ANALYSIS OF BENTHIC COMMUNITY STRUCTURE AND ITS APPLICATION TO MFL DEVELOPMENT IN THE WEEKI WACHEE AND CHASSAHOWITZKA RIVERS

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Prepared for:
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Table 3-5 Revised 4/9/07

Errata and Notes – (M.Heyl)

Tables 3-3, 3-4 Units :
dominance = Sqrt (% occurrence / % composition)

Table 3-5 revised after final report.
Log10 Numers of taxa – Depth is not significant (see Appendix pp38-41

Table 3-6   "X" is salinity
All linear significant
All quad significant
Only abundance polynomial is significant

Appendiix A
"BH2" = benthic diversity to base 2
L10ABUND = log 10 total benthic abundance
L10BS = log 10 benthic species (Richness)

Appendix D   (See page 27 – PCA results for basis.
O = oligotrophic 0-7 ppt
M = mesotrophic 7 – 18 ppt
P = polyhaline   18- 29 ppt
E = euhaline   > 29

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1.0 INTRODUCTION

The Southwest Florida Water Management District (District) is one of five water management districts charged with protecting and managing the State of Florida's water resources. One of the District's legislatively mandated responsibilities is to establish minimum flows and levels for surface water bodies including freshwater streams and the freshwater inflow to estuarine waters.

The objectives of this project are to quantify relationships between physical parameters, especially salinity, to the responses of benthic macroinvertebrates in the Weeki Wachee and Chassahowitzka Rivers.

1.1 Minimum Flows and Levels

Minimum flows and levels (MFLs) are the "*… flow below which significant harm occurs to the water resources or ecology of the area*" (SWFWMD, 2001). Specifically, minimum flows are defined in Florida Statutes (372.042) as "the limit at which further withdrawals would be significantly harmful to the water resources or ecology of the area". MFLs may vary both seasonally and spatially within a river.

The general approach to developing an MFL is to establish defensible *quantitative* relationships between key ecological components of the system in question (*e.g*., freshwater inflow and salinity) and a resource of concern (*e.g.,* benthic macroinvertebrates). The rationale for this approach is that the inflow regime and the resultant salinity distributions affect the structure and function of biological communities.

1.2 Benthic Macroinvertebrates

Benthic (bottom-dwelling) organisms are small but important invertebrates that include organisms such as aquatic insects, worms, snails, clams, and shrimp. The benthos live in or on the substrates of rivers, estuaries, etc. Benthic macroinvertebrates are generally sessile, although some species may undergo migrations into the water column (*e.g*., amphipod crustaceans) or produce planktonic larvae (*e.g.,* polychaete worms). As a group, however, they are relatively sedentary and are considered to be effective integrators of a variety of environmental factors, including salinity (Boesch and Rosenberg, 1981; U.S.E.P.A., 1999). Unlike the more mobile nekton, most benthic invertebrates lack the mobility to escape large or rapid fluctuations in environmental conditions.

Benthic invertebrates occupy a variety of niches *vis a vis* energy transfer. Benthic organisms process organic material as detritivores, suspension feeders, and deposit feeders, forming an essential link in the transfer of energy to secondary consumers including other benthic organisms, finfish, and avifauna. Tubiculous and fossorial benthic invertebrates may fulfill an important role in reworking sediments. In this role as bioturbators, they may bring suspended sediments into contact with the water column thereby translocating nutrients and pollutants and oxygenating sediments.

1.3 Relationships Between Flow and Benthos

With respect to supporting MFL development, the benthos is an important biotic resource that is responsive to changes in flow regimes. Flow is an influential component of riverine and estuarine systems and changes in flow can potentially affect many ecological and environmental variables.

Flow affects the volume and velocity of the river, which directly affects benthos (Figure 1-1). Under extremely high flows, benthic organisms may be physically washed out of the system. Some aquatic insects take advantage of flowing water by undergoing "drift". Aquatic drift can reduce overcrowding and facilitate feeding. Additionally, flow affects salinity, dissolved oxygen, sediments, and nutrients, which also affect the abundance and distribution of the benthos (Figure 1-1).

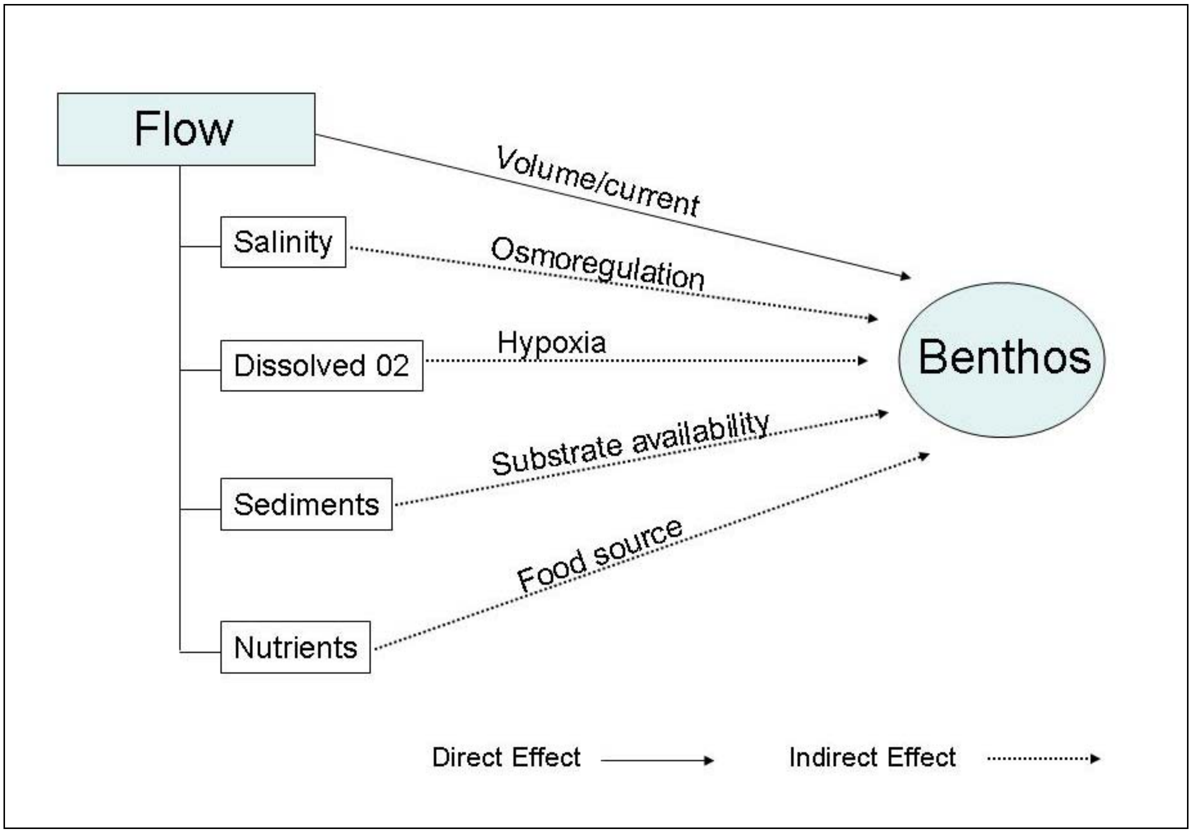

Figure 1-1. Conceptual diagram showing the direct (solid line) and indirect (dashed line) effects of flow on benthos.

Salinity is the most important physical factor affecting the biota of tidal rivers. Salinity is largely influenced by the amount of freshwater inflow entering an estuary, and it is typically negatively correlated with flow. Salinity can affect the distribution and abundance of individual species, and the overall composition of the benthic community. During high flow periods, salinity at a particular location is expected to be lower and may provide new habitat for the more motile species that are intolerant of elevated salinities. During low flow periods, saline waters may penetrate further upstream, facilitating habitat expansion for estuarine species. Generally, the salinity gradient will shift upstream and downstream based on flow conditions.

Benthic organisms are limited in their distribution within a tidal river by the physiological challenges and stresses associated with variable salinity environments. Osmotic limitations restrict the ability of many freshwater species from using habitats in downstream portions that are tidally influenced. Marine species also face osmotic problems, which restrict access to upstream freshwater habitats. True estuarine species typically tolerate a wide-range of salinities, although they may have discrete "preferences" for optimal reproduction and growth.

In summary, salinity is less of an acute stressor and more a chronic stressor for estuarine invertebrates. For example, the common isopod *Cyathura polita* can complete its life cycle over salinities ranging from 0 to 30 ppt. Northern populations are, however, capable of osmoregulation in distilled water for up to 12 hours (Kelly and Burbanck, 1976).

Changes in the timing and amount of freshwater inflow may alter the salinity regime such that shifts in dominant species occur. The physical environment may become less favorable for some species and more favorable for others. That is, the "preferred" salinity regime may now occur at a different time, in a different location, or occupy a smaller area of the system than currently. For example, the displacement of a particular salinity regime could move it to a reach of the river where the sedimentary factors are unfavorable (*cf.* "stationary" *vs.* "dynamic" habitats of Browder and Moore, 1981). Since sediment type is also a key abiotic factor affecting the structure of benthic communities, community structure could be altered. Changes in freshwater inflow then may have profound effects in terms of energy flow within the system as well as the physical reworking of the sediments.

Flow also affects dissolved oxygen concentrations by modifying residence times and by physically altering stratification conditions. Increased residence times may be associated with decreased dissolved oxygen.

Freshwater flow affects both concentrations and loadings of other water quality constituents (Boynton and Kemp, 2000; Gillanders and Kingsford, 2002). Dissolved constituents such as ions, dissolved nutrients, and metals may be diluted at higher flows and concentrated at lower flows (FDER, 1985; Grabe, 1989). The magnitude and timing of freshwater inflows affects the amount of nutrients and organic matter that enters a waterway. Thus, increased productivity may occur some time after a period of increased flows (Kalke and Montagna, 1989; Bate *et al*., 2002). Sediment loads downstream are also increased during high flows (e. g, the Mississippi River delta). Loadings of contaminants, including metals and organic compounds that bind to smaller particles (Seidemann, 1991) are often associated with increased sediment loads. Additionally, increased sedimentation may suffocate sediment dwelling organisms.

Freshwater inflow will also affect stream current velocities. Current velocity affects substrate composition by influencing the available parent material as well as organic inputs. The main components of substrate composition are grain-size, the interstitial spaces between the grains, and the presence or absence of organic detritus. Larger grained sediments drop out from the current first, and are deposited furthest upstream. Finer grained sediments are carried further downstream, with the finest sediments being carried the furthest. Organic inputs may be of various sizes, ranging from fallen trees to small organic fragments. The interstices, or the small spaces between larger grained substrate material, form micro-habitats that are used by particular benthic organisms; the interstitial spaces also provide an area for the finer grained organic matter to collect. Generally, abundance and diversity increase with increasing sediment grain size and the abundance of benthic organisms may be high where the organic content is relatively high (Gray, 1981; Grizzle, 1984).

Residence time affects the ability of phytoplankton to take up nutrients, as well as the ability for secondary producers to consume phytoplankton, and this extends to other consumers as well. Higher flows are associated with increased nutrient loading. Lower flows permit a longer residence time for chlorophyll and nutrients. During high flow conditions, flushing is more rapid and residence time in the river is reduced (Peterson and Festa, 1984; Jassby *et al*., 1995; Flannery *et al*., 2002).

1.4 Quantitative Responses of Benthos to Changes in Freshwater Inflow

Janicki Environmental, Inc. (2006) developed a suite of quantitative tools for the District capable of supporting the development of MFLs. The expected quantitative responses of benthic organisms to changes in freshwater inflow were defined. These quantitative responses are expected to integrate all of the direct influences of flow changes and the indirect influences of flow changes (*e.g*., salinity changes, dissolved oxygen concentration changes). Quantitative responses were derived in an unbiased manner from a large (>2,000 samples) database extending over two decades from 12 Southwest Florida tidal rivers.

The species that make up estuarine benthic communities exist in a continual state of change, but the basic structure of the community may be observed to have a relatively predictable response signal above the often high degree of natural variability.

Species distribution (presence/absence response patterns) within a tidal river may be limited by the physiological challenges and stresses associated with variable flow environments. True estuarine species are typically euryhaline and have adaptations that allow them to live within a wide range of salinity conditions.

Species abundances are also affected by the stresses caused by altered flows. Such changes may affect the success of individual animals within a species, consequently affecting the overall abundance of that species. For example, while the distribution of a given species may be determined by salinity, species able to tolerate saline conditions may still be affected by salinity-related stressors. Species typically have an optimal salinity that is somewhere within the range of salinity that they may be able to inhabit. The salinity in which the early life stages of certain species develop, may impact their growth and survival rates. It will also affect the availability of prey and where adults of the species congregate and forage. Species abundance responses are expected to be more affected by differences in collection methodologies between monitoring programs, and particular care must be used when analyzing such data across programs.

Community structure, which integrates species presence and abundance, is also dependant upon the salinity regime. Responses in the benthic community are expected to be the composite result of the affects of salinity on all the individual species within the community, as described previously. Community responses include derived metrics such as taxa richness and diversity and their responses to changes in freshwater inflow.

1.5 Study Areas

The following provides a brief description of the two study areas.

1.5.1 Weeki Wachee River

The Weeki Wachee River (Figure 1-2) originates at Weeki Wachee Spring and enters the Gulf of Mexico just south of Bayport. Weeki Wachee Springs, approximately 12 km upstream contributes the majority of the river’s flow. Little Spring and the Mud River contribute additional inflow. Mud River flow is higher in total dissolved solids and conductivity than Weeki Wachee Spings because of the higher mineral content of Salt

Springs (Rosenau *et al*., 1977; SWFWMD, unpublished data). Rosenau et al. (1977) reported that conductivity at Weeki Wachee Spring was <300 μmhos cm^{-1} whereas in Mud Spring conductivity, at a depth of 19 m, was 23,000 μmhos cm^{-1}. Average monthly flows of the Weeki Wachee River at Brooksville (1993 to 2003) have ranged from 135 cfs (May) to 167 cfs (September) (*cf*. Figure 1-3). Frazer et al. (2001) reported flows of around 106 cfs during 1998-2001. For the period 1966-2005, the interquartile daily flows ranged from 146 to 191 cfs. Rosenau et al. (1977) listed three flows ranging between 83 and 128 cfs for Mud Spring.

Frazer *et al*. (2001) found salinities >0.5 ppt occurring as far upstream as river kilometer (RKM) 7.6 (their transect 12); Clewell et al. (2002) found saline waters penetrating approximately 3.5 km upstream. Freshwater outflow contributes to reduced salinities at least 5 km offshore (Dixon, 1986). Frazer et al. (2001) showed that salinities at the rivers mouth varied widely during their study period, ranging from around 15 ppt in August to almost 30 ppt in November and May (Figure 1-3).

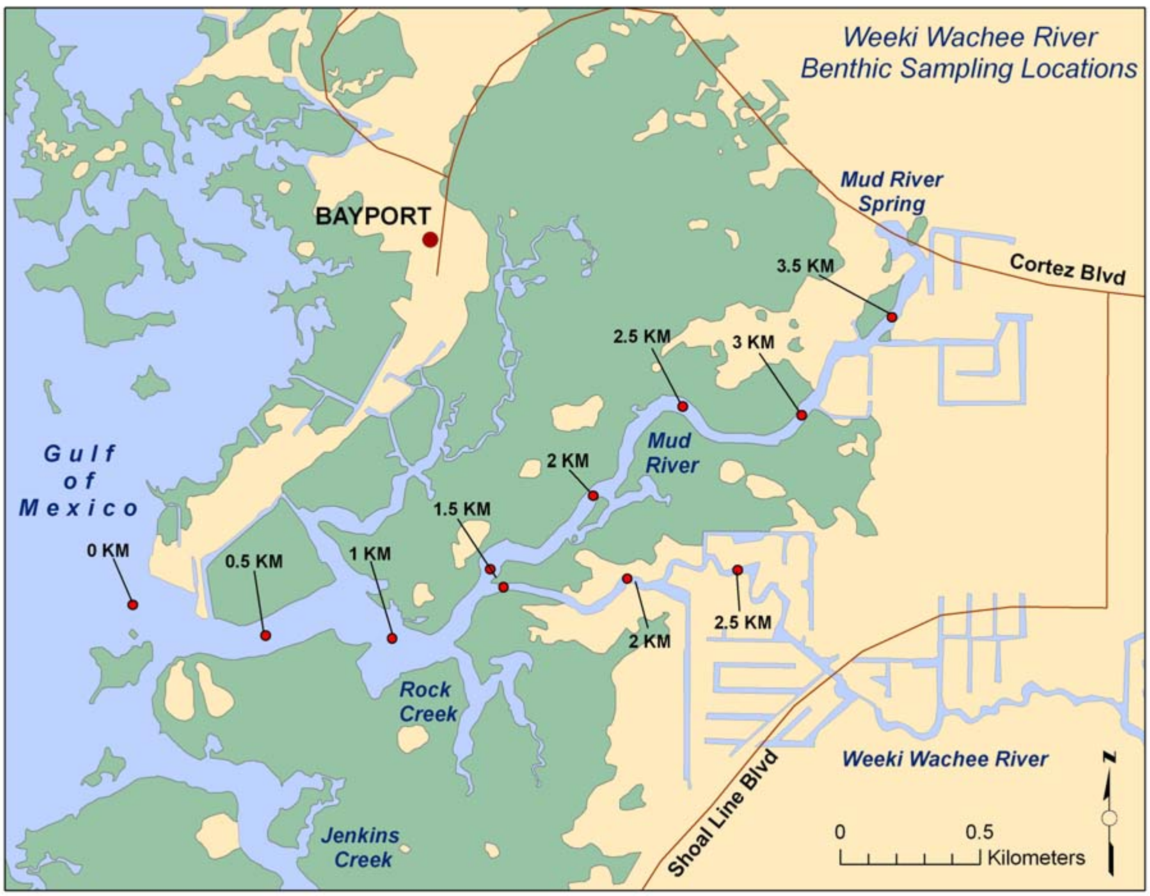

Figure 1-2. The Weeki Wachee and Mud rivers.

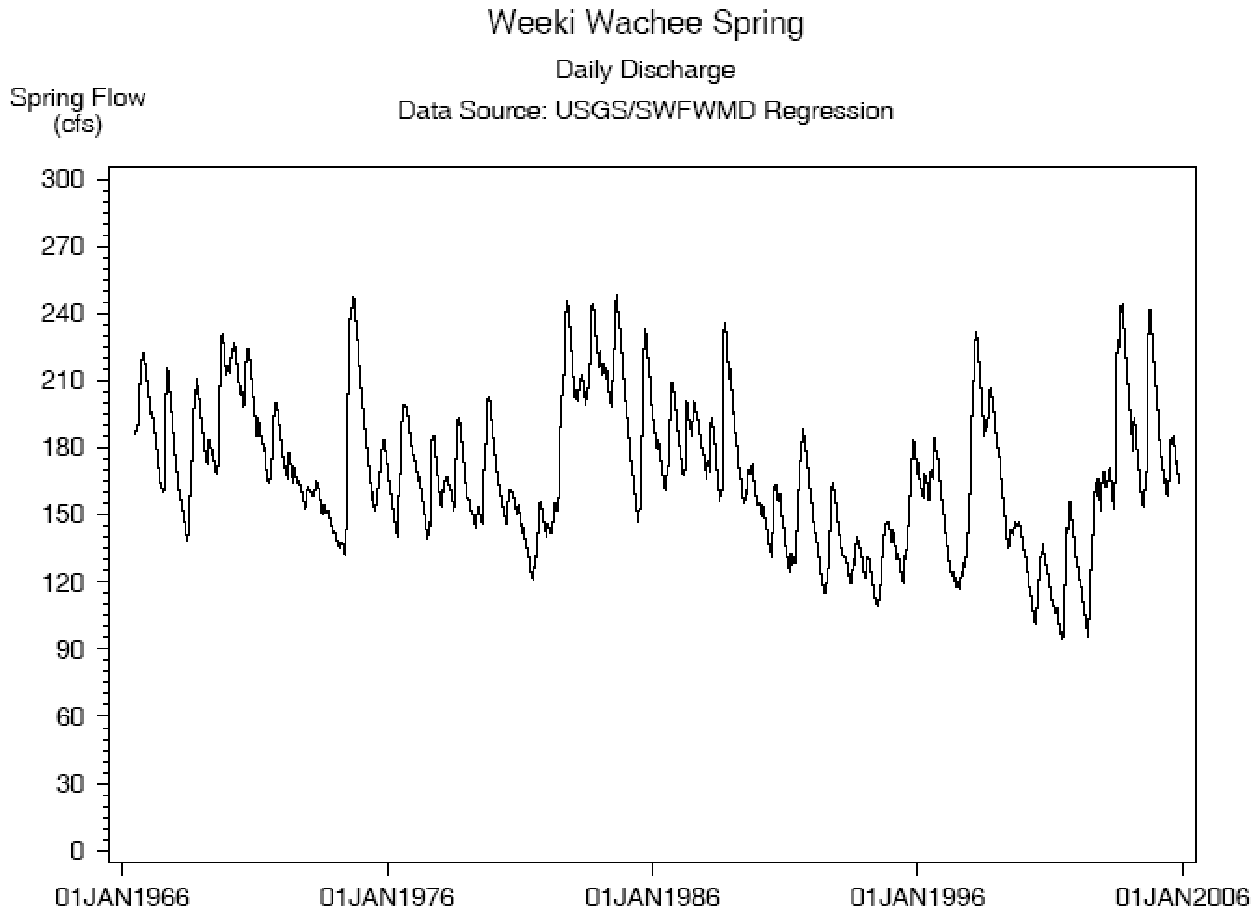

Figure 1-3. Mean daily discharge at Weeki Wachee Spring, 1966-2005.

The river channel is approximately 20 meters wide in the freshwater reaches, widening to 120 meters downstream of RKM 2 (Clewell et al., 2002).

Submersed aquatic vegetation (SAV) is prevalent in the upper portion of Weeki Wachee River. Common freshwater species include *Hydrilla verticillata*, *Najas guadalupensis*, *Vallisneria americana* and *Sagittaria kurziana,* and the algae *Lyngbya* sp. and *Chaetomorpha* sp. (Frazer et al., 2001; Clewell et al., 2002). Frazer et al. (2001) reported that estuarine coverage by SAV is minimal with patches near the river's mouth (Frazer et al., 2001). Mote Marine Laboratory (unpublished data) reported *Ruppia maritima, Zanichellia palustris*, *Myriophyllum spicatum*, and *Potamogeton pectinatus* from the lower two kilometers of the river.

1.5.2 Chassahowitzka River

The Chassahowitzka River is also a spring-fed river that originates in Citrus County and enters the Gulf of Mexico at Chassahowitzka Bay (Figure 1-4). The river is an "Outstanding Florida Water" and a major resource is the 30,000 acre Chassahowitzka National Wildlife Refuge, managed by the U.S. Fish and Wildlife Service.

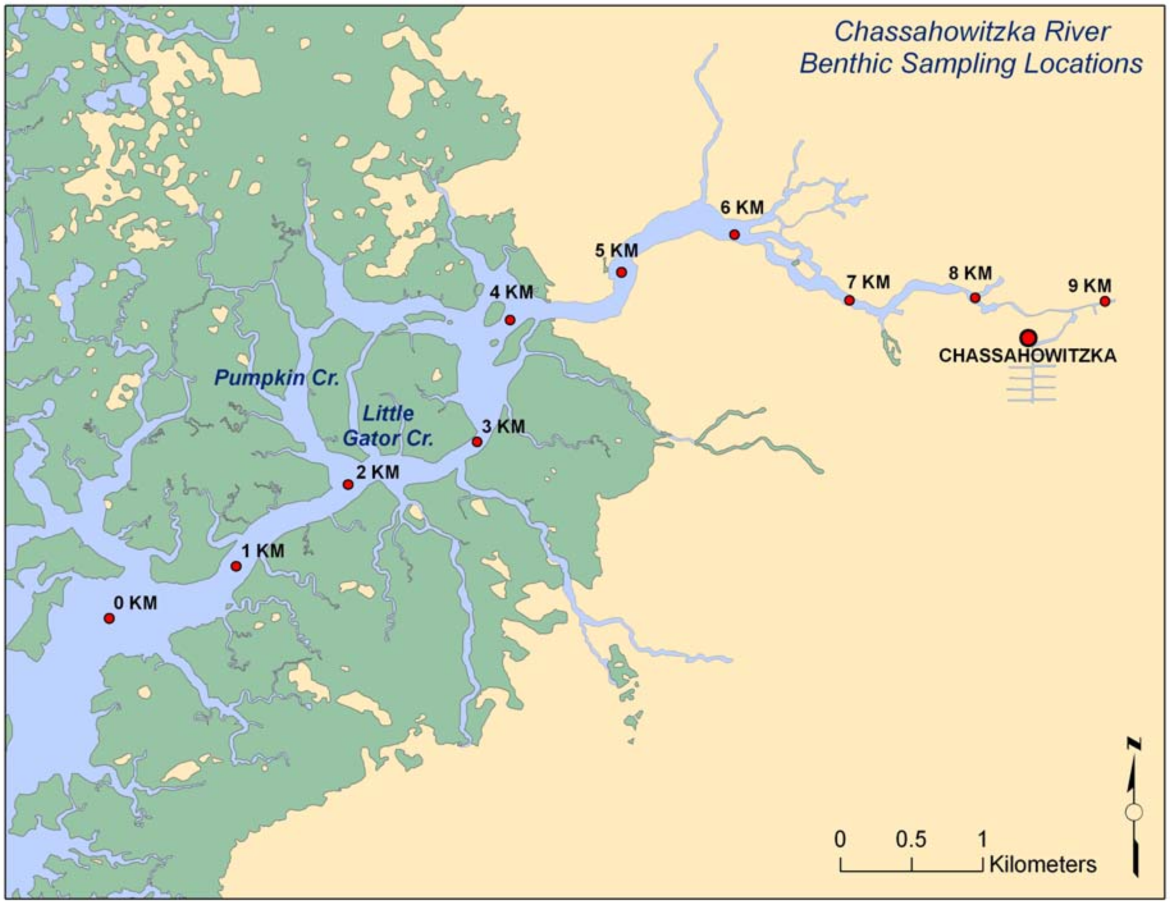

Figure 1-4. The Chassahowitzka River.

The river is tidal to the headspring, approximately 8 kilometers upstream from the mouth. The salinity of the discharges from the spring complex ranges from freshwater up to 16 ppt (Baird Spring). Frazer *et al*. (2001) showed that the river headwaters are oligohaline year-round. Salinities at the river's mouth range widely, from <10 (August and November) to almost 25 ppt (May).

Chassahowitzka Springs is estimated to contribute 50% of the flow. Monthly mean flows at have ranged from 31.8 to 197 cfs (mean=140 cfs; data from 1930-1972 cited in Yobbi and Knochnemus, 1989). Frazer *et al*. (2001) reported a mean flow of approximately 140 cfs during their three year study. Flows measured at Hommasassa from 1999 through 2005 ranged from 25 to 87 cfs, with a median of 59 cfs (Figure 1-5). Yobbi (1992) observed that there is a seasonal component to the springs discharge. Lowest flows occur during June and July and the greatest flows occur during early fall.

Frazer et al. (2001) observed that there is little residential development along this river, with approximately a dozen single family residences in the lower reach. The upper reach of the Chassahowitzka is relatively narrow but broadens considerably (to 175 m) downstream. Wolfe (1990) describes the coastal vegetation as primarily hammock forest, which includes *Quercus virginiana, Juniperus silicicola*, and *Sabal palmetto* among the characteristic species. *Taxodium distichum* is common along the spring runs that join with the river. *Typha* spp. and *Phragmites australis* are found at the lower salinities. There is an extensive marsh system (*Cladium jamaicense and Juncus roemerianus)* near the mouth of the river.

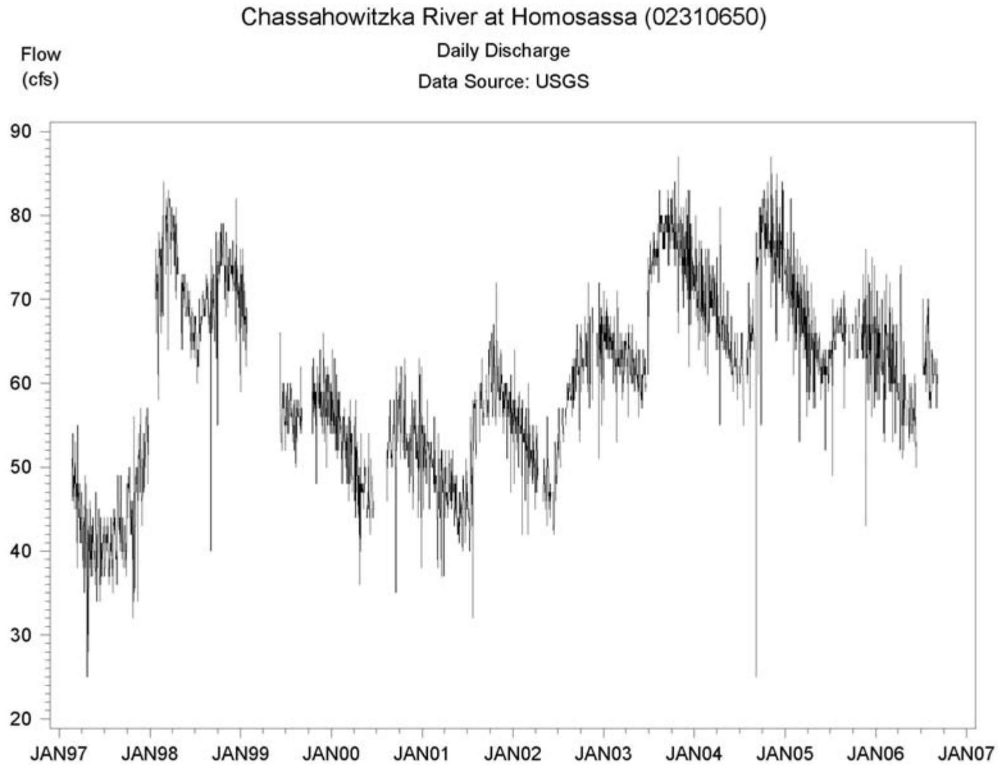

Figure 1-5. Mean daily discharge of the Chassahowitzka River at Homosassa, 1997-2006.

Submersed aquatic vegetation occurs throughout most of the river, gradually declining downstream. Common freshwater macrophytes include *Vallisneria americana*, *Potamogeton pectinatus*, *Najas guadalupensis*, *Myriophyllum spicatum*, and *Hydrilla verticillata* (Frazer et al., 2001; Clewell et al., 2004). Filamentous macroalgae, including *Lyngbya* sp. and *Chaetomorpha* sp., are also abundant.

In the coastal area, SAV beds are well-developed, and may cover as much as 90% of the substrate where water depths are <2m (McNulty et al., 1972; Wolfe, 1990). Species include *Thallassia testudinum, Syringodium filiforme, Halodule wrightii*, and, in lower salinity areas, *Ruppia maritima* (Iverson and Bittaker, 1986).

2.0 METHODS

In this section the field, laboratory, and data analysis methods are summarized.[1]

2.1 Field Methods

2.1.1 Weeki Wachee River

Directed sampling, in which sampling locations were chosen based upon prior knowledge and local experience, took place during the 1984 benthic survey of the Weeki Wachee River (Culter, 1986). Culter (1986) established four stations at RKM -5.8, -0.2, 1.3, and 2.4 (Figure 2-1) for his 1984 survey. Samples were collected during February, May, August and November 1984 and October 1985.

[1] ["left bank / right bank refer to looking upstream]

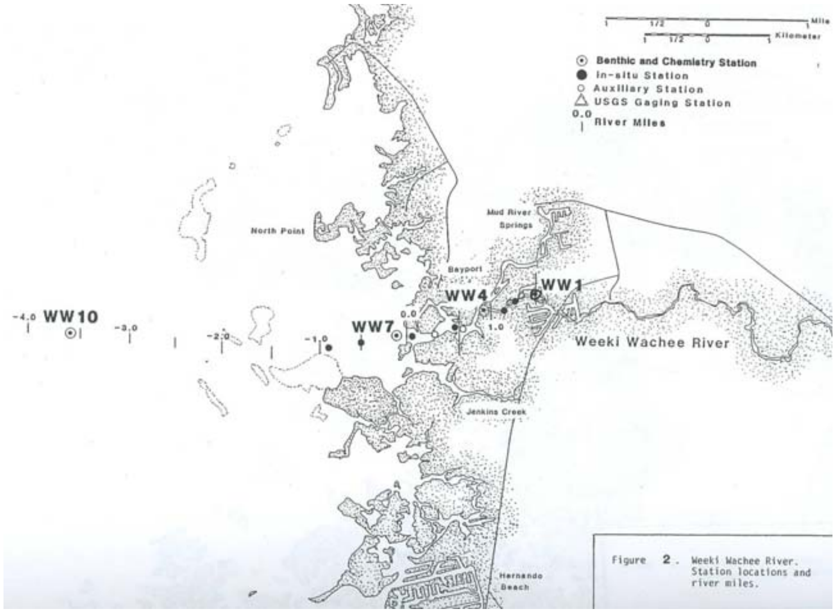

Figure 2-1. Location of benthic sampling stations in the Weeki Wachee River, 1984-1985 (Culter 1986).

During 1984 and 1985, the benthos was sampled by divers using a box corer (12.5 cm x 12.5 cm x 15 cm; area=0.156 cm^2) (Culter, 1986). Ten field replicates were collected at each site during each survey period.

The March 2005 survey of the Weeki Wachee and Mud rivers employed a stratified directed design (Janicki Environmental, 2005). The Weeki Wachee proper was stratified into lower (mouth to RKM 1.4) and upper strata (RKM 1.4 to RKM 2.4) (Figure 2-2). The Mud River (2.4 km in length) was treated as a third stratum (Figure 2-2).

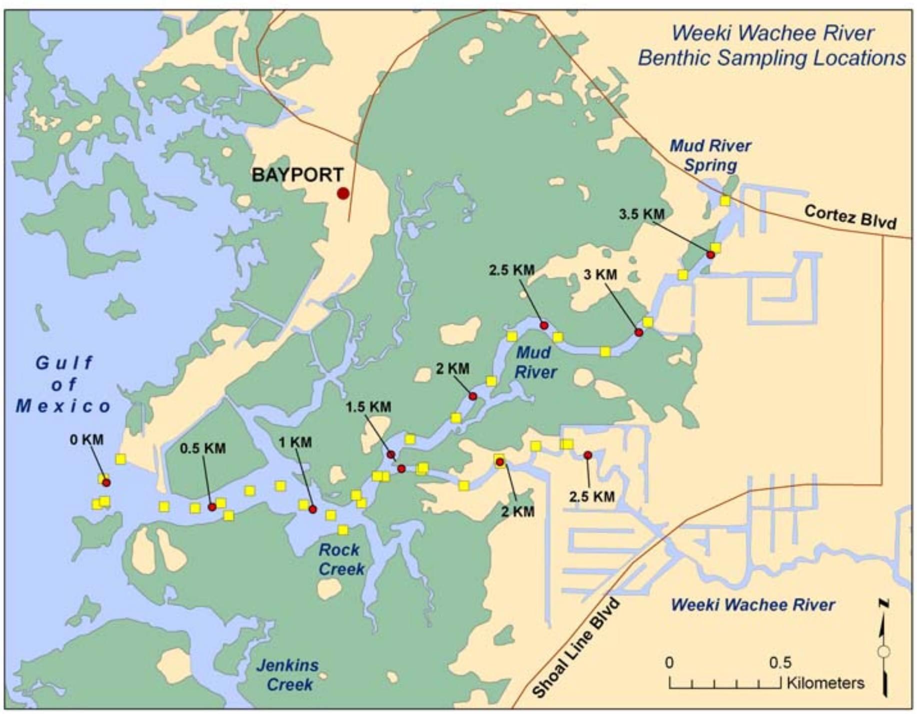

Figure 2-2. Location of benthic sampling stations in the Weeki Wachee River and Mud River, March 2005.

Ten evenly-spaced (every 140 m) transects were established in the lower stratum and five (every 200 m) in the upper stratum; ten evenly-spaced transects (every 240 m) were established in the Mud River. Samples were collected from the Weeki Wachee along both the centerline of the river and from alternating north and south sides of the river. Samples were only collected along the centerline of the Mud River.

For the March 2005 survey, a 7.62 cm diameter hand core sampler (area= 45.6 cm^2) was used to collect the soft sediment infauna and a sweep net was used to collect epifaunal organisms associated with submerged aquatic vegetation (SAV). A total of 40 infaunal and four sweep net samples were collected. For each of the surveys, aliquots of sediment for grain size, organic content, etc analyses were taken from a separate core. These samples were labeled and stored on ice until transferred to Mote Marine Laboratory for analysis.

All macroinvertebrate samples were processed in a similar manner. Each sample was bagged with an internal label; magnesium sulfate solution was added to relax the organisms. Samples were later sieved (0.5 mm mesh) to remove finer-grained particles of sediment and meiofauna. They were then fixed in a 10% solution of buffered formalin and Rose Bengal stain.

2.1.2 Chassahowitzka River

The Chassahowitzka River was surveyed during both the dry (May) and wet seasons (September) of 2005 for infaunal and SAV associated epifaunal macroinvertebrates and during the dry season only for SAV.

A 7.63 cm diameter core sampler was used to collect the soft sediment infauna and a sweep net was used to collect SAV-associated epifauna.

The Chassahowitzka River was stratified for the infaunal and epifaunal sampling into lower (RKM 0.7 TO RKM 2.7) and upper (RKM 2.7 to RKM 8.7) strata (Figure 2-3). Transects were established every RKM in the lower stratum and every 0.5 RKM in the upper.

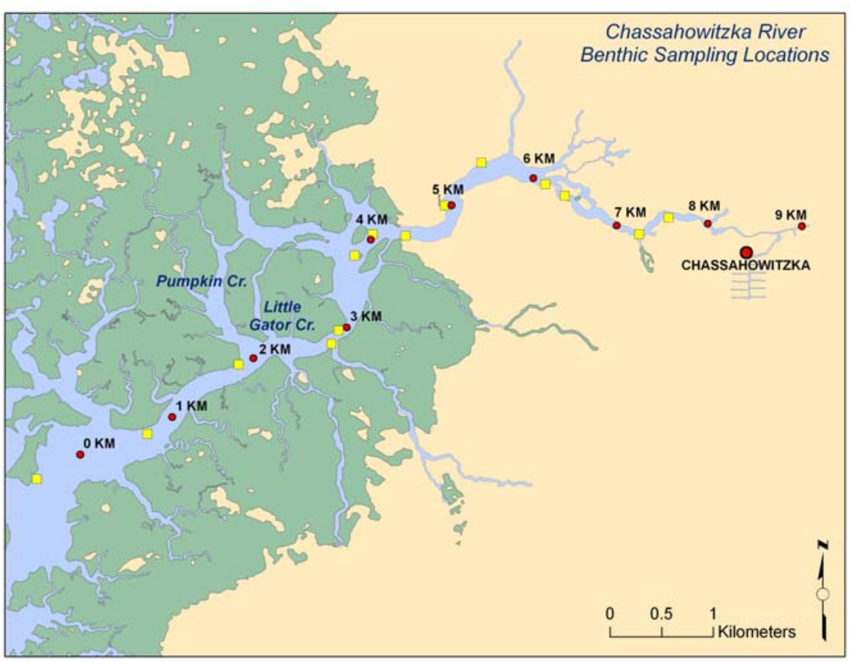

Figure 2-3. Location of benthic sampling stations in the Chassahowitzka River, May and September 2005.

Three core samples and three sweep net samples were collected in the lower stratum. Eleven cores and sweep net samples were collected from the upper stratum.

Macroinvertebrate samples were field processed following the procedures described above for the Weeki Wachee River. Aliquots of sediment for grain size, organic content, etc analyses were taken from a separate core.

Seventeen transects (every 0.5 km) were established for SAV. Ten quadrats were sampled for SAV using the method outlined by Braun-Blanquet (1932). Ten quadrats were located haphazardly along each of the 17 transects. Quadrat size was 0.25 m^2. The Braun-Blanquet scale for coverage is as follows:

0: species not present
1: species <5% of total
2: species=5-10% of total
3: species=10-25% of total
4: species=25-50% of total
5: species=50-90% of total
6: species>90% of total

Other information/data collected at each sampling location included:

- sample depth;
- latitude and longitude;
- qualitative descriptions of land-use, including types of riparian vegetation, along the shoreline proximate to each sampling location; and
- relevant field notes on habitat, ambient environmental conditions.

2.2 Laboratory Methods

Macroinvertebrate samples were transferred from the fixative to a preservative (a solution of 50% to 70% isopropanol or ethanol) after at least 48 hours. All organisms were sorted from the samples, to at least 90% recovery, under a dissecting microscope. Macroinvertebrates were identified to the lowest practical identification level—typically genus or species. If an animal was a member of one of the “minor” taxonomic groups, such as the Nemertea, identifications might only be to that higher taxonomic level. Additionally, if an organism was damaged or a juvenile, identifications to the genus or species level could not always be made. All of the organisms were counted in core samples and sweep net samples collected from the Weeki Wachee and Mud rivers; organisms collected in sweep net samples from the Chassahowitzka River were counted as present. Sediment samples were analyzed for grain-size composition, skewness, kurtosis, percentage of organic matter (as loss on ignition; Dean, 1974).

Dixon (personal communication) summarized the grain size analysis methods. Grain-size distribution was measured by a laser diffraction instrument (Coulter LS-200), capable of measurement between 0.4 and 2000 μm equivalent spherical diameters. As the instrument is sensitive only to 2,000 μm, sediments were first sieved through a 2 mm mesh prior to diffraction analysis. Total percent sand, silt and clay were calculated as the sum of volume percent between 2000 and 62.5 μm, 62.5 and 3.91 μm, and 3.91 to 0.04 μm, respectively, using the Wentworth size scales and a ϕ value of 8.0 as the silt-clay boundary. Geometric distributional statistics were computed from the logarithmic center of each size grouping since sediment distributions are typically more log-normal than normal.

2.3 Data Analysis Techniques

2.3.1. Primary Data

The primary data analyzed include:

- river location (kilometer);
- hydrographic data – conductivity (salinity), temperature, DO, and pH;
- sediment size data; and
- benthic macroinvertebrate count data.

The benthic macroinvertebrate data have been used to estimate the following derived, univariate community metrics:

- Species (taxa) richness - the number of distinct species (taxa) identifiable in a sample. This is the simplest representation of "diversity". Every effort was made to ensure that comparable levels of identification were employed.

- Species diversity (Shannon-Wiener diversity, H') - the metric that incorporates both numbers of taxa and the distribution of those organisms within a sample (evenness). For example, one may consider two samples each with 10 distinct taxa. In the first sample there is a single individual of each of the 10 taxa and the evenness is high. In the second sample, if one taxon is represented by 100 organisms and the remaining nine taxa are represented by single individuals, evenness is lower. The former sample would have the higher diversity value.

- Total abundance – the sum of the individual taxon counts in a sample.

2.3.2. Univariate Analyses

Spearman rank correlation analysis has been used to assess the relationship between two variables. Also, multiple regression methods have been employed to estimate the quantitative relationships between a series of independent variables and a dependent variable. Logistic regression was used to quantify the relationship between salinity and the probability of occurrence of individual benthic macroinvertebrate taxa. Janicki Environmental, Inc. (2006) employed univariate logistic regression (Huisman et al., 1993, Peeters and Gardiniers, 1998, Ysebaert et al., 2002) to estimate the probability of occurrence as a function of salinity for selected taxa from 12 Southwest Florida tidal rivers. The "optimum" or "preferred" salinity was that with the highest probability of occurrence for that taxon. A "tolerance" range was calculated as the salinity or sediment type $\pm$75% of the optimum (Peeters and Gardiniers, 1998).

2.3.3. Multivariate Analyses

Two multivariate analysis techniques have been applied to assess how salinity and other abiotic variables relate to the benthic macroinvertebrate community structure. These include:

- Cluster Analysis - Numerical classification analysis ("cluster" analysis) was used to evaluate the spatial and temporal structure of the benthic community in the Weeki Wachee, Mud, and Chassahowitzka rivers. Particular attention was paid to whether salinity was a factor that could help explain the observed biotic structure. This approach might be able to distinguish differences in species distributions across what was essentially a narrow range of salinities (salinities >18 ppt accounted for<10% of the observations). Bray-Curtis was chosen to compute the similarity matrix and group average clustering was used to represent the relationships as a dendrogram (tree diagram). Because somewhat different sampling methods were used in Weeki Wachee River in 1984-1985 than were used in the Weeki Wachee, Mud, and Chassahowitzka rivers in 2005, data were converted to percent composition (Boesch, 1977) when the data from the three rivers were analyzed together.

The Chassahowitzka River data from 2005 were also analyzed separately. For this analysis, taxon counts were used rather than relative abundance. These counts were 4th root transformed in order to permit a greater number of taxa to influence the results (Clarke and Warwick, 2001). The use of untransformed data yields results strongly influenced by the most abundant taxa. Cao et al. (1998) argue that "rare" taxa may be more sensitive to environmental perturbation than common species. Therefore, an analytical approach that is more responsive to the "community" rather than to only a few dominant taxa was desirable. Thorne et al. (1999) have also demonstrated that the 4th root transformation is preferred because it represents a "good compromise between untransformed and binary data". Therefore the 4th root transformation was employed when organism densities were used for the multivariate analyses. A small constant (0.1) was added to all sample counts prior to the 4th root transformation. This is done *a priori* to diminish the effect of samples which are "outliers" and have little resemblance to other samples (PRIMER 2006). "Meaningful" groups of samples were defined using a "variable" stopping rule. Boesch (1977) suggests that variable stopping rules may be more appropriate than "fixed" stopping rules for ecological data.

- Principal Components Analysis **-** Principal Components Analysis (PCA) was used to by Janicki Environmental, Inc. (2006) to identify salinity and sediment classes based upon the ranges of over which the taxa occur for data collected from 12 Southwest Florida tidal rivers over a >20 year period. Bulger et al. (1993) pioneered this methodology for developing taxa-specific salinity classes for mid-Atlantic estuarine nekton. The results of this analysis are one of the tools that are used to evaluate benthic responses to altered salinity regimes.

To further assess the differences identified by either the cluster analyses or the PCAs two additional analyses were performed:

- The "Analysis of Similarities" (ANOSIM) is a nonparametric test that determines whether or not two or more groups of species differ (Clarke and Warwick, 2001).

- The "Similarity Percentage" (SIMPER) analysis was also used to identify objectively those taxa that explain relatively large proportions of the differences between groups of samples (*e.g.,* salinity class) (Clarke and Warwick, 2001; PRIMER, 2006).

3.0 RESULTS

3.1 Abiotic Characteristics

The following sections describe the abiotic characteristics of the two study areas. The District provided a long-term (1984-1986; 1994-2005) database for hydrographic variables in both the Weeki Wachee and Mud rivers. The available hydrographic and sediment data that were collected coincident with the benthic samples are summarized in Table 3-1.

Table 3-1. Summary of mean and range (in parentheses) of bottom water abiotic variables and sediment characteristics coincident with benthic sample collections in the Weeki Wachee, Mud, and Chassahowitzka rivers, 1984-1985 and 2005.

Variable	Weeki Wachee 1984-1985 (n=24)	Weeki Wachee 2005 (n=32)	Mud 2005 (n=10)	Chassahowitzka 2005 Dry Season (n=11)	Chassahowitzka 2005 Wet Season (n=11)
Temperature (°C)	24.1 (13.4-30.7)	18.9 (15.2-22.4)	21.8 (21.1-23.0)	26.1 (24.8-27.4)	26.3 (24.5-28.6)
Salinity (ppt)	13.1 (0.0-30.8)	4.9 (0.3-11.4)	8.0 (4.1-16.4)	3.9 (1.2-7.5)	5.1 (0.8-15.5)
Dissolved Oxygen (mg/L)	7.0 (4.9-10.1)	7.1 (6.3-9.2)	8.4 (3.1-11.4)	10.5 (6.1-18.6)	6.1 (3.7-9.5)
pH	No Data	8.19 (7.88-8.51)	8.07 (7.41-8.36)	8.27 (7.91-8.87)	7.69 (7.45-8.04)
Sediment % Silt+Clay	12.9 (9.2-17.9)	18.8 (4.8-32.7)	19.6 (5.8-32.0)	23.0 (9.7-56.6)	28.4 (8.6-52.9)
Sediment Mean Grain Size ϕ	No Data	2.8 (1.1-3.5)	3.0 (2.3-3.4)	1.4 (0.7-2.9)	2.1 (0.7-3.7)
Sample Depth (m)	2.5 (1.0-4.5)	0.7 (0.2-2.0)	0.8 (0.2-1.2)	1.8 (0.4-4.6)	1.6 (0.6-3.6)

- **Weeki Wachee and Mud River**

The mean water temperatures were generally similar being lowest in the 2005 samples from both Weeki Wachee and Mud River. Mean salinities were highest during in Weeki Wachee during the 1984-1985 sampling period. The DO in all study areas and sampling periods were well above hypoxic conditions. There was little difference across study areas in pH and sediment size.

To examine further the temporal variability in salinity in the Weeki Wachee the mean salinity by year and calendar month was estimated and summarized in Figure 3-1. With the exception of 1994 and 1995 when somewhat higher salinities were observed, the year-to-year variability in salinity was minimal. The within-year variability in salinity was also relatively small. Salinities were lowest during January and November; March salinities were less than those of July-November; December salinities were greater than those of January.

Spatial variation in observed salinities in the Weeki Wachee were also examined and summarized in Figure 3-2. Salinity varied longitudinally as expected with higher salinity downstream and lower salinity at upstream stations.

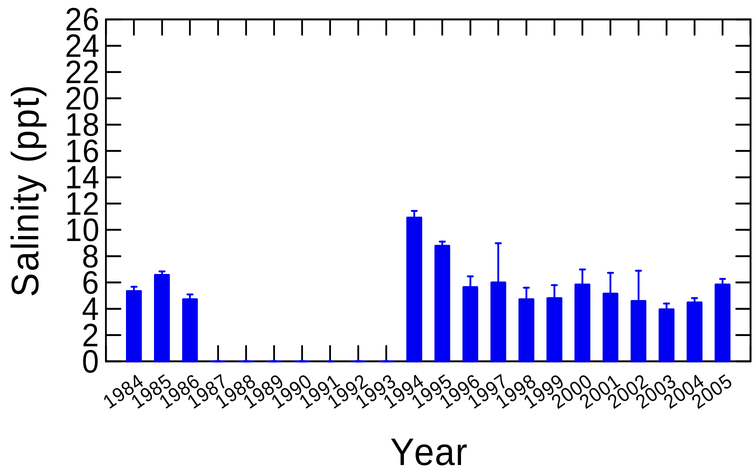

Figure 3-1. Mean (standard error) salinity in the Weeki Wachee River, by year, 1984-1986 and 1994-2005, river kilometers 0 to 4.

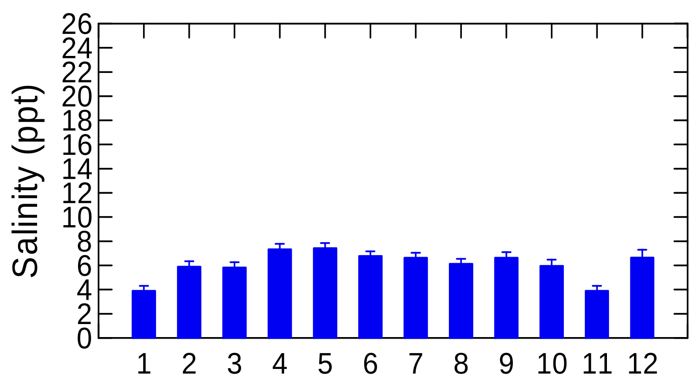

Figure 3-2. Mean (standard error) salinity in the Weeki Wachee River, by month, 1984-1986 and 1994-2005, river kilometers 0 to 4.

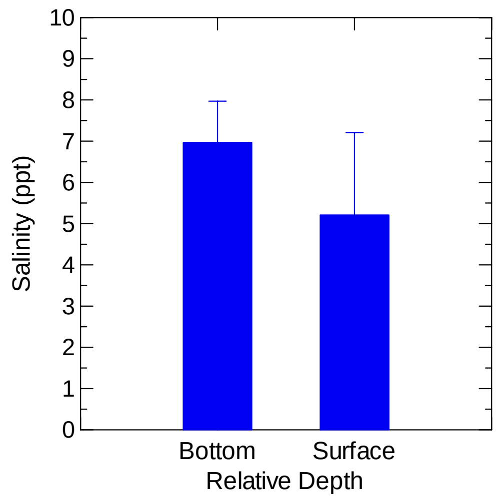

Figure 3-3. Mean (+standard error) surface and bottom salinity in the Weeki Wachee River, 1984-1986 and 1994-2005.

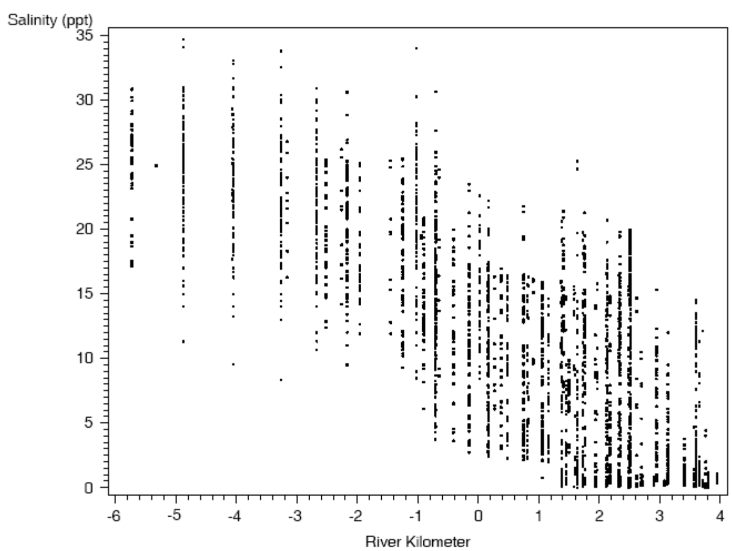

Figure 3-4. Salinity from all depths *vs*. river kilometer in the Weeki Wachee River, 1984-1986 and 1994-2005.

The ionic composition of the Mud River is similar to that of seawater (Table 3-2). It seems unlikely that any differences are ecologically significant, although it is worth noting that they could be. Zalizniak et al. (2006) found that, for Australian freshwater invertebrates exposed to different ionic compositions of inland saline waters, sublethal effects were detected. The salinity of the Mud River is more similar to that of the nearshore gulf waters than it is to Weeki Wachee River water. Therefore, any reductions in flow that would allow the upstream migration of gulf water into the Mud River likely would not significantly affect the benthic community of the Mud River.

Table 3-2. Ionic composition (percent) of Mud River water *vs*. "typical" seawater (from Pearse and Gunter, 1957).		
Variable	**Mud River**	**Seawater**
Chlorides	56.28 to 56.76	55.04
Sodium	29.43 to 29.95	30.62
Sulfates	6.93 to 7.19	7.68
Magnesium	3.83 to 3.85	3.69
Calcium	1.42 to 1.66	1.15
Potassium	1.35 to 1.36	1.10

Most of the sediment samples collected with the infaunal samples were fine sands (ϕ=2 to 3) (Figure 3-5). The percentage of fine-grained sediments (silts and clays) ranged from 4.8% to 33% (Figure 3-5). These statistics summarize the sediments collected with soft-sediment infaunal core samples. They may not necessarily be representative of the typical substrate in specific parts of the river. As Culter (1986) noted, and we also found while sampling, there are parts of the river where currents are swifter, and the sediments have been scoured leaving a limestone substratum. This area begins just upstream of the river's confluence with the Mud River. Mud River sediments were also fine and very-fine sands (ϕ=2 to <4) (Figure 3-6). The percentage of fine-grained sediments (silts and clays) varied widely and ranged from 5.8% to 32%. The finest grained sediments were found near the river's head.

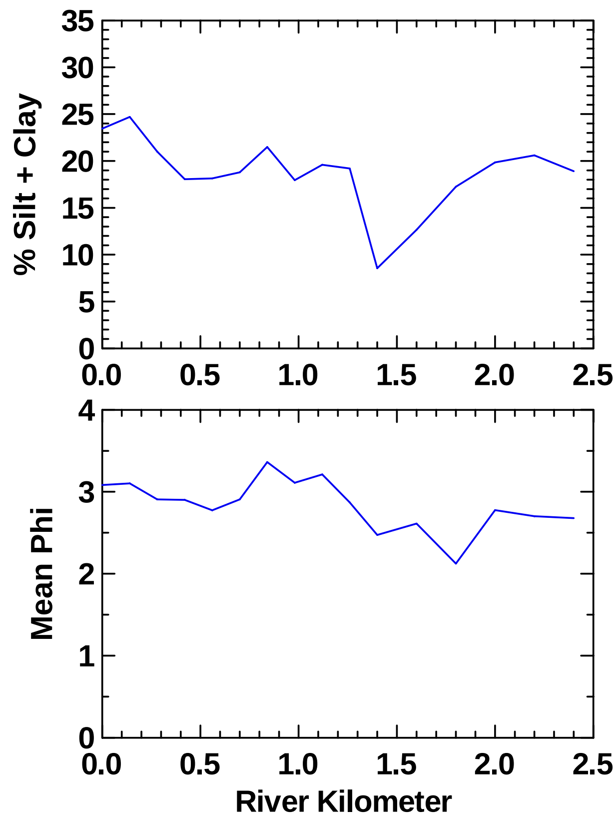

Figure 3-5. Longitudinal variation in sediment grain size (mean phi) and percent silt+clay in sediments in the Weeki Wachee River, March 2005.

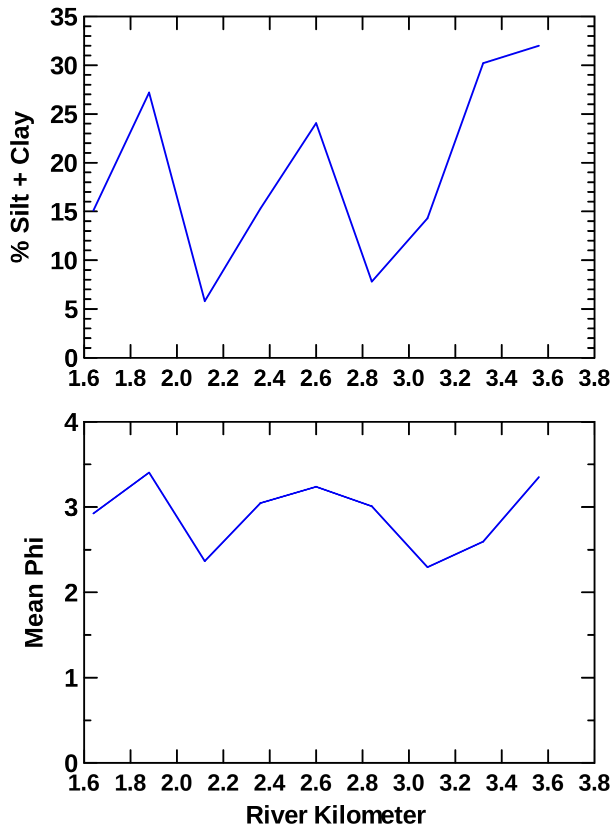

Figure 3-6. Longitudinal variation in sediment grain size (mean phi) and percent silt+clay in sediments in the Mud River, March 2005.

3.1.2 Chassahowitzka River

Salinities in the Chassahowitzka River ranged from 0.8 to 15.6 ppt; there were no significant differences between wet and dry seasons ($F_{1,26}$=0.1; p=0.7). Dissolved oxygen concentrations ranged from 3.7 to 18.6 ppm and the dry season concentrations were generally indicative of supersaturation (Table 3-1).

Sediments were fine and very-fine sands (ϕ=2 to <4) downstream of RKM 5 and medium and coarse sand-sized sediments upstream (Figure 3-7). The percentage of fine-grained sediments (silts and clays) generally decreased from >30% near the mouth to 15% at RKM 8, with the exception of a value >50% at RKM 4.5.

3.2 Biota

3.2.1 Dominant Infaunal Taxa

Dominant taxa were identified for each river for each sampling method. Dominants are identified by their dominance score which is calculated as *Dominance Score = (% occurrence * % composition)*$^{-0.5}$.

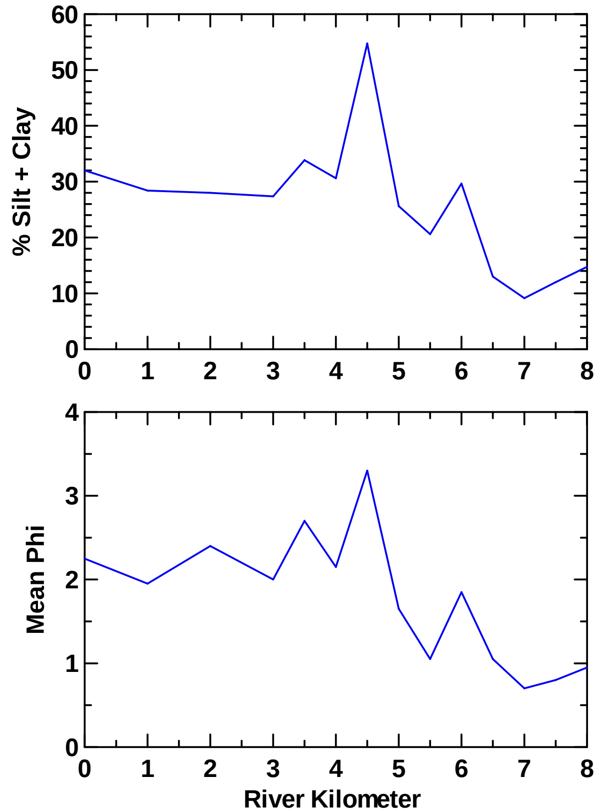

Figure 3-7. Longitudinal variation in sediment grain size (mean phi) and percent silt+clay in sediments in the Chassahowitzka River, May and September 2005.

The polychaete *Laeonereis culveri* and the amphipod *Gammarus mucronatus,* were among the top-ranked dominant taxa in each of the three rivers (Table 3-3). *Grandidierella bonnieroides*, oligochaete worms, *Hargeria rapax*, *Laeonereis culveri, Gammarus mucronatus*, and *Polypedilum scalaneum* had Dominance scores >30 in at least one of the rivers.

3.2.2 Dominant Epifaunal Taxa

The amphipods *Gammarus mucronatus*, and *Grandidierella bonnieroides* were the top-ranked Dominant taxa in both the Weeki Wachee and Mud rivers (Table 3-4). The asellote isopod *Uromunna reynoldsi* was a dominant in the Weeki Wachee River but not in the Mud River. The tanaid *Hargeria rapax* and the mysid *Taphromysis bowmani* were each ranked among the top ten dominants in the Mud River and were not ranked in the Weeki Wachee.

Chassahowitzka River epifauna were only counted as present. Taxa that occurred most frequently (>70% of the samples) included (Table 3-4):

- wet season: *Grandidierella bonnieroides*, *Gammarus mucronatus*, *Laeonereis culveri*, *Polymesoda caroliniana*, and undientifed insects;
- dry season: *Gammarus mucronatus*, hydrobiid gastropods, and *Grandidierella bonnieroides.*

The two seasons differed mainly in the absence of *Laeonereis culveri* and unidentified insects during the dry season sampling.

Table 3-3. Ten dominant macroinvertebrate taxa identified from infaunal samples collected in the Weeki Wachee, Mud, and Chassahowitzka rivers.				
TAXON	**Weeki Wachee 1984-1985**	**Weeki Wachee 2005**	**Mud 2005**	**Chassahowitzka 2005**
ANNELIDA				
Capitella capitata			12.0	
Hobsonia floridana		11.8		
Laeonereis culveri	15.8	36.4	9.1	29.9
Leitoscoloplos robustus			12.6	
Oligochaeta-genera undetermined				41.7
Tubificidae-genera undetermined	13.0	10.9	23.2	
Limnodriloides sp.		15.9		
MOLLUSCA				
Parastarte triquetra			15.5	
Tellina mera		7.0		
Hydrobiidae-genera undetermined	12.4	7.7	13.7	
CRUSTACEA				
TANAIDACEA				
Hargeria rapax	34.6		21.9	8.8
CUMACEA				
Almyracuma proximoculi	19.2			
ISOPODA				
Ericsonella attenuata	9.4			
Ericsonella filiformis				3.6
Mesanthura florida				17.3
Uromunna reynoldsi	10.9			
AMPHIPODA				
Cerapus benthophilus	24.8			
Cerapus sp. A		11.8		
Corophiidae-genera undetermined				17.6
Gammarus mucronatus	14.3	29.8	39.2	13.3
Grandidierella bonnieroides	42.4		59.3	28.1
Melita nitida complex		19.1		5
INSECTA				
Insecta-genera undetermined				11.6
Polypedilum scalaneum		36.2	11.0	

Table 3-4. Ten dominant macroinvertebrate taxa identified from epifaunal samples collected in the Weeki Wachee and Mud rivers and the ten most frequently occurring taxa in epifaunal samples collected in the Chassahowitzka River (by season.					
TAXON	Weeki Wachee March 2005	Mud March 2005		Chassahowitzka May 2005 (Wet Season)	Chassahowitzka September 2005 (Dry Season)
ANNELIDA					
Capitella capitata		8.9			
Laeonereis culveri	27.6			78.6	
Leitoscoloplos robustus		12.8			
Limnodrilus hoffmeisteri	12.6				
MOLLUSCA					
Polymesoda caroliniana	13.8			71.4	21.4
Hydrobiidae-genera undetermined	12.7	25.9		57.1	71.4
CRUSTACEA					
MYSIDACEA					
Americamysis almyra				57.1	21.4
Taphromysis bowmani		16.1		57.1	57.1
TANAIDACEA					42.9
Hargeria rapax		17.5			
CUMACEA					
Almyracuma proximoculi					21.4
ISOPODA					
Cassidinidea ovalis	9.2				
Cyathura polita	13.4				
Edotea montoa	9.7	11.0		50.0	35.7
Ericsonella filiformis					35.7
Uromunna reynoldsi	47.0				
AMPHIPODA					
Ampelisca abdita					21.4
Cerapus sp. A		8.4			
Gammarus mucronatus	51.5	66.1		85.7	100.0
Grandidierella bonnieroides	48.1	51.4		92.9	71.4
Monocorophium acherusicum		8.4			
Corophiidae-genera undetermined				57.1	28.6
DECAPODA					
Palaemonetes pugio					28.6
Xanthidae				50.0	
INSECTA				71.4	

3.2.3 Relationships Univariate Community Metrics and Abiotic Variables

Three univariate metrics of community structure were calculated:

- species (or taxa) richness);
- species (taxa) diversity; and
- total numbers of individuals m^{-2} (quantitative core samples only).

Species (taxa) richness is the number of distinct species (taxa) identifiable in a sample. Species (taxa) richness is the simplest representation of “diversity”. Every effort was made to ensure that comparable levels of identification were employed.

Species diversity (Shannon-Wiener diversity, H’) is a metric that incorporates both numbers of taxa and the distribution of those organisms within a sample (evenness). For example, one may consider two samples each with 10 distinct taxa. In the first sample there is a single individual of each of the 10 taxa and the evenness is high. In the second sample, if one taxon is represented by 100 organisms and the remaining nine taxa are represented by single individuals, evenness is lower. The former sample would have the higher diversity value.

To examine the relationship between the abiotic variables and infaunal species richness, diversity, and total numbers of individuals m^{-2}, a stepwise multiple regression (Neter et al., 1985) was applied to the 2005 data. Abiotic variables were normalized to their z-score to address differences in scale and range of the different abiotic variables (Digby and Kempton, 1987).

Statistically significant relationships were found between numbers of taxa, Shannon-Wiener diversity, and total numbers of individuals with various combinations of water quality and sediment variables (Table 3-5; Appendix A) in these regression analyses. Each of the equations explained between 19% and 37% of the total variance, therefore variables other than those examined contribute significantly to the total variance observed in these three community metrics.

Salinity, temperature, and sediment size (i.e., the percentage of silt+clay in the sediments and sediment mean ϕ) contributed significantly to the observed variance in the three community metrics (Table 3-5). The numbers of taxa and numbers of individuals were negatively associated with temperature and the percentage of silt+clay. The number of taxa and diversity were negatively related to salinity. Neither dissolved oxygen nor pH contributed significantly to the observed variance in any of the three community metrics.

To examine further the relationship between salinity and the three community metrics, a series of simple regressions were applied to the 2005 infaunal data. These analyses indicate that the three community metrics did not vary in a linear fashion with variation in salinity as none of the three community metrics displayed a significant linear relationship with salinity (Table 3-6). Quadratic regression equations explained a significant proportion of the observed variation in both the number of taxa and the Shannon-Wiener diversity index. A polynomial regression explained more of the variation in the total abundance than either linear or quadratic regression equations (Table 3-6).

Table 3-5. Results of stepwise multiple regression analyses that examine the

relationship between three univariate community metrics (log_{10} N+1 numbers of taxa, Shannon-Wiener diversity, and log_{10} N+1 total numbers of organisms) and normalized values of bottom water and sediment abiotic variables. Benthic infaunal data analyzed were collected during 2005 from the Weeki Wachee,, Mud, and Chassahowitzka rivers. Significance is defined at a $p < 0.05$. [Table revised after Final Report]			
Variable	**Log_{10} Numbers of Taxa**	**Shannon-Wiener Diversity**	**Log_{10} Numbers of Individuals**
Adjusted Squared Multiple R^2	0.34	0.18	0.25
Intercept	0.98	2.28	3.99
Depth	NS	NS	NS
Temperature	NS	NS	NS
Salinity	0.10	0.30	0.09
pH	NS	NS	NS
Dissolved Oxygen	NS	NS	NS
% Silt+Clay	-0.08	NS	-0.21
Sediment Mean ϕ	NS	NS	NS

Table 3-6. Results of simple linear, quadratic, and polynomial regression analyses that examine the relationship between three univariate community metrics (log_{10} N+1 numbers of taxa, Shannon-Wiener diversity, and log_{10} N+1 total numbers of organisms) and salinity in the Weeki Wachee, Mud, and Chassahowitzka rivers- combined 2005 infaunal data. Diagnostic plots for each of the regressions in Table 3-6 are provided in Appendix B.

Equation	**Equation**	**r^2**
	Log_{10} (Number of Taxa)	
Linear	Y= 0.783+ 0.038X	0.56
Quadratic	Y= 0.66+ 0.114X - 0.007X^2	0.62
Polynomial	Y= 0.57+ 0.227X - 0.033X^2 + 0.0016X^3	0.66
	Log_{10} (Total Abundance/m^2)	
Linear	Y= 3.63+ 0.064X	0.35
Quadratic	Y= 3.42+ 0.197X - 0.012X^2	0.40
Polynomial	Y= 2.98+ 0.73X - 0.136X^2 + 0.007X^3	0.52
	Shannon-Wiener Diversity (H’)	
Linear	Y= 1.77+ 0.096X	0.46
Quadratic	Y= 1.41+ 0.326X - 0.021X^2	0.55
Polynomial	Y= 1.26 + 0.67X - 0.101X^2 + 0.005X^3	0.58

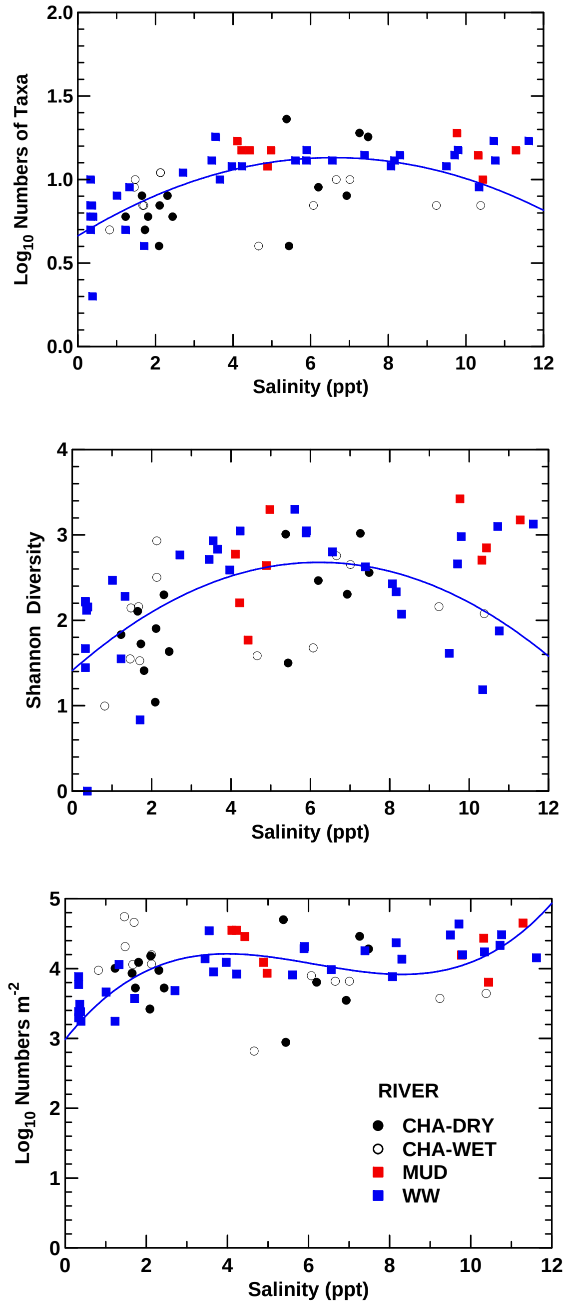

Figure 3-8. Relationships between numbers of taxa, Shannon-Wiener diversity, and total numbers of benthic organisms m^{-2} and salinity, Weeki Wachee, Mud, and Chassahowitzka rivers, 2005.

3.2.4 Multivariate Community Structure

To examine the multivariate structure of the benthic macroinvertebrate communities in the Weeki Wachee and Chassahowitzka rivers, two data analysis techniques were applied: cluster analysis and PCA.

3.2.4.1 Cluster Analysis

Numerical classification analysis ("cluster" analysis) was used to evaluate the spatial and temporal structure of the benthic community in the Weeki Wachee, Mud, and Chassahowitzka rivers. Particular attention was paid to whether salinity was a factor that could help explain the observed biotic structure. This approach might be able to distinguish differences in species distributions across what was essentially a narrow range of salinities (salinities >18 ppt accounted for<10% of the observations).

Prior to running the cluster analysis, samples were stratified by year, river, wet or dry season, and river kilometer. The mean percent composition of the taxa within each of these categories was then calculated and used in the computation of the similarity matrix. The Bray-Curtis similarity metric was chosen to compute the similarity matrix. Because somewhat different sampling methods were used in Weeki Wachee River in 1984-1985 than were used in the Weeki Wachee, Mud, and Chassahowitzka rivers in 2005, data were converted to percent composition (Boesch, 1977).

Group-average clustering was used to represent the similarity matrix a dendrogram (tree diagram) (Figure 3-9, Appendix C). "Meaningful" groups of stations-dates were defined using a "variable" stopping rule. Boesch (1977) suggests that variable stopping rules may be more appropriate than "fixed" stopping rules for ecological data.

Inspection of the dendrogram shown in Figure 3-9 indicates three major groups of samples designated as A, B, and C. Group A was composed of the 1984-1985 samples collected at the offshore Weeki Wachee River sampling station (Station WW10). These samples were dominated by the polychaete *Aricidea philbinae* and unidentified tubificid oligochaetes (Table 3-7). Group B was composed primarily of Weeki Wachee and Mud River samples, with several wet season Chassahowitzka River samples. *Grandidierella bonnieroides* and *Hargeria rapax* were the characteristic taxa. Group C was composed of Chassahowitzka River samples including all of the dry season samples from that river. Characteristic taxa in the Group C samples included oligochaete worms and *Grandidierella bonnieroides*.

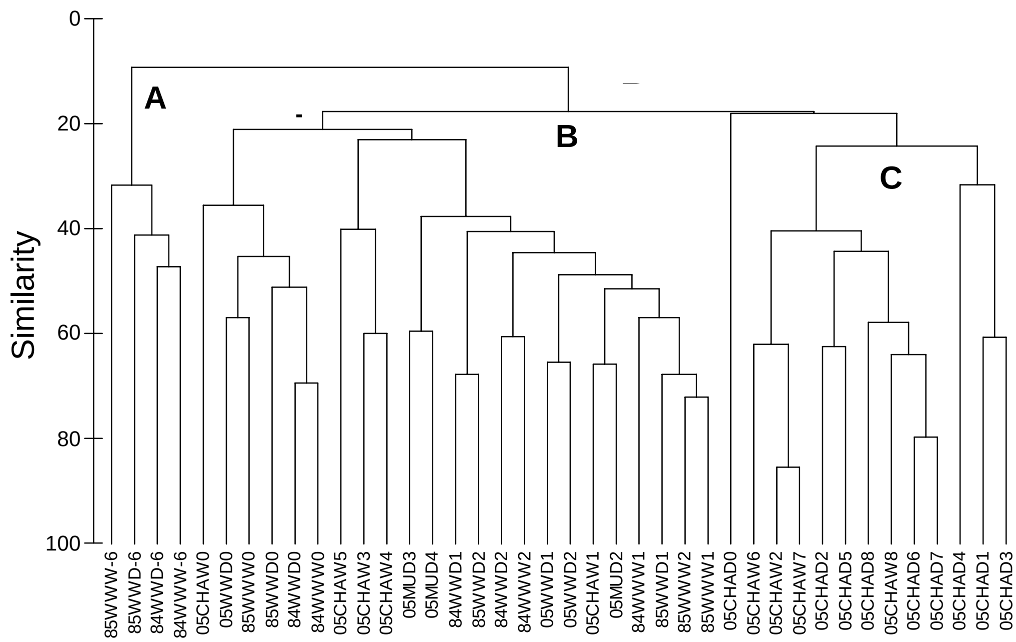

Figure 3-9. Dendrogram showing the similarity among samples from the Weeki Wachee, Mud, and Chassahowitzka rivers, 1984-1985 and 2005. Appendix C presents an ordered listing of samples.

The results demonstrate that the benthos of the offshore waters of the Weeki Wachee River differ due to the higher salinity in this area (Table 3-8). The offshore samples contained higher relative abundances of *Aricidea philbinae* and Tubificidae than found in the samples from the Weeki Wachee and Mud rivers. The results also demonstrate that the benthos of the Chassahowitzka River differs from that of the Weeki Wachee and Mud rivers. The benthos of the Chassahowitzka River differed from that of the Weeki Wachee and Mud rivers primarily due to the higher relative abundances of oligochaetes and *Laeonereis culveri* in the Chassahowitzka River (Table 3-7). This difference is likely related to the difference in the percentage silt+clay between the Weeki Wachee/Mud and Chassahowitzka sediments (Table 3-8).

Table 3-7. Comparison of mean percent composition for those taxa that contributed primarily to the differences between the adjacent groups identified by the cluster analysis of the Bray-Curtis similarity matrix.

Taxa	Group A	Group B	% Contribution to Difference Between Groups A and B
Grandidierella bonnieroides	3.3	27.1	14
Aricidea philbinae	23.6	0.0	13
Hargeria rapax	4.2	16.0	9
Tubificidae	15.5	3.4	8
Laeonereis culveri	0.7	11.3	6
Chone cf. americana	9.5	0.0	5
Taxa	**Group B**	**Group C**	**% Contribution to Difference Between Groups B and C**
Oligochaeta	0.0	24.9	15
Grandidierella bonnieroides	27.1	13.8	13
Hargeria rapax	16.0	2.6	10
Laeonereis culveri	11.3	10.8	9
Corophiidae	0.0	9.3	6

Table 3-8. Mean abiotic conditions for each of the groups of samples identified by the cluster analysis of the Bray-Curtis similarity matrix.

Variable	Cluster Group		
	A	B	C
Temperature (^{o}C)	24.5	21.1	26.1
Salinity (ppt)	24.8	6.9	4.1
Depth (m)	2.9	1.2	1.7
Dissolved Oxygen (mg/L)	7.2	7.3	8.4
pH	No Data	8.2	8.0
% Silt+Clay	10.4	18.4	25.6
Mean ϕ	No Data	2.8	1.8

3.2.4.2 Principal Components Analysis

Principal Components Analysis (PCA) has been used extensively to examine benthic community structure and how it relates to various environmental conditions (Boesch, 1977; Gauch, 1982). Bulger et al. (1993) employed a PCA methodology to develop taxa-specific salinity classes for mid-Atlantic estuarine nekton.

Janicki Environmental, Inc. (2006) used PCA to identify salinity and sediment classes based upon the ranges of over which the taxa occur for data collected from 12 Southwest Florida tidal rivers over a 20-year period. The results of this analysis provide one of the tools that can be used to evaluate benthic community responses to altered salinity regimes.

Four salinity classes were identified from the Southwest Florida tidal river PCA (Janicki Environmental, 2006) (Figure 3-10):

- 0 to 7 ppt – operationally defined as oligohaline;
- >7 to 18 ppt – operationally defined as mesohaline;
- >18-29 ppt – operationally defined as polyhaline; and
- >29 ppt – operationally defined as euhaline.

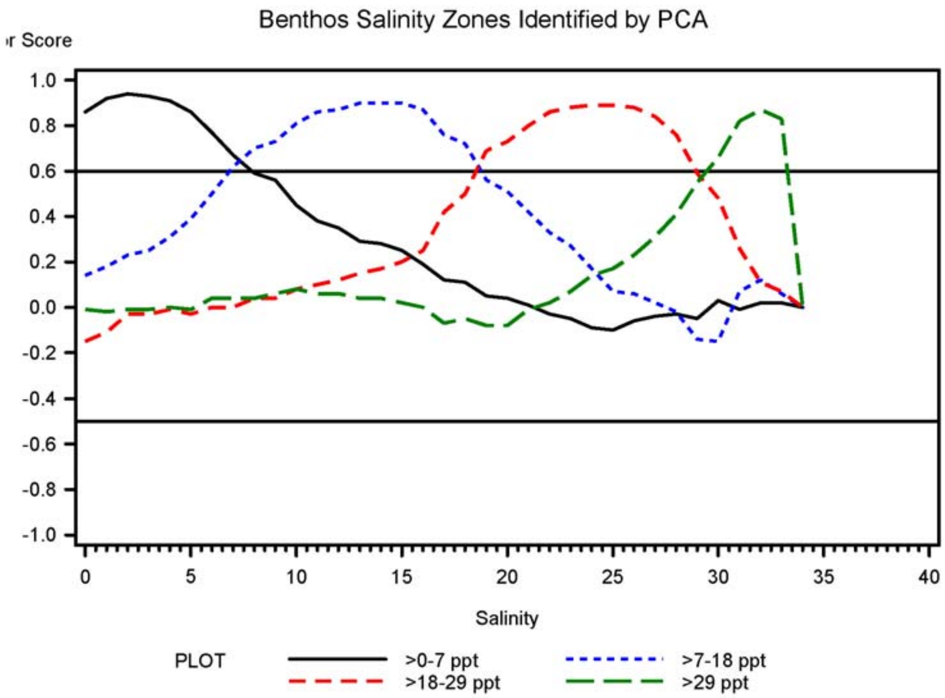

Figure 3-10. Salinity classes identified for Southwest Florida tidal rivers, based upon the distribution of the benthos (From: Janicki Environmental, Inc. 2006).

To examine the applicability of this salinity classification system to the benthic communities of the Weeki Wachee and Mud rivers, each of the samples from this study were assigned to one of the four salinity classes. The benthic infauna from samples within each salinity class were examined. Specifically, Analysis of Similarities (ANOSIM) was applied to determine whether the identified groups differed in their composition (Table 3-9, Appendix D). This analysis shows all pair-wise comparisons of the benthic communities within the four salinity classes are significant with the exception of the polyhaline-euhaline pair. This suggests that the salinity classification based on the 12 Southwest Florida tidal rivers is applicable to the Weeki Wachee and Mud rivers and provides a potential tool for the establishment of a minimum flow in these water bodies.

Table 3-9. Comparison of the benthic community structure in the four salinity classes defined by the PCA of data collected from 12 Southwest Florida tidal rivers (Janicki Environmental, 2006). Rho values that compare classes are presented. *p < 0.05; **p< 0.01; NS=not significant.

Salinity Class	Oligohaline	Mesohaline	Polyhaline	Euhaline
Oligohaline				
Mesohaline	0.15**	-		
Polyhaline	0.53**	0.52**	-	
Euhaline	0.88*	0.74*	-0.01 NS	

The differences in the benthic communities between adjacent salinity classes that were significantly different (oligohaline vs. mesohaline and polyhaline vs. euhaline) were examined using a SIMPER analysis technique and presented in Table 3-10 (Appendix C). As salinity increased from oligohaline to mesohaline conditions the proportional contribution of the tanaid *Hargeria rapax* increased while that of the polychaete *Laeonereis culveri* decreased. As salinity increased to polyhaline conditions the proportions that *Hargeria rapax* and *Grandidierella bonnieroides* made to the assemblage declined and that of *Aricidea philbinae* (Polychaeta), tubificid oligochaetes, and nemerteans increased (Table 3-10).

Table 3-10. Comparison of mean percent composition in the Weeki Wachee and Mud rivers for those taxa that contributed primarily to the differences between the adjacent salinity classes identified by the PCA of the benthic communities in 12 Southwest Florida tidal rivers (Janicki Environmental, 2006).			
Taxa	**Oligohaline**	**Mesohaline**	**% Contribution to Difference Between Oligohaline and Mesohaline Classes**
Hargeria rapax	8.5	26.3	17
Grandidierella bonnieroides	29.8	23.5	15
Laeonereis culveri	17.6	2.2	12
Gammarus mucronatus	8.2	7.3	7
Taxa	**Polyhaline**	**Euhaline**	**% Contribution to Difference Between Polyhaline and Euhaline Classes**
Hargeria rapax	26.3	4.6	15
Grandidierella bonnieroides	23.5	13.6	13
Aricidea philbinae	0.9	15.7	9
Tubificidae	3.0	11.6	7
Nemertea	0.2	7.5	5
Almyracuma proximoculi	0.7	6.8	4

The utility of the salinity classification developed from the 12 Southwest Florida tidal rivers (Janicki Environmental, 2006) to describe how salinity affects the structure of the benthic community of the Chassahowitzka River was also examined. As before, each of the samples from this study were assigned to one of the four salinity classes and the benthic infauna from samples within each salinity class were examined. No samples were taken from areas with salinity greater than 18 ppt, therefore only the oligohaline and mesohaline classes are represented in this analysis. Since all of the Chassahowitzka River infaunal samples were collected by the same size core, the analyses were based on densities of organisms rather than percent composition. The densities were transformed to reduce the relative importance of abundant, ubiquitous taxa.

An ANOSIM test showed that benthic community structure differed significantly between the oligohaline and mesohaline classes (ANOSIM test R statistic = 0.47; p= 0.016) (Appendix D). As salinity increased from oligohaline to mesohaline conditions, the densities of oligochaete worms, *Laeonereis culveri*, and *Grandidierella bonnieroides* decreased and that of the amphipod *Gammarus mucronatus* increased (Table 3-11). Therefore, as was observed for the Weeki Wachee and Mud rivers, the salinity classification based on the 12 Southwest Florida tidal rivers is applicable to the Chassahowitzka River and provides a potential tool for the establishment of a minimum flow in this river.

Table 3-11. Comparison of mean abundance in the Chassahowitzka River for those taxa that contributed primarily to the differences between the oligohaline and mesohaline salinity classes identified by the PCA of the benthic communities in 12 Southwest Florida tidal rivers (Janicki Environmental, 2006).			
Taxa	**Oligohaline**	**Mesohaline**	**% Contribution to Difference Between**

			Oligohaline and Mesohaline Classes
Oligochaeta	3.9	2.2	10
Laeonereis culveri	3.0	2.2	10
Grandidierella bonnieroides	3.1	2.7	9
Insecta	2.3	2.5	8
Polymesoda carolina	2.0	2.2	8
Gammarus mucronatus	1.2	2.9	7

3.2.5 Relationships Between Salinity and the Probability of Occurrence of Selected Taxa

The effect of salinity on benthic community structure also depends upon how the distributions of individual taxa vary with changes in salinity. Logistic regression has been used to quantify the relationship between salinity and the probability of occurrence of estuarine biota (Huisman et al., 1993; Peeters and Gardiniers, 1998; Ysebaert et al., 2002). Janicki Environmental (2006) employed univariate logistic regression to estimate the probability of occurrence as a function of salinity for selected taxa from 12 Southwest Florida tidal rivers. The “optimum” or “preferred” salinity was that with the highest probability of occurrence for that taxon. A “tolerance” range was calculated as the salinity range coincident with the 25th and 75th percent probability of occurrence (Peeters and Gardiniers, 1998).

Figure 3-11 presents a summary of the salinity preference data derived from the univariate logistic regressions for series of selected benthic taxa. These eight taxa include six taxa among the ranked dominants in the Weeki Wachee river system and three of the ranked dominants from the Chassahowitzka River samples (Figure 3-3). These eight taxa have also identified as being preferred prey items for fishes by Peebles (2005). Appendix E presents the results of the logistic regression analyses.

Most of the taxa examined have wide salinity tolerances (Figure 3-11). For example, the salinity tolerances of *Almyracuma proximoculi* and *Grandidierella bonnieroides* extend from oligohaline to polyhaline salinities. *Gammarus mucronatus* prefers polyhaline salinities but clearly tolerates mesohaline and euhaline salinities. Several taxa, including *Polypedilum scalaneum, Polymesoda caroliniana,* and *Laeonereis culveri*, have relatively low salinity optima (< 10 ppt). The narrowest salinity tolerance ranges were found for *Tagelus plebeius* (mesohaline) and *Hargeria rapax* (euhaline).

Flow reductions sufficient to alter the salinity regimes of the Weeki Wachee and Chassahowitzka rivers could contribute to an upriver shift in the distributions of several of benthic taxa, particularly those with the narrower tolerance ranges (e.g, *Polypedilum scalaneum, Tagelus plebeius,* and *Hargeria rapax*). A relatively large alteration of the salinity regime of the Weeki Wachee River could, for example, shift a favorable salinity regime for an infaunal species upstream to that part of the river where hard substrates predominate and the spatial extents of a preferred habitat would be reduced.

SALINITY PREFERENCES

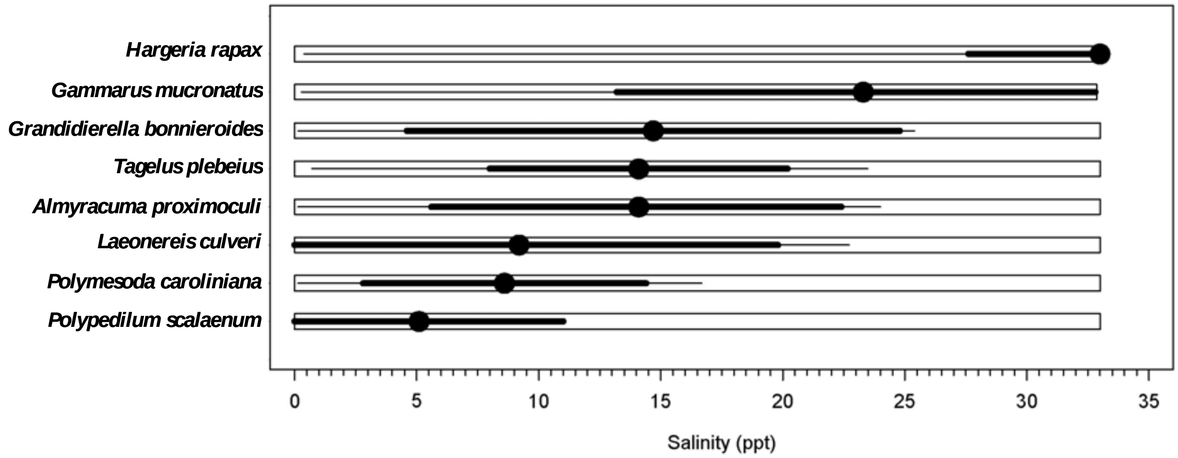

Figure 3-11. Summary of salinity optimum (circle), tolerance range (solid bar), 10th to 90th percentile probability of occurrence (thin line), and model domain (open bar) of salinity for eight selected benthic taxa derived from Janicki Environmental (2006).

4.0 CONCLUSIONS

The establishment of a minimum flow for a tidal river is typically based on establishing a quantitative relationship between freshwater inflow (or a surrogate such as salinity) and an important component of the ecosystem—such as benthic macroinvertebrates. The preferred approach then, is to develop quantitative and predictive relationships that explain a significant portion of variability in the ecosystem component of interest.

Laeonereis culveri and *Gammarus mucronatus,* were among the dominant taxa in each of the three rivers. Other taxa dominant in at least one of the rivers included: *Grandidierella bonnieroides*, oligochaete worms, *Hargeria rapax*, and larvae of *Polypedilum scalaneum*.

The benthos of the Weeki Wachee River shifted along the river's longitudinal axis. The offshore stations sampled in 1984-1985 were clearly different in composition from those of the river proper. During March 2005, the upstream and downstream strata differed somewhat in community structure. The tanaid *Hargeria rapax* was proportionately more abundant in the lower river and the amphipod *Grandidierella bonnieroides* was proportionately more abundant in the upper stratum.

The benthos of the Chassahowitzka River differs from that of the Weeki Wachee and Mud rivers. Oligochaetes and *Laeonereis culveri* were proportionately more abundant in the Chassahowitzka River. The between-river difference appears to be related to the difference in the percentage silt+clay between the two rivers. The benthos of the Chassahowitzka River also differed seasonally. *Laeonereis culveri* was relatively more abundant during the wet season and oligochaetes, unidentified corophiid amphipods, and *Ampelisca abdita* were more abundant during the dry season.

Several abiotic factors, including the percentage silt+clay content of the sediments and water temperature, affected the benthic community structure in the study rivers. Both numbers of taxa and numbers of individuals were negatively associated with temperature and the percentage silt+clay in the sediment.

Salinity also contributed significantly to the variation in benthic community structure. The benthic macroinvertebrate communities in the Weeki Wachee and Mud rivers were exposed to a relatively narrow range of salinities (0 to 16.5 ppt) during the 2005 sampling periods. However, the 1984-1985 sampling in the Weeki Wachee River included an offshore station where salinities ranged from 23 to 30.8 ppt. The longer-term record of salinities in the Weeki Wachee River, however, shows considerable longitudinal variation. Since the observed variation in the discharge from Weeki Wachee Spring is relatively small, much of the observed salinity variation likely reflects tidal effects. Within the Chassahowitzka River, the wet and dry season salinities measured during collection of the benthic samples were very similar.

Data analysis indicated that three community metrics (the number of taxa, the Shannon-Wiener diversity index, and total abundance) did not vary linearly with variation in salinity. Quadratic regression equations explained a significant proportion of the observed variation in both the number of taxa and the Shannon-Wiener diversity index, while a polynomial relationship explained more of the variation in the total abundance than either linear or quadratic relationships.

As salinity increased from oligohaline to mesohaline conditions, the proportional contribution of the tanaid *Hargeria rapax* increased while that of the polychaete *Laeonereis culveri* decreased. As salinity increased further to polyhaline conditions (mainly 1984-1985 data from the Weeki Wachee River), the relative abundance of *Hargeria rapax* and *Grandidierella bonnieroides* declined and that of *Aricidea philbinae*, tubificid oligochaetes, and nemerteans increased.

Numerical classification provided some insight on how salinity might be related to community structure, even within the narrow range of salinities encountered. The three groups of stations and dates were organized along a salinity gradient. The mean salinities of the three groups were 4.1, 6.9, and 24.8 ppt, respectively. The lowest salinity group was essentially a Chassahowitzka River group of samples in which unidentified nemerteans and unidentified oligochaetes were predominant. The other two groups were composed primarily of Weeki Wachee and Mud river samples. *Hargeria rapax* was proportionately more abundant in the higher salinity (offshore) group and *Grandidierella bonnieroides* and *Polypedilum scalaneum* were proportionately more abundant in the lower salinity (river) group.

The relationships between the probability of occurrence of specific benthic taxa indicated that taxa characteristic of the study rivers, such as *Laeonereis culveri* and *Almyracuma cf. proximoculi,* to be among the dominant taxa more sensitive to salinity changes. Each appear to have relatively low salinity optima (<15 ppt) and their distribution would likely shift upstream in these rivers should salinities increase. Other taxa that have relatively low salinity optima include *Polypedilum scalaneum* and *Polymesoda caroliniana* and their distributions would also likely shift upstream with increasing salinity in the study rivers. *Tagelus plebeius,* with an optimum salinity in the 13 to 16 ppt range, could penetrate further upriver. A dominant mesohaline species such as *Hargeria rapax*, might also expand its distribution as higher salinities move upstream.

The substratum of the Weeki Wachee River varies along its longitudinal axis. The fine sand-sized sediments that provide habitat for many infaunal species are replaced in some upstream areas by exposed limestone (Culter, 1986; S. Grabe, personal observations). This bedrock diminishes the amount of finer-grained sands that infauna require. If the salinities typically encountered over downstream finer sediments migrated upstream over the limestone substrate, the effect of this salinity change would be modified.

5.0 APPLICATION OF STUDY RESULTS TO MFL DETERMINATION

Estuarine benthic macroinvertebrates are typically tolerant of wide ranges in salinity over daily and seasonal time scales. Given the relatively narrow range of salinities normally encountered in these rivers, subtle changes in salinity alone may not have a detectable effect on the benthos in them. The study results clearly demonstrate that the structure of the benthic communities in the study rivers is influenced by variation in salinity conditions. Changes in the availability of habitat within two salinity ranges, oligohaline (<7 ppt) and mesohaline (7-18 ppt), could result in an appreciable change in the benthic communities of the study rivers. As salinities in these rivers increase from mesohaline to polyhaline (18-29 ppt) conditions, the probability of occurrence of a number of important benthic taxa would likely change. Based on these observations, it is recommended that reductions in flow that result in a significant reduction in the availability of the oligohaline and mesohaline habitats be considered in the determination of minimum flows in the Weeki Wachee and Chassahowitzka rivers.

6.0 LITERATURE CITED

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APPENDIX A.

STEPWISE REGRESSION OUTPUT

Dependent Variable L10BS
Minimum tolerance for entry into model = 0.000000

Forward stepwise with Alpha-to-Enter=0.150 and Alpha-to-Remove=0.150
Step # 1 R = 0.460 R-Square = 0.212
Term entered: SALINITY

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.0964	0.0227	0.4602	1.00000	1	18.0069	0.0001
Out		Part. Corr.						
2	PH	0.0494	.	.	0.75197	1	0.1618	0.6888
3	DEPTH	0.0019	.	.	0.96340	1	0.0002	0.9875
4	DO	0.0334	.	.	0.86332	1	0.0737	0.7869
6	TEMP	-0.3573	.	.	0.92851	1	9.6601	0.0028
7	SILTCLAY	-0.4356	.	.	0.98946	1	15.4589	0.0002
8	MEANPHI	0.2596	.	.	0.99678	1	4.7700	0.0325

Step # 2 R = 0.601 R-Square = 0.361
Term entered: SILTCLAY

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.1048	0.0207	0.5002	0.98946	1	25.5821	0.0000
7	SILTCLAY	-0.0790	0.0201	-0.3888	0.98946	1	15.4589	0.0002
Out		Part. Corr.						
2	PH	-0.0622	.	.	0.70924	1	0.2526	0.6170
3	DEPTH	0.0480	.	.	0.95483	1	0.1501	0.6997
4	DO	-0.0747	.	.	0.81886	1	0.3646	0.5481
6	TEMP	-0.2036	.	.	0.74628	1	2.8120	0.0984
8	MEANPHI	0.0822	.	.	0.80055	1	0.4425	0.5083

Step # 3 R = 0.623 R-Square = 0.388
Term entered: TEMP

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.0925	0.0217	0.4415	0.87672	1	18.1465	0.0001
6	TEMP	-0.0387	0.0231	-0.1884	0.74628	1	2.8120	0.0984
7	SILTCLAY	-0.0625	0.0221	-0.3080	0.79527	1	8.0093	0.0062
Out		Part. Corr.						
2	PH	-0.1466	.	.	0.62044	1	1.4064	0.2400
3	DEPTH	0.1915	.	.	0.67698	1	2.4359	0.1235
4	DO	-0.0540	.	.	0.80922	1	0.1874	0.6665
8	MEANPHI	-0.0609	.	.	0.48197	1	0.2379	0.6274

Step # 4 R = 0.641 R-Square = 0.410
Term entered: DEPTH

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
3	DEPTH	0.0376	0.0241	0.1821	0.67698	1	2.4359	0.1235
5	SALINITY	0.0783	0.0233	0.3738	0.74360	1	11.2769	0.0013
6	TEMP	-0.0616	0.0271	-0.2995	0.52912	1	5.1502	0.0266
7	SILTCLAY	-0.0563	0.0222	-0.2772	0.76951	1	6.4189	0.0138
Out		Part. Corr.						
2	PH	-0.0909	.	.	0.55485	1	0.5250	0.4714
4	DO	0.0233	.	.	0.68492	1	0.0341	0.8540
8	MEANPHI	-0.0556	.	.	0.48145	1	0.1955	0.6599

2 case(s) deleted due to missing data.

Eigenvalues of unit scaled X'X

1	2	3	4	5
1.6572	1.1688	1.0224	0.8605	0.2912

Condition indices

1	2	3	4	5
1.0000	1.1907	1.2731	1.3878	2.3857

.

Variance proportions

	1	2	3	4	5
CONSTANT	0.0029	0.0023	0.8191	0.1610	0.0147
DEPTH	0.1150	0.0702	0.0312	0.2533	0.5303
SALINITY	0.0010	0.5039	0.0034	0.0105	0.4812
TEMP	0.1471	0.0339	0.0091	0.0012	0.8088
SILTCLAY	0.1156	0.0058	0.0620	0.4651	0.3515

Dep Var: L10BS   N: 69   Multiple R: 0.6406   Squared multiple R: 0.4103

Adjusted squared multiple R: 0.3735   Standard error of estimate: 0.1640

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	0.9809	0.0199	0.0000	.	49.3885	0.0000
DEPTH	0.0376	0.0241	0.1821	0.6770	1.5607	0.1235
SALINITY	0.0783	0.0233	0.3738	0.7436	3.3581	0.0013
TEMP	-0.0616	0.0271	-0.2995	0.5291	-2.2694	0.0266
SILTCLAY	-0.0563	0.0222	-0.2772	0.7695	-2.5336	0.0138

Effect	Coefficient	Lower 95%	Upper 95%
CONSTANT	0.9809	0.9412	1.0206
DEPTH	0.0376	-0.0105	0.0856
SALINITY	0.0783	0.0317	0.1249
TEMP	-0.0616	-0.1158	-0.0074
SILTCLAY	-0.0563	-0.1007	-0.0119

Correlation matrix of regression coefficients

	CONSTANT	DEPTH	SALINITY	TEMP	SILTCLAY
CONSTANT	1.0000				
DEPTH	-0.0406	1.0000			
SALINITY	0.0596	-0.3897	1.0000		
TEMP	0.0661	-0.5394	0.4720	1.0000	
SILTCLAY	-0.1055	0.1800	-0.2841	-0.4640	1.0000

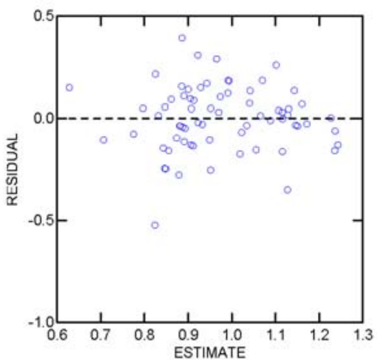
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	1.1973	4	0.2993	11.1338	0.0000
Residual	1.7206	64	0.0269		

*** WARNING ***
Case 15 has large leverage (Leverage = 0.3228)
Case 64 is an outlier (Studentized Residual = -3.5717)

Durbin-Watson D Statistic 1.7145
First Order Autocorrelation 0.0974

Plot of residuals against predicted values



2 case(s) deleted due to missing data.
Step # 0 R = 0.000 R-Square = 0.000

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
Out		Part. Corr.						
2	PH	-0.2827	.	.	1.00000	1	5.8192	0.0186
3	DEPTH	0.1111	.	.	1.00000	1	0.8370	0.3635
4	DO	-0.2424	.	.	1.00000	1	4.1820	0.0448
5	SALINITY	0.4177	.	.	1.00000	1	14.1621	0.0004
6	TEMP	-0.2397	.	.	1.00000	1	4.0843	0.0473
7	SILTCLAY	-0.1498	.	.	1.00000	1	1.5383	0.2192
8	MEANPHI	0.1991	.	.	1.00000	1	2.7646	0.1010

Dependent Variable BH2
Minimum tolerance for entry into model = 0.000000

Forward stepwise with Alpha-to-Enter=0.150 and Alpha-to-Remove=0.150
Step # 1 R = 0.418 R-Square = 0.174
Term entered: SALINITY

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.2876	0.0764	0.4177	1.00000	1	14.1621	0.0004
Out		Part. Corr.						
2	PH	-0.0947	.	.	0.75197	1	0.5979	0.4422
3	DEPTH	0.0349	.	.	0.96340	1	0.0807	0.7773
4	DO	-0.1042	.	.	0.86332	1	0.7242	0.3978
6	TEMP	-0.1462	.	.	0.92851	1	1.4418	0.2341
7	SILTCLAY	-0.2132	.	.	0.98946	1	3.1432	0.0809
8	MEANPHI	0.1933	.	.	0.99678	1	2.5625	0.1142

Step # 2 R = 0.460 R-Square = 0.212
Term entered: SILTCLAY

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.3013	0.0756	0.4377	0.98946	1	15.8784	0.0002
7	SILTCLAY	-0.1300	0.0733	-0.1947	0.98946	1	3.1432	0.0809
Out		Part. Corr.						
2	PH	-0.1534	.	.	0.70924	1	1.5668	0.2152
3	DEPTH	0.0566	.	.	0.95483	1	0.2089	0.6492
4	DO	-0.1603	.	.	0.81886	1	1.7152	0.1949
6	TEMP	-0.0591	.	.	0.74628	1	0.2278	0.6348
8	MEANPHI	0.1128	.	.	0.80055	1	0.8370	0.3636

2 case(s) deleted due to missing data.

Eigenvalues of unit scaled X'X

1	2	3
1.1194	1.0227	0.8579

Condition indices

1	2	3
1.0000	1.0462	1.1422

Variance proportions

	1	2	3
CONSTANT	0.1466	0.5820	0.2714
SALINITY	0.2580	0.3857	0.3563
SILTCLAY	0.4763	0.0012	0.5225

Dep Var: BH2   N: 69   Multiple R: 0.4605   Squared multiple R: 0.2120

Adjusted squared multiple R: 0.1881   Standard error of estimate: 0.6134

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	2.2893	0.0741	0.0000	.	30.8779	0.0000
SALINITY	0.3013	0.0756	0.4377	0.9895	3.9848	0.0002
SILTCLAY	-0.1300	0.0733	-0.1947	0.9895	-1.7729	0.0809

Effect	Coefficient	Lower 95%	Upper 95%
CONSTANT	2.2893	2.1412	2.4373
SALINITY	0.3013	0.1503	0.4523
SILTCLAY	-0.1300	-0.2764	0.0164

Correlation matrix of regression coefficients

	CONSTANT	SALINITY	SILTCLAY
CONSTANT	1.0000		
SALINITY	0.0317	1.0000	
SILTCLAY	-0.0856	-0.1027	1.0000

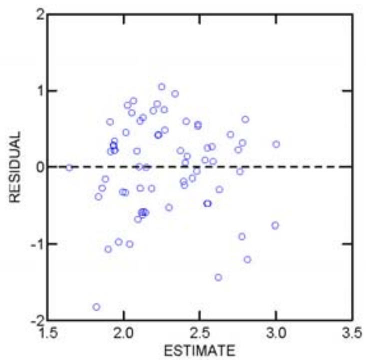
Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	6.6822	2	3.3411	8.8792	0.0004
Residual	24.8348	66	0.3763		

*** WARNING ***
Case 64 is an outlier (Studentized Residual = -3.2530)

Durbin-Watson D Statistic 2.3279
First Order Autocorrelation -0.1770

Plot of residuals against predicted values




```
2 case(s) deleted due to missing data.
Step # 0 R =  0.000 R-Square =  0.000
```

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
Out		Part. Corr.						
2	PH	0.0178	.	.	1.00000	1	0.0213	0.8844
3	DEPTH	-0.0247	.	.	1.00000	1	0.0410	0.8401
4	DO	0.1885	.	.	1.00000	1	2.4672	0.1210
5	SALINITY	0.1628	.	.	1.00000	1	1.8246	0.1813
6	TEMP	-0.3314	.	.	1.00000	1	8.2667	0.0054
7	SILTCLAY	-0.4724	.	.	1.00000	1	19.2472	0.0000
8	MEANPHI	0.0616	.	.	1.00000	1	0.2552	0.6151

Dependent Variable L10ABUND
Minimum tolerance for entry into model = 0.000000

Forward stepwise with Alpha-to-Enter=0.150 and Alpha-to-Remove=0.150
Step # 1 R = 0.472 R-Square = 0.223
Term entered: SILTCLAY

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
7	SILTCLAY	-0.1976	0.0451	-0.4724	1.00000	1	19.2472	0.0000
Out		Part. Corr.						
2	PH	-0.1214	.	.	0.93409	1	0.9881	0.3238
3	DEPTH	0.0320	.	.	0.98752	1	0.0677	0.7956
4	DO	0.0837	.	.	0.93865	1	0.4653	0.4975
5	SALINITY	0.2410	.	.	0.98946	1	4.0706	0.0477
6	TEMP	-0.1778	.	.	0.84224	1	2.1533	0.1470
8	MEANPHI	-0.1812	.	.	0.81094	1	2.2403	0.1392

Step # 2 R = 0.518 R-Square = 0.268
Term entered: SALINITY

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.0922	0.0457	0.2136	0.98946	1	4.0706	0.0477
7	SILTCLAY	-0.2068	0.0443	-0.4943	0.98946	1	21.8087	0.0000
Out		Part. Corr.						
2	PH	-0.0038	.	.	0.70924	1	0.0009	0.9758
3	DEPTH	-0.0124	.	.	0.95483	1	0.0100	0.9206
4	DO	0.1873	.	.	0.81886	1	2.3629	0.1291
6	TEMP	-0.1055	.	.	0.74628	1	0.7318	0.3954
8	MEANPHI	-0.2162	.	.	0.80055	1	3.1867	0.0789

Step # 3 R = 0.550 R-Square = 0.302
Term entered: MEANPHI

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.1013	0.0452	0.2347	0.97679	1	5.0156	0.0285
7	SILTCLAY	-0.2453	0.0486	-0.5864	0.79467	1	25.4616	0.0000
8	MEANPHI	-0.0882	0.0494	-0.2067	0.80055	1	3.1867	0.0789
Out		Part. Corr.						
2	PH	0.0305	.	.	0.69248	1	0.0597	0.8077
3	DEPTH	-0.0996	.	.	0.82986	1	0.6408	0.4264
4	DO	0.1323	.	.	0.74745	1	1.1409	0.2895
6	TEMP	-0.3193	.	.	0.44929	1	7.2656	0.0090

Step # 4 R = 0.611 R-Square = 0.374
Term entered: TEMP

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
5	SALINITY	0.0585	0.0460	0.1357	0.86074	1	1.6184	0.2079
6	TEMP	-0.1686	0.0625	-0.3978	0.44929	1	7.2656	0.0090
7	SILTCLAY	-0.2190	0.0474	-0.5235	0.76110	1	21.3145	0.0000
8	MEANPHI	-0.1916	0.0608	-0.4490	0.48197	1	9.9272	0.0025
Out		Part. Corr.						
2	PH	-0.0856	.	.	0.61475	1	0.4652	0.4977
3	DEPTH	0.0442	.	.	0.67625	1	0.1233	0.7266
4	DO	0.1049	.	.	0.73922	1	0.7005	0.4058

Step # 5 R = 0.598 R-Square = 0.358
Term removed: SALINITY

	Effect	Coefficient	Std Error	Std Coef	Tol.	df	F	'P'
In								
1	Constant							
6	TEMP	-0.1960	0.0590	-0.4626	0.50987	1	11.0412	0.0015
7	SILTCLAY	-0.2069	0.0467	-0.4946	0.79313	1	19.6329	0.0000
8	MEANPHI	-0.2021	0.0605	-0.4735	0.49092	1	11.1382	0.0014
Out		Part. Corr.						
2	PH	-0.1596	.	.	0.91958	1	1.6718	0.2007
3	DEPTH	0.1005	.	.	0.79273	1	0.6532	0.4220
4	DO	0.0412	.	.	0.84530	1	0.1089	0.7425
5	SALINITY	0.1570	.	.	0.86074	1	1.6184	0.2079

2 case(s) deleted due to missing data.

Eigenvalues of unit scaled X'X

1	2	3	4
2.0282	1.0138	0.6515	0.3065

Condition indices

1	2	3	4
1.0000	1.4144	1.7643	2.5723

Variance proportions

	1	2	3	4
CONSTANT	0.0007	0.9351	0.0641	0.0001
TEMP	0.0927	0.0052	0.1160	0.7860
SILTCLAY	0.0961	0.0197	0.8748	0.0094
MEANPHI	0.0925	0.0039	0.0648	0.8388

Dep Var: L10ABUND   N: 69   Multiple R: 0.5981   Squared multiple R: 0.3578

Adjusted squared multiple R: 0.3281   Standard error of estimate: 0.3498

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P(2 Tail)
CONSTANT	3.9910	0.0423	0.0000	.	94.3587	0.0000
TEMP	-0.1960	0.0590	-0.4626	0.5099	-3.3228	0.0015
SILTCLAY	-0.2069	0.0467	-0.4946	0.7931	-4.4309	0.0000
MEANPHI	-0.2021	0.0605	-0.4735	0.4909	-3.3374	0.0014

Effect	Coefficient	Lower 95%	Upper 95%
CONSTANT	3.9910	3.9065	4.0755
TEMP	-0.1960	-0.3138	-0.0782
SILTCLAY	-0.2069	-0.3002	-0.1137
MEANPHI	-0.2021	-0.3230	-0.0811

Correlation matrix of regression coefficients

	CONSTANT	TEMP	SILTCLAY	MEANPHI
CONSTANT	1.0000			
TEMP	0.0176	1.0000		
SILTCLAY	-0.0935	-0.1482	1.0000	
MEANPHI	-0.0201	0.6282	0.2415	1.0000

Analysis of Variance

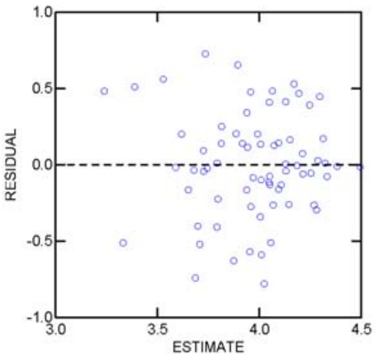
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	4.4303	3	1.4768	12.0694	0.0000
Residual	7.9531	65	0.1224		

*** WARNING ***

Case 63 has large leverage (Leverage = 0.4421)

```
Durbin-Watson D Statistic          1.7085
First Order Autocorrelation        0.0921
```

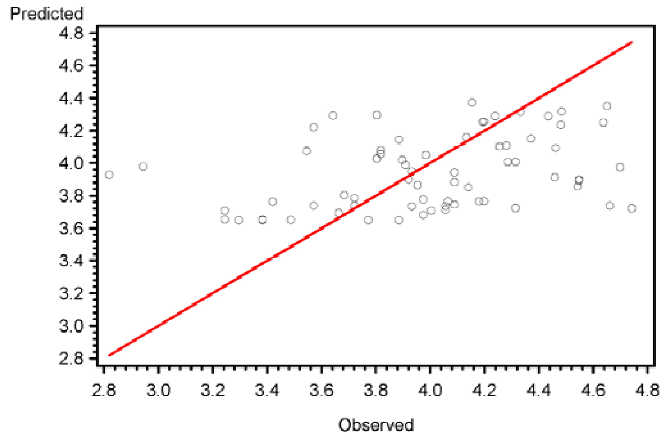
Plot of residuals against predicted values



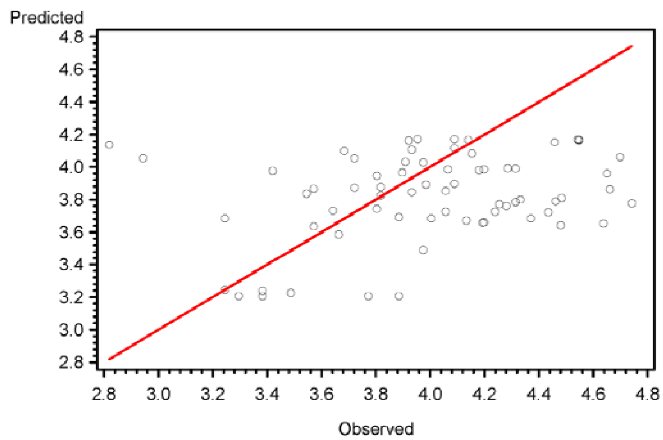
APPENDIX B.

RESULTS OF REGRESSIONS OF NUMBERS OF TAXA, SHANNON-WIENER DIVERSITY, AND TOTAL ABUNDANCE OF BENTHIC ORGANISMS ON SALINITY
Weeki Wachee, Mud, and Chassahowitzka Rivers, 2005

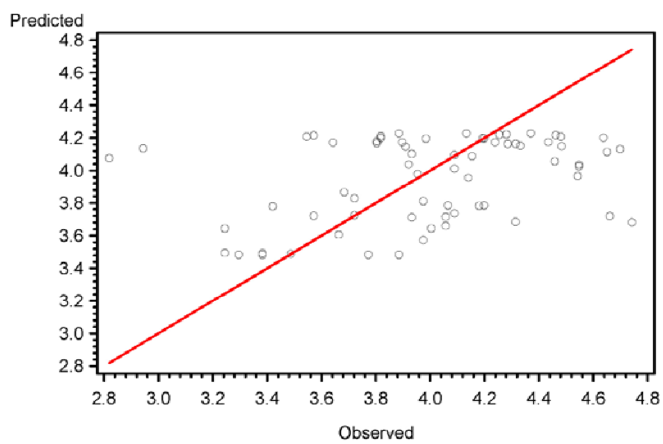
Predicted versus Observed Salinity
Regression - Log(Abundance) = Salinity

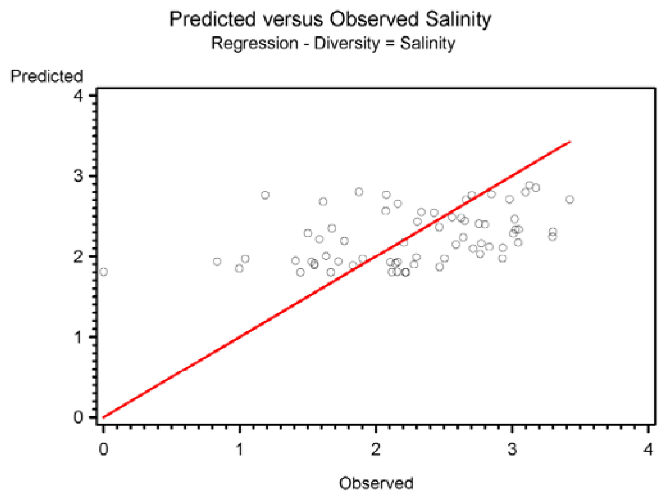


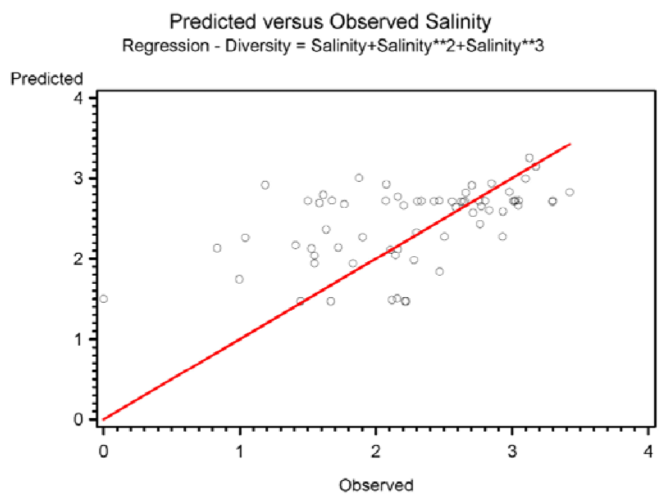
Predicted versus Observed Salinity
Regression - Log(Abundance) = Salinity+Salinity**2+Salinity**3

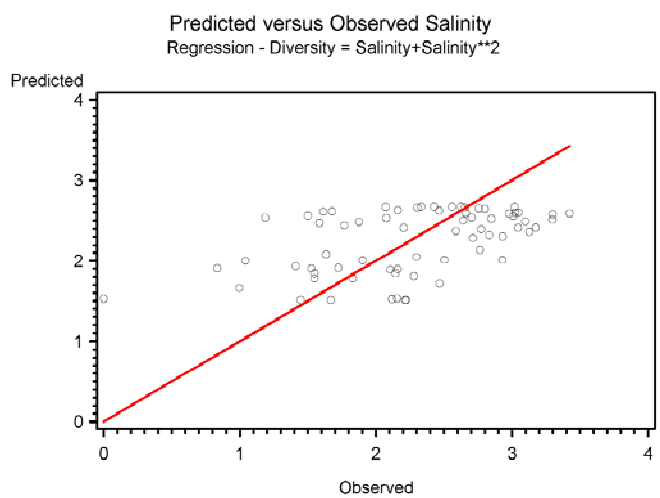


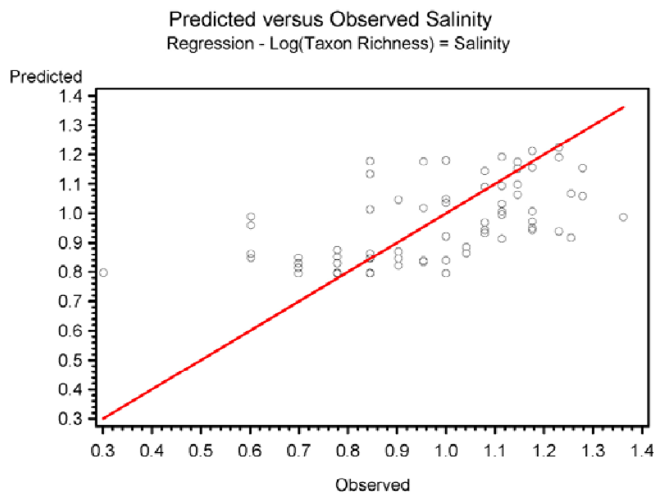
Predicted versus Observed Salinity
Regression - Log(Abundance) = Salinity+Salinity**2

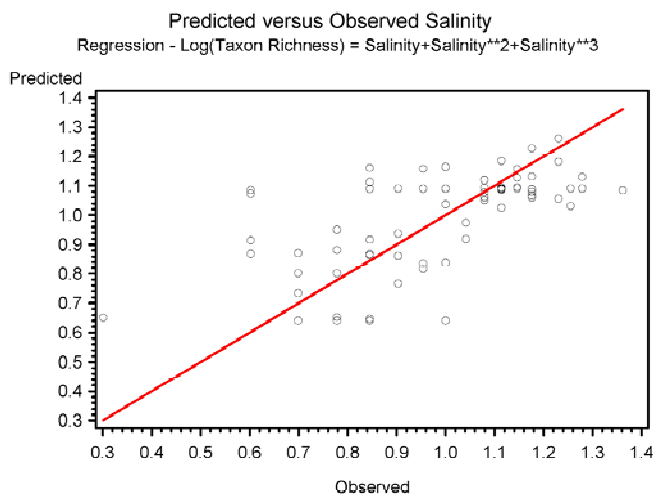


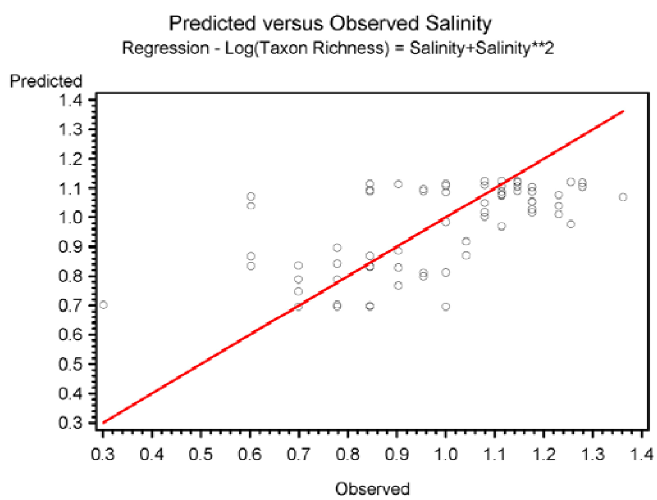

Predicted versus Observed Salinity
Regression - Diversity = Salinity
Predicted
Observed

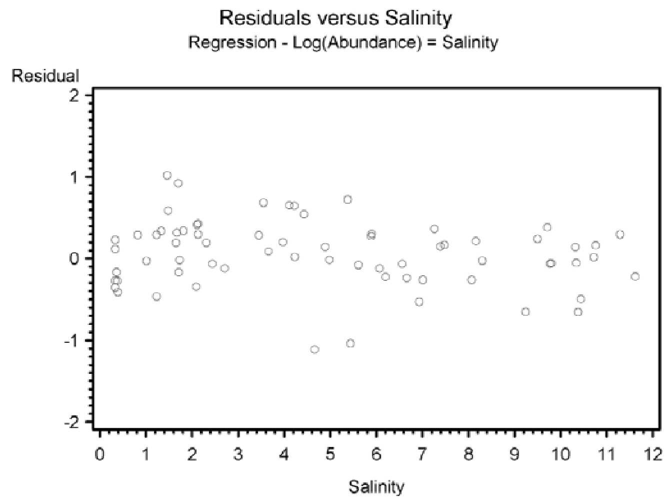

Predicted versus Observed Salinity
Regression - Diversity = Salinity+Salinity**2+Salinity**3
Predicted
Observed

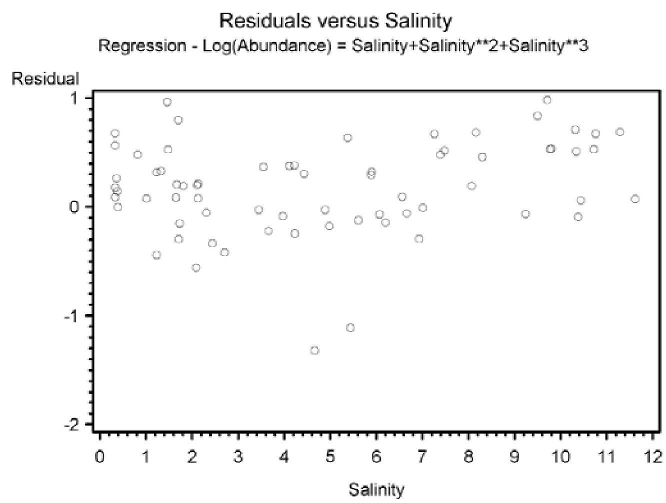

Predicted versus Observed Salinity
Regression - Diversity = Salinity+Salinity**2
Predicted
Observed

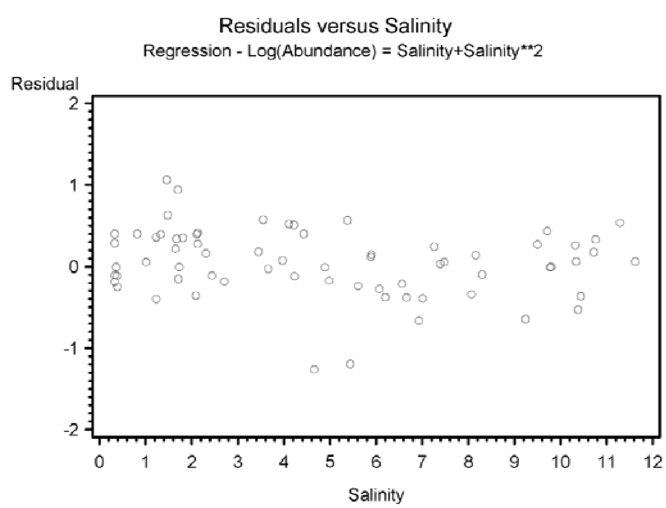

Predicted versus Observed Salinity
Regression - Log(Taxon Richness) = Salinity
Predicted
Observed

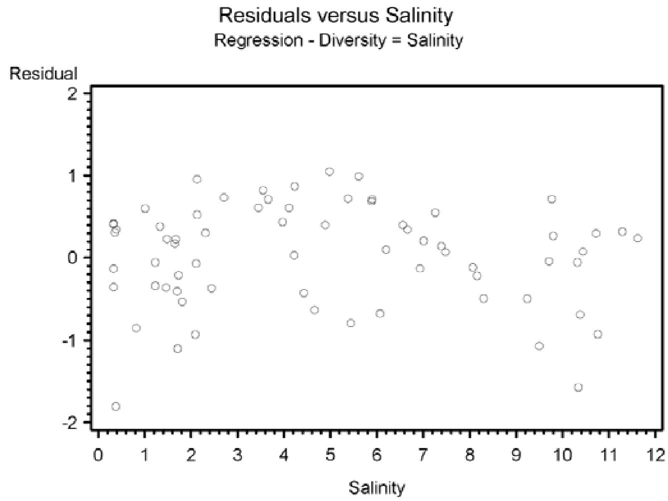

Predicted versus Observed Salinity
Regression - Log(Taxon Richness) = Salinity+Salinity**2+Salinity**3
Predicted
Observed

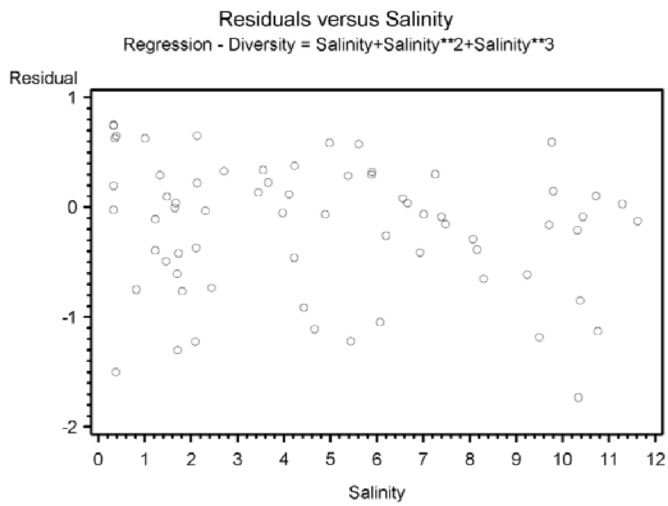

Predicted versus Observed Salinity
Regression - Log(Taxon Richness) = Salinity+Salinity**2
Predicted
Observed

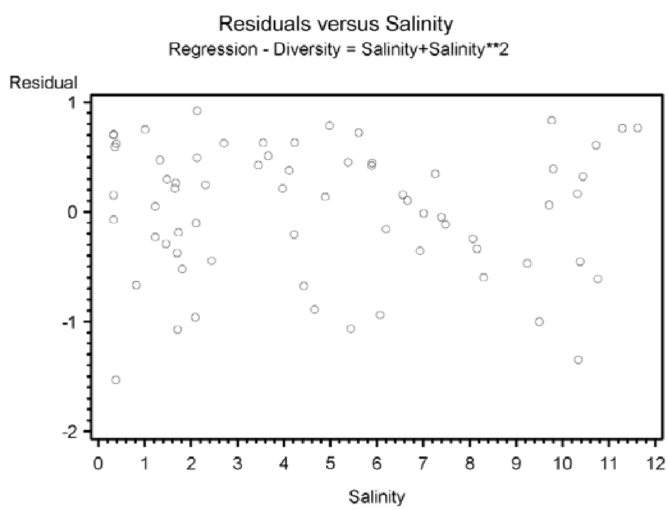

Residuals versus Salinity
Regression - Log(Abundance) = Salinity
Residual
2
1
0
-1
-2
0 1 2 3 4 5 6 7 8 9 10 11 12
Salinity


Residuals versus Salinity
Regression - Log(Abundance) = Salinity+Salinity**2+Salinity**3
Residual
1
0
-1
-2
0 1 2 3 4 5 6 7 8 9 10 11 12
Salinity

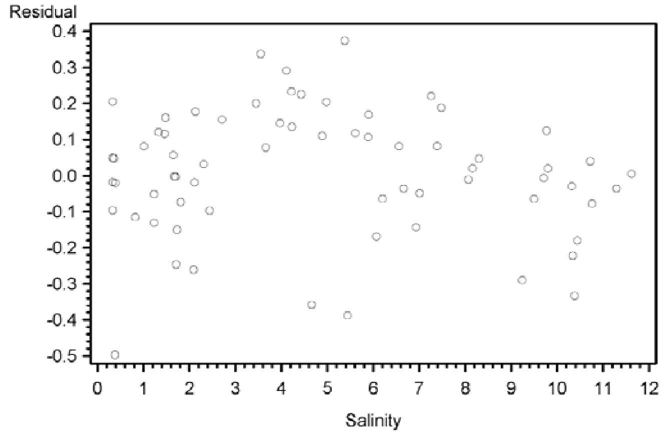

Residuals versus Salinity
Regression - Log(Abundance) = Salinity+Salinity**2
Residual
2
1
0
-1
-2
0 1 2 3 4 5 6 7 8 9 10 11 12
Salinity


Residuals versus Salinity
Regression - Diversity = Salinity
Residual
2
1
0
-1
-2
0 1 2 3 4 5 6 7 8 9 10 11 12
Salinity

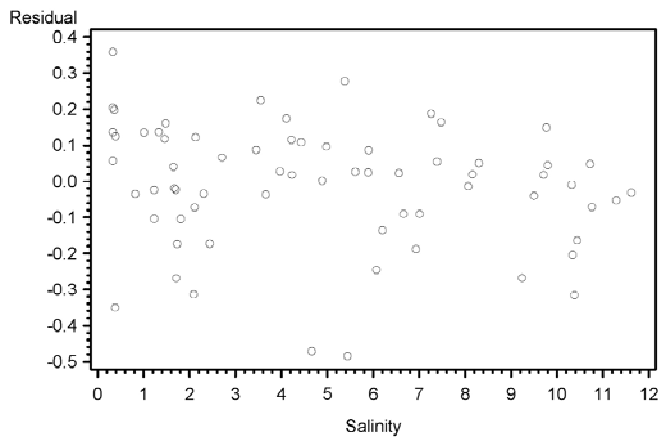

Residuals versus Salinity
Regression - Diversity = Salinity+Salinity**2+Salinity**3
Residual
1
0
-1
-2
0 1 2 3 4 5 6 7 8 9 10 11 12
Salinity


Residuals versus Salinity
Regression - Diversity = Salinity+Salinity**2
Residual
1
0
-1
-2
0 1 2 3 4 5 6 7 8 9 10 11 12
Salinity

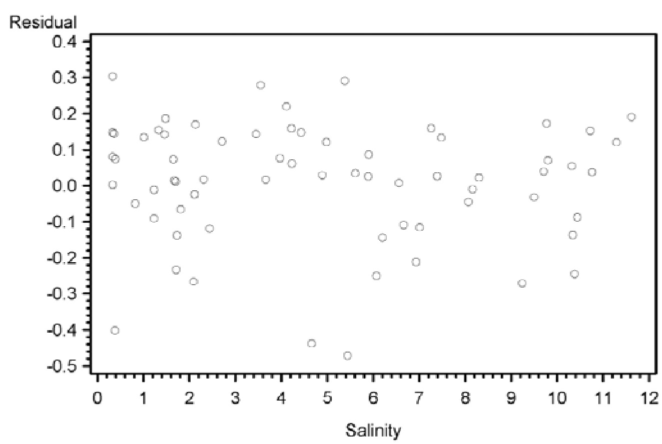
Residuals versus Salinity
Regression - Log(Taxon Richness) = Salinity



Residuals versus Salinity
Regression - Log(Taxon Richness) = Salinity+Salinity**2+Salinity**3



Residuals versus Salinity
Regression - Log(Taxon Richness) = Salinity+Salinity**2



APPENDIX C.

CLUSTER ANALYSIS OUTPUT

SAMPLE CODE
Year – 2 digits
River – 2 digits
Season – 1 digit
River Kilometer

SAMPLE	GROUP
85WWW-6	A
85WWD-6	A
84WWD-6	A
84WWW-6	A
05CHAW0	A
05WWD0	A
85WWW0	A
85WWD0	A
84WWWD0	A
84WWW0	A
05CHAW5	B
05CHAW3	B
05CHAW4	B
05MUD3	B
05MUD4	B
84WWD1	B
85WWD2	B
84WWD2	B
84WWW2	B
05WWD1	B
05WWD2	B
05CHAW1	B
05MUD2	B
84WWW1	B
85WWD1	B
85WWW2	B
85WWW1	B
05CHAD0	B
05CHAW6	C
05CHAW2	C
05CHAW7	C
05CHAD2	C
05CHAD5	C
05CHAD8	C
05CHAW8	C
05CHAD6	C
05CHAD7	C
05CHAD4	C
05CHAD1	C
05CHAD3	C

APPENDIX D.

ANOSIM AND SIMPER ANALYSIS OUTPUT
Weeki Wachee and Mud Rivers

ANOSIM
Analysis of Similarities

One-Way Analysis
Global Test
Sample statistic (Global R): 0.196
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
M, O	0.104	1.5	Very large	999	14
M, P	0.443	0.2	12620256	999	1
M, E	0.603	6.3	32	32	2
O, P	0.396	0.2	491796152	999	1
O, E	0.695	3.6	56	56	2
P, E	-0.007	62.5	8	8	5

SIMPER

Similarity Percentages - species contributions

One-Way Analysis

Group M
Average similarity: 28.44

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
GRANBONN	21.02	11.49	1.10	40.39	40.39
HARGRAPA	22.12	9.04	0.68	31.80	72.19

Group O
Average similarity: 26.09

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
GRANBONN	23.56	12.82	1.00	49.15	49.15
LAEOCULV	14.90	4.82	0.57	18.46	67.62

Group P
Average similarity: 20.13

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
ARICPHIL	15.71	6.22	0.58	30.89	30.89
TUBIFICID	11.60	4.73	0.71	23.48	54.37

Group E
Less than 2 samples in group

Groups M & O
Average dissimilarity = 76.60

	Group M	Group O				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
HARGRAPA	22.12	6.14	10.86	0.99	14.18	14.18
GRANBONN	21.02	23.56	10.04	1.28	13.10	27.28
LAEOCULV	4.01	14.90	7.52	0.71	9.81	37.10
OLIGO	1.08	11.61	6.03	0.57	7.87	44.97
GAMMMUCR	7.27	7.13	4.62	0.93	6.03	50.99

Groups M & P
Average dissimilarity = 84.55

	Group M	Group P				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
HARGRAPA	22.12	4.59	10.66	0.96	12.61	12.61
GRANBONN	21.02	13.58	10.47	1.34	12.38	24.99
ARICPHIL	0.68	15.71	7.83	1.06	9.26	34.25
TUBIFICID	2.37	11.60	5.59	1.10	6.61	40.86
NEMERTEA	0.46	7.48	3.82	0.45	4.52	45.38
LAEOCULV	4.01	5.58	3.81	0.64	4.50	49.89
ALMYPROX	1.01	6.81	3.62	0.63	4.28	54.17

Groups O & P
Average dissimilarity = 85.74

	Group O	Group P				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
GRANBONN	23.56	13.58	11.18	1.31	13.03	13.03
LAEOCULV	14.90	5.58	7.95	0.78	9.28	22.31
ARICPHIL	0.00	15.71	7.85	1.05	9.16	31.47
OLIGO	11.61	0.00	5.80	0.54	6.77	38.24
TUBIFICID	2.60	11.60	5.72	1.10	6.67	44.91
HARGRAPA	6.14	4.59	4.28	0.74	4.99	49.90
NEMERTEA	0.68	7.48	3.96	0.46	4.61	54.51

Groups M & E
Average dissimilarity = 87.93

	Group M	Group E				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
CERIMUSC	0.05	23.03	11.49	81.90	13.07	13.07
TRANCONR	0.59	23.36	11.38	6.95	12.95	26.02
GRANBONN	21.02	0.00	10.51	1.22	11.95	37.97
HARGRAPA	22.12	16.46	9.70	1.28	11.03	49.00
CHONAMER	0.00	12.25	6.12	4144.16	6.96	55.97

Groups O & E
Average dissimilarity = 94.56

	Group O	Group E				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
GRANBONN	23.56	0.00	11.78	1.24	12.46	12.46
TRANCONR	0.00	23.36	11.68	40244932.80	12.35	24.81
CERIMUSC	0.00	23.03	11.52	29580179.58	12.18	36.99
LAEOCULV	14.90	0.24	7.37	0.68	7.80	44.79
HARGRAPA	6.14	16.46	7.26	2.61	7.68	52.47

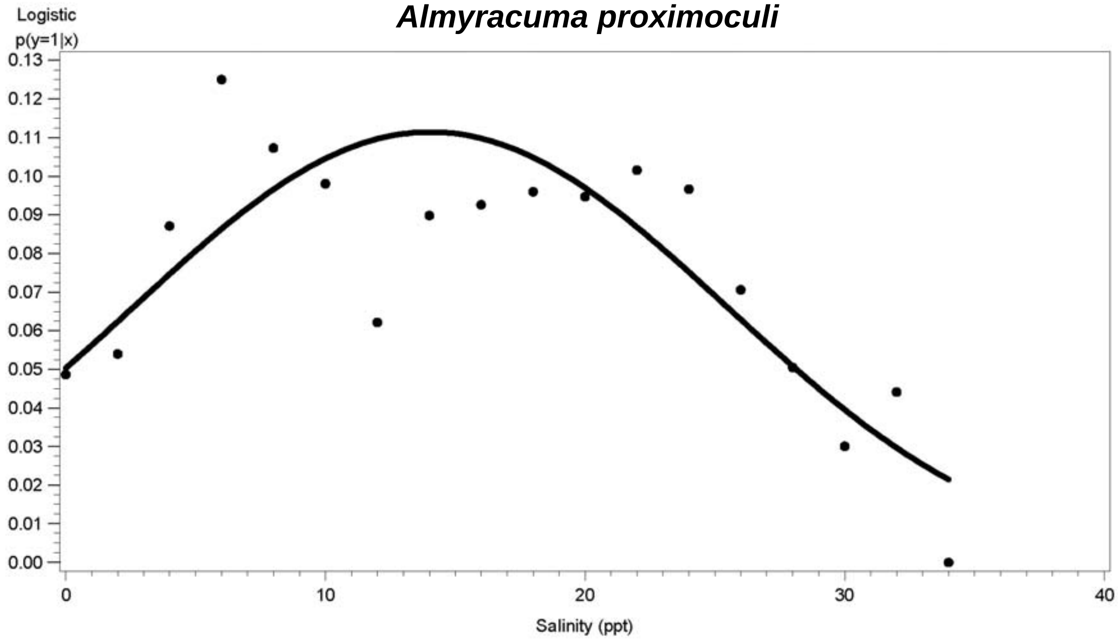
Groups P & E
Average dissimilarity = 80.18

	Group P	Group E				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
CERIMUSC	0.13	23.03	11.45	101.27	14.28	14.28
TRANCONR	1.03	23.36	11.16	13.82	13.92	28.20
HARGRAPA	4.59	16.46	7.09	3.96	8.84	37.04
ARICPHIL	15.71	11.11	7.06	1.95	8.80	45.84
GRANBONN	13.58	0.00	6.79	0.68	8.47	54.31

APPENDIX E.

LOGISTIC REGRESSION ANALYSIS OUTPUT

Almyracuma proximoculi

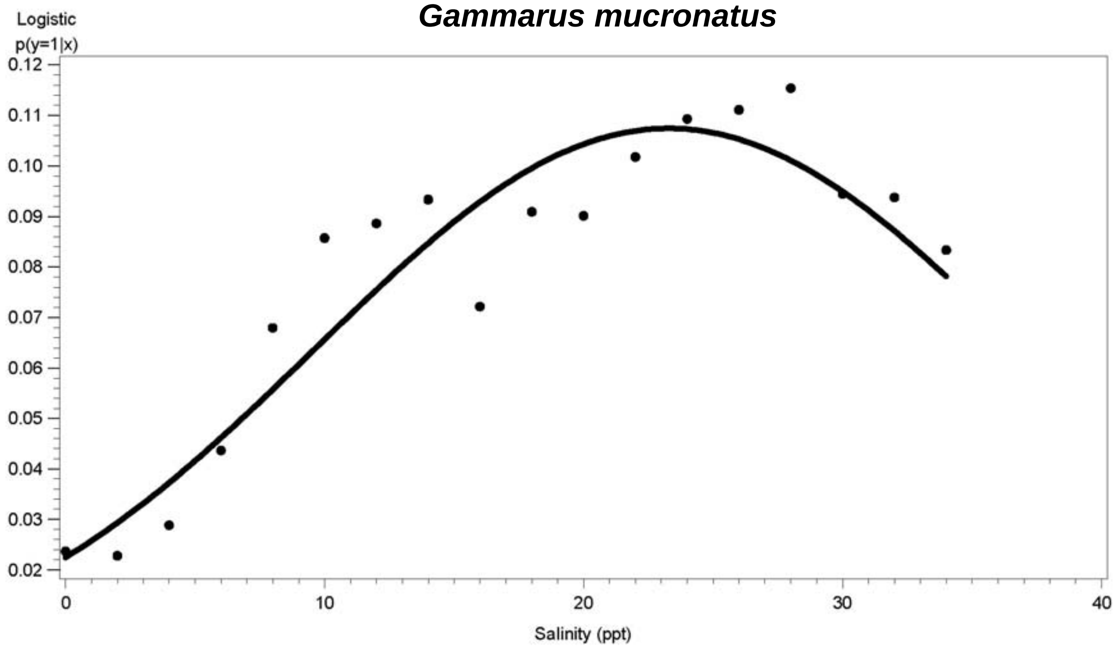


Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=<.001 x2=<.001

Association Statistics: 55% Concordant 36.6% Discordant 8.4% Ties Sample Size: Present in 137 of 1932 samples

Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)

Gammarus mucronatus

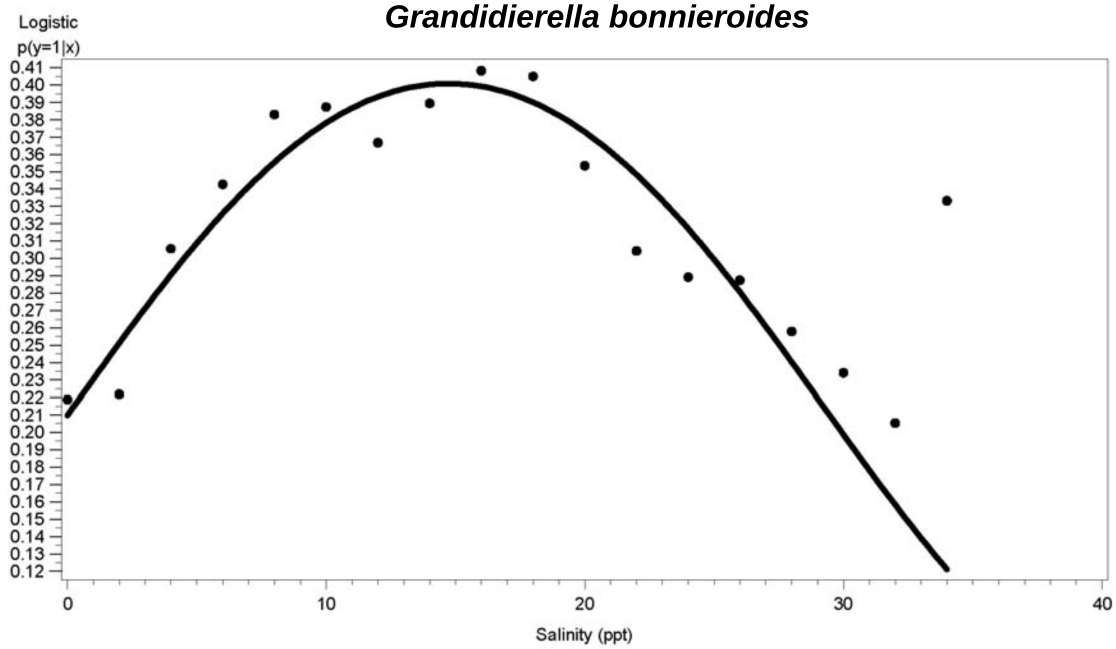


Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=<.001 x2=0.017

Association Statistics: 64.5% Concordant 27.2% Discordant 8.3% Ties Sample Size: Present in 103 of 1850 samples

Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)

Grandidierella bonnieroides

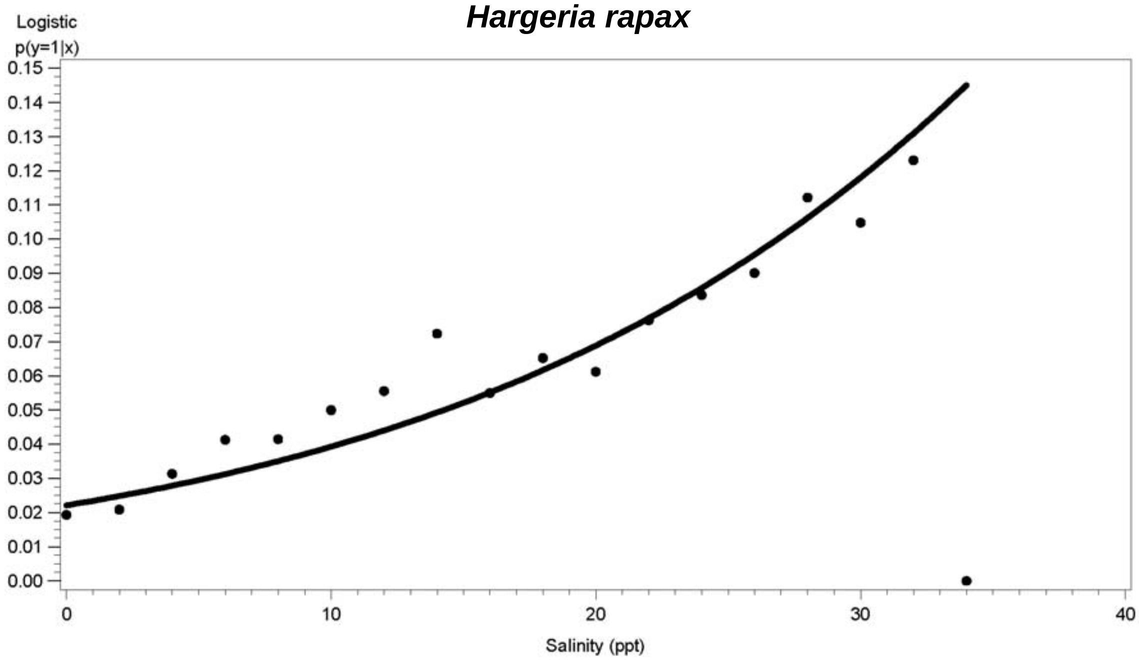


Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=<.001 x2=<.001

Association Statistics: 58.9% Concordant 38.5% Discordant 2.6% Ties Sample Size: Present in 594 of 2092 samples

Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)

Hargeria rapax

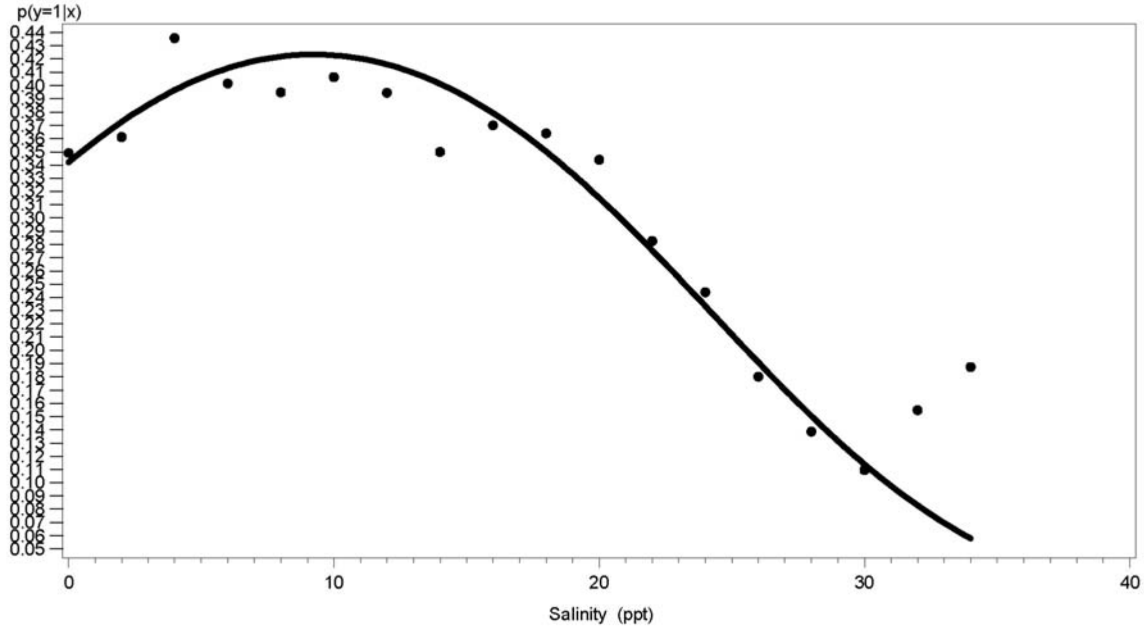


Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=<.001

Association Statistics: 63.3% Concordant 27.4% Discordant 9.3% Ties Sample Size: Present in 84 of 1870 samples

Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)

Laeonereis culveri

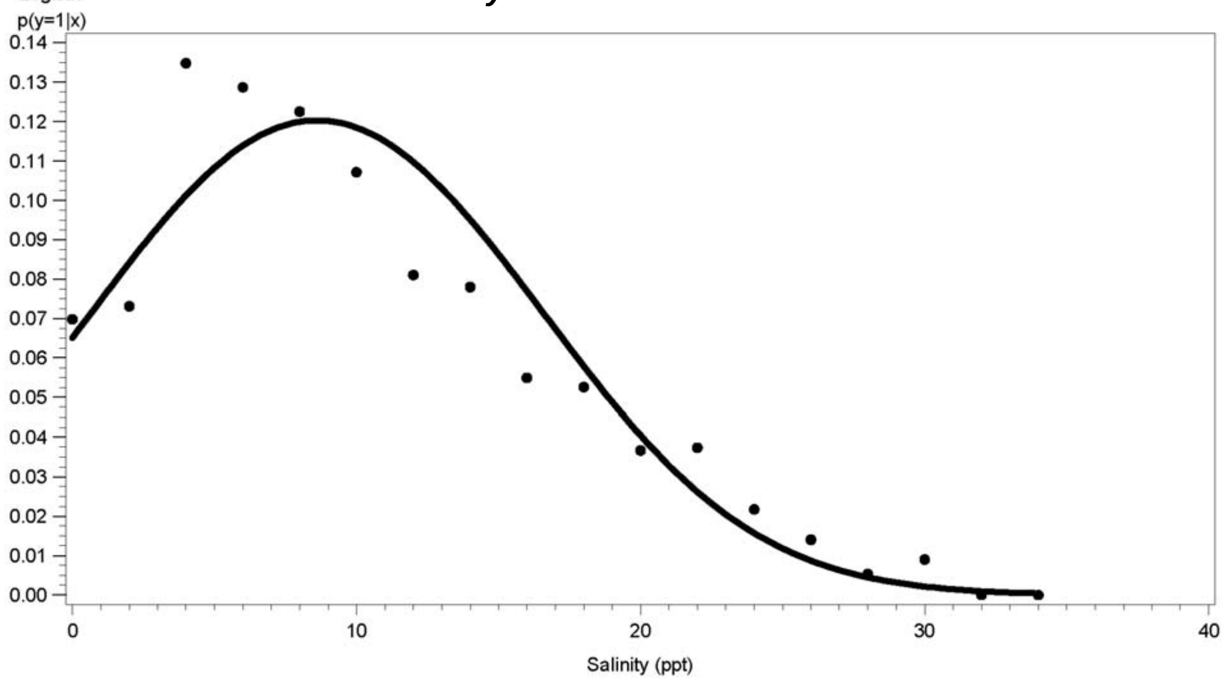


Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=<.001 x2=<.001

Association Statistics: 57.3% Concordant 38.1% Discordant 4.6% Ties Sample Size: Present in 656 of 1995 samples

Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)

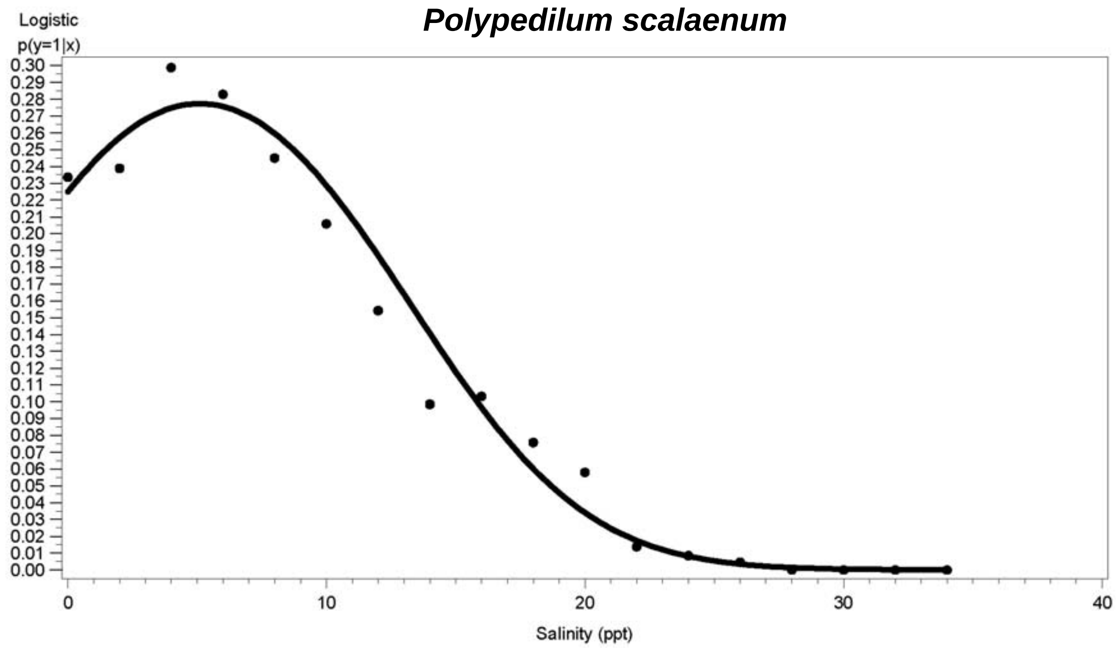
Polymesoda caroliniana



Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=<.001 x2=<.001

Association Statistics: 63.9% Concordant 29.2% Discordant 6.9% Ties Sample Size: Present in 114 of 1752 samples

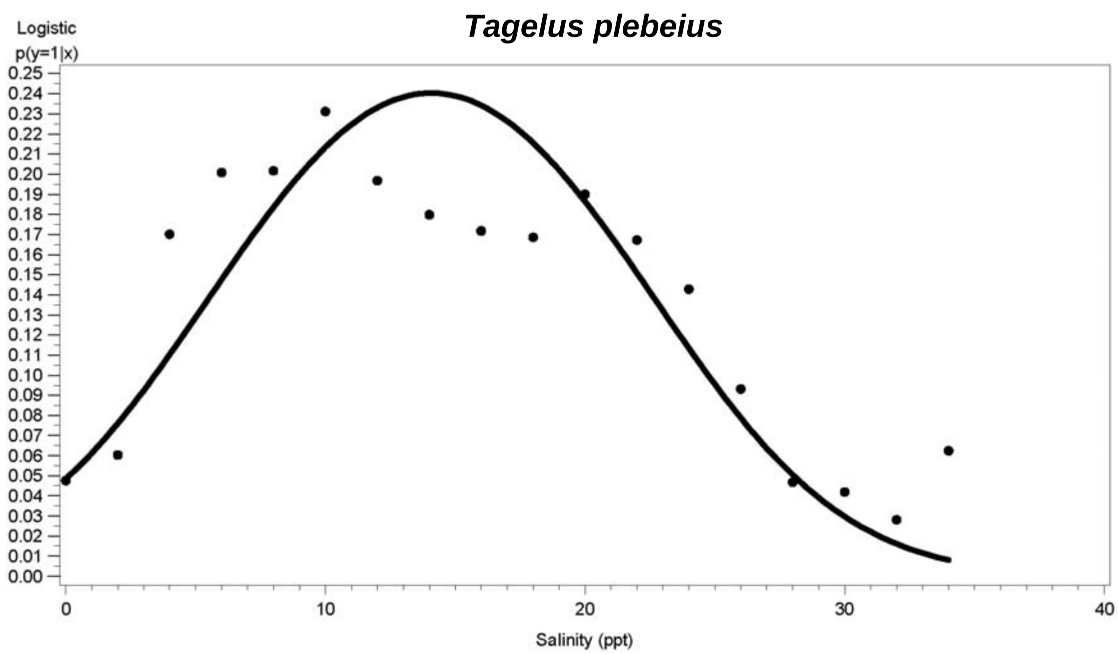
Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)



Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=0.002 x2=<.001

Association Statistics: 67.9% Concordant 26.2% Discordant 5.9% Ties Sample Size: Present in 300 of 1780 samples

Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)



Probability Statistics: Prob > Chi-Square: Intercept=<.001 x=<.001 x2=<.001

Association Statistics: 69.1% Concordant 29% Discordant 1.9% Ties Sample Size: Present in 218 of 1988 samples

Note: Observed p(y) is summarized (solid dots) over a moving window of width=4 for Salinity (ppt)