

# **Standard Operating Procedure for Mysid Laboratory Analysis**

**LG408**

---

**Revision 3, July 2019**

## Table of Contents

<u>Section Number</u>	<u>Subject</u>	<u>Page</u>
1.0	SCOPE AND APPLICATION.....	1
2.0	SUMMARY OF METHOD.....	1
3.0	SAMPLE COLLECTION AND PRESERVATION.....	1
4.0	APPARATUS .....	1
5.0	REAGENTS.....	2
6.0	ANALYTICAL PROCEDURE – MYSID SAMPLE ANALYSIS .....	2
7.0	CALCULATION OF MYSID BIOMASS.....	9
8.0	CALCULATIONS AND REPORTING.....	9
9.0	QUALITY CONTROL AND METHODS PRECISION.....	11
10.0	SAFETY AND WASTE DISPOSAL.....	12
11.0	REFERENCES.....	12
	FIGURES.....	14
	APPENDIX 1: FORMS .....	23

Disclaimer: Mention of trade names or commercial products does not constitute endorsement or recommendation of use.

## Standard Operating Procedure for Mysid Laboratory Analysis

### 1.0 SCOPE AND APPLICATION

- 1.1 This standard operating procedure is used to identify, sex, enumerate, and measure mysids from the Great Lakes.

### 2.0 SUMMARY OF METHOD

- 2.1 The method involves macroscopic and microscopic examination of mysid samples. The entire sample is examined for mysids in a sorting tray. Marsupia of up to 25 gravid female mysids are examined under a stereoscopic microscope for number and stage of brood. Gravid females may have been separated from the bulk sample in the field (see SOP for sampling). Up to 125 mysids (including up to 25 gravid females) are photographed for digital length measurement.

### 3.0 SAMPLE COLLECTION AND PRESERVATION

- 3.1 See U. S. EPA GLNPO Standard Operating Procedure (SOP) for Mysid Sample Collection and Preservation (LG409). See the WQS SOP Master List for the current survey to locate the correct version.

### 4.0 APPARATUS

- 4.1 Most supplies can be acquired from biological supply companies (such as Wildlife Supply). The supplies needed are as follows:

Dissecting microscope with 6x to 50x magnification

600-mL graduated cylinder

Sieve with 200- $\mu$ m mesh

Sieve with 100- $\mu$ m or finer mesh

Funnel (mouth larger than sieve)

Glass sorting tray

Forceps

Microprobe

50-mL Beaker

250 to 500-mL glass jars

Scintillation or similar vials

Retort stand

Clamp holder

Round burette clamp

Digital camera with ability to take at least 16 megapixel w/ macro, or 10 megapixel with super-macro  
ImageJ or similar software (<http://imagej.nih.gov/ij/>)

## 5.0 REAGENTS

5.1 Reagents can be ordered through chemical supply companies.

5.2 The reagents needed are as follows:

Ethanol (95% Ethyl Alcohol solution)

## 6.0 ANALYTICAL PROCEDURE – MYSID SAMPLE ANALYSIS

### 6.1 Bench Data Sheet

6.1.1 Data sheet (see Appendix 1) records include: Lake, Collection Date, Sample ID, Station, Replicate, Lab Analyst, Processing Date, Location, Camera Name, Analysis Type, Prior Analyst and date [for QA or training counts]. This information should be on the field sheet and sample bottle. Also record processing information associated with this specific lab analysis including: lab analyst, processing date, location of processing, name of camera used, type of analysis (Regular, Quality Assurance, Training Solo, or Training Verification), and (if applicable) lab analyst and processing date for previous lab analysis of this sample. The bench data sheet also includes (1) data on gravid female mysids, with detailed data on up to 25 (separated in field/lab, embryo development stage, embryo count, and picture ID); (2) data on all other mysids in the sample, with detailed data on up to 100 (count and photo IDs of mysids in each of three categories: juvenile, male, and non-gravid female); and (3) count of “extra” mysids (mysids in addition to the 100) which are intact, just heads, just tails, and the total of these [calculated as # intact + max(# just heads, # just tails)]. The total number of mysids in the sample is the sum of the number of gravid females, the measured animals, and the extra animals. Check boxes on presence of other photographs and mysid species, with notes on these. Other notes. Place to record initials and date of entry into electronic database.

### 6.2 Initial Sample Processing Prep

6.2.1 The steps of sample preparation listed in this section involve working with formalin and must be performed under an operating fume hood while wearing proper personal protective equipment (eyes and hands). A few specific steps do not involve formalin and may be performed outside a fume hood.

6.2.2 Process samples one at a time. Open sample jar and pour contents through a 200- $\mu$ m mesh or finer sieve over funnel into beaker or formalin waste receptacle. Using a squirt bottle, rinse the emptied sample jar with water to collect any remaining contents and pour rinse water with contents through sieve. Rinse the sample jar thoroughly with water into the sieve to remove any residual organisms adhering to walls of the sample jar. Recap the sample jar, and set aside until section 6.8. The re-sealed sample jar may be set aside outside the fume hood.

- 6.2.3 Pick up each individual cryovials with forceps, and thoroughly rinse off formalin residue while holding over the sieve, before setting aside in a beaker or other holder (until section 6.5). If necessary, sealed and rinsed-off cryovials may be set aside outside the fume hood.
- 6.2.4 Thoroughly rinse remaining sample contents with water while in sieve (over waste beaker/receptacle) in fume hood. Be sure to rinse off all formalin residue from sample and sieve.
- 6.2.5 Transfer rinsed-off sieve contents to the sorting tray. Be sure to thoroughly rinse sieve contents into the sorting tray using a squirt bottle. Set the sorting tray aside temporarily (until section 6.4). This step may be done outside the fume hood.

### 6.3 Cryovials Processing Prep

- 6.3.1 The steps of sample preparation listed in this section involve working with formalin and must be performed under an operating fume hood while wearing proper personal protective equipment (eyes and hands). A few specific steps in 6.3 do not involve formalin and may be performed outside a fume hood (These include: 6.3.5, 6.3.7).
- 6.3.2 Cryovials contain mysids which were suspected in the field to be gravid females. Occasionally, some of these mysids are not gravid females (mistaken in the field). Place any such mysids back into the sorting tray with the rest of the sample. Make a note on the bench sheet if some cryovials contained animals that were not gravid females. The cryovials are used because embryos can fall out of a gravid female's marsupium during sample transportation, and then we cannot know which gravid female produced the embryos which we find loose in the main sample jar. Storing gravid females in individual cryovials allows any embryos that come loose during transportation to be tracked to a particular gravid female.
- 6.3.3 Process cryovials one at a time. Open a cryovial, empty its contents into a 50-mL beaker, and rinse the cryovial out into the beaker. It can take several mL of water to rinse out both the adult mysid and all loose embryos. Ensure enough water is used. Visually inspect the beaker (still under the fume hood) to estimate how many embryos have fallen out of the marsupium. Ensure through the rest of the process that these embryos do not get left behind in beakers, sieves, etc.
- 6.3.4 Pour and rinse the mysid, her embryos, and the formalin-water from the 50-mL beaker through a 100- $\mu$ m or finer mesh sieve into the formalin waste beaker/receptacle. Rinse gently but thoroughly with water.
- 6.3.5 Transfer rinsed-off sieve contents to an individual covered petri dish with water squirt bottle. Be sure to rinse out all embryos loose in sieve. Cap petri dish and set aside temporarily (until section 6.5). This step may be done outside the fume hood.
- 6.3.6 Recap cryovial, and set aside until section 6.8. The cryovial may be set aside outside the fume hood.

6.3.7 Repeat steps 6.3.2 – 6.3.7 for each cryovial from the sample.

#### 6.4 Sample Processing Steps

6.4.1 Scan the sample for any gravid females and unusual mysids and remove them carefully to separate petri dishes, making sure no embryos are lost in the process. It is best to place a glass sorting tray over a black or white surface. A black surface is better for spotting the whole animal, whereas a white surface is better for spotting eyes.

6.4.2 Remove any unusual mysids that might be neither *Mysis diluviana* nor *Hemimysis anomala* (see Fig. 1) and bring these animals to the attention of the project manager or team leader. They will confirm the taxonomic identification and decide if the animal should be sent out for further taxonomic evaluation. Be sure to note on datasheet if other mysid species than *Mysis diluviana* are confirmed as present in sample. Currently, two species are present in the Great Lakes (*Mysis diluviana* and *Hemimysis anomala*) but almost all mysids in GLNPO samples are expected to be *Mysis diluviana*. Pothoven et al. (2007) will be helpful for describing species-specific characters that differentiate *Mysis diluviana* and *Hemimysis anomala*. Also relevant is Audzijonyte and Väinölä (2005), who describe the *Mysis relicta* complex (*M. diluviana* was formerly grouped with *M. relicta*). Three other mysid species are spreading across Europe and may therefore arrive to the Great Lakes at some time and we therefore added pictures of these species. For more details on species-specific characters of *Katamysis warpachowski*, *Limnomysis benedeni*, and *Paramysis lacustris* (synonym: *Mesomysis czerniavskiy*), Sars (1893) is a good reference (see also: fig. 1). The general reference for mysid biology is Mauchline (1980).

6.4.3 Identify gravid females as animals with an embryo-filled marsupium. An embryo-filled marsupium on a preserved gravid female is typically an obvious opaque whitish color. Place these gravid females into individual covered petri dishes (up to 25 total individual gravid females for the sample).

6.4.4 If the total number of gravid females (from cryovials and the sorting tray) exceeds 25 for the sample, place gravid females in excess of 25 together in a separate petri dish. Animals in excess of 25 should be photographed and measured but do not need to be further processed.

#### 6.5 Gravid mysid processing

6.5.1 Examine the individual gravid female mysids in each petri dish from steps 6.3.5 and 6.4.3 under a dissecting scope.

6.5.2 The total number of gravid females in cryovials [separated in the field] should have been marked in the field sheet and should also be noted in the bench sheet where specified.

6.5.3 Processing of gravid females from the cryovials.

- 6.5.3.1 Number the gravid female petri dishes and fill in gravid data table on the datasheet according to this numbering.
  - 6.5.3.2 For each of the females from a cryovial (6.3.5), the “Field/Lab” column of the gravid data table on the bench datasheet should be labeled as “Field.”
  - 6.5.3.3 Note whether the pouch looks full or not. Some mysid embryos may have escaped, and mysids are suspected to loose embryos through the embryo development stages in the marsupium (Mauchline 1980).
  - 6.5.3.4 Remove embryos from the marsupium with gentle pressure using a microprobe. Record the number of embryos present (both the ones found loose in the cryovials and the ones removed under the microprobe).
  - 6.5.3.5 Record the stage of the embryos present (E = early, M = mid, L = late, Fig. 2, 3, Mauchline 1980). Early stage embryos are spherical or slightly elongated and contained within the egg membrane which is shed at the end of this stage. These are stage-I embryos (and are often referred to as “eggs”). Mid stage embryos resemble commas. Some may have slight eye development. The thoracic legs and antennae develop during this stage and the eyes become pigmented but are covered by a thin membrane. These are stage-II embryos (and are often referred to as “larvae”). Late stage embryos resemble adults and have developed eyes that are not constrained by a membrane. These are stage-III embryos (and are often referred to as “eyed larvae”).
  - 6.5.3.6 Set mysid with embryos in covered petri dish aside (with number maintained) until photographed (section 6.8).
- 6.5.4 Process lab-separated gravid females.
- 6.5.4.1 Number the gravid female petri dishes with the first number following the last number for field-separated gravid females (see step 6.5.3.1) and fill in gravid data table on the datasheet according to this numbering.
  - 6.5.4.2 For each of the females from the sorting tray, the “Field/Lab” column of the gravid data table on the bench datasheet should be labeled as “Lab.”
  - 6.5.4.3 Continue processing as in 6.5.3.3 – 6.5.3.6.
  - 6.5.4.4 Set aside numbered petri dishes of individual gravid females (until section 6.8).

- 6.6 Enumeration of juveniles, males and non-gravid females.
- 6.6.1 Stir/swirl sorting tray contents to randomly distribute mysids for sorting. Scan from one end of the tray, carefully removing all mysids (whole or partial) while progressing towards the other end of the tray. Make sure that all mysids, even small ones, are removed as you progress through the tray. Do NOT first remove the most visible mysids. This will ensure that the measured individuals are not a biased subsample of the mysids in the sample. The first 100 whole mysids of each species removed from the tray in this way should be sorted into petri dishes by species and maturity/sex.
- 6.6.2 Maturity/Sex of mysids
- 6.6.2.1 Animals smaller than 10 mm (*M. diluviana*) or 4 mm (*H. anomala*) (standard length: rostrum tip to base of telson) can be considered “juvenile” without further investigation of sexual characteristics. It is difficult to distinguish young *M. diluviana* males from females when they are <10 mm long (Pothoven et al. 2004).
- 6.6.2.2 Assign sex when possible for *M. diluviana*  $\geq$  10 mm. Determine sex using characteristics of an adult (bifurcated and developed 4<sup>th</sup> pleopod in males; obvious marsupium in females; Fig. 4, 5).
- 6.6.2.3 If sex characters are not obvious and the animal is larger than 10 mm, closely examine the animal under a microscope for a thickened 4<sup>th</sup> pleopod in early stages of development (sort with males if present), or for expanded flaps of the lamellae on the 6<sup>th</sup> and 7<sup>th</sup> thoracic legs in early stages of marsupium development (sort with females if present). Sometimes the pleopods may be tight against the abdomen; run a fine probe or forceps along the abdomen starting at the uropoda and the pleopods will usually become evident.
- 6.6.2.4 If neither development of marsupium nor slight development of 4<sup>th</sup> pleopod is evident, sort the mysid with juvenile mysids.
- 6.6.2.5 Note any unusual features of the animals.
- 6.6.3 Any mysids remaining after the first 100 are removed should be identified to species and counted, and the number recorded in the data sheet under “Extras: intact”. Any partial mysids (abdomens or cephalothoraxes) should also be counted and recorded as “Extras: heads” or “Extras:tails”. The number in each group should be noted on the appropriate part of the bench data sheet.
- 6.6.4 Note any unusual features of the sample or the animals in the sample (for example condition of the preservation).



- 6.7 Photographing mysids for length measurements (first 100 animals removed from the sample).
- 6.7.1 Make legible labels for photographs (see Appendix 1) which include sample ID number, photograph date, analyst surname, and whether the photograph is of juveniles (“Juv”), males (“Male”), or females (“Fem”). For gravid female mysids note gravid ID number, which corresponds to number in row of datasheet table.
  - 6.7.2 Over a dark countertop or surface, place a flat, transparent, metric ruler and the label. Place the petri dish (transparent) on top of the ruler and label. The ruler should have a sliver of white backing behind at least 10 mm of the edge in the center of the ruler to make tick-marks visible. Arrange the mysids so they are all visible in the field of view from a camera mounted on a retort stand or other device at close range (< 14 cm high; Fig. 6). It is best if no mysids are over white or light-colored surfaces, do not overlap each other, and are lying flat against the surface of the petri dish. (Fig. 7). There may be some animals that are twisted in such way that it is impossible to lay them flat, those animals should still be photographed, but will not be measured.
  - 6.7.3 Record the filename of the photograph as displayed on the camera (e.g., “3574.jpg”) in the appropriate field of the datasheet for gravid females and for random 100 subsample.
  - 6.7.4 Take separate pictures for each species in sample.
  - 6.7.5 Transfer photographs to a computer hard drive and an external or cloud backup drive. Picture names should be maintained as recorded in data notebook, but should have a prefix consisting of a sample ID. Pictures should be placed in folders titled by the sample ID number-
- 6.8 Re-Preserving and Archiving Mysid Samples
- 6.8.1 Re-preserve sorting tray contents in sample jar with 95% Ethanol for a final concentration of 70%. Leave room for mysid vials and cryovials (see below).
  - 6.8.2 Preserve each petri dish’s mysids in their own, labeled vial (groups of mysids) or cryovial (individual gravid female mysids and embryos), with 95% ethanol (for a minimum final concentration of 70% ethanol).
  - 6.8.3 Vials should be labeled (see Appendix 1) with sample ID number, date of processing, analyst initials and one of, “Juv,” “Mal,” “Fem,” or, “Extra.” Gravid female cryovials should be labeled sample ID and the female gravid ID number (e.g., “13GS27D95 FGr 1). If a vial or cryovial contains a species other than *Mysis diluviana*, include species name on the label. Labels should be resistant to ethanol.
  - 6.8.4 Insert cryovials and vials into sample Jar. Fill the jar with 70% ethanol so that the individual tubes are covered. If possible, use a smaller sample jar (e.g. 250 or 500 mL) for archiving.

- 6.8.5 Seal the sample jar lid with parafilm. Important to avoid minimize evaporation of ethanol.
- 6.8.6 Jars of processed samples should be labeled (see Appendix 1) with the sample ID number, survey, lake, station, date of collection, target organisms (“Mysids”), date of processing, and analyst surname.
- 6.8.7 Recording keeping
  - 6.8.7.1 When a sample has been processed, an entry should be made in an electronic sample-status file, with the sample ID and date of completion. The lab analyst surname should be entered into a sample database table (see 8.4).
  - 6.8.7.2 Handwritten data sheets (see Appendix 1) should be scanned and stored with the electronic data files in a folder for each survey.

## 6.9 Mysid Digital Measurement

- 6.9.1 Open the image file in ImageJ. View photo at a magnification appropriate for accuracy.
- 6.9.2 Use the straight line tool to measure the pixel distance of 1 cm on the ruler. Be sure the length of this line is exactly perpendicular to the tick marks of the ruler; this can be done by measuring the distance at the edge of the ruler or along the baseline of the tick marks. Be sure the measuring line begins and ends on the same side of the tick marks. Go to Analysis>Set Scale. Set known distance to “10” and units to “mm” and click OK. Measurements will now be recorded directly in scaled mm. Record the measurement for the scale line (press “M” on keyboard) and draw it on photograph with measurement number for future reference (press “Alt-D” on keyboard). Note that a line will draw simply by pressing “D”, but “Alt-D” is required to print a number on the photograph when using ImageJ.
- 6.9.3 To measure a mysid, use the segmented line tool to draw a line from the tip of the rostrum to the end of the abdomen (same as the base of the telson; Fig. 8a-b). Use as few segments as possible to capture the curvature of the dorsal surface of the mysid (e.g., Fig. 8c). Capturing this dorsal curve is a more consistent method than using a set number of segments which require subjective determination of where to place points and thus introduce substantial variability. Vertices of the line should be along the dorsal surface of the mysid. Recognize that the dorsal surface will not always be on the edge of the mysid as photographed, as it is nearly impossible to get all the mysids in a photograph to lay perfectly on their lateral sides (e.g., Fig. 8d). Record the distance of the line in ImageJ (press “M” on the keyboard). After recording the distance, draw the measurement taken on the photograph for a record (press “Alt-D” on the keyboard). This allows mistakes in recording measurements to be noticed both during and after the measurement process. Note that a line will draw simply by pressing “D”, but “Alt-D” is required to print a number on the photograph (ImageJ).

- 6.9.4 Go through the mysids systematically to ensure measurement of all mysids and no duplication of measurements. The total number of measurements should be 1 more than the number recorded on the bench sheet (1 scale measurement + # mysids). If this is not the case, look through each mysid measurement in order to ensure no mysids were measured twice (missing a number on the photo) or that no mysids were missed (a number appears more than once on the photo). In the event of a disagreement of number of mysids in photograph and number recorded on bench sheet, believe the photographed number, especially if more than recorded on bench sheet, unless a specific note on the bench sheet addresses this discrepancy (i.e., “one 3 mm mysid not in photograph”).
- 6.9.5 If an animal cannot be measured, note that on the data file. This occur when the animal is damaged or is not laid flat against the bottom of the petri dish.
- 6.9.6 Save the photograph in ImageJ as a tif file with the same name as the original jpg file. This will preserve the measurement lines used. Saving the file as a tif is better than a jpg file because the tif file preserves the measurement lines. A tif file is also the default image type for ImageJ.
- 6.9.7 Save the measurement results in ImageJ as a \*.txt file with the same name as the saved photograph (this is the photo ID, e.g., “G1404\_003\_245”).

## 7.0 CALCULATION OF MYSID BIOMASS

- 7.1 Biomass of individual mysids will be calculated using this L-W regression:

$$\ln(W, \text{ dry wt in g}) = -12.27 + 2.72 \ln(L, \text{ mm}) \quad n = 241, r^2 = 0.86 \quad (\text{Johannsson 1995}).$$

This equation is derived for the length measure used here (tip of rostrum to end of abdomen/base of telson), even though the original reference states otherwise (Johannsson pers. comm.). The equation for tip of rostrum to cleft of telson is:  $\ln(W, \text{ dry wt, g}) = -12.55 + 2.72 \ln(L, \text{ mm})$  (Rudstam et al. 2008)

## 8.0 CALCULATIONS AND REPORTING

- 8.1 Mysid data are reported as number in the sample, areal density (#/m<sup>2</sup>) and area biomass (g DW/m<sup>2</sup>) which are calculated as follows:
- 8.1.1 Number in sample = total number of intact animals (gravid+random sample+extra) + the maximum of the number of heads and the number of tails. This is a minimum number of mysids in the sample. Number in sample of separate groups is calculated by assigning the extra animals to groups based on proportions in the random sample. Gravid females are counted in the whole samples and not included in the extra or random samples.

8.1.2 Areal density:

$$D_a = \frac{N}{A}$$

where:

N =Number of organisms in sample

D<sub>a</sub> =Density of organisms in numbers per square meter

A=Area of the mouth of the net (m<sup>2</sup>) = 0.7854 m<sup>2</sup> for 1-m diameter net

8.1.3 Areal biomass:  $B_a = D_a b_{av}$

where:

b<sub>av</sub> =Average mass of an individual mysid in the sample

B<sub>a</sub> =Biomass of organisms in grams per square meter

Note the average mass is calculated using the calculated individual mass measurements, not from the average length in the sample.

- 8.2 Two measures of lengths are common in mysids, (1) from the tip of the rostrum to either the end of the abdomen as used here, or (2) from the tip of the rostrum to the end of cleft of the telson. Because it is easier to measure to the end of the abdomen on pictures, and because Canada's DFO has a long history of measuring to the end of the abdomen in the Great Lakes, we use this measurement for mysids samples. Note that mysids in GLNPO zooplankton samples are measured from the tip of the rostrum to the cleft of the telson (SOP LG 403rev 08). There is a strong relationship between the two measures (Fig. 9):

$$[\text{Length to end of abdomen}] = 0.8895 * [\text{Length to cleft of telson}]$$

(Source Ted Schaner and Michael Connerton,  $R^2 = 0.9938$ ,  $N=16$ ).

8.3 Data Entry

- 8.3.1 Data are transferred to an excel spreadsheet, proof-read, and entered into an ACCESS database. Information on individual mysid, on sample collection and on analysis procedure are entered in separate data tables. Test queries in the database includes checks that all samples included in the individual database have entries in the sample and analysis tables and that all lengths entered are within expected ranges. Animals smaller than 10 mm are classified as juveniles. Animals > 11 mm are classified as males or females. Animals >11 mm that were classified as juveniles because of lack of obvious sex characteristics are classified as females in the database. Animals between 10 and 11 mm may be classified as juveniles, males or females in the database.

- 8.3.2 All density and biomass calculations are made in the ACCESS database.
- 8.3.3 All field sheet and bench sheets should be scanned and stored on a secure cloud storage. Original field and bench sheet will be kept with the grantee until both samples and these original sheets are submitted to EPA for long-term archiving.
- 8.3.4 Backup/duplication must be made of all data submitted to EPA. Data should also be backed up on a secure cloud-based storage device (e.g. CornellBox for Cornell University).

## 9.0 QUALITY CONTROL AND METHODS PRECISION

### 9.1 Duplicate analyses

- 9.1.1 Ten percent of all samples taken a given year are analyzed in duplicate by a second analyst. The second analyst will scan the remaining sample, count, sex and stage the animals separated by the first analyst, and re-measure lengths on one photograph. Thus, all mysids, and separately all gravid females, are recounted by the second analyst but only a subset are re-measured.
- 9.1.2 These data are not combined with the primary data as only one picture is used for QA length measurements. Instead the counts of total number of mysids, of gravid females, and the average length from one photographs from both analysts are reported in a separate QA table in the data submissions.
- 9.1.3 It is expected that the counts and measurements by two analysts should be no more than 10% different from each other. The QA parameters used for mysids are total counts, counts of gravid females, and average lengths.
- 9.1.4 If there are discrepancies, the project leader will discuss with the analysts and determine if samples need to be recounted, resorted, or pictures re-measured. If the animals are small, even small differences in length measurements may result in lower agreement. Explanations for failed QA data should be given in the submission letter.

### 9.2 Training

- 9.2.1 New analysts will be trained under direct supervision of an experienced analyst on at least 3 samples, at least one of which is from spring, and one of which is from summer. All three embryo stages should be viewed. Other species that may be present (*H. anomala*, other possible invaders) should be viewed using voucher specimens.
- 9.2.2 This phase should be continued until the new analyst recalls the processing steps, accurately determines species, sex and embryo stages, captures good photographs, and takes accurate digital measurements.

- 9.2.3 Then, the next two samples analyzed by the new analyst will also be assessed by the experienced analyst and their results compared as per section 9.1.3 and 9.1.5. If both samples are within the 10% limit in all criteria, then the new analyst can analyze samples on their own.
- 9.2.4 The most difficult aspects of training are the determination of sex for immature animals  $\geq 10$  mm in length and the digital measurement technique (placing anchor points of segmented line). Be sure that the trainee is confident and accurate with these procedures before training is complete.
- 9.2.5 When available, samples from other surveys can be used to train new analysts.

## 10.0 SAFETY AND WASTE DISPOSAL

- 10.1 Proper PPE should be worn in the laboratory while handling and preparing samples for analyses (this should include appropriate gloves and safety glasses when handling formalin (37% formaldehyde). Follow all laboratory waste disposal guidelines regarding the disposal of formalin (37% formaldehyde) and ethanol (95% ethyl alcohol) solutions.

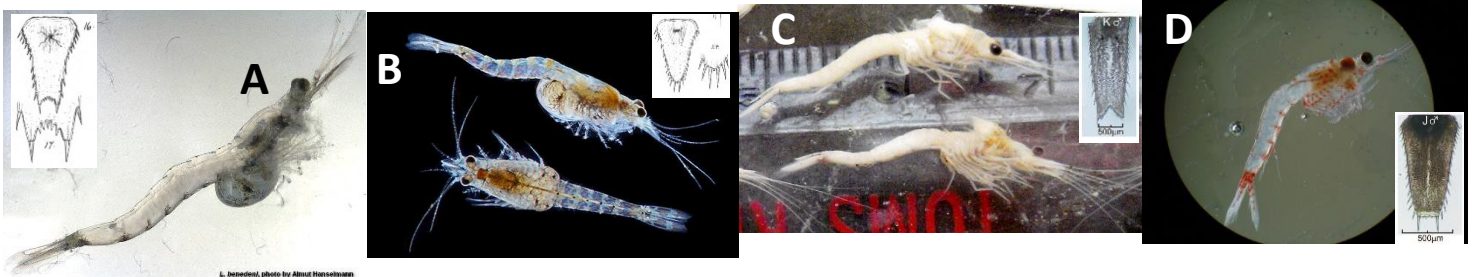
## 11.0 REFERENCES

- 11.1 Audzijonyte, A., Väinölä, R., 2005. Diversity and distributions of circumpolar fresh- and brackish-water *Mysis* (Crustacea : Mysida): descriptions of *M. relicta* Lovén, 1862, *M. salemaai n. sp.*, *M. segerstralei n. sp.* and *M. diluviana n. sp.*, based on molecular and morphological characters. *Hydrobiologia* 544, 89-141.
- 11.2 Borza, P., 2014. Life history of invasive Ponto-Caspian mysids (Crustacea:Mysida): A comparative study. *Limnologica* 44, 9-17.
- 11.3 Grossnickle, N.E. and A.M. Beeton. 1979. Antennal scale length as a measure of relative size in the opossum, shrimp, *Mysis relicta* Lovén. *Crustaceana* 36: 141-146.
- 11.4 Johannsson, O.E., 1995. Response of *Mysis relicta* population dynamics and productivity to spatial and seasonal gradients in Lake Ontario. *Can. J. Fish. Aquat. Sci.* 52, 1509-1522.
- 11.5 Kelleher, B., G. Van der Velde, K. J. Whittmann, M. A. Faasse, and A. Bij de Vaate. 1999. Current status of the freshwater Mysidae in the Netherlands, with records of *Limnomysis benedeni* Czerniavsky, 1882, a Pontocaspian species in the Dutch Rhine branches. *Bulletin Zoologisch Museum University van Amsterdam* 16: 89-94.
- 11.6 Mauchline, J., 1980. The biology of mysids and euphausiids. In: Blaxter, J.H.S., Russell, F.S., Young, M.s (Eds.), *Advances in Marine Biology*, pp. 1-369.
- 11.7 Pothoven, S.A., G. L. Fahnenstiel, and H.A. Vanderploeg. 2004. Spatial distribution, biomass and population dynamics of *Mysis relicta* in Lake Michigan. *Hydrobiologia* 522: 291-299.

- 11.8 Pothoven, S. A., I. A. Grigorovich, G. L. Fahnenstiel, and M. D. Balcer. 2007. Introduction of the Ponto-Caspian bloody-red mysid *Hemimysis anomala* into the Lake Michigan Basin. *Journal of Great Lakes Research* 33: 285-292.
- 11.9 Rudstam, L.G., Schaner, T., Gal, G., Boscarino, B.T., O'Gorman, R., Warner, D.M., Johannsson, O.E., Bowen, K., 2008. Hydroacoustic measures of *Mysis relicta* abundance and distribution in Lake Ontario. *Aquatic Ecosystem Health and Management* 11: 355-367.
- 11.10 Sars, G. O. 1893. *Crustacea Caspia*, contributions to the knowledge of the carcinological fauna of the Caspian Sea. Part I, Mysidae. Imprimerie de L'academie Imperiale des Sciences, St. Petersburg.
- 11.11 Walsh, M.G., Boscarino, B.T., Marty, J., Johannsson, O.E., 2012. *Mysis diluviana* and *Hemimysis anomala*: Reviewing the roles of a native and invasive mysid in the Laurentian Great Lakes region. *J. Great Lakes Res.* 38 (Supplement 2): 1-6.

FIGURES

FIGURE 1: GREAT LAKES MYSIDS AND TWO POTENTIAL INVADERS



Pictures of Mysids that are or may be in the Great Lakes, with respective insets of species telson. A) *Limnomysis benedeni*, B) *Katamysis warpachowski*, C) *Mysis diluviana*, D) *Hemimysis anomala*. Form is more important than color or transparency here; most will lack color (as *M. diluviana* in C) when preserved.

The following observations are based on voucher specimens held at Cornell and taxonomic resources:

- *Limnomysis benedeni* telson has a rounded, U-shaped cleft, with spinulose concave lateral margins. Brood pouch laterally wider and with markings on lateral surfaces of brood pouch, rather than ventral surface as in *M. diluviana*. Antennal scales tapering in lanceolate form, with narrow base; dorsal texture of scale corrugated like a sea-shell. (A)
- *Katamysis warpachowski* have a convexly-rounded posterior margin of telson. *K. warpachowski* thoracic legs stubby, and held tightly to ventral surface of thorax; thoraxes dorso-ventrally compact. *K. warpachowski* uropoda margins are with short setae. Texture of uropod akin to *L. benedeni* antennal scale, but less pronounced (B)
- *Mysis diluviana* have a V-shaped cleft in their telson, with straight-to-convex lateral margins. Antennal scales fully setose margins. Uropoda are also setose all round. (C)
- *Hemimysis anomala* have straight, posterior, setose margin of telson. *Hemimysis* also have the proximal half of the lateral edge of their antennal scales non-setose. (D)
- *Paramysis lacustris* (Syn: *Mesomysis czerniavskyi*) are the most similar to *Mysis diluviana* in appearance. The major difference is in the antennal scale (Sars, 1893, Plate 5). The outer margins are smooth and non-setose, forming into a spiniform projection at anterior end, which is somewhat anteriorly truncated. Anterior and interior margins of antennal scales are setose.

See Pothoven et al (2007) for more information about *M. diluviana* and *H. anomala* characters, and Sars (1893) for more information about *K. warpachowski* and *L. benedeni* characters. *L. benedeni* and *K. warpachowski* photographs by Almut Hanselmann; *M. diluviana* photograph by Toby Holda; *H. anomala* photograph by NOAA GLERL; telson drawings from Sars (1893), and telson photographs from Pothoven et al (2007).

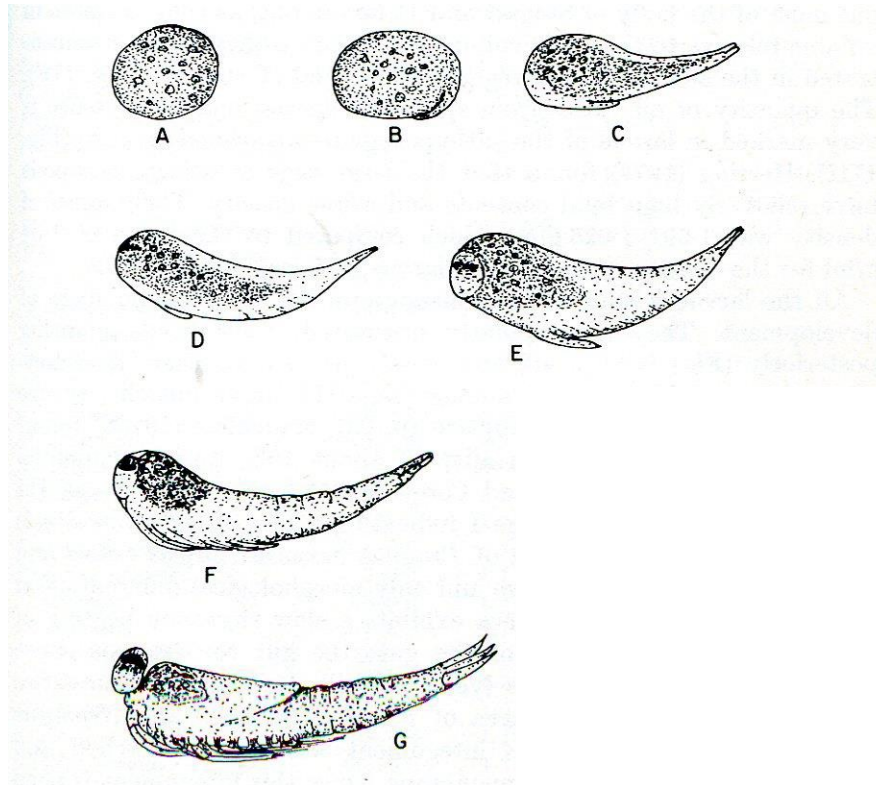


**FIGURE 2: PHOTOGRAPHS MYSID EMBRYO STAGES**



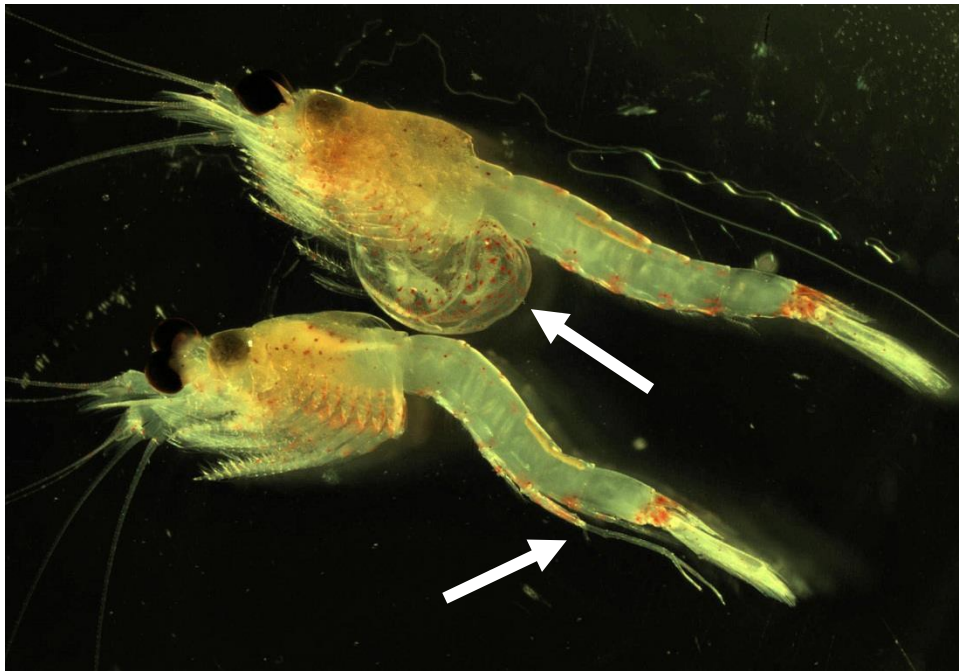
Pictures from Kelly Bowen and Toby Holda

FIGURE 3: DRAWINGS MYSID EMBRO STAGES



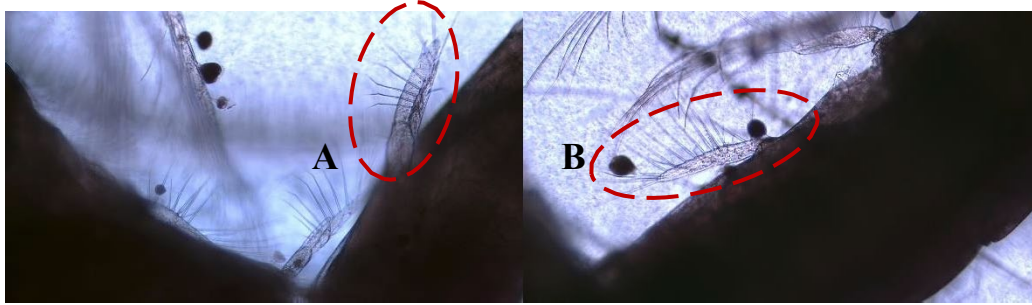
From Mauchline (1980) Fig 2.17. Larvae of a mysid. A. egg-shaped embryo (stage I or Early stage), B embryo with developing abdomen (stage I, Early stage), C-F stage II (Mid-stage) larvae at successive stages of development, G stage III (Late stage) larva after the first larval molt and with the eyes stalked. Stages I, II, and III correspond respectively to early, mid, and late stages in this protocol.

**FIGURE 4: FEMALE (UPPER ANIMAL) AND MALE HEMIMYSIS**



Arrows point at the brood pouch (marsupium) for the female and the enlarged fourth pleopod for the male.

**FIGURE 5: EARLY DEVELOPMENT OF SEX CHARACTERISTICS IN *MYSIS DILUVIANA***



Pleopods of mysids – view of ventral surface of abdomen. Fourth (A) pleopod of early-development immature male (common level of development at lengths 9-12 mm). Undeveloped pleopod (B) as in female mysids for first-fifth pleopods, and as in male mysids for first, second, and fifth pleopods. Compare A and B. A is bifurcate, while B is not bifurcate. Note that this distinction can be quite subtle. If the angle is different, this bifurcate characteristic may appear merely as a thickness to the pleopod (so rotation is required to confirm sex in that case). Note that focal distance is important. Fully developed male fourth pleopod is similar to the one shown for *Hemimysis* in Figure 4.

**FIGURE 6: CAMERA STAND**

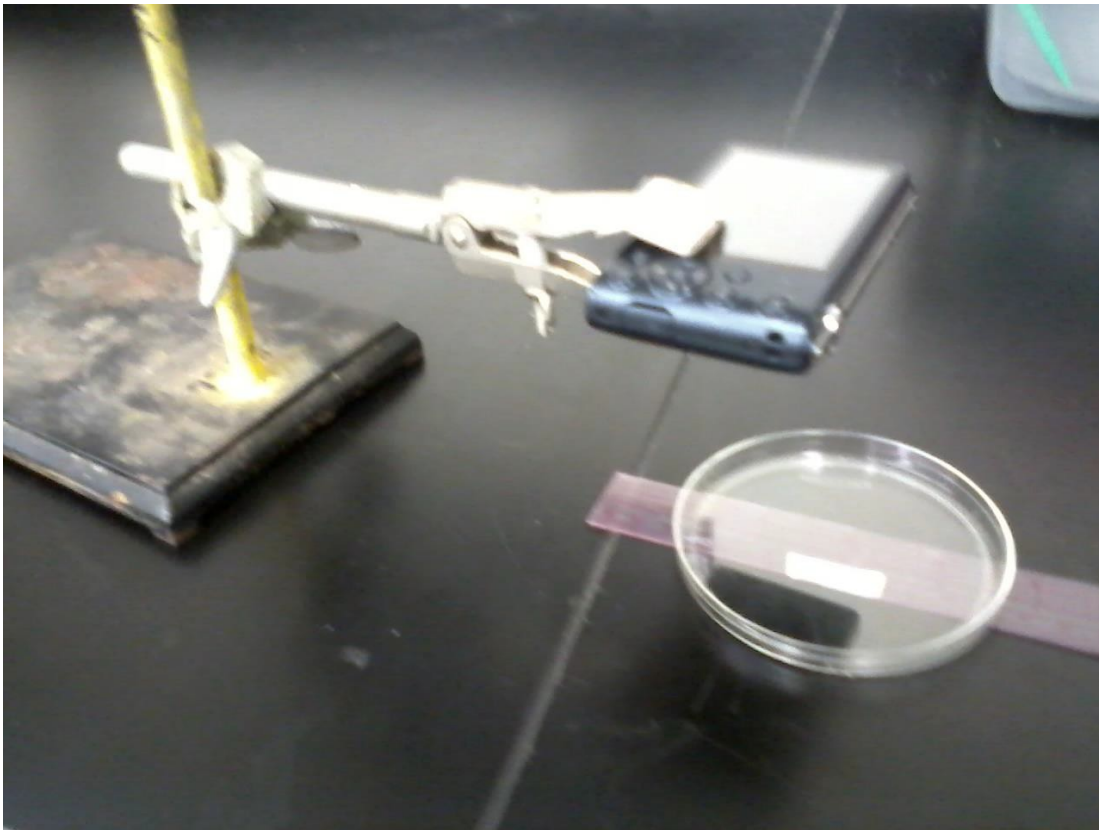
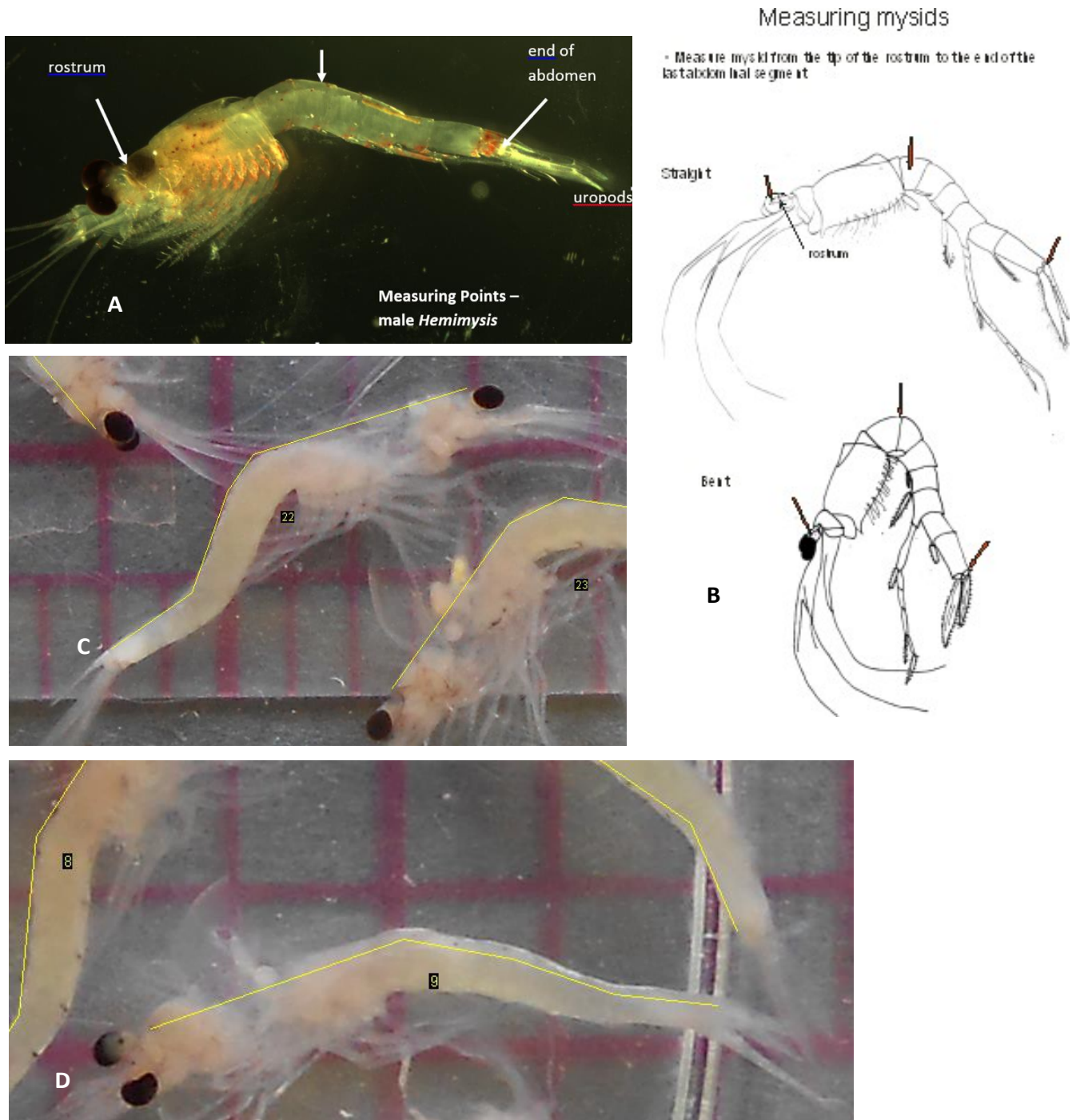


FIGURE 7: MYSID PHOTOGRAPH SETUP



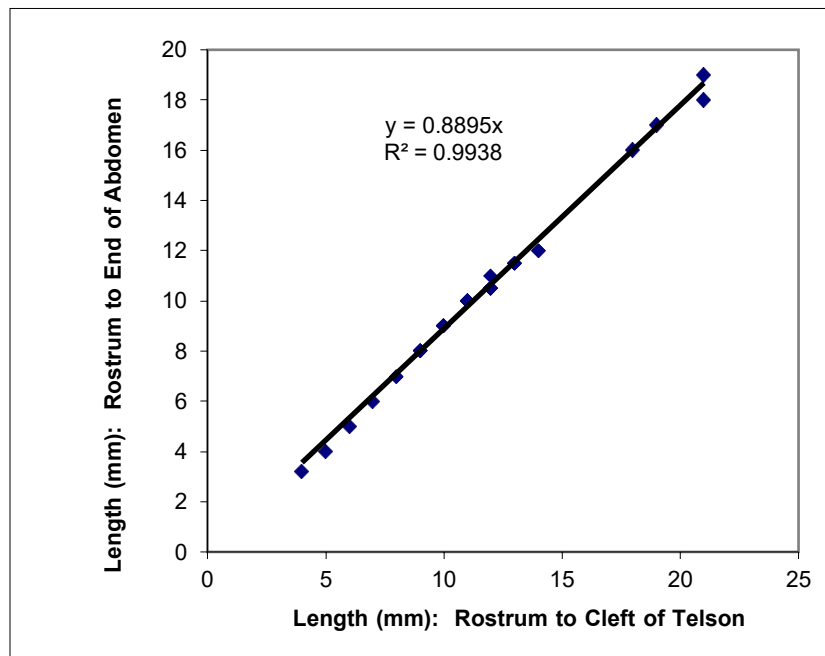
*Mysis diluviana* arranged in petri dish. Note the dark background, the shading of the dish from glare where there are mysids, and the spacing of mysids so they do not overlap. Also, note the label format.

FIGURE 8: MYSID LENGTH MEASUREMENTS



Photos A and B from Kelly Bowen. Photos C and D from Toby Holda.

FIGURE 9: RELATIONSHIP BETWEEN COMMON MYSIS LENGTH METRICS



Regression of mysid length as measured from tip of rostrum to end of abdomen as a function of mysid length as measured from tip of rostrum to cleft of telson (see Fig. 1). From Ted Schaner and Michael Connerton.



**APPENDIX 1: FORMS**

**Bench Data Sheet Format .....24**  
**Label Formats .....25**

**Bench Data Sheet Format**

Lake: \_\_\_\_\_ Coll Date: \_\_\_\_\_ Sample ID: \_\_\_\_\_ Station: \_\_\_\_\_ Rep: \_\_\_\_\_

Lab Analyst: \_\_\_\_\_ Process Date: \_\_\_\_\_ Location: \_\_\_\_\_ Camera Name: \_\_\_\_\_

Analysis Type:  R  QA  TS  TV Prior analyst and date (if applicable): \_\_\_\_\_

**Data on Gravid female *Mysis diluviana* in sample:**

Data on photographed Gravid females (up to 25)

ID	From Vial?	EmbStg	BrdCnt	PicID	PchFull?
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					

ID	From Vial?	EmbStg	BrdCnt	PicID	PchFull?
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					

# Loose Embryos E\_\_M\_\_L\_\_ Pic ID \_\_\_\_\_

# Vial Yes \_\_\_\_\_ # Vial No \_\_\_\_\_ # >25 \_\_\_\_\_

Total Number Gravids (Sum 3 above) \_\_\_\_\_

**Data on all *Mysis diluviana* in sample (except gravid females):**

Random Subsample Counts (up to 100 total) and Photo IDs

Sex & Maturity	<i>Mysis</i> <10 mm	Male	Non-Gravid Female	Total
Count				
Pic ID				

Count "Extra" mysids: # Intact: \_\_\_\_\_ # just heads \_\_\_\_\_ # just tails : \_\_\_\_\_

Total \_\_\_\_\_ (sum of # intact and max of (# heads, # tails)

Total Mysids in Sample (Sum of totals for Gravid Females, Random Subsample, and "Extra") \_\_\_\_\_

Other photographs?  Y  N

If yes, list Pic ID with description and count of items in these photographs:

Other species present?  Y  N (Species name if yes: \_\_\_\_\_)

Details (Pic ID, "Extra count, etc.):

Other Notes:

Entered into Analysis Data Table by (initials) \_\_\_\_\_ on (date) \_\_\_\_\_

---

**Label Formats**

**Photograph Labels:**

[Sample ID]	[Analyst Surname]
[Processing Date]	Pic [Picture # for sample] [Sex/maturity of mysids]

*Example:*

12GA11S75 Holda
11-24-14 Pic1 Juv

**Vial Labels:**

[Sample ID]	[Analyst Surname]
[Processing Date]	[Vial Type]

*Example:*

12GA11S75 Holda
11-24-14Sized

**Cryovial/Female Gravid Photo Labels:**

[Sample ID] FGGr [Gravid ID]
------------------------------

*Example:*

12GA11S75 FGGr 11
-------------------

**Processed Sample Jar Labels:**

Mysids	[Sample ID]
[Station ID]	[Collection Date]
[Processing Date]	[Analyst Surname]

*Example:*

Mysids	12GA11S75
MI 40	4-2-12
11-24-14	Holda