# A MULTIVARIATE STATISTICAL ANALYSIS OF RELATIONSHIPS BETWEEN FRESHWATER INFLOWS AND MOLLUSK DISTIBUTIONS IN TIDAL RIVERS IN SOUTHWEST FLORIDA

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## Abstract

The estuaries and rivers of the western coast of Florida, bordering the Gulf of Mexico, has been under intense study for some time with a goal to identify relationships between inflows, salinity, and natural resources. The mollusks have been show to be especially sensitive to salinity in many past studies, in many parts of the world. Several recent studied supported by the Southwest Florida Water Management District have focused on mollusk distributions for six tidal rivers: Peace River, Alafia River, Myakka River, Weeki Wachee River, Shell Creek, and the Shakett Creek Dona/Roberts Bay system. The purpose of the current project is to perform an inter-river, multivariate analysis that examines relationships between freshwater inflows, physicochemical variables that are affected by freshwater inflows (e.g. salinity, dissolved oxygen), and the distribution of mollusk populations in tidal rivers of southwest Florida.

The design of all studies consists of mollusks being sampled along transects within each river system. The transects run lengthwise originating at the mouth of each river, heading upstream. To enable all of the rivers to be compared simultaneously, the measure of distance along each transect was standardized by grouping all stations along each transect into two-kilometer (2-km) segments. Community structure of mollusk species was analyzed using non-metric multi-dimensional scaling (MDS). Relationships between mollusk communities and environmental factors were identified by using a mulitvariate procedure that matches biotic (i.e., mollusc community structure) with environmental (i.e., sediments, temperature, dissolved oxygen, salinity and, pH) variables. Analyses were constrained to variables that were common to all data sets.

In this limited analysis of southwest Florida mollusk communities, it is concluded that mollusk species are controlled more by water quality rather than the sediment they live in or on. The most important variable correlated with mollusk communities is salinity, which is a proxy for freshwater inflow. It is almost impossible to directly link community changes in response to inflow changes, because not replicates over time were carried out in the rivers sampled. Although total mollusk abundance was not a good indicator of inflow effects, certain indicator species have been identified however, that characterize salinity ranges in southwest Florida rivers. *Corbicula fluminea, Rangia cuneata*, and *Neritina usnea* were the only common species that occurred at salinities below 1 psu. Although, *C. fluminea* was the best indicator of freshwater habitat, because densities were highest below 2 psu, it is an introduced bivalve species. *Rangia cuneata*, a bivalve, has been noted as an indicator of a fresh- to brackish-water with an estimated tolerance of up to 20 psu in other studies as well. *Neritina usnea* is a gastropod and is also common in fresh- to brackish-water salinities. These salinity ranges may be useful in predicting mollusk community reactions to alterations in salinity that result from actual or simulated changes in freshwater inflow.

## Introduction

The Southwest Florida Water Management District (the District) has completed individual studies of mollusk distributions for six tidal rivers in southwest Florida located between the Springs Coast, and Charlotte Harbor, and includes Tampa Bay (Figure 1). A consistent methodology was used in these studies and the District has the complete data files for these projects: Peace River, Alafia River, Myakka River, Weeki Wachee River, Shell Creek, and the Shakett Creek Dona/Roberts Bay system (Table 1). The District also has extensive data for freshwater inflows and physicochemical variables (e.g. salinity, dissolved oxygen, pH) in these systems that cover the period of mollusk data collection. As yet, however, there has not been an effort that combines data from these tidal rivers to describe and quantify factors that affect mollusk distributions in tidal rivers in the region.

The purpose of the current project is to perform an inter-river, multivariate analysis that examines relationships between freshwater inflows and the distribution of mollusk populations in tidal rivers of southwest Florida. Relationships between mollusk distributions and physicochemical variables that are affected by freshwater inflows (e.g. salinity, dissolved oxygen) will also be evaluated. The overall purpose of the project will be to better define the physical and chemical requirements of mollusk species that inhabit tidal river systems in southwest Florida.

Understanding the relationship between salinity and other environmental parameters that relate to mollusk distributions is important to evaluate the freshwater flow requirements needed to protect the natural resources in these tidal river systems. The approach used in this project was to collect the data from the six tidal river systems in one place, organize the data into compatible file formats, and analyze the combined data sets.

Table 1. Reports on the mollusks of tidal rivers of southwest Florida.

| River System | Report |
|---|---|
| Peace River | Mote Marine Laboratory. 2002. Benthic Macroinvertebrate and Mollusk indicators. Mote Marine Laboratory Technical Report 744, Sarasota, Fl. |
| Alafia River | Mote Marine Laboratory. 2003. An Investigation of Relationships between Freshwater Inflows and Benthic Macroinvertebrates in the Alafia River Estuary. Mote Marine Laboratory Technical Report 912, Sarasota, Fl. |
| Shell Creek | Estevez, E.D. 2004. Molluscan Bio-indicators of the Tidal Shell Creek, Florida. Mote Marine Laboratory Technical Report 971, Sarasota, Fl. |
| Myakka River Dona/Roberts Bay | Estevez, E.D. 2004. Molluscan Bio-indicators of the Tidal Myakka River and Inshore Waters of Venice, Florida. Mote Marine Laboratory Technical Report 990, Sarasota, Fl. |
| Weeki Wachee River | Estevez, E.D. 2005. Letter Report for mollusk surveys of the Weeki Wachee and Mud River. Letter Report submitted by Mote Marine Laboratory to the Southwest Florida Water Management District. Brooksville, Fl. |

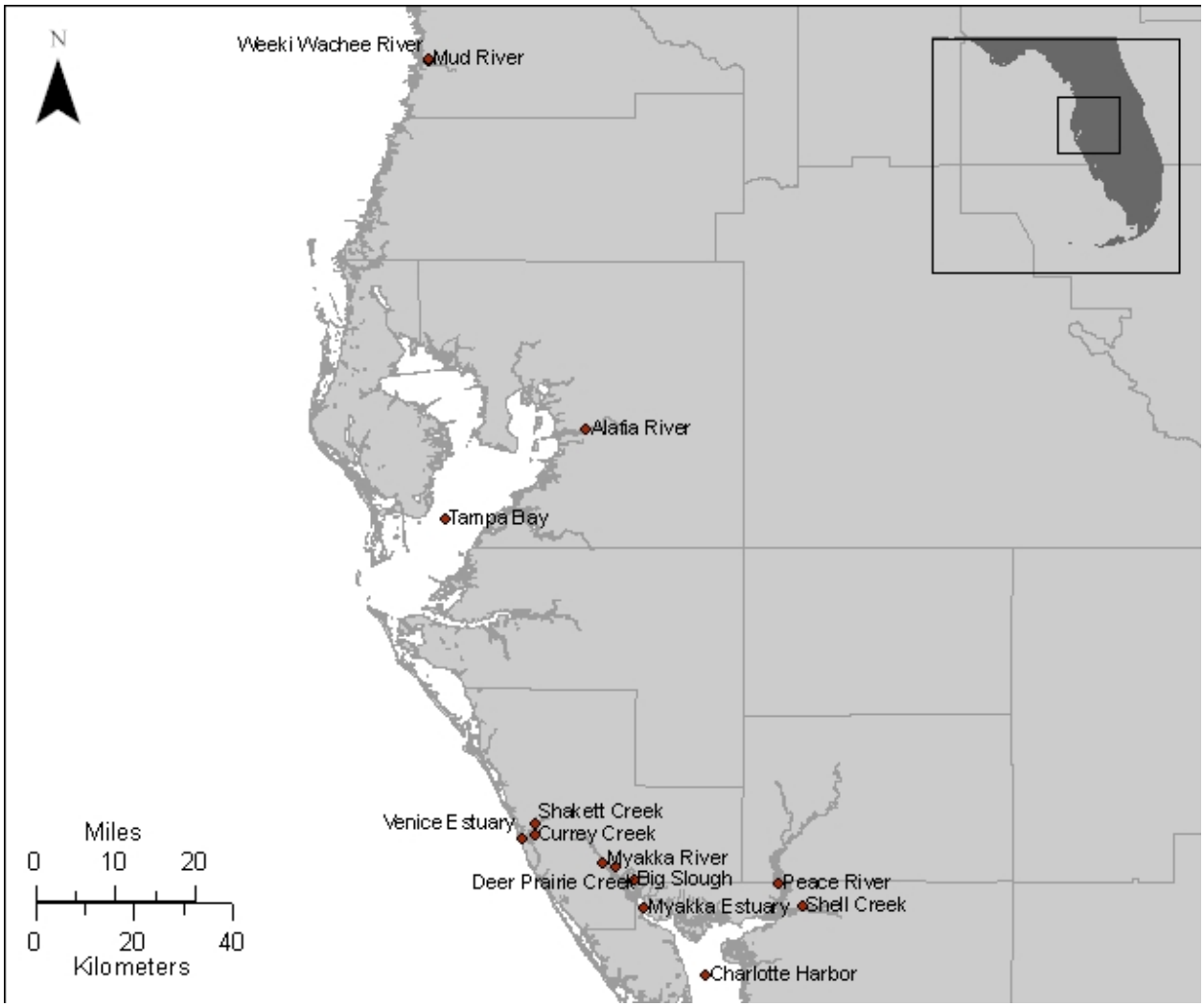


Figure 1. Map of the west coast of Florida showing the study sites.

## Methods

### Study Area

Data on mollusks that were extracted from the reports listed in Table 1, which were provided by the Mote Marine Laboratory (MML) (MML 2002, 2003, 2004; Estevez 2004a, 2004b). The data set was quite complex, and had to be concatenated, merged, and formatted prior to multivariate analysis.

The first step in data base creation was to determine the relationship between site designations in the data set and if there were any differences in the actual sampling designs in the different rivers and if there were aggregation relationships among the rivers (Table 2).

Table 2. Location of site names in the mollusk data set within river systems, and sampling year.

| Estuary | River System | Site (or creek) | Year | Photo Map Figure |
|---|---|---|---|---|
| Tampa Bay | Alafia | Alafia | 2001 | 3 |
| Charlotte Harbor | Myakka | Big Slough | 2004 | |
| Charlotte Harbor | Myakka | Blackburn | 2004 | 4 |
| Charlotte Harbor | Myakka | Deer Prairie | 2004 | 4 |
| Charlotte Harbor | Myakka | Myakka | 2004 | 4 |
| Charlotte Harbor | Peace | Peace | 1999 | 5 |
| Charlotte Harbor | Peace | Peace | 2000 | 5 |
| Charlotte Harbor | Peace | Shell | 2004 | 6 |
| Venice | Dona/Roberts Bay | Currey | 2004 | 7 |
| Venice | Dona/Roberts Bay | Shakett | 2004 | 7 |
| Weeki Wachee | Weeki Wachee | Mud River | 2005 | 8 |
| Weeki Wachee | Weeki Wachee | Weeki Wachee | 2005 | 8 |

The study sites are all located on the west coast of Florida (Figure 1). They group into four areas: Weeki Wachee River estuary, Alafia River in Tampa Bay, Curry River and Shakett River located in the Dona/Roberts Bay estuary, and Charlotte Harbor estuary. Most of the sites were in the Charlotte Harbor estuary (Figure 2).

The Alafia River is about 80 km long, and the watershed area is about 1062 $km^2$. All mollusk samples were collected from the main channel of the river (Figure 3).

The Myakka River (Figure 4) has three areas where mollusks have been sampled. Big Slough is near the 14 km marker, Deer Prairie Creek is near the 19 km marker, and Blackburn Canal is near the 32 km marker.

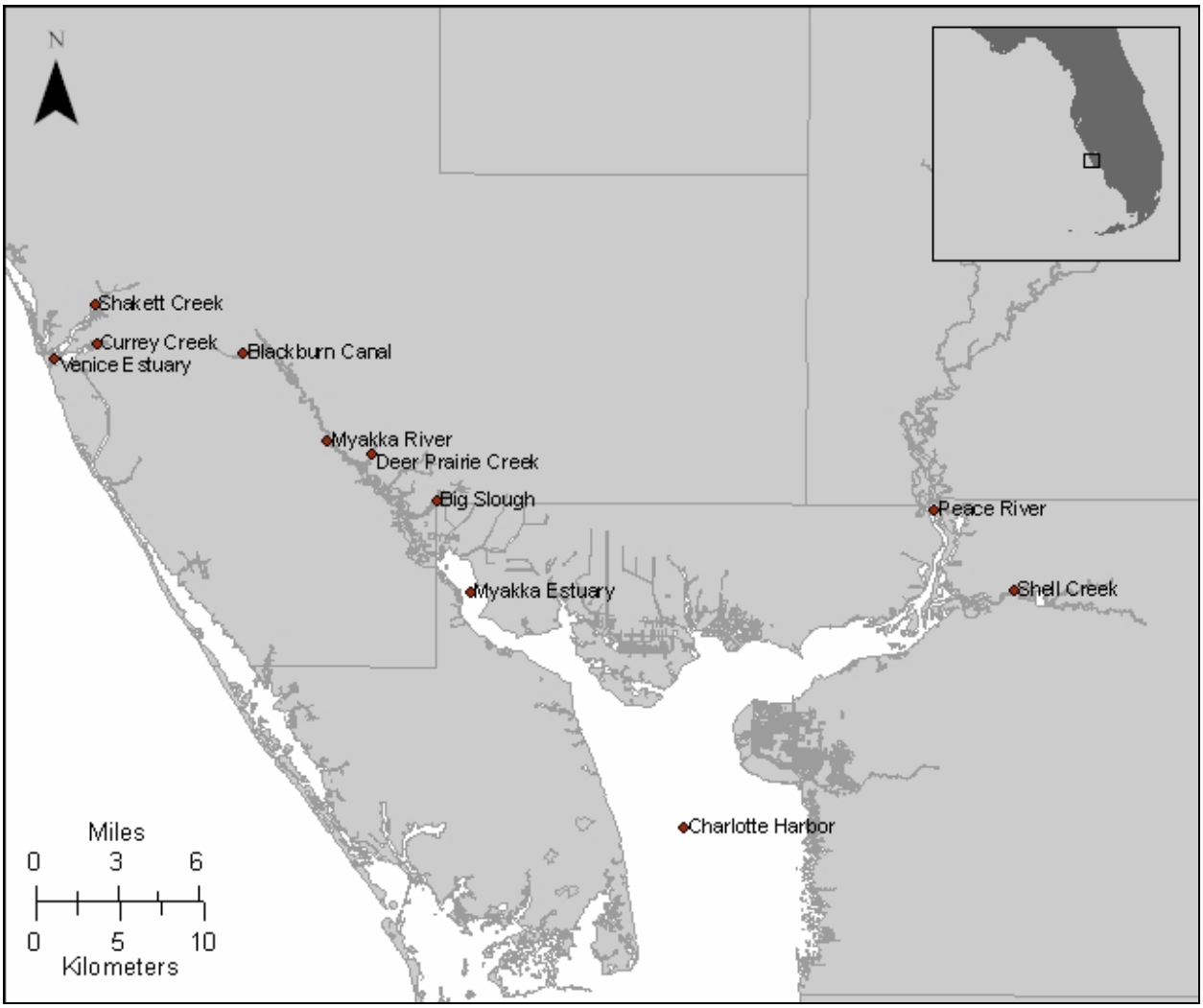


Figure 2. Map of Charlotte Harbor estuary showing locations of rivers and creeks connected to it.

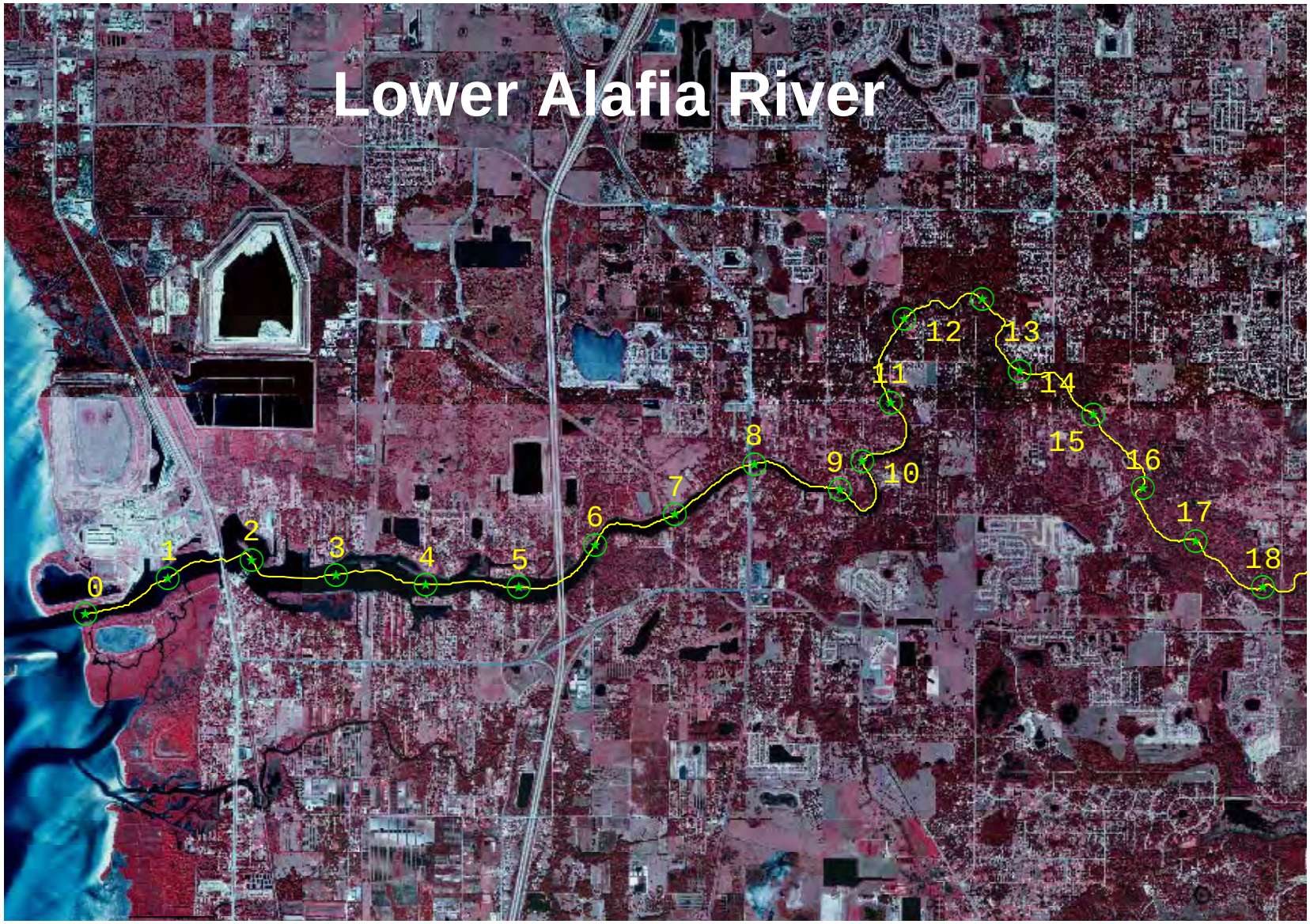


Figure 3. Alafia River photomap with centerline and distances.


Figure 4. Myakka River photomap with centerline and distance markers in kilometers.

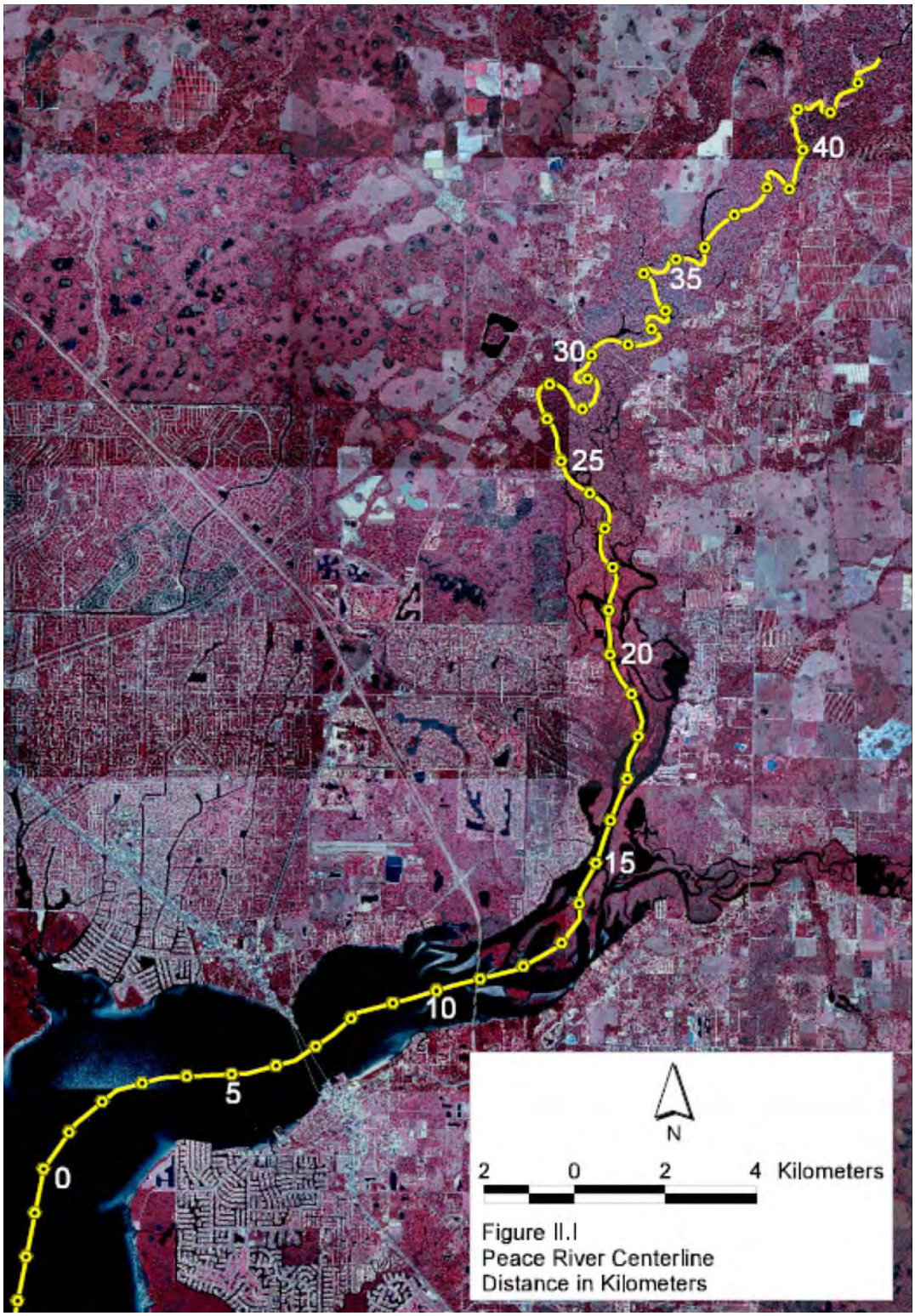


Figure 5. Peace River photomap with centerline distances in kilometers.

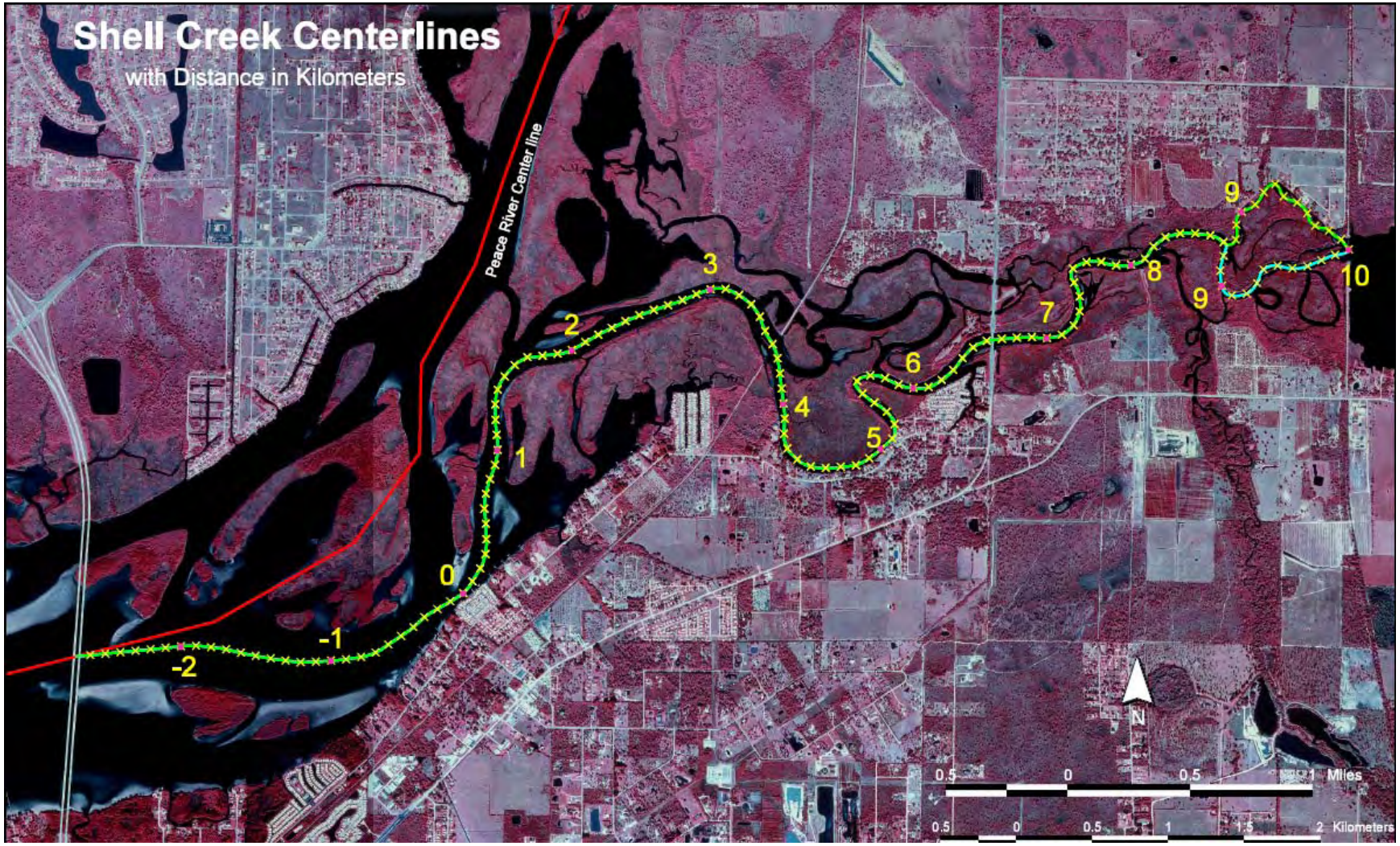


Figure 6. Shell Creek photomap showing centerline km markers.

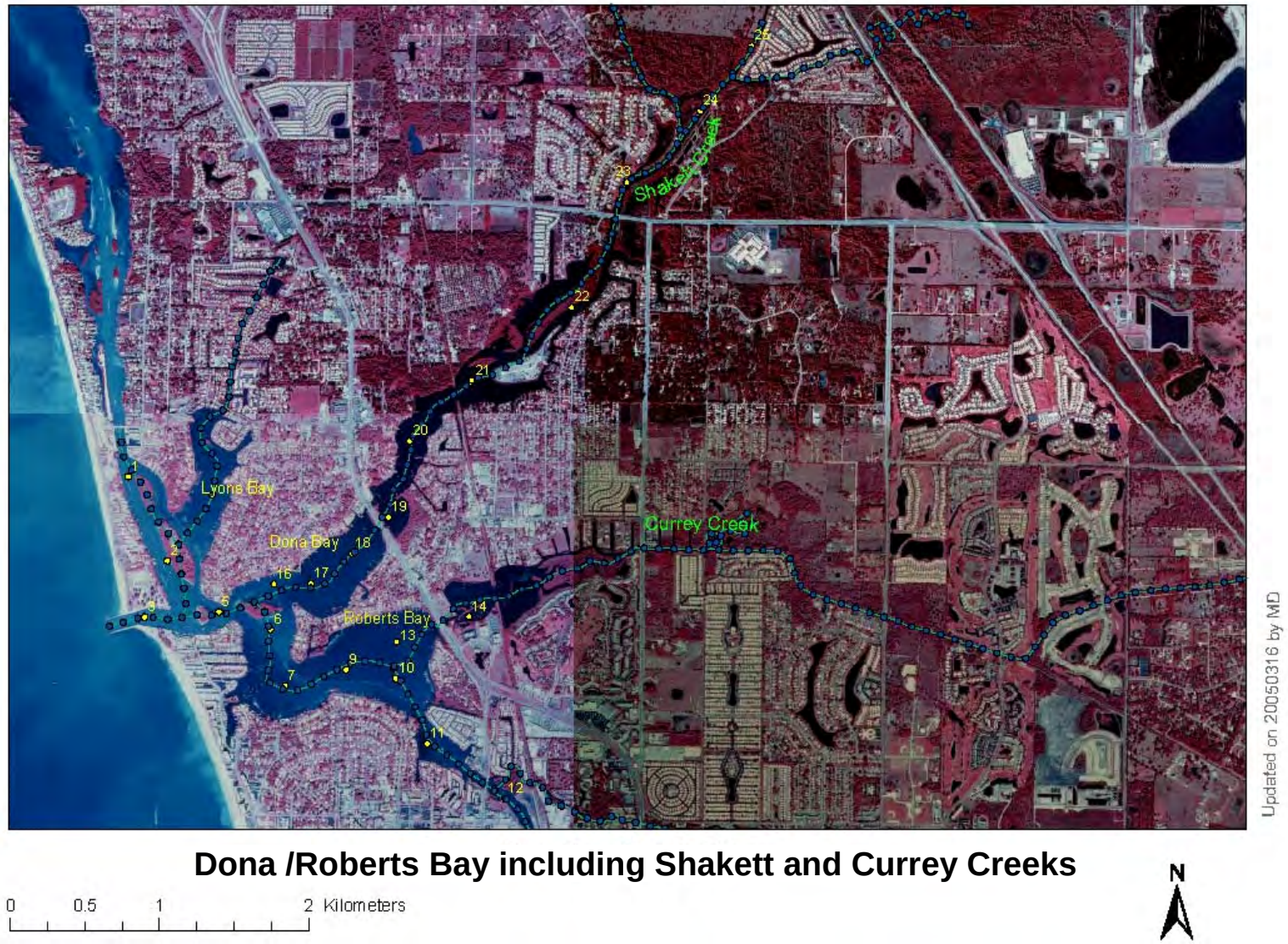


Figure 7. Dona/Roberts Bay photomap showing centerline km markers in Shakett and Currey Creeks .

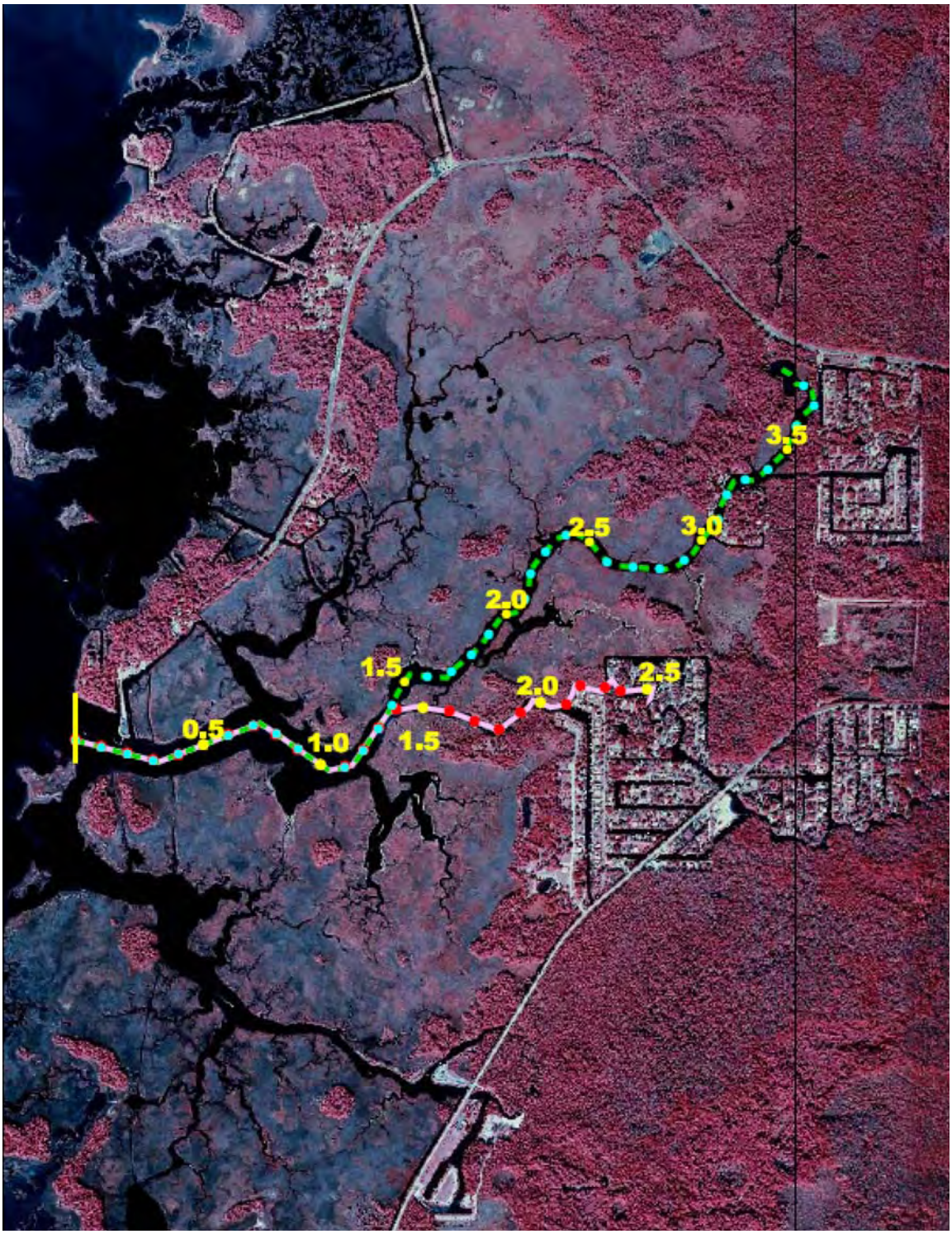


Figure 8. The Weeki Wachee River system showing centerline km markers, and the center line for the Mud River Tributary to the north.

The Peace River (Figure 5) includes Shell Creek near the 15 km marker. The Peace River ecosystem has been sampled three times. Twice in the Peace River itself, and once just in Shell Creek (Figure 6).

Shakett and Currey Creeks are located in the Dona/Roberts Bay complex in the region designated as the Venice Estuary (Figure 7). Shakett Creek ends in Dona Bay and Currey Creek ends in Roberts Bay.

The Weeki Wachee River is a small, spring-fed system in which the penetration of brackish water is generally less than 2.5 km upstream from the river mouth (Figure 8). Mud river, which is also spring-fed, joins the Weeki Wachee about 1.4 km upstream of the river mouth. While the upsream reaches of the Weeki Wachee are fresh, the Mud River receives flow from brackish springs and salinity in the Mud River increases upstream toward the river head.

Mollusca Data

The sampling design employed by Mote Marine Laboratory (MML) consists of mollusks being sampled along transects within each river system (MML 2002, 2003, 2004; Estevez 2004a, 2004b). The transects run lengthwise originating at the mouth of each river, heading upstream, hence distance and station names increase with marine influence having the lowest numbers and freshwater influence having the highest numbers (Figures 3 - 8). The content of the original data sets varied with each river system, however they all contained the distance along the river transect where samples were taken and the mollusc species found. These distances represented the stations within the river site, and a total of 180 such stations were sampled across all sites. At each sampling location, mollusks were sampled systematically across the river channel perpendicular to the river centerline so that samples were collected from mid-channel, shallow subtidal, and intertidal areas.

For each sampling event, the variables reported included the size of the sampling device, the number of juvenile mollusks, the number of live mollusks, the number of dead mollusks, size of shells and whether the samples were taken from the subtidal or intertidal area of the river system. For all statistical analyses in the current study, mollusk counts from the subtidal and intertidal zones of each station were combined. Several sampling devices were used, but all the data reported on here is from one sized 0.464 $m^2$. The raw counts were converted to abundance of individuals per square meter (i.e., $n/m^2$) for all analyses, e.g., species richness, frequency or occurrence, and multivariate analyses.

For the current study, analysis was focused on the data relating to live mollusks. Without shell dating and knowledge of shell transport information after death, it is very difficult to correlate the presence of empty shells of dead mollusks with freshwater inflow and other physiographic information. However, the dead shells do provide information on historical communities, so are listed in this report.

Samples from multiple years of sampling were found only from the Peace River (Table 2). For the purpose of the current study, the sampling stations at Peace River were averaged over the two years they were sampled (1999 and 2000).

To enable all of the rivers to be compared simultaneously, the measure of distance along each transect (Figs. 3 - 8) had to be reduced and standardized. To do this, the distance of each sampling station from each transect was aggregated into two-kilometer (2-km) segment bins. This was performed by rounding the actual distance from the mouth of the river (in kilometers) to increments of two. Each segment was numbered as the midpoint of the actual distance, thus a segment labeled 2 km would encompass stations found at 1.0 km to 2.9 km of a transect. Overall, 67 new stations, or 2-km segments, were created for analysis (Table 3). While this approach was necessary to ensure comparability over the spatial extent of river systems, it created an unbalanced sampling design, because more than one sampling station occurred within many new 2-km segments. Thus, species abundance were averaged for each new 2-km segment prior to analysis to ensure a balanced sampling design.

The scientific names of all the species were verified and made to be consistent across all data sets. In addition, the full taxonomic description was verified. The convention for species names and taxonomy used in the current study is based on the Species 2000 website, http://www.sp2000.org/. The Species 2000 lists are prepared with cooperation with the Integrated Taxonomic Information System (ITIS). The specific source was the Annual Check List 2006.

Hill's number one (N1) diversity index was used to report species diversity (Hill, 1973). Hill's N1 is the exponential form ($e^{H'}$) of the Shannon-Weaver diversity index H′. N1 was used because it has units of numbers of species, and is easier to interpret than most other diversity indices (Ludwig and Reynolds, 1988).

A second measure of diversity, taxonomic distinctness ($\Delta^*$) was calculated. Taxonomic distinctness addresses the problems associated with measures of species richness and other diversity indices because it is based not just on species abundances, but also the taxonomic distance through classification of every pair of individuals (Warwick and Clark 1995). For example, a sample with two clams is very different from a sample with one clam and one snail, even though both have a richness measure of 2. The $\Delta^*$ statistic was calcuated using Primer software (Clarke and Warwick, 2001).

Table 3. Aggregation of Mote Marine Laboratory (MML) sampling data for the current analyses. For each river-site, the MML stations were placed in 2-km bins where all stations within the 2-km bin were treated as replicates and averaged.

| River | Site | 2-km Bin Name | Number of MML Stations |
|---|---|---|---|
| Alafia | Alafia | 0 | 2 |
| Alafia | Alafia | 2 | 3 |
| Alafia | Alafia | 4 | 4 |
| Alafia | Alafia | 6 | 4 |
| Alafia | Alafia | 8 | 4 |
| Alafia | Alafia | 10 | 4 |
| Alafia | Alafia | 12 | 3 |
| Alafia | Alafia | 16 | 1 |
| Alafia | Alafia | 18 | 1 |
| Dona/Roberts | Currey | 2 | 3 |
| Dona/Roberts | Currey | 4 | 2 |
| Dona/Roberts | Shakett | 0 | 1 |
| Dona/Roberts | Shakett | 2 | 4 |
| Dona/Roberts | Shakett | 4 | 4 |
| Dona/Roberts | Shakett | 6 | 3 |
| Myakka | BigSlough | 2 | 2 |
| Myakka | Blackburn | 0 | 1 |
| Myakka | DeerPrairie | 2 | 2 |
| Myakka | DeerPrairie | 4 | 1 |
| Myakka | Myakka | -0 | 2 |
| Myakka | Myakka | 2 | 2 |
| Myakka | Myakka | 4 | 2 |
| Myakka | Myakka | 6 | 2 |
| Myakka | Myakka | 8 | 2 |
| Myakka | Myakka | 10 | 2 |
| Myakka | Myakka | 12 | 2 |
| Myakka | Myakka | 14 | 3 |
| Myakka | Myakka | 16 | 1 |
| Myakka | Myakka | 18 | 2 |
| Myakka | Myakka | 20 | 3 |
| Myakka | Myakka | 22 | 2 |
| Myakka | Myakka | 24 | 1 |
| Myakka | Myakka | 26 | 3 |
| Myakka | Myakka | 28 | 2 |
| Myakka | Myakka | 30 | 2 |
| Myakka | Myakka | 32 | 2 |
| Myakka | Myakka | 36 | 2 |
| Myakka | Myakka | 38 | 3 |
| Myakka | Myakka | 40 | 1 |
| Peace | Peace | 0 | 1 |
| Peace | Peace | 2 | 1 |

| River | Site | 2-km Bin Name | Number of MML Stations |
|---|---|---|---|
| Peace | Peace | 4 | 1 |
| Peace | Peace | 6 | 1 |
| Peace | Peace | 8 | 4 |
| Peace | Peace | 10 | 4 |
| Peace | Peace | 12 | 4 |
| Peace | Peace | 14 | 4 |
| Peace | Peace | 16 | 5 |
| Peace | Peace | 18 | 5 |
| Peace | Peace | 20 | 4 |
| Peace | Peace | 22 | 5 |
| Peace | Peace | 24 | 4 |
| Peace | Peace | 26 | 5 |
| Peace | Peace | 28 | 4 |
| Peace | Peace | 30 | 4 |
| Peace | Peace | 32 | 4 |
| Peace | Peace | 34 | 3 |
| Peace | Peace | 36 | 1 |
| Shell | Shell | 0 | 2 |
| Shell | Shell | 2 | 4 |
| Shell | Shell | 4 | 4 |
| Shell | Shell | 6 | 3 |
| Shell | Shell | 8 | 4 |
| WeekiWachee | MudRiver | 2 | 2 |
| WeekiWachee | MudRiver | 4 | 1 |
| WeekiWachee | WeekiWachee | 0 | 2 |
| WeekiWachee | WeekiWachee | 2 | 4 |
| Total Number of segment bins and stations | | 67 | 180 |

Multivariate Analyses

Community structure of mollusk species was analyzed by non-metric multi-dimensional scaling (MDS). MDS is a statistical tool that can be used to compare many variables (multivariate data) from different stations at once rather than a single variable (univariate data). In the current study, MDS was used to compare abundances of individuals of each species for each river-site-segment combination. Thus, the data was organized into a matrix where each row was a station, i.e., a river-site-segment combination (Table 3) and each column was a species abundance variable. The distance between river-site-segment combinations in the MDS plot can be related to community similarities or differences between rivers, sites, and segments. All multivariate statistical analysis was performed using Primer software (Clarke and Warwick, 2001).

Analysis is a multi-step procedure. First, data is transformed using the natural logarithm plus 1 (i.e., ln+1). Then, the data matrix of species and river-site-segment combinations, is converted to a Bray-Curtis similarity matrix for each station. Differences and similarities among communities were

highlighted based on cluster analysis calculated from the similarity matrix. The MDS scores for each river-segment combination is calculated from the similarity matrix, and then plotted in 2-dimensional space. Overlying the MDS plot with a cluster of samples with the same similarity score allows visualization of station similarities. Often a subset of variables, i.e., a subset of species in the present case, can explain much of the spatial pattern in an MDS plot. The BVSTEP procedure in the Primer software package finds the smallest subset of species that explains the same overall pattern as the whole data set.

Physicochemical Variables

Physicochemical data for each tidal river system were provided by the Southwest Florida Water Management District. Profiles of temperature, dissolved oxygen, salinity and pH were taken along all transects. Profiles were measured at different dates at various distances along the transects of each river. Multiple samples were taken along the transects within a 2 - 13 year period. The length of period and actual years sampled varied with each river (Table 4). As with the mollusc data, the distance along each transect was converted into two kilometer segments. The four water quality parameters measured (temperature, dissolved oxygen, salinity and pH) were all averaged by transect segment and river. Water chemistry samples were taken in all of the rivers, however parameters measured in the rivers were inconsistent between rivers. This inconsistency meant that no single variable was measured in all of the rivers. For this reason, use of the water chemistry data in this current study was limited.

Principle Components Analysis (PCA), a parametric multivariate method, was used to determine differences between river-segment combinations. As with MDS, the distance between river-segment combinations in the PCA plot can be related to actual similarities or differences in water quality between river-segment combinations.

Table 4. Period when water quality profiles were taken in each river system.

| River System | Site (or creek) | Start of Period | End of Period |
|---|---|---|---|
| Alafia | Alafia | Jan 1999 | Dec 2003 |
| Myakka | Myakka | Feb 1998 | Mar 2005 |
| Peace | Peace | Aug 1996 | Dec 2004 |
| Shell | Shell | Feb 1991 | Dec 2004 |
| Venice | Curry | Aug 2003 | May 2005 |
| Venice | Shakett | Aug 2003 | May 2005 |
| Weeki Wachee | Mud River | July 2003 | May 2005 |
| Weeki Wachee | Weeki Wachee | July 2003 | May 2005 |

Sediment

Samples along each transect were also analyzed by MML for sediment characteristics. The parameters available were sediment grain size distributions (median, mean, % sand, %silt, % clay, skewness, kurtosis), sediment moisture, and the proportion of organic material present in the sediment.

Relating Mollusks and Environmental Factors

Relationships between mollusk communities and environmental factors were investigated using the Biota-Environment (BIO-ENV) procedure. The BIO-ENV procedure is a multivariate method that matches biotic (i.e., mollusc community structure) with environmental variables (Clarke and Warwick 2001). This is carried out by calculating weighted Spearman rank correlations ($\rho_w$) between sample ordinations from all of the environmental variables and an ordination of biotic variables (Clarke and Ainsworth, 1993). Correlations are then compared to determine the best match. The BIO-ENV procedure uses different numbers of abiotic sample variables in calculating correlations to investigate the different levels of environmental complexity. For this study, the mollusk species abundance MDS ordination was compared with all physicochemical and sediment variables. Any river-segment combination that did not have all sediment, physiochemical (temperature, dissolved oxygen, salinity and pH ) variables as well as any mollusc data were omitted from this analysis because multivariate analysis can only be performed when all variables are present. The significance of relationships were tested using RELATE, a non-parametric form of the mantel test. The BIO-ENV and RELATE procedures were calculated with Primer software (Clarke and Warwick 2001).

Salinity was used as a proxy for distance from a freshwater source because salinity increases as distance from the freshwater source increases. Salinity was directly compared with individual species abundances, total mollusk abundances and mollusk diversity.

The relationship between macrofauna characteristics and salinity were examined with a non-linear model, which was used successfully in Texas estuaries (Montagna et al., 2002). The assumption behind the model is that there is an optimal range for salinity and values decline prior to and after meeting this maximum value. That is, the relationship resembles a bell-shaped curve. The shape of this curve can be predicted with a three-parameter, log normal model:

$$Y = a \times \exp( -0.5 \times (\ln(X / c) / b)^2)$$

The model was used to characterize the nonlinear relationship between a biological characteristic (Y) and salinity (X) and inflow (X). The three parameters characterize different attributes of the curve, where *a* is the maximum value, *b* is the skewness or rate of change of the response as a function of salinity, and *c* the location of the peak response value on the salinity axis. The model was fit to data using the Regression Wizard in SigmaPlot, which uses the Marquardt-Levenberg algorithm to find coefficients (parameters) of the independent variables that give the best fit between the equation and the data (Systat, 2006).

## Results

### Physical Environments

With the exception of Mud River, salinity decreases with distance from the river or creek mouth in all the river systems (Figure 9). The transect in each river was a different length and covered different salinity ranges, thus a km segment number in one river did not correspond to a similar salinity range in another system (Figure 10). The transects of the Alafia, Myakka and Peace Rivers were at least 20 km long and had mean salinity ranges between 20 and 25 psu. Although the Shakett and Weeki Wachee River transects covered less than 8 km, they also covered a mean salinity range of at least 15 psu. The transects in Currey and Shakett Creeks and Mud River did not extend to freshwater, as did the transects on the other river systems. A salinity barrier on Shakett Creek truncates this river and structurally isolates a freshwater zone under most flow conditions. As described earlier, the Mud River is an unusual system that is fed by brackish springs and salinity increases toward the river head. Only two transect segments were sampled in each of Currey Creek and the Mud River.

Principal Components (PC) analysis was used to compare the physical environments among the river systems. Only six of the eight river/creek systems could be analyzed because of a lack of sufficient data for two of the river systems (Mud River and Currey Creek). The PC analysis reduces the four environmental variables of salinity, temperature, pH, and dissolved oxygen (DO) to just two axes or PCs. The first (PC1) and second (PC2) principal components of the physicochemical data explain 47.9 % and 25.3 % of the variation within the data set respectively (total 73.1 %; Figure 11a). PC1 is dominated by by salinity differences and PC2 is dominated by temperature and dissolved oxygen. This means that PC1 represents changes over distance along the transects or between rivers, and PC2 represents temporal change, e.g., seasonal changes, in water properties with higher temperatures and lower DO in summer compared to winter.

The PC analysis demonstrates the differences between the different water bodies (Figure 11b). The Weeki Wachee, Shakett, Myakka are all distinct water bodies. The differences are primarily a result of separation along the PC2 axis. Whereas the Shakett and Myakka had similar temperature and DO conditions, they were distinct from the Weeki Wachee in this regard. However, separation along PC1 indicates the Shakett and Myakka had distinct salinity regimes, but different from the Weeki Wachee system. The Peace, Alfia, and Shell rivers were very similar to one another with respect to their physical characteristics.

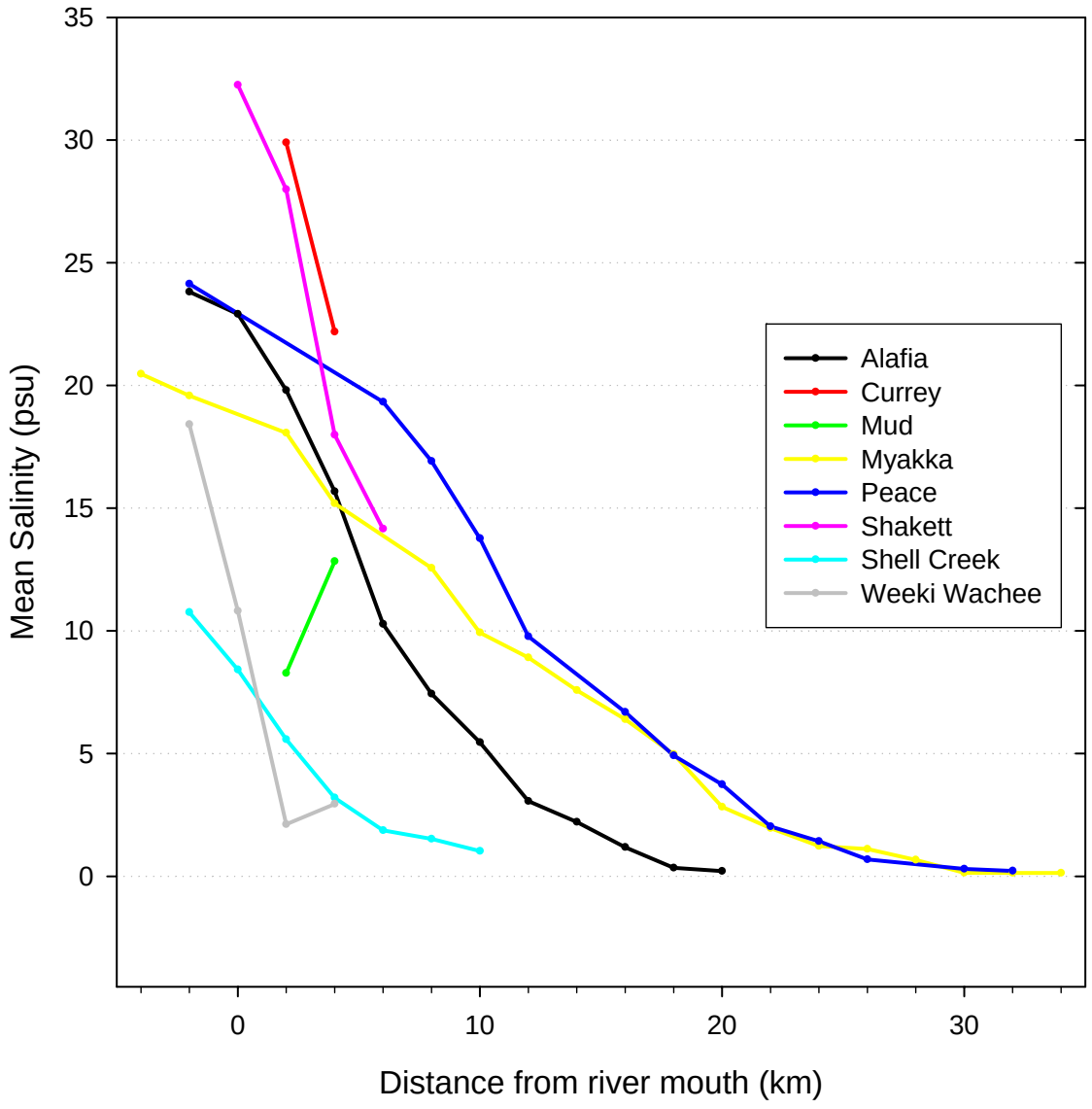


Figure 9. Mean salinity along transects at each creek /site system

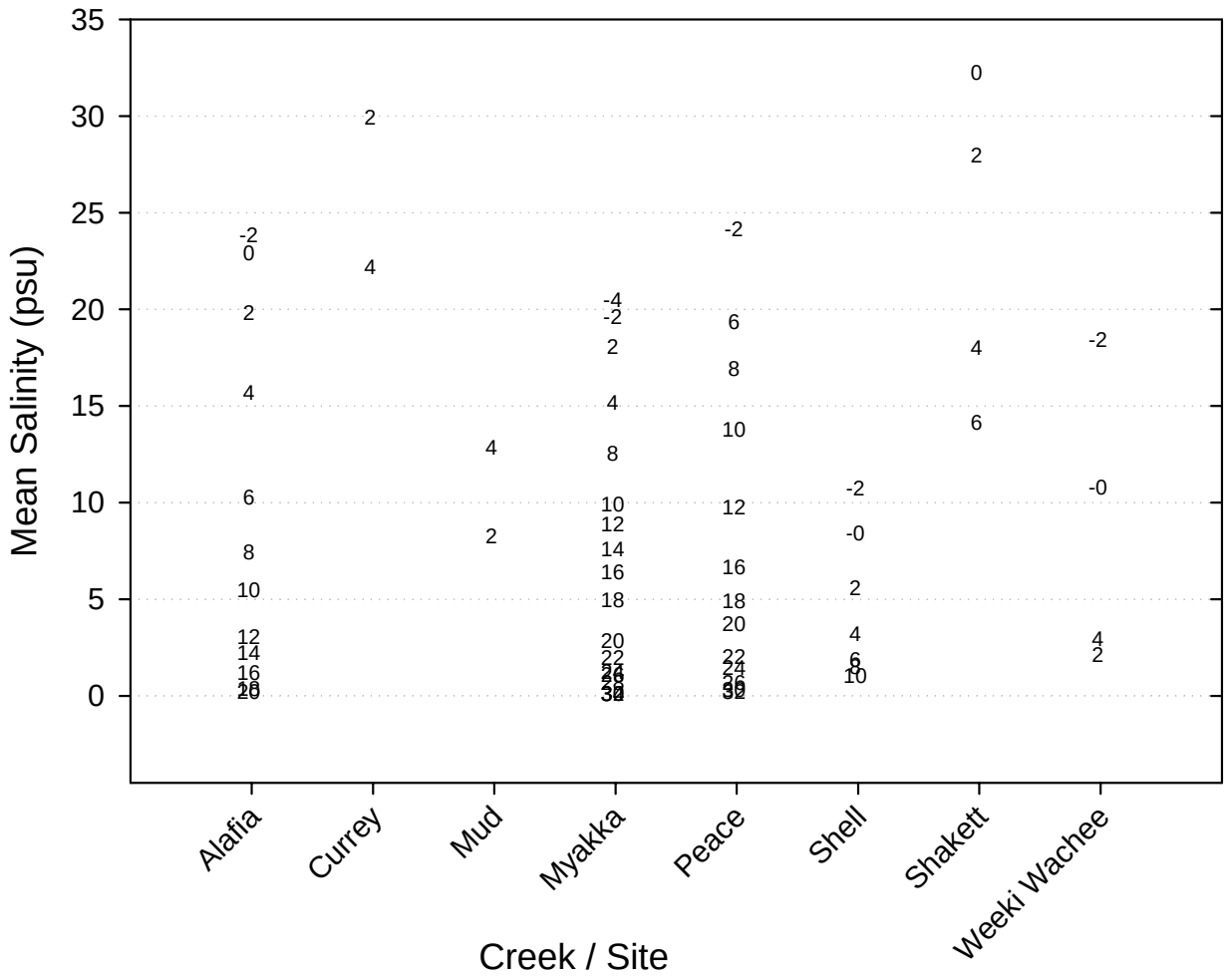


Figure 10. Salinity for each transect segment for each creek / site. The number value represents the distance in 2-km segments upstream from the mouth of the river.

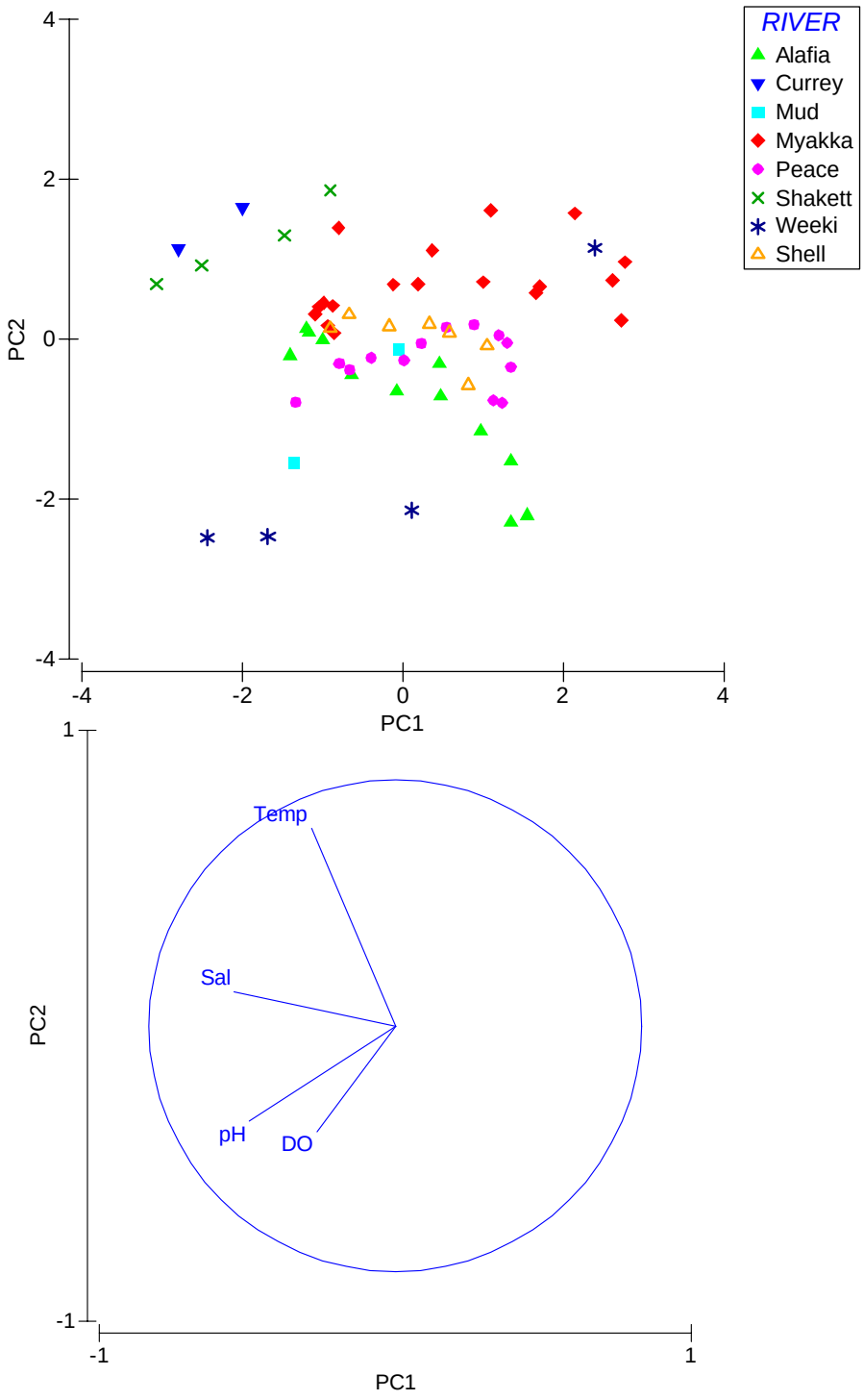


Figure 11. Principal Components Analysis of water quality in southwest Florida rivers. A. Principal Component variable loadings (bottom). B. Transect segment-river station scores (top).

Taphonomy

Examining the fossil shells or death-assemblages, i.e., taphonomy, is a good technique to understand the derivation of extant benthic communities. A total of 58 dead species were found, two of which were Brachiopoda and not Mollusca (Table 5). The total taxonomic list is presented for completeness only. However, 23 more species were found among dead shells than live shells. The total abundance was similar with an average of 95 $m^{-2}$ dead shells compared to an average of 82 $m^{-2}$ live shells. The proportion of dead shells to live shells was similar overall because a paired-difference test was not significantly different ($p = 0.7822$). The dead shells are interesting because more species exist in this region than were found live. This does not mean that species have gone extinct or are now longer found in the environment. Shells are transported after death, and the age of the shells are unknown, therefore the remainder of this current report focuses on the living fauna.

Mollusca Community Structure

A total of 35 species were found in all the live specimens from all of the rivers sampled (Table 5). Two species, *Glotttidia pyramidata* and an unidentified species, were actually brachipods, and not mollusks. So, there were actually only 33 species of Mollusca. Of these, 25 species were bivalves and eight species were gastropods. Two families of bivalves, Tellinidae and Mytilidae, were represented by four species each, and there were three species of Veneridae. Otherwise, all families were represented by only one or two species.

The dominant species was the Asian Clam, *Corbicula fluminea*, which is an exotic species that was introduced to Florida waters (Table 6). The large number of *Corbicula* was largely due to very high densities of this species in the tidal freshwater reaches of the Peace River. A total of 1,036 individuals were found among all samples, and the average abundance was 33 individuals $m^{-2}$ were found among the 27 different river-segment samples. This represented 40% of total average abundance. The next four most dominant species were *Polymesoda caroliniana* (11 %), *Rangia cuneata* (8 %), *Tagelus plebius* (6 %), and *Amygdalum papyrium* (5%). These top five most abundant mollusks were bivalves and comprised 70 % of all species found. The dominant gastropod, *Neritina usnea,* was the sixth ranked species in dominance (4% of total average abundance). The second most dominant species, *P. Caroliniana*, was found most often, 35 times in the river-segment samples

Dominance patterns were different in different rivers (Table 7). For example, *C. flumninea* was dominant only in the Peace and Myakka rivers. In contrast, *P. carolinian* was dominant in Shell Creek and Big Slough, the second dominant in Deer Praire, Myakkaand Weeki Wachee. *Rangia cuneata* was dominant in Deer Praire and was the only organism found in Blackburn. *Tagelus plebeius* was co-dominant in Weeki Wachee, and dominant in Mud and Currey creeks. *Geukensia granosissima* was dominant in the Alafia River, and *Crassostrea virginica* was co-dominant in Weeki Wachee and dominant in Shakett Creek. However, the distribution of *C. virginica* in the Weeki Wachee River was largely limited to individuals located near the river mouth.

Similarity in mollusk communities among the river-segment sites was generally low (Figure 12). The Bray-Curtis similarity matrix is most easily visualized in the multidimensional scaling (MDS) plot (Figure 13). All of the river-segment combinations are found in associations of groups of no

more than 15 % similarity. At the 15% similarity level there are three groups, two smaller groups with low station numbers (i.e., more mare conditions), and there is one large group. At the 25% similarity level, the large group splits into 4 smaller groups. Although the pattern of river-segment groupings is based on 35 species, it is being driven by just seven species: *Corbicula fluminea, Crassostrea virginica, Littoraria irrorata, Neritina usnea, Polymesoda caroliniana, Rangia cuneata*, and *Tagelus plebeius* (BVSTEP, rho > 0.95, r = 0.96). These species drive the trend that downstream segments close to marine sources (with low 2-k segment numbers) tend to group to the left and higher segment numbers groups the right.

The four groups at the 25% level within the large central group at the 15% similarity level(Figure 13), can be explained based on the distribution of three species (Figure 14). From left to right, the station groups are dominated by *Crassostrea virginica, Littoraria irrorata*, and *Corbicula fluminea*. The is a small cluster of seven river-segment combinations from downstream reaches of the Peace, Shakett and Weeki Wachee systems, which were dominated by high densities of *Crassostrea virginica*. The largest cluster of river-segment combinations and nearly wholly bounded by the 25 % similarity level in the center, is a group of mid to lower segments, and included segments from all rivers and this cluster is dominated by high densities of *Polymesoda caroliniana*. Other species that were common in this large group of stations were *Littoraria irrorata* and *Tagelus plebeius*. Finnally, in the right hand corner of the large center group is a cluster of freshwater stations in the Myakka and Peace rivers that all have very high densities of *Corbicula fluminea*. *Neritina usnea* and *Rangia cuneata* were alos dominant in this cluster.

Three stations were distinct from all the three clusters described above. The Blackburn-0 km station segment had only a few mollusks, the Peace-6 km station was dominated by just one specie, the clam *Macoma constricta*. The Shakett-0 km station had high densities of *Tagelus plebeius*.

The 16 km segment of the transect in the Alafia River was 100 % different from all of the other stations. This station had only one mollusk, an unidentified Planorbidae, which was not found elsewhere. The station was so different from all others, it is not included in the MDS plot in Figure 13).

Table 5. Taxonomic list of all live and dead species found. Abundance of all dead and live individuals found per $m^2$ averaged over all samples (i.e., river-site-segment combinations). Abbreviations: PH = Phylum, CL = Class, OR = Order, and FA = Family.

| PH | CL | OR | FA | Species | Dead | Live |
|---|---|---|---|---|---|---|
| Brachiopoda | | | | | | |
| | | | | Brachiopoda (unidentified) | 0 | 0.008 |
| | Lingulida | | | | | |
| | | Lingulidata | | | | |
| | | | Lingulidae | | | |
| | | | | *Glottidia pyramidata* | 0.016 | 0.064 |
| Mollusca | | | | | | |
| | | | | Mollusca (unidentified) | 0.016 | 0.023 |
| | Gastropoda | | | | | |
| | | Pulmonata | | | | |
| | | | Ellobium | | | |
| | | | | *Melampus* sp. | 0.055 | 0 |
| | | Basommatophora | | | | |
| | | | Planorbidae | | | |
| | | | | Planorbidae (unidentified) | 0.208 | 0.032 |
| | | Neotaeniogloassa | | | | |
| | | | Littorinidae | | | |
| | | | | *Littoraria irrorata* | 0.469 | 1.811 |
| | | | Epitoniidae | | | |
| | | | | *Epitonium rupicola* | 0.031 | 0 |
| | | | Calyptraeidae | | | |
| | | | | *Crepidula fornicata* | 0.318 | 0 |
| | | | Naticidae | | | |
| | | | | *Polinices duplicatus* | 0.133 | 0.048 |
| | | | Cerithiidae | | | |
| | | | | *Cerithium atratum* | 0.495 | 0 |
| | | | Triphoridae | | | |
| | | | | *Triphora melanura* | 0.031 | 0 |
| | | Cephalaspidea | | | | |
| | | | Bullidae | | | |
| | | | | *Bulla striata* | 0.073 | 0 |
| | | | Haminoeidae | | | |
| | | | | *Haminoea succinea* | 0.851 | 1.062 |
| | | Neogastropoda | | | | |
| | | | Conidae | | | |
| | | | | *Conus* sp. | 0.010 | 0 |
| | | | Nassariidae | | | |
| | | | | *Nassarius vibex* | 2.944 | 1.395 |
| | | | Melongenidae | | | |
| | | | | *Melongena corona* | 0.247 | 0.153 |

| PH | CL | OR | FA | Species | Dead | Live |
|---|---|---|---|---|---|---|
| | | | Muricidae | | | |
| | | | | *Eupleura* sp. | 0.021 | 0 |
| | | | | *Urosalpinx tampaensis* | 0.042 | 0 |
| | | Neritopsina | | | | |
| | | | Neritidae | | | |
| | | | | *Neritina usnea* | 5.990 | 3.028 |
| | Bivalvia | | | | | |
| | | | | Bivalvia (unidentified) | 0.062 | 0.317 |
| | | Myoida | | | | |
| | | | Pholadidae | | | |
| | | | | *Cyrtopleura* sp. | 0 | 0.008 |
| | | Veneroida | | | | |
| | | | Cardiidae | | | |
| | | | | *Laevicardium mortoni* | 0.497 | 0.131 |
| | | | Corbiculidae | | | |
| | | | | *Corbicula fluminea* | 23.306 | 33.107 |
| | | | | *Polymesoda caroliniana* | 13.281 | 9.052 |
| | | | Dreissenidae | | | |
| | | | | *Mytilopsis leucophaeata* | 6.093 | 0.796 |
| | | | Lasaeidae | | | |
| | | | | *Mysella planulata* | 0.492 | 0.137 |
| | | | Lucinidae | | | |
| | | | | *Anodontia alba* | 0.062 | 0 |
| | | | | *Lucina pectinata* | 0.203 | 0.011 |
| | | | Mactridae | | | |
| | | | | *Mulinia lateralis* | 0.923 | 1.734 |
| | | | | *Rangia cuneata* | 11.418 | 6.619 |
| | | | | *Spisula solidissima similis* | 0.031 | 0 |
| | | | Pharidae | | | |
| | | | | *Ensis minor* | 0.031 | 0 |
| | | | Pisidiidae | | | |
| | | | | *Musculium partumeium* | 0.031 | 0.011 |
| | | | | *Pisidium* sp. | 0.008 | 0 |
| | | | Semelidae | | | |
| | | | | *Abra aequalis* | 0.008 | 0 |
| | | | Solecurtidae | | | |
| | | | | *Tagelus plebeius* | 5.604 | 4.553 |
| | | | Solenidae | | | |
| | | | | *Solen viridis* | 0.016 | 0 |

| PH | CL | OR | FA | Species | Dead | Live |
|---|---|---|---|---|---|---|
| | | | Tellinidae | | | |
| | | | | *Macoma constricta* | 0.515 | 2.662 |
| | | | | *Macoma tenta* | 0.102 | 0.056 |
| | | | | *Tellina versicolor* | 0.325 | 2.741 |
| | | | | *Tellina* sp. | 1.265 | 0.139 |
| | | | Veneridae | | | |
| | | | | *Anomalocardia auberiana* | 1.369 | 0.075 |
| | | | | *Chione cancellata* | 2.051 | 0.348 |
| | | | | *Cyclinella tenuis* | 0.161 | 0.059 |
| | | | | *Macrocallista nimbosa* | 0.016 | 0 |
| | | | | *Mercenaria campechiensis* | 0.130 | 0 |
| | | | | Veneridae (unidentified) | 0.016 | 0 |
| | | Arcoida | | | | |
| | | | Arcidae | | | |
| | | | | *Anadara transversa* | 0.122 | 0.064 |
| | | | Noetiidae | | | |
| | | | | *Noetia ponderosa* | 0.016 | 0 |
| | | Mytiloida | | | | |
| | | | Mytilidae | | | |
| | | | | *Amygdalum papyrium* | 0.261 | 4.268 |
| | | | | *Brachidontes modiolus* | 0 | 0.127 |
| | | | | *Geukensia granosissima* | 1.201 | 2.793 |
| | | | | *Ischadium recurvum* | 1.861 | 1.780 |
| | | Ostreoida | | | | |
| | | | Ostreidae | | | |
| | | | | *Crassostrea virginica* | 9.923 | 2.626 |
| | | | | *Ostrea frons* | 0.445 | 0 |
| | | | Pectinidae | | | |
| | | | | *Argopecten irradians* | 0.224 | 0 |
| | | | Anomiidae | | | |
| | | | | *Anomia simplex* | 0.916 | 0 |
| | | Pterioida | | | | |
| | | | Pinnidae | | | |
| | | | | *Atrina serrata* | 0.010 | 0 |
| **Total** | | | | | **94.945** | **81.837** |

Table 6. Species dominance based on average abundance. Total number of live individuals found and the frequency of number of times found among all unaggregated samples, average abundance among the 67 samples (i.e., river, site, 2-km segment combinations), and percent composition of the total community abundance.

| **Species** | **Total** | **Frequency** | **Abundance (n $m^{-2}$)** | **Percent (%)** |
|---|---|---|---|---|
| *Corbicula fluminea* | 1,036 | 27 | 33.107 | 40.454 |
| *Polymesoda caroliniana* | 344 | 35 | 9.052 | 11.061 |
| *Rangia cuneata* | 225 | 28 | 6.619 | 8.088 |
| *Tagelus plebeius* | 180 | 28 | 4.553 | 5.563 |
| *Amygdalum papyrium* | 150 | 11 | 4.268 | 5.215 |
| *Neritina usnea* | 109 | 26 | 3.028 | 3.700 |
| *Geukensia granosissima* | 173 | 9 | 2.793 | 3.413 |
| *Tellina versicolor* | 96 | 8 | 2.741 | 3.349 |
| *Macoma constricta* | 85 | 5 | 2.662 | 3.253 |
| *Crassostrea virginica* | 137 | 17 | 2.626 | 3.208 |
| *Littoraria irrorata* | 94 | 19 | 1.811 | 2.213 |
| *Ischadium recurvum* | 92 | 15 | 1.780 | 2.176 |
| *Mulinia lateralis* | 130 | 13 | 1.734 | 2.119 |
| *Nassarius vibex* | 47 | 11 | 1.395 | 1.705 |
| *Haminoea succinea* | 33 | 3 | 1.062 | 1.297 |
| *Mytilopsis leucophaeata* | 40 | 5 | 0.796 | 0.973 |
| *Chione cancellata* | 11 | 3 | 0.348 | 0.426 |
| Bivalvia (unidentified) | 20 | 4 | 0.317 | 0.387 |
| *Melongena corona* | 8 | 5 | 0.153 | 0.187 |
| *Tellina* sp. | 10 | 4 | 0.139 | 0.170 |
| *Mysella planulata* | 17 | 1 | 0.137 | 0.167 |
| *Laevicardium mortoni* | 6 | 3 | 0.131 | 0.161 |
| *Brachidontes modiolus* | 17 | 4 | 0.127 | 0.155 |
| *Anomalocardia auberiana* | 7 | 3 | 0.075 | 0.092 |
| *Anadara transversa* | 3 | 2 | 0.064 | 0.079 |
| *Glottidia pyramidata* | 4 | 1 | 0.064 | 0.079 |
| *Cyclinella tenuis* | 3 | 3 | 0.059 | 0.072 |
| *Macoma tenta* | 5 | 2 | 0.056 | 0.069 |
| *Polinices duplicatus* | 2 | 2 | 0.048 | 0.059 |
| Planorbidae (unidentified) | 1 | 1 | 0.032 | 0.039 |
| Mollusca (unidentified) | 3 | 2 | 0.023 | 0.028 |
| *Lucina pectinata* | 1 | 1 | 0.011 | 0.013 |
| *Musculium partumeium* | 1 | 1 | 0.011 | 0.013 |
| Brachiopoda | 1 | 1 | 0.008 | 0.010 |
| *Cyrtopleura* sp. | 1 | 1 | 0.008 | 0.010 |

Table 7. Dominance of all species as a percentage of all the average number of individuals found in each site (river or creek) sampled.

| | River or Creek | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Alafia** | **Big Slough** | **Blackburn** | **Currey** | **Deer Prairie** | **Mud** | **Myakka** | **Peace** | **Shakett** | **Shell** | **Weeki** |
| *Corbicula fluminea* | 1.23 | 0 | 0 | 0 | 4.65 | 0 | 42.12 | 53.32 | 0 | 0.26 | 1.25 |
| *Polymesoda caroliniana* | 19.07 | 40 | 0 | 1.9 | 44.19 | 21.74 | 17.23 | 3.51 | 2.13 | 46.59 | 21.25 |
| *Rangia cuneata* | 0 | 24 | 100 | 0 | 51.16 | 0 | 8.86 | 5.79 | 0 | 30.90 | 0 |
| *Tagelus plebeius* | 3.69 | 28 | 0 | 34.18 | 0 | 30.43 | 9.54 | 1.36 | 24.63 | 19.31 | 23.75 |
| *Crassostrea virginica* | 21.88 | 0 | 0 | 5.7 | 0 | 26.09 | 0 | 1.06 | 27.59 | 0 | 25 |
| *Geukensia granosissima* | 29.44 | 0 | 0 | 0 | 0 | 0 | 6.22 | 0.22 | 0 | 0 | 0 |
| *Amygdalum papyrium* | 1.23 | 0 | 0 | 0 | 0 | 0 | 0 | 8.28 | 0 | 0 | 0 |
| *Neritina usnea* | 5.89 | 8 | 0 | 0 | 0 | 0 | 0.45 | 4.95 | 1.31 | 0.77 | 0 |
| *Ischadium recurvum* | 0 | 0 | 0 | 1.9 | 0 | 0 | 0.45 | 2.52 | 16.26 | 1.02 | 15.0 |
| *Littoraria irrorata* | 4.53 | 0 | 0 | 1.27 | 0 | 8.69 | 7.92 | 0.47 | 2.46 | 0.51 | 8.75 |
| *Macoma constricta* | 0 | 0 | 0 | 0 | 0 | 13.04 | 0 | 5.16 | 0 | 0 | 0 |
| *Chione cancellata* | 0 | 0 | 0 | 27.85 | 0 | 0 | 0 | 0 | 6.9 | 0 | 0 |
| *Tellina versicolor* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.42 | 0 | 0 | 0 |
| *Mulinia lateralis* | 1.71 | 0 | 0 | 3.8 | 0 | 0 | 2.49 | 2.44 | 0 | 0.13 | 0 |
| *Nassarius vibex* | 0 | 0 | 0 | 3.8 | 0 | 0 | 0.11 | 2.63 | 0.99 | 0 | 0 |
| *Mytilopsis leucophaeata* | 3.56 | 0 | 0 | 0 | 0 | 0 | 3.85 | 0 | 0 | 0.51 | 0 |
| *Haminoea succinea* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.1 | 0 | 0 | 0 |
| *Laevicardium mortoni* | 0 | 0 | 0 | 10.76 | 0 | 0 | 0 | 0 | 2.46 | 0 | 0 |
| *Tellina* sp. | 0 | 0 | 0 | 1.27 | 0 | 0 | 0 | 0 | 6.9 | 0 | 2.5 |
| Bivalvia (unidentified) | 4.35 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| *Anomalocardia auberiana* | 0 | 0 | 0 | 1.27 | 0 | 0 | 0 | 0 | 3.94 | 0 | 0 |
| *Anadara transversa* | 0 | 0 | 0 | 3.8 | 0 | 0 | 0 | 0.06 | 0 | 0 | 0 |
| *Melongena corona* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.27 | 0 | 0 | 2.5 |
| *Mysella planulata* | 2.24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cyclinella tenuis* | 0 | 0 | 0 | 1.27 | 0 | 0 | 0.11 | 0 | 1.97 | 0 | 0 |
| *Macoma tenta* | 0.66 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.99 | 0 | 0 |
| *Brachidontes modiolus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 |
| *Lucina pectinata* | 0 | 0 | 0 | 1.27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mollusca (unidentified) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.01 | 0.99 | 0 | 0 |
| Planorbidae (unidentified) | 0.53 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Glottidia pyramidata* | 0 | 0 | 0 | 0 | 0 | 0 | 0.45 | 0 | 0 | 0 | 0 |
| *Polinices duplicatus* | 0 | 0 | 0 | 0 | 0 | 0 | 0.11 | 0.06 | 0 | 0 | 0 |
| *Cyrtopleura* sp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.49 | 0 | 0 |
| *Musculium partumeium* | 0 | 0 | 0 | 0 | 0 | 0 | 0.08 | 0 | 0 | 0 | 0 |
| Brachiopoda | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.02 | 0 | 0 | 0 |

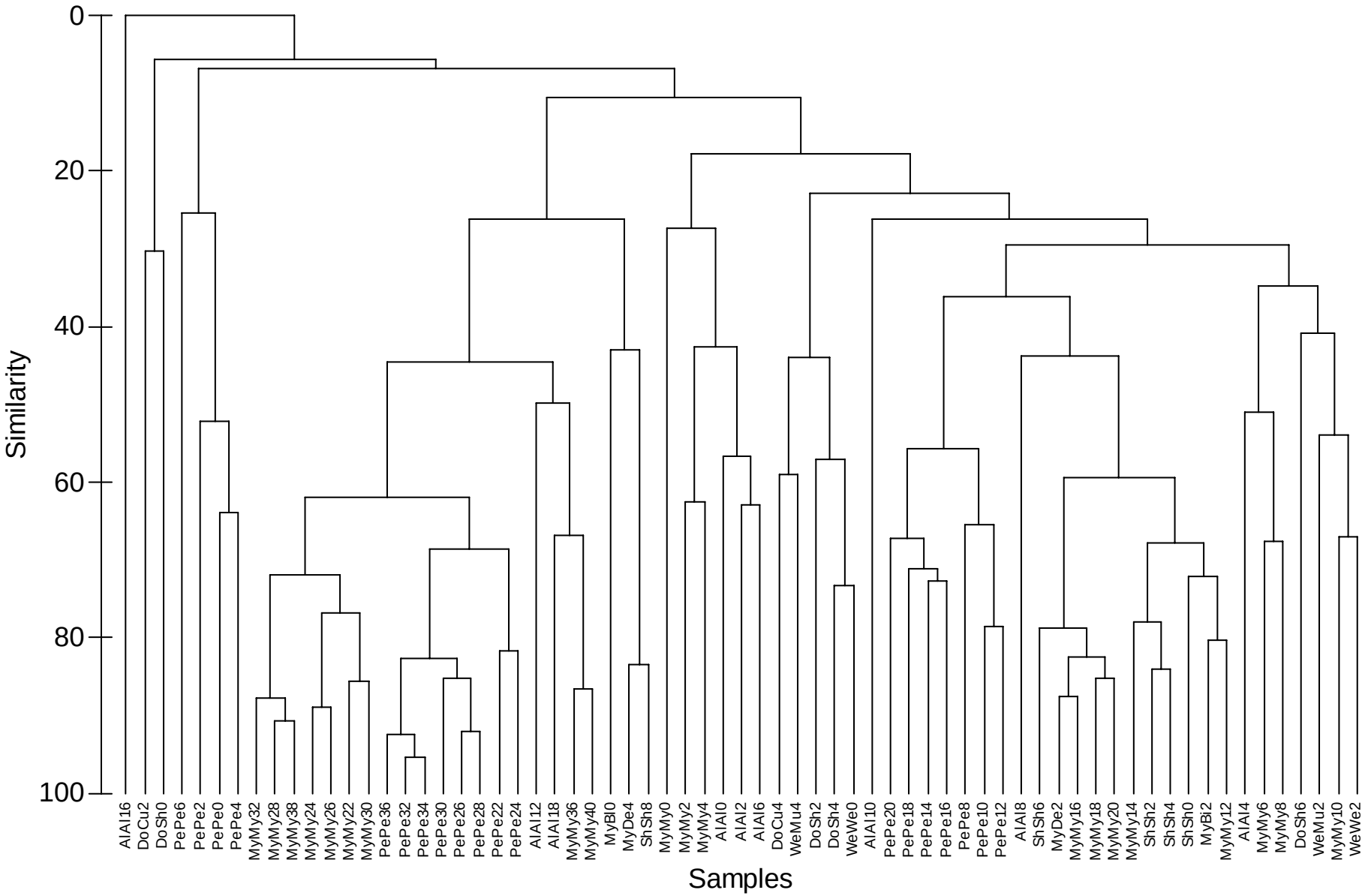


Figure 12. Bray-Curtis similarity indices for each station (i.e., river, site, 2-km segment combination).

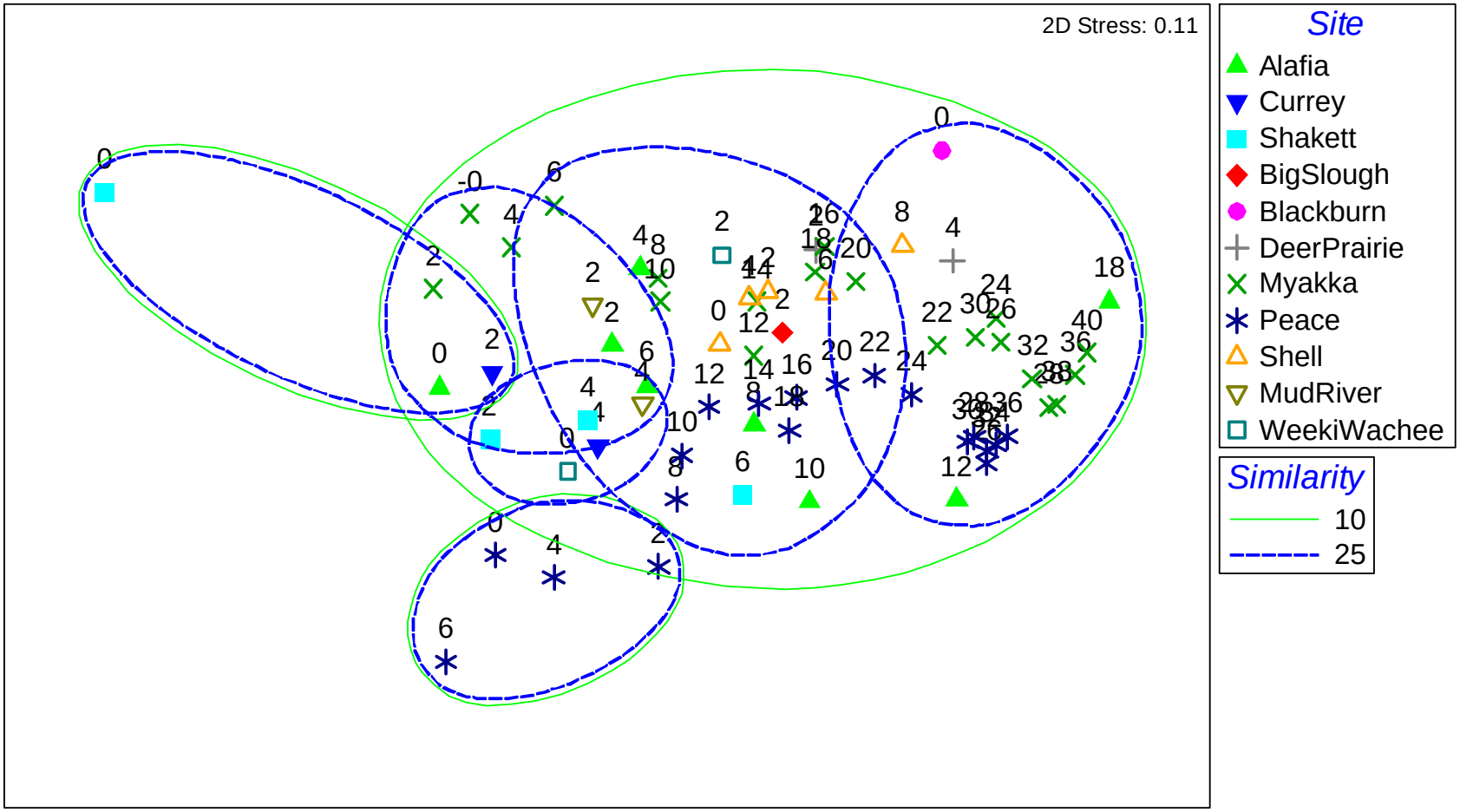


Figure 13. Relationships between mollusk communities from multi-dimensional scaling (MDS) analysis. Symbols represent the river or creek site with shape and color, and the km segment number is listed above the river symbol. Segment 16 from the Alafia River is outside the range of this plot.

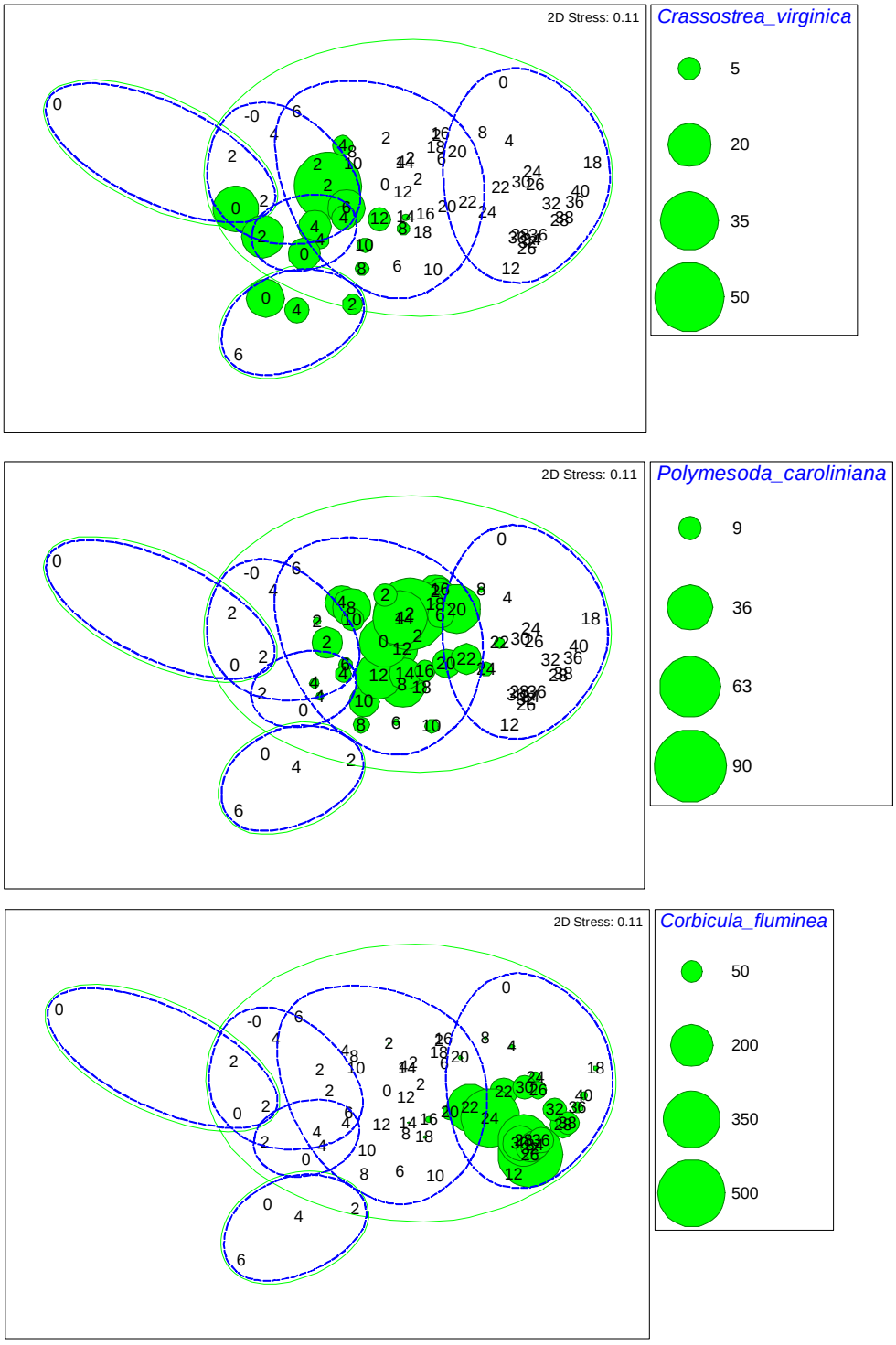


Figure 14. Abundance of three species (as bubbles) driving similarities among samples in the MDS plot in Figure 13.

Mollusca Diversity

Diversity characteristics were calculated for each river-site-segment combination. Hill's diversity index, N1, typically increased or was high in segments from 0 km to 2 km, then decreased to 10 km, then increased again, peaking in the 20 km to 24 km range, and decreased again toward the freshwater source (Figure 15). However, N1 is influenced by sample size, so it is best to compare metrics that do not have these problems, such as the taxonomic distinctness index, $\Delta^*$ (Figure 16). The trend for $\Delta^*$ is different, with a large range in the 0 km to 14 km range, and then an abrupt decreasing trend from 14 km to 40 km. The two rivers with the longest segments, Myakka and Peace, look different for N1, but similar for $\Delta^*$. Shell Creek is interesting because it has the highest $\Delta^*$ diversity, but the second to lowest N1 diversity compared to other rivers in the 0 km to 10 km range. Overall, the trend for N1 is a double peak at 2 km and 22 km, whereas the overall trend for $\Delta^*$ is one single peak around 12 km.

Univariate measures of diversity are difficult to compare among the rivers and river-sites because there was an uneven sampling effort of segments among these locations and there is a strong change of changing diversity along the salinity gradient (Figures 15 - 16). . However, most sites were sampled from the 0 km to 8 km range, so this portion of each transect can be averaged to compare sites (Table 8). An one-way, block analysis of variance was calculated to test for differences between sites. All measures were different among sites. Total abundance (N) was different at the $p = 0.0087$ level. Species richness (S) was barely significant for site differences ($p = 0.0470$). The number of dominant species (N1) was different among sites ($p = 0.0130$), and so was taxonomic distinctness ($\Delta^*$) different among sites ($p = 0.0015$). Hill's diversity index, N1, ranges from 1.2 dominant species in the Peace River to 5.5 in Big Slough. Most other sites have N1 values of 3 - 4. Taxonomic distinctness index, $\Delta^*$, ranges from 33 at Shakett Creek to 78 at Shell Creek. The $\Delta^*$ is only 40 for Big Slough, even though it has the highest number of species (11) and dominant species (5.5). Shell, Weeki Wachee, Alafia, and Currey are the most diverse sites.

Table 8. Diversity characteristics by river or creek site averaged over segments 0 km - 8 km. A. Aggregated by sites, i.e., rivers or creeks within river systems. B. Aggregated by river systems. Abbreviations: S = species richness, i.e., number of species, N = abundance of individuals $m^{-2}$, N1 = Hill's diversity index of number of dominant species, $\Delta^*$ = taxonomic distinctness, -std = standard deviation.

| Site | Segments | S | S-std | N | N-std | N1 | N1-std | $\Delta^*$ | $\Delta^*$-std |
|---|---|---|---|---|---|---|---|---|---|
| Alafia | 5 | 5.4 | 1.7 | 74.8 | 43.6 | 3.4 | 1.1 | 59.1 | 3.6 |
| Big Slough | 1 | 11.0 | | 48.1 | | 5.5 | | 39.7 | |
| Blackburn | 1 | 5.0 | | 8.6 | | 4.5 | | 50.3 | |
| Currey | 2 | 4.0 | 2.8 | 74.9 | 0.7 | 2.0 | 1.3 | 58.6 | 30.2 |
| Deer Prairie | 2 | 6.0 | 5.7 | 27.8 | 27.1 | 3.7 | 2.7 | 35.0 | 32.4 |
| Mud | 2 | 4.0 | 0.0 | 12.4 | 6.9 | 3.7 | 0.1 | 53.0 | 8.8 |
| Myakka | 5 | 3.8 | 3.1 | 22.4 | 16.1 | 2.7 | 1.7 | 37.5 | 24.6 |
| Peace | 5 | 1.6 | 0.5 | 56.3 | 26.4 | 1.2 | 0.3 | 17.9 | 16.3 |
| Shakett | 4 | 3.5 | 0.6 | 86.8 | 64.7 | 2.3 | 0.7 | 33.2 | 4.9 |
| Shell | 5 | 2.4 | 0.5 | 225.8 | 162.0 | 1.3 | 0.2 | 78.3 | 2.6 |
| Weeki Wachee | 2 | 4.5 | 0.7 | 21.6 | 10.7 | 3.1 | 0.9 | 62.4 | 7.4 |

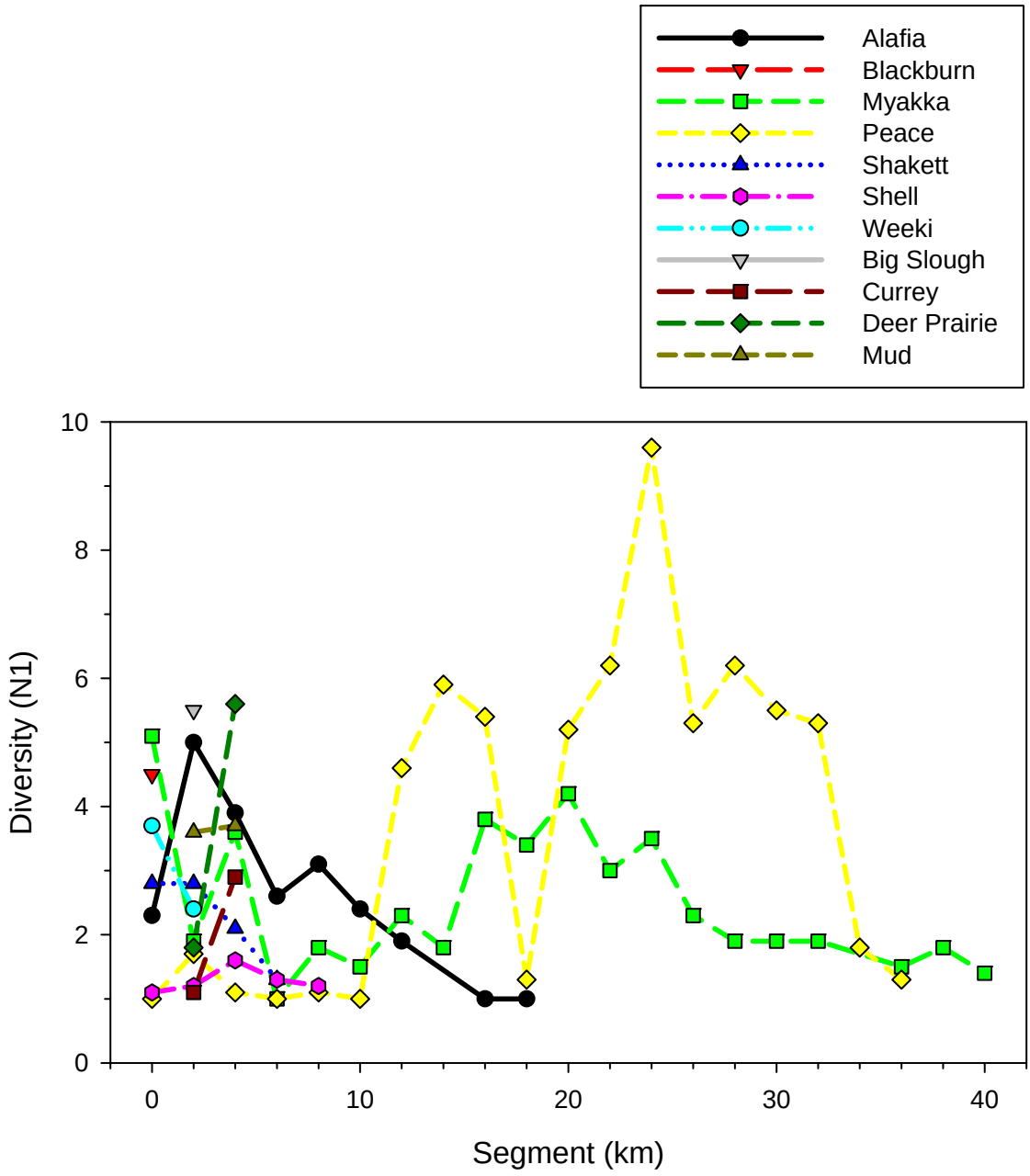


Figure 15. Diversity calculated as Hill's N1, the number of dominant species in segment site combinations.

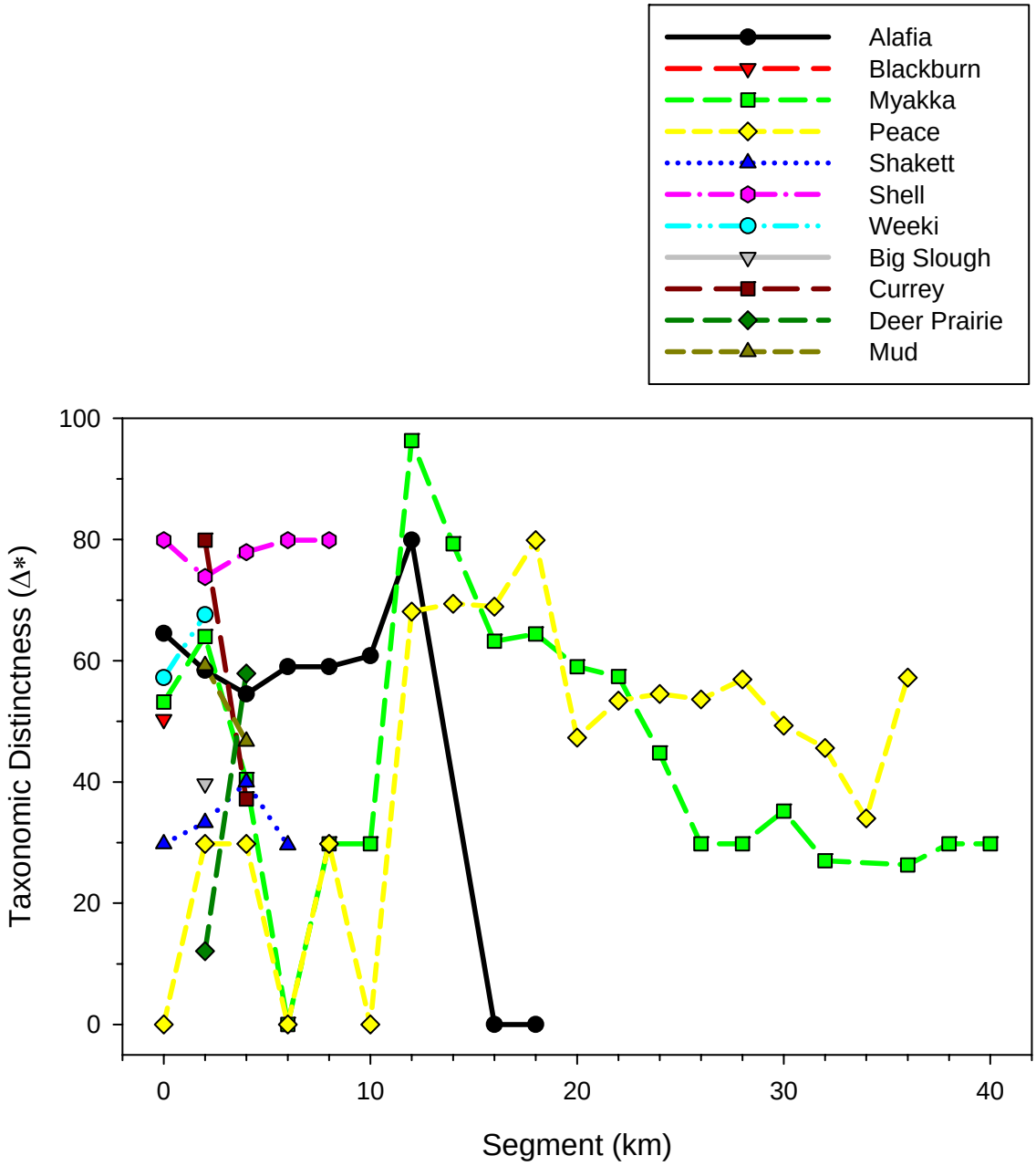


Figure 16. Diversity calculated as taxonomic distinctness (Δ*), the taxonomic distance through phylogenetic classification of every pair of individuals.

Mollusk-Environment Relationships

There are at least two approaches to relating mollusks to the environment, but in all cases salinity is used as the surrogate for inflow. One approach is to relate (by univariate or multivariate models) salinity with abundance, diversity, or community structure. The second approach is to examine the relationship between abundance and salinity to identify those species or species groups that might have optimal, or highest abundance, within specific salinity ranges.

For the first approach, a multivariate analysis (the BIO-ENV procedure) was used to identify the combinations of environmental variables that could predict mollusk abundance. Out of 62 transect-segments sampled for water quality and 67 transect-segments sampled for molluscs, there were only 45 common transect-segments that could be analyzed using BIO-ENV because of missing data in the other 17. Salinity, temperature, and pH were the environmental variables that correlated the highest with the mollusk community distributions ($\rho_w = 0.612$; Table 9). The RELATE procedure was used to determine that this correlation was significant ($p < 0.001$). The single variable that correlated the highest with mollusk communities was salinity ($\rho_w = 0.576$). In fact, salinity was the only variable that fit the community distributions in all the tests. The water quality variables had higher correlations with the mollusk communities than any single, or combination of, sediment characteristics. Of the sediment variables, median and mean grain size fit best, but all sediment variables always were selected after Salinity, temperature, and pH. It is therefore obvious that overlying water properties, especially salinity values, have more control on the mollusk communities than the sediment characteristics.

Table 9. Top ten correlations between mollusk species abundance (i.e., the resemblance matrix used for the similarity (Figure 12) and multi-dimensional scaling plot (Figure 13)) and normalized environmental data from Biota-Environment (BIOENV) analysis.

| No. of Variables | Correlation ($\rho_w$) | Variables Selected |
|---|---|---|
| 3 | 0.619 | Salinity, Temperature, pH |
| 2 | 0.608 | Salinity, pH |
| 4 | 0.594 | Salinity, Temperature, pH, Median grain size |
| 4 | 0.579 | Salinity, Temperature, pH, Mean grain size |
| 1 | 0.566 | Salinity |
| 2 | 0.559 | Salinity, Temperature |
| 4 | 0.555 | Salinity, Temperature, pH, Kurtosis grain size |
| 4 | 0.554 | Salinity, Temperature, pH, %Clay |
| 4 | 0.552 | Salinity, Temperature, pH, %Solids |
| 4 | 0.552 | Salinity, Temperature, pH, %Silt |

In the second approach, total mollusk abundance did not correlate with salinity among all river sites (Figure 17b). The highest abundances occurred at low salinities, but this is attributed to the large population of *Corbicula fluminea* that occurred in the Peace River at low salinities. Mollusk

diversity increased with salinity, particularly as salinity increased from 0 to 2 psu, but the correlation was weak (Figure 17a). Hill's N1 values were consistently close to one where mean salinity was close to one, however, as salinity and overall N1 increased, so too did the range of N1 values.

Two rivers, the Myakka and Peace, were sampled in long transects (Figure 9). Examining distributions along salinity gradients in these two rivers alone would remove bias to differences in systems (Figures 16, 18 and 19). In both rivers there was a strong relationship between diversity and abundance with salinity where the abundance and diversity increased with increasing salinity, then peaked, and then declined. This curve is similar to a 3-parameter log normal distribution, which was found to fit total macrofauna abundance in a Texas estuary (Montagna et al., 2002), so the data was fit to that non-linear model. The relationship between salinity and diversity was stronger in the Peace River than the Myakka River based on the probability level (P) and goodness of fit parameter ($R^2$) (Table 10).

The ten dominant species were examined for correlations with salinity (Table 11). *Corbicula fluminea* was only found where mean salinities were lower than 7 psu, but was most common where mean salinities were less than or equal to 2 psu (Figure 20a), but the fitted maximum salinity value (parameter c in Table 10) was 0.6 psu. *C. fluminea* was also only found in abundances higher than 10 $m^{-2}$ in the Myakka and Peace Rivers. *Polymesoda caroliniana* was found in all river systems but occurred where salinities were between 1 and 20 psu (Figure 20b) and peaked at salinity values of 5 psu (Table 10). Both *P. caroliniana* and *C. fluminea* are in the same family (Corbiculidae). *Rangia cuneata* and *Tagelua plebius* were found in low to moderate salinities and had calculated salinity peaks at 4 and 7 psu respectively (Figure 21). , *Crassostrea virginica* and *Geukensia granosissima* were generally found at higher salinities (Figure 22) and had calculated salinity peaks at 24 and 10 psu respectivley. *Mulinia lateralis* and *Neritina usnea* had different distributions (Figure 23). *Mulinia* ranged from 5 tp 15 ppt, and the model calculated a peak at 14 psu. According to the model, *N. usnea* abundance did not change with salinity (P = 0.43). *Littoriaria irrorata* and *Ischadium recurvum* were found over a wide range of salinities (Figure 24), and peak salinities were calculated as 14 and 12 psu respectively. Two other species not figured, *Amygdalum papyrium* and *Tellina versicolor* were all found in less than 9 segments so therefore a reasonable salinity range could not be estimated.

Table 10. Parameters from nonlinear regression to predict mollusk characteristics from salinity. These parameters are represented on lines in Figures 16, 18 - 24. Probability (P) that model fits the data, per cent of variance explained by data ($R^2$), parameters for maximum biological value (*a*), rate of change (*b*), and maximum salinity value (*c*), and standard deviation for parameters in parentheses. N1 = Hill's diversity index, and n = abundance (individuals per $m^2$), all species are n $m^{-2}$.

| **Variable** | **P** | **$R^2$** | **a** | **b** | **c** |
|---|---|---|---|---|---|
| Myakka N1 | 0.1658 | 0.26 | 3.11 (0.36) | 2.45 (0.65) | 2.15 (0.86) |
| Myakka n | 0.0682 | 0.36 | 54.9 (7.9) | 2.63 (0.84) | 0.59 (0.41) |
| Peace N1 | 0.0098 | 0.64 | 7.29 (1.02) | 1.61 (0.31) | 0.99 (0.28) |
| Peace n | 0.0013 | 0.77 | 218 (24.8) | 1.44 (0.20) | 1.05 (0.20) |
| *C. fluminea* | 0.0001 | 0.31 | 178 (43.2) | 0.78 (0.19) | 0.63 (0.18) |
| *P. caroliniana* | 0.0001 | 0.32 | 28.8 (5.1) | 0.66 (0.13) | 4.89 (0.63) |
| *R. cuneata* | 0.0001 | 0.38 | 27.3 (4.8) | 0.49 (0.08) | 3.69 (0.31) |
| *T. plebius* | 0.0003 | 0.28 | 15.4 (3.0) | 0.48 (0.12) | 7.30 (0.90) |
| *G. granosissima* | 0.0001 | 0.77 | 156 (11.9) | 0.006 (3e-7) | 10.3 (3e-6) |
| *C. virginica* | 0.0001 | 0.33 | 19.3 (4.2) | 0.18 (0.04) | 22.4 (1.0) |
| *M. lateralis* | 0.0001 | 0.37 | 324 (53.3) | 0.006 (3e-7) | 13.6 (8e-6) |
| *N. usnea* | 0.4320 | 0.03 | 4.92 (1.71) | 2.96 (2.77) | 0.45 (1.33) |
| *L. irrorata* | 0.0001 | 0.33 | 6.43 (1.28) | 0.31 (0.07) | 13.8 (0.98) |
| *I. recurvum* | 0.0169 | 0.16 | 5.68 (1.81) | 0.31 (0.11) | 12.3 (1.3) |

Table 11. Salinity Range of twelve most abundant species

| **Species** | **Salinity Range (psu)** | **Transect segments with sp. present** |
|---|---|---|
| *Corbicula fluminea* | < 7 (most ≤ 2 ) | 20 |
| *Polymesoda caroliniana* | 1 to 20 | 32 |
| *Rangia cuneata* | < 16 (most ≤ 10 ) | 23 |
| *Tagelus plebeius* | > 2 | 25 |
| *Geukensia granosissima* | 10 to 24 | 5 |
| *Amygdalum papyrium* | 2 to 20 | 8 (7 in Peace R.) |
| *Crassostrea virginica* | > 7 | 13 |
| *Mulinia lateralis* | > 2 | 10 |
| *Neritina usnea* | < 18 | 20 |
| *Tellina versicolor* | 2 to 18 | 7 (all in Peace R.) |
| *Littoraria irrorata* | > 2 | 17 |
| *Ischadium recurvum* | > 6 | 11 |

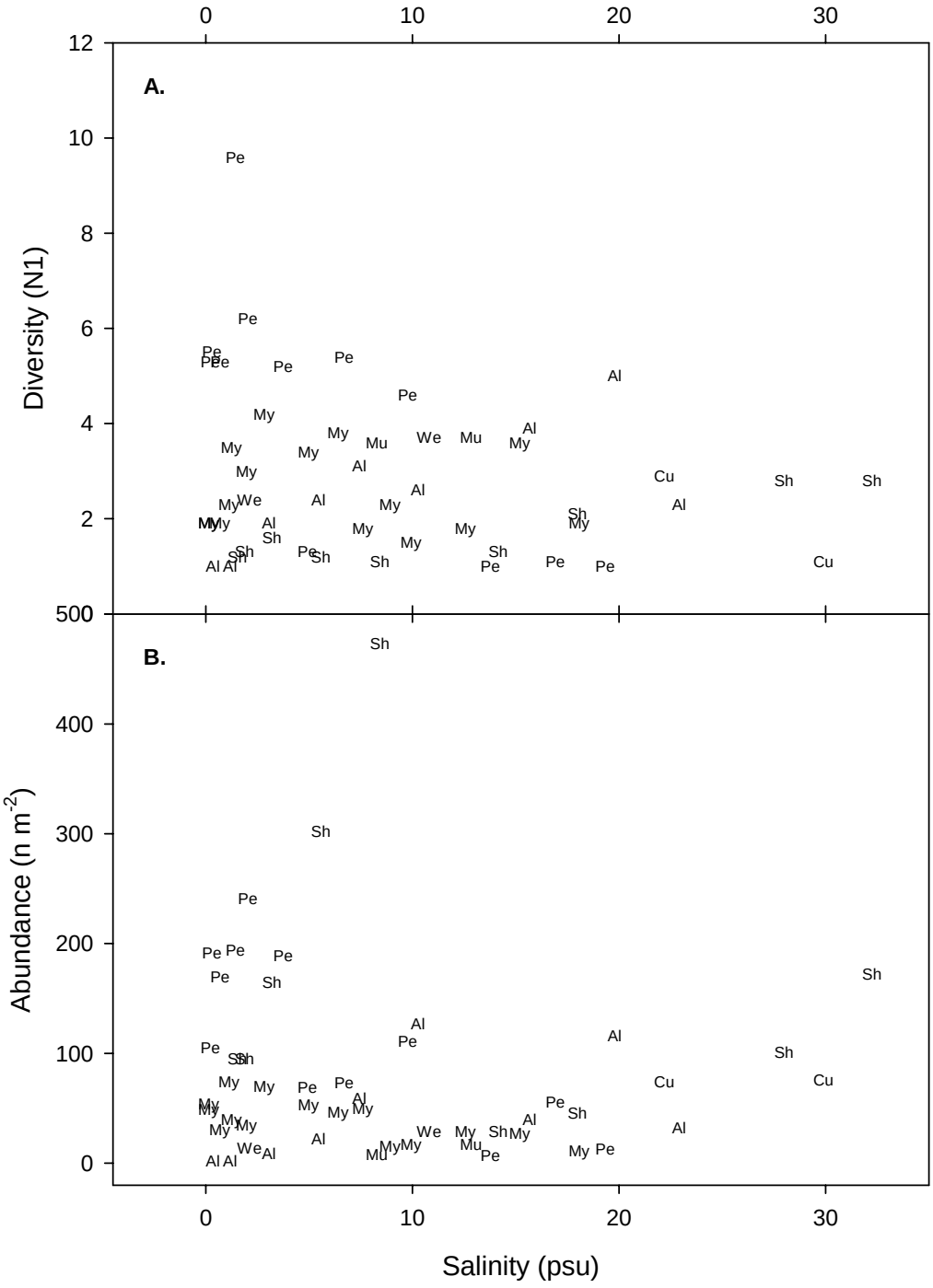


Figure 17. Relationship between salinity and total mollusks at all sites. A. Hill's N1 diversity index (top). B. Abundance (bottom). Key to abbreviations: Al = Alafia River, Bi = Big Slough, Bl = Blackburn Creek, Cu = Currey Creek, De = Deer Praire Creek, My = Myakka River, Pe = Peace River, Sh = Shakett Creek, She = Shell Creek, We = Weeki Wachee River.

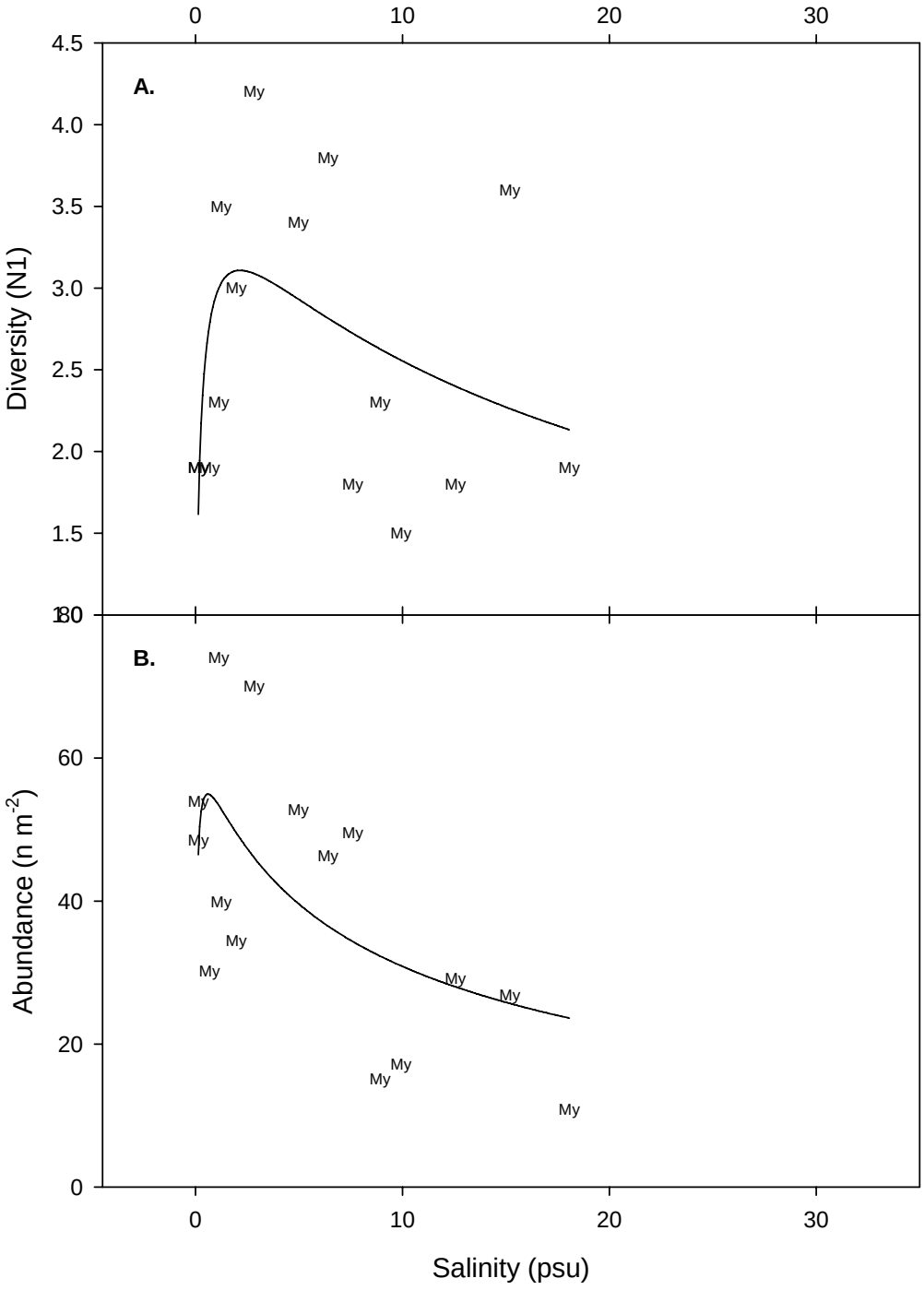


Figure 18. Relationship between salinity and total mollusks at Myakka (My) River sites. A. Hill's N1 diversity index (top). Line is fit with the log normal, 3-parameter model.

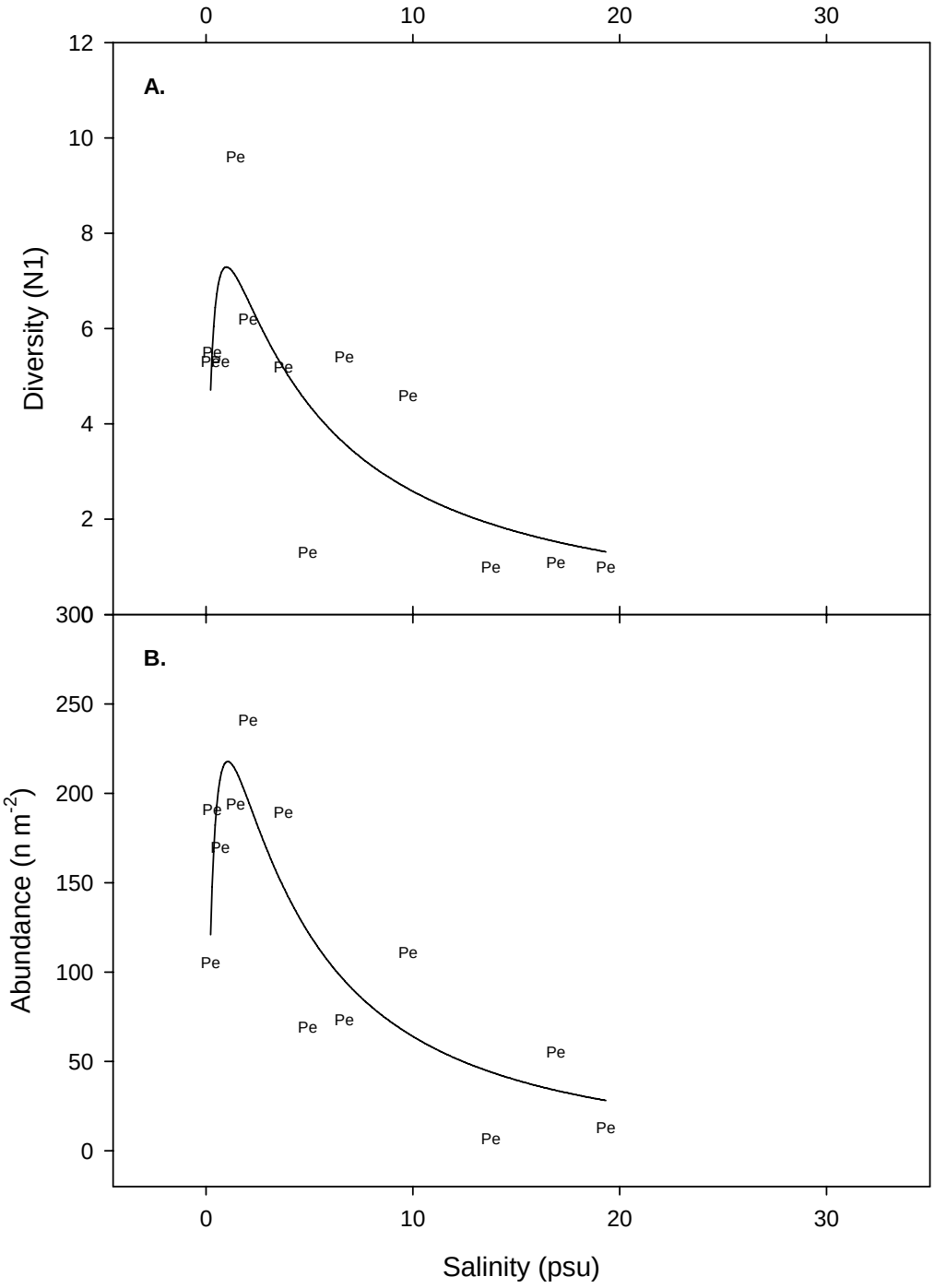


Figure 19. Relationship between salinity and total mollusks at Peace (Pe) River sites. A. Hill's N1 diversity index (top). Line is fit with the log normal, 3-parameter model.

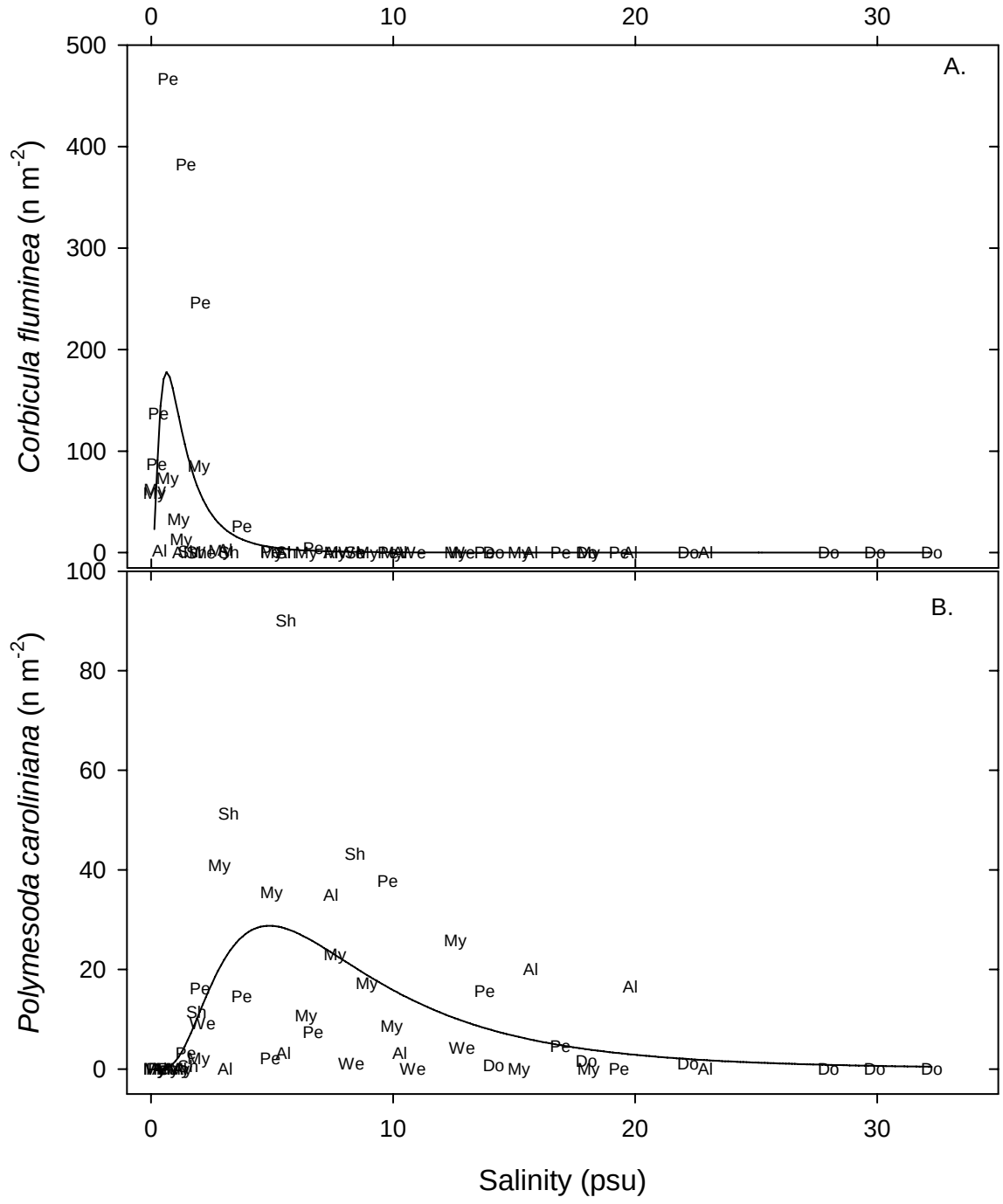


Figure 20. Relationship between salinity and species abundance. A. *Corbicula fluminea*, and B. *Polymesoda caroliniana*. Key: Al = Alafia River, Cu = Currey Creek, Do = Dona/Roberts Bay, My = Myakka River, Pe = Peace River, Sh = Shakett Creek, She = Shell Creek, We = Weeki Wachee River.

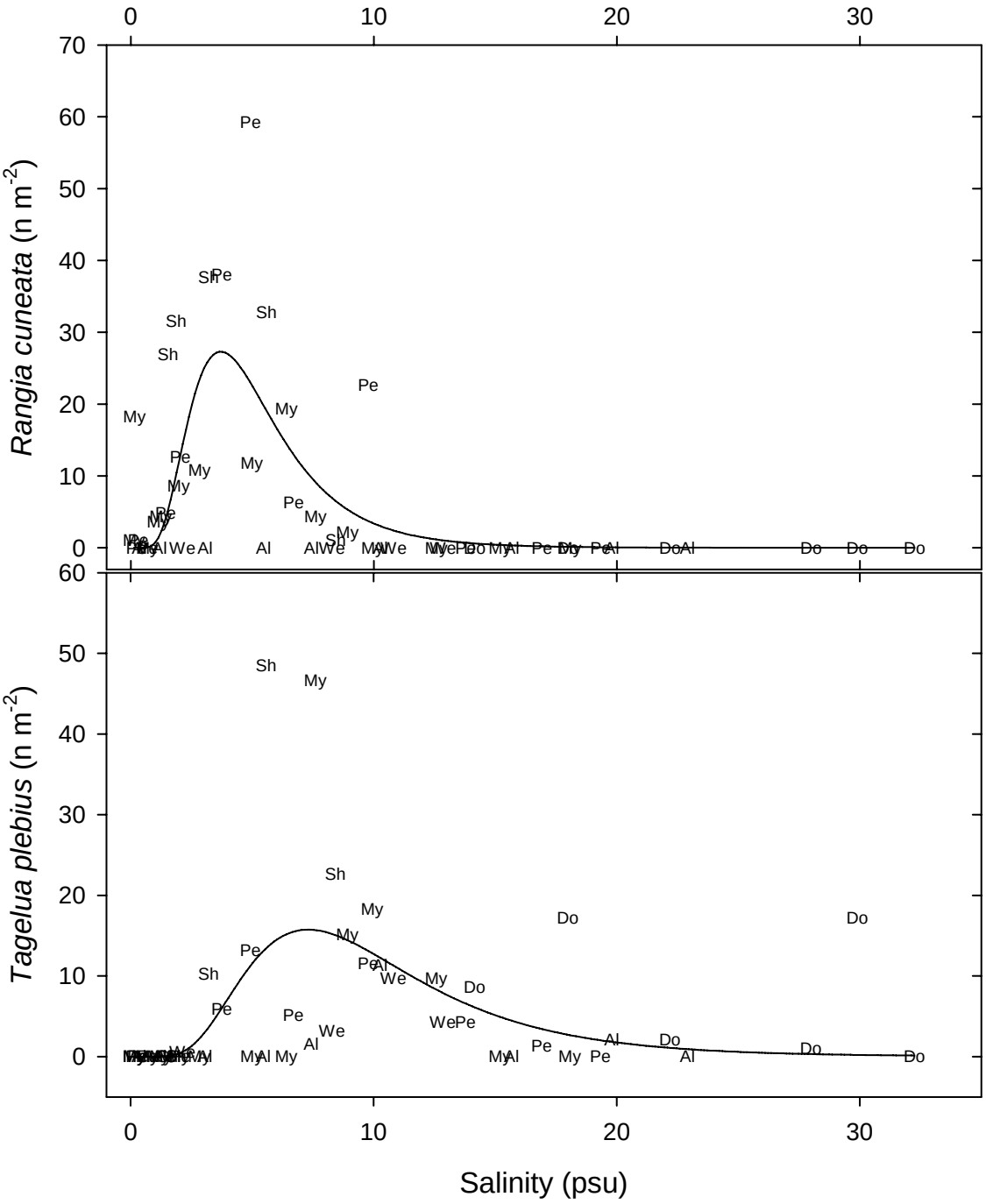


Figure 21. Relationship between salinity and species abundance. A. *Rangia cuneata*, and B. *Tagelus plebius*. Key: Al = Alafia River, Cu = Currey Creek, Do = Dona/Roberts Bay, My = Myakka River, Pe = Peace River, Sh = Shakett Creek, She = Shell Creek, We = Weeki Wachee River.

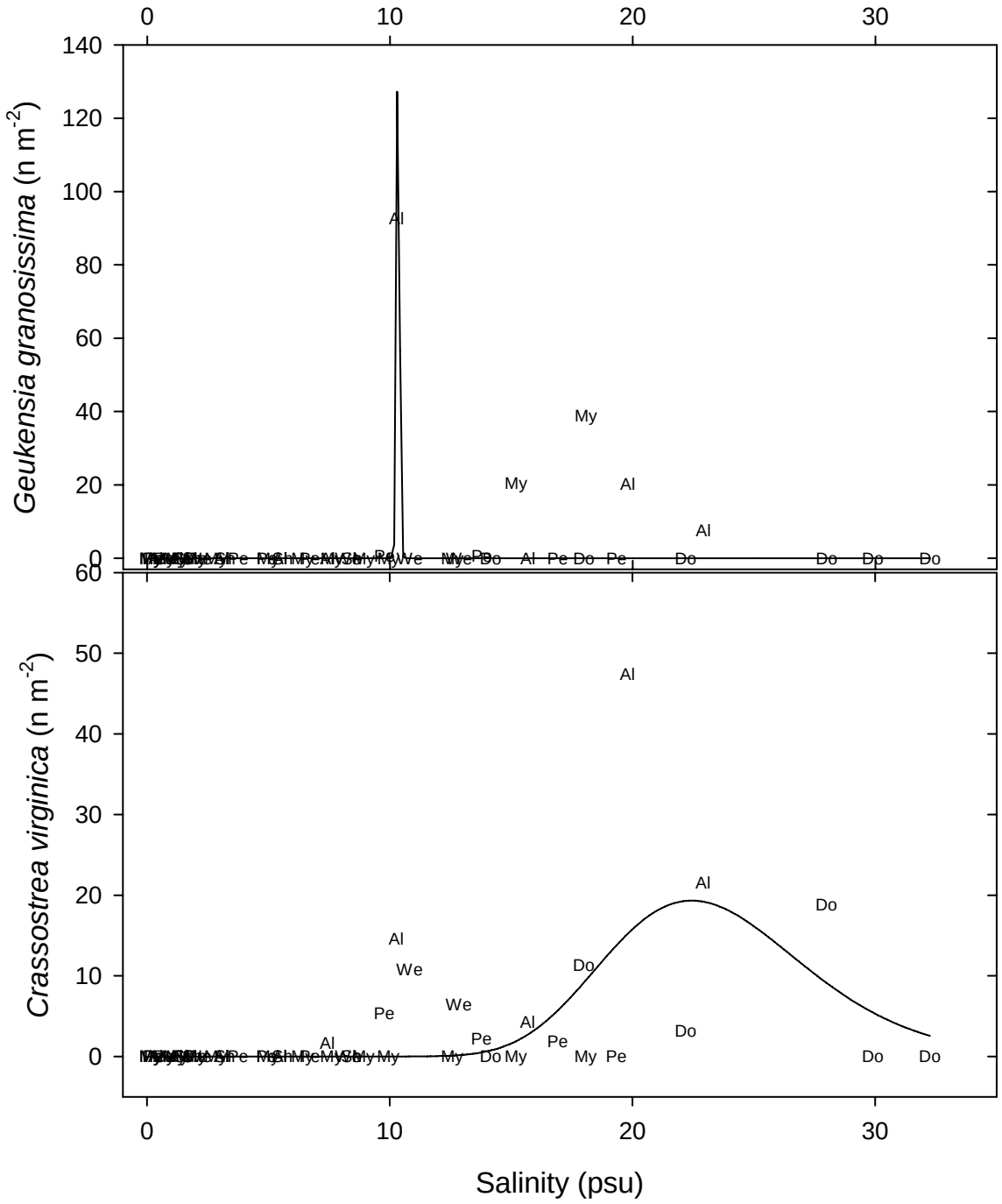


Figure 22. Relationship between salinity and species abundance. A. *Geukensia granosissima*, and B. *Crassostrea virginica*. Key: Al = Alafia River, Cu = Currey Creek, Do = Dona/Roberts Bay, My = Myakka River, Pe = Peace River, Sh = Shakett Creek, She = Shell Creek, We = Weeki Wachee River.

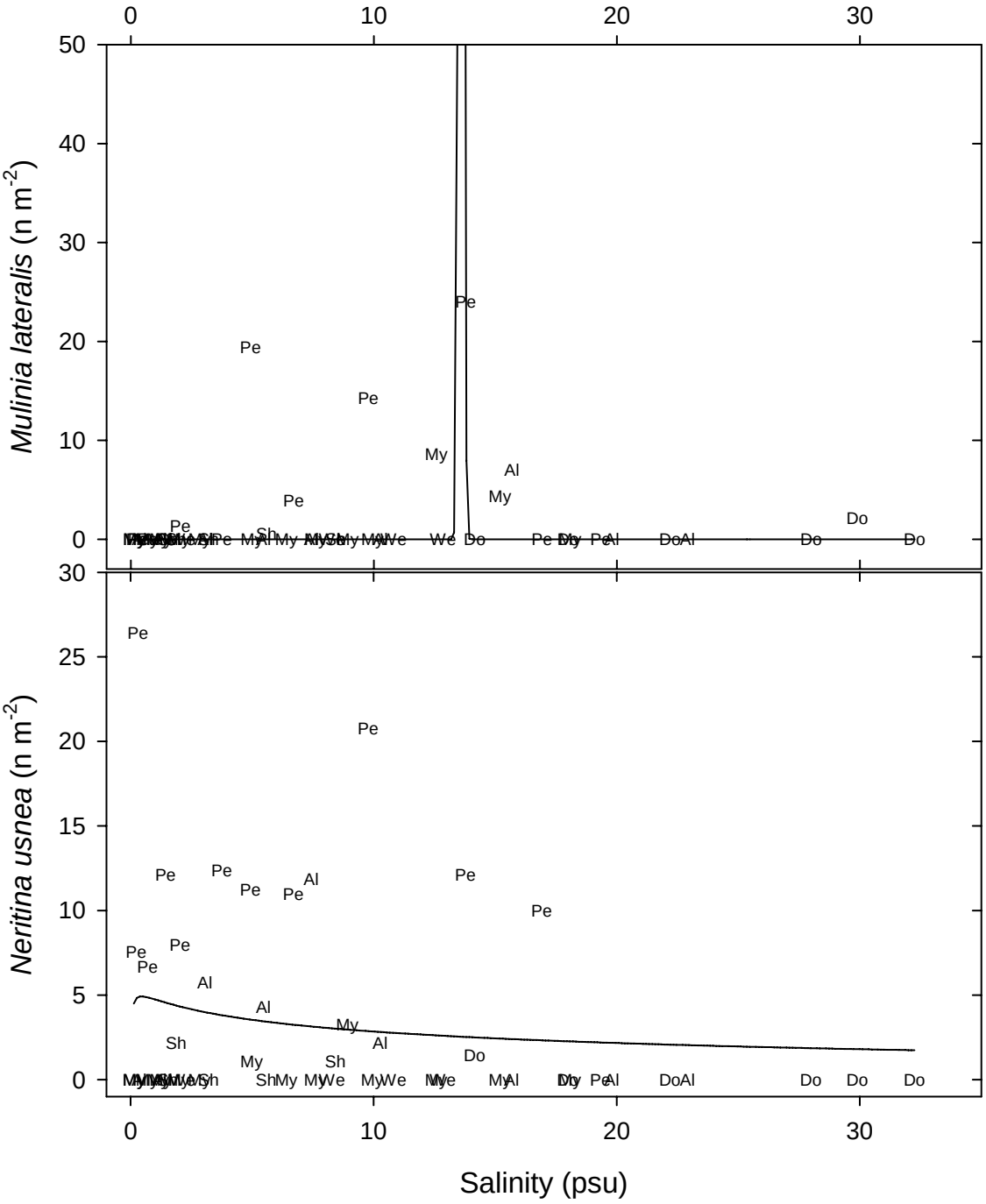


Figure 23. Relationship between salinity and species abundance. A. *Mulinea lateralis*, and B. *Neritina usnea*. Key: Al = Alafia River, Cu = Currey Creek, Dona/Roberts Bay, My = Myakka River, Pe = Peace River, Sh = Shakett Creek, She = Shell Creek, We = Weeki Wachee River.

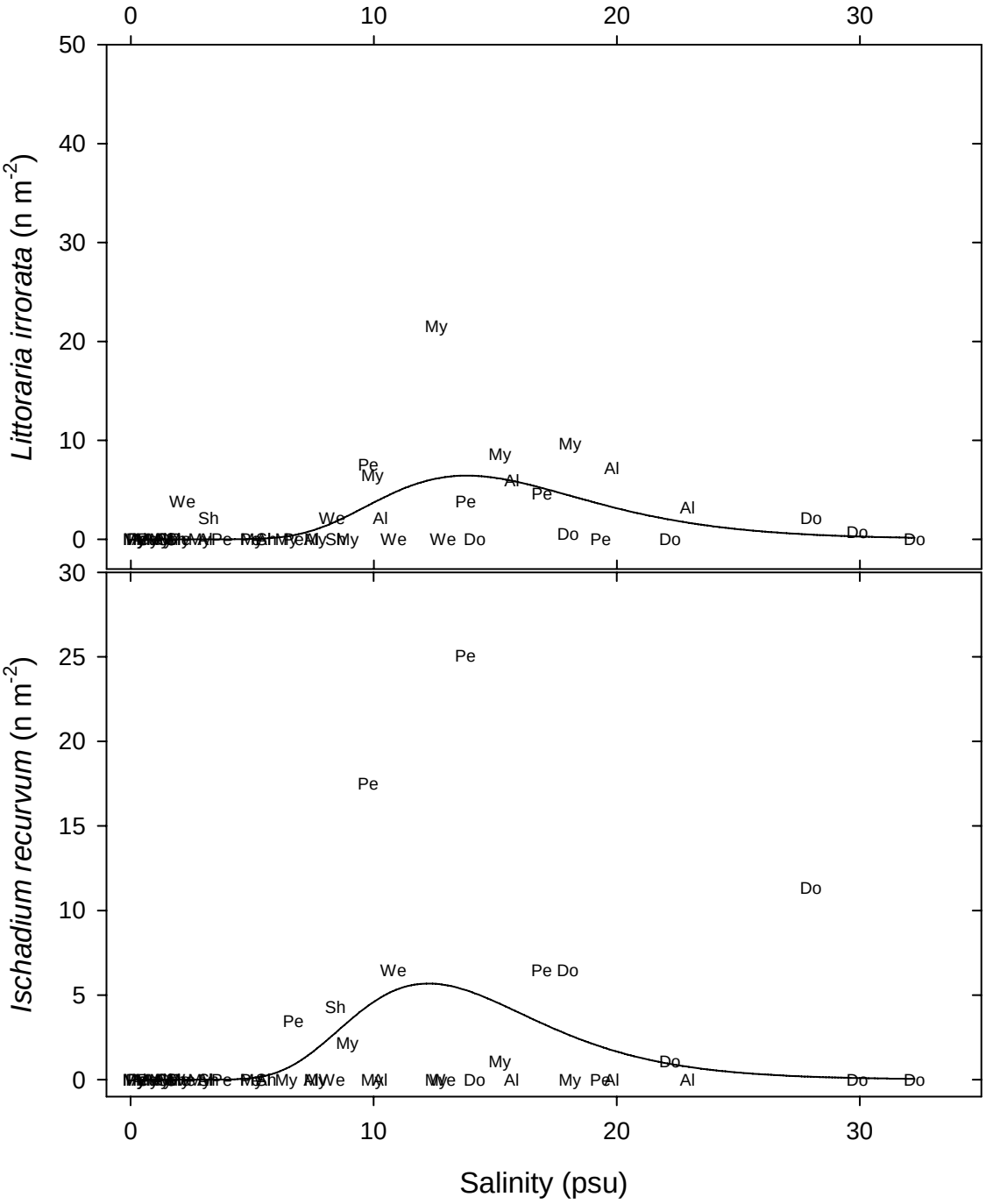


Figure 24. Relationship between salinity and species abundance. A. *Littoraria* irrorata, and B. *Ischadium recurvum*. Key: Al = Alafia River, Cu = Currey Creek, Dona/Roberts Bay, My = Myakka River, Pe = Peace River, Sh = Shakett Creek, She = Shell Creek, We = Weeki Wachee River.

## Discussion

The overall purpose of this project was to better define the physical and chemical requirements of mollusk species that inhabit tidal river systems in southwest Florida. To meet this purpose, an inter-river analysis was performed to examine relationships between freshwater inflows and the distribution of mollusk populations. Although the available data of mollusk species abundances and water quality were useful, the data was from independent investigations without regard to some larger, regional scale design and analysis. Thus, the data did not fit well into a sampling design that could be used toward the purpose of this report. The most important factor that inhibited a more comprehensive interpretation was that the mollusk samples were not taken in the same year (Table 2) and not always the same season. Two exceptions to this lack of synoptic sampling were the Myakka and Dona/Roberts Bay systems. The lack of synoptic sampling is important because the physical environment of an estuary is quite variable and strongly reacts to the different atmospheric events over short-term (e.g., storms) and long-term (e.g., seasonal or yearly weather cycles) temporal scales. Mollusks, as indicators of environmental change, are affected by these physical changes in an estuary. Therefore, by taking samples at different times, especially different years, the ability to compare the mollusk communities between estuarine rivers is impaired. In a stable estuarine river system, replicates could help to mitigate this problem, however, apart from the Peace River, there were no replicates reported. The water quality variables were also sampled over different time periods depending on the river sampled. This is not as great a problem as with the mollusk samples because many replicates were taken, which allows estimating the average conditions in a system. Caution has to be used when interpreting the current analysis because a poor assumption, that mollusk communities do not change over time, had to be made to allow the comparisons of rivers at a regional scale.

There was little similarity in the mollusk communities among all the rivers as most stations shared 25% or less species in common (Figures 12 and 13). Although sampling occurred over different years, there were community similarities at similar transect segments along each river. There were upstream clusters, downstream clusters, and larger clusters of intermediate range transects. The segments with the most similar mollusk communities occurred in the most upstream segments of the Peace, Myakka and Alafia Rivers. These segments had the most stable and lowest mean salinities (Figures 9 and 10), likely resulting from the minimal tidal influence in these areas. Further downstream, decreased and more variable freshwater influences, allows different species and communities to persist compared to stable upstream waters. Other factors such as tides, waves, currents, and inshore geomorphology create diversity both within and between estuarine river systems. This increase in physical diversity between rivers results in the higher differences in mollusk communities between rivers downstream than upstream.

The highest correlations between mollusk communities and any combination of physical variables (sediment or water quality), were dominated by water quality variables, especially salinity (Table 9). From this, it can be concluded that salinity differences is more important than sediment differences in regulating mollusk community habitats in southwest Florida. This conclusion by the way, is a conclusion that is robust, because it is independent of the problem of a lack of synoptic samples. The combinations with the highest correlations almost always included salinity, temperature and pH. The best single physical indicator of mollusk communities was salinity (Table 9). Because salinity is a direct indicator for freshwater inflow, this means that freshwater inflow is

the most important factor controlling mollusk communities. It also means that to assess the effects of freshwater inflow on mollusk communities in southwest Florida, confounding factors, e.g., sediment type, water temperature, are less important than the effects of freshwater inflow.

Species ranges were estimated by comparing mean salinity values for each transect-segment with abundances of mollusk species in those same segments (Figures 20 to 24, Table 11). *Corbicula fluminea*, *Rangia cuneata*, and *Neritina usnea* were the only common species that occurred at salinities below 1 psu. However *C. fluminea* was the best indicator of freshwater habitat, because densities were highest below 2 psu. *C. fluminea* is an introduced bivalve species can survive salinities up to 13 psu (Morton and Tong, 1985) however mostly occur in freshwater (Aguirre and Poss, 1999). *R. cuneata* has been noted as an indicator of a fresh- to brackish-water with an estimated tolerance of up to 20 psu (Swingle and Bland, 1974; Montagna and Kalke, 1995). *N. usnea* is a gastropod also common in fresh- to brackish-water salinities. *Polymesoda caroliniana* is a native brackish water bivalve (Gainey and Greenberg, 1977) also from the Corbiculidae family. In this current study, *P. caroliniana* was present at salinities between 1 and 20 psu. *P. caroliniana* is a good indicator because it is present in all creeks/sites. *T. plebius, Crassostrea virginica, Mulinea lateralis, Littoriaria irrorata,* and *Ischadium recurvum* are also good indicators for bracksish to seawater salinities. Total mollusk abundance and aggregated mollusk species diversity do not make good indicators for freshwater inflow across all rivers (Figure 17), but is useful within rivers (Figures 16, 18 and 19). In addition, there is evidence of seriation in the mollusk communities as evidence of the trend of transect numbers increasing from left to right in the MDS analysis (Figure 14).

In this limited analysis of southwest Florida mollusk communities, it is concluded that mollusk species are controlled more by water quality rather than the sediment they live in or on. The most important variable correlated with mollusk communities is salinity, which is a proxy for freshwater inflow. It is almost impossible to directly link community changes in response to inflow changes, because not replicates over time were carried out in the rivers sampled. Certain indicator species have been identified however, that characterize salinity ranges in southwest Florida rivers. These salinity ranges may be useful in predicting mollusk community reactions to alterations in salinity that result from actual or simulated changes in freshwater inflow.

Taking all samples in the same month as well as taking replicate samples over time would greatly improve the ability to accurately determine the relationships of mollusk communities relative to those in other rivers. Synchronization of sampling and sample replication would also improve the ability to accurately correlate between mollusk communities and freshwater inflows. The use of transect-segments in this study design is still appropriate however.

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