). Fuller et al. (2011) and Mills and Sommer (1995) provide ecological information on *Cordylophora*.

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Cnidaria Hatschek, 1888							X	X	X	X	X	X	X	X	X	X	X	Slobodkin and Bossert (2010)	
	Hydrozoa Owen, 1843						X	X	X	X	X	X	X	X	X	X	X	Slobodkin and Bossert (2010)	
		Hydroida Johnston, 1836					X	X	X		X	X	X	X	X	X	X	Slobodkin and Bossert (2010)	
			Capitata Kühn, 1913				X	X	X		X	X	X	X	X	X	X	Slobodkin and Bossert (2010)	
				Hydridae Dana, 1846			X	X	X		X	X	X	X	X	X	X	Slobodkin and Bossert (2010)	
					Hydra Linnaeus, 1758		X	X	X		X	X	X	X	X	X	X	Slobodkin and Bossert (2010)	
			Anthomedusae Haeckel, 1879				X	X	X	X	X	X	X	X			X	Slobodkin and Bossert (2010)	
				Clavidae McCrady, 1859			X	X	X	X	X	X	X	X			X	Slobodkin and Bossert (2010)	
					Cordylophora Allman, 1844		X	X	X	X	X	X	X	X			X	Fuller et al. (2011); Mills and Sommer (1995); Ruiz et al. (1999)	Non-native invasive species, in brackish and coastal freshwaters, but spreading inland

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Limnomedusae Kramp, 1938				X	X	X		X	X	X		X	X	X	Slobodkin and Bossert (2010)	
				Olindiasidae Haeckel, 1879			X	X	X		X	X	X		X	X	X	Slobodkin and Bossert (2010)	
					Craspedacusta Lankester, 1880		X	X	X		X	X	X		X	X	X	Slobodkin and Bossert (2010)	
						Craspedacusta sowerbyi Lankester, 1880	X	X	X		X	X	X		X	X	X	Slobodkin and Bossert (2010)	

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Mills, C. E., and F. Sommer. 1995. Invertebrate introductions in marine habitats: two species of hydromedusae (Cnidaria) native to the Black Sea, *Maeotias inexspectata* and *Blackfordia virginica*, invade San Francisco Bay. Marine Biology **122**:279-288.

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Slobodkin, L. B., and P. E. Bossert. 2010. Cnidaria. [pp. 125-142]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.

Additional Sources of Information on Cnidaria

Fuller, P., E. Maynard, & D. Raikow. 2011. *Cordylophora caspia*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. Revision Date: 8/15/2009. Accessed 14 February 2011 at URL: http://nas.er.usgs.gov/queries/FactSheet.asp?SpeciesID=1060

Phylum: Platyhelminthes
Standard Effort Level I: Class
Standard Effort Level II: Class
Standard Taxonomic Reference: Kolasa and Tyler (2010)
Reviewed by:

Platyhelminthes are identified only to class level using Kolasa and Tyler (2010). Most characters for separating taxa are internal, and there is some confusion regarding the identity of many taxa. Many turbellarians cannot be accurately placed to order even by experts (Dr. John Holleman, personal communication).

Taxonomic Hierarchy		Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Platyhelminthes Gegenbauer, 1859		X	X	X	X	X	X	X	X	X	X	X	Kolasa and Tyler (2010)	
	Turbellaria Ehrenberg, 1821	X	X	X	X	X	X	X	X	X	X	X	Kolasa and Tyler (2010)	

Literature Cited

Kolasa, J., and S. Tyler. 2010. Turbellarians and Nemertea. [pp. 143-161]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.

Phylum: Nemertea
Standard Effort Level I: Genus
Standard Effort Level II: Genus
Standard Taxonomic Reference: Kolasa and Tyler (2010)
Reviewed by:

Freshwater nemerteans are monogeneric, and are identified using Kolasa and Tyler (2010).

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Order	Family	Genus	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Nemertea Schultze, 1851					X	X	X		X	X	X	X	X	X	X	Kolasa and Tyler (2010)	
	Enopla Schultze, 1851				X	X	X		X	X	X	X	X	X	X	Kolasa and Tyler (2010)	
		Hoplonemertea Hubrecht, 1879			X	X	X		X	X	X	X	X	X	X	Kolasa and Tyler (2010)	
			Tetrastemmatidae Hubrecht, 1879		X	X	X		X	X	X	X	X	X	X	Kolasa and Tyler (2010)	
				Prostoma Duges, 1828	X	X	X		X	X	X	X	X	X	X	Kolasa and Tyler (2010)	

Literature Cited

Kolasa, J., and S. Tyler. 2010. Turbellarians and Nemertea. [pp. 143-161]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.

Phylum: Nemata
Standard Effort Level I: excluded from benthic datasets
Standard Effort Level II: excluded from benthic datasets
Standard Taxonomic Reference: Poinar (2010)
Reviewed by:

Nematoda is now considered to be a junior synonym of Nemata Cobb, 1919 (Brusca and Brusca, 2003). Nematodes are typically left at phylum. The vast majority of freshwater nematodes are not large enough to be considered "macroinvertebrates". Typically, the only "macro" nematodes encountered in benthic samples are in the family Mermithidae, which are parasitic on dipterans and ephemeropterans. As they are parasites, they are of little ecological importance. (See STE Rules section 3.4.3)

Taxonomic Hierarchy	Habitat				Distribution							Literature Cited	Comments
Phylum	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Nemata Cobb, 1919	X	X	X	X	X	X	X	X	X	X	X	Poinar (2010)	Fresh and brackish; excluded from benthic datasets

Literature Cited

Brusca, R.C. and G.J. Brusca. 2003. Invertebrates, 2nd ed. Sinauer Associates, Sunderland, MA. 936 pp.

Poinar, G. O., Jr. 2010. Nematoda and Nematomorpha. [pp. 237-276]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.

Phylum: Nematomorpha
Standard Effort Level I: excluded from benthic datasets
Standard Effort Level II: excluded from benthic datasets
Standard Taxonomic Reference: Poinar (2010)
Reviewed by:

Nematomorphans are typically excluded from SWAMP bioassessment datasets. As they are parasites of terrestrial insects, and do not feed as free living adults, they are of little ecological importance (See STE Rules Section 3.4.3).

Taxonomic Hierarchy	Habitat				Distribution							Literature Cited	Comments
Phylum	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Nematomorpha Vejdovsky, 1886	X	X	X		X	X	X	X	X	X	X	Poinar (2010)	Excluded from benthic datasets

Literature Cited

Poinar, G. O., Jr. 2010. Nematoda and Nematomorpha. [pp. 237-276]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.

Phylum: Entoprocta
Standard Effort Level I: Genus
Standard Effort Level II: Genus
Standard Taxonomic Reference: Wood (2010)
Reviewed by:

Entoprocta are generally identified using Wood (2010). Entoprocts are not typically enumerated as a quantitative part of benthic samples, as they are colonial and sessile. However, their presence in samples should be noted, as they are non-native invasive species in the western US (Eng, 1977), and are tolerant to a variety of organic pollutants, low oxygen, and high TDS (Wood, 2010).

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Phylum	Order	Family	Subfamily	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Entoprocta Nitsche, 1870						X	X	X		X				X			Wood (2010)	
	Urnatellida Annandale, 1915					X	X	X		X				X			Eng (1977)	
		Urnatellidae Annandale, 1915				X	X	X		X				X			Eng (1977)	
				Urnatella Leidy, 1851		X	X	X		X				X			Eng (1977)	
					Urnatella gracilis Leidy, 1851	X	X	X		X				X			Eng (1977)	

Literature Cited

Eng, L.L. 1977. The freshwater entoproct *Urnatella gracilis* Leidy, in the Delta-Mendota Canal, California. Wasmann Journal of Biology **39**:56-62

Wood, T. S. 2010. Bryozoans. [pp. 437-454]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.
.

Phylum: Ectoprocta
Standard Effort Level I: Class
Standard Effort Level II: Class
Standard Taxonomic Reference: Wood (2010)
Reviewed by:

Ectoprocta (formerly Bryozoa) are generally identified using Wood (2010). Ectoprocts are not typically enumerated as a quantitative part of benthic samples, as they are colonial and most taxa are sessile. However, their presence in samples should be noted, as they are indicators of clean, well oxygenated water.

Taxonomic Hierarchy		Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Ectoprocta Nitsche, 1869		X	X	X	X	X	X	X	X	X	X	X	Wood (2010)	
	Phylactolaemata Allman, 1856	X	X	X	X	X	X	X	X	X	X	X	Wood (2010)	

Literature Cited

Wood, T. S. 2010. Bryozoans. [pp. 437-454]. In: J. H. Thorp and A. P. Covich (editors), Ecology and classification of North American freshwater invertebrates, third edition, xiv + 1021 pp. Academic Press, San Diego, CA.
.

Phylum: Mollusca
Standard Effort Level I: Genus
Standard Effort Level II: Genus/ Species
Standard Taxonomic Reference: Dillon (2006), Burch (1972), Nedeau et al. (2006)
Reviewed by:

The freshwater mollusks of western North America have a long and convoluted taxonomic history, with much confusion in the literature. Snails should be identified using Perez et al. (2004) and Dillon (2006). Since the 1980s most workers have followed Burch's (1982, and various iterations thereafter) book on freshwater gastropods, wherein he made many taxonomic changes. However, Burch's work was not peer reviewed, nor published in the peer reviewed literature. For that reason, we follow Hubendick (1951) for the genus *Lymnaea*, Clarke (1981) for *Valvata*, Henderson (1929) for *Juga*, and Baker (1945) for the Planorbidae and Wethington (2004) for the Physidae.

For the bivalves, the Burch (1972) keys remain the best available for the sphaericean clams. Freshwater mussels west of the continental divide are separated using Nedeau et al. (2006).

The freshwater snails, clams and mussels are ecologically significant, and their taxonomic relationships are poorly understood. Immature animals are not identifiable due to the tremendous amount of convergence in juvenile forms, and many groups cannot be identified beyond genus level without dissection. Non-native invasive species, particularly the asian clam, *Corbicula*, and the New Zealand Mudsnail, *Potamopyrgus* are ecological threats. Montana State University provides a webpage with useful information on the taxonomy and ecology of *Potamopyrgus*.

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Mollusca Linnaeus, 1758							X	X	X		X	X	X	X	X	X	X	Burch (1975); Nedeau et al. (2006)	
	Bivalvia Linnaeus, 1758						X	X	X		X	X	X	X	X	X	X	Burch (1975);	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																		Nedeau et al. (2006)	
		Palaeoheterodonta Newell, 1965					X	X	X		X	X	X	X	X	X	X	Burch (1975); Nedeau et al. (2006)	
			Unionoida Stoliczka, 1871				X	X	X		X	X	X	X	X	X	X	Burch (1975); Nedeau et al. (2006)	
				Unionidae Fleming, 1828			X	X	X		X	X	X	X	X	X	X	Burch (1975); Nedeau et al. (2006)	
					Anodonta Lamarck, 1799		X	X			X	X	X	X	X	X	X	Burch (1975); Nedeau et al. (2006)	
					Gonidea Conrad, 1857		X	X			X	X	X		X			Burch (1975); Nedeau et al. (2006)	
						Gonidea angulata (Lea, 1838)	X	X			X	X	X		X			Burch (1975); Nedeau et al. (2006)	
				Margaritiferidae Haas, 1940			X	X			X	X	X	X	X	X		Burch (1975); Nedeau et al. (2006)	
					Margaritifera Schumacher, 1817		X	X			X	X	X	X	X	X		Burch (1975); Nedeau et al. (2006)	
						Margaritifera falcata (Gould, 1850)	X	X			X	X	X	X	X	X		Burch (1975); Nedeau et	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																		al. (2006)	
		Heterodonta Neumayr, 1884					X	X			X	X	X	X	X	X	X		
			Veneroida Adams and Adams, 1856				X	X			X	X	X	X	X	X	X		
				Corbiculidae Gray, 1847			X	X			X	X	X	X	X	X	X		
					Corbicula Megerle von Mühlfeld, 1811		X	X			X	X	X	X	X	X	X	Hanna (1966)	Non-native invasive species
				Sphaeriidae Deshayes, 1854			X	X	X		X	X	X	X	X	X	X	Burch (1972)	
					Musculium Link, 1807		X	X	X		X	X	X	X	X	X		Burch (1972)	
						Musulium lacustre (Müller, 1774)	X	X	X		X	X	X					Burch (1972)	
						Musulium partumeium (Say, 1822)	X	X	X		X	X	X	X	X	X		Burch (1972)	
						Musulium secuirs Prime, 1851	X	X	X		X	X	X					Burch (1972)	
					Pisidium Pfeiffer, 1821		X	X	X		X	X	X	X	X	X	X	Burch (1972)	
						Pisidium casertanum (Poli, 1795)	X	X	X		X	X	X	X	X	X	X	Burch (1972)	
						Pisidium compressum Prime, 1852	X	X	X		X	X	X	X	X	X	X	Burch (1972)	
						Pisidium conventus Clessin, 1877	X	X	X				X					Burch (1972)	
						Pisidium ferrugineum Prime, 1852	X	X	X				X	X				Burch (1972)	
						Pisidium idahoense Roper, 1890	X	X	X		X		X					Burch (1972)	
						Pisidium insigne Gabb, 1868	X	X	X				X	X				Burch (1972)	
						Pisidium lilljeborgi Clessin, 1886	X	X	X		X	X	X	X				Burch (1972)	
						Pisidium nitidum Jenyns, 1832	X	X	X		X	X	X	X	X	X		Burch (1972)	
						Pisidium rotundatum Prime, 1851	X	X	X				X	X				Burch (1972)	
						Pisidium subtruncatum Malam, 1855	X	X	X		X	X	X	X				Burch (1972)	
						Pisidium ultramontanum Prime, 1865	X	X	X			X	X					Burch (1972)	
						Pisidium variabile Prime, 1852	X	X	X		X	X	X	X	X	X		Burch (1972)	
						Pisidium ventricosum Prime, 1851	X	X	X				X	X				Burch (1972)	
						Pisidium walkeri Sterki, 1895	X	X	X					X		X		Burch (1972)	
					Sphaerium Scopoli, 1777		X	X	X		X	X	X	X	X	X	X	Burch (1972)	
						Sphaerium nitidum Clessin, 1876	X	X	X				X	X				Burch (1972)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Sphaerium occidentale (Gould, 1850)	X	X	X			X	X	X				Burch (1972)	
						Sphaerium patella (Gould, 1850)	X	X	X		X	X	X					Burch (1972)	
						Sphaerium striatum (Lamarck, 1818)	X	X	X		X	X	X	X	X	X	X	Burch (1972)	
	Gastropoda Cuvier, 1797						X	X	X		X	X	X	X	X	X			
		Prosobranchia Milne-Edwards, 1848					X	X	X		X	X	X	X					
			Architaenioglossa Haller, 1892				X	X	X		X	X	X	X					
				Viviparidae Gray, 1847			X	X	X		X	X	X	X					
					Bellamya Jousseaume, 1886		X	X	X		X	X	X	X					
						Bellamya chinensis (Gray, 1817)	X	X	X		X	X	X	X	X	X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
						Bellamya japonica (von Martens, 1861)	X	X	X		X	X	X		X	X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
				Ampullariidae Gray, 1824			X	X	X		X				X	X			
					Marisa Gray, 1824		X	X	X		X				X	X			
						Marisa cornuarietis (Linnaeus, 1758)	X	X	X		X				X	X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
					Pomacea Perry, 1810		X	X	X		X				X	X			
						Pomacea bridgesii (Reeve, 1856)	X	X	X		X				X	X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
						Pomacea canaliculata (Lamarck, 1828)	X	X	X		X				X	X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
						Pomacea paludosa (Say, 1829)	X	X	X		X				X	X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
			Neotaenioglossa Ponder and Lindberg, 1997				X	X	X		X			X	X	X			
				Thiaridae Gill, 1871			X	X	X		X			X	X	X			
					Melanoides Olivier, 1904		X	X	X		X			X	X	X			

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Melanoides tuberculatus (Müller, 1774)	X	X	X		X			X	X	X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
					Tarebia Adams and Adams, 1854		X	X	X		X			X		X			
						Tarebia granifera (Lamarck, 1822)	X	X	X		X			X		X		Perez et al. (2004)	Non-native invasive species
			Sorbeoconcha Ponder and Lindberg, 1997				X	X			X	X	X						
				Pleuroceridae Fischer, 1885			X	X			X	X	X						
					Juga Adams and Adams, 1854		X	X			X	X	X						
						Juga acutifilosa (Stearns, 1890)	X	X			X	X	X					Perez et al. (2004); Dillon (2006)	
						Juga bulbosa (Gould, 1847)	X	X			X	X						Perez et al. (2004); Dillon (2006)	
						Juga hemphilli (Henderson, 1935)	X	X				X	X					Perez et al. (2004); Dillon (2006)	formerly listed as *Goniobasis hemphilli* Henderson, 1935
						Juga interioris (Goodrich, 1944)	X	X							X				
						Juga laurae (Goodrich, 1944)	X	X							X				
						Juga nigrina (Lea, 1856)	X	X			X	X						Perez et al. (2004); Dillon (2006)	
						Juga plicifera (Lea, 1838)	X	X			X	X	X					Perez et al. (2004); Dillon (2006)	
						Juga silicula (Gould, 1847)	X	X			X	X	X					Perez et al. (2004); Dillon (2006)	
			Hypsogastropoda Ponder & Lindberg, 1997				X	X	X	X	X	X	X	X	X	X			
				Amnicolidae Tryon, 1863			X	X			X	X	X	X	X				
					Amnicola Gould and Haldeman, 1840		X	X			X	X	X	X	X				

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Amnicola limosa (Say, 1817)	X	X			X	X	X	X	X			Perez et al. (2004)	
					Colligyrus Hershler, 1999		X	X			X	X							
						Colligyrus convexus Hershler, Frest, Liu and Johannes, 2003	X	X			X								
						Colligyrus greggi (Pilsbry, 1935)	X	X				X						Perez et al. (2004); Dillon (2006)	
				Cochliopidae Tryon, 1866			X	X	X	X	X			X	X	X			
					Eremopyrgus Hershler, 1999		X	X							X				
						Eremopyrgus eganensis Hershler, 1999	X	X							X			Perez et al. (2004)	
					Ipnobius Hershler, 2001		X	X			X								
						Ipnobius robustus (Hershler, 1989)	X	X			X							Perez et al. (2004)	
					Tryonia Stimpson, 1865		X	X	X	X	X			X	X	X		Perez et al. (2004)	
				Hydrobiidae Simpson, 1865			X	X			X	X	X	X	X	X			
					Pyrgulopsis Call and Pilsbry, 1886		X	X			X	X	X	X	X	X		Perez et al. (2004); Dillon (2006)	
				Lithoglyphidae Tryon, 1863			X	X			X	X	X	X	X				
					Fluminicola Stimpson, 1865		X	X			X	X	X	X	X			Perez et al. (2004); Dillon (2006)	
				Hypsogastropoda *Incertae sedis*			X	X			X	X	X		X	X			family associations for these genera not yet determined
					Potamopyrgus Stimpson, 1856		X	X			X			X		X			
						Potamopyrgus antipodarum (Gray, 1843)	X	X			X			X		X		Perez et al. (2004); Dillon (2006)	Non-native invasive species
					Pristinicola Hershler, Frest, Johannes, Bowler and Thompson, 1994		X	X			X	X	X						

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Pristinicola hemphilli (Pilsbry, 1890)	X	X			X	X	X					Perez et al. (2004); Dillon (2006)	
				Assimineidae Adams and Adams, 1856			X			X	X								
					Assiminea Fleming, 1828		X			X	X								
						Assiminea californica (Tryon, 1875)	X			X	X							Perez et al. (2004)	
						Assiminea infima Berry, 1947	X			X	X							Perez et al. (2004)	
				Pomatiopsidae Stimpson, 1865			X			X	X	X	X						
					Pomatiopsis Tryon, 1862		X			X	X	X	X					Perez et al. (2004)	
			Heterostropha Fischer, 1885				X	X	X		X	X	X						
				Valvatidae Gray, 1840			X	X	X		X	X	X						
					Valvata Müller, 1774		X	X	X		X	X	X						
						Valvata humeralis Say, 1829	X	X	X		X	X	X					Perez et al. (2004); Dillon (2006)	
						Valvata tricarinata (Say, 1817)	X	X	X		X	X	X					Perez et al. (2004); Dillon (2006)	
						Valvata utahensis Call, 1844	X	X	X		X	X	X	X				Perez et al. (2004); Dillon (2006)	
						Valvata virens Tryon, 1863	X	X	X		X							Perez et al. (2004); Dillon (2006)	
		Pulmonata Cuvier in Blainville, 1814					X	X	X		X	X	X	X	X	X	X		
			Basommatophora Keferstein in Bronn, 1864				X	X	X		X	X	X	X	X	X	X		
				Lymnaeidae Rafinesque, 1815			X	X	X		X	X	X	X	X	X	X		
					Fisherola Hannibal, 1912		X	X	X			X	X						
						Fisherola nuttalli (Haldeman, 1841)	X	X	X			X	X					Perez et al. (2004); Dillon (2006)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Lanx Clessin, 1882		X	X	X		X	X	X					Henderson (1929)	
						Lanx alta (Tryon, 1869)	X	X	X		X	X						Henderson (1929)	
						Lanx hannai Walker, 1925	X	X	X		X							Henderson (1929)	Found in NW CA outside of Central Valley
						Lanx klamathensis Hannibal, 1912	X	X	X			X						Henderson (1929)	
						Lanx nuttallii (Haldeman, 1841)	X	X	X				X					Henderson (1929)	
						Lanx patelloides (Lea, 1856)	X	X	X		X	X						Perez et al. (2004); Dillon (2006)	
						Lanx subrotundatus (Tryon, 1869)	X	X	X			X						Perez et al. (2004); Dillon (2006); Henderson (1929)	
					Lymnaea Lamarck, 1799		X	X	X		X	X	X	X	X	X	X	Perez et al. (2004); Dillon (2006)	
				Physidae Fitzinger, 1833			X	X	X		X	X	X	X	X	X	X		
					Aplexa Fleming, 1820		X		X				X	X					
						Aplexa elongata (Say, 1821)	X		X				X	X				Wethington (2004); Dillon (2006)	
					Physa Draparnaud, 1801		X	X	X		X	X	X	X	X	X	X		
						Physa acuta Draparnaud, 1805	X	X	X		X	X	X	X	X	X	X	Wethington (2004); Dillon (2006)	
						Physa gyrina Say 1821	X	X	X		X	X	X	X	X	X	X	Wethington (2004); Dillon (2006)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Physa pomilia (Conrad, 1834)	X	X	X		X	X	X		X	X	X	Wethington (2004); Dillon (2006)	
						Physa zionis Pilsbry, 1926	X	X						X				Wethington (2004); Dillon (2006)	
				Ellobiidae Pfeiffer, 1854			X	X	X		X	X	X						
					Ovatella Bivona, 1832		X	X	X		X	X	X						
						Ovatella myosotis (Draparnaud, 1801)	X	X	X		X	X	X					Hanna (1966)	Non-native invasive species
				Planorbidae Rafinesque, 1815			X	X	X		X	X	X	X	X	X	X		
					Biomphalaria Preston, 1910		X	X	X							X			
						Biompholaria havanensis (Pfieffer, 1839)	X	X	X							X		Perez et al. (2004); Dillon (2006)	
					Ferrissia Walker, 1903		X	X	X		X	X		X	X			Perez et al. (2004); Dillon (2006)	Ancylidae synonymized into Planorbidae (Albrecht et al, 2006)
					Gyraulus Charpentier, 1837		X	X	X		X	X	X	X	X	X			
						Gyraulus circumstriatus (Tryon, 1866)	X	X	X		X	X	X	X	X	X		Perez et al. (2004); Dillon (2006)	
						Gyraulus crista (Linnaeus, 1758)	X	X	X		X	X	X	X	X			Perez et al. (2004); Dillon (2006)	
						Gyraulus deflectus (Say, 1824)	X	X	X		X	X	X	X	X	X		Perez et al. (2004); Dillon (2006)	
						Gyraulus parvus (Say, 1817)	X	X	X		X	X	X	X				Perez et al. (2004); Dillon (2006)	
					Helisoma Swainson, 1840		X	X	X		X	X	X	X	X	X	X		

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Phylum	Class	Subclass	Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Helisoma anceps (Menke, 1830)	X	X	X		X	X	X	X	X	X	X	Perez et al. (2004); Dillon (2006)	
						Helisoma newberryi (Lea, 1858)	X	X	X		X	X	X					Perez et al. (2004); Dillon (2006)	
						Helisoma subcrenatum (Carpenter, 1857)	X	X	X		X	X		X				Perez et al. (2004); Dillon (2006)	Non-native invasive species
					Menetus Adams and Adams, 1855		X	X	X		X	X	X						
						Menetus opercularis (Gould, 1847)	X	X	X		X	X	X					Perez et al. (2004); Dillon (2006)	
					Micromenetus Baker, 1945		X	X	X										
						Micromenetus dilatatus (Gould, 1841)	X	X	X		X	X	X					Perez et al. (2004); Dillon (2006)	
					Promenetus Baker, 1935		X	X	X					X					
						Promenetus exacuous (Say, 1821)	X	X	X			X	X	X				Perez et al. (2004); Dillon (2006)	
						Promenetus umbilicatellus (Cockerell, 1887)	X	X	X			X	X	X				Perez et al. (2004); Dillon (2006)	
					Vorticifex Meek in Dall, 1870		X	X	X										
						Vorticifex effusa (Lea, 1856)	X	X	X		X	X						Perez et al. (2004); Dillon (2006)	
						Vorticifex solida (Dall 1870)	X	X	X		X				X			Perez et al. (2004); Dillon (2006)	may be a synonym of *V. effusa* (Lea)

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Phylum: Annelida
Standard Effort Level I: Class
Standard Effort Level II: Oligochaeta and Branchiobdella to class, Hirudinea to genus, Polychaeta to species.
Standard Taxonomic References: Kathman and Brinkhurst (1998), Govedich et al. (2010), Klemm (1972), Foster (1972)
Reviewed by:

Annelids are generally identified using Kathman and Brinkhurst (1998) or Govedich et al. (2010). Hirudinea can be identified using Govedich et al. (2010), Klemm (1972) and Klemm (1995). Polychaetes are best identified using Foster (1972). Branchiobdella are typically excluded from SWAMP bioassessment samples as they are commensals on crayfish (see STE Rules section 3.4.3).

Taxonomic Hierarchy									Habitat				Distribution							Literature Cited	Comments
Phylum	Subphylum	Class	Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Annelida Lamarck, 1809									X	X	X		X	X	X	X	X	X	X		
		Hirudinea Lamarck, 1818							X	X	X		X	X	X	X	X	X	X		
				Arhynchobdellida Blanchard, 1894					X	X	X		X	X	X	X	X	X	X		
						Haemopidae Richardson, 1969/Hirudinidae Whitman, 1886			X	X	X		X	X	X	X	X	X	X	Klemm (1972); Klemm (1995)	
						Erpobdellidae Blanchard, 1894			X	X	X		X	X	X	X	X	X	X	Klemm (1972); Klemm (1995)	
							Dina Blanchard, 1892						X	X		X					
							Erpobdella de Blainville, 1818						X	X	X	X					
							Mooreobdella Pawlowski, 1955						X								
				Rhynchobdellida Blanchard, 1894					X	X	X		X	X	X	X	X	X	X		
						Glossiphoniidae Vailliant, 1890			X	X	X		X	X	X	X	X	X	X	Klemm (1972); Klemm (1995)	
							Helobdella Blanchard, 1896		X	X	X		X	X	X	X	X	X			
							Placobdella Blanchard, 1893		X	X	X						X	X			
						Piscicolidae Johnston, 1865			X	X	X		X	X	X	X	X	X	X	Klemm (1972); Klemm (1995)	

Taxonomic Hierarchy									Habitat				Distribution							Literature Cited	Comments
Phylum	Subphylum	Class	Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Branchiobdella Holt, 1965							X	X	X		X	X	X	X	X	X			Excluded from benthic datasets
		Polychaeta Grube, 1850							X	X	X	X	X	X	X				X		
				Canalipalpata Rouse and Fauchald, 1997					X	X	X	X	X	X	X				X		
						Serpulidae Johnston, 1865			X	X	X	X	X						X		
							Ficopomatus Southern, 1921		X	X	X	X	X						X		
								Ficopotamus enigmaticus (Fauvel, 1922)	X	X	X	X	X						X	Foster (1972)	Fresh and brackish
						Sabellidae Malmgren, 1867			X	X	X		X	X	X						
							Manayunkia Leidy, 1859		X	X	X		X	X	X						
								Manayunkia speciosa Leidy, 1858	X	X	X		X	X	X					Foster (1972)	
			Palpata Rouse and Fauchald, 1997						X	X	X	X	X	X	X						
				Aciculata *Rouse and Fauchald, 1997*					X	X	X	X	X	X	X						
					Phylodocida Dales, 1962				X	X	X	X	X	X	X						
						Nereididae Johnston, 1865			X	X	X	X	X	X	X						
							Lycastoides Johnson, 1903		X	X			X								
								Lycastoides alticola Johnston, 1903	X	X			X							Foster (1972)	Only known from a single mountain stream in Sierra Laguna.
							Neanthes Kinberg, 1866		X	X	X		X	X	X						
								Neanthes limnicola (Johnson, 1901)	X	X	X		X	X	X					Foster (1972)	
							Nereis Linnaeus, 1758		X	X	X	X	X	X	X						
								Nereis succinea Frey & Leuckart, 1847	X	X	X	X	X	X	X					Foster (1972)	Fresh and brackish
							Namanereis Chamberlin, 1919		X	X	X		X								
								Namanereis hawaiiensis (Johnson, 1903)	X	X	X		X							Foster (1972)	Native to Hawai'i, found in a pond in southern CA
			Scolecida Benham, 1894						X	X	X		X	X	X						

Taxonomic Hierarchy									Habitat				Distribution							Literature Cited	Comments
Phylum	Subphylum	Class	Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Aeolosomatidae Beddard, 1895			X	X	X		X	X	X						
							Aeolosoma Ehrenberg, 1828		X	X	X		X	X	X						
	Clitellata Michaelsen, 1919								X	X	X	X	X	X	X	X	X	X	X		
		Oligochaeta Grube, 1850							X	X	X	X	X	X	X	X	X	X	X	Kathman and Brinkhurst (1998); Brinkhurst and Gelder (2001)	

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Klemm, D. 1995. Identification Guide to the Freshwater Leeches (Annelida: Hirudinea) of Florida and other Southern States. State of Florida, Department of Environmental Protection, Division of Water Facilities, Tallahassee.

Acari: Mites
Standard Effort Level I&II: Genus (where possible)
Standard Taxonomic Reference: Smith, Cook and Smith (2010)
Reviewed by:

This section remains relatively unchanged since the previous revision of the STE. The standard text is the chapter in Thorp and Covich (Smith et al., 2010), which provides keys to mature and immature specimens. Cook (1974) is an excellent source for detailed illustrations to supplement the newer key. The information in the table below reflects material identified from benthic samples from California streams (primarily by the ABL, SNARL and Jon Lee Consulting), and should not be taken as an authoritative list. An "X" in the distribution column refers to published records, while a "?" refers to known but unpublished records.

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Acari Leach, 1817						X	X	X		X	X	X	X	X	X	X	Smith, Cook and Smith (2010), Cook (1974)	
	Sarcoptiformes Reuter, 1909					X	X	X		X	X	X	X	X	X	X	Smith, Cook and Smith (2010), Cook (1974)	
		Oribatei Dugès, 1833				X	X	X		X	X	X	X	X	X	X	Smith, Cook and Smith (2010), Cook (1974)	Oribatid mites are presently excluded from benthic datasets; this is subject to change in the future as there are some aquatic oribatids
	Trombidiformes Reuter, 1909					X	X	X		X	X	X	X	X	X	X	Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Acalyptonotidae Walter, 1911			X					X						Smith, Cook and Smith (2010), Cook (1974); Smith (1983)	
				Acalyptonotus Walter, 1911		X					X						Smith, Cook and Smith (2010), Cook (1974); Smith (1983)	
			Anisitsiellidae Koenike, 1910			X				X	X	X					Smith, Cook and Smith (2010), Cook (1974)	
				Bandakia Thor, 1913		X					X						Smith, Cook and Smith (2010), Cook (1974); Smith (1979)	
				Utaxatax Habeeb, 1964		X				X	X	X					Smith, Cook and Smith (2010), Cook (1974); Smith (1979)	
			Arrenuridae Thor, 1900			X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Arrenurus Dugés, 1834		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Athienemanniidae Viets, 1922			X				X	X	X	X				Smith, Cook and Smith (2010), Cook (1974); Smith (1992)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Chelomideopsis Romijn, 1920		X				?	X	X					Smith, Cook and Smith (2010), Cook (1974); Smith (1992)	Smith (1992) provides a key to species
				Platyhydracarus Smith, 1989		X				X	X	X	X				Smith, Cook and Smith (2010), Cook (1974); Smith (1989)	
			Aturidae Thor, 1900			X				X					X		Smith, Cook and Smith (2010), Cook (1974)	
				Aturus Kramer 1875		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Axonopsis Piersig, 1893		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Brachypoda Lebert, 1879		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Erebaxonopsis Motas and Tanasachi, 1947		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Estellacarus Habeeb, 1954		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Ljania Thor, 1898		X				?							Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Lethaxona Viets, 1932		X					X						Smith, Cook and Smith (2010), Cook (1974)	
				Phreatobrachypoda Cook, 1963		X				X	X						Smith, Cook and Smith (2010), Cook (1974)	
				Stygalbiella Cook, 1974		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Woolastookia Habeeb, 1954		X				X							Smith, Cook and Smith (2010), Cook (1974)	
			Chappuisididae Motas and Tanasachi, 1946			X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Chappuisides Szalay, 1943		X				X							Smith, Cook and Smith (2010), Cook (1974)	
			Eylaidae Leach, 1815			X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Eylais Latreille, 1796		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Feltriidae Viets, 1926			X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Feltria Koenike, 1892		X				X							Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Frontipodopsidae Viets, 1931			X				X		X					Smith, Cook and Smith (2010), Cook (1974)	
				Frontipodopsis Walter, 1919		X				X		X					Smith, Cook and Smith (2010), Cook (1974)	
			Hydrodromidae Viets, 1936			X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Hydrodroma Koch, 1837		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Hydrovolziidae Thor, 1905			X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Hydrovolzia Thor, 1905		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Hydryphantidae Thor, 1900			X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Cyclothyas Lundblad, 1941		X					X						Smith, Cook and Smith (2010), Cook (1974)	
				Partnunia Piersig, 1896		X				?	X						Smith, Cook and Smith (2010), Cook (1974)	
				Protzia Piersig, 1896		X				?							Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Thyas Koch, 1836		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Thyopsis Piersig, 1899		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Thyopsoides Smith and Cook, 1999		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Wandesia Schechtel, 1912		X				X							Smith, Cook and Smith (2010), Cook (1974)	
			Hygrobatidae Koch, 1842			X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Atractides Koch, 1837		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Corticacarus Lundblad, 1936		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Hygrobates Koch, 1837		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Mesobates Thor, 1901		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Lebertiidae Thor, 1900			X				X							Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Estelloxus Habeeb, 1963		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Lebertia Neuman, 1880		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Scutolebertia Smith, 1991		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Limnesiidae Thor, 1900			X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Kawamuracarus Uchida, 1937		X									X		Smith, Cook and Smith (2010), Cook (1974)	
				Limnesia Koch, 1836		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Neomamersa Lundblad, 1953		X									X		Smith, Cook and Smith (2010), Cook (1974)	
				Neotyrrellia Lundblad, 1938		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Tyrrellia Koenike, 1896		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Limnocharidae Grube, 1859			X				X							Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Limnochares Latreille, 1796		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Neolimnochares Lundblad, 1937		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Mideopsidae Koenike, 1910			X				?	X	X					Smith, Cook and Smith (2010), Cook (1974)	
				Mideopsis Neuman, 1880		X				?	X	X					Smith, Cook and Smith (2010), Cook (1974)	
			Momoniidae Viets, 1926			X				X	X	X					Smith, Cook and Smith (2010), Cook (1974)	
				Momonia Halbert, 1906		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Stygomomonia Szalay, 1943		X				X	X	X					Smith, Cook and Smith (2010), Cook (1974)	
			Omartacaridae Cook, 1963			X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Omartacarus Cook, 1963		X				X							Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Nudomideopsidae Smith, 1990			X					X						Smith, Cook and Smith (2010), Cook (1974), Smith (1983)	
				Neomideopsis Smith, 1983		X					X						Smith, Cook and Smith (2010), Cook (1974); Smith (1983)	
				Nudomideopsis Szalay, 1945		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Paramideopsis Smith, 1983		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Oxidae Viets, 1926			X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Frontipoda Koenike, 1891		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Oxus Kramer, 1877		X				X							Smith, Cook and Smith (2010), Cook (1974)	
			Pionidae Thor, 1900			X				X				X			Smith, Cook and Smith (2010), Cook (1974)	
				Nautarachna Moniez, 1988		X				X				X			Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Pontarachnidae Koenike, 1910			X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Pontarachna Philippi, 1840		X				X							Smith, Cook and Smith (2010), Cook (1974)	
			Sperchontidae Thor, 1900			X				?	X						Smith, Cook and Smith (2010), Cook (1974)	
				Sperchon Kramer, 1877		X				?	X						Smith, Cook and Smith (2010), Cook (1974)	
				Sperchonopsis Piersig, 1896		X				?	X						Smith, Cook and Smith (2010), Cook (1974)	
			Thermacaridae Sokolow, 1927			X				X							Smith, Cook and Smith (2010), Cook (1974)	
					Thermacarus Sokolow, 1927	X				X							Smith, Cook and Smith (2010), Cook (1974)	
			Torrenticolidae Piersig, 1902			X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Neoatractides Lundblad, 1941		X									X		Smith, Cook and Smith (2010), Cook (1974)	
				Pseudotorrenticola Walter, 1906		X				?							Smith, Cook and Smith (2010), Cook (1974)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Subclass	Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Testudacarus Walter, 1928		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Torrenticola Piersig, 1896		X				?							Smith, Cook and Smith (2010), Cook (1974)	
			Uchidastygacaridae Inamura, 1956			X				X	X						Smith, Cook and Smith (2010), Cook (1974)	
				Uchidastygacarus Inamura, 1956		X					X						Smith, Cook and Smith (2010), Cook (1974)	
				Yachatsia Cook, 1963		X				X	X						Smith, Cook and Smith (2010), Cook (1974)	
			Unionicolidae Oudemans, 1909			X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Koenikea Wolcott, 1900		X				?							Smith, Cook and Smith (2010), Cook (1974)	
				Neumania Lebert, 1879		X				X							Smith, Cook and Smith (2010), Cook (1974)	
				Unionicola Haldeman, 1842		X				?							Smith, Cook and Smith (2010), Cook (1974)	some are parasites of Mollusca

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Subphylum: Crustacea
Standard Effort Level I: Genus
Standard Effort Level II: Genus/Species
Standard Taxonomic Reference: Rogers (2005)
Reviewed by:

The Crustacea are best separated using the keys in Rogers (2005) and literature cited therein. Crustaceans are important in bioassessment. Mysids, amphipods and isopods are sensitive to many pollutants and heavy metals, most crayfish and freshwater crabs are invasive species, and there are several state and federally protected species.

In Napa, Sonoma and Marin counties in California, many streams and rivers are occupied by the California Freshwater Shrimp (*Syncaris pacifica*), which is both a state and federally protected species. In temporary pools and streams in the Agate Desert area in southern Oregon, and California's Great Central Valley and the southern California coastal counties are five federally protected fairy shrimp and one federally protected tadpole shrimp. These animals are protected under the state and federal Endangered Species Acts and the California Environmental Quality Act. Any individual, private company or agency that violates these laws may be subject to substantial fines, imprisonment, or both.

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Crustacea Brünnich, 1772												X		X	X	X	X	X	X	X		
	Branchiopoda Latreille, 1817											X		X	X	X	X	X	X	X		
		Sarsostraca Tasch, 1969												X	X	X	X	X	X	X		
				Anostraca Sars, 1867								X		X	X	X	X	X	X	X	Eriksen and Belk (1999); Rogers (2002)	
					Artemiina Weekers, Murugan, Vanfleteren, Belk, and Dumont (2002)							X		X	X	X	X	X	X	X		
							Artemiidae Growchowski, 1896					X		X	X	X	X	X	X	X		
								Artemia Leach, 1819				X		X	X	X	X	X	X	X		

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Artemia franciscana Kellogg, 1906			X		X	X	X	X	X	X	X	Eriksen and Belk (1999)	Salt lakes
									Artemia monica Verrill, 1869			X		X							Eriksen and Belk (1999)	Reported only from Mono Lake
					Anostracina Weekers, Murugan, Vanfleteren, Belk, and Dumont (2002)							X		X	X	X	X	X	X	X	Eriksen and Belk (1999)	
							Branchinectidae Daday, 1910					X		X	X	X	X	X	X	X	Eriksen and Belk (1999)	
								Branchinecta Verrill, 1869				X		X	X	X	X	X	X	X	Eriksen and Belk (1999)	
									Branchinecta campestris Lynch, 1960			X		X	X	X					Rogers (2006)	Freshwater layers on salt lakes
									Branchinecta coloradensis Packard, 1874			X		X	X	X	X	X	X		Belk and Rogers (2002)	Temporary wetlands
									Branchinecta conservatio Eng, Belk and Eriksen, 1990			X		X							Eriksen and Belk (1999)	Listed under the Federal Endangered Species Act; Temporary wetlands
									Branchinecta cornigera Lynch, 1958			X			X	X						Temporary wetlands
									Branchinecta dissimilis Lynch, 1972			X		X	X						Belk and Rogers (2002)	Temporary wetlands
									Branchinecta gigas Lynch, 1937			X		X	X	X	X	X				Temporary wetlands
									Branchinecta hiberna Rogers and Fugate, 2001			X		X	X						Rogers and Fugate (2001)	Temporary wetlands

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Branchinecta kaibabensis Belk and Fugate, 2000			X		X	X						Belk and Fugate (2000)	Temporary wetlands
									Branchinecta lindahli Packard, 1883			X		X	X	X	X	X	X	X	Eriksen and Belk (1999)	Temporary wetlands
									Branchinecta longiantenna Eng, Belk and Eriksen, 1990			X		X							Eriksen and Belk (1999)	Listed under the Federal Endangered Species Act; Temporary wetlands
									Branchinecta lynchi Eng, Belk and Eriksen, 1990			X		X	X						Eriksen and Belk (1999)	Listed under the Federal Endangered Species Act; Temporary wetlands
									Branchinecta mackini Dexter, 1956			X		X	X	X	X	X		X	Eriksen and Belk (1999)	Temporary wetlands
									Branchinecta mesovallensis Belk and Fugate, 2000			X		X							Eriksen and Belk (1999)	Temporary wetlands
									Branchinecta oriena Belk and Rogers, 2002			X		X				X			Belk and Rogers (2002)	Temporary wetlands
									Branchinecta oterosanvinceti Obregon-Barboza, Maeda-Martinez, Garcia-Velazco, and Dumont 2002			X								?		Temporary wetlands
									Branchinecta packardi Pearse, 1912			X					X		X	X		Temporary wetlands
									Branchinecta paludosa (Muller, 1798)			X					X					Temporary wetlands

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Branchinecta sandiegonensis Fugate, 1993			X		X						X	Eriksen and Belk (1999)	Listed under the Federal Endangered Species Act; Temporary wetlands
							Chirocephalidae Daday, 1910					X		X	X	X	X	X	X			Temporary wetlands
								Eubranchipus Verrill, 1870				X		X	X	X			X			Temporary wetlands
									Eubranchipus bundyi Forbes, 1876			X		X	X	X	X		X		Eriksen and Belk (1999); Hill et al. (1997)	Temporary wetlands
									Eubranchipus oregonus Creaser, 1930			X		X	X						Eriksen and Belk (1999); Hill et al. (1997)	Temporary wetlands
									Eubranchipus serratus Forbes, 1976			X		X	X	X	X	X			Eriksen and Belk (1999); Hill et al. (1997)	Temporary wetlands
								Linderiella Brtek, 1964				X		X								Eriksen and Belk (1999)	Temporary wetlands
									Linderiella occidentalis (Dodds, 1923)			X		X								Eriksen and Belk (1999)	Temporary wetlands
									Linderiella santarosae Thiery and Fugate, 1994			X		X								Eriksen and Belk (1999)	Temporary wetlands
							Streptocephalidae Daday, 1910					X		X	X	X	X	X	X	X		Eriksen and Belk (1999)	Temporary wetlands
								Streptocephalus Baird, 1852				X		X	X	X	X	X	X	X		Eriksen and Belk (1999)	Temporary wetlands

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Streptocephalus dorothae Mackin, 1942			X		X			X		X	X	Eriksen and Belk (1999)	Temporary wetlands
									Streptocephalus mackini Moore, 1966			X							X	X	Eriksen and Belk (1999)	Temporary wetlands
									Streptocephalus sealii Ryder, 1879			X		X	X	X			X		Eriksen and Belk (1999)	Temporary wetlands
									Streptocephalus texanus Packard, 1871			X		X			X	X	X	X	Eriksen and Belk (1999)	Temporary wetlands
									Streptocephalus woottoni Eng, Belk and Eriksen, 1990			X		X							Eriksen and Belk (1999)	Listed under the Federal Endangered Species Act; Temporary wetlands
								Phallocryptus Birabén, 1951					X								Rogers (2003)	Temporary wetlands
									Phallocryptus sublettei (Sissom, 1976)			X					X				Rogers (2003)	Temporary wetlands
							Thamnocephalidae Packard, 1883					X		X				X	X	X		Temporary wetlands
								Thamnocephalus Packard, 1877				X		X				X	X	X		Temporary wetlands
									Thamnocephalus mexicanus Linder, 1941			X							X	X		Temporary wetlands
									Thamnocephalus platyurus Packard, 1877			X		X				X	X	X	Eriksen and Belk (1999)	Temporary wetlands
		Phyllopoda Preuss, 1951										X		X	X	X	X	X	X	X	Rogers (2005)	Temporary wetlands

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Notostraca Sars, 1867								X		X	X	X	X	X	X	X	Rogers (2001)	Temporary wetlands and temporary streams
							Triopsidae Keilhack, 1909					X		X			X	X	X	X	Rogers (2001)	Temporary wetlands and temporary streams
								Lepidurus Leach, 1819				X		X	X	X	X	X	X	X	Rogers (2001)	Temporary wetlands and temporary streams
									Lepidurus bilobatus Packard, 1883			X		X	X			X			Rogers (2001)	Temporary wetlands and temporary streams
									Lepidurus couesii Packard, 1875			X			X	X					Rogers (2001)	Temporary wetlands and temporary streams
									Lepidurus cryptus Rogers, 2001			X		X	X			X			Rogers (2001)	Temporary wetlands and temporary streams
									Lepidurus lemmoni Holmes, 1894			X		X	X	X		X	X	X	Rogers (2001)	Temporary wetlands and temporary streams

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Lepidurus packardi Simon, 1886			X		X							Rogers (2001)	Listed under the Federal Endangered Species Act; temporary wetlands and temporary streams
								Triops Schrank, 1803				X		X	X	X	X	X	X	X	Rogers (2005)	Temporary wetlands
									Triops longicaudatus (LeConte, 1846)			X		X	X	X		X	X	X	Rogers (2005)	Temporary wetlands
				Laevicaudata Linder, 1945								X		X	X	X	X	X	X	X	Martin and Belk (1988)	Temporary wetlands
							Lynceidae Baird, 1845					X		X	X	X	X	X	X	X	Martin and Belk (1988)	Temporary wetlands
								Lynceus Müller, 1776				X		X	X	X	X	X	X	X	Martin and Belk (1988)	Temporary wetlands
									Lynceus brachyurus Müller, 1776			X		X	X	X	X	X	X	X	Martin and Belk (1988)	Temporary wetlands
									Lynceus brevifrons (Packard, 1877)			X							X	X	Martin and Belk (1988)	Temporary wetlands
									Lynceus mucronatus (Packard, 1875)			X				X					Martin and Belk (1988)	Temporary wetlands
				Diplostraca Gerstaecker, 1866								X		X	X	X	X	X	X	X	Rogers (2005)	Temporary wetlands
					Spinicaudata Linder, 1945							X		X	X	X	X	X	X	X	Wootton and Mattox (1958)	Temporary wetlands
							Cyzicidae Stebbing, 1910					X		X	X	X	X	X	X	X	Wootton and Mattox (1958)	Temporary wetlands

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
								Cyzicus Audouin, 1837				X		X	X	X	X	X	X	X	Wootton and Mattox (1958)	Temporary wetlands
								Eocyzicus Daday de Deés, 1915				X		X			X	X	X	X	Wootton and Mattox (1958)	Temporary wetlands
									Eocyzicus digueti (Richard, 1895)			X		X			X	X	X	X	Wootton and Mattox (1958)	Temporary wetlands
							Leptestheriidae Daday, 1923					X		X			X	X	X	X	Martin and Cash-Clark (1993)	Temporary wetlands
								Leptestheria G.O. Sars, 1898				X		X			X	X	X	X	Martin and Cash-Clark (1993)	Temporary wetlands
									Leptestheria compleximanus (Packard, 1877)			X		X			X	X	X	X	Martin and Cash-Clark (1993)	Temporary wetlands
					Cladocera Latreille, 1829					X	X	X		X	X	X	X	X	X	X		Excluded from benthic datasets
	Maxillopoda Dahl, 1956									X	X	X	X	X	X	X	X	X	X	X		
		Branchiura Thorell, 1864								X	X	X	X	X	X	X	X	X	X	X	Cressey (1972)	Parasites; excluded from benthic datasets
				Arguloida Yamaguti, 1963						X	X	X	X	X	X	X	X	X	X	X	Cressey (1972)	Parasites; excluded from benthic datasets
							Argulidae Leach, 1819			X	X	X	X	X	X	X	X	X	X	X	Cressey (1972)	Parasites; excluded from benthic datasets

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
								Argulus Müller, 1785		X	X	X	X	X	X	X	X	X	X	X	Cressey (1972)	Parasites; excluded from benthic datasets
		Copepoda Milne-Edwards, 1840								X	X	X	X	X	X	X	X	X	X	X		Excluded from benthic datasets
	Ostracoda Latreille, 1802									X	X	X	X	X	X	X	X	X	X	X		
	Malacostraca Latreille, 1802									X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	
		Eumalacostraca Grobben, 1892								X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	
			Peracarida Calman, 1904							X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	
				Mysida Haworth, 1825						X	X	X	X	X	X	X		X			Rogers (2005)	
							Mysidae Haworth, 1825			X	X	X	X	X	X	X		X			Rogers (2005)	
								Acanthomysis Czerniavsky, 1882		X	X	X	X	X	X	X					Rogers (2005)	
									Acanthomysis aspera Ii, 1964	X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
									Acanthomysis hwanhaiensis Ii, 1964	X	X	X	X	X							Rogers (2005)	Fresh and brackish water
								Alienacanthomysis Holmquist, 1981		X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
									Alienacanthomysis macropsis (Tattersall, 1932	X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
								Deltamysis Bowman and Orsi, 1992		X	X	X	X	X							Bowman and Orsi (1992)	Fresh and brackish water
									Deltamysis homquistae Bowman and Orsi, 1992	X	X	X	X	X							Bowman and Orsi (1992)	Fresh and brackish water
								Hyperacanthomysis Fukuoka and Murano, 2000		X	X	X	X	X							Fukoka and Murano (2000)	Fresh and brackish water

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Hyperacanthomysis longirostris (Ii, 1964)	X	X	X	X	X							Fukoka and Murano (2000)	Fresh and brackish water
								Mysis Latreille, 1803		X	X	X		X	X	X		X			Rogers (2005)	alpine lakes
									Mysis diluviana Audzijonyte & Vainola, 2005	X	X	X		X	X	X		X			Rogers (2005)	alpine lakes
								Neomysis Czerniavsky, 1882		X	X	X		X	X	X					Rogers (2005)	
									Neomysis kadiakensis Ortmann, 1908	X	X	X		X	X	X					Rogers (2005)	
									Neomysis mercedis Homes, 1896	X	X	X		X	X	X					Rogers (2005)	
				Amphipoda Latreille, 1816						X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	
							Anisogammaridae Bousfield, 1977			X	X	X		X	X	X					Bousfield & Morino (1992)	
								Ramellogammarus Bousfield, 1979		X	X	X		X	X	X					Bousfield & Morino (1992)	
									Ramellogammarus californicus Bousfield and Morino, 1992	X	X	X		X							Bousfield & Morino (1992)	
									Ramellogammarus campestris Bousfield and Morino, 1992	X	X	X	X		X						Bousfield & Morino (1992)	Fresh and brackish water
									Ramellogammarus columbianus Bousfield and Morino, 1992	X	X	X	X	X	X	X					Bousfield & Morino (1992)	Fresh and brackish water
									Ramellogammarus littoralis Bousfield and Morino, 1992	X	X	X	X		X						Bousfield & Morino (1992)	Fresh and brackish water
									Ramellogammarus oregonensis (Shoemaker, 1944)	X	X	X	X	X	X	X					Bousfield & Morino (1992)	Fresh and brackish water
									Ramellogammarus ramellus (Weckel, 1907)	X	X	X	X	X	X						Bousfield & Morino (1992)	Fresh and brackish water
									Ramellogammarus setosus Bousfield and Morino, 1992	X	X	X				X					Bousfield & Morino (1992)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Ramellogammarus similimanus (Bousfield, 1961)	X	X	X			X						Bousfield & Morino (1992)	
									Ramellogammarus vancouverensis Bousfield, 1979	X	X	X	X			X					Bousfield & Morino (1992)	Fresh and brackish water
							Corophiidae Dana, 1849			X	X	X	X	X	X	X					Bousfield & Hoover (1997)	Fresh and brackish water
								Americorophium Bousfield and Hoover, 1997		X	X	X	X	X	X	X					Bousfield & Hoover (1997)	Fresh and brackish water
									Americorophium spinicorne (Stimpson, 1857)	X	X	X	X	X	X	X					Bousfield & Hoover (1997)	Fresh and brackish water
									Americorophium salmonis (Stimpson, 1857)	X	X	X	X		X	X					Bousfield & Hoover (1997)	Fresh and brackish water
									Americorophium stimpsoni (Shoemaker, 1941)	X	X	X	X	X	X						Bousfield & Hoover (1997)	Fresh and brackish water
							Crangonyctidae Bousfield, 1973			X	X			X	X	X	X	X	X	X	Rogers (2005)	
								Crangonyx Bate, 1859		X	X			X	X	X	X	X	X	X	Rogers (2005)	
								Stygobromus Cope, 1872		X	X			X	X	X	X	X	X	X	Rogers (2005)	
									Stygonyx Bousfield and Holsinger, 1989	X	X				X						Bousfield and Holsinger (1989)	
									Stygonyx courtneyi Bousfield and Holsinger, 1989	X	X				X						Bousfield and Holsinger (1989)	
							Gammaridae Latreille, 1802			X	X	X		X	X	X	X	X	X	X	Rogers (2005)	
								Gammarus Fabricius, 1775		X	X	X		X	X	X	X	X	X	X	Rogers (2005)	
							Haustoriidae Strebbing, 1906					X				X					Bousfield (1958)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
								Diporeia Bousfield, 1989				X				X					Bousfield (1958)	
									Diporeia erythrophthalma (Waldron, 1953)			X				X					Bousfield (1958)	
							Hyalellidae Bulycheva, 1957			X	X	X		X	X	X	X	X	X	X	Gonzales and Watling (2002)	
								Hyalella S.I. Smith, 1874		X	X	X		X	X	X	X	X	X	X	Gonzales and Watling (2002)	
							Talitridae Rafinesque, 1815				X	X		X	X	X	X	X	X	X	Rogers (2005)	Terrestrial to amphibious
								Arctitalitus Hurley, 1975			X	X		X	X	X	X	X	X	X	Rogers (2005)	Terrestrial to amphibious
									Arctitalitus sylvaticus (Haswell, 1879)		X	X		X	X	X	X	X	X	X	Rogers (2005)	Terrestrial to amphibious
								Talitroides Bonner, 1898			X	X		X	X	X	X	X	X	X	Rogers (2005)	Terrestrial to amphibious
									Talitroides alluaudi Chevreux, 1898		X	X		X	X	X	X	X	X	X	Morino and Ortal (1993)	Terrestrial to amphibious
									Talitroides topitotum Burt, 1934		X	X		X	X	X	X	X	X	X	Rogers (2005)	Terrestrial to amphibious
				Isopoda Latreille, 1817						X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	Fresh and brackish water
					Flabellifera Sars, 1882					X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
							Sphaeromatidae Latreille, 1825			X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
								Gnorimosphaeroma Menzies, 1954		X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Asellota Latreille, 1802					X	X	X		X	X	X	X	X			Williams (1970, 1976)	
							Asellidae Latreille, 1802			X	X	X		X	X	X		X			Williams (1970, 1976); Toft et al. (2002)	
								Asellus Geoffrey, 1764		X	X	X		X	X	X					Toft et al. (2002)	
									Asellus hilgendorfi Bouvallius, 1886	X	X	X		X	X	X					Magniez and Toft (2000); Toft et al. (2002)	Non-native invasive species
								Bowmanasellus Lewis, 2008		X				X							Lewis (2008)	
									Bowmanasellus sequoiae (Bowmann, 1975)	X				X							Lewis (2008); Bowman (1975)	known only from Lilburn Cave, Tulare Co.
								Caecidotea Packard, 1871		X	X	X		X	X	X	X	X			Williams (1970, 1972)	
									Caecidotea communis (Say, 1818)	X	X	X				X	X				Williams (1970, 1972)	
									Caecidotea occidentalis (Williams, 1970)	X	X	X		X	X	X					Williams (1970, 1972); Bowman (1974)	
									Caecidotea racovitzai (Williams)	X	X	X		X		X					Williams (1970, 1972)	Introduced in CA
									Caecidotea tomalensis (Harford, 1877)	X	X	X		X							Bowman (1973)	
								Calasellus Bowman, 1981		X	X	X		X							Bowman (1981)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Calasellus californicus (Miller, 1933)	X	X	X		X							Miller (1933); Bowman (1981)	
									Calasellus longus (Bowman, 1981)	X	X	X		X							Bowman (1981)	
								Columbasellus Lewis, Martin and Wetzer, 2003		X	X	X				X					Lewis et al. (2003)	
									Columbasellus acheron Lewis, Martin and Wetzer, 2003	X	X	X				X					Lewis et al. (2003)	
								Oregonasellus Lewis, 2008		X					X						Lewis (2008)	
									Oregonasellus elliotti Lewis, 2008	X					X						Lewis (2008)	known only from Malheur Cave, Harney Co.
								Salmasellus Bowman, 1975		X	X	X			X	X					Lewis (2001)	
									Salmasellus howarthi Lewis, 2001	X	X	X			X	X					Lewis (2001)	
							Munnidae Sars, 1897			X	X	X		X							Rogers (2005)	
						Oniscidea Latreille, 1802				X	X	X		X	X	X	X	X	X	X	Rogers (2005)	
								Ligiidae Leach, 1814		X	X	X		X	X	X	X				Rogers (2005)	
									Ligium Brandt, 1833	X	X	X					X				Rogers (2005)	
					Tanaidacea Dana, 1849					X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
							Leptochelidae Lana, 1973			X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
									Leptochelia Dana, 1849	X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water; undescribed species known

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Tanaidae Dana, 1849			X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water
								Sinelobus Sieg, 1980		X	X	X	X	X	X	X					Rogers (2005)	Fresh and brackish water; undescribed species known
			Eucarida Calman, 1904							X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	Fresh and brackish water
				Decapoda Latreille, 1802						X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	Fresh and brackish water
						Caridea Dana, 1852				X	X	X	X	X	X	X	X	X	X	X	Rogers (2005)	Fresh and brackish water
							Atyidae de Hann, 1849			X	X	X		X							Rogers (2005)	
								Syncaris Holmes, 1900		X	X	X		X							Martin and Wicksten (2004); Rogers (2005)	
									Syncaris pacifica (Holmes, 1895)	X	X	X		X							Martin and Wicksten (2004); Rogers (2005)	Listed under the Federal and California State Endangered Species Acts
									Syncaris pasadenae (Kingsley, 1897)	X	X	X		X							Martin and Wicksten (2004); Rogers (2005)	Possibly extinct

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Palaemonidae Rafinesque, 1815			X	X	X	X	X	X	X		X	X	X	Holthuis (1952); Jayachandran (2001); Rogers (2005)	
								Exopalaemon Holthuis, 1950		X	X	X	X	X	X	X					Jayachandran (2001); Rogers (2005)	Fresh and brackish water
									Exopalaemon carinicauda (Holthuis, 1950)	X			X	X							Jayachandran (2001)	Fresh and brackish water
									Exopalaemon modestus (Heller, 1862)	X	X	X		X	X	X					Jayachandran (2001)	
								Palaemon Webber, 1795		X	X	X		X	X						Holthuis (1952)	
									Palaemon macrodactylus Rathbun, 1902	X	X	X		X	X						Jayachandran (2001)	
								Palaemonetes Heller, 1869		X	X	X		X	X	X		X	X	X	Holthuis (1952)	
									Palaemonetes kadiakensis Rathbun, 1902	X	X	X		X							Holthuis (1952); Rogers (2005)	
									Palaemonetes paludosus Gibbes, 1850	X	X	X		X	X	X		X	X	X	Holthuis (1952)	
							Astacidea Latreille, 1802										X					
								Astacidae Latreille, 1802		X	X	X		X	X	X	X	X	X		Riegel (1959); Rogers (2005)	
								Pacifastacus Bott, 1950		X	X	X		X	X	X	X	X	X		Riegel (1959); Rogers (2005)	
									Pacifastacus connectens (Faxon, 1914)	X	X	X			X		X				Riegel (1959); Rogers (2005)	
									Pacifastacus fortis (Faxon, 1914)	X	X	X		X							Riegel (1959); Rogers (2005)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Pacifastacus gambelii (Girard, 1852)	X	X	X		?	X	X		X			Riegel (1959); Rogers (2005)	
									Pacifastacus lenisculus klamathensis (Stimpson, 1859)	X	X	X		X	X	X					Riegel (1959); Rogers (2005)	
									Pacifastacus lenisculus lenisculus (Dana, 1852)	X	X	X		X	X	X		X	X		Riegel (1959); Rogers (2005)	
									Pacifastacus lenisculus trowbridgii (Stimpson, 1857)	X	X	X			X	X					Riegel (1959); Rogers (2005)	
									Pacifastacus nigrescens (Stimpson, 1857)	X	X	X		X							Riegel (1959); Rogers (2005)	May be extinct
								Cambaridae Hobbs, 1942		X	X	X		X	X	X	X	X	X	X	Riegel (1959); Rogers (2005)	
									Orconectes Cope, 1872	X	X	X		X	X	X	X	X	X	X	Riegel (1959); Rogers (2005)	
									Orconectes neglectus neglectus (Faxon, 1885)	X	X	X			X		X				Riegel (1959); Rogers (2005)	
									Orconectes virilis (Hagen, 1870)	X	X	X		X			X		X		Riegel (1959); Rogers (2005)	
									Procambarus Ortmann, 1905	X	X	X		X	X		X	X	X	X	Riegel (1959); Rogers (2005)	
									Procambarus clarkii (Girard, 1852)	X	X	X		X	X		X	X	X	X	Riegel (1959); Rogers (2005)	
							Brachyura Latreille, 1802															
								Geothelphusidae Ortmann, 1893		X	X	X						X			Rogers (2005)	
									Geothelphusa Stimpson, 1858	X	X	X						X			Rogers (2005)	
									Geothelpusa dehaani (White, 1874)	X	X	X						X			Rogers (2005)	
								Grapsidae MacLeay, 1838		X	X	X	X	X							Rogers (2005)	Fresh and brackish water

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Subphylum	Class	Subclass	Superorder	Order	Suborder	Infraorder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
								Eriocheir de Haan, 1835		X	X	X	X	X							Rogers (2005)	Fresh and brackish water
									Eriocheir sinensis Milne-Edwards, 1854	X	X	X	X	X							Rogers (2005)	Fresh and brackish water
							Ocypodidae Rafinesque, 1815			X			X	X							Rogers (2005)	
								Uca Leach, 1814		X			X	X							Rogers (2005)	
									Uca crenulata (Lockington, 1877)	X			X	X							Rogers (2005)	
							Panopeidae Ortmann, 1893			X			X	X	X						Rogers (2005)	
								Rhithropanopeus Rathbun, 1898		X			X	X	X						Rogers (2005)	
									Rhithropanopeus harrisii (Gould, 1841)	X			X	X	X						Rogers (2005)	

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Additional Sources of Information on Crustacea

Large Branchiopod Bibliography, updated 6 March 2006. Accessed 14 February 2011 at URL: http://www3.uakron.edu/biology/bibintro.html

Collembola: Springtails
Standard Effort Level I: Excluded from benthic datasets
Standard Effort Level II: Excluded from benthic datasets
Standard Taxonomic Reference: Christiansen and Snider (2008)
Reviewed by:

Collembolans can be identified to genus using the key in Merritt, Cummins and Berg (Christiansen and Snider, 2008) or DeWalt et al. (2010). Collembola live neustonically or near any aquatic or moist habitat including stream and pond margins, intertidal pools, watersoaked wood and carpet. Collembola are excluded from benthic datasets.

Taxon	Habitat				Distribution							Literature Cited	Comments
Class	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Collembola Lubbock, 1869		X	X	X	X	X	X		X	X	X	Christiansen and Snider (1996); Hilsenhoff (2001)	Excluded from benthic datasets

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Christiansen, K. A., and R. J. Snider. 2008. Chapter 10: Aquatic Collembola. [pp. 165-179]. In: R. W. Merritt, K. W. Cummins and M. B. Berg (editors), An introduction to the aquatic insects of North America, fourth edition, xvi + 1158 pp. + 39 color plates. Kendall/Hunt Publishing Company, Dubuque, Iowa.

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Additional Sources of Information on Collembola

Checklist of the Collembola of the World, updated 31 January 2011. Accessed 14 February 2011 at URL: http://www.collembola.org/

Ephemeroptera: Mayflies
Standard Effort Level I: Genus
Standard Effort Level II: Species (where possible)
Standard Taxonomic Reference: Waltz and Burian (2008)
Reviewed by:

Nymphs can be identified to genus using the key in Merritt, Cummins and Berg (Waltz and Burian, 2008). Considerable reorganization of the baetid genera has taken place since the key was published (Lugo-Ortiz and McCafferty, 1998). A mayfly workshop was given by the Northwest Biological Assessment Workgroup in 2005. The manual created by Jacobus and Randolph (2005) serves as a very useful supplementary text with numerous provisional keys and unpublished distributional and habitat information for western mayflies. There are two useful websites on Ephemeroptera: Mayfly Central, hosted by Purdue University, maintains the Mayflies of North America checklist and has distributional information, and; Ephemeroptera Galactica, hosted by the Museum Collections of Aquatic Entomology at Florida A&M University, has a bibliography that offers many mayfly paper PDFs.

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Ephemeroptera Hyatt and Arms, 1891				X	X	X		X	X	X	X	X	X	X	Waltz and Burian (2008)	for keys to families and genera
	Acanthametropodidae Edmunds in Edmunds, Allen and Peters, 1963			X	X						X					
		Analetris Edmunds in Edmunds and Koss, 1972		X	X						X					
			Analetris eximia Edmunds in Edmunds and Koss, 1972	X	X						X					
	Ameletidae McCafferty, 1991			X	X			X	X	X	X	?	X			
		Ameletus Eaton, 1885		X	X			X	X	X		?	X		Zloty (1996)	Not all species described as nymphs -- best to leave identifications at *Ameletus* sp.
	Ametropodidae Bengtsson, 1913			X	X			X	X	X						
		Ametropus Albarda, 1878		X	X			X	X	X					Allen and Edmunds (1976); McCafferty	nymphs for both North American species are known and keyed in Allen and Edmunds (1976)

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
															(2001)	
			Ametropus ammophilus Allen and Edmunds, 1976	X	X			X	X	X						
			Ametropus neavei McDunnough, 1928	X	X						X					
	Baetidae Leach, 1815			X				X	X	X	X	X	X	X	Waltz and Burian (2008); Lugo-Ortiz and McCafferty (1998); Morihara and McCafferty (1979)	Morihara and McCafferty (1979) is still useful because it contains good descriptions.
		Acentrella Bengtsson, 1912		X				X	X	X		X	X	X	Jacobus and McCafferty (2006)	
			Acentrella insignificans (McDunnough, 1926)	X	X			X	X	X	X	X	X		Jacobus and McCafferty (2006)	
			Acentrella turbida (McDunnough, 1924)	X	X			X	X	X	X		X		Jacobus and McCafferty (2006)	
		Acerpenna Waltz and McCafferty, 1987		X	X			~~X~~	X							
			Acerpenna pygmaea (Hagen, 1861)	X	X				X						Meyer and McCafferty (2007)	
		Apobaetis Day, 1955		X	X			X	X						Meyer and McCafferty (2004)	Occurs in warm water streams; Tuolumne River in CA Central Valley
			Apobaetis etowah (Traver, 1935)	X	X			X	X						Meyer and McCafferty (2004)	Occurs in warm water streams; Tuolumne River in CA Central Valley

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Baetis Leach, 1815		X	X			X	X	X	X	X	X		Wiersema et al. (2004); Morihara and McCafferty (1979)	The nymphs for several species remain undescribed.
			Baetis adonis Traver, 1935	X	X			X	X							
			Baetis alius Day, 1954	X	X			X	X	X			X			
			Baetis bicaudatus Dodds, 1923	X	X			X	X	X	X					
			Baetis brunneicolor McDunnough, 1925	X	X					X						
			Baetis diablus Day, 1954	X	X			X	~~X~~							nymph stage unknown; possibly syn. of *B. tricaudatus*
			Baetis flavistriga McDunnough, 1921	X	X			X	X	X	X					
			Baetis magnus McCafferty and Waltz, 1986	X	X			X					X			
			Baetis notos Allen and Murvosh, 1987	X	X			X	X	X	X		X			
			Baetis palisadi Mayo, 1952	X	X			X								nymph stage unknown
			Baetis piscatoris Traver, 1935	X	X			X								nymph stage unknown
			Baetis tricaudatus Dodds, 1923	X	X			X	X	X	X	X	X	X		
		Baetodes Needham and Murphy, 1924		X	X				X				X		Cohen and Allen (1978); McCafferty and Provonsha (1993)	
			Baetodes alleni McCafferty and Provonsha, 1993	X	X								X		Cohen and Allen (1978); McCafferty and Provonsha (1993)	
			Baetodes arizonensis Koss, 1972	X	X								X		Cohen and Allen (1978); McCafferty and Provonsha (1993); Koss (1972)	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Baetodes bibranchius McCafferty and Provonsha, 1993	X	X				X						Cohen and Allen (1978); McCafferty and Provonsha (1993)	
			Baetodes edmundsi Koss, 1972	X	X								X		Cohen and Allen (1978); McCafferty and Provonsha (1993); Koss (1972)	
		Callibaetis Eaton, 1881		X	X	X		X	X	X	X	X				No published nymph key to species
			Camelobaetidius Demoulin, 1966	X	X			X	X	X	X	X	X		Lugo-Ortiz and McCafferty (1995); McCafferty and Randolph (2000)	
			Camelobaetidius kickapoo McCafferty, 2000	X	X								X		Lugo-Ortiz and McCafferty (1995); McCafferty and Randolph (2000)	
			Camelobaetidius maidu Jacobus and McCafferty, 2005	X	X			X							Jacobus and McCafferty (2005)	
			Camelobaetidius mexicanus (Traver and Edmunds, 1968)	X	X				X						Lugo-Ortiz and McCafferty (1995); McCafferty and Randolph (2000)	
			Camelobaetidius musseri (Traver and Edmunds, 1968)	X	X							X			Lugo-Ortiz and McCafferty (1995); McCafferty and	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
															Randolph (2000)	
			Camelobaetidius warreni (Traver and Edmunds, 1968)	X	X			X	X	X	X	X			Lugo-Ortiz and McCafferty (1995); McCafferty and Randolph (2000)	widespread species; *C. similis* Lugo-Ortiz and McCafferty is considered to be a synonym
		Centroptilum Eaton, 1869		X	X			X	X	X	X	X	X			No published nymph key to species
		Cloeodes Traver, 1938		X	X			X	X				X		Waltz and McCafferty (1987)	This key includes nymphs of all three southwestern species
			Cloeodes excogitatus Waltz & MacCafferty, 1987	X	X			X	X				X		Waltz and McCafferty (1987)	
			Cloeodes macrolamellus Waltz and McCafferty, 1987										X		Waltz and McCafferty (1987)	
			Cloeodes peninsulus Waltz and McCafferty, 1987										X		Waltz and McCafferty (1987)	
		Diphetor Waltz and McCafferty, 1987		X	X			X	X	X	X				Morihara and McCafferty (1979)	only one North American species
			Diphetor hageni (Eaton, 1885)	X	X			X	X	X	X				Morihara and McCafferty (1979)	only one North American species
		Fallceon Waltz and McCafferty, 1987		X	X			X	X	X	X	X	X			with the additions of new species in the genus, best to leave at *Fallceon*
			Fallceon eatoni (Kimmins, 1934)	X	X			X					X		McCafferty (2006)	
			Fallceon quilleri (Dodds, 1923)	X	X			X	X	X	X	X			Morihara and McCafferty (1979)	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Fallceon sonora (Allen and Murvosh, 1987)	X	X			X							Morihara and McCafferty (1979)	may be a synonym of F. eatoni (Kimmins, 1934)
			Fallceon thermophilos (McDunnough, 1926)	X	X			X							Morihara and McCafferty (1979)	*Acerpenna sulfurus* and *A. thermophilos* were both synonymized and moved to *Fallceon* in McCafferty and Meyer (2008)
		Heterocloeon McDunnough, 1925		X	X				X	X						
			Heterocloeon anoka (Daggy, 1945)	X	X				X	X					McCafferty et al. (2005); Guenther and McCafferty (2005)	Guenther and McCafferty (2005) placed this species into *Iswaeon* McCafferty and Webb (2005)
		Moribaetis Waltz and McCafferty, 1985													McCafferty (2007)	specimens found in Oak Creek, AZ
			Moribaetis mimbresaurus McCafferty, 2007	X	X								X		McCafferty (2007)	specimens found in Oak Creek, AZ
		Paracloeodes Day, 1955		X	X			X	X							only one species known in the west
			Paracloeodes minutus (Daggy, 1945)	X	X			X	X							only one species known in the west
		Plauditus Lugo-Ortiz and McCafferty, 1998		X	X			?	X						Lugo-Ortiz and McCafferty (1998)	
			Plauditus punctiventris (McDunnough, 1923)	X	X			?	X						Lugo-Ortiz and McCafferty (1998)	
		Procloeon Bengtsson, 1915		X	X			X	X	X						No published nymph key to species
		Pseudocloeon Klapalek, 1905		X	X			X	X	X	X		X		McCafferty and Waltz (1995) Lugo-Ortiz et al. (1999)	Species formerly in *Labiobaetis*

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Pseudocloeon apache (McCafferty & Waltz, 1995)	X	X			?	X	X	X		X		McCafferty and Waltz (1995) Lugo-Ortiz et al. (1999)	
			Pseudocloeon dardanum (McDunnough, 1923)	X	X					X						
			Pseudocloeon propinquum (Walsh, 1863)	X	X			X	X	X	X				McCafferty and Waltz (1995) Lugo-Ortiz et al. (1999)	
	Baetiscidae Banks, 1900			X	X										Pescador and Berner (1981)	
		Baetisca Walsh, 1863		X	X							X			Baumann and Kondratieff (2000)	
			Baetisca columbiana Edmunds, 1960	X	X					X						
			Baetisca lacustris McDunnough, 1932	X	X							X			Baumann and Kondratieff (2000)	record from Humboldt River
	Caenidae Newman, 1853			X				X	X	X	X	X	X	X		Other genera are possible in the Southwest; revision of the family by Sun and McCafferty
		Brachycercus Curtis, 1834		X							X				Sun and McCafferty (2008)	
			Brachycercus harrisella Curtis, 1834	X							X				Sun and McCafferty (2008)	
		Caenis Stephens, 1835		X				X	X	X	X	X	X	X	Provonsha (1990)	Mature nymphs may be identified to species
			Caenis amica Hagen, 1861	X				X	X	X	X		X		Provonsha (1990)	
			Caenis bajaensis Allen and Murvosh, 1983	X				X					X	X	Provonsha (1990)	
			Caenis latipennis Banks, 1907	X				X	X	X		X	X		Provonsha (1990)	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Caenis punctata McDunnough, 1931	X				X							Provonsha (1990)	
			Caenis tardata McDunnough, 1931	X												
			Caenis youngi Roemhild, 1984	X				X	X	X						
		Susperatus Sun and McCafferty, 2008		X							X					
			Susperatus prudens (McDunnough, 1931)	X												
			Susperatus tuberculatus (Soldan, 1986)	X							X					
	Ephemerellidae Klapalek, 1909			X				X	X	X					Jacobus and McCafferty (2008)	revised key in Jacobus and McCafferty (2008)
		Attenella Edmunds, 1971		X				X	X	X	X				Allen and Edmunds (1961a)	
			Attenella attenuata (McDunnough, 1925)	X	X			X							Allen and Edmunds (1961a)	
			Attenella delantala (Mayo, 1952)	X	X			X	X	X					Allen and Edmunds (1961a)	
			Attenella margarita (Needham, 1927)	X	X			X	X	X	X				Allen and Edmunds (1961a)	
			Attenella soquele (Day, 1954)	X	X			X	X	X					Allen and Edmunds (1961a)	
		Caudatella Edmunds, 1959		X	X			X	X						Jacobus (2010); Allen and Edmunds (1961b)	Jacobus (2010) provides an updated key to nymphs
			Caudatella columbiella (McDunough, 1935)	X	X			X							Jacobus (2010); Allen and Edmunds (1961b)	removed from synonymy with *Caudatella heterocaudata* (McDunnough)
			Caudatella edmundsi (Allen, 1959)	X	X			X	X	X					Jacobus (2010); Allen and Edmunds	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
															(1961b)	
			Caudatella heterocaudata (McDunnough, 1929)	X	X			X	X	X					Jacobus (2010); Allen and Edmunds (1961b)	
			Caudatella hystrix (Traver, 1934)	X	X			X	X	X					Jacobus (2010); Allen and Edmunds (1961b)	*Caudatella cascadia* (Allen and Edmunds) now a synonym
			Caudatella jacobi (McDunnough, 1939)	X	X			X	X	X					Jacobus (2010); Allen and Edmunds (1961b)	
		Drunella Needham, 1905		X	X			X	X	X	X	X	X	X	Allen and Edmunds (1962)	
			Drunella coloradensis (Dodds, 1923)	X	X			X	X	X	X	X	X	X	Allen and Edmunds (1962)	
			Drunella doddsii (Needham, 1927)	X	X			X	X	X	X	X			Allen and Edmunds (1962)	
			Drunella flavilinea (McDunnough, 1926)	X	X			X	X	X				X	Allen and Edmunds (1962)	
			Drunella grandis (Eaton, 1884)	X	X			X	X	X	X	X	X		Allen and Edmunds (1962)	
			Drunella pelosa (Mayo, 1951)	X	X			X	X	X					Allen and Edmunds (1962)	
			Drunella spinifera (Needham, 1927)	X	X			X	X	X	X				Allen and Edmunds (1962)	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Ephemerella Walsh, 1863		X	X			X	X	X	X	X	X		Allen and Edmunds (1965); Jacobus and McCafferty (2003)	
			Ephemerella alleni Jensen and Edmunds, 1966	X	X				X	X					Allen and Edmunds (1965); Jacobus and McCafferty (2003)	
			Ephemerella aurivillii (Bengtsson, 1908)	X	X			X	X	X					Allen and Edmunds (1965); Jacobus and McCafferty (2003)	
			Ephemerella dorothea Needham, 1908	X	X			X	X	X	X	X	X		Allen and Edmunds (1965); Jacobus and McCafferty (2003)	*Ephemerella infrequens McDunnough recently synonymized with E. dorothea*
			Ephemerella excrucians Walsh, 1862	X	X			X	X	X	X	X	X		Allen and Edmunds (1965); Jacobus and McCafferty (2003)	*Ephemerella inermis Eaton recently synonymized with E. excrucians*
			Ephemerella maculata Traver, 1934	X	X			X	X						Allen and Edmunds (1965); Jacobus and McCafferty (2003)	
			Ephemerella tibialis McDunnough, 1924	X	X			X	X	X	X	X	X		Allen and Edmunds (1963b); Jacobus and McCafferty (2008)	formerly *Serratella tibialis* (McDunnough)

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Ephemerella velmae Allen and Edmunds, 1963	X	X			X	X						Allen and Edmunds (1963b); Jacobus and McCafferty (2008)	formerly *Serratella velmae* (Allen and Edmunds, 1963)
			Ephemerella verruca Allen and Edmunds, 1965	X	X				X	X					Allen and Edmunds (1965); Jacobus and McCafferty (2003)	
		Eurylophella Tiensuu, 1935		X	X	X		X	X	X					Allen and Edmunds (1963a)	
			Eurylophella lodi (Mayo, 1952)	X	X	X		X	X	X					Allen and Edmunds (1963a)	
		Matriella Jacobus, 2008		X	X			X	X	X					Jacobus (2008)	
			Matriella teresa (Traver, 1934)	X	X			X	X	X					Jacobus (2008)	formerly *Serratella teresa* (Traver)
		Serratella Edmunds, 1959		X				X	X	X		X	X	X	Allen and Edmunds (1963b)	
			Serratella levis (Day, 1954)	X	X			X	X						Allen and Edmunds (1963b)	
			Serratella micheneri (Traver, 1934)	X	X			X	X	X			X	X	Allen and Edmunds (1963b)	
		Timpanoga Needham, 1927		X	X			X	X	X	X	X			Allen and Edmunds (1959)	
			Timpanoga hecuba (Eaton, 1884)	X	X			X	X	X	X	X			Allen and Edmunds (1959)	Two dubious subspecies recognized
	Ephemeridae Latreille, 1810			X				X	X	X		X			McCafferty (1975)	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Ephemera Linnaeus, 1758		X						X	X	X			McCafferty (1975)	*Ephemera compar* Hagen, 1875 from Colorado is considered to be extinct
			Ephemera simulans Walker, 1853	X					X	X	X	X			McCafferty (1975)	
		Hexagenia Walsh, 1863		X				X	X	X	X	X			McCafferty (1975)	
			Hexagenia bilineata (Say, 1824)	X						X					McCafferty (1975)	
			Hexagenia limbata (Serville, 1829)	X				X	X	X	X	X			McCafferty (1975)	
	Heptageniidae Needham in Needham and Betten, 1901			X				X			X				Wang and McCafferty (2004); Webb and McCafferty (2008)	
		Anepeorus McDunnough, 1925		X							X					
			Anepeorus rusticus McDunnough, 1925	X							X					
		Cinygma Eaton, 1885		X	X			X	X	X						nymphs cannot be reliably separated at present
		Cinygmula McDunnough, 1933		X	X			X	X	X	X		X			nymphs cannot be reliably separated at present
		Ecdyonurus Eaton, 1868		X	X			X	X	X	X	X	X		McCafferty (2004); Bednarik and Edmunds (1980)	mature nymphs may be separated using labral characters
			Ecdyonurus criddlei (McDunnough, 1927)	X	X			X	X	X	X	X	X		McCafferty (2004); Bednarik and Edmunds (1980)	species formerly in *Heptagenia,* then *Nixe*
			Ecdyonurus simplicoides (McDunnough, 1924)	X	X			?	X	X	X		X		McCafferty (2004); Bednarik and	species formerly in *Heptagenia,* then *Nixe*

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
															Edmunds (1980)	
			Epeorus Eaton, 1881	X	X			X	X	X	X	X	X	X		The key in Edmunds and Allen (1964) includes only species found in the Rocky Mts. The nymph stage is unknown for several western species.
			Heptagenia Walsh, 1863	X	X			X	X	X	X	X	X			nymphs cannot be reliably separated at present
			Ironodes Traver, 1935	X	X			X	X					X		nymphs cannot be reliably separated at present
			Leucrocuta Flowers, 1980	X	X			?	X		X					immature *Leucrocuta/Nixe/Ecdyonurus* difficult to separate
			Leucrocuta jewetti (Allen, 1966)	X	X			?	X							
			Leucrocuta petersi (Allen, 1966)	X	X						X					
			Mccaffertium Bednarik, 1979	X	X			X	X	X	X	X			Wang and McCafferty (2004)	formerly a subgenus of *Stenonema*
			Mccaffertium terminatum (Walsh, 1862)	X	X			X	X	X	X	X			Bednarik and McCafferty (1979); Wang and McCafferty (2004)	
			Nixe Flowers, 1980	X	X			X	X							immature *Leucrocuta/Nixe/Ecdyonurus* difficult to separate
			Nixe kennedyi (McDunnough, 1924)	X	X			X	X						McCafferty (2004)	nymph not described for this species
			Rhithrogena Eaton, 1881	X	X			X	X	X	X	X	X	X		nymphs cannot be reliably separated at present

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
	Isonychiidae Burks, 1953			X	X			X	X			X	X			
		Isonychia Eaton, 1871		X	X			X	X		X	X	X			Although distribution is useful for identifying *Isonychia* in the West, reliable species identifications require rearing
			Isonychia campestris McDunnough, 1931	X	X						X					
			Isonychia intermedia (Eaton, 1885)	X	X							X	X			
			Isonychia velma Needham, 1932	X	X			X	X						Day (1952)	
	Leptohyphidae Edmunds and Traver, 1954			X	X			X	X	X	X	X	X	X	Wiersema and McCafferty (2000); Allen (1978)	several new genera were erected in Wiersema and McCafferty (2000) but no species key to nymphs was included; David Baumgardner has completed his phylogeny for the family -- several genera will fall to synonymy once it is published
		Asioplax Wiersema and McCafferty, 2000		X	X			X	X		X				Wiersema and McCafferty (2000); Allen (1978)	
			Asioplax edmundsi (Allen, 1967)	X	X			X	X		X				Wiersema and McCafferty (2000); Allen (1978)	
		Homoleptohyphes Allen and Murvosh, 1987		X	X			X					X		Wiersema and McCafferty (2000); Allen (1978)	
			Homoleptohyphes dimorphus (Allen, 1967)	X	X			X					X		Wiersema and McCafferty (2000); Allen (1978)	

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Homoleptohyphes mirus (Allen, 1967)	X	X								X		Wiersema and McCafferty (2000); Allen (1978)	
			Homoleptohyphes quercus (Kilgore and Allen, 1973)	X	X								X		Wiersema and McCafferty (2000); Allen (1978)	
		Leptohyphes Eaton, 1882		X	X						X		X		Baumgardner and McCafferty (2010); Wiersema and McCafferty (2000); Allen (1978)	Baumgardner and McCafferty's revision revalidates several species and provides a key to nymphs
			Leptohyphes apache Allen, 1967	X	X						X	X	X		Baumgardner and McCafferty (2010)	species revalidated
			Leptohyphes ferruginus Allen and Brusca, 1973	X	X								X		Baumgardner and McCafferty (2010)	species revalidated
			Leptohyphes lestes Allen and Brusca, 1973	X	X								X		Baumgardner and McCafferty (2010)	
			Leptohyphes zalope Traver, 1958	X	X						~~X~~		X		Baumgardner and McCafferty (2010)	
		Tricoryhyphes Allen and Murvosh, 1987		X	X								X		Wiersema and McCafferty (2000); Allen (1978)	
			Tricoryhyphes condylus (Allen, 1967)	X	X								X		Wiersema and McCafferty (2000); Allen (1978)	
		Tricorythodes Ulmer, 1920		X	X			X	X	X		X	X	X	Wiersema and McCafferty	

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Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
															(2000); Allen (1978)	
			Tricorythodes explicatus (Eaton, 1892)	X	X			X	X	X	X	X	X	X	Wiersema and McCafferty (2000); Allen (1978)	*Tricorythodes minutus* Traver is now a jr. synonym
			Tricorythodes fictus Traver, 1935	X	X			X							Wiersema and McCafferty (2000); Allen (1978)	
		Vaccupernius Wiersema and McCafferty, 2000		X	X								X		Wiersema and McCafferty (2000); Allen (1978)	
			Vaccupernius packeri (Allen, 1967)	X	X								X		Wiersema and McCafferty (2000); Allen (1978)	
	Leptophlebiidae Banks, 1900			X				X	X	X	X	X	X	X		
		Choroterpes Eaton, 1881		X	X			X	X		X	X	X	X	McCafferty (1992)	
		Leptophlebia Westwood, 1840		X	X	X		X	X	X					Burian (2000)	
		Neochoroterpes Allen, 1974		X									X		Henry (1993)	
			Neochoroterpes kossi (Allen, 1974)	X									X		Henry (1993)	
		Paraleptophlebia Lestage, 1917		X				X	X	X	X	X	X	X		Most species undescribed as nymphs; best to leave at genus. See Edmunds and McCafferty (1996) for discussion of species with tusks.
		Thraulodes Ulmer, 1920		X	X								X		Traver and Edmunds (1967)	
			Thraulodes brunneus Koss, 1966	X	X								X		Traver and Edmunds (1967)	

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Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Thraulodes gonzalesi Traver and Edmunds, 1967	X	X								X		Traver and Edmunds (1967)	
			Thraulodes tenulineus Lugo-Ortiz and McCafferty, 1996	X	X								X		Traver and Edmunds (1967)	
		Traverella Edmunds, 1948		X	X					X	X	X	X		Allen (1973)	
			Traverella albertana (McDunnough, 1931)	X	X					X	X	X	X		Allen (1973)	
	Oligoneuriidae Ulmer, 1914			X							X		X		Edmunds et al. (1958)	
		Homoeoneuria Eaton, 1881		X							X					
			Homoeoneuria alleni Pescador and Peters, 1980	X							X					
		Lachlania Hagen, 1868		X							X		X		Edmunds et al. (1958)	
			Lachlania saskatchewanensis Ide, 1941	X							X		X		Edmunds et al. (1958)	
	Polymitarcyidae Banks, 1900			X				X	X	X	X	X			McCafferty (1975)	
		Ephoron Williamson, 1802		X				X	X	X	X	X			McCafferty (1975)	
			Ephoron album (Say, 1824)	X				X	X	X	X	X			McCafferty (1975)	
	Potamanthidae Albarda in Selys-Longchamps, 1888			X	X			X							McCafferty and Meyer (2007)	
		Anthopotamus McCafferty and Bae, 1990		X	X			X							McCafferty and Meyer (2007)	
			Anthopotamus verticis (Say, 1839)	X	X			X							McCafferty and Meyer (2007)	
	Pseudironidae Edmunds and Traver, 1854			X	X			X			X					
		Pseudiron McDunnough, 1931		X	X			X			X					
			Pseudiron centralis McDunnough, 1931	X	X			X			X					
	Siphlonuridae Ulmer, 1920			X				X	X	X	X	X	X	X		
		Edmundsius Day, 1953		X	X			X								

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Edmundsius agilis Day 1953	X	X			X								
		Parameletus Bengtsson, 1908		X	X					X	X					
			Parameletus columbiae McDunnough, 1938	X	X					X	X					
		Siphlonurus Eaton, 1868		X				X			X					

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Odonata: Damselflies and Dragonflies
Standard Effort Level I: Genus
Standard Effort Level II: Species (where possible)
Standard Taxonomic Reference(s): Tennessen (2008), Westfall and May (1996), Needham, Westfall and May (2000)
Reviewed by:

Updated keys to families and genera are given in the odonate chapter of Merritt, Cummins and Berg (Tennessen, 2008). Generic and species keys to adults and immatures are given for damselflies in Westfall and May (1996) and dragonflies in Needham, Westfall and May (2000). See Tennessen and Paulson's (2007) workshop manual and Rehn (2000) for more detailed ecological and distributional information on Californian and Pacific Coast odonates. Kennedy (1917) and Paulson and Garrison (1977) provided considerable distributional information for the Pacific Coast region. *Aeshna* has been revised and many species placed into the genus *Rhionaeshna* (von Ellenrieder, 2002).

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Odonata Fabricius, 1793					X	X	X		X	X	X	X	X	X	X		
	Zygoptera Selys, 1854				X	X	X		X	X	X	X	X	X	X	Westfall and May (1996)	Has keys for all damselflies found in the region
		Calopterygidae Selys, 1850			X	X			X	X	X	X	X	X	X	Westfall and May (1996)	
			Calopteryx Leach, 1815		X	X			X	X	X	X				Westfall and May (1996)	
				Calopteryx aequabilis Say, 1839	X	X			X	X	X	X				Westfall and May (1996)	Relatively intolerant of pollution; rare in Northern CA
			Hetaerina Hagen in Selys, 1853		X	X			X			X	X	X	X	Westfall and May (1996)	
				Hetaerina americana (Fabricius, 1798)	X	X			X			X	X	X	X	Westfall and May (1996)	Relatively intolerant of pollution
				Hetaerina vulnerata Hagen in Selys, 1853	X	X						X		X		Westfall and May (1996)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Coenagrionidae Kirby, 1890			X				X			X				Westfall and May (1996)	
			Amphiagrion Selys, 1876		X	X	X		X	X	X	X	X	X	X	Westfall and May (1996)	
				Amphiagrion abbreviatum (Selys, 1876)	X	X	X		X	X	X	X	X	X	X	Westfall and May (1996)	
			Apanisagrion Kennedy, 1920		X									X		Westfall and May (1996)	
				Apanisagrion lais (Brauer, 1876)	X									X		Westfall and May (1996)	
			Argia Rambur, 1842		X	X			X	X	X	X	X	X	X	Westfall and May (1996)	
				Argia agrioides Calvert, 1895	X	X			X	X				X	X	Westfall and May (1996)	
				Argia alberta Kennedy, 1918	X	X			X	X		X	X	X		Westfall and May (1996)	prefers seeps and springs
				Argia emma Kennedy, 1915	X	X			X	X	X	X	X			Westfall and May (1996)	
				Argia fumipennis (Burmeister, 1839)	X									X		Westfall and May (1996)	
				Argia hinei Kennedy, 1918	X	X			X				X	X	X	Westfall and May (1996)	
				Argia immunda (Hagen, 1861)	X	X			X				X	X		Westfall and May (1996)	
				Argia lacrimans (Hagen, 1861)	X									X		Westfall and May (1996)	
				Argia lugens (Hagen, 1861)	X	X			X	X		X		X	X	Westfall and May (1996)	
				Argia moesta (Hagen, 1861)	X	X			X			X	X	X	X	Westfall and May (1996)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Argia munda Calvert, 1902	X									X		Westfall and May (1996)	
				Argia nahuana Calvert, 1902	X	X			X	X		X	X	X	X	Westfall and May (1996)	
				Argia oenea Hagen in Selys, 1865	X									X	X	Westfall and May (1996)	
				Argia pallens Calvert, 1902	X									X		Westfall and May (1996)	
				Argia pima Garrison, 1994	X									X		Westfall and May (1996)	
				Argia plana Calvert, 1902	X									X		Westfall and May (1996)	
				Argia sabino Garrison, 1994	X									X		Westfall and May (1996)	
				Argia sedula (Hagen, 1861)	X	X	X		X				X	X		Westfall and May (1996)	
				Argia tarascana Calvert, 1902	X									X		Westfall and May (1996)	
				Argia tezpi Calvert, 1902	X									X	X	Westfall and May (1996)	
				Argia tonto Calvert, 1902	X									X		Westfall and May (1996)	
				Argia translata Hagen in Selys, 1865	X								X	X		Westfall and May (1996)	
				Argia vivida Hagen in Selys, 1865	X	X			X	X	X	X	X	X	X	Westfall and May (1996)	
			Coenagrion Kirby, 1890		X		X		X	X	X	X	X	X		Westfall and May (1996)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Coenagrion resolutum (Hagen in Selys, 1876)	X		X		X	X	X	X	X	X		Westfall and May (1996)	Uncommon in northern Sierra lakes and bogs; larvae hard to distinguish from *Enallagma* sp.
			Coenagrion Kirby, 1890/*Enallagma* Selys, 1875		X		X									Westfall and May (1996)	some specimens incompletely separable
			Enallagma Selys, 1875		X	X	X		X	X	X	X	X	X	X	Westfall and May (1996)	
				Enallagma anna Williamson, 1900	X	X			X	X		X	X	X		Westfall and May (1996)	
				Enallagma basidens Calvert, 1902	X	X			X					X		Westfall and May (1996)	
				Enallagma boreale (Selys, 1875)	X		X		X	X	X	X	X	X		Westfall and May (1996)	
				Enallagma carunculatum Morse, 1895	X	X	X		X	X	X	X	X	X	X	Westfall and May (1996)	
				Enallagma civile (Hagen, 1861)	X	X	X		X			X	X	X	X	Westfall and May (1996)	
				Enallagma clausum Morse, 1895	X	X	X		X	X	X	X	X			Westfall and May (1996)	
				Enallagma cyathigerum (Charpentier, 1840)	X		X		X	X	X	X	X	X	X	Westfall and May (1996)	
				Enallagma ebrium (Hagen, 1861)	X						X	X				Westfall and May (1996)	
				Enallagma eiseni Calvert, 1895	X										X	Westfall and May (1996)	
				Enallagma novaehispaniae Calvert, 1902	X										X	Westfall and May (1996)	
				Enallagma praevarum (Hagen, 1861)	X	X	X		X			X	X	X	X	Westfall and May (1996)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Enallagma semicirculare Selys, 1876	X									X		Westfall and May (1996)	
			Hesperagrion Calvert, 1902		X									X		Westfall and May (1996)	
				Hesperagrion heterodoxum (Selys, 1868)	X									X		Westfall and May (1996)	
			Ischnura Charpentier, 1840		X	X	X		X	X	X	X	X	X	X	Westfall and May (1996)	
				Ischnura barberi Currie, 1903	X	X	X		X			X		X		Westfall and May (1996)	
				Ischnura cervula Selys, 1876	X	X	X		X	X	X	X	X	X	X	Westfall and May (1996)	
				Ischnura damula Calvert, 1902	X							X		X		Westfall and May (1996)	
				Ischnura demorsa (Hagen, 1861)	X							X		X		Westfall and May (1996)	
				Ischnura denticollis (Burmeister, 1839)	X				X	X		X	X	X	X	Westfall and May (1996)	
				Ischnura erratica Calvert, 1895	X		X		X	X	X					Westfall and May (1996)	
				Ischnura gemina (Kennedy, 1917)	X		X		X							Westfall and May (1996)	CA endemic
				Ischnura hastata (Say, 1839)	X	X	X		X					X		Westfall and May (1996)	
				Ischnura perparva McLachlan in Selys, 1876	X	X	X		X	X	X	X	X	X		Westfall and May (1996)	
				Ischnura ramburii (Selys, 1842)	X		X		X					X	X	Westfall and May (1996)	
			Nehalennia Selys, 1850		X		X		X		X					Westfall and May (1996)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Nehalennia irene (Hagen, 1861)	X		X		X		X					Westfall and May (1996)	
			Telebasis Selys, 1865		X	X	X		X			X		X	X	Westfall and May (1996)	
				Telebasis incolumis Williamson and Williamson, 1930	X										X	Westfall and May (1996)	
				Telebasis salva (Hagen, 1861)	X	X	X		X			X		X	X	Westfall and May (1996)	Widespread but uncommon at low to middle elevations
			Zoniagrion Kennedy, 1917		X	X			X							Westfall and May (1996)	
				Zoniagrion exclamationis (Selys, 1876)	X	X			X							Westfall and May (1996)	Monotypic; CA endemic
		Lestidae Needham, 1903			X	X	X		X	X	X	X		X	X	Westfall and May (1996)	
			Archilestes Selys, 1862		X	X	X		X	X	X	X		X	X	Westfall and May (1996)	
				Archilestes californica McLachlan, 1895	X	X	X		X	X	X			X	X	Westfall and May (1996)	
				Archilestes grandis (Rambur, 1842)	X	X			X			X		X	?	Westfall and May (1996)	
			Lestes Leach, 1815		X	X	X		X	X	X		X	X		Westfall and May (1996)	
				Lestes alacer Hagen, 1861	X									X		Westfall and May (1996)	
				Lestes congener Hagen, 1861	X	X	X		X	X	X	X	X	X		Westfall and May (1996)	
				Lestes disjunctus Selys, 1862	X		X		X	X	X	X	X	X		Westfall and May (1996)	*Lestes disjunctus disjunctus* Selys
				Lestes dryas Kirby, 1890	X		X		X	X	X	X	X	X		Westfall and May (1996)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Lestes stultus Hagen, 1861	X		X		X	X						Westfall and May (1996)	
				Lestes unguiculatus Hagen, 1861	X		X		X	X	X	X	X			Westfall and May (1996)	
		Platystictidae Laidlaw, 1924												X		Westfall and May (1996); Hoekstra and Garrison (1999)	very restricted locality
			Palaemnema Selys, 1860											X		Westfall and May (1996); Hoekstra and Garrison (1999)	very restricted locality
				Palaemnema domina Calvert, 1905	X									X		Westfall and May (1996); Hoekstra and Garrison (1999)	very restricted locality
	Anisoptera Selys, 1854				X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	Has keys for all dragonflies found in the region
		Aeshnidae Selys, 1858			X	X	X		X	X	X	X	X	X		Needham, Westfall and May (2000)	
			Aeshna Fabricius, 1775		X	X	X		X			X				Needham, Westfall and May (2000)	Even late instar larvae are difficult to distinguish and should be left at genus
			Aeshna Fabricius, 1775/Rhionaeshna von Ellenrieder, 2002		X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000); von Ellenrieder (2002)	
			Rhionaeshna von Ellenrieder, 2002		X	X	X		X	X	X	X	X	X		von Ellenrieder (2002)	generic separation may require identifying to species first
			Anax Leach, 1815		X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Anax junius (Drury, 1773)	X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
				Anax walsinghami McLachlan, 1882	X	X			X			X	X	X	X	Needham, Westfall and May (2000)	
			Oplonaeschna Selys, 1883		X				X			X		X		Needham, Westfall and May (2000)	
				Oplonaeschna armata (Hagen, 1861)	X				X			X		X		Needham, Westfall and May (2000)	Only one CA record from Water Canyon in Inyo County
			Remartinia Navás, 1911		X									X		Needham, Westfall and May (2000)	
				Remartinia luteipennis (Burmeister, 1839)	X									X		Needham, Westfall and May (2000)	
		Cordulegastridae Calvert, 1893			X	X			X	X	X	X	X	X		Needham, Westfall and May (2000)	
			Cordulegaster Leach, 1815		X	X			X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Cordulegaster diadema Selys, 1868	X							X		X		Needham, Westfall and May (2000)	
				Cordulegaster dorsalis Hagen in Selys, 1858	X				X	X	X	X	X			Needham, Westfall and May (2000)	
		Corduliidae Selys, 1871			X	X	X		X	X	X	X	X			Needham, Westfall and May (2000)	Corduliids unlikely in samples collected by SWAMP protocols
			Cordulia Leach, 1815		X		X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Cordulia shurtleffii Scudder, 1861	X		X		X	X	X	X	X			Needham, Westfall and May (2000)	Northern CA ponds and lakes at higher elevations
			Epitheca Charpentier, 1840		X	X	X		X	X	X					Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Epitheca canis (McLachlan, 1886)	X	X	X		X	X	X					Needham, Westfall and May (2000)	Lakes and slower sections of Northern CA streasm
				Epitheca spinigera (Selys, 1871)	X				X	X	X					Needham, Westfall and May (2000)	as *Tetragoneuria* in some lists
			Somatochlora Selys, 1871		X		X		X	X	X	X	X			Needham, Westfall and May (2000)	boggy marshes and lakes at higher elevation in Northern CA; uncommon
				Somatochlora albicincta (Burmeister, 1839)	X				X	X	X					Needham, Westfall and May (2000)	
				Somatochlora minor Calvert, 1898	X					X	X					Needham, Westfall and May (2000)	
				Somatochlora semicircularis (Selys, 1871)	X				X	X	X	X	X			Needham, Westfall and May (2000)	
		Gomphidae Rambur, 1842			X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
			Erpetogomphus Selys, 1858		X				X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
				Erpetogomphus compositus Hagen in Selys, 1858	X	X			X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
				Erpetogomphus crotalinus (Hagen in Selys, 1854)	X									X		Needham, Westfall and May (2000)	
				Erpetogomphus designatus Hagen in Selys, 1858	X								X	X		Needham, Westfall and May (2000)	
				Erpetogomphus lampropeltis Kennedy, 1918	X				X					X		Needham, Westfall and May (2000)	
			Gomphus Leach, 1815		X	X	X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Gomphus externus Hagen in Selys, 1858	X							X				Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Gomphus graslinellus Walsh, 1862	X						X					Needham, Westfall and May (2000)	
				Gomphus kurilis (Hagen in Selys, 1858)	X	X	X		X	X	X		X			Needham, Westfall and May (2000)	Northern CA streams; rarely in lakes
				Gomphus lynnae Paulson, 1983	X					X	X					Needham, Westfall and May (2000)	
			Octogomphus Selys, 1873		X				X	X	X		X		X	Needham, Westfall and May (2000)	
				Octogomphus specularis Hagen, 1859	X				X	X	X		X		X	Needham, Westfall and May (2000)	Monotypic
			Ophiogomphus Selys, 1854		X	X			X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Ophiogomphus arizonicus Kennedy, 1917	X									X		Needham, Westfall and May (2000)	
				Ophiogomphus bison Selys, 1873	X	X			X	X		?	X			Needham, Westfall and May (2000)	
				Ophiogomphus morrisoni Selys, 1879	X	X			X	X		X	X			Needham, Westfall and May (2000)	
				Ophiogomphus occidentis Hagen, 1883	X	X			X	X	X	X	X			Needham, Westfall and May (2000)	
				Ophiogomphus severus Hagen, 1874	X				X	X	X	X	X			Needham, Westfall and May (2000)	
			Progomphus Selys, 1854		X	X			X	X		X		X		Needham, Westfall and May (2000)	
				Progomphus borealis McLachlan in Selys, 1873	X	X			X	X		X		X		Needham, Westfall and May (2000)	
			Stylurus Needham, 1897		X	X			X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Stylurus intricatus (Hagen in Selys, 1858)	X	X			X				X	X		Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Stylurus olivaceus (Selys, 1873)	X	X			X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Stylurus plagiatus (Selys, 1854)	X	X			X					X		Needham, Westfall and May (2000)	
		Libellulidae Selys, 1850			X				X			X				Needham, Westfall and May (2000)	
			Brachymesia Kirby, 1889		X		X		X					X		Needham, Westfall and May (2000)	
				Brachymesia furcata (Hagen, 1861)	X		X		X					X		Needham, Westfall and May (2000)	Southern California ponds
				Brachymesia gravida (Calvert, 1890)	X									X		Needham, Westfall and May (2000)	
			Brechmorhoga Kirby, 1894		X	X			X			X		X		Needham, Westfall and May (2000)	
				Brechmorhoga mendax (Hagen, 1861)	X	X			X			X		X		Needham, Westfall and May (2000)	Deep crenulations in labial palps make this key to Cordullidae in Merritt and Cummins
				Brechmorhoga pertinax (Hagen, 1861)	X									X		Needham, Westfall and May (2000)	stray only? Not a breeding population
			Dythemis Hagen, 1861		X									X	X	Needham, Westfall and May (2000)	
				Dythemis fugax Hagen, 1861	X									X		Needham, Westfall and May (2000)	
				Dythemis nigrescens Calvert, 1899	X									X	X	Needham, Westfall and May (2000)	
				Dythemis velox Hagen, 1861	X									X		Needham, Westfall and May (2000)	
			Erythemis Hagen, 1861		X				X	X	X	X	X	X		Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Erythemis collacata (Hagen, 1861)	X				X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Erythemis simplicicollis (Say, 1839)	X									X		Needham, Westfall and May (2000)	
				Erythemis vesiculosa (Fabricius, 1775)	X									X		Needham, Westfall and May (2000)	
			Erythrodiplax Brauer, 1868		X				?					X	X	Needham, Westfall and May (2000)	
				Erythrodiplax basifusca (Calvert, 1895)	X									X	X	Needham, Westfall and May (2000)	
				Erythrodiplax funerea (Hagen, 1861)	X				?					X		Needham, Westfall and May (2000)	
			Ladona Needham, 1897		X		X		X	X	X	X				Needham, Westfall and May (2000)	
				Ladona julia (Uhler, 1857)	X		X		X	X	X	X				Needham, Westfall and May (2000)	
			Leucorrhinia Brittinger, 1850		X		X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Leucorrhinia borealis Hagen, 1890	X						X	X				Needham, Westfall and May (2000)	
				Leucorrhinia glacialis Hagen, 1890	X		X		X	X	X		X			Needham, Westfall and May (2000)	
				Leucorrhinia hudsonica (Selys, 1850)	X		X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Leucorrhinia intacta (Hagen, 1861)	X		X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Leucorrhinia proxima Calvert, 1890	X		X		X		X	X				Needham, Westfall and May (2000)	
			Libellula Linnaeus, 1758		X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Libellula comanche Calvert, 1861	X	X			X	X		X	X	X		Needham, Westfall and May (2000)	restricted to seeps and springs
				Libellula composita (Hagen, 1873)	X	X			X	X			X	X		Needham, Westfall and May (2000)	restricted to seeps and springs
				Libellula croceipennis Selys, 1868	X	X			X				X			Needham, Westfall and May (2000)	restricted to seeps and springs
				Libellula forensis Hagen, 1861	X	X			X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Libellula luctuosa Burmeister, 1839	X				X	X	X			X		Needham, Westfall and May (2000)	
				Libellula nodisticta Hagen, 1861	X	X			X	X		X	X	X		Needham, Westfall and May (2000)	prefers seeps and springs
				Libellula pulchella Drury, 1773	X		X		X	X	X		X	X		Needham, Westfall and May (2000)	
				Libellula quadrimaculata Linnaeus, 1758	X		X		X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Libellula saturata Uhler, 1857	X	X	X		X	X		X	X	X	X	Needham, Westfall and May (2000)	
			Macrodiplax Brauer, 1868		X		X		X					X	X	Needham, Westfall and May (2000)	
				Macrodiplax balteata (Hagen, 1861)	X		X		X					X	X	Needham, Westfall and May (2000)	desert spring-fed marshes
			Macrothemis Hagen, 1868		X									X		Needham, Westfall and May (2000)	
				Macrothemis inacuta Calvert, 1898	X									X		Needham, Westfall and May (2000)	
			Orthemis Hagen, 1861		X		X		X			X	X	X		Needham, Westfall and May (2000)	
				Orthemis discolor (Burmeister, 1839)	X		X							X		Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Orthemis ferruginea (Fabricius, 1775)	X		X		X			X	X	X		Needham, Westfall and May (2000)	
			Pachydiplax Brauer, 1868		X		X		X	X	X	X	X	X		Needham, Westfall and May (2000)	monotypic
				Pachydiplax longipennis (Burmeister, 1839)	X				X	X	X	X	X	X		Needham, Westfall and May (2000)	monotypic
			Paltothemis Karsch, 1890		X	X			X			X		X	X	Needham, Westfall and May (2000)	
				Paltothemis lineatipes Karsch, 1890	X	X			X			X		X	X	Needham, Westfall and May (2000)	Deep crenulations in labial palps make this key to Cordullidae in Merritt and Cummins
			Pantala Hagen, 1861		X				X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
				Pantala flavescens (Fabricius, 1798)	X				X	X	X		X	X		Needham, Westfall and May (2000)	
				Pantala hymenaea (Say, 1839)	X				X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
			Perithemis Hagen, 1861		X		X		X					X		Needham, Westfall and May (2000)	
				Perithemis domitia (Drury, 1773)	X									X		Needham, Westfall and May (2000)	
				Perithemis intensa Kirby, 1889	X		X		X					X		Needham, Westfall and May (2000)	
				Perithemis tenera (Say, 1839)	X									X		Needham, Westfall and May (2000)	
			Plathemis Hagen, 1861		X	X	X		X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Plathemis lydia (Drury, 1773)	X	X	X		X	X	X	X	X	X		Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Plathemis subornata Hagen, 1861	X	X	X		X	X			X	X		Needham, Westfall and May (2000)	
			Pseudoloeon Kirby, 1889		X									X		Needham, Westfall and May (2000)	monotypic
				Pseudoleon superbus (Hagen, 1861)	X									X		Needham, Westfall and May (2000)	monotypic
			Sympetrum Newman, 1833		X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
				Sympetrum corruptum (Hagen, 1861)	X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
				Sympetrum costiferum (Hagen, 1861)	X		X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Sympetrum danae (Sulzer, 1776)	X		X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Sympetrum illotum (Hagen, 1861)	X	X	X		X	X	X		X			Needham, Westfall and May (2000)	
				Sympetrum internum Montgomery, 1911	X	X	X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Sympetrum madidum (Hagen, 1861)	X	X	X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Sympetrum obtrusum (Hagen, 1867)	X		X		X	X	X	X	X			Needham, Westfall and May (2000)	
				Sympetrum occidentale Bartenev, 1911	X	X	X		X	X	X		X	X		Needham, Westfall and May (2000)	three recognized subspecies
				Sympetrum pallipes (Hagen, 1874)	X	X	X		X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Sympetrum signiferum Cannings and Garrison, 1991	X									X		Needham, Westfall and May (2000)	
				Sympetrum vicinum (Hagen, 1861)	X		X		X	X	X					Needham, Westfall and May (2000)	

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Tramea Hagen, 1861		X	X	X		X	X	X	X	X	X	X	Needham, Westfall and May (2000)	
				Tramea calverti Muttkowski, 1895	X	X	X		?							Needham, Westfall and May (2000)	unpublished record for CA
				Tramea lacerata Hagen, 1861	X	X	X		X	X	X	X	X	X		Needham, Westfall and May (2000)	
				Tramea onusta Hagen, 1861	X	X	X		X			X	X	X	X	Needham, Westfall and May (2000)	
		Macromiidae Needham, 1903			X				X							Needham, Westfall and May (2000)	
			Macromia Rambur, 1842		X				X	X	X		X	X		Needham, Westfall and May (2000)	
				Macromia magnifica McLachlan in Selys, 1874	X				X	X	X		X	X		Needham, Westfall and May (2000)	Northern CA foothills and coast ranges
		Petaluridae Needham, 1901			X				X	X	X		X			Needham, Westfall and May (2000)	
			Tanypteryx Kennedy, 1917														
				Tanypteryx hageni (Selys, 1879)	X				X	X	X		X			Needham, Westfall and May (2000)	Rare and localized in seeps and springs

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Plecoptera: Stoneflies
Standard Effort Level I: Genus
Standard Effort Level II: Species (where possible)
Standard Taxonomic Reference: Stewart and Stark (2002); Stewart and Stark (2008)
Reviewed by: John B. Sandberg

Nymphs may be identified to family and genus using Stewart and Stark (2002) or the chapter in Merritt, Cummins and Berg (Stewart and Stark, 2008), which also provides keys to adults. Although species keys exist for the adults of many families and genera in the West, many nymphs remain undescribed. Early instar nymphs of Capniidae can be very difficult to identify to genus; it is recommended that only mature nymphs be identified beyond Capniidae. Many genera and species of Capniidae, Leuctridae, Nemouridae and Taeniopterygidae are underrepresented in benthic samples because they emerge during the winter months or they prefer ephemeral habitats. A current species list and distribution for stoneflies, The North American Stonefly List (Stark et al.), is currently maintained on the Illinois Natural History Survey website.

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Plecoptera Burmeister, 1839						X	X	X		X	X	X	X	X	X	X	Stewart and Stark (2008); Stewart and Stark (2002)	has keys to all families and genera in North America
	Euholognatha Zwick, 1969					X	X	X		X	X	X	X	X	X	X		
		Capniidae Klapalek, 1905				X	X			X	X	X	X	X	X	X		Nymphs notoriously difficult to separate even to genus; immature specimens are best left at Capniidae
			Bolshecapnia Ricker, 1965			X	X			X							Stewart and Stark (2002)	
					Bolshecapnia maculata (Jewett, 1954)	X	X			X								known only from CA
			Capnia Pictet, 1841			X	X			X	X	X	X	X	X	X	Nelson and Baumann (1989)	nymphs are not separable to species
			Capnura Banks, 1900			X	X			X	X	X	X	X	X			nymphs not separable to species

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Eucapnopsis Okamoto, 1922			X	X			X	X	X	X	X	X			only one North American species
					Eucapnopsis brevicauda Claassen, 1924	X	X			X	X	X	X	X	X			only one North American species
			Isocapnia Banks, 1938			X	X			X	X	X	X				Zenger and Baumann, 2004	nymphs not separable to species
			Mesocapnia Rauser, 1968			X	X			X	X	X	X		X			nymphs not separable to species
			Paracapnia Hanson, 1946			X	X			X	X	X					Stark and Baumann (2004)	nymphs not separable to species
			Utacapnia Nebeker and Gaufin, 1967			X	X			X	X	X	X	X				nymphs not separable to species
		Leuctridae Klapalek, 1905											X					
			Calileuctra Shepard and Baumann, 1995			X	X			X							Shepard and Baumann (1995)	found in intermittant streams; nymphs not separable to species
			Despaxia Ricker, 1943			X	X			X	X	X						monotypic
					Despaxia augusta (Banks, 1907)	X	X			X	X	X						monotypic
			Megaleuctra Neave, 1934			X	X			X	X	X						nymphs not separable to species
			Moselia Ricker, 1943			X	X			X	X	X		X				monotypic
					Moselia infuscata (Claassen, 1923)	X	X			X	X	X		X				monotypic
			Paraleuctra Hanson, 1941			X	X			X	X	X	X	X				nymphs not separable to species
			Perlomyia Banks, 1906			X	X			X	X	X	X					nymphs not separable to species
			Pomoleuctra Stark and Kyzar, 2000			X	X			X	X						Stark and Kyzar (2001)	formerly in *Paraleuctra;* nymphs not separable to species
		Nemouridae Newman, 1853											X					
			Amphinemura Ris, 1902			X	X						X		X			nymphs not separable to species

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Lednia Ricker, 1952			X	X			X		X					Kondratieff, Lechleitner and Zuellig (2006); Baumann and Kondratieff (2010)	nymphs inseparable except by distribution
					Lednia borealis Baumann and Kondratieff, 2010	X	X					X					Baumann and Kondratieff (2010)	Kondratieff et al. (2006) reported this species as *Lednia tumana*
					Lednia sierra Baumann and Kondratieff, 2010	X	X			X							Baumann and Kondratieff (2010)	
			Malenka Ricker, 1952			X	X			X	X	X	X	X	X	X		nymphs not separable to species; genus currently being revised, many new species likely to be described
			Nanonemoura Baumann and Fiala, 2001			X	X				X							monotypic; known only from type locality
					Nanonemoura wahkeena (Jewett, 1954)	X	X				X							monotypic; known only from type locality
			Nemoura Pictet, 1841			X	X			X								
					Nemoura spiniloba Jewett, 1954	X	X			X								only one species in western North America
			Ostrocerca Ricker, 1952			X	X			?	X	X						nymphs not separable to species; unpublished record for CA
			Podmosta Ricker, 1952			X	X			X	X	X	X	X				nymphs not separable to species
			Prostoia Ricker, 1952			X	X			X	X	X	X	X				only one species in western North America
					Prostoia besametsa (Ricker, 1952)	X	X			X	X	X	X	X				only one species in western North America
			Soyedina Ricker, 1952			X	X			X	X	X		X				nymphs not separable to species
			Visoka Ricker, 1952			X	X			X	X	X						monotypic
					Visoka cataractae (Neave, 1933)	X	X			X	X	X						monotypic

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Zapada Ricker, 1952			X	X			X	X	X	X	X			Baumann et al. (1977)	key to nymphs of three species (cinctipes, columbiana and frigida); remaining species key to oregonensis group
					Zapada cinctipes (Banks, 1897)	X	X			X		X	X	X			Baumann et al. (1977)	
					Zapada columbiana (Claassen, 1923)	X	X			X	X	X	X				Baumann et al. (1977)	
					Zapada frigida (Claassen, 1923)	X	X			X	X	X	X	X			Baumann et al. (1977)	
				Zapada oregonensis group sensu Baumann et al. (1977)						X	X	X		X			Baumann et al. (1977)	includes *Z. cordillera* (Baumann and Gaufin, 1971), *Z. haysi* (Ricker, 1952) and *Z. oregonensis* (Claassen, 1923)
					Zapada cordillera (Baumann and Gaufin, 1971)	X	X			X	X	X					Baumann et al. (1977)	*Z. oregonensis* group sensu Baumann et al. (1977)
					Zapada haysi (Ricker, 1952)	X	X			X	X	X	X	X			Baumann et al. (1977)	*Z. oregonensis* group sensu Baumann et al. (1977)
					Zapada oregonensis (Claassen, 1923)	X	X			X	X	X		X			Baumann et al. (1977)	*Z. oregonensis* group sensu Baumann et al. (1977)
		Taeniopterygidae Klapalek, 1905				X	X			X	X	X	X	X	X			
			Doddsia Needham and Claassen, 1925			X	X			X	X	X	X	X				monotypic
					Doddsia occidentalis (Banks, 1900)	X	X			X	X	X	X	X				monotypic
			Oemopteryx Klapalek, 1902			X	X			X			X					nymphs not separable to species
			Taenionema Banks, 1905			X	X			X	X	X	X	X	X		Stewart (2009)	tentative key of nymphs to species
					Taenionema californicum (Needham and Claassen, 1925)	X	X			X								
					Taenionema grinnelli (Banks, 1918)	X	X			X								

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Taenionema jacobii Stanger and Baumann, 1993	X	X								X			
					Taenionema jeanae Baumann and Nelson, 2007	X	X			X							Baumann and Nelson (2007)	
					Taenionema jewetti Stanger and Baumann, 1993	X	X				X	X						
					Taenionema kincaidi (Hoppe, 1938)	X	X			X	X	X		X				
					Taenionema oregonense (Needham and Claassen, 1925)	X	X				X	X						
					Taenionema pacificum (Banks, 1900)	X	X			X	X	X	X		X			
					Taenionema pallidum (Banks, 1902)	X	X			X	X	X	X	X				
					Taenionema raynorium (Claassen, 1937)	X	X			X								
					Taenionema uinta Stanger and Baumann, 1993	X	X				X		X	X				
					Taenionema umatilla Stanger and Baumann, 1993	X	X				X							
			Taeniopteryx Pictet, 1841			X	X			X	X	X	X					
					Taeniopteryx nivalis (Fitch, 1847)	X	X			X	X	X	X					only species in region
	Systellognatha Enderlein, 1909					X	X						X					
		Chloroperlidae Okamoto, 1912				X	X			X	X	X	X	X	X	X	Surdick (1985)	
			Alloperla Banks, 1906			X	X			X	X	X	X	X				nymphs not separable to species
			Bisancora Surdick, 1981			X	X			X	X					X		nymphs not separable to species
			Haploperla Navas, 1934			X	X			X	X	X				X		
					Haploperla chilnualna (Ricker, 1952)	X	X			X	X	X				X		only species in western North America
			Kathroperla Banks, 1920			X	X			X	X	X		X			Stark and Surdick (1987)	nymphs not separable to species
			Paraperla Banks, 1906			X	X			X	X	X	X					nymphs not separable to species
			Plumiperla Surdick, 1985			X	X			X	X	X	X	X				nymphs not separable to species
			Sasquaperla Stark and Baumann, 2001			X	X			X							Stark and Baumann (2001)	monotypic
					Sasquaperla hoopa Stark and Baumann, 2001	X	X			X							Stark and Baumann (2001)	monotypic

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Suwallia Ricker, 1943			X	X			X	X	X	X	X	X		Alexander (1999)	nymphs not separable to species
			Sweltsa Ricker, 1943			X	X			X	X	X	X	X	X		Stark and Stewart (2005)	nymphs not separable to species; 9 of 21 western species nymphs described
			Triznaka Ricker, 1952			X	X			X	X	X	X	X				nymphs not separable to species
			Utaperla Ricker, 1952			X	X						X	X				
					Utaperla sopladora Ricker, 1952	X	X						X	X				only species found in western North America
		Peltoperlidae Claassen, 1931				X	X			X	X	X		X				
			Sierraperla Jewett, 1954			X	X			X	X			X				monotypic
					Sierraperla cora (Needham and Smith, 1916)	X	X			X	X			X				monotypic
			Soliperla Ricker, 1952			X	X			X	X	X		X			Stark (1983	nymphal key includes only 4 of the 7 known species
			Yoraperla Ricker, 1952			X	X			X	X	X		X			Stark and Nelson (1994)	nymphs for all four North American species described and keyed
					Yoraperla brevis (Banks, 1907)	X	X				X						Stark and Nelson (1994)	
					Yoraperla mariana (Ricker, 1943)	X	X			X	X	X					Stark and Nelson (1994)	
					Yoraperla nigrisoma (Banks, 1948)	X	X			X	X	X		X			Stark and Nelson (1994)	
					Yoraperla siletz Stark and Nelson, 1994	X	X			X	X	X					Stark and Nelson (1994)	
		Perlidae MacLachlan, 1886				X	X			X	X	X	X	X	X			
			Acroneuria Pictet, 1841			X	X						X				Baumann, Gaufin and Surdick (1977)	
					Acroneuria abnormis (Newman, 1838)	X	X						X				Baumann, Gaufin and Surdick (1977)	
			Anacroneuria Klapalek, 1909			X	X								X			

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Anacroneuria wipukupa Baumann and Olson, 1984	X	X								X			only species found in North America
			Calineuria Ricker, 1954			X	X			X	X	X						
					Calineuria californica (Banks, 1905)	X	X			X	X	X						only species found in North America
			Claassenia Wu, 1934			X	X			X	X	X	X		X			
					Claassenia sabulosa (Banks, 1900)	X	X			X	X	X	X		X			only species found in North America
			Doroneuria Needham and Claassen, 1922			X	X			X	X	X	X	X				Utah record based on nymphs only
					Doroneuria baumanni Stark and Gaufin, 1974	X	X			X	X	X		X				*D. theodora* (Needham and Claassen) is found in BC, ID, MT and WY
			Hesperoperla Banks, 1938			X	X			X	X	X	X	X	X			mature nymphs of the two species easily separable
					Hesperoperla hoguei Baumann and Stark, 1980	X	X			X							Baumann and Stark (1980)	
					Hesperoperla pacifica (Banks, 1900)	X	X			X	X	X	X	X	X		Stewart and Stark (2002)	
		Perlodidae Klapalek, 1912				X	X			X	X	X	X	X	X	X		
			Baumannella Stark and Stewart, 1985			X	X			X							Stark and Stewart (1985)	monotypic
					Baumannella alameda (Needham and Claassen, 1925)	X	X			X							Stark and Stewart (1985)	monotypic
			Calliperla Banks, 1947			X	X			X	X	X						monotypic
					Calliperla luctuosa (Banks, 1906)	X	X			X	X	X						monotypic
			Cascadoperla Szczytko and Stewart, 1979			X	X			X	X	X					Szczytko and Stewart (1979)	monotypic
					Cascadoperla trictura (Hoppe, 1938)	X	X			X	X	X					Szczytko and Stewart (1979)	monotypic
			Chernokrilus Ricker, 1952			X	X			X	X							nymphs are not separable to species

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Chernokrilus misnomus (Claassen, 1925)	X	X			X	X						Stewart and Stark (1984); Kondratieff, Baumann and Lee (2007)	*Chernokrilus erratus* (Claassen) is now a jr. synonym
			Cosumnoperla Szczytko and Bottorff, 1987			X	X										Szczytko and Bottorff (1987)	
					Cosumnoperla hypocrena Szczytko and Bottorff, 1987	X	X			X							Szczytko and Bottorff (1987)	
					Cosumnoperla sequoia Bottorff, 2007	X	X			X							Bottorff (2007)	gives characters for nymph
			Cultus Ricker, 1952			X	X			X	X	X	X		X			
			Diura Billberg, 1820			X	X			X	X		X	X				
					Diura knowltoni (Frison, 1937)	X	X			X	X		X	X				
			Frisonia Ricker, 1943			X	X			X	X	X		X				
					Frisonia picticeps (Hanson, 1942)	X	X			X	X	X		X				
			Isogenoides Klapalek, 1912			X	X			X		X	X		X		Sandberg and Stewart (2005)	key given for nymphs to all species of *Isogenoides*
					Isogenoides colubrinus (Hagen, 1874)	X	X			X			X		X		Sandberg and Stewart (2005)	
					Isogenoides elongatus (Hagen, 1874)	X	X					X	X		X		Sandberg and Stewart (2005)	
					Isogenoides zionensis Hanson, 1949	X	X						X		X		Sandberg and Stewart (2005)	
			Isoperla Banks, 1906			X	X			X	X	X	X	X	X	X	Szczytko and Stewart (1979); Bottorff et al. (1990); Szczytko and Stewart (2002)	not all western *Isoperla* species described as nymphs

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Kogotus Ricker, 1952			X	X			X	X	X	X					immature specimens have secondary lacinial tooth which makes ID to genus difficult; immature *Kogotus* and *Rickera* difficult to separate
					Kogotus modestus (Banks, 1908)	X	X						X					
					Kogotus nonus (Needham and Claassen, 1925)	X	X			X	X	X						only species in the region
			Kogotus Ricker, 1952/*Rickera* Jewett, 1954			X	X			X	X	X						many specimens of these genera, especially early instars inseparable
			Megarcys Klapalek, 1912			X	X			X	X	X	X	X				nymphs not separable to species
			Oroperla Needham, 1933			X	X			X								monotypic
					Oroperla barbara Needham, 1933	X	X			X								monotypic
			Osobenus Ricker, 1952			X	X			X	X	X						monotypic
					Osobenus yakimae (Hoppe, 1938)	X	X			X	X	X						monotypic
			Perlinodes Needham and Claassen, 1925			X	X			X	X	X						monotypic
					Perlinodes aurea (Smith, 1917)	X	X			X	X	X						monotypic
			Pictetiella Illies, 1966			X	X					X	X				Stark and Kondratieff (2004)	Stark and Kondratieff provide characters to separate the nymphs although distribution also diagnostic
					Pictetiella expansa (Banks, 1920)	X	X						X				Stark and Kondratieff (2004)	found in scattered localities CO, UT, MT, WY, ID
					Pictetiella lechleitneri Stark and Kondratieff, 2004	X	X					X					Stark and Kondratieff (2004)	found only in Mt. Ranier, WA area
			Rickera Jewett, 1954			X	X			X	X	X		X				monotypic; immature specimens difficult to separate from *Kogotus*
					Rickera sorpta (Needham and Claassen, 1925)	X	X			X	X	X		X				monotypic
			Salmoperla Baumann and Lauck, 1987			X	X			X								monotypic
					Salmoperla sylvanica Baumann and Lauck, 1987	X	X			X								monotypic

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Setvena Ricker, 1952			X	X				X	X					Stewart and Stanger (1985)	key to nymphs of all three known species
					Setvena tibialis (Banks, 1914)	X	X				X	X						
					Setvena wahkeena Stewart and Stanger, 1985	X	X				X							
			Skwala Ricker, 1952			X	X			X	X	X	X	X	X		Zwick (1989)	nymphs of the two species are not separable
			Susulus Bottorff and Stewart, 1989			X	X			X							Bottorff et al. (1989)	monotypic
					Susulus venustus (Jewett, 1965)	X	X			X							Bottorff et al. (1989)	monotypic
		Pteronarcyidae Enderlein, 1909				X	X			X	X	X	X	X	X			
			Pteronarcella Banks, 1900			X	X			X	X	X	X	X	X		Baumann et al. (1977); Stewart and Stark (2002)	Stewart and Stark suggest that key in Baumann et al. does not work to separate nymphs of the two species
			Pteronarcys Newman, 1838			X	X			X	X	X	X	X	X		Baumann et al. (1977)	key to nymphs of both species
					Pteronarcys californica Newport, 1851	X	X			X	X	X	X		X			
					Pteronarcys princeps Banks, 1907	X	X			X	X	X	X	X				

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Hemiptera (Suborder Heteroptera): True Bugs
Standard Effort Level I: Genus
Standard Effort Level II: Species
Standard Taxonomic Reference(s): Polhemus (2008)
Reviewed by:

Keys to families and genera are provided in Merritt, Cummins and Berg (Polhemus, 2008). The best regional text for all families remains Menke (ed.) (1979), which gives keys to all genera and species then known to occur in California. Stonedahl and Lattin (1986) surveyed the Corixidae for Oregon and Washington. Polhemus and Polhemus (2002) discussed the distributions of aquatic bugs in the Great Basin. This revision of the STE includes only those families which are truly aquatic (all Nepomorpha, except Gelastocoridae and Ochteridae) and excludes all Gerromorpha and Leptopodomorpha. The Notonectidae and Pleidae, which are included in this list, are generally rejected from benthic datasets.

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Hemiptera Linnaeus, 1758								X	X	X	X	X	X	X	X	X	X	X		
	Heteroptera Latreille, 1810							X	X	X	X	X	X	X	X	X	X	X	Polhemus (2008); Menke et al. (1979)	Polhemus provides keys to families and genera; Menke et al. provide keys to species, many of these keys will work for the entire western US
		Gerromorpha Popov, 1971							X	X		X	X	X	X	X	X	X		
			Gerridae Leach, 1815						X	X		X	X	X	X	X	X	X		Excluded from benthic datasets
			Hebridae Amyot and Serville, 1843						X	X		X	X			X	X			Excluded from benthic datasets
			Hydrometridae Bilberg, 1820						X	X		X	X				X			Excluded from benthic datasets

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Macroveliidae McKinstry, 1942						X	X		X	X		X	X	X			Excluded from benthic datasets
			Mesoveliidae Douglas and Scott, 1867						X	X		X								Excluded from benthic datasets
			Veliidae Amyot and Serville, 1843						X	X		X	X	X	X	X	X			Excluded from benthic datasets
		Nepomorpha Popov, 1968						X	X	X		X	X	X	X	X	X			
			Belostomatidae Leach, 1815					X				X			X		X		Menke (1979)	
						Abedus Stal, 1862		X				X			X		X		Menke (1979)	
							Abedus breviceps Stal, 1862	X									X		Menke (1979)	
							Abedus herberti Hidalgo, 1935	X							X		X		Menke (1979)	
							Abedus indentatus (Haldeman, 1854)	X				X							Menke (1979)	
							Abedus ovatus Stal, 1862	X									X		Menke (1979)	
							Abedus parkeri Menke, 1966	X									X		Menke (1979)	
							Abedus vicinus Mayr, 1871	X									X		Menke (1979)	
						Belostoma Latreille, 1807		X				X	X		X	X	X		Menke (1979)	
							Belostoma bakeri Montandon, 1913	X				X	X		X	X	X		Menke (1979)	
							Belostoma confusum Lauck, 1959	X									X		Menke (1979)	
							Belostoma flumineum Say, 1832	X				X	X			X	X		Menke (1979)	
							Belostoma saratogae Menke, 1958	X				X							Menke (1979)	known only from Saratoga Spring, Death Valley, CA
							Belostoma subspinosum (Palisot, 1820)	X				X					X		Menke (1979)	
						Lethocerus Mayr, 1853		X				X	X	X	X	X	X		Goodwyn (2006)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Lethocerus americanus (Leidy, 1847)	X				X	X	X	X	X			Goodwyn (2006)	
							Lethocerus angustipes (Mayr, 1871)	X				X				X			Goodwyn (2006)	known in the U.S. only from Saratoga Spring, Death Valley, CA; also found in Mexico
							Lethocerus medius (Guerin-Meneville, 1857)	X									X		Goodwyn (2006)	
			Corixidae Leach, 1815					X	X	X	X	X	X	X	X	X	X	X	Polhemus (2008); Hungerford (1948); Lauck (1979); Stonedahl and Lattin (1986)	
				Corixinae Enderlein, 1915				X	X	X	X	X	X	X	X	X	X	X	Polhemus (2008); Hungerford (1948); Lauck (1979); Stonedahl and Lattin (1986)	
					Corixini Enderlein, 1915			X	X	X	X	X	X	X	X	X	X	X	Polhemus (2008); Hungerford (1948); Lauck (1979); Stonedahl and Lattin (1986)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Arctocorisa Wallengren, 1894		X		X		?			X				Hungerford (1948)	high elevation ponds
							Arctocorisa sutilis (Uhler, 1876)	X		X		?			X				Hungerford (1948)	unpublished record for CA
						Callicorixa White, 1873		X				X	X	X	X	X			Stonedahl and Lattin (1986)	
							Callicorixa alaskensis Hungerford, 1926	X						X	X				Stonedahl and Lattin (1986)	
							Callicorixa audeni Hungerford, 1928	X				X	X	X	X	X			Stonedahl and Lattin (1986)	
							Callicorixa scudderi Jansson, 1979	X					X	X					Stonedahl and Lattin (1986)	
							Callicorixa vulnerata (Uhler, 1861)	X				X	X	X	X				Stonedahl and Lattin (1986)	
						Cenocorixa Hungerford, 1948		X				X	X	X	X				Hungerford (1948)	
							Cenocorixa andersoni Hungerford, 1948	X					X	X					Hungerford (1948)	
							Cenocorixa bifida (Hungerford, 1926)	X						X	X				Hungerford (1948)	as *C. bifida hungerfordi* Lansbury, 1960
							Cenocorixa blaisdelli (Hungerford, 1930)	X				X		X					Hungerford (1948)	
							Cenocorixa expleta (Uhler, 1895)	X						X					Hungerford (1948)	
							Cenocorixa kuiterti Hungerford, 1948	X				X							Hungerford (1948)	
							Cenocorixa utahensis (Hungerford, 1925)	X					X	X	X	X	X		Hungerford (1948)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Cenocorixa wileyae (Hungerford, 1926)	X				X	X	X	X	X	X		Hungerford (1948)	
						Corisella Lundblad, 1928		X				X	X	X	X	X	X		Hungerford (1948)	
							Corisella decolor (Uhler, 1871)	X				X	X		X	X			Hungerford (1948)	
							Corisella edulis (Champion, 1901)	X					X		X	X	X		Hungerford (1948)	
							Corisella inscripta (Uhler, 1894)	X				X	X	X	X	X	X		Hungerford (1948)	
							Corisella tarsalis (Fieber, 1851)	X				X			X	X			Hungerford (1948)	
						Hesperocorixa Kirkaldy, 1908		X				X	X	X	X	X	X		Hungerford (1948)	
							Hesperocorixa atopodonta (Hungerford, 1927)	X					X	X					Hungerford (1948)	
							Hesperocorixa escheri (Heer, 1853)	X						X					Hungerford (1948)	dubious species?
							Hesperocorixa kennicotti (Uhler, 1897)	X						X					Hungerford (1948)	
							Hesperocorixa laevigata (Uhler, 1893)	X				X	X	X	X	X	X		Hungerford (1948)	
							Hesperocorixa nitida (Fieber, 1851)	X						X					Hungerford (1948)	
							Hesperocorixa vulgaris (Hungerford, 1925)	X				X	X	X					Hungerford (1948)	
						Morphocorixa Jaczewski, 1931		X									X		Hungerford (1948)	
							Morphocorixa lundbladi (Jaczewski, 1931)	X									X		Hungerford (1948)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Palmacorixa Abbott, 1912		X				?							Hungerford (1948)	unpublished record for CA
							Palmacorixa buenoi Abbott, 1913	X				?							Hungerford (1948)	unpublished record for CA
						Pseudocorixa Jaczewski, 1931		X									X		Hungerford (1948)	
							Pseudocorixa beameri (Hungerford, 1928)	X									X		Hungerford (1948)	
						Ramphocorixa Abbott, 1912		X									X		Hungerford (1948)	
							Ramphocorixa rotundocephala Hungerford, 1927	X									X		Hungerford (1948)	
						Sigara Fabricius, 1775		X				X	X	X	X	X	X		Hungerford (1948)	
							Sigara decoratella (Hungerford, 1926)	X						X					Hungerford (1948)	
							Sigara alternata (Say, 1825)	X					X	X		X	X		Hungerford (1948)	
							Sigara grossolineata Hungerford, 1948	X				X	X	X	X				Hungerford (1948)	
							Sigara krafti Stonedahl, 1984	X					X	X					Stonedahl (1984)	
							Sigara mckinstryi Hungerford, 1948	X				X	X	X					Hungerford (1948)	
							Sigara nevadensis (Walley, 1936)	X							X	X			Hungerford (1948)	
							Sigara omani (Hungerford, 1930)	X				X	X	X		X	X		Hungerford (1948)	
							Sigara vallis Lauck, 1966	X				X							Lauck (1979)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Sigara vandykei Hungerford, 1948	X				X		X					Hungerford (1948)	
							Sigara washingtonensis Hungerford, 1948	X				X	X	X	X	X	X		Hungerford (1948)	
						Trichocorixa Kirkaldy, 1908		X			X	X			X	X	X		Hungerford (1948)	
							Trichocorixa arizonensis Sailer, 1948	X									X		Hungerford (1948)	
							Trichocorixa calva (Say, 1832)	X				X				X	X		Hungerford (1948)	
							Trichocorixa reticulata (Guerin-Meneville, 1857)	X			X	X				X			Hungerford (1948)	
							Trichocorixa uhleri Sailer, 1948	X								X	X		Hungerford (1948)	
							Trichocorixa verticalis (Fieber, 1851)	X			X	X			X	X	X		Hungerford (1948)	
					Graptocorixini Hungerford, 1948			X				X	X		X	X	X	X	Hungerford (1948)	
						Graptocorixa Hungerford, 1930		X				X	X		X	X	X		Hungerford (1948)	
							Graptocorixa abdominalis (Say, 1832)	X							X	X	X	X	Hungerford (1948)	
							Graptocorixa californica (Hungerford, 1925)	X				X	X						Hungerford (1948)	
							Graptocorixa gerhardi (Hungerford, 1925)	X									X		Hungerford (1948)	
							Graptocorixa serrulata (Uhler, 1897)	X					X			X	X		Hungerford (1948)	
							Graptocorixa uhleri (Hungerford, 1925)	X				X				X			Hungerford (1948)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Graptocorixa uhleroidea Hungerford, 1938	X				X							Hungerford (1948)	
						Neocorixa Hungerford, 1925		X									X		Hungerford (1948)	only one species found in North America
							Neocorixa snowi Hungerford, 1925	X									X		Hungerford (1948)	only species found in North America
			Gelastocoridae Kirkaldy, 1897									X	X	X	X	X	X			Shoredwellers; excluded from benthic datasets
			Naucoridae Leach, 1815					X				X	X		X	X	X			
				Ambrysinae Usinger, 1941				X				X	X		X	X	X			
						Ambrysus Stal, 1861		X				X	X			X	X		Polhemus (1979); La Rivers (1951)	
							Ambrysus amargosus La Rivers, 1953	X								X			Polhemus (1979); La Rivers (1951)	Ash Meadows, NV; Listed under the Federal Endangered Species Act
							Ambrysus arizonus La Rivers, 1951	X								X			Polhemus (1979); La Rivers (1951)	
							Ambrysus californicus Montandon, 1897	X				X							Polhemus (1979); La Rivers (1951)	
							Ambrysus circumcinctus Montandon, 1910	X									X		Polhemus (1979); La Rivers (1951)	
							Ambrysus funebris La Rivers, 1949	X				X				X			Polhemus (1979); La Rivers (1951)	Death Valley, CA

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Ambrysus melanopterus Stal, 1862	X									X		Polhemus (1979); La Rivers (1951)	
							Ambrysus mormon Montandon, 1909	X				X	X		X	X	X		Polhemus (1979); La Rivers (1951)	
							Ambrysus occidentalis La Rivers, 1951	X				X					X		Polhemus (1979); La Rivers (1951)	
							Ambrysus pulchellus Montandon, 1897	X									X		Polhemus (1979); La Rivers (1951)	
							Ambrysus puncticollis Stal, 1876	X				X					X		Polhemus (1979); La Rivers (1951)	
							Ambrysus relictus Polhemus and Polhemus, 1994	X								X			Polhemus and Polhemus (1994)	Ash Meadows, NV
							Ambrysus thermarum La Rivers, 1953	X									X			
							Ambrysus woodburyi Usinger, 1946	X							X	X	X		Polhemus (1979); La Rivers (1951)	
				Limnocorinae Stal, 1876				X								X			Polhemus (1979); La Rivers (1951)	
							Limnocoris Stal, 1860	X								X			Polhemus (1979); La Rivers (1951)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Limnocoris moapensis (La Rivers, 1950)	X								X			Sites and Willig (1994)	Moapa Warm Springs, NV
				Naucorinae Stal, 1876				X				X				X	X		Polhemus (1979); La Rivers (1951)	
						Pelocoris Stal, 1876		X				X				X	X		Polhemus (1979); La Rivers (1951)	
							Pelocoris biimpressus Montandon, 1898	X				X				X	X		Polhemus and Sites (1995)	*P. shoshone* La Rivers, 1948 now a synonym
			Nepidae Latreille, 1802					X				X	X				X		Sites and Polhemus (1994)	
				Nepinae Douglas and Scott, 1865				X									X		Sites and Polhemus (1994)	
					Curictini Menke and Stange, 1964			X									X		Sites and Polhemus (1994)	
						Curicta Stal, 1861		X									X		Sites and Polhemus (1994)	
							Curicta pronotata Kuitert, 1949	X									X		Sites and Polhemus (1994)	
				Ranatrinae Douglas and Scott, 1865				X				X	X			X	X		Sites and Polhemus (1994)	

Taxonomic Hierarchy								Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Ranatra Fabricius, 1790		X				X	X			X	X		Sites and Polhemus (1994)	
							Ranatra brevicollis Montandon, 1910	X				X							Sites and Polhemus (1994)	
							Ranatra fusca Palisot, 1820	X				X	X			X			Sites and Polhemus (1994)	
							Ranatra montezuma Polhemus, 1976	X									X		Sites and Polhemus (1994)	known only from Montezuma's Well, AZ
							Ranatra quadridentata Stal, 1862	X				X					X		Sites and Polhemus (1994)	
			Notonectidae Latreille, 1802									X	X	X	X	X	X			Excluded from benthic datasets
			Ochteridae Kirkaldy, 1906									X					X		Polhemus (1996)	Excluded from benthic datasets
			Pleidae Fieber, 1851									X			?				Polhemus (2008)	
		Leptopodomorpha Popov, 1971										X	X	X		X	X			
			Leptopodidae Amyot and Serville, 1843									X		X		X				Excluded from benthic datasets
			Saldidae Amyot and Serville, 1843									X			X				Polhemus (2008)	Excluded from benthic datasets
	Homoptera Latreille (1810)																			Excluded from benthic datasets

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Megaloptera: Dobsonflies and Alderflies
Standard Effort Level I: Genus
Standard Effort Level II: Genus
Standard Taxonomic Reference: Flint, Evans and Neunzig (2008)
Reviewed by:

Larvae may be identified to genus using the key in Merritt, Cummins and Berg (Flint, Evans and Neunzig, 2008). Early instar corydalids are best left at family since head color patterns generally do not develop until later instars. Evans's (1972) unpublished dissertation provides a species key to western megalopteran larvae, although the key does not include one species of *Sialis* and three species of *Protochauliodes*. Keys to adults as well as distributional and ecological information may found in the sources listed below. The Bibliography of the Neuropterida website is a useful resource and provides many downloadable PDFs of Megaloptera and Neuroptera literature.

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Megaloptera Latreille, 1802				X	X	X		X	X	X	X	X	X	X		
	Corydalidae Leach, 1815			X	X			X	X	X	X	X	X	X	Flint, Evans and Neunzig (2008)	
		Corydalus Latreille, 1802		X	X			X			X	X	X	X	Contreras-Ramos (1998)	*C. cognatus* (Hagen) is a synonym of *C. texanus* Banks
			Corydalus bidenticulatus Contreras-Ramos, 1998	X	X								X		Contreras-Ramos (1998)	single record from Huachuca Mts., Miller Canyon
			Corydalus texanus Banks, 1903	X	X			X			X	X	X	X	Contreras-Ramos (1998)	
		Dysmicohermes Munroe, 1953		X	X			X	X	X					Evans (1972)	
		Neohermes Banks, 1908		X	X			X	X			X	X		Evans (1972)	
		Orohermes Evans, 1984		X	X			X	X						Evans (1972)	
			Orohermes crepusculus (Chandler, 1954)	X	X			X	X						Evans (1972)	
		Protochauliodes van der Weele, 1909		X	X			X	X	X					Evans (1972)	mostly found in intermittent streams; larvae not described for all species

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
	Sialidae Leach, 1815			X	X	X		X	X	X		X				
		Sialis Latreille, 1802		X	X	X		X	X	X	X	X			Evans (1972); Whiting (1991)	key to mature larvae, but lacking *S. bilobata*

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Neuroptera: Spongillaflies
Standard Effort Level I: Genus
Standard Effort Level II: Genus
Standard Taxonomic Reference: Flint, Evans and Neunzig (2008)
Reviewed by:

Larvae may be identified to genus using the key in Merritt, Cummins and Berg (Flint, Evans and Neunzig, 2008). Bowles (2006) provides a species key to larvae, although there is only one species in each genus found in the region. Larvae feed on and live in conjunction with freshwater sponges. Several other Neuroptera families occur in the region and their larvae occasionally show up in benthic samples. Tauber (1991) provides a key to North American Neuroptera larvae. The Bibliography of the Neuropterida website is a useful resource and provides many downloadable PDFs of Megaloptera and Neuroptera literature.

Taxonomic Hierarchy				Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Neuroptera Linnaeus, 1758				X	X	X		X	X	X			X		Flint, Evans and Neunzig (2008); Parfin and Gurney (1956); Brown (1974)	
	Sisyridae Handlirsch, 1906			X	X	X		X	X	X			X		Flint, Evans and Neunzig (2008); Parfin and Gurney (1956); Brown (1974)	
		Climacia MacLachlan, 1869		X		X		X	X						Flint, Evans and Neunzig (2008); Parfin and Gurney (1956); Brown (1974)	only one species in the region
			Climacia californica Chandler, 1953	X		X		X	X						Chandler (1953); Whaley et al. (2004)	only species in the region
		Sisyra Burmeister, 1839		X		X			X	X			X		Flint, Evans and Neunzig (2008); Parfin and Gurney (1956); Brown (1974)	only one species in the region
			Sisyra vicaria Walker, 1853	X		X			X	X			X		Grigarick (1975)	only species in the region

Literature Cited

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Parfin, S. I. and A. B. Gurney. 1956. The spongilla-flies, with special reference to those of the Western Hemisphere (Sisyridae, Neuroptera). Proceedings of the United States National Museum **105**(3360): 421-529.

Tauber, C. A. 1991. Order Neuroptera. [pp. 126-143]. In: F. W. Stehr (editor), Immature Insects, Volume 2. Kendall/Hunt, Dubuque, Iowa.

Whaley, D. K., E. A. Boyd, D. G. Johnson, and R. S. Zack. 2004. A new state record for *Climacia californica* Chandler (Neuroptera: Sisyridae) in Washington. Pan-Pacific Entomologist **79**(3/4): 247-248.

Additional Sources of Information on Neuroptera

Bibliography of the Neuropterida, version 8.00. Maintained by the Department of Entomology at Texas A&M University, updated 6 April 2009. Accessed 14 February 2011 at URL: http://lacewing.tamu.edu/Bibliography/index.html

Trichoptera: Caddisflies
Standard Effort Level I: Genus
Standard Effort Level II: Species (where possible)
Standard Taxonomic Reference: Wiggins (1996)
Reviewed by:

Keys to families and genera are given in Wiggins (1996) as well as the chapters in Merritt, Cummins and Berg (Wiggins and Currie, 2008; Morse and Holzenthal, 2008). Wiggins (2004) provides updated family keys for larvae, pupae and adults as well as providing a wealth of behavioral and ecological information. Distributional information comes from original sources. Blinn and Ruiter (2005, 2006) give a preliminary checklist for Arizona. The Trichoptera World Checklist website maintained by John C. Morse at Clemson University is a good source for information.

Larvae for many genera are not identifiable to species because some remain undescribed. Larvae and pupae can sometimes be reared to adults and thus identified to species, but only if living specimens are collected. For preserved specimens, well-developed, pharate pupae can sometimes be identified to species by using the metamorphotype method (Milne, 1938). In this case, the genitalia of a pharate pupa can be observed through the pupal cuticle and the specimen identified using keys and descriptions of the adult. DNA is also a useful tool for making larval and female associations provided specimens are preserved in 80% (or better) non-denatured ethanol and frozen or sent immediately for analysis.

There are presently 19 recognized species groups of *Rhyacophila* known from the region covered by this list. Of these, 14 species groups have at least one representative species described as larvae in the peer-reviewed literature. These citations can be found in the list under the literature cited column for each species group. Ross (1956) and Schmid (1970) assigned most of the known *Rhyacophila* species to species groups based on adult characteristics. Three as yet unpublished but disseminated works (Wold, 1973; Smith, draft key and Wisseman, draft key) have further dealt with *Rhyacophila*, illustrating or describing larvae for most of the species groups. Associative material now exists for 4 of the remaining 5 species groups, leaving only larvae from the *Rhyacophila viquaea* group as undescribed and unassociated. Since the metamorphotype method may be used to identify pupae to any of these species groups based on the adult morphology and taxonomy, all 19 species groups names have been included in this version of the STE List (see STE Rules, section 3.2.3). However, at this time we recommend not using the following species group names for larval identifications until formal descriptions appear in the peer reviewed literature: *ecosa* group, *rayneri* group, *vemna* group, *viquaea* group and *vofixa* group.

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Trichoptera Leach, 1813						X	X	X		X	X	X	X	X	X	X	Wiggins and Currie (2008); Morse and Holzenthal (2008); Wiggins (1996)	
	Spicipalpia Weaver, 1984					X	X	X		X	X	X	X	X	X	X	Wiggins (1996)	
		Glossosomatidae *Wallengren, 1891*				X	X			X	X	X	X	X	X		Wiggins (1996)	
			Agapetus Curtis, 1834			X	X			X	X	X			X		Wiggins (1996)	
			Anagapetus Ross, 1938			X	X			X	X	X					Wiggins (1996); Ruiter (2004)	
			Culoptila Mosely, 1954			X	X								X		Wiggins (1996); Blahnik and Holzenthal (2006)	
			Glossosoma Curtis, 1834			X	X			X	X	X		X	X		Wiggins (1996)	
			Protoptila Banks, 1904			X	X			X	X	X			X		Wiggins (1996)	
		Hydrobiosidae Ulmer, 1905				X	X								X		Wiggins (1996)	
			Atopsyche Banks, 1905			X	X							X	X		Wiggins (1996)	
		Hydroptilidae Stephens, 1836				X	X	X		X	X	X	X	X	X		Wiggins (1996); Blickle (1979)	except for monotypic forms, larvae not identifiable to species
			Agraylea Curtis, 1834			X	X	X		X	X	X	X				Wiggins (1996)	Primarily lotic, will sometimes be found in slow-moving sections of streams
			Alisotrichia Flint, 1964			X	X						X		X		Wiggins	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																	(1996)	
					Alisotrichia arizonica (Blickle & Denning, 1977)	X	X						X		X			
			Dibusa Ross, 1939			X	X			?							Wiggins (1996)	Based on an undescribed species collected by D.G. Denning; larvae probably similar to the eastern species *D. angata* Ross
			Hydroptila Dalman, 1819			X	X	X		X	X	X	X	X	X		Wiggins (1996)	
			Ithytrichia Eaton, 1873			X	X			X			X		X		Wiggins (1996)	
			Leucotrichia Mosely, 1934			X	X			X	X		X	X	X		Wiggins (1996)	
			Mayatrichia Mosely, 1937			X	X						X		X		Wiggins (1996)	
			Metrichia Ross, 1938			X	X								X		Wiggins (1996)	found in small springs and seeps
			Neotrichia Morton, 1905			X	X			X			X		X		Wiggins (1996)	
				Neotrichia canixa group Marshall, 1979		X	X			?					X			larvae in the *canixa* group have lateral horns on the head as illustrated in Fig 3.9g in Wiggins (1996); the ABL has seen specimens from several localities in Northern California
			Nothotrichia Flint, 1967			X	X			X							Harris and Armitage (1997)	larval association made with a metamorphotype male; the description is in progress
					Nothotrichia shasta Harris and Armitage, 1997	X	X			X							Harris and Armitage (1997)	larval association made with a metamorphotype male; the description is in progress
			Ochrotrichia Mosely, 1934			X	X			X	X	X	X	X	X		Wiggins (1996)	many undescribed species in CA alone

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Oxyethira Eaton, 1873			X	X	X		X	X	X	X		X		Wiggins (1996)	
			Palaeagapetus Ulmer, 1912			X	X			X	X	X					Wiggins (1996)	occur in cold water seeps in liverworts
			Stactobiella Martynov, 1924			X	X			X	X	X	X		X		Wiggins (1996)	occur in small, rapid streams
			Zumatrichia Mosely, 1937			X	X								X		Wiggins (1996)	occur in fast-flowing, large rivers
					Zumatrichia notosa (Ross, 1944)	X	X								X			
		Rhyacophilidae Stephens, 1836				X	X			X	X	X					Wiggins (1996)	
			Himalopsyche Banks, 1940			X	X			X	X	X					Wiggins (1996)	only one species in Nearctic
					Himalopsyche phryganea (Ross, 1941)	X	X			X	X	X						only one species in Nearctic
			Rhyacophila Pictet, 1834			X	X			X	X	X	X	X	X		Wiggins (1996)	
				Rhyacophila alberta group sensu Schmid (1970)		X	X			X	X	X	X				Schmid (1970), Smith (1968)	
				Rhyacophila angelita group sensu Schmid (1970)		X	X			X	X	X	X	X	X		Schmid (1970), Flint (1962), Smith (1968)	
				Rhyacophila betteni group sensu Schmid (1970)		X	X			X	X	X					Schmid (1970), Smith (1968)	
				Rhyacophila brunnea group sensu Smith and Manuel (1984)		X	X			X	X	X	X	X			Smith and Manuel (1984)	
				Rhyacophila coloradensis group sensu Schmid (1970)		X	X			X	X	X	X		X		Schmid (1970), Smith (1968), Peck and Smith (1977)	
				Rhyacophila ecosa group sensu Schmid (1970)		X	X			X	X	X					Schmid (1970)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Rhyacophila grandis group sensu Schmid (1970)		X	X			X	X	X					Schmid (1970), Smith (1984)	
				Rhyacophila hyalinata group sensu Schmid (1970)		X	X			X	X		X				Schmid (1970), Smith (1968)	
				Rhyacophila lieftincki group sensu Schmid (1970)		X	X			X	X	X					Schmid (1970), Smith (1984)	
					Rhyacophila arnaudi Denning, 1948	X	X			X	X	X						sole representative of *lieftincki* group in SAFIT region
				Rhyacophila nevadensis group sensu Schmid (1970)		X	X			X	X	X		X			Schmid (1970), Smith (1985)	
				Rhyacophila oreta group sensu Schmid (1970)		X	X			X	X	X	X				Schmid (1970), Smith (1968)	
				Rhyacophila rayneri group sensu Ross (1956)		X	X			X						X	Ross (1956)	larvae associated, but unpublished
					Rhyacophila rayneri Ross, 1951	X	X			X						X		only species in this group
				Rhyacophila rotunda group sensu Schmid (1970)		X	X			X			X	X	X		Schmid (1970), Smith (1968)	
				Rhyacophila sibirica group sensu Ross (1956)		X	X			X	X	X	X				Schmid (1970), Smith (1968)	
				Rhyacophila vagrita group sensu Schmid (1970)		X	X				X	X	X				Schmid (1970), Smith (1968)	
					Rhyacophila vagrita Milne, 1936	X	X				X	X	X					
				Rhyacophila vemna group sensu Schmid (1970)		X	X				X	X					Schmid (1970)	larvae unknown, most likely similar to *brunnea* group but bigger
				Rhyacophila verrula group sensu Schmid (1970)		X	X			X	X	X	X				Schmid (1970), Smith (1968)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Rhyacophila viquaea group sensu Schmid (1970)	X	X			X	X	X					Schmid (1970)	larva unknown
					Rhyacophila vofixa group sensu Schmid (1970)	X	X			X	X	X	X				Schmid (1970)	larvae associated, but unpublished
	Annulipalpia Martynov, 1924																	
		Hydropsychidae Curtis, 1835				X	X			X	X	X		X	X		Wiggins (1996)	
			Arctopsyche MacLachlan, 1868			X	X			X	X	X	X	X			Wiggins (1996); Givens and Smith (1980)	Occur in cold, fast streams; key to larvae in Givens and Smith (1980)
					Arctopsyche californica Ling, 1938	X	X			X							Givens and Smith (1980)	
					Arctopsyche grandis (Banks, 1900)	X	X			X	X	X	X	X			Givens and Smith (1980)	
					Arctopsyche ladogensis (Kolenati, 1859)	X	X						X					
			Cheumatopsyche Wallengren, 1891			X	X			X	X	X	X	X	X		Wiggins (1996)	Occur in warmer streams; relatively tolerant of pollution; larvae not presently identifiable to species
			Diplectrona Westwood, 1840			X	X			X							Wiggins (1996)	CA endemic; known from only a couple sites in Southern CA
					Diplectrona californica Banks, 1914	X	X			X								CA endemic; known from only a couple sites in Southern CA
			Homoplectra Ross, 1938			X	X			X	X						Wiggins (1996)	Occur in intermittent spring seeps, headwaters of mountain streams

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Hydropsyche Pictet, 1834			X	X			X	X	X	X	X	X		Wiggins (1996); Schefter and Wiggins (1984); Geraci, Zhou, Morse and Kjer (2010)	Some authors split this genus into *Hydropsyche* (s. str.) and *Ceratopsyche* Ross and Unzicker, 1977 (Schefter and Wiggins use the term *Hydropsyche morosa* group); Geraci et al (2010) presented DNA evidence to show that *Ceratopsyche* is not a good genus.
			Macrostemum Kolenati, 1859			X	X						X					
					Macrostemum zebratum (Hagen, 1861)	X	X						X					
			Parapsyche Betten 1934			X	X			X	X	X		X			Wiggins (1996); Givens and Smith (1980)	Occur in small, cold streams; only two of the five known western species described as larvae
			Smicridea MacLachlan, 1871			X	X			X					X		Wiggins (1996)	Often abundant in southwestern streams
		Philopotamidae Stephens, 1829				X	X			X	X		X	X	X		Wiggins (1996)	
			Chimarra Stephens, 1829			X	X			X	X		X	X	X		Wiggins (1996)	
			Dolophilodes Ulmer, 1909			X	X			X	X	X		X			Wiggins (1996)	larvae not separable from *Sisko* at this time
			Dolophilodes Ulmer, 1909/Sisko Ross 1956			X	X			X	X	X	X	X			Blahnik (2005)	larvae inseparable between these two genera at this time
			Sisko Ross, 1956			X	X				X						Blahnik (2005)	larvae unassociated; 2 species removed from *Dolophilodes*
			Wormaldia MacLachlan, 1865			X	X			X	X	X	X	X	X		Wiggins (1996)	
		Polycentropodidae Ulmer, 1903				X	X	X		X	X	X			X		Wiggins (1996)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Nyctiophylax Brauer, 1865			X	X	X			X						Wiggins (1996)	some authors use genus *Paranyctiophylax* for North American species
					Nyctiophylax moestus Banks, 1911	X	X	X			X							Occur in lakes and slow-moving sections of streams
			Polycentropus Curtis, 1835			X	X	X		X	X	X	X		X		Wiggins (1996); Wiggins (1973)	Wiggins (1973) reported *Polycentropus* in temporary pools
			Polyplectropus Ulmer, 1905			X	X								X		Wiggins (1996)	Occur in small, cool streams
					Polyplectropus charlesi (Ross, 1941)	X	X								X			
		Psychomyiidae Walker, 1852				X	X			X	X	X	X		X		Wiggins (1996)	
			Psychomyia Latreille, 1829			X	X			X	X	X	X		X		Wiggins (1996)	
			Tinodes Curtis, 1834			X	X			X	X		X	X	X		Wiggins (1996)	Larvae probably occur only in lotic waters where they build silken tubes of sand, often near the stream margin
		Xiphocentronidae Brauer, 1870 Ross, 1949				X	X								X		Wiggins (1996)	
			Cnodocentron Schmid, 1982			X	X								X		Wiggins (1996); Moulton and Stewart (1997)	
					Cnodocentron yavapai Moulton and Stewart, 1997	X	X								X		Wiggins (1996); Moulton and Stewart (1997)	The type locality is a small, spring-fed stream with a dense canopy; larvae build silken tubes on rocks
	Integrapalpia Martynov, 1924					X	X	X		X	X	X	X	X	X	X		
		Apataniidae Wallengren, 1886				X	X	X		X	X	X	X	X	X		Wiggins (1996)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Allomyia Banks, 1916			X	X			X	X	X	X	X			Wiggins (1996)	cold springs, Sierra Nevada Mts.
			Apatania Kolenati, 1848			X	X	X									Wiggins (1996)	larvae in far North or at high elevations may live in lakes; only 1 of the 4 species described as larvae
			Moselyana Denning, 1949			X	X				X	X					Wiggins (1996)	monotypic; larvae live in seeps
					Moselyana comosa (Denning, 1949)	X	X				X	X					Wiggins (1996)	monotypic; larvae live in seeps
			Pedomoecus Ross, 1947			X	X			X	X	X					Wiggins (1996)	monotypic
					Pedomoecus sierra Ross, 1947	X	X			X	X	X					Wiggins (1996)	monotypic
		Brachycentridae Ulmer, 1903				X	X			X	X	X	X	X	X			
			Amiocentrus Ross, 1938			X	X			X	X		X	X			Wiggins (1996)	monotypic
					Amiocentrus aspilus (Ross, 1938)	X	X			X	X		X	X			Wiggins (1996)	monotypic
			Brachycentrus Curtis, 1834			X	X			X	X	X	X	X	X		Wiggins (1996); Flint (1984)	larvae are identifiable to species
					Brachycentrus americanus (Banks, 1899)	X	X			X	X	X	X		X		Flint (1984)	
					Brachycentrus echo (Ross, 1947)	X	X			X			X				Flint (1984)	
					Brachycentrus occidentalis Banks, 1911	X	X			X	X	X	X	X	X		Flint (1984)	
			Eobrachycentrus Wiggins, 1965			X	X				X	X					Wiggins (1996)	monotypic
					Eobrachycentrus gelidae Wiggins, 1965	X	X				X	X					Wiggins (1996)	monotypic
			Micrasema MacLachlan, 1876			X	X			X	X	X	X		X		Wiggins (1996); Chapin (1978)	
		Calamoceratidae Ulmer, 1905				X	X			X	X						Wiggins (1996)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Heteroplectron MacLachlan, 1871			X	X			X	X						Wiggins (1996)	larvae live in slower moving waters with woody debris
					Heteroplectron californicum MacLachlan, 1871	X	X			X	X						Wiggins (1996)	larvae live in slower moving waters with woody debris
			Phylloicus Müller, 1880			X	X								X		Wiggins (1996)	2 species found in AZ; no species key for larvae
		Goeridae Ulmer, 1903				X	X			X	X	X					Wiggins (1996)	
			Goera Stephens, 1829			X	X			X	X						Wiggins (1996)	one species in Western North America
					Goera archaon Ross, 1947	X	X			X	X							one species in Western North America
			Goeracea Denning, 1968			X	X				X	X					Wiggins (1996); Wiggins (1973)	key to larvae and pupae of both species
					Goeracea genota (Ross, 1941)	X	X				X	X					Wiggins (1996)	
					Goeracea oregona Denning, 1968	X	X			X	X						Wiggins (1996)	
			Lepania Ross, 1941			X	X				X	X					Wiggins (1996)	monotypic
					Lepania cascada Ross, 1941	X	X				X	X					Wiggins (1996)	monotypic
		Helicopsychidae Ulmer, 1906				X	X	X		X	X	X	X		X		Wiggins (1996); Johanson (2002)	
			Helicopsyche von Siebold, 1856			X	X	X		X	X	X	X		X		Wiggins (1996); Johanson (2002)	larvae not adequately separable to species
		Lepidostomatidae Ulmer, 1903				X	X	X					X				Wiggins (1996); Weaver	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																	(1988)	
					Lepidostoma Rambur, 1842	X	X	X		X	X	X	X	X	X		Wiggins (1996); Weaver (1988)	
		Leptoceridae Leach, 1815				X	X	X		X	X	X	X	X			Wiggins (1996)	
					Ceraclea Stephens, 1829	X	X	X		X	X	X		X			Wiggins (1996); Resh (1976); Morse (1975)	some species feed on freshwater sponges
					Mystacides Berthold, 1879	X	X	X		X	X	X	X				Wiggins (1996); Yamamoto and Wiggins (1964)	Yamamoto and Wiggins provided a larval key to species
					Mystacides alafimbriata Hill-Griffin, 1912	X				X	X	X	X					
					Mystacides interjecta (Banks, 1914)	X				?								unconfirmed record for CA; *Mystacides longicornis* (Linnaeus, 1758) is a junior synonym
					Mystacides sepulchralis (Walker, 1852)	X				X								
					Nectopsyche Müller, 1879	X	X	X		X	X	X	X	X	X		Wiggins (1996)	larvae of Western species are incompletely separable
					Oecetis MacLachlan, 1877	X	X	X		X	X		X		X		Wiggins (1996); Floyd (1995)	
					Triaenodes MacLachlan, 1865	X	X	X			X	X	X		X		Wiggins (1996); Glover (1996)	Holzenthal and Andersen (2004) consider *Ylodes* as a subgenus of *Triaenodes*
		Limnephilidae Kolenati, 1848				X	X			X	X	X	X	X	X		Wiggins (1996)	monotypic
					Allocosmoecus Banks, 1943	X	X			X	X	X					Wiggins (1996)	monotypic

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Allocosmoecus partitus Banks, 1943	X	X			X	X	X					Wiggins (1996)	monotypic
			Amphicosmoecus Schmid, 1955			X	X			X	X		X				Wiggins (1996)	monotypic
					Amphicosmoecus canax (Ross, 1947)	X	X			X	X		X		X			monotypic
			Anabolia Stephens, 1837			X	X								X			
					Anabolia bimaculata (Walker, 1852)	X	X						X		X			
			Asynarchus MacLachlan, 1880			X	X	X		X	X	X	X				Wiggins (1996)	
			Chyranda Ross, 1944			X	X			X	X	X	X	X			Wiggins (1996)	monotypic
					Chyranda centralis (Banks, 1900)	X	X			X	X	X	X	X				monotypic
			Clistoronia Banks, 1916			X		X		X	X	X	X		X		Wiggins (1996)	Larvae live in ponds and lakes at higher elevations
			Clostoeca Banks, 1943			X		X		X	X	X					Wiggins (1996)	monotypic; larvae live in small seepage areas
					Clostoeca disjuncta (Banks, 1914)	X		X		X	X	X					Wiggins (1996)	monotypic; larvae live in small seepage areas
			Crenophylax Ruiter and Nishimoto, 2007			X	X								X		Ruiter and Nishimoto (2007)	
					Crenophylax sperryi (Banks, 1943)	X	X								X		Ruiter and Nishimoto (2007)	moved from *Limnephilus sperryi* (Banks)
			Cryptochia Ross, 1950			X	X			X							Wiggins (1996); Wisseman and Anderson (1987)	Larvae live in small, cold spring streams at or above water's edge
			Desmona Denning, 1954			X	X	X		X	X	X					Wiggins (1996); Wiggins and Wisseman (1990)	Larvae live in small streams and seepage areas

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Dicosmoecus MacLachlan, 1875			X	X	X		X	X	X	X	X			Wiggins (1996); Wiggins and Richardson (1982)	Larvae may be identified to species using Wiggins and Richardson (1982)
					Dicosmoecus atripes (Hagen, 1875)	X	X	X		X	X	X	X	X			Wiggins and Richardson (1982)	
					Dicosmoecus gilvipes (Hagen, 1875)	X	X	X		X	X	X	X	X			Wiggins and Richardson (1982)	
					Dicosmoecus pallicornis Banks, 1943	X	X	X		X				X			Wiggins and Richardson (1982)	
			Ecclisocosmoecus Schmid, 1964			X	X				X	X					Wiggins (1996); Ross (1950)	Only one North American species
					Ecclisocosmoecus scylla (Milne, 1935)	X	X				X	X					Wiggins (1996); Ross (1950)	Only one North American species
			Ecclisomyia Banks, 1907			X	X			X	X	X	X	X			Wiggins (1996)	
			Eocosmoecus Wiggins and Richardson, 1989			X	X				X	X					Wiggins (1996); Wiggins and Richardson (1989)	Two species occur in Western North America, both described as larvae
					Eocosmoecus frontalis (Banks, 1943)	X	X				X	X					Wiggins (1996)	
					Eocosmoecus schmidi (Wiggins, 1975)	X	X					X					Wiggins (1996)	
			Glyphopsyche Banks, 1904			X	X	X		X	X	X					Wiggins (1996)	Three species occur in US; only one in SAFIT region

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Glyphopsyche irrorata (Fabricius, 1781)	X	X	X		X	X	X					Wiggins (1996)	
			Grammotaulius Kolenati, 1848			X	X	X			X		X				Wiggins (1996)	Found in ponds and small streams
					Grammotaulius betteni Hill-Griffin, 1912	X	X	X			X						Wiggins (1996)	Found in ponds and small streams
					Grammotaulius lorettae Denning, 1941	X	X	X					X				Wiggins (1996)	Found in ponds and small streams
			Halesochila Banks, 1907			X		X		?	X	X					Wiggins (1996)	monotypic; unpublished record for CA
					Halesochila taylori (Banks, 1904)	X		X		?	X	X					Wiggins (1996)	monotypic; unpublished record for CA
			Hesperophylax Banks, 1916			X	X	X		X	X	X	X	X	X		Wiggins (1996); Parker and Wiggins (1985)	larvae described for all but one Western species (*H. minutus*)
			Homophylax Banks, 1900			X	X			X	X	X	X	X			Wiggins (1996)	Larvae easily confused with *Psychoglypha*
			Hydatophylax Wallengen, 1891			X	X			X	X	X	X				Wiggins (1996); Schmid (1950); Ruiter (1999)	the Utah population has likely been extirpated
					Hydatophylax hesperus (Banks, 1914)	X	X			X	X	X	X				Wiggins (1996); Schmid (1950); Ruiter (1999)	the Utah population has likely been extirpated
			Lenarchus Martynov, 1914			X		X		X	X	X	X	X			Wiggins (1996)	
			Limnephilus Leach, 1815			X	X	X		X	X	X	X		X		Wiggins (1996)	
			Nemotaulius Banks, 1906			X		X			X		X				Wiggins (1996)	only one Nearctic species

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Nemotaulius hostilis (Hagen, 1873)	X		X			X		X					only one Nearctic species
			Onocosmoecus Banks, 1943			X	X	X		X	X	X	X	X			Wiggins (1996)	
			Philarctus MacLachlan, 1880			X	X	X			X						Wiggins (1996)	only one North American species
					Philarctus bergrothi McLachlan, 1880	X	X	X			X							only one North American species
			Philocasca Ross, 1941			X	X					X					Wiggins (1996)	
			Pseudostenophylax Martynov, 1909			X	X			X	X						Wiggins (1996)	only one species in western North America
					Pseudostenophylax edwardsi (Banks, 1920)	X	X			X	X							only one species in western North America
			Psychoglypha Ross, 1944			X	X			X	X	X	X				Wiggins (1996)	larvae may be confused with *Homophylax*
			Psychoronia Banks, 1916			X							X					
					Psychoronia costalis (Banks, 1901)	X							X					
			Pycnopsyche Banks, 1905			X	X					X					Wiggins (1996)	
					Pycnopsyche guttifer (Walker, 1852)	X	X					X						only species in SAFIT region
		Odontoceridae Wallengren, 1891				X	X			X					X		Wiggins (1996)	
			Marilia Müller, 1880			X	X			X					X		Wiggins (1996)	
			Namamyia Banks, 1905			X	X			X	X						Wiggins (1996)	monotypic
					Namamyia plutonis Banks, 1905	X	X			X	X							monotypic
			Nerophilus Banks, 1899			X	X			X	X						Wiggins (1996)	monotypic
					Nerophilus californicus (Hagen, 1861)	X	X			X	X							monotypic
			Parthina Denning, 1954			X	X			X	X				X		Wiggins (1996)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Phryganeidae Leach, 1815				X	X	X		X	X	X	X				Wiggins (1996); Wiggins (1998)	Wiggins (1998) reviews larvae and adults for the family
			Agrypnia Curtis, 1835			X	X	X		X	X	X	X				Wiggins (1996)	
			Banksiola Martynov, 1924			X	X	X		X	X		X				Wiggins (1996)	transcontinental; but only species known in western North America
					Banksiola crotchi Banks, 1943	X	X	X		X	X		X					
			Phryganea Linnaeus, 1758			X		X		X	X		X				Wiggins (1996)	
					Phryganea cinerea Walker, 1852	X		X		X	X		X					
			Ptilostomis Kolenati, 1859			X	X	X				X					Wiggins (1996)	
					Ptilostomis ocellifera (Walker, 1852)	X	X	X				X						
			Yphria Milne, 1934			X	X			X	X						Wiggins (1996)	monotypic
					Yphria californica (Banks, 1907)	X	X			X	X							monotypic
		Rossianidae Gall, 1996				X	X					X					Wiggins (1996)	small, cold mountain streams
			Goereilla Denning, 1968			X	X					X					Ruiter (1999)	
					Goereilla baumanni Denning, 1971	X	X					X					Ruiter (1999)	
			Rossiana Denning, 1953			X	X					X					Wiggins (1996)	small, cold mountain streams
					Rossiana montana Denning, 1953	X	X					X					Wiggins (1996)	small, cold mountain streams
		Sericostomatidae Stephens, 1836				X	X			X	X				X		Wiggins (1996)	
			Agarodes Banks, 1899			X	X						X				Wiggins (1996)	
					Agarodes hesperus (Banks, 1914)	X	X						X				Wiggins (1996)	
			Gumaga Tsuda, 1938			X	X			X	X		X		X		Wiggins (1996)	

Taxonomic Hierarchy						Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Uenoidae Iwata, 1927				X	X			X	X	X		X	X		Wiggins (2005)	
			Farula Milne, 1936			X	X			X	X	X					Wiggins (2005)	small, cold mountain streams
			Neophylax MacLachlan, 1871			X	X			X	X	X	X	X			Vineyard et al. (2005); Wiggins (2004)	larvae may be identified to species
					Neophylax occidentis Banks, 1924	X	X			X	X		X	X				
					Neophylax rickeri Milne, 1935	X	X			X	X	X						
					Neophylax smithi Vineyard and Wiggins, 1987	X	X					X						
					Neophylax splendens Denning, 1948	X	X			X	X	X	X					
			Neothremma Dodds and Hisaw, 1925			X	X			X	X	X	X				Wiggins (2004)	small to medium turbulent mountain streams
			Oligophlebodes Ulmer, 1905			X	X			X	X	X	X		X		Wiggins (2004)	small, turbulent mountain streams

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Lepidoptera: Moths and Butterflies
Standard Effort Level I: Genus for *Parapoynx* and *Petrophila*, otherwise Order
Standard Effort Level II: Genus for *Parapoynx* and *Petrophila*, otherwise Order
Standard Taxonomic Reference: Solis (2008)
Reviewed by:

Larvae of *Parapoynx* and *Petrophila* may be identified to genus using the key in Merritt, Cummins and Berg (Solis, 2008). This key and others for aquatic Lepidoptera should be used with caution for specimens collected in bioassessment samples. Careful collecting of Lepidoptera larvae to preserve case integrity and to record host-plant association is required to eliminate accidentals (e.g., terrestrial or riparian taxa). The key presented in Stehr and Martinat (1987) is a more complete guide to the families of North American Lepidoptera.

Taxonomic Hierarchy					Habitat				Distribution							Literature Cited	Comments
Order	Family	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Lepidoptera Linnaeus, 1758					X	X	X		X	X	X		X	X	X	Solis (2008); Stehr and Martinat (1987)	
	Pyralidae Latreille, 1802				X	X	X		X	X	X		X	X	X		
		Parapoynx Hübner, 1825			X	X			X								
		Petrophila Guilding, 1830			X	X	X		X	X	X		X	X	X		

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Coleoptera: Beetles
Standard Effort Level I: Genus
Standard Effort Level II: Species (where possible)
Standard Taxonomic Reference: White and Roughley (2008), Larson et al. (2000) (Dytiscidae), Shepard (2002) (Elmidae)
Reviewed by: Eric G. Chapman (Haliplidae), Doug Post (Dytiscidae)

Aquatic beetles (larvae and adults) can generally be identified to genus using the keys in Merritt, Cummins and Berg (White and Roughley, 2008). Adults can be identified to genus using the keys in Arnett and Thomas (2001) and Arnett et al. (2002). Although designed for the Florida beetle fauna, Epler (1999) is a useful resource. Larson et al. (2000) should be used for all generic dytiscid identifications. For specimens from the Southwest, other supplementary references may be required for species identifications. Post (2005) put together an excellent guide to California dytiscids. Challet and Brett (1998) is very useful for dytiscid distributions within California. An undescribed elmid genus, which is being described by Cheryl Barr, is known to occur throughout the Pacific Northwest. Shepard (1993) gives some habitat and ecological information for this genus. Brown (1972a) is still the best source for elmid species keys. The keys in White and Roughley (2008) for the Chrysomelidae, Staphylinidae and Curculionidae should be used with caution since each of these families have very few truly aquatic representatives – none benthic – but many terrestrial genera. The inclusion of these genera in White and Roughley (2008) for these families makes the assumption that the specimens being keyed are definitely aquatic, thus excluding the possibility of accidental terrestrials. This caveat also applies to a number of other families that have riparian or strictly terrestrial adults.

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Coleoptera Linnaeus, 1758							X	X	X		X	X	X	X	X	X	X	White and Roughley (2008)	Keys for families and genera
	Myxophaga Crowson, 1955						X				X				X	X			
		Hydroscaphidae LeConte, 1874					X				X				X	X			found in thin films of water

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Hydroscapha LeConte, 1874		X				X				X	X		Maier, Ivie, Johnson and Maddison (2010)	found in thin films of water; Maier et al. (2010) described a new species from Idaho and suggest that many other undescribed species may be found in the Western USA
						Hydroscapha natans LeConte, 1874	X				X				X	X		Shepard (2003)	found in thin films of water
		Microsporidae Crotch, 1873									X					X		Shepard (2003)	shoredwellers
					Sphaerius Waltl, 1838						X					X		Shepard (2003)	shoredwellers
	Adephaga Schellenberg, 1806						X	X			X	X	X	X	X	X	X		
		Amphizoidae LeConte, 1853					X	X			X	X	X	X	X	X		Kavanaugh (1986)	
					Amphizoa LeConte, 1854		X	X			X	X	X					Kavanaugh (1986)	
						Amphizoa insolens LeConte, 1853	X	X			X	X	X					Kavanaugh (1986)	
						Amphizoa lecontei Matthews, 1872	X	X				X	X	X	X	X		Kavanaugh (1986)	
						Amphizoa striata Van Dyke, 1927	X	X				X	X					Kavanaugh (1986)	
		Carabidae Latreille, 1802									X	X	X	X	X	X	X		Many species are riparian; only two listed as "semi-aquatic"
				Omophronini Bonelli, 1810							X	X	X	X		X			
					Omophron Latreille, 1802						X							Benschoter and Cook (1956)	Shoredwellers; excluded from benthic datasets
				Pogonini Laporte, 1834															
					Thalassotrechus van Dyke, 1918						X								Excluded from benthic datasets
						Thalassotrechus barbarae (Horn, 1892)					X								intertidal dweller; *Thalassotrechus nigripennis* van Dyke, 1918 is a junior synonym

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Dytiscidae Leach, 1815							X	X	X		X	X	X	X	X	X	X	Larson et al. (2000); Post (2005); Challet and Brett (1998)	Larson et al. (2000) should be considered as the standard text for this family.
	Copelatinae Van den Branden, 1885						X		X		X	X				X		Larson et al. (2000)	
					Copelatus Erichson, 1832		X		X		X	X				X		Larson et al. (2000)	
						Copelatus chevrolati renovatus Guignot, 1952	X		X		X					X		Larson et al. (2000)	
						Copelatus glyphicus (Say, 1823)	X		X		X	X						Larson et al. (2000)	
	Hydrotrupinae Roughley, 2000						X	X			X	X						Larson et al. (2000)	monotypic; Pacific Coast of CA and OR; also Sierra Nevada Mts.
					Hydrotrupes Sharp, 1882		X	X			X	X						Larson et al. (2000)	monotypic; Pacific Coast of CA and OR; also Sierra Nevada Mts.
						Hydrotrupes palpalis Sharp, 1882	X	X			X	X						Larson et al. (2000)	monotypic; Pacific Coast of CA and OR; also Sierra Nevada Mts.
	Laccophilinae Bedel, 1881						X		X		X	X	X	X	X	X		Larson et al. (2000); Zimmerman (1970)	
					Laccophilus Leach, 1817		X		X		X	X	X	X	X	X		Larson et al. (2000); Zimmerman (1970)	
						Laccophilus biguttatus Kirby, 1837	X		X		X			X				Larson et al. (2000); Zimmerman (1970)	
						Laccophilus fasciatus terminalis Sharp, 1882	X		X		X			X				Larson et al. (2000); Zimmerman	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																		(1970)	
						Laccophilus horni van den Branden, 1885	X									X		Larson et al. (2000); Zimmerman (1970)	
						Laccophilus maculosus decipiens LeConte, 1852	X		X		X	X	X	X	X			Larson et al. (2000); Zimmerman (1970)	
						Laccophilus maculosus shermani Leech, 1944	X									X		Larson et al. (2000); Zimmerman (1970)	
						Laccophilus mexicanus atristernalis Crotch, 1873	X				X	X		X	X			Larson et al. (2000); Zimmerman (1970)	
						Laccophilus mexicanus mexicanus Aube, 1838	X				X			X		X		Larson et al. (2000); Zimmerman (1970)	
						Laccophilus oscillator Sharp, 1882	X									X		Larson et al. (2000); Zimmerman (1970)	
						Laccophilus pictus Laporte de Castelnau, 1835	X									X	X	Larson et al. (2000); Zimmerman (1970)	
						Laccophilus quadrilineatus quadrilineatus Horn, 1871	X				X					X		Larson et al. (2000); Zimmerman (1970)	
						Laccophilus salvini Sharp, 1882	X									X		Larson et al. (2000); Zimmerman (1970)	

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Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Laccophilus sonorensis Zimmerman, 1970	X				X					X		Larson et al. (2000); Zimmerman (1970)	
						Laccophilus vacaensis Young, 1953	X									X		Larson et al. (2000); Zimmerman (1970)	
			Hydroporinae Erichson, 1837				X				X	X	X		X	X	X	Larson et al. (2000)	
				Laccornini Wolfe and Roughley, 1990			X	X				X	X					Larson et al. (2000)	
					Laccornis Gozis, 1914		X	X				X	X					Larson et al. (2000)	
						Laccornis pacificus Leech, 1940	X	X				X	X					Larson et al. (2000)	
				Methlini Van den Branden, 1885			X				X							Larson et al. (2000)	
					Celina Aubé, 1837		X				X							Larson et al. (2000)	
						Celina occidentalis Young, 1979	X				X							Larson et al. (2000)	
				Hydrovatini Sharp, 1882			X				X							Larson et al. (2000)	
					Hydrovatus Motschulsky, 1853		X				X							Larson et al. (2000)	
						Hydrovatus brevipes Sharp, 1882	X				X			X				Larson et al. (2000)	
						Hydrovatus davidis Young, 1956	X				X							Larson et al. (2000)	
				Hyphydrini Sharp, 1882			X		X		X		X			X		Larson et al. (2000)	
					Desmopachria Babington, 1841		X		X		X		X			X		Larson et al. (2000)	
						Desmopachria convexa (Aube, 1838)	X		X				X					Larson et al. (2000)	
						Desmopachria dispersa (Crotch, 1873)	X				X							Larson et al. (2000)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Desmopachria latissima (LeConte, 1851)	X				X							Larson et al. (2000)	
						Desmopachria mexicana Sharp, 1882	X				X					X		Larson et al. (2000)	
						Desmopachria portmanni (Clark, 1862)	X									X		Larson et al. (2000)	
				Bidessini Sharp, 1882			X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	
					Liodessus Guignot, 1939		X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	
						Liodessus obscurellus (LeConte, 1852)	X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	widespread in the West
						Liodessus saratogae Miller, 1998	X				X							Larson et al. (2000)	Death Valley, CA
					Neoclypeodytes Young, 1967		X				X	X			X	X	X	Miller (2001); Larson et al. (2000)	
						Neoclypeodytes amybethae Miller, 2001	X									X		Miller (2001); Larson et al. (2000)	
						Neoclypeodytes challeti Miller, 2001	X										X	Miller (2001); Larson et al. (2000)	
						Neoclypeodytes cinctellus (LeConte, 1852)	X	X			X				X	X	X	Miller (2001); Larson et al. (2000)	
						Neoclypeodytes fryii (Clark 1862)	X									X	X	Miller (2001); Larson et al. (2000)	
						Neoclypeodytes haroldi Miller, 2001	X									X		Miller (2001); Larson et al. (2000)	
						Neoclypeodytes leachi (Leech, 1948)	X				X	X						Miller (2001); Larson et al. (2000)	
						Neoclypeodytes ornatellus (Fall, 1917)	X		X		X	X						Miller (2001); Larson et al. (2000)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Neoclypeodytes pictodes (Sharp, 1882)	X				X							Miller (2001); Larson et al. (2000)	
						Neoclypeodytes plicipennis (Crotch, 1873)	X				X					X		Miller (2001); Larson et al. (2000)	
						Neoclypeodytes quadripustulatus (Fall, 1917)	X				X							Miller (2001); Larson et al. (2000)	
						Neoclypeodytes roughleyi Miller, 2001	X				X							Miller (2001); Larson et al. (2000)	
					Uvarus Guignot, 1939		X	X			X	X		X		X	X	Larson et al. (2000)	
						Uvarus amandus (LeConte, 1852)	X	X						X		X		Larson et al. (2000)	
						Uvarus subtilis (LeConte, 1852)	X	X			X	X		X		X	X	Larson et al. (2000)	
				Hydroporini Erichson, 1837			X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	
					Hydroporus Clairville, 1806		X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	
						Hydroporus axillaris LeConte, 1851	X	X			X	X	X	X				Larson et al. (2000)	
						Hydroporus carri Larson, 1975	X	X				X		X				Larson et al. (2000)	
						Hydroporus despectus Sharp, 1882	X		X		X		X	X				Larson et al. (2000)	
						Hydroporus fortis LeConte, 1851	X		X		X	X	X		X			Larson et al. (2000)	
						Hydroporus fuscipennis Schaum, 1868	X		X				X	X				Larson et al. (2000)	
						Hydroporus geniculatus Thomson, 1854	X							X					
						Hydroporus klamathensis Larson and Roughley, 2000	X				X	X						Larson et al. (2000)	
						Hydroporus leechi Gordon, 1981	X		X		X							Larson et al.	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																		(2000)	
						Hydroporus longiusculus Gemminger & Harold, 1868	X				X	X	X		X	X		Larson et al. (2000)	
						Hydroporus mannerheimi Balfour-Browne, 1944	X		X		X	X	X					Larson et al. (2000)	
						Hydroporus notabilis LeConte, 1850	X					X		X				Larson et al. (2000)	
						Hydroporus occidentalis Sharp, 1882	X		X		X		X	X				Larson et al. (2000)	
						Hydroporus pervicinus Fall, 1923	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Hydroporus signatus Mannerheim, 1853	X						X					Larson et al. (2000)	
						Hydroporus simplex Gordon, 1981	X		X		X							Larson et al. (2000)	
						Hydroporus sinuatipes Fall, 1923	X		X		X	X	X					Larson et al. (2000)	
						Hydroporus striola (Gyllenhal, 1827)	X						X					Larson et al. (2000)	
						Hydroporus subpubescens LeConte, 1852	X		X		X	X	X					Larson et al. (2000)	
						Hydroporus tademus Leech, 1949	X		X		X	X	X					Larson et al. (2000)	
						Hydroporus tenebrosus LeConte, 1850	X		X				X	X	X			Larson et al. (2000)	
						Hydroporus transpunctatus Chandler, 1941	X		X		X		X	X		X		Larson et al. (2000)	
						Hydroporus tristis (Paykull, 1798)	X		X			X	X					Larson et al. (2000)	
						Hydroporus zackii Larson and Roughley, 2000	X		X						X			Larson et al. (2000)	springs in Ash Meadows, Nye Co.
					Hydrocolus Roughley and Larson, 2000		X						X	X				Larson et al. (2000)	
						Hydrocolus paugus (Fall, 1923)	X						X	X				Larson et al. (2000)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Hygrotus Stephens, 1828		X		X		X	X	X	X	X	X		Larson et al. (2000), Miller, Wolfe and Biström (2006)	Miller et al. (2006) have shown this genus to be paraphyletic thus it will likely be split into at least 2 genera *(Hygrotus* and *Coelambus)*
						Hygrotus acaroides (LeConte, 1855)	X					X						Larson et al. (2000)	
						Hygrotus artus Fall, 1919	X				X							Larson et al. (2000)	
						Hygrotus bruesi (Fall, 1928)	X					X		X	X			Larson et al. (2000)	
						Hygrotus collatus (Fall, 1919)	X				X				X	X		Larson et al. (2000)	
						Hygrotus curvipes (Leech, 1938)	X				X							Larson et al. (2000)	
						Hygrotus dissimilis Gemminger and Harold, 1868	X		X			X						Larson et al. (2000)	
						Hygrotus femoratus (Fall, 1901)	X									X		Larson et al. (2000)	known from 2 males from NM and AZ; may be synonym of *H. nubilis* (LeConte)
						Hygrotus fontinalis Leech, 1966	X				X							Larson et al. (2000)	
						Hygrotus fraternus (LeConte, 1852)	X				X					X	X	Larson et al. (2000)	
						Hygrotus hydropicus (LeConte, 1852)	X				X	X					X	Larson et al. (2000)	
						Hygrotus impressopunctatus (Schaller, 1783)	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Hygrotus infuscatus (Sharp, 1882)	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Hygrotus intermedius (Fall, 1919)	X		X		X	X	X					Larson et al. (2000)	
						Hygrotus lutescens (LeConte, 1852)	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Hygrotus marklini (Gyllenhal, 1813)	X		X					X		X		Larson et al. (2000)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
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						Hygrotus masculinus (Crotch, 1874)	X		X		X	X	X	X	X			Larson et al. (2000)	
						Hygrotus nigrescens (Fall, 1919)	X		X		X	X	X	X	X			Larson et al. (2000)	
						Hygrotus nubilis (LeConte, 1855)	X		X							X		Larson et al. (2000)	
						Hygrotus obscureplagiatus (Fall, 1919)	X		X		X	X	X					Larson et al. (2000)	
						Hygrotus patruelis (LeConte, 1855)	X		X		X			X	X			Larson et al. (2000)	
						Hygrotus pedalis (Fall, 1901)	X				X							Larson et al. (2000)	
						Hygrotus picatus (Kirby, 1837)	X		X				X	X				Larson et al. (2000)	
						Hygrotus sayi Balfour-Browne, 1944	X		X		X	X	X	X	X			Larson et al. (2000)	
						Hygrotus semivittatus (Fall, 1919)	X		X		X	X	X		X	X		Larson et al. (2000)	
						Hygrotus sharpi (van den Branden, 1885)	X				X					X	X	Larson et al. (2000)	
						Hygrotus thermarum (Darlington, 1928)	X				X	X			X			Larson et al. (2000)	
						Hygrotus tumidiventris (Fall, 1919)	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Hygrotus turbidus (LeConte, 1855)	X		X		X	X	X					Larson et al. (2000)	
						Hygrotus unguicularis (Crotch, 1874)	X		X		X	X	X	X				Larson et al. (2000)	
						Hygrotus wardii (Clark, 1862)	X									X		Larson et al. (2000)	
					Nebrioporus Régimbart, 1906		X		X				X	X				Larson et al. (2000)	
						Nebrioporus macronychus (Shirt and Angus, 1992)	X		X				X	X				Larson et al. (2000)	
					Neoporus Guignot, 1931		X	X	X			X	X			X		Larson et al. (2000)	
						Neoporus arizonicus (Fall, 1917)	X	X	X							X		Larson et al.	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
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																		(2000)	
						Neoporus dimidiatus (Gemminger and Harold, 1868)	X		X							X		Larson et al. (2000)	
						Neoporus undulatus (Say, 1823)	X		X			X	X					Larson et al. (2000)	
					Oreodytes Seidlitz, 1887		X	X	X		X	X	X	X	X	X		Larson et al. (2000); Zimmerman (1985)	
						Oreodytes abbreviatus (Fall, 1923)	X				X							Larson et al. (2000); Zimmerman (1985)	
						Oreodytes alaskanus (Fall, 1926)	X		X				X					Larson et al. (2000); Zimmerman (1985)	
						Oreodytes angustior (Hatch, 1928)	X	X				X	X					Larson et al. (2000); Zimmerman (1985)	
						Oreodytes congruus (LeConte, 1878)	X	X			X	X	X	X	X			Larson et al. (2000); Zimmerman (1985)	
						Oreodytes crassulus (Fall, 1923)	X	X			X	X	X	X				Larson et al. (2000); Zimmerman (1985)	
						Oreodytes humboltensis Zimmerman, 1985	X				X							Larson et al. (2000); Zimmerman (1985)	
						Oreodytes laevis (Kirby, 1837)	X		X				X					Larson et al. (2000); Zimmerman (1985)	

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						Oreodytes obesus cordillerensis Larson, 1990	X	X			?		X	X				Larson et al. (2000); Zimmerman (1985)	formerly *O. rivalis* (Gyllenhal)
						Oreodytes obesus obesus (LeConte, 1866)	X	X			X	X						Larson et al. (2000); Zimmerman (1985)	formerly *O. rivalis* (Gyllenhal)
						Oreodytes picturatus (Horn, 1883)	X	X			X	X	X		X			Larson et al. (2000); Zimmerman (1985)	
						Oreodytes quadrimaculatus (Horn, 1883)	X	X			X	X	X		X			Larson et al. (2000); Zimmerman (1985)	
						Oreodytes rhyacophilus Zimmerman, 1985	X				X							Larson et al. (2000); Zimmerman (1985)	
						Oreodytes scitulus bisulcatus (Fall, 1923)	X	X			X							Larson et al. (2000); Zimmerman (1985)	
						Oreodytes scitulus scitulus (LeConte, 1855)	X	X			?			X				Larson et al. (2000); Zimmerman (1985)	
						Oreodytes sierrae Zimmerman, 1985	X				X							Larson et al. (2000); Zimmerman (1985)	
						Oreodytes snoqualmie (Hatch, 1933)	X		X				X					Larson et al. (2000); Zimmerman (1985)	
						Oreodytes subrotundus (Fall, 1923)	X				X							Larson et al. (2000);	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
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																		Zimmerman (1985)	
					Sanfilippodytes Franciscolo, 1979		X	X	X		X	X				X		Larson et al. (2000)	*Sanfilippodytes* currently undergoing revision; best to leave specimens at genus
						Sanfilippodytes adelardi (Rochette, 1983)	X				X							Larson et al. (2000)	
						Sanfilippodytes barbarae (Fall, 1932)	X				X							Larson et al. (2000)	
						Sanfilippodytes barbarensis (Wallis, 1933)	X				X							Larson et al. (2000)	
						Sanfilippodytes belfragei (Sharp, 1882)	X				X							Larson et al. (2000)	
						Sanfilippodytes bidessoides (Leech, 1941)	X				X							Larson et al. (2000)	
						Sanfilippodytes corvallis (Fall, 1923)	X					X						Larson et al. (2000)	
						Sanfilippodytes hardyi (Sharp, 1882)	X				X							Larson et al. (2000)	
						Sanfilippodytes kingi (Clark, 1862)	X									X		Larson et al. (2000)	
						Sanfilippodytes latebrosus (LeConte, 1852)	X				X							Larson et al. (2000)	
						Sanfilippodytes malkini (Hatch, 1951)	X				X	X						Larson et al. (2000)	
						Sanfilippodytes pacificus (Fall, 1923)	X						?					Larson et al. (2000)	unconfirmed record for WA
						Sanfilippodytes palliatus (Horn, 1883)	X				X							Larson et al. (2000)	
						Sanfilippodytes rossi (Leech, 1941)	X				X							Larson et al. (2000)	
						Sanfilippodytes setifer Roughley & Larson, 2000	X		X		X							Larson et al. (2000)	
						Sanfilippodytes terminalis (Sharp, 1882)	X	X			X							Larson et al. (2000)	
						Sanfilippodytes veronicae (Rochette, 1983)	X				X							Larson et al. (2000)	

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						Sanfilippodytes vilis (LeConte, 1852)	X				X							Larson et al. (2000)	
						Sanfilippodytes williami (Rochette, 1986)	X				X					X		Larson et al. (2000)	
						Stictotarsus Zimmermann, 1919	X	X	X		X			X		X	X	Larson et al. (2000); Zimmerman (1975, 1982); Angus (2010)	Angus (2010) erected Boreonectes to include the "griseostriatus group" of species. This includes 8 species in this list. Further work is necessary to refine this list and place the remaining species into possibly additional new genera.
						Stictotarsus aequinoctialis (Clark, 1862)	X		X					X		X		Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus coelamboides (Fall, 1923)	X				X							Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus corvinus (Sharp, 1887)	X									X		Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus decemsignatus (Clark, 1862)	X									X		Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus deceptus (Fall, 1932)	X	X			X						X	Larson et al. (2000); Zimmerman (1975, 1982)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Stictotarsus dolerosus (Leech, 1945)	X				X							Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus eximius (Motschulsky, 1859)	X				X							Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus expositus (Fall, 1923)	X				X							Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus funereus (Crotch, 1873)	X				X							Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus griseostriatus (DeGeer, 1774)	X		X		X	X	X	X	X	X		Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus panaminti (Fall, 1923)	X				X							Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus roffi (Clark, 1862)	X									X		Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus spenceri (Leech, 1945)	X							X				Larson et al. (2000); Zimmerman (1975, 1982)	
						Stictotarsus spectabilis (Zimmerman, 1982)	X									X		Larson et al. (2000); Zimmerman (1975, 1982)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Stictotarsus striatellus (LeConte, 1852)	X		X		X	X	X	X	X	X	X	Larson et al. (2000); Zimmerman (1975, 1982)	
						Stygoporus Larson and Labonte, 1994						X						Larson et al. (2000)	monotypic; stygobiontic
						Stygoporus oregonensis Larson and Labonte, 1994						X						Larson et al. (2000)	monotypic; stygobiontic
			Colymbetinae Erichson, 1837				X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	
				Agabini Thomson, 1867			X	X	X		X	X	X	X	X	X	X	Larson et al. (2000), Nilsson (2000)	Nilsson (2000) has rearranged the species within this group, moving many species between *Agabus* and *Ilybius* as well as using the generic concepts of *Ilybiosoma* and *Platambus.* He did not create a revised key. We suggest leaving the list as presented in Larson et al. (2000) until a new North American key is devised.
					Agabinus Crotch, 1873		X	X			X	X	X	X				Larson et al. (2000)	
						Agabinus glabrellus (Motschulsky, 1859)	X	X			X	X	X	X				Larson et al. (2000)	
						Agabinus sculpturellus Zimermann, 1919	X	X			X	X						Larson et al. (2000)	
					Agabus Leach, 1817		X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	
						Agabus ambiguus (Say, 1823)	X	X					X					Larson et al. (2000)	
						Agabus ajax Fall, 1922	X							X				Larson et al. (2000)	
						Agabus ancillus Fall, 1922	X		X			X	X					Larson et al.	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																		(2000)	
						Agabus antennatus Leech, 1939	X							X				Larson et al. (2000)	
						Agabus anthracinus Mannerheim, 1852	X		X		X		X	X				Larson et al. (2000)	
						Agabus apache Young, 1981	X		X							X		Larson et al. (2000)	
						Agabus approximatus Fall, 1922	X	X			X	X		X	X	X		Larson et al. (2000)	
						Agabus austinii Sharp, 1882	X	X			X	X	X	X	X			Larson et al. (2000)	
						Agabus austrodiscors Larson, 1996	X	X			X							Larson et al. (2000)	
						Agabus bifarius (Kirby, 1837)	X		X				X					Larson et al. (2000)	
						Agabus bjorkmanae Hatch, 1939	X	X			X	X	X	X	X			Larson et al. (2000)	
						Agabus brevicollis LeConte, 1857	X	X			X							Larson et al. (2000)	
						Agabus canadensis Fall, 1922	X		X				X					Larson et al. (2000)	
						Agabus confertus LeConte, 1861	X	X			X	X	X					Larson et al. (2000)	
						Agabus cordatus (LeConte, 1853)	X	X						X		X		Larson et al. (2000)	
						Agabus discors LeConte, 1861	X		X		X	X	X					Larson et al. (2000)	
						Agabus disintegratus (Crotch, 1873)	X		X		X	X		X	X	X		Larson et al. (2000)	
						Agabus erichsoni Gemminger and Harold, 1868	X		X		X			X				Larson et al. (2000)	
						Agabus euryomus Larson, 1996	X		X		X	X						Larson et al. (2000)	
						Agabus griseipennis LeConte, 1859	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Agabus hoppingi Leech, 1942	X	X			X							Larson et al. (2000)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Agabus hypomelas Mannerheim, 1843	X		X		X	X	X	X				Larson et al. (2000)	
						Agabus ilybiiformis (Zimmermann, 1928)	X	X			X	X						Larson et al. (2000)	
						Agabus jimzim Larson, 1996	X									X		Larson et al. (2000)	
						Agabus klamathensis Larson & Leech, 1989	X	X			X	X						Larson et al. (2000)	
						Agabus kootenai Larson, 1991	X		X		X	X	X	X				Larson et al. (2000)	
						Agabus lineelus LeConte, 1861	X		X		X							Larson et al. (2000)	
						Agabus lugens LeConte, 1852	X	X			X	X		X				Larson et al. (2000)	
						Agabus lutosus LeConte, 1853	X		X		X	X	X				X	Larson et al. (2000)	
						Agabus minnesotensis Wallis, 1933	X	X						X	X			Larson et al. (2000)	
						Agabus morosus LeConte, 1852	X	X			X	X						Larson et al. (2000)	
						Agabus obliteratus nectris Leech, 1942	X	X				X	X					Larson et al. (2000)	
						Agabus obliteratus obliteratus LeConte, 1859	X	X			X			X	X	X		Larson et al. (2000)	
						Agabus oblongulus Fall, 1922	X		X			X	X					Larson et al. (2000)	
						Agabus obsoletus LeConte, 1858	X	X			X				X			Larson et al. (2000)	
						Agabus pandurus Leech, 1942	X	X			X							Larson et al. (2000)	
						Agabus perplexus Sharp, 1882	X	X			X	X	X					Larson et al. (2000)	
						Agabus pisobius Leech, 1949	X		X				X					Larson et al. (2000)	
						Agabus punctulatus Aube, 1838	X		X		X	X	X	X		X		Larson et al. (2000)	
						Agabus regularis (LeConte, 1852)	X				X						X	Larson et al. (2000)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Agabus roguus Larson, 1997	X	X				X						Larson et al. (2000)	Curry Co., OR
						Agabus rumppi Leech, 1964	X	X			X				X			Larson et al. (2000)	
						Agabus sasquatch Larson, 1991	X		X		X				X			Larson et al. (2000)	
						Agabus semipunctatus (Kirby, 1837)	X		X				X					Larson et al. (2000)	
						Agabus semivittatus LeConte, 1852	X	X			X			X	X	X		Larson et al. (2000)	
						Agabus seriatus (Say, 1823)	X	X			X	X	X	X	X	X		Larson et al. (2000)	
						Agabus smithi Brown, 1930	X					X	X					Larson et al. (2000)	
						Agabus strigulosus (Crotch, 1873)	X		X		X	X	X	X	X			Larson et al. (2000)	
						Agabus tristis Aube, 1838	X		X		X	X	X	X		X		Larson et al. (2000)	
						Agabus vandykei Leech, 1942	X		X		X	X						Larson et al. (2000)	
						Agabus vancouverensis Leech, 1937	X		X				X					Larson et al. (2000)	
						Agabus versimilis Brown, 1932	X		X			X	X	X				Larson et al. (2000)	
						Agabus walsinghami (Crotch, 1873)	X	X			X	X	X					Larson et al. (2000)	
						Ilybius Erichson, 1832	X		X		X	X	X	X	X	X		Larson et al. (2000); Larson (1987)	
						Ilybius angustior (Gyllenhal, 1808)	X		X					X		X		Larson et al. (2000); Larson (1987)	
						Ilybius biguttulus (German, 1824)	X		X					X				Larson et al. (2000); Larson (1987)	
						Ilybius fraterculus LeConte, 1862	X		X		X	X	X	X	X	X		Larson et al. (2000); Larson (1987)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Ilybius picipes (Kirby, 1837)	X		X				X					Larson et al. (2000); Larson (1987)	
						Ilybius quadrimaculatus Aube, 1838	X		X		X	X	X					Larson et al. (2000); Larson (1987)	
						Ilybius subaeneus Erichson, 1837	X		X					X				Larson et al. (2000); Larson (1987)	
				Colymbetini Erichson, 1837			X	X	X		X	X	X	X	X	X		Larson et al. (2000)	
					Colymbetes Clairville, 1806		X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Colymbetes crotchi Sharp, 1882	X		X		X							Larson et al. (2000)	
						Colymbetes densus LeConte, 1859	X		X		X	X						Larson et al. (2000)	two recognized subspecies with possible intergrades
						Colymbetes incognitus Zimmerman, 1981	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Colymbetes sculptilis Harris, 1829	X		X					X				Larson et al. (2000)	
						Colymbetes strigatus LeConte, 1851	X		X		X							Larson et al. (2000)	
					Rhantus Dejean, 1833		X	X	X		X	X	X	X	X	X		Larson et al. (2000); Zimmerman (1975)	
						Rhantus anisonychus Crotch, 1873	X				X			X				Larson et al. (2000); Zimmerman (1975)	
						Rhantus atricolor (Aube, 1838)	X									X		Larson et al. (2000); Zimmerman (1975)	
						Rhantus binotatus (Harris, 1828)	X				X	X	X	X	X	X		Larson et al. (2000); Zimmerman	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																		(1975)	
						Rhantus consimilis Motschulsky, 1859	X		X		X	X	X	X	X			Larson et al. (2000); Zimmerman (1975)	
						Rhantus gutticollis (Say, 1834)	X	X			X	X	X	X	X	X		Larson et al. (2000); Zimmerman (1975)	
						Rhantus sericans Sharp, 1882	X		X		X	X	X	X				Larson et al. (2000); Zimmerman (1975)	
						Rhantus sinuatus (LeConte, 1862)	X		X				X					Larson et al. (2000); Zimmerman (1975)	
						Rhantus suturellus (Harris, 1828)	X		X				X					Larson et al. (2000); Zimmerman (1975)	
						Rhantus wallisi Hatch, 1953	X		X		X	X	X					Larson et al. (2000); Zimmerman (1975)	
				Coptotomini Van den Branden, 1885			X				X	X	X	X	X			Larson et al. (2000)	
					Coptotomus Say, 1834		X		X		X	X	X	X	X			Larson et al. (2000)	
						Coptotomus longulus longulus LeConte, 1852	X		X		X	X	X	X	X			Larson et al. (2000)	
			Dytiscinae Leach, 1815				X	X	X		X	X	X	X	X	X	X	Larson et al. (2000)	
				Dytiscini Leach, 1817			X				X	X	X	X	X	X		Larson et al. (2000)	
					Dytiscus Linnaeus, 1758		X		X		X	X	X	X	X	X		Larson et al. (2000)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Dytiscus alaskanus Balfour-Browne, 1944	X		X				X	X				Larson et al. (2000)	
						Dytiscus circumcinctus Ahrens, 1811	X		X				X					Larson et al. (2000)	
						Dytiscus cordieri Aube, 1838	X		X		X	X	X					Larson et al. (2000)	
						Dytiscus dauricus Gebler, 1832	X		X		X	X	X	X	X	X		Larson et al. (2000)	
						Dytiscus habilis Say, 1834	X		X							X		Larson et al. (2000)	
						Dytiscus hatchi Wallis, 1950	X		X		X	X	X					Larson et al. (2000)	
						Dytiscus hybridus Aube, 1838	X		X			X						Larson et al. (2000)	
						Dytiscus marginicollis LeConte, 1845	X		X		X	X	X	X	X	X		Larson et al. (2000)	
				Hydaticini Sharp, 1882			X		X		X	X	X	X				Larson et al. (2000)	
					Hydaticus Leach, 1817		X		X		X	X	X	X				Larson et al. (2000)	
						Hydaticus aruspex Clark, 1864	X		X		X	X	X	X				Larson et al. (2000)	
				Acilini Thomson, 1867			X		X		X	X	X	X	X			Larson et al. (2000)	
					Acilius Leach, 1817		X		X		X	X	X	X	X			Bergsten&Miller (2006)	
						Acilius abbreviatus Mannerheim, 1843	X		X		X	X	X	X	X			Bergsten&Miller (2006)	
					Graphoderus Dejean, 1833		X		X		X		X	X				Larson et al. (2000)	
						Graphoderus liberus (Say, 1825)	X		X		?		X					Larson et al. (2000)	CA record unpublished
						Graphoderus occidentalis Horn, 1883	X		X		X	X	X	X				Larson et al. (2000)	
						Graphoderus perplexus Sharp, 1882	X		X		X		X	X				Larson et al. (2000)	
					Thermonectus Dejean, 1837		X		X		X	X		X		X		Larson et al.	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																		(2000)	
						Thermonectus intermedius Crotch, 1873	X		X		X	X		X				Larson et al. (2000)	
						Thermonectus marmoratus Hope, 1832	X	X			X			X				Larson et al. (2000)	
						Thermonectus nigrofasciatus nigrofasciatus Aube, 1838	X		X							X		Larson et al. (2000)	
						Thermonectus sibleyi Goodhue-McWilliams, 1981	X		X							X		Larson et al. (2000)	
				Eretini Crotch, 1873			X				X			X		X		Larson et al. (2000)	
					Eretes LaPorte, 1833		X				X			X		X		Larson et al. (2000)	
						Eretes sticticus (Linnaeus, 1767)	X				X			X		X		Miller (2002)	
				Cybistrini Sharp, 1882			X		X		X				X			Larson et al. (2000)	
					Cybister Curtis, 1827		X		X		X			X	X			Larson et al. (2000)	
						Cybister ellipticus LeConte, 1851	X		X		X							Larson et al. (2000)	
						Cybister explanatus LeConte, 1851	X		X		X			X	X			Larson et al. (2000)	
		Gyrinidae MacLeay, 1825					X	X	X		X	X	X	X	X	X	X		adults not benthic and so are excluded from benthic sets
			Gyrininae Régimbart				X	X	X		X	X	X	X	X	X	X		
				Enhydrini Régimbart			X	X	X		X					X	X		
					Dineutus MacLeay, 1825		X	X	X		X					X	X	Leech and Chandler (1956); Wood (1962)	no recent published key for North American species
				Orectochilini Régimbart, 1882			X	X			X					X			
					Gyretes Brullé, 1835		X	X			X					X		Babin (2004)	
				Gyrinini Régimbart			X	X	X		X	X	X	X	X	X		Oygur and Wolfe (1992)	
					Gyrinus Müller, 1764		X	X	X		X	X	X	X	X	X		Oygur and Wolfe (1992)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Haliplidae Aube, 1836					X		X		X	X	X	X	X	X	X	Leech and Chandler (1956)	the species keys in Usinger are the still the most recent
					Apteraliplus Chandler, 1943		X		X		X	X	X						vernal pools only; this genus may be sunk under *Haliplus* at some point
						Apteraliplus parvulus (Roberts, 1913)	X		X		X	X	X						vernal pools only; this genus may be sunk under *Haliplus* at some point
					Brychius Thomson, 1859		X				X			X				Mousseau and Roughley (2007)	
						Brychius hornii Crotch, 1873	X				X	X		X				Mousseau and Roughley (2007)	*B. albertanus* Carr 1928 now jr. synonym of *B. hornii*
						Brychius pacificus Carr, 1928	X				X							Mousseau and Roughley (2007)	
					Haliplus Latreille, 1802		X				X			X			X		
						Haliplus concolor LeConte, 1852	X				X	X	X				X		
						Haliplus cylindricus Roberts, 1913	X				X					X			
						Haliplus distinctus Wallis, 1933	X				X			X				Kenner (2005)	
						Haliplus dorsomaculatus Zimmermann, 1924	X				X	X							
						Haliplus eremicus Wells, 1989	X								X	X		Wells (1989)	possibly a synonym of *H. mimeticus* Matheson
						Haliplus gracilis Roberts, 1913	X				X	X	X						
						Haliplus leechi Wallis, 1933	X				X	X	X	X					
						Haliplus longulus LeConte, 1859	X				X	X	X					Kenner (2005)	
						Haliplus mimeticus Matheson, 1912	X				X							Leech (1957)	
						Haliplus robertsi Zimmermann, 1924	X				X	X	X	X		X			
						Haliplus rugosus Roberts, 1913	X				?						X		records probably only for Baja and not CA
						Haliplus subguttatus Roberts,	X				X	X	X					Leech (1964)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
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						1913													
						Haliplus tumidus LeConte, 1880	X									X		van Vondel and Spangler (2008)	
					Peltodytes Régimbart, 1878		X				X			X			X	Leech and Chandler (1956)	
						Peltodytes callosus (LeConte, 1852)	X	X	X		X	X	X	X	X		X	Leech and Chandler (1956)	
						Peltodytes dispersus Roberts, 1913	X	X	X		?			X		X			unconfirmed record for CA
						Peltodytes mexicanus (Wehncke, 1883)	X									X		van Vondel and Spangler (2008)	
						Peltodytes simplex (LeConte, 1852)	X	X	X		X				X		X	Leech and Chandler (1956)	
		Noteridae Thomson, 1860					X		X		X							Leech (1970)	
					Suphisellus Crotch, 1873		X		X		X							Leech (1970)	
						Suphisellus bicolor (Say, 1831)	X		X		X							Leech (1970)	
	Polyphaga Emery, 1886						X	X			X	X	X	X	X	X	X		
		Chrysomelidae Latreille, 1802									X								Excluded from benthic datasets
		Curculionidae Latreille, 1801									X								Excluded from benthic datasets
		Dryopidae Billberg, 1820					X				X	X	X	X	X	X		Brown (1972a)	larvae are primarily terrestrial; occasionally in headwater seeps
					Dryops Oliver, 1791		X	X			X					X		Brown (1972a)	riparian; seldomly taken in benthic samples
						Dryops arizonensis Schaeffer, 1905	X	X			X					X		Brown (1972a)	
					Helichus Erichson, 1847		X	X			X	X	X	X	X	X	X	Brown (1972a); Nelson (1989)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Helichus columbianus Brown, 1931	X	X			X	X	X		X	X	X	Brown (1972a); Nelson (1989); Nelson (1981)	
						Helichus striatus LeConte, 1852	X	X			X	X	X	X	X	X		Brown (1972a); Nelson (1989)	
						Helichus suturalis LeConte, 1852	X	X			X					X	X	Brown (1972a); Nelson (1989)	
						Helichus triangularis Musgrave, 1935	X	X								X		Brown (1972a); Nelson (1989)	
					Postelichus Nelson, 1989		X	X			X			X		X	X	Brown (1972a); Nelson (1989)	
						Postelichus confluentus (Hinton, 1935)	X	X								X		Brown (1972a); Nelson (1989)	
						Postelichus immsi (Hinton, 1937)	X	X			X			X		X		Brown (1972a); Nelson (1989)	
						Postelichus productus (LeConte, 1852)	X	X			X						X	Brown (1972a); Nelson (1989)	
		Elmidae Curtis, 1830					X	X	X		X	X	X	X	X	X	X	Shepard (2002); White and Roughley (2008); Brown (1972a)	Shepard's updated generic key to elmid adults includes *Xenelmis* but not the undescribed genus known to occur in the Pacific Northwest; a manuscript describing 3 species from this new genus has been submitted for publication by Cheryl Barr as of 1/23/2011
			Larainae Boving and Craighead, 1930				X	X	X		X	X	X	X					
				Laraini LeConte, 1861			X	X	X		X	X	X	X				Spangler (1987)	
					Lara LeConte, 1852		X	X	X		X	X	X	X					adults usually terrestrial, may be taken in benthic samples

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Lara avara LeConte, 1852	X	X	X		X			X				Brown (1972a)	
						Lara gehringi Darlington, 1929	X	X	X		X	X	X					Brown (1972a)	may be a synonym of *Lara avara*
			Elminae Curtis, 1830				X	X	X		X	X	X		X	X	X		
				Elmini Curtis, 1830			X	X	X		X	X	X		X	X	X		
					Ampumixis Sanderson, 1954		X	X			X	X							monotypic
						Ampumixis dispar (Fall, 1925)	X	X			X	X							monotypic
					Atractelmis Chandler, 1954		X	X			X	X							monotypic
						Atractelmis wawona Chandler, 1954	X	X			X	X							monotypic
					Cleptelmis Sanderson, 1954		X	X			X	X		X				Shepard (1998)	monotypic
						Cleptelmis addenda (Fall, 1907)	X	X			X	X	X	X				Shepard (1998)	monotypic
					Cylloepus Erichson, 1847		X	X								X			
						Cylloepus abnormis (Horn, 1870)	X	X								X		Brown (1972a)	
						Cylloepus parkeri Sanderson, 1953	X	X								X		Brown (1972a)	
					Dubiraphia Sanderson, 1954		X	X	X		X					X			
						Dubiraphia brunnescens (Fall, 1925)	X	X	X		X							Brown (1972a)	Lake Co., Clear Lake
						Dubiraphia giulianii (van Dyke, 1949)	X	X	?		X							Brown (1972a); Shepard (1993)	may be a synonym of *Dubiraphia brunnescens*
					Heterelmis Sharp, 1882		X	X			X					X	X		
						Heterelmis glabra (Horn, 1870)	X	X								X		Brown (1972b)	
						Heterelmis obesa Sharp, 1882	X	X			X					X		Brown (1972b)	
						Heterelmis stephani Brown, 1972	X	X								X		Brown (1972b)	
					Heterlimnius Hinton, 1935		X	X			X	X	X	X					
						Heterlimnius corpulentus (LeConte, 1874)	X	X			X		X	X				Brown (1972a)	
						Heterlimnius koebelei (Martin, 1927)	X	X			X	X	X					Brown (1972a)	
					Hexacylloepus Hinton, 1940		X	X								X			unpublished records for AZ
					Huleechius Brown, 1981		X	X								X	X	Brown (1981)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Huleechius marroni Brown, 1981	X	X									X	Brown (1981)	
						Huleechius marroni carolus Brown, 1981	X	X								X		Brown (1981)	
					Macrelmis Motschulsky, 1859		X	X								X		Brown (1972a)	
						Macrelmis moestus (Horn, 1870)	X	X								X		Brown (1972a)	may be a synonom of *Macrelmis texanus* Schaeffer, 1911
					Microcylloepus Hinton, 1935		X	X			X			X		X			there are unpublished records of additional species in the SW
						Microcylloepus formicoideus Shepard, 1990	X	X			X							Shepard (1990)	occurs in Death Valley only
						Microcylloepus moapus fraxinus La Rivers, 1949	X	X							X			Brown (1972a)	warm springs in SE Nevada
						Microcylloepus moapus moapus La Rivers, 1949	X	X							X			Brown (1972a)	warm springs in SE Nevada
						Microcylloepus similis (Horn, 1870)	X	X			X			X		X		Shepard (1993)	widespread in the West
						Microcylloepus thermarum (Darlington, 1928)	X	X							X			Brown (1972a)	warm springs in NW Nevada
					Narpus Casey, 1893		X	X			X	X	X	X		X		Brown (1972a)	
						Narpus angustus Casey, 1893	X	X			X	X	X					Brown (1972a)	
						Narpus arizonicus (Brown, 1930)	X	X								X		Brown (1972a)	
						Narpus concolor (LeConte, 1881)	X	X			X	X	X	X				Brown (1972a)	
					Neocylloepus Brown, 1970		X	X								X		Shepard (2002); White and Roughley (2008)	undetermined species
					Neoelmis Musgrave, 1935		X	X								X		Shepard (2002); White and Roughley (2008)	undetermined species
					Optioservus Sanderson, 1954		X	X			X	X	X	X	X	X		Shepard (2002); White (1978); Shepard (1993)	several Western species may not be valid

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Optioservus canus Chandler, 1954	X	X			X							Shepard (2002); White (1978); Shepard (1993)	several Western species may not be valid
						Optioservus castanipennis (Fall, 1925)	X	X						X				Shepard (2002); White (1978); Shepard (1993)	several Western species may not be valid
						Optioservus divergens (LeConte, 1874)	X	X			X			X	X	X		Shepard (2002); White (1978); Shepard (1993)	several Western species may not be valid
						Optioservus heteroclitus White, 1978	X	X			X							Shepard (2002); White (1978); Shepard (1993)	several Western species may not be valid
						Optioservus quadrimaculatus (Horn, 1870)	X	X			X	X	X	X	X			Shepard (2002); White (1978); Shepard (1993)	several Western species may not be valid
						Optioservus seriatus (LeConte, 1874)	X	X			X	X		X				Shepard (2002); White (1978); Shepard (1993)	several Western species may not be valid
					Ordobrevia Sanderson, 1953		X	X			X	X						Shepard (2002); White and Roughley (2008)	only one species in North America
						Ordobrevia nubifera (Fall, 1901)	X	X			X	X						Shepard (2002); White and Roughley (2008)	only one species in North America
					Rhizelmis Chandler, 1954		X	X			X	X						Shepard (2002); White and Roughley (2008)	monotypic
						Rhizelmis nigra Chandler, 1954	X	X			X	X						Shepard (2002); White and Roughley (2008)	monotypic

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Stenelmis Dufour, 1835		X	X			X	X	X		X	?		Shepard (2002); White and Roughley (2008)	*S. occidentalis* is the only species occurring outside of Nevada springs
						Stenelmis calida Chandler, 1949	X	X							X			Schmude (1999)	Key designed for *Stenelmis* of the Nevada springs, but will serve for the West in general
						Stenelmis lariversi Schmude, 1999	X	X							X			Schmude (1999)	Key designed for *Stenelmis* of the Nevada springs, but will serve for the West in general
						Stenelmis moapa LaRivers, 1949	X	X							X			Schmude (1999)	Key designed for *Stenelmis* of the Nevada springs, but will serve for the West in general
						Stenelmis occidentalis Schmude and Brown, 1991	X	X				X			X	?		Schmude (1999)	Key designed for *Stenelmis* of the Nevada springs, but will serve for the West in general
					Xenelmis Hinton, 1936		X	X								X		Shepard (2002); Brown (1985); Brown (1981)	
						Xenelmis sandersoni Brown, 1985	X	X								X		Shepard (2002); Brown (1985); Brown (1981)	only species from the USA; larvae still not included in generic keys, but easily identifiable
				Macronychini Steffan, 1961			X	X			X	X	X	X	X	X	X		
					Zaitzevia Champion, 1923		X	X			X	X	X	X	X	X	X	Brown (1972a); Brown (2001)	
						Zaitzevia parvula (Horn, 1870)	X	X			X	X	X	X	X	X	X	Brown (2001)	
						Zaitzevia posthonia Brown, 2001	X	X			X	X	X						

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Epimetopidae Zaitzev, 1908					X									X		van Tassel (2002)	
					Epimetopus Lacordaire, 1854		X									X		van Tassel (2002)	
		Eulichadidae Crowson, 1973					X				X							Brown (1972a)	formerly placed in the Ptilodactylidae; adults terrestrial
					Stenocolus LeConte, 1853		X				X							Brown (1972a)	adults terrestrial
						Stenocolus scutellaris LeConte, 1853	X				X							Brown (1972a)	adults terrestrial
		Georissidae Laporte, 1840									X							Shepard (2003)	shoredwellers; excluded from benthic datasets; some authors consider this a subfamily of Hydrophilidae
		Heteroceridae MacLeay, 1825									X	X	X	X	X	X		Pacheco (1964); Pacheco (1978); Shepard (1993)	Excluded from benthic datasets; larvae and adults shoredwellers; Pacheco (1964) revised the family describing many new genera but few authors follow his classification. The Pacheco names are given in the comments column.
		Hydraenidae Mulsant, 1844					X	X	X		X	X	X	X	X	X	X		
			Hydraeninae d'Orchymont, 1919				X	X	X		X	X	X	X	X	X	X		
				Hydraenini Perkins, 1980			X	X	X		X	X	X	X	X	X	X		
					Hydraena Kugelann, 1794		X	X	X		X	X	X	X	X	X	X	Perkins (1980); Perkins (2001)	Found along stream margins, also some lentic situations
						Hydraena alternata Perkins, 1980	X	X								X		Perkins (1980)	Known from extreme southeastern AZ
						Hydraena arenicola Perkins, 1980	X	X	X		X	X						Perkins (1980)	
						Hydraena arizonica Perkins, 1980	X	X								X		Perkins (1980)	
						Hydraena bituberculata Perkins, 1980	X	X								X		Perkins (1980)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Hydraena californica Perkins, 1980	X	X			X							Perkins (1980)	
						Hydraena circulata Perkins, 1980	X	X			X	X	X	X		X	X	Perkins (1980)	
						Hydraena leechi Perkins, 1980	X	X								X		Perkins (1980)	
						Hydraena mignymixys Perkins, 1980	X	X			X							Perkins (1980)	
						Hydraena nigra Hatch, 1965	X	X			X	X		X	X			Perkins (1980)	
						Hydraena occidentalis Perkins, 1980	X	X	X		X	X	X					Perkins (1980)	
						Hydraena pacifica Perkins, 1980	X	X	X		X	X	X	X	X			Perkins (1980)	
						Hydraena petila Perkins, 1980	X	X			X							Perkins (1980)	
						Hydraena sierra Perkins, 1980	X	X			X	X						Perkins (1980)	
						Hydraena tuolumne Perkins, 1980	X	X			X							Perkins (1980)	
						Hydraena vandykei d'Orchymont, 1923	X	X			X							Perkins (1980)	
						Hydraena yosemitensis Perkins, 1980	X	X			X							Perkins (1980)	
					Limnebius Leach, 1815						X	X	X	X		X	X	Perkins (1980); Perkins (2001)	
						Limnebius alutaceous (Casey, 1886)					X	X	X					Perkins (1980); Perkins (2001)	
						Limnebius arenicolus Perkins, 1980					X	X					X	Perkins (1980); Perkins (2001)	
						Limnebius leechi Perkins, 1980					X							Perkins (1980); Perkins (2001)	Coastal Ranges
						Limnebius piceus (Horn, 1872)					X						X	Perkins (1980); Perkins (2001)	
						Limnebius sinuatus Sharp, 1882										X		Perkins (1980); Perkins (2001)	
						Limnebius utahensis Perkins, 1980								X				Perkins (1980); Perkins (2001)	
			Ochthebiinae Perkins, 1980				X	X	X	X	X	X	X	X	X	X	X		

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Gymnocthebius d'Orchymont, 1943							X	X				X	X	Perkins (1980); Perkins (2001)	
						Gymnochthebius falli Perkins, 1980										X		Perkins (1980); Perkins (2001)	
						Gymnochthebius fossatus (LeConte, 1855)										X		Perkins (1980); Perkins (2001)	
						Gymnochthebius laevipennis (LeConte, 1878)					X	X					X	Perkins (1980); Perkins (2001)	
						Gymnochthebius oppositus Perkins, 1980											X	Perkins (1980); Perkins (2001)	
					Neochthebius d'Orchymont, 1932						X	?	?					Perkins (1980); Perkins (2001)	intertidal, found in rock crevises from CA to BC
						Neochthebius vandykei (Knisch, 1924)					X	?	?					Perkins (1980); Perkins (2001)	intertidal, found in rock crevises from CA to BC
					Ochthebius Leach, 1815		X	X	X		X	X	X	X	X	X	X	Perkins (1980); Perkins (2001)	dwellers of stream and pond margins
						Ochthebius apache Perkins, 1980	X									X			
						Ochthebius arenicolus Perkins, 1980	X				X	X					X		
						Ochthebius arizonicus Perkins, 1980	X									X			
						Ochthebius aztecus Sharp, 1887	X	X			X	X		X	X				
						Ochthebius biinicisus Perkins, 1980	X	X		X	X	X							
						Ochthebius bisinuatus Perkins, 1980	X				X	X	X						
						Ochthebius borealis Perkins, 1980	X	X	X		X	X	X		X		X		
						Ochthebius brevipennis Perkins, 1980	X		X		X	X	X						
						Ochthebius californicus Perkins, 1980	X				X				X				
						Ochthebius costipennis Fall, 1901	X				X								
						Ochthebius crassalus Perkins,	X				X								

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						1980													
						Ochthebius crenatus Hatch, 1965	X				X	X							
						Ochthebius cribricollis LeConte, 1850	X		X		X	X	X		X				
						Ochthebius discretus LeConte, 1878	X	X			X	X	X	X	X		X		
						Ochthebius gruwelli Perkins, 1980	X	X	X		X						X		
						Ochthebius interruptus LeConte, 1852	X	X	X	X	X	X	X				X		
						Ochthebius lecontei Perkins, 1980	X						X	X	X				
						Ochthebius leechi Wood and Perkins, 1978	X	X			X								
						Ochthebius lineatus LeConte, 1852	X				X	X	X	X	X	X	X		
						Ochthebius madrensis Perkins, 1980	X	X								X			
						Ochthebius marinus (Paykull, 1798)	X				X	X	X	X	X				
						Ochthebius martini Fall, 1919	X	X			X								
						Ochthebius mimicus Brown, 1933	X					X	X						
						Ochthebius orbus Perkins, 1980	X				X	X							
						Ochthebius pacificus Perkins, 1980	X				X	X	X		X				
						Ochthebius puncticollis LeConte, 1852	X	X			X			X		X	X		
						Ochthebius recticulus Perkins, 1980	X				X								known only from Wilbur Hot Springs, CA
						Ochthebius rectus LeConte, 1878	X		X	X	X	X	X		X	X			
						Ochthebius rectusalus Perkins, 1980	X		X	X	X		X				X		
						Ochthebius richmondi Perkins, 1980	X				X		X						
						Ochthebius sculptoides Perkins, 1980	X	X			X	X		X	X				
						Ochthebius sculptus LeConte,	X	X	X		X	X					X		

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						1878													
						Ochthebius sierrensis Perkins, 1980	X				X								
						Ochthebius similis Sharp, 1882	X	X								X			
						Ochthebius spanglerorum Wood and Perkins, 1978	X							X					
						Ochthebius tubus Perkins, 1980	X	X			X					X	X		
						Ochthebius uniformis Perkins, 1980	X		X		X	X	X						
		Hydrophilidae Latreille, 1802					X	X	X		X	X	X	X	X	X	X	Smetana (1988); Leech and Chandler (1956)	Smetana's keys are more recent, but do not cover the SW USA
					Ametor Semenov, 1900		X	X			X	X	X					Smetana (1988); Leech and Chandler (1956)	
						Ametor latus (Horn, 1873)	X	X			X	X	X					Smetana (1988); Leech and Chandler (1956)	
						Ametor scabrosus (Horn, 1873)	X	X			X	X	X					Smetana (1988); Leech and Chandler (1956)	
					Anacaena Thomson, 1859		X	X	X		X							Leech and Chandler (1956)	
						Anacaena limbata (Fabricius, 1792)	X	X	X		X							Smetana (1988); Leech and Chandler (1956)	This name probably represents a species complex
						Anacaena signaticollis (Fall, 1924)	X				X							Leech and Chandler (1956)	
					Berosus Leach, 1817		X				X							Miller (1965a); van Tassel (1963); Leech and Chandler (1956)	van Tassel's (1966) revision of *Berosus* remains unpublished; species keys should be used with caution

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Berosus fraternus LeConte, 1855	X				X					X		Leech and Chandler (1956)	*B. californicus* now a synonym
						Berosus hatchi Miller, 1965	X					X	X					Miller (1965a)	
						Berosus infuscatus LeConte, 1855	X				X					X		Leech and Chandler (1956)	
						Berosus ingeminatus d'Orchymont, 1946	X				X							Leech and Chandler (1956)	
						Berosus maculosus Mannerheim, 1853	X				X							Leech and Chandler (1956)	
						Berosus metalliceps Sharp, 1882	X				X							Leech and Chandler (1956)	
						Berosus notapeltatus van Tassell, 1963	X									X		van Tassell (1963)	
						Berosus oregonensis Miller, 1965	X					X						Miller (1965a)	
						Berosus punctatissimus LeConte. 1852	X				X					X		Leech and Chandler (1956)	
						Berosus sayi Hansen, 1999	X				X							Leech and Chandler (1956)	*Berosus striatus* is a junior synonym
						Berosus stylifera Horn 1873	X				X					X		Leech and Chandler (1956)	
						Chaetarthria Stephens, 1833	X				X							Miller (1974)	
						Chaetarthria bicolor Sharp, 1882	X				X					X		Miller (1974)	
						Chaetarthria hespera Miller, 1974	X				X			X		X		Miller (1974)	
						Chaetarthria leechi Miller, 1974	X				X							Miller (1974)	
						Chaetarthria magna Miller, 1974	X				X							Miller (1974)	
						Chaetarthria nigrella (LeConte, 1861)	X				X		X					Miller (1974)	
						Chaetarthria ochra Miller, 1974	X				X					X		Miller (1974)	
						Chaetarthria pallida (LeConte, 1861)	X				X	X		X	X	X		Miller (1974)	
						Chaetarthria punctulata Sharp, 1882	X				X					X	X	Miller (1974)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Chaetarthria pusilla Sharp, 1882	X				X					X	X	Miller (1974)	
						Chaetarthria spinata Miller, 1974	X				X							Miller (1974)	
						Chaetarthria truncata Miller, 1974	X				X							Miller (1974)	
						Chaetarthria utahensis Miller, 1974	X							X				Miller (1974)	
					Crenitis Bedel, 1881		X	X	X		X	X	X		X	X		Miller (1965); Smetana (1988)	
						Crenitis alticola (Fall, 1924)	X	?	?		X	X	X			X		Miller (1965)	
						Crenitis dissimilis (Horn, 1873)	X				X							Miller (1965); Smetana (1988)	
						Crenitis malkini Miller, 1965	X	X				X						Miller (1965); Smetana (1988)	
						Crenitis morata (Horn, 1890)	X		X		X							Smetana (1988)	
						Crenitis palpalis Miller, 1965	X				X	X						Miller (1965)	
						Crenitis paradigma (d'Orchymont, 1942)	X		X			X	X					Smetana (1988)	
						Crenitis rufiventris (Horn, 1873)	X		X		X					X		Smetana (1988)	
						Crenitis seriellus (Fall, 1924)	X				X								
						Crenitis snoqualmie Miller, 1965	X	?				X	X					Miller (1965)	
					Cymbiodyta Bedel, 1880		X	X	X		X	X	X		X	X	X	Smetana (1974)	
						Cymbiodyta acuminata Fall, 1924	X						X					Smetana (1974)	
						Cymbiodyta arizonica Smetana, 1974	X									X		Smetana (1974)	
						Cymbiodyta columbiana Leech, 1948	X	X	X		X	X	X					Smetana (1974)	
						Cymbiodyta dorsalis (Motschulsky, 1859)	X	X	X		X	X	X	X	X	X	X	Smetana (1974)	
						Cymbiodyta fraterculus (Sharp, 1882)	X									X		Smetana (1974)	
						Cymbiodyta howdeni Smetana, 1974	X									X		Smetana (1974)	
						Cymbiodyta imbellis (LeConte, 1861)	X	X	X		X	X						Smetana (1974)	
						Cymbiodyta leechi Miller, 1964	X				X	X	X					Smetana (1974)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Cymbiodyta minima Notman, 1919	X					X	X					Smetana (1974)	
						Cymbiodyta occidentalis Smetana, 1974	X	?	X		X							Smetana (1974)	
						Cymbiodyta pacifica Leech, 1948	X	X	X		X	X	X					Smetana (1974)	
						Cymbiodyta pseudopacifica Smetana, 1974	X				X							Smetana (1974)	
						Cymbiodyta puella Smetana, 1974	X	X	X		X							Smetana (1974)	
						Cymbiodyta punctatostriata (Horn, 1873)	X	X	?		X							Smetana (1974)	
						Cymbiodyta seriata Smetana, 1974	X	X								X		Smetana (1974)	
						Cymbiodyta vindicata Fall, 1924	X						X					Smetana (1974)	
						Enochrus Thomson, 1859	X	X	X		X	X	X		X	X	X	Gundersen (1978); Gundersen (1977)	
						Enochrus aridus Gundersen, 1977	X				X					X		Gundersen (1978); Gundersen (1977)	
						Enochrus californicus (Horn, 1890)	X		X		X	X	X	X			X	Gundersen (1978); Gundersen (1977)	
						Enochrus carinatus carinatus (LeConte, 1855)	X				X							Gundersen (1978); Gundersen (1977)	
						Enochrus carinatus fucatus (Horn, 1873)	X									X		Gundersen (1978); Gundersen (1977)	
						Enochrus cristatus (LeConte, 1855)	X				X	X	X	X	X	X	X	Gundersen (1978); Gundersen (1977)	
						Enochrus cuspidatus (LeConte, 1878)	X				X	X			X			Gundersen (1978); Gundersen (1977)	
						Enochrus diffusus (LeConte, 1855)	X				X	X	X	X	X			Gundersen (1978); Gundersen (1977)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Enochrus fimbriatus (Melsheimer, 1844)	X				X			X	X			Gundersen (1978); Gundersen (1977)	as *E. perplexus* (LeConte, 1855) in some lists
						Enochrus hamiltoni Leech, 1950	X	X	X		X	X	X	X	X			Gundersen (1978); Gundersen (1977)	Several different color morphs exist
						Enochrus ochraceus (Melsheimer, 1844)	X		X		X							Gundersen (1978); Gundersen (1977)	
						Enochrus piceus piceus Miller, 1964	X				X	X	X	X	X	X		Gundersen (1978); Gundersen (1977)	
						Enochrus piceus glabrus Gundersen, 1977	X									X		Gundersen (1978); Gundersen (1977)	
						Enochrus pygmaeus pectoralis (LeConte, 1855)	X				X			X	X	X	X	Gundersen (1978); Gundersen (1977)	
						Enochrus pygmaeus pygmaeus (Fabricius, 1792)	X				X					X	X	Gundersen (1978); Gundersen (1977)	
					Helochares Mulsant, 1844		X				X								
						Helochares normatus (LeConte, 1861)	X				X								as *H. maculicollis* Mulsant, 1844
					Hemiosus Sharp, 1882		X	X								X		van Tassell (1964)	recorded from Gila River, AZ
						Hemiosus exilis (LeConte, 1851)	X	X								X		van Tassell (1964)	recorded from Gila River, AZ
					Hydrochara Berthold, 1827		X				X		X	X	X	X	X	Smetana (1980); Leech and Chandler (1956)	
						Hydrochara lineata LeConte, 1855	X				X			X	X	X	X	Smetana (1980); Leech and Chandler (1956)	
						Hydrochara obtusata (Say, 1823)	X						X					Smetana (1980)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Hydrochara rickseckeri Horn, 1895	X				X							Smetana (1980); Leech and Chandler (1956)	Listed as a species of concern; may become federally listed
					Hydrobius Leach, 1815		X				X								
						Hydrobius fuscipes (Linnaeus, 1758)	X				X								
					Hydrophilus Geoffrey, 1762		X				X							Leech and Chandler (1956)	
						Hydrophilus insularis Laporte, 1840	X				X							Leech and Chandler (1956)	
						Hydrophilus triangularis Say, 1823	X		X		X							Leech and Chandler (1956)	
					Laccobius Erichson, 1837		X				X								
						Laccobius acutipenis Miller, 1965	X				X							Cheary (1971)	
						Laccobius agilis Randall, 1838	X				X	X	X	X					
						Laccobius borealis Cheary, 1971	X				X	X	X	X	X			Cheary (1971)	
						Laccobius bruesi Cheary, 1971	X				X							Cheary (1971)	
						Laccobius californicus d'Orchymont,1942	X				X	X	X						
						Laccobius carri d'Orchymont, 1942	X				X	X		X	X				
						Laccobius chandleri Cheary, 1971	X							X				Cheary (1971)	
						Laccobius columbianus Miller, 1965	X							X				Cheary (1971)	
						Laccobius ellipticus LeConte, 1855	X				X	X	X		X		X		
						Laccobius hardyi Cheary, 1971	X							X		X	X	Cheary (1971)	
						Laccobius insolitus d'Orchymont, 1942	X				X								
						Laccobius leechi Cheary, 1971	X				X					?		Cheary (1971)	
						Laccobius mexicanus d'Orchymont, 1942	X				X			X		X	X	Cheary (1971)	
						Laccobius nevadensis Miller, 1965	X				X	X			X			Miller (1965b)	
						Laccobius occidentalis Cheary, 1971	X				X							Cheary (1971)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Laccobius oregonensis Cheary, 1971	X					X						Cheary (1971)	
						Laccobius pacificus Miller, 1965	X				X	X	X					Miller (1965b)	
						Laccobius piceus Fall, 1921	X				X							Cheary (1971)	
						Laccobius tridentipenis Cheary, 1971	X				X							Cheary (1971)	
						Laccobius truncatipenis Miller, 1965	X				X	X	X					Miller (1965b)	
					Paracymus Thompson, 1867		X				X							Wooldridge (1966)	*P. securus* not in this key, but all US species are
						Paracymus communis Wooldridge, 1966	X				X					X		Wooldridge (1966)	
						Paracymus confusus Wooldridge, 1966	X									X		Wooldridge (1966)	
						Paracymus elegans (Fall, 1901)	X				X							Wooldridge (1966)	
						Paracymus ellipsis (Fall, 1910)	X				X					X		Wooldridge (1966)	
						Paracymus restrictus Wooldridge, 1966	X				X							Wooldridge (1966)	
						Paracymus securus Wooldridge, 1975	X										X	Wooldridge (1975)	
						Paracymus subcupreus (Say, 1825)	X				X							Wooldridge (1966)	
						Paracymus tarsalis Miller, 1963	X				X							Wooldridge (1966)	
					Tropisternus Solier, 1834		X				X							Leech and Chandler (1956)	
						Tropisternus californicus (LeConte, 1855)	X				X	X					X	Leech and Chandler (1956)	
						Tropisternus columbianus Brown, 1931	X	X	X		X	X	X				X	Leech and Chandler (1956)	
						Tropisternus ellipticus (LeConte, 1855)	X		X		X	X	X	X	X	X	X	Leech and Chandler (1956)	
						Tropisternus lateralis (Fabricius, 1775)	X	X	X		X	X	X	X	X	X	X	Leech and Chandler (1956)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
						Tropisternus obscurus Sharp, 1882	X										X	Leech and Chandler (1956)	
						Tropisternus orvus Leech, 1945	X				X	X			X			Leech and Chandler (1956)	
						Tropisternus salsamentus Fall, 1901	X				X	X					X	Leech and Chandler (1956)	
						Tropisternus sublaevis (LeConte, 1855)	X				X			X	X	X	X	Leech and Chandler (1956)	
		Helophoridae Thomson, 1859					X				X	X	X	X	X	X	X	Smetana (1985)	some authors consider this as a subfamily of Hydrophilidae
					Helophorus Fabricius, 1775		X				X	X	X	X	X	X	X	Smetana (1985)	
		Hydrochidae Thomson, 1859					X												some authors consider this as a subfamily of Hydrophilidae
					Hydrochus Leach, 1817		X				X	X	X			X	X		Hellman (1975) revised the genus, describing a number of new species for North America. This work remains unpublished and the new names, two of which are found in AZ, remain unavailable.
		Lampyridae Latreille, 1817							X										larvae are shoredwellers, not truly aquatic; excluded from benthic datasets
					Pyractomena Dejean, 1833				X										emergent vegetation of ponds and marshes
		Limnichidae Erichson, 1846									X	X	X	X	X	X		Wooldridge (1975, 1986); Shepard (1993)	larvae and adults shoredwellers; excluded from benthic datasets

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
		Lutrochidae Kasap and Crowson, 1975					X	X								X		Brown (1972a); Brown and Murvosh (1970)	adults terrestrial; excluded from benthic datasets
		Psephenidae Lacordaire, 1854					X	X			X	X	X		X	X	X	Brown (1972a); Shepard (1993)	
			Eubriinae Lacordaire, 1857				X	X			X	X						Brown (1972a); Shepard (1993)	
					Acneus Horn, 1880		X	X			X	X						Brown (1972a); Shepard (1993)	larvae not separable to species
			Eubrianacinae Jacobson, 1913				X	X			X	X			X			Brown (1972a); Shepard (1993)	
					Eubrianax Kiesenwetter, 1874		X	X			X	X			X			Brown (1972a); Shepard (1993)	
						Eubrianax edwardsii (LeConte, 1874)	X	X			X	X			X			Brown (1972a); Shepard (1993)	only one species in Nearctic Region
			Psepheninae Lacordaire, 1854				X	X			X	X	X		X	X		Brown (1972a); Brown and Murvosh (1974)	
					Psephenus Haldeman, 1853		X	X			X	X	X		X	X		Brown (1972a); Brown and Murvosh (1974)	larvae to genus, except *P. falli* Casey which is widespread outside of AZ
		Ptilodactylidae Laporte, 1836					X				X				X			Brown (1972a)	adults terrestrial; larvae found mainly in seeps and headwater streams
					Anchycteis Horn, 1880		X				X				X			Brown (1972a)	adults terrestrial; larvae found mainly in seeps and headwater streams
						Anchycteis velutina Horn, 1880	X				X				X			Brown (1972a)	adults terrestrial; larvae found mainly in seeps and headwater streams

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Araeopidius Cockerell, 1906		X				X							Lawrence (1991)	adults terrestrial; larvae found mainly in seeps and headwater streams
						Araeopidius monochus LeConte, 1874	X				X							Lawrence (1991)	adults terrestrial; larvae found mainly in seeps and headwater streams
		Scirtidae Fleming, 1821					X		X		X	X	X	X		X		Tetrault (1967)	adults terrestrial and excluded from benthic datasets; larvae to genus only; all lentic, some lotic in slower microhabitats
					Cyphon Paykull, 1799		X		X		X	X	X	X				Tetrault (1967)	many undescribed species including some in the SAFIT region
					Elodes Latreille, 1796		X		X		X	X	X		X			Tetrault (1967)	
					Herthania Klausnitzer, 2006		X		X		X	X	X	X	X			Klausnitzer (2006)	genus erected for several *Cyphon* species, including two in the SAFIT region; larvae undescribed but probably inseparable from *Cyphon*
					Prionocyphon Redtenbacher, 1858		X		X							X		Leech and Chandler (1956)	Leech and Chandler in Usinger (1956) reported this genus from Western AZ; as no other papers have corroborated this record, it is likely an error.
					Scirtes Illiger, 1807		X		X		X					X		Tetrault (1967)	
		Scarabaeidae Latreille, 1802							X		X							Rogers (1997)	Excluded from benthic datasets
			Aphodiinae Leach, 1815						X		X							Rogers (1997)	

Taxonomic Hierarchy							Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Family	Subfamily	Tribe	Genus	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
					Aphodius Illiger, 1798				X		X							Rogers (1997)	
						Aphodius alternatus Horn, 1887			X		X							Rogers (1997)	surface dweller in some vernal pools
		Staphylinidae Latreille, 1802									X	X	X	X	X	X	X		Excluded from benthic datasets

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Diptera: True Flies
Standard Effort Level I: Genus (where possible) -- Chironomidae to family
Standard Effort Level II: Genus/species (where possible) -- Chironomidae to genus or species group (where noted)
Standard Taxonomic Reference: Merritt, Cummins and Berg (2008)
Reviewed by:

Keys to families and genera are given in Merritt, Cummins and Berg (Courtney and Merritt, 2008 – larvae; Merritt and Webb, 2008 – pupae and adults; Byers and Gelhaus, 2008 – Tipulidae; Adler and Currie, 2008 – Simuliidae; Wallace and Walker, 2008 – Culicidae; Ferrington, Berg and Coffman, 2008 – Chironomidae). Stone et al. (1983) is a good source for distributional information. See also McAlpine et al. (1981, 1987, 1989) for additional keys, illustrations, biological and phylogenetic information and bibliographic references for all Diptera families. The Simuliidae have recently been revised for North America (Adler et al., 2004) When identifying chironomids, it may be helpful to have a number of additional texts at hand including Wiederholm (1983), Wiederholm (1986), and Epler (2001). The latter text, although designed for use in North and South Carolina, is well illustrated and has up-to-date keys for many Nearctic genera. It also contains useful information on the hazards of midge larva identification including ecology, nomenclature, slide-mounting, and quality assurance.

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
Diptera Latreille, 1817										X	X	X	X	X	X	X	X	X	X	X	Courtney and Merritt (2008); Merritt and Webb (2008)	keys to families and genera
	Nematocera Berthold, 1827									X	X	X		X	X	X	X	X	X	X		
		Tipulomorpha Brues, Melander, Carpenter and Morton, 1954								X	X	X		X	X	X	X	X	X	X		
			Tanyderidae Osten Sacken, 1880							X	X			X	X		X				Alexander (1967)	
							Protanyderus Osten Sacken, 1859			X	X			X	X		X				Alexander (1967)	only genus in western USA

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Tipulidae Latreille, 1802							X	X			X	X	X	X	X	X	X	Byers and Gelhaus (2008), Gelhaus (2000)	also a number of terrestrial forms; Gelhaus key identifies many of these
				Limoniinae Speiser, 1909						X	X			X	X	X	X	X	X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Antocha Osten Sacken, 1859			X	X			X	X	X					Byers and Gelhaus (2008), Gelhaus (2000)	
									Antocha monticola Alexander, 1917	X	X			X	X	X					Byers and Gelhaus (2008), Gelhaus (2000)	only species known from Western US
							Cryptolabis Osten Sacken, 1859			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	larvae are often confused with *Limnophila*
							Dicranota Zetterstedt, 1838			X	X			X	X	X	X				Byers and Gelhaus (2008), Gelhaus (2000)	
							Erioptera Meigen, 1800			X	X			X	X	X	X	X	X		Byers and Gelhaus (2008), Gelhaus (2000)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Gonomyia Meigen, 1818			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Hesperoconopa Alexander, 1948			X	X			X	X	X	X				Byers and Gelhaus (2008), Gelhaus (2000)	
							Hexatoma Latreille, 1809			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Limnophila Macquart, 1834			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Limonia Meigen, 1800			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Molophilus Curtis, 1833			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Ormosia Rondani, 1856			X	X			X	X	X	X				Byers and Gelhaus (2008), Gelhaus (2000)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Paradelphomyia Alexander, 1936			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Pedicia Latreille, 1809			X	X			X	X	X	X				Byers and Gelhaus (2008), Gelhaus (2000)	
							Pilaria Sintenis, 1889			X	X				X	X					Byers and Gelhaus (2008), Gelhaus (2000)	
							Pseudolimnophila Alexander, 1919			X	X			X			X				Byers and Gelhaus (2008), Gelhaus (2000)	
							Rhabdomastix Skuse, 1890			X	X			X	X	X	X		X		Byers and Gelhaus (2008), Gelhaus (2000)	
							Ulomorpha Osten Sacken, 1869			X	X			X	X	X					Byers and Gelhaus (2008), Gelhaus (2000)	
				Tipulinae Latreille, 1802						X	X			X	X	X	X	X	X	X	Byers and Gelhaus (2008), Gelhaus (2000)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Holorusia Loew, 1863			X	X			X	X	X			X		Byers and Gelhaus (2008), Gelhaus (2000)	monotypic
									Holorusia hespera Arnaud and Byers, 1990	X	X			X	X	X			X		Byers and Gelhaus (2008), Gelhaus (2000), Arnaud and Byers (1990)	monotypic
							Prionocera Loew, 1844			X	X			X	X						Byers and Gelhaus (2008), Gelhaus (2000), Arnaud and Byers (1990)	
									Prionocera oregonica Alexander, 1943	X	X			X	X						Byers and Gelhaus (2008), Gelhaus (2000), Arnaud and Byers (1990)	
							Tipula Linnaeus, 1758			X	X			X	X	X	X	X	X	X	Byers and Gelhaus (2008), Gelhaus (2000)	
		Blephariceromorpha Rohdendorf, 1961								X	X			X	X	X	X	X				
			Blephariceridae Schiner, 1862							X	X			X	X	X	X	X			Courtney and Merritt (2008); Hogue (1973)	Hogue provides keys to mature larvae, pupae and adults

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Blepharicerinae Schiner, 1862						X	X			X	X	X	X	X			Hogue (1973)	
					Blepharicerini Loew, 1862					X	X			X	X	X	X	X			Hogue (1973)	
							Agathon Röder, 1890			X	X			X	X	X		X			Hogue (1973)	
									Agathon comstocki (Kellogg, 1903)	X	X			X	X	X		X			Hogue (1973)	
									Agathon doanei (Kellogg, 1900)	X	X			X							Hogue (1973)	
									Agathon elegantulus von Röder, 1890	X	X			X	X	X		X			Hogue (1973)	
									Agathon aylmeri group sensu Hogue, 1970	X	X			X		X		X	X		Hogue (1973); Hogue (1987)	these species were transfered from *Dioptopsis* to *Agathon* in Hogue (1987); some or all still appear under *Dioptopsis* in some lists
									Agathon arizonica (Alexander, 1958)	X	X			X					X		Hogue (1973); Hogue (1987)	*Agathon alpina* (Hogue, 1966) is a junior synonym
									Agathon aylmeri (Garrett, 1923)	X	X			X		X					Hogue (1973); Hogue (1987)	
									Agathon dismalea (Hogue, 1970)	X	X			X	X			X			Hogue (1973); Hogue (1987)	
									Agathon markii (Garrett, 1925)	X	X			X		X					Hogue (1973); Hogue (1987)	
									Agathon sequoiarum (Alexander, 1952)	X	X			X		X					Hogue (1973); Hogue (1987)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Bibiocephala Osten Sacken, 1874			X	X			X	X		X				Hogue (1973)	
									Bibiocephala grandis Osten Sacken, 1874	X	X			X	X		X				Hogue (1982)	*Bibiocephala nigripes* is a junior synonym
							Blepharicera Macquart, 1843			X	X			X	X		X				Hogue (1973)	
									Blepharicera jordani (Kellogg, 1903)	X	X			X	X						Hogue (1973)	
									Blepharicera kalmiopsis Jacobson and Courtney, 2009	X	X			X	X						Jacobson and Courtney (2009); Hogue (1973)	
									Blepharicera micheneri (Alexander, 1959)	X	X			X							Hogue (1973)	
									Blepharicera ostensackeni (Kellogg, 1903)	X	X			X	X						Hogue (1973)	
									Blepharicera zionensis Alexander, 1959	X	X			X	X		X					
							Philorus Kellog, 1903			X	X			X							Hogue (1973)	
									Philorus californicus Hogue, 1964	X	X			X							Hogue (1973)	
									Philorus jacinto Hogue, 1966	X	X			X							Hogue (1973)	
									Philorus vanduzeei Alexander, 1966	X	X			X							Hogue (1973)	
									Philorus yosemite (Osten Sacken, 1877)	X	X			X							Hogue (1973)	
			Deuterophlebiidae Edwards, 1922							X	X			X	X	X	X				Courtney (1990)	
							Deuterophlebia Edwards, 1922			X	X			X	X	X	X				Courtney (1990)	all species keyed
									Deuterophlebia coloradensis Pennak, 1945	X	X			X	X	X	X				Courtney (1990)	
									Deuterophlebia inyoensis Kennedy, 1960	X	X			X	X	X					Courtney (1990)	Mono County
									Deuterophlebia nielsoni Kennedy, 1958	X	X			X							Courtney (1990)	Mono & Tuolumne Counties

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Deuterophlebia personata Courtney, 1990	X	X			X	X	X					Courtney (1990)	
									Deuterophlebia shasta Wirth, 1951	X	X			X	X						Courtney (1990)	Siskiyou & El Dorado Counties
									Deuterophlebia vernalis Courtney, 1990	X	X					X					Courtney (1990)	
		Psychodomorpha Henning, 1968								X	X			X	X	X			X	X		
			Psychodidae Bigot, 1854							X	X			X	X	X			X	X		
							Maruina Müller, 1895			X	X			X	X	X					Hogue (1973)	
									Maruina lanceolata (Kincaid, 1899)	X	X			X	X	X				X	Hogue (1973)	2 other spp. Known from eastern Colorado
							Psychoda Latreille, 1796			X	X			X	X	X			X			
							Pericoma Walker, 1856/*Telmatoscopus* Eaton, 1904			X	X			X	X	X			X	X		larvae of these genera incompletely separable
		Ptychopteromorpha Wood and Borkent, 1986								X	X			X	X	X	X	X	X			
			Ptychopteridae Brauer, 1869							X	X			X	X	X	X	X	X			found in seeps or stream margins
							Bittacomorpha Westwood, 1835			X	X	X		X	X	X			X			rarely shows up in benthic samples; larvae inseparable
							Bittacomorphella Alexander, 1916			X	X			X	X	X		X				rarely shows up in benthic samples; larvae inseparable
							Ptychoptera Meigen, 1800			X	X	X		X	X	X	X	X	X			most commonly encountered of the three genera (in benthic samples)
		Culicomorpha Henning, 1948												X	X	X	X	X	X	X		

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Ceratopogonidae Grassi, 1900							X	X			X	X	X	X	X	X	X	Courtney and Merritt (2008); Glukova (1979)	A number of additional genera may be encountered in benthic samples; larvae and pupae in need of revision
				Ceratopogoninae Grassi, 1900						X	X			X	X	X	X	X	X	X		
							Alluaudomyia Kieffer, 1913			X	X			X								
							Bezzia Kieffer, 1899/*Palpomyia* Meigen, 1818			X	X			X		X		X				
							Ceratopogon Meigen, 1800			X	X			X	X							
							Culicoides Latreille, 1809			X	X			X	X	X		X	X	X		
							Monohelea Kieffer, 1917			X	X			X					X			
							Nilobezzia Kieffer, 1921			X	X			X								
							Probezzia Kieffer, 1906			X	X			X					X			
							Serromyia Meigen, 1818			X	X			X								
							Sphaeromias Curtis, 1829			X	X			X								
							Stilobezzia Kieffer, 1901			X	X			X					X			
				Dasyheleinae Lenz, 1934						X	X			X					X	X		
							Dasyhelea Kieffer, 1911			X	X			X					X	X		
				Forcipomyiinae Lenz, 1934						X	X			X	X	X			X	X		
							Atrichopogon Kieffer, 1906			X	X			X		X						
							Forcipomyia Meigen, 1818			X	X			X	X	X			X	X		
			Chaoboridae Cook, 1965									X		X	X	X						
							Chaoborus Lichtenstein, 1800					X		X	X	X						
							Eucorethra Underwood, 1903			X	X			X	?	?						occasionally found in benthic samples; associated with cold springs
									Eucorethra underwoodi Underwood, 1903	X	X			X	?	?						occasionally found in benthic samples; associated with cold springs

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Mochlonyx Loew, 1844					X		X	X							
			Chironomidae Macquart, 1838							X	X			X	X	X	X	X	X	X		
				Chironominae Macquart, 1838						X	X			X	X	X	X	X	X	X		
					Chironomini Macquart, 1838					X	X			X	X	X	X	X	X	X		
							Apedilum Townes, 1945			X	X			X				X	X			
							Chernovskiia Saether, 1977			X	X			X								
									Chernovskiia orbicus (Townes, 1945)	X	X			X								
							Chironomus Meigen, 1803			X	X			X	X	X	X	X	X			
							Cladopelma Kieffer, 1921			X	X			X								
							Cryptochironomus Kieffer, 1918			X	X			X		X					Saether (2009)	Saether's larval key includes all species in SAFIT region
							Cryptotendipes Lenz, 1941			X	X			X								
							Cyphomella Saether, 1977			X	X								X			
									Cyphomella gibbera Saether, 1977	X	X								X			
							Demeijerea Kruseman, 1933			X	X				X		X					
									Demeijerea brachialis (Coquillett, 1901)	X	X				X		X					
							Demicryptochironomus Lenz, 1941			X	X			X								
							Dicrotendipes Kieffer, 1913			X	X			X	X	X	X	X	X			
							Endochironomus Kieffer, 1918			X	X			X	X	X					Grodhaus (1987)	
									Endochironomus nigricans (Johannsen, 1905)	X	X			X	X	X						
							Endotribelos Grodhaus, 1987			X	X			X							Grodhaus (1987)	
									Endotribelos hesperium (Sublette, 1960)	X	X			X								
							Glyptotendipes Kieffer, 1913			X	X			X	X	X	X					
							Goeldichironomus Fittkau, 1965			X	X			X								
							Harnischia Kieffer, 1921			X	X			X			X				Sublette (1960)	
									Harnischia curtilamellata (Malloch, 1915)	X	X			X							Sublette (1960)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
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							Kiefferulus Goetghebuer, 1922			X	X			X	X							
							Lauterborniella Thienemann and Bause in Bause, 1913			X	X			X								monotypic
									Lauterborniella agrayloides (Kieffer, 1911)	X	X			X								monotypic
							Microchironomus Kieffer, 1918			X	X			X								
									Microchironomus nigrovittatus (Malloch, 1915)	X	X			X								
							Microtendipes Kieffer, 1915			X	X			X	X	?					Wiederholm (1983)	two species groups recognized
								Microtendipes pedellus group sensu Pinder and Reiss (1983)		X	X			X	X	?					Wiederholm (1983)	
								Microtendipes rydalensis group sensu Pinder and Reiss (1983)		X	X			X							Wiederholm (1983)	
							Nilothauma Kieffer, 1921			X	X			X								
							Pagastiella Brundin, 1949			X	X			X								
							Parachironomus Lenz, 1921			X	X			X								
							Paracladopelma Harnisch, 1923			X	X			X								
									Paracladopelma alphaeus (Sublette, 1960)	X	X			X								
							Paralauterborniella Lenz, 1941			X	X			X								monotypic
									Paralauterborniella nigrohalteris (Malloch, 1915)	X	X			X								monotypic
							Paratendipes Kieffer, 1911			X	X			X					?			
							Phaenopsectra Kieffer, 1921			X	X			X	X	X			X			
							Polypedilum Kieffer, 1912			X	X			X	X	X		X	X			
							Robackia Saether, 1977			X	X			X							Wiederholm (1983); Epler (2001)	two species, easily separable
									Robackia claviger (Townes, 1945)	X	X					X					Wiederholm (1983); Epler (2001)	
									Robackia demeijeri (Kruseman, 1933)	X	X			X							Wiederholm (1983); Epler (2001)	
							Sergentia Kieffer, 1922			X	X			X		X	X	X				

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
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									Sergentia albescens (Townes, 1945)	X	X			X		X	X	X				
							Stenochironomus Kieffer in Kieffer and Thienemann, 1919			X	X			X	X	X						
							Stictochironomus Kieffer in Kieffer and Thienemann, 1919			X	X			X								
							Synendotendipes Grodhaus, 1987			X	X			X							Grodhaus (1987)	
									Synendotendipes luski Grodhaus, 1987	X	X			X								
							Tribelos Townes, 1945			X	X			X							Grodhaus (1987)	
							Xenochironomus Kieffer, 1921			X	X			X								
									Xenochironomus xenolabis (Kieffer, 1916)	X	X			X								only species known from North America
					Pseudochironomini Saether, 1977					X	X			X								
							Pseudochironomus Malloch, 1915			X	X			X		X	X	X				
									Pseudochironomus richardsoni Malloch, 1915	X	X			X		X	X	X				
					Tanytarsini Goetghebuer, 1937					X	X			X			X					
							Caladomyia Säwedal, 1981			X				X							Lothrop and Mulla (1995); Sawedahl (1981)	Sawedahl (1981) has larval figures; likely synonymous with Tanytarsus according to Peter Cranston
									Caladomyia pistra Sublette and Sasa, 1994	X				X							Lothrop and Mulla (1995)	
							Cladotanytarsus Kieffer, 1921			X	X			X					X			
							Constempellina Brundin, 1947			X	X			X								
							Micropsectra Kieffer, 1909			X	X			X			X		X			
							Micropsectra Kieffer, 1909/Tanytarsus Wulp, 1874			X	X			X			X		X			for immature or indeterminate larvae with long lauterborn stalks
							Paratanytarsus Thienemann and Bause in Bause, 1913			X	X			X								
									Paratanytarsus grimmii (Schneider, 1885)	X	X			?		?					Langton et al. (1988)	parthenogenetic with apparently world-wide distribution

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Rheotanytarsus Thienemann and Bause in Bause, 1913			X	X			X								
									Rheotanytarsus hamatus Sublette and Sasa, 1994	X	X								X		Sublette, Stevens and Shannon (1998)	
							Stempellina Thienemann and Bause in Bause, 1913			X	X			X								
							Stempellinella Brundin, 1947			X	X			X								may be synonymous with *Zavrelia*
							Sublettea Roback, 1975			X	X			X								
									Sublettea coffmani (Roback, 1975)	X	X			X								
							Tanytarsus Wulp, 1874			X	X			X								*Nimbocera* now a synonym of *Tanytarsus*
							Zavrelia Kieffer in Bause, 1913			X	X			X								
				Diamesinae Kieffer, 1923						X	X											
					Boreoheptagyini Brundin, 1966					X	X											
							Boreoheptagyia Brundin, 1966			X	X			?		X						
									Boreoheptagyia lurida (Garrett, 1925)	X	X			?		X						
					Diamesini Kieffer, 1923					X	X			X	X	X	X	X	X			
							Diamesa Meigen in Gistl, 1835			X	X			X	X	X	X	X	X			
							Pagastia Oliver, 1959			X	X			?			X					
									Pagastia partica (Roback, 1957)	X	X						X					
							Potthastia Kieffer, 1922			X	X			?								
								Potthastia gaedii group sensu Oliver, 1983		X	X			?								
								Potthastia longimana group sensu Oliver, 1983		X	X			?								
							Protanypus Kieffer, 1906			X	X			?								unpublished record but verified by Peter Cranston
							Pseudodiamesa Goetghebuer, in Goetghebuer and Lenz, 1939			X	X			X	X							
							Sympotthastia Pagast, 1947			X	X			X	X							
									Sympotthastia diastena (Sublette, 1964)	X	X			X	X							

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
				Orthocladiinae Edwards, 1929						X												
							Acricotopus Kieffer, 1921			X				?								
							Brillia Kieffer, 1913			X	X											
							Bryophaenocladius Thienemann, 1934			X	X						X					
							Cardiocladius Kieffer, 1912			X	X			X				X	X			
							Chaetocladius Kieffer, 1911			X	X				X							
							Chasmatonotus Loew, 1864							X	X	X						
							Clunio Haliday, 1855							X								inter tidal
									Clunio californiensis Hashimoto, 1974					X								inter tidal
							Corynoneura Winnertz, 1846			X	X			?			X					
									Corynoneura diara (Roback, 1957)	X	X						X					
							Cricotopus Wulp, 1874			X	X			X			X					
								Cricotopus bicinctus group sensu Cranston et al., 1983		X	X			X								
								Cricotopus trifascia group sensu Cranston et al., 1983		X	X			X								
									Cricotopus nostocicola Wirth, 1957	X	X			X	X						Wirth (1957); Ashe and Murray (1980)	found in blue-green alga *Nostoc*
							Diplocladius Kieffer in Kieffer and Thienemann, 1908			X	X			?								ABL has larvae from Lake Davis project
							Doithrix Saether and Sublette, 1983			X	X			X								
							Epoicocladius Zavrel in Šulc and Zavrel, 1924															
							Eretmoptera Kellog, 1900							X								inter tidal; larvae unknown
									Eretmoptera browni Kellogg, 1900					X								inter tidal; larvae unknown
							Eukiefferiella Thienemann, 1926			X	X			X					X		Bode (1983); (Epler 2001)	*E. similis* group sensu Bode, 1983 is *Cardiocladius*
									Eukiefferiella brehmi group sensu Bode, 1983	X	X			X							Bode (1983); (Epler	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
																					2001)	
									Eukiefferiella brevicalcar group sensu Bode, 1983	X	X			X							Bode (1983); (Epler 2001)	
									Eukiefferiella claripennis group sensu Bode, 1983	X	X			X					X		Bode (1983); (Epler 2001)	
									Eukiefferiella cyanea group sensu Bode, 1983	X	X				?						Bode (1983); (Epler 2001)	
									Eukiefferiella coerulescens group sensu Bode, 1983	X	X			X					X		Bode (1983); (Epler 2001)	
									Eukiefferiella devonica group sensu Bode, 1983	X	X			X							Bode (1983); (Epler 2001)	
									Eukiefferiella gracei group sensu Bode, 1983	X	X			X							Bode (1983); (Epler 2001)	
									Eukiefferiella pseudomontana group sensu Bode, 1983	X	X			X							Bode (1983); (Epler 2001)	
								Euryhapsis Oliver, 1981		X	X			X								
								Georthocladius Strenzke, 1941		X	X			X								
								Gymnometriocnemus Goetghebuer, 1932		X	X			?								
								Heleniella Gowin, 1943		X	X			X								
								Heterotanytarsus Spärck, 1923		X	X			?								
								Heterotrissocladius Spärck, 1923		X	X			X								
									Heterotrissocladius marcidus group sensu Cranston et al., 1983	X	X			X								
									Heterotrissocladius subpilosus group sensu Cranston et al., 1983	X	X											

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Hydrobaenus Fries, 1830			X	X			X			X					
							Krenosmittia Thienemann and Krüger, 1939			X	X			X								
							Limnophyes Eaton, 1875			X	X			X	X				X			
							Lopescladius Oliveira, 1967			X	X			?								
							Mesocricotopus Brundin, 1956			X	X			?								unpublished record but verified by Peter Cranston
							Metriocnemus Wulp, 1874			X	X			X								
							Nanocladius Kieffer, 1913			X	X			X								
							Oliveiriella Wiedenbrug and Fittkau, 1997			X	X								X		Krestian, Kosnicki, Spindler, Stringer and Epler (2009)	
							Onconeura Andersen and Saether, 2005			X	X								X		Krestian, Kosnicki, Spindler, Stringer and Epler (2009)	
							Orthocladius Wulp, 1874			X	X			X	X	X	X	X	X			genus except for *O. (Symposiocladius) lignicola* (Kieffer, 1915)
									Orthocladius lignicola (Kieffer, 1915)	X	X			X	X							
							Orthocladius complex			X	X			X	X	X	X	X	X	X		equivalent to *Cricotopus/Orthocladius* used by some labs; *Orthocladius* complex is used to trap other genera such as *Paratrichocladius* which is very difficult to separate
							Parachaetocladius Wülker, 1959			X	X			X								

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
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							Paracladius Hirvenoja 1973			X	X								X			
							Parakiefferiella Thienemann, 1936			X	X			X								
							Parametriocnemus Goetghebuer, 1932			X	X			X								
							Paraphaenocladius Thienemann in Spärck, 1924			X	X			X					X			
							Paratrichocladius Santos Abreu, 1918			X	X			X			X					
							Parorthocladius Thienemann, 1935			X	X				X							unnamed species
							Platysmittia Saether, 1982			X	X			?								larva from Lake Davis project
							Psectrocladius Kieffer, 1926			X	X			X			X					
							Pseudorthocladius Goetghebuer, 1932			X	X			X								
							Pseudosmittia Goetghebuer, 1932			X	X			X					X			
							Psilometriocnemus Saether, 1969			X	X			X								
							Rheocricotopus Thienemann and Harnisch, 1932			X	X			X								
							Rheosmittia Brundin in Cranston and Saether, 1986			X	X			X								
							Smittia Holmgren, 1869			X	X			X			X					
							Symbiocladius Kieffer, 1925				X			X			X					phoretic on mayflies
							Synorthocladius Thienemann, 1935			X	X			X								
							Tempisquitoneura Epler, 1995			X	X						X	X	X		Lester, Krestian and Epler (2003); Krestian et al. (2009)	larvae phoretic on Corydalidae
									Tempisquitoneura merrillorum Epler, 1995	X	X						X	X	X		Lester, Krestian and Epler (2003); Krestian et al. (2009)	larvae phoretic on Corydalidae
							Tethymyia Wirth, 1949							X								inter tidal
							Thalassosmittia Strenzke and Remmert, 1957							?								inter tidal
							Thienemanniella Kieffer, 1911			X	X			X								

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Tokunagaia Saether, 1973			X	X			?								ABL has larvae from Lake Davis project
							Tvetenia Kieffer, 1922			X	X			X					X		Bode (1983)	
								Tvetenia bavarica group sensu Bode, 1983		X	X			X							Bode (1983)	
								Tvetenia discoloripes group sensu Bode, 1983		X	X			X					X		Bode (1983)	
							Xylotopus Oliver, 1982			X	X						X					
									Xylotopus par (Coquillett, 1901)	X	X						X					only species known from North America
							Zalutschia Lipina, 1939			X	X						X					
									Zalutschia xethis (Roback, 1957)	X	X						X					
				Podonominae Thienemann, 1937						X	X										Brundin (1983); Brundin (1986)	found in headwater streams
					Boreochlini Brundin, 1966					X	X										Brundin (1983); Brundin (1986)	
							Boreochlus Edwards in Edwards and Thienemann, 1938			X	X			X		X					Brundin (1983); Brundin (1986)	larvae are inseparable to species
							Paraboreochlus Thienemann, 1939			X	X			X							Brundin (1983); Brundin (1986)	ABL has a pupa from Lake Davis area; confirmed by Peter Cranston
					Podonomini Thienemann, 1937					X	X										Brundin (1983); Brundin (1986)	
							Parochlus Enderlein, 1912			X	X			X	?	?					Brundin (1983); Brundin (1986)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
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									Parochlus kiefferi (Garrett, 1925)	X	X			X	?	?					Brundin (1983); Brundin (1986)	only species known from North America
				Prodiamesinae Saether, 1976						X	X			?							Saether (1983); Saether (1986)	
							Monodiamesa Kieffer, 1922			X	X			?							Saether (1983); Saether (1986)	
							Odontomesa Pagast, 1947			X	X			?							Saether (1983); Saether (1986)	
							Prodiamesa Kieffer, 1906			X	X			?							Saether (1983); Saether (1986)	
				Tanypodinae Thienemann and Zavřel, 1916						X	X			X	X	X	X	X	X	X		
					Coelotanypodini Roback, 1982					X	X			?								
							Clinotanypus Kieffer, 1913			X	X			X								
					Macropelopiini Fittkau, 1962					X	X			X	X	X	X			X		
							Alotanypus Roback, 1971			X	X			X		X	X			X		
							Apsectrotanypus Fittkau, 1962			X	X			X	X	X		X	X			
							Bilyjomyia Niitsuma and Watson, 2009			X	X			X	X	X		X	X		Nitsuma and Watson (2009)	new genus erected for *Apsectrotanypus algens* (Coquillet)
									Bilyjomyia algens (Coquillett, 1902)	X	X			X	X	X		X	X		Nitsuma and Watson (2009)	formerly *Apsectrotanypus algens* (Coquillet)
							Brundiniella Roback, 1978			X	X			X	X	X	X					monotypic
									Brundiniella eumorpha (Sublette, 1964)	X	X			X	X	X	X					monotypic
							Derotanypus Roback, 1971			X	X			X	X	X	X	X				

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Derotanypus aclines (Sublette, 1964)	X	X			X	X	X	X	X				
							Macropelopia Thienemann in Thienemann and Kieffer, 1916			X	X			?								
							Psectrotanypus Kieffer, 1909			X	X			X	X	X	X	X				
							Radotanypus Fittkau and Murray, 1985			X	X			X	X							
					Natarsiini Roback and Moss, 1978					X	X			X								
							Natarsia Fittkau, 1962			X	X			X								
					Pentaneuriini Fittkau, 1962					X	X			X	X	X		X	X			
							Ablabesmyia Johannsen, 1905			X	X			X	X	X	X	X	X			
							Conchapelopia Fittkau, 1957			X	X			X	X	X	?		X			Thienemannimyia group
							Hayesomyia Murray and Fittkau, 1985			X	X			X		X						Thienemannimyia group
							Helopelopia Roback, 1971			X	X			?								Thienemannimyia group; ABL has a pupa from Lake Davis
							Krenopelopia Fittkau, 1962			X	X			?	X							
							Labrundinia Fittkau, 1962			X	X			X					X			
							Larsia Fittkau, 1962			X	X			X								
							Meropelopia Roback, 1971			X	X			X								Thienemannimyia group
							Monopelopia Fittkau, 1962			X	X			X								
							Nilotanypus Kieffer, 1923			X	X			X					X			
							Paramerina Fittkau, 1962			X	X			X	X	X	X					
							Pentaneura Philippi, 1865			X	X			X	X	X			X			
							Reomyia Roback, 1986			X	X				X							
							Rheopelopia Fittkau, 1962			X	X			?								Thienemannimyia group; Peter Cranston has reared specimens from CA

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
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							Thienemannimyia group Fittkau, 1962			X	X			X	X	X		X	X			includes *Arctopelopia, Conchapelopia, Hayesomyia, Helopelopia, Meropelopia, Rheopelopia, Thienemannimyia*
								Thienemannimyia Fittkau, 1957		X	X			X	X	X		X	X			Thienemannimyia group
								Xenopelopia Fittkau, 1962		X	X			X								
								Zavrelimyia Fittkau, 1962		X	X			X	X	X						
						Procladiini Roback, 1971				X	X											
								Djalmabatista Fittkau, 1968		X	X			?								ABL has larvae from several CA localities
								Procladius Skuse, 1889		X	X			X	X	X	X	X	X			
						Tanypodini Kieffer, 1906				X	X											
								Tanypus Meigen, 1803		X	X						X					
					Telmatogetoninae Brundin, 1966																	intertidal
								Telmatogeton Schiner, 1866														intertidal
			Culicidae Stephens, 1829							X	X	X		X	X	X	X	X	X	X		
								Aedes Meigen, 1818				X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Aedes aegypti (Linneaus, 1762)			X								X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Aedes cinereus Meigen, 1918			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

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									Aedes vexans (Meigen, 1830)			X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
							Anopheles Meigen, 1818				X	X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Anopheles earlei Vargus, 1943			X					X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Anopheles franciscanus McCracken, 1904		X	X		X	X		X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Anopheles freeborni Aitken, 1939		X	X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Anopheles hermsi Barr and Guptavanij, 1989		X	X		X							Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Anopheles judithae Zavortnik, 1969		X	X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Anopheles occidentalis Dyar and Knab, 1906		X	X		X	X						Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Anopheles punctipennis (Say, 1823)		X	X		X	X	X					Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
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							Culex Linnaeus, 1758					X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex anips Dyar, 1916			X		X						X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex apicalis Adams, 1903			X		X	X		X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex arizonensis Bohart, 1948			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex boharti Brookman and Reeves, 1950			X		X	X	X		X		X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex coronator Dyar and Knab, 1906			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex erythrothorax Dyar, 1907			X		X			X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex interrogator Dyar and Knab, 1906			X		X					X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex pipiens pipiens Linneaus, 1758			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

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									Culex quinquefasciatus Say, 1823			X		X			X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex reevesi Wirth, 1948			X		X							Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex restuans Theobald, 1901			X		X	X		X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex salinarius Coquillett, 1904			X			X						Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex stigmatosoma Dyar, 1907			X		X	X	X		X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex tarsalis Coquillett, 1896			X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex territans Walker, 1856			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culex thriambus Dyar, 1921			X		X			X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culiseta Felt, 1904			X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Culiseta impatiens (Walker, 1848)			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culiseta incidens (Thompson, 1869)			X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culiseta inornata (Williston, 1893)			X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culiseta minnesotae Barr, 1957			X			X	X	X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culiseta morsitans (Theobald, 1901)			X			X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Culiseta particeps (Adams, 1903)			X		X	X	X		X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
							Coquillettidia Dyar, 1905					X		X	X	X					Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Coquillettidia peturbans (Walker, 1856)			X		X	X	X					Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
							Ochlerotatus Lynch Arribálzaga, 1891					X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Ochlerotatus aboriginis (Dyar, 1917)			X		X	X						Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus aloponotum (Dyar, 1917)			X			X	X					Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus bicristatus (Thurman and Winkler, 1950)			X		X							Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus burgeri (Zavortnik)			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus campestris (Dyar and Knab, 1907)			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus cataphylla (Dyar, 1916)			X		X	X	X	X	X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus clivis (Lanzaro and Eldridge, 1992)			X		X							Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus communis (DeGeer, 1776)			X		X	X	X	X	X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus deserticola (Zavortnik, 1969)			X		X							Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

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Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Ochlerotatus dorsalis (Meigen, 1830)			X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus epactius (Dyar and Knab, 1908)			X		X			X	X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus excrucians (Walker, 1856)			X			X		X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus fitchii (Felt and Young, 1904)			X		X	X	X	X	X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus flavescens (Müller, 1764)			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus hendersoni (Cockerell, 1918)			X					X	X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus hexodontus Dyar, 1916			X		X	X	X	X	X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus impiger (Walker, 1848)			X			X	X	X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus implicatus (Vockeroth, 1954)			X		X	X	X	X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

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Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Ochlerotatus increpitus (Dyar, 1916)			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus intrudens (Dyar, 1919)			X			X	X	X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus japonicus japonicus (Theobald, 1901)			X				X					Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus melanimon (Dyar, 1924)			X		X	X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus monticola (Belkin and McDonald, 1957)			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus muelleri (Dyar, 1920)			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus nevadensis Chapman and Barr, 1964			X			X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus nigromaculatus (Ludlow, 1906)			X		X	X	X	X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus niphadopsis (Dyar and Knabb, 1917)			X		X	X		X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

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									Ochlerotatus papago (Zavortnik, 1970)			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus provocans (Walker, 1848)			X			X	X					Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus pullatus (Coquillett, 1904)			X		X	X		X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus purpureipes (Aitken, 1941)			X		X					X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus schizopinax (Dyar, 1929)			X		X	X		X	X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus sierrensis (Ludlow, 1905)			X		X	X		X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus sollicitans sollicitans (Walker, 1856)			X		X					X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus sollicitans idahoensis (Theobald, 1903)			X			X	X	X	X			Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus squamiger (Coquillett, 1902)			X		X						X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

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Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Ochlerotatus sticticus (Meigen, 1838)			X		X	X	X	X				Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus taeniorhynchus (Wiedemann, 1821)			X		X					X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus tahoensis (Dyar, 1916)			X		X							Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus thelcter (Dyar, 1918)			X		X					X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus trivittatus (Coquillett, 1902)			X					X		X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus varipalpus (Coquillett, 1902)			X					X		X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus ventrovittus (Dyar, 1916)			X		X	X		X		X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Ochlerotatus washinoi (Lanzaro and Eldridge, 1992)			X		X	X						Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
							Orthopodomyia Theobald, 1904					X		X	X		X		X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Orthopodomyia kummi Edwards, 1939			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Orthopodomyia signifera (Coquillett, 1896)			X		X	X		X		X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
							Psorophora Robineau-Desvoidy, 1827					X		X			X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Psorophora columbiae (Dyar and Knab, 1906)			X		X				X	X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Psorophora discolor (Coquillett, 1903)			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Psorophora howardii Coquillett, 1901			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Psorophora signipennis (Coquillett, 1904)			X		X			X	X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
							Toxorhynchites Theobald, 1901					X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Toxorhynchites moctezuma Dyar and Knab, 1906			X							X		Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus

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Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Uranotaenia Lynch Arribálzaga, 1891					X		X				X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
									Uranotaenia anhydor anhydor Dyar, 1907			X		X				X	X	X	Darsie and Ward (2005)	Only fourth instar larvae can be reliably identified beyond genus
			Dixidae Schiner, 1868							X	X	X		X	X	X			X		Cook (1983)	
							Dixa Meigen, 1818			X	X			X		X			X		Cook (1983)	
							Dixella Dyar and Shannon, 1924			X	X	X		X	X	X					Cook (1983)	
							Meringodixa Nowell, 1951			X	X			X							Cook (1983)	
									Meringodixa chalonensis (Nowell, 1951)	X	X			X							Cook (1983)	
			Simuliidae Newman, 1834							X	X			X	X	X	X	X	X	X	Adler et al. (2004)	
				Parasimuliinae Smart, 1945						X	X			X	X	X					Adler et al. (2004)	streams in coniferous forests dominated by western hemlock
							Parasimulium Malloch, 1914			X	X			X	X	X					Adler et al. (2004)	streams in coniferous forests dominated by western hemlock; larvae not separable to species
									Parasimulium species "A", Adler, Currie and Wood, 2004	X	X				X						Adler et al. (2004)	streams in coniferous forests dominated by western hemlock; larva unknown
									Parasimulium crosskeyi Peterson, 1977	X	X				X	X					Adler et al. (2004)	streams in coniferous forests dominated by western hemlock; larva indistinguishable from *P. stonei*

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Parasimulium furcatum Malloch, 1914	X	X			X	X	X					Adler et al. (2004)	streams in coniferous forests dominated by western hemlock; larva unknown
									Parasimulium melanderi Stone, 1963	X	X					X					Adler et al. (2004)	known from 1 specimen from WA and 2 from British Columbia; larva unknown; possibly subterranean
									Parasimulium stonei Peterson, 1977	X	X			X	X	X					Adler et al. (2004)	streams in coniferous forests dominated by western hemlock; larva indistinguishable from *P. crosskeyi*
				Simuliinae Newman, 1834						X	X			X	X	X	X	X	X		Adler et al. (2004)	
					Prosimuliini Enderlein, 1921					X	X			X	X	X	X	X	X		Adler et al. (2004)	
							Twinnia Stone and Jamnback, 1955			X	X			X	X	X	X				Adler et al. (2004)	headwater streams; impoundment outflows
									Twinnia hirticornis Wood, 1978	X	X			X	X						Adler et al. (2004)	headwater streams; impoundment outflows
									Twinnia nova (Dyar and Shannon, 1927)	X	X					X	X				Adler et al. (2004)	headwater streams; impoundment outflows
							Helodon Enderlein, 1921			X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Helodon pleuralis (Malloch, 1914)	X	X					X					Adler et al. (2004)	
									Helodon clavatus (Peterson, 1970)	X	X					X					Adler et al. (2004)	
									Helodon beardi Adler, Currie and Wood, 2004	X	X			X	X				X		Adler et al. (2004)	

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Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Helodon chaos Adler, Currie and Wood, 2004	X	X			X	X	X					Adler et al. (2004)	
									Helodon diadelphus Adler, Currie and Wood, 2004	X	X				X	X					Adler et al. (2004)	
									Helodon mccreadiei Adler, Currie and Wood, 2004	X	X			X	X	X					Adler et al. (2004)	
									Helodon newmani Adler, Currie and Wood, 2004	X	X			X	X						Adler et al. (2004)	
									Helodon onchyodactylus (Dyar & Shannon, 1927)	X	X			X	X	X	X				Adler et al. (2004)	
									Helodon protus Adler, Currie and Wood, 2004	X	X				X	X					Adler et al. (2004)	
									Helodon susanae (Peterson, 1970)	X	X			X	X	X	X				Adler et al. (2004)	
									Helodon trochus Adler, Currie and Wood, 2004	X	X							X			Adler et al. (2004)	
							Prosimulium Roubaud, 1906			X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Prosimulium caudatum Shewell, 1959	X	X			X	X	X					Adler et al. (2004)	
									Prosimulium constrictistylum Peterson, 1970	X	X							X			Adler et al. (2004)	
									Prosimulium davesi Peterson and Defoliart, 1960	X	X				X	X	X				Adler et al. (2004)	
									Prosimulium dicentum Dyar and Shannon, 1927	X	X			X	X	X					Adler et al. (2004)	
									Prosimulium dicum Dyar and Shannon, 1927	X	X			X	X	X			X		Adler et al. (2004)	
									Prosimulium doveri Sommerman, 1962	X	X					X					Adler et al. (2004)	
									Prosimulium esselbaughi Sommerman, 1964	X	X			X	X	X		X			Adler et al. (2004)	
									Prosimulium exigens Dyar and Shannon, 1927	X	X			X	X	X	X	X	X		Adler et al. (2004)	

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									Prosimulium flaviantennus (Stains and Knowlton, 1940)	X	X			X			X		X		Adler et al. (2004)	
									Prosimulium formosum Shewell, 1959	X	X			X	X	X			X		Adler et al. (2004)	
									Prosimulium frohnei Sommerman, 1958	X	X			X							Adler et al. (2004)	
									Prosimulium fulvithorax Shewell, 1959	X	X			X	X	X					Adler et al. (2004)	
									Prosimulium fulvum (Coquillett, 1902)	X	X			X	X	X	X				Adler et al. (2004)	
									Prosimulium idemai Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	
									Prosimulium imposter Peterson, 1970	X	X			X	X				X		Adler et al. (2004)	
									Prosimulium longirostrum Adler, Currie and Wood, 2004	X	X				X						Adler et al. (2004)	
									Prosimulium minifulvum Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	
									Prosimulium rusticum Adler, Currie and Wood, 2004	X	X								X		Adler et al. (2004)	
									Prosimulium secretum Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	
									Prosimulium shewelli Peterson and Defoliart, 1960	X	X			X			X				Adler et al. (2004)	
									Prosimulium travisi Stone, 1952	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Prosimulium uinta Peterson and Defoliart, 1960	X	X								X		Adler et al. (2004)	
									Prosimulium unicum (Twinn, 1938)	X	X								X		Adler et al. (2004)	
					Simuliini Newman, 1834					X	X			X	X	X	X	X	X		Adler et al. (2004)	
							Greniera Doby and David, 1959			X	X			X	X	X	X				Adler et al. (2004)	rarely encountered

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									Greneria "species F", Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	rarely encountered
									Greneria humeralis Currie, Adler and Wood, 2004	X	X			X	X	X					Adler et al. (2004)	rarely encountered
									Greneria denaria (Davies, Peterson and Wood, 1962)	X	X					X	X				Adler et al. (2004)	rarely encountered
							Stegopterna Enderlein, 1930			X	X			X	X	X	X	X	X		Adler et al. (2004)	shallow mountain streams
									Stegopterna acra Currie, Adler and Wood, 2004	X	X			X			X	X	X		Adler et al. (2004)	shallow mountain streams
									Stegopterna permutata (Dyar and Shannon, 1927)	X	X			X		X					Adler et al. (2004)	shallow mountain streams
									Stegopterna xantha Currie, Adler and Wood, 2004	X	X			X	X	X					Adler et al. (2004)	shallow mountain streams
							Tlalocomyia Wygodzinsky and Diaz Najera, 1970			X	X			X	X	X			X		Adler et al. (2004)	shallow mountain streams, seeps
									Tlalocomyia andersoni Currie, Adler and Wood, 2004	X	X			X	X						Adler et al. (2004)	shallow mountain streams, seeps
									Tlalocomyia osbornii (Stains and Knowlton, 1943)	X	X			X	X	X			X		Adler et al. (2004)	shallow mountain streams, seeps
									Tlalocomyia ramifera Currie, Adler and Wood, 2004	X	X				X	X					Adler et al. (2004)	shallow mountain streams, seeps
									Tlalocomyia stewarti (Coleman, 1953)	X	X			X							Adler et al. (2004)	shallow mountain streams, seeps
							Gigantodax Enderlein, 1925			X	X								X		Adler et al. (2004)	small, high-elevation springs
									Gigantodax adleri Moulton, 1996	X	X								X		Adler et al. (2004)	small, high-elevation springs
							Metacnephia Crosskey, 1969			X	X			X			X				Adler et al. (2004)	high elevation lake outlets and streams
									Metacnephia coloradensis Peterson and Kondratieff, 1995	X	X			X							Adler et al. (2004)	high elevation lake outlets and streams
									Metacnephia jeanae (Defoliart and Peterson, 1960)	X	X			X			X				Adler et al. (2004)	high elevation lake outlets and streams

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									Metacnephia villosa (Defoliart and Peterson, 1960)	X	X			X			X				Adler et al. (2004)	high elevation lake outlets and streams
							Simulium Latreille, 1802			X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium anduzei Vargas and Diaz Najera, 1948	X	X								X		Adler et al. (2004)	
									Simulium apricarium Adler, Currie and Wood, 2004	X	X			X			X		X		Adler et al. (2004)	
									Simulium argus Wiliston, 1893	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium baffinense Twinn, 1936	X	X						X				Adler et al. (2004)	
									Simulium balteatum Adler, Currie and Wood, 2004	X	X			X	X	X					Adler et al. (2004)	
									Simulium bivittatum Malloch, 1914	X	X				X	X	X		X		Adler et al. (2004)	
									Simulium brevicercum Knowlton and Rowe, 1934	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									*Simulium bricenoi V*argas, Martinez Palacios and Diaz Najera, 1946	X	X								X		Adler et al. (2004)	
									Simulium canadensis Hearle,1932	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium canonicolum (Dyar and Shannon, 1927)	X	X						X	X	X		Adler et al. (2004)	
									Simulium carbunculum Adler, Currie and Wood, 2004	X	X			X			X	X	X		Adler et al. (2004)	
									Simulium chromatinum Adler, Currie and Wood, 2004	X	X								X		Adler et al. (2004)	
									Simulium chromocentrum Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	
									Simulium clarum (Dyar and Shannon, 1927)	X	X			X							Adler et al. (2004)	
									Simulium conicum Adler, Currie and Wood, 2004	X	X			X	X	X					Adler et al. (2004)	

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									Simulium craigi Stone and Snoddy, 1969	X	X			X			X		X		Adler et al. (2004)	
									Simulium curiei Adler and Wood, 1991	X	X			X	X	X	X		X		Adler et al. (2004)	
									Simulium decorum Walker, 1948	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium defoliarti Stone and Peterson, 1958	X	X			X	X	X					Adler et al. (2004)	
									Simulium donovani Vargas, 1943	X	X			X	X			X	X		Adler et al. (2004)	
									Simulium encisoi Vargas and Diaz Najera, 1949	X	X			X				X	X		Adler et al. (2004)	
									Simulium exculatum Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	
									Simulium freemani Vargas and Diaz Najera, 1949	X	X						X		X		Adler et al. (2004)	
									Simulium griseum Coquillett, 1898	X	X			X			X		X		Adler et al. (2004)	
									Simulium hechti Vargas, Martinez Palacios and Diaz Najera, 1946	X	X			X	X	X		X	X		Adler et al. (2004)	
									Simulium hippovorum Malloch, 1914	X	X			X	X	X		X	X		Adler et al. (2004)	
									Simulium hunteri Malloch, 1914	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium infernale Adler, Currie and Wood, 2004	X	X			X			X				Adler et al. (2004)	
									Simulium iriartei Vargas, Martinez Palacios and Diaz Najera, 1946	X	X								X		Adler et al. (2004)	
									Simulium irritatum Lugger, 1897	X	X						X				Adler et al. (2004)	
									Simulium jacumbae Dyar and Shannon, 1927	X	X			X	X	X		X	X		Adler et al. (2004)	
									Simulium joculator Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Simulium longithallum Diaz Najera and Vulcano, 1962	X	X								X		Adler et al. (2004)	
									Simulium meridionale Riley, 1887	X	X			X				X			Adler et al. (2004)	
									Simulium merritti Adler, Currie and Wood, 2004	X	X						X				Adler et al. (2004)	
									Simulium modicum Adler, Currie and Wood, 2004	X	X			X		X					Adler et al. (2004)	
									Simulium mysterium Adler, Currie and Wood, 2004	X	X			X							Adler et al. (2004)	
									Simulium nebulosum Currie and Adler, 1986	X	X			X	X	X					Adler et al. (2004)	
									Simulium negativum Adler, Currie and Wood, 2004	X	X						X	X			Adler et al. (2004)	
									Simulium notatum Adams, 1904	X	X								X		Adler et al. (2004)	
									Simulium paynei Vargas, 1942	X	X								X		Adler et al. (2004)	
									Simulium petersoni Stone and Defoliart, 1959	X	X			X	X		X	X			Adler et al. (2004)	
									Simulium pilosum (Knowlton and Rowe, 1934)	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium piperi Dyar and Shannon, 1927	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium pugetense (Dyar and Shannon, 1927)	X	X			X	X	X					Adler et al. (2004)	
									Simulium quadratum (Stains and Knowlton, 1943)	X	X			X		X	X				Adler et al. (2004)	
									Simulium rostratum (Lundstrom, 1911)	X	X				X	X					Adler et al. (2004)	
									Simulium saxosum Adler, Currie and Wood, 2004	X	X			X	X	X					Adler et al. (2004)	
									Simulium silvestre (Rubtsov, 1956)	X	X			X	X	X					Adler et al. (2004)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Simulium tescorum Stone and Boreham, 1965	X	X			X	X	X		X	X		Adler et al. (2004)	
									Simulium twinni Stains and Knowlton, 1940	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium tribulatum Lugger, 1897	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium vandalicum Dyar and Shannon, 1927	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium venator Dyar and Shannon, 1927	X	X			X	X		X	X			Adler et al. (2004)	
									Simulium venustum Say, 1823	X	X			X					X		Adler et al. (2004)	
									Simulium virgatum Coquillett, 1902	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium vittatum Zetterstadt, 1838	X	X			X	X	X	X	X	X		Adler et al. (2004)	
									Simulium wyomingense Stone and Defoliart, 1959	X	X			X	X		X				Adler et al. (2004)	
									Simulium zephyrus Adler, Currie and Wood, 2004	X	X				X	X					Adler et al. (2004)	
			Thaumaleidae Bezzi, 1913							X	X			X							Wirth and Stone (1956)	second genus *Trichothaumalea* is found in British Columbia
									Thaumalea Ruthe, 1831	X	X			X							Wirth and Stone (1956)	
	Brachycera Zetterstedt, 1842													X	X	X	X	X	X	X		
		Tabanomorpha Hennig, 1948								X	X			X	X	X	X	X	X	X		
			Athericidae Stuckenberg, 1973							X	X			X							Webb (1977)	
									Atherix Meigen, 1844	X	X			X							Webb (1977)	

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
									Atherix pachypus Bigot, 1887	X	X			X							Webb (1977)	Three species known from the USA. *A. pachypus* is the name used for the western species
			Oreoleptidae Zloty, Sinclair and Pritchard, 2005							X	X			?	?						Zloty, Sinclair and Pritchard (2005)	unpublished records for Oregon, California
							Oreoleptis Zloty, Sinclair and Pritchard, 2005			X	X			?	?						Zloty, Sinclair and Pritchard (2005)	unpublished records for Oregon, California
									Oreoleptis torrenticola Zloty, Sinclair and Pritchard, 2005	X	X			?	?						Zloty, Sinclair and Pritchard (2005)	unpublished records for Oregon, California
			Pelecorhynchidae Enderlein, 1922							X	X			X	X	X						
							Bequaertomyia Brennan, 1935			?	?			X	?	?						unknown biology; possibly not aquatic
							Glutops Burgess, 1878			X	X			X	X	X						
			Stratiomyidae Giebel, 1856							X	X			X	X	X		X	X	X		
							Caloparyphus James, 1939			X	X			X	X	X			X		Sinclair (1989)	early instars inseparable from *Euparyphus*
							Caloparyphus James, 1939/*Euparyphus* Gerstäcker, 1857			X	X			X							Sinclair (1989)	use this name for all early instars of *Caloparyphus* and *Euparyphus*
							Euparyphus Gerstäcker, 1857			X	X			X	X	X			X		Sinclair (1989)	spiracular stalk doesn't develop until final instar
							Hedriodiscus Enderlein, 1914/*Odontomyia* Meigen, 1932			X	X			X	X	X			X			
							Myxosargus Brauer, 1882			X	X			X					X			
							Nemotelus Geoffrey, 1762			X	X			X	X	X		X		X		
							Stratiomys Geoffrey, 1762			X	X			X	X	X						

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
			Tabanidae Latreille, 1802											X							Courtney and Merritt (2008); Middlekauff and Lane (1980)	also many terrestrial genera
							Apatolestes Williston, 1885			X	X			X	X	X			X	X		
							Atylotus Osten Sacken, 1876/*Tabanus* Linnaeus, 1758			X	X			X	X	X			X			incompletely separable, except by habitat; most specimens from lotic habitats will be *Tabanus*
							Chrysops Meigen, 1800			X	X			X	X	X		X	X			
							Haematopota Meigen, 1800			X	X			X								
							Hybomitra Enderlein, 1922			X	X			X	X	X			X			
							Silvius Meigen, 1820			X	X			X	X	X		X				
							Tabanus Linnaeus, 1758															see note for *Atylotus/Tabanus*
		Asilomorpha Rohdendorf, 1961								X	X			X								
			Empididae Latreille, 1804							X	X			X	X	X						only a few genera are aquatic
				Clinocerinae Collin, 1928						X	X			X		X						
							Clinocera Meigen, 1800			X	X			X		X						
							Roederiodes Coquillett, 1901			X	X			X								feed on simuliid pupae
							Trichoclinocera Collin, 1941			X	X			?								
							Wiedemannia Zetterstedt, 1838			X	X			?		X						feed on simuliid pupae
				Empidinae Latreille, 1804						X	X			X	X	X						
							Oreogeton Schiner, 1860			X	X			X	X	X						feed on simuliid larvae
				Hemerodromiinae Schiner, 1862						X	X			X	X	X						
							Chelifera Macquart, 1823/*Metachela* Coquillet, 1903			X	X			X	X	X					MacDonald and Harkrider (1999)	larvae are inseparable at this time

Taxonomic Hierarchy										Habitat				Distribution							Literature Cited	Comments
Order	Suborder	Infraorder	Family	Subfamily	Tribe	Genus group	Genus	Species group	Species	Benthic	Lotic	Lentic	Estuarine	CA	OR	WA	UT	NV	AZ	Baja		
							Hemerodromia Meigen, 1822			X	X			X	X	X						
							Neoplasta Coquillett, 1895			X	X			X	X	X					MacDonald and Harkrider (1999)	
			Dolichopodidae Latreille, 1809							X	X			X	X	X		X	X			larvae and pupae should be identified to family
		Muscomorpha Crampton, 1944								X	X			X	X	X		X	X	X		
			Canacidae Enderlein, 1935											X								intertidal dwellers
			Phoridae Curtis, 1833																			larvae and pupae should be identified to family
			Syrphidae Latreille, 1802							X	X	X		X	X	X		X	X	X		larvae and pupae should be identified to family
			Sciomyzidae Fallén, 1820							X	X	X		X	X	X		X	X	X		larvae and pupae should be identified to family
			Ephydridae Zetterstedt, 1837							X	X	X		X	X	X		X	X	X	Courtney and Merritt (2008)	The key in Merritt, Cummins and Berg is incomplete. Larvae should be left at family unless reared or identified using a more complete key.
			Muscidae Latreille, 1802							X	X	X		X	X	X		X	X	X		larvae and pupae should be identified to family

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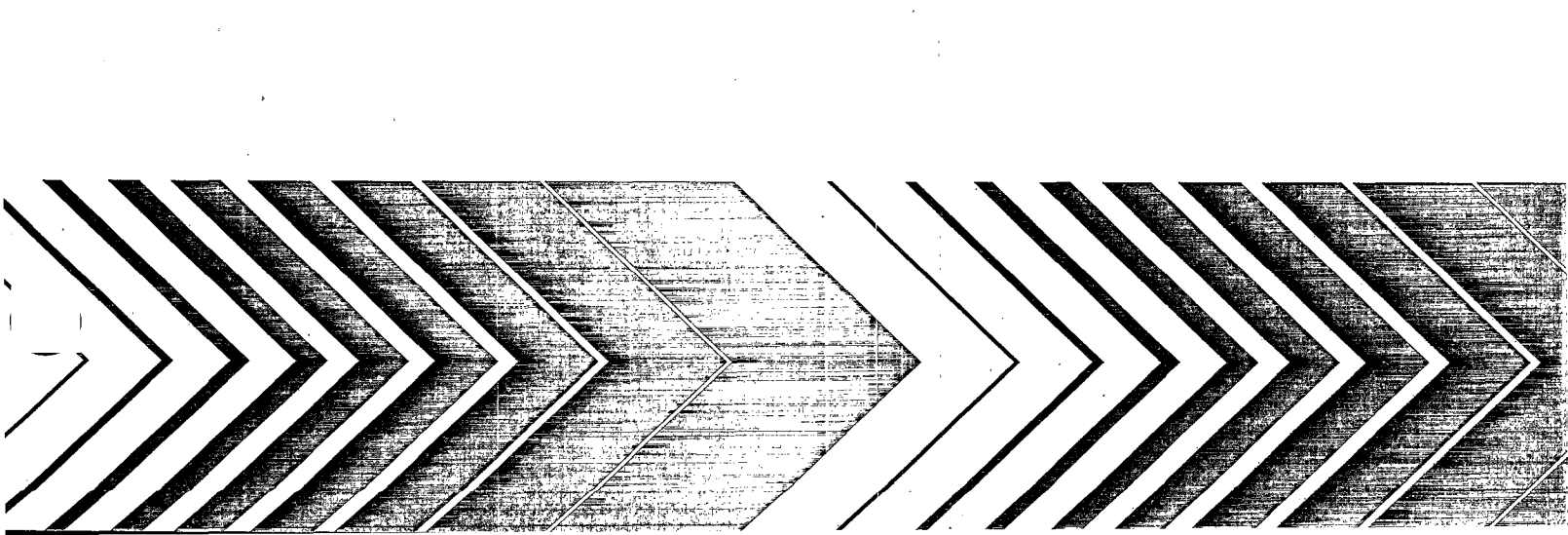
Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates

Second Edition



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Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates

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Disclaimer

This guidance is designed to describe procedures for testing freshwater organisms in the laboratory to evaluate the potential toxicity or bioaccumulation of chemicals in whole sediments. This guidance document has no immediate or direct regulatory consequence. It does not in itself establish or affect legal rights or obligations, or represent a determination of any party's liability. The USEPA may change this guidance in the future.

This guidance document has been reviewed in accordance with USEPA Policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Foreword

Sediment contamination is a widespread environmental problem that can potentially pose a threat to a variety of aquatic ecosystems. Sediment functions as a reservoir for common chemicals such as pesticides, herbicides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and metals such as lead, mercury, and arsenic. In-place contaminated sediment can result in depauperate benthic communities, while disposal of contaminated dredged material can potentially exert adverse effects on both pelagic and benthic systems. Historically, assessment of sediment quality has been limited to chemical characterizations. The United States Environmental Protection Agency (USEPA) is developing methodologies to calculate chemical-specific sediment quality guidelines (referred to as equilibrium partitioning sediment guidelines or ESGs) for use in the Agency's regulatory programs. However, quantifying contaminant concentrations alone cannot always provide enough information to adequately evaluate potential adverse effects that arise from interactions among chemicals, or that result from time-dependent availability of sediment-associated contaminants to aquatic organisms. Because relationships between bioavailability and concentrations of chemicals in sediment are not fully understood, determination of contaminated sediment effects on aquatic organisms may require the use of controlled toxicity and bioaccumulation tests.

As part of USEPA's Contaminated Sediment Management Strategy, Agency programs have agreed to use consistent methods to determine whether sediments have the potential to affect aquatic ecosystems. More than ten federal statutes provide authority to many USEPA program offices to address the problem of contaminated sediment. The sediment test methods in this manual will be used by USEPA to make decisions under a range of statutory authorities concerning such issues as: dredged material disposal, registration of pesticides, assessment of new and existing industrial chemicals, Superfund site assessment, and assessment and cleanup of hazardous waste treatment, storage, and disposal facilities. The use of uniform sediment testing procedures by USEPA programs is expected to increase data accuracy and precision, facilitate test replication, increase the comparative value of test results, and ultimately increase the efficiency of regulatory processes requiring sediment tests.

This second edition of the manual is a revision to USEPA (1994a; EPA 600/R-94/024). Primary revisions to the first edition of the manual include:

Section 14: This new section describes methods for evaluating sublethal effects of sediment-associated contaminants with the amphipod *Hyalella azteca*. See also associated revisions to Sections 1.3, 2, 4.3, 7.1.3, and 10.3. Section 11 also outlines methods for measuring growth and survival as primary endpoints in 10-d tests with *Hyalella azteca.*

Section 15: This new section describes methods for evaluating sublethal effects of sediment-associated contaminants with the midge *Chironomus tentans*. See also associated revisions to Sections 1.3, 2, 4.3, 7.1.3, 10.4, and Appendix C.

Section 2.1.2.1.1: Additional detail has been included on test acceptability (i.e., control vs. reference sediment).

Foreword (continued)

Section 6.2.2: The range of acceptable light intensity for culture and testing has been revised from 500 lux to 1000 lux to 100 to 1000 lux.

Sections 7.2, 8.2, 8.3.2, 8.4.4.7: Additional detail has been added to sections on formulated sediments, sediment storage, sediment spiking, and interstitial water sampling.

Sections 9.14, 10.3, and 17.4: The requirement to conduct monthly reference-toxicity tests has been modified to recommend the conduct of reference-toxicity tests periodically to assess the sensitivity of the test organisms.

Sections 9.14.2 and 17.4.3: These revised sections now state that before conducting tests with contaminated sediment, it is strongly recommended that the laboratory conduct the tests with control sediment(s). Results of these preliminary studies should be used to determine if use of the control sediment and other test conditions (i.e., water quality) result in acceptable performance in the tests as outlined in Tables 11.3, 12.3, 13.4, 14.3, and 15.3.

Section 10.3.2: Diatoms are no longer used to culture *Hyalella azteca* following procedures of USEPA (1993).

Section 11: *In Section11.2.2 (and associated sections and tables):* The recommended feeding level of 1.5 mL of YCT/day/beaker in the 10-d *Hyalella azteca* sediment toxicity test in the first edition of the manual has been revised to 1.0 ml of YCT/day/beaker. This change was made to make the 10-d test described in Section 11 consistent with the feeding level recommended in the 42-d test with *Hyalella azteca* described in Section 14. *In Section 11.3:* Additional guidance has been included in the revised manual regarding acclimation of test organisms to temperature (see also Section 12.3, 13.3, 14.3, and 15.3). *In Section 11.3.6.1.1:* Acceptable concentrations of dissolved oxygen in overlying water are now expressed in mg/L rather than in a percentage of saturation. See also Sections 10, 12, 13, 14, and 15.

Sections 12.3.8 and 15.3.8: The recommendation is now made to measure ash-free dry weight of *Chironomus tentans* instead of dry weight. See also Sections 13.3.8 for *Lumbriculus variegatus* and 14.3.7 for *Hyalella azteca*.

Section 13.3.7: This section outlines additional guidance on depuration of *Lumbriculus variegatus* in bioaccumulation testing.

Section 17.6: This revised section now includes summaries of the results of round-robin tests using the methods for long-term toxicity tests outlined in Sections 14 and 15.

Appendix A in the first edition of the manual (USEPA, 1994) was not included in this edition (summary of a workshop designed to develop consensus for the 10-d toxicity test and bioaccumulation methods). This information has been cited by reference in this current edition of the manual.

For additional guidance on the technical considerations in the manual, please contact Teresa Norberg-King, USEPA, Duluth, MN (218/529-5163, fax -5003, email norberg-king.teresa@epa.gov) or Chris Ingersoll, USGS, Columbia, MO (573/876-1819, fax -1896, email chris_ingersoll@usgs.gov).

Abstract

Procedures are described for testing freshwater organisms in the laboratory to evaluate the potential toxicity or bioaccumulation of chemicals in whole sediments. Sediments may be collected from the field or spiked with compounds in the laboratory. Toxicity methods are outlined for two organisms, the amphipod *Hyalella azteca* and the midge *Chironomus tentans.* Toxicity tests with amphipods or midges are conducted for 10 d in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is renewed daily and test organisms are fed during the toxicity tests. The endpoints in the 10-d toxicity test with *H. azteca* and *C. tentans* are survival and growth. Procedures are primarily described for testing freshwater sediments; however, estuarine sediments (up to 15‰ salinity) can also be tested in 10-d sediment toxicity tests with *H. azteca.* Guidance is also provided for conducting long-term sediment toxicity tests with *H. azteca* and *C. tentans.* The long-term sediment exposures with *H. azteca* are started with 7- to 8-d-old amphipods. On Day 28 of the sediment exposure, amphipods are isolated from the sediment and placed in water-only chambers where reproduction is measured on Day 35 and 42. Endpoints measured in the amphipod test include survival (Day 28, 35, and 42), growth (on Day 28 and 42), and reproduction (number of young/female produced from Day 28 to 42). The long-term sediment exposures with *C. tentans* start with newly hatched larvae (<24-h old) and continue through emergence, reproduction, and hatching of the F_1 generation (about 60-d sediment exposures). Survival and growth are determined at 20 d. Starting on Day 23 to the end of the test, emergence and reproduction of *C. tentans* are monitored daily. The number of eggs/female is determined for each egg mass, which is incubated for 6 d to determine hatching success. The procedures described in Sections 14 and 15 include measurement of a variety of lethal and sublethal endpoints with *Hyalella azteca* and *Chironomus tentans*; minor modifications of the basic methods can be used in cases where only a subset of these endpoints is of interest. Guidance for conducting 28-d bioaccumulation tests with the oligochaete *Lumbriculus variegatus* is also provided in the manual. Overlying water is renewed daily and test organisms are not fed during bioaccumulation tests. Methods are also described for determining bioaccumulation kinetics of different classes of compounds during 28-d exposures with *L. variegatus.*

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Acknowledgments

This document is a general purpose testing manual for freshwater sediments. This manual is a revision to a previously published edition of this manual (USEPA, 1994a). The approaches described in this manual were developed from ASTM (1999a), ASTM (1999b), ASTM (1999c), ASTM (1999d), Ankley et al. (1993), Phipps et al. (1993), USEPA (1994b), USEPA (1994c), Ingersoll et al. (1995), Ingersoll et al. (1998), Sibley et al. (1996), Sibley et al. (1997a), Sibley et al. (1997b), and Benoit et al. (1997).

This second edition of the manual reflects the consensus of the Freshwater Sediment Toxicity Assessment Committee and the U.S. Environmental Protection Agency (USEPA) Program Offices. Members of the Freshwater Sediment Toxicity Assessment Committee for the second edition of this manual included G.A. Burton, Wright State University, Dayton, OH; T.D. Dawson, Integrated Laboratory Systems (ILS), Duluth, MN; F.J. Dwyer, U.S. Fish & Wildlife Service (USFWS), Columbia, MO; C.G. Ingersoll, U.S. Geological Survey (USGS), Columbia, MO; D.S. Ireland, USEPA, Washington, D.C.; N.E. Kemble, USGS, Columbia, MO; D.R. Mount, USEPA, Duluth, MN; T.J. Norberg-King, USEPA, Duluth, MN; P.K. Sibley, University of Guelph, Guelph, Ontario, and Leanne Stahl, USEPA, Washington, D.C.

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Section 1
Introduction

1.1 Significance of Use

1.1.1 Sediment provides habitat for many aquatic organisms and is a major repository for many of the more persistent chemicals that are introduced into surface waters. In the aquatic environment, most anthropogenic chemicals and waste materials including toxic organic and inorganic chemicals eventually accumulate in sediment. Mounting evidence exists of environmental degradation in areas where USEPA Water Quality Criteria (WQC; Stephan et al., 1985) are not exceeded, yet organisms in or near sediments are adversely affected (Chapman, 1989). The WQC were developed to protect organisms in the water column and were not intended to protect organisms in sediment. Concentrations of chemicals in sediment may be several orders of magnitude higher than in the overlying water; however, bulk sediment concentrations have not been strongly correlated to bioavailability (Burton, 1991). Partitioning or sorption of a compound between water and sediment may depend on many factors, including aqueous solubility, pH, redox, affinity for sediment organic carbon and dissolved organic carbon, grain size of the sediment, sediment mineral constituents (oxides of iron, manganese, and aluminum), and the quantity of acid volatile sulfides in sediment (Di Toro et al., 1990, 1991). Although certain chemicals are highly sorbed to sediment, these compounds may still be available to the biota. Contaminated sediments may be directly toxic to aquatic life or can be a source of contaminants for bioaccumulation in the food chain.

1.1.2 Assessments of sediment quality have commonly included sediment chemical analyses and surveys of benthic community structure. Determination of sediment chemical concentrations on a dry weight basis alone offers little insight into predicting adverse biological effects because bioavailability may be limited by the intricate partitioning factors mentioned above. Likewise, benthic community surveys may be inadequate because they sometimes fail to discriminate between effects of contaminants and those that result from unrelated non-contaminant factors, including water-quality fluctuations, physical parameters, and biotic interactions. To obtain a direct measure of sediment toxicity or bioaccumulation, laboratory tests have been developed in which surrogate organisms are exposed to sediments under controlled conditions. Sediment toxicity tests have evolved into effective tools that provide direct, quantifiable evidence of biological consequences of sediment contamination that can only be inferred from chemical or benthic community analyses. To evaluate sediment quality nationwide, USEPA developed the National Sediment Inventory (NSI), which is a compilation of existing sediment quality data and protocols used to evaluate the data. The NSI was used to produce the first biennial report to Congress on sediment quality in the United States as required under the Water Resources Development Act of 1992 (USEPA, 1997a; 1997b; 1997c). USEPA's evaluation of the data shows that sediment contamination exists in every region and state of the country and various waters throughout the United States contain sediment that is sufficiently contaminated with toxic pollutants to pose potential risks to fish and to humans and wildlife who eat fish. The use of consistent sediment testing methods described in this manual will provide high quality data needed for the NSI, future reports to Congress, and regulatory programs to prevent, remediate, and manage contaminated sediments (USEPA, 1998).

1.1.3 The objective of a sediment test is to determine whether chemicals in sediment are harmful to or are bioaccumulated by benthic organisms. The tests can be used to measure interactive toxic effects of complex chemical mixtures in sediment. Furthermore, knowledge of specific pathways of interactions among sediments and test organisms is not necessary to conduct the tests (Kemp and Swartz, 1988). Sediment tests can be used to (1) determine the relationship between toxic effects and bioavailability; (2) investigate interactions among chemicals; (3) compare the sensitivities of different organisms; (4) determine spatial and temporal distribution of contamination; (5) evaluate dredged material; (6) measure toxicity as part of product licensing or safety testing or chemical approval; (7) rank areas for cleanup, and (8) set cleanup goals and estimate the effectiveness of remediation or management practices.

1.1.4 A variety of standard methods have been developed for assessing the toxicity of contaminants associated with sediments using amphipods, midges, polychaetes, oligochaetes, mayflies, or cladocerans (i.e., ASTM,1999a; ASTM,1999b; ASTM, 1999c; ASTM, 1999d; USEPA, 1994a; USEPA, 1994b; Environment Canada, 1997a; Environment Canada, 1997b). Several endpoints are suggested in these methods to measure effects of contaminants in sediment including survival, growth, behavior, or reproduction; however, survival of test organisms in

10-d exposures is the endpoint most commonly reported. These short-term exposures which only measure effects on survival can be used to identify high levels of contamination, but may not be able to identify moderately contaminated sediments (Sibley et al., 1996; Sibley et al., 1997a; Sibley et al., 1998; Benoit et al., 1997; Ingersoll et al., 1998). Sublethal endpoints in sediment tests may also prove to be better estimates of responses of benthic communities to contaminants in the field (Kemble et al., 1994) The first edition of this manual (USEPA, 1994a) described 10-d toxicity tests with the amphipod *Hyalella azteca* and midge *Chironomus tentans* (Section 11, 12). This second edition of the manual now outlines approaches for evaluating sublethal endpoints in longer-term sediment exposures with these two species (Section 14, 15). Guidance is also presented in Section 13 regarding sediment bioaccumulation testing with the oligochaete *Lumbriculus variegatus*.

1.1.5 Results of toxicity tests on sediments spiked at different concentrations of chemicals can be used to establish cause and effect relationships between chemicals and biological responses. Results of toxicity tests with test materials spiked into sediments at different concentrations may be reported in terms of an LC50 (median lethal concentration), an EC50 (median effect concentration), an IC50 (inhibition concentration), or as a NOEC (no observed effect concentration) or LOEC (lowest observed effect concentration). In some cases, results of bioaccumulation tests may also be reported in terms of a Biota-sediment Accumulation Factor (BSAF) (Ankley et al., 1992a; Ankley et al., 1992b).

1.1.6 Evaluating effect concentrations for chemicals in sediment requires knowledge of factors controlling their bioavailability. Similar concentrations of a chemical in units of mass of chemical per mass of sediment dry weight often exhibit a range in toxicity in different sediments (Di Toro et al., 1990; Di Toro et al., 1991). Effect concentrations of chemicals in sediment have been correlated to interstitial water concentrations, and effect concentrations in interstitial water are often similar to effect concentrations in water-only exposures. The bioavailability of nonionic organic compounds in sediment is often inversely correlated with the organic carbon concentration. Whatever the route of exposure, these correlations of effect concentrations to interstitial water concentrations indicate that predicted or measured concentrations in interstitial water can be used to quantify the exposure concentration to an organism. Therefore, information on partitioning of chemicals between solid and liquid phases of sediment is useful for establishing effect concentrations (Di Toro et al., 1991).

1.1.7 Field surveys can be designed to provide either a qualitative reconnaissance of the distribution of sediment contamination or a quantitative statistical comparison of contamination among sites. Surveys of sediment toxicity or bioaccumulation are usually part of more comprehensive analyses of biological, chemical, geological, and hydrographic data. Statistical correlations may be improved and sampling costs may be reduced if subsamples are taken simultaneously for sediment tests, chemical analyses, and benthic community structure.

1.1.8 Table 1.1 lists several approaches the USEPA has considered for the assessment of sediment quality (USEPA, 1992c). These approaches include (1) equilibrium partitioning, (2) tissue residues, (3) interstitial water toxicity, (4) benthic community structure, (5) whole-sediment toxicity and sediment-spiking tests, (6) Sediment Quality Triad, and (7) sediment quality guidelines (see Chapman, 1989 and USEPA, 1989a; USEPA, 1990a; USEPA, 1990b; USEPA, 1992b for a critique of these methods). The sediment assessment approaches listed in Table 1.1 can be classified as numeric (e.g., equilibrium partitioning), descriptive (e.g., whole-sediment toxicity tests), or a combination of numeric and descriptive approaches (e.g., Effect Range Median; USEPA, 1992c). Numeric methods can be used to derive chemical-specific equilibrium partitioning sediment guidelines (ESGs) or other sediment quality guidelines (SQGs). Descriptive methods such as toxicity tests with field-collected sediment cannot be used alone to develop numerical ESGs or other SQGs for individual chemicals. Although each approach can be used to make site-specific decisions, no one single approach can adequately address sediment quality. Overall, an integration of several methods using the weight of evidence is the most desirable approach for assessing the effects of contaminants associated with sediment (Long and Morgan, 1990; MacDonald et al., 1996; Ingersoll et al., 1996; 1997). Hazard evaluations integrating data from laboratory exposures, chemical analyses, and benthic community assessments provide strong complementary evidence of the degree of pollution-induced degradation in aquatic communities (Chapman et al., 1992; Chapman et al., 1997; Burton, 1991).

1.2 Program Applicability

1.2.1 The USEPA has authority under a variety of statutes to manage contaminated sediments (Table 1.2 and USEPA, 1990e). USEPA's Contaminated Sediment Management Strategy (USEPA, 1998) establishes the following four goals for contaminated sediments and describes actions that the Agency intends to take to accomplish these goals: (1) to prevent further contamination of sediments that may cause unacceptable ecological or human health risks; (2) when practical, to clean up existing sediment contamination that adversely affects the Nation's waterbodies or their uses, or that causes other significant effects on human health or the environment; (3) to ensure that sediment dredging and the disposal of dredged material continue to be managed in an environmentally sound manner; and (4) to develop and consistently apply methodologies for analyzing contaminated sediments. The Agency plans to employ its pollution prevention and source control programs to address the first goal. To accomplish the second goal, USEPA will consider a range of risk management alternatives to reduce the volume and effects of existing contaminated sediments, including *in-situ* containment and contaminated

Table 1.1 Sediment Quality Assessment Procedures[1]

Method	Type			Approach
	Numeric	Descriptive	Combination	
Equilibrium Partitioning	*			A sediment quality value for a given contaminant is determined by calculating the sediment concentration of the contaminant that corresponds to an interstitial water concentration equivalent to the USEPA water-quality criterion for the chemical.
Tissue Residues	*			Safe sediment concentrations of specific chemicals are established by determining the sediment chemical concentration that results in acceptable tissue residues.
Interstitial Water Toxicity	*	*	*	Toxicity of interstitial water is quantified and identification evaluation procedures are applied to identify and quantify chemical components responsible for sediment toxicity.
Benthic Community Structure		*		Environmental degradation is measured by evaluating alterations in benthic community structure.
Whole-sediment Toxicity and Sediment Spiking	*	*	*	Test organisms are exposed to sediments that may contain known or unknown quantities of potentially toxic chemicals. At the end of a specified time period, the response of the test organisms is examined in relation to a specified endpoint. Dose-response relationships can be established by exposing test organisms to sediments that have been spiked with known amounts of chemicals or mixtures of chemicals.
Sediment Quality Triad	*	*	*	Sediment chemical contamination, sediment toxicity, and benthic community structure are measured on the same sediment sample. Correspondence between sediment chemistry, toxicity, and field effects is used to determine sediment concentrations that discriminate conditions of minimal, uncertain, and major biological effects.
Sediment Quality Guidelines	*	*	*	The sediment concentration of contaminanants associated with toxic responses measured in laboratory exposures or in field assessments (i.e., Apparent Effect Threshold (AET), Effect Range Median (ERM), Probable Effect Level (PEL)).

[1] Modified from USEPA (1992c)

sediment removal. Finally, the Agency is developing tools for use in pollution prevention, source control, remediation, and dredged material management to meet the collective goals. These tools include national inventories of sediment quality and environmental releases of contaminants, numerical assessment guidelines to evaluate contaminant concentrations, and standardized bioassays to evaluate the bioaccumulation and toxicity potential of sediment samples.

1.2.2 The Clean Water Act (CWA) is the single most important law dealing with environmental quality of surface waters in the United States. The objective of the CWA is to restore and maintain the chemical, physical, and biological integrity of the Nation's waters (CWA, Section 101). Federal and state monitoring programs traditionally have focused on evaluating water column problems caused by point source dischargers. Findings in the National Sediment Quality Survey, Volume I of the first biennial report to Congress on sediment quality in the U.S., indicate that this focus needs to be expanded to include sediment quality impacts (Section 1.1.2 and USEPA, 1997a).

1.2.3 The Office of Water (OW), the Office of Prevention, Pesticides, and Toxic Substances (OPPTS), the Office of Solid Waste (OSW), and the Office of Emergency and Remedial Response (OERR) are all committed to the principle of consistent tiered testing described in the Contaminated Sediment Management Strategy (USEPA, 1998). Agency-wide consistent testing is desirable because all USEPA programs will use standard methods to evaluate health risk and produce comparable data. It will also provide the basis for uniform cross-program decision-making within the USEPA. Each program will, however, retain the flexibility of deciding whether identified risks would trigger regulatory actions.

1.2.4 Tiered testing refers to a structured, hierarchical procedure for determining data needs relative to decision-making that consists of a series of tiers, or levels, of investigative intensity. Typically, increasing tiers in a tiered testing framework involve increased information and decreased uncertainty (USEPA, 1998). Each EPA program office intends to develop guidance for interpreting the tests conducted within the tiered framework and to explain how information within each tier would trigger

Table 1.2 Statutory Needs for Sediment Quality Assessment[1]

Law[2]	Area of Need
CERCLA	• Assessment of need for remedial action with contaminated sediments; assessment of degree of cleanup required, disposition of sediments
CWA	• National Pollutant Discharge Elimination System (NPDES) permitting, especially under Best Available Technology (BAT) in water-quality-limited water • Section 403(c) criteria for ocean discharges; mandatory additional requirements to protect marine environment • Section 301(g) waivers for publicly owned treatment works (POTWs) discharging to marine waters • Section 404 permits for dredge and fill activities (administered by the U.S. Army Corps of Engineers [USACE])
FIFRA	• Reviews of uses for new and existing chemicals • Pesticide labeling and registration
MPRSA	• Permits for ocean dumping
NEPA	• Preparation of environmental impact statements for projects with surface water discharges
TSCA	• Section 5: Premanufacture notification reviews for new industrial chemicals • Sections 4, 6, and 8: Reviews for existing industrial chemicals
RCRA	• Assessment of suitability (and permitting of) on-land disposal or beneficial use of contaminated sediments considered "hazardous"

[1] Modified from Dickson et al., 1987 and Southerland et al., 1992.

[2] CERCLA Comprehensive Environmental Response, Compensation and Liability Act (Superfund).
CWA Clean Water Act.
FIFRA Federal Insecticide, Fungicide, and Rodenticide Act.
MPRSA Marine Protection, Resources and Sanctuary Act.
NEPA National Environmental Policy Act.
TSCA Toxic Substances Control Act.
RCRA Resource Conservation and Recovery Act.

regulatory action. Depending on statutory and regulatory requirements, the program specific guidance will describe decisions based on a weight of evidence approach, a pass-fail approach, or comparison to a reference site. The following two approaches are currently being used by USEPA: (1) the Office of Water-U.S. Army Corps of Engineers dredged material testing framework and (2) the OPPTS ecological risk assessment tiered testing framework. USEPA-USACE (1998a) describes the dredged material testing framework and Smrchek and Zeeman (1998) summarizes the OPPTS testing framework. A tiered testing framework has not yet been chosen for Agency-wide use, but some of the components have been identified to be standardized. These components include toxicity tests, bioaccumulation tests, sediment quality guidelines, and other measurements that may have ecological significance, including benthic community structure evaluation, colonization rate, and *in situ* sediment testing within a mesocosm (USEPA, 1992a).

1.3 Scope and Application

1.3.1 A variety of standard methods have been previously developed for assessing the toxicity of chemicals in sediments using amphipods, midges, polychaetes, oligochaetes, mayflies, or cladocerans (USEPA, 1994a; USEPA, 1994b; ASTM, 1999a; ASTM, 1999b; ASTM; 1999c; ASTM, 1999d; Environment Canada, 1997a; Environment Canada, 1997b). Several endpoints are suggested in these methods to measure effects of chemicals in sediment including survival, growth, behavior, or reproduction; however, survival of test organisms in 10-d exposures is the endpoint most commonly reported. These short-term exposures which only measure effects on survival can be used to identify high levels of contamination, but might not be able to identify moderate levels of contamination in sediments (Benoit et al., 1997; Ingersoll et al., 1998; Sibley et al., 1996; Sibley et al., 1997a; Sibley et al., 1997b; Sibley et al., 1998).

1.3.2 Procedures described in Sections 11 and 12 for conducting 10-d sediment toxicity tests with the amphipod *H. azteca* (measuring survival) and the midge *C. tentans* (measuring survival and growth) were described in the first edition of the manual (USEPA, 1994a). Section 14 of this second edition of the manual now describes a method for determining potential sublethal effects of contaminants associated with sediment on *H. azteca,* including effects on reproduction based on a procedure described by Ingersoll et al. (1998). Section 15 of this second edition of the manual now describes a method for determining sublethal endpoints in sediment tests based on a life-cycle test with *C. tentans* described by Benoit et al. (1997), Sibley et al. (1996), and Sibley et al. (1997a). Procedures are primarily described for testing freshwater sediments; however, estuarine sediments (up to 15‰ salinity) can also be tested in 10-d sediment tests with *H. azteca*.

1.3.2.1 The decision to conduct 10-d or long-term toxicity tests with *H. azteca* or *C. tentans* depends on the goal of the assessment. In some instances, sufficient information may be gained by measuring sublethal endpoints in 10-d tests. In other instances, the 10-d tests could be used to screen samples for toxicity before long-term tests are conducted. While the long-term tests are needed to determine direct effects on reproduction, measurement of growth in these toxicity tests may serve as an indirect estimate of reproductive effects of chemicals associated with sediments (Section 14.4.5 and 15.4.6.2). Additional studies are ongoing to more thoroughly evaluate the relative sensitivity between lethal and sublethal endpoints measured in 10-d tests and between sublethal endpoints measured in the long-term tests. Results of these studies and additional applications of the methods described in Sections 14 and 15 will provide data that can be used to assist in determining where application of long-term tests will be most appropriate.

1.3.2.2 Use of sublethal endpoints for assessment of contaminant risk is not unique to toxicity testing with sediments. Numerous regulatory programs require the use of sublethal endpoints in the decision-making process (Pittinger and Adams, 1997) including: (1) Water Quality Criteria (and State Standards); (2) National Pollution Discharge Elimination System (NPDES) effluent monitoring (including chemical-specific limits and sublethal endpoints in toxicity tests); (3) Federal Insecticide, Rodenticide and Fungicide Act (FIFRA) and the Toxic Substances Control Act (TSCA; tiered assessment includes several sublethal endpoints with fish and aquatic invertebrates); (4) Superfund Comprehensive Environmental Response, Compensation and Liability Act (CERCLA); (5) Organization of Economic Cooperation and Development (OECD; sublethal toxicity testing with fish and invertebrates); (6) European Economic Community (EC; sublethal toxicity testing with fish and invertebrates); and (7) the Paris Commission (behavioral endpoints).

1.3.3 Guidance for conducting 28-d bioaccumulation tests with the oligochaete *Lumbriculus variegatus* is also provided in this manual (Section 13). Overlying water is renewed daily and organisms are not fed during bioaccumulation tests. Methods are also described for determining bioaccumulation kinetics of different classes of compounds during 28-d exposures with *L. variegatus.*

1.3.4 Additional research and methods development are now in progress to (1) refine sediment Toxicity Identification Evaluation (TIE) procedures (Ankley and Thomas, 1992), (2) refine sediment spiking procedures, (3) develop *in situ* toxicity tests to assess sediment toxicity and bioaccumulation under field conditions, (4) evaluate relative sensitivity of endpoints measured in toxicity tests, (5) develop methods for additional species, (6) evaluate relationships between toxicity and bioaccumulation, and (7) produce additional data on confirmation of responses in laboratory tests with natural populations of benthic organisms. This information will be described in future editions of this manual or other USEPA manuals.

1.3.4.1 This methods manual serves as a companion to the marine sediment testing method manuals (USEPA, 1994b; USEPA, 1999).

1.3.5 Procedures described in this manual are based on the following documents: ASTM (1999a), ASTM (1999b), ASTM (1999c), ASTM (1999d), Ankley et al. (1993), Phipps et al. (1993), Call et al. (1994), USEPA (1991a), USEPA (1994a), USEPA (1994b), Ingersoll et al. (1995), Ingersoll et al. (1998), Sibley et al. (1996), Sibley et al. (1997a), Sibley et al. (1997b), and Benoit et al. (1997). This manual outlines specific test methods for evaluating the toxicity of sediments in 10-d exposures with *H. azteca* and *C. tentans.* The manual also outlines general guidance on procedures for evaluating the effects of sediment contaminants in long-term exposures with *H. azteca* and *C. tentans* and bioaccumulation of contaminants in sediment with *L. variegatus.* Some issues that may be considered in interpretation of test results are the subject of continuing research, including the influence of feeding on bioavailability, nutritional requirements of the test organisms, additional performance criteria for organism health, and confirmation of responses in laboratory tests with natural benthic populations. As additional research is completed on these and other test species, the results will be incorporated into future editions of this manual. See Section 4 for additional details.

1.3.6 General procedures described in this manual might be useful for conducting tests with other aquatic organisms; however, modifications may be necessary. Altering the procedures described in this manual may alter bioavailability and produce results that are not directly comparable with results of acceptable procedures. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms (e.g., *Diporeia* spp., *Tubifex tubifex, Hexagenia* spp.). If tests are conducted with procedures different from those described in this manual, additional tests are required to determine comparability of results.

1.3.6.1 Methods have been described for culturing and testing indigenous species that may be as sensitive or more sensitive than the species recommended in this manual. However, the USEPA currently allows the use of indigenous species only where state regulations require their use or prohibit importation of the recommended species. Where state regulations prohibit importation or use of the recommended test species, permission should be requested from the appropriate regulatory agency before using indigenous species.

1.3.6.2 Where states have developed culturing and testing methods for indigenous species other than those recommended in this manual, data comparing the sensitivity of the substitute species and one or more of the recommended species must be obtained with sediments or reference toxicants to ensure that the species selected are at least as sensitive and appropriate as the recommended species.

1.3.7 Selection of Test Organisms

1.3.7.1 The choice of a test organism has a major influence on the relevance, success, and interpretation of a test. Test organism selection should be based on both environmental relevance and practical concerns (DeWitt et al., 1989; Swartz, 1989). Ideally, a test organism should (1) have a toxicological database demonstrating relative sensitivity and discrimination to a range of chemicals of concern in sediment; (2) have a database for interlaboratory comparisons of procedures (e.g., round-robin studies); (3) be in contact with sediment (e.g., water column vs. benthic organism); (4) be readily available through culture or from field collection; (5) be easily maintained in the laboratory; (6) be easily identified; (7) be ecologically or economically important; (8) have a broad geographical distribution, be indigenous (either present or historical) to the site being evaluated, or have a niche similar to organisms of concern (e.g., similar feeding guild or behavior to the indigenous organisms); (9) be tolerant of a broad range of sediment physico-chemical characteristics (e.g., grain size); and (10) be compatible with selected exposure methods and endpoints (Table 1.3, ASTM, 1998d). The method should also be (11) peer reviewed (e.g., journal articles, ASTM guides) and (12) confirmed with responses with natural populations of benthic organisms (Sections 1.3.7.9 and 1.3.8.5).

1.3.7.2 Of these criteria (Table 1.3), a database demonstrating relative sensitivity to chemicals, contact with sediment, ease of culture in the laboratory, interlaboratory comparisons, tolerance to varying sediment physicochemical characteristics, and confirmation with responses of natural benthic populations were the primary criteria used for selecting *H. azteca, C. tentans,* and *L. variegatus* for the current edition of this manual. Many organisms that might be appropriate for sediment testing do not now meet these selection criteria because historically little emphasis has been placed on developing standardized testing procedures for benthic organisms. A similar database must be developed in order for other organisms to be included in future editions of this manual (e.g., mayflies [*Hexagenia* spp.], other midges [*C. riparius*], other amphipods [*Diporeia* spp.], cladocerans [*Daphnia magna, Ceriodaphnia dubia*], or mollusks).

1.3.7.3 An important consideration in the selection of specific species for test method development is the existence of information concerning relative sensitivity of the organisms both to single chemicals and complex mixtures. A number of studies have evaluated the sensitivity of *H. azteca, C. tentans* and *L. variegatus,* relative to one another, as well as other commonly tested freshwater species. For example, Ankley et al. (1991b) found *H. azteca* to be as, or slightly more, sensitive than *Ceriodaphnia dubia* to a variety of sediment elutriate and pore-water samples. In that study, *L. variegatus* were less sensitive to the samples than either the amphipod or the cladoceran. West et al. (1993) found the rank sensitivity of the three species to the lethal effects of copper in sediments could be ranked (from greatest to least): *H. azteca* > *C. tentans* > *L. variegatus*. In short-term (48 to 96 h) exposures, *L. variegatus* generally was less sensitive than *H. azteca, C. dubia,* or *Pimephales promelas* to cadmium, nickel, zinc, copper, and lead (Schubauer-Berigan et al., 1993). Of the latter three species, no one was consistently the most sensitive to all five metals.

1.3.7.3.1 In a study of Great Lakes sediment, *H. azteca, C. tentans,* and *C. riparius* were among the most sensitive and discriminatory of 24 organisms tested (Burton and Ingersoll, 1994; Burton et al., 1996a; Ingersoll et al., 1993). Kemble et al. (1994) found the rank sensitivity of four species to metal-contaminated sediments to be (from greatest to least): *H. azteca* > *C. riparius* > *Oncorhynchus mykiss* (rainbow trout) > *Daphnia magna.* The relative sensitivity of the three endpoints evaluated in the *H. azteca* test with Clark Fork River sediments was (from greatest to least): length > sexual maturation > survival.

1.3.7.3.2 In 10-d water-only and whole-sediment tests, *H. azteca* and *C. tentans* were more sensitive than *D. magna* to fluoranthene (Suedel et al., 1993).

1.3.7.3.3 Water-only tests also have been conducted for 10 d with a number of chemicals using the three species described in this manual (Phipps et al., 1995; Table 1.4). All tests were flow-through exposures using a soft natural water (Lake Superior) with measured chemical concentrations that, other than the absence of sediment, were conducted under conditions (e.g., temperature, photoperiod, feeding) similar to those being described for the standard 10-d sediment test. In general, *H. azteca* was more sensitive to copper, zinc, cadmium, nickel and lead than either *C. tentans* or *L. variegatus*. *Chironomus tentans* and *H. azteca* exhibited a similar sensitivity to several of the pesticides tested. *Lumbriculus variegatus* was not tested with several of the pesticides; however, in other studies with whole sediments contaminated by DDT and associated metabolites, and in short-term (96-h) experiments with organophosphate insecticides (diazinon, chlorpyrifos), *L. variegatus* has proven to be far less sensitive than either *H. azteca* or *C. tentans*. These results highlight two important points germane to the methods in this manual. First, neither of the two test species selected for estimating sediment toxicity *(H. azteca, C. tentans)* was consistently more sensitive to all chemicals, indicating the importance of using multiple test organisms when performing sediment assessments. Second, *L. variegatus* appears to be relatively insensitive to most of the test chemicals, which perhaps is a positive attribute for an organism used in bioaccumulation tests.

1.3.7.3.4 Using the data from Table 1.4, sensitivity of *H. azteca, C. tentans* and *L. variegatus* can be evaluated relative to other freshwater species. For this analysis, acute and chronic toxicity data from water quality criteria (WQC) documents for copper, zinc, cadmium, nickel, lead, DDT, dieldrin and chlorpyrifos, and toxicity information from the AQUIRE database (AQUIRE, 1992) for DDD and DDE, were compared to assay results for the three species (Phipps et al., 1995). The sensitivity of *H. azteca*

Table 1.3 Rating of Selection Criteria for Freshwater Sediment Toxicity Testing Organisms[1]

Criterion	*Hyalella azteca*	*Diporeia* spp.	*Chironomus tentans*	*Chironomus riparius*	*Lumbriculus variegatus*	*Tubifex tubifex*	*Hexagenia* spp.	Mollusks	*Daphnia* spp. and *Ceriodaphnia* spp.
Relative sensitivity toxicity database	+	-	+	-	+	-	-	-	-
Round-robin studies conducted	+	-	+	-	-	-	-	-	-
Contact with sediment	+	+	+	+	+	+	+	+	-
Laboratory culture	+	-	+	+	+	+	-	-	+
Taxonomic identification	+/-	+/-	+/-	+/-	+	+	+	+	+
Ecological importance	+	+	+	+	+	+	+	+	+
Geographical distribution	+	+/-	+	+	+	+	+	+	+/-
Sediment physico-chemical tolerance	+	+	+/-	+	+	+	-	+	NA
Response confirmed with benthic populations	+	+	+	+	+	+	+	-	+
Peer reviewed	+	+	+	+	+	+	+	-	+/-
Endpoints[2] monitored	S, G, M, R	S, B, A	S, G, E, R	S, G, E	B, S, R	S, R	S, G	B	S, G, R

[1] A "+" or "-" rating indicates a positive or negative attribute
[2] S = Survival, G = Growth, B = Bioaccumulation, A = Avoidance, R = Reproduction, M = Maturation, E = Emergence, NA = not applicable

Table 1.4 Water-only, 10-d LC50 (µg/L) Values for *Hyalella azteca, Chironomus tentans,* and *Lumbriculus variegatus* [1]

Chemical	*H. azteca*	*C. tentans*	*L. variegatus*
Copper	35	54	35
Zinc	73	1,125[2]	2,984
Cadmium	2.8[3]	NT[4]	158
Nickel	780	NT	12,160
Lead	<16	NT	794
p,p′-DDT	0.07	1.23	NT
p,p′-DDD	0.17	0.18	NT
p,p′-DDE	1.39	3.0	>3.3
Dieldrin	7.6	1.1	NT
Chlorpyrifos	0.086	0.07	NT

[1] Chemicals tested at ERL-Duluth in soft water—hardness 45 mg/L as $CaCO_3$ at pH 7.8 to 8.2 (Phipps et al., 1995).
[2] 50% mortality at highest concentration tested.
[3] 70% mortality at lowest concentration tested.
[4] NT = not tested.

to metals and pesticides, and *C. tentans* to pesticides was comparable to chronic toxicity data generated for other test species. This was not completely unexpected given that the 10-d exposures used for these two species are likely more similar to chronic partial life-cycle tests than the 48- to 96-h exposures traditionally defined as acute in WQC documents. Interestingly, in some instances (e.g., dieldrin, chlorpyrifos), LC50 data generated for *H. azteca* or *C. tentans* were comparable to or lower than any reported for other freshwater species in the WQC documents. This observation likely is a function not only of the test species, but of the test conditions; many of the tests on which early WQC were based were static, rather than flow-through, and utilized unmeasured contaminant concentrations.

1.3.7.4 Relative species sensitivity frequently varies among chemicals; consequently, a battery of tests including organisms representing different trophic levels may be needed to assess sediment quality (Craig, 1984;

Williams et al., 1986a; Long et al., 1990; Ingersoll et al., 1990; Burton and Ingersoll, 1994; Burton et al., 1996a; USEPA, 1989c). For example, Reish (1988) reported the relative toxicity of six metals (As, Cd, Cr, Cu, Hg, and Zn) to crustaceans, polychaetes, pelecypods, and fishes and concluded that no single species or group of test organisms was the most sensitive to all of the metals.

1.3.7.5 Measurable concentrations of ammonia are common in the pore water of many sediments and have been found to be a common cause of toxicity in pore water (Jones and Lee, 1988; Ankley et al., 1990; Schubauer-Berigan and Ankley, 1991). Acute toxicity of ammonia to *H. azteca*, *C. tentans*, and *L. variegatus* has been evaluated in several studies. As has been found for many other aquatic organisms, the toxicity of ammonia to *C. tentans* and *L. variegatus* has been shown to be dependent on pH. Four-day LC50 values for *L. variegatus* in water-column (no sediment) exposures ranged from 6.6 to 390 mg/L total ammonia as pH was increased from 6.3 to 8.6 (Schubauer-Berigan et al., 1995). For *C. tentans*, 4-d LC50 values ranged from 82 to 370 mg/L total ammonia over a similar pH range (Schubauer-Berigan et al., 1995). Ankley et al. (1995) reported that the toxicity of ammonia to *H. azteca* (also in water-only exposures) showed differing degrees of pH-dependence in different test waters. Toxicity was not pH dependent in soft reconstituted water, with 4-d LC50 values of about 20 mg/L at pH ranging from 6.5 to 8.5. In contrast, ammonia toxicity in hard reconstituted water exhibited substantial pH dependence with LC50 values decreasing from >200 to 35 mg/L total ammonia over the same pH range. Borgmann and Borgmann (1997) later showed that the variation in ammonia toxicity across these waters could be attributed to differences in sodium and potassium content, which appear to influence the toxicity of ammonia to *H. azteca*.

1.3.7.5.1 Although these studies provide benchmark concentrations that may be of concern in sediment pore waters, additional studies by Whiteman et al. (1996) indicated that the relationship between water-only LC50 values and those measured in sediment exposures differs among organisms. In sediment exposures, the 10-d LC50 for *L. variegatus* and *C. tentans* occurred when sediment pore water reached about 150% of the LC50 determined from water-only exposures. However, experiments with *H. azteca* showed that the 10-d LC50 was not reached until pore water concentrations were nearly 10 times the water-only LC50, at which time the ammonia concentration in the overlying water was equal to the water-only LC50. The authors attribute this discrepancy to avoidance of sediment by *H. azteca*. Thus, although it appears that water-only LC50 values may provide suitable screening values for potential ammonia toxicity, higher concentrations may be necessary to actually induce ammonia toxicity in sediment exposures, particularly for *H. azteca*. Further, these data underscore the importance of measuring the pH of pore water when ammonia toxicity may be of concern. Ankley and Schubauer-Berigan (1995) and Besser et al. (1998) describe procedures for conducting toxicity identification evaluations (TIEs) for pore-water or whole-sediment samples to determine whether ammonia is contributing to the toxicity of sediment samples.

1.3.7.6 Sensitivity of a species to chemicals is also dependant on the duration of the exposure and the endpoints evaluated. Sections 14.4 and 15.4 describe results of studies which demonstrate the utility of measuring sublethal endpoints in sediment toxicity tests with *H. azteca* and *C. tentans.*

1.3.7.7 The sensitivity of an organism to chemicals should be balanced with the concept of discrimination (Burton and Ingersoll, 1994; Burton et al., 1996). The response of a test organism should provide discrimination between different levels of contamination.

1.3.7.8 The sensitivity of an organism is related to the route of exposure and biochemical response to chemicals. Sediment-dwelling organisms can receive exposure from three primary sources: interstitial water, sediment particles, and overlying water. Food type, feeding rate, assimilation efficiency, and clearance rate will control the dose of chemicals from sediment. Benthic invertebrates often selectively consume different particle sizes (Harkey et al., 1994) or particles with higher organic carbon concentrations, which may have higher chemical concentrations. Grazers and other collector-gatherers that feed on aufwuchs, or surface films, and detritus may receive most of their body burden directly from materials attached to sediment or from actual sediment ingestion. In amphipods (Landrum, 1989) and clams (Boese et al., 1990), uptake through the gut can exceed uptake across the gills of certain hydrophobic compounds. Organisms in direct contact with sediment may also accumulate chemicals by direct adsorption to the body wall or by absorption through the integument (Knezovich et al., 1987).

1.3.7.9 Despite the potential complexities in estimating the dose that an animal receives from sediment, the toxicity and bioaccumulation of many chemicals in sediment such as Kepone®, fluoranthene, organochlorines, and metals have been correlated with either the concentration of these chemicals in interstitial water or, in the case of nonionic organic chemicals, in sediment on an organic-carbon normalized basis (Di Toro et al., 1990; Di Toro et al., 1991). The relative importance of whole sediment and interstitial water routes of exposure depends on the test organism and the specific chemical (Knezovich et al., 1987). Because benthic communities contain a diversity of organisms, many combinations of exposure routes can be important. Therefore, behavior and feeding habits of a test organism can influence its ability to accumulate chemicals from sediment and should be considered when selecting test organisms for sediment testing.

1.3.7.10 The response of *H. azteca* and *C. tentans* in laboratory toxicity studies has been compared with the response of natural benthic populations.

1.3.7.10.1 Chironomids were not found in sediment samples that decreased growth of *C. tentans* by 30% or

more in 10-d laboratory toxicity tests (Giesy et al., 1988). Wentsel et al. (1977a, 1977b, 1978) reported a correlation between responses of *C. tentans* in laboratory tests and the abundance of *C. tentans* in metal-contaminated sediments.

1.3.7.10.2 Canfield et al. (1994, 1996, 1998) evaluated the composition of benthic invertebrate communities in sediments for the following areas: (1) three Great Lakes Areas of Concern (AOC; Buffalo River, NY; Indiana Harbor, IN; Saginaw River, MI), (2) the upper Mississippi River, and (3) the Clark Fork River located in Montana. Results of these benthic community assessments were compared to sediment chemistry and toxicity (28-d sediment exposures with *H. azteca* which monitored effects on survival, growth, and sexual maturation). Good concordance was evident between measures of laboratory toxicity, sediment contamination, and benthic invertebrate community composition in extremely contaminated samples. However, in moderately contaminated samples, less concordance was observed between the composition of the benthic community and either laboratory toxicity test results or sediment contaminant concentration. Laboratory sediment toxicity tests better identified chemical contamination in sediments compared to many of the commonly used measures of benthic invertebrate community composition. Benthic measures may reflect other factors such as habitat alteration in addition to responding to contaminants. Canfield et al. (1994, 1996, 1998) identified the need to better evaluate noncontaminant factors (i.e., TOC, grain size, water depth, habitat alteration) in order to better interpret the response of benthic invertebrates to sediment contamination.

1.3.7.10.3 The results from laboratory sediment toxicity tests were compared to colonization of artificial substrates exposed *in situ* to Great Lakes sediment (Burton and Ingersoll, 1994; Burton et al., 1996a). Survival or growth of *H. azteca* and *C. tentans* in 10- to 28-d laboratory exposures were negatively correlated to percent chironomids and percent tolerant taxa colonizing artificial substrates in the field. Schlekat et al. (1994) reported generally good agreement between sediment tests with *H. azteca* and benthic community responses in the Anacostia River, Washington, D.C.

1.3.7.10.4 Sediment toxicity to amphipods in 10-d toxicity tests, field contamination, and field abundance of benthic amphipods were examined along a sediment contamination gradient of DDT (Swartz et al., 1994). Survival of *Eohaustorius estuarius, Rhepoxynius abronius,* and *H. azteca* in laboratory toxicity tests was positively correlated to abundance of amphipods in the field and, along with the survival of *H. azteca*, was negatively correlated to DDT concentrations. The threshold for 10-d sediment toxicity in laboratory studies was about 300 µg DDT (+metabolites)/g organic carbon. The threshold for abundance of amphipods in the field was about 100 µg DDT (+metabolites)/g organic carbon. Therefore, correlations between toxicity, contamination, and field populations indicate that short-term sediment toxicity tests can provide reliable evidence of biologically adverse sediment contamination in the field, but may be underprotective of sublethal effects.

1.3.8 Selection of Organisms for Sediment Bioaccumulation Testing

1.3.8.1 Several studies have demonstrated that hydrophobic organic compounds are bioaccumulated from sediment by freshwater infaunal organisms, including larval insects *(C. tentans,* Adams et al., 1985; Adams, 1987; *Hexagenia limbata,* Gobas et al., 1989), oligochaetes *(Tubifex tubifex* and *Limnodrilus hoffmeisteri,* Oliver, 1984; Oliver, 1987; Connell et al., 1988), and by marine organisms (polychaetes, *Nephtys incisa;* mollusks, *Mercenaria mercenaria, Yoldia limatula;* Lake et al., 1990). Consumers of these benthic organisms may bioaccumulate or biomagnify chemicals. Therefore, in addition to sediment toxicity, it may be important to examine the uptake of chemicals by aquatic organisms from contaminated sediments.

1.3.8.2 Various species of organisms have been suggested for use in studies of chemical bioaccumulation from aquatic sediments. Several criteria should be considered before a species is adopted for routine use in these types of studies (Ankley et al., 1992a; Call et al., 1994). These criteria include (1) availability of organisms throughout the year, (2) known chemical exposure history, (3) adequate tissue mass for chemical analyses, (4) ease of handling, (5) tolerance of a wide range of sediment physico-chemical characteristics (e.g., particle size), (6) low sensitivity to chemicals associated with sediment (e.g., metals, organics), (7) amenability to long-term exposures without adding food, (8) and ability to accurately reflect concentrations of chemicals in field-exposed organisms (e.g., exposure is realistic). With these criteria in mind, the advantages and disadvantages of several potential freshwater taxa for bioaccumulation testing are discussed below.

1.3.8.3 Freshwater clams provide an adequate tissue mass, are easily handled, and can be used in long-term exposures. However, few non-exotic freshwater species are available for testing. Exposure of clams is uncertain because of valve closure. Furthermore, clams are filter feeders and may accumulate lower concentrations of chemicals compared with detritivores (Lake et al., 1990). Chironomids can be readily cultured, are easy to handle, and reflect appropriate routes of exposure. However, their rapid life cycle makes it difficult to perform long-term exposures with hydrophobic compounds; also, chironomids can readily biotransform organic compounds such as benzo[a]pyrene (Harkey et al., 1994). Larval mayflies reflect appropriate routes of exposure, have adequate tissue mass for residue analysis, and can be used in long-term tests. However, mayflies cannot be continuously cultured in the laboratory and consequently are not always available for testing. Furthermore, the background concentrations of chemicals and health of field-collected individuals may be uncertain. Amphipods (e.g., *H. azteca)* can be cultured in the laboratory, are easy to handle, and reflect appropriate routes of exposure. However, their size

may be insufficient for residue analysis and *H. azteca* are sensitive to chemicals in sediment. Fish (e.g., fathead minnows) provide an adequate tissue mass, are readily available, are easy to handle, and can be used in long-term exposures. However, the route of exposure is not appropriate for evaluating the bioavailability of sediment-associated chemicals to benthic organisms.

1.3.8.4 Oligochaetes are infaunal benthic organisms that meet many of the test criteria listed above. Certain oligochaete species are easily handled and cultured, provide reasonable biomass for residue analyses, and are tolerant of varying sediment physical and chemical characteristics. Oligochaetes are exposed to chemicals via all appropriate routes of exposure, including pore water and ingestion of sediment particles. Oligochaetes need not be fed during long-term bioaccumulation exposures (Phipps et al., 1993). Various oligochaete species have been used in toxicity and bioaccumulation evaluations (Chapman et al., 1982a, Chapman et al., 1982b; Wiederholm, 1987; Kielty et al., 1988a; Kielty et al., 1988b; Phipps et al., 1993), and field populations have been used as indicators of the pollution of aquatic sediments (Brinkhurst, 1980; Spencer, 1980; Oliver, 1984; Lauritsen, 1985; Robbins et al., 1989; Ankley et al., 1992b; Brunson et al., 1993; Brunson et al., 1998). An additional desirable characteristic of *Lumbriculus variegatus* in bioaccumulation tests is that this species does not biotransform PAHs (Harkey et al., 1994).

1.3.8.5 The response of *L. variegatus* in laboratory bioaccumulation studies has been confirmed with natural populations of oligochaetes.

1.3.8.5.1 Total PCB concentrations in laboratory-exposed *L. variegatus* were similar to concentrations measured in field-collected oligochaetes from the same sites (Ankley et al., 1992b). PCB homologue patterns also were similar between laboratory-exposed and field-collected oligochaetes. The more highly chlorinated PCBs tended to have greater bioaccumulation in the field-collected organisms. In contrast, total PCBs in laboratory-exposed *(Pimephales promelas)* and field-collected *(Ictalurus melas)* fish revealed poor agreement in bioaccumulation relative to the sediment concentrations at the same sites.

1.3.8.5.2 Chemical concentrations measured in *L. variegatus* after 28-d exposures to sediment in the laboratory were compared to chemical concentrations in field-collected oligochaetes from the 13 pools of the upper Mississippi River where these sediments were collected (Brunson et al., 1998). Chemical concentrations were relatively low in sediments and tissues from the pools evaluated. Only polycyclic aromatic hydrocarbons (PAHs) and total polychlorinated biphenyls (PCBs) were frequently measured above detection limits. A positive correlation was observed between lipid-normalized concentrations of PAHs detected in laboratory-exposed *L. variegatus* and field-collected oligochaetes across all sampling locations. Rank correlations for concentrations of individual compounds between laboratory-exposed and field-collected oligochaetes were strongest for benzo(e)pyrene, perylene, benzo(b,k)-fluoranthene, and pyrene (Spearman rank correlations > 0.69). About 90% of the paired PAH concentrations in laboratory-exposed and field-collected oligochaetes were within a factor of three of one another indicating laboratory results could be extrapolated to the field with a reasonable degree of certainty.

1.4 Performance-based Criteria

1.4.1 USEPA's Environmental Monitoring Management Council (EMMC) recommended the use of performance-based methods in developing chemical analytical standards (Williams, 1993). Performance-based methods were defined by EMMC as a monitoring approach that permits the use of appropriate methods that meet pre-established demonstrated performance standards (Section 9.2).

1.4.2 The USEPA Office of Water's Office of Science and Technology and Office of Research and Development held a workshop on September 16-18, 1992 in Washington, DC to provide an opportunity for experts in the field of sediment toxicology and staff from USEPA's Regional and Headquarters program offices to discuss the development of standard freshwater and marine sediment testing procedures (USEPA, 1992a; USEPA, 1994a). Workgroup participants reached a consensus on several culturing and testing methods. In developing guidance for culturing freshwater test organisms to be included in the USEPA methods manual for sediment tests, it was agreed that no single method should be required to culture organisms. However, the consensus at the workshop was that since the success of a test depends on the health of the cultures, having healthy test organisms of known quality and age for testing was the key consideration. A performance-based criteria approach was selected as the preferred method through which individual laboratories should evaluate culture methods rather than by control-based criteria. This method was chosen to allow each laboratory to optimize culture methods and minimize effects of test organism health on the reliability and comparability of test results. See Tables 11.3, 12.3, 13.4, 14.3, and 15.3 for a listing of performance criteria for culturing and testing.

Section 2
Summary of Method

2.1 Method Description and Experimental Design

2.1.1 Method Description

2.1.1.1 This manual describes procedures for testing freshwater organisms in the laboratory to evaluate the potential toxicity or bioaccumulation of chemicals associated with whole sediments. Sediments may be collected from the field or spiked with compounds in the laboratory. Toxicity methods are outlined for two organisms, the amphipod *Hyalella azteca* and the midge *Chironomus tentans*. Methods are described for conducting 10-d toxicity tests with amphipods (Section 11) or midges (Section 12). Toxicity tests are conducted for 10 d in 300-mL chambers containing 100 mL of sediment and 175 mL of overlying water. Overlying water is added daily and test organisms are fed during the toxicity tests. The endpoints in the 10-d toxicity test with *H. azteca* and *C. tentans* are survival and growth. Procedures are primarily described for testing freshwater sediments; however, estuarine sediments (up to 15 ‰ salinity) can also be tested in 10-d toxicity tests with *H. azteca*.

2.1.1.2 Guidance is also described in the manual for conducting long-term sediment toxicity tests with *H. azteca* (Section 14) and *C. tentans* (Section 15). The long-term sediment exposures with *H. azteca* are started with 7- to 8-d-old amphipods. On Day 28, amphipods are isolated from the sediment and placed in water-only chambers where reproduction is measured on Day 35 and 42. Endpoints measured in the long-term amphipod test include survival (Day 28, 35, and 42), growth (Day 28 and 42), and reproduction (number of young per female produced from Day 28 to 42). The long-term sediment exposures with *C. tentans* start with newly hatched larvae (<24-h old) and continues through emergence, reproduction, and hatching of the F_1 generation (about 60-d exposures). Survival and growth are determined at 20 d. Starting on Day 23 to the end of the test, emergence and reproduction of *C. tentans* are monitored daily. The number of eggs per female is determined for each egg mass, which is incubated for 6 d to determine hatching success.

2.1.1.3 Guidance for conducting 28-d bioaccumulation tests with the oligochaete *Lumbriculus variegatus* is also provided in the manual. The overlying water is added daily and the test organisms are not fed during bioaccumulation tests. Section 13 also describes procedures for determining bioaccumulation kinetics of different classes of compounds during 28-d exposures with *L. variegatus*.

2.1.2 Experimental Design

The following section is a general summary of experimental design. See Section 16 for additional detail.

2.1.2.1 Control and Reference Sediment

2.1.2.1.1 Sediment tests include a control sediment (sometimes called a negative control). A control sediment is a sediment that is essentially free of contaminants, is used routinely to assess the acceptability of a test, and is not necessarily collected near the site of concern. Any contaminants in control sediment are thought to originate from the global spread of pollutants and do not reflect any substantial input from local or nonpoint sources (ASTM, 1999c). A control sediment provides a measure of test acceptability, evidence of test organism health, and a basis for interpreting data obtained from the test sediments. A reference sediment is typically collected near an area of concern (e.g., a disposal site) and is used to assess sediment conditions exclusive of material(s) of interest. Testing a reference sediment provides a site-specific basis for evaluating toxicity.

2.1.2.1.1.1 In general, the performance of test organisms in the negative control is used to judge the acceptability of a test, and either the negative control or reference sediment may be used to evaluate performance in the experimental treatments, depending on the purpose of the study. Any study in which organisms in the negative control do not meet performance criteria must be considered questionable because it suggests that adverse factors affected the test organisms. Key to avoiding this situation is using only control sediments that have a demonstrated record of performance using the same test procedure. This includes testing of new collections from sediment sources that have previously provided suitable control sediment.

2.1.2.1.1.2 Because of the uncertainties introduced by poor performance in the negative control, such studies should be repeated to insure accurate results. However, the scope or sampling associated with some studies may make it difficult or impossible to repeat a study. Some researchers have reported cases where performance in

the negative control is poor, but performance criteria are met in a reference sediment included in the study design. In these cases, it might be reasonable to infer that other samples that show good performance are probably not toxic; however, any samples showing poor performance should not be judged to have shown toxicity, since it is unknown whether the adverse factors that caused poor control performance might have also caused poor performance in the test treatments.

2.1.2.1.2 Natural geomorphological and physico-chemical characteristics such as sediment texture may influence the response of test organisms (DeWitt et al., 1988). The physico-chemical characteristics of test sediment must be within the tolerance limits of the test organism. Ideally, the limits of a test organism should be determined in advance; however, controls for factors such as grain size and organic carbon can be evaluated if the recommended limits are approached or exceeded in a test sediment. See Section 10.1 for information on physico-chemical requirements of test organisms. If the physico-chemical characteristics of a test sediment exceed the tolerance limits of the test organism, it may be desirable to include a control sediment that encompasses those characteristics. The effects of some sediment characteristics (e.g., grain size or total organic carbon) on sediment test results may be addressed with regression equations (DeWitt et al., 1988; Ankley et al., 1994a). The use of formulated sediment can also be used to evaluate physico-chemical characteristics of sediment on test organisms (Walsh et al., 1991; Suedel and Rodgers, 1994; Kemble et al., 1999; USEPA, 1998).

2.1.2.2 The experimental design depends on the purpose of the study. Variables that need to be considered include the number and type of control sediments, the number of treatments and replicates, and water-quality characteristics.

2.1.2.2.1 The purpose of the study might be to determine a specific endpoint such as an LC50 and may include a control sediment, a positive control, a solvent control, and several concentrations of sediment spiked with a chemical (see Section 8.3.2).

2.1.2.2.2 The purpose of the study might be to determine whether field-collected sediments are toxic, and may include controls, reference sediments, and test sediments. Controls are used to evaluate the acceptability of the test (Tables 11.3, 12.3, 13.4, 14.3, 15.3) and might include a control sediment, a formulated sediment (Section 7.2), a sand substrate (for *C. tentans*; Section 12.2, 15.2), or water-only exposures (for *H. azteca*; Section 14.3.7.8). Testing a reference sediment provides a site-specific basis for evaluating toxicity of the test sediments. Comparisons of test sediments to multiple reference or control sediments representative of the physical characteristics of the test sediment (i.e., grain size, organic carbon) may be useful in these evaluations. A summary of field sampling design is presented by Green (1979). See Section 16 for additional guidance on experimental design and statistics.

2.1.2.3 If the purpose of the study is to conduct a reconnaissance field survey to identify contaminated sites for further investigation, the experimental design might include only one sample from each site to allow for maximum spatial coverage. The lack of replication at a site usually precludes statistical comparisons (e.g., analysis of variance [ANOVA]) among sites, but these surveys can be used to identify contaminated sites for further study or may be evaluated using regression techniques (Sokal and Rohlf, 1981; Steel and Torrie, 1980).

2.1.2.4 In other instances, the purpose of the study might be to conduct a quantitative sediment survey of chemistry and toxicity to determine statistically significant differences between effects among control and test sediments from several sites. The number of replicates per site should be based on the need for sensitivity or power (Section 16). In a quantitative survey, replicates (separate samples from different grabs collected at the same site) would need to be taken at each site. Chemical and physical characterizations of each of these grabs would be required for each of these replicates used in sediment testing. Separate subsamples might be used to determine within-sample variability or to compare test procedures (e.g., comparative sensitivity among test organisms), but these subsamples cannot be considered to be true field replicates for statistical comparisons among sites (ASTM, 1999a).

2.1.2.5 Sediments often exhibit high spatial and temporal variability (Stemmer et al., 1990a). Therefore, replicate samples may need to be collected to determine variance in sediment characteristics. Sediments should be collected with as little disruption as possible; however, subsampling, compositing, or homogenization of sediment samples may be necessary for some experimental designs.

2.1.2.6 Site locations might be distributed along a known pollution gradient, in relation to the boundary of a disposal site, or at sites identified as being contaminated in a reconnaissance survey. Both spatial and temporal comparisons can be made. In pre-dredging studies, a sampling design can be prepared to assess the contamination of samples representative of the project area to be dredged. Such a design should include subsampling of cores taken to the project depth.

2.1.2.7 The primary focus of the physical and experimental test design, and statistical analysis of the data, is the experimental unit. The experimental unit is defined as the smallest physical entity to which treatments can be independently assigned (Steel and Torrie, 1980) and to which air and water exchange between test chambers is kept to a minimum. As the number of test chambers per treatment increases, the number of degrees of freedom and the power of a significance test increase, and therefore, the width of the confidence interval on a point estimate, such as an LC50, decreases (Section 16). Because of factors that might affect test results, all test chambers should be treated as similarly as possible. Treatments should be randomly assigned to individual test chamber

locations. Assignment of test organisms to test chambers should be impartial (Davis et al., 1998).

2.2 Types of Tests

2.2.1 Methods for conducting 10-d toxicity tests are outlined for two organisms, the amphipod *H. azteca* (Section 11) and the midge *C. tentans* (Section 12). The manual primarily describes methods for testing freshwater sediments; however, the methods described can also be used for testing *H. azteca* in estuarine sediments in 10-d tests (up to 15‰ salinity).

2.2.2 Guidance for conducting long-term toxicity tests is also outlined for *H. azteca* (Section 14) and *C. tentans* (Section 15).

2.2.3 Guidance for conducting 28-d bioaccumulation tests with the oligochaete *L. variegatus* is described in Section 13. Procedures are also described for determining bioaccumulation kinetics of different classes of compounds during 28-d exposures with *L. variegatus*.

2.3 Test Endpoints

2.3.1 Endpoints measured in the 10-d toxicity tests are survival and growth. Length or weight is reported as the average of the surviving organisms at the end of the test (Sections 11 and 12). From these data, biomass can also be calculated (dry weight of surviving organisms divided by the initial number of organisms). The rationale for evaluating biomass in toxicity testing is as follows: small differences in either growth or survival may not be statistically significantly different from the control; however, a combined estimate of biomass may increase the statistical power of the test. Although USEPA (1994c, d) describes procedures for reporting biomass as a measure of growth in effluent toxicity tests, the approach has not yet been routinely applied to sediment testing. Therefore, biomass is not listed as a primary endpoint in the methods described in Sections 11, 12, 14, and 15.

2.3.2 Endpoints measured in the long-term *H. azteca* exposures include survival (Day 28, 35, and 42), growth (Day 28 and 42), and reproduction (number of young per female produced from Day 28 to 42). The long-term sediment exposures with *C. tentans* start with newly hatched larvae (<24-h old) and continue through emergence, reproduction, and hatching of the F_1 generation (about 60-d exposures). Survival is determined at 20 d. Starting on Day 23 to the end of the test, emergence and reproduction of *C. tentans* are monitored daily. The number of eggs per female is determined for each egg mass, which is incubated for 6 d to determine hatching success.

2.3.2.1 The long-term toxicity test methods for *Hyalella azteca* and *Chironomus tentans* (Sections 14 and 15) can be used to measure effects on reproduction as well as long-term survival and growth. Reproduction is a key variable influencing the long-term sustainability of populations (Rees and Crawley, 1989) and has been shown to provide valuable and sensitive information in the assessment of sediment toxicity (Derr and Zabik, 1972; Wentsel et al., 1978; Williams et al., 1987; Postma et al., 1995; Sibley et al., 1996, 1997a; Ingersoll et al., 1998). Further, as concerns have emerged regarding the environmental significance of chemicals that can act directly or indirectly on reproductive endpoints (e.g., endocrine disrupting compounds), the need for comprehensive reproductive toxicity tests has become increasingly important. Reproductive endpoints measured in sediment toxicity tests with *H. azteca* and *C. tentans* tend to be more variable compared with those for survival or growth (Section 14.4.6 and 15.4.6). Hence, additional replicates would be required to achieve the same statistical power as for survival and growth endpoints (Section 16). The procedures described in Sections 14 and 15 include measurement of a variety of lethal and sublethal endpoints; minor modifications of the basic methods can be used in cases where only a subset of these endpoints is of interest (Sections 14.1.3 and 15.1.2).

2.3.3 Endpoints measured in bioaccumulation tests are tissue concentrations of contaminants and for some types of studies, lipid content. Behavior of test organisms should be qualitatively observed daily in all tests (e.g., avoidance of sediment).

Section 3
Definitions

3.1 Terms

The following terms were defined in Lee (1980), NRC (1989), USEPA (1989c), USEPA-USACE (1991), USEPA-USACE (1998a), ASTM (1999a), ASTM (1999b), or ASTM (1999h).

3.1.1 Technical Terms

3.1.1.1 ***Bioaccumulation.*** The net accumulation of a substance by an organism as a result of uptake from all environmental sources.

3.1.1.2 ***Bioaccumulation factor.*** Ratio of tissue residue to contaminant source concentration at steady state.

3.1.1.3 ***Bioaccumulation potential.*** Qualitative assessment of whether a contaminant is bioavailable.

3.1.1.4 ***Bioconcentration.*** The net assimilation of a substance by an aquatic organism as a result of uptake directly from aqueous solution.

3.1.1.5 ***Bioconcentration factor (BCF).*** Ratio of tissue residue to water contaminant concentration at steady state.

3.1.1.6 ***Biota-sediment accumulation factor (BSAF).*** The ratio of tissue residue to source concentration (e.g., sediment at steady state normalized to lipid and sediment organic carbon).

3.1.1.7 ***Clean.*** Denotes a sediment or water that does not contain concentrations of test materials which cause apparent stress to the test organisms or reduce their survival.

3.1.1.8 ***Concentration.*** The ratio of weight or volume of test material(s) to the weight or volume of sediment or water.

3.1.1.9 ***Contaminated sediment.*** Sediment containing chemical substances at concentrations that pose a known or suspected threat to environmental or human health.

3.1.1.10 ***Control sediment.*** A sediment that is essentially free of contaminants and is used routinely to assess the acceptability of a test. Any contaminants in control sediment may originate from the global spread of pollutants and do not reflect any substantial input from local or nonpoint sources. Comparing test sediments to control sediments is a measure of the toxicity of a test sediment beyond inevitable background contamination. Control sediment is also called a **negative control** because no toxic effects are anticipated in this treatment.

3.1.1.11 ***Depuration.*** Loss of a substance from an organism as a result of any active (e.g., metabolic breakdown) or passive process when the organism is placed into an uncontaminated environment. Contrast with Elimination.

3.1.1.12 ***Effect concentration (EC)***. The toxicant concentration that would cause an effect in a given percentage of the test population. Identical to LC when the observable adverse effect is death. For example, the EC50 is the concentration of toxicant that would cause a specified effect in 50% of the test population.

3.1.1.13 ***Elimination.*** General term for the loss of a substance from an organism that occurs by any active or passive means. The term is applicable either in a contaminated environment (e.g., occurring simultaneously with uptake) or in a clean environment. Contrast with Depuration.

3.1.1.14 ***Equilibrium partitioning sediment guidelines (ESGs).*** Numerical concentrations of chemical contaminants in sediment at or below which direct lethal or sublethal toxic effects on benthic organisms are not expected. ESGs are based on the theory that an equilibria exists among contaminant concentration in sediment pore water, contaminant associated with a binding phase in sediment, and biota. ESGs are derived by assigning a protective water-only effects concentration to the pore water (such as a Final Chronic Value), and expressing the associated equilibrium sediment concentration in terms of the principal binding phase that limits contaminant bioavailability (e.g., total organic carbon for nonionic organics or acid volatile sulfides for metals).

3.1.1.15 ***Formulated sediment.*** Mixtures of materials used to mimic the physical components of a natural sediment.

3.1.1.16 ***Inhibition concentration (IC).*** The toxicant concentration that would cause a given percent reduction in a non-quantal measurement for the test population. For

example, the IC25 is the concentration of toxicant that would cause a 25% reduction in growth for the test population, and the IC50 is the concentration of toxicant that would cause a 50% reduction.

3.1.1.17 ***Interstitial water or pore water.*** Water occupying space between sediment or soil particles.

3.1.1.18 ***k_1.*** Uptake rate coefficient from the aqueous phase, with units of g-water x g-tissue^{-1} x time^{-1}. Contrast with k_s.

3.1.1.19 ***k_2.*** Elimination rate constant, with units of time^{-1}.

3.1.1.20 ***k_s.*** Sediment uptake rate coefficient from the sediment phase, with units of g-sediment x g-tissue^{-1} x time^{-1}. Contrast with k_1.

3.1.1.21 ***K_{oc}.*** Organic carbon-water partitioning coefficient.

3.1.1.22 ***K_{ow}.*** Octanol-water partitioning coefficient.

3.1.1.23 ***Kinetic Bioaccumulation Model.*** Any model that uses uptake and/or elimination rates to predict tissue residues.

3.1.1.24 ***Lethal concentration (LC).*** The toxicant concentration that would cause death in a given percentage of the test population. Identical to EC when the observable adverse effect is death. For example, the LC50 is the concentration of toxicant that would cause death in 50% of the test population.

3.1.1.25 ***Lowest observed effect concentration (LOEC).*** The lowest concentration of a toxicant to which organisms are exposed in a test that causes an adverse effect on the test organisms (i.e., where a significant difference exists between the value for the observed response and that for the controls).

3.1.1.26 ***No observed effect concentration (NOEC).*** The highest concentration of a toxicant to which organisms are exposed in a test that causes no observable adverse effect on the test organisms (i.e., the highest concentration of a toxicant in which the value for the observed response is not statistically significantly different from the controls).

3.1.1.27 ***Overlying water.*** The water placed over sediment in a test chamber during a test.

3.1.1.28 ***Reference sediment.*** A whole sediment near an area of concern used to assess sediment conditions exclusive of material(s) of interest. The reference sediment may be used as an indicator of localized sediment conditions exclusive of the specific pollutant input of concern. Such sediment would be collected near the site of concern and would represent the background conditions resulting from any localized pollutant inputs as well as global pollutant input. This is the manner in which reference sediment is used in dredged material evaluations.

3.1.1.29 ***Reference-toxicity test.*** A test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

3.1.1.30 ***Sediment.*** Particulate material that usually lies below water. Formulated particulate material that is intended to lie below water in a test.

3.1.1.31 ***Spiked sediment.*** A sediment to which a material has been added for experimental purposes.

3.1.1.32 ***Steady state.*** An equilibrium or "constant" tissue residue resulting from the balance of the flux of compound into and out of the organism. Operationally determined by no statistically significant difference in tissue residue concentrations from three consecutive sampling periods.

3.1.1.33 ***Whole sediment.*** Sediment and associated pore water that have had minimal manipulation. The term **bulk sediment** has been used synonymously with whole sediment.

3.1.2 Grammatical Terms

The words "must," "should," "may," "can," and "might" have very specific meanings in this manual.

3.1.2.1 "Must" is used to express an absolute requirement, that is, to state that a test ought to be designed to satisfy the specified conditions, unless the purpose of the test requires a different design. "Must" is only used in connection with the factors that directly relate to the acceptability of a test.

3.1.2.2 "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although a violation of one "should" is rarely a serious matter, violation of several will often render the results questionable.

3.1.2.3 Terms such as "is desirable," "is often desirable," and "might be desirable" are used in connection with less important factors.

3.1.2.4 "May" is used to mean "is (are) allowed to," "can" is used to mean "is (are) able to," and "might" is used to mean "could possibly." Thus, the classic distinction between "may" and "can" is preserved, and "might" is never used as a synonym for either "may" or "can."

Section 4
Interferences

4.1 General Introduction

4.1.1 Interferences are characteristics of a sediment or sediment test system, aside from those related to sediment-associated chemicals of concern, that can potentially affect test organism survival, growth, or reproduction. These interferences can potentially confound interpretation of test results in two ways: (1) false-positive response, i.e., toxicity is observed in the test when contamination is not present at concentrations known to elicit a response, or there is more toxicity than expected; and (2) false-negative response, i.e., no toxicity or bioaccumulation is observed when contaminants are present at concentrations known to elicit a response, or there is less toxicity or bioaccumulation than expected.

4.1.2 There are three categories of interfering factors that can cause false-negative or false-positive responses: (1) those characteristics of sediments affecting survival independent of chemical concentration (i.e., noncontaminant factors), (2) changes in chemical bioavailability as a function of sediment manipulation or storage, and (3) the presence of indigenous organisms. Although test procedures and test organism selection criteria were developed to minimize these interferences, this section describes the nature of these interferences.

4.1.3 Because of the heterogeneity of natural sediments, extrapolation from laboratory studies to the field can sometimes be difficult (Table 4.1; Burton, 1991). Sediment collection, handling, and storage procedures may alter bioavailability and concentration of chemicals of concern by changing the physical, chemical, or biological characteristics of the sediment. Maintaining the integrity of a field-collected sediment during removal, transport, mixing, storage, and testing is extremely difficult and may complicate the interpretation of effects. Direct comparisons of organisms exposed in the laboratory and in the field would be useful to verify laboratory results. However, spiked sediment may not be representative of contaminated sediment in the field. Mixing time (Stemmer et al., 1990a), aging (Word et al., 1987; Landrum, 1989; Landrum and Faust, 1992) and the chemical form of the material can affect responses of test organisms in spiked sediment tests.

4.1.4 Laboratory testing with field-collected sediments may be useful in estimating cumulative effects and interactions of multiple chemicals in a sample. Tests with field samples usually cannot discriminate between effects of individual chemicals. Most sediment samples contain a complex matrix of inorganic and organic chemicals with many unidentified compounds. The use of Toxicity

Table 4.1 Advantages and Disadvantages for Use of Sediment Tests[1]

Advantages
• Sediment tests measure bioavailable fraction of contaminant(s).
• Sediment tests provide a direct measure of benthic effects, assuming no field adaptation or amelioration of effects.
• Limited special equipment is required for testing.
• Ten-day toxicity test methods are rapid and inexpensive.
• Legal and scientific precedence exists for use; ASTM standard guides are available.
• Sediment tests measure unique information relative to chemical analyses or benthic community analyses.
• Tests with spiked chemicals provide data on cause-effect relationships.
• Sediment toxicity tests can be applied to all chemicals of concern.
• Tests applied to field samples reflect cumulative effects of contaminants and contaminant interactions.
• Toxicity tests are amenable to confirmation with natural benthos populations.
Disadvantages
• Sediment collection, handling, and storage may alter bioavailability.
• Spiked sediment may not be representative of field contaminated sediment.
• Natural geochemical characteristics of sediment may affect the response of test organisms.
• Indigenous animals may be present in field-collected sediments.
• Route of exposure may be uncertain and data generated in sediment toxicity tests may be difficult to interpret if factors controlling the bioavailability of contaminants in sediment are unknown.
• Tests applied to field samples may not discriminate effects of individual chemicals.
• Few comparisons have been made of methods or species.
• Only a few chronic methods for measuring sublethal effects have been developed or extensively evaluated.
• Laboratory tests have inherent limitations in predicting ecological effects.

[1] Modified from Swartz (1989)

Identification Evaluations (TIE) in conjunction with sediment tests with spiked chemicals may provide evidence of causal relationships and can be applied to many chemicals of concern (Ankley and Thomas, 1992; Adams et al., 1985; USEPA, 1996b). Sediment spiking can also be used to investigate additive, antagonistic, or synergistic effects of specific chemical mixtures in a sediment sample (Swartz et al., 1988).

4.1.5 Spiked sediment may not be representative of contaminated sediment in the field. Mixing time (Stemmer et al., 1990b) and aging (Word et al., 1987; Landrum, 1989; and Landrum and Faust, 1992) of spiked sediment can affect responses of organisms.

4.1.6 Most assessments of contaminated sediment rely on short-term-lethality testing methods (e.g., ≤10 d; USEPA-USACE, 1977; USEPA-USACE, 1991; Sections 11 and 12). Short-term-lethality tests are useful in identifying "hot spots" of sediment contamination but may not be sensitive enough to evaluate moderately contaminated areas. Sediment quality assessments using sublethal responses of benthic organisms, such as effects on growth and reproduction, have been used to successfully evaluate moderately contaminated areas (Scott, 1989; Kemble et al., 1994; Ingersoll et al., 1998; Sections 14 and 15).

4.1.7 Despite the interferences discussed in this section, existing sediment test methods that include measurement of sublethal endpoints may be used to provide a rapid and direct measure of effects of contaminants on benthic communities (e.g., Canfield et al., 1996). Laboratory tests with field-collected sediment can also be used to determine temporal, horizontal, or vertical distribution of contaminants in sediment. Most tests can be completed within two to four weeks. Legal and scientific precedents exist for use of toxicity and bioaccumulation tests in regulatory decision-making (e.g., USEPA, 1986a). Furthermore, sediment tests with complex contaminant mixtures are important tools for making decisions about the extent of remedial action for contaminated aquatic sites and for evaluating the success of remediation activities.

4.2 Noncontaminant Factors

4.2.1 Results of sediment tests can be used to predict effects that may occur with aquatic organisms in the field as a result of exposure under comparable conditions. Yet motile organisms might avoid exposure in the field. Photoinduced toxicity caused by ultraviolet (UV) light may be important for some compounds associated with sediment (e.g., polycyclic aromatic hydrocarbons (PAHs); Davenport and Spacie, 1991; Ankley et al., 1994b). Fluorescent light does not contain UV light, but natural sunlight does. Lighting can therefore affect toxicological responses and is an important experimental variable for photoactivated chemicals. However, lighting typically used to conduct laboratory tests does not include the appropriate spectrum of ultraviolet radiation to photoactivate compounds (Oris and Giesy, 1985; Ankley et al., 1994b). Therefore, laboratory tests may not account for toxicity expressed by this mode of action.

4.2.2 Natural geomorphological and physico-chemical characteristics such as sediment texture may influence the response of test organisms (DeWitt et al., 1988). The physico-chemical characteristics of test sediment need to be within the tolerance limits of the test organism. Ideally, the limits of the test organism should be determined in advance; however, control samples reflecting differences in factors such as grain size and organic carbon can be evaluated if the limits are exceeded in the test sediment (Section 10.1). The effects of sediment characteristics can also be addressed with regression equations (DeWitt et al., 1988; Ankley et al., 1994a). Effects of physico-chemical characteristics of sediment on test organisms can also be evaluated by using formulated sediment for testing (Section 7.2; Walsh et al., 1991; Suedel and Rodgers, 1994; Kemble et al., 1999). See Sections 11.4, 12.4, 13.4, 14.4, and 15.4 for a discussion of the relationships between grain size of sediment and responses of test organisms.

4.2.3 A weak relationship was evident between mean reproduction of *H. azteca* in the 42-d test and grain size (Section 14.4.3; Ingersoll et al., 1998). Additional study is needed to better evaluate potential relationships between reproduction of *H. azteca* and the physical characteristics of the sediment. The weak relationship between grain size of sediment and reproduction may have been due to the fact that some of the samples with higher amounts of sand also had higher concentrations of organic chemicals compared with other samples (Ingersoll et al., 1998). *Hyalella azteca* tolerated a wide range in sediment particle size and organic matter in 10- to 28-d tests measuring effects on survival or growth (Ankley et al., 1994a; Suedel and Rodgers, 1994; Ingersoll et al., 1996; Ingersoll et al., 1998; Kemble et al., 1999; Section 14.4.3).

4.2.3.1 Until additional studies have been conducted which substantiate this lack of a correlation between physical characteristics of sediment and reproduction measured in the 42-d *H. azteca* test, it would be desirable to test control or reference sediments which are representative of the physical characteristics of field-collected sediments. Formulated sediments could be used to bracket the ranges in physical characteristics expected in the field-collected sediments being evaluated (Section 7.2). Addition of YCT should provide a minimum amount of food needed to support adequate survival, growth, and reproduction of *H. azteca* in sediments low in organic matter (Section 14.2). Without addition of food, *H. azteca* can starve during exposures (McNulty et al., 1999) making it impossible to differentiate effects of chemicals from other sediment characteristics.

4.2.4 Additional potential interferences of tests are described in Sections 11.4, 12.4, 13.4, 14.4, and 15.4.

4.3 Changes in Bioavailability

4.3.1 Sediment toxicity tests are meant to serve as an indicator of contaminant-related toxicity that might be expected under field or natural conditions. Some studies have indicated differences between results of laboratory testing and results of field testing of sediments using *in situ* exposures (Sasson-Brickson and Burton, 1991).

4.3.2 Sediment collection, handling, and storage procedures may alter contaminant bioavailability and concentration by changing the physical, chemical, or biological characteristics of the sediment. Manipulations such as mixing, homogenization, and sieving may temporarily disrupt the equilibrium of organic compounds in sediment. Similarly, oxidation of anaerobic sediments increases the availability of certain metals (Di Toro et al., 1990). Because the availability of contaminants can be a function of the degree of manipulation, this manual recommends that handling, storage, and preparation of the sediment for testing be as consistent as possible. If sieving is performed, it is done primarily to remove predatory organisms and large debris. This manipulation most likely results in a worst-case condition of heightened bioavailability yet eliminates predation as a factor that might confound test results. When sediments are sieved, it may be desirable to take samples before and after sieving (e.g., pore-water metals or DOC, AVS, TOC) to document the influence of sieving on sediment chemistry. USEPA does not recommend sieving freshwater sediments on a routine basis. See USEPA (1999) and ASTM (1999b).

4.3.3 Testing sediments at temperatures different from the field might affect contaminant solubility, partitioning coefficients, or other physical and chemical characteristics. Interaction between sediment and overlying water and the ratio of sediment to overlying water can influence bioavailability (Stemmer et al., 1990b).

4.3.4 The addition of food, water, or solvents to the test chambers might obscure the bioavailability of contaminants in sediment or might provide a substrate for bacterial or fungal growth (Harkey et al., 1997). Without addition of food, the test organisms may starve during exposures (Ankley et al., 1994a; McNulty et al., 1999). However, the addition of food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987, Harkey et al., 1994) depending on the amount of food added, its composition (e.g., TOC), and the chemical(s) of interest.

4.3.5 Depletion of aqueous and sediment-sorbed contaminants resulting from uptake by an organism or absorption to a test chamber can also influence availability. In most cases, the organism is a minor sink for contaminants relative to the sediment. However, within the burrow of an organism, sediment desorption kinetics might limit uptake rates. Within minutes to hours, a major portion of the total chemical can be inaccessible to the organisms because of depletion of available residues. The desorption of a particular compound from sediment may range from easily reversible (labile; within minutes) to irreversible (non-labile; within days or months; Karickhoff and Morris, 1985). Interparticle diffusion or advection and the quality and quantity of sediment organic carbon can also affect sorption kinetics.

4.3.6 The route of exposure may be uncertain, and data from sediment tests may be difficult to interpret if factors controlling the bioavailability of contaminants in sediment are unknown. Bulk-sediment chemical concentrations may be normalized to factors other than dry weight. For example, concentrations of nonionic organic compounds might be normalized to sediment organic-carbon content (USEPA, 1992c) and certain metals normalized to acid volatile sulfides (Di Toro et al., 1990). Even with the appropriate normalizing factors, determination of toxic effects from ingestion of sediment or from dissolved chemicals in the interstitial water can still be difficult (Lamberson and Swartz, 1988).

4.4 Presence of Indigenous Organisms

4.4.1 Indigenous organisms may be present in field-collected sediments. An abundance of the same organism or organisms taxonomically similar to the test organism in the sediment sample may make interpretation of treatment effects difficult. For example, growth of amphipods, midges, or mayflies may be reduced if high numbers of oligochaetes are in a sediment sample (Reynoldson et al., 1994). Previous investigators have inhibited the biological activity of sediment with sieving, heat, mercuric chloride, antibiotics, or gamma irradiation (see ASTM, 1999b). However, further research is needed to determine effects on contaminant bioavailability or other modifications of sediments from treatments such as those used to remove or destroy indigenous organisms.

Section 5
Health, Safety, and Waste Management

5.1 General Precautions

5.1.1 Development and maintenance of an effective health and safety program in the laboratory requires an ongoing commitment by laboratory management and includes (1) the appointment of a laboratory health and safety officer with the responsibility and authority to develop and maintain a safety program, (2) the preparation of a formal written health and safety plan, which is provided to each laboratory staff member, (3) an ongoing training program on laboratory safety, and (4) regular safety inspections.

5.1.2 This manual addresses procedures that may involve hazardous materials, operations, and equipment, but it does not purport to address all of the safety problems associated with their use. It is the responsibility of the user to establish appropriate safety and health practices, and determine the applicability of regulatory limitations before use. While some safety considerations are included in this manual, it is beyond the scope of this manual to encompass all safety requirements necessary to conduct sediment tests.

5.1.3 Collection and use of sediment may involve substantial risks to personal safety and health. Contaminants in field-collected sediment may include carcinogens, mutagens, and other potentially toxic compounds. Inasmuch as sediment testing is often begun before chemical analyses can be completed, worker contact with sediment needs to be minimized by (1) using gloves, laboratory coats, safety glasses, face shields, and respirators as appropriate, (2) manipulating sediment under a ventilated hood or in an enclosed glove box, and (3) enclosing and ventilating the exposure system. Personnel collecting sediment samples and conducting tests should take all safety precautions necessary for the prevention of bodily injury and illness that might result from ingestion or invasion of infectious agents, inhalation or absorption of corrosive or toxic substances through skin contact, and asphyxiation because of lack of oxygen or presence of noxious gases.

5.1.4 Before beginning sample collection and laboratory work, personnel should determine that all required safety equipment and materials have been obtained and are in good condition.

5.2 Safety Equipment

5.2.1 Personal Safety Gear

5.2.1.1 Personnel should use appropriate safety equipment, such as rubber aprons, laboratory coats, respirators, gloves, safety glasses, face shields, hard hats, and safety shoes.

5.2.2 Laboratory Safety Equipment

5.2.2.1 Each laboratory should be provided with safety equipment such as first aid kits, fire extinguishers, fire blankets, emergency showers, and eye wash stations.

5.2.2.2 All laboratories should be equipped with a telephone to enable personnel to summon help in case of emergency.

5.3 General Laboratory and Field Operations

5.3.1 Laboratory personnel should be trained in proper practices for handling and using chemicals that are encountered during procedures described in this manual. Routinely encountered chemicals include acids, organic solvents, and standard materials for reference-toxicity tests. Special handling and precautionary guidance in Material Safety Data Sheets should be followed for reagents and other chemicals purchased from supply houses.

5.3.2 Work with some sediment may require compliance with rules pertaining to the handling of hazardous materials. Personnel collecting samples and performing tests should not work alone.

5.3.3 It is advisable to wash exposed parts of the body with bactericidal soap and water immediately after collecting or manipulating sediment samples.

5.3.4 Strong acids and volatile organic solvents should be used in a fume hood or under an exhaust canopy over the work area.

5.3.5 An acidic solution should not be mixed with a hypochlorite solution because hazardous vapors might be produced.

5.3.6 To prepare dilute acid solutions, concentrated acid should be added to water, not vice versa. Opening a bottle of concentrated acid and adding concentrated acid to water should be performed only under a fume hood.

5.3.7 Use of ground-fault systems and leak detectors is strongly recommended to help prevent electrical shocks. Electrical equipment or extension cords not bearing the approval of Underwriter Laboratories should not be used. Ground-fault interrupters should be installed in all "wet" laboratories where electrical equipment is used.

5.3.8 All containers should be adequately labeled to identify their contents.

5.3.9 Good housekeeping contributes to safety and reliable results.

5.4 Disease Prevention

5.4.1 Personnel handling samples that are known or suspected to contain human wastes should be given the opportunity to be immunized against hepatitis B, tetanus, typhoid fever, and polio. Thorough washing of exposed skin with bactericidal soap should follow handling these samples.

5.5 Safety Manuals

5.5.1 For further guidance on safe practices when handling sediment samples and conducting toxicity tests, check with the permittee and consult general industrial safety manuals including USEPA (1986b) and Walters and Jameson (1984).

5.6 Pollution Prevention, Waste Management, and Sample Disposal

5.6.1 It is the laboratory's responsibility to comply with the federal, state, and local regulations governing the waste management, particularly hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

5.6.2 Guidelines for the handling and disposal of hazardous materials should be strictly followed. The federal government has published regulations for the management of hazardous waste and has given the states the option of either adopting those regulations or developing their own. If states develop their own regulations, they are required to be at least as stringent as the federal regulations. As a handler of hazardous materials, it is a laboratory's responsibility to know and comply with the applicable state regulations. Refer to The Bureau of National Affairs Inc., (1986) for the citations of the federal requirements.

5.6.3 Substitution of nonhazardous chemicals and reagents should be encouraged and investigated whenever possible. For example, use of a nonhazardous compound for a positive control in reference-toxicity tests is advisable. Reference-toxicity tests with copper can provide appropriate toxicity at concentrations below regulated levels.

Section 6
Facilities, Equipment, and Supplies

6.1 General

6.1.1 Before a sediment test is conducted in any test facility, it is desirable to conduct a "nontoxicant" test with each test species in which all test chambers contain a control sediment (sometimes called the negative control) and clean overlying water. Survival, growth, or reproduction of the test organisms will demonstrate whether facilities, water, control sediment, and handling techniques are adequate to result in acceptable species-specific control numbers. Evaluations may also be made on the magnitude of between-chamber variance in a test. See Section 9.14.

6.2 Facilities

6.2.1 The facility must include separate areas for culturing test organisms and sediment testing to reduce the possibility of contamination by test materials and other substances, especially volatile compounds. Holding, acclimation, and culture chambers should not be in a room where sediment tests are conducted, stock solutions or sediments are prepared, or equipment is cleaned. Test chambers may be placed in a temperature-controlled recirculating water bath, environmental chamber, or equivalent facility with temperature control. An enclosed test system is desirable to provide ventilation during tests to limit exposure of laboratory personnel to volatile substances.

6.2.2 Light of the quality and luminance normally obtained in the laboratory is adequate (about 100 to 1000 lux using wide-spectrum fluorescent lights; e.g., cool-white or daylight) has been used successfully to culture and test organisms. Lux is the unit selected for reporting luminance in this manual. Multiply units of lux by 0.093 to convert to units of foot candles. Multiply units of lux by 6.91×10^{-3} to convert to units of $\mu E/m^2/s$ (assuming an average wavelength of 550 nm ($\mu mol^{-2}\ s^{-1} = W\ m \times \lambda[nm] \times 8.36 \times 10^{-3}$); ASTM, 1999g). Luminance should be measured at the surface of the water in test chambers. A uniform photoperiod of 16L:8D can be achieved in the laboratory or in an environmental chamber using automatic timers.

6.2.3 During phases of rearing, holding, and testing, test organisms should be shielded from external disturbances such as rapidly changing light or pedestrian traffic.

6.2.4 The test facility should be well ventilated and free of fumes. Laboratory ventilation systems should be checked to ensure that return air from chemistry laboratories or sample handling areas is not circulated to culture or testing rooms, or that air from testing rooms does not contaminate culture rooms. Air pressure differentials between rooms should not result in a net flow of potentially contaminated air to sensitive areas through open or loose-fitting doors. Air used for aeration must be free of oil and fumes. Oil-free air pumps should be used where possible. Filters to remove oil, water, and bacteria are desirable. Particles can be removed from the air using filters such as BALSTON® Grade BX (Balston, Inc., Lexington, MA) or equivalent, and oil and other organic vapors can be removed using activated carbon filters (e.g., BALSTON® C-1 filter), or equivalent.

6.3 Equipment and Supplies

6.3.1 Equipment and supplies that contact stock solutions, sediment, or overlying water should not contain substances that can be leached or dissolved in amounts that adversely affect the test organisms. In addition, equipment and supplies that contact sediment or water should be chosen to minimize sorption of test materials from water. Glass, type 316 stainless steel, nylon, high-density polyethylene, polypropylene, polycarbonate, and fluorocarbon plastics should be used whenever possible to minimize leaching, dissolution, and sorption. Concrete and high-density plastic containers may be used for holding and culture chambers, and in the water-supply system. These materials should be washed in detergent, acid rinsed, and soaked in flowing water for a week or more before use. Cast-iron pipe should not be used in water-supply systems because colloidal iron will be added to the overlying water and strainers will be needed to remove rust particles. Copper, brass, lead, galvanized metal, and natural rubber must not contact overlying water or stock solutions before or during a test. Items made of neoprene rubber and other materials not mentioned above should not be used unless it has been shown that their use will not adversely affect survival, growth, or reproduction of the test organisms.

6.3.2 New lots of plastic products should be tested for toxicity by exposing organisms to them under ordinary test conditions before general use.

6.3.3 General Equipment

6.3.3.1 Environmental chamber or equivalent facility with photoperiod and temperature control (20°C to 25°C).

6.3.3.2 Water purification system capable of producing at least 1 mega-ohm water (USEPA, 1991a).

6.3.3.3 Analytical balance capable of accurately weighing to 0.01 mg.

6.3.3.4 Reference weights, Class S—for documenting the performance of the analytical balance(s). The balance(s) should be checked with reference weights that are at the upper and lower ends of the range of the weighings made when the balance is used. A balance should be checked at the beginning of each series of weighings, periodically (such as every tenth weight) during a long series of weighings, and after taking the last weight of a series.

6.3.3.5 Volumetric flasks and graduated cylinders—Class A, borosilicate glass or nontoxic plastic labware, 10 to 1000 mL for making test solutions.

6.3.3.6 Volumetric pipets—Class A, 1 to 100 mL.

6.3.3.7 Serological pipets—1 to 10 mL, graduated.

6.3.3.8 Pipet bulbs and fillers—PROPIPET® or equivalent.

6.3.3.9 Droppers, and glass tubing with fire polished edges, 4- to 6-mm ID—for transferring test organisms.

6.3.3.10 Wash bottles—for rinsing small glassware, instrument electrodes and probes.

6.3.3.11 Glass or electronic thermometers—for measuring water temperature.

6.3.3.12 National Bureau of Standards Certified thermometer (see USEPA Method 170.1; USEPA, 1979b).

6.3.3.13 Dissolved oxygen (DO), pH/selective ion, and specific conductivity meters and probes for routine physical and chemical measurements are needed. Unless a test is being conducted to specifically measure the effect of DO or conductivity, a portable field-grade instrument is acceptable.

6.3.3.14 See Table 6.1 for a list of additional equipment and supplies. Appendix C outlines additional equipment and supplies needed for conducting the long-term exposures with *C. tentans.*

6.3.4 Water-delivery System

6.3.4.1 The water-delivery system used in water-renewal testing can be one of several designs (Appendix A). The system should be capable of delivering water to each replicate test chamber. Mount and Brungs (1967) diluters have been successfully modified for sediment testing. Other diluter systems have also been useful (Ingersoll and Nelson, 1990; Maki, 1977; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998). The water-delivery system should be calibrated before the test by determining the flow rate of the overlying water. The general operation of the system should be visually checked daily throughout the length of the test. If necessary, the water-delivery system should be adjusted during the test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%.

6.3.4.2 The overlying water can be replaced manually (e.g., siphoning); however, manual systems take more time to maintain during a test. In addition, automated systems generally result in less suspension of sediment compared to manual renewal.

6.3.5 Test Chambers

6.3.5.1 Test chambers may be constructed in several ways and of various materials, depending on the experimental design and the contaminants of interest. Clear silicone adhesives, suitable for aquaria, sorb some organic compounds that might be difficult to remove. Therefore, as little adhesive as possible should be in contact with the test material. Extra beads of adhesive should be on the outside of the test chambers rather than on the inside. To leach potentially toxic compounds from the adhesive, all new test chambers constructed using silicone adhesives should be held at least 48 h in overlying water before use in a test.

6.3.5.2 Test chambers for specific tests are described in Sections 11, 12, 13, 14, and 15.

6.3.6 Cleaning

6.3.6.1 All nondisposable sample containers, test chambers, and other equipment that have come in contact with sediment should be washed after use in the manner described below to remove surface contaminants.

1. Soak 15 min in tap water and scrub with detergent, or clean in an automatic dishwasher.
2. Rinse twice with tap water.
3. Carefully rinse once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases. To prepare a 10% solution of acid, add 10 mL of concentrated acid to 90 mL of deionized water.
4. Rinse twice with deionized water.
5. Rinse once with full-strength, pesticide-grade acetone to remove organic compounds (use a fume hood or canopy). Hexane might also be used as a solvent for removing nonionic organic compounds. However, acetone is preferable if only one organic solvent is used to clean equipment.
6. Rinse three times with deionized water.

Table 6.1 Equipment and Supplies for Culturing and Testing Specific Test Organisms[1]

A. Biological Supplies

Brood stock of test organisms
Active dry yeast (HA)
Cerophyl® (dried cereal leaves; HA)
Trout food pellets (HA)
Tetrafin® or Tetramin® goldfish food (CT)
Trout starter (LV)
Helisoma sp. snails (optional; LV)
Algae (e.g., *Selenastrum capricornutum, Chlorella;* CT)
Diatoms (e.g., *Navicula* sp; HA)

B. Glassware

Culture chambers
Test chambers (300-mL high-form lipless beaker; HA and CT)
Test chambers (15.8- x 29.3- x 11.7-cm, W x L x H; LV)
Juvenile holding beakers (e.g., 1 L; HA)
Crystallizing dishes or beakers (200 to 300 mL; CT)
Erlenmeyer flasks (250 and 500 mL; CT)
Larval rearing chambers (e.g., l9 L capacity; CT)
1/4" glass tubing (for aspirating flask; CT)
Glass bowls (20-cm diameter; LV)
Glass vials (10 mL; LV)
Wide-bore pipets (4- to 6-mm ID)
Glass disposable pipets
Burettes (for hardness and alkalinity determinations)
Graduated cylinders (assorted sizes, 10 mL to 2 L)

C. Instruments and Equipment

Dissecting microscope
Stainless-steel sieves (e.g., U.S. Standard No. 25, 30 35, 40, 50 mesh)
Delivery system for overlying water (See Appendix B for a listing of equipment needed for water delivery systems)
Photoperiod timers
Light meter
Temperature controllers
Thermometer
Continuous recording thermometers
Dissolved oxygen meter
pH meter
Ion-specific meter
Ammonia electrode (or ammonia test kit)
Specific-conductance meter
Drying oven
Desiccator
Balance (0.01 mg sensitivity)

C. Instruments and Equipment

Blender
Refrigerator
Freezer
Light box
Hemacytometer (HA)
Paper shredder, cutter, or scissors (CT, LV)
Tissue homogenizer (LV)
Electric drill with stainless steel auger (diameter 7.6 cm, overall length 38 cm, auger bit length 25.4 cm (Section 8.3)

D. Miscellaneous

Ventilation system for test chambers
Air supply and airstones (oil free and regulated)
Cotton surgical gauze or cheese cloth (HA)
Stainless-steel screen (no. 60 mesh, for test chambers)
Glass hole-cutting bits
Silicon adhesive caulking
Plastic mesh (110-µm mesh opening; Nytex® 110; HA)
Aluminum weighing pans (Sigma Chemical Co., St. Louis, MO)
Fluorescent light bulbs
Nalgene bottles (500 mL and 1000 mL for food preparation and storage)
Deionized water
Air line tubing
White plastic dish pan
"Coiled-web material" (3-M, St. Paul, MN; HA)
White paper toweling (for substrate; CT)
Brown paper toweling (for substrate; LV)
Screening material (e.g., Nitex® (110 mesh), window screen, or panty hose; CT)
Water squirt bottle
Dissecting probes (LV)
Dental picks (LV)
Shallow pans (plastic (light-colored), glass, stainless steel)

E. Chemicals

Detergent (nonphosphate)
Acetone (reagent grade)
Hexane (reagent grade)
Hydrochloric acid (reagent grade)
Chloroform and methanol (LV)
Copper Sulfate, Potassium Chloride
Reagents for reconstituting water
Formalin (or Notox®)
Sucrose

HA = *Hyalella azteca*
CT = *Chironomus tentans*
LV = *Lumbriculus variegatus*
[1] Appendix C outlines additional equipment and supplies for the long-term exposures with *C. tentans.*

6.3.6.2 All test chambers and equipment should be thoroughly rinsed or soaked with the dilution water immediately before use in a test.

6.3.6.3 Many organic solvents (e.g., methylene chloride) leave a film that is insoluble in water. A dichromate-sulfuric acid cleaning solution can be used in place of both the organic solvent and the acid (see ASTM, 1999e), but the solution might attack silicone adhesive and leave chromium residues on glass. An alternative to use of dichromate-sulfuric acid could be to heat glassware for 8 h at 450°C.

Section 7
Water, Formulated Sediment, Reagents, and Standards

7.1 Water

7.1.1 Requirements

7.1.1.1 Water used to test and culture organisms should be uniform in quality. Acceptable water should allow satisfactory survival, growth, or reproduction of the test organisms. Test organisms should not show signs of disease or apparent stress (e.g., discoloration, unusual behavior). If problems are observed in the culturing or testing of organisms, it is desirable to evaluate the characteristics of the water. See USEPA (1991a) and ASTM (1999a) for a recommended list of chemical analyses of the water supply.

7.1.2 Source

7.1.2.1 A natural water is considered to be of uniform quality if monthly ranges of the hardness, alkalinity, and specific conductance are less than 10% of their respective averages and if the monthly range of pH is less than 0.4. Natural waters should be obtained from an uncontaminated well or spring, if possible, or from a surface-water source. If surface water is used, the intake should be positioned to (1) minimize fluctuations in quality and contamination, (2) maximize the concentration of dissolved oxygen, and (3) ensure low concentrations of sulfide and iron. Municipal water supplies may be variable and may contain unacceptably high concentrations of materials such as copper, lead, zinc, fluoride, chlorine, or chloramines. Chlorinated water should not be used for culturing or testing because residual chlorine and chlorine-produced oxidants are toxic to many aquatic organisms. Use of tap water is discouraged unless it is dechlorinated and passed through a deionizer and carbon filter (USEPA, 1991a).

7.1.2.2 For site-specific investigations, it is desirable to have the water-quality characteristics of the overlying water as similar as possible to the site water. For certain applications the experimental design might require use of water from the site where sediment is collected.

7.1.2.3 Water that might be contaminated with facultative pathogens may be passed through a properly maintained ultraviolet sterilizer equipped with an intensity meter and flow controls or passed through a filter with a pore size of 0.45 µm or less.

7.1.2.4 Water might need aeration using air stones, surface aerators, or column aerators. Adequate aeration will stabilize pH, bring concentrations of dissolved oxygen and other gases into equilibrium with air, and minimize oxygen demand and concentrations of volatiles. Excessive aeration may reduce hardness and alkalinity of hard water (e.g., 280 mg/L hardness as $CaCO_3$; E.L. Brunson, USGS, Columbia, MO, personal communication). The concentration of dissolved oxygen in source water should be between 90 to 100% saturation to help ensure that dissolved oxygen concentrations are acceptable in test chambers.

7.1.3 Reconstituted Water

7.1.3.1 Ideally, reconstituted water should be prepared by adding specified amounts of reagent-grade chemicals to high-purity distilled or deionized water (ASTM, 1999e; USEPA, 1991a). Problems have been observed with use of reconstituted water in long-term exposures with *H. azteca* (Section 7.1.3.4.3). In some applications, acceptable high-purity water can be prepared using deionization, distillation, or reverse-osmosis units (Section 6.3.3.2; USEPA, 1991a). In some applications, test water can be prepared by diluting natural water with deionized water (Kemble et al., 1994) or by adding salts to relatively dilute natural waters.

7.1.3.2 Deionized water should be obtained from a system capable of producing at least 1 mega-ohm water. If large quantities of high quality deionized water are needed, it may be advisable to supply the laboratory grade water deionizer with preconditioned water from a mixed-bed water treatment system. Some investigators have observed that holding reconstituted water prepared from deionized water for several days before use in sediment tests may improve performance of test organisms.

7.1.3.3 Conductivity, pH, hardness, dissolved oxygen, and alkalinity should be measured on each batch of reconstituted water. The reconstituted water should be aerated before use to adjust pH and dissolved oxygen to the acceptable ranges (e.g., Section 7.1.3.4.1). USEPA (1991a) recommends using a batch of reconstituted water for two weeks.

7.1.3.4 Reconstituted Fresh Water (Smith et al., 1997)

7.1.3.4.1 To prepare 100 L of reconstituted fresh water, use the reagent-grade chemicals as follows:

1. Place about 75 L of deionized water in a properly cleaned container.

2. Add 5 g of $CaSO_4$ and 5 g of $CaCl_2$ to a 2-L aliquot of deionized water and mix (e.g., on a stir plate) for 30 min or until the salts dissolve.

3. Add 3 g of $MgSO_4$, 9.6 g $NaHCO_3$, and 0.4 g KCl to a second 2-L aliquot of deionized water and mix on a stir plate for 30 min.

4. Pour the two 2-L aliquots containing the dissolved salts into the 75 L of deionized water and fill the carboy to 100 L with deionized water.

5. Aerate the mixture for at least 24 h before use.

6. The water quality of the reconstituted water should be approximately the following: hardness, 90 to 100 mg/L as $CaCO_3$, alkalinity 50 to 70 mg/L as $CaCO_3$, conductivity 330 to 360 mS/cm, and pH 7.8 to 8.2.

7.1.3.4.2 This reconstituted fresh water (reformulated moderately hard reconstituted water) described by Smith et al. (1997) and described in the first edition of this manual (USEPA, 1994a) has been used successfully in 10-d round-robin testing with *H. azteca, C. tentans,* and *C. riparius* (Section 17). This reconstituted water has a higher proportion of chloride to sulfate compared to the reconstituted waters described in ASTM (1999e) and USEPA (1991a).

7.1.3.4.3 McNulty et al. (1999) and Kemble et al. (1998, 1999) observed poor survival of *H. azteca* in tests conducted 14 to 28 d using a variety of reconstituted waters including the reconstituted water described by Smith et al. (1997). Borgmann (1996) described a reconstituted water that was used successfully to maintain *H. azteca* in culture; however, some laboratories have not had success with reproduction of the *H. azteca* when using this reconstituted water in the 42-d test (T.J. Norberg-King, USEPA, Duluth, MN, personal communication). Research is ongoing to develop additional types of reconstituted waters suitable for *H. azteca*. Until an acceptable reconstituted water has been developed for long-term exposures with *H. azteca,* a natural water demonstrated to support adequate survival, growth, and reproduction of amphipods is recommended for use in long-term *H. azteca* exposures (Section 14.2; Ingersoll et al., 1998; Kemble et al., 1998, 1999).

7.1.3.5 Synthetic Seawater

7.1.3.5.1 Reconstituted salt water can be prepared by adding commercial sea salts, such as FORTY FATHOMS®, HW MARINEMIX®, INSTANT OCEAN®, or equivalent to deionized water.

7.1.3.5.2 A synthetic seawater formulation called GP2 is prepared with reagent grade chemicals that can be diluted with deionized water to the desired salinity (USEPA, 1994d).

7.1.3.5.3 Ingersoll et al. (1992) describe procedures for culturing *H. azteca* at salinities up to 15 ‰. Reconstituted salt water was prepared by adding INSTANT OCEAN® salts to a 25:75 (v/v) mixture of freshwater (hardness 283 mg/L as $CaCO_3$) and deionized water that was held at least two weeks before use. Synthetic seawater was conditioned by adding 6.2 mL of Frit-zyme® #9 nitrifying bacteria (*Nitromonas* sp. and *Nitrobacter* sp.; Fritz Chemical Company, Dallas, TX) to each liter of water. The cultures were maintained by using renewal procedures; 25% of the culture water was replaced weekly. *Hyalella azteca* have been used to evaluate the toxicity of estuarine sediments up to 15 ‰ salinity in 10-d exposures (Nebeker and Miller, 1988; Roach et al., 1992; Winger et al., 1993; Ingersoll et al., 1996).

7.2 Formulated Sediment

7.2.1 General Requirements

7.2.1.1 Formulated sediments are mixtures of materials that mimic the physical components of natural sediments. Formulated sediments have not been routinely applied to evaluate sediment contamination. A primary use of formulated sediment could be as a control sediment. Formulated sediments allow for standardization of sediment testing or provide a basis for conducting sediment research. Formulated sediment provides a basis by which any testing program can assess the acceptability of their procedures and facilities. In addition, formulated sediment provides a consistent measure evaluating performance-based criteria necessary for test acceptability. The use of formulated sediment eliminates interferences caused by the presence of indigenous organisms. For toxicity tests with sediments spiked with specific chemicals, the use of a formulated sediment eliminates or controls the variation in sediment physico-chemical characteristics and provides a consistent method for evaluating the fate of chemicals in sediment. See USEPA (1999) and ASTM (1999b) for additional detail regarding uses of formulated sediment.

7.2.1.2 A formulated sediment should (1) support the survival, growth, or reproduction of a variety of benthic invertebrates, (2) provide consistent acceptable biological endpoints for a variety of species, and (3) be composed of materials that have consistent characteristics. Consistent material characteristics include (1) consistency of materials from batch to batch, (2) contaminant concentrations below concentrations of concern, and (3) availability to all individuals and facilities (Kemble et al., 1999).

7.2.1.3 Physico-chemical characteristics that might be considered when evaluating the appropriateness of a formulated sediment include percent sand, percent clay, percent silt, organic carbon content, cation exchange

capacity (CEC), oxidation reduction potential (redox), pH, and carbon:nitrogen:phosphorus ratios.

7.2.2 Sources of Materials

7.2.2.1 A variety of methods describe procedures for making formulated sediments. These procedures often use similar constituents; however, they often include either a component or a formulation step that would result in variation from test facility to test facility. In addition, most of the procedures have not been subjected to standardization and consensus approval or round-robin (ring) testing. The procedure outlined by Kemble et al. (1999) below was evaluated in round-robin testing with *Hyalella azteca* and *Chironomus tentans* (Section 17.6).

7.2.2.2 Most formulated sediments include sand and clay/silt that meet certain specifications; however, some may be quite different. For example, three sources of clay and silt include Attagel® 50, ASP® 400, and ASP® 400P. Table 7.1 summarizes the characteristics of these materials. The percentage of clay ranges from 56.5 to 88.5 and silt ranges from 11.5 to 43.5. These characteristics should be evaluated when considering the materials to use in a formulated sediment.

Table 7.1 Characteristics of Three Sources of Clays and Silts Used in Formulated Sediments

Characteristic	Attagel® 50	ASP® 400	ASP® 400P
% Sand	0.0	0.01	0.0
% Clay	88.50	68.49	56.50
% Silt	11.50	31.50	43.50
Soil class	Clay	Clay	Silty clay

Note: Table 7.3 lists suppliers for these materials.

7.2.2.3 A critical component of formulated sediment is the source of organic carbon. Many procedures have used peat as the source of organic carbon. Other sources of organic carbon listed in Table 7.2 have been evaluated including humus, potting soil, maple leaves, composted cow manure, rabbit chow, cereal leaves, chlorella, trout chow, Tetramin®, Tetrafin®, and alpha cellulose. Only peat, humus, potting soil, composted cow manure, and alpha cellulose have been used successfully without fouling the overlying water in sediment testing (Kemble et al., 1999). The other sources of organic carbon listed in Table 7.2 caused dissolved oxygen concentrations to fall to unacceptable levels (Kemble et al., 1999). Kemble et al. (1999) reported that conditioning of formulated sediment was not necessary when alpha cellulose was used as a source of organic carbon to prepare sediment for use as a negative control. In addition, alpha cellulose is a consistent source of organic carbon that is relatively biologically inactive and low in concentrations of chemicals of concern. It is one of three forms of cellulose (alpha, beta, and gamma) that differ in their degree of polymerization. Alpha cellulose has the highest degree of polymerization and is the chief constituent of paper pulp. The beta and gamma forms have a much lower degree of polymerization and are known as hemicellulose. Hence, compared with other sources of organic carbon, alpha cellulose would not serve as a food source, but would serve as an organic carbon constituent for sediment to add texture or to provide a partitioning compartment for chemicals. Using alpha cellulose as a source of organic carbon for sediment-spiking studies has not been adequately evaluated. Recent work conducted by J. Besser (USGS, Columbia, MO, unpublished data) indicated that using alpha cellulose as a source or organic carbon in 21-d studies resulted in some generation of sulfide in the pore water, which may affect the bioavailability of metals spiked in sediment.

Table 7.2. Carbon, Nitrogen, Phosphorus Levels for Various Sources of Organic Carbon (Kemble et al., 1998a)

Organic carbon Source	Carbon (%)	Nitrogen (mg/g)	Phosphorus (µg/g)
Peat	47	4	0.4
Maple leaves 1	42	6	1.3
Maple leaves 2	47	3	1.7
Cow manure	30	11	8.2
Rabbit chow	40	18	0.2
Humic acid	40	3	ND[1]
Cereal leaves	47	4	0.4
Chlorella	40	41	5.7
Trout chow	43	36	11.0
Tetramin®	37	45	9.6
Tetrafin®	36	29	8.6
Alpha cellulose	30	0.7	ND

[1] Not detected.

7.2.2.4 An important consideration in the selection of an organic carbon source may be the ratio of carbon to nitrogen to phosphorus. As demonstrated in Table 7.2, percentage carbon ranged from 30 to 47, nitrogen ranged from 0.7 to 45 mg/g, and phosphorus ranged from below detection to 11 µg/g for several different carbon sources. These characteristics should be evaluated when considering the materials to use in a formulated sediment.

7.2.3 Procedure

7.2.3.1 A summary of various procedures that have been used to formulate sediment are listed below. Suppliers of various components are listed in Table 7.3.

1. Walsh et al. (1981): (1) Wash sand (Mystic White No. 85, 45, and 18—New England Silica Inc.; Note: Mystic White sands are no longer available. Kemble et al. (1999) found White Quartz sand to be an acceptable substitute; Table 7.3) and sieve into three grain sizes: coarse (500 to 1500 mm); medium (250 to 499 mm); and fine (63 to 249 mm). (2) Obtain clay and silt from Engelhard Corp. (3) Mill and sieve peat moss through an 840-mm screen. (4) Mix constituents dry in the following quantities: coarse sand (0.6%); medium sand (8.7%); fine sand (69.2%); silt (10.2%); clay (6.4%); and organic matter (4.9%).

Table 7.3 Sources of Components Used in Formulated Sediments

Component	Sources
Sand	• White Quartz sand #1 dry, #2, #3—New England Silica, Inc., South Windsor, CT (Note: Mystic White sands are no longer available. Kemble et al. (1999) found White Quartz sand to be an acceptable substitute).
	• Product No. 33094, BDH Chemical, Ltd., Poole, England
Kaolinite	• ASP 400, ASP 400P, ASP 600, ASP 900—Englehard Corporation, Edison, NJ
	• Product No. 33059, BDH Chemical, Ltd., Poole, England
Montmorillonite	• W.D. Johns, Source Clays, University of Missouri, Columbia, MO
Clay	• Lewiscraft Sculptor's Clay, available in hobby and artist supply stores
Humus	• Sims Bark Co., Inc., Tuscumbia, AL
Alpha cellulose	• Sigma Co., St. Louis, MO
Peat	• D.L. Browning Co., Mather, WI
	• Joseph Bentley, Ltd., Barrow-on-Humber, South Humberside, England
	• Mellinger's, North Lima, OH
Potting soil	• Zehr's No Name Potting Soil, Mississauga, Ontario
Humic acid	• Aldrich Chemical Co, Milwaukee, WI
Cow manure	• A.H. Hoffman, Inc., Landisville, PA
Dolomite	• Ward's Natural Science Establishment, Inc., Rochester, NY

2. Harrahy and Clements (1997): (1) Rinse peat moss then soak for 5 d in deionized water renewing water daily. (2) After acclimation for 5 d, remove all water and spread out to dry. (3) Grind moss and sieve using the following sieve sizes: 1.18 mm (discard these particles); 1.00 mm (average size 1.09 mm); 0.85 mm (average size 0.925); 0.60 (average size 0.725); 0.425 mm (average size 0.5125 mm); retainer (average size 0.2125 mm). (4) Use a mixture of sizes that provides an average particle size of 840 mm. (5) Wash medium quartz sand and dry. (6) Obtain clay and silt using ASP 400 (Englehard Corp). (7) Mix constituents dry in the following quantities: sand (850 g); silt and clay (150 g); dolomite (0.5 g); sphagnum moss (22 g); and humic acid (0.1g). (8) Mix sediment for an hour on a rolling mill and store dry until ready for use.

3. Hanes et al. (1991): (1) Sieve sand and retain two particle sizes (90 to 180 um and 180 to 250 um) which are mixed in a ratio of 2:1. (2) Dry potting soil for 24 h at room temperature and sieve through a 1-mm screen. Clay is commercially available sculptors clay. (3) Determine percent moisture of clay and soil after drying for 24 h at 60 to 100°C (correct for percent moisture when mixing materials). (4) Mix constituents by weight in the following ratios: sand mixture (42%); clay (42%); and soil (16%). (5) Autoclave after mixing in a foil-covered container for 20 min. Mixture can be stored indefinitely if kept covered after autoclaving.

4. Naylor (1993): (1) Sieve acid-washed sand to obtain a 40- to 100-mm size. (2) Obtain clay as kaolin light. (3) Grind and sieve peat moss using a 2-mm screen (peat moss which is allowed to dry out will not rehydrate and will float on the water surface). (4) Adjust for the use of moist peat moss by determining moisture content (dry 5 samples of peat at 60°C until constant weight is achieved). (5) Mix constituents by weight in the following percentages: sand (69%); kaolin (20%); peat (10% [adjust for moisture content]); and $CaCO_3$ (1%). (6) Mix for 2 h in a soil shaker and store in sealed containers.

5. Suedel and Rodgers (1994): (1) Sieve sand (Mystic White #18 and 90; Note: Mystic White sands are no longer available. Kemble et al. (1999) found White Quartz sand to be an acceptable substitute; Table 7.3) to provide three different size fractions: coarse (2.0 to 0.5 mm), medium (0.5 to 0.25 mm) and fine (0.25 to 0.05 mm). (2) Ash silt (ASP 400), clay (ASP 600 and 900), montmorillonite clay, and dolomite at 550°C for 1 h to remove organic matter. (3) Dry humus (70°C) and mill to 2.0 mm. (4) Add dolomite as 1% of the silt requirement. (5) Age materials for 7 d in flowing water before mixing. (6) Mix constituents to mimic the desired characteristics of the sediment of concern.

6. Kemble et al. (1999) describe procedures for making a variety of formulated sediments ranging in grain size and organic carbon. A sediment with 19% sand and 2% organic carbon was produced by combining: (1) 219 grams of sand (White Quartz #1 dry), (2) 1242 grams of a silt-clay mixture (ASP 400), (3) 77.3 grams of alpha cellulose, (4) 0.15 grams of humic

acid, and (5) 7.5 grams of dolomite (the dolomite is a source of bicarbonate buffering that occurs naturally in soils and sediments). Steps for processing the sand before use include: (1) rinsing sand with gentle mixing in well water (hardness 283 mg/L as $CaCO_3$, alkalinity 255 mg/L as $CaCO_3$, pH 7.8) until the water runs clear, (2) rinsing the sand for 5 min with deionized water, and (3) air drying the sand. Constituents are mixed for 1 h on a rolling mill and stored dry until ready for use (i.e., no conditioning required). When formulated sediments are made with a high silt-clay content, the alkalinity and hardness of the pore water may drop due to cation exchange. Gentle mixing of the formulated sediment with overlying water before use in testing reduces this change in the water quality characteristics of the pore water.

7.3 Reagents

7.3.1 Data sheets should be followed for reagents and other chemicals purchased from supply houses. The test material(s) should be at least reagent grade, unless a test using a formulated commercial product, technical-grade, or use-grade material is specifically needed. Reagent containers should be dated when received from the supplier, and the shelf life of the reagent should not be exceeded. Working solutions should be dated when prepared and the recommended shelf life should not be exceeded.

7.4 Standards

7.4.1 Appropriate standard methods for chemical and physical analyses should be used when possible. For those measurements for which standards do not exist or are not sensitive enough, methods should be obtained from other reliable sources.

Section 8
Sample Collection, Storage, Manipulation, and Characterization

8.1 Collection

8.1.1 Before the preparation or collection of sediment, a procedure should be established for the handling of sediment that might contain unknown quantities of toxic chemicals (Section 5).

8.1.2 Sediments are spatially and temporally variable (Stemmer et al., 1990a). Replicate samples should be collected to determine variance in sediment characteristics. Sediment should be collected with as little disruption as possible; however, subsampling, compositing, or homogenization of sediment samples might be necessary for some experimental designs. Sampling can cause loss of sediment integrity, change in chemical speciation, or disruption of chemical equilibrium (ASTM, 1999b). A benthic grab or core should be used rather than a dredge to minimize disruption of the sediment sample. Sediment should be collected from a depth that will represent expected exposure. For example, oligochaetes may burrow 4 to 15 cm into sediment. Samples collected for evaluations of dredged material should include sediment cores to the depth of removal. Surveys of the toxicity of surficial sediment are often based on cores of the upper 2 cm sediment depth.

8.1.3 Exposure to direct sunlight during collection should be minimized, especially if the sediment contains photolytic compounds. Sediment samples should be cooled to 4°C in the field before shipment (ASTM, 1999b). Dry ice can be used to cool samples in the field; however, sediments should never be frozen. Monitors can be used to measure temperature during shipping (e.g., TempTale Temperature Monitoring and Recording System, Sensitech, Inc., Beverly, MA).

8.1.4 For additional information on sediment collection and shipment see USEPA (1999) and ASTM (1999b).

8.2 Storage

8.2.1 Since the contaminants of concern and influencing sediment characteristics are not always known, it is desirable to hold the sediments after collection in the dark at 4°C. Traditional convention has held that toxicity tests should be started as soon as possible following collection from the field, although actual recommended storage times range from two weeks (ASTM, 1999b) to less than eight weeks (USEPA-USACE, 1998a). Discrepancies in recommended storage times reflected a lack of data concerning the effects of long-term storage on the physical, chemical, and toxicological characteristics of the sediment. However, numerous studies have recently been conducted to address issues related to sediment storage (Dillon et al., 1994; Becker and Ginn, 1995; Carr and Chapman, 1995; Moore et al., 1996; Sarda and Burton, 1995; Sijm et al., 1997; DeFoe and Ankley, 1998). The conclusions and recommendations offered by these studies vary substantially and appear to depend primarily upon the type or class of contaminant(s) present. Considered collectively, these studies suggest that the recommended guidance that sediments be tested sometime between the time of collection and 8 weeks storage is appropriate. Additional guidance is provided below.

8.2.2 Extended storage of sediments that contain high concentrations of labile chemicals (e.g., ammonia, volatile organics) may lead to a loss of these chemicals and a corresponding reduction in toxicity. Under these circumstances, the sediment should be tested as soon as possible after collection, but not later than within two weeks (Sarda and Burton, 1995). Sediments that exhibit low-level to moderate toxicity can exhibit considerable temporal variability in toxicity, although the direction of change is often unpredictable (Carr and Chapman, 1995; Moore et al., 1996; DeFoe and Ankley, 1998). For these types of sediments, the recommended storage time of <8 weeks may be most appropriate. In some situations, a minimum storage period for low-to-moderately contaminated sediments may help reduce variability. For example, DeFoe and Ankley (1998) observed high variability in survival during early testing periods (e.g., <2 weeks) in sediments with low toxicity. DeFoe and Ankley (1998) hypothesized that this variability partially reflected the presence of indigenous predators that remained alive during this relatively short storage period. Thus, if predatory species are known to exist, and the sediment does not contain labile contaminants, it may be desirable to store the sediment for a short period before testing (e.g., 2 weeks) to reduce potential for interferences from indigenous organisms. Sediments that contain comparatively stable compounds (e.g., high molecular weight compounds such as PCBs) or which exhibit a moderate-to-high level of toxicity, typically do not vary appreciably in toxicity in relation to storage duration (Moore et al., 1996; DeFoe and Ankley, 1998). For these sediments, long-term storage (e.g., >8 weeks) can be undertaken.

8.2.3 Researchers may wish to conduct additional characterizations of sediment to evaluate possible effects of storage. Concentrations of chemicals of concern could be measured periodically in pore water during the storage period and at the start of the sediment test (Kemble et al., 1994). Ingersoll et al. (1993) recommend conducting a toxicity test with pore water within two weeks from sediment collection and at the start of the sediment test. Freezing might further change sediment properties such as grain size or chemical partitioning and should be avoided (ASTM, 1999b; Schuytema et al., 1989). Sediment should be stored with no air over the sealed samples (no head space) at 4°C before the start of a test (Shuba et al.,1978). Sediment may be stored in containers constructed of suitable materials as outlined in Section 6.

8.3 Manipulation

8.3.1 Homogenization

8.3.1.1 Samples tend to settle during shipment. As a result, water above the sediment should not be discarded but should be mixed back into the sediment during homogenization. Sediment samples should not be sieved to remove indigenous organisms unless there is a good reason to believe indigenous organisms may influence the response of the test organism. However, large indigenous organisms and large debris can be removed using forceps. Reynoldson et al. (1994) observed reduced growth of amphipods, midges, and mayflies in sediments with elevated numbers of oligochaetes and recommended sieving sediments suspected to have high numbers of indigenous oligochaetes. If sediments must be sieved, it may be desirable to analyze samples before and after sieving (e.g., pore-water metals, DOC, AVS, TOC) to document the influence of sieving on sediment chemistry.

8.3.1.2 If sediment is collected from multiple field samples, the sediment can be pooled and mixed by stirring or using a rolling mill, feed mixer, or other suitable apparatus (see ASTM, 1999b). Homogenization of sediment can be accomplished using a variable-speed hand-held drill outfitted with a stainless-steel auger (diameter 7.6 cm, overall length 38 cm, auger bit length 25.4 cm; Part No. 800707, Augers Unlimited, Exton, PA; Kemble et al., 1994).

8.3.2 Sediment Spiking

8.3.2.1 Test sediment can be prepared by manipulating the properties of a control sediment. Mixing time (Stemmer et al., 1990a) and aging (Word et al., 1987; Landrum, 1989; Landrum and Faust, 1992) of spiked sediment can affect bioavailability of chemicals in sediment. Many studies with spiked sediment are often started only a few days after the chemical has been added to the sediment. This short time period may not be long enough for sediments to equilibrate with the spiked chemicals (Section 8.3.2.2.3). Consistent spiking procedures should be followed in order to make interlaboratory comparisons. See USEPA (1999) and ASTM (1999b) for additional detail regarding sediment spiking.

8.3.2.1.1 The cause of sediment toxicity and the magnitude of interactive effects of chemicals can be estimated by spiking a sediment with chemicals or complex waste mixtures (Lamberson and Swartz, 1992). Sediments spiked with a range of concentrations can be used to generate either point estimates (e.g., LC50) or a minimum concentration at which effects are observed (lowest observed effect concentration; LOEC). Results of tests may be reported in terms of a BSAF (Ankley et al., 1992b). The influence of sediment physico-chemical characteristics on chemical toxicity can also be determined with sediment-spiking studies (Adams et al., 1985).

8.3.2.2 The test material(s) should be at least reagent grade, unless a test using a formulated commercial product, technical-grade, or use-grade material is specifically needed. Before a test is started, the following should be known about the test material: (1) the identity and concentration of major ingredients and impurities, (2) water solubility in test water, (3) log Kow, BCF (from other test species), persistence, hydrolysis, and photolysis rates of the test substances, (4) estimated toxicity to the test organism and to humans, (5) if the test concentration(s) are to be measured, the precision and bias of the analytical method at the planned concentration(s) of the test material, and (6) recommended handling and disposal procedures. Addition of test material(s) to sediment may be accomplished using various methods, such as a (1) rolling mill, (2) feed mixer, or (3) hand mixing (ASTM, 1999b; USEPA, 1999). Modifications of the mixing techniques might be necessary to allow time for a test material to equilibrate with the sediment. Mixing time of spiked sediment should be limited from minutes to a few hours, and temperature should be kept low to minimize potential changes in the physico-chemical and microbial characteristics of the sediment (ASTM, 1999b). Duration of contact between the chemical and sediment can affect partitioning and bioavailability (Word et al., 1987). Care should be taken to ensure that the chemical is thoroughly and evenly distributed in the sediment. Analyses of sediment subsamples are advisable to determine the degree of mixing homogeneity (Ditsworth et al., 1990). Moreover, results from sediment-spiking studies should be compared to the response of test organisms to chemical concentrations in natural sediments (Lamberson and Swartz, 1992).

8.3.2.2.1 Organic chemicals have been added: (1) directly in a dry (crystalline) form; (2) coated on the inside walls of the container (Ditsworth et al., 1990); or (3) coated onto silica sand (e.g., 5% w/w of sediment) which is added to the sediment (D.R. Mount, USEPA, Duluth, MN, personal communication). In techniques 2 and 3, the chemical is dissolved in solvent, placed in a glass spiking container (with or without sand), then the solvent is slowly evaporated. The advantage of these three approaches is that no solvent is introduced to the sediment, only the chemical being spiked. When testing spiked sediments, procedural blanks (sediments that have been handled in the same way, including solvent addition and evaporation, but contain no added chemical) should be tested in addition to regular negative controls.

8.3.2.2.2 Metals are generally added in an aqueous solution (ASTM, 1999b; Carlson et al., 1991; Di Toro et al., 1990). Ammonia has also been successfully spiked using aqueous solutions (Besser et al., 1998). Inclusion of spiking blanks is recommended.

8.3.2.2.3 Sufficient time should be allowed after spiking for the spiked chemical to equilibrate with sediment components. For organic chemicals, it is recommended that the sediment be aged at least one month before starting a test. Two months or more may be necessary for chemicals with a high log Kow (e.g., >6; D.R. Mount, USEPA, Duluth, MN, personal communication). For metals, shorter aging times (1 to 2 weeks) may be sufficient. Periodic monitoring of chemical concentrations in pore water during sediment aging is highly recommended as a means to assess the equilibration of the spiked sediments. Monitoring of pore water during spiked sediment testing is also recommended.

8.3.2.3 Direct addition of a solvent (other than water) to the sediment should be avoided if possible. Addition of organic solvents may dramatically influence the concentration of dissolved organic carbon in pore water. If an organic solvent is to be used, the solvent should be at a concentration that does not affect the test organism. Further, both solvent control and negative control sediments must be included in the test. The solvent control must contain the highest concentration of solvent present and must be from the same batch used to make the stock solution (see ASTM, 1999e).

8.3.2.4 If the test contains both a negative control and a solvent control, the survival, growth, or reproduction of the organisms tested should be compared. If a statistically significant difference is detected between the two controls, only the solvent control may be used for meeting the acceptability of the test and as the basis for calculating results. The negative control might provide additional information on the general health of the organisms tested. If no statistically significant difference is detected, the data from both controls should be used for meeting the acceptability of the test and as the basis for calculating the results (ASTM, 1999f). If performance in the solvent control is markedly different from that in the negative control, it is possible that the data are compromised by experimental artifacts and may not accurately reflect the toxicity of the chemical in natural sediments.

8.3.3 Test Concentration(s) for Laboratory Spiked Sediments

8.3.3.1 If a test is intended to generate an LC50, a toxicant concentration series (0.5 or higher) should be selected that will provide partial mortalities at two or more concentrations of the test chemical. The LC50 of a particular compound may vary depending on physical and chemical sediment characteristics. It may be desirable to conduct a range-finding test in which the organisms are exposed to a control and three or more concentrations of the test material that differ by a factor of ten. Results from water-only tests could be used to establish concentrations to be tested in a whole-sediment test based on predicted pore-water concentrations (Di Toro et al., 1991).

8.3.3.2 Bulk-sediment chemical concentrations might be normalized to factors other than dry weight. For example, concentrations of nonpolar organic compounds might be normalized to sediment organic-carbon content, and simultaneously extracted metals might be normalized to acid volatile sulfides (Di Toro et al., 1990; Di Toro et al., 1991).

8.3.3.3 In some situations it might be necessary to simply determine whether a specific concentration of test material is toxic to the test organism, or whether adverse effects occur above or below a specific concentration. When there is interest in a particular concentration, it might only be necessary to test that concentration and not to determine an LC50.

8.4 Characterization

8.4.1 All sediments should be characterized and at least the following determined: pH and ammonia of the pore water, organic carbon content (total organic carbon, TOC), particle size distribution (percent sand, silt, clay), and percent water content (ASTM, 1999a; Plumb, 1981). See Section 8.4.4.7 for methods to isolate pore water.

8.4.2 Other analyses on sediments might include biological oxygen demand, chemical oxygen demand, cation exchange capacity, Eh, total inorganic carbon, total volatile solids, acid volatile sulfides, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, as well as interstitial water analyses for various physicochemical parameters.

8.4.3 Macrobenthos may be evaluated by subsampling the field-collected sediment. If direct comparisons are to be made, subsamples for toxicity testing should be collected from the same sample to be used for analysis of sediment physical and chemical characterizations. Qualitative descriptions of the sediment can include color, texture, and presence of macrophytes or animals. Monitoring the odor of sediment samples should be avoided because of potential hazardous volatile chemicals.

8.4.4 Analytical Methodology

8.4.4.1 Chemical and physical data should be obtained using appropriate standard methods whenever possible. For those measurements for which standard methods do not exist or are not sensitive enough, methods should be obtained from other reliable sources.

8.4.4.2 The precision, accuracy, and bias of each analytical method used should be determined in the appropriate matrix: that is, sediment, water, tissue. Reagent blanks and analytical standards should be analyzed, and recoveries should be calculated.

8.4.4.3 Concentration of spiked test material(s) in sediment, interstitial water, and overlying water should be

measured as often as practical during a test. If possible, the concentration of the test material in overlying water, interstitial water and sediments should be measured at the start and end of a test. Measurement of test material(s) degradation products might also be desirable.

8.4.4.4 Separate chambers should be set up at the start of a test and destructively sampled during and at the end of the test to monitor sediment chemistry. Test organisms and food should be added to these extra chambers.

8.4.4.5 Measurement of test material(s) concentration in water can be accomplished by pipeting water samples from about 1 to 2 cm above the sediment surface in the test chamber. Overlying water samples should not contain any surface debris, any material from the sides of the test chamber, or any sediment.

8.4.4.6 Measurement of test material(s) concentration in sediment at the end of a test can be taken by siphoning most of the overlying water without disturbing the surface of the sediment, then removing appropriate aliquots of the sediment for chemical analysis.

8.4.4.7 Interstitial water

8.4.4.7.1 Interstitial water (pore water), defined as the water occupying the spaces between sediment or soil particles, is often isolated to provide either a matrix for toxicity testing or to provide an indication of the concentration or partitioning of contaminants within the sediment matrix. Draft USEPA equilibrium partitioning sediment guidelines (ESGs) are based on the presumption that the concentration of chemicals in the interstitial water are correlated directly to their bioavailability and, therefore, their toxicity (Di Toro et al., 1991). Of additional importance is contaminants in interstitial waters can be transported into overlying waters through diffusion, bioturbation, and resuspension processes (Van Rees et al., 1991). The usefulness of interstitial water sampling for determining chemical contamination or toxicity will depend on the study objectives and nature of the sediments at the study site.

8.4.4.7.2 Isolation of sediment interstitial water can be accomplished by a wide variety of methods, which are based on either physical separation or on diffusion/equilibrium. The common physical-isolation procedures can be categorized as: (1) centrifugation, (2) compression/squeezing, or (3) suction/vacuum. Diffusion/equilibrium procedures rely on the movement (diffusion) of pore-water constituents across semipermeable membranes into a collecting chamber until an equilibrium is established. A description of the materials and procedures used in the isolation of pore water is included in the reviews by Bufflap and Allen (1995a), ASTM (1999b), and USEPA (1999).

8.4.4.7.3 When relatively large volumes of water are required (>20 mL) for toxicity testing or chemical analyses, appropriate quantities of sediment are generally collected with grabs or corers for subsequent isolation of the interstitial water. Several isolation procedures, such as centrifugation (Ankley and Scheubauer-Berigan, 1994), squeezing (Carr and Chapman, 1995) and suction (Winger and Lasier, 1991; Winger et al., 1998), have been used successfully to obtain adequate volumes for testing purposes. Peepers (dialysis) generally do not produce sufficient volumes for most analyses; however, larger sized peepers (500-mL volume) have been used for collecting interstitial water *in situ* for chemical analyses and organism exposures (Burton, 1992; Sarda and Burton, 1995).

8.4.4.7.4 There is no one superior method for the isolation of interstitial water used for toxicity testing and associated chemical analyses. Factors to consider in the selection of an isolation procedure may include: (1) volume of pore water needed, (2) ease of isolation (materials, preparation time, and time required for isolation), and (3) artifacts in the pore water caused by the isolation procedure. Each approach has unique strengths and limitations (Bufflap and Allen, 1995a, 1995b; Winger et al., 1998), which vary with sediment characteristics, chemicals of concern, toxicity test methods, and desired test resolution (i.e., data quality objectives). For suction or compression separation, which uses a filter or a similar surface, there may be changes to the characteristics of the interstitial water compared with separation using centrifugation (Ankley et al., 1994; Horowitz et al., 1996). For most toxicity test procedures, relatively large volumes of interstitial water (e.g., liters) are frequently needed for static or renewal exposures with the associated water chemistry analyses. Although centrifugation can be used to generate large volumes of interstitial water, it is difficult to use centrifugation to isolate water from coarser sediment. If smaller volumes of interstitial water are adequate and logistics allow, it may be desirable to use peepers, which establish an equilibrium with the pore water through a permeable membrane. If logistics do not allow placement of peeper samplers, an alternative procedure could be to collect cores that can be sampled using side port suctioning or centrifugation (G.A. Burton, Wright State University, personal communication). However, if larger samples of interstitial water are needed, it would be necessary to collect multiple cores as quickly as possible using an inert environment and to centrifuge samples at ambient temperatures. See USEPA (1999) and ASTM (1999b) for additional detail regarding isolation of interstitial water.

Section 9
Quality Assurance and Quality Control

9.1 Introduction

9.1.1 Developing and maintaining a laboratory quality assurance (QA) program requires an ongoing commitment by laboratory management and also includes the following: (1) appointment of a laboratory quality assurance officer with the responsibility and authority to develop and maintain a QA program, (2) preparation of a Quality Assurance Project Plan with Data Quality Objectives, (3) preparation of written descriptions of laboratory Standard Operating Procedures (SOPs) for test organism culturing, testing, instrument calibration, sample chain-of-custody, laboratory sample tracking system, and (4) provision of adequate, qualified technical staff and suitable space and equipment to assure reliable data. Additional guidance for QA can be obtained in USEPA (1989d) and in USEPA (1994e).

9.1.2 QA practices within a testing laboratory should address all activities that affect the quality of the final data, such as (1) sediment sampling and handling, (2) the source and condition of the test organisms, (3) condition and operation of equipment, (4) test conditions, (5) instrument calibration, (6) replication, (7) use of reference toxicants, (8) record keeping, and (9) data evaluation.

9.1.3 Quality control (QC) practices, on the other hand, consist of the more focused, routine, day-to-day activities carried out within the scope of the overall QA program. For more detailed discussion of quality assurance, and general guidance on good laboratory practices related to testing see FDA (1978), USEPA (1979a), USEPA (1980a), USEPA (1980b), USEPA (1991a), USEPA (1994c), USEPA (1994d), USEPA (1995), DeWoskin (1984), and Taylor (1987).

9.2 Performance-based Criteria

9.2.1 USEPA Environmental Monitoring Management Council (EMMC) recommended the use of performance-based methods in developing standards for chemical analytical methods (Williams, 1993). Performance-based methods were defined by EMMC as a monitoring approach that permits the use of appropriate methods that meet pre-established demonstrated performance standards. Minimum required elements of performance, such as precision, reproducibility, bias, sensitivity, and detection limits should be specified, and the method should be demonstrated to meet the performance standards.

9.2.2 Participants at a September 1992 USEPA sediment toxicity workshop arrived at a consensus on several culturing and testing methods for freshwater organisms (Appendix A of USEPA, 1994a). In developing guidance for culturing test organisms to be included in this manual for sediment tests, it was generally agreed that no single method must be used to culture organisms. Success of a test relies on the health of the culture from which organisms are taken for testing. Having healthy organisms of known quality and age for testing is the key consideration relative to culture methods. Therefore, a performance-based criteria approach is the preferred method through which individual laboratories should evaluate culture health rather than using control-based criteria. Performance-based criteria were chosen to allow each laboratory to optimize culture methods while providing organisms that produce reliable and comparable test results. See Tables 11.3, 12.3, 13.4, 14.3 and 15.3 for a listing of performance criteria for culturing and testing.

9.3 Facilities, Equipment, and Test Chambers

9.3.1 Separate areas for test organism culturing and testing must be provided to avoid loss of cultures due to cross-contamination. Ventilation systems should be designed and operated to prevent recirculation or leakage of air from chemical analysis laboratories or sample storage and preparation areas into test organism culturing or sediment testing areas, and from sediment testing laboratories and sample preparation areas into culture rooms.

9.3.2 Equipment for temperature control should be adequate to maintain recommended test-water temperatures. Recommended materials should be used in the fabricating of the test equipment that comes in contact with the sediment or overlying water.

9.3.3 Before a sediment test is conducted in a new facility, a “noncontaminant” test should be conducted in which all test chambers contain a control sediment and overlying water. This information is used to demonstrate that the facility, control sediment, water, and handling procedures provide acceptable responses of test organisms (See Section 9.14).

9.4 Test Organisms

9.4.1 The organisms should appear healthy, behave normally, feed well, and have low mortality in cultures, during holding (e.g., <20% for 48 h before the start of a test), and in test controls. The species of test organisms should be positively identified to species.

9.5 Water

9.5.1 The quality of water used for organism culturing and testing is extremely important. Overlying water used in testing and water used in culturing organisms should be uniform in quality. Acceptable water should allow satisfactory survival, growth, or reproduction of the test organisms. Test organisms should not show signs of disease or apparent stress (e.g., discoloration, unusual behavior). See Section 7 for additional details.

9.6 Sample Collection and Storage

9.6.1 Sample holding times and temperatures should conform to conditions described in Section 8.

9.7 Test Conditions

9.7.1 It is desirable to measure temperature continuously in at least one chamber during each test. Temperatures should be maintained within the limits specified for each test. Dissolved oxygen, alkalinity, water hardness, conductivity, ammonia, and pH should be checked as prescribed in Sections 11.3, 12.3, 13.3, 14.3 and 15.3.

9.8 Quality of Test Organisms

9.8.1 It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (Section 9.16). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity to select chemicals. The requirement in the first edition of this manual for laboratories to conduct monthly reference-toxicity tests (USEPA, 1994a) has not been included as a requirement in this second edition for testing sediments because of the inability of reference-toxicity tests to identify stressed populations of test organisms (McNulty et al., 1999). Physiological measurements such as lipid content might also provide useful information regarding the health of the cultures.

9.8.2 It is desirable to determine the sensitivity of test organisms obtained from an outside source. The supplier should provide data with the shipment describing the history of the sensitivity of organisms from the same source culture. The supplier should also certify the species identification of the test organisms and provide the taxonomic references or name(s) of the taxonomic expert(s) consulted.

9.8.3 All organisms in a test must be from the same source (Section 10.2.2). Organisms may be obtained from laboratory cultures or from commercial or government sources (Table 10.1). The test organisms used should be identified using an appropriate taxonomic key, and verification should be documented (Pennak, 1989; Merritt and Cummins, 1996). Obtaining organisms from wild populations should be avoided unless organisms are cultured through several generations in the laboratory. In addition, the ability of the wild population of sexually reproducing organisms to cross breed with the existing laboratory population should be determined (Duan et al.,1997). Sensitivity of the wild population to select chemicals (e.g., Table 1.4) should also be documented.

9.9 Quality of Food

9.9.1 Problems with the nutritional suitability of the food will be reflected in the survival, growth, or reproduction of the test organisms in cultures or in sediment tests.

9.9.2 Food used to culture organisms used in bioaccumulation tests must be analyzed for compounds to be measured in the bioaccumulation tests.

9.10 Test Acceptability

9.10.1 Tables 11.3, 12.3, 13.4, 14.3 and 15.3 outline requirements for acceptability of the tests. An individual test may be conditionally acceptable if temperature, dissolved oxygen, and other specified conditions fall outside specifications, depending on the degree of the departure and the objectives of the tests (see test condition summaries in Tables 11.1, 12.1, 13.1, 14.1, and 15.1). The acceptability of a test will depend on the experience and professional judgment of the laboratory analyst and the reviewing staff of the regulatory authority. Any deviation from test specifications should be noted when reporting data from a test.

9.11 Analytical Methods

9.11.1 All routine chemical and physical analyses for culture and testing water, food, and sediment should include established quality assurance practices outlined in USEPA methods manuals (USEPA, 1979a; USEPA, 1979b; USEPA, 1991a; USEPA, 1994b).

9.11.2 Reagent containers should be dated when received from the supplier, and the shelf life of the reagent should not be exceeded. Working solutions should be dated when prepared and the recommended shelf life should not be exceeded.

9.12 Calibration and Standardization

9.12.1 Instruments used for routine measurements of chemical and physical characteristics such as pH, dissolved oxygen, temperature, and conductivity should be calibrated before use each day according to the instrument manufacturer's procedures as indicated in the general section on quality assurance (see USEPA Methods 150.1, 360.1, 170.1, and 120.1; USEPA, 1979b). Calibration data should be recorded in a permanent log.

9.12.2 A known-quality water should be included in the analyses of each batch of water samples (e.g., water hardness, alkalinity, conductivity). It is desirable to include certified standards in the analysis of water samples.

9.13 Replication and Test Sensitivity

9.13.1 The sensitivity of sediment tests will depend in part on the number of replicates/treatment, the significance level selected, and the type of statistical analysis. If the variability remains constant, the sensitivity of a test will increase as the number of replicates is increased. The minimum recommended number of replicates varies with the objectives of the test and the statistical method used for analysis of the data (Section 16).

9.14 Demonstrating Acceptable Performance

9.14.1 Intralaboratory precision, expressed as a coefficient of variation (CV) of the range in response for each type of test to be used in a laboratory, can be determined by performing five or more tests with different batches of test organisms using the same reference toxicant at the same concentrations with the same test conditions (e.g., the same test duration, type of water, age of test organisms, feeding) and the same data analysis methods. This should be done to gain experience for the toxicity tests and to serve as a point of reference for future testing. A reference-toxicity concentration series (0.5 or higher) should be selected that will provide partial mortalities at two or more concentrations of the test chemical (Section 8.3.3). Information from previous tests can be used to improve the design of subsequent tests to optimize the dilution series selected for future testing.

9.14.2 Before conducting tests with potentially contaminated sediment, it is strongly recommended that the laboratory conduct the tests with control sediment(s) alone. Results of these preliminary studies should be used to determine if use of the control sediment and other test conditions (i.e., water quality) result in acceptable performance in the tests as outlined in Tables 11.1, 12.1, 13.1, 14.1, and 15.1.

9.14.3 Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control sediment or test sediments and recovery could be determined after 1 h (Tomasovic et al., 1994).

9.15 Documenting Ongoing Laboratory Performance

9.15.1 Outliers, which are data falling outside the control limits, and trends of increasing or decreasing sensitivity are readily identified. If the reference-toxicity results from a given test fall outside the “expected” range (e.g., +2 SD), the sensitivity of the organisms and the credibility of the test results may be suspect. In this case, the test procedure should be examined for defects and should be repeated with a different batch of test organisms (Section 16).

9.15.2 A sediment test may be acceptable if specified conditions of a reference-toxicity test fall outside the expected ranges (Section 9.10.2). Specifically, a sediment test should not be judged unacceptable if the LC50 for a given reference-toxicity test falls outside the expected range or if mortality in the control of the reference-toxicity test exceeds 10%. All the performance criteria outlined in Tables 11.3, 12.3, 13.4, 14.3, and 15.3 must be considered when determining the acceptability of a sediment test. The acceptability of the sediment test would depend on the experience and judgment of the investigator and the regulatory authority.

9.15.3 Performance should improve with experience, and the control limits should gradually narrow, as the statistics stabilize. However, control limits of +2 SD, by definition, will be exceeded 5% of the time, regardless of how well a laboratory performs. For this reason, good laboratories that develop very narrow control limits may be penalized if a test result that falls just outside the control limits is rejected *de facto*. The width of the control limits should be considered in decisions regarding rejection of data (Section 17).

9.16 Reference Toxicants

9.16.1 Historically, reference-toxicity testing has been thought to provide three types of information relevant to the interpretation of toxicity test data: (1) an indication of the relative “health” of the organisms used in the test; (2) a demonstration that the laboratory can perform the test procedure in a reproducible manner; and (3) information to indicate whether the sensitivity of the particular strain or population in use at a laboratory is comparable to those in use in other facilities. With regard to the first type of information, recent work by McNulty et al. (1999) suggests that reference-toxicity tests may not be effective in identifying stressed populations of test organisms. In addition, reference-toxicity tests recommended for use with sediment toxicity tests are short-term, water column tests, owing in part to the lack of a standard sediment for reference-toxicity testing. Because the test procedures for reference-toxicity tests are not the same as for the sediment toxicity tests of interest, the applicability of reference-toxicity tests to demonstrate ability to reproducibly perform the sediment test procedures is greatly reduced. Particularly for the long-term sediment toxicity tests with *H. azteca* and *C. tentans*, performance of control organisms over time may be a better indicator of success in handling and testing these organisms (Sections 14 and 15).

9.16.2 Although the requirement for monthly testing has been removed in this second edition of the manual, periodic reference-toxicity testing should still be conducted as an indication of overall comparability of results among laboratories (at a minimum, six tests over a 3-year period should be conducted to evaluate potential differences in life stage or genetic strain of test organisms). In

particular, reference-toxicity tests should be performed more frequently when organisms are obtained from outside sources, when there are changes in culture practices, or when brood stock from an outside source is incorporated into a laboratory culture.

9.16.3 Reference toxicants such as sodium chloride (NaCl), potassium chloride (KCl), cadmium chloride ($CdCl_2$), and copper sulfate ($CuSO_4$) are suitable for use. No one reference toxicant can be used to measure the sensitivity of test organisms with respect to another toxicant with a different mode of action (Lee, 1980). However, it may be unrealistic to test more than one or two reference toxicants routinely. KCl has been used successfully in round-robin water-only exposures with *H. azteca* and *C. tentans* (Section 17).

9.16.4 Test conditions for conducting reference-toxicity tests with *H. azteca, C. tentans,* and *L. variegatus* are outlined in Tables 9.1 and 9.2. Reference-toxicity tests can be conducted using one organism/chamber or multiple organisms in each chamber. Some laboratories have observed low control survival when more than one midge/chamber is tested in water-only exposures.

9.17 Record Keeping

9.17.1 Section 16.1 outlines recommendations for record keeping (i.e., data files, chain-of-custody).

Table 9.1 Recommended Test Conditions for Conducting Reference-toxicity Tests with One Organism/Chamber

Parameter	Conditions
1. Test type:	Water-only test
2. Dilution series:	Control and at least 5 test concentrations (0.5 dilution factor)
3. Toxicant:	NaCl, KCl, Cd, or Cu
4. Temperature:	23 ± 1°C
5. Light quality:	Wide-spectrum fluorescent lights
6. Illuminance:	About 100 to 1000 lux
7. Photoperiod:	16L:8D
8. Renewal of water:	None
9. Age of organisms:	*H. azteca:* 7- to 14-d old (1- to 2-d range in age) *C. tentans:* second- to third-instar larvae (about 10-d-old larvae)[1] *L. variegatus:* adults
10. Test chamber:	30-mL plastic cups (covered with glass or plastic)
11. Volume of water:	20 mL
12. Number of organisms/chamber:	1
13. Number of replicate chambers/treatment:	10 minimum
14. Feeding:	*H. azteca:* 0.1 mL YCT (1800 mg/L stock) on Day 0 and 2 *C. tentans:* 0.25 mL Tetrafin® (4 g/L stock) on Day 0 and 2 *L. variegatus:* not fed
15. Substrate:	*H. azteca:* Nitex® screen (110 mesh) *C. tentans:* sand (monolayer) *L. variegatus:* no substrate
16. Aeration:	None
17. Dilution water:	Culture water, well water, surface water, site water, or reconstituted water
18. Test chamber cleaning:	None
19. Water quality:	Hardness, alkalinity, conductivity, dissolved oxygen, and pH at the beginning and end of a test. Temperature daily.
20. Test duration:	96 h
21. Endpoint:	Survival (LC50)
22. Test acceptability:	90% control survival

[1] Age requirement: All animals must be third or second instar with at least 50% of the organisms at third instar.

Table 9.2 Recommended Test Conditions for Conducting Reference-toxicity Tests with More Than One Organism/Chamber

	Parameter	Conditions
1.	Test type:	Water-only test
2.	Dilution series:	Control and at least 5 test concentrations (0.5 dilution factor)
3.	Toxicant:	NaCl, KCl, Cd, or Cu
4.	Temperature:	23 ± 1°C
5.	Light quality:	Wide-spectrum fluorescent lights
6.	Illuminance:	About 100 to 1000 lux
7.	Photoperiod:	16L:8D
8.	Renewal of water:	None
9.	Age of organisms:	*H. azteca:* 7- to 14-d old (1- to 2-d range in age) *C. tentans:* second to third instar (about 10-d-old larvae)[1] *L. variegatus:* adults
10.	Test chamber:	250-mL glass beaker (covered with glass or plastic)
11.	Volume of water:	100 mL (minimum)
12.	Number of organisms/chamber:	10 minimum
13.	Number of replicate chambers/treatment:	3 minimum
14.	Feeding:	*H. azteca:* 0.5 mL YCT (1800 mg/L stock) on Day 0 and 2 *C. tentans:* 1.25 mL Tetrafin® (4 g/L stock) on Day 0 and 2 *L. variegatus:* not fed
15.	Substrate:	*H. azteca:* Nitex® screen (110 mesh) *C. tentans:* sand (monolayer) *L. variegatus:* no substrate
16.	Aeration:	None
17.	Dilution water:	Culture water, well water, surface water, site water or reconstituted water
18.	Test chamber cleaning:	None
19.	Water quality:	Hardness, alkalinity, conductivity, dissolved oxygen, and pH at the beginning and end of a test. Temperature daily.
20.	Test duration:	96 h
21.	Endpoint:	Survival (LC50)
22.	Test acceptability:	90% control survival

[1] Age requirement: All animals must be third or second instar with at least 50% of the organisms at third instar.

Section 10
Collecting, Culturing, and Maintaining Test Organisms

10.1 Life Histories

10.1.1 *Hyalella azteca*

10.1.1.1 *Hyalella azteca* inhabit permanent lakes, ponds, and streams throughout North and South America (de March, 1981; Pennak, 1989). Occurrence of *H. azteca* is most common in warm (20°C to 30°C for much of the summer) mesotrophic or eutrophic lakes that support aquatic plants. These amphipods are also found in ponds, sloughs, marshes, rivers, ditches, streams, and springs, but in lower numbers. *Hyalella azteca* have achieved densities of >10,000/m^2 in preferred habitats (de March, 1981).

10.1.1.2 *Hyalella azteca* are epibenthic detritivores that burrow into the sediment surface. Hargrave (1970a) reported that *H. azteca* selectively ingest bacteria and algae. The behavior and feeding habits of *H. azteca* make them excellent test organisms for sediment assessments.

10.1.1.3 Reproduction by *H. azteca* is sexual. The adult males are larger than females and have larger second gnathopods (de March, 1981). Males pair with females by grasping the females (amplexus) with their gnathopods while on the backs of the females. After feeding together for 1 to 7 d the female is ready to molt and the two organisms separate for a short time while the female sheds her old exoskeleton. Once the exoskeleton is shed, the two organisms reunite and copulation occurs. The male places sperm near the marsupium of the female and her pleopods sweep the sperm into the marsupium. The organisms again separate and the female releases eggs from her oviducts into the marsupium where they are fertilized. *Hyalella azteca* average about 18 eggs/brood (Pennak, 1989) with larger organisms having more eggs (Cooper, 1965).

10.1.1.4 The developing embryos and newly hatched young are kept in the marsupium until the next molt. At 24°C to 28°C, hatching ranges from 5 to 10 d after fertilization (Embody, 1911; Bovee, 1950; Cooper, 1965). The time between molts for females is 7 to 8 d at 26°C to 28°C (Bovee, 1950). Therefore, about the time embryos hatch, the female molts and releases the young. *Hyalella azteca* average 15 broods in 152 d (Pennak, 1989). Pairing of the sexes is simultaneous with embryo incubation of the previous brood in the marsupium. *Hyalella azteca* have a minimum of nine instars (Geisler, 1944). There are 5 to 8 pre-reproductive instars (Cooper, 1965) and an indefinite number of post-reproductive instars. The first five instars form the juvenile stage of development, instar stages 6 and 7 form the adolescent stage when sexes can be differentiated, instar stage 8 is the nuptial stage, and all later instars are the adult stages of development (Pennak, 1989).

10.1.1.5 *Hyalella azteca* have been successfully cultured at illuminance of about 100 to 1000 lux (Ingersoll and Nelson, 1990; Ankley et al., 1991a; Ankley et al., 1991b). *Hyalella azteca* avoid bright light, preferring to hide under litter and feed during the day.

10.1.1.6 Temperatures tolerated by *H. azteca* range from 0 to 33°C (Embody, 1911; Bovee, 1949; Sprague, 1963). At temperatures less than 10°C the organisms rest and are immobile (de March, 1977; de March, 1978). At temperatures of 10°C to 18°C, reproduction can occur. Juveniles grow more slowly at colder temperatures and become larger adults. Smaller adults with higher reproduction are typical when organisms are grown at 18°C to 28°C. The highest rates of reproduction occur at 26°C to 28°C (de March, 1978) while lethality occurs at 33°C to 37°C (Bovee, 1949; Sprague, 1963).

10.1.1.7 *Hyalella azteca* are found in waters of widely varying types. *Hyalella azteca* can inhabit saline waters up to 29 ‰; however, their distribution in these saline waters has been correlated to water hardness (Ingersoll et al., 1992). *Hyalella azteca* inhabit water with high Mg concentrations at conductivities up to 22,000 µS/cm, but only up to 12,000 µS/cm in Na-dominated waters (Ingersoll et al., 1992). De March (1981) reported *H. azteca* were not collected from locations where calcium was less than 7 mg/L. *Hyalella azteca* have been cultured in reconstituted salt water with a salinity up to 15‰ (Ingersoll et al., 1992; Winger and Lasier, 1993). In laboratory studies, Sprague (1963) reported a 24-h LC50 for dissolved oxygen at 20°C of 0.7 mg/L. Pennak and Rosine (1976) reported similar findings. Nebeker et al. (1992) reported 48-h and 30-d LC50s for *H. azteca* of less than 0.3 mg/L dissolved oxygen. Weight and reproduction of *H. azteca* were reduced after 30-d exposure to 1.2 mg/L dissolved oxygen.

10.1.1.8 *Hyalella azteca* tolerate a wide range of substrates. Ingersoll et al. (1996) reported that *H. azteca* tolerated sediments ranging from more than 90% silt- and clay-sized particles to 100% sand-sized particles without detrimental effects on either survival or growth. *Hyalella azteca* tolerated a wide range in grain size and organic matter in 10- to 42-d tests with formulated sediment (Suedel and Rodgers, 1994; Ingersoll et al., 1998). Ankley et al. (1994a) evaluated the effects of natural sediment physico-chemical characteristics on the results of 10-d laboratory toxicity tests with *H. azteca, C. tentans,* and *L. variegatus*. Tests were conducted with and without the addition of exogenous food. Survival of organisms was decreased in tests without added food. Physico-chemical sediment characteristics including grain size and TOC were not significantly correlated to the response of *H. azteca* in either fed or unfed tests. See Sections 4.2.3 and 14.4 for additional detail regarding studies of the influence of grain size in long-term sediment toxicity tests with *H. azteca*.

10.1.2 *Chironomus tentans*

10.1.2.1 *Chironomus tentans* have a holarctic distribution (Townsend et al., 1981) and are commonly found in eutrophic ponds and lakes (Flannagan, 1971; Driver, 1977). Midge larvae are important in the diet of fish and waterfowl (Sadler, 1935; Siegfried, 1973; Driver et al., 1974; McLarney et al., 1974). Larvae of *C. tentans* usually penetrate a few cm into sediment. In both lotic and lentic habitats with soft bottoms, about 95% of the chironomid larvae occur in the upper 10 cm of substrates, and very few larvae are found below 40 cm (Townsend et al., 1981). Larvae were found under the following conditions in British Columbia lakes by Topping (1971): particle size <0.15 mm to 2.0 mm, temperature 0 to 23.3°C, dissolved oxygen 0.22 to 8.23 mg/L, pH 8.0 to 9.2, conductivity 481 to 4,136 µmhos/cm, and sediment organic carbon 1.9 to 15.5%. Larvae were absent from lakes if hydrogen sulfide concentration in overlying water exceeded 0.3 mg/L. Abundance of larvae was positively correlated with conductivity, pH, amount of food, percentages of particles in the 0.59 to 1.98 mm size range, and concentrations of Na, K, Mg, Cl, SO_4, and dissolved oxygen. Others (e.g., Curry, 1962; Oliver, 1971) have reported a temperature range of 0 to 35°C and a pH range of 7 to 10.

10.1.2.2 *Chironomus tentans* are aquatic during the larval and pupal stages. The life cycle of *C. tentans* can be divided into four distinct stages: (1) an egg stage, (2) a larval stage, consisting of four instars, (3) a pupal stage, and (4) an adult stage. Mating behavior has been described by Sadler (1935) and others (ASTM, 1999a). Males are easily distinguished from females because males have large, plumose antennae and a much thinner abdomen with visible genitalia. The male has paired genital claspers on the posterior tip of the abdomen (Townsend et al., 1981). The adult female weighs about twice as much as the male, with about 30% of the female weight contributed by the eggs. After mating, adult females oviposit a single transparent, gelatinous egg mass directly into the water. At the USEPA Office of Research and Development Laboratory (Duluth, MN), the females oviposit eggs within 24 h after emergence. Egg cases contain a variable number of eggs from about 500 to 2000 eggs/eggcase (J. Jenson, ILS, Duluth, MN, personal communication) and will hatch in 2 to 4 d at 23°C. Under optimal conditions larvae will pupate and emerge as adults after about 21 d at 23°C. Larvae begin to construct tubes (or cases) on the second or third day after hatching. The cases lengthen and enlarge as the larvae grow with the addition of small particles bound together with threads from the mouths of larvae (Sadler, 1935). The larvae draw food particles inside the tubes and also feed in the immediate vicinity of either end of the open-ended tubes with their caudal extremities anchored within the tube. The four larval stages are followed by a black-colored pupal stage (lasting about 3 d) and emergence to a terrestrial adult (imago) stage. The adult stage lasts for 3 to 5 d, during which time the adults mate during flight and the females oviposit their egg cases (2 to 3 d post-emergence; Sadler, 1935).

10.1.2.3 Grain size tolerance of *C. tentans* in sediment testing is described in Section 12.4.3 for 10-d exposures and in Section 15.4.3 for long-term exposures.

10.1.3 *Lumbriculus variegatus*

10.1.3.1 *Lumbriculus variegatus* inhabit a variety of sediment types throughout the United States and Europe (Chekanovskaya, 1962; Cook, 1969; Spencer, 1980; Brinkhurst, 1986). *Lumbriculus variegatus* typically tunnel in the upper aerobic zone of sediments of reservoirs, rivers, lakes, ponds, and marshes. When not tunneling, they bury their anterior portion in sediment and undulate their posterior portion in overlying water for respiratory exchange.

10.1.3.2 Adults of *L. variegatus* can reach a length of 40 to 90 mm, diameter of 1.0 to 1.5 mm, and wet weight of 5 to 12 mg (Call et al., 1991; Phipps et al., 1993). Lipid content is about 1.0% (wet weight, Ankley et al., 1992b; Brunson et al., 1993; Brunson et al., 1998). *Lumbriculus variegatus* most commonly reproduce asexually, although sexual reproduction has been reported (Chekanovskaya, 1962). Newly hatched worms have not been observed in cultures (Call et al., 1991; Phipps et al., 1993). Cultures consist of adults of various sizes. Populations of laboratory cultures double (number of organisms) every 10 to 14 d at 20°C (Phipps et al., 1993).

10.1.3.3 *Lumbriculus variegatus* tolerate a wide range of substrates. Ankley et al. (1994a) evaluated the effects of natural sediment physico-chemical characteristics on the results of 10-d laboratory toxicity tests with *H. azteca, C. tentans,* and *L. variegatus.* Tests were conducted with and without the addition of exogenous food. Survival and reproduction of organisms was decreased in tests without added food. Physico-chemical sediment characteristics including grain size and TOC were not significantly correlated to reproduction or growth of *L. variegatus* in either fed or unfed tests.

10.2 General Culturing Procedures

10.2.1 Acceptability of a culturing procedure is based in part on performance of organisms in culture and in the sediment test (Section 1.4 and 9.2). No single technique for culturing test organisms is required. What may work well for one laboratory may not work as well for another laboratory. While a variety of culturing procedures are outlined in Section 10.3 for *H. azteca,* in Section 10.4 for *C. tentans,* and in Section 10.5 for *L. variegatus,* organisms must meet the test acceptability requirements listed in Tables 11.3, 12.3, 13.4, 14.3, and 15.3.

10.2.2 All organisms in a test must be from the same source. Organisms may be obtained from laboratory cultures or from commercial or government sources (Table 10.1). The test organism used should be identified using an appropriate taxonomic key, and verification should be documented. Obtaining organisms from wild populations should be avoided unless organisms are cultured through several generations in the laboratory. In addition, the ability of the wild population of sexually reproducing organisms to crossbreed with the existing laboratory population should be determined (Duan et al. ,1997). Sensitivity of the wild population to select chemicals (e.g., Table 1.4) should also be documented.

Table 10.1 Sources of Starter Cultures of Test Organisms

Source	Species
U.S. Environmental Protection Agency Mid-Continent Ecological Division 6201 Congdon Boulevard Duluth, MN 55804 Teresa Norberg-King (218/529-5163, fax -5003) email: norberg-king.teresa@epa.gov	*H. azteca* *C. tentans* *L. variegatus*
U.S. Environmental Protection Agency Environmental Monitoring System Laboratory 26 W. Martin Luther Dr. Cincinnati, OH 45244 Jim Lazorchak (513/569-7076, fax -7609) email: lazorchak.jim@epa.gov	*H. azteca* *L. variegatus*
Columbia Environmental Research Center U.S. Geological Survey 4200 New Haven Road Columbia, MO 65201 Eugene Greer (573/876-1820, fax -1896) email: eugene_greer@usgs.gov	*H. azteca* *C. tentans* *L. variegatus*
Great Lakes Environmental Research Laboratory, NOAA 2205 Commonwealth Boulevard Ann Arbor, MI 48105-1593 Peter Landrum (313/741-2276, fax -2055) email: landrum@glerl.noaa.gov	*L. variegatus*
Wright State University Institute for Environmental Quality Dayton, OH 45435 Allen Burton (937/775-2201, fax -4997) email: aburton@wright.edu	*H. azteca* *C. tentans* *L. variegatus*
Michigan State University Department of Fisheries and Wildlife No. 13 Natural Resources Building East Lansing, MI 48824-1222 John Giesy (517/353-2000, fax 517/432-1984) email: jgiesy@aol.com	*H. azteca* *C. tentans* *L. variegatus*

10.2.3 Test organisms obtained from commercial sources should be shipped in well-oxygenated water in insulated containers to maintain temperature during shipment. Temperature and dissolved oxygen of the water in the shipping containers should be measured on arrival to determine if the organisms might have been subjected to low dissolved oxygen or temperature fluctuations. The temperature of the shipped water should be gradually adjusted to the desired culture temperature at a rate not exceeding 2°C per 24 h. Additional reference-toxicity testing is suggested if organisms are not cultured at the testing laboratory (Section 9.16).

10.2.4 A group of organisms should not be used for a test if they appear to be unhealthy, discolored, or otherwise stressed (e.g., >20% mortality for 48 h before the start of a test). If the organisms fail to meet these criteria, the entire batch should be discarded and a new batch should be obtained. All organisms should be as uniform as possible in age and life stage. Test organisms should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and as quickly as possible.

10.2.5 *H. azteca, C. tentans,* and *L. variegatus* can be cultured in a variety of waters. Water of a quality sufficient to culture fathead minnows (*Pimephales promelas*) or cladocerans will generally be adequate.

10.2.5.1 Variable success has been reported using reconstituted waters to culture or test *H. azteca* in long-term exposures (i.e., >10 d; See Section 7.1.3 for details).

10.2.5.2 Organisms can be cultured using either static or renewal procedures. Renewal of water is recommended to limit loss of the culture organisms from a drop in dissolved oxygen or a buildup of waste products. In renewal systems, there should be at least one volume addition/d of culture water to each chamber. In static systems, the overlying water volume should be changed at least weekly by siphoning down to a level just above the substrate and slowly adding fresh water. Extra care should be taken to ensure that proper water quality is maintained in static systems. For example, aeration is needed in static systems to maintain dissolved oxygen at >2.5 mg/L.

10.2.5.3 A recirculating system using an under-gravel filter has been used to culture amphipods and midges (P.V. Winger, USGS, Athens, GA, personal communication). The approach for using a recirculating system to culture organisms has been described by New et al. (1974), Crandall et al. (1981), and Rottmann and Campton (1989). Under-gravel filters can be purchased from aquarium suppliers and consist of an elevated plate with holes that fit on the bottom of an aquarium. The plate has a standpipe to which a pump can be attached. Gravel or

an artificial substrate (e.g., plastic balls or multi-plate substrates) is placed on the plate. The substrates provide surface area for microorganisms that use nitrogenous compounds. A simple example of a recirculating system is two aquaria positioned one above the other with a total volume of 120 L. The bottom aquarium contains the under-gravel filter system, gravel, or artificial substrate, and a submersible pump. The top aquarium is used for culture of animals and has a hole in the bottom with a standpipe for returning overflow water to the bottom aquarium. Water lost to evaporation is replaced weekly, and water is replaced at one- to two-month intervals. Cultures fed foods such as Tetramin® or Tetrafin® should include limestone gravel to help avoid depression in pH. Recirculating systems require less maintenance than static systems.

10.2.6 Cultures should be maintained at 23°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux (USEPA, 1994a; ASTM, 1999a). Cultures should be observed daily. Water temperature should be measured daily or continuously, and dissolved oxygen should be measured weekly. It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity to select chemicals. The previous requirement for laboratories to conduct monthly reference-toxicity tests (USEPA 1994a) has not been included as a requirement in this second edition for testing sediments due to the inability of reference-toxicity tests to identify stressed populations of test organisms (Section 9.16; McNulty et al., 1999). Culture water hardness, alkalinity, ammonia, and pH should be measured at least quarterly. If amphipods are cultured using static conditions, it is desirable to measure water quality more frequently. If reconstituted water is used to culture organisms, water quality should be measured on each batch of reconstituted water. Culture procedures should be evaluated and adjusted as appropriate to restore or maintain the health of the culture.

10.3 Culturing Procedures for *Hyalella azteca*

10.3.1 The culturing procedures described below are based on methods described in USEPA (1991a), Ankley et al. (1994a), Call et al. (1994), Tomasovic et al. (1994), Greer (1993), Ingersoll and Nelson (1990), Ingersoll et al. (1998), ASTM (1999a) and USEPA (1994a). The culturing procedure must produce 7- to 14-d-old amphipods to start a 10-d sediment test (Table 11.3). The 10-d test with *H. azteca* should start with a narrow range in size or age of *H. azteca* (1- to 2-d range in age) to reduce potential variability in growth at the end of the 10-d test. This narrower range would be easiest to obtain using known-age organisms (i.e., Section 10.3.2, 10.3.4) instead of sieving the cultures (Section 10.3.5) to obtain similar-sized amphipods (i.e., amphipods within a range of 1- to 2-d old will be more uniform in size than organisms within the range of 7 d). The culturing procedure must produce 7- to 8-d-old amphipods to start a long-term test with *H. azteca* (Table 14.3).

10.3.2 The following procedure described by Call et al. (1994) and USEPA (1991a) can be used to obtain known-age amphipods to start a test. Mature amphipods (50 organisms >30-d old at 23°C) are held in 2-L glass beakers containing 1 L of aerated culture water and cotton gauze as a substrate. Amphipods are fed 10 mL of a yeast-Cerophyl®-trout chow (YCT) mixture (Appendix B) and 10 mL of the green algae *Selenastrum capricornutum* (about 3.5×10^7 cells/mL). Five mL of each food is added to each culture daily, except for renewal days, when 10 mL of each food is added.

10.3.2.1 Water in the culture chambers is changed weekly. Survival of adults and juveniles and production of young amphipods should be measured at this time. The contents of the culture chambers are poured into a translucent white plastic or white enamel pan. After the adults are removed, the remaining amphipods will range in age from <1- to 7-d old. Young amphipods are transferred with a pipet into a 1-L beaker containing culture water and are held for one week before starting a toxicity test. Organisms are fed 10 mL of YCT and 10 mL of green algae on start-up day, and 5 mL of each food each following day (Appendix B). Survival of young amphipods should be >80% during this one-week holding period. Records should be kept on the number of surviving adults, number of breeding pairs, and young production and survival. This information can be used to develop control charts that are useful in determining whether cultures are maintaining a vigorous reproductive rate indicative of culture health. Some of the adult amphipods can be expected to die in the culture chambers, but mortality greater than about 50% should be cause for concern. Reproductive rates in culture chambers containing 60 adults can be as high as 500 young per week. A decrease in reproductive rate may be caused by a change in water quality, temperature, food quality, or brood stock health. Adult females will continue to reproduce for several months.

10.3.3 A second procedure for obtaining known-age amphipods is described by Borgmann et al. (1989). Known-age amphipods are cultured in 2.5-L chambers containing about 1 L of culture water and between 5 and 25 adult *H. azteca.* Each chamber contains pieces of cotton gauze presoaked in culture water. Once a week the test organisms are isolated from the gauze and collected using a sieve. Amphipods are then rinsed into petri dishes where the young and adults are sorted. The adults are returned to the culture chambers containing fresh water and food.

10.3.4 A third procedure for obtaining known-age amphipods is described by Greer (1993), Tomasovic et al. (1994), and Ingersoll et al. (1998). Mass cultures of mixed-age amphipods are maintained in 80-L glass aquaria containing about 50 L of water (Ingersoll and Nelson, 1990). A flaked food (e.g., Tetrafin®) is added to each culture chamber receiving daily water renewals to provide about 20 g dry solids/50 L of water twice weekly in an 80-L culture chamber. Additional flaked food is added when

most of the flaked food has been consumed. Laboratories using static systems should develop lower feeding rates specific to their systems. Each culture chamber has a substrate of maple leaves and artificial substrates (six 20-cm diameter sections per 80-L aquaria of nylon "coiled-web material"; 3-M, St. Paul, MN). Before use, leaves are soaked in 30‰ salt water for about 30 d to reduce the occurrence of planaria, snails, or other organisms in the substrate. The leaves are then flushed with water to remove the salt water and residuals of naturally occurring tannic acid before placement in the cultures.

10.3.4.1 To obtain known-age amphipods, a U.S. Standard Sieve #25 (710-µm mesh) is placed underwater in a chamber containing mixed-age amphipods. A #25 sieve will retain mature amphipods, and immature amphipods will pass through the mesh. Two or three pieces of artificial substrate (3-M coiled-web material) or a mass of leaves with the associated mixed-age amphipods are quickly placed into the sieve. The sieve is brought to the top of the water in the culture chamber keeping all but about 1 cm of the sieve under water. The artificial substrates or leaves are then shaken under water several times to dislodge the attached amphipods. The artificial substrates or leaves are taken out of the sieve and placed back in the culture chamber. The sieve is agitated in the water to rinse the smaller amphipods back into the culture chamber. The larger amphipods remaining in the sieve are transferred with a pipet into a dish and then placed into a shallow glass pan (e.g., pie pan) where immature amphipods are removed. The remaining mature amphipods are transferred using a pipet into a second #25 sieve which is held in a glass pan containing culture water.

10.3.4.2 The mature amphipods are left in the sieve in the pan overnight to collect any newborn amphipods that are released. After 24 h, the sieve is moved up and down several times to rinse the newborn amphipods (<24-h old) into the surrounding water in the pan. The sieve is removed from the pan, and the mature amphipods are placed back into their culture chamber or placed in a second pan containing culture water if additional organisms are needed for testing. The newborn amphipods are moved with a pipet and placed in a culture chamber with flowing water during a grow-out period. The newborn amphipods should be counted to determine if adequate numbers have been collected for the test.

10.3.4.3 Isolation of about 1500 (750 pairs) adults in amplexus provided about 800 newborn amphipods in 24 h and required about six man-hours of time. Isolation of about 4000 mixed-age adults (some in amplexus and others not in amplexus) provided about 800 newborn amphipods in 24 h and required less than one man-hour of time. The newborn amphipods should be held for 6 to 13 d to provide 7- to 14-d-old organisms to start a 10-d test (Section 11) or should be held for 7 d to provide 7- to 8-d-old organisms to start a long-term test (Section 14). The neonates are held in a 2-L beaker for 6 to 13 d before the start of a test. On the first day of isolation, the neonates are fed 10 mL of YCT (1800 mg/L stock solution) and 10 mL of *Selenastrum capricornutum* (about 3.5×10^7 cells/mL). On the third, fifth, seventh, ninth, eleventh, and thirteenth days after isolation, the amphipods are fed 5 mL of both YCT and *S. capricornutum*. Amphipods are initially fed a higher volume to establish a layer of food on the bottom of the culture chamber. If dissolved oxygen drops below 4 mg/L, about 50% of the water should be replaced (Ingersoll et al., 1998).

10.3.5 Laboratories that use mixed-age amphipods for testing must demonstrate that the procedure used to isolate amphipods will produce test organisms that are 7- to 14-d old. For example, amphipods passing through a U.S. Standard #35 sieve (500 µm), but stopped by a #45 sieve (355 µm) averaged 1.54 mm (SD 0.09) in length (P.V. Winger, USGS, Athens, GA, unpublished data). The mean length of these sieved organisms corresponds to that of 6-d-old amphipods (Figure 10.1). After holding for 3 d before testing to eliminate organisms injured during sieving, these amphipods would be about 9 d old (length 1.84 mm, SD 0.11) at the start of a toxicity test.

10.3.5.1 Ingersoll and Nelson (1990) describe the following procedure for obtaining mixed-age amphipods of a similar size to start a test. Smaller amphipods are isolated from larger amphipods using a stack of U.S. Standard sieves: #30 (600 µm), #40 (425 µm), and #60 (250 µm). Sieves should be held under water to isolate the amphipods. Amphipods may float on the surface of the water if they are exposed to air. Artificial substrate or leaves are placed in the #30 sieve. Culture water is rinsed through the sieves and small amphipods stopped by the #60 sieve are washed into a collecting pan. Larger amphipods in the #30 and #40 sieves are returned to the culture chamber. The smaller amphipods are then placed in 1-L beakers containing culture water and food (about 200 amphipods per beaker) with gentle aeration.

10.3.5.2 Amphipods should be held and fed at a rate similar to the mass cultures for at least 2 d before the start of a test to eliminate animals injured during handling.

10.3.6 See Section 10.2.6 for procedures used to evaluate the health of cultures.

10.4 Culturing Procedures for *Chironomus tentans*

10.4.1 The culturing methods described below are based on methods described in USEPA (1991a), Ankley et al. (1994a), Call et al. (1994), Greer (1993), ASTM (1999a), and USEPA (1994a). A *C. tentans* 10-d survival and growth test must be started with second- to third-instar larvae (about 10-d-old larvae; Section 12; Figure 10.2). At a temperature of 23°C, larvae should develop to the third instar by 9 to 11 d after hatching (about 11 to 13 d post-oviposition). The instar of midges at the start of a test can be determined based on head capsule width (Table 10.2) or based on weight or length at sediment test initiation. Average length of midge larvae should be 4 to 6 mm, while average dry weight should be 0.08 to 0.23 mg/individual. A *C. tentans* long-term test must be started with larvae less than 24 h old (see Section 15.3 for a

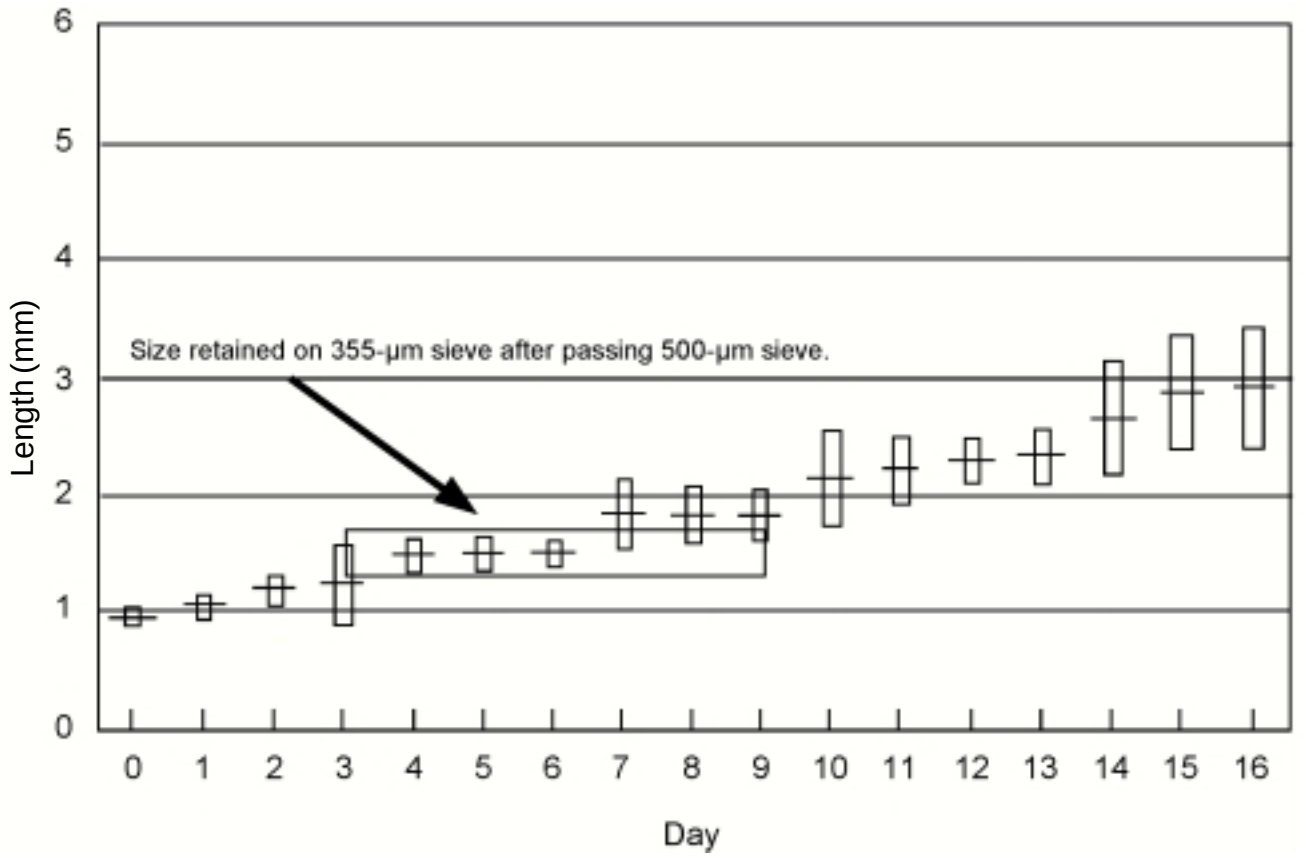


Figure 10.1 Mean length (+/- 2SD) and relative age of *Hyalella azteca* collected by sieving in comparison with length of known-age organisms. P.V. Winger, USGS, Athens, GA, unpublished data.

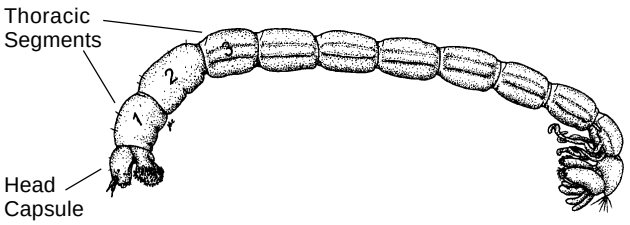


Figure 10.2. *Chironomus tentans* larvae. Note thoracic segments which are used to measure instars. (Reprinted from Clifford, 1991 with kind permission from the University of Alberta Press.)

Table 10.2 *Chironomus tentans* Instar and Head Capsule Widths[1]

Instar	Days after hatching	Mean (mm)	Range (mm)
First	1 to 4.4	0.10	0.09 to 0.13
Second	4.4 to 8.5	0.20	0.18 to 0.23
Third	8.5 to 12.5	0.38	0.33 to 0.45
Fourth	≥12.5	0.67	0.63 to 0.71

[1] T.J. Norberg-King, USEPA, Duluth, MN, unpublished data.

description of an approach for obtaining *C. tentans* larvae less than 24 h old).

10.4.2 Historically, third-instar *C. tentans* were frequently referred to as the second instar in the published literature. When *C. tentans* larvae were measured daily, the *C. tentans* raised at 22°C to 24°C were third instar, not second instar, by 9 to 11 d after hatching (T.J. Norberg-King, USEPA, Duluth, MN, unpublished data).

10.4.3 Both silica sand and shredded paper toweling have been used as substrates to culture *C. tentans.* Either substrate may be used if a healthy culture can be maintained. Greer (1993) used sand or paper toweling to culture midges; however, sand was preferred due to the ease in removing larvae for testing. Sources of sand are listed in Section 7.

10.4.3.1 Paper towels are prepared according to a procedure adapted from Batac-Catalan and White (1982). Plain white kitchen paper towels are cut into strips. Cut toweling is loosely packed into a blender with culture water and blended for a few seconds. Small pieces should be available to the organism; blending for too long will result in a fine pulp that will not settle in a culture tank. Blended towels can then be added directly to culture tanks, eliminating any conditioning period for the substrate. A mass of the toweling sufficient to fill a 150-mL beaker is placed into a blender containing 1 L of deionized water, and blended for 30 sec or until the strips are broken apart in the form of a pulp. The pulp is then sieved using a 710-µm

sieve and rinsed well with deionized water to remove the shortest fibers.

10.4.3.2 Dry shredded paper toweling loosely packed into a 2-L beaker will provide sufficient substrate for about ten 19-L chambers (USEPA, 1991a). The shredded toweling placed in a 150-mL beaker produces enough substrate for one 19-L chamber. Additional substrate can be frozen in deionized water for later use.

10.4.4 Five egg cases will provide a sufficient number of organisms to start a new culture chamber. Egg cases should be held at 23°C in a glass beaker or crystallizing dish containing about 100 to 150 mL of culture water (temperature change should not exceed 2°C per d). Food is not added until the embryos start to hatch (in about 2 to 4 d at 23°C) to reduce the risk of oxygen depletion. About 200 to 400 larvae are then placed into each culture chamber. Crowding of larvae will reduce growth. See Section 10.4.5.1 or 10.4.6.1 for a description of feeding rates. Larvae should reach the third instar by about 10 d after median hatch (about 12 to 14 d after the time the eggs were laid; Table 10.2).

10.4.5 *Chironomus tentans* are cultured in soft water at the USEPA laboratory in Duluth (USEPA, 1993c) in glass aquaria (19.0-L capacity, 36 x 21 x 26 cm high). A water volume of about 6 to 8 L in these flow-through chambers can be maintained by drilling an overflow hole in one end 11 cm from the bottom. The top of the aquarium is covered with a mesh material to trap emergent adults. Pantyhose with the elasticized waist is positioned around the chamber top and the legs are cut off. Fiberglass-window screen glued to a glass strip (about 2- to 3-cm wide) rectangle placed on top of each aquarium has also been used by Call et al. (1994). About 200 to 300 mL of 40-mesh silica sand is placed in each chamber.

10.4.5.1 The stocking density of the number of *C. tentans* eggs should be about 600 eggs per 6 to 8 L of water. Dawson et al. (1999) found that the cultures in 15-L aquaria and 7 L of water were self-regulating in density regardless of the initial number of eggs stocked in each tank. However, tanks with a higher initial stocking density (i.e., 1400 eggs/tank) increased the time of peak adult emergence to 30 to 33 d, whereas tanks with lower stocking densities (600 or 1000 eggs/tank) had peak emergence at 22 to 25 d after hatching.

10.4.5.2 Fish food flakes (i.e., Tetrafin®) are added to each culture chamber to provide a final food concentration of about 0.04 mg dry solids/mL of culture water. A stock suspension of the solids is blended with distilled water to form an initial slurry. It is then filtered through a 200-micron Nitex screen and diluted with distilled water to form a 56 g dry solids/L final slurry (Appendix B). The larvae in each tank are fed 2.5 mL of slurry (140 mg of Tetrafin per day) from Day 0 to Day 7 and 5 mL of slurry (280 mg Tetrafin per day) from Day 8 on. Feeding is done after the water renewal process is completed. The stock suspension should be well mixed immediately before removing an aliquot for feeding. Each batch of food should be refrigerated and can be used for up to two weeks (Appendix B). Laboratories using static systems should develop lower feeding rates specific to their systems.

10.4.6 *Chironomus tentans* are cultured by Greer (1993) in Rubbermaid® 5.7-L polyethylene cylindrical containers. The containers are modified by cutting a semicircle into the lid 17.75 cm across by 12.5 cm. Stainless-steel screen (20 mesh/0.4 cm) is cut to size and melted to the plastic lid. The screen provides air exchange, retains emerging adults, and is a convenient way to observe the culture. Two holes about 0.05 cm in diameter are drilled through the uncut portion of the lid to provide access for an air line and to introduce food. The food access hole is closed with a No. 00 stopper. Greer (1993) cultures midges under static conditions with moderate aeration, and about 90% of the water is replaced weekly. Each 5.7-L culture chamber contains about 3 L of water and about 25 mL of fine sand. Eight to 10 chambers are used to maintain the culture.

10.4.6.1 Midges in each chamber are fed 6 mL/d of a 100 g/L suspension of fish food flakes (e.g., Tetrafin®) on Tuesday, Wednesday, Thursday, Friday, and Sunday. A 6-mL chlorella suspension (deactivated "Algae-Feast® *Chlorella,*" Earthrise Co., Callpatria, CA) is added to each chamber on Saturday and on Monday. The chlorella suspension is prepared by adding 5 g of dry chlorella powder/L of water. The mixture should be refrigerated and can be used for up to two weeks.

10.4.6.2 The water should be replaced more often if animals appear stressed (e.g., at surface or pale color at the second instar) or if the water is cloudy. Water is replaced by first removing emergent adults with an aspirator. Any growth on the sides of the chamber should be brushed off before water is removed. Care should be taken not to pour or siphon out the larvae when removing the water. Larvae will typically stay near the bottom; however, a small-mesh sieve or nylon net can be used to catch any larvae that float out. After the chambers have been cleaned, temperature-adjusted culture water is poured back into each chamber. The water should be added quickly to stir up the larvae. Using this procedure, the approximate size, number, and the general health of the culture can be observed.

10.4.7 Adult emergence will begin about three weeks after hatching at 23°C. Once adults begin to emerge, they can be gently siphoned into a dry aspirator flask on a daily basis. An aspirator can be made using a 250- or 500-mL Erlenmeyer flask, a two-hole stopper, some short sections of 0.25-inch glass tubing, and Tygon® tubing for collecting and providing suction (Figure 10.3). Adults should be aspirated with short inhalations to avoid injuring the organisms. The mouthpiece on the aspirator should be replaced or disinfected between use. Sex ratio of the adults should be checked to ensure that a sufficient number of males are available for mating and fertilization. One male may fertilize more than one female. However, a

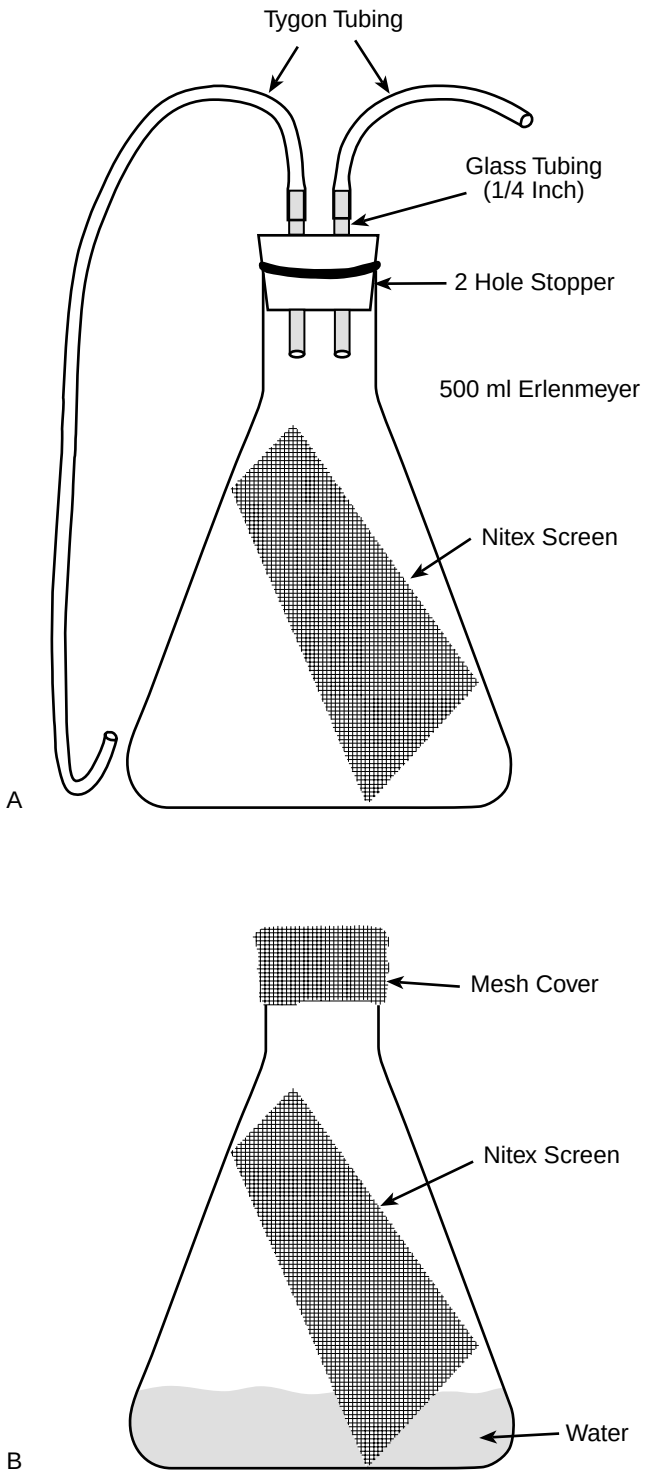


Figure 10.3 Aspirator chamber (A) and reproduction and oviposit chamber (B) for adult midges.

ratio of one male to three females improves fertilization success.

10.4.7.1 A reproduction and oviposit chamber may be prepared in several different ways (Figure 10.3). Culture water (about 50 to 75 mL) can be added to the aspiration flask in which the adults were collected (Figure 10.3; Batac-Catalan and White, 1982). The USEPA Office of Research and Development Laboratory (Duluth, MN; USEPA, 1991a) uses a 500-mL collecting flask with a length of Nitex® screen positioned vertically and extending into the culture water (Figure 10.3). The Nitex® screen is used by the females to position themselves just above the water during oviposition. The two-hole stopper and tubing of the aspirator should be replaced by screened material or a cotton plug for good air exchange in the oviposition chamber.

10.4.7.2 Greer (1993) uses an oviposition box to hold emergent adults. The box is constructed of a 5.7-L chamber with a 20-cm tall cylindrical chamber on top. The top chamber is constructed of stainless-steel screen (35 mesh/2.54 cm) melted onto a plastic lid with a 17.75-cm hole. A 5-cm hole is cut into the side of the bottom chamber and a #11 stopper is used to close the hole. Egg cases are removed by first sliding a piece of plexiglass between the top and bottom chambers. Adult midges are then aspirated from the bottom chamber. The top chamber with plexiglass is removed from the bottom chamber and a forceps is used to remove the egg cases. The top chamber is put back on top of the bottom chamber, the plexiglass is removed, and the aspirated adults are released from the aspirator into the chamber through the 5-cm hole.

10.4.8 About two to three weeks before the start of a test, at least 3 to 5 egg cases should be isolated for hatching using procedures outlined in Section 10.4.4.

10.4.9 Records should be kept on the time to first emergence and the success of emergence for each culture chamber. It is also desirable to monitor growth and head capsule width periodically in the cultures. See Section 10.2.6 for additional detail on procedures for evaluating the health of the cultures.

10.5 Culturing Procedures for *Lumbriculus variegatus*

10.5.1 The culturing procedures described below are based on methods described in Phipps et al. (1993), USEPA (1991a), Call et al. (1994), Brunson et al. (1998), and USEPA (1994a). Bioaccumulation tests are started with adult organisms.

10.5.2 *Lumbriculus variegatus* are generally cultured with daily renewal of water (57- to 80-L aquaria containing 45 to 50 L of water).

10.5.3 Paper towels can be used as a substrate for culturing *L. variegatus* (Phipps et al., 1993). Substrate is prepared by cutting unbleached brown paper towels into strips either with a paper shredder or with scissors. Cut toweling is loosely packed into a blender with culture water and blended for a few seconds. Small pieces should be available to the organisms; blending for too long will result in a fine pulp that will not settle in culture tanks. Blended towels can then be added directly to culture tanks, eliminating any conditioning period for the substrate. The paper towel substrate is renewed with blended towels when thin or bare areas appear in the cultures. The substrate in the chamber will generally last for about two months.

10.5.4 Oligochaetes probably obtain nourishment from ingesting the organic matter in the substrate (Pennak, 1989). *Lumbriculus variegatus* in each of the culture chambers are fed a 10-mL suspension of 6 g of trout starter 3 times/week. The particles will temporarily disperse on the surface film, break through the surface tension, and settle out over the substrate. Laboratories using static systems should develop lower feeding rates specific to their systems. Food and substrate used to culture oligochaetes should be analyzed for compounds to be evaluated in bioaccumulation tests. If the concentration of the test compound is above the detection level and the food is not measured, the test may be invalidated. Recent studies in other laboratories, for example, have indicated elevated concentrations of PCBs in substrate and/or food used for culturing the oligochaete (J. Amato, AScI Corporation, Duluth, MN, personal communication).

10.5.5 Phipps et al. (1993) recommend starting a new culture with 500 to 1000 worms. Conditioned paper toweling should be added when the substrate in a culture chamber is thin.

10.5.6 On the day before the start of a test, oligochaetes can be isolated by transferring substrate from the cultures into a beaker using a fine-mesh net. Additional organisms can be removed using a glass pipet (20-cm long, 5-mm i.d.; Phipps et al., 1993). Water can be slowly trickled into the beaker. The oligochaetes will form a mass and most of the remaining substrate will be flushed from the beaker. On the day the test is started, organisms can be placed in glass or stainless-steel pans. A gentle stream of water from the pipet can be used to spread out clusters of oligochaetes. The remaining substrate can be siphoned from the pan by allowing the worms to reform in a cluster on the bottom of the pan. For bioaccumulation tests, aliquots of worms to be added to each test chamber can be transferred using a blunt dissecting needle or dental pick. Excess water can be removed during transfer by touching the mass of oligochaetes to the edge of the pan. The mass of oligochaetes is then placed in a tared weigh boat, quickly weighed, and immediately introduced into the appropriate test chamber. Organisms should not be blotted with a paper towel to remove excess water (Brunson et al., 1998).

10.5.7 The culture population generally doubles (number of organisms) in about 10 to 14 d. See Section 10.2.6 for additional detail on procedures for evaluating the health of the cultures.

Section 11
Test Method 100.1
Hyalella azteca 10-d Survival and Growth Test for Sediments

11.1 Introduction

11.1.1 *Hyalella azteca* (Saussure) have many desirable characteristics of an ideal sediment toxicity testing organism including relative sensitivity to contaminants associated with sediment, short generation time, contact with sediment, ease of culture in the laboratory, and tolerance to varying physico-chemical characteristics of sediment. Their response has been evaluated in interlaboratory studies and has been confirmed with natural benthic populations. Many investigators have successfully used *H. azteca* to evaluate the toxicity of freshwater sediments (e.g., Nebeker et al., 1984a; Borgmann and Munwar, 1989; Ingersoll and Nelson, 1990; Ankley et al., 1991a; Ankley et al., 1991b; Burton et al., 1989; Winger and Lasier, 1993; Kemble et al., 1994). *H. azteca* has been used for a variety of sediment assessments (Ankley et al., 1991; West et al., 1993; Hoke et al., 1994, 1995; West et al., 1994). *Hyalella azteca* can also be used to evaluate the toxicity of estuarine sediments (up to 15 ‰ salinity; Nebeker and Miller, 1988; Roach et al., 1992; Winger et al., 1993). Endpoints typically monitored in 10-d sediment toxicity tests with *H. azteca* include survival and growth.

11.1.2 A test method for conducting a 10-d sediment toxicity test is described in Section 11.2 for *H. azteca.* Methods outlined in Appendix A of USEPA (1994a) and in Section 11.1.1 were used for developing test method 100.1. Results of tests using procedures different from the procedures described in Section 11.2 may not be comparable, and these different procedures may alter contaminant bioavailability. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

11.2 Recommended Test Method for Conducting a 10-d Sediment Toxicity Test with *Hyalella azteca*

11.2.1 Recommended conditions for conducting a 10-d sediment toxicity test with *H. azteca* are summarized in Table 11.1. A general activity schedule is outlined in Table 11.2. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 16). The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. When variability remains constant, the sensitivity of a test increases as the number of replicates increase.

11.2.2 The recommended 10-d sediment toxicity test with *H. azteca* must be conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux (Table 11.1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten 7- to 14-d-old amphipods are used to start a test. The 10-d test should start with a narrow range in size or age of *H. azteca* (i.e., 1- to 2-d range in age) to reduce potential variability in growth at the end of a 10-d test (Section 10.3.1). The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing (Section 16). Amphipods in each test chamber are fed 1.0 mL of YCT food daily (Appendix B). The first edition of the manual (USEPA, 1994a) recommended a feeding level of 1.5 mL of YCT daily; however, this feeding level was revised to 1.0 mL to be consistent, with the feeding level in the long-term test with *H. azteca* (Section 14). Each chamber receives 2 volume additions/d of overlying water. Water renewals may be manual or automated. Appendix A describes water-renewal systems that can be used to deliver overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are summarized in Table 11.3.

11.3 General Procedures

11.3.1 Sediment into Test Chambers

11.3.1.1 The day before the sediment test is started (Day -1) each sediment should be thoroughly homogenized and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the degree of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is

Table 11.1 Test Conditions for Conducting a 10-d Sediment Toxicity Test with *Hyalella azteca*

	Parameter	Conditions
1.	Test type:	Whole-sediment toxicity test with renewal of overlying water
2.	Temperature:	23 ± 1°C
3.	Light quality:	Wide-spectrum fluorescent lights
4.	Illuminance:	About 100 to 1000 lux
5.	Photoperiod:	16L:8D
6.	Test chamber:	300-mL high-form lipless beaker
7.	Sediment volume:	100 mL
8.	Overlying water volume:	175 mL
9.	Renewal of overlying water:	2 volume additions/d (Appendix A); continuous or intermittent (e.g., 1 volume addition every 12 h)
10.	Age of organisms:	7- to 14-d old at the start of the test (1- to 2-d range in age)
11.	Number of organisms/chamber:	10
12.	Number of replicate chambers/treatment:	Depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 16).
13.	Feeding:	YCT food, fed 1.0 mL daily (1800 mg/L stock) to each test chamber. The first edition of the manual (USEPA, 1994a) recommended a feeding level of 1.5 mL of YCT daily; however, this feeding level was revised to 1.0 mL to be consistent with the feeding level in the long-term tests with *H. azteca* (Section 14).
14.	Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L.
15.	Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16.	Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen (Appendix A).
17.	Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
18.	Test duration:	10 d
19.	Endpoints:	Survival and growth
20.	Test acceptability:	Minimum mean control survival of 80% and measurable growth of test organisms in the control sediment. Additional performance-based criteria specifications are outlined in Table 11.3.

Table 11.2 General Activity Schedule for Conducting a 10-d Sediment Toxicity Test with *Hyalella azteca* [1]

Day	Activity
-7	Separate known-age amphipods from the cultures and place in holding chambers. Begin preparing food for the test. There should be a 1- to 2-d range in age of amphipods used to start the test.
-6 to -2	Feed and observe isolated amphipods (Section 10.3), monitor water quality (e.g., temperature and dissolved oxygen).
-1	Feed and observe isolated amphipods (Section 10.3), monitor water quality. Add sediment into each test chamber, place chambers into exposure system, and start renewing overlying water.
0	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia). Transfer 10 7- to 14-day-old amphipods into each test chamber. Release organisms under the surface of the water. Add 1.0 mL of YCT into each test chamber. Archive 20 test organisms for length determination or archive 80 test organisms for dry weight determination. Observe behavior of test organisms.
1 to 8	Add 1.0 mL of YCT food to each test chamber. Measure temperature and dissolved oxygen. Observe behavior of test organisms.
9	Measure total water quality.
10	Measure temperature and dissolved oxygen. End the test by collecting the amphipods with a sieve (Section 11.3.7.1). Count survivors and prepare organisms for weight or length measurements.

[1] Modified from Call et al., 1994

Table 11.3 Test Acceptability Requirements for a 10-d Sediment Toxicity Test with *Hyalella azteca*

A. It is recommended for conducting a 10-d test with *Hyalella azteca* that the following performance criteria be met:

1. Age of *H. azteca* at the start of the test must be between 7- to 14-d old. The 10-d test should start with a narrow range in size or age of *H. azteca* (i.e., 1- to 2-d range in age) to reduce potential variability in growth at the end of a 10-d test (Section 10.3.1).
2. Average survival of *H. azteca* in the control sediment must be greater than or equal to 80% at the end of the test. Growth of test organisms should be measurable in the control sediment at the end of the 10-d test (i.e., relative to organisms at the start of the test).
3. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

B. Performance-based criteria for culturing *H. azteca* include the following:

1. It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.
2. Laboratories should track parental survival in the cultures and record this information using control charts if known-age cultures are maintained. Records should also be kept on the frequency of restarting cultures and the age of brood organisms.
3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly: pH, hardness, alkalinity, and ammonia. Dissolved oxygen in the cultures should be measured weekly. Temperature of the cultures should be recorded daily. If static cultures are used, it may be desirable to measure water quality more frequently.
4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

1. All organisms in a test must be from the same source.
2. Storage of sediments collected from the field should follow guidance outlined in Section 8.2.
3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
5. Test organisms must be cultured and tested at 23°C (±1°C).
6. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.
7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

11.3.1.2 Each test chamber should contain the same amount of sediment, determined either by volume or by weight. Overlying water is added to the chambers on Day -1 in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon® with a handle attached) placed above the sediment to dissipate the force of the water. A test begins when the organisms are added to the test chambers (Day 0).

11.3.2 Renewal of Overlying Water

11.3.2.1 Renewal of overlying water is required during a test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Hardness, alkalinity and ammonia concentrations in the water above the sediment, within a treatment, typically should not vary by more than 50% during the test. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water-delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998; Wall et al., 1998; Leppanen and Maier, 1998). The water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms or food on Day 0. Appendix A describes water-renewal systems that can be used for conducting sediment tests.

11.3.2.2 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static

tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the pore water.

11.3.3 Acclimation

11.3.3.1 Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

11.3.3.2 Culturing of organisms and toxicity assessment are typically conducted at 23°C. However, occasionally there is a need to perform evaluations at temperatures different than that recommended. Under these circumstances, it may be necessary to acclimate organisms to the desired test temperature to prevent thermal shock when moving immediately from the culture temperature to the test temperature (ASTM, 1999a). Acclimation can be achieved by exposing organisms to a gradual change in temperature; however, the rate of change should be relatively slow to prevent thermal shock. A change in temperature of 1°C every 1 to 2 h has been used successfully in some studies (P.K. Sibley, University of Guleph, Guelph, Ontario, personal communication; APHA, 1989). Testing at temperatures other than 23°C needs to be preceded by studies to determine expected performance under alternate conditions.

11.3.4 Placing Organisms in Test Chambers

11.3.4.1 Test organisms should be handled as little as possible. Amphipods should be introduced into the overlying water below the air-water interface. Test organisms can be pipetted directly into overlying water. The size of the test organisms at the start of the test should be measured using the same measure (length or weight) that will be used to assess their size at the end of the test. For length, a minimum of 20 organisms should be measured. For weight measurement, a larger sample size (e.g., 80) may be desirable because of the relative small mass of the organisms. This information can be used to determine consistency in the size of the organisms used to start a test.

11.3.5 Feeding

11.3.5.1 For each beaker, 1.0 mL of YCT is added from Day 0 to Day 9. Without addition of food, the test organisms may starve during exposures. However, the addition of the food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987; Harkey et al., 1994). Furthermore, if too much food is added to the test chamber or if the mortality of test organisms is high, fungal or bacterial growth may develop on the sediment surface. Therefore, the amount of food added to the test chambers is kept to a minimum.

11.3.5.2 Suspensions of food should be thoroughly mixed before aliquots are taken. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding should be suspended for one or more days. A drop in dissolved oxygen below 2.5 mg/L during a test may indicate that the food added is not being consumed. Feeding should be suspended for the amount of time necessary to increase the dissolved oxygen concentration (ASTM, 1999a). If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface should be made daily.

11.3.6 Monitoring a Test

11.3.6.1 All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

11.3.6.2 Measurement of Overlying Water-quality Characteristics

11.3.6.2.1 Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of a test. Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to composite water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water. Water quality should be measured on each batch of water prepared for the test.

11.3.6.2.2 Dissolved oxygen should be measured daily and should be maintained at a minimum of 2.5 mg/L. If a probe is used to measure dissolved oxygen in overlying water, it should be thoroughly inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination. Aeration can be used to maintain dissolved oxygen in the overlying water above 2.5 mg/L (i.e., about 1 bubble/second in the overlying water). Dissolved oxygen and pH can be measured directly in the overlying water with a probe.

11.3.6.2.3 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.

11.3.7 Ending a Test

11.3.7.1 Any of the surviving amphipods in the water column or on the surface of the sediment can be pipetted from the beaker before sieving the sediment. Immobile organisms isolated from the sediment surface or from sieved material should be considered dead. A #40 sieve (425-µm mesh) can be used to remove amphipods from sediment. Alternatively, Kemble et al. (1994) suggest sieving of sediment using the following procedure: (1) pour about half of the overlying water through a #50- (300-µm) U.S. standard mesh sieve, (2) swirl the remaining water to suspend the upper 1 cm of sediment, (3) pour this slurry through the #50-mesh sieve and wash the contents of the sieve into an examination pan, (4) rinse the coarser sediment remaining in the test chamber through a #40- (425-µm) mesh sieve and wash the contents of this second sieve into a second examination pan. Surviving test organisms are removed from the two pans and counted. If growth (length) is to be measured (Ingersoll and Nelson, 1990), the organisms can be preserved in 8% sugar formalin solution. The sugar formalin solution is prepared by adding 120 g of sucrose to 80 mL of formalin, which is then brought to a volume of 1 L using deionized water. This stock solution is mixed with an equal volume of deionized water when used to preserve organisms. NoTox® (Earth Safe Industries, Belle Mead, NJ) can be used as a substitute for formalin (Unger et al., 1993).

11.3.7.2 A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 5 min/replicate). Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control or test sediments, and recovery could be determined after 1 h (Tomasovic et al., 1994).

11.3.8 Test Data

11.3.8.1 Survival and growth are measured at the end of the 10-d sediment toxicity test with *H. azteca.* Growth of amphipods is often a more sensitive toxicity endpoint compared to survival (Burton and Ingersoll, 1994; Kemble et al., 1994; Becker et al., 1995; Ingersoll et al., 1996; Ingersoll et al., 1998; Steevens and Benson, 1998). The duration of the 10-d test starting with 7- to 14-d-old amphipods is not long enough to determine sexual maturation or reproductive effects. The 42-d test (Section 14) is designed to evaluate additional sublethal endpoints in sediment toxicity tests with *H. azteca*. See Section 14.4.5.3 for a discussion of measuring dry weight vs. length of *H. azteca*.

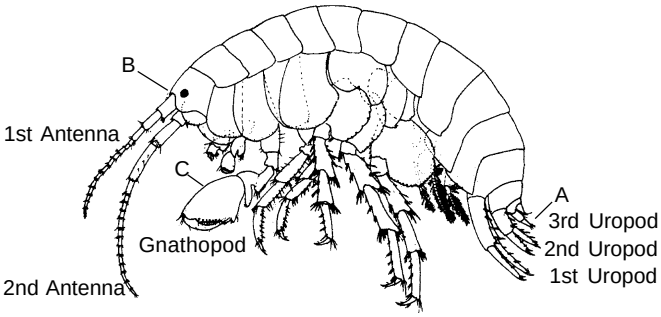


Figure 11.1 *Hyalella azteca*. (A) denotes the uropods; (B) denotes the base of the first antennae; (C) denotes the gnathopod used for grasping females. Meaurement of length is made from base of the 3rd uropod (A) to (B). Females are recognized by the presence of egg cases or the absence of an enlarged gnathopod. (Reprinted from Cole and Watkins, 1997 with kind permission from Kluwer Academic Publishers.)

11.3.8.2 Amphipod body length (±0.1 mm) can be measured from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface (Figure 11.1). Ingersoll and Nelson (1990) describe the use of a digitizing system and microscope to measure lengths of *H. azteca.* Kemble et al. (1994) also photographed invertebrates (at a magnification of 3.5X) and measured length using a computer-interfaced digitizing tablet. Antennal segment number can also be used to estimate length or weight of amphipods (E.L. Brunson, USGS, Columbia, MO, personal communication). Wet or dry weight measurements have also been used to estimate growth of *H. azteca* (ASTM, 1999a). If test organisms are to be used for an evaluation of bioaccumulation, it is not advisable to dry the sample before conducting the residue analysis. If conversion from wet weight to dry weight is necessary, aliquots of organisms can be weighed to establish wet to dry weight conversion factors. A consistent procedure should be used to remove the excess water from the organisms before measuring wet weight.

11.3.8.3 Dry weight of amphipods should be determined by pooling all living organisms from a replicate and drying the sample at about 60°C to 90°C to a constant weight. The sample is brought to room temperature in a desiccator and weighed to the nearest 0.01 mg to obtain mean weight per surviving organism per replicate (see Section 14.3.7.6) The first edition of this manual (USEPA, 1994a) recommended dry weight as a measure of growth for both *H. azteca* and *C. tentans*. For *C. tentans*, this recommendation was changed in the current edition to ash-free dry weight (AFDW) instead of dry weight, with the intent of reducing bias introduced by gut contents (Sibley et al., 1997a). However, this recommendation was not extended to include *H. azteca*. Studies by Dawson et al. (personal communication, T.D. Dawson, Integrated Laboratory Systems, Duluth, MN) have indicated that the ash content of *H. azteca* is not greatly decreased by purging organisms in clean water before weighing, suggesting that sediment does not comprise a large portion of the overall dry weight. In addition, using AFDW further decreases an

already small mass, potentially increasing measurement error. For this reason, dry weight continues to be the recommended endpoint for estimating growth of *H. azteca* via weight (growth can also be determined via length).

11.4 Interpretation of Results

11.4.1 Section 16 describes general information for interpretation of test results. The following sections describe species-specific information that is useful in helping to interpret the results of sediment toxicity tests with *H. azteca.*

11.4.2 Age Sensitivity

11.4.2.1 The sensitivity of *H. azteca* appears to be relatively similar up to at least 24- to 26-d-old organisms (Collyard et al., 1994). For example, the toxicity of diazinon, Cu, Cd, and Zn was similar in 96-h water-only exposures starting with 0- to 2-d-old organisms through 24- to 26-d-old organisms (Figure 11.2). The toxicity of alkylphenol ethoxylate (a surfactant) tended to increase with age. In general, this suggests that tests started with 7- to 14-d-old amphipods would be representative of the sensitivity of *H. azteca* up to at least the adult life stage.

11.4.3 Grain Size

11.4.3.1 *Hyalella azteca* are tolerant of a wide range of substrates. Physico-chemical characteristics (e.g., grain size or TOC) of sediment were not significantly correlated to the response of *H. azteca* in toxicity tests in which organisms were fed (Section 10.1.1.8; Ankley et al., 1994a).

11.4.4 Isolating Organisms at the End of a Test

11.4.4.1 Quantitative recovery of young amphipods (e.g., 0- to 7-d old) is difficult given their small size (Figure 11.3, Tomasovic et al., 1994). Recovery of older and larger amphipods (e.g., 21-d old) is much easier. This was a primary reason for deciding to start 10-d tests with 7- to 14-d-old amphipods (organisms are 17- to 24-d old at the end of the 10-d test).

11.4.5 Influence of Indigenous Organisms

11.4.5.1 Survival of *H. azteca* in 28-d tests was not reduced in the presence of oligochaetes in sediment samples (Reynoldson et al., 1994). However, growth of amphipods was reduced when high numbers of oligochaetes were placed in a sample. Therefore, it is important to determine the number and biomass of indigenous organisms in field-collected sediment in order to better interpret growth data (Reynoldson et al., 1994; DeFoe and Ankley, 1998). Furthermore, presence of predators may also influence the response of test organisms in sediment (Ingersoll and Nelson, 1990).

11.4.6 Ammonia toxicity

11.4.6.1 Section 1.3.7.5 addresses interpretative guidance for evaluating toxicity associated with ammonia in sediment.

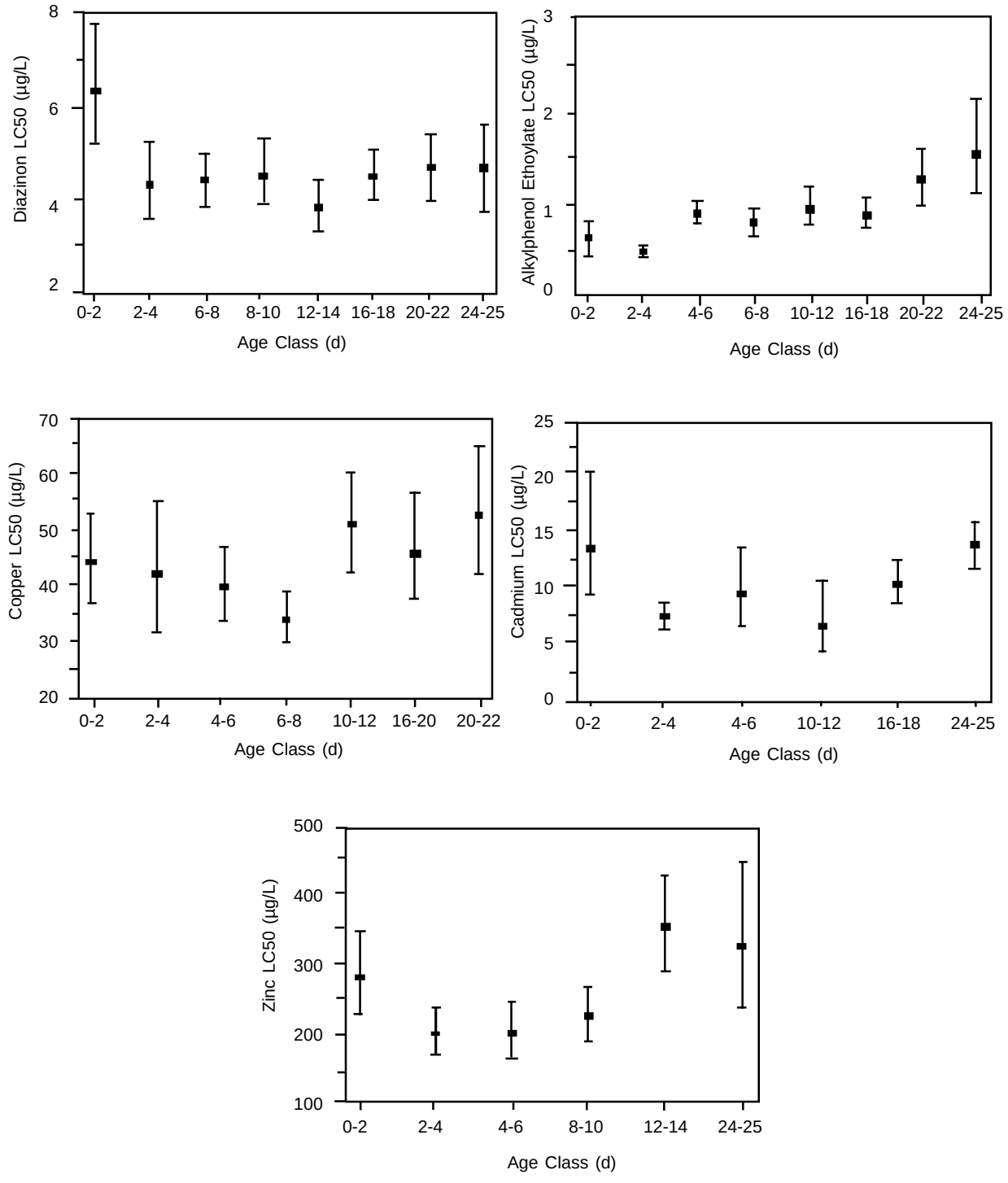


Figure 11.2 Lifestage sensitivity of *Hyalella azteca* in 96-h water-only exposures.

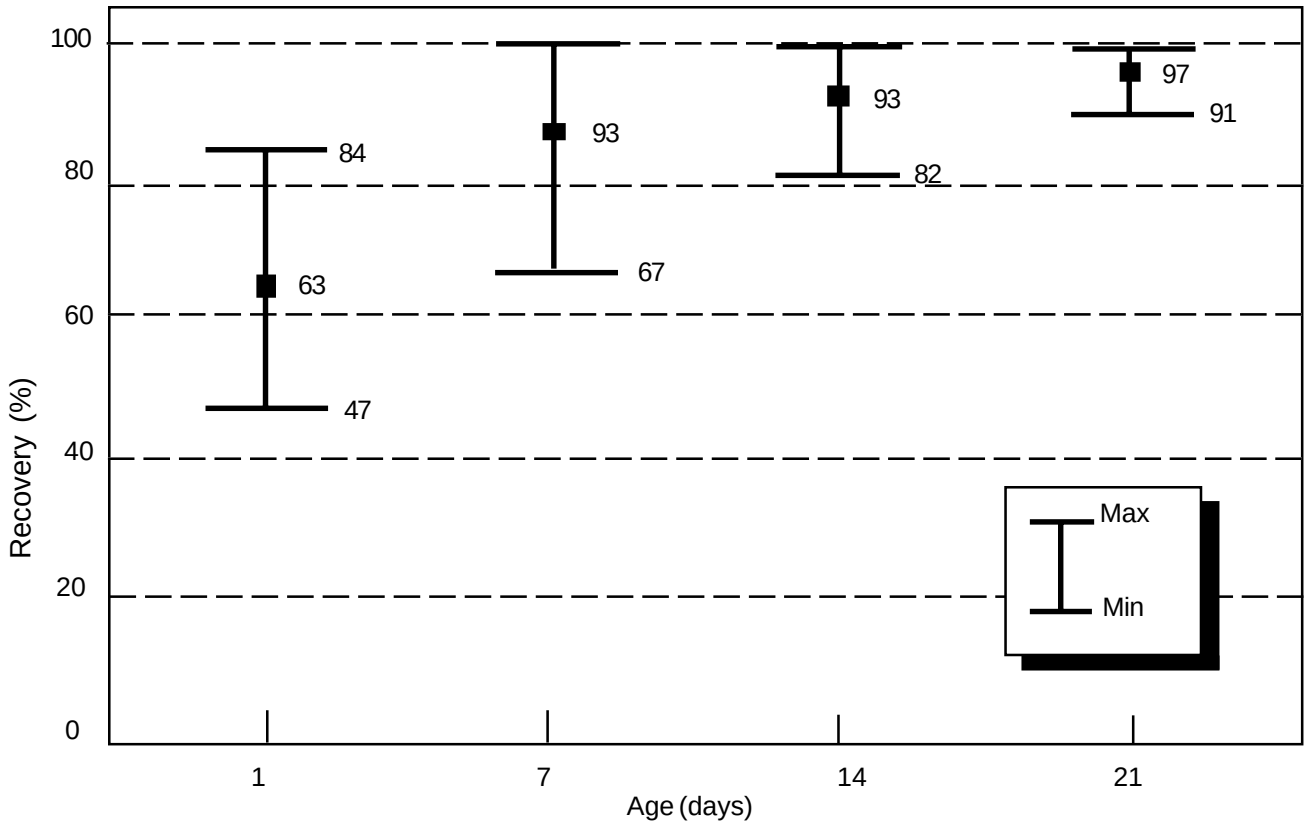


Figure 11.3 Average recovery of different age *Hyalella azteca* from sediment by 7 individuals.

Section 12
Test Method 100.2
Chironomus tentans 10-d Survival and Growth Test for Sediments

12.1 Introduction

12.1.1 *Chironomus tentans* (Fabricius) have many desirable characteristics of an ideal sediment toxicity testing organism including relative sensitivity to contaminants associated with sediment, contact with sediment, ease of culture in the laboratory, tolerance to varying physicochemical characteristics of sediment, and short generation time. Their response has been evaluated in interlaboratory studies and has been confirmed with natural benthic populations. Many investigators have successfully used *C. tentans* to evaluate the toxicity of freshwater sediments (e.g., Wentsel et al., 1977; Nebeker et al., 1984a; Nebeker et al., 1988; Adams et al., 1985; Giesy et al., 1988; Hoke et al., 1990; West et al., 1993; Ankley et al., 1993; Ankley et al., 1994a; Ankley et al.,1994b). *C. tentans* has been used for a variety of sediment assessments (West et al., 1993; Hoke et al., 1994, 1995; West et al., 1994; Ankley et al., 1994c). Endpoints typically monitored in 10-d sediment toxicity tests with *C. tentans* include survival and growth (ASTM, 1999a).

12.1.2 A specific test method for conducting a 10-d sediment toxicity test is described in Section 12.2 for *C. tentans.* Methods outlined in Appendix A of USEPA (1994a) and in Section 12.1.1 were used for developing test method 100.2. Results of tests using procedures different from the procedures described in Section 12.2 may not be comparable and these different procedures may alter contaminant bioavailability. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

12.2 Recommended Test Method for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans*

12.2.1 Recommended conditions for conducting a 10-d sediment toxicity test with *C. tentans* are summarized in Table 12.1. A general activity schedule is outlined in Table 12.2. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 16). The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. When variability remains constant, the sensitivity of a test increases as the number of replicates increases.

12.2.2 The recommended 10-d sediment toxicity test with *C. tentans* must be conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux (Table 12.1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten second- to third-instar midges (about 10-d old) are used to start a test (Section 10.4.1). The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 16). Midges in each test chamber are fed 1.5 mL of a 4-g/L Tetrafin® suspension daily. Each test chamber receives 2 volume additions/d of overlying water. Water renewals may be manual or automated. Appendix A describes water-renewal systems that can be used to deliver overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are summarized in Table 12.3.

12.3 General Procedures

12.3.1 Sediment into Test Chambers

The day before the sediment test is started (Day -1) each sediment should be thoroughly homogenized and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the extent of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

12.3.1.1 Each test chamber should contain the same amount of sediment, determined either by volume or by weight. Overlying water is added to the chambers in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides

Table 12.1 Recommended Test Conditions for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans*

Parameter	Conditions
1. Test type:	Whole-sediment toxicity test with renewal of overlying water
2. Temperature:	23 ± 1°C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illuminance:	About 100 to 1000 lux
5. Photoperiod:	16L:8D
6. Test chamber:	300-mL high-form lipless beaker
7. Sediment volume:	100 mL
8. Overlying water volume:	175 mL
9. Renewal of overlying water:	2 volume additions/d (Appendix A); continuous or intermittent (e.g., one volume addition every 12 h)
10. Age of organisms:	Second- to third-instar larvae (about 10-d-old larvae; all organisms must be third instar or younger with at least 50% of the organisms at third instar; Section 10.4.1)
11. Number of organisms/chamber:	10
12. Number of replicate chambers/treatment:	Depends on the objective of the test. Eight replicates are recommended for routine testing (see Section 16).
13. Feeding:	Tetrafin® goldfish food, fed 1.5 mL daily to each test chamber (1.5 mL contains 6.0 mg of dry solids)
14. Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L.
15. Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16. Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen (Appendix A).
17. Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
18. Test duration:	10 d
19. Endpoints:	Survival and growth (ash-free dry weight, AFDW)
20. Test acceptability:	Minimum mean control survival must be 70%, with minimum mean weight/ surviving control organism of 0.48 mg AFDW. Performance-based criteria specifications are outlined in Table 12.3.

of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon with a handle attached) placed above the sediment to dissipate the force of the water. Renewal of overlying water is started on Day -1. A test begins when the organisms are added to the test chambers (Day 0).

12.3.2 Renewal of Overlying Water

12.3.2.1 Renewal of overlying water is required during a test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Hardness, alkalinity and ammonia concentrations in the water above the sediment, within a treatment, typically should not vary by more than 50% during the test. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water-delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998; Wall et al., 1998; Leppanen and Maier, 1998). Each water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms or food on Day 0. Appendix A describes water-renewal systems that can be used for conducting sediment tests.

12.3.2.2 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et

Table 12.2 General Activity Schedule for Conducting a 10-d Sediment Toxicity Test with *Chironomus tentans* [1]

Day	Activity
-14	Isolate adults for production of egg cases.
-13	Place newly deposited egg cases into hatching dishes.
-12	Prepare a larval rearing chamber with new substrate.
-11	Examine egg cases for hatching success. If egg cases have hatched, transfer first-instar larvae and any remaining unhatched embryos from the crystallizing dishes into the larval rearing chamber. Feed organisms.
-10	Same as Day -11.
-9 to -2	Feed and observe midges (Section 10.4). Measure water quality (e.g., temperature and dissolved oxygen).
-1	Add food to each larval rearing chamber and measure temperature and dissolved oxygen. Add sediment into each test chamber, place chamber into exposure system, and start renewing overlying water.
0	Measure total water quality (temperature, pH, hardness, alkalinity, dissolved oxygen, conductivity, ammonia). Remove third-instar larvae from the culture chamber substrate. Add 1.5 mL of Tetrafin® (4.0 g/L) into each test chamber. Transfer 10 larvae into each test chamber. Release organisms under the surface of the water. Archive 20 test organisms for instar determination and weight or length determination. Observe behavior of test organisms.
1 to 8	Add 1.5 mL of food to each test chamber. Measure temperature and dissolved oxygen. Observe behavior of test organisms.
9	Measure total water quality.
10	Measure temperature and dissolved oxygen. End the test by collecting the midges with a sieve. Measure weight or length of surviving larvae.

[1] Modified from Call et al., 1994

al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the interstitial water.

12.3.3 Acclimation

12.3.3.1 Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

12.3.3.2 Culturing of organisms and toxicity assessment are typically conducted at 23°C. However, occasionally there is a need to perform evaluations at temperatures different than that recommended. Under these circumstances, it may be necessary to acclimate organisms to the desired test temperature to prevent thermal shock when moving immediately from the culture temperature to the test temperature (ASTM, 1999a). Acclimation can be achieved by exposing organisms to a gradual change in temperature; however, the rate of change should be relatively slow to prevent thermal shock. A change in temperature of 1°C every 1 to 2 h has been used successfully in some studies (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication; APHA, 1989). Testing at temperatures other than 23°C needs to be preceded by studies to determine expected performance under alternate conditions.

12.3.4 Placing Organisms in Test Chambers

12.3.4.1 Test organisms should be handled as little as possible. Midges should be introduced into the overlying water below the air-water interface. Test organisms can be pipetted directly into overlying water. Developmental stage of the test organisms should be documented from a subset of at least 20 organisms used to start the test (Section 10.4.1). Developmental stage can be determined from head capsule width (Table 10.2), length (4 to 6 mm), or dry weight (0.08 to 0.23 mg/individual). It is desirable to measure size at test initiation using the same measure as will be used to assess growth at the end of the test.

12.3.5 Feeding

12.3.5.1 For each beaker, 1.5 mL of Tetrafin® is fed from Day 0 to Day 9. Without addition of food, the test organisms may starve during exposures. However, the addition of the food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987; Harkey et al., 1994). Furthermore, if too much food is added to the test chamber or if the mortality of test organisms is high, fungal or bacterial growth may develop on the sediment surface. Therefore, the amount of food added to the test chambers is kept to a minimum.

12.3.5.2 Suspensions of food should be thoroughly mixed before aliquots are taken. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding should be suspended for one or more days. A drop in dissolved oxygen below 2.5 mg/L during a test may indicate that the food

Table 12.3 Test Acceptability Requirements for a 10-d Sediment Toxicity Test with *Chironomus tentans*

A. It is recommended for conducting a 10-d test with *C. tentans* that the following performance criteria be met:

1. Tests must be started with second- to third-instar larvae (about 10-d-old larvae; see Section 10.4.1).
2. Average survival of *C. tentans* in the control sediment must be greater than or equal to 70% at the end of the test.
3. Average size of *C. tentans* in the control sediment must be at least 0.48 mg AFDW at the end of the test.
4. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

B. Performance-based criteria for culturing *C. tentans* include the following:

1. It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.
2. Laboratories should keep a record of time to first emergence for each culture and record this information using control charts. Records should also be kept on the frequency of restarting cultures.
3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly: pH, hardness, alkalinity, and ammonia. Dissolved oxygen in the cultures should be measured weekly. Temperature of the cultures should be recorded daily. If static cultures are used, it may be desirable to measure water quality more frequently.
4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

1. All organisms in a test must be from the same source.
2. Storage of sediments collected from the field should follow guidance outlined in Section 8.2.
3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
5. Test organisms must be cultured and tested at 23°C (±1°C).
6. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.
7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

added is not being consumed. Feeding should be suspended for the amount of time necessary to increase the dissolved oxygen concentration (ASTM, 1999a). If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface should be made daily.

12.3.6 Monitoring a Test

12.3.6.1 All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

12.3.6.2 Measurement of Overlying Water-Quality Characteristics

12.3.6.2.1 Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of a test. Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to composite water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water. Water quality should be measured on each batch of water prepared for the test.

12.3.6.2.2 Water-only exposures evaluating the tolerance of *C. tentans* larvae to depressed DO have indicated that significant reductions in weight occurred after 10-d exposure to 1.1 mg/L DO, but not at 1.5 mg/L (V. Mattson, USEPA, Duluth, MN, personal communication). This finding concurs with the observations during method development at the USEPA laboratory in Duluth that excursions of DO as low as 1.5 mg/L did not seem to have an effect on midge survival and development (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication). Based on these findings, it appears that periodic depressions of DO below 2.5 mg/L (but not below 1.5 mg/L) are not likely to adversely affect test results, and thus should not be a reason to discard test data. Nonetheless, tests should be managed toward a goal of DO > 2.5 mg/L to insure satisfactory performance. If the

DO level of the water falls below 2.5 mg/L for any one treatment, aeration is encouraged and should be done in all replicates for the duration of the test. Occasional brushing of screens on outside of beakers will help maintain the exchange of water during renewals using the exposure system described by Benoit et al. (1993). If a probe is used to measure DO in overlying water, it should be thoroughly inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination. Aeration can be used to maintain dissolved oxygen in the overlying water above 2.5 mg/L (i.e., about 1 bubble/second in the overlying water).

12.3.6.2.3 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.

12.3.7 Ending a Test

12.3.7.1 Immobile organisms isolated from the sediment surface or from sieved material should be considered dead. A #40 sieve (425-µm mesh) can be used to remove midges from sediment. Alternatively, Kemble et al. (1994) suggest sieving of sediment using the following procedure: (1) pour about half of the overlying water through a #50- (300-µm) U.S. standard mesh sieve, (2) pour about half of the sediment through the #50-mesh sieve and wash the contents of the sieve into an examination pan, (3) rinse the coarser sediment remaining in the test chamber through a #40- (425-µm) mesh sieve and wash the contents of this second sieve into a second examination pan. Surviving midges can then be isolated from these pans. See Section 12.3.8.1 and 12.3.8.2 for the procedures for measuring weight or length of midges.

12.3.7.2 A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 5 min/replicate). Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control sediment and recovery could be determined after 1 h (Tomasovic et al., 1994).

12.3.8 Test Data

12.3.8.1 Ash-free dry weight (AFDW) and survival are the endpoints measured at the end of the 10-d sediment toxicity test with *C. tentans*. The 10-d method for *C. tentans* in the first edition of this manual (USEPA, 1994a), as well as most previous research, has used dry weight as a measure of growth. However, Sibley et al. (1997b) found that the grain size of sediments influences the amount of sediment that *C. tentans* larvae ingest and retain in their gut. As a result, in finer-grain sediments, a substantial portion of the measured dry weight may be comprised of sediment rather than tissue. While this may not represent a strong bias in tests with identical grain size distributions in all treatments, most field assessments are likely to have varying grain size among sites. This will likely create differences in dry weight among treatments that are not reflective of true somatic growth. For this reason, weight of midges should be measured as ash-free dry weight (AFDW) instead of dry weight. AFDW will more directly reflect actual differences in tissue weight by reducing the influence of sediment in the gut. The duration of the 10-d test starting with third-instar larvae is not long enough to determine emergence of adults. Average size of *C. tentans* in the control sediment must be at least 0.6 mg at the end of the test (0.48 mg AFDW) (Ankley et al., 1993; ASTM, 1999a; Section 17.5). If test organisms are to be used for an evaluation of bioaccumulation, it is not advisable to dry the sample before conducting the residue analysis. If conversion from wet weight to dry weight is necessary, aliquots of organisms can be weighed to establish wet to dry weight conversion factors. A consistent procedure should be used to remove the excess water from the organisms before measuring wet weight.

12.3.8.2 For determination of AFDW, first pool all living larvae in each replicate and dry the sample to a constant weight (e.g., 60°C for 24 h). Note that the weigh boats should be ashed before use to eliminate weighing errors due to the pan oxidizing during ashing. The sample is brought to room temperature in a dessicator and weighed to the nearest 0.01 mg to obtain mean weights per surviving organism per replicate. The dried larvae in the pan are then ashed at 550°C for 2 h. The pan with the ashed larvae is then reweighed and the tissue mass of the larvae is determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan. In rare instances where preservation is required, an 8% sugar formalin solution can be used to preserve samples (USEPA, 1994a), but the effects of preservation on the weights and lengths of the midges have not been sufficiently studied. Pupae or adult organisms must not be included in the sample to estimate ash-free dry weight. If head capsule width is to be measured, it should be measured on surviving midges at the end of the test before ash-free dry weight is determined.

12.3.8.3 Measurement of length is optional. Separate replicate beakers should be set up to sample lengths of midges at the end of an exposure. An 8% sugar formalin solution can be used to preserve samples for length measurements (Ingersoll and Nelson, 1990). The sugar formalin solution is prepared by adding 120 g of sucrose to 80 mL of formalin, which is then brought to a volume of 1 L using deionized water. This stock solution is mixed with an equal volume of deionized water when used to preserve organisms. NoTox® (Earth Safe Industries, Belle Mead, NJ) can be used as a substitute for formalin (Unger et al., 1993). Midge body length (±0.1 mm) can be measured from the anterior of the labrum to the posterior of the last abdominal segment (Smock, 1980). Kemble et al. (1994) photographed midges at magnification of 3.5X and measured the images using a computer-interfaced digitizing tablet. A digitizing system and microscope can

also be used to measure length (Ingersoll and Nelson, 1990).

12.4 Interpretation of Results

12.4.1 Section 16 describes general information for interpretation of test results. The following sections describe species-specific information that is useful in helping to interpret the results of sediment toxicity tests with *C. tentans*.

12.4.2 Age Sensitivity

12.4.2.1 Midges are perceived to be relatively insensitive organisms in toxicity assessments (Ingersoll, 1995). This conclusion is based on measuring survival of fourth-instar larvae in short-term water-only exposures, a procedure that may underestimate the sensitivity of midges to toxicants. The first and second instars of chironomids are more sensitive to contaminants than the third or fourth instars. For example, first-instar *C. tentans* larvae were 6 to 27 times more sensitive than fourth-instar larvae to acute copper exposure (Nebeker et al., 1984b; Gauss et al., 1985; Figure 12.1) and first-instar *C. riparius* larvae were 127 times more sensitive than second-instar larvae to acute cadmium exposure (Williams et al., 1986b; Figure 12.1). In chronic tests with first-instar larvae, midges were often as sensitive as daphnids to inorganic and organic compounds (Ingersoll et al., 1990). Sediment tests should be started with uniform age and size midges because of the dramatic differences in sensitivity of midges by age. Whereas third-instar midges are not as sensitive as younger organisms, the larger larvae are easier to handle and isolate from sediment at the end of a test.

12.4.2.2 DeFoe and Ankley (1998) studied a variety of contaminated sediments and showed that the sensitivity of *C. tentans* 10-d tests is greatly increased by measurement of growth in addition to survival. Growth of midges in 10-d sediment tests was found to be a more sensitive endpoint than survival of *Hyalella azteca* (DeFoe and Ankley, 1998). In cases where sensitivity of organisms before the third instar is of interest, the long-term sediment exposures can be used, since they begin with newly hatched larvae (Section 15).

12.4.3 Physical characteristics of sediment

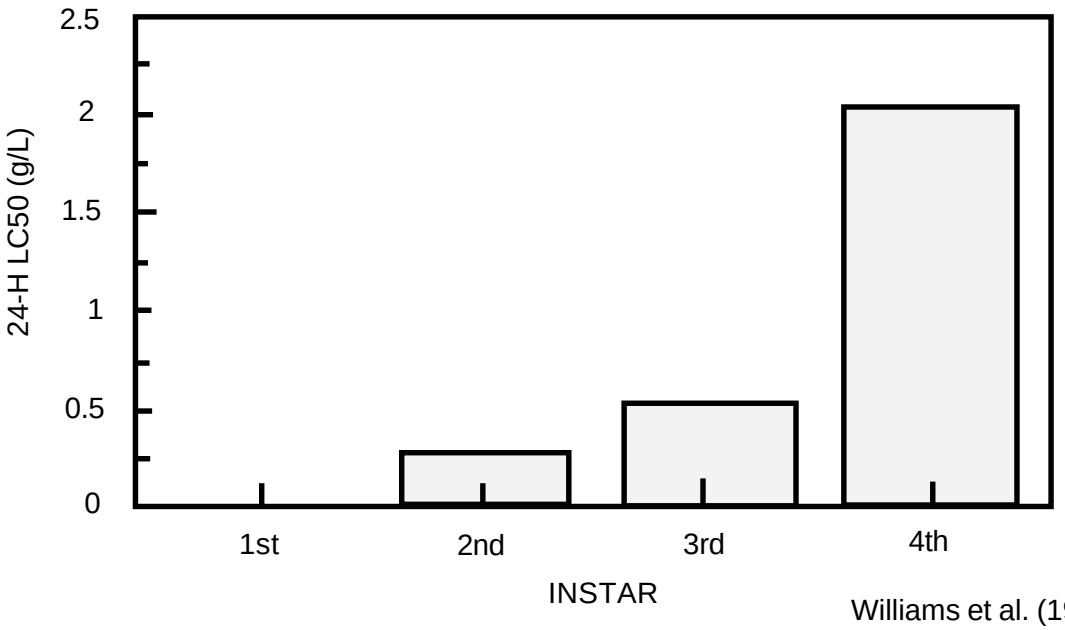
12.4.3.1 Grain Size

12.4.3.1.1 Larvae of *C. tentans* appear to be tolerant of a wide range of particle size conditions in substrates. Several studies have shown that survival is not affected by particle size in natural sediments, sand substrates, or formulated sediments in both 10-d and long-term exposures (Ankley et al., 1994; Suedel and Rodgers, 1994; Sibley et al., 1997b, 1998). Ankley et al. (1994a) found that growth of *C. tentans* larvae was weakly correlated with sediment grain size composition, but not organic carbon, in 10-d tests using 50 natural sediments from the Great Lakes. However, Sibley et al. (1997b) found that the correlation between grain size and larval growth disappeared after accounting for inorganic material contained within larval guts and concluded that growth of *C. tentans* was not related to grain size composition in either natural sediments or sand substrates. Avoiding confounding influences of gut contents on weight is the impetus for recommending ash-free dry weight (instead of dry weight) as the index of growth in the 10-day and long-term *C. tentans* tests. Failing to do so could lead to erroneous conclusions regarding the toxicity of the test sediment (Sibley et al., 1997b). Procedures for correcting for gut contents are described in Section 12.3.8. Emergence, reproduction (mean eggs/female), and hatch success were also not affected by the particle size composition of substrates in long-term tests with *C. tentans* (Sibley et al., 1998; Section 15).

12.4.3.2 Organic Matter

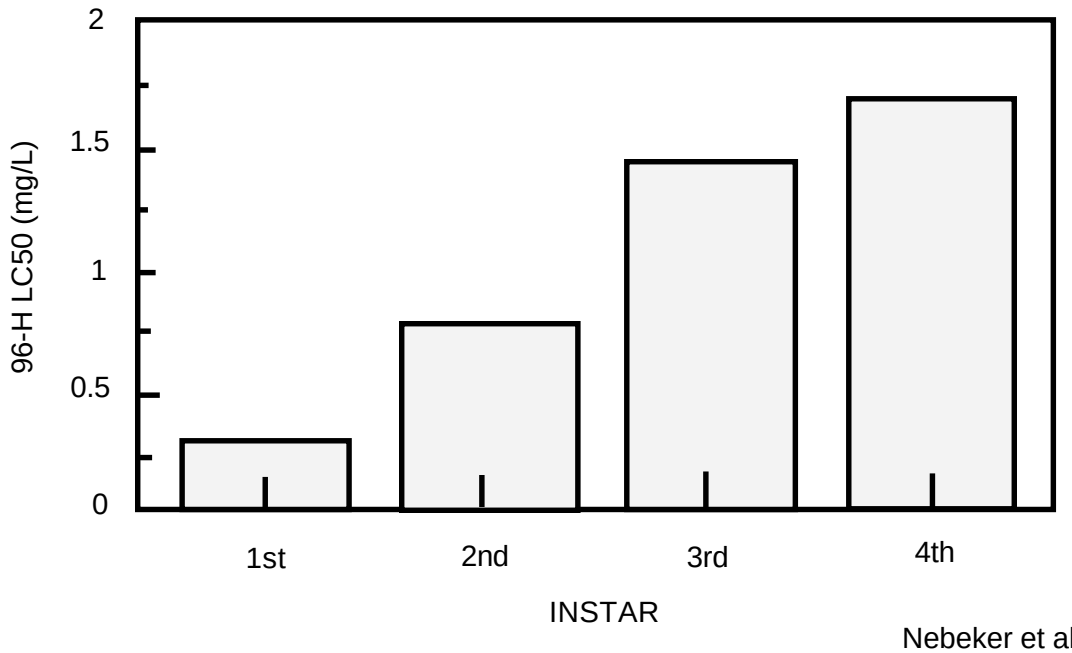
12.4.3.2.1 Based on 10-d tests, the content of organic matter in sediments does not appear to affect survival of *C. tentans* larvae in natural and formulated sediments, but may be important with respect to larval growth. Ankley et al. (1994a) found no relationship between sediment organic content and survival or growth in 10-d bioassays with *C. tentans* in natural sediments. Suedel and Rodgers (1994) observed reduced survival in 10-d tests with a formulated sediment when organic matter was <0.91%; however, supplemental food was not supplied in this study, which may influence these results relative to the 10-d test procedures described in this manual. Lacey et al. (1999) found that survival of *C. tentans* larvae was generally not affected in 10-d tests by either the quality or quantity of synthetic (alpha-cellulose) or naturally derived (peat, maple leaves) organic material spiked into a formulated sediment, although a slight reduction in survival below the acceptability criterion (70%) was observed in a natural sediment diluted with formulated sediment at an organic matter content of 6%. In terms of larval growth, Lacey et al. (1999) did not observe any systematic relationship between the level of organic material (e.g., food quantity) and larval growth for each carbon source. Although a significant reduction in growth was observed at the highest concentration (10%) of the leaf treatment in the food quantity study, significantly higher larval growth was observed in this treatment when the different carbon sources were compared at about equal concentrations (effect of food quality). In the latter study, the following gradient of larval growth was established in relation to the source of organic carbon: peat < natural sediment < alpha-cellulose < leaves. Since all of the treatments received a supplemental source of food, these data suggest that both the quality and quantity of organic carbon in natural and formulated sediments may represent an important confounding factor for the growth endpoint in tests with *C. tentans* (Lacey et al., 1999). However, it is important to note that these data are based on 10-d tests; the applicability of these data to long-term testing has not been evaluated (Section 15).

A. *Chironomus riparius:* Cadmium



Williams et al. (1985)

B. *Chironomus tentans*: Copper



Nebeker et al. (1964)

Figure 12.1 Lifestage sensitivity of chironomids.

12.4.4 Isolating Organisms at the End of a Test

12.4.4.1 Quantitative recovery of larvae at the end of a 10-d sediment test should not be a problem. The larvae are red and typically greater than 5 mm long.

12.4.5 Influence of Indigenous Organisms

12.4.5.1 The influence of indigenous organisms on the response of *C. tentans* in sediment tests has not been reported. Survival of a closely related species, *C. riparius* was not reduced in the presence of oligochaetes in sediment samples (Reynoldson et al., 1994). However, growth of *C. riparius* was reduced when high numbers of oligochaetes were placed in a sample. Therefore, it is important to determine the number and biomass of indigenous organisms in field-collected sediment in order to better interpret growth data (Reynoldson et al., 1994; DeFoe and Ankley, 1998). Furthermore, presence of predators may also influence the response of test organisms in sediment (Ingersoll and Nelson, 1990).

12.4.6. Sexual Dimorphism

12.4.6.1 Differences in size between males and females of a closely related midge species (*Chironomus riparius*) had little effect on interpretation of growth-related effects in sediment tests (<3% probability of making a Type I error [nontoxic sample classified as toxic] due to sexual dimorphism; Day et al., 1994). Therefore, sexual dimorphism will probably not be a confounding factor when interpreting growth results measured in sediment tests with *C. tentans*.

12.4.7 Ammonia Toxicity

12.4.7.1 Section 1.3.7.5 addresses interpretative guidance for evaluating toxicity associated with ammonia in sediment.

Section 13
Test Method 100.3
Lumbriculus variegatus Bioaccumulation Test for Sediments

13.1 Introduction

13.1.1 *Lumbriculus variegatus* (Oligochaeta) have many desirable characteristics of an ideal sediment bioaccumulation testing organism including contact with sediment, ease of culture in the laboratory, and tolerance to varying physico-chemical characteristics of sediment. The response of *L. variegatus* in laboratory exposures has been confirmed with natural benthic populations. Many investigators have successfully used *L. variegatus* in toxicity or bioaccumulation tests. Toxicity studies have been conducted in water-only tests (Bailey and Liu, 1980; Hornig, 1980; Ewell et al., 1986; Nebeker et al., 1989; Ankley et al., 1991a; Ankley et al., 1991b), in effluent tests (Hornig, 1980), and in whole-sediment tests (Nebeker et al., 1989; Ankley et al., 1991a; Ankley et al., 1991b; Ankley et al., 1992a; Call et al., 1991; Carlson et al., 1991; Phipps et al., 1993; West et al., 1993). Several studies have reported the use of *L. variegatus* to examine bioaccumulation of chemicals from sediment (Schuytema et al., 1988; Nebeker et al., 1989; Ankley et al., 1991b; Call et al., 1991; Carlson et al., 1991; Ankley et al., 1993; Kukkonen and Landrum, 1994; and Brunson et al., 1993, 1998). However, interlaboratory studies have not yet been conducted with *L. variegatus.*

13.1.2 Additional research is needed on the standardization of bioaccumulation procedures with sediment. Therefore, Section 13.2 describes general guidance for conducting a 28-d sediment bioaccumulation test with *L. variegatus*. Methods outlined in Appendix A of USEPA (1994a) and in Section 13.1.1 were used for developing this general guidance. Results of tests using procedures different from the procedures described in Section 13.2 may not be comparable, and these different procedures may alter bioavailability. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

13.2 Procedure for Conducting Sediment Bioaccumulation Tests with *Lumbriculus variegatus*

13.2.1 Recommended test conditions for conducting a 28-d sediment bioaccumulation test with *L. variegatus* are summarized in Table 13.1. Table 13.2 outlines procedures for conducting sediment toxicity tests with *L. variegatus*. A general activity schedule is outlined in Table 13.3. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 16). The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. When variability remains constant, the sensitivity of a test increases as the number of replicates increases.

13.2.2 The recommended 28-d sediment bioaccumulation test with *L. variegatus* can be conducted with adult oligochaetes at 23°C with a 16L:8D photoperiod at a illuminance of about 100 to 1000 lux (Table 13.1). Test chambers can be 4 to 6 L that contain 1 to 2 L of sediment and 1 to 4 L of overlying water. The number of replicates/treatment depends on the objective of the test. Five replicates are recommended for routine testing (Section 16). To minimize depletion of sediment contaminants, the ratio of total organic carbon in sediment to dry weight of organisms should be about 50:1. A minimum of 1 g/replicate with up to 5 g/replicate should be tested. Oligochaetes are not fed during the test. Each chamber receives 2 volume additions/d of overlying water. Appendix A and Brunson et al., (1998) describe water-renewal systems that can be used to deliver overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are outlined in Table 13.4.

13.2.2.1 Before starting a 28-d sediment bioaccumulation test with *L. variegatus,* a toxicity screening test can be conducted for at least 4 d using procedures outlined in Table 13.2 (Brunson et al., 1993). The preliminary toxicity screening test is conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux. Test chambers are 300-mL high-form lipless beakers containing

Table 13.1 Recommended Test Conditions for Conducting a 28-d Sediment Bioaccumulation Test with *Lumbriculus variegatus*

Parameter	Conditions
1. Test type:	Whole-sediment bioaccumulation test with renewal of overlying water
2. Temperature:	23 ± 1°C
3. Light quality:	Wide-spectrum fluorescent lights
4. Illuminance:	About 100 to 1000 lux
5. Photoperiod:	16L:8D
6. Test chamber:	4- to 6-L aquaria with stainless steel screens or glass standpipes
7. Sediment volume:	1 L or more depending on TOC
8. Overlying water volume:	1 L or more depending on TOC
9. Renewal of overlying water:	2 volume additions/d (Appendix A); continuous or intermittent (e.g., one volume addition every 12 h)
10. Age of test organisms:	Adults
11. Loading of organisms in chamber:	Ratio of total organic carbon in sediment to organism dry weight should be no less than 50:1. Minimum of 1 g/replicate. Preferably 5 g/replicate.
12. Number of replicate chambers/treatment:	Depends on the objective of the test. Five replicates are recommended for routine testing (see Section 16).
13. Feeding:	None
14. Aeration:	None, unless DO in overlying water drops below 2.5 mg/L
15. Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16. Test chamber cleaning:	If screens become clogged during the test, gently brush the *outside* of the screen (Appendix A).
17. Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
18. Test duration:	28 d
19. Endpoint:	Bioaccumulation
20. Test acceptability:	Performance-based criteria specifications are outlined in Table 13.4.

100 mL of sediment and 175 mL of overlying water. Ten adult oligochaetes/replicate are used to start a test. Four replicates are recommended for toxicity screening tests. Oligochaetes are not fed during the test. Each chamber receives 2 volume additions/d of overlying water. Appendix A and Brunson et al. (1998) describe water-renewal systems that can be used to deliver overlying water. Overlying water should be similar to the water to be used in the bioaccumulation test. Endpoints monitored at the end of a toxicity test are number of organisms and behavior. Numbers of *L. variegatus* in the toxicity screening test should not be significantly reduced in the test sediment relative to the control sediment. Test organisms should burrow into test sediment. Avoidance of test sediment by *L. variegatus* may decrease bioaccumulation.

13.3 General Procedures

13.3.1 Sediment into Test Chambers

13.3.1.1 The day before the sediment test is started (Day -1) each sediment should be thoroughly homogenized and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the extent of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

13.3.1.2 Each test chamber should contain the same amount of sediment, determined either by volume or by weight. Overlying water is added to the chambers in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon® with a handle attached) placed above the sediment to dissipate the force of the water. Renewal of overlying water is started on Day -1. A test begins when the organisms are added to the test chambers (Day 0).

13.3.2 Renewal of Overlying Water

13.3.2.1 Renewal of overlying water is recommended during a test. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Hardness, alkalinity and ammonia

Table 13.2 Recommended Test Conditions for Conducting a Preliminary 4-d Sediment Toxicity Screening Test with *Lumbriculus variegatus*

	Parameter	Conditions
1.	Test type:	4-d whole-sediment toxicity test with renewal of overlying water
2.	Temperature:	23 ± 1°C
3.	Light quality:	Wide-spectrum fluorescent lights
4.	Illuminance:	About 100 to 1000 lux
5.	Photoperiod:	16L:8D
6.	Test chamber:	300-mL high-form lipless beaker
7.	Sediment volume:	100 mL
8.	Overlying water volume:	175 mL
9.	Renewal of overlying water:	2 volume additions/d (Appendix A); continuous or intermittent (e.g., one volume addition every 12 h)
10.	Age of test organisms:	Adults
11.	Number of organisms/chamber:	10
12.	Number of replicate chambers/treatment:	4 minimum
13.	Feeding:	None
14.	Aeration:	None, unless DO in overlying water drops below 2.5 mg/L
15.	Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16.	Test chamber cleaning:	If screens become clogged during the test, gently brush the *outside* of the screen.
17.	Overlying water quality:	Hardness, alkalinity, conductivity, pH, and ammonia at the beginning and end of a test. Temperature and dissolved oxygen daily.
18.	Test duration:	4 d (minimum; up to 10 d)
19.	Endpoints:	Number of organisms and behavior. There should be no significant reduction in number of organisms in a test sediment relative to the control.
20.	Test acceptability:	Performance-based criteria specifications are outlined in Table 13.4.

concentrations in the water above the sediment, within a treatment, should not vary by more than 50% during the test. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water-delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998; Wall et al., 1998; Leppanen and Maier, 1998). Each water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms on Day 0 (Appendix A).

13.3.2.2 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the interstitial water.

13.3.3 Acclimation

13.3.3.1 Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

13.3.3.2 Culturing of organisms and toxicity assessment are typically conducted at 23°C. However, occasionally there is a need to perform evaluations at temperatures different than that recommended. Under these circumstances, it may be necessary to acclimate organisms to the desired test temperature to prevent thermal shock

Table 13.3 General Activity Schedule for Conducting a 28-d Sediment Bioaccumulation Test with *Lumbriculus variegatus*

A. Conducting a 4-d Toxicity Screening Test (conducted before the 28-d bioaccumulation test)

Day	*Activity*
-1	Isolate worms for conducting toxicity screening test. Add sediment into each test chamber, place chambers into exposure system, and start renewing overlying water.
0	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia). Transfer 10 worms into each test chamber. Measure weight of a subset of 20 organisms used to start the test. Observe behavior of test organisms.
1 to 2	Measure temperature and dissolved oxygen. Observe behavior of test organisms.
3	Same as Day 1. Measure total water quality.
4	Measure temperature and dissolved oxygen. End the test by collecting the oligochaetes with a sieve and determine weight of survivors. Bioaccumulation tests should not be conducted with *L. variegatus* if a test sediment significantly reduces number of oligochaetes relative to the control sediment or if oligochaetes avoid the sediment.

B. Conducting a 28-d Bioaccumulation Test

Day	*Activity*
-1	Isolate worms for conducting bioaccumulation test. Add sediment into each test chamber, place chambers into exposure system, and start renewing overlying water.
0	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia). Transfer appropriate amount of worms (based on weight) into each test chamber. Sample a subset of worms used to start the test for residue analyses. Observe behavior of test organisms.
1 to 6	Measure temperature and dissolved oxygen. Observe behavior of test organisms.
7	Same as Day 1. Measure total water quality.
8 to 13	Same as Day 1
14	Same as Day 7
15 to 20	Same as Day 1
21	Same as Day 7
22 to 26	Same as Day 1
27	Measure total water quality.
28	Measure temperature and dissolved oxygen. End the uptake by collecting the worms with a sieve. Separate any indigenous organisms from *L. variegatus.* Determine the weight of survivors. Eliminate the gut contents of surviving worms in water for 6 to 8 h. Longer purging periods (not to exceed 24 hours) may be used if all target analytes have Log K_{ow} >5 (Section 13.3.7.3).

when moving immediately from the culture temperature to the test temperature (ASTM, 1999a). Acclimation can be achieved by exposing organisms to a gradual change in temperature; however, the rate of change should be relatively slow to prevent thermal shock. A change in temperature of 1°C every 1 to 2 h has been used successfully in some studies (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication). Testing at temperatures other than 23°C needs to be preceded by studies to determine expected performance under alternate conditions.

13.3.4 Placing Organisms in Test Chambers

13.3.4.1 Isolate oligochaetes for starting a test as described in Section 10.5.6. A subset of *L. variegatus* at the start of the test should be sampled to determine starting concentrations of chemicals of concern. Mean group weights should be measured on a subset of at least 100 organisms used to start the test. The ratio of total organic carbon in sediment to dry weight of organisms at the start of the test should be no less than 50:1.

13.3.4.2 Oligochaetes added to each replicate should not be blotted to remove excess water (Section 10.5.6). Oligochaetes can be added to each replicate at about 1.33 X of the target stocking weight (Brunson et al., 1998). This additional 33% should account for the excess weight from water in the sample of nonblotted oligochaetes at the start of the test.

13.3.5 Feeding

13.3.5.1 *Lumbriculus variegatus* should not be fed during a bioaccumulation test.

Table 13.4 Test Acceptability Requirements for a 28-d Sediment Bioaccumulation Test with *Lumbriculus variegatus*

A. It is recommended for conducting a 28-d test with *L. variegatus* that the following performance criteria be met:

1. Numbers of *L. variegatus* in a 4-d toxicity screening test should not be significantly reduced in the test sediment relative to the control sediment.
2. Test organisms should burrow into test sediment. Avoidance of test sediment by *L. variegatus* may decrease bioaccumulation.
3. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

B. Performance-based criteria for culturing *L. variegatus* include the following:

1. It may be desirable for laboratories to periodically perform 96-h water-only reference toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.
2. Laboratories should monitor the frequency with which the population is doubling in the culture (number of organisms) and record this information using control charts (doubling rate would need to be estimated on a subset of animals from a mass culture). Records should also be kept on the frequency of restarting cultures. If static cultures are used, it may be desirable to measure water quality more frequently.
3. Food used to culture organisms should be analyzed before the start of a test for compounds to be evaluated in the bioaccumulation test.
4. Laboratories should record the following water-quality characteristics of the cultures at least quarterly and the day before the start of a sediment test: pH, hardness, alkalinity, and ammonia. Dissolved oxygen in the cultures should be measured weekly. Temperature of the cultures should be recorded daily.
5. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
6. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

1. All organisms in a test must be from the same source.
2. Storage of sediments collected from the field should follow guidance outlined in Section 8.2.
3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and/or the appropriate solvent controls must be included in a test. The concentration of solvent used must not affect test organisms adversely.
5. Test organisms must be cultured and tested at 23°C (±1°C).
6. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C
7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

13.3.6 Monitoring a Test

13.3.6.1 All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

13.3.6.2 Measurement of Overlying Water-quality Characteristics

13.3.6.2.1 Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of a test. Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to composite water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water. Water quality should be measured on each batch of water prepared for the test.

13.3.6.2.2 Dissolved oxygen should be measured daily and should be above 2.5 mg/L. If a probe is used to measure dissolved oxygen in overlying water, it should be thoroughly inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination. Aeration can be used to maintain dissolved oxygen in the overlying water above 2.5 mg/L (i.e., about 1 bubble/second in the overlying water). Dissolved oxygen and pH can be measured directly in the overlying water with a probe.

13.3.6.2.3 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.

13.3.7 Ending a Test

13.3.7.1 Sediment at the end of the test can be sieved through a fine-meshed screen sufficiently small to retain the oligochaetes (e.g., U.S. standard sieve #40 (425-µm mesh) or #60 (250-µm mesh). The sieved material should be quickly transferred to a shallow pan to keep oligochaetes from moving through the screen. Immobile organisms should be considered dead.

13.3.7.2 The sediment contribution to the body weight of *Lumbriculus variegatus* is reported to be about 20% of the wet weight and the contribution to chemical concentrations ranges from 0 to 11% in two laboratory studies (Kukkonen and Landrum, 1994; 1995). Analyses by Mount et al. (1998) suggest that under certain conditions substantially larger errors may occur if gut contents are included in samples for tissue analysis. Accordingly, after separating the organisms from the sediment, test animals are held in clean water to allow the worms to purge their guts of sediment. To initiate gut purging, live oligochaetes are transferred from the sieved material to a 1-L beaker containing overlying water only. Oligochaetes should not be placed in clean sediment to eliminate gut contents. Clean sediment can add to the dry weight of the oligochaetes, which would result in a dilution of chemical concentrations on a dry weight basis. Further, purging in clean sediment is thought to accelerate depuration of chemical from tissues (Kukkonen and Landrum, 1994). The elimination beakers may need to be aerated to maintain dissolved oxygen above 2.5 mg/L.

13.3.7.3 The first edition of this manual (USEPA, 1994a) specified a 24-h holding period for gut purging, based on the findings of Call et al. (1991) who reported that *L. variegatus* clear more than 90% of their gut contents in 24 h. Kukkonen and Landrum (1995) reported *L. variegatus* will purge out the intestinal contents in 10 h in water, and more recently, Mount et al. (1999) found that gut purging of *L. variegatus* was essentially complete in only 6 h . Shorter purging periods may be preferable to reduce depuration of chemical from tissue during holding in clean water, particularly for compounds with log K_{ow} < 5 (Figure 13.1). Mount et al. (1999) estimated that after a 6-h purging period, compounds with log K_{ow} > 3.85 would remain at >90% of their initial concentrations, but after 24 h, only compounds with log K_{ow} > 5 would be at >90% of the initial concentration in tissue. For this reason, it is recommended that the purging period last 6 to 8 h. Longer purging periods (not to exceed 24 hours) may be used if all target analytes have log K_{ow} > 5.

13.3.7.4 Field-collected sediments may include indigenous oligochaetes. The behavior and appearance of indigenous oligochaetes are usually different from *L. variegatus.* It may be desirable to test extra chambers without the addition of *L. variegatus* to check for the presence of indigenous oligochaetes in field-collected sediment (Phipps et al., 1993). Bioaccumulation of chemicals by indigenous oligochaetes exposed in the same chamber with introduced *L. variegatus* in a 28-d test has been evaluated (Brunson et al., 1993). Peak concentrations of select PAHs and DDT in this study were similar in the indigenous oligochaetes and *L. variegatus* exposed in the same chamber for 28 d.

13.3.7.5 Care should be taken to isolate at least the minimum amount of tissue mass from each replicate chamber needed for analytical chemistry.

13.3.8 Test Data

13.3.8.1 Sensitivity of tissue analyses is dependent largely on the mass of tissue available and the sensitivity of the analytical procedure. To obtain meaningful results from bioaccumulation tests, it is essential that desired detection limits be established before testing, and that the test design allow for sufficient tissue mass. Tissue masses required for various analyses at selected lower limits of detection are listed in Table 13.5. Detection limits for individual PAHs in tissue are listed in Table 13.6. For most chemicals, a minimum mass of 1 g/replicate (wet weight) and preferably 5 g/replicate (wet weight) should be tested. Again, however, to insure results will be meaningful, required masses for analytes of interest to the study should be specifically evaluated before the study is designed.

13.3.8.2 If an estimate of dry weight is needed, a subsample should be dried to a constant weight at about 60 to 90°C. The sample is brought to room temperature in a desiccator and weighed to the nearest 0.01 mg. *Lumbriculus variegatus* typically contain about 1% lipid (wet weight). It may be desirable to determine ash-free dry weight (AFDW) of oligochaetes instead of dry weight. Measurement of AFDW is recommended over dry weight for *C. tentans* due to the contribution of sediment in the gut to the weight of midge (Section 12.3.8; Sibley et al., 1997b). Additional data are needed to determine the contribution of sediment in the gut of *L. variegatus* to body weight before a definitive recommendation can be made to measure AFDW of oligochaetes routinely.

13.3.8.3 Depending on specific study objectives, total lipids can be measured on a subsample of the total tissue mass of each thawed replicate sample. Gardner et al. (1985) describe procedures for measuring lipids in 1 mg of tissue. Different methods of lipid analysis can yield different results (Randall et al., 1991). The analytical method used for lipid analysis should be calibrated against the chloroform-methanol extraction method described by Folch et al. (1957) and Bligh and Dyer (1959).

13.3.8.3.1 A number of studies have demonstrated that lipids are the major storage site for organic chemicals in a variety of organisms (Roberts et al., 1977; Oliver and Niimi, 1983; de Boer, 1988). Because of the importance of

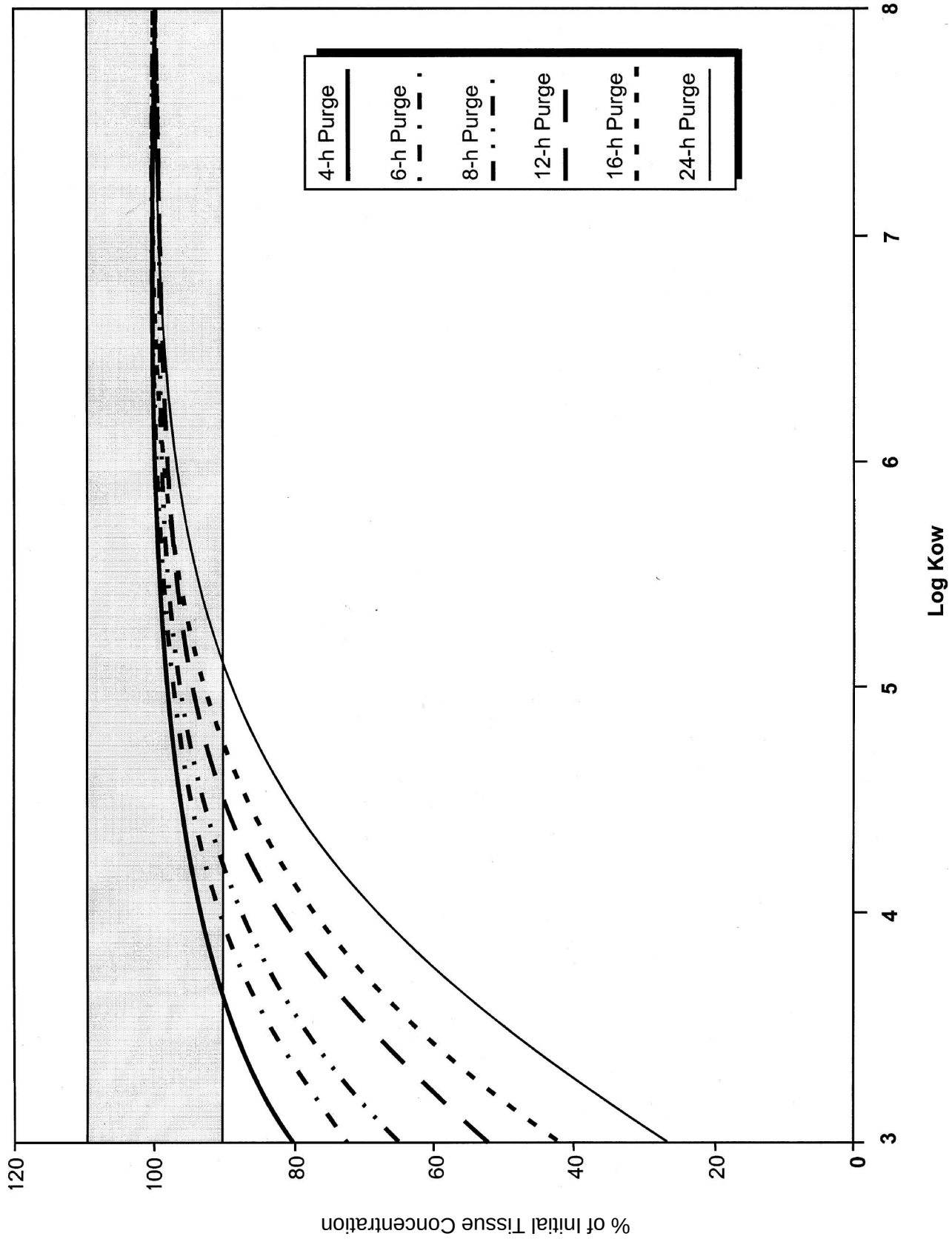


Figure 13.1 Predicted depuration of nonionic organic chemicals from tissue of *Lumbriculus variegatus* as a function of K_{ow} and duration of depuration, assuming no contribution of sediment in the gut. Shaded area represents $\pm$10% of tissue concentration at the beginning of the depuration period (Mount et al., 1999).

Table 13.5 Grams of *Lumbriculus variegatus* Tissue (Wet Weight) Required for Various Analytes at Selected Lower Limits of Detection

Analyte	Grams of Tissue 1.0	2.0	5.0
	Lower Limit of Detection (µg/g)		
PCBs			
PCB (total[1])	0.600	0.300	0.120
PCB (congener[2])			
Level of chlorination			
mono-trichloro	0.025	0.0125	0.005
tetra-hexachloro	0.050	0.025	0.010
hepta-octachloro	0.075	0.0375	0.015
nona-decachloro	0.125	0.0625	0.025
Organochlorine pesticides [1]			
p,p´ DDE	0.050	0.025	0.010
p,p´ - DDD	0.050	0.025	0.010
p,p´ - DDT	0.050	0.025	0.010
o,p´ - DDE	0.050	0.025	0.010
o,p´ DDD	0.050	0.025	0.010
o,p´ DDT	0.050	0.025	0.010
Alpha-chlordane	0.050	0.025	0.010
Gamma-chlordane	0.050	0.025	0.010
Dieldrin	0.050	0.025	0.010
Endrin	0.050	0.025	0.010
Heptachlorepoxide	0.050	0.025	0.010
Oxychlordane	0.050	0.025	0.010
Mirex	0.050	0.025	0.010
Trans - nonachlor	0.050	0.025	0.010
Toxaphene	0.600	0.300	0.120
PAHs [3]			
PAHs	0.012	0.006	0.002
Dioxins [4]			
TCDD (ng/g)	0.020	0.010	0.004
Inorganic [5]			
Cadmium	0.005	0.0025	0.001
Copper	0.005	0.0025	0.001
Lead	0.005	0.0025	0.001
Zinc	0.005	0.0025	0.001

[1] Schmitt et al., 1990
[2] USEPA, 1990c
[3] Vassilaros et al., 1982
[4] USEPA, 1990d
[5] Schmitt and Finger, 1987

Table 13.6 Detection Limits (ng) of Individual PAHs by HPLC-FD[1]

Analyte	Detection Limit (ng)
Benzo(a)pyrene	0.01
Pyrene	0.03
Benzo(k)fluoranthene	0.03
Dibenz(a,h)anthracene	0.03
Anthracene	0.10
Benz(a)anthracene	0.10
Benzo(e)pyrene	0.10
Benzo(b)fluoranthene	0.10
Benzo(g,h,i)perylene	0.10
3-Methyleholanthrene	0.10

[1] Obana et al., 1981

lipids, it may be desirable to normalize bioaccumulated concentrations of nonpolar organics to the tissue lipid concentration. Lipid concentration is one of the factors required in deriving the BSAF (Section 16). However, the difficulty with using this approach is that each lipid method generates different lipid concentrations (see Kates (1986) for discussion of lipid methodology). The differences in lipid concentrations directly translate to a similar variation in the lipid-normalized chemical concentrations or BSAF.

13.3.8.3.2 For comparison of lipid-normalized tissue residues or BASFs, it is necessary to either promulgate a standard lipid technique or to intercalibrate the various techniques. Standardization of a single method is difficult because the lipid methodology is often intimately tied in with the extraction procedure for chemical analysis. As an interim solution, the Bligh-Dyer lipid method (Bligh and Dyer, 1959) is recommended as a temporary "intercalibration standard" (ASTM, 1999c).

13.3.8.3.3 The potential advantages of Bligh-Dyer include its ability to extract neutral lipids not extracted by many other solvent systems and the wide use of this method (or the same solvent system) in biological and toxicological studies (e.g., Roberts et al., 1977; Oliver and Niimi, 1983; de Boer, 1988; Landrum, 1989). Because the technique is independent of any particular analytical extraction procedure, it will not change when the extraction technique is changed. Additionally, the method can be modified for small tissue sample sizes as long as the solvent ratios are maintained (Herbes and Allen, 1983; Gardner et al., 1985).

13.3.8.3.4 If the Bligh-Dyer method is not the primary lipid method used, the chosen lipid analysis method should be compared with Bligh-Dyer for each tissue type. The chosen lipid method can then be converted to "Bligh-Dyer" equivalents and the lipid-normalized tissue

residues reported in "Bligh-Dyer equivalents." In the interim, it is suggested that extra tissue of each species be frozen for future lipid analysis in the event that a different technique proves more advantageous (ASTM, 1999c).

13.4 Interpretation of Results

13.4.1 Section 16 describes general information for interpretation of test results. The following sections describe species-specific information that is useful in helping to interpret the results of sediment bioaccumulation tests with *L. variegatus.*

13.4.2 Duration of Exposure

13.4.2.1 Because data from bioaccumulation tests often will be used in ecological or human health risk assessments, the procedures are designed to generate quantitative estimates of steady-state tissue residues. Eighty percent of steady state is used as the general criterion (ASTM, 1999c). Because results from a single or few species often will be extrapolated to other species, the procedures are designed to maximize exposure to sediment-associated chemicals so as not to systematically underestimate residues in untested species.

13.4.2.2 A kinetic study can be conducted to estimate steady-state concentrations instead of conducting a 28-d bioaccumulation test (e.g., sample on Day 1, 3, 7, 14, 28; Brunson et al., 1993; USEPA-USACE, 1991). A kinetic test conducted under the same test conditions outlined above, can be used when 80% of steady state will not be obtained within 28 d or when more precise estimates of steady-state tissue residues are required. Exposures shorter than 28 d may be used to determine whether compounds are bioavailable (i.e., bioaccumulation potential).

13.4.2.3 DDT reportedly reached 90% of steady state by Day 14 of a 56-d exposure with *L. variegatus*. However, low molecular weight PAHs (e.g., acenaphthylene, fluorene, phenanthrene) generally peaked at Day 3 and tended to decline to Day 56 (Brunson et al., 1993). In general, concentrations of high molecular weight PAHs (e.g., benzo[b]fluoranthene, benzo[e]pyrene, indeno-[1,2,3-c,d]pyrene) either peaked at Day 28 or continued to increase during the 56-d exposure.

13.4.3 Influence of Indigenous Organisms

13.4.3.1 Field-collected sediments may include indigenous oligochaetes. Phipps et al. (1993) recommend testing extra chambers without the addition of *L. variegatus* to check for the presence of indigenous oligochaetes in field-collected sediment.

13.4.4 Sediment Toxicity in Bioaccumulation Tests

13.4.4.1 Toxicity or altered behavior of organisms in a sample may not preclude use of bioaccumulation data; however, information on adverse effects of a sample should be included in the report.

13.4.4.2 Grain Size.

13.4.4.2.1 *Lumbriculus variegatus* are tolerant of a wide range of substrates. Physico-chemical characteristics (e.g., grain size) of sediment were not significantly correlated to the growth or reproduction of *L. variegatus* in 10-d toxicity tests (see Section 10.1.3.3; Ankley et al., 1994a).

13.4.4.3 Sediment Organic Carbon

13.4.4.3.1 Reduced growth of *L. variegatus* may result from exposure to sediments with low organic carbon concentrations (G.T. Ankley, USEPA, Duluth, MN, personal communication). For this reason, reduced growth observed in bioaccumulation tests could be caused by either direct toxicity or insufficient nutrition of the sediment. Testing additional replicate chambers with supplemental food could be used to help make this distinction, although the effect of added food on accumulation of chemicals would need to be considered in the test interpretation.

13.4.4.4 Ammonia Toxicity

13.4.4.4.1 Section 1.3.7.5 addresses interpretative guidance for evaluating toxicity associated with ammonia in sediment.

Section 14
Test Method 100.4
Hyalella azteca 42-d Test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth, and Reproduction

14.1 Introduction

14.1.1 *Hyalella azteca* are routinely used to assess the toxicity of chemicals in sediment (Section 11; Nebeker et al., 1984; Dillon and Gibson,1986; Burton et al., 1989; Burton et al., 1992; Ingersoll and Nelson, 1990; Borgmann and Munawar, 1989; Ankley et al., 1994; Winger and Lazier, 1994; Suedel and Rodgers, 1994; Day et al., 1995; Kubitz et al.,1996). Test duration and endpoints recommended in previously developed standard methods for sediment testing with *H. azteca* include 10-d survival (Section 11; USEPA, 1994a) and 10- to 28-d survival and growth (ASTM, 1999a; Environment Canada, 1998a). Short-term exposures which only measure effects on survival can be used to identify high levels of contamination, but may not be able to identify marginally contaminated sediments. The method described in this section can be used to evaluate potential effects of contaminated sediment on survival, growth, and reproduction of *H. azteca* in a 42-d test.

14.1.2 Section 14.2 describes general guidance for conducting a 42-d test with *H. azteca* that can be used to evaluate the effects of contaminants associated with sediments on survival, growth and reproduction. Refinements of these methods may be described in future editions of this manual after additional laboratories have successfully used the method (Section 17.6). The 42-d test with *H. azteca* has not been adequately evaluated in water with elevated salinity (Section 1.3.2).

14.1.3 The procedure outlined in Section 14.2 is based on procedures described in Ingersoll et al. (1998). The sediment exposure starts with 7- to 8-d-old amphipods. On Day 28, amphipods are isolated from the sediment and placed in water-only chambers where reproduction is measured on Day 35 and 42. Typically, amphipods are first in amplexus at about Day 21 to 28 with release of the first brood between Day 28 to 42. Endpoints measured include survival (Day 28, 35 and 42), growth (as length or dry weight measured on Day 28 and 42), and reproduction (number of young/female produced from Day 28 to 42). The procedures described in Table 14.1 include measurement of a variety of lethal and sublethal endpoints; minor modifications of the basic methods can be used in cases where only a subset of these endpoints is of interest.

14.1.3.1 Several designs were considered for measuring reproduction in sediment exposures based on the reproductive biology of *H. azteca* (Ingersoll et al., 1998). The first design considered was a continuation of the 28-d sediment exposures described in Ingersoll et al. (1996) for an additional two weeks to determine the number of young produced in the first brood. The limitation of this design is the difficulty in quantitatively isolating young amphipods from sediment (Tomasovic et al., 1995). A second design considered was extension of the 28-d sediment exposure for an additional month or longer until several broods are released. These multiple broods could then be isolated from the sediment. The limitation of this second design is that specific effects on reproduction could not be differentiated from reduced survival of offspring and it would still be difficult to isolate the young amphipods from sediment. A third design considered, and the one described in this manual, was to expose amphipods in sediment until a few days before the release of the first brood. The amphipods could then be sieved from the sediment and held in water to determine the number of young produced (Ingersoll et al., 1998). This test design allows a quantitative measure of reproduction. One limitation to this design is that amphipods might recover from effects of sediment exposure during this holding period in clean water (Landrum and Scavia, 1983; Kane Driscoll et al., 1997); however, amphipods are exposed to sediment during critical developmental stages before release of the first brood in clean water.

14.1.4 The method has been used to evaluate a formulated sediment and field-collected sediments with low to moderate concentrations of contaminants (Ingersoll et al., 1998). Survival of amphipods in these sediments was typically >85% after the 28-d sediment exposures and the 14-d holding period in water to measure reproduction (Ingersoll et al., 1998). The method outlined in 14.2 has also been evaluated in round-robin testing with 8 to 12 laboratories (Section 17.6). After the 28-d sediment exposures in a control sediment (West Bearskin), survival was >80% for >88% of the laboratories; length was >3.2 mm/individual for >71% of the laboratories; and dry weight was >0.15 mg/individual for >66% of the laboratories. Reproduction from Day 28 to Day 42 was >2 young/female for >71% of the laboratories participating in the round-robin testing. Reproduction was more variable within and among laboratories; hence, more replicates might be

Table 14.1 Test Conditions for Conducting a 42-d Sediment Toxicity Test with *Hyalella azteca* (modified from USEPA 1994a and ASTM 1999a).

	Parameter	Conditions
1.	Test type:	Whole-sediment toxicity test with renewal of overlying water
2.	Temperature:	23 ± 1°C
3.	Light quality:	Wide-spectrum fluorescent lights
4.	Illuminance:	About 100 to 1000 lux
5.	Photoperiod:	16L:8D
6.	Test chamber:	300-mL high-form lipless beaker
7.	Sediment volume:	100 mL
8.	Overlying water volume:	175 mL in the sediment exposure from Day 0 to Day 28 (175 to 275 mL in the water-only exposure from Day 28 to Day 42)
9.	Renewal of overlying water:	2 volume additions/d (Appendix A); continuous or intermittent (e.g., one volume addition every 12 h)
10.	Age of organisms:	7- to 8-d old at the start of the test
11.	Number of organisms/chamber:	10
12.	Number of replicate chambers/treatment:	12 (4 for 28-d survival and growth and 8 for 35- and 42-d survival, growth, and reproduction). Reproduction is more variable than growth or survival; hence, more replicates might be needed to establish statistical differences among treatments (See Section 14.2.3).
13.	Feeding:	YCT food, fed 1.0 mL (1800 mg/L stock) daily to each test chamber.
14.	Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L.
15.	Overlying water:	Culture water, well water, surface water or site water. Use of reconstituted water is not recommended.
16.	Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen (Appendix A).
17.	Overlying water quality:	Hardness, alkalinity, conductivity, and ammonia at the beginning and end of a sediment exposure (Day 0 and 28). Temperature daily. Conductivity weekly. Dissolved oxygen (DO) and pH three times/ week. Concentrations of DO should be measured more often if DO drops more than 1 mg/L since the previous measurement.
18.	Test duration:	42 d
19.	Endpoints:	28-d survival and growth; 35-d survival and reproduction; and 42-d survival, growth, reproduction, and number of adult males and females on Day 42
20.	Test acceptability:	Minimum mean control survival of 80% on Day 28. Additional performance-based criteria specifications are outlined in Table 14.3 based on results of round-robin testing (Sections 14.1.4 and 17.6).

needed to establish statistical differences among treatments with this endpoint.

14.1.5 Growth of *H. azteca* in sediment tests often provides unique information that can be used to discriminate toxic effects of exposure to contaminants (Brasher and Ogle, 1993; Borgmann, 1994; Kemble et al., 1994; Ingersoll et al., 1996; Kubitz et al., 1996; Milani et al., 1996; Steevens and Benson, 1998). Either length or weight can be measured in sediment tests with *H. azteca*. However, additional statistical options are available if length is measured on individual amphipods, such as nested analysis of variance which can account for variance in length between replicates (Steevens and Benson, 1998). Ongoing water-only studies testing select contaminants will provide additional data on the relative sensitivity and variability of sublethal endpoints in toxicity tests with *H. azteca* (Ingersoll et al., 1998).

14.1.6 Results of tests using procedures different from the procedures described in Section 14.2 may not be comparable, and these different procedures may alter contaminant bioavailability. Comparisons of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

14.2 Procedure for Conducting a *Hyalella azteca* 42-d Test for Measuring the Effects of Sediment-associated Contaminants on Survival, Growth, and Reproduction

14.2.1 Conditions for evaluating sublethal endpoints in a sediment toxicity test with *H. azteca* are summarized in Table 14.1. A general activity schedule is outlined in Table 14.2. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 16). When variability remains constant, the sensitivity of a test increases as the number of replicates increase.

14.2.2 The 42-d sediment toxicity test with *H. azteca* is conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux (Table 14.1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten amphipods in each test chamber are fed 1.0 mL of YCT daily (Appendix B). Each test chamber receives 2 volume additions/d of overlying water. Water renewals may be manual or automated. Appendix A describes water-renewal systems that can be used to deliver overlying water. Overlying water should be a source of water that has been demonstrated to support survival, growth, and reproduction of *H. azteca* in culture. McNulty et al. (1999) and Kemble et al. (1999) observed poor survival of *H. azteca* in tests conducted 14 to 28 d using a variety of reconstituted waters including the reconstituted water (reformulated moderately hard reconstituted water) described in Smith et al. (1997) and described in the first edition of this manual (USEPA, 1994a). Borgmann (1996) described a reconstituted water that was used successfully to maintain *H. azteca* in culture; however, some laboratories have not had success when using this reconstituted

Table 14.2 General Activity Schedule for Conducting a 42-d Sediment Toxicity Test with *Hyalella azteca*

Day	Activity
Pre-Test	
-8	Separate known-age amphipods from the cultures and place in holding chambers. Begin preparing food for the test. The <24-h amphipods are fed 10 mL of YCT (1800 mg/L stock solution) and 10 mL of *Selenastrum capricornutum* (about 3.0 x 10^7 cells/mL) on the first day of isolation and 5 mL of both YCT and *S. capricornutum* on the 3rd and 5th d after isolation.
-7	Remove adults and isolate <24-h-old amphipods (if procedures outlined in Section 10.3.4 are followed).
-6 to -2	Feed and observe isolated amphipods (Section 10.3), monitor water quality (e.g., temperature and dissolved oxygen).
-1	Feed and observe isolated amphipods (Section 10.3), monitor water quality. Add sediment into each test chamber, place chambers into exposure system, and start renewing overlying water.
Sediment Test	
0	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia). Transfer ten 7- to 8-d-old amphipods into each test chamber. Release organisms under the surface of the water. Add 1.0 mL of YCT (1800 mg/L stock) into each test chamber. Archive 20 test organisms for length determination or archive 80 test organisms for dry weight determination. Observe behavior of test organisms.
1 to 27	Add 1.0 mL of YCT to each test beaker. Measure temperature daily, conductivity weekly, and dissolved oxygen (DO) and pH three times/week. Observe behavior of test organisms.
28	Measure temperature, dissolved oxygen, pH, hardness, alkalinity, conductivity and ammonia. End the sediment-exposure portion of the test by collecting the amphipods with a #40-mesh sieve (425-µm mesh; U.S. standard size sieve). Use four replicates for growth measurements: count survivors and preserve organisms in sugar formalin for growth measurements. Use eight replicates for reproduction measurements: place survivors in individual replicate water-only beakers and add 1.0 mL of YCT to each test beaker/d and 2 volume additions/d (Appendix A) of overlying water.
Reproduction Phase	
29 to 35	Feed daily (1.0 mL of YCT). Measure temperature daily, conductivity weekly, and DO and pH three times a week. Measure hardness and alkalinity weekly. Observe behavior of test organisms.
35	Record the number of surviving adults and remove offspring. Return adults to their original individual beakers and add food.
36 to 41	Feed daily (1.0 mL of YCT). Measure temperature daily, conductivity weekly, and DO and pH three times a week. Measure hardness and alkalinity weekly. Observe behavior of test organisms.
41	Measure total water quality (pH, temperature, dissolved oxygen, hardness, alkalinity, conductivity, ammonia).
42	Record the number of surviving adults and offspring. Surviving adult amphipods on Day 42 are preserved in sugar formalin solution. The number of adult males in each beaker is determined from this archived sample. This information is used to calculate the number of young produced per female per replicate from Day 28 to Day 42.

water in the 42-d test (T.J. Norberg-King, USEPA, Duluth, MN, personal communication). For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are summarized in Table 14.3.

14.2.3 The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. A total of 12 replicates, each containing ten 7- to 8-d-old amphipods, are tested for each treatment. Starting the test with substantially younger or older organisms may compromise the reproductive endpoint. For the total of 12 replicates the assignment of beakers is as follows: 12 replicates are set up on Day -1 of which 4 replicates are used for 28-d growth and survival endpoints and the other 8 replicates are used for measurement of survival and reproduction on Day 35 and for measurement of survival, reproduction, or growth on Day 42.

14.3 General Procedures

14.3.1 Sediment into Test Chambers

14.3.1.1 The day before the sediment test is started (Day -1) each sediment should be thoroughly homogenized and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the degree of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is

Table 14.3 Test Acceptability Requirements for a 42-d Sediment Toxicity Test with *Hyalella azteca*

A. It is recommended for conducting the 42-d test with *H. azteca* that the following performance criteria be met:

1. Age of *H. azteca* at the start of the test should be 7- to 8-d old. Starting a test with substantially younger or older organisms may compromise the reproductive endpoint.
2. Average survival of *H. azteca* in the control sediment on Day 28 should be greater than or equal to 80%.
3. Laboratories participating in round-robin testing (Section 17.6) reported after 28-d sediment exposures in a control sediment (West Bearskin), survival >80% for >88% of the laboratories; length >3.2 mm/individual for >71% of the laboratories; and dry weight >0.15 mg/individual for >66% of the laboratories. Reproduction from Day 28 to Day 42 was >2 young/female for >71% of the laboratories participating in the round-robin testing. Reproduction was more variable within and among laboratories; hence, more replicates might be needed to establish statistical differences among treatments with this endpoint.
4. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the sediment exposure, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

B. Performance-based criteria for culturing *H. azteca* include the following:

1. It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.
2. Laboratories should track parental survival in the cultures and record this information using control charts if known-age cultures are maintained. Records should also be kept on the frequency of restarting cultures and the age of brood organisms.
3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly: pH, hardness, alkalinity, and ammonia. Dissolved oxygen in the cultures should be measured weekly. Temperature of the cultures should be recorded daily. If static cultures are used, it may be desirable to measure water quality more frequently.
4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.
5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

1. All organisms in a test must be from the same source.
2. Storage of sediments collected from the field should follow guidance outlined in Section 8.2.
3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.
5. Test organisms must be cultured and tested at 23°C (±1°C).
6. The mean of the daily test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.
7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

14.3.1.2 Each test chamber should contain the same amount of sediment, determined either by volume or by weight. Overlying water is added to the chambers on Day -1 in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon with a handle attached) placed above the sediment to dissipate the force of the water. Renewal of overlying water is started on Day -1. A test begins when the organisms are added to the test chambers (Day 0).

14.3.2 Renewal of Overlying Water

14.3.2.1 Renewal of overlying water is required during a test. At any particular time during a test, flow rates through any two test chambers should not differ by more than 10%. Hardness, alkalinity and ammonia concentrations in the water above the sediment, within a treatment, typically should not vary by more than 50% during the test. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water-delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998; Wall et al., 1998; Leppanen and Maier, 1998). The water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms or food on Day 0. Appendix A describes water-renewal systems that can be used for conducting sediment tests.

14.3.2.2 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the pore water.

14.3.3 Acclimation

14.3.3.1 Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

14.3.3.2 Culturing of organisms and toxicity assessment are typically conducted at 23°C. However, occasionally there is a need to perform evaluations at temperatures different than that recommended. Under these circumstances, it may be necessary to acclimate organisms to the desired test temperature to prevent thermal shock when moving immediately from the culture temperature to the test temperature (ASTM, 1999a). Acclimation can be achieved by exposing organisms to a gradual change in temperature; however, the rate of change should be relatively slow to prevent thermal shock. A change in temperature of 1°C every 1 to 2 h has been used successfully in some studies (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication; APHA, 1989). Testing at temperatures other than 23°C needs to be preceded by studies to determine expected performance under alternate conditions.

14.3.4 Placing Organisms in Test Chambers

14.3.4.1 Test organisms should be handled as little as possible. Amphipods should be introduced into the overlying water below the air-water interface. Test organisms can be pipetted directly into overlying water. The size of the test organisms at the start of the test should be measured using the same measure (length or weight) that will be used to assess their size at the end of the test. For length, a minimum of 20 organisms should be measured. For weight measurement, a larger sample size (e.g., 80) may be desirable because of the relatively small mass of the organisms. This information can be used to determine consistency in the size of the organisms used to start a test.

14.3.5 Feeding

14.3.5.1 For each beaker, 1.0 mL of YCT is added from Day 0 to Day 42. Without addition of food, the test organisms may starve during exposures. However, the addition of the food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987; Harkey et al., 1994). Furthermore, if too much food is added to the test chamber, or if the mortality of test organisms is high, fungal or bacterial growth may develop on the sediment surface. Therefore, the amount of food added to the test chambers is kept to a minimum.

14.3.5.2 Suspensions of food should be thoroughly mixed before aliquots are taken. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding should be suspended for one or more days. A drop in dissolved oxygen below 2.5 mg/L during a test may indicate that the food added is not being consumed. Feeding should be suspended for the amount of time necessary to increase the

dissolved oxygen concentration (ASTM, 1999a). If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface should be made daily.

14.3.6 Monitoring a Test

14.3.6.1 All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

14.3.6.2 Measurement of Overlying Water-quality Characteristics

14.3.6.2.1 Conductivity, pH, DO, hardness, alkalinity, and ammonia should be measured in all treatments at the beginning and at the end of the sediment exposure portion of the test. Water-quality characteristics should also be measured at the beginning and end of the reproductive phase (Day 29 to Day 42). Conductivity should be measured weekly, whereas pH and DO should be measured three times/week (Section 14.3.6.2.2). Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to composite water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water.

14.3.6.2.2 Dissolved oxygen should be measured three times/week and should be at a minimum of 2.5 mg/L. If a probe is used to measure dissolved oxygen in overlying water, it should be thoroughly inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination. Aeration can be used to maintain dissolved oxygen in the overlying water above 2.5 mg/L (i.e., about 1 bubble/second in the overlying water). Dissolved oxygen and pH can be measured directly in the overlying water with a probe.

14.3.6.2.3 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.

14.3.7 Ending a Test

14.3.7.1 Endpoints monitored include 28-d survival and growth of amphipods and 35-d and 42-d survival, growth, and reproduction (number of young/female) of amphipods. Growth or reproduction of amphipods may be a more sensitive toxicity endpoint compared to survival (Burton and Ingersoll, 1994; Kemble et al., 1994; Ingersoll et al., 1998).

14.3.7.2 On Day 28, 4 of the replicate beakers/sediment are sieved with a #40-mesh sieve (425-µm mesh; U.S. standard size sieve) to remove surviving amphipods for growth determinations. Any of the surviving amphipods in the water column or on the surface of the sediment can be pipetted from the beaker before sieving the sediment. The sediment in each beaker should be sieved in two separate aliquots (i.e., most of the amphipods will probably be found in the surface aliquot). Immobile organisms isolated from the sediment surface or from sieved material should be considered dead. Surviving amphipods from these 4 replicates can be preserved in separate vials containing 8% sugar formalin solution if length of amphipods is to be measured (Ingersoll and Nelson, 1990). The sugar formalin solution is prepared by adding 120 g of sucrose to 80 mL of formalin which is then brought to a volume of 1 L using deionized water. This stock solution is mixed with an equal volume of deionized water when used to preserve organisms. NoTox® (Earth Safe Industries, Belle Mead, NJ) can be used as a substitute for formalin (Unger et al., 1993).

14.3.7.3 A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 5 min/replicate). Laboratories should demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control or test sediments, and recovery could be determined after 1 h (Tomasovic et al., 1994).

14.3.7.4 Growth of amphipods can be reported as either length or weight; however, additional statistical options are available if length is measured on individual organisms (Section 14.4.5.3).

14.3.7.5 Amphipod body length (±0.1 mm) can be measured from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface (Figure 11.1). Kemble et al. (1994) describe the use of a digitizing system and microscope to measure lengths of *H. azteca*. Kemble et al. (1994) also photographed invertebrates (at a magnification of 3.5X) and measured length using a computer-interfaced digitizing tablet.

14.3.7.6 Dry weight of amphipods in each replicate can be determined on Day 28 and 42. If both weight and length are to be determined, weight should be measured after length on the preserved samples. Gaston et al. (1995) and Duke et al. (1996) have shown that biomass or length of several aquatic invertebrates did not significantly change after two to four weeks of storage in 10% formalin. If test organisms are to be used for an evaluation of bioaccumulation, it is not advisable to dry the sample before conducting the residue analysis. If conversion from wet weight to dry weight is necessary, aliquots of organisms can be weighed to establish wet to dry weight conversion factors. A consistent procedure should be used to remove the excess water from the organisms before measuring wet weight.

14.3.7.7 Dry weight of amphipods can be determined as follows: (1) transferring the archived amphipods from a replicate out of the sugar formalin solution into a crystallizing dish; (2) rinsing amphipods with deionized water; (3) transferring these rinsed amphipods to a preweighed aluminum pan; (4) drying these samples for 24 h at 60°C; and (5) weighing the pan and dried amphipods on a balance to the nearest 0.01 mg. Average dry weight of individual amphipods in each replicate is calculated from these data. Due to the small size of the amphipods, caution should be taken during weighing (10 dried amphipods after a 28-d sediment exposure may weigh less than 2.5 to 3.5 mg). Weigh pans need to be carefully handled using powder-less gloves and the balance should be calibrated with standard weights with each use. Use of small aluminum pans (e.g., 7 x 22 x 7 mm, Sigma Chemical Company, St. Louis, MO) will help reduce variability in measurements of dry weight. Weigh boats can also be constructed from sheets of aluminum foil.

14.3.7.8 The first edition of this manual (USEPA, 1994a) recommended dry weight as a measure of growth for both *H. azteca* and *C. tentans*. For *C. tentans*, this recommendation was changed in the current edition to ash-free dry weight (AFDW) instead of dry weight, with the intent of reducing bias introduced by gut contents (Sibley et al., 1997a). However, this recommendation was not extended to include *H. azteca*. Studies by Dawson et al. (personal communication, T.D. Dawson, Integrated Laboratory Systems, Duluth, MN) have indicated that the ash content of *H. azteca* is not greatly decreased by purging organisms in clean water before weighing, suggesting that sediment does not comprise a large portion of the overall dry weight. In addition, using AFDW further decreases an already small mass, potentially increasing measurement error. For this reason, dry weight continues to be the recommended endpoint for estimating growth of *H. azteca* via weight (growth can also be determined via length).

14.3.7.9 On Day 28, the remaining 8 beakers/sediment are also sieved and the surviving amphipods in each sediment beaker are placed in 300-mL water-only beakers containing 150 to 275 mL of overlying water and a 5-cm x 5-cm piece of Nitex screen (Nylon Bolting cloth; 44% open area and 280-um aperture, Wildlife Supply Company, Saginaw, MI; Ingersoll et al., 1998). In a subsequent study, improved reproduction of *H. azteca* was observed when the Nitex screen was replaced with a 3-cm x 3-cm piece of the nylon "Coiled-web material" described in Section 10.3.4 for use in culturing amphipods (T.J. Norberg-King, USEPA, personal communication). Each water-only beaker receives 1.0 mL of YCT stock solution and about two volume additions of water daily.

14.3.7.10 Reproduction of amphipods is measured on Day 35 and Day 42 in the water-only beakers by removing and counting the adults and young in each beaker. On Day 35, the adults are then returned to the same water-only beakers. Adult amphipods surviving on Day 42 are preserved in sugar formalin. The number of adult females is determined by simply counting the adult males (mature male amphipods will have an enlarged second gnathopod) and assuming all other adults are females (cf., Figure 11.1). The number of females is used to determine number of young/female/beaker from Day 28 to Day 42. Growth can also be measured for these adult amphipods.

14.4 Interpretation of Results

14.4.1 Data Analysis

14.4.1.1 Endpoints measured in the 42-d *H. azteca* test include survival (Day 28, 35, and 42), growth (as length or dry weight on Day 28 and 42), and reproduction (number of young/female produced from Day 28 to 42). Section 16 describes general information regarding statistical analysis of these data, including both point estimates (i.e., LC50s) and hypothesis testing (i.e., ANOVA). The following sections describe species-specific information that is useful in helping to interpret the results of 42-d sediment toxicity tests with *H. azteca.*

14.4.2 Age Sensitivity

14.4.2.1 The sensitivity of *H. azteca* appears to be relatively similar up to at least 24- to 26-d-old organisms (Collyard et al., 1994). For example, the toxicity of diazinon, Cu, Cd, and Zn was similar in 96-h water-only exposures starting with 0- to 2-d-old organisms through 24- to 26--d-old organisms (Figure 11.2). The toxicity of alkylphenol ethoxylate (a surfactant) tended to increase with age. In general, this suggests that tests started with 7-d to 8-d-old amphipods would be representative of the sensitivity of *H. azteca* up to at least the adult life stage.

14.4.3 Grain Size

14.4.3.1 *Hyalella azteca* tolerate a wide range in sediment grain size and organic matter in 10- to 28-d tests measuring effects on survival or growth (Ankley et al., 1994; Suedel and Rodgers, 1994; Ingersoll et al., 1996; Kemble et al., 1999). Using the method outlined in Section 14.2, no significant correlations were observed between the survival, growth, or reproduction of *H. azteca* and the physical characteristics of the sediment (grain size ranging from predominantly silt to predominantly sand), TOC (ranging from 0.3 to 9.6%), water content (ranging from 19 to 81%; Ingersoll et al., 1998). Additionally, no significant correlations were observed between these biological endpoints and the water-quality characteristics (i.e., hardness, alkalinity, ammonia) of pore water or overlying water in the sediments evaluated by Ingersoll et al. (1998). Weak trends were observed between reproduction of amphipods and percent clay, percent silt, and percent sand. Additional study is needed to better evaluate potential relationships between reproduction of *H. azteca* and these physical characteristics of the sediment. The weak relationship between the sediment grain size and reproduction may have been due to the fact that samples with higher amounts of sand also had higher concentrations of organic contaminants compared to other samples evaluated in Ingersoll et al. (1998).

14.4.3.2 Until additional studies have been conducted which substantiate this lack of a correlation between physical characteristics of sediment and the reproductive endpoints measured in the long-term sediment test with *H. azteca*, it would be desirable to test control or reference sediments which are representative of the physical characteristics of field-collected sediments. Formulated sediments could be used to bracket the ranges in physical characteristics expected in the field-collected sediments being evaluated (Section 7.2). Addition of YCT should provide a minimum amount of food needed to support adequate survival, growth, and reproduction of *H. azteca* in sediments low in organic matter. Without addition of food, *H. azteca* can starve during exposures (McNulty et al., 1999) making it impossible to differentiate effects of contaminants from other sediment characteristics.

14.4.4 Influence of Indigenous Organisms

14.4.4.1 Survival of *H. azteca* in 28-d tests was not reduced in the presence of oligochaetes in sediment samples (Reynoldson et al., 1994). However, growth of amphipods was reduced when high numbers of oligochaetes were placed in a sample. Therefore, it is important to determine the number and biomass of indigenous organisms in field-collected sediments in order to better interpret growth data (Reynoldson et al., 1994; DeFoe and Ankley, 1998). Furthermore, presence of predators may also influence response of test organisms in sediment (Ingersoll and Nelson, 1990).

14.4.5 Relationships between Growth and Reproductive Endpoints

14.4.5.1 Natural or anthropogenic stressors that affect growth of invertebrates may also affect reproduction, because of a minimum size needed for reproduction (Rees and Crawley, 1989; Ernsting et al., 1993; Moore and Dillon, 1993; Enserink et al., 1995; Moore and Farrar, 1996; Sibley et al., 1996, 1997a). Ingersoll et al. (1998) reported a significant correlation between reproduction from Day 28 to 42 and length of *H. azteca* on Day 28 when data are plotted by the mean of each treatment (Figure 14.1a; Spearman rank correlation of 0.59, p=0.0001). Based on 28-d lengths, smaller amphipods (<3.5 mm) tended to have lower reproduction and larger amphipods (>4.3 mm) tended to have higher reproduction; however, the range in reproduction was wide for amphipods 3.5 to 4.3 mm in length. Based on 42-d lengths, there was a weaker correlation between length and reproduction (i.e., reproduction and length measured in paired replicates; Figure 14.1b, Spearman rank correlation of 0.49, p=0.0001). Similarly, plotting data by individual replicates (data not shown) did not improve the relationship between 42-d length and reproduction compared to the plots by the mean of each treatment (Figure 14.1b; Ingersoll et al., 1998).

14.4.5.2 Weaker relationships were observed between reproduction and dry weight measured on Day 28 (Figure 14.2a, Spearman rank correlation of 0.44, p = 0.0037, n = 42) or dry weight measured on Day 42 (Figure 14.2b, Spearman rank correlation 0.34, p = 0.0262, n = 42). Round-robin studies (Section 17.6) have generated additional data that will be used to further evaluate relationships between growth and reproduction of *H. azteca* in sediment tests using the procedures outlined in Section 14.2.

14.4.5.3 A significant correlation was evident between length and dry weight of amphipods (Figure 14.3, Spearman rank of 0.80, p=0.0001) indicating that either length or weight could be measured in sediment tests with *H. azteca*. However, additional statistical options are available if length is measured on individual amphipods, such as nested ANOVA which can account for variance in length within replicates (Steevens and Benson, 1998). Analyses are ongoing to evaluate the ability of length vs. weight to discriminate between contaminated and uncontaminated samples in a database described in Ingersoll et al. (1996).

14.4.5.4 The relatively variable relationship between growth and reproduction probably reflects the fact that most of these comparisons were made within a fairly narrow range in length (3.5 to 5.0 mm; Figure 14.1) or dry weight (0.25 to 0.50 mg; Figure 14.2). Other investigators have reported a similar degree of variability in reproduction of *H. azteca* within a narrow range of length or weight, with stronger correlations observed over wider ranges (Hargrave, 1970b; Strong, 1972; Wen, 1993; Moore and Farrar, 1996). The degree of correlation between growth and reproduction may also be dependent on the genetic strain of *H. azteca* evaluated (Strong, 1972; France, 1992).

14.4.5.5 The proportion of males to females within a treatment or by replicate was not correlated to young production, but may have contributed to a variation in reproduction (Ingersoll et al., 1998). Wen (1993) reported that when two or three males were placed in a beaker with one female *H. azteca*, the frequency of successful amplexus was reduced, possibly from aggression between the males. Future study is needed to determine if increasing the number of amphipods/beaker would result in a more consistent proportion of males to females within a beaker and would reduce variability in reproduction.

14.4.5.6 Reproduction was often more variable than growth (Ingersoll et al., 1998). The coefficient of variation (CV) was typically <10% for growth and >20% for reproduction. This difference in variation affects the statistical power of the comparisons and the number of replicates required for a test. For example, detection of a 20% difference between treatment means at a statistical power of 0.8 would require about 4 replicates at a CV of 10% and 14 replicates at a CV of 20% (Figure 16.5). Fewer replicates would be required if detection of larger differences among treatment means were of interest. Ongoing water-only studies testing select contaminants will hopefully provide additional data on the relative sensitivity and variability of sublethal endpoints in toxicity tests with *H. azteca* (Ingersoll et al., 1998).

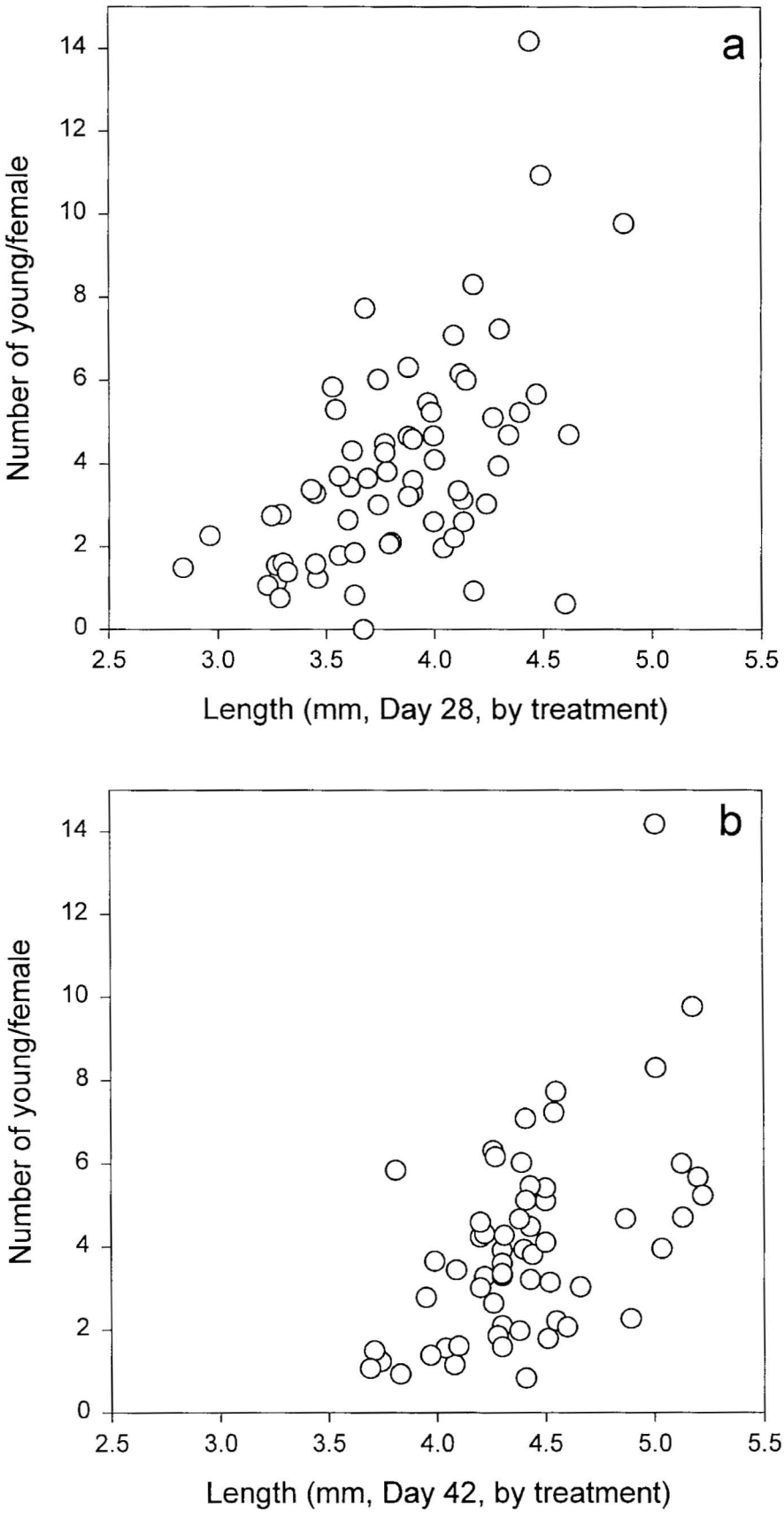


Figure 14.1 **Relationships between *Hyalella azteca* length and reproduction by (a) treatment means for 28-d length or (b) treatment means for 42-d length.**

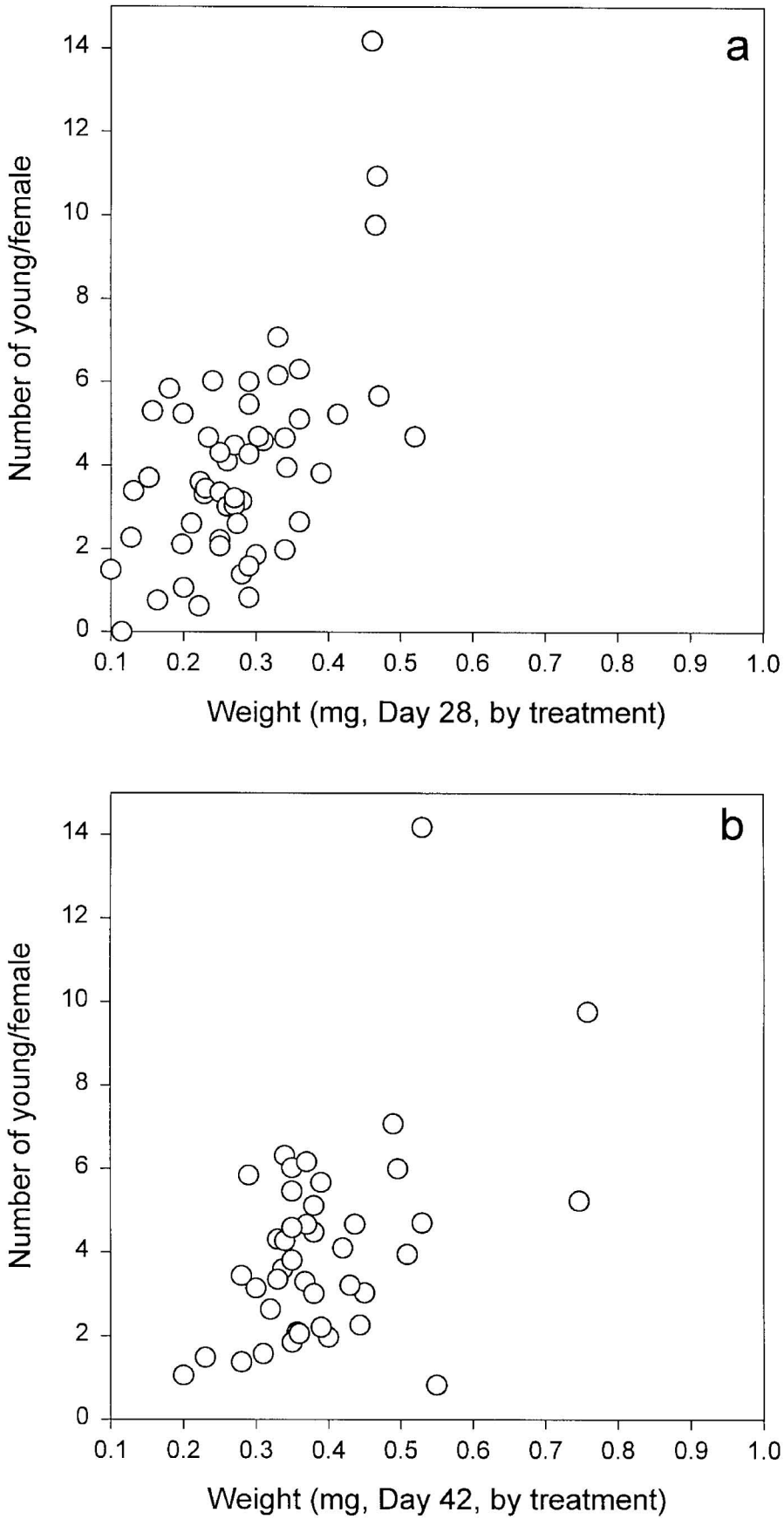


Figure 14.2 **Relationships between *Hyalella azteca* dry weight and reproduction by (a) treatment means for 28-d dry weight or (b) treatment means for 42-d dry weight.**

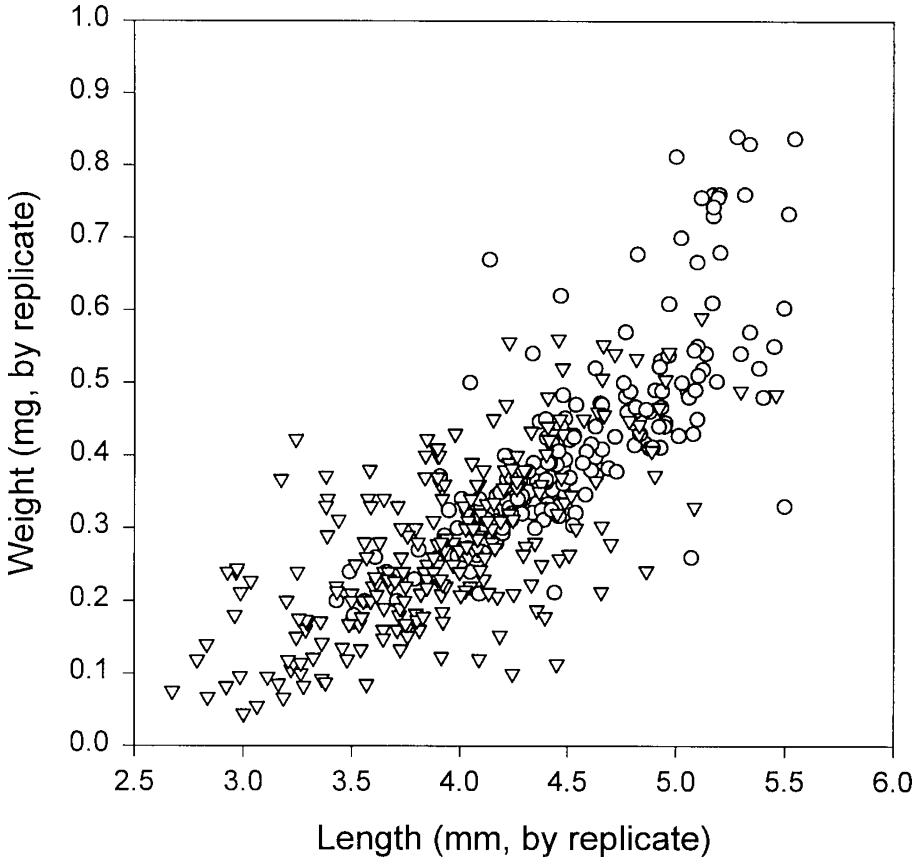


Figure 14.3 Relationship between *Hyalella azteca* length and dry weight. Triangles are data for Day 28 and circles are data for Day 42 (Ingersoll et al., 1998).

14.4.5.7 The 8-replicate design recommended in this manual (Table 14.1) is a compromise between logistical constraints and statistical considerations. Laboratories experienced with this method have shown CVs of 25 to 50% (Ingersoll et al., 1998), though some higher values were observed during the round-robin testing (Section 17.6), in which most labs had not previously performed the test.

14.4.5.8 As discussed above, the number of replicates can be adjusted according to the needs of a particular study. For example, Kubitz et al. (1996) recommended a two-step process for assessing growth in sediment tests with *H. azteca*. Using this process, a limited number of replicates would be tested in a screening step. Samples identified as possibly affecting reproduction could then be tested in a confirmatory step with additional replicates. This two-step analysis conserves laboratory resources and increases statistical power when needed to discriminate sublethal effects. A similar approach could be applied to evaluate reproductive effects of contaminants in sediment where a limited number of replicates could be initially tested to evaluate potential effects. Samples identified as possibly toxic based on reproduction could then be reevaluated using an increased number of replicates. However, the use of sediments stored for extended periods of time may introduce variability in results between the two studies (Section 8.2).

14.4.6 Relative Endpoint Sensitivity

14.4.6.1 Measurement of sublethal endpoints in sediment tests with *H. azteca* can provide unique information that has been used to discriminate toxic effects of exposure to contaminants. Table 14.4 compares the relative sensitivity of survival and growth endpoints in 14- and 28-d tests with *H. azteca* (Ingersoll et al., 1996, 1998). When 14-d and 28-d tests were conducted concurrently measuring both survival and growth, both tests identified 34% of the samples as toxic and 53% of the samples as not toxic (N=32). Both tests identified an additional 6% of the samples as toxic. Survival or growth endpoints identified a similar percentage of samples as toxic in both the 14- and 28-d tests. However, the majority of the samples used to make these comparisons were highly contaminated. Additional exposures conducted with moderately contaminated sediment might exhibit a higher percentage of sublethal effects in the 28-d test compared to the 14-d test.

14.4.6.2 When both survival and growth were measured in 14-d tests (N=25), only 4% of the samples reduced

Table 14.4 Percentage of Paired Tests or Paired Endpoints Identifying Samples as Toxic in *Hyalella azteca* 14-d or 28-d Tests. See USEPA (1996a) and Ingersoll et al. (1996) for a description of this database.

Comparisons	Tox/tox[1] (%)	Not/not[2] (%)	Tox/not[3] (%)	Not/tox[4] (%)	N[5]
Survival or growth: 14 d/28 d	34	53	6	6	32
Survival: 14 d/28 d	25	66	0	10	32
Growth: 14 d/28 d	8	64	12	16	25
14 d: survival/growth	4	60	20	16	25
28 d: survival/growth	16	52	14	18	44

[1] Tox/tox: samples toxic (significant reduction relative to the control $p<0.05$) with both tests (or both endpoints).

[2] Not/not: samples not toxic with both tests (or both endpoints).

[3] Tox/not: samples toxic to the first but not the second test (or endpoint).

[4] Not/tox: samples not toxic to the first but toxic to the second test (or endpoint).

[5] N: number of samples

both survival and growth; however, 20% reduced survival only and 16% reduced growth only (60% did not reduce survival or growth). Hence, if survival was the only endpoint measured in 14-d tests, 16% of the toxic samples would be incorrectly classified. Similar percentages are also observed for the 28-d tests. When both survival and growth were measured in the 28-d test (N=44), 16% of the samples reduced both survival and growth, 14% reduced survival only, 18% reduced growth only, and 52% did not reduce survival or growth.

14.4.6.3 The endpoint comparisons in Table 14.4 represent only samples where both survival and growth could be measured. If a sample was extremely toxic, it would not be included in this comparison since growth could not be measured. Moderately contaminated sediments that did not severely reduce survival could have a reduced growth. For example, in 28-d tests with sediments from the Clark Fork River, growth was a more sensitive endpoint compared to survival or maturation. Only 13% of the samples reduced survival and 20% of the samples reduced maturation; however, growth was reduced in 53% of the samples (Kemble et al., 1994).

14.4.6.4 Other investigators have reported measurement of growth in tests with *H. azteca* often provides unique information that can help discriminate toxic effects of exposure to contaminants in sediment (Kubitz et al., Milani et al., 1996; Steevens and Benson, 1998) or water (Brasher and Ogle, 1993; Borgmann, 1994). Similarly, in sediment tests with the midge *C. tentans*, sublethal endpoints are often more sensitive than survival as indicators of contaminant stress (Section 12 and 15). In contrast, Borgmann et al. (1989) reported that growth or reproduction did not add additional information beyond measurement of survival of *H. azteca* in water-only exposures with cadmium or pentachlorophenol. Similarly, Day et al. (1995) reported that weight did not add additional information beyond measurement of survival in 28-d tests with *H. azteca*. Ramirez-Romero (1997) reported that reproduction of *H. azteca* was not affected by exposure to sublethal concentrations of fluoranthene in sediment when exposures were started with juvenile amphipods. Brasher and Ogle (1993) started exposures with adult amphipods and observed the sensitivity of reproduction compared to survival of *H. azteca* was dependent on the chemical tested (reproduction more sensitive to selenite and survival more sensitive to selenate in water-only exposures). Long-term exposures starting with juvenile amphipods would likely be more appropriate to assess effects of contaminants on reproduction (i.e., Carr and Chapman, 1992; Nebeker et al., 1992).

14.4.7 Future Research

14.4.7.1 Additional studies are needed to further evaluate the use of reconstituted water and ammonia on long-term exposures with *H. azteca*. Section 1.3.8.5 addresses interpretative guidance for evaluating toxicity associated with ammonia in sediment. Ongoing water-only toxicity tests with select chemicals (i.e., cadmium, DDD and fluoranthene) should generate data that can be used to better determine the relative sensitivity of survival, reproduction, and growth endpoints in tests with *H. azteca* (Ingersoll et al., 1998). These water-only studies will also be used to evaluate potential recovery of amphipods after transfer into clean water to measure reproduction. In addition to studies evaluating the relative sensitivity of endpoints, research is also needed to evaluate the ability of these laboratory endpoints to estimate responses of benthic organisms exposed in the field to chemicals in sediments (Canfield et al., 1996).

Section 15
Test Method 100.5
Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chironomus tentans*

15.1 Introduction

15.1.1 The midge *Chironomus tentans* has been used extensively in the short-term assessment of chemicals in sediments (Wentsel et al., 1977; Nebeker et al., 1984; Giesy et al., 1988; West et al., 1994), and standard methods have been developed for testing with this midge using 10-d exposures (Ingersoll et al., 1995; USEPA, 1994a; ASTM, 1999a). *Chironomus tentans* is a good candidate for long-term toxicity testing because it normally completes its life cycle in a relatively short period of time (25 to 30 d at 23°C), and a variety of developmental (growth, survivorship) and reproductive (fecundity) endpoints can be monitored. In addition, emergent adults can be readily collected so it is possible to transfer organisms from the sediment test system to clean, overlying water for direct quantification of reproductive success.

15.1.2 The long-term sediment toxicity test with the midge, *Chironomus tentans*, is a life-cycle test in which the effects of sediment exposure on survival, growth, emergence, and reproduction are assessed (Benoit et al., 1997). Procedures for conducting the long-term test with *C. tentans* are described in Section 15.2. The test is started with newly hatched larvae (<24-h old) and continues through emergence, reproduction, and hatching of the F_1 generation. Survival is determined at 20 d and at the end of the test (about 50 to 65 d). Growth is determined at 20 d, which corresponds to the 10-d endpoint in the 10-d *C. tentans* growth test started with 10-d-old larvae (Section 12). From Day 23 to the end of the test, emergence and reproduction are monitored daily. The number of eggs is determined for each egg case, which is incubated for 6 d to determine hatching success. Each treatment of the life-cycle test is ended separately when no additional emergence has been recorded for 7 consecutive days (the 7-d criterion). When no emergence is recorded from a treatment, ending of that treatment should be based on the control sediment using this 7-d criterion. Appendix C and Table 6.1 outline equipment and supplies needed to conduct this test. The procedures described in Table 15.1 include measurement of a variety of lethal and sublethal endpoints; minor modifications of the basic methods can be used in cases where only a subset of these endpoints is of interest.

15.1.3 The method outlined in Section 15.2 has been evaluated in round-robin testing with 10 laboratories using two clean sediments (Section 17.6). In the preliminary round-robin with 1.5 mL of Tetrafin/d as a food source, 90% of labs met the survival criterion (>70%), 100% of labs met the growth criterion (>0.48 mg AFDW), 70% of labs met the emergence criterion (>50%), 90% of labs met the reproduction criterion (>800 eggs/female), and 88% of labs met the percent hatch criterion (>80%). Reproduction was generally more variable than growth or survival within and among laboratories; hence, more replicates might be needed to establish statistical significance of small decreases in reproduction.

15.1.4 Growth and other sublethal endpoints in sediment tests with *C. tentans* often provide unique information that can be used to discriminate toxic effects of exposure to contaminants. See Section 15.4.6 for additional details.

15.1.5 Results of tests using procedures different from the procedures described in Section 15.2 may not be comparable and these different procedures may alter contaminant bioavailability. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting sediment tests with aquatic organisms. If tests are conducted with procedures different from the procedures described in this manual, additional tests are required to determine comparability of results (Section 1.3).

15.2 Procedure for Conducting a Life-cycle Test for Measuring the Effects of Sediment-associated Contaminants on *Chironomus tentans*

15.2.1 Conditions for conducting a long-term sediment toxicity test with *C. tentans* are summarized in Table 15.1. A general activity schedule is outlined in Table 15.2. Decisions concerning the various aspects of experimental design, such as the number of treatments, number of test chambers/treatment, and water-quality characteristics should be based on the purpose of the test and the methods of data analysis (Section 16). When variability

Table 15.1 Test Conditions for Conducting a Long-term Sediment Toxicity Test with *Chironomus tentans*

	Parameter	Conditions
1.	Test type:	Whole-sediment toxicity test with renewal of overlying water
2.	Temperature:	23 ±1°C
3.	Light quality:	Wide-spectrum fluorescent lights
4.	Illuminance:	About 100 to 1000 lux
5.	Photoperiod:	16L:8D
6.	Test chamber:	300-mL high-form lipless beaker
7.	Sediment volume:	100 mL
8.	Overlying water volume:	175 mL
9.	Renewal of overlying water:	2 volume additions/d (Appendix A); continuous or intermittent (e.g., one volume addition every 12 h)
10.	Age of organisms:	< 24-h-old larvae
11.	Number of organisms/chamber:	12
12.	Number of replicate chambers/treatment:	16 (12 at Day -1 and 4 for auxiliary males on Day 10)
13.	Feeding:	Tetrafin® goldfish food, fed 1.5 mL daily to each test chamber starting Day -1 (1.0 mL contains 4.0 mg of dry solids)
14.	Aeration:	None, unless dissolved oxygen in overlying water drops below 2.5 mg/L
15.	Overlying water:	Culture water, well water, surface water, site water, or reconstituted water
16.	Test chamber cleaning:	If screens become clogged during a test, gently brush the *outside* of the screen (Appendix A).
17.	Overlying water quality:	Hardness, alkalinity, conductivity, and ammonia at the beginning, on Day 20, and at the end of a test. Temperature daily (ideally continuously). Dissolved oxygen (DO) and pH three times/week. Conductivity weekly. Concentrations of DO should be measured more often if DO has declined by more than 1 mg/L since previous measurement.
18.	Test duration:	About 50 to 65 d; each treatment is ended separately when no additional emergence has been recorded for seven consecutive days. When no emergence is recorded from a treatment, termination of that treatment should be based on the control sediment using this 7-d criterion.
19.	Endpoints:	20-d survival and weight; female and male emergence, adult mortality, the number of egg cases oviposited, the number of eggs produced, and the number of hatched eggs. Potential sublethal endpoints are listed in Table 15.4.
20.	Test acceptability:	Average size of *C. tentans* in the control sediment at 20 d must be at least 0.6 mg/surviving organism as dry weight or 0.48 mg/surviving organism as AFDW. Emergence should be greater than or equal to 50%. Experience has shown that pupae survival is typically >83% and adult survival is >96%. Time to death after emergence is <6.5 d for males and <5.1 d for females. The mean number of eggs/egg case should be greater than or equal to 800 and the percent hatch should be greater than or equal to 80%. See Sections 15.1.3 and 17.6 for a summary of performance in round-robin testing.

remains constant, the sensitivity of a test increases as the number of replicates increases.

15.2.2 The long-term sediment toxicity test with *C. tentans* is conducted at 23°C with a 16L:8D photoperiod at an illuminance of about 100 to 1000 lux (Table 15.1). Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Each test chamber receives 2 volume additions/d of overlying water. Water renewals may be manual or automated. Appendix A describes water-renewal systems that can be used to deliver overlying water. Overlying water should be a source of water that has been demonstrated to support survival, growth, and reproduction of *C. tentans* in culture. For site-specific evaluations, the characteristics of the overlying water should be as similar as possible to the site where sediment is collected. Requirements for test acceptability are summarized in Table 15.3.

15.2.3 The number of replicates and concentrations tested depends in part on the significance level selected and the type of statistical analysis. For routine testing, a total of 16 replicates, each containing 12, <24-h-old larvae are tested for each treatment. For the total of 16 replicates the assignment of beakers is as follows: initially, 12 replicates are set up on Day -1 of which 4 replicates are used for 20-d growth and survival endpoints and 8

Table 15.2 General Activity Schedule for Conducting a Long-term Sediment Toxicity Test with *Chironomus tentans*

Day	**Activity**
Pre-Test	
-4	Start reproduction flask with cultured adults (1:3 male:female ratio). For example for 15 to 25 egg cases, 10 males and 30 females are typically collected. Egg cases typically range from 600 to 1500 eggs/case.
-3	Collect egg cases (a minimum of 6 to 8) and incubate at 23°C.
-2	Check egg cases for viability and development.
-1	1. Check egg cases for hatch and development. 2. Add 100 mL of homogenized test sediment to each replicate beaker and place in corresponding treatment holding tank. After sediment has settled for at least 1 h, add 1.5 mL Tetrafin slurry (4g/L solution) to each beaker. Overlying water renewal begins at this time.
Sediment Test	
0	1. Transfer all egg cases to a crystallizing dish containing control water. Discard larvae that have already left the egg cases in the incubation dishes. Add 1.5 mL food to each test beaker with sediment before the larvae are added. Add 12 larvae to each replicate beaker (beakers are chosen by random block assignment). Let beakers sit (outside the test system) for 1 h following addition of the larvae. After this period, gently immerse all beakers into their respective treatment holding tanks. 2. Measure temperature, pH, hardness, alkalinity, dissolved oxygen, conductivity and ammonia at start of test.
1-End	On a daily basis, add 1.5 mL food to each beaker. Measure temperature daily. Measure the pH and dissolved oxygen three times a week during the test. Measure conductivity weekly. If the DO has declined more than 1 mg/L since previous reading, increase frequency of DO measurements and aerate if DO continues to be less than 2.5 mg/L. Measure hardness, alkalinity, conductivity, ammonia, temperature, pH, and dissolved oxygen at the end of the test.
6	For auxiliary male production, start reproduction flask with culture adults (e.g., 10 males and 30 females; 1:3 male to female ratio).
7-10	Follow set-up schedule for auxiliary male beakers (4 replicates/treatment) described above for Day -3 to Day 0.
19	In preparation for weight determinations, ash weigh pans at 550°C for 2 h. Note that the weigh pans should be ashed before use to eliminate weighing errors due to the pan oxidizing during ashing of samples.
20	1. Randomly select four replicates from each treatment and sieve the sediment to recover larvae for growth and survival determinations. Pool all living larvae per replicate and dry the sample to a constant weight (e.g., 60°C for 24 h). 2. Install emergence traps on each of the remaining reproductive replicate beakers. 3. Measure temperature, pH, hardness, alkalinity, dissolved oxygen, conductivity and ammonia.
21	The sample with dried larvae is brought to room temperature in a dessicator and weighed to the nearest 0.01 mg . The dried larvae in the pan are then ashed at 550°C for 2 h. The pan with the ashed larvae is then reweighed and the tissue mass of the larvae determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan.
Chronic Measurements	
23-End	On a daily basis, record emergence of males and females, pupal, and adult mortality, and time to death for previously collected adults. Each day, transfer adults from each replicate to a corresponding reproduction/oviposition (R/O) chamber. Transfer each primary egg case from the R/O chamber to a corresponding petri dish to monitor incubation and hatch. Record each egg case oviposited, number of eggs produced (using either the ring or direct count methods), and number of hatched eggs. If it is difficult to estimate the number of eggs in an egg case, use a direct count to determine the number of eggs; however the hatchability data will not be obtained for this egg case.
28	Place emergence traps on auxiliary male replicate beakers.
33-End	Transfer males emerging from the auxiliary male replicates to individual inverted petri dishes. The auxiliary males are used for mating with females from corresponding treatments from which most of the males had already emerged or in which no males emerged.
40-End	After 7 d of no recorded emergence in a given treatment, end the treatment by sieving the sediment to recover larvae, pupae, or pupal exuviae. When no emergence occurs in a test treatment, that treatment can be ended once emergence in the control sediment has ended using the 7-d criterion.

replicates for determination of emergence and reproduction. It is typical for males to begin emerging 4 to 7 d before females. Therefore, additional males, referred to as auxiliary males, need to be available during the prime female emergence period for each respective chamber/sediment. To provide these males, 4 additional replicates are stocked with 12, <24-h-old larvae 10 d following initiation of the test. Midges in each test chamber are fed 1.5 mL of a 4-g/L Tetrafin® suspension daily. Endpoints monitored include 20-d survival and weight, emergence, time to death (adults), reproduction, and egg hatchability.

Table 15.3 Test Acceptability Requirements for a Long-term Sediment Toxicity Test with *Chironomus tentans*

A. It is recommended for conducting a long-term test with *C. tentans* that the following performance criteria be met:

1. Tests must be started with less than 1-d- (<24-h) old larvae. Starting a test with substantially older organisms may compromise the emergence and reproductive endpoint.

2. Average survival of *C. tentans* in the control sediment should be greater than or equal to 70% on Day 20 and greater than 65% at the end of the test.

3. Average size of *C. tentans* in the control sediment at 20 d must be at least 0.6 mg/surviving organism as dry weight or 0.48 mg/surviving organism as AFDW. Emergence should be greater than or equal to 50%. Experience has shown that pupae survival is typically >83% and adult survival is >96%. Time to death after emergence is <6.5 d for males and <5.1 d for females. The mean number of eggs/egg case should be greater than or equal to 800 and the percent hatch should be greater than or equal to 80%. See Sections 15.1.3 and 17.6 for a summary of performance in round-robin testing.

4. Hardness, alkalinity, and ammonia in the overlying water typically should not vary by more than 50% during the test, and dissolved oxygen should be maintained above 2.5 mg/L in the overlying water.

B. Performance-based criteria for culturing *C. tentans* include the following:

1. It may be desirable for laboratories to periodically perform 96-h water-only reference-toxicity tests to assess the sensitivity of culture organisms (Section 9.16.2). Data from these reference-toxicity tests could be used to assess genetic strain or life-stage sensitivity of test organisms to select chemicals.

2. Laboratories should keep a record of time to first emergence for each culture and record this information using control charts. Records should also be kept on the frequency of restarting cultures.

3. Laboratories should record the following water-quality characteristics of the cultures at least quarterly: pH, hardness, alkalinity, and ammonia. Dissolved oxygen in the cultures should be measured weekly. Temperature of the cultures should be recorded daily. If static cultures are used, it may be desirable to measure water quality more frequently.

4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms.

5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

C. Additional requirements:

1. All organisms in a test must be from the same source.

2. Storage of sediments collected from the field should follow guidance outlined in Section 8.2.

3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.

4. Negative-control sediment and appropriate solvent controls must be included in a test. The concentration of solvent used must not adversely affect test organisms.

5. Test organisms must be cultured and tested at 23°C (±1°C).

6. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.

7. Natural physico-chemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.

15.3 General Procedures

15.3.1 Collection of Egg Cases

15.3.1.1 Egg cases are obtained from adult midges held in a sex ratio of 1:3 male:female. Ten males and 30 females will produce between 15 to 25 egg cases. Adults should be collected four days before starting a test (Appendix C, Figure C.3). The day after collection of adults, 6 to 8 of the larger “C” shaped egg cases are transferred to a petri dish with culture water and incubated at 23°C (Appendix C, Figure C.2). Hatching typically begins around 48 h and larvae typically leave the egg case 24 h after the first hatch. The number of eggs in each egg case will vary, but typically ranges from 600 to 1500 eggs. It should be noted that mating may have occurred in culture tanks before males and females are placed into flasks for collecting eggs.

15.3.2 Hatching of Eggs

15.3.2.1 Hatching of eggs should be complete by about 72 h. Hatched larvae remain with the egg case for about 24 h and appear to use the gelatinous component of the egg case as an initial source of food (Sadler, 1935; Ball and Baker, 1995). After the first 24-h period with larvae hatched, transfer the egg cases from the incubation petri dish to another dish with clean test water. Larvae having already left the egg case in the incubation petri dish are discarded since their precise age and time away from the gelatinous food source is unknown. The action of transferring the egg case stimulates the remaining larvae to leave the egg case within a few hours. These are the larvae that are used to start the test.

Table 15.4 Endpoints for a Long-term Sediment Toxicity Test with *Chironomus tentans*

Lethal	Sublethal		
Survival	Growth	Emergence	Reproduction
Larvae (20 d)	Larvae	Total/Percent	Sex Ratio
Larvae (End)		Cumulative (Rate)	Time to Oviposition
Pupae		Time to First	Mean Eggs/Female
Adults		Time to Death	Egg Cases/Treatment
			Egg Hatchability

15.3.3 Sediment into Test Chambers

15.3.3.1 The day before the sediment test is started (Day -1) each sediment should be thoroughly homogenized and added to the test chambers (Section 8.3.1). Sediment should be visually inspected to judge the extent of homogeneity. Excess water on the surface of the sediment can indicate separation of solid and liquid components. If a quantitative measure of homogeneity is required, replicate subsamples should be taken from the sediment batch and analyzed for TOC, chemical concentrations, and particle size.

15.3.3.2 Each test chamber should contain the same amount of sediment, determined either by volume or by weight. Overlying water is added to the chambers in a manner that minimizes suspension of sediment. This can be accomplished by gently pouring water along the sides of the chambers or by pouring water onto a baffle (e.g., a circular piece of Teflon with a handle attached) placed above the sediment to dissipate the force of the water. Renewal of overlying water is started on Day -1. A test begins when the organisms are added to the test chambers (Day 0).

15.3.4 Renewal of Overlying Water

15.3.4.1 Renewal of overlying water is required during a test. Two volume additions of overlying water (continuous or intermittent) should be delivered to each test chamber daily. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Hardness, alkalinity and ammonia concentrations in the water above the sediment, within a treatment, typically should not vary by more than 50% during the test. Mount and Brungs (1967) diluters have been modified for sediment testing, and other automated water-delivery systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998; Wall et al., 1998; Leppanen and Maier, 1998). Each water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms on Day 0. Appendix A describes water-renewal systems that can be used for conducting sediment tests.

15.3.4.2 In water-renewal tests with one to four volume additions of overlying water/d, water-quality characteristics generally remain similar to the inflowing water (Ingersoll and Nelson, 1990; Ankley et al., 1993); however, in static tests, water quality may change profoundly during the exposure (Shuba et al., 1978). For example, in static whole-sediment tests, the alkalinity, hardness, and conductivity of overlying water more than doubled in several treatments during a four-week exposure (Ingersoll and Nelson, 1990). Additionally, concentrations of metabolic products (e.g., ammonia) may also increase during static exposures, and these compounds can either be directly toxic to the test organisms or may contribute to the toxicity of the contaminants in the sediment. Furthermore, changes in water-quality characteristics such as hardness may influence the toxicity of many inorganic (Gauss et al., 1985) and organic (Mayer and Ellersieck, 1986) contaminants. Although contaminant concentrations are reduced in the overlying water in water-renewal tests, organisms in direct contact with sediment generally receive a substantial proportion of a contaminant dose directly from either the whole sediment or from the interstitial water.

15.3.5 Acclimation

15.3.5.1 Test organisms must be cultured and tested at 23°C. Ideally, test organisms should be cultured in the same water that will be used in testing. However, acclimation of test organisms to the test water is not required.

15.3.5.2 Culturing of organisms and toxicity assessment are typically conducted at 23°C. However, occasionally there is a need to perform evaluations at temperatures different than that recommended. Under these circumstances, it may be necessary to acclimate organisms to the desired test temperature to prevent thermal shock when moving immediately from the culture temperature to the test temperature (ASTM, 1999a). Acclimation can be achieved by exposing organisms to a gradual decline in temperature; however, the rate of decline should be relatively slow to prevent thermal shock. A decline in temperature of 1°C every 1 to 2 h has been used successfully in some studies (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication; APHA, 1989). Testing at temperatures other than 23°C needs to be preceded by

studies to determine expected performance under alternate conditions.

15.3.6 Placing Organisms in Test Chambers

15.3.6.1 Test organisms should be handled as little as possible. To start the test, larvae are collected with a Pasteur pipet from the bottom of the incubation dish with the aid of a dissecting microscope. Test organisms are pipetted directly into overlying water and care should be exercised to release them under the surface of the water. Transferring the larvae to exposure chambers within 4 h of emerging from the egg case reportedly improves survival (Benoit et al., 1997). Laboratory personnel should practice transferring first-instar midge larvae before tests with sediment are conducted.

15.3.7 Feeding

15.3.7.1 Each beaker receives a daily addition of 1.5 mL of Tetrafin® (4 mg/mL dry solids). Without addition of food, the test organisms may starve during exposures. However, the addition of the food may alter the availability of the contaminants in the sediment (Wiederholm et al., 1987; Harkey et al., 1994). Furthermore, if too much food is added to the test chamber, or if the mortality of test organisms is high, fungal or bacterial growth may develop on the sediment surface. Therefore, the amount of food added to the test chambers is kept to a minimum.

15.3.7.1 Suspensions of food should be thoroughly mixed before aliquots are taken. If excess food collects on the sediment, a fungal or bacterial growth may develop on the sediment surface, in which case feeding should be suspended for one or more days. A drop in dissolved oxygen below 2.5 mg/L during a test may indicate that the food added is not being consumed. Feeding should be suspended for the amount of time necessary to increase the dissolved oxygen concentration (ASTM, 1999a). If feeding is suspended in one treatment, it should be suspended in all treatments. Detailed records of feeding rates and the appearance of the sediment surface should be made daily.

15.3.8 Monitoring a Test

15.3.8.1 All chambers should be checked daily and observations made to assess test organism behavior such as sediment avoidance. However, monitoring effects on burrowing activity of test organisms may be difficult because the test organisms are often not visible during the exposure. The operation of the exposure system should be monitored daily.

15.3.8.2 Measurement of Overlying Water-quality Characteristics

15.3.8.2.1 Conductivity, hardness, alkalinity, and ammonia should be measured in all treatments at the beginning of the test, on Day 20, and at the end of the test. Dissolved oxygen (DO) and pH measurements should be taken at the beginning of a test and at least three times a week until the end of the test. Conductivity should be measured weekly. Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessary to composite water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water. Water quality should be measured on each batch of water prepared for the test.

15.3.8.2.2 Routine chemistries on Day 0 should be taken before organisms are placed in the test beakers. Dissolved oxygen and pH can be measured directly in the overlying water with a probe. However, for DO it is important to allow the probe time to equilibrate in the overlying water in an effort to accurately measure concentrations of DO. If a probe is used for measurements in overlying water, it should be inspected between samples to make sure that organisms are not attached and should be rinsed between samples to minimize cross contamination.

15.3.8.2.3 Water-only exposures evaluating the tolerance of *C. tentans* larva to depressed DO have indicated that significant reductions in weight occurred after 10-d exposure to 1.1 mg/L DO, but not at 1.5 mg/L (V. Mattson, USEPA, Duluth, MN, personal communication). This finding concurs with the observations during method development at the USEPA laboratory in Duluth that excursions of DO as low as 1.5 mg/L did not seem to have an effect on midge survival and development (P.K. Sibley, University of Guelph, Guelph, Ontario, personal communication). Based on these findings, periodic depressions of DO below 2.5 mg/L (but not below 1.5 mg/L) are not likely to adversely affect test results, and thus should not be a reason to discard test data. Nonetheless, tests should be managed toward a goal of DO >2.5 mg/L to insure satisfactory performance. If the DO level of the water falls below 2.5 mg/L for any one treatment, aeration is encouraged and should be done in all replicates for the duration of the test (i.e., about 1 bubble/second in the overlying water). Occasional brushing of screens on outside of beakers will help maintain the exchange of water during renewals.

15.3.8.2.4 Temperature should be measured at least daily in at least one test chamber from each treatment. The temperature of the water bath or the exposure chamber should be continuously monitored. The daily mean test temperature must be within ±1°C of 23°C. The instantaneous temperature must always be within ±3°C of 23°C.

15.3.8.3 Monitoring Survival and Growth

15.3.8.3.1 At 20 d, 4 of the initial 12 replicates are selected for use in growth and survival measurements. Using a #40 sieve (425-µm mesh) to remove larvae from sediment, collect the *C. tentans* and record data on record sheet (Appendix D). Any immobile organisms isolated from the sediment surface or from sieved material should be considered dead. Often *C. tentans* larvae tend to lose their coloration within 15 to 20 min of death and may become rigidly elongate. Surviving larvae are kept separated by replicate for weight measurements; if pupae are

recovered (<1% occurrence at recommended testing conditions), these organisms are included in survival data but not included in the growth data. A consistent amount of time should be taken to examine sieved material for recovery of test organisms (e.g., 5 min/replicate).

15.3.8.3.2 The 10-d method for *C. tentans* in the first edition of this manual (USEPA, 1994a), as well as most previous research, has used dry weight as a measure of growth. However, Sibley et al. (1997b) found that the grain size of sediments influences the amount of sediment that *C. tentans* larvae ingest and retain in their gut. As a result, in finer-grain sediments, a substantial portion of the measured dry weight may be comprised of sediment rather than tissue. While this may not represent a strong bias in tests with identical grain size distributions in all treatments, most field assessments are likely to have varying grain size among sites. This will likely create differences in dry weight among treatments that are not reflective of true somatic growth. For this reason, weight of midges should be measured as ash-free dry weight (AFDW) instead of dry weight. AFDW will more directly reflect actual differences in tissue weight by reducing the influence of sediment in the gut. If test organisms are to be used for an evaluation of bioaccumulation, it is not advisable to dry the sample before conducting the residue analysis. If conversion from wet weight to dry weight is necessary, aliquots of organisms can be weighed to establish wet to dry weight conversion factors. A consistent procedure should be used to remove the excess water from the organisms before measuring wet weight.

15.3.8.3.3 The AFDW of midges should be determined for the growth endpoint. All living larvae per replicate are combined and dried to a constant weight (e.g., 60°C for 24 h). Note that the weigh boats should be ashed before use to eliminate weighing errors due to the pan oxidizing during ashing. The sample is brought to room temperature in a desiccator and weighed to the nearest 0.01 mg to obtain mean weights per surviving organism per replicate. The dried larvae in the pan are then ashed at 550°C for 2 h. The pan with the ashed larvae is then reweighed and the tissue mass of the larvae is determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan. For rare instances in which preservation is required, an 8% sugar formalin solution can be used to preserve samples (USEPA, 1994a), but the effects of preservation on the weight and lengths of the midges have not been sufficiently studied. The sugar formalin solution is prepared by adding 120 g of sucrose to 80 mL of formalin which is then brought to a volume of 1 L using deionized water. This stock solution is mixed with an equal volume of deionized water when used to preserve organisms. NoTox® (Earth Safe Industries, Belle Mead, NJ) can be used as a substitute for formalin (Unger et al., 1993).

15.3.8.4 Monitoring Emergence

15.3.8.4.1 Emergence traps are placed on the reproductive replicates on Day 20 (emergence traps for the auxiliary beakers are added at the corresponding 20-d time interval for those replicates; Appendix C, Figures C.1 and C.4). At 23 °C, emergence in control sediments typically begins on or about Day 23 and continues for about 2 weeks. However, in contaminated sediments, the emergence period may be extended by several weeks.

15.3.8.4.2 Two categories are recorded for emergence: complete emergence and partial emergence. Complete emergence occurs when an organism has shed the pupal exuviae completely and escapes the surface tension of the water. If complete emergence has occurred but the adult has not escaped the surface tension of the water, the adult will die within 24 h. Therefore, 24 h should elapse before this death is recorded. Partial emergence occurs when an adult has only partially shed the pupal exuviae. These adults will also die, an event which can be recorded after 24 h. Pupae at the sediment surface or the air-water interface may emerge successfully during the 24-h period. However, cannibalism of sediment bound pupae by larvae may also occur. Data are recorded on data sheets provided as shown in example data sheet (Appendix D).

15.3.8.4.3 Between Day 23 and the end of the test, emergence of males and females, pupal and adult mortality, and time to death for adults is recorded daily for the reproductive replicates. On Day 30 (20-d-old organisms), emergence traps are placed on the auxiliary beakers to collect the additional males for use with females emerging from the reproduction replicates (Table 15.2; Appendix C, Figures C.1 and C.4). Data are recorded on data sheets provided as shown in the example data sheet (Appendix D).

15.3.8.5 Collecting Adults for Reproduction

15.3.8.5.1 Adults are collected daily from individual traps using the aspirator and collector dish (Appendix C, Figure C.2). With the collector dish nearby, the emergence trap is quickly moved from the beaker onto the dish. With the syringe plunger fully drawn, the glass collector tube is inserted through the screened access hole of the collector dish and the adults gently aspirated into the syringe barrel. Aspirated adults can easily be seen through the translucent plastic of the syringe. The detachable portion of the aspirator unit is then replaced with a reproduction/oviposit (R/O) chamber. This exchange can be facilitated by placing the thumb of the hand holding the syringe over the barrel entry port until the R/O chamber is in place. With the R/O chamber in place, and the plunger on a solid surface, the barrel of the syringe is pushed gently downward which forces the adults to move up into the R/O unit. Adults remaining on the transfer apparatus may be prodded into the R/O chamber by gently tapping the syringe. The transfer process is completed by quickly moving the R/O chamber to a petri dish containing clean water. At all times during

the transfer process, it is important to ensure that the adults are stationary to minimize the possibility of escape.

15.3.8.5.2 At about Day 33 to the end of the test, the auxiliary males may be needed to support reproduction in females. Males that emerge from the auxiliary male replicates are transferred to individual inverted petri dishes (60 x 15 mm dishes without water and with air holes drilled in top of the dish; see Appendix C for a listing of equipment.) Each male may be used for mating with females from corresponding treatments for up to 5 d. Males may be used for breeding with more than one new emergent female. Males from a different replicate within the same sediment treatment may be paired with females of replicates where no males have emerged. Data can be recorded on data sheets provided in Appendix D.

15.3.8.6 Monitoring Reproduction

15.3.8.6.1 Each R/O unit is checked daily for dead adults and egg cases. Dead organisms are removed. In situations where many adults are contained within an R/O chamber, it may be necessary to assume that a dead adult is the oldest male or female in that replicate for the purpose of recording time to death. To remove dead adults and egg cases from the R/O chamber, one side of the chamber is carefully lifted just enough to permit the insertion of a transfer pipet or tweezers.

15.3.8.6.2 For each emerged female, at least one male, obtained from the corresponding reproductive replicate, from another replicate of that treatment, or from the auxiliary male beakers, is transferred into the R/O unit using an aspirator. Females generally remain sexually receptive up to 3 d if they have not already mated. Benoit et al. (1997) have shown that over 90% of females will oviposit within 1 d of fertilization; however, a few will require as long as 72 h to oviposit. A female will lay a single primary egg case, usually in the early morning (Sadler, 1935). A second, generally smaller egg case may be laid; however these second egg cases are prone to fungus and the viability of embryos is typically poor. These second egg cases do not need to be counted, or recorded, and the numbers of eggs are not included in the egg counts because eggs in second egg cases typically have lower viability.

15.3.8.7 Counting Eggs, Egg Case Incubation, and Hatch Determination

15.3.8.7.1 Primary egg cases from the R/O chamber are transferred to a separate and corresponding petri dish (60 x 15 mm with about 15 mL of water) to monitor incubation and hatch. The number of eggs should be estimated in each egg case by using a "ring method" as follows: (1) for each egg case, the mean number of eggs in five rings is determined; (2) these rings should be selected at about equal distances along the length of the egg case; (3) the number of eggs/ring multiplied by the number of number of rings in the egg case will provide an estimate of the total number of eggs. This can be done in about 5 min or less for each egg case. Accuracy of estimating versus a direct count method is very close, roughly 95% (Benoit et al., 1997). The ring method is best suited to the “C” shaped egg cases.

15.3.8.7.2 When the integrity of an egg case precludes estimation by the ring method (egg case is convoluted or distorted), the eggs should be counted directly. Each egg case is placed into a 5-cm glass culture tube containing about 2 mL of 2 N sulfuric acid (H_2SO_4) and left overnight. The acid dissolves the gelatinous matrix surrounding the eggs but does not affect the structural integrity of the eggs themselves. After digestion, the eggs are collected with a Pasteur pipet and spread across a microscope slide for counting under a dissecting microscope. Counting can be simplified by drawing a grid on the underside of the slide. The direct count method requires a minimum of 10 min to complete and does not permit determination of hatching success.

15.3.8.7.3 Following estimated egg counts, each egg case is transferred to a 60- x 15-mm plastic petri dish containing 15 mL overlying water and incubated at 23°C until hatching is complete. Although the time required to initiate hatching at this temperature is about 2 d, the period of time required to bring about complete hatch may be as long as 6 d. Therefore, hatching success is determined after 6 d of incubation. Hatching success is determined by subtracting the number of unhatched eggs remaining after the 6 d period from the number of eggs originally estimated for that egg case. Unhatched eggs either remain in the gelatinous egg case or are distributed on the bottom of the petri dish.

15.3.8.7.4 Depending on the objectives of the study, reproductive output in *C. tentans* may be expressed as: (1) number of eggs/female or (2) number of offspring/female. The former approach estimates reproductive output (fecundity) in terms of the number of eggs deposited by a female (secondary egg cases are not included) and does not take into account survival of hatched eggs. This approach has been shown to adequately discriminate contaminant (Sibley et al., 1996) and noncontaminant (Sibley et al., 1997a) stressors. Since this approach does not require monitoring egg masses for hatchability, the time and labor involved in conducting the life-cycle test is reduced. However, studies that require estimates of demographic parameters, or include population modeling, will need to determine the number of viable offspring per female (Sibley et al., 1997a). This will require determination of larval hatch (see Section 15.3.8.7.3). Although larval hatch is listed as a potential endpoint by itself in this manual (Table 15.4), the sensitivity of this endpoint has not been fully assessed.

15.3.9 Ending a Test

15.3.9.1 The point at which the life-cycle test is ended depends upon the sediments being evaluated. In clean sediments, the test typically requires 40 to 50 d from initial setup to completion. However, test duration will increase in the presence of environmental stressors which

act to reduce growth and delay emergence (Sibley et al., 1997a). Where a strong gradient of sediment contamination exists, emergence patterns between treatments will likely become asynchronous, in which case each treatment needs to be ended separately. For this reason, emergence is used as a guide to decide when to end a test.

15.3.9.2 For treatments in which emergence has occurred, the treatment (not the entire test) is ended when no further emergence is recorded over a period of 7 d (the 7-d criterion). At this time, all beakers of the treatment are sieved through a #40-mesh screen (425 µm) to recover remaining larvae, pupae, or pupal castes. When no emergence is recorded in a treatment at any time during the test, that treatment can be ended once emergence in the control sediment has ended using the 7-d criterion.

15.4 Interpretation of Results

15.4.1 Data Analysis

15.4.1.1 Endpoints measured in the *C. tentans* test include survival, growth, emergence and reproduction. Section 16 describes general information regarding statistical analysis of these data, including both point estimates (i.e., LC50s) and hypothesis testing (i.e., ANOVA). The following sections describe species-specific information that is useful in helping to interpret the results of long-term sediment toxicity tests with *C. tentans*.

15.4.2 Age Sensitivity

15.4.2.1 Midges are perceived to be relatively insensitive organisms in toxicity assessments (Ingersoll, 1995). This conclusion is based on the practice of measuring survival of fourth-instar larvae in short-term water-only exposures, a procedure that may underestimate the sensitivity of midges to toxicants. The first and second instars of chironomids are more sensitive to contaminants than the third or fourth instars. For example, first-instar *C. tentans* larvae were 6 to 27 times more sensitive than fourth-instar larvae to acute copper exposure (Nebeker et al., 1984b; Gauss et al., 1985; Figure 12.1) and first-instar *C. riparius* larvae were 127 times more sensitive than second-instar larvae to acute cadmium exposure (Williams et al., 1986b; Figure 12.1). In long-term tests with first-instar larvae, midges were often as sensitive as daphnids to inorganic and organic compounds (Ingersoll et al., 1990). Sediment tests should be started with uniform age and size midges because of the dramatic differences in sensitivity of midges by age.

15.4.3 Physical Characteristics of Sediment

15.4.3.1 Grain Size

15.4.3.1.1 Larvae of *C. tentans* appear to be tolerant of a wide range of particle size conditions in substrates. Several studies have shown that survival is not affected by particle size in natural sediments, sand substrates, or formulated sediments in both 10-d and long-term exposures (Ankley et al., 1994; Suedel and Rodgers, 1994; Sibley et al., 1997b, 1998). Ankley et al. (1994a) found that growth of *C. tentans* larvae was weakly correlated with sediment grain size composition, but not organic carbon, in 10-d tests using 50 natural sediments from the Great Lakes. However, Sibley et al. (1997b) found that the correlation between grain size and larval growth disappeared after accounting for inorganic material contained within larval guts and concluded that growth of *C. tentans* was not related to grain size composition in either natural sediments or sand substrates. Avoiding confounding influences of gut contents on weight is the impetus for recommending ash-free dry weight (instead of dry weight) as the index of growth in the 10-day and long-term *C. tentans* tests. Failing to do so could lead to erroneous conclusions regarding the toxicity of the test sediment (Sibley et al., 1997b). Procedures for correcting for gut contents are described in Section 15.3.8.3. Emergence, reproduction (mean eggs/female), and hatch success were also not affected by the particle size composition of substrates in long-term tests with *C. tentans* (Sibley et al., 1998).

15.4.3.2 Organic Matter

15.4.3.2.1 Based on 10-d tests, the content of organic matter in sediments does not appear to affect survival of *C. tentans* larvae in natural and formulated sediments, but may be important with respect to larval growth. Ankley et al. (1994a) found no relationship between sediment organic content and survival or growth in 10-d bioassays with *C. tentans* in natural sediments. Suedel and Rodgers (1994) observed reduced survival in 10-d tests with a formulated sediment when organic matter was <0.91%; however, supplemental food was not supplied in this study, which may influence these results relative to the 10-d test procedures described in this manual. Lacey et al. (1999) found that survival of *C. tentans* larvae was generally not affected in 10-d tests by either the quality or quantity of synthetic (alpha-cellulose) or naturally derived (peat, maple leaves) organic material spiked into a formulated sediment, although a slight reduction in survival below the acceptability criterion (70%) was observed in a natural sediment diluted with formulated sediment at an organic matter content of 6%. In terms of larval growth, Lacey et al. (1999) did not observe any systematic relationship between the level of organic material (e.g., food quantity) and larval growth for each carbon source. Although a significant reduction in growth was observed at the highest concentration (10%) of the leaf treatment in the food quantity study, significantly higher larval growth was observed in this treatment when the different carbon sources were compared at about equal concentrations (effect of food quality). In the latter study, the following gradient of larval growth was established in relation to the source of organic carbon: peat < natural sediment < alpha-cellulose < leaves. Since all of the treatments received a supplemental source of food, these data suggest that both the quality and quantity of organic carbon in natural and formulated sediments may represent an important confounding factor for the growth endpoint in tests with *C. tentans* (Lacey et al., 1999). However, it is

important to note that these data are based on 10-d tests; the applicability of these data to long-term testing has not been evaluated.

15.4.4 Isolating Organisms at the End of a Test

15.4.4.1 Quantitative recovery of larvae at the end of a sediment test should not be a problem. The larvae are red and typically greater than 5 mm long and are readily retained on the #40-mesh sieve.

15.4.5 Influence of Indigenous Organisms

15.4.5.1 The influence of indigenous organisms on the response of *C. tentans* in sediment tests has not been reported. Survival of a closely related species, *C. riparius* was not reduced in the presence of oligochaetes in sediment samples (Reynoldson et al., 1994). However, growth of *C. riparius* was reduced when high numbers of oligochaetes were placed in a sample. Therefore, it is important to determine the number and biomass of indigenous organisms in field-collected sediment in order to better interpret growth data (Reynoldson et al., 1994; DeFoe and Ankley, 1998). Furthermore, the presence of predators may also influence the response of test organisms in sediment (Ingersoll and Nelson, 1990).

15.4.6 Relationship Between Endpoints

15.4.6.1 Relationship Between Growth and Emergence Endpoints

15.4.6.1.1 An important stage in the life cycle of *C. tentans* is the emergence of adults from pupal forms. Emergence has been used in many studies as an indicator of contaminant stress (Wentsel et al., 1978; Pascoe et al., 1989; Sibley et al., 1996). The use of emergence as an endpoint in this context is based upon the understanding that larval growth and emergence are intimately related such that environmental factors that affect larval development may also affect emergence success. Implicit in the relationship between growth and emergence is the notion of a weight threshold that needs to be attained by larvae in order for emergence to take place (Hilsenhoff,1966; Liber et al., 1996; Sibley et al., 1997a). For example, based on evaluations conducted in clean control sediment, Liber et al. (1996) and Sibley et al. (1997a) showed that a minimum tissue mass threshold of approximately 0.6 mg dry weight or 0.48 mg ash-free dry weight was required before pupation and emergence could take place (Figure 15.1). Further, Sibley et al. (1997a) found that maximum emergence (e.g., >60%) in this sediment occurred only after larvae had attained a tissue mass of about 0.8 mg dry weight. This value corresponds closely to that suggested by Ankley et al. (1994a) as an acceptability criterion for growth in control sediments in 10-d tests with *C. tentans*.

15.4.6.2 Relationship Between Growth and Reproduction Endpoints

15.4.6.2.1 Natural or anthropogenic stressors that affect growth of invertebrates may also affect reproduction, because of a minimum threshold body mass needed for reproduction (Rees and Crawley, 1989; Ernsting et al., 1993; Moore and Dillon, 1993; Sibley et al., 1996,1997a). Sibley et al. (1996,1997a) reported a significant relationship between growth (dry weight) of larval *C. tentans* and reproductive output (mean number of eggs) of adults in relation to both food and contaminant (zinc) stressors (Figure 15.2). The form that this relationship may take depends upon the range of stress to which the larvae are exposed and may be linear or sigmoidal. The latter relationship is typically characterized by an upper maximum determined by competitive factors (i.e., food and space availability) and a lower minimum determined primarily by emergence thresholds (See Section 15.4.6.1; Sibley et al., 1997a).

15.4.6.2.2 Embryo viability (percent hatch of eggs) has been shown to evaluate the toxicity for waterborne chemicals (Williams et al.,1986b; Pascoe et al.,1989). However, percent hatch has not been used extensively as an endpoint to assess toxicity in contaminated sediments. Sibley et al. (1996) found that the viability of embryos was not affected at any of the zinc treatments for which egg masses were produced; >87% of all eggs eventually hatched. Additional information regarding the measurement of embryo viability in round-robin testing is presented in Section 17.6.

15.4.6.2.3 In contrast to *H. azteca* (Section 14.4), length is not commonly utilized as a growth endpoint in *C. tentans*. However, length may represent a useful alternative to weight. For example, recent studies (P.K. Sibley, University of Guelph, Guelph, Ontario, unpublished data) found a significant relationship (r^2=0.99; p <0.001) between ash-free dry weight and length in larvae of *C. tentans* reared in clean control sediment (Figure 15.3). This suggests that either weight or length could be used to assess growth in *C. tentans*. However, the relationship between length and emergence or reproductive endpoints has not been evaluated.

15.4.6.3 Relationship Between Growth and Population Endpoints

15.4.6.3.1 Few studies have attempted to quantitatively define the relationship between larval growth and population-level processes. However, an accurate understanding of the ecological relevance of growth as an endpoint in sediment toxicity tests can only be achieved in terms of its effect, if any, on population-level processes. Sibley et al. (1997a) found a significant relationship between larval growth and the intrinsic rate of population increase in *C. tentans* in relation to a food stressor (Figure 15.4). When applied in a theoretical population model, it was further demonstrated that changes in larval growth resulting from the stressor gradient were significantly correlated to the predicted number of offspring recruited to subsequent generations.

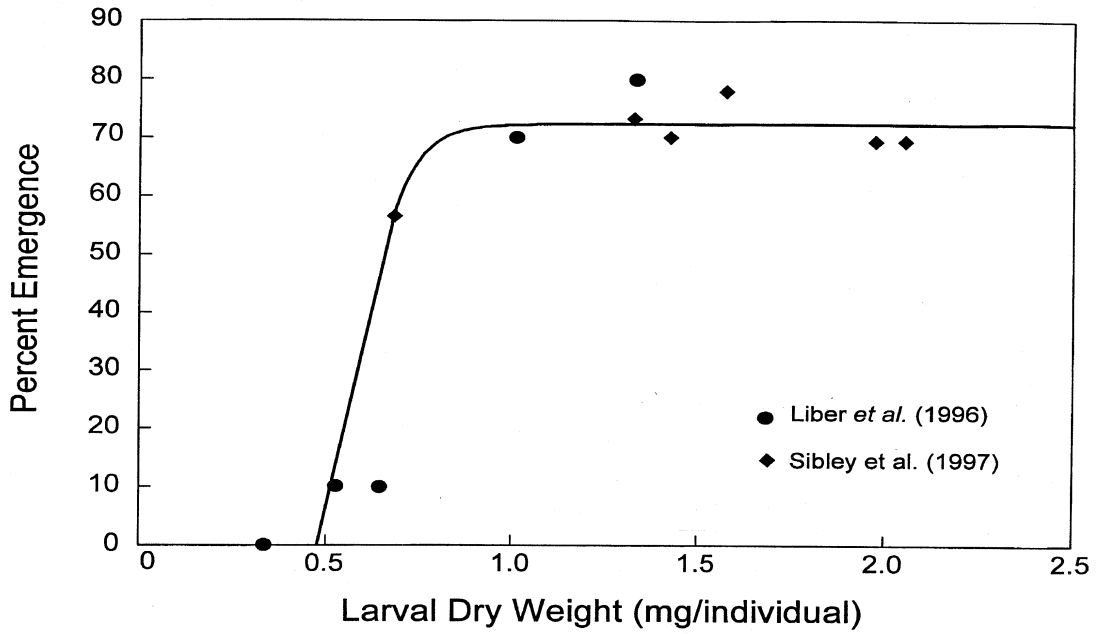


Figure 15.1 Relationship between weight and emergence of *Chironomus tentans.*

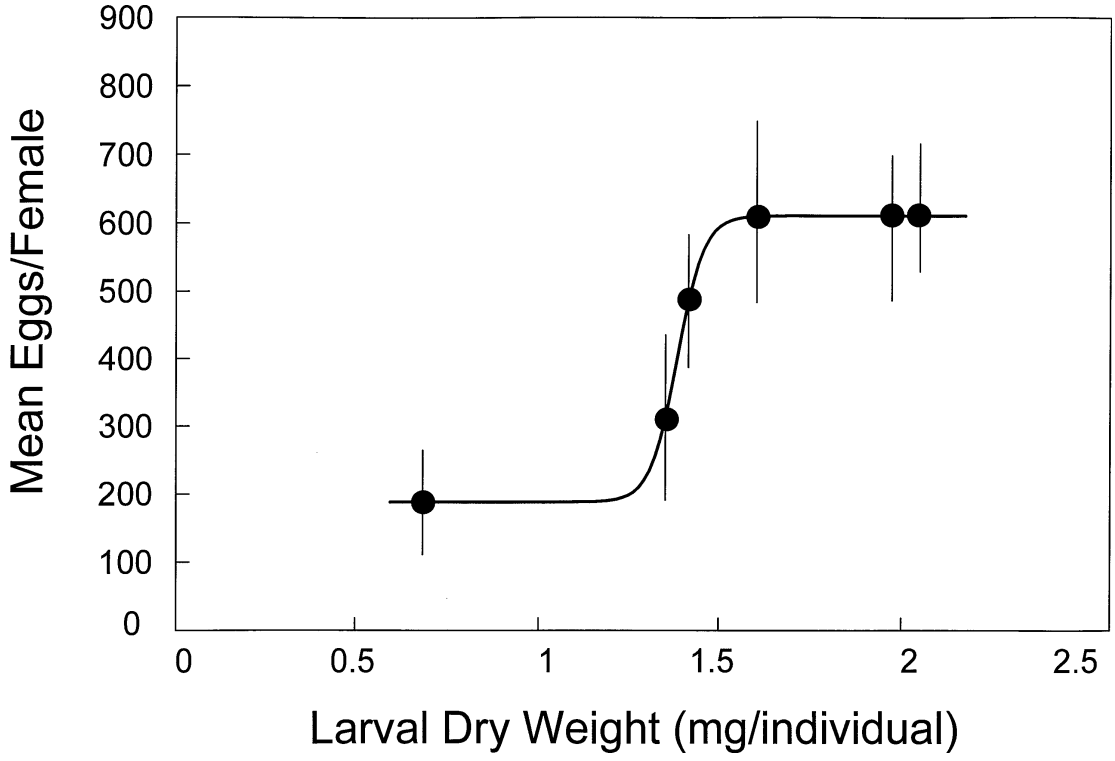


Figure 15.2 Relationship between weight and reproduction of *Chironomus tentans.*

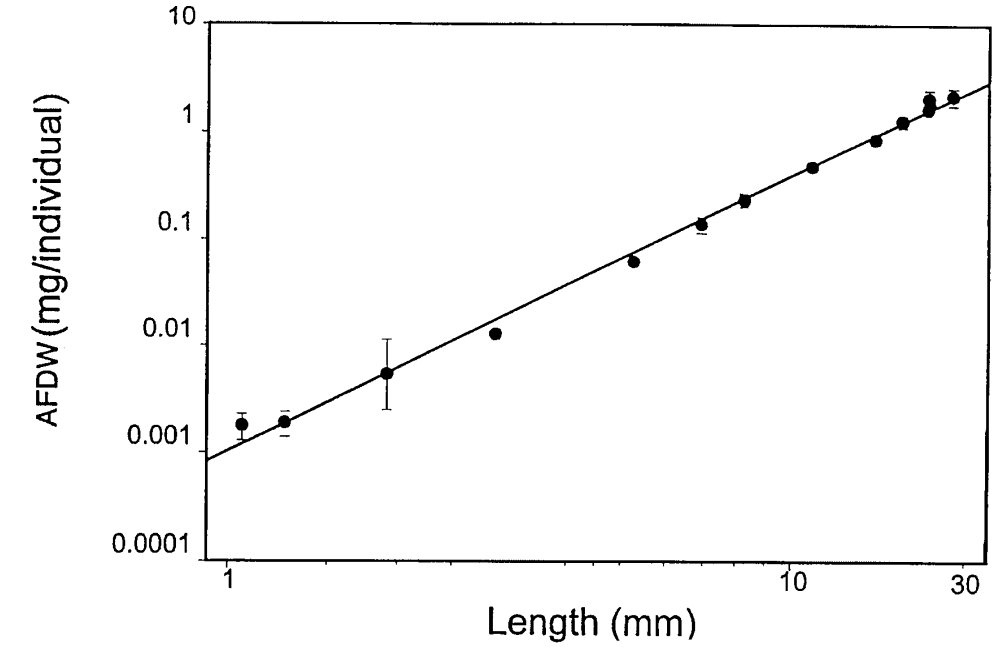


Figure 15.3 Relationship between ash-free dry weight (AFDW) and length of *Chironomus tentans.*

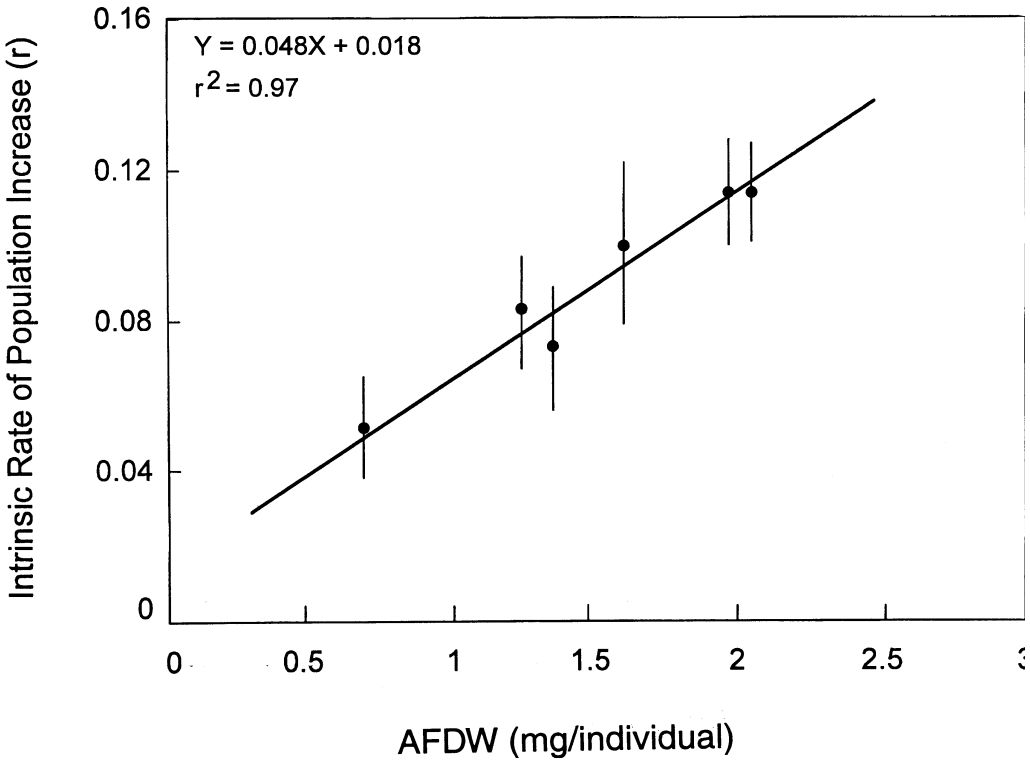


Figure 15.4 Relationship between ash-free dry weight (AFDW) and intrinsic rate of natural increase of *Chironomus tentans.*

15.4.6.4 Relative Endpoint Variability

15.4.6.4.1 Based on coefficient of variation (CV) determined from a control sediment (West Bearskin), the following variability has been documented for the various endpoints in the *C. tentans* life-cycle test (Sibley et al., 1996; Benoit et al., 1997): Survival (<20%), growth as dry weight (<15%), emergence (<30%), reproduction as mean eggs/female (<20%), percent hatch (<10%). Additional information regarding the variation in these endpoints in round-robin testing is presented in Section 17.6.

15.4.6.5 Relative Endpoint Sensitivity

15.4.6.5.1 Measurement of sublethal endpoints (e.g., growth) can often provide unique information in addition to measuring survival. A comparison of lethal and sublethal endpoints relative to toxicity identification is presented in Table 14.4 for *H. azteca*. However, few studies have compared the relative sensitivity of the various endpoints in the *C. tentans* life cycle or in 10-d tests. Sibley et al. (1997a) found that larval *C. tentans* exposed to a gradient of food stress did not experience significant effects on survival, yet did experience a significant reduction in growth and reproduction. Further, the proportion of larvae hatching in this study was high (>80%) and not systematically related to treatment, suggesting that percent hatch may be a relatively insensitive endpoint to sediment-associated contaminants. This is consistent with the findings of another study using zinc-spiked sediments; no effect on embryo viability was observed for those treatments in which egg masses were produced (Sibley et al. 1996). Although the responses observed in the feeding study were not due to a contaminant stressor per se, the sublethal endpoints were clearly better able to discriminate the presence of the stressor than was lethality. Ankley and DeFoe (1998) studied a variety of contaminated sediments and found that the sensitivity of *C. tentans* 10-d tests is greatly increased by measurement of growth in addition to survival. Growth of midge in these 10-d sediment tests was found to be a more sensitive endpoint than survival of *Hyalella azteca*.

15.4.7 Future Research

15.4.7.1 Additional studies using known concentration gradients in sediment, should be conducted to better differentiate the relative sensitivity between lethal and sublethal endpoints and between sublethal endpoints in the long-term *C. tentans* test. Additional studies also are needed to further evaluate the influence of ammonia on long-term exposures with *C. tentans*. Section 1.3.8.5 addresses interpretative guidance for evaluating toxicity associated with ammonia in sediment. Planned water-only toxicity tests with select chemicals (i.e., cadmium, DDD, and fluoranthene) should generate data that can be used to better determine the relative sensitivity of survival, reproduction, and growth endpoints in tests with *C. tentans*. In addition to studies evaluating the relative sensitivity of endpoints, research is also needed to evaluate the ability of these laboratory endpoints to estimate responses of benthic organisms exposed in the field to chemicals in sediments.

Section 16
Data Recording, Data Analysis and Calculations, and Reporting

16.1 Data Recording

16.1.1 Quality assurance project plans with data quality objectives and standard operating procedures should be developed before starting a test. Procedures should be developed by each laboratory to verify and archive data (USEPA, 1994e).

16.1.2 A file should be maintained for each sediment test or group of tests on closely related samples (Section 9). This file should contain a record of the sample chain-of-custody; a copy of the sample log sheet; the original bench sheets for the test organism responses during the sediment test(s); chemical analysis data on the sample(s); control data sheets for reference toxicants; detailed records of the test organisms used in the test(s), such as species, source, age, date of receipt, and other pertinent information relating to their history and health; information on the calibration of equipment and instruments; test conditions used; and results of reference-toxicity tests. Original data sheets should be signed and dated by the laboratory personnel performing the tests. A record of the electronic files of data should also be included in the file.

16.1.3 Example data sheets are included in Appendix D.

16.2 Data Analysis

16.2.1 Statistical methods are used to make inferences about populations, based on samples from those populations. In most sediment toxicity and bioaccumulation tests, test organisms are exposed to chemicals in sediment to estimate the response of the population of laboratory organisms. The organism response to these sediments is usually compared with the response to a control or reference sediment, or in some analyses of bioaccumulation test data, with a fixed standard such as a Food and Drug Administration (FDA) action level. In any toxicity or bioaccumulation test, summary statistics such as means and standard errors for response variables (e.g., survival, chemical concentrations in tissue) should be provided for each treatment (e.g., pore-water concentration, sediment).

16.2.1.1 Types of Data.

16.2.1.1.1 Two types of data can be obtained from sediment toxicity or bioaccumulation tests. The most common endpoint in toxicity testing is mortality, which is a dichotomous or categorical type of data. Other endpoints measured in sublethal evaluations include growth and reproduction (Sections 14 and 15) or tissue concentrations (e.g., in sediment bioaccumulation tests conducted with oligochaetes (Section 13) or with polychaetes and mollusks; USEPA, 1994b). Growth, reproduction, and bioaccumulation endpoints are representative of continuous data.

16.2.1.2 Sediment Testing Scenarios

16.2.1.2.1 Sediment tests are conducted to determine whether contaminants in sediment are harmful to or are bioaccumulated in benthic organisms. Sediment tests are commonly used in studies designed to (1) evaluate dredged material, (2) assess site contamination in the environment (e.g., to rank areas for cleanup), and (3) determine effects of specific contaminants, or combinations of contaminants, through the use of sediment-spiking techniques. Each of these broad study designs has specific statistical design and analytical considerations, which are detailed below.

16.2.1.2.2 Dredged Material Evaluation. In these studies, each site is compared individually with a reference sediment. The statistical procedures appropriate for these studies are generally pairwise comparisons. Additional information on toxicity testing of dredged material and analysis of data from dredged material evaluations is available in USEPA-USACE (1998a).

16.2.1.2.3 Site Assessment of Field Contamination. Surveys of sediment toxicity or bioaccumulation often are included in more comprehensive analyses of biological, chemical, geological, and hydrographic data. Statistical correlation can be improved and costs may be reduced if subsamples are taken simultaneously for sediment toxicity or bioaccumulation tests, chemical analyses, and benthic community structure determinations. There are several statistical approaches to field assessments, each with a specific purpose. If the objective is to compare the response or residue level at all sites individually to a control sediment, then the pairwise comparison approach described below is appropriate. If the objective is to

compare among all sites in the study area, then a multiple comparison procedure that employs an experiment-wise error rate is appropriate. If the objective is to compare among groups of sites, then orthogonal contrasts are a useful data analysis technique.

16.2.1.2.4 Sediment-spiking Experiments. Sediments spiked with known concentrations of chemicals can be used to establish cause-and-effect relationships between chemicals and biological responses. Results of toxicity tests with test materials spiked into sediments at different concentrations may be reported in terms of an LC50, EC50, IC50, NOEC, or LOEC. Results of bioaccumulation tests with either field or spiked samples may be reported in terms of a BSAF (biota sediment accumulation factor; ASTM, 1999c). The statistical approach outlined above for spiked-sediment toxicity tests also applies to the analysis of data from sediment dilution experiments or water-only reference-toxicity tests.

16.2.2 Experimental Design

16.2.2.1 The guidance outlined below on the analysis of sediment toxicity and bioaccumulation test data is adapted from a variety of sources including ASTM (1999c), USEPA (1991a), USEPA (1994a), USEPA (1994b), and USEPA-USACE (1998a). The objectives of a sediment toxicity or bioaccumulation test are to quantify contaminant effects on or accumulation in test organisms exposed to natural or spiked sediments or dredged materials and to determine whether these effects are statistically different from those occurring in a control or reference sediment. Each experiment consists of at least two treatments: the control and one or more test treatment(s). The test treatment(s) consist(s) of the contaminated or potentially contaminated sediment(s). A control sediment is always required to ensure that no contamination is introduced during the experiment setup and that test organisms are healthy. A control sediment is used to judge the acceptability of the test (Tables 11.3, 12.3, 13.4, 14.3, 15.3). Some designs also require a reference sediment that represents an environmental condition or potential treatment effect of interest. Controls are used to evaluate the acceptability of the test and might include a control sediment, a sand substrate (for *C. tentans*; Section 12.2, 15.2), or water-only exposures (for *H. azteca*; Section 14.3.7.8). Testing a reference sediment provides a site-specific basis for evaluating toxicity of the test sediments. Comparisons of test sediments to multiple reference or control sediments representative of the physical characteristics of the test sediment (i.e., grain size, organic carbon) may be useful in these evaluations (Section 2.1.2).

16.2.2.2 Experimental Unit

16.2.2.2.1 During toxicity testing, each test chamber to which a single application of treatment is applied is an experimental unit. During bioaccumulation testing, however, the test organism may be the experimental unit if individual members of the test species are evaluated and they are large enough to provide sufficient biomass for chemical analysis. The important concept is that the treatment (sediment) is applied to each experimental unit as a discrete unit. Experimental units should be independent and should not differ systematically.

16.2.2.3 Replication

16.2.2.3.1 Replication is the assignment of a treatment to more than one experimental unit. The variation among replicates is a measure of the within-treatment variation and provides an estimate of within-treatment error for assessing the significance of observed differences between treatments.

16.2.2.4 Minimum Detectable Difference (MDD)

16.2.2.4.1 As the minimum difference between treatments which the test is required or designed to detect decreases, the number of replicates required to meet a given significance level and power increases. Because no consensus currently exists on what constitutes a biologically acceptable MDD, the appropriate statistical minimum significant difference should be a data quality objective (DQO) established by the individual user (e.g., program considerations) based on their data requirements, the logistics and economics of test design, and the ultimate use of the sediment toxicity or bioaccumulation test results.

16.2.2.5 Minimum Number of Replicates

16.2.2.5.1 Eight replicates are recommended for 10-d freshwater sediment toxicity testing (Section 11 and 12) and five replicates are recommended for 10-d marine testing (USEPA, 1994b). However, four replicates per treatment are the absolute minimum number of replicates for a 10-d sediment toxicity test. A minimum of five replicates per treatment is recommended for bioaccumulation testing (Section 13). It is always prudent to include as many replicates in the test design as are economically and logistically possible. USEPA 10-d sediment toxicity testing methods recommend the use of 10 organisms per replicate for freshwater testing or 20 organisms per replicate for 10-d marine testing. An increase in the number of organisms per replicate in all treatments is allowable only if (1) test performance criteria for the recommended number of replicates are achieved and (2) it can be demonstrated that no change occurs in contaminant availability due to the increased organism loading. See Tables 14.1 and 15.1 for a description of the number of replicates and test organisms/replicate recommended for long-term testing of *Hyalella azteca* or *Chironomus tentans*.

16.2.2.6 Randomization

16.2.2.6.1 Randomization is the unbiased assignment of treatments within a test system and to the exposure chambers ensuring that no treatment is favored and that observations are independent. It is also important to (1) randomly select the organisms (but not the number of organisms) for assignment to the control and test treatments (e.g., a bias in the results may occur if all of the largest animals are placed in the same treatment),

(2) randomize the allocation of sediment (e.g., do not take all the sediment in the top of a jar for the control and the bottom for spiking), and (3) randomize the location of exposure units.

16.2.2.7 Pseudoreplication

16.2.2.7.1 The appropriate assignment of treatments to the replicate exposure chambers is critical to the avoidance of a common error in design and analysis termed "pseudoreplication" (Hurlbert, 1984). Pseudoreplication occurs when inferential statistics are used to test for treatment effects even though the treatments are not replicated or the replicates are not statistically independent (Hurlbert, 1984). The simplest form of pseudoreplication is the treatment of subsamples of the experimental unit as true replicates. For example, two aquaria are prepared, one with control sediment and the other with test sediment, and 10 organisms are placed in each aquarium. Even if each organism is analyzed individually, the 10 organisms only replicate the biological response and do not replicate the treatment (i.e., sediment type). In this case, the experimental unit is the 10 organisms and each organism is a subsample. A less obvious form of pseudoreplication is the potential systematic error due to the physical segregation of exposure chambers by treatment. For example, if all the control exposure chambers are placed in one area of a room and all the test exposure chambers are in another, spatial effects (e.g., different lighting, temperature) could bias the results for one set of treatments. Random physical intermixing of the exposure chambers or randomization of treatment location may be necessary to avoid this type of pseudoreplication. Pseudoreplication can be avoided or reduced by properly identifying the experimental unit, providing replicate experimental units for each treatment, and applying the treatments to each experimental unit in a manner that includes random physical intermixing (interspersion) and independence. However, avoiding pseudoreplication completely may be difficult or impossible given resource constraints.

16.2.2.8 Optimum Design of Experiments

16.2.2.8.1 An optimum design is one which obtains the most precise answer for the least effort. It maximizes or minimizes one of many optimality criteria, which are formal, mathematical expressions of certain properties of the model that are fit to the data. Optimum design of experiments using specific approaches described in Atkinson and Donev (1992) has not been formally applied to sediment testing; however, it might be desirable to use the approaches in experiments. The choice of optimality criterion depends on the objective of the test, and composite criteria can be used when a test has more than one goal. A design is optimum only for a specific model, so it is necessary to know beforehand which models might be used (Atkinson and Donev, 1992).

16.2.2.9 Compositing Samples

16.2.2.9.1 Decisions regarding compositing of samples depend on the objective of the test. Compositing is used primarily in bioaccumulation experiments when the biomass of an individual organism is insufficient for chemical analysis. Compositing consists of combining samples (e.g., organisms, sediment) and chemically analyzing the mixture rather than the individual samples. The chemical analysis of the mixture provides an estimate of the average concentration of the individual samples making up the composite. Compositing also may be used when the cost of analysis is high. Each organism or sediment sample added to the composite should be of equal size (i.e., wet weight) and the composite should be completely homogenized before taking a sample for chemical analysis. If compositing is performed in this manner, the value obtained from the analysis of the composite is the same as the average obtained from analyzing each individual sample (within any sampling and analytical errors). If true replicate composites (not subsample composites) are made, the variance of the replicates will be less than the variance of the individual samples, providing a more precise estimate of the mean value. This increases the power of a test between means of composites over a test between means of individuals or samples for a given number of samples analyzed. If compositing reduces the actual number of replicates, however, the power of the test will also be reduced. If composites are made of individuals or samples varying in size, the value of the composite and the mean of the individual organisms or sediment samples are no longer equivalent. The variance of the replicate composites will increase, decreasing the power of any test between means. In extreme cases, the variance of the composites can exceed the population variance (Tetra Tech, 1986). Therefore, it is important to keep the individuals or sediment samples comprising the composite equivalent in size. If sample sizes vary, consult the tables in Schaeffer and Janardan (1978) to determine if replicate composite variances will be higher than individual sample variances, which would make compositing inappropriate.

16.2.3 Hypothesis Testing and Power

16.2.3.1 The purpose of a toxicity or bioaccumulation test is to determine if the biological response to a treatment sample differs from the response to a control sample. Figure 16.1 presents the possible outcomes and decisions that can be reached in a statistical test of such a hypothesis. The null hypothesis is that no difference exists among the mean control and treatment responses. The alternative hypothesis of greatest interest in sediment tests is that the treatments are toxic, or contain concentrations of bioaccumulatable compounds, relative to the control or reference sediment.

16.2.3.2 Statistical tests of hypotheses can be designed to control for the chances of making incorrect decisions. In Figure 16.1, alpha (α) represents the probability of making a Type I statistical error. A Type I statistical error in this testing situation results from the false conclusion

Decision	TR =Control	TR > Control
TR =Control	Correct 1 - α	Type II Error β
TR > Control	Type I Error α	Correct 1 - β (Power)

Treatment response (TR), Alpha (α) represents the probability of making a Type I statistical error (false positive); beta (β) represents the probability of making a Type II statistical error (false negative).

Figure 16.1 Treatment response for a Type I and Type II error.

that the treated sample is toxic or contains chemical residues not found in the control or reference sample. Beta (β) represents the probability of making a Type II statistical error, or the likelihood that one erroneously concludes there are no differences among the mean responses in the treatment, control or reference samples. Traditionally, acceptable values for α have ranged from 0.1 to 0.01 with 0.05 or 5% used most commonly. This choice should depend upon the consequences of making a Type I error. Historically, having chosen α, environmental researchers have ignored β and the associated power of the test (1-β).

16.2.3.3 Fairweather (1991) presents a review of the need for, and the practical implications of, conducting power analyses in environmental monitoring studies. This review also includes a comprehensive bibliography of recent publications on the need for, and use of, power analyses in environmental study design and data analysis. The consequences of a Type II statistical error in environmental studies should never be ignored and may, in fact, be one of the most important criteria to consider in experimental designs and data analyses that include statistical hypothesis testing. To paraphrase Fairweather (1991), "The commitment of time, energy and people to a false positive (a Type I error) will only continue until the mistake is discovered. In contrast, the cost of a false negative (a Type II error) will have both short- and long-term costs (e.g., ensuing environmental degradation and the eventual cost of its rectification)."

16.2.3.4 The critical components of the experimental design associated with the testing of hypotheses outlined above are (1) the required MDD between the treatment and control or reference responses, (2) the variance among treatment and control replicate experimental units, (3) the number of replicate units for the treatment and control samples, (4) the number of animals exposed within a replicate exposure chamber, and (5) the selected probabilities of Type I (α) and Type II (β) errors.

16.2.3.5 Sample size or number of replicates may be fixed due to cost or space considerations or may be varied to achieve *a priori* probabilities of α and β. The MDD should be established ahead of time based upon biological and program considerations. The investigator has little control of the variance among replicate exposure chambers. However, this variance component can be minimized by selecting test organisms that are as biologically similar as possible and maintaining test conditions within prescribed quality control (QC) limits.

16.2.3.6 The MDD is expressed as a percentage change from the mean control response. To test the equality of the control and treatment responses, a two-sample *t* test with its associated assumptions is the appropriate parametric analysis. If the desired MDD, the number of replicates per treatment, the number of organisms per replicate and an estimate of typical among replicate variability, such as the coefficient of variation (CV) from a control sample, are available, it is possible to use a graphical approach as in Figure 16.2 to determine how likely it is that a 20% reduction will be detected in the treatment response relative to the control response. The CV is defined as 100% x (standard deviation divided by the mean). In a test design with 8 replicates per treatment and with an α level of 0.05, high power (i.e., >0.8) to detect a 20% reduction from the control mean occurs only if the CV is 15% or less (Figure 16.2). The choice of these variables also affects the power of the test. If 5 replicates are used per treatment (Figure 16.3), the CV needs to be 10% or lower to detect a 20% reduction in response relative to the control mean with a power of 90%.

16.2.3.7 Relaxing the α level of a statistical test increases the power of the test. Figure 16.4 duplicates Figure 16.2 except that α is 0.10 instead of 0.05. Selection of the appropriate α level of a test is a function of the costs associated with making Type I and II statistical errors. Evaluation of Figure 16.2 illustrates that with a CV of 15% and an α level of 0.05, there is an 80% probability (power) of detecting a 20% reduction in the mean treatment response relative to the control mean. However, if α is set at 0.10 (Figure 16.4) and the CV remains at 15%, then there is a 90% probability (power) of detecting a 20% reduction relative to the control mean. The latter example would be preferable if an environmentally conservative analysis and interpretation of the data is desirable.

16.2.3.8 Increasing the number of replicates per treatment will increase the power to detect a 20% reduction in treatment response relative to the control mean (Figure 16.5). Note, however, that for less than 8 replicates per treatment it is difficult to have high power (i.e., >0.80) unless the CV is less than 15%. If space or cost limit the number of replicates to fewer than 8 per treatment, then it may be necessary to find ways to reduce the among replicate variability and consequently the CV. Options that are available to increase the power of the test include selecting more uniform organisms to reduce biological variability or increasing the α level of the test. For CVs in the range of 30% to 40%, even 8 replicates per treatment is inadequate to detect small reductions (<20%) in response relative to the control mean.

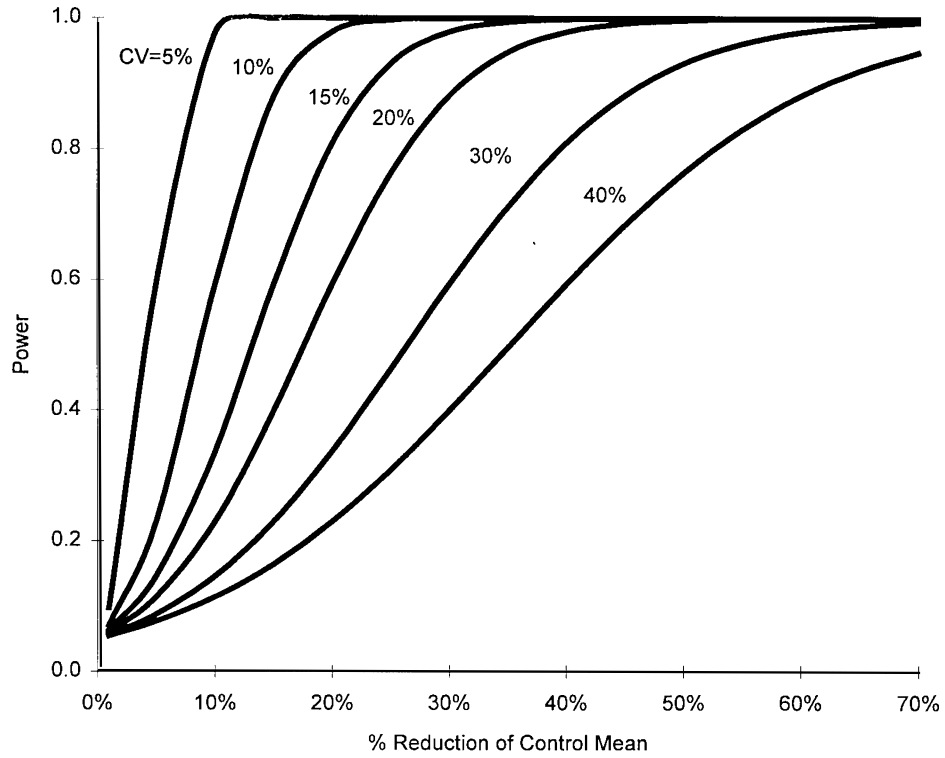


Figure 16.2 **Power of the test vs. percent reduction in treatment response relative to the control mean at various CVs (8 replicates, alpha = 0.05 [one-tailed]).**

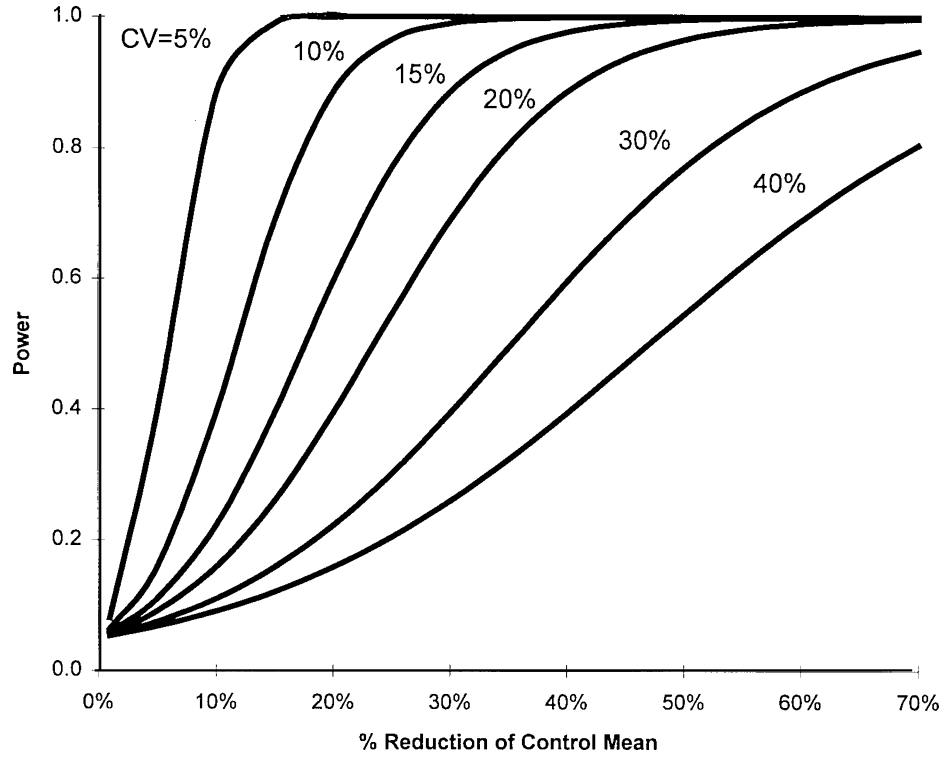


Figure 16.3 **Power of the test vs. percent reduction in treatment response relative to the control mean at various CVs (5 replicates, alpha = 0.05 [one-tailed]).**

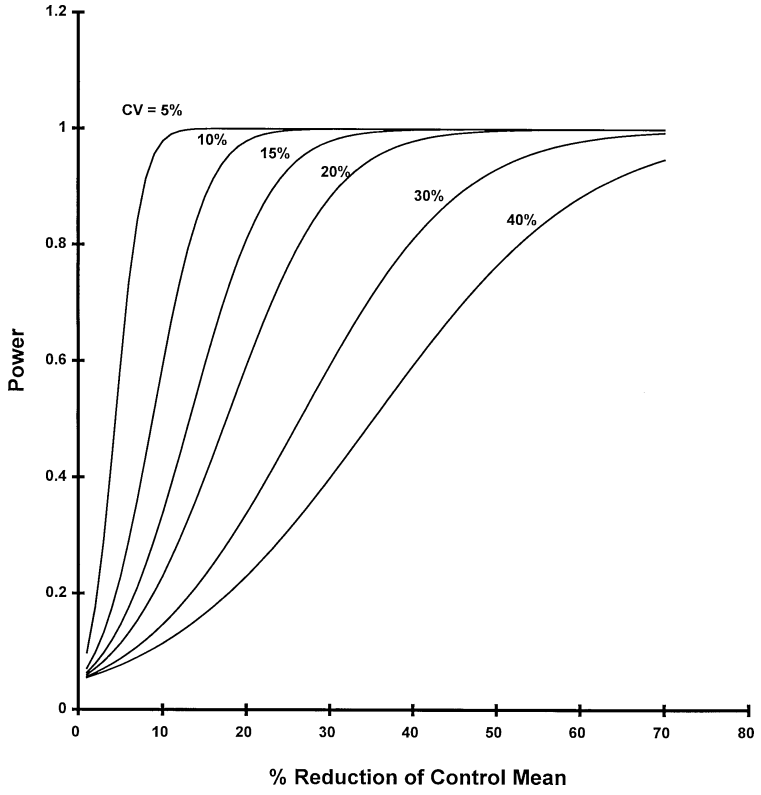


Figure 16.4 Power of the test vs. percent reduction in treatment response relative to the control mean at various CVs (8 replicates, alpha = 0.10 [one-tailed]).

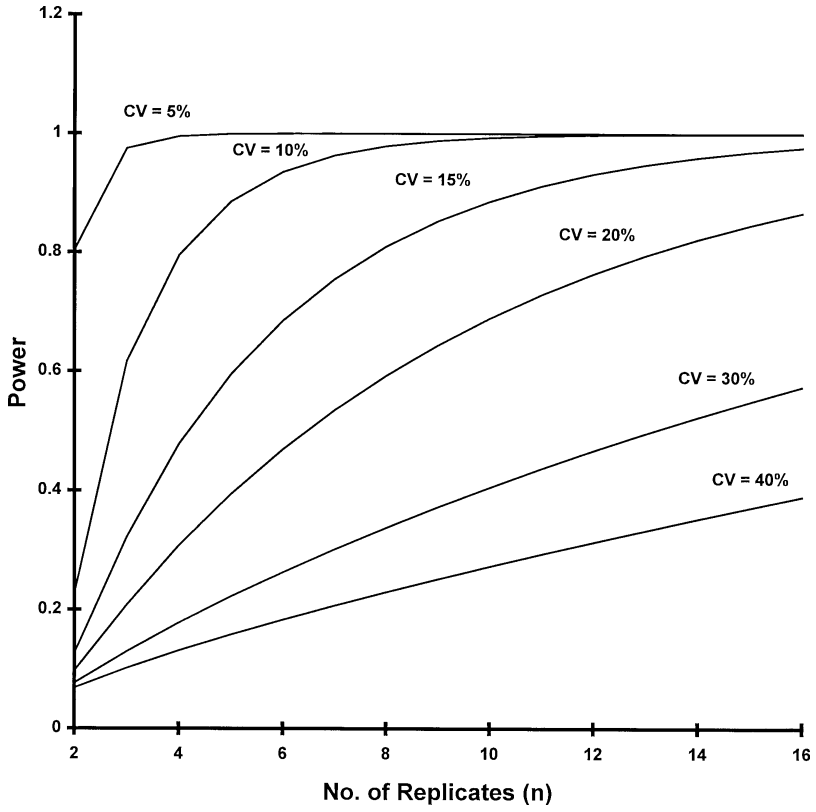


Figure 16.5 Effect of CV and number of replicates on the power to detect a 20% decrease in treatment response relative to the control mean (alpha = 0.05 [one-tailed]).

16.2.3.9 The effect of the choice of α and β on number of replicates for various CVs, assuming the combined total probability of Type I and Type II statistical errors is fixed at 0.25, is illustrated in Figure 16.6. An α of 0.10 therefore establishes a β of 0.15. In Figure 16.6, if $\alpha = \beta = 0.125$, the number of replicates required to detect a difference of 20% relative to the control is at a minimum. As α or β decrease, the number of replicates required to detect the same 20% difference relative to the control increases. However, the curves are relatively flat over the range of 0.05 to 0.20, and their shape will change dramatically if the combined total $\alpha + \beta$ is changed. Limiting the total of $\alpha + \beta$ to 0.10 greatly increases the number of replicates necessary to detect a preselected percentage reduction in mean treatment response relative to the control mean.

16.2.4 Comparing Means

16.2.4.1 Figure 16.7 outlines a decision tree for analysis of survival, growth, or reproduction data subjected to hypothesis testing. In the tests described herein, samples or observations refer to replicates of treatments. Sample size n is the number of replicates (i.e., exposure chambers) in an individual treatment, not the number of organisms in an exposure chamber. Overall sample size N is the combined total number of replicates in all treatments. The statistical methods discussed in this section are described in general statistics texts such as Steel and Torrie (1980), Sokal and Rohlf (1981), Dixon and Massey (1983), Zar (1984), and Snedecor and Cochran (1989). It is recommended that users of this manual have at least one of these texts and associated statistical tables on hand. A nonparametric statistics text such as Conover (1980) might also be helpful.

16.2.4.2 Mean

16.2.4.2.1 The sample mean ($\bar{x}$) is the average value, or $\Sigma x_i/n$ where

n = number of observations (replicates)

x_i = ith observation

Σx_i = every x summed = $x_1 + x_2 + x_3 + \ldots + x_n$

16.2.4.3 Standard Deviation

16.2.4.3.1 The sample standard deviation (s) is a measure of the variation of the data around the mean and is equivalent to $\sqrt{s^2}$. The sample variance, s^2, is given by the following "machine" or "calculation" formula:

$$s^2 = \frac{\sum_{i=1}^{n} x_i^2 - \left(\bar{x}\right)^2}{n-1}$$

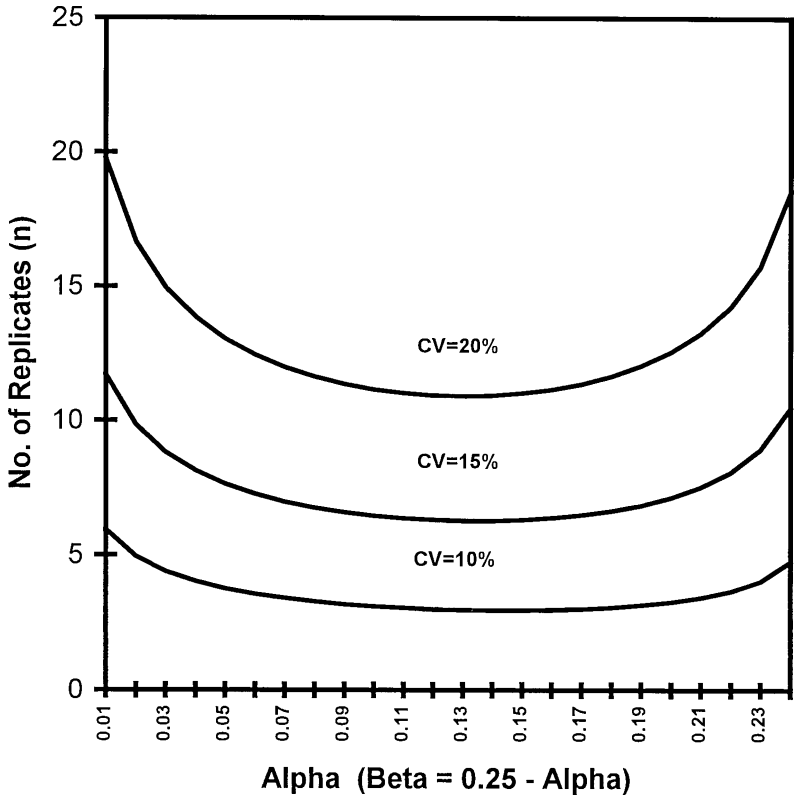


Figure 16.6 Effect of alpha and beta on the number of replicates at various CVs (assuming combined alpha + beta = 0.25).

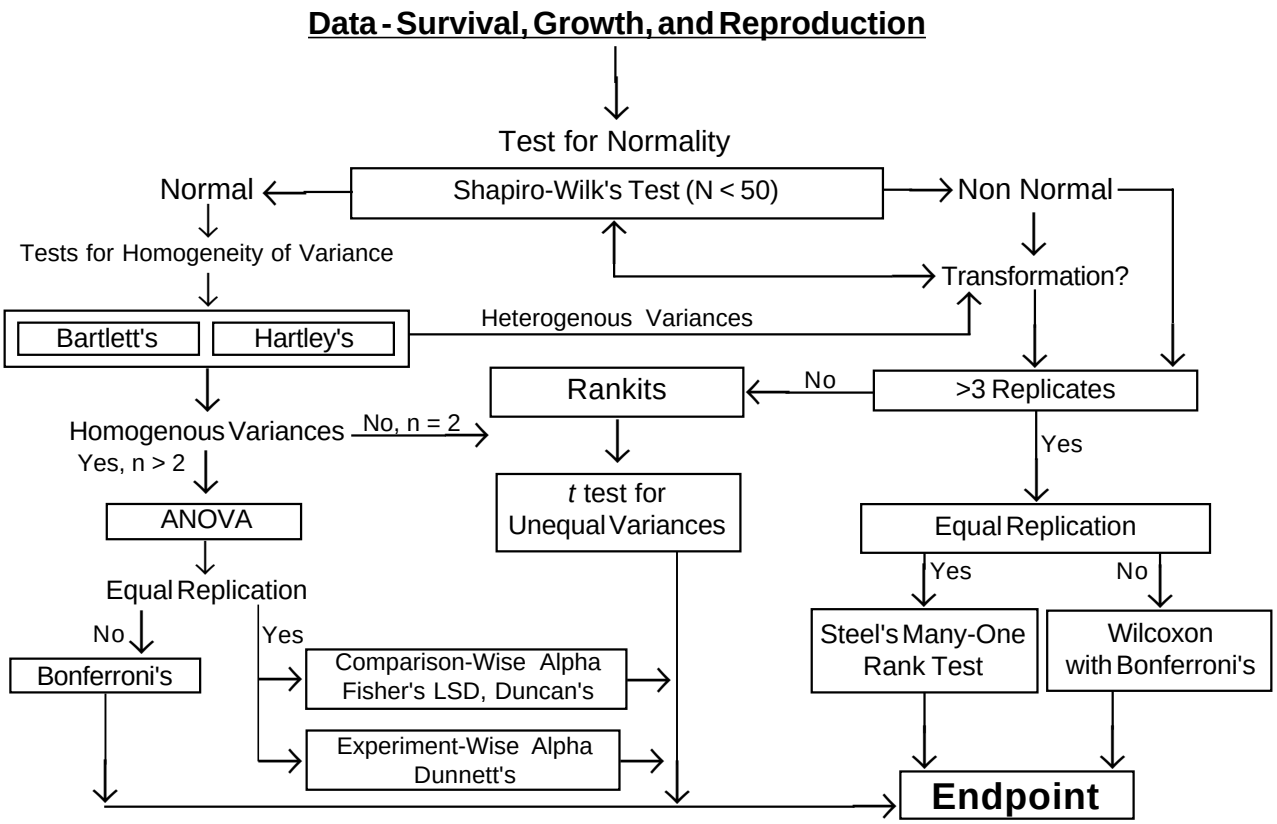


Figure 16.7 Decision tree for analysis of survival, growth, and reproduction data subjected to hypothesis testing.

16.2.4.4 Standard Error of the Mean

16.2.4.4.1 The standard error of the mean (SE, or $s/\sqrt{n}$) estimates variation among sample means rather than among individual values. The SE is an estimate of the standard deviation among means that would be obtained from several samples of n observations each. Most of the statistical tests in this manual compare means with other means (e.g., dredged sediment mean with reference mean) or with a fixed standard (e.g., FDA action level; ASTM, 1999c). Therefore, the "natural" or "random" variation of sample means (estimated by SE), rather than the variation among individual observations (estimated by s), is required for the tests.

16.2.4.5 Tests of Assumptions

16.2.4.5.1 In general, parametric statistical analyses such as *t* tests and analysis of variance are appropriate only if (1) there are independent, replicate experimental units for each treatment, (2) the observations within each treatment follow a normal distribution, and (3) variances for both treatments are equal or similar. The first assumption is an essential component of experimental design. The second and third assumptions can be tested using the data obtained from the experiment. Therefore, before conducting statistical analyses, tests for normality and equality of variances should be performed.

16.2.4.5.2 Outliers. Extreme values and systematic departures from a normal distribution (e.g., a log-normal distribution) are the most common causes of departures from normality or equality of variances. An outlier is an inconsistent or questionable data point that appears unrepresentative of the general trend exhibited by the majority of the data. Outliers may be detected by tabulation of the data, by plotting, or by analysis of residuals. An explanation should be sought for any questionable data points. Without an explanation, data points should only be discarded with extreme caution. If there is no explanation, the analysis should be performed both with and without the outlier, and the results of both analyses should be reported. An appropriate transformation, such as the arc sine-square root transformation, will normalize many distributions (USEPA, 1985). Problems with outliers can usually be solved only by using nonparametric tests, but careful laboratory practices can reduce the frequency of outliers.

16.2.4.5.3 Tests for Normality. The most commonly used test for normality for small sample sizes (N<50) is the Shapiro-Wilk's test. This test determines if residuals are normally distributed. Residuals are the differences between individual observations and the treatment mean. Residuals, rather than raw observations, are tested because subtracting the treatment mean removes any differences among treatments. This scales the observations so that the mean of residuals for each treatment and over

all treatments is zero. The Shapiro-Wilk's test provides a test statistic W, which is compared to values of W expected from a normal distribution. W will generally vary between 0.3 and 1.0, with lower values indicating greater departure from normality. Because normality is desired, one looks for a high value of W with an associated probability greater than the pre-specified α level.

16.2.4.5.3.1 Table 16.1 provides α levels to determine whether departures from normality are significant. Normality should be rejected when the probability associated with W (or other normality test statistic) is less than α for the appropriate total number of replicates (N) and design. A balanced design means that all treatments have an equal number (n) of replicate exposure chambers. A design is considered unbalanced when the treatment with the largest number of replicates (n_{max}) has at least twice as many replicates as the treatment with the fewest replicates (n_{min}). Note that higher α levels are used when the number of replicates is small, or when the design is unbalanced, because these are the cases in which departures from normality have the greatest effects on *t* tests and other parametric comparisons. If data fail the test for normality, even after transformation, nonparametric tests should be used for additional analyses (See Section 16.2.4.8 and Figure 16.7).

16.2.4.5.3.2 Tables of quantiles of W can be found in Shapiro and Wilk (1965), Gill (1978), Conover (1980), USEPA (1989c) and other statistical texts. These references also provide methods of calculating W, although the calculations can be tedious. For that reason, commonly available computer programs or statistical packages are preferred for the calculation of W.

16.2.4.5.4 Tests for Homogeneity of Variances. There are a number of tests for equality of variances. Some of these tests are sensitive to departures from normality, which is why a test for normality should be performed first. Bartlett's test or other tests such as Levene's test or Cochran's test (Winer, 1971; Snedecor and Cochran, 1989) all have similar power for small, equal sample sizes (n=5) (Conover et al., 1981). The data must be normally distributed for Bartlett's test. Many software packages for *t* tests and analysis of variance (ANOVA) provide at least one of the tests.

Table 16.1 Suggested α Levels to Use for Tests of Assumptions

Test	Number of Observations[1]	α When Design Is Balanced	α When Design Is Unbalanced[2]
Normality	N = 2 to 9	0.10	0.25
	N = 10 to 19	0.05	0.10
	N = 20 or more	0.01	0.05
Equality of variances	n = 2 to 9	0.10	0.25
	n = 10 or more	0.05	0.10

[1] N = total number of observations (replicates) in all treatments combined; n = number of observations (replicates) in an individual treatment

[2] $n_{max} \bullet 2\, n_{min}$

16.2.4.5.4.1 If no tests for equality of variances are included in the available statistical software, Hartley's F_{max} can easily be calculated:

$$F_{max} = (\text{larger of } s_1^2, s_2^2) / (\text{smaller of } s_1^2, s_2^2)$$

When F_{max} is large, the hypothesis of equal variances is more likely to be rejected. F_{max} is a two-tailed test because it does not matter which variance is expected to be larger. Some statistical texts provide critical values of F_{max} (Winer, 1971; Gill, 1978; Rohlf and Sokal, 1981).

16.2.4.5.4.2 Levels of α for tests of equality of variances are provided in Table 16.1. These levels depend upon number of replicates in a treatment (n) and allotment of replicates among treatments. Relatively high α's (i.e., $\geq$0.10) are recommended because the power of the above tests for equality of variances is rather low (about 0.3) when n is small. Equality of variances is rejected if the probability associated with the test statistic is less than the appropriate α.

16.2.4.6 Transformations of the Data

16.2.4.6.1 When the assumptions of normality or homogeneity of variance are not met, transformations of the data may remedy the problem, so that the data can be analyzed by parametric procedures, rather than by a nonparametric technique. The first step in these analyses is to transform the responses, expressed as the proportion surviving, by the arc sine-square root transformation. The arc sine-square root transformation is commonly used on proportionality data to stabilize the variance and satisfy the normality requirement. If the data do not meet the assumption of normality and there are four or more replicates per group, then the nonparametric test, Wilcoxon Rank Sum test, can be used to analyze the data. If the data meet the assumption of normality, Bartlett's test or Hartley's *F* test for equality of variances is used to test the homogeneity of variance assumption. Failure of the homogeneity of variance assumption leads to the use of a modified *t* test, and the degrees of freedom for the test are adjusted.

16.2.4.6.2 The arc sine-square root transformation consists of determining the angle (in radians) represented by a sine value. In this transformation, the proportion surviving is taken as the sine value, the square root of the sine value is calculated, and the angle (in radians) for the square root of the sine value is determined. When the proportion surviving is 0 or 1, a special modification of the transformation should be used (Bartlett, 1937). An example of the arc sine-square root transformation and modification are provided below.

1. Calculate the response proportion (RP) for each replicate within a group, where

   RP = (number of surviving organisms)/(number exposed)

2. Transform each RP to arc sine, as follows:

   a. For RPs greater than zero or less than one:

      Angle (in radians) = *arc sine* $\sqrt{(RP)}$

   b. Modification of the arc sine when RP = 0.

      Angle (in radians) = *arc sine* $\sqrt{\frac{1}{4n}}$

      where n = number of animals/treatment rep.

   c. Modification of the arc sine when RP = 1.0.

      Angle = 1.5708 radians-(radians for RP = 0)

16.2.4.7 Two Sample Comparisons (N=2)

16.2.4.7.1 The true population mean (μ) and standard deviation (σ) are known only after sampling the entire population. In most cases, samples are taken randomly from the population, and the s calculated from those samples is only an estimate of σ. Student's *t*-values account for this uncertainty. The degrees of freedom for the test, which are defined as the sample size minus one (n-1), should be used to obtain the correct *t*-value. Student's *t*-values decrease with increasing sample size because larger samples provide a more precise estimate of μ and σ.

16.2.4.7.2 When using a *t* table, it is crucial to determine whether the table is based on one-tailed probabilities or two-tailed probabilities. In formulating a statistical hypothesis, the alternative hypothesis can be one-sided (one-tailed test) or two-sided (two-tailed test). The null hypothesis (H_0) is always that the two values being analyzed are equal. A one-sided alternative hypothesis (H_a) is that there is a specified relationship between the two values (e.g., one value is greater than the other) versus a two-sided alternative hypothesis (H_a) which is that the two values are simply different (i.e., either larger or smaller). A one-tailed test is used when there is an *a priori* reason to test for a specific relationship between two means, such as the alternative hypothesis that the treatment mortality or tissue residue is greater than the control mortality or tissue residue. In contrast, the two-tailed test is used when the direction of the difference is not important or cannot be assumed before testing.

16.2.4.7.3 Since control organism mortality or tissue residues and sediment chemical concentrations are presumed lower than reference or treatment sediment values, conducting one-tailed tests is recommended in most cases. For the same number of replicates, one-tailed tests are more likely to detect statistically significant differences between treatments (e.g., have a greater power) than are two-tailed tests. This is a critical consideration when dealing with a small number of replicates (such as 8/treatment). The other alternative for increasing statistical power is to increase the number of replicates, which increases the cost of the test.

16.2.4.7.4 There are cases when a one-tailed test is inappropriate. When no *a priori* assumption can be made as to how the values vary in relationship to one another, a two-tailed test should be used. An example of an alternative two-sided hypothesis is that the reference sediment total organic carbon (TOC) content is different (greater or lesser) from the control sediment TOC. A two-tailed test should also be used when comparing tissue residues among different species exposed to the same sediment and when comparing bioaccumulation factors (BAFs) or biota-sediment accumulation factors (BSAFs).

16.2.4.7.5 The *t*-value for a one-tailed probability can be found in a two-tailed table by looking up *t* under the column for twice the desired one-tailed probability. For example, the one-tailed *t*-value for $\alpha = 0.05$ and df = 20 is 1.725, and is found in a two-tailed table using the column for $\alpha = 0.10$.

16.2.4.7.6 The usual statistical test for comparing two independent samples is the two-sample *t* test (Snedecor and Cochran, 1989). The *t*-statistic for testing the equality of means $\overline{x}_1$ and $\overline{x}_2$ from two independent samples with n_1 and n_2 replicates and unequal variances is

$$t = (\bar{x}_1 - \bar{x}_2) / \sqrt{s_1^2 / n_1 + s_2^2 / n_2}$$

where s_1^2 and s_2^2 are the sample variances of the two groups. Although the equation assumes that the variances of the two groups are unequal, it is equally useful for situations in which the variances of the two groups are equal. This statistic is compared with the Student's *t* distribution with degrees of freedom (df) given by Satterthwaite's (1946) approximation:

$$df = \frac{(s_1^2 / n_1 + s_2^2 / n_2)^2}{(s_1^2 / n_1)^2 / (n_1 - 1) + (s_2^2 / n_2)^2 / (n_2 - 1)}$$

This formula can result in fractional degrees of freedom, in which case one should round the degree of freedom down to the nearest integer in order to use a *t* table. Using this approach, the degrees of freedom for this test will be less than the degrees of freedom for a *t* test assuming equal variances. If there are unequal numbers of replicates in the treatments, the *t* test with Bonferroni's adjustment can be used for data analysis (USEPA, 1994c; USEPA, 1994d). When variances are equal, an *F* test for equality is unnecessary.

16.2.4.8 Nonparametric Tests

16.2.4.8.1 Tests such as the *t* test, which analyze the original or transformed data and which rely on the properties of the normal distribution, are referred to as parametric tests. Nonparametric tests, which do not require normally distributed data, analyze the ranks of data and generally compare medians rather than means. The me-

dian of a sample is the middle or 50th percentile observation when the data are ranked from smallest to largest. In many cases, nonparametric tests can be performed simply by converting the data to ranks or normalized ranks (rankits) and conducting the usual parametric test procedures on the ranks or rankits.

16.2.4.8.2 Nonparametric tests are useful because of their generality, but have less statistical power than corresponding parametric tests when the parametric test assumptions are met. If parametric tests are not appropriate for comparisons because the normality assumption is not met, data should be converted to normalized ranks (rankits). Rankits are simply the z-scores expected for the rank in a normal distribution. Thus, using rankits imposes a normal distribution over all the data, although not necessarily within each treatment. Rankits can be obtained by ranking the data, then converting the ranks to rankits using the following formula:

$$rankit = z_{[(rank - 0.375) / (N + 0.25)]}$$

where z is the normal deviate and N is the total number of observations. Alternatively, rankits may be obtained from standard statistical tables such as Rohlf and Sokal (1981).

16.2.4.8.3 If normalized ranks are calculated, the ranks should be converted to rankits using the formula above. In comparisons involving only two treatments (N=2), there is no need to test assumptions on the rankits or ranks; simply proceed with a one-tailed *t* test for unequal variances using the rankits or ranks.

16.2.4.9 Analysis of Variance (N>2)

16.2.4.9.1 Some experiments are set up to compare more than one treatment with a control, whereas others may also be interested in comparing the treatments with one another. The basic design of these experiments is the same as for experiments evaluating pairwise comparisons. After the applicable comparisons are determined, the data must be tested for normality to determine whether parametric statistics are appropriate and whether the variances of the treatments are equal. If normality of the data and equal variances are established, then an analysis of variance (ANOVA) may be performed to address the hypothesis that all the treatments, including the control, are equal. If normality or equality of variance are not established, then transformations of the data might be appropriate, or nonparametric statistics can be used to test for equal means. Tests for normality of the data should be performed on the treatment residuals. A residual is defined as the observed value minus the treatment mean, that is, $r_{ik} = o_{ik}$ - (k^{th} treatment mean). Pooling residuals provides an adequate sample size to test the data for normality.

16.2.4.9.2 The variances of the treatments should also be tested for equality. Currently there is no easy way to test for equality of the treatment means using analysis of variance if the variances are not equal. In a toxicity test with several treatments, one treatment may have 100% mortality in all of its replicates, or the control treatment may have 100% survival in all of its replicates. These responses result in 0 variance for a treatment that results in a rejection of equality of variance in these cases. No transformation will change this outcome. In this case, the replicate responses for the treatment with 0 variance should be removed before testing for equality of variances. Only those treatments that do not have 0 replicate variance should be used in the ANOVA to get an estimate of the within treatment variance. After a variance estimate is obtained, the means of the treatments with 0 variance can be tested against the other treatment means using the appropriate mean comparison. Equality of variances among the treatments can be evaluated with the Hartley F_{max} test or Bartlett's test. The option of using nonparametric statistics on the entire set of data is also an alternative.

16.2.4.9.3 If the data are not normally distributed or the variances among treatments are not homogeneous, even after data transformation, nonparametric analyses are appropriate. If there are four or more replicates per treatment and the number of replicates per treatment is equal, the data can be analyzed with Steel's Many-One Rank test. Unequal replication among treatments requires data analysis with the Wilcoxon Rank Sum test with Bonferroni's adjustment. Steel's Many-One Rank test is a nonparametric test for comparing treatments with a control. This test is an alternative to the Dunnett's test, and may be applied to data when the normality assumption has not been met. Steel's test requires equal variances across treatments and the control, but is thought to be fairly insensitive to deviations from this condition (USEPA, 1991a). Wilcoxon's Rank Sum test is a nonparametric test to be used as an alternative to the Steel's test when the number of replicates are not the same within each treatment. A Bonferroni's adjustment of the pairwise error rate for comparison of each treatment versus the control is used to set an upper bound of alpha on the overall error rate. This is in contrast to the Steel's test with a fixed overall error rate for alpha. Thus, Steel's test is a more powerful test (USEPA, 1991a).

16.2.4.9.4 Different mean comparison tests are used depending on whether an α percent comparison-wise error rate or an α percent experiment-wise error rate is desired. The choice of a comparison-wise or experiment-wise error rate depends on whether a decision is based on a pairwise comparison (comparison-wise) or from a set of comparisons (experiment-wise). For example, a comparison-wise error rate would be used for deciding which stations along a gradient were acceptable or not acceptable relative to a control or reference sediment. Each individual comparison is performed independently at a smaller α (than that used in an experiment-wise comparison), such that the probability of making a Type I error in the entire series of comparisons is not greater than the chosen experiment-wise α level of the test. This results in a more conservative test when comparing any particular sample to the control or reference. However, if several samples were taken from the same area and the decision to accept or reject the area was based upon all comparisons

with a reference, then an experiment-wise error rate should be used. When an experiment-wise error rate is used, the power to detect real differences between any two means decreases as a function of the number of treatment means being compared to the control treatment.

16.2.4.9.5 The recommended procedure for pairwise comparisons that have a comparison-wise α error rate and equal replication is to do an ANOVA followed by a one-sided Fisher's Least Significant Difference (LSD) test (Steel and Torrie, 1980). A Duncan's mean comparison test should give results similar to the LSD. If the treatments do not contain equal numbers of replicates, the appropriate analysis is the *t* test with Bonferroni's adjustment. For comparisons that maintain an experiment-wise α error rate, Dunnett's test is recommended for comparisons with the control.

16.2.4.9.6 Dunnett's test has an overall error rate of α, which accounts for the multiple comparisons with the control. Dunnett's procedure uses a pooled estimate of the variance, which is equal to the error value calculated in an ANOVA.

16.2.4.9.7 To perform the individual comparisons, calculate the *t* statistic for each treatment and control combination, as follows:

$$t_i = \frac{(\overline{Y_1} - \overline{Y_i})}{S_w \sqrt{(1/n_1) + (1/n_i)}}$$

where $\overline{Y_i}$ = mean for each treatment

$\overline{Y_1}$ = mean for the control

S_w = square root of the within mean square

n_1 = number of replicates in the control

n_i = number of replicates for treatment "i"

To quantify the sensitivity of the Dunnett's test, the minimum significant difference (MSD=MDD) may be calculated with the following formula:

$$MSD = d\ S_w \sqrt{(1/n_1) + (1/n)}$$

where d = Critical value for the Dunnett's Procedure

S_w = The square root of the within mean square

n = The number of replicates per treatment, assuming an equal number of replicates at all treatment concentrations

n_1 = Number of replicates in the control

16.2.5 Methods for Calculating LC50s, EC50s, and ICps

16.2.5.1 Figure 16.8 outlines a decision tree for analysis of point estimate data. USEPA manuals (USEPA, 1991a; USEPA, 1994c; USEPA, 1994d) discuss in detail the mechanics of calculating LC50 (or EC50) or ICp values using the most current methods. The most commonly used methods are the Graphical, Probit, trimmed Spearman-Karber and the Linear Interpolation Methods. Methods for evaluating point estimate data using logistic regression are outlined in Snedecor and Cochran (1989). In general, results from these methods should yield similar estimates. Each method is outlined below, and recommendations are presented for the use of each method.

16.2.5.2 Data for at least five test concentrations and the control should be available to calculate an LC50, although each method can be used with fewer concentrations. Survival in the lowest concentration must be at least 50%, and an LC50 should not be calculated unless at least 50% of the organisms die in at least one of the serial dilutions. When less than 50% mortality occurs in the highest test concentration, the LC50 is expressed as greater than the highest test concentration.

16.2.5.3 Due to the intensive nature of the calculations for the estimated LC50 and associated 95% confidence interval using most of the following methods, it is recommended that the data be analyzed with the aid of computer software. Computer programs to estimate the LC50 or ICp values and associated 95% confidence intervals using the methods discussed below (except for the Graphical Method) were developed by USEPA and can be obtained by sending a diskette with a written request to USEPA, National Exposure Research Laboratory, 26 W.

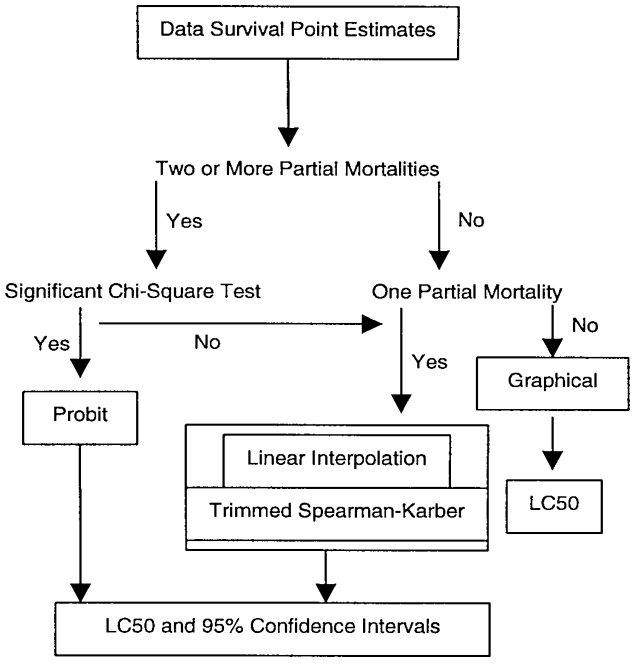


Figure 16.8 Decision tree for analysis of point estimate data.

Martin Luther King Drive, Cincinnati, OH 45268 or call 513/569-7076.

16.2.5.4 Graphical Method

16.2.5.4.1 This procedure estimates an LC50 (or EC50) by linearly interpolating between points of a plot of observed percentage mortality versus the base 10 logarithm ($\log_{10}$) of treatment concentration. The only requirement for its use is that treatment mortalities bracket 50%.

16.2.5.4.2 For an analysis using the Graphical Method, the data should first be smoothed and adjusted for mortality in the control replicates. The procedure for smoothing and adjusting the data is detailed in the following steps: Let p_0, p_1, ..., p_k denote the observed proportion mortalities for the control and the k treatments. The first step is to smooth the p_i if they do not satisfy $p_0 - p_i - ... - p_k$. The smoothing process replaces any adjacent p_i's that do not conform to $p_0 - p_1 - ... - p_k$ with their average. For example, if p_i is less than p_{i-1}, then

$$p_{i-1}^s = p_i^s = (p_i + p_{i-1})/2$$

where p_i^s = the smoothed observed proportion mortality for concentration *i.*

Adjust the smoothed observed proportion mortality in each treatment for mortality in the control group using Abbott's formula (Finney, 1971). The adjustment takes the form:

$$p_i^a = (p_i^s - p_0^s)/(1 - p_0^s)$$

where p_0^s = the smoothed observed proportion mortality for the control

p_i^s = the smoothed observed proportion mortality for concentration *i.*

16.2.5.5 The Probit Method

16.2.5.5.1 This method is a parametric statistical procedure for estimating the LC50 (or EC50) and the associated 95% confidence interval (Finney, 1971). The analysis consists of transforming the observed proportion mortalities with a Probit transformation, and transforming the treatment concentrations to $\log_{10}$. Given the assumption of normality for the $\log_{10}$ of the tolerances, the relationship between the transformed variables mentioned above is about linear. This relationship allows estimation of linear regression parameters, using an iterative approach. A Probit is the same as a z-score: for example, the Probit corresponding to 70% mortality is $z_{.70}$ or = 0.52. The LC50 is calculated from the regression and is the concentration associated with 50% mortality or z = 0. To obtain a reasonably precise estimate of the LC50 with the Probit Method, the observed proportion mortalities must bracket 0.5 and the $\log_{10}$ of the tolerance should be normally distributed. To calculate the LC50 estimate and associated 95% confidence interval, two or more of the observed proportion mortalities must be between zero and one. The original percentage of mortalities should be corrected for control mortality using Abbott's formula (Section 16.2.5.4.1; Finney, 1971) before the Probit transformation is applied to the data.

16.2.5.5.2 A goodness-of-fit procedure with the Chi-square statistic is used to determine whether the data fit the Probit model. If many data sets are to be compared to one another, the Probit Method is not recommended, because it may not be appropriate for many of the data sets. This method also is only appropriate for percent mortality data sets and should not be used for estimating endpoints that are a function of the control response, such as inhibition of growth or reproduction. Most computer programs that generate Probit estimates also generate confidence interval estimates for the LC50. These confidence interval estimates on the LC50 might not be correct if replicate mortalities are pooled to obtain a mean treatment response (USEPA-USACE, 1998a). This can be avoided by entering the Probit-transformed replicate responses and doing a least-squares regression on the transformed data.

16.2.5.6 The Trimmed Spearman-Karber Method

16.2.5.6.1 The trimmed Spearman-Karber Method is a modification of the Spearman-Karber, nonparametric statistical procedure for estimating the LC50 and the associated 95% confidence interval (Hamilton et al., 1977). This procedure estimates the trimmed mean of the distribution of the $\log_{10}$ of the tolerance. If the log tolerance distribution is symmetric, this estimate of the trimmed mean is equivalent to an estimate of the median of the log tolerance distribution. Use of the trimmed Spearman-Karber Method is only appropriate for lethality data sets when the requirements for the Probit Method are not met (USEPA, 1994c; USEPA, 1994d).

16.2.5.6.2 To calculate the LC50 estimate with the trimmed Spearman-Karber Method, the smoothed, adjusted, observed proportion mortalities must bracket 0.5. To calculate a confidence interval for the LC50 estimate, one or more of the smoothed, adjusted, observed proportion mortalities must be between zero and one.

16.2.5.6.3 Smooth the observed proportion mortalities as described for the Probit Method. Adjust the smoothed observed proportion mortality in each concentration for mortality in the control group using Abbott's formula (see Probit Method, Section 16.2.5.5). Calculate the amount of trim to use in the estimation of the LC50 as follows:

$$\text{Trim} = \max(p_1^a, 1 - p_k^a)$$

where p_1^a = the smoothed, adjusted proportion mortality for the lowest treatment concentration, exclusive of the control.

p_k^a = the smoothed, adjusted proportion mortality for the highest treatment concentration.

k = the number of treatment concentrations, exclusive of the control.

16.2.5.7 Linear Interpolation Method

16.2.5.7.1 The Linear Interpolation Method calculates a toxicant concentration that causes a given percent reduction (e.g., 25%, 50%, etc.) in the endpoint of interest and is reported as an ICp value (IC = Inhibition Concentration; where p = the percent effect). The procedure was designed for general applicability in the analysis of data from chronic toxicity tests and for the generation of an endpoint from a continuous model that allows a traditional quantitative assessment of the precision of the endpoint, such as confidence limits for the endpoint of a single test or a mean and coefficient of variation for the endpoints of multiple tests.

16.2.5.7.2 As described in USEPA (1994c; 1994d), the Linear Interpolation Method of calculating an ICp assumes that the responses (1) are monotonically nonincreasing, where the mean response for each higher concentration is less than or equal to the mean response for the previous concentration, (2) follow a piecewise linear response function, and (3) are from a random, independent, and representative sample of test data. If the data are not monotonically nonincreasing, they are adjusted by smoothing (averaging). In cases where the responses at the low toxicant concentrations are much higher than in the controls, the smoothing process may result in a large upward adjustment in the control mean. In the Linear Interpolation Method, the smoothed response means are used to obtain the ICp estimate reported for the test. No assumption is made about the distribution of the data except that the data within a group being resampled are independent and identically distributed.

16.2.5.7.3 The Linear Interpolation Method assumes a linear response from one concentration to the next. Thus, the IC is estimated by linear interpolation between two concentrations whose responses bracket the response of interest, the (p) percent reduction from the control.

16.2.5.7.4 If the assumption of monotonicity of test results is met, the observed response means ($\overline{Y}_i$) should stay the same or decrease as the toxicant concentration increases. If the means do not decrease monotonically, the responses are “smoothed” by averaging (pooling) adjacent means. Observed means at each concentration are considered in order of increasing concentration, starting with the control mean ($\overline{Y}_1$). If the mean observed response at the lowest toxicant concentration ($\overline{Y}_2$) is equal to or smaller than the control mean ($\overline{Y}_1$), it is used as the response. If it is larger than the control mean, it is averaged with the control, and this average is used for both the control response (M_1) and the lowest toxicant concentration response (M_2). This mean is then compared to the mean observed response for the next higher toxicant concentration ($\overline{Y}_3$). Again, if the mean observed response for the next higher toxicant concentration is smaller than the mean of the control and the lowest toxicant concentration, it is used as the response. If it is higher than the mean of the first two, it is averaged with the mean of the first two, and the resulting mean is used as the response for the control and two lowest concentrations of toxicant. This process is continued for data from the remaining toxicant concentrations. Unusual patterns in the deviations from monotonicity may require an additional step of smoothing. Where $\overline{Y}_i$ decrease monotonically, the $\overline{Y}_i$ become M_i without smoothing.

16.2.5.7.5 To obtain the ICp estimate, determine the concentrations C_J and C_{J+1} that bracket the response M_1 (1 - p/100), where M_1 is the smoothed control mean response and p is the percent reduction in response relative to the control response. These calculations can easily be done by hand or with a computer program as described below. The linear interpolation estimate is calculated as follows:

$$ICp = C_J + [\, M_1(1 - p/100) - M_J \,] \; \frac{(C_{J+1} - C_J)}{(M_{J+1} - M_J)}$$

where C_J = tested concentration whose observed mean response is greater than $M_1(1 - p/100)$.

C_{J+1} = tested concentration whose observed mean response is less than $M_1(1 - p/100)$.

M_1 = smoothed mean response for the control.

M_J = smoothed mean response for concentration J.

M_{J+1} = smoothed mean response for concentration J + 1.

p = percent reduction in response relative to the control response.

IC*p* = estimated concentration at which there is a percent reduction from the smoothed mean control response.

16.2.5.7.6 Standard statistical methods for calculating confidence intervals are not applicable for the ICp. The bootstrap method, as proposed by Efron (1982), is used to obtain the 95% confidence interval for the true mean. In the bootstrap method, the test data Y_{ji} is randomly resampled with replacement to produce a new set of data Y_{ji}* that is statistically equivalent to the original data, but which produces a new and slightly different estimate of the IC*p* (IC*p**). This process is repeated at least 80 times (Marcus and Holtzman, 1988), resulting in multiple “data” sets, each with an associated ICp* estimate. The distribution of the ICp* estimates derived from the sets of resampled data approximates the sampling distribution of the ICp estimate. The standard error of the ICp is estimated by the standard deviation of the individual ICp* estimates. Empirical confidence intervals are derived from the quantiles of the ICp* empirical distribution. For ex-

ample, if the test data are resampled a minimum of 80 times, the empirical 2.5% and the 97.5% confidence limits are about the second smallest and second largest ICp* estimates (Marcus and Holtzman, 1988). The width of the confidence intervals calculated by the bootstrap method is related to the variability of the data. When confidence intervals are wide, the reliability of the IC estimate is in question. However, narrow intervals do not necessarily indicate that the estimate is highly reliable, because of undetected violations of assumptions and the fact that the confidence limits based on the empirical quantiles of a bootstrap distribution of 80 samples may be unstable.

16.2.6 Analysis of Bioaccumulation Data

16.2.6.1 In some cases, body burdens will not approach steady-state body burdens in a 28-d test (ASTM, 1999c). Organic compounds exhibiting these kinetics will probably have a log Kow >5, be metabolically refractory (e.g., highly chlorinated PCBs, dioxins), or have low depuration rates. Additionally, tissue residues of several heavy metals may gradually increase over time so that 28 d is inadequate to approach steady-state. Depending on the goals of the study and the adaptability of the test species to long-term testing, it may be necessary to conduct an exposure longer than 28 d (or a kinetic study) to obtain a sufficiently accurate estimate of steady-state tissue residues of these compounds.

16.2.6.2 Biotic Sampling

16.2.6.2.1 In the long-term studies, the exposure should continue until steady-state body burdens are attained. ASTM (1999c) recommends a minimum of five sampling periods (plus t_0) when conducting water exposures to generate bioconcentration factors (BCFs). Sampling in a geometric progression is also recommended with sampling times reasonably close to S/16, S/8, S/4, S/2, and S, where S is the time to steady state. This sampling design assumes a fairly accurate estimate of time to steady state, which is often not the case with sediment exposures.

16.2.6.2.2 To document steady state from sediment exposures, placing a greater number of samples at and beyond the predicted time to steady state is recommended. With a chemical expected to reach steady state within 28 to 50 d, samples should be taken at Day 0, 7, 14, 21, 28, 42, 56, and 70. If the time to steady state is much greater than 42 d, then additional sampling periods at two-week intervals should be added (e.g., Day 84). Slight deviations from this schedule (e.g., Day 45 versus Day 42) are not critical, though for comparative purposes, samples should be taken at t_{28}. An estimate of time to steady state may be obtained from the literature or estimated from structure-activity relationships, though these values should be considered the minimum times to steady state.

16.2.6.2.3 This schedule increases the likelihood of statistically documenting that steady state has been obtained although it does not document the initial uptake phase as well. If an accurate estimate of the sediment uptake rate coefficient (Ks) is required, additional sampling periods are necessary during the initial uptake phase (e.g., Day 0, 2, 4, 7, 10, 14).

16.2.6.3 Abiotic Samples

16.2.6.3.1 The bioavailable fraction of the contaminants as well as the nutritional quality of the sediment are more prone to depletion in extended tests than during the 28-d exposures. To statistically document whether such depletions have occurred, replicate sediment samples should be collected for physical and chemical analysis from each sediment type at the beginning and the end of the exposure. Archiving sediment samples from every biological sampling period also is recommended.

16.2.6.4 Short-term Uptake Tests

16.2.6.4.1 Compounds may attain steady state in the oligochaete, *Lumbriculus variegatus,* in less than 28 d (Kukkonen and Landrum, 1993). However, before a shorter test is used, it must be ascertained that the analytes of interest do indeed achieve steady state in *L. variegatus* in <28 d. Biotic and abiotic samples should be taken at Day 0 and 10 following the same procedure used for the 28-d tests. If time-series biotic samples are desired, sample on Day 0, 1, 3, 5, 7, and 10.

16.2.6.5 Estimating Steady State

16.2.6.5.1 In tests where steady state cannot be documented, it may be possible to estimate steady-state concentrations. Several methods have been published that can be used to predict steady-state chemical concentrations from uptake and depuration kinetics (Spacie and Hamelink, 1982; Davies and Dobbs, 1984). All of these methods were derived from fish exposures and most use a linear uptake, first-order depuration model that can be modified for uptake of chemicals from sediment. To avoid confusing uptake from water versus sediment, Ks, the sediment uptake rate coefficient, is used instead of K1. The Ks coefficient has also been referred to as the uptake clearance rate (Landrum et al., 1989). Following the recommendation of Stehly et al. (1990), the gram sediment and gram tissue units are retained in the formulation:

$$Ct(t) = Ks \times Cs / K2 \times (1 - e^{-K2 \times t})$$

where
- Ct = chemical concentration in tissue at time t
- Cs = chemical concentration in sediment
- Ks = uptake rate coefficient in tissue (g sed g^{-1} day^{-1})
- K2 = depuration constant (day^{-1})
- t = time (days)

As time approaches infinity, the maximum or equilibrium chemical concentration within the organism (Ct_{max}) becomes

$$Ct_{max} = Cs \times Ks / K2$$

Correspondingly, the bioaccumulation factor (BAF) for a compound may be estimated from

$$BAF = Ks / K2$$

16.2.6.5.2 This model assumes that the sediment concentration and the kinetic coefficients are invariant. Depletion of the sediment concentrations in the vicinity of the organism would invalidate the model. Further, the rate coefficients are conditional on the environment and health of the test organisms. Thus, changes in environmental conditions such as temperature or changes in physiology such as reproduction will also invalidate the model. Despite these potential limitations, the model can provide estimates of steady-state tissue residues.

16.2.6.5.3 The kinetic approach requires an estimate of Ks and K2, which are determined from the changes in tissue residues during the uptake phase and depuration phase, respectively. The uptake experiment should be short enough that an estimate of Ks is made during the linear portion of the uptake phase to avoid an unrealistically low uptake rate due to depuration. The depuration phase should be of sufficient duration to smooth out any loss from a rapidly depurated compartment such as loss from the voiding of feces. Unless there is reason to suspect that the route of exposure will affect the depuration rate, it is acceptable to use a K2 derived from a water exposure. For further discussion of this method for bioconcentration studies in fish, see Davies and Dobbs (1984), Spacie and Hamelink (1982), and ASTM (1999b). For application of this procedure for sediment, see ASTM (1999c). Recent studies of the accumulation of sediment-associated chemicals by benthos suggest that the kinetics for freshly dosed sediments may require a more complex formulation to estimate the uptake clearance constant than that presented above (Landrum, 1989).

16.2.6.5.4 This model predicts that equilibrium would be reached only as time becomes infinite. Therefore, for practical reasons, apparent steady state is defined here as 95% of the equilibrium tissue residue. The time to reach steady state can be estimated by

$$S = \ln[1 / (1.00-0.95)] / K2 = 3.0 / K2$$

where S = time to apparent steady state (days)

Thus, the key information is the depuration rate of the compound of interest in the test species or phylogenetically related species. Unfortunately, little of this data has been generated for benthic invertebrates. When no depuration rates are available, the depuration rate constant for organic compounds can then be estimated from the relationship between Kow and K2 for fish species (Spacie and Hamelink, 1982):

$$K2 = \text{antilog}[1.47-0.414 \times \log(Kow)]$$

The relationship between S and K2 and between K2 and Kow is summarized in Table 16.2. Estimated time (days) to reach 95% of chemical steady-state tissue residue (S) and depuration rate constants (K2) are calculated from octanol-water partition coefficients using a linear uptake, first-order depuration model (Spacie and Hamelink, 1982). The K2 values are the amount depurated (decimal fraction of tissue residue lost per day). Table 16.2 may be used to make a rough estimate of the exposure time to reach steady-state tissue residues if a depuration rate constant for the compound of interest from a phylogenetically similar species is available. If no depuration rate is available, then the table may be used for estimating the S of organic compounds from the Kow value. However, as these data were developed from fish bioconcentration data, its applicability to the kinetics of uptake from sediment-associated chemicals is unknown. The portion of organics readily available for uptake may be small in comparison to the total sediment organic concentration (Landrum, 1989). Therefore S values generated by this model should be considered as minimum time periods.

16.2.6.5.5 Using a linear uptake, first-order depuration model to estimate exposure time to reach steady-state body burden for metals is problematical for a number of reasons. The kinetics of uptake may be dependent upon a small fraction of the total sediment metal load that is bioavailable (Luoma and Bryan, 1982). Depuration rates may be more difficult to determine, as metals bound to proteins may have very low exchange rates (Bryan, 1976). High exposure concentrations of some metals can lead to the induction of metal binding proteins, like metallothionein, which detoxify metals. These metal-protein complexes within the organism have extremely low exchange rates with the environment (Bryan, 1976). Thus, the induction of metal binding proteins may result in decreased depuration rate constants in organisms exposed to the most polluted sediments. Additionally, structure-activity relationships that exist for organic chemicals (e.g., relationship between Kow and BCFs) are not well developed for metals.

Table 16.2 Estimated Time to Obtain 95 Percent of Steady-state Tissue Residue

Log Kow	K2	S (days)
1	0.114	0.2
2	0.44	0.5
3	0.17	1.4
4	0.0065	3.5
5	0.0025	9.2
6	0.00097	24
7	0.00037	61
8	0.00014	160
9	0.00006	410

16.3 Data Interpretation

16.3.1 Sediments spiked with known concentrations of chemicals can be used to establish cause and effect relationships between chemicals and biological responses. Results of toxicity tests with test materials spiked into sediments at different concentrations may be reported in terms of an LC50 (median lethal concentration), an EC50 (median effect concentration), an IC50 (inhibition concentration), or as a NOEC (no observed effect concentration) or LOEC (lowest observed effect concentration; Section 3). Consistent spiking procedures should be followed in order to make interlaboratory comparisons (Section 8.3).

16.3.2 Evaluating effect concentrations for chemicals in sediment requires knowledge of factors controlling the bioavailability. Similar concentrations of a chemical in units of mass of chemical per mass of sediment dry weight often exhibit a range in toxicity in different sediments (Di Toro et al., 1991; USEPA, 1992c). Effect concentrations of chemicals in sediment have been correlated to interstitial water concentrations, and effect concentrations in interstitial water are often similar to effect concentrations in water-only exposures. The bioavailability of nonionic organic compounds are often inversely correlated with the organic carbon concentration of the sediment. Whatever the route of exposure, the correlations of effect concentrations to interstitial water concentrations indicate that predicted or measured concentrations in interstitial water can be useful for quantifying the exposure concentration to an organism. Therefore, information on partitioning of chemicals between solid and liquid phases of sediment can be useful for establishing effect concentrations.

16.3.3 Toxic units can be used to help interpret the response of organisms to multiple chemicals in sediment. A toxic unit is the concentration of a chemical divided by an effect concentration. For example, a toxic unit of exposure can be calculated by dividing the measured concentration of a chemical in pore water by the water-only LC50 for the same chemical (Ankley et al., 1991a). Toxicity expressed as toxic units may be summed and this may provide information on the toxicity of chemical mixtures (Ankley et al., 1991a).

16.3.4 Field surveys can be designed to provide either a qualitative reconnaissance of the distribution of sediment contamination or a quantitative statistical comparison of contamination among sites (Burton and Ingersoll, 1994). Surveys of sediment toxicity are usually part of more comprehensive analyses of biological, chemical, geological, and hydrographic data. Statistical correlation can be improved and costs reduced if subsamples are taken simultaneously for sediment toxicity or bioaccumulation tests, chemical analyses, and benthic community structure.

16.3.5 Descriptive methods, such as toxicity tests with field-collected sediment, should not be used alone to evaluate sediment contamination. An integration of several methods using the weight of evidence is needed to assess the effects of contaminants associated with sediment (Long and Morgan, 1990; Ingersoll et al., 1996, 1997; MacDonald et al., 1996). Hazard evaluations integrating data from laboratory exposures, chemical analyses, and benthic community assessments provide strong complementary evidence of the degree of pollution-induced degradation in aquatic communities (Chapman et al., 1992, 1997; Burton, 1991; Canfield et al., 1994, 1996, 1998).

16.3.6 Toxicity Identification Evaluation (TIE) procedures can be used to help provide insights as to specific contaminants responsible for toxicity in sediment (USEPA, 1991b; Ankley and Thomas, 1992). For example, the toxicity of contaminants such as metals, ammonia, hydrogen sulfide, and nonionic organic compounds can be identified using TIE procedures.

16.3.7 Interpretation of Comparisons of Tissue Residues

16.3.7.1 If the mean control tissue residues at Day 28 are not significantly greater than the Day 0 tissue residues, it can be concluded that there is no significant contamination from the exposure system or from the control sediment. If there is significant uptake, the exposure system or control sediment should be reevaluated as to suitability. Even if there is a significant uptake in the controls, it is still possible to compare the controls and treatments as long as the contaminant concentrations in the test tissue residues are substantially higher. However, if control values are high, the data should be discarded and the experiment conducted again after determining the source of contamination.

16.3.7.2 Comparisons of the 28-d control (or reference) tissue residues and 28-d treatment tissue residues determines whether there was statistically significant bioaccumulation due to exposure to test sediments. Comparisons between control and reference tissue residues at Day 28 determine whether there was a statistically significant bioaccumulation due to exposure to the reference sediment. If no significant difference is detected when treatment tissue residues are compared to a set criterion value (e.g., FDA action level) with a one-tailed test, the residues must be considered equivalent to the value even though numerically the mean treatment tissue residue may be smaller.

16.3.7.3 BAFs and BSAFs

16.3.7.3.1 Statistical comparisons between ratios such as BAFs or BSAFs are difficult due to computation of error terms. Since all variables used to compute BAFs and BSAFs have errors associated with them, it is necessary to estimate the variance as a function of these errors. This can be accomplished using approximation techniques such as the propagation of error (Beers, 1957) or a Taylor series expansion method (Mood et al., 1974). BAFs and BSAFs can then be compared using these estimates of the variance. ASTM (1999c) provides examples of this approach.

16.3.7.4 Comparing Tissue Residues of Different Compounds

16.3.7.4.1 In some cases, it is of interest to compare the tissue residues of different compounds. For example, Rubinstein et al. (1987) compared the uptake of thirteen different PCB congeners to test for differences in bioavailability. Because the values for the different compounds are derived from the same tissue samples, they are not independent and tend to be correlated, so standard *t* tests and ANOVAs are inappropriate. A repeated measures technique (repeated testing of the same experimental unit) should be used where the experimental unit (individual) is considered as a random factor and the different compounds as a second factor. See Rubinstein et al. (1987) and Lake et al. (1990) for an example of the application of repeated measures to bioaccumulation data.

16.4 Reporting

16.4.1 The record of the results of an acceptable sediment test should include the following information either directly or by referencing available documents:

16.4.1.1 Name of test and investigator(s), name and location of laboratory, and dates of start and end of test.

16.4.1.2 Source of control or test sediment, and method for collection, handling, shipping, storage and disposal of sediment.

16.4.1.3 Source of test material, lot number if applicable, composition (identities and concentrations of major ingredients and impurities if known), known chemical and physical properties, and the identity and concentration(s) of any solvent used.

16.4.1.4 Source and characteristics of overlying water, description of any pretreatment, and results of any demonstration of the ability of an organism to survive or grow in the water.

16.4.1.5 Source, history, and age of test organisms; source, history, and age of brood stock, culture procedures; and source and date of collection of the test organisms, scientific name, name of person who identified the organisms and the taxonomic key used, age or life stage, means and ranges of weight or length, observed diseases or unusual appearance, treatments used, and holding procedures.

16.4.1.6 Source and composition of food; concentrations of test material and other contaminants; procedure used to prepare food; and feeding methods, frequency and ration.

16.4.1.7 Description of the experimental design and test chambers, the depth and volume of sediment and overlying water in the chambers, lighting, number of test chambers and number of test organisms/treatment, date and time test starts and ends, temperature measurements, dissolved oxygen concentration (µg/L) and any aeration used before starting a test and during the conduct of a test.

16.4.1.8 Methods used for physical and chemical characterization of sediment.

16.4.1.9 Definition(s) of the effects used to calculate LC50 or EC50s, biological endpoints for tests, and a summary of general observations of other effects.

16.4.1.10 A table of the biological data for each test chamber for each treatment, including the control(s), in sufficient detail to allow independent statistical analysis.

16.4.1.11 Methods used for statistical analyses of data.

16.4.1.12 Summary of general observations on other effects or symptoms.

16.4.1.13 Anything unusual about the test, any deviation from these procedures, and any other relevant information.

16.4.2 Published reports should contain enough information to clearly identify the methodology used and the quality of the results.

Section 17
Precision and Accuracy

17.1 Determining Precision and Accuracy

17.1.1 Precision is a term that describes the degree to which data generated from replicate measurements differ and reflects the closeness of agreement between randomly selected test results. Accuracy is the difference between the value of the measured data and the true value and is the closeness of agreement between an observed value and an accepted reference value. Quantitative determination of precision and accuracy in sediment testing of aquatic organisms is difficult or may be impossible in some cases, as compared to analytical (chemical) determinations. This is due, in part, to the many unknown variables that affect organism response. Determining the accuracy of a sediment test using field samples is not possible since the true values are not known. Since there is no acceptable reference material suitable for determining the accuracy of sediment tests, the accuracy of the test methods has not been determined (Section 17.2).

17.1.2 Sediment tests exhibit variability due to several factors (Section 9). Test variability can be described in terms of two types of precision, either single laboratory (intralaboratory or repeatability; Section 17.5.1) precision or multi-laboratory (interlaboratory or reproducibility; Section 17.5.2, 17.5.3 and 17.6) precision. Intralaboratory precision reflects the ability of trained laboratory personnel to obtain consistent results repeatedly when performing the same test on the same organism using the same toxicant. Interlaboratory precision (also referred to as round-robin or ring tests) is a measure of the reproducibility of a method when tests are conducted by a number of laboratories using that method and the same organism and samples. Generally, intralaboratory results are less variable than interlaboratory results (USEPA, 1991a; USEPA, 1991c; USEPA, 1994b; USEPA, 1994c; Hall et al., 1989; Grothe and Kimerle, 1985).

17.1.3 A measure of precision can be calculated using the mean and relative standard deviation (percent coefficient of variation, or CV% = standard deviation/mean x 100) of the calculated endpoints from the replicated endpoints of a test. However, precision reported as the CV should not be the only approach used for evaluating precision of tests and should not be used for the NOEC levels derived from statistical analyses of hypothesis testing. The CVs can be very high when testing extremely toxic samples. For example, if there are multiple replicates with no survival and one with low survival, the CV might exceed 100%, yet the range of response is actually quite consistent. Therefore, additional estimates of precision should be used, such as range of responses, and minimum detectable differences (MDD) compared to control survival or growth. Several factors can affect the precision of the test, including test organism age, condition and sensitivity; handling and feeding of the test organisms; overlying water quality; and the experience of the investigators in conducting tests. For these reasons, it is recommended that trained laboratory personnel conduct the tests in accordance with the procedures outlined in Section 9. Quality assurance practices should include the following: (1) single laboratory precision determinations that are used to evaluate the ability of the laboratory personnel to obtain precise results using reference toxicants for each of the test organisms and (2) preparation of control charts (Section 17.4) for each reference toxicant and test organism. The single laboratory precision determinations should be made before conducting a sediment test and should be periodically performed as long as whole-sediment tests are being conducted at the laboratory.

17.1.4 Intralaboratory precision data are routinely calculated for test organisms using water-only 96-h exposures to a reference toxicant, such as potassium chloride (KCl). Intralaboratory precision data should be tracked using a control chart. Each laboratory's reference-toxicity data will reflect conditions unique to that facility, including dilution water, culturing, and other variables (Section 9). However, each laboratory's reference-toxicity CVs should reflect good repeatability.

17.1.5 Interlaboratory precision (round-robin) tests have been completed with both *Hyalella azteca* and *Chironomus tentans* using 4-d water-only tests and 10-d whole-sediment tests described in Section 11.2 and 12.2 (Section 17.5). Section 17.6 describes results of round-robin evaluations with long-term sediment toxicity tests described in Sections 14 and 15 for *H. azteca* and *C. tentans.*

17.2 Accuracy

17.2.1 The relative accuracy of toxicity tests cannot be determined since there is no acceptable reference material. The relative accuracy of the reference-toxicity tests can only be evaluated by comparing test responses to control charts.

17.3 Replication and Test Sensitivity

17.3.1 The sensitivity of sediment tests will depend in part on the number of replicates per concentration, the probability levels (alpha and beta) selected, and the type of statistical analysis. For a specific level of variability, the sensitivity of the test will increase as the number of replicates is increased. The minimum recommended number of replicates varies with the objectives of the test and the statistical method used for analysis of the data (Section 16).

17.4 Demonstrating Acceptable Laboratory Performance

17.4.1 Intralaboratory precision, expressed as a coefficient of variation (CV), can be determined by performing five or more tests with different batches of test organisms, using the same reference toxicant, at the same concentrations, with the same test conditions (e.g., the same test duration, type of water, age of test organisms, feeding), and same data analysis methods. A reference-toxicity concentration series (dilution factor of 0.5 or higher) should be selected that will provide partial mortalities at two or more concentrations of the test chemical (Section 9.14, Table 9.1, 9.2). See Section 9.16 for additional detail on reference-toxicity testing.

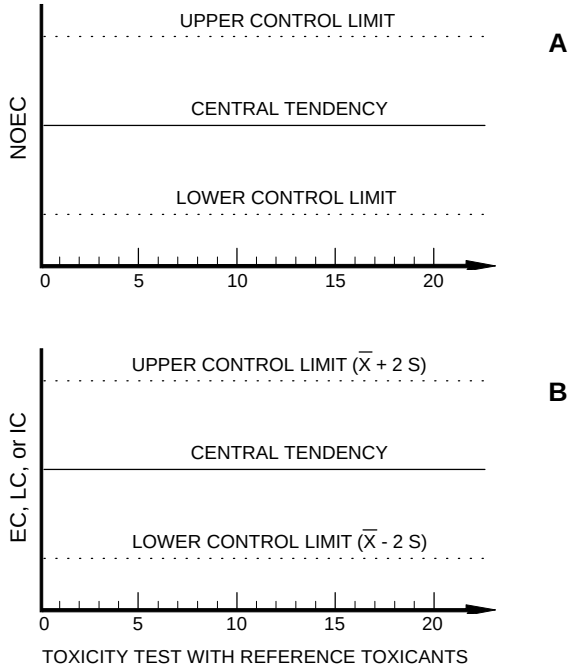
17.4.2 It is desirable to determine the sensitivity of test organisms obtained from an outside source. The supplier should provide data with the shipment describing the history of the sensitivity of organisms from the same source culture.

17.4.3 Before conducting tests with potentially contaminated sediment, it is strongly recommended that the laboratory conduct the tests with control sediment(s) alone. Results of these preliminary studies should be used to determine if use of the control sediment and other test conditions (i.e., water quality) result in acceptable performance in the tests as outlined in Tables 11.1, 12.1, 13.1, 14.1, and 15.1.

17.4.4 A control chart should be prepared for each combination of reference toxicant and test organism. Each control chart should include the most current data. Endpoints from five tests are adequate for establishing the control charts. In this technique, a running plot is maintained for the values (X_i) from successive tests with a given reference toxicant (Figure 17.1), and the endpoints (LC50, NOEC, ICp) are examined to determine if they are within prescribed limits. Control charts as described in USEPA (1991a) and USEPA (1993b) are used to evaluate the cumulative trend of results from a series of samples. The mean and upper and lower control limits (±2 SD) are recalculated with each successive test result. After two years of data collection, or a minimum of 20 data points, the control (cusum) chart should be maintained using only the 20 most recent data points.

17.4.5 The outliers, which are values falling outside the upper and lower control limits, and trends of increasing or decreasing sensitivity, are readily identified using control charts. With an alpha of 0.05, one in 20 tests would be expected to fall outside of the control limits by chance alone. During a 30-d period, if two reference-toxicity tests out of a total of the previous 20 fall outside the control limits, the sediment toxicity tests conducted during the time in which the second reference-toxicity test failed are suspect and should be considered as provisional and subject to careful review.

17.4.5.1 A sediment test may be acceptable if specified conditions of a reference-toxicity test fall outside the expected ranges (Section 9). Specifically, a sediment test should not necessarily be judged unacceptable if the LC50 for a given reference-toxicity test falls outside the expected range or if mortality in the control of the reference-toxicity test exceeds 10% (Tables 9.1 and 9.2). All the performance criteria outlined in Tables 11.3, 12.3, 13.4,



$$\bar{x} = \frac{\sum_{i=1} x_i}{n}$$

$$s = \sqrt{\frac{\sum_{i=1}^{n} x_i^2 - \frac{(\sum_{i=1}^{n} x_i)^2}{n}}{n-1}}$$

where x_1 = Successive toxicity values of toxicity tests.
n = Number of tests.
$\bar{x}$ = Mean toxicity value.
S = Standard deviation.

Figure 17.1 Control (cusum) charts: (A) hypothesis testing results; and (B) point estimates (LC, EC, or IC).

14.3, and 15.3 must be considered when determining the acceptability of a sediment test. The acceptability of the sediment test would depend on the experience and judgment of the investigator and the regulatory authority.

17.4.6 If the value from a given test with the reference toxicant falls more than two standard deviations (SD) outside the expected range, the sensitivity of the organisms and the overall credibility of the test system may be suspect (USEPA, 1991a). In this case, the test procedure should be examined for defects and should be repeated with a different batch of test organisms.

17.4.7 Performance should improve with experience, and the control limits for point estimates should gradually narrow. However, control limits of ±2 SD, by definition, will be exceeded 5% of the time, regardless of how well a laboratory performs. Highly proficient laboratories that develop a very narrow control limit may be unfairly penalized if a test that falls just outside the control limits is rejected *de facto*. For this reason, the width of the control limits should be considered in determining whether or not an outlier is to be rejected. This determination may be made by the regulatory authority evaluating the data.

17.4.8 The recommended reference-toxicity test consists of a control and five or more concentrations in which the endpoint is an estimate of the toxicant concentration that is lethal to 50% of the test organisms in the time period prescribed by the test. The LC50 is determined by an appropriate procedure, such as the trimmed Spearman-Karber Method, Probit Method, Graphical Method, or the Linear Interpolation Method (Section 16).

17.4.9 The point estimation analysis methods recommended in this manual have been chosen primarily because they are well-tested, well-documented, and are applicable to most types of test data. Many other methods were considered in the selection process, and it is recognized that the methods selected are not the only possible methods of analysis of toxicity data.

17.5 Precision of Sediment Toxicity Test Methods: Evaluation of 10-d Sediment Tests and Reference-toxicity Tests

17.5.1 Intralaboratory Performance

17.5.1.1 Intralaboratory performance of the *Hyalella azteca* and *Chironomus tentans* 10-d tests (as described in Tables 11.1 and 12.1) was evaluated at the USEPA Office of Research and Development Laboratory (Duluth, MN) using one control sediment sample in June 1993. In this study, five individuals simultaneously conducted the 10-d whole-sediment toxicity tests as described in Tables 11.1 and 12.1 with the exception of the feeding rate of 1.0 mL rather than 1.5 mL for *C. tentans*. The results of the study are presented in Table 17.1. The mean survival for *H. azteca* was 90.4% with a CV of 7.2% and the mean survival for *C. tentans* was 93.0% with a CV of 5.7%. All of the individuals met the survival performance criteria of 80% for *H. azteca* (Table 11.3) or 70% for *C. tentans* (Table 12.3).

17.5.2 Interlaboratory Precision: 1993 Evaluation of the 10-d Sediment Tests and the Reference-toxicity Tests

17.5.2.1 Interlaboratory precision using reference-toxicity tests or 10-d whole-sediment toxicity tests using the methods described in this manual (Tables 9.1, 9.2, 11.1, and 12.1) were conducted by federal government laboratories, contract laboratories, and academic laboratories that had demonstrated experience in sediment toxicity testing for a first time in 1993 (Section 17.5.2.2 and Burton et al., 1996b) and a second time in 1996/1997 (the "1996/1997 study"; Section 17.5.3). In the 1993 study the only exception to the methods outlined in Table 9.1 and 9.2 was that 80% rather than the current recommendation of 90% survival was used to judge the acceptability of the reference-toxicity tests. The 1993 round-robin study was conducted in two phases for each test organism. The experimental design for the 1993 round-robin study required each laboratory to conduct 96-h water-only reference-toxicity tests in Phase 1 and 10-d whole-sediment tests in Phase 2 with *Hyalella azteca* or *Chironomus tentans* over a period of six months. Criteria for selection of participants in the 1993 round-robin study were that the laboratories: (1) had existing cultures of the test organisms, (2) had experience conducting tests with the organisms, and (3) would participate voluntarily. The test methods for the reference-toxicity tests and the whole-sediment toxicity tests were similar among laboratories. Standard operating procedures detailing the test methods were provided to all participants. Culture methods were not specified and were not identical across laboratories.

Table 17.1 Intralaboratory Precision for Survival of *Hyalella azteca* and *Chironomus tentans* in 10-d Whole-sediment Toxicity Tests, June 1993[1]

	Percent Survival	
Individual	*H. azteca*	*C. tentans*
A	85	85
B	93	93
C	90	93
D	84	94
E	100	100
N	5	5
Mean	90.4	93.0
CV	7.2%	5.7%

[1] Test sample was from a control sediment (T.J. Norberg-King, USEPA, Duluth, MN, personal communication). The test was conducted at the same time by five individuals at the USEPA Office of Research and Development Laboratory (Duluth, MN). The source of overlying water was from Lake Superior.

Table 17.2 Participants in 1993 Round-robin Studies[1]

	Chironomus tentans			*Hyalella azteca*	
Laboratory	96-h KCl Test Dec 92	96-h KCl Test May 93	10-d Sediment Test May 93	96-h KCl Test Oct 92	10-d Sediment Test Mar 93
A	Y	N	N	Y	N
B	Y	Y	Y	Y	Y
C	Y	N	Y	Y	Y
D	Y	Y	Y	N	N
E	Y	Y	Y	Y	Y
F	Y	Y	Y	Y	Y
G	Y	Y	Y	Y	Y
H	Y	N	N	Y	N
I	Y	Y	Y	—[2]	Y
J	Y	Y	Y	Y	Y
K	—[3]	—[3]	—[3]	Y	Y
L	—[4]	—[4]	—[4]	Y	Y
N	10	7	8	10	9

[1] Y = Laboratory participated in testing sediment samples.
[2] Test in January 1993.
[3] Participated using *C. riparius* only.
[4] Did not intend to participate with *C. tentans.*

Table 17.3 Interlaboratory Precision for *Hyalella azteca* 96-h LC50s from Water-only Static Acute Toxicity Tests Using a Reference Toxicant (KCl) (October 1992)

Laboratory	KCl LC50 (mg/L)	Confidence Intervals Lower	Confidence Intervals Upper	Percent Control Survival
A	372	352	395	100
B	321	294	350	98
C	232	205	262	100
D	—[1]	—[1]	—[1]	—[1]
E	325	282	374	100
F	276	240	316	98
G	297	267	331	73
H	336	317	356	100
I	142[2]	101	200	93
J	337	286	398	100
L	250	222	282	100
N	10			10
Mean 1	289.0[3]			96.2%
CV 1	23.0%[3]			8.3%
N	9			9
Mean 2	305.0[4]			98.8
CV 2	14.2%[4]			2.1%

[1] Laboratory did not participate in *H. azteca* test in October.
[2] Results are from a retest in January using three concentrations only; results excluded from analysis.
[3] Mean 1 and CV 1 include all data points
[4] Mean 2 and CV 2 exclude data points for all sediment samples from laboratories that did not meet minimum control survival of ≥80%.

17.5.2.2 In the second series of round-robin tests conducted in 1996/1997, 10-d and long-term toxicity testing methods were evaluated with *Hyalella azteca* and *Chironomus tentans*. Results from these interlaboratory comparisons conducted in 1996/1997 are presented in detail in Sections 17.5.3 and 17.6. The second series of interlaboratory comparisons conducted in 1996/1997 did not restrict testing to laboratories with experience. As in 1993, the participants in the 1996/1997 round-robin study included government, contract, and academic laboratories. In the 1996/1997 study, no water-only reference-toxicity tests were conducted.

17.5.2.3 Ten laboratories participated in the *H. azteca* reference-toxicity test in the 1993 study (Table 17.2). The results from the tests with KCl are summarized in Table 17.3. The test performance criteria of ≥80% control survival was met by 90% of the laboratories resulting in a mean control survival of 98.8% (CV = 2.1%). The mean LC50 was 305 mg/L (CV = 14.2%) and the LC50s ranged from 232 to 372 mg/L KCl.

17.5.2.4 In the 10-d whole-sediment tests with *H. azteca*, nine laboratories tested the three sediments described above and five laboratories tested a fourth sediment from a heavily contaminated site in the 1993 study (Table 17.4). All laboratories completed the tests; however, Laboratory C had 75% survival, which was below the acceptable test criteria for survival (Table 11.3). For these tests, the CV was calculated using the mean percent survival for the eight laboratories that met the performance criteria for the test. The CV for survival in the control sediment (RR 3) was 5.8% with a mean survival of 94.5% and survival ranging from 86% to 100%. For sediments RR 2 and RR 4, the mean survival was 3.3% and 4.3%, respectively (Table 17.4). For RR 2, survival ranged from 0% to 24% (CV = 253%) and for RR 4, the survival ranged from 0% to 11% (CV = 114%). Survival in the moderately contaminated sediment (RR 1) was 54.2% with survival ranging from 23% to 76% (CV = 38.9%). When the RR 1 data for each laboratory were compared to the control for that laboratory, the range for the minimum detectable difference (MDD) between the test sediments and the control sediment ranged from 5 to 24% with a mean of 11% (SD = 6).

17.5.2.5 The Phase 1 *C. tentans* reference-toxicity test was conducted with KCl on two occasions in the 1993 study (Tables 17.5 and 17.6). Both tests were conducted in 20 mL of test solution in 30-mL beakers using 10 replicates per treatment with 1 organism per beaker. Animals were fed 0.25 mL of a 4 g/L solution of Tetrafin® on Day 0 and Day 2 (Table 9.1). For the first reference-toxicity test comparison, 10 laboratories participated, and

Table 17.4 Interlaboratory Precision for Survival of *Hyalella azteca* in 10-d Whole-sediment Toxicity Tests Using Four Sediments (March 1993)

	Mean Percent Survival (SD) in Sediment Samples							
Laboratory	RR 1		RR 2		RR 3 (Control)		RR 4	
A	—[1]		—[1]		—[1]		—[1]	
B	76.2	(20.7)	2.5	(7.1)	97.5	(4.6)	11.2	(13.6)
C	57.52[2]	(14.9)	1.2[2]	(0)	75.0[2]	(17.7)	1.2[2]	(0)
D	—[1]		—[1]		—[1]		—[1]	
E	46.2	(17.7)	0	(0)	97.5	(7.1)	—	
F	72.5	(12.8)	23.7	(18.5)	98.7	(3.5)	0	(0)
G	50.0	(28.3)	0	(0)	100	(0)	3.3	(5.2)
H	—[1]		—[1]		—[1]		—[1]	
I	73.7	(32.0)	0	(0)	86.2	(10.6)	—	
J	65.0	(9.3)	0	(0)	96.2	(5.2)	2.5	(7.1)
K	22.5	(18.3)	0	(0)	95.0	(5.3)	—	
L	27.5	(16.7)	0	(0)	86.2	(18.5)	—	
N	9		9		9		5	
Mean 1[3]	54.6		3.0		93.0		3.6	
CV 1	36.2%		256%		9.0%		121%	
N	8		8		8		4	
Mean 2[4]	54.2		3.3		94.5		4.3	
CV 2	38.9%		253%		5.8%		114%	

[1] Laboratory did not participate in *H. azteca* test in March.
[2] Survival in control sediment (RR 3) below minimum acceptable level.
[3] Mean 1 and CV 1 include all data points.
[4] Mean 2 and CV 2 exclude data points for all sediment samples from laboratories that did not meet minimum control survival of ≥80%.

eight laboratories met the survival criteria of the round robin, which was ≥80% survival (Table 17.5). The mean LC50 for the eight laboratories that met the survival criterion was 4.25 g/L (CV of 51.8%). The LC50s ranged from 1.25 to 6.83 g/L. Length and instar were determined for a subset of organisms at the start of the tests for some of the laboratories. When length was correlated with the LC50, the larger animals were less sensitive than the smaller animals. The effect level was significantly correlated ($r^2 = 0.78$) with the organism size, which ranged from 1.56 mm to 10.87 mm (ages of animals ranged from 7- to 13-d post-deposition). The majority of these animals were the third instar, with the smallest animals in their first instar and the largest animals a mix of third and fourth instar (Table 17.5) as determined by head capsule width.

17.5.2.6 For the second Phase 1 KCl reference-toxicity tests with *C. tentans,* seven laboratories participated in the 1993 study (Table 17.6). The test conditions were identical to those in the previous reference-toxicity test except that a minimum size was specified rather than using initial age of the animals. Each laboratory was instructed to start the test when larvae were at least 0.4 to 0.6 mm long. Therefore, a more consistent size of test organisms was used in this test. Six out of the seven laboratories met the ≥80% control survival criterion with a mean LC50 of 5.37 g/L (CV = 19.6%). The LC50s ranged from 3.61 to 6.65 g/L.

17.5.2.7 Eight laboratories participated in the 10-d whole-sediment testing with *C. tentans*. The same three sediments used in the *H. azteca* whole-sediment test were used for this test in the 1993 study (Table 17.7). All test conditions were those as described in Table 12.1 with the exception of the feeding rate of 1.0 mL rather than 1.5 mL for *C. tentans.* Three laboratories did not meet the control criteria for acceptable tests of ≥70% survival in the control (RR 3) sediment (Table 12.3). For the five laboratories that successfully completed the tests, the mean survival in the control sediment (RR 3) was 92.0% (CV of 8.3%) and survival ranged from 81.2% to 98.8%. For the RR 2 sediment sample, the mean survival among the five laboratories was 3.0% (CV = 181%) and for the RR 1 sediment sample, the mean survival was 86.8% (CV = 13.5%). A significant effect on survival was not evident for the RR 1 sample, but growth was affected (Table 17.8). When the RR 1 data for each laboratory were compared to the control for that laboratory, the MDD for survival among laboratories ranged from 2.3 to 12.1% with a mean of 8% (SD = 4).

17.5.2.8 For *C. tentans,* growth in 10-d tests is a sensitive indicator of sediment toxicity (Ankley et al., 1993) and growth was also measured in the round-robin comparison in the 1993 study (Table 17.8). Using the data from five laboratories with acceptable control survival in the control sediment (RR 3), the mean weight of *C. tentans* for the control sediment (RR 3) was 1.254 mg (CV

Table 17.5 Interlaboratory Precision for *Chironomus tentans* 96-h LC50s from Water-only Static Acute Toxicity Tests Using a Reference Toxicant (KCl) (December 1992)

Laboratory	KCl LC50 (g/L)	Confidence Interval		Control Survival (%)	Mean Length (mm)	Instar at Start of Test	Age at Start of Test (day)
		Lower	Upper				
A	6.19	5.37	7.13	75[1]	10.87	3,4	1
B	6.83	6.38	7.31	100	10.43	3	13
C	5.00	4.16	6.01	100	5.78	3	11
D	3.17	2.29	4.40	100	5.86	3	11
E	2.00[2]	—[2]	—	80	6.07	3	11
F	1.25	—[3]	—	80	1.56	1	12
G	6.28	5.26	7.50	95	7.84	3	11
H	2.89	2.39	3.50	95	6.07	3	7
I	6.66	6.01	7.24	100	—[4]	—[4]	10
J	1.77	0.59	5.26	65[1]	4.42	2,3	7
N	10			10	8		10
Mean 1[5]	4.20			89.0	6.6		10.3
CV 1	52.7%			14.5%	46.6%		17.9%
N	8			8	7		8
Mean 2[6]	4.25			93.8	6.2		10.75
CV 2	51.8%			9.3%	39.5%		15.2%

[1] Control survival below minimum acceptable level.
[2] Unable to calculate LC50 with trimmed Spearman Karber; no confidence interval could be calculated.
[3] Confidence intervals cannot be calculated as no partial mortalities occurred.
[4] No animals were measured.
[5] Mean 1 and CV 1 include all data points.
[6] Mean 2 and CV 2 exclude data points for all samples from laboratories that did not meet minimum control survival of ≥80%.

Table 17.6 Interlaboratory Precision for *Chironomus tentans* 96-h LC50s from Water-only Static Acute Toxicity Tests Using a Reference Toxicant (KCl) (May 1993)

Laboratory	KCl LC50 (g/L)	Confidence Interval		Control Survival (%)	Age at Start of Test (day)
		Lower	Upper		
A	—[1]	—	—	—	—
B	6.65	—[2]	—	90	12
C	—[1]	—	—	—	—
D	5.30	4.33	6.50	55[3]	10
E	5.11	4.18	6.24	100	11
F	3.61	2.95	4.42	90	10
G	5.36	4.43	6.49	93	12
H	—[1]	—	—	—	
I	5.30	4.33	6.52	95	10-11
J	6.20	4.80	7.89	100	13
N	7			7	7
Mean 1[4]	5.36			89	11.1
CV 1	17.9%			17.5%	9.46%
N	6			6	6
Mean 2[5]	5.37			94.7	11.2
CV 2	19.6%			4.8%	9.13%

[1] Did not participate in reference-toxicity test in April.
[2] Confidence intervals cannot be calculated as no partial mortalities occurred.
[3] Control survival below minimum acceptable level.
[4] Mean 1 and CV 1 include all data points.
[5] Mean 2 and CV 2 exclude data points for all samples from laboratories that did not meet minimum control survival of ≥70%.

Table 17.7 Interlaboratory Precision for Survival of *Chironomus tentans* in 10-d Whole-sediment Toxicity Tests Using Three Sediments (May 1993)

	Mean Percent Survival (SD) in Sediment Samples					
Laboratory	RR 1		RR 2		RR 3 (Control)	
A	—[1]		—[1]		—[1]	
B	67.5	(14.9)	2.5	(7.1)	98.8	(3.5)
C	15.0[2]	(12.0)	0[2]	(0)	62.5[2]	(26.0)
D	60.0[2]	(20.0)	0[2]	(0)	66.3[2]	(27.7)
E	85.0	(11.9)	0	(0)	93.8	(9.2)
F	87.5[2]	(12.5)	0[2]	(0)	43.8[2]	(30.2)
G	90.0	(13.1)	12.5	(3.5)	87.5	(10.3)
H	—[1]		—[1]		—[1]	
I	97.5	(4.6)	0	(0)	98.8	(3.5)
J	93.8	(11.8)	0	(0)	81.2	(8.3)
N	8		8		8	
Mean 1[3]	74.5		1.88		79.1	
CV 1	36.7%		233%		25.1%	
N	5		5		5	
Mean 2[4]	86.8		3.0		92.0	
CV 2	13 5%		181%		8.3%	

[1] Did not participate in *C. tentans* test in May.
[2] Survival in control sediment (RR 3) below minimum acceptable level.
[3] Mean 1 and CV 1 include all data points.
[4] Mean 2 and CV 2 exclude data points for all sediment samples from laboratories that did not meet minimum control survival of ≥70%.

Table 17.8 Interlaboratory Precision for Growth of *Chironomus tentans* in 10-d Whole-sediment Toxicity Tests Using Three Sediments (May 1993)

	Growth—Dry Weight in mg (SD) in Sediment Samples					
Laboratory	RR 1		RR 2		RR 3 (Control)	
A	—[1]		—[1]		—[1]	
B	0.370	(0.090)	0	(0)	1.300	(0.060)
C	0.883[2]	(0.890)	0[2]	(0)	0.504[2]	(0.212)
D	0.215[2]	(0.052)	0[2]	(0)	1.070[2]	(0.107)
E	0.657	(0.198)	0	(0)	0.778	(0.169)
F	0.210[2]	(0.120)	0[2]	(0)	0.610[2]	(0.390)
G	0.718	(0.114)	0	(0)	1.710	(0.250)
H	—[1]		—[1]		—[1]	
I	0.639	(0.149)	0	(0)	1.300	(0.006)
J	0.347	(0.050)	0	(0)	1.180	(0.123)
N	8		8		8	
Mean 1[3]	0.505		—		1.056	
CV 1	49.9%		—		38.3%	
N	5		5		5	
Mean 2[4]	0.546		—		1.254	
CV 2	31.9%		—		26.6%	

[1] Did not participate in testing in May.
[2] Survival in control sediment (RR 3) below minimum acceptable level.
[3] Mean 1 and CV 1 include all data points.
[4] Mean 2 and CV 2 exclude data points for all sediment samples from laboratories that did not meet minimum control survival of ≥70%.

= 26.6%). The *C. tentans* in the moderately contaminated sediment (RR 1) had a mean weight of 0.546 mg (CV = 31.9%). No growth measurements were obtained for *C. tentans* in sediment RR 2 because of the high mortality. The mean minimum detectable difference for growth among laboratories meeting the survival performance criteria was 11% (SD = 5) and the MDD ranged from 4.8 to 23.6% when the RR 1 data were compared to the RR 3 data.

17.5.3 Interlaboratory Precision: 1996/1997 Evaluation of 10-d Sediment Tests

17.5.3.1 ***The 1996/1997 Precision Evaluation: 10-d Whole-sediment Toxicity Testing***. The results of the 10-d toxicity interlaboratory comparisons conducted in 1996/1997 are presented in Tables 17.9 to 17.12. A total of 18 laboratories participated in the 1996/1997 study; however, not all samples were tested by all laboratories. Laboratories performed the tests during a specified time period and followed methods outlined in Tables 11.1 and 12.1. Field samples were pretested to identify moderately toxic samples. Samples were prepared and subsampled at one time to increase consistency among the subsamples. Samples were shipped to the testing laboratories by express mail. Laboratories used their own water supplies and were asked to use moderately hard water (hardness about 100 mg/L as $CaCO_3$). The following samples were evaluated in the 10-d toxicity tests: a field control sediment from West Bearskin Lake, MN (WB), a formulated sediment (FS, formulated with alpha-cellulose; Kemble et al., 1999), two contaminated sediments (Little Scioto River, OH (LS); Defoe Creek site, Keweenaw, MI (DC)), and FS spiked with three concentrations of cadmium (0.3, 1.0, and 3.0 mg/kg Cd). The LS sample was primarily contaminated with polycyclic aromatic hydrocarbons and the DC sample was primarily contaminated with copper. Some laboratories did not conduct tests on all samples due to logistical constraints. In addition, ash-free dry weight (AFDW) was not measured by laboratories which did not have access to a muffle furnace.

17.5.3.2 ***The 1996/1997 Precision Evaluation – Hyalella azteca***. Eighteen laboratories participated in the 1996/1997 *H. azteca* 10-d comparison (Table 17.9). A total of 82% of the laboratories had acceptable survival (≥80%) and for these tests the average survival (and CV) was 92% (CV=5%) in the WB control sediment and 89% (CV=12%) in the formulated sediment (FS). The two contaminated field sediments (DC, LS) were moderately toxic, with the mean survival of 45% (CV=38%) in DC sediment and 57% (CV=49%) in LS sediment. The mean MDDs of the two contaminated samples for all laboratories relative to the WB control sediment were low (14% for both the DC and the LS sediments). The range of MDDs relative to the WB control sediment among all laboratories was 8 to 23% for the DC sediment and 2 to 22% for the LS sediment. A dose response effect was observed with the Cd-spiked formulated sediments. Moderate toxicity was observed in the 1 mg/kg Cd sample with a mean survival of 49% (CV=40%). The mean MDD and range for the 1 mg/kg Cd sample for all laboratories was 16% (5.7 to 26%). It is apparent from the MDDs that some laboratories had low variability while others had only moderate levels of variability.

17.5.3.3 ***The 1996/1997 Precision Evaluation – Chironomus tentans***. Eighteen laboratories participated in the 1996/1997 *C. tentans* 10-d survival and growth comparison (Table 17.10) with the same samples used in the toxicity test as described above. A total of 15 laboratories (89%) had acceptable survival (≥70%), and for these tests, the mean survival was 89% (CV=9.4%) in the WB control sediment and 88% (CV=10.2%) in the formulated sediment (FS). The two contaminated field sediments were only slightly toxic to the midge (mean survival of 80% (CV=16%) for the DC sediment and 71% (CV=33%) for LS sediment). The mean MDDs relative to the WB control sediment, across all laboratories for the two contaminated samples were low (12% for the DC sediment and 11% for LC sediment). The range of MDDs relative to the WB control sediment among laboratories were 6.1 to 22% for the DC sediment and 5.1 to 18% for LS sediment. No toxicity was observed for survival in the cadmium tests. The mean survival of midge in the 1 mg/kg Cd treatment was 92% (CV=5.6%). The mean MDD and range for the 1 mg/kg Cd sample was 12% (6.9 to 30%). It is apparent from the MDDs that some laboratories had low variability while others had slightly lower variability.

17.5.3.4 Growth of *C. tentans* was evaluated by up to 16 laboratories in 1996/1997, depending on the sample and whether or not they had capabilities to determine AFDW. For dry weight analyses, 12 of 15 laboratories had acceptable dry weight (≥0.6 mg/individual) and survival >70% in the WB control sediment, while 12 of 15 of the laboratories had acceptable dry weight and survival in the formulated sediment (FS; Table 17.11). For AFDW, 7 of 11 laboratories had acceptable weight (≥0.48 mg/individual) and survival >70% in WB control sediment (field control) (WB) and 7 of 11 laboratories reported acceptable weight in the formulated sediment (FS; Table 17.12). For the midges, the mean dry weight was 1.39 mg/organism (CV=33%) in the WB control sediment and 1.50 mg/organism (CV=31%) in the formulated sediment (FS) for laboratories that met the control survival in WB control sediment. For AFDW, mean AFDW was 0.92 mg/organism (CV=30%) in the WB control sediment and 1.161 mg/organism (CV=33%) in the formulated sediment (FS). Exposure to the contaminated DC sediment reduced the weight of the midge (mean weight of 0.49 mg/organism (CV=60%) as dry weight, while the mean weight of 0.24 mg/organism (CV=45%) was determined for the AFDW), yet exposure to LS sediment did not reduce weight of midges (1.45 mg dry weight (CV=45%); 0.86 mg AFDW (CV=27%)). The mean MDDs relative to WB control sediment, across all laboratories for the two contaminated samples, were low (0.17 mg/organism dry weight for the DC sediment and 0.28 mg dry weight for LS sediment). The range of MDDs among laboratories for dry weight was 0.04 to 0.53 mg/organism for DC sediment and 0.09 to 1.04 mg/organism for LS sediment. The AFDW data exhibited a similar pattern. Mean MDD as AFDW was 0.12 mg for the DC sediment and 0.16 mg for the LS sediment. The range

Table 17.9 Interlaboratory Precision for Survival (%) of *Hyalella azteca* in 10-d Whole-sediment Toxicity Tests (1996/1997)

	Mean Percent Survival (SD) in Sediment Samples and Cd-spiked Control Sediment													
	Sediment								Cadmium -FS Spikes (mg/kg)					
Laboratory	WB		DC		LS		FS		0.3-Cd		1-Cd		3-Cd	
A	71[a]	(23.0)	0[a]		NT[b]		40[a]	(37.8)	NT		NT		NT	
B	75[a]	(24.5)	49[a]	(27.5)	84[a]	(30.7)	90[a]	(7.6)	NT		NT		NT	
C	NT	NT	NT		NT		95[c]	(5.8)	90[c]	(14.1)	73[c]	(9.6)	0[c]	
E	85	(15.1)	31	(19.6)	71	(34.4)	83	(14.9)	68	(9.6)	83	(9.6)	3	(5.0)
F	94	(5.2)	31	(18.1)	19	(16.4)	60	(20.0)	40	(8.2)	28	(5.0)	3	(5.0)
G	83	(15.8)	38	(15.8)	28	(12.8)	90	(9.3)	NT		NT		NT	
H	95	(7.6)	61	(19.6)	64	(20.7)	99	(3.5)	NT		NT		NT	
I	95	(5.4)	33	(13.8)	85	(9.3)	99	(3.5)	83	(20.6)	28	(17.1)	0	
K	95	(7.6)	79	(9.9)	94	(7.4)	100	(0)	98	(5.0)	60	(8.2)	0	
M	86	(17.7)	23	(21.9)	50	(22.7)	85	(16.9)	80	(14.1)	65	(19.2)	0	
N	91	(6.4)	48	(10.4)	29	(23.6)	85	(14.1)	100	(0)	70	(8.2)	3	(5.0)
O	91	(8.4)	50	(14.1)	74	(10.6)	95	(5.4)	78	(22.2)	55	(26.5)	0	
P	88	(7.1)	56	(27.2)	60	(27.3)	85	(10.7)	83	(16.2)	48	(16.2)	0	
Q	91	(8.4)	20	(16.0)	84	(22.0)	96	(5.2)	98	(5.0)	23	(28.7)	0	
S	68[a]	(17.5)	34[a]	(24.5)	80[a]	(23.9)	70[a]	(25.1)	NT		NT		NT	
U	94	(7.4)	60	(30.2)	63	(21.2)	95	(5.4)	88	(12.6)	38	(15.0)	0	
V	95	(10.0)	35	(20.8)	75	(20.8)	93	(15.0)	93	(5.0)	40	(14.1)	0	
X	99	(3.5)	59	(12.5)	0		85	(15.1)	NT		NT		NT	
N-1[d]	17		17		16		17		11		11		11	
Mean-1	88		42		60		85		83		49		1	
SD-1	9.1		18.9		27.4		15.7		17.2		19.4		1.4	
CV-1	10.3		45.6		45.7		18.4		20.9		39.7		171.3	
N-2[e]	14		14		14		14		11		11		11	
Mean- 2	92		45		57		89		83		49		1	
SD- 2	4.6		17.1		27.9		10.4		17.2		19.4		1.4	
CV-2	5.0		38.3		49.1		11.6		20.9		39.7		171.3	

[a] Control survival below acceptable level of 80% in WB sediment.

[b] NT = not tested.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD-1 and CV-1 include all data (except Laboratory C) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the control performance acceptability criteria in WB sediment.

Table 17.10 Interlaboratory Precision for Survival (%) of *Chironomus tentans* in 10-d Whole-sediment Toxicity Tests (1996/1997)

	Mean Percent Survival (SD) in Sediment Samples and Cd-Spiked Control Sediment													
	Sediment								Cadmium -FS Spikes (mg/kg)					
Laboratory	WB		DC		LS		FS		0.3-Cd		1-Cd		3-Cd	
A	81	(13.6)	79	(6.4)	NT[a]		88	(10.35)	NT		NT		NT	
B	100	(0)	89	(9.1)	93	(8.9)	90	(7.56)	NT		NT		NT	
C	NT		NT		NT		98[b]	(5.00)	98[b]	(5.0)	95[b]	(5.8)	85[b]	(19.1)
E	94	(7.4)	93	(11.7)	84	(13.0)	96	(5.18)	83	(17.1)	85	(5.8)	73	(9.6)
F	99	(3.5)	84	(10.6)	84	(7.4)	88	(8.86)	95	(5.8)	93	(9.6)	98	(5.0)
G	85	(10.7)	76	(20.7)	19	(27.5)	74	(24.46)	NT		NT		NT	
H	96	(7.4)	93	(7.1)	94	(7.4)	100	(0)	NT		NT		NT	
I	90	(7.6)	83	(13.9)	74	(10.6)	86	(14.08)	85	(12.9)	93	(9.6)	83	(15.0)
J	38[c]	(25.5)	25[c]	(20.7)	83[c]	(13.9)	48[c]	(35.76)	23[c]	(22.2)	63[c]	(28.7)	40[c]	(24.5)
K	96	(5.2)	84	(10.6)	NT		98	(4.63)	NT		95	(10.0)	NT	
L	84	(13.0)	70	(13.1)	86	(11.9)	86	(13.02)	NT		NT		NT	
N	83	(12.8)	46	(32.9)	86	(11.9)	91	(17.27)	88	(12.6)	95	(5.8)	70	(8.2)
O	51[c]	(21.0)	61[c]	(18.1)	91[c]	(8.4)	51[c]	(14.58)	85[c]	(5.8)	90[c]	(8.2)	95[c]	(5.8)
P	78	(10.4)	70	(17.7)	41	(24.2)	88	(13.89)	93	(9.6)	93	(9.6)	73	(9.6)
Q	91	(8.4)	93	(8.9)	94	(11.9)	99	(3.54)	98	(5.0)	98	(5.0)	98	(5.0)
R	82	(3.4)	71	(15.4)	56	(13.2)	77	(5.89)	81	(8.0)	83	(11.8)	72	(29.4)
S	75	(14.1)	75	(27.8)	60	(15.1)	71	(18.08)	NT		NT		NT	
X	100	(0)	89	(12.5)	51	(21.7)	98	(7.07)	NT		NT		NT	
N-1[d]	17		17		15		17		9		10		9	
Mean-1	84		75		68		84		81		89		78	
SD-1	16.9		18.0		27.9		15.5		22.6		10.2		18.4	
CV-1	20.0		23.9		41.0		18.5		27.8		11.4		23.6	
N-2[e]	15		15		13		15		7		8		7	
Mean-2	89		80		71		89		89		92		81	
SD-2	8.3		12.5		23.6		9.1		6.5		5.2		12.3	
CV-2	9.4		15.7		33.3		10.2		7.3		5.6		15.2	

[a] NT = not tested.

[b] Not included in any mean as WB control sediment was not tested.

[c] Control survival below acceptable level of 70% in WB sediment.

[d] N-1, Mean-1, SD-1 and CV-1 include all data (except Laboratory C) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the control performance acceptability criteria in WB sediment.

Table 17.11 Interlaboratory Precision for Growth (mg/Individual dry weight) of *Chironomus tentans* in 10-d Whole-sediment Toxicity Tests (1996/1997)

	Mean Growth as Dry Weight (SD) in Sediment Samples and Cd-Spiked Control Sediment													
	Sediment								Cadmium -FS Spikes (mg/kg)					
Laboratory	WB		DC		LS		FS		0.3-Cd		1-Cd		3-Cd	
A	0.94	(0.15)	0.38	(0.09)	NT[a]		1.22	(0.27)	NT		NT		NT	
B	1.02	(0.06)	0.24	(0.03)	0.90	(0.34)	1.37	(0.12)	NT		NT		NT	
C	NT		NT		NT		0.86[b]	(0.12)	0.83[b]	(0.14)	0.83[b]	(0.14)	0.20[b]	(0.09)
E	2.47	(0.30)	1.05	(0.21)	2.69	(0.42)	2.29	(0.51)	3.44	(0.29)	2.42	(0.41)	2.90	(0.58)
F	1.69	(0.17)	0.41	(0.13)	1.62	(0.29)	2.43	(0.40)	2.48	(0.26)	2.50	(0.29)	1.02	(0.43)
H	0.92	(0.12)	0.24	(0.05)	0.93	(0.06)	1.29	(0.21)	NT		NT		NT	
I	1.55	(0.27)	0.37	(0.17)	1.80	(0.40)	1.74	(0.49)	2.58	(0.25)	2.05	(0.57)	2.05	(0.50)
J	0.90[c]	(0.83)	0.15[c]	(0.06)	0.91[c]	(0.69)	0.36[c]	(0.23)	1.02[c]	(0.87)	0.42[c]	(0.25)	0.18[c]	(0.05)
K	1.48	(0.12)	0.20	(0.03)	NT		1.68	(0.18)	NT		1.29	(0.05)	NT	
N	0.22[d]	(0.11)	0.06[d]	(0.02)	0.30[d]	(0.06)	0.32[d]	(0.10)	0.35[d]	(0.17)	0.27[d]	(0.04)	0.12[d]	(0.02)
O	0.99[c]	(0.17)	0.07[c]	(0.03)	0.81[c]	(0.07)	1.37[c]	(0.29)	0.67[c]	(0.09)	0.55[c]	(0.06)	0.15[c]	(0.02)
P	1.36	(0.18)	1.01	(0.21)	0.87	(0.31)	0.99	(0.29)	1.63	(0.68)	1.54	(0.18)	1.11	(0.03)
Q	1.01	(0.29)	0.21	(0.09)	1.31	(0.27)	1.08	(0.17)	1.06	(0.15)	1.16	(0.18)	1.16	(0.10)
R	1.31	(0.29)	0.58	(0.28)	1.06	(0.36)	1.51	(0.34)	1.25	(0.38)	1.37	(0.28)	0.70	(0.24)
S	1.73	(0.29)	0.48	(0.21)	2.36	(0.35)	1.26	(0.80)	NT			NT	NT	
X	0.97	(0.10)	0.68	(0.14)	0.95	(0.36)	1.09	(0.22)	NT			NT	NT	
N-1[e]	15		15		13		15		9		10		9	
Mean-1	1.24		0.41		1.27		1.33		1.61		1.36		1.04	
SD-1	0.51		.31		0.67		0.58		1.02		0.80		0.93	
CV-1	41.6		75.3		53.1		43.3		63.3		58.7		69.6	
N-2[f]	12		12		10		12		6		7		6	
Mean-2	1.39		0.49		1.45		1.50		2.1		1.76		1.49	
SD-2	0.45		0.29		0.65		0.47		0.92		0.56		0.83	
CV-2	33.2		60.2		45.1		31.1		44.2		31.5		55.3	

[a] NT = not tested.

[b] Not included in any mean as WB control sediment was not tested.

[c] Control survival below acceptable level of 70% in WB sediment.

[d] Control weight below acceptable level of 0.60 mg/organism in WB sediment.

[e] N-1, Mean-1, SD-1 and CV-1 include all data (except Laboratory C) whether control met acceptable limits or not in WB control sediment.

[f] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the control performance acceptability criteria in WB sediment.

Table 17.12 Interlaboratory Precision for Growth (mg/Individual as ash-free dry weight) of *Chironomus tentans* in 10-d Whole-sediment Toxicity Tests (1996/1997)

Laboratory	Mean Growth as Ash-free Dry Weight (SD) in Sediment Samples and Cd-spiked Control Sediment													
	Sediment								Cadmium -FS Spikes (mg/kg)					
	WB		DC		LS		FS		0.3-Cd		1-Cd		3-Cd	
B	0.79	(0.03)	0.18	(0.03)	0.69	(0.07)	1.04	(0.09)	NT[a]		NT		NT	
C	NT		NT		NT		0.20[b]	(0.05)	0.19[b]	(0.03)	0.23[b]	(0.12)	0.03[b]	(0.03)
E	0.25[c]	(0.09)	0.10[c]	(0.03)	0.2[c]	(0.07)	0.24[c]	(0.06)	0.48[c]	(0.12)	0.27[c]	(0.08)	0.38[c]	(0.18)
F	0.50	(0.11)	0.13	(0.12)	0.73	(0.16)	1.14	(0.39)	0.94	(0.10)	1.00	(0.31)	0.45	(0.24)
I	1.35	(0.26)	0.32	(0.13)	1.16	(0.27)	1.99	(1.50)	2.01	(0.19)	1.56	(0.35)	1.55	(0.41)
K	1.06	(0.09)	0.17	(0.02)	NT		1.12	(0.09)	NT		0.91	(0.03)	NT	
L	1.07	(0.28)	0.34	(0.09)	1.13	(0.23)	1.11	(0.18)	NT		NT		NT	
O	0.30[c,d]	(0.05)	0.01[c,d]	(0.01)	0.26[c,d]	(0.06)	0.60[c,d]	(0.15)	0.22[c,d]	(0.03)	0.16[c,d]	(0.03)	0.03[c,d]	(0.01)
P	0.36[d]	(0.33)	0.29[d]	(0.03)	0.18[d]	(0.10)	0.15[d]	(0.05)	0.46[d]	(0.41)	0.29[d]	(0.07)	0.21[d]	(0.05)
Q	0.76	(0.24)	0.15	(0.08)	0.78	(0.16)	0.79	(0.12)	0.74	(0.12)	0.78	(0.22)	0.78	(0.04)
R	0.88	(0.27)	0.40	(0.16)	0.64	(0.17)	0.94	(0.20)	0.74	(0.21)	0.86	(0.22)	0.46	(0.17)
X	0.15[d]	(0.04)	0.20[d]	(0.09)	0.49[d]	(0.21)	0.30[d]	(0.18)	NT		NT		NT	
N-1[e]	11		11		10		11		7		8		7	
Mean-1	0.677		0.208		0.630		0.856		0.799		0.729		0.551	
S D-1	0.39		0.12		0.35		0.53		0.58		0.47		0.50	
CV-1	58.1		56.1		54.9		61.8		73.1		64.6		90.2	
N-2[f]	7		7		6		7		4		5		4	
Mean-2	0.916		0.241		0.855		1.161		1.108		1.022		0.810	
SD-2	0.27		0.11		0.23		0.39		0.61		0.31		0.52	
CV-2	29.8		45.0		26.8		33.2		55.0		30.4		63.8	

[a] NT = not tested.

[b] Not included in any mean as WB control sediment was not tested.

[c] Control weight below acceptable weight criteria of 0.48 mg/organism in WB sediment.

[d] Control survival below acceptable level of 70% in WB sediment.

[e] N-1, Mean-1, SD-1 and CV-1 include all data (except Laboratory C) whether control met acceptable limits or not in WB sediment.

[f] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the control performance acceptability criteria in WB sediment.

of MDDs for AFDW across laboratories was 0.03 to 0.22 mg for the DC sediment and 0.04 to 0.25 mg for LS sediment. No toxicity relative to weight was observed in the cadmium tests. The mean dry weight of midge in the 1 mg/kg Cd treatment was 1.76 mg/organism (CV=32%). The mean MDD and range for the 1 mg/kg Cd sample was 0.28 mg/organism (0.09 to 0.57). The AFDW for the 1 mg/kg sample was 1.022 mg/organism (CV=30%) with MDDs of 0.19 mg (0.04 to 0.36).

17.5.4 These round-robin tests conducted in 1993 (Section 17.5.2) and in 1996/1997 (Section 17.5.3) exhibited similar or better precision compared to many chemical analyses and effluent toxicity test methods (USEPA, 1991a; USEPA, 1991c). The success rate for test initiation and completion of the USEPA's round-robin evaluations is a good indication that a well equipped and trained staff will be able to successfully conduct these tests. This is an important consideration for any test performed routinely in any regulatory program.

17.6 Precision of Sediment Toxicity Test Methods: Evaluation of Long-term Sediment Tests

17.6.1 Interlaboratory precision evaluations of the long-term *H. azteca* and *C. tentans* tests, using the methods described in Sections 14 and 15, were conducted by federal government, contract, and academic laboratories that had demonstrated experience in sediment toxicity testing, although only two of the laboratories had prior experience with the long-term test methods described in this manual. This round-robin study was conducted in two phases: a Preliminary Round-robin (PRR) and a Definitive Round-robin (DRR). The objective of the PRR was to provide participating laboratories with an opportunity to become acquainted with the techniques necessary to conduct the two tests and to solicit commentary and recommendations regarding potential improvements for the definitive evaluation. Criteria for selection of participants in both phases were that the laboratories: (1) had existing cultures of the test organisms, (2) had experience conducting 10-d tests with the organisms, and (3) would participate voluntarily. Methods for conducting toxicity tests were similar among laboratories, and each laboratory was supplied with detailed operating procedures outlining these methods. Methods for culturing were not specified and were not identical across laboratories (as long as each laboratory started with the appropriate age test organisms). The PRR (phase 1) included the WB control sediment (West Bearskin, MN; WB) and the formulated sediment (FS) in which alpha-cellulose represented the primary carbon source (Kemble et al., 1999; Table 17.13). The DRR (phase 2) also included a copper-contaminated sediment from Cole Creek, Keweenaw, MI (CC), and a PAH-contaminated sediment from the Little Scioto River, OH (LS). In addition to the WB control sediment and the FS sediment described above, an additional sediment, in which peat (PE) represented the primary carbon source, was also tested (Table 17.13).

17.6.2 Twelve laboratories participated in the PRR with *H. azteca*. In these tests, 100% of laboratories passed the acceptability criterion for survival (≥80%) in the WB control sediment at 28 d (Table 17.14) with survival ranges of 83 to 98% at 28 d, 71 to 93% at 35 d and 63 to 92% at 42 d. In the formulated sediment (FS), 80% of the laboratories met the survival criterion at 28 d (range: 47 to 98%). Survival ranges in FS sediment at 35 d were 48 to 98% and at 42 d the survival ranges were 48 to 98%. For growth measured as length in the WB sediment, 92% of the laboratories reported the mean length of the organisms to be ≥3.2 mm at 28 d (range: 3.07 to 5.64 mm). For the FS sediment, 100% of the laboratories reported length ≥3.2 mm with lengths ranging from 3.54 to 5.44 mm. For growth measured as dry weight, >66% of the laboratories met the minimum weight criterion (≥0.15 mg/organism) in WB (range: 0.10 to 1.16 mg/individual). In the FS samples, 100% of the laboratories met this growth criterion, with weight ranges from 0.15 to 0.90 mg/individual. The criterion for reproductive output for *H. azteca* (≥2 young/female) was met by 78% of laboratories in the WB (range: 0 to 27 young/female). In the FS samples, 89% of the laboratories met the reproductive requirement with ranges of 0.62 to 22 young/female.

17.6.3 Ten laboratories participated in the PRR with *C. tentans*. In these tests, 90% of laboratories passed the acceptability criterion for survival at 20 d (≥70%) in WB (range: 67 to 96%; Table 17.14), and in the FS sediment, 60% of the laboratories met the acceptability criterion (range: 42 to 83%). For growth measured as dry weight, 100% of laboratories passed the criterion (≥0.6 mg/individual) in WB (range: 1.45 to 3.78 mg/individual). For the FS samples, 86% of the laboratories passed the criterion (range: 0.50 to 3.40 mg/individual). For growth as AFDW, 100% of the laboratories passed the criterion of ≥0.48 mg in the WB (range: 0.86 to 3.22 mg/individual) (Table 17.14). In the FS sediment, 88% of the laboratories met the growth criterion (as dry weight) with ranges of weights from 0.42 to 2.72 mg/individual. The criterion for emergence (≥50%) was met by 70% of the laboratories in WB sediment. In the FS, 50% of the laboratories met the emergence criterion. The criterion for reproductive output in *C. tentans* (≥800 eggs/female) was exceeded by 90% of laboratories in WB control sediment (range: 504 to 1240 eggs/female). In FS, 86% of laboratories met this criterion in the FS (range: 0 to 1244 eggs/female). The suggested criterion for percent hatch (≥80%) was met by 88% of laboratories in WB (range: 0 to 98%), and in FS, 67% of laboratories (range: 0 to 98.7%).

17.6.4 In both the *H. azteca* and *C. tentans* tests, the results of the PRR demonstrated that the majority of laboratories met the acceptability criteria for those endpoints for which criteria had been established (e.g., survival and growth). The highest proportion of failures in the midge test occurred with post-pupation endpoints (emergence, percent hatch) and may reflect the fact that the criteria developed for these endpoints are based on evaluations conducted at a single laboratory (Sibley et al., 1996; Sibley et al., 1997b; Benoit et al., 1997). In the PRR, some laboratories experienced unacceptably low oxygen

Table 17.13 Physical Characteristics of the Sediments Used in the Preliminary and Definitive Round-robin Evaluations of Long-term Methods for Sediment Toxicity Testing (Section 17.6).

Sediment	Total Organic Carbon (%)	Water Content	Particle Size (%)			Sediment Type
			Sand	Clay	Silt	
FS[a] (a high sand/low TOC)	2.2	31	74	16	11	Sandy Loam
WB	3.3	31	74	16	10	Sandy Loam
PE	10	ND[a]	ND	ND	ND	Clay

[a] ND = not determined

Table 17.14 Percentage of Laboratories Meeting Performance Levels for the Following Endpoints in the WB Control Sediment Evaluated in the Long-term Round-robin Tests.

Performance Level	Preliminary Round	Definitive Round
	Hyalella azteca	
28-d survival ≥ 80%	100	88
28-d growth ≥ 3.2 mm length	92	71
28-d growth ≥0.15 mg dry weight	66	88
28- to 42-d reproduction (≥ 2 young/female)	78	71
	Chironomus tentans	
20-d survival ≥70%	90	63
20-d growth ≥0.6 mg (dry weight)	100	63
20-d growth ≥0.48 mg (ash-free dry weight)	100	67
Emergence ≥50%	70	50
Number of eggs/egg case ≥ 800	90	63
Percentage hatch ≥80%	88	57

Table 17.15 **Interlaboratory Comparison of Day 28 Percent Survival (Mean ± SD) of *H. azteca* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).**

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	100	(0)	97	(4.9)	94	(6.7)	94	(7.9)	NT [a]	
F	62[b]	(33.0)	84[b]	(21.1)	90[b]	(20.9)	38[b]	(35.2)	93[b]	(23.0)
H	93	(9.6)	85	(5.8)	98	(5.0)	NT		68	(37.8)
K	95	(10.0)	98	(4.5)	96	(6.7)	NT			NT
L	83	(12.2)	88	(8.7)	84	(12.4)	78	(14.2)	54	(40.6)
N	89	(16.8)	92	(8.4)	91	(6.7)	NT		NT	
Q	98	(5.0)	93	(9.6)	80	(27.1)	90	(14.4)	88	(9.6)
U	100	(0)	100	(0)	98	(5.0)	NT		NT	
X	NT		NT		NT		83[c]	(10.7)	93[c]	(7.5)
N-1[d]	8		8		8		4		4	
Mean-1	90		92		91		75		75	
SD-1	12.8		6.0		6.4		25.6		17.8	
CV (%)-1	14.3		6.5		7.0		34.6		23.6	
N-2[e]	7		7		7		3		3	
Mean-2	94		93		91		87		70	
SD-2	6.4		5.5		6.9		8.7		16.8	
CV (%)- 2	6.8		5.9		7.5		9.9		24.1	

[a] NT = not tested

[b] Control survival below acceptable level of 80% in WB sediment.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD-1 and CV-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control performance acceptability criteria in WB sediment.

levels during evaluation of the *C. tentans* test which was attributed to high feeding rates. To address this issue, the feeding rate for the DRR of the *C. tentans* test was reduced from 1.5 to 1.0 mL/d of Tetrafin.

17.6.5 In total, eight laboratories participated in the DRR with *H. azteca*; however all laboratories did not test all sediments. Mean survival for those laboratories that met the control survival test acceptability criteria at 28 d in the WB control sediment was 94% (CV=6.8%). In FS, the mean survival was 87% (CV=9.9%), and in the PE it was 70% (CV=24%; Table 17.15). Mean survival at 35 d with laboratories that met the ≥80% control survival criterion at 28 d was as follows: WB had 92% survival (CV=7.2%), FS had 88% survival (CV=15.1%) and PE had survival of 63% (CV=34.0%; Table 17.16). Mean survival at 42 d with laboratories that met the ≥80% 28-d control survival criterion was as follows: WB had 92% survival (CV=7.4%), FS had 84% survival (CV=14.1%) and PE had 60% survival (CV=38.2% with 3 laboratories; Table 17.16). At 28 d, 88% of the laboratories met the control survival criteria in the WB control sediment (Table 17.14). When acceptable 28-d control survival was reported in WB sediment, 71% of the laboratories met the length criterion (≥3.2 mm) for *H. azteca* (Table 17.14). For those laboratories that met the 28-d survival criterion and the growth criterion, the mean growth (measured as length) of *H. azteca* at 28 d was 4.17 mm (CV=12.4%) in WB, 3.51 mm (CV=22.6%) in the FS and 3.24 mm (CV=36.6%) in the PE (Table 17.18). For growth measured as dry weight for the WB control sediment, 88% of the laboratories met the weight criterion of ≥0.15 mg/individual when acceptable 28-d control survival was reported (Table 17.19) The mean growth of *H. azteca* (mg/individual dry weight) in each sample where 28-d control survival and growth was met was: 0.25 mg (CV=27.8%) in WB, 0.30 mg (CV=68.6%) in FS, and 0.18 mg (CV=34.0%; Table 17.19) in PE. For the WB control sediment, 71% of the laboratories met the reproduction criteria (≥2 young/female) when acceptable 28-d control survival was reported (Table 17.14). The mean reproduction from 28 to 42 d for laboratories that met both the reproduction criteria and 28-d survival criteria was 3.13 young/female (CV=48.9%) for WB. For the FS, only one laboratory that had acceptable survival in WB control sediment at 28 d also had acceptable reproduction at 42 d, with a mean of 2.3 young/female. For the PE sediment, the only laboratory that had acceptable survival did not have acceptable young production, as only 0.08 young/female were obtained (Table 17.20).

Table 17.16 **Interlaboratory Comparison of Day 35 Percent Survival (Mean ± SD) of *H. azteca* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).**

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	98	(7.1)	96	(5.2)	96	(5.2)	98	(4.6)	NT[a]	
F	70[b]	(31.6)	73[b]	(31.5)	86[b]	(27.2)	33[b]	(38.5)	86[b]	(35.0)
H	95	(7.6)	96	(7.4)	95	(5.4)	NT		46	(26.7)
K	91	(18.1)	96	(5.2)	90	(10.7)	NT		NT	
L	78	(10.4)	83	(8.9)	84	(13.0)	73	(17.5)	57	(37.4)
N	93	(7.1)	88	(11.7)	83	(7.6)	NT		NT	
Q	94	(9.2)	86	(27.7)	88	(13.9)	93	(8.9)	88	(12.8)
U	95	(7.6)	98	(4.6)	86	(10.6)	NT		NT	
X	NT		NT		NT		74[c]	(16.9)	95[c]	(5.4)
N-1[d]	8		8		8		4		4	
Mean-1	89		89		88		74		69	
SD-1	9.8		8.9		4.9		21.0		30.0	
CV (%)1	11.0		10.0		5.6		30.4		40.1	
N-2[e]	7		7		7		3		3	
Mean-2	92		92		89		88		63	
SD-2	6.6		6.2		5.2		13.2		21.5	
CV (%) -2	7.2		6.7		5.9		15.1		34.0	

[a] NT = not tested

[b] Control survival below acceptable level of 80% in WB sediment at 28 d.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

17.6.6 Overall, nine laboratories participated in the DRR with *C. tentans* but not all laboratories tested all sediments. Mean survival (with CV in parentheses) for those laboratories that met the control criterion of ≥80% survival at 20 d was 85% (CV=5%) for WB sediment. In addition, mean survival at 28 d, in the FS was 86% (CV=14.4%) and, in the PE sediment was 75% (CV=13.9%) (Table 17.21). In total, 63% of the laboratories met the acceptability criterion for survival (≥70%) for the WB control sediment in the *C. tentans* test (Table 17.14). For laboratories reporting dry weights, the mean growth of *C. tentans* at 20 d (criterion of ≥0.60 mg/individual dry weight and ≥70% survival) was 1.45 mg (CV=58.6%) for WB sediment. In addition, mean growth (as dry weight) was 1.63 mg/individual (CV=20.9%) for the FS and 1.43 mg/individual (CV=47.9%) for the PE sediment (Table 17.22). For laboratories reporting weights as AFDW, the mean growth of *C. tentans* at 20 d (criterion of ≥0.48 mg/individual AFDW and ≥70% survival) was 0.81 mg (CV=53.3%) for WB, 1.05 mg/individual (CV=18.1%) for FS, and 0.64 mg/individual (CV=12.7%) for PE (Table 17.23). For growth as dry weight in the WB control sediment, 63% of the laboratories met the acceptability criterion for survival and growth (as dry weight) in the *C. tentans* test, while for AFDW, 67% of the laboratories met the test acceptability criterion of ≥0.48 mg/AFDW per individual (Table 17.14). Mean percent emergence for those laboratories that met the emergence criterion of ≥50% reported emergence in WB control sediment as 69.8% (CV=29.5%). In addition, mean emergence was 50.5% in FS (CV=68.6%) and 55.8% in PE (CV=30.3%) sediment (Table 17.24). In total 50% of the laboratories met the acceptability criterion for both 20-d survival and emergence in the WB control sediment (Table 17.14). The success rate for the number of eggs /case and the control survival criterion was 63% in WB. Mean number of eggs/female was 1118 eggs/case (CV=15.0%) in WB. The FS and PE sediments had 1024 eggs/case (CV=30.4%) and 867 eggs/case (CV=29.3%), respectively (Table 17.25). The mean percent hatch for laboratories with acceptable control survival and acceptable number of eggs/case was 90% (CV=10.8%) for WB control sediment (Table17.26), and 57% of the laboratories that tested these

Table 17.17 Interlaboratory Comparison of Day 42 Percent Survival (Mean ± SD) of *H. azteca* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	95	(7.6)	93	(7.1)	95	(5.4)	93	(8.9)	NT[a]	
F	61[b]	(31.8)	68[b]	(33.7)	85[b]	(26.7)	30[b]	(37.6)	83[b]	(33.7)
H	90	(9.3)	90	(9.3)	93	(8.9)	NT		40	(26.2)
K	91	(18.1)	96	(5.2)	88	(12.8)	NT		NT	
L	75	(10.7)	83	(8.9)	84	(13.0)	70	(16.0)	55	(36.7)
N	89	(8.4)	81	(17.3)	79	(10.7)	NT		NT	
Q	93	(11.7)	81	(30.9)	88	(13.9)	89	(13.6)	85	(16.0)
U	93	(8.9)	95	(5.4)	86	(10.6)	NT		NT	
X	NT		NT		NT		43[c]	(23.2)	84[c]	(9.2)
N-1[d]	8		8		8		4		4	
Mean-1	86		86		87		70		65	
SD-1	11.7		9.6		5.1		28.6		21.8	
CV (%)-1	13.6		11.2		5.8		40.7		33.3	
N-2[e]	7		7		7		3		3	
Mean-2	92		88		87		84		60	
SD-2	6.6		6.6		5.4		12.1		22.9	
CV (%)2	7.4		7.5		6.2		14.1		38.2	

[a] NT = not tested

[b] Control survival below acceptable level of 80% in WB sediment at 28 d.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV (%)-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

sediments met the test acceptability criteria for hatchability.

17.6.7 In total, the proportion of laboratories that met the various endpoint criteria in WB control sediment in the DRR was higher for *H. azteca* than it was for *C. tentans*. The most likely reason for the lower success with *C. tentans* in the DRR was the reduction in feeding rate (from 1.5 to 1.0 ml of Tetrafin/beaker/d) relative to the PRR. In the PRR with *C. tentans*, the proportion of laboratories meeting the various endpoint criteria was generally higher (see Table 17.14), particularly for post-pupation endpoints (emergence, reproduction, and percent hatch). Therefore, this manual recommends that the higher feeding rate of 1.5 ml/beaker/d be used in long-term tests with *C. tentans* (Section 15).

17.6.8 In the DRR, mean survival (CV in parentheses) of *H. azteca* in the LS sediment (contaminated with PAHs; using only values where the 28-d control survival criterion was met) was 91% (CV=7.5%) at 28 d, was 89% (CV=5.9%) at 35 d and 87% (CV=6.2%) at 42 d (Tables 17.15 to 17.17). Mean survival of *C. tentans* at 20 d in the LS sediment was 40% (CV=82.6%; Table 17.21). The growth of *H. azteca* in LS sediment resulted in a mean length of 4.37 mm (CV=10.1%; Table 17.18) and a mean dry weight of 0.31 mg/individual (CV=38.2%; Table 17.19). Mean growth of *C. tentans* in LS was 1.72 mg/individual (CV=66.2%) as dry weight (Table 17.22) and 2.31 mg/individual (CV=59.1%) as AFDW (Table 17.23). For both species, all growth endpoints were highest for LS relative to the other sediments evaluated, except for *H. azteca* dry weight which had a comparable mean as the other four sediments. The mean proportion of *C. tentans* larvae emerging from LS was 35.7% (CV=71.2%; Table 17.24). This value was roughly half of the emergence from the control sediments. Mean reproductive output of *H. azteca* in LS sediment, for those laboratories with acceptable control survival, was 3.08 young/female (CV of 41.0%; Table 17.20). The mean reproductive output of *C. tentans* in the LS sediment for laboratories that met the control survival criteria was 980 eggs/female (CV=20.1%; Table 17.25), which was similar to the WB, FS, and PE sediments. Mean percent hatch of *C. tentans* eggs was 94% (CV=6.5%) for the laboratories that met at least 70% control survival (Table 17.26).

Table 17.18. Interlaboratory Comparison of Day 28 Length (Mean mm/Individual ± SD) of *H. azteca* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	4.15	(0.23)	4.00	(0.11)	4.29	(0.16)	2.96	(0.03)	NT[a]	
F	3.02[b,c]	(0.28)	4.66[b,c]	(0.17)	5.23[b,c]	(0.41)	3.70[b,c]	(0.30)	5.03[b,c]	(0.06)
H	3.77	(0.32)	2.72	(0.14)	3.77	(0.17)	NT		2.40	(0.41)
K	4.18	(0.12)	4.39	(0.29)	4.95	(0.22)	NT		NT	
L	5.02	(0.11)	4.97	(0.27)	4.62	(0.40)	4.07	(0.39)	4.08	(0.64)
N	NR		NR		NR		NR		NR	
Q	3.11[c]	(0.10)	3.17[c]	(0.18)	4.29[c]	(0.45)	4.51[c]	(0.46)	3.27[c]	(0.03)
U	3.74	(0.08)	3.99	(0.17)	4.21	(0.13)	4.21	NA	NT	
X	NT		NT		NT		3.25[d]	(0.20)	3.35[d]	(0.21)
N-1[e]	7		7		7		5		4	
Mean-1	3.86		3.99		4.40		3.81		3.69	
SD-1	0.68		0.76		0.50		0.66		0.99	
CV (%)-1	17.8		20.1		10.9		17.3		30.4	
N-2[f]	5		5		5		2		2	
Mean-2	4.17		4.01		4.37		3.51		3.24	
SD-2	0.52		0.83		0.44		0.79		1.19	
CV (%)-2	12.4		20.6		10.1		22.6		36.6	

[a] NT = not tested; NR = not reported; NA = not applicable.

[b] Control survival below acceptable level of 80% in WB sediment at 28 d.

[c] Length below acceptable level of 3.2 mm in length in WB control sediment.

[d] Not included in any mean as WB control sediment was not tested.

[e] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[f] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

17.6.9 Across all laboratories that met the 28-d survival criterion of ≥80% for *H. azteca*, the mean survival in the contaminated CC sediment sample was 93% (CV=5.9%) at 28 d, 92% (CV=7.2%) at 35 d, and 88% at 42 d (CV=7.5%; Tables 17.15 to 17.17). Mean survival of *C. tentans* at 20 d for laboratories that met the 20-d control survival criteria was 75% (CV=30.9%; Table 17.21). In CC sediment, the mean growth of *H. azteca* was 4.01 mm (CV=20.6%) as length (Table 17.18) and 0.24 mg/individual (CV=75.2%) as dry weight (Table 17.19). Mean growth of *C. tentans* in CC sediment was 0.68 mg/individual (CV=66.0%) as dry weight (Table 17.22) and 0.37 mg/individual (CV=49.6%) as AFDW (Table 17.23). The growth was reduced about 50% in the CC sediment in comparison to the WB, FS, and PE sediments for *C. tentans* only. The mean proportion of *C. tentans* larvae to emerge from CC sediment was 38% (CV=60.5%; Table 17.24). Similar to the LS sediment sample, this emergence was reduced to about half of that observed in the control sediments. Mean reproductive output of *H. azteca* in CC sediment, for those laboratories with acceptable 28-d control survival, was 1.64 young/female (CV=103.3%) in contrast to the mean for WB of 3.13 young/female (CV=48.9%; Table 17.20). The mean reproductive output of *C. tentans* eggs in the CC sediment for laboratories that met the 20-d control survival criteria was 621 eggs/female (CV=52.4%) (Table 17.25) which was the lowest egg production for all sediments, which averaged between 404-1194 eggs/female. The mean percent hatch of *C. tentans* eggs was 69% (CV=49.5%) for the laboratories that met at least 70% control survival (Table 17.26); all other sediments had percent hatches for survival averaging 90 to 94%.

17.6.10 For the chronic *H. azteca* test, the mean MDD for survival relative to the WB control sediment for the CC sediment across all laboratories was only 7.7% (2.4 to 19.5%) at 28 d and 12.8% (6.4 to 28l.7%) at day 42. The MDDs for survival of amphipods were also small in the LS sediment: 10.8% (3.3 to 26%) at 28 d and 11.5% (5.7 to 26%) at 42 d. The mean MDDs relative to WB control sediment were also low for the 28-d amphipod weights as the mean MDD for the CC sediment relative to WB control sediment was 0.06 mg (0.04 to 0.14 mg) and the mean MDD

Table 17.19. Interlaboratory Comparison of Day 28 Dry Weight (Mean mg/Individual ± SD) of *H. azteca* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	0.29	(0.04)	0.23	(0.02)	0.34	(0.07)	0.12	(0.02)	NT[a]	
F	0.01[b,c]	(0.01)	0.49[b,c]	(0.04)	0.78[b,c]	(0.18)	0.11[b,c]	(0.15)	0.73[b,c]	(0.10)
H	0.25	(0.06)	0.10	(0)	0.20	(0)	NT		0.15	(0.06)
K	0.31	(0.04)	0.56	(0.05)	0.58	(0.09)	NT		NT	
L	0.36	(0.04)	0.41	(0.07)	0.32	(0.12)	0.40	(0.10)	0.24	(0.05)
N	0.23	(0.10)	0.09	(0.03)	0.25	(0.09)	NT		NT	
Q	0.16	(0.04)	0.09	(0.01)	0.31	(0.09)	0.39	(0.06)	0.13	(0.01)
U	0.19	(0.02)	0.21	(0.03)	0.27	(0.04)	NT		NT	
X	NT		NT		NT		0.22[d]	(0.17)	0.42[d]	(0.37)
N-1[e]	8		8		8		4		4	
Mean-1	0.22		0.27		0.38		0.23		0.31	
SD-1	0.11		0.19		0.20		0.16		0.28	
CV (%)-1	48.8		69.6		52.1		71.2		90.0	
N-2[f]	7		7		7		3		3	
Mean-2	0.25		0.24		0.31		0.30		0.18	
SD-2	0.07		0.18		0.12		0.21		0.06	
CV (%)-2	27.8		75.2		38.2		68.6		34.0	

[a] NT = not tested.

[b] Control survival below acceptable level of 80% in WB sediment at 28 d.

[c] Weight below test acceptable criteria of 0.15 mg/organism in WB control sediment.

[d] Not included in any mean as WB control sediment was not tested.

[e] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[f] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

for length was 0.26 mm (0.18 to 0.33 mm). The mean MDD for LS sediment for amphipod growth as weight was 0.10 mg (0.05 to 0.16 mg) and length of 0.33 mm (0.14 to 0.44 mm). The mean MDD for the mean number of young per female was 1.92 (0.09 to 2.4) in CC sediment and 2.06 (0.57 to 3.1) in LS sediment relative to WB control sediment.

17.6.11 The summary of the MDDs relative to the WB control sediment for CC and LS samples and the chronic *C. tentans* test is discussed by endpoint. For percent survival at 20 d, the mean MDDs relative to WB control sediment for CC and LS sediments were 14.4% (range of 5.9 to 19.1%) and 15.6% (5.8 to 25.3%), respectively. For 20 d dry weights, the mean MDDs were 24.9% (CC) and 64.2% (LS) with ranges of 15.6 to 30.4% and 25.1 to 126.9%, respectively. The mean MDD and range for the AFDW relative to the WB control sediment was 29.9% (22.9 to 44.6%) for the CC sediment and 68.7% (22.9 to 125.0%) for LS sediment. For emergence the mean MDD for the CC sediment was 19.4% (10.5 to 25.0%) and the mean LS MDD was 17.9 (8.2 to 23.0%). The number of eggs produced had a mean MDD relative to the WB control sediment of 19.4% (11.0 to 29.3%) for the CC sediment and 24.4% (11.9 to 37.4%) for LS sediment, while hatch had a mean MDD of 42.2% (7.4 to 77.3%) for the CC sediment and 30.5% for LS sediment (9.3 to 53.7%).

17.6.12 These chronic round-robin tests exhibited similar or better precision compared to many chemical analyses and effluent toxicity test methods (USEPA, 1991a; USEPA, 1991c). The success rate for test initiation and completion of the USEPA's round-robin evaluations is a good indication that a well equipped and trained staff will be able to successfully conduct these tests. These are very important considerations for any test performed routinely in any regulatory program.

Table 17.20 Interlaboratory Comparison of Reproduction (Mean Number of Young/Female ± SD) of *H. azteca* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	5.7	(3.1)	4.2	(2.2)	4.2	(1.6)	2.3	(2.9)	NT[a]	
F	4.0[b]	(4.7)	7.5[b]	(7.6)	19.4[b]	(4.4)	5.4[b]	(2.1)	16.5[b]	(9.4)
H	2.3	(2.6)	0.3	(0.2)	1.2	(1.3)	NT		0.08	(1.8)
K	3.3	(1.9)	1.2	(1.4)	4.1	(4.5)	NT		NT	
L	NA[a]		NA		NA		NA		NA	
N	2.0	(1.5)	0.2	(0.7)	2.2	(1.3)	NT		NT	
Q	0.09[c]	(0.1)	0.04[c]	(0.04)	0.6[c]	(0.9)	0.2[c]	(0.2)	0.3[c]	(0.4)
U	2.4	(1.5)	2.4	(1.7)	3.5		NT		NT	
X	NT		NT		NT		0.12[d]	(0.73)	0.5[d]	(0.7)
N-1[e]	7		7		7		3		3	
Mean-1	2.8		2.2		5.0		2.6		5.9	
SD-1	1.8		2.7		6.5		2.7		9.2	
CV (%)-1	62.6		121.6		128.2		100.5		157.3	
N-2[f]	5		5		5		1		1	
Mean-2	3.13		1.64		3.08		2.3		0.08	
SD-2	1.53		1.69		1.27		--		--	
CV (%)-2	48.9		103.3		41.0		--		--	

[a] NT = not tested; NA = not applicable: young count not reported per female.

[b] Survival below test acceptable criteria in WB control sediment at 28 d.

[c] Reproduction below test acceptable criteria in WB control sediment of 2 young/female.

[d] Not included in any mean as WB control sediment was not tested.

[e] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[f] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

Table 17.21 **Interlaboratory Comparison of Day 20 Percent Survival (Mean ± SD) of *C. tentans* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).**

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	94	(8)	98	(4)	19	(13)	94	(8)	NT[a]	
F	79	(16)	40	(4)	17	(7)	81	(8)	65	(10)
H	44[b]	(4)	69[b]	(21)	42[b]	(23)	40[b]	(10)	NT	
I	54[b]	(8)	44[b]	(14)	15[b]	(12)	NT		56[b]	(10)
K	79	(14)	74	(7)	58	(15)	NT		NT	
N	48[b]	(14)	50[b]	(18)	60[b]	(21)	NT		NT	
Q	77	(8)	69	(10)	16	(4)	71	(11)	75	(18)
V	98	(4)	94	(8)	90	(4)	98	(4)	85	(14)
X	NT		NT		NT		75[c]	(30)	63[c]	(5)
N-1[d]	8		8		8		5		4	
Mean-1	72		67		40		77		71	
SD-1	20.6		21.7		28.0		23.2		12.9	
CV (%)-1	28.7		32.3		70.6		30.2		18.3	
N-2[e]	5		5		5		4		3	
Mean-2	85		75		40		86		75	
SD-2	9.8		23.2		33.1		12.4		10.5	
CV (%)-2	11.5		30.9		82.6		14.4		13.9	

[a] NT = not tested.

[b] Survival below test acceptable criteria of 70% in WB control sediment at 20 d.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

Table 17.22 **Interlaboratory Comparison of Dry Weight (Mean mg/Individual ± SD) of *C. tentans* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).**

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	1.16	(0.09)	0.71	(0.17)	0.83	(0.32)	1.85	(0.76)	NT[a]	
F	0.94	(0.28)	0.33	(0.07)	3.49	(1.23)	1.84	(0.30)	1.15	(0.19)
H	2.18[b]	(0.13)	0.88[b]	(0.22)	2.85[b]	(0.58)	2.43[b]	(0.30)	NT	
I	1.96[b]	(0.49)	2.00[b]	(0.84)	2.31[b]	(1.17)	NT		2.65	(1.49)
K	1.45	(0.32)	0.71	(0.16)	2.05	(0.29)	NT		NT	
N	1.33[b]	(0.91)	0.99[b]	(0.63)	1.39[b]	(0.66)	NT		NT	
Q	0.79	(0.25)	0.26	(0.04)	1.57	(0.60)	1.13	(0.24)	0.93	(0.45)
V	2.90	(0.73)	1.39	(0.34)	0.66	(0.24)	1.71	(0.52)	2.21	(0.38)
X	NT		NT		NT		1.41[c]	(0.26)	1.83[c]	(0.23)
N-1[d]	8		8		8		5		4	
Mean-1	1.59		0.91		1.89		1.79		1.74	
SD-1	0.71		0.57		0.98		0.46		0.83	
CV (%)-1	44.7		62.6		51.6		25.8		47.7	
N-2[e]	5		5		5		4		3	
Mean-2	1.45		0.68		1.72		1.63		1.43	
SD-2	0.85		0.45		1.14		0.34		0.68	
CV (%)-2	58.6		66.0		66.2		20.9		47.9	

[a] NT = not tested.

[b] Survival below test acceptable criteria of 70% in WB control sediment at 20 d.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

Note: All dry weight measurements for WB sediment were above the acceptable level of 0.6 mg/organism as dry weight.

Table 17.23 Interlaboratory Comparison of Ash-free Dry Weight (Mean mg/Individual ± SD) of *C. tentans* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	0.87	(0.12)	0.54	(0.17)	4.22	(1.80)	1.13	(0.31)	NT[a]	
F	0.65	(0.18)	0.22	(0.03)	2.38	(0.84)	1.18	(0.20)	0.69	(0.19)
H	1.74[b]	(0.13)	0.69[b]	(0.19)	1.93[b]	(0.43)	1.89[b]	(0.40)	NT	
I	NM [a]		NM		NM		NM		NM	
K	1.16	(0.28)	0.51	(0.09)	1.44	(0.29)	NT		NT	
N	0.78[b]	(0.31)	0.99[b]	(0.48)	0.71[b]	(0.47)	NT		NT	
Q	0.57	(0.27)	0.20	(0.03)	1.20	(0.50)	0.83	(0.15)	0.58	(0.26)
V	NM		NM		NM		NM		NM	
X	NT		NT		NT		0.30[c]	(0.04)	0.53[c]	(0.11)
N-1[d]	6		6		6		4		2	
Mean-1	0.96		0.53		1.98		1.26		0.64	
SD-1	0.43		0.30		1.24		0.58		0.08	
CV (%)-1	45.0		56.7		62.6		35.7		12.2	
N-2[e]	4		4		4		3		2	
Mean-2	0.81		0.37		2.31		1.05		0.64	
SD-2	0.43		0.18		1.36		0.19		0.08	
CV (%)-2	53.3		49.6		59.1		18.1		12.7	

[a] NT = not tested; NM = not measured.

[b] Survival below test acceptable criteria of 70% in WB control sediment at 20 d.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

Note: All dry weight measurements for WB sediment above acceptable level of 0.48 mg/organism as AFDW.

Table 17.24 Interlaboratory Comparison of Percent Emergence (Mean ± SD) of *C. tentans* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	65.6	(14.4)	41.7	(19.9)	18.8	(18.8)	75	(21.8)	NT[a]	
F	20.8[b]	(7.7)	5.2[b]	(8.8)	12.5[b]	(16.6)	29.2[b]	(14.1)	31.2[b]	(15.3)
H	28.2[b,c]	(8.9)	28.2[b,c]	(13.3)	46.9[b,c]	(15.4)	26.0[b,c]	(14.4)	NT	
I	11.8[b,c]	(12.0)	22.9[b,c]	(19.2)	5.6[b,c]	(4.1)	NT		8.3[b,c]	(10.7)
K	57.3	(18.6)	24.0	(13.7)	49.0	(10.4)	NT		NT	
N	30.2[b,c]	(17.8)	11.5[b,c]	(6.2)	32.3[b,c]	(10.4)	NT		NT	
Q	56.3	(13.9)	16.7	(10.0)	10.4	(8.6)	26.0	(14.3)	43.8	(20.8)
V	100	(0)	67.7	(16.9)	64.6	(13.2)	NT		67.7	(9.4)
X	NT		NT		NT		46.5[d]	(20.2)	50.7[d]	(24.2)
N-1[e]	8		8		8		4		4	
Mean-1	46.3		27.2		30.0		39.1		37.8	
SD-1	29.1		19.7		21.6		24.0		24.8	
CV (%)-1	62.8		72.4		71.9		61.5		65.7	
N-2[f]	4		4		4		2		2	
Mean-2	69.8		37.5		35.7		50.5		55.8	
SD-2	20.6		22.7		25.4		34.6		16.9	
CV (%)-2	29.5		60.5		71.2		68.6		30.3	

[a] NT = not tested.

[b] Emergence below test acceptable criteria of 50% in WB control sediment.

[c] Survival below test acceptable criteria of 70% in WB control sediment at 20 d.

[d] Not included in any mean as WB control sediment was not tested.

[e] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[f] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

Table 17.25 Interlaboratory Comparison of the Number of Eggs/Female (Mean ± SD) in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	1258	(429)	523	(124)	1025	(366)	1260	(178)	NT[a]	
F	998	(243)	444	NA	722	(711)	671	(133)	721	(200)
H	1397[b]	(408)	919[b]	(306)	1069[b]	(580)	995[b]	(615)	NT	
I	1261[b]	(225)	538[b]	(117)	NT		NT		988[b]	(290)
K	1023	(177)	538	(117)	835	(86)	NT		NT	
N	1047[b]	(410)	484[b]	(345)	728[b]	(479)	NT		NT	
Q	978	(168)	404	(204)	1190	(126)	1141	(391)	720	(105)
V	1333	(227)	1194	(63)	1127	(191)	NT		1160	(120)
X	NT		NT		NT		828[c]	(286)	827[c]	(214)
N-1[d]	8		8		7		5		5	
Mean-1	1162		631		951		1017		897	
SD-1	168		277		193		255		216	
CV (%)-1	14.4		43.9		20.1		25.1		24.1	
N-2[e]	5		5		5		3		4	
Mean-2	1118		621		980		1024		867	
SD-2	168		325		197		311		254	
CV (%)-2	15.0		52.4		20.1		30.4		29.3	

[a] NT = not tested; NA = not applicable.

[b] Survival below test acceptable criteria of 70% in WB control sediment at 20 d.

[c] Not included in any mean as WB control sediment was not tested.

[d] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[e] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

Note: The number of eggs acceptable criteria (≥800 eggs) was above acceptable level for all laboratories in WB sediment.

Table 17.26 **Interlaboratory Comparison of Percent Hatch (Mean ± SD) of *C. tentans* in a Long-term Sediment Exposure Using Five Sediments (WB = West Bearskin, CC = Cole Creek, LS = Little Scioto River, FS = Formulated Sediment (using alpha-cellulose as organic carbon source), and PE = Formulated Sediment (using peat moss as organic carbon source)).**

	Sediment									
Laboratory	WB		CC		LS		FS		PE	
E	80	(17.0)	37	(33.0)	51	(39.0)	77	(16.1)	NT	
F	99	(0.2)	97	NA	99	NA	97	(2.3)	99	(0.4)
H	93[b]	(3.5)	80[b]	(24.6)	71[b]	(36.5)	74[b]	(49.2)	NT	
I	NM[a]		NM		NM		NM		NM	
K	62[c]	(23.5)	78[c]	(38.5)	74[c]	(14.0)	NT		NT	
N	68[b,c]	(35.8)	47[b,c]	(47.3)	54[b,c]	(40.8)	NT		NT	
Q	80	(35.2)	31	(53.3)	95	(3.2)	89	(19.4)	88	(18.3)
V	91	(8.4)	81	(33.0)	87	(10.8)	NT		96	(1.7)
X	NT		NT		NT		60[d]	(44.0)	80[d]	(27.1)
N-1[e]	7		7		7		4		3	
Mean-1	82		64		76		84		94	
SD-1	13.5		25.6		18.9		10.7		6.0	
CV (%)-1	16.6		39.8		24.9		12.7		6.4	
N-2[f]	4		4		4		3		3	
Mean-2	90		69		94		93		94	
SD-2	9.7		34.3		6.1		5.5		6.0	
CV (%)-2	10.8		49.5		6.5		5.9		6.4	

[a] NT = not tested; NM = not measured; NA = not applicable.

[b] Survival below test acceptable criteria of 70% in WB control sediment at 20 d.

[c] Hatch below test acceptable criteria of 80% in WB control sediment.

[d] Not included in any mean as WB control sediment was not tested.

[e] N-1, Mean-1, SD1 and CV (%)-1 include all data (except Laboratory X) whether control met acceptable limits or not in WB sediment.

[f] N-2, Mean-2, SD-2 and CV-2 include only data for sediment samples from laboratories that met the 28-d control survival performance acceptability criteria in WB sediment.

Section 18
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Appendix A
Exposure Systems

A.1 Renewal of overlying water is recommended during sediment tests (Section 11.3, 12.3, 13.3, 14.3, 15.3). The overlying water can be replaced manually (e.g., siphoning) or automatically. Automatic systems require more equipment and initially take more time to build, but manual addition of water takes more time during a test. In addition, automated systems generally result in less suspension of sediment compared to manual renewal of water.

A.2 At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%. Mount and Brungs (1967) diluters have been modified for sediment testing, and other diluter systems have also been used (Maki, 1977; Ingersoll and Nelson, 1990; Benoit et al., 1993; Zumwalt et al., 1994; Brunson et al., 1998; Wall et al., 1998; Leppanen and Maier, 1998). The water-delivery system should be calibrated before a test is started to verify that the system is functioning properly. Renewal of overlying water is started on Day -1 before the addition of test organisms or food on Day 0. Water-delivery systems are described by Benoit et al. (1993) in Section A.3 and by Zumwalt et al. (1994) in Section A.4. A 60-mL syringe with a mesh screen over the end can be used to manually remove and replace overlying water (J. Lazorchak, USEPA, Cincinnati, OH, personal communication).

A.3 Benoit et al. (1993) describe a sediment testing intermittent-renewal (STIR) system (stationary or portable) for invertebrate toxicity testing with sediment. The STIR system has been used to conduct both short-term and long-term sediment toxicity tests with amphipods and midges (Sections 11, 12, 14, 15). Either stationary or portable systems enable the maintenance of acceptable water quality (e.g., dissolved oxygen) by automatically renewing overlying water in sediment tests at rates ranging from 1 to 21 volume renewals/d. The STIR system not only reduces the labor associated with renewal of overlying water but also affords a gentle exchange of water that results in virtually no sediment suspension. Both gravity-operated systems can be installed in a compact vented enclosure. The STIR system has been used for conducting 10-d whole-sediment tests with *Chironomus tentans, Hyalella azteca* and *Lumbriculus variegatus.*

A.3.1 STIR systems described in Benoit et al. (1982) can be modified to conduct sediment tests and at the same time maintain their original capacity to deliver varying concentrations of toxicants for water-only toxicity tests. A STIR system (stationary or portable) solely for sediment toxicity tests was designed, which offers a simple, inexpensive approach for the automated renewal of variable amounts of overlying water (Figures A.1 and A.2). This system is described below. The system can be built as a two-unit system (Section A.3.2) or with more exposure treatments (Section A.3.4). All exposure systems consist of exposure holding tanks, head tanks, head tank support stands, and a water bath (Section A.3.2 and A.3.3). The automated delivery system includes design descriptions for a support stand, water renewal supply, and water-delivery apparatus (Section A.3.4).

A.3.2 Two-unit Portable STIR System Construction (Figures A.1 and A.2)

A.3.2.1 Exposure Holding Tanks (2) (Figure A.3).

1. Outer diameter: 15.8 cm wide x 29.3 cm long x 11.7 cm high

2. Cutting dimensions: (double-strength glass, 3 mm)

2 Bottoms:	15.8 cm x 29.3 cm
4 Sides:	11.4 cm x 28.7 cm
4 Ends:	11.4 cm x 15.8 cm

3. Hole: 1.6 cm centered between sides and 7.2 cm from bottom edge of 11.4 cm high end piece.

4. Standpipe Height: 10.3 cm above inside of tank bottom.

A.3.2.2 Head Tanks (2) (4-L capacity; Figure A.3)

1. Outer diameter: 15.8 cm wide x 24 cm long x 14.5 cm high

2. Cutting dimensions: (acrylic plastic, 6 mm)

2 Bottoms:	15.8 cm x 24 cm
4 Sides:	13.9 cm x 22.8 cm
4 Ends:	13.9 cm x 15.8 cm

3. Acrylic plastic sheets should be cut with a smooth cutting fine toothed table saw blade. Dimension cut pieces can most easily be glued together with Weld-On® #16 clear-thickened cement for acrylic

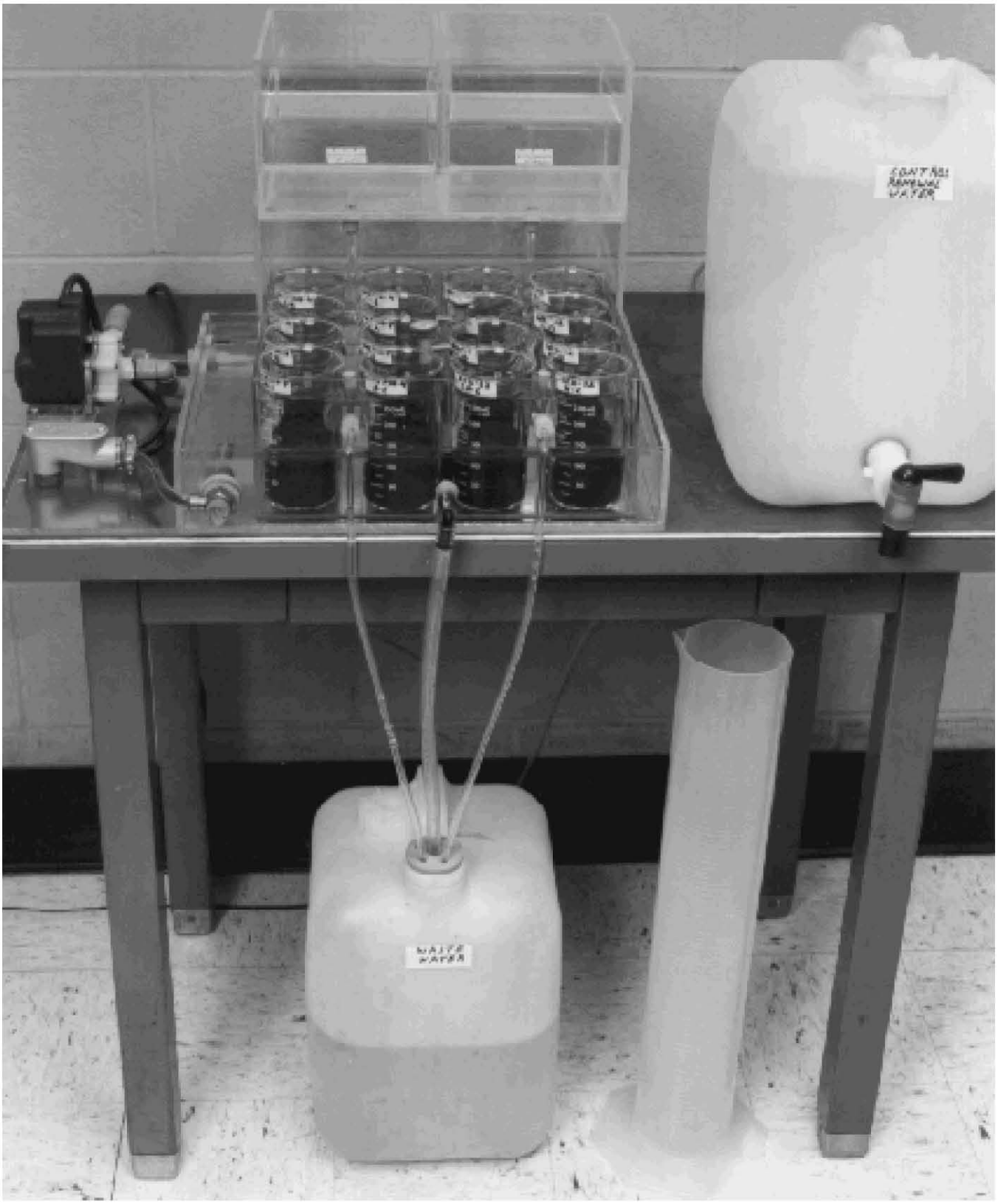


Figure A.1 Portable table top STIR system described in Benoit et al. (1993).

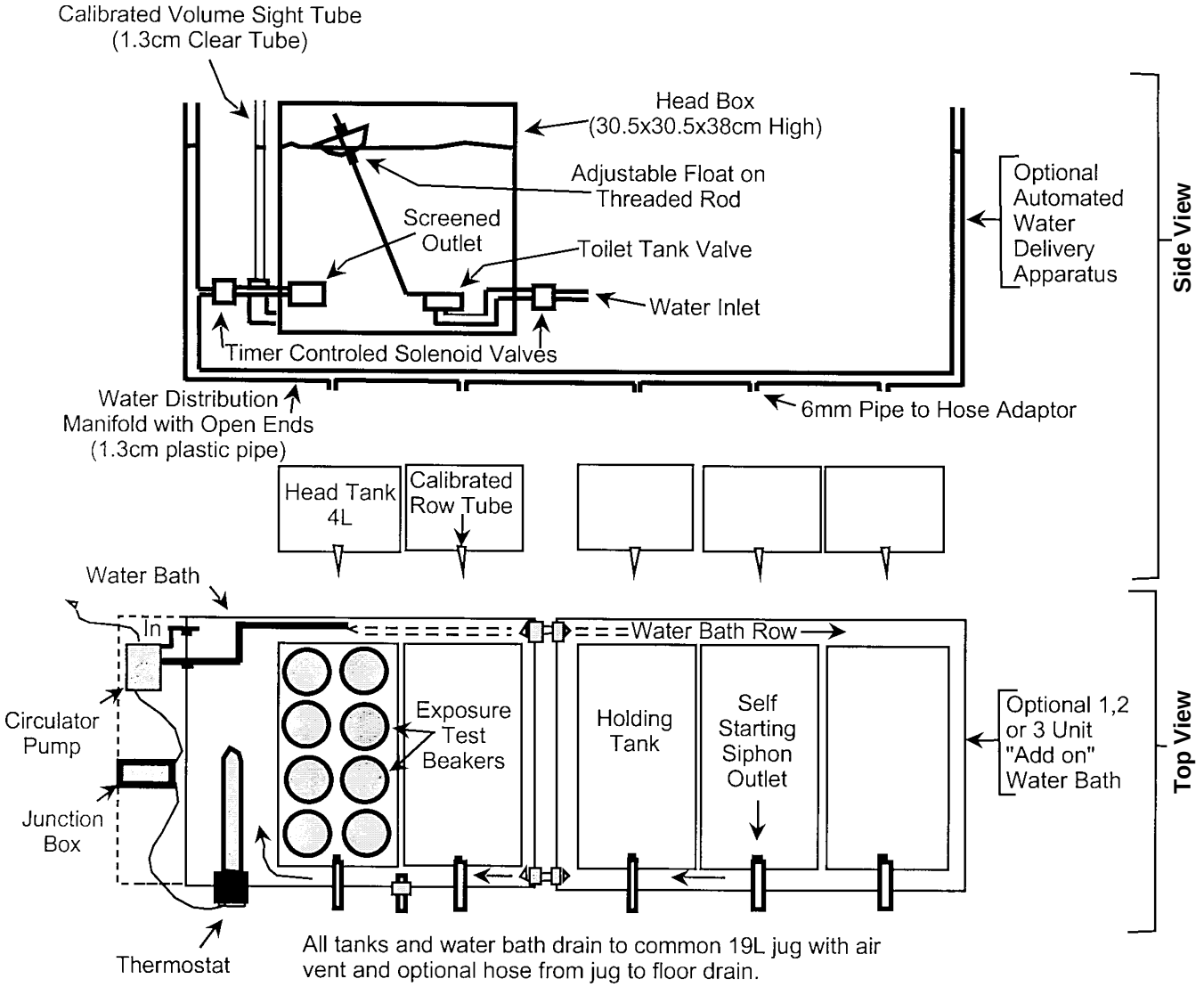


Figure A.2 Portable table top STIR system with several additional options as described in Benoit et al. (1993).

plastic (Industrial Polychemical Service, P.O. Box 471, Gardena, CA, 90247).

4. Hole: 1.6 cm centered between sides and 2 cm from front edge of 24-cm-long bottom piece. Holes can most easily be drilled in acrylic plastic by using a wood spade bit and drill press.

5. Flow Tubes: 10-mL pipet tip initially cut off at the 6-mL mark and inserted flush with top of #0 stopper. Top of stopper should be inserted nearly flush with head tank bottom. With 2 L of water in head tank, calibrate flow tube to deliver 32 mL/min.

A.3.2.3 Head Tank Support Stand *(1) (Figure A.3)*

1. Outer diameter: 16.7 cm wide x 33.7 cm long x 17.8 cm high

2. Cutting dimensions: (acrylic plastic, 6 mm)

1 Bottom:	16.7 cm x 33.7 cm
2 Sides:	17.2 cm x 32.5 cm
2 Ends:	17.2 cm x 16.7 cm

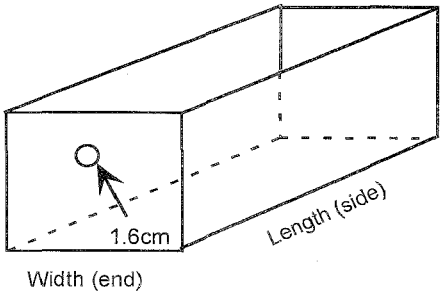
3. Size is such that both head tanks fit into support stand for storage and transport.

A.3.2.4 Water Bath *(1) (Figure A.3)*

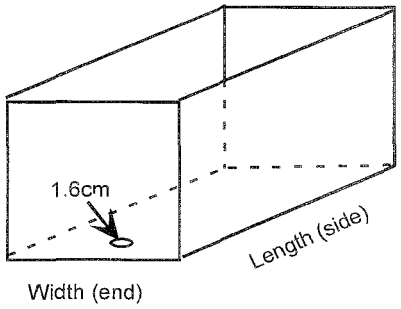
1. Outer diameter: 33 cm wide x 40.6 cm long x 7.4 cm high

2. Cutting dimensions: (acrylic plastic, 6 mm)

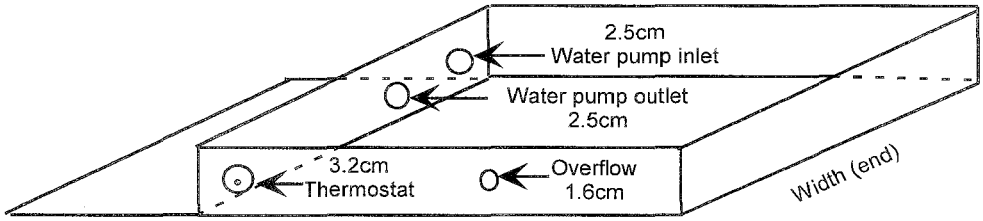
1 Bottom:	33 cm x 55.9 cm
2 Ends:	33 cm x 6.8 cm
2 Sides:	39.4 cm x 6.8 cm



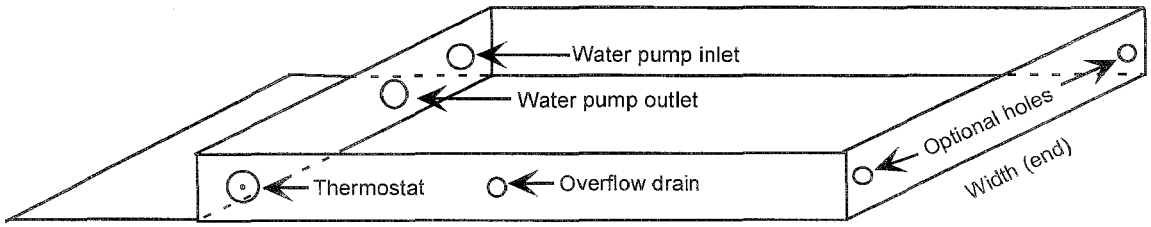
Exposure Holding Tank



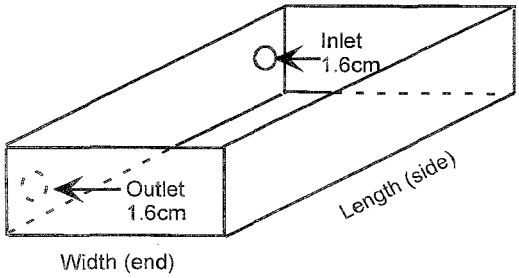
Head Tank



Basic Water Bath



Basic Water Bath with Optional Holes for Water Bath



Add-on Water Bath for One Additional Unit

Figure A.3 Tanks for the STIR system in Benoit et al. (1993).

3. Holes:
    a. Overflow drain; 1.6 cm centered 2.9 cm from bottom edge of 39.4-cm-long side piece and 17.8 cm from right edge.
    b. Thermostat; 3.2 cm centered 2.5 cm from bottom edge of 39.4-cm-long side piece and 3.2 cm from left edge.
    c. Water pump outlet; 2.5 cm centered 2.5 cm from bottom edge of 33-cm-long end piece and 8.3 cm from back edge.
    d. Water pump inlet; 2.5 cm centered 2.5 cm from bottom edge of 33-cm-long end piece and 2.0 cm from back edge.
4. A small 90° elbow made of glass or plastic is attached to the water pump inlet tube and turned downward so the circulator pump will not pick up air at the water surface.
5. The bottom piece for the water bath includes 15.3-cm extension for motor mount and the thermostat electrical junction box.
6. Motor Mount: 5.1 cm wide x 11.4 cm long x 3.8 cm thick mount made from 6 pieces of 6-mm acrylic plastic. Four of these pieces are glued together. The other two pieces are glued together, motor attached to the edge with two screws and the two pieces (with motor attached) are then screwed to the top of the four pieces. The entire unit is then glued to water bath extension after 6-mm PVC piping is attached and secured with stoppers to the inlet and outlet water bath holes.
7. Thermostat Conduit Junction Box: (1.3-cm small left back (SLB)) is attached to the water bath extension by screwing a 1.3-cm PVC plug into junction box and securing this plug with a screw, countersunk up through the bottom and into the PVC plug.

A.3.2.5 Latex Rubber Mold

A.3.2.5.1 If you plan to construct a substantial number of exposure test beakers, as described in Benoit et al. (1993), then it would be to your advantage to make a latex rubber mold to give support to the underside of the glass when drilling holes. It significantly reduces the number of broken beakers. Liquid latex, with hardener that can be purchased from the local hardware store is commonly used to coat the handles of tools. The rubber mold is constructed as follows:

1. Mix latex with hardener as per instructions.
2. Fill one exposure test beaker with the mixture.
3. Suspend one 5-cm eye bolt (5-mm diameter) with nut on end so that the eye is protruding just above the top of the mixture.
4. Allow the latex plenty of time to "set up."
5. With proper eye protection and wearing heavy gloves, gently break the beaker with a small hammer and remove all of the glass from the mold.
6. Using a long drill bit for wood, drill an air vent hole through the mold from top through bottom.
7. When using the mold, wet the mold and the beaker with water before inserting. Place the beaker, with pre-marked location of holes, on its side in a 3.5-L stainless steel pan filled with coolant water so that the beaker is just below the surface. The beaker is then held in position with one hand while the other hand operates the drill press. Operator should wear proper eye protection.
8. After the two holes are drilled, the mold can be easily removed, with some effort, by inserting the eye bolt into the handle of a securely attached "C" clamp and physically pulling the beaker from the mold.

A.3.3 Suggested Options for More Exposure Treatments (examples given are for a three-unit treatment system)

A.3.3.1 Exposure Holding Tanks and Head Tanks

A.3.3.1.1 Same dimensions as for two-unit system except that three (3) of each should be made.

A.3.3.2 Head Tank Support Stand (1) (Figure A.3)

1. Outer diameter: 16.7 cm wide x 49.5 cm long x 17.8 cm high
2. Cutting dimensions: (acrylic plastic, 6 mm)

1 Bottom:	16.7 cm x 49.5 cm
2 Sides:	17.2 cm x 48.3 cm
2 Ends:	17.2 cm x 16.7 cm

3. Size is such that the three head tanks will fit into the support stand for storage and transport.

A.3.3.3 Water Bath (1) (Figure A.3)

1. Outer diameter: 33 cm wide x 56.4 cm long x 7.4 cm high
2. Cutting dimensions: (acrylic plastic, 6 mm)

1 Bottom:	33 cm x 71.7 cm
2 Ends:	33 cm x 6.8 cm
2 Sides:	55 cm x 6.8 cm

3. Holes: All hole sizes and locations are the same as for the two-unit system except that overflow drain is located 25.7 cm from right edge of 55-cm side. Also, two optional 1.6-cm holes centered 2.5 cm from bottom edge of 33-cm-long end piece and 1.8 cm

from corner edges are shown in the drawing for future additions of "add-on" water baths.

4. Motor mount and junction box installations are the same as for two-unit system.

A.3.3.4 "Add-on" Water Bath (example given is for one additional unit treatment system; Figure A.3)

1. Outer diameter: 18.5 cm wide x 33 cm long x 8 cm high

2. Cutting dimensions: (acrylic plastic, 6 mm)

1 Bottom:	18.5 cm x 33 cm
2 Ends:	17.3 cm x 7.4 cm
2 Sides:	33 cm x 7.4 cm

3. Holes: Inlet and outlet holes (1.6 cm) are centered 2.5 cm from bottom edge of 33-cm long side piece and 1.8 cm from corner edges.

4. The above holes will match the previously drilled holes in the main water bath. The "add-on" water bath is connected using #2 stoppers and 6.4-cm lengths of clear plastic tubing (1.3-cm diameter). The circulator pump outlet tubing (Tygon®) in the main water bath is extended through the inlet connection as shown in Figure A.2. Circulating water is then forced into the "add-on" bath and flows back to the main water bath by gravity.

5. Note that the walls of the "add-on" bath are 6 mm higher than the main water bath to accommodate the small head of water that builds up.

6. "Add-on" water baths tend to run a little warmer (0.2°C) than main water bath test temperatures.

A.3.4 Optional Automated Water-delivery Apparatus for Table Top STIR Systems (examples given are for a three-unit treatment system)

A.3.4.1 Support Stand

A.3.4.1.1 A stand to support the automated water-delivery apparatus, shown in Figure A.2, can be made from bolted slotted angle iron bolted with corner braces. A convenient size to construct is 30 cm wide x 85 cm long x 43 cm high. The head box in Figure A.2 sits on top of the stand, and the water distribution manifold as shown in Figure A.2 is placed directly under the top of the stand with two 1.3-cm conduit hangers. A small portion of each angle iron cross piece is cut away to allow the pipe to be clamped into the conduit hanger. This also keeps the manifold up high enough for sufficient clearance between the head tanks and the 6-mm pipe to hose adapters as shown in Figure A.2.

A.3.4.2 Water Renewal Supply

A.3.4.2.1 If tests will be conducted in the local water supply, then the head box water inlet shown in Figure A.2 is simply plumbed into the supply line. However, if the tests are conducted with transported water or with reconstituted water, the head box water inlet can be connected to a Nalgene® drum with flexible Tygon® tubing. With a four-volume test beaker water renewal flow rate per day, both 114-L and 208-L Nalgene® drums will hold a 5-d supply for a 3-unit treatment system and a 5-unit treatment system, respectively. If the water supply drum is located below the head box, then an open air water pump such as a March® model MDXT pump (PFC Equipment Corp., Minneapolis, MN 55440) can be used between the drum and head box.

A.3.4.3 Operation of Water-delivery Apparatus

A.3.4.3.1 The head box water inlet solenoid valve (Figure A.2) and the open air water pump (if needed) are connected to the same timer control switch. The head box water outlet solenoid valve is connected to another separate timer control switch. With four test beaker renewals/d and a 3-unit treatment system, the head box toilet float valve is pre-adjusted to allow the head box to fill to the 12-L mark on the sight tube (Figure A.2).

A.3.4.3.2 With head box filled, the renewal cycle begins when the first timer opens the head box outlet solenoid valve. The distribution manifold is quickly flooded and the 12 L of renewal water divided equally to each of the three 4-L head tanks. Since the timers have a minimum setting of one hour on-off periods, the first timer is set to shut off the head box outlet solenoid valve one hour after it opens.

A.3.4.3.3 About 30 min later, the second timer is set to open the head box water inlet solenoid valve (and pump if needed). As head box water volume reaches the 12-L mark, the pre-adjusted toilet tank valve stops the water flow. One hour after they come on, the second timer will shut off the solenoid valve inlet and water pump.

A.3.4.3.4 The automated system is then ready for the next renewal cycle that is set to begin 12 h after the first cycle. Head box volume dimensions are such that up to five-unit treatment systems can be tested simultaneously as shown in Figure A.2.

A.3.5 A criticism of the system described by Benoit et al. (1993) is that the (up to) 8 beakers placed in each holding tank are not true replicates because of the potential for exchange of water overlying the sediments among the beakers. However, this concern is largely semantic with regard to actual test results. The rationale for this position is described below. The data described below are unpublished data from USEPA Duluth (G.T. Ankley, USEPA, Duluth, MN, personal communication).

A.3.5.1 Beakers within a test tank should contain an aliquot of the *same* homogenized sediment and the same test species. The replication is intended to reflect variability

in the biology (e.g., health) of the organism, as well as placement and recovery of the animals from the test sediments (i.e., operator variability). To treat even completely separate tanks containing homogenized sediment from the same source as true replicates (of the sediment "treatment") is inaccurate and is pseudoreplication. Hence, because the same sediment is tested in each beaker in a particular tank, and because the replication is focused on defining variability in the biology of the organism (and the operator), this is essentially a nonissue from a theoretical standpoint.

A.3.5.2 From a practical standpoint, it is important to determine the potential influence of one beaker on another over the course of a test. To determine this, a study was designed (which is not advocated) in which treatments were mixed within a tank. In the first experiment, four beakers of highly metal-contaminated sediment from the Keweenaw Waterway, MI, were placed in the same tank as four beakers containing clean sediment from West Bearskin Lake, MN. This was done in two tanks; in one tank, 10 amphipods *(Hyalella azteca)* were added to each beaker, while in the other tank, 10 midges *(Chironomus tentans)* were placed in each beaker. Controls for the experiment consisted of the West Bearskin sediments assayed in separate "clean" tanks. The four contaminated beakers were placed "upstream" of the four clean beakers to attempt to maximize possible exchange of contaminant. At the end of the test, organism survival (and growth for *C. tentans)* was measured in two of the beakers from each site and sediment Cu concentrations were determined in the other two beakers from each site. The Keweenaw sediments contained concentrations of Cu in excess of 9,000 µg/g (dry wt), and were toxic to both test species (Table A.1). Conversely, survival of both *C. tentans* and *H. azteca* was high in the West Bearskin sediments from the Keweenaw tank, and was similar to survival in West Bearskin sediments held in separate tanks. Most important, there was no apparent increase in Cu concentrations in the West Bearskin sediments held in the Keweenaw tank (Table A.1).

A.3.5.3 A similar design was used to determine transfer of contaminants among beakers containing sediments spiked with the organochlorine pesticide dieldrin. In this experiment, sediment from Airport Pond, MN, was spiked with dieldrin and placed in the same tank as clean unspiked Airport Pond sediments. Two different concentrations were assayed as follows: (1) in the midge test, sediment concentrations were about 150 µg dieldrin/g (dry weight) and (2) in the amphipod test, sediments contained in excess of 450 µg dieldrin/g sediment. The control for the experiment again consisted of clean Airport Pond sediment held in a separate tank. The spiked sediments were toxic to both test species, and survival of organisms held in the clean Airport Pond sediments was similar in the two different tanks. However, there was an effect on the growth of *C. tentans* from the clean Airport Pond sediment assayed in the tank containing the spiked sediment. This corresponded to the presence of measurable dieldrin concentrations in unspiked Airport Pond sediments in the tank with the mixed treatments (Table A.2). The concentrations of dieldrin in the unspiked sediment, although detectable, were on the order of 5,000-fold lower than the spiked sediments, indicating relatively minimal transfer of pesticide.

A.3.5.4 Using a similar design, an investigation was made to evaluate if extremely low dissolved oxygen (DO) concentrations, due to sediment oxygen demand, in four beakers in a test system would result in a decrease in DO in other beakers in the tank. In this experiment, trout chow was added to each of four beakers containing clean Pequaywan Lake sediment, and placed in a test tank with four beakers containing Pequaywan Lake sediment without exogenous organic carbon. Again, the control consisted of Pequaywan Lake sediment held in a separate tank under otherwise identical test conditions. Assays were conducted, without organisms, for 10 d. At this time, DO concentrations were very low in the beakers containing trout chow-amended sediment (ca., 1 mg/L, n = 4). However, overlying water DO concentrations in the

Table A.1 Sediment Copper Concentrations and Organism Survival and Growth at the End of a 10-d Test with West Bearskin Sediment in an Individual Tank Versus 10-d Cu Concentrations and Organism Survival and Growth in West Bearskin Sediment Tested in the Same Tank as Keweenaw Waterway Sediment[1]

Sediment	Tank	Species	Survival (%)	Dry wt (mg/organism)	Cu (µg/g)
WB[2]	1	Amphipod	90	ND[3]	22.4
WB	2	Amphipod	100	ND	13.8
KW[4]	2	Amphipod	20	ND	9397.0
WB	3	Midge	95	1.34	12.3
WB	4	Midge	100	1.33	15.6
KW	4	Midge	5	ND	9167.0

[1] All values are the mean of duplicate observations (G.T. Ankley, USEPA, Duluth, MN, unpublished data)
[2] West Bearskin
[3] Not determined
[4] Keweenaw Waterway

Table A.2 Sediment Dieldrin Concentrations and Organism Survival and Growth at the End of a 10-d Test with Airport Pond Sediment in an Individual Tank Versus 10-d Dieldrin Concentrations and Organism Survival and Growth in Airport Pond Sediment Tested in the Same Tank as Dieldren-spiked Airport Pond Sediment[1]

Sediment	Tank	Species	Survival (%)	Dry wt (mg/organism)	Dieldrin (µg/g)
AP[2]	1	Amphipod	75	ND[3]	<0.01
AP	2	Amphipod	80	ND	0.07
DAP[4]	2	Amphipod	20	ND	446.4
AP	3	Midge	85	1.71	<0.01
AP	4	Midge	85	0.13	0.04
DAP	4	Midge	0	ND	151.9

[1] All values are the mean of duplicate observations (G.T. Ankley, USEPA, Duluth, MN, unpublished data)
[2] Airport Pond
[3] Not determined
[4] Dieldren-spiked Airport Pond

"untreated" vs. the "treated" beakers in a separate tank were similar, i.e., 6.8 vs. 6.9 mg/L, respectively. This indicates that from a practical standpoint, even under extreme conditions of mixed treatments (which again, is *not* recommended), interaction between beakers within a tank is minimal.

A.3.5.5 One final observation germane to this issue is worth noting. If indeed beakers of homogenized sediment within a test tank do not serve as suitable replicates, this should be manifested by a lack of variability among beakers with regard to biological assay results. This has not proven to be the case. For example, in a recent amphipod test with a homogenized sediment from the Keweenaw Waterway in which all eight replicates were held in the same tank, mean survival for the test was 76%; however, survival in the various beakers ranged from 30 to 100%, with a standard deviation of 21%. Clearly, if the test system were biased so as to reduce variability (i.e., result in unsuitable replicates due to common overlying water), this type of result would not be expected.

A.3.5.6 In summary, in both a theoretical and practical sense, use of the system described by Benoit et al. (1993) results in valid replicates that enable the evaluation of variability due to factors related to differences in organism biology and operator effects. To achieve this, it is important that treatments not be mixed within a tank; rather, the replicates should be generated from the same sediment sample. Given this, and the fact that it is difficult to document interaction between beakers using even unrealistic (and unrecommended) designs, leads to the conclusion that variability of replicates from the test system can be validly used for hypothesis testing.

A.4 Zumwalt et al. (1994) also describe a water-delivery system that can accurately deliver small volumes of water (50 mL/cycle) to eight 300-mL beakers to conduct sediment tests. The system was designed to be comparable with the system described by Benoit et al. (1993). This water-delivery system has been used in a variety of applications (i.e., Kemble et al., 1998a,b; Ingersoll et al., 1998).

A.4.1 Eight 35-mL polypropylene syringes equipped with 18-gauge needles are suspended from a splitting chamber (Figure A.4). The system is suspended above eight beakers and about 1 L of water/cycle is delivered manually or automatically to the splitting chamber. Each syringe fills and empties 50 mL into each beaker and the 600 mL of excess water empties out an overflow in the splitting chamber (Section A.4.3.1). The volume of water delivered per day can be adjusted by changing either the cycling rate or the size of the syringes. The system has been used to renew overlying water in whole-sediment toxicity tests with *H. azteca* and *C. tentans*. Variation in delivery of water among 24 beakers was less than 5%. The system is inexpensive (<$100), easy to build (<8 h), and easy to calibrate (<15 min).

A.4.2 Water-Splitting Chamber

A.4.2.1 The glass water-splitting chamber is 14.5 cm wide, 30 cm long, and 6.5 cm high (inner diameter). Eight 3.8-cm holes and one 2.5-cm hole are drilled in a 15.5 cm x 30.5 cm glass bottom before assembly (Figure A.4 and Table A.3). The glass bottom is made from 4.8- (3/16 inch) or 6.4-mm (1/4 inch) plate glass. An easy way to position the 3.8-cm holes is to place the eight 300-mL beakers (2 wide x 4 long) under the bottom plate and mark the center of each beaker. The 2.5-cm hole for overflow is centered at one end of the bottom plate between the last two holes and endplate (Figure A.4). After drilling the holes in the bottom plate, the side (6.5 x 30.5 cm) and end (6.5 x 14.5 cm) plates are cut from 3.2-mm (1/8 inch) double-strength glass and the splitting box is assembled using silicone adhesive. Sharp glass edges should be sanded smooth using a whetstone or a piece of carborundum wheel. After the splitting chamber has dried for 24 h, four 12-mm (outer diameter) stainless-steel tubes (7 cm long) are glued to each corner of the splitting chamber (the surface of the steel tubes is scored with rough emery paper to allow better adhesion of the silicone). These tubes are used as sleeves for attaching the legs to the splitting chamber. The legs of the splitting chamber are threaded stainless-steel rods (9.5 mm [3/8 inch] diameter, 36 cm long). The location of the tubes depends on the way that the beakers are to be accessed in the waterbath. If the tubes are placed on the side of the splitting chamber, a 3.2-mm-thick x 2-cm-wide x 7-cm-long spacer is required so beakers and the optional waterbath can be slid out the ends (Figure A.4). If the sleeves and legs are attached to the ends of the splitting chamber, the beakers and waterbath can be removed from the side. The legs are inserted into the 12-mm tubes and secured using nylon nuts or wingnuts. The distance between the tips of the needles to the surface of the water in the 300-mL beakers is about 2 cm. Four 1-L beakers could also be placed under the splitting chamber.

A.4.2.2 A #7 silicone stopper drilled with a 21-mm (outer diameter) core borer is used to hold each 35-mL polypropylene syringe (45 mL total capacity) in place. Glass syringes could be used if adsorption of contaminants on the surface of the syringe is of concern. A dilute soap solution can be used to help slide the syringe into the #7 stopper (until the end of the syringe is flush with the top of stopper). Stoppers and syringes are inserted into 3.8-cm holes and are visually leveled. A #5 silicone stopper drilled with an 8-mm (outer diameter) core borer is placed in the 2.5 cm overflow hole. An 8-mm (outer diameter) glass tube (7.5 cm long) is inserted into the stopper. Only 3 mm of the overflow tube should be left exposed above the stopper. This overflow drain is placed about 3 mm lower than the top of the syringes. A short piece of 6.4-mm (1/4 inch; inner diameter) tubing can be placed on the lower end of drain to collect excess water from the overflow.

A.4.2.3 The splitting chamber is leveled by placing a level on top of the chamber and adjusting the nylon nuts. Eighteen-gauge needles are attached to the syringes.

Figure A.4 Water splitting chamber described in Zumwalt et al. (1994).

Table A.3 Materials Needed for Constructing a Zumwalt et al. (1994) Delivery System

Equipment
Drill press
Glass drill bits (2.54 cm [1 inch] and 3.8 cm [1.5 inch])
Cork boring set
Table-top saw equipped with a carborundum wheel
Small level (about 30 cm long)
Supplies
300-mL beakers (lipless, tall form; e.g., Pyrex Model 1040)
Stainless-steel screen (50- x 50-mesh)
9.5-mm (3/8 inch x 16) stainless-steel threaded rod
9.5-mm (3/8 inch x 16) nylon wingnuts
9.5-mm (3/8 inch x 16) nylon nuts
35-mL Mono-ject syringes (Sherwood Medical, St. Louis, MO)
18-gauge Mono-ject stainless-steel hypodermic needles
Silicone stoppers (#0, 5, and 7)
Plate glass (6.4 mm [1/4 inch], 4.8 mm [3/16 inch], 3.2 mm [1/8 inch])
Glass tubing (8-mm outer diameter)
Stainless-steel tubing (12-mm outer diameter)
Silicone adhesive (without fungicide)
5-way stainless-steel gang valves and Pasteur pipets (14.5 cm [5.75 inch])

About 6 mm of the needle should remain after the sharp tip has been cut off using a carborundum wheel. Jagged edges left in the bore of the needle can be smoothed using a small sewing needle or stainless-steel wire.

A.4.2.4 When about 1 L of water is delivered to the splitting chamber, the top of each syringe should be quickly covered with water. The overflow tube will quickly drain excess water to a level just below the tops of the syringes. The syringes should empty completely in about 4 min. If water remains in a syringe, the needle should be checked to ensure that it is clean and does not have any jagged edges.

A.4.3 Calibration and Delivery of Water to the Splitting Chamber

A.4.3.1 Flow adjustments can be made by sliding either the stoppers or syringes up or down to deliver more or less water. A splitting chamber with eight syringes can be calibrated in less than 15 min. Delivery of water to the splitting chamber can be as simple as manually adding about 1 L of water/cycle. Water can be added automatically to the splitting chamber using a single cell or a Mount and Brungs (1967) diluter that delivers about 1 L/cycle on a time delay. About 50 mL will be delivered to each of the 8 beakers/cycle and 600 mL will flow out the overflow. A minimum of about 1 L/cycle should be dumped into the splitting chamber to ensure each syringe fills to the top. If the quantity of water is limited at a laboratory, the excess water that drains through the overflow can be collected and recycled.

A.4.4 Waterbath and Exposure Beakers

A.4.4.1 The optional waterbath surrounding the beakers is made from 3.2-mm (1/8-inch) double-strength glass and is 15.8 cm wide x 29.5 cm long x 11.7 cm high (Figure A.4 [Figure A.3 in the Benoit et al., 1993 system]). Before the pieces are assembled, a 1.4-cm hole is drilled in one of the end pieces. The hole is 7.2 cm from the bottom and centered between each side of the end piece. A glass tube inserted through a #0 silicone stopper can be used to drain water from the waterbath. A notch is made in each 300-mL beaker by making two cuts with a carborundum wheel 1.9 cm apart to the 275 mL level. The beaker is etched across the bottom of the cuts, gently tapped to remove the cut section, and the notch is covered with 50- x 50-mesh stainless-steel screen using silicone adhesive. The waterbath illustrated in Figure A.4 is optional if the splitting chambers and beakers are placed in a larger waterbath to collect waste water. This smaller waterbath could be used to collect waste water and a surrounding larger waterbath could be used for temperature control.

A.4.5 Operation and Maintenance

A.4.5.1 Maintenance of the system is minimal. The syringes should be checked daily to make sure that all of the water is emptying with each cycle. As long as the syringe empties completely, the rate of flow out of the syringes is not important because a set volume of water is delivered from each syringe. If the syringe does not empty completely with each cycle, the needle tip should be replaced or cleaned with a thin wire or sewing needle. If the screens on the beakers need to be cleaned, a toothbrush can be used to brush the outside of screens.

A.4.5.2 Overlying water can be aerated by suspending Pasteur pipets (e.g., Pyrex disposable 14.5-cm [5.75 inch] length) about 3 cm above the sediment surface in the beakers. Five-way stainless-steel gang valves are suspended from the splitting chamber using stainless-steel hooks. Latex tubing (3.2-mm [1/8 inch] inner diameter) is used to connect valves and pipets. Flow rate of air should be maintained at about 2 to 3 bubbles/s and the pipets can be placed on the outside of the beakers when samples of overlying water are taken during a test.

A.4.5.3 The splitting chambers were used to deliver water in a toxicity test with the midge *Chironomus tentans* exposed to metal-contaminated sediments (Zumwalt et al., 1994). Ten third-instar midges were exposed in 300-mL beakers containing 100 mL of sediment and 175 mL of overlying water at 23°C. Midges in each beaker received a daily suspension of 4 mg Tetrafin® flake food and survival and growth were measured after 10 d. Splitting chambers delivered 50 mL/cycle of overlying water to each of the eight replicate beakers/sediment sample. One liter of water was delivered with a single-cell diluter to each splitting chamber 4 times/d. This cycle rate resulted in 1.1 volume additions of overlying water/d to each beaker ([4 cycles/d x 50-mL volume/cycle]/175 mL of overlying water). The variation in delivery of water between 24 beakers was less than 5%.

A.4.5.4 Hardness, alkalinity, and conductivity in water overlying the sediments averaged about 20% higher than inflowing water. These water-quality characteristics tended to be more similar to inflowing water at the end of the

exposure compared with the beginning of the exposure. The average pH was about 0.3 units lower than inflowing water. Ammonia in overlying water ranged from 0.20 to 0.83 mg/L. The dissolved oxygen content was about 1 mg/L lower than inflowing water at the beginning of the exposure and was about 2 to 3 mg/L lower than inflowing water by the end of the exposure. Survival and growth of midges were reduced with exposure to metal-contaminated sediments. Water delivered at a similar rate to a second set of beakers using a system described by Benoit et al. (1993) resulted in similar overlying water quality and similar toxic effects on midges.

A.4.5.5 The system has been used to deliver 33 ‰ salt water to exposure chambers for 10 d. Precipitation of salts on the tips of the needles reduced flow from the syringes. Use of a larger bore needle (16-gauge) reduced clogging problems; however, daily brushing of the needle tips is required. Use of larger bore needles with 300-mL beakers containing 100 mL of sediment and 175 mL of overlying water results in some suspension of sediment in the overlying water. This suspension of sediment can be eliminated if the stream of water from the larger bore needle falls on a baffle (e.g., a piece of glass) at the surface of the water in the beaker.

A.5 Brunson et al. (1998) describe a water-delivery system for use with larger exposure chambers in the *Lumbriculus variegatus* sediment exposures (Section 13). Exposures of oligochaetes by Brunson et al. (1998) were conducted for 28 d in 4-L glass beakers containing 1 L of sediment and 3 L of overlying water. Four replicate chambers were tested for each sediment sample evaluated. Each beaker was calibrated to 4 L using a glass standpipe that exited through the beaker wall and was held in place with a silicon stopper. Beakers received 2 volume additions (6 L) of overlying water per day. Water was delivered using a modified Mount and Brungs diluter system that was designed to deliver 1 L/cycle (Ingersoll and Nelson, 1990). An in-line flow splitter was attached to each delivery line to split the water flow evenly to each of four beakers. These splitters were constructed of 1/4 inch PVC pipe with four silicone stoppers and 14-gauge stainless-steel hypodermic needles with the points and connector ends cut off the needles (Figure A.5). Glass stands were used to support the splitters, keeping them level to maintain a constant volume delivery to each beaker (+ 5%).

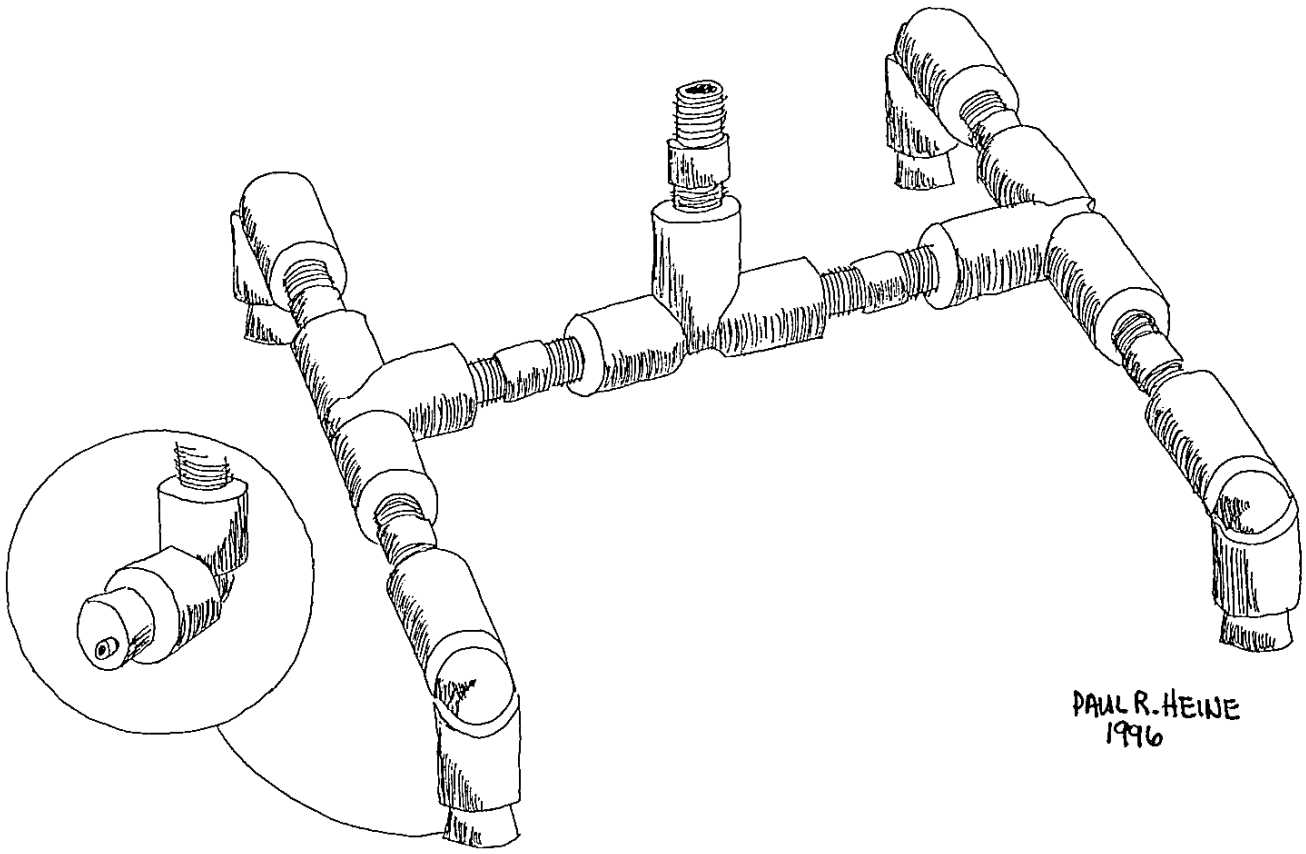


Figure A.5. Diagram of in-line flow splitter used to deliver overlying water in the sediment exposures of *Lumbriculus variegatus* (Brunson et al., 1998).

Appendix B
Food Preparation

B.1 Yeast, Cerophyl®, and Trout Chow (YCT) for Feeding the Cultures and *Hyalella azteca*

B.1.1 Food should be stored at 4°C and used within two weeks from preparation; however, once prepared, YCT can be frozen until use.

B.1.2 Digested trout chow is prepared as follows:

1. Preparation of trout chow requires one week. Use 1/8 inch pellets prepared according to current U.S. Fish and Wildlife Service specifications. Suppliers of trout chow include Zeigler Bros., Inc., P.O. Box 95, Gardners, PA, 17324 (717/780-9009); Glencoe Mills, 1011 Elliott, Glencoe, MN, 55336 (320/864-3181); and Murray Elevators, 118 West 4800 South, Murray, UT 84107 (800/521-9092).

2. Add 5.0 g of trout chow pellets to 1 L of deionized water. Mix well in a blender and pour into a 2-L separatory funnel or similar container. Digest before use by aerating continuously from the bottom of the vessel for one week at ambient laboratory temperature. Water lost due to evaporation is replaced during digestion. Because of the offensive odor usually produced during digestion, the vessel should be placed in a ventilated area.

3. At the end of the digestion period, allow material to settle for a minimum of 1 h. Filter the supernatant through a fine mesh screen (e.g., Nitex® 110 mesh). Combine with equal volumes of the supernatant from Cerophyl® and yeast preparation (below). The supernatant can be used fresh, or it can be frozen until use. Discard the remaining particulate material.

B.1.3 Yeast is prepared as follows:

1. Add 5.0 g of dry yeast, such as Fleishmann's® Yeast, Lake State Kosher Certified Yeast, or equivalent, to 1 L of deionized water.

2. Stir with a magnetic stirrer, shake vigorously by hand, or mix with a blender at low speed, until the yeast is well dispersed.

3. Combine the yeast suspension immediately (do not allow to settle) with equal volumes of supernatant from the trout chow (above) and Cerophyl® preparations (below). Discard excess material.

B.1.4 Cerophyl® is prepared as follows:

1. Place 5.0 g of dried, powdered cereal or alfalfa leaves, or rabbit pellets, in a blender. Cereal leaves are available as "Cereal Leaves" from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO, 63178 (800/325-3010); or as Cerophyl®, from Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, NY, 14692-9012 (716/359-2502). Dried, powdered alfalfa leaves may be obtained from health food stores, and rabbit pellets are available at pet shops.

2. Add 1 L of deionized water.

3. Mix in a blender at high speed for 5 min, or stir overnight at medium speed on a magnetic stir plate.

4. If a blender is used to suspend the material, place in a refrigerator overnight to settle. If a magnetic stirrer is used, allow to settle for 1 h. Decant the supernatant and combine with equal volumes of supernatant from trout chow and yeast preparations (above). Discard excess material.

B.1.5 Combined yeast-Cerophyl-trout chow (YCT) is mixed as follows:

1. Thoroughly mix equal (e.g., 300 mL) volumes of the three foods as described above.

2. Place aliquots of the mixture in small (50 mL to 100 mL) screw-cap plastic bottles.

3. Freshly prepared food can be used immediately, or it can be frozen until needed. Thawed food is stored in the refrigerator between feedings and is used for a maximum of two weeks. Do not store YCT frozen over three months.

4. It is advisable to measure the dry weight of solids in each batch of YCT before use. The food should contain 1.7 to 1.9 g solids/L.

B.2 Algal Food

B.2.1 Starter cultures of the green algae, *Selenastrum capricornutum* are available from the following sources: American Type Culture Collection (Culture No. ATCC 22662), 12301 Parklawn Drive, Rockville, MD 10852, or Culture Collection of Algae, Botany Department, University of Texas, Austin, TX 78712.

B.2.2 Algal Culture Medium for the green algae is prepared as follows (USEPA, 1993a):

1. Prepare stock nutrient solutions using reagent grade chemicals as described in Table B.1.

Table B.1 Nutrient Stock Solutions for Maintaining Algal Stock Cultures

Stock solution	Compound	Amount dissolved in 500 mL deionized water
1. Macronutrients		
A.	$MgCl_2•6H_2O$	6.08 g
	$CaCl_2•2H_2O$	2.20 g
	$NaNO_3$	12.75 g
B.	$MgSO_4•7H_2O$	7.35 g
C.	K_2HPO_4	0.522 g
D.	$NaHCO_3$	7.50 g
2. Micronutrients		
	H_3BO_3	92.8 mg
	$MnCl_2•4H_2O$	208.0 mg
	$ZnCl_2$	l.64 mg[1]
	$FeCl_3•6H_2O$	79.9 mg
	$CoCl_2•6H_2O$	0.714 mg[2]
	$Na_2MoO_4•2H_2O$	3.63 mg[3]
	$CuCl_2•2H_2O$	0.006 mg[4]
	$Na_2EDTA•2H_2O$	l50.0 mg
	Na_2SeO_4	1.196 mg[5]

[1]$ZnCl_2$—Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to micronutrient stock.

[2]$CoCl_2•6H_2O$—Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to micronutrient stock.

[3]$Na_2MoO_4•2H_2O$—Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to micronutrient stock.

[4]$CuCl_2•2H_2O$—Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to micronutrient stock.

[5]Na_2SeO_4—Weigh out 119.6 mg and dilute to 100 mL. Add 1 mL of this solution to micronutrient stock.

2. Add 1 mL of each stock solution, in the order listed in Table B.1, to about 900 mL of deionized water. Mix well after the addition of each solution. Dilute to 1 L, mix well. The final concentration of macronutrients and micronutrients in the culture medium is listed in Table B.2.

3. Immediately filter the medium through a 0.45 µm pore diameter membrane at a vacuum of not more than 380 mm (15 in.) mercury, or at a pressure of not more than one-half atmosphere (8 psi). Wash the filter with 500 mL deionized water before use.

4. If the filtration is carried out with sterile apparatus, the filtered medium can be used immediately, and no further sterilization steps are required before the inoculation of the medium. The medium can also be sterilized by autoclaving after it is placed in the culture vessels. Unused sterile medium should not be stored more than one week before use, because there may be substantial loss of water by evaporation.

B.2.3 Algal Cultures

B.2.3.1 Two types of algal cultures are maintained: (1) stock cultures and (2) “food” cultures.

Table B.2 Final Concentration of Macronutrients and Micronutrients in the Algal Culture Medium

Macronutrient	Concentration (mg/L)	Element	Concentration (mg/L)
$NaNO_3$	25.5	N	4.20
$MgCl_2•6H_2O$	12.2	Mg	2.90
$CaCl_2•2H_2O$	4.41	Ca	1.20
$MgSO_4•7H_2O$	14.7	S	1.91
K_2HPO_4	1.04	P	0.186
$NaHCO_3$	15.0	Na	11.0
		K	0.469
		C	2.14

Micronutrient	Concentration (µg/L)	Element	Concentration (µg/L)
H_3BO_3	185	B	32.5
$MnCl_2•4H_2O$	416	Mn	115
$ZnCl_2$	3.27	Zn	1.57
$CoCl_2•6H_2O$	1.43	Co	0.354
$CuCl_2•2H_2O$	0.012	Cu	0.004
$Na_2MoO_4•2H_2O$	7.26	Mo	2.88
$FeCl_3•6H_2O$	160	Fe	33.1
$Na_2EDTA•2H_2O$	300	—	—
Na_2SeO_4	2.39	Se	0.91

B.2.3.2 Establishing and Maintaining Stock Cultures of Algae

1. Upon receipt of the "starter" culture of *S. capricornutum* (usually about 10 mL), a stock culture is started by aseptically transferring 1 mL to each of several 250-mL culture flasks containing 100 mL algal culture medium (prepared as described above). The remainder of the starter culture can be held in reserve for up to six months in a refrigerator (in the dark) at 4°C.

2. The stock cultures are used as a source of algae to initiate "food" cultures. The volume of stock culture maintained at any one time will depend on the amount of algal food required for culture. Stock culture volume may be rapidly "scaled up" to several liters using 4-L serum bottles or similar vessels containing 3 L of growth medium.

3. Culture temperature is not critical. Stock cultures may be maintained at 25°C in environmental chambers with cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of about 4300 lux).

4. Cultures are mixed twice daily by hand.

5. Stock cultures can be held in the refrigerator until used to start "food" cultures, or can be transferred to new medium weekly. One to 3 mL of 7-d-old algal stock culture, containing about 1.5 X 10^6 cells/mL are transferred to each 100 mL of fresh culture medium. The inoculum should provide an initial cell density of about 10,000 to 30,000 cells/mL in the new stock cultures. Aseptic techniques should be used in maintaining the stock algal cultures, and care should be exercised to avoid contamination by other microorganisms.

6. Stock cultures should be examined microscopically weekly at transfer for microbial contamination. Reserve quantities of culture organisms can be maintained for 6 to 12 months if stored in the dark at 4°C. It is advisable to prepare new stock cultures from "starter" cultures obtained from established outside sources of organisms every four to six months.

B.2.3.3 Establishing and Maintaining "S. capricornutum Food" Cultures

1. "*S. capricornutum* food" cultures are started 7 d before use. About 20 mL of 7-d-old algal stock culture (described in the previous paragraph), containing 1.5 X 10^6 cells/mL are added to each liter of fresh algal culture medium (e.g., 3 L of medium in a 4-L bottle or 18 L in a 20-L bottle). The inoculum should provide an initial cell density of about 30,000 cells/mL. Aseptic techniques should be used in preparing and maintaining the cultures, and care should be exercised to avoid contamination by other microorganisms. However, sterility of food cultures is not as critical as in stock cultures because the food cultures are used in 7 to 10 d. A one-month supply of algal food can be grown at one time and stored in the refrigerator.

2. Food cultures may be maintained at 25°C in environmental chambers with the algal stock cultures or cultures of other organisms if the illumination is adequate (continuous "cool-white" fluorescent lighting of about 4300 lux).

3. Cultures are mixed continuously on a magnetic stir plate (with a medium size stir bar), in a moderately aerated separatory funnel, or are manually mixed twice daily. If the cultures are placed on a magnetic stir plate, heat generated by the stirrer might elevate the culture temperature several degrees. Caution should be taken to prevent the culture temperature from rising more than 2 to 3°C.

B.2.3.4 Preparing Algal Concentrate of S. capricornutum for Use as Food

1. An algal concentrate of *S. capricornutum* containing 3.0 to 3.5 X 10^7 cells/mL is prepared from food cultures by centrifuging the algae with a plankton or bucket-type centrifuge, or by allowing the cultures to settle in a refrigerator for at least one week and siphoning off the supernatant.

2. The cell density (cells/mL) in the concentrate is measured with an electronic particle counter, microscope and hemocytometer, fluorometer, or spectrophotometer and used to determine the dilution (or further concentration) required to achieve a final cell count of 3.0 to 3.5 X 10^7 cells/mL.

3. Assuming a cell density of about 1.5 X 10^6 cells/mL in the algal food cultures at 7 d, and 100% recovery in the concentration process, a 3-L culture at 7 to 10 d will provide 4.5 X 10^9 algal cells.

4. Algal concentrate can be stored in the refrigerator for one month.

5. Cultures of *Hyalella azteca* are fed 10 mL/L on renewal/harvest days and 5 mL/L on all other days (USEPA, 1993c).

B.2.3.5 Cell Counts

1. Several types of automatic electronic and optical particle counters are available to rapidly count cell number (cells/mL) and mean cell volume (MCV; μm^3/cell). The Coulter Counter is widely used and is discussed in detail in USEPA (1978). When the Coulter Counter is used, an aliquot

(usually 1 mL) of the test culture is diluted 10X to 20X with a 1% sodium chloride electrolyte solution, such as Coulter ISOTON®, to facilitate counting. The resulting dilution is counted using an aperture tube with a 100-µm diameter aperture. Each cell (particle) passing through the aperture causes a voltage drop proportional to its volume. Depending on the model, the instrument stores the information on the number of particles and the volume of each, and calculates the mean cell volume. The following procedure is used:

A. Mix the algal culture in the flask thoroughly by swirling the contents of the flask about six times in a clockwise direction, and then six times in the reverse direction; repeat the two-step process at least once.

B. At the end of the mixing process, stop the motion of the liquid in the flask with a strong brief reverse mixing action, and quickly remove 1 mL of cell culture from the flask with a sterile pipet.

C. Place the aliquot in a counting beaker, and add 9 mL (or 19 mL) of electrolyte solution (such as Coulter ISOTON®).

D. Determine the cell density (and MCV, if desired).

2. Manual microscope counting methods for cell counts are determined using a Sedgwick-Rafter, Palmer-Maloney, hemocytometer, inverted microscope, or similar methods. For details on microscope counting methods, see APHA (1992) and USEPA (1973). Whenever feasible, 400 cells per replicate are counted to obtain ±10% precision at the 95% confidence level. This method has the advantage of allowing for the direct examination of the condition of the cells.

B.3 Tetrafin® Food (or Other Fish Flake Food) for Culturing and Testing *Chironomus tentans*

B.3.1 Food should be stored at 4°C and used within two weeks from preparation or can be frozen until use. If it is frozen, it should be reblended, once thawed, to break up any clumps

1. Blend the Tetrafin® food in deionized water for 1 to 3 min or until very finely ground.

2. Filter slurry through an #110 Nitex screen to remove large particles. Place aliquot of food in 100- to 500-mL screw-top plastic bottles. It is desirable to determine dry weight of solids in each batch of food before use. Food should be held for no longer than two weeks at 4°C. Food can be frozen before use, but it is desirable to use fresh food.

3. Tetrafin® food is added to each culture chamber to provide about 0.04 mg dry solids/mL of culture water. A stock suspension of the solids is prepared in culture water such that a total volume of 5.0 mL of food suspension is added daily to each culture chamber. For example, if a culture chamber volume is 8 L, 300 mg of food would be added daily by adding 5 mL of a 56 g/L stock suspension (USEPA, 1993).

4. In a sediment test, Tetrafin® food (4.0 g/L) is added at 1.5 mL daily to each test chamber.

Appendix C
Supplies and Equipment for Conducting the *Chironomus tentans* Life-cycle Sediment Toxicity Test

C.1 General

C.1.1 Section 15 outlines the methods for conducting a *Chironomus tentans* life-cycle sediment toxicity test. This Appendix describes the equipment needed to conduct this test.

C.2 Emergence Traps (Figure C.1)

C.2.1 These traps are needed from Day 20 to the end of the test. These traps fit on the top of the lipless glass beakers with the narrow end up. These are 5-ounce plastic cups with 14-mesh nylon screen glued to the cup in place of the plastic bottom.

C.3 Reproduction/Oviposit Chambers (R/O; Figure C.2)

C.3.1 These R/O chambers use emergence traps and are needed once adults begin to emerge. Emergence traps are used to store adults collected daily, and are placed in a 100- X 20-mm petri dish that contains about 50 mL of overlying water. When emergence occurs, the emergence traps containing adults are removed and placed onto a petri dish. At least one male for each emergent female is added, and the R/O chamber (Figure C.2) is placed back into the test system or into environmental chambers maintained at the appropriate temperature and lighting. A new emergence trap is then placed on top of the lipless beaker. The R/O chambers are kept in this manner to collect the egg masses and track mortality of adults. If space is not a limiting factor, maintaining one R/O chamber per pair of organisms is encouraged. Where space is limited, many adults may be kept in a single R/O chamber, and the chambers may be double stacked (Double Stack Support Stand described in Section C.8) using a larger plastic (9-ounce) cup that serves as a stand for the second level of the emergence trap. The egg masses are removed by lifting the edge of the cup enough to permit transfer with a pipet.

C.4 Adult Collector Dish (Figure C.3)

C.4.1 This is used as a tray which is placed under the emergence trap or reproduction/oviposit (R/O) chambers to provide access to adults and to facilitate transfer of the males and females as needed. This dish is constructed of large petri dishes, i.e., 100- X 20-mm glass dishes or 100- X 20-mm plastic dishes. A 2.54-cm hole is cut in the middle and covered with 58-mesh opening nylon screen. Two slits are cut within the screen at 90 degree angles to each other. This facilitates insertion of the aspirator tube without risk of the adults flying away.

C.5 Aspirator (Figure C.3)

C.5.1 This is used to collect and transfer adults from the reproduction/oviposit (R/O) chambers. A 60-cc syringe is modified by cutting the end with the tip off and adding a retainer to hold the emergence traps and reproductive/oviposit chambers. The retainer is a 7-cm diameter plastic lid (from 270-mL wide mouth glass jar) and a large stopper is used to hold the syringe. The stopper and the lid is drilled with a hole saw of about 1 inch. The large stopper is glued to the lid. This retainer is then attached to the syringe. To facilitate transferring the animals, prepare two tubes, one about 16 cm in length and one about 4 cm (6-mm ID) and place these in a stopper (i.e., No. 5, 5.5 or 6) that has been drilled with two holes. Fasten a section (about 70 cm) of tygon tubing onto the short piece of glass and cover the tube with a piece of thin stainless steel screen (250-µm mesh) before inserting the tube into the rubber stopper. Adults should be stationary in trap to minimize the possibility of escape.

C.6 Auxiliary Male Holding Dish

C.6.1 When emergence begins in the auxiliary beakers, the males are transferred individually to inverted 60- X 15-mm plastic petri dishes with several small holes (3 mm in diameter) drilled in the top. A thin layer of overlying water (about 5 mL) is added and renewed until the males are needed for the reproduction chambers. These males are held in the test system for temperature control, and can be used for up to 5 d after collection.

C.7 Egg Hatching Chamber

C.7.1 Petri dishes, 60- X 15-mm plastic, are used to incubate (23°C) egg masses in approximately 15 mL of water. Hatch is monitored for 6 d. Hatch success is determined by subtracting the number of unhatched eggs at the end of 6 d from the initial estimate of the egg mass.

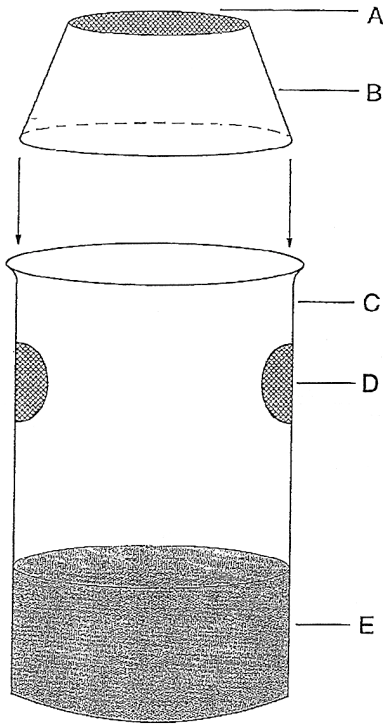


Figure C.1. Emergence trap used in the life-cycle *Chironomus tentans* sediment test. A: the nylon screen; B: the inverted plastic cups; C: the 300-mL lipless exposure beaker; D: the water exchange screen ports; E: test sediment.

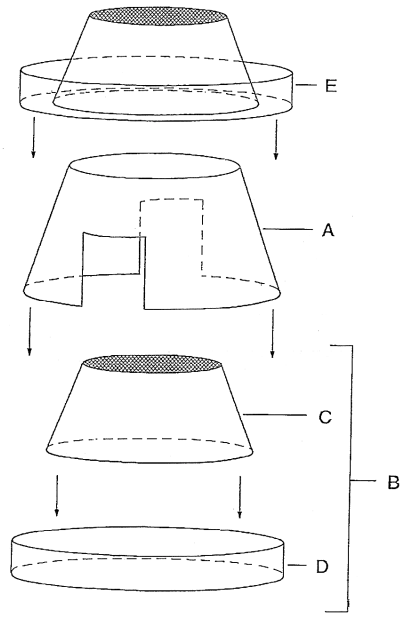


Figure C.2. The reproduction/oviposit chamber with the double stack support stand. A: the notched, inverted 270-ml (9-oz) plastic cup used to allow double stacking; B: the reproduction/oviposit (R/O) unit (C and D); C: inverted, 120-mL (4-oz) plastic cup with nylon screen; D: one-half of petri dish (100 X 20 mm) with 50 mL of overlying water; E: the reproduction/oviposit (R/O) chamber.

C.8 Supplies and Sources

A. Emergence Trap/Reproduction Oviposit Chamber.

1. 120-mL (5-ounce) plastic cups, Plastics Inc., St. Paul, MN 55164.

2. 1400-mesh opening (micron) nylon screen (mesh count = 14/inch), Monodur® 1400 Farbric Corporation, 7160 Northland Circle, Minneapolis, MN 55428, 612/535-3220.

B. Double Stack Support Stand: 270-mL (9-ounce) plastic cups, Solo Inc, Urbana, IL, 61801-2895.

C. Aspirator.

1. 60-cc syringe, 1 each, B-D® No. 309663, Becton and Dickinson & Company, Franklin Lakes, NJ 07417-1884.

2. 7-cm diameter plastic lid, 1 each.

3. Rubber stopper, 1 each, size 10, 10.5, or 11.

4. Rubber stopper, 1 each, size 5.5 or 6.

5. Glass tubing, 6-mm I.D., 1- 16 cm long, 1- 4 cm long.

6. Nalgene 6-mm plastic connector for mouth piece.

7. Stainless-steel screen, 250-µm mesh.

D. Auxillary Male Holding Chamber: 60- X 15-mm petri dish with 3-mm holes drilled, Falcon 1007 B-D®, Becton and Dickinson and Company, Franklin Lakes, NJ 07417-1884.

E. Egg Hatching Chambers: 60- X 15-mm petri dish, Falcon 1007 B-D®, Becton and Dickinson and Company, Franklin Lakes, NJ 07417-1884.

F. Adult Collector Dish:

1. 100- X 20-mm glass petri dish with a 2.54-cm access hole, Corning Glassware Corning, New York or 100- X 20-mm plastic petri dish with a 2.54-cm access hole, Falcon 1005 B-D®, Becton and Dickinson and Company, Franklin Lakes, NJ 07417-1884.

2. 58-mesh opening nylon screen, cut with slits at 90° angles to each other, Monodur®,Farbric Corporation, 7160 Northland Circle, Minneapolis, MN 55428, 612/535-3220.

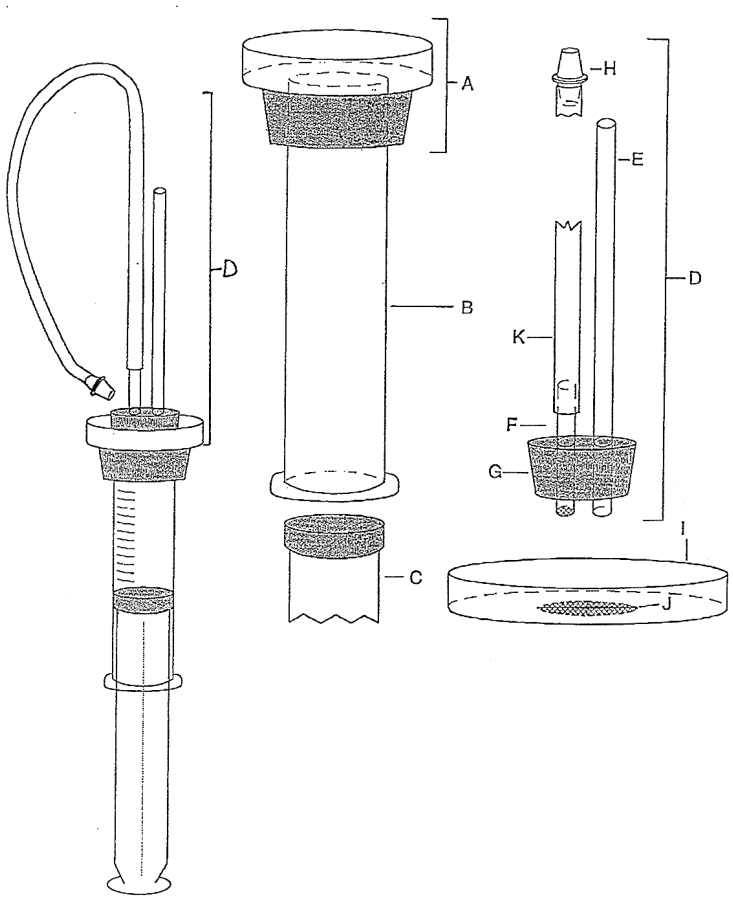


Figure C.3. Adult collection/transfer equipment. A: transfer retainer unit showing inverted plastic cover and rubber stopper glued inside of it; B: 60-cc syringe; C: plunger; D: detachable aspirator unit; E: long glass collector tube; F: short glass tube to serve as connector for inhaler tube; note stainless steel screen attached to end through stopper; G: 2-hole rubber stopper; H: nalgene plastic connector attached to tygon tubing and used as a mouthpiece to provide slight suction; I: collector dish, one-half of glass or plastic petri dish; J: petri dish with hole access that is screen covered and slotted; K: tygon tubing attached to glass tubing (F).

C.9 Construction of an Adult Midge Emergence Trap for Use in a "Zumwalt" Exposure System in Life-cycle Sediment Tests

C.9.1 The construction of the emergence trap described in Figure C.4 is an alternate design to the trap illustrated in Figures C.1 and C.2. The emergence trap illustrated in Figure C.4 is designed to fit under the exposure system described by Zumwalt et al. (1994; Section A.4). The level of the syringes will need to be raised about 1 1/2 inches using the threaded steel rods supporting the upper chamber.

C.9.2 Cut a 2 1/2-inch plexiglass tube into 1 1/4-inch-long pieces using a bandsaw or miter box and a handsaw.

C.9.3 Drill a 1/2-inch hole in the side (middle) of the 1 1/4-inch ring of plexiglass. Cut a small board to fit inside of the 1 1/4-inch ring to help support the plexiglass when drilling. The 1/2-inch drill bit should be dulled to help prevent the bit from digging in too fast.

C.9.4 Drill three 1/16-inch holes in the plexiglass ring spaced evenly around the ring and 1/4 inch off the bottom of the ring.

C.9.5 Trace around the stainless-steel screen. Cut out screen and place on top of the plexiglass ring. Use a propane-soldering torch or glass-blowing torch to heat up one end of a 1/4-inch or 3/8-inch threaded steel rod (about 12 to 15 inches long so that one end remains cool). Press the hot end of the steel rod against the screen and plexiglass until the screen melts into the plexiglass (usually a few seconds). Repeat the process until the screen is completely melted to the top of the plexiglass ring.

C.9.6. Bend 4-mm glass tubing (outer diameter) over a propane-soldering torch or glass-blowing torch and cut the tubing with a glass wheel or etch the tubing with a file to break. This glass tube is only to be used if beakers need

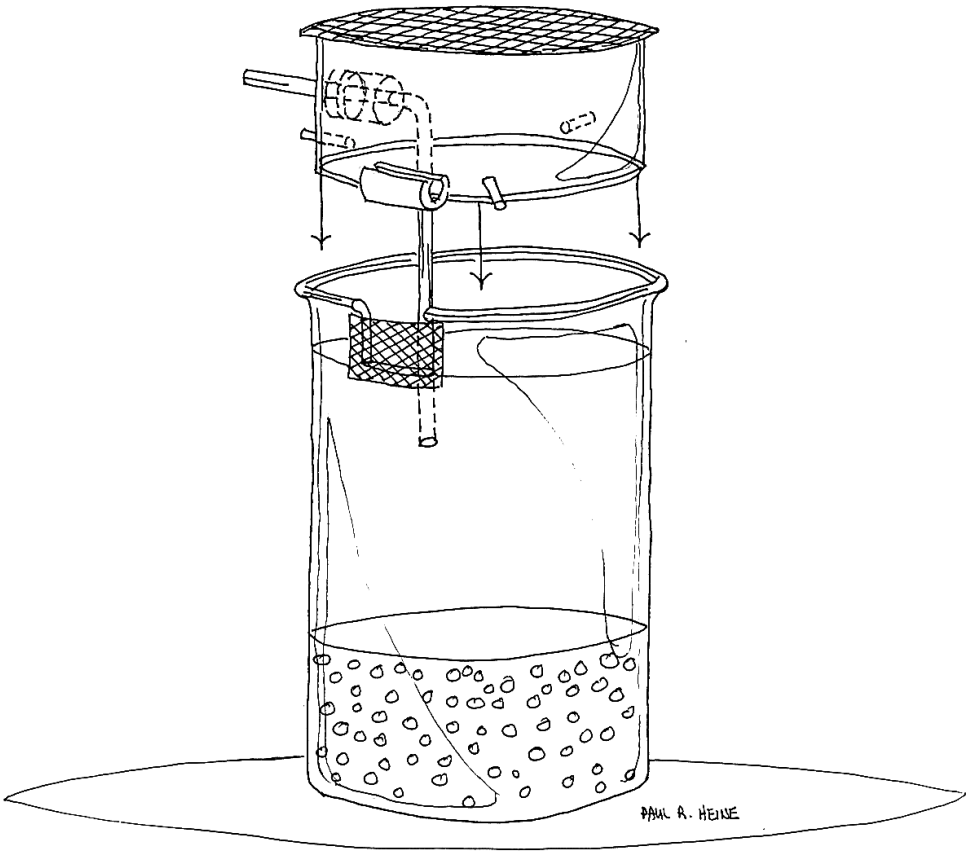


Figure C.4 Emergence traps that can be used with the Zumwalt water-delivery system described in Section A.4.

to be aerated during the midge exposure. An air line is connected to each tube and a gang valve is used to regulate air flow (about 1 bubble/second). The glass tube extends below the bottom of the plexiglass tube into the surface of the overlying water. A 4-mm slot will need to be cut in the petri dish in order to slide the petri dish under the emergence trap to remove adult midges from the test beakers (Figure C.2). The emergence trap capped with this petri dish can then be set on a 300-mL beaker to remove the adults with an aspirator as illustrated in Figure C.3.

C.9.7 Press 3/8-inch-long pins into the three 1/16-inch holes drilled in the side of the plexiglass tube. These pins make the plexiglass tube stable on the top of the beaker.

C.9.8 If the plexiglass tubes are used in beakers with a notch at the top (i.e., the beakers described in Zumwalt et al., 1994; Section A.4), a 2-cm length of 1/8-inch inner diameter latex tubing will need to be slit lengthwise and then slipped onto the bottom of the plexiglass tube. This tubing is then lined up with the notch in the beakers to prevent emerging midges from escaping. This piece of tubing is not needed if beakers described in Benoit et al. (1993) are used (i.e., beakers with holes drilled in the side).

C.9.9 Supplies

A. McMaster Carr, P.O. Box 4355, Chicago, IL 60680-4355, 708/833-0300 (part number and materials).

1. 8486 K 115, Acrylic tube 2 1/2-inch outer diameter and 1/8-inch wall.

2. 9226 T 84, 16- X 16-inch stainless wire cloth (0.018-wire diameter).

3. 90145 A 417, 1/16-inch diameter stainless dowel pins 3/8 inch long.

B. Thomas Scientific, P.O. Box 99, Swedesboro,NJ 08085-0099, 609/467-2000: 8747-E17, #00 silicone stopper.

Appendix D
Sample Data Sheets

Culture Aquarium	Date of Egg Mass Deposition	Date 4th Instar Larvae Were Weighed	Age of Weighed 4th Instar Larvae	Mean Dry Weight of 4th Instar Larvae (n = 10)	Date of Observed First Emergent Adult	Total Number of Egg Masses Produced	General Comments	Initials of Culturist
A								
B								
C								
D								
E								
F								

Figure D.1 Data sheet for the evaluation of a *Chironomus tentans* culture.

Position # _____. Tank # _____. Set up Date ___ / ___ / ___. Init. _____.

Embryo Deposition Date ___/ ___ / ___.
Embryo Hatch Date (day 0) ___/ ___/ ___.
Number of larvae used to initiate tank _____.
or number of egg cases used _____.
Date 10 Days Old Post Hatch ___/ ___/ ___.
First Emergence Date ___/ ___ / ___.
Substrate Type _____.
Food Type _____. Conc. _____. Date Made _____.

Emergence Data (Performed 3 x Per Week)

Date	# of	# of	Total	Comments	Init.

Chemistries (Performed Weekly)

Date	pH	D.O.	Ammonia	Temperature	Init.

Figure D.2 QA/QC data sheet for *Chironomus tentans* culture.

10-d Old *C. tentans* Body Lengths		
Date:		
Tank #	Length	Init.
Mean		

10-d Old *C. tentans* Head Capsule Widths		
Date:		
Tank #	Width	Init.
% 2nd Instar		
% 3rd Instar		
% 4th Instar		

***C. tentans* Dry Weight Data**						
Date	Tank #	Pan + 10 Organisms	Pan Only	Difference	Weight/ Organism (mg)	Init.
Mean						

Figure D.3 QA/QC data sheets for *Chironomus tentans* culture.

Brood Stock Source_____

Test Type (circle one)[1]: SU SM RU RM FU FM

No. of Animals Tested Per Replicate_____

No. of Replicates_____

Method of LC50 Estimate_____

Reference Toxicant ($CuSO_4$ or KCl)_____

Reference Toxicant Supplier and Lot No.

_____

Reference Toxicant Purity_____

Test Initiation Date_____

Toxicologist_____

Exposure Duration (Hr)	Number of Mortalities											
	Control		Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5	
	A	B	A	B	A	B	A	B	A	B	A	B
0												
24												
48												
72												
96												

Current Test 96-h LC50 = _____

Number of Reference-toxicity Test Used
to Determine Cumulative Mean 96-h LC50_____

Mean 96-h LC50 for All Tests to Date_____

Acceptability of Current Test[2] Yes_____ No_____

---

[1] SU = Static unmeasured
SM = Static measured
RU = Renewal unmeasured
RM = Renewal measured
FU = Flow-through unmeasured
FM = Flow-through measured

[2] Based on two standard deviations around the cumulative mean 96 h-LC50

Figure D.4 Data sheet for performing reference-toxicity tests.

Sediment Sample Source_____

Date of Test Initiation_____

Toxicologist Conducting Test_____

Test Day	Test Replicate Sampled	Temperature (^{o}C)	Dissolved Oxygen (mg/L)	pH	Hardness (mg/L)	Alkalinity (mg/L)	Specific Conductance (umhos/cm)	Total Ammonia (mg/L)
0								
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Figure D.5 Data sheet for temperature and overlying water chemistry measurements.

Daily Checklist for Sediment Tests

Dissolved Oxygen
Minimum Acceptable Concentration
(40% of Saturation at Target Temp)

Study Code _____

Study Name _____

Building _____ Diluter _____ Waterbath _____ Target temperature _____ °C

Study Director _____ Acceptable Range _____ °C to _____ °C

Lead Technician _____ Month _____ = _____ mg/L

Day of Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Day of Study																															
Diluter Operation																															
Number of Cycles																															
Time of Day																															
Temperature																															
Air Pressure																															
Aeration																															
Brush Screens																															
Clean Needles																															
Feeding																															
Total Water Quality																															
Partial Water Quality																															
Initials																															

Comments _____

_____

_____

_____

Approved by _____ Date _____

Figure D.6 Data sheet for daily checklist for sediment tests.

Water Quality Data Sheet

Study Code ____________ Date __________ Test Day _________ Study Director ________________ Investigator ____________________________

Study Name ____

	Dissolved Oxygen (mg/L)	Temp. °C	Salinity (ppt)	Conductivity (umhos or uS)	pH					Alkalinity (ppm $CaCO_3$)					Hardness (ppm $CaCO_3$) ___ ml	Turbidity (NTU) ___ ml	Total Ammonia as NH_3 (mg/L) ___ ml	ml of ISA	Other ______	______
Volume of Sample =	___ml	___ml	___ml	___ml	___ml			___ml											___ml	___ml
Sample Code						(ml Titrant x mult. factor =)					(ml Titrant x mult. factor =)									
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
							x		=			x		=						
Meter #																				
Initials																				

Comments ________

Approved by ________________ Date ___________

Figure D.7 Data sheet for water quality parameters.

CHEMISTRIES

Page ____ of ____

Test Type ______________________ Sample Info ______________ Water Type ______________________

Organism ______________________ ______________ Experimenter ______________________

Test Dates ______________________ ______________ Test System ______________________

I.D.	Day	-1	0	1	2	3	4	5	6	7	8	9	10	Remarks
pH														
DO (mg/L)														
Temp ^{0}C														
Hard/Alk														
pH														
DO (mg/L)														
Temp ^{0}C														
Hard/Alk														
pH														
DO (mg/L)														
Temp ^{0}C														
Hard/Alk														
pH														
DO (mg/L)														
Temp ^{0}C														
Hard/Alk														

Figure D.8 Chemistry data sheet.

Study Director _____

Study Code _____

Study Name _____

Daily Comment Sheet

Day_____ Date_____-_____-_____ Initials_____

Day_____ Date_____-_____-_____ Initials_____

Day_____ Date_____-_____-_____ Initials_____

Day_____ Date_____-_____-_____ Initials_____

Day_____ Date_____-_____-_____ Initials_____

Figure D.9 Daily comment data sheet.

Weight Data Form

Test Dates	Species	
Test Material	Weighing Date	Food
Location	Oven Temp (°C)	Age Organisms
Analyst	Drying Time (h)	Initial No/Rep

Sample	Replicate	Wt. of Oven Dried Pan (mg)	Wt. of Pan + Oven Dried Organisms (mg)	Dried Wt. of Organisms (mg)	Number of Survivors	Mean wt per Survivor	Sample Mean

Figure D.10 Weight data sheet.

Date: ______________________ **Test:** ______________________

Species: ______________________ **Investigator:** ______________________

Facility: ______________________

Treatment (Site)	Rep	Number Surviving	Pan Weight	Pan + Larvae	Dry Weight		Pan + Ash	Ash-free Dry Wt	
					Total	Indiv.		Total	Indiv.
	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								
	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								
	1								
	2								
	3								
	4								
	5								
	6								
	7								
	8								

Figure D.11 Data sheets for *Chironomus tentans* tests.

At termination of test:

1. Sieve sediment from each beaker and record the number of recovered larvae in the "survival" column.

2. Place all larvae from one replicate in a pre-ashed and pre-weighed aluminum weigh pan.

3. Dry larvae at 60°C for at least 24 hr.

4. Weigh pan + larvae and record weight under appropriate column of data sheet.

5. Ash pan + larvae at 550°C for 2 hr. Let cool to room temperature.

6. Weigh pan + ashed material.

7. Remove ash (e.g. with a small brush) and weigh pan.

8. Calculate dry weight as the difference between the pan+larvae weight and the pan weight.

9. Calculate ash-free dry weight as the difference between the pan+larvae weight and the pan+ash weight.

Figure D.12 Instructions for terminating a *Chironomus tentans* test.

Date: _____ Test: _____ Species: _____

Investigator: _____ Facility: _____

Treatment	Rep	Larvae Number	Dead Pupae			Date of Emergence				Date of Egg Mass	Egg Counts		Number Eggs Not Hatched	Date Adult Died
			No ID	♂	♀	Partial ♂	Partial ♀	Complete ♂	Complete ♀		Live	Acid		
		1												
		2												
		3												
		4												
		5												
		6												
		7												
		8												
		9												
		10												
		11												
		12												

Figure D.13 Data sheet for the *Chironomus tentans* life-cycle test.

Comments (Adult transfers, mate pairings etc.)

Data Summary

No. of larvae recovered at end of test:	Total Larvae:	Number Dead Pupae:
No. Dead/Escaped Adults:	Total Emerged Adults:	Total Egg Mass:

Date: 01/28/96 **Test:** Mississippi River **Species:** *C. tentans*

Investigator: _____ **Facility:** _____

	Rep	Larvae Number	Dead Pupae No ID	♂	♀	Date of Emergence ♂ Partial	♀ Partial	♂ Complete	♀ Complete	Date of Egg Mass	Egg Counts Live	Acid	Number Eggs Not Hatched	Date Adult Died
7RR-A	A	1						2/23[a]						2/23
		2						2/24[b]						
		3							2/26	2/28		644		3/3
		4						2/25						3/2
		5						2/27						
		6							2/27	2/28	722		38	3/4
		7						2/27[c]						3/6
		8	3/4											
		9						3/6[d]						
		10							3/8[e]	3/9	1004		31	3/13
		11	3/15											
		12												

Comments (Adult transfers, mate pairings etc.)

[a] Fully emerged; dead on water	[e] 3/8 ♂ transferred from 7SR-A on 3/8	
[b] 2/24 ♂ transferred to 7RR-B on 2/24		
[c] 2/27 ♂ transferred to 7RR-B on 3/2		
[d] 3/6 ♂ transferred to 7RR-C on 3/6		

Data Summary

No. of larvae recovered at end of test: 0	**Total Larvae: 11**	**Number Dead Pupae: 2**
No. Dead/Escaped Adults: 1/0	**Total Emerged Adults: 9**	**Total Egg Masses: 3**

Figure D.14 Example entries for a *Chironomus tentans* life-cycle test data sheet.

Copy of a sample data sheet that will be used to record all information pertaining to emergence and reproduction of *C. tentans* during the life-cycle test. For clarity, consistency, and ease of data interpretation, it is important that each lab fill out this sheet as illustrated. A brief interpretation of each recording (column) is provided below.

I Data Sheet Requirement. One data sheet is needed for each replicate. Thus, a treatment having 8 reproduction replicates will have 8 data sheets (survival and growth data are recorded on separate sheets). All emergence and reproduction data for a replicate are recorded on the corresponding data sheet.

II Recording Pupae, Emergence, and Egg Mass Data. Record all pupae, emergence, and egg mass data as dates.

III Column Heading Interpretation

***Station/Site and Replicate*.** Enter name of sample and corresponding replicate (e.g., 7RR-A).

***Larvae #*.** These numbers correspond to the 12 larvae placed in each replicate.

***Dead Pupae*.** If it is not possible to determine the gender of the dead pupae, enter the date found in the **"No ID"** column. Otherwise, enter the date found in either the male or female column.

***Date of Emergence*.** If an adult has not completely shed the pupal exuviae, enter the date found under the **"partial emergence"** category as a male or female. If emergence is complete but the adult is dead (typically floating on the water surface), record date under **"complete emergence"** category as a male or female and enter a footnote as indicated in **"footnote a"** in comments section of data sheet.

Partially emerged adults, and those that have emerged completely but were unable to escape the surface tension of the water, usually die within 24 hr. In both cases, the date of death should be recorded as one day later under the **"Date Adult Died"** column.

***Date of Egg Mass*.** Record the date on which the egg mass was collected from the replicate.

***Egg Counts*.** Enter number of eggs counted using either the acid-digestion (direct count) or ring method (indirect count).

***Number Eggs Not Hatched*.** Enter the number of unhatched eggs from each oviposited egg mass for which an indirect count (ring method) was determined.

***Date Adult Died*.** Enter the date that the adult died (be sure to follow transferred adults).

IV Comments Section. All comments concerning adult transfers and emergence patterns should be recorded in this section as footnotes (see footnotes a-e on sample data sheet).

V Data Summary Section. At termination of each replicate, record the **Number of Larvae Recovered at End of Test** after sieving and determine the number of **Total Larvae** alive during the test. Also record the **Number Dead Pupae**, **Number Dead/Escaped Adults, Total Emerged Adults,** and number of **Total Egg Masses** by summing the appropriate columns.

VI Example Entries for *C. tentans* Data-Sheet 7RR-A

Example #1. On 2/23/95 a male emerged from this replicate. This is recorded under the "Male" category of the "Complete Emergence" column on the first line. This male was fully emerged but was dead and floating on the water surface. This is recorded as footnote "a" in the "Comments" section and the date of death recorded under the "Date Adult Died" column.

Example #2. A female emerged from this replicate on 2/26/95 which is recorded under the "Female" heading of the "Complete Emergence" column. This female produced an egg mass on 2/28/96 which is recorded under the "Date of Egg Mass" column.

Example #3. A dead pupae was recorded on 3/4/95. Since the sex was not determined, it was recorded under the "No ID" heading of the "Dead Pupae" column. Pupal sex may be determined by examining the genitalia under a dissecting microscope (the genitalia can be seen through the pupal exuviae which is usually, but not always, transparent).

Example #4. A male emerged on 2/24/95 in 7 RR-A and was transferred to replicate 7RR-B. This is shown as footnote "b". Recording this type of data helps to keep track of where males are and the number of times they have reproduced.

A male from 7SR-A (one of the stand-by replicates) was transferred to 7 RR-A on 3/8/95. This is recorded as footnote "e" on the 7RR-A data sheet. For completeness, a corresponding footnote on the 7 SR-A data sheet should be made regarding this transfer.

D.15 Instructions for completing the *Chironomus tentans* life-cycle test data sheet.

Appendix C

Field Logs (Samples)

QAPP/ MRPP Sampling Field Log

Page _ of _

Project Name:	Date:	Time Start:	Time End:
Sampler:			

Sampling Event Type:	☐ Water Quality Sampling	☐ Bioassessment Sampling (Algae)	☐ Bioassessment Sampling (Benthic Macroinvertegrate)

Weather and Observations

Date Rain Predicted to Occur:	Predicted % chance of rain:

Estimate storm start:_____ (date and time)	Estimate storm duration:_____ (hours)	Estimate time since last storm: _____ (days or hours)	Rain gauge reading: _____ (inches)

Field Device Calibration

Meter ID No./Desc.: Calibration Date/Time:	Meter ID No./Desc.: Calibration Date/Time:
Meter ID No./Desc.: Calibration Date/Time:	Meter ID No./Desc.: Calibration Date/Time:
Meter ID No./Desc.: Calibration Date/Time:	Meter ID No./Desc.: Calibration Date/Time:

Field Measurements

Sample Location Description	Matrix	Parameter	Reading (include units)	Time

Grab Samples Collected

Sample Location Description	Matrix	Parameter/ Specimen		Time

Additional Field Observations

Note: Describe significant field observations. Describe reasons for sampling that was not successful. Add additional sheets as needed.

REACH DOCUMENTATION

Standard Reach Length (wetted width ≤ 10 m) = 150 m Distance between transects = 15 m
Alternate Reach Length (wetted width >10 m) = 250 m Distance between transects = 25 m

Project Name:	Date: / / 2011	Sample Collection Time:
Stream Name:	Site Name/ Description:	
Site Code:	Crew Members:	
Latitude (actual – decimal degrees): ºN	datum: NAD83	
Longitude (actual – decimal degrees): ºW	*other:*	GPS Device:

AMBIENT WATER QUALITY MEASUREMENTS

turbidity and silica are optional; calibration date required

Temp (Deg C)		pH		Alkalinity (mg/L)		Turbidity (ntu)	
		cal. date				cal. date	
Dissolved O^2 (mg/L)		Specific. Conduct (uS/cm)		Salinity. (ppt)		Silica (mg/L)	
cal. date		cal. date		cal. date		cal. date	

REACH LENGTH

Actual Length (m) *(see reach length guidelines at top of form)*	
Explanation:	

DISCHARGE MEASUREMENTS

1st measurement = left bank (looking downstream)

check if discharge measurements not possible (explain in field notes section) ☐

VELOCITY AREA METHOD (preferred)

cal. date

Transect Width (m):

	Distance from Left Bank (cm)	Depth (cm)	Velocity (ft/sec)		Distance from Left Bank (cm)	Depth (cm)	Velocity (ft/sec)
1				11			
2				12			
3				13			
4				14			
5				15			
6				16			
7				17			
8				18			
9				19			
10				20			

BUOYANT OBJECT METHOD (use ONLY if velocity area method not possible)

	Float 1	Float 2	Float 3
Distance (m)			
Float Time (sec)			

Float Reach Cross Section

width (m) depth(cm)	Upper Section	Middle Section	Lower Section
Width			
Depth 1			
Depth 2			
Depth 3			
Depth 4			
Depth 5			

NOTABLE FIELD CONDITIONS (check one box per topic)

Evidence of recent rainfall (enough to increase surface runoff)	NO	☐	minimal	☐	>10% flow increase	☐
Evidence of fires in reach or immediately upstream (<500 m)	NO	☐	< 1 year	☐	< 5 years	☐
Dominant landuse/ landcover in area surrounding reach	Agriculture	☐	Forest	☐	Rangeland	☐
	Urban/ Industrial	☐	Suburb/Town	☐	Other	☐

ADDITIONAL COBBLE EMBEDDEDNESS MEASURES

(carry over from transect forms if needed to attain target count of 25; measure in %)

1	2	3	4	5	6	7	8	9	10	11	12	13
14	15	16	17	18	19	20	21	22	23	24	25	

Site Code:	Date: __ __ / __ __ / 2011	

SLOPE and BEARING FORM (transect based - for Full PHAB only)

AUTOLEVEL	
CLINOMETER	
HANDLEVEL	
OTHER	

Starting Transect	MAIN SEGMENT (record percent of inter-transect distance in each segment if supplemental segments are used)						SUPPLEMENTAL SEGMENT (record percent of inter-transect distance in each segment if supplemental segments are used)					
	Stadia rod measurements		Slope (%) or Elevation Difference cm ☐ % ☐	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)	Stadia rod measurements		Slope or Elevation Difference cm ☐ % ☐	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)
K												
J												
I												
H												
G												
F												
E												
D												
C												
B												
A												
additional calculation area												

ADDITIONAL HABITAT CHARACTERIZATION

High Gradient ☐ Low Gradient ☐

Parameter	Optimal	Suboptimal	Marginal	Poor
Epifaunal Substrate/ Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover (50% for low-gradient streams); mix of submerged logs, undercut banks, cobble or other stable habitat	40-70% mix of stable habitat (30-50% for low-gradient streams); well-suited for full colonization potential	20-40% mix of stable habitat (10-30% in low-gradient streams); substrate frequently disturbed or removed	Less than 20% stable habitat (10% in low-gradient streams); lack of habitat is obvious; substrate unstable or lacking
Score:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition (<20% in low-gradient streams)	Some new increase in bar formation, mostly from gravel, sand, or fine sediment; 5-30% of the bottom affected (20-50% in low-gradient streams)	Moderate deposition of new gravel, sand, or fine sediment on bars; 30-50% of the bottom affected (50 - 80% in low-gradient streams)	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently (>80% in low-gradient streams)
Score:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern	Some channelization present, (e.g., bridge abutments); evidence of past channelization (> 20yrs) may be present but recent channelization not present	Channelization may be extensive: embankments or shoring structures present on both banks; 40 to 80% of stream reach disrupted	Banks shored with gabian or cement; Over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely
Score:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Site Code:	Site Name:		Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect A**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

TAKE PHOTOGRAPHS
(check box if taken & record photo code)

Downstream (**optional**)	☐
Upstream (**required**)	☐

Inter-Transect: AB

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m): / Bankfull Height (m):	**Transect B**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: BC

Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect C**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: CD	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m): / Bankfull Height (m):	**Transect D**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: DE	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m): / Bankfull Height (m):	**Transect E**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: EF	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m): / Bankfull Height (m):	**Transect F**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

Riparian Vegetation
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

Instream Habitat Complexity

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

Densiometer Readings (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

Human Influence
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

Bank Stability
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

TAKE PHOTOGRAPHS
(check box if taken & record photo code)

Downstream (**required**)	☐
Upstream (**required**)	☐

Inter-Transect: FG	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:		Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):	**Transect G**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: GH	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m):	Bankfull Height (m):

Transect H

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: HI	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m): / Bankfull Height (m):	**Transect I**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: IJ	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m): / Bankfull Height (m):	**Transect J**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

Inter-Transect: JK	Wetted Width (m):

Inter-Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes

0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
UD = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

FLOW HABITATS
(% between transects, total=100%)

Channel Type	%
Cascade/ Falls	
Rapid	
Riffle	
Run	
Glide	
Pool	
Dry	

Site Code:	Site Name:	Date: __ __ / __ __ / 2011
Wetted Width (m):	Bankfull Width (m): / Bankfull Height (m):	**Transect K**

Transect Substrates

Position	Dist from LB (m)	Depth (cm)	mm/ size class	% Cobble Embed.	CPOM	Microalgae Thickness Code	Macroalgae Attached	Macroalgae Unattached	Macrophytes
Left Bank					P A		P A D	P A D	P A D
Left Center					P A		P A D	P A D	P A D
Center					P A		P A D	P A D	P A D
Right Center					P A		P A D	P A D	P A D
Right Bank					P A		P A D	P A D	P A D
	Note: Substrate sizes can be recorded either as direct measures of the median axis of each particle or one of the size class categories listed on the supplemental page (direct measurements preferred)								

Microalgae Thickness Codes
0 = No microalgae present, Feels rough, not slimy;
1 = Present but not visible, Feels slimy;
2 = Present and visible but <1mm; Rubbing fingers on surface produces a brownish tint on them, scraping leaves visible trail.
3 = 1-5mm;
4 = 5-20mm;
5 = >20mm;
U = Cannot determine if microalgae present, substrate too small or covered with silt (formerly Z code).
D = Dry, not assessed

RIPARIAN VEGETATION
(facing downstream)

0 = Absent (0%) 3 = Heavy (40-75%)
1 = Sparse (<10%) 4 = Very Heavy (>75%)
2 = Moderate (10-40%)

Vegetation Class	Left Bank	Right Bank
Upper Canopy (>5 m high)		
Trees and saplings >5 m high	0 1 2 3 4	0 1 2 3 4
Lower Canopy (0.5 m-5 m high)		
All vegetation 0.5 m to 5 m	0 1 2 3 4	0 1 2 3 4
Ground Cover (<0.5 m high)		
Woody shrubs & saplings <0.5 m	0 1 2 3 4	0 1 2 3 4
Herbs/ grasses	0 1 2 3 4	0 1 2 3 4
Barren, bare soil/ duff	0 1 2 3 4	0 1 2 3 4

INSTREAM HABITAT COMPLEXITY

0 = Absent (0%)
1 = Sparse (<10%)
2 = Moderate (10-40%)
3 = Heavy (40-75%)
4 = Very Heavy (>75%)

Filamentous Algae	0 1 2 3 4
Aquatic Macrophytes/ Emergent Vegetation	0 1 2 3 4
Boulders	0 1 2 3 4
Woody Debris >0.3 m	0 1 2 3 4
Woody Debris <0.3 m	0 1 2 3 4
Undercut Banks	0 1 2 3 4
Overhang. Vegetation	0 1 2 3 4
Live Tree Roots	0 1 2 3 4
Artificial Structures	0 1 2 3 4

DENSIOMETER READINGS (0-17)
count covered dots

Center Left	
Center Upstream	
Center Right	
Center Downstream	
Optional	
Left Bank	
Right Bank	

HUMAN INFLUENCE
(circle only the closest to wetted channel)

0 = Not Present;
B = On Bank;
C = Between Bank & 10m from Channel;
P = >10m+<50m from Channel;
Channel (record Yes or No)

	Left Bank	Channel	Right Bank
Walls/ Rip-rap/ Dams	P C B 0	Y N	0 B C P
Buildings	P C B 0	Y N	0 B C P
Pavement/ Cleared Lot	P C B 0		0 B C P
Road/ Railroad	P C B 0	Y N	0 B C P
Pipes (Inlet/ Outlet)	P C B 0	Y N	0 B C P
Landfill/ Trash	P C B 0	Y N	0 B C P
Park/ Lawn	P C B 0		0 B C P
Row Crop	P C B 0		0 B C P
Pasture/ Range	P C B 0		0 B C P
Logging Operations	P C B 0		0 B C P
Mining Activity	P C B 0	Y N	0 B C P
Vegetation Management	P C B 0		0 B C P
Bridges/ Abutments	P C B 0	Y N	0 B C P
Orchards/ Vineyards	P C B 0		0 B C P

BANK STABILITY
(score zone 5m upstream and 5m downstream of transect between bankfull - wetted width)

Left Bank	eroded	vulnerable	stable
Right Bank	eroded	vulnerable	stable

TAKE PHOTOGRAPHS
(check box if taken & record photo code)

Downstream (**required**) ☐

Upstream (**optional**) ☐

Site Code:	Date: __ __ / __ __ / 2011	**FULL FORM**

BENTHIC INVERTEBRATE SAMPLES

Collection Method (indicate standard or margin-center-margin)			Replicate	# jars
RWB (standard)	**RWB** (MCM)	**TRC**	**1**	
RWB (standard)	**RWB** (MCM)	**TRC**	**2**	
RWB (standard)	**RWB** (MCM)	**TRC**		
RWB (standard)	**RWB** (MCM)	**TRC**		

Field Notes/ Comments:

Chemistry Equipment ID

Analyte	Equipment
pH	
temperature	
dissolved oxygen	
specific conductance	
salinity	
alkalinity	
turbidity	
silica	
Velocity	

ALGAE SAMPLES

Collection Method (circle one or write new method if applicable)	SWAMP EMAP	SWAMP EMAP	SWAMP EMAP	SWAMP EMAP
Collection Device (sum # of transects per device)	**Rep. 1**	**Rep. 2**	**Rep.**	**Rep.**
Rubber Delimiter (area=12.6cm^2)				
PVC Delimiter (area=12.6cm^2)				
Syringe Scrubber (area=5.3cm^2)				
Other area=				
Number of transects sampled (0-11)				
Composite Volume (mL)				
Assemblage ID volume **(diatoms)** (50 mL tube)				
Assemblage ID volume **(soft algae)** (50 mL tube)				
Check if Qualitative Algae sample was collected with soft algae/diatom sample (required even if macroalgae not visible)	☐	☐	☐	☐
Check if a water chem. **integrated** sample was collected (chl, AFDM)	☐	☐	☐	☐
Chlorophyll a volume use GF/F filter (25 mL **(preferred volume)**				
Ash Free Dry Mass (AFDM) volume use GF/F filter (25 mL **(preferred vol)**				

Water and Sediment Chemistry Samples

Check if a WATER chemistry **grab** sample was collected (nutrients, SSC, etc.)	☐
Check if a DUPLICATE WATER chemistry **grab** sample was collected	☐
Check if a SEDIMENT chemistry sample was collected	☐
Check if a DUPLICATE SEDIMENT chemistry sample was collected	☐

Sediment Collection Device: SCOOP CORE GRAB

Material: Stainless Steel Polyethylene Polycarbonate Other

Sediment Collection Depth (cm): 2 or 5

Create Lab Collection records for each checked box for integrated and grab water chemistry samples

ADDITIONAL PHOTOGRAPHS

Description	Photo Code	Description	Photo Code

Flow Habitat Type	DESCRIPTION
Cascades	Short, high gradient drop in stream bed elevation often accompanied by boulders and considerable turbulence
Falls	High gradient drop in elevation of the stream bed associated with an abrupt change in the bedrock
Rapids	Sections of stream with swiftly flowing water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles
Riffles	Shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence; (< 0.5 m deep, > 0.3 m/s).
Runs	Long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool; (> 0.5 m deep, > 0.3 m/s). A **step-run** is a series of runs separated by short riffles or flow obstructions that cause discontinuous breaks in slope
Glides	A section of stream with little or no turbulence, but faster velocity than pools; (< 0.5 m deep, < 0.3 m/s)
Pools	A reach of stream that is characterized by deep, low-velocity water and a smooth surface; (> 0.5 m deep, < 0.3 m/s)

Size Class Code	Size Class Range	Size Class Description	Common Size Reference
RS	> 4 m	bedrock, smooth	larger than a car
RR	> 4 m	bedrock, rough	larger than a car
XB	1 - 4 m	boulder, large	meter stick to car
SB	25 cm - 1.0 m	boulder, small	basketball to meter stick
CB	64 - 250 mm	cobble	tennis ball to basketball
GC	16 - 64 mm	gravel, coarse	marble to tennis ball
GF	2 – 16 mm	gravel, fine	ladybug to marble
SA	0.06 – 2 mm	sand	gritty to ladybug
FN	< 0.06 mm	fines	not gritty
HP	< 0.06 mm	hardpan (consolidated fines)	
WD	NA	wood	
RC	NA	concrete/ asphalt	
OT	NA	other	

BANK STABILITY

Although this measure of the degree of erosive potential is subjective, it can provide clues to the erosive potential of the banks within the reach. Assign the category whose description best fits the conditions in the area between the wetted channel and bankfull channel (see figure below)

Eroded	Banks show obvious signs of erosion from the current or previous water year; banks are usually bare or nearly bare
Vulnerable	Banks have some vegetative protection (usually annual growth), but not enough to prevent erosion during flooding
Stable	Bank vegetation has well-developed roots that protect banks from erosion; alternately, bedrock or artificial structures (e.g., concrete/ rip-rap) prevent bank erosion

CPOM/ COBBLE EMBEDDEDNESS

CPOM: Record presence (P) or absence (A) of coarse particulate organic matter (>1.0 mm particles) within 1 cm of each substrate particle

Cobble Embeddedness: Visually estimate % embedded by fine particles (record to nearest 5%)

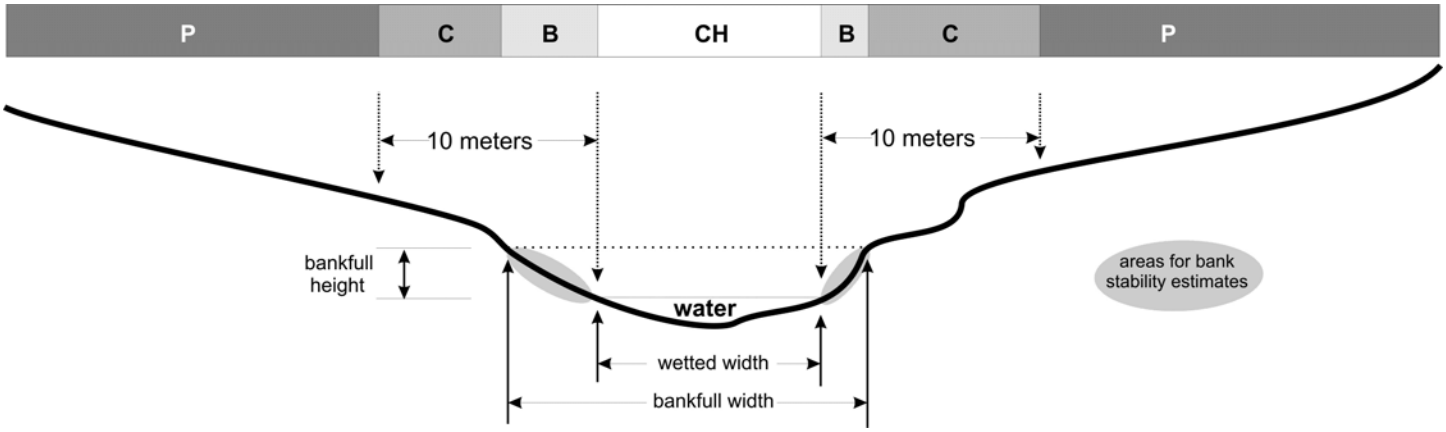
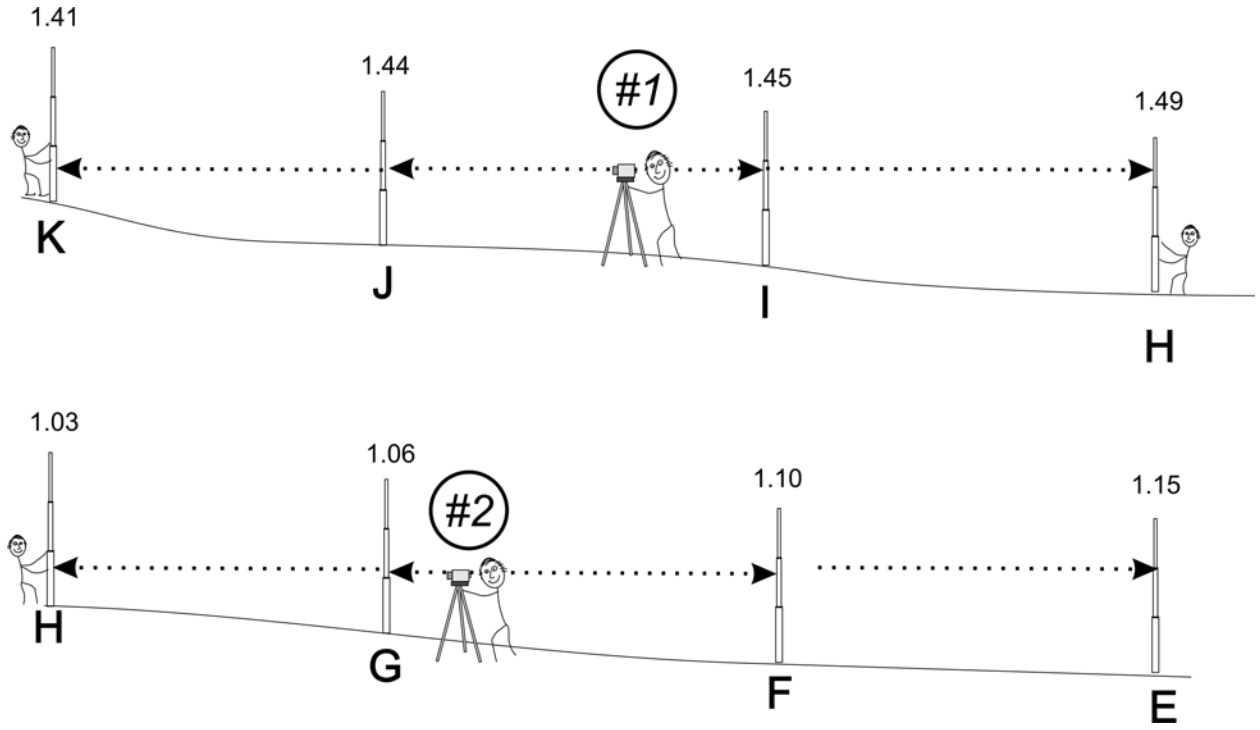


Figure 1. Cross-sectional diagram of stream transect indicating regions for assessing human influence measures:

- The measurement zone extends 5 meters upstream and 5 meters downstream of each transect
- Record one category for each bank and for the wetted channel (3 values possible)
- In reaches with wide banks, region "C" may be entirely overlapped by region "B"; in these cases, circle "B"
- Region "P" extends from 10 meters to the distance that can be seen from the channel, but not greater than 50 m

SLOPE and BEARING FORM **EXAMPLE**	AUTOLEVEL	X
	CLINOMETER	
	HANDLEVEL	

Starting Transect	MAIN SEGMENT (record percent of inter-transect distance in each segment if supplemental segments are used)						SUPPLEMENTAL SEGMENT (record percent of inter-transect distance in each segment if supplemental segments are used)					
	Stadia rod measurements		Slope (%) or Elevation Difference cm ■ % □	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)	Stadia rod measurements		Slope or Elevation Difference cm □ % □	Segment Length (m)	Bearing (0°-359°)	Percent of Total Length (%)
K	1.41											
J	1.44		3	15	140	100						
I	1.45		1	15	145	100						
H	1.49	1.03	4	15	150	100						
G		1.06	3	15	143	100						
F		1.10	4	15	187	100						
E		1.15	5	15	195	100						



1. Level the autolevel at Position #1
2. Place base of stadia rod at water level every time
3. Sight to stadia rod at Transect K, then Transect J
4. Rotate scope and sight to Transects I and H.
5. Move level to Position #2 and re-level
6. Re-sight to stadia rod at Transect H, then Transect G
7. Rotate scope and sight to Transects F and E

Note: Sites will vary in the number of separate level positions needed to survey the reach.

Appendix D

Example Chain of Custody

CHAIN OF CUSTODY EXAMPLE FROM EPA

Survey				**Samplers:** Signature					
Station Number	Station Location	Date	Time	Sample Type			Seq. No.	No. Of Containers	Analysis Required
				Water		Air			
				Comp	Grab.				
Relinquished by: Signature			**Received by:** Signature						**Date/Time**
Relinquished by: Signature			**Received by:** Signature						**Date/Time**
Relinquished by: Signature			**Received by:** Signature						**Date/Time**
Relinquished by: Signature			**Received by Mobile Laboratory for Field analysis:** Signature						**Date/Time**
Dispatched by:		**Date/Time**	**Received for Laboratory by:** Signature						**Date/Time**
Method of Shipment:									

Appendix E

Comprehensive List of Pesticides and Herbicides for Water Quality Monitoring

APPENDIX E. Comprehensive List of Pesticides and Herbicides for Water Quality Monitoring

Based on analysis of the specified chemical family, the following comprehensive list identifies the individual pesticides/ herbicides that may be detected in water quality samples.

Organochlorine Pesticides (sediment)	Organochlorine Pesticides (water)	Organophosphate (water)	Pyrethroids (sediment)
Aldrin alpha-HCH beta-HCH cis-Chlordane trans-Chlordane Dacthal DDD (o,p') DDD (p,p') DDE (o,p') DDE (p,p') DDMU (p,p') DDT (o,p') DDT (p,p') Dieldrin Endosulfan I Endosulfan II Endosulfan sulfate Endrin delta-HCH gamma-HCH Heptachlor Heptachlor epoxide Hexachlorobenzene Methoxychlor Mirex Nonachlor, cis Nonachlor, trans Oxadiazon Oxychlordane Tedion Toxaphene	Aldrin alpha-HCH beta-HCH cis-Chlordane trans-Chlordane Dacthal DDD (o,p') DDD (p,p') DDE (o,p') DDE (p,p') DDMU (p,p') DDT (o,p') DDT (p,p') delta-HCH Dieldrin Endosulfan I Endosulfan II Endosulfan sulfate Endrin Endrin Aldehyde Endrin Ketone gamma-HCH Heptachlor Heptachlor epoxide Hexachlorobenzene Methoxychlor Mirex cis-Nonachlor trans-Nonachlor Oxadiazon Oxychlordane Tedion Toxaphene	Aspon Azinphos ethyl Carbophenothion Chlorfenvinphos Chlorpyrifos Chlorpyrifos methyl Ciodrin Coumaphos Demeton-S Diazinon Naled Dichlofenthion Dichlorvos Dicrotophos Dimethoate Dioxathion Disulfoton Ethion Famphur Fenchlorophos Fenitrothion Fensulfothion Fenthion Fonofos Azinphos methyl Leptophos Malathion Methidathion Parathion, ethyl Parathion, methyl Molinate Phorate Mevinphos Phosmet Phosphamidon Ethoprop Sulfotep Bolstar Terbufos Tetrachlorvinphos Thiobencarb Thionazin Tokuthion Merphos Trichlorfon Trichloronate	Bifenthrin Cyfluthrin, Total Cypermethrin, Total Deltamethrin Esfenvalerate/ Fenvalerate, Total lambda-Cyhalothrin, Total cis-Permethrin *trans-Permethrin*