Water and Sediment Quality Survey of Threatened and Endangered Freshwater Mussel Habitat in the Chipola River Basin, Florida

by

Jon M. Hemming[1*], Parley Winger[2], Heath Rauschenberger[3], Karen Herrington[4], Peter Durkee[1], and Dan Scollan[1]

[1] U. S. Fish and Wildlife Service, Division of Ecological Services, 1601 Balboa Avenue, Panama City, Florida 32405

[2] U. S. Geological Survey, Patuxent Wildlife Research Center Athens, Warnell School of Forest Resources, University of Georgia, Athens, GA 30602

[3] U. S. Fish and Wildlife Service, Division of Ecological Services, 6620 Southpoint Drive South, Jacksonville, FL 32216-0958

[4] U. S. Fish and Wildlife Service, Fisheries Resource Office, 1601 Balboa Avenue, Panama City, Florida 32405

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ABSTRACT

Reduced habitat quality may be contributing to the decline of freshwater mussels in southeastern rivers. As part of an ongoing evaluation of the quality of freshwater mussel habitat in Gulf Coastal Rivers, the Chipola River was assessed during 2006 and 2007. Sediment samples were collected at 8 sites along the Chipola River on May 2-3, 2006 and analyzed for contaminants and tested in the laboratory for toxicity to *Hyalella azteca* using 29-d exposures to solid-phase sediment and 96-h exposures to sediment porewater. In addition, benthic macroinvertebrate populations were sampled at each site. Using the Sediment Quality Triad approach (chemistry, toxicity and in-situ benthic assemblages) in assessing habitat quality, 3 sites had lower habitat quality. Sites 1, 4 and 8 were considered impaired, with Site 4 being most impaired. *H. azteca* survival in porewater exposures was reduced and trace elements were elevated in sediments at these sites; however, concentrations were not considered exceedingly high. Water quality samples collected did not violate of the State of Florida's water quality standards. The lack of concordance among the test metrics (in-situ benthic assemblages were not impaired) at these sites suggests marginal habitat impairment.

Key Words: Water quality, sediment quality, macroinvertebrates, Chipola River, freshwater mussels

Acknowledgements

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INTRODUCTION

Freshwater Mussels

Animals classified as freshwater mussels (Family Unionidae) have been described as vital components of aquatic ecosystems, both ecologically and economically. These bivalve mollusks can have a large influence on total benthic biomass and are important participants in nutrient cycling and sediment dynamics (Newton 2003). However, both density and species diversity of these organisms in North America have declined to a large extent during the past century (Newton 2003). Unionid mussels are one of the most rapidly declining faunal group in the United States and constitute the largest group of federally listed endangered or threatened invertebrates. Over 70 percent of the 297 species and subspecies are listed as endangered, threatened, or of special concern (Williams *et al.* 1993, Neves 1997).

Although the causal factors for unionid declines are largely unknown, contributing factors may include sedimentation, disease, predation, changes in fish communities (used as larval host), alterations of river channels, commercial exploitation, environmental contamination, and introduction of exotic species (Fuller 1974, Havlik and Marking 1987, NNMCC 1998, Augspurger *et al.* 2003, Bogan 1993, Mummert *et al*. 2003, Newton 2003). However, most studies that have reported unionid declines provide only anecdotal evidence of casual mechanisms (Newton *et al.* 2003). Although causation has not been established, exposure to contaminants may have contributed to significant mussel losses (Newton 2003). Descriptions of localized mortality have been provided for chemical spills and other discrete point source discharges; however, range-wide decreases in mussel density and diversity may have resulted from the more insidious effects of chronic, low-level contamination (Naimo 1995, Newton 2003, Newton *et al.* 2003). As stated by Augspurger and others (2003), freshwater mussel experts often report chemical contaminants as factors limiting to unionids (Richter *et al.* 1997). They also noted the differential sensitivity of freshwater mussels that results in tolerances to some organic solvents and pesticides (Keller 1993, Keller and Ruessler 1997), but also high sensitivity of early life stages to contaminants such as chlorine (Goudreau *et al.*

1993), metals (Keller and Zam 1991, Jacobson *et al.* 1993), and ammonia (Goudreau *et al.* 1993, Horne and McIntosh 1979).

Newton (2003) described aspects of unionid life history that may make them important sentinels of habitat integrity. Adult mussels are large bodied, long-lived (30–130 years), sediment-dwelling invertebrate organisms. The exposure to the surrounding environment is greatly enhanced by their filter-feeding strategy. They are consequently exposed to contaminants that have been dissolved in water, associated with suspended particles, and deposited in bottom sediments (Newton 2003). Unfortunately, most toxicity data for freshwater mussels is from water-only exposures despite reports that sediment-associated contaminants contributed to declines of mollusks in several large rivers (Sparks and Sandusky 1981, Wilson *et al.* 1995).

The current challenge and focus in unionid ecotoxicology lies in the improvement of laboratory toxicological evaluations, particularly with respect to sublethal effects at developmental life stages. The link between these laboratory findings and field observations of long-term chronic exposures and multiple stressors may prove essential to the recovery of freshwater mussel species in North America and elsewhere (Newton 2003).

Freshwater Mussel Recovery

As described in the U.S. Fish and Wildlife Service (Service) freshwater mussel recovery plan, the fat threeridge (*Amblema neislerii*), shinyrayed pocketbook (*Lampsilis subangulata*), Gulf moccasinshell (*Medionidus penicillatus*), Ochlockonee moccasinshell (*Medionidus simpsonianus*), oval pigtoe (*Pleurobema pyriforme*), Chipola slabshell (*Elliptio chipolaensis*), and purple bankclimber (*Elliptoideus sloatianus*) freshwater mussel species have suffered population declines. Historically, these species of the eastern Gulf Slope rivers were known to have rich populations. The reduction and fragmentation of the freshwater mussel populations in these systems have resulted in species vulnerability to extinction. These rivers drain the Apalachicolan Region which extends from the Escambia River eastward to the Suwannee River system and includes portions of southeast Alabama, west-central and southwest Georgia, and north Florida.

Collectively, these rivers comprise the area's predominant drainage.

Within the eastern Gulf Slope drainage, the Chipola River Basin stretches from Alabama southward to the eastern Florida panhandle (Figure 1). The Chipola drainage provides important habitat for four federally listed endangered and one federally listed threatened freshwater mussels, including the Chipola slabshell, fat threeridge, Gulf moccasinshell, oval pigtoe, and shinyrayed pocketbook. Species richness (number of federally listed threatened or endangered species) of imperiled taxa appears to remain steady, however abundabce and distribution of these species cannot be ascertained from the available data (Figures 2-4).

The Service's goal is to restore viable populations of the Chipola slabshell, fat threeridge, Gulf moccasinshell, oval pigtoe, and shinyrayed pocketbook within their historical ranges. This survey included the identification of potential threats that have historically limited or currently limit freshwater mussel populations. Reduction or elimination of those limiting factors will allow for the successful re-establishment of these mussel populations so that their protection under the Endangered Species Act will no longer be required (USFWS 2003).

The objective of this Chipola River drainage survey was to determine water and sediment quality differences among sites. This comparative assessment of habitat quality was conducted to reveal factors that may be limiting freshwater mussel success. In completing the water quality risk assessment, two factors and three tasks outlined in the Service's Recovery Plan were addressed. To address these points, information was gathered and used in the ranking of sites needing protection, restoration, and/or eventual re-introduction of listed species. The aspects of the Recovery Plan addressed in this study are:

- Factor D – inadequacy of existing regulatory mechanisms (compliance).
- Factor E – factors affecting its [listed species] continued existence.
- Task 1.3.3 – Determine mechanisms and impacts of present and foreseeable threats to the species at the micro- and macro-habitat level, and watershed basis.
- Task 1.3.5 – Investigate the need for management, including habitat

improvement, based on new data such as … information on the impacts of existing threats.

- Task 3.5 – Identify and prioritize streams, stream reaches, and watersheds in need of protection from further threats to these species and their host fishes.

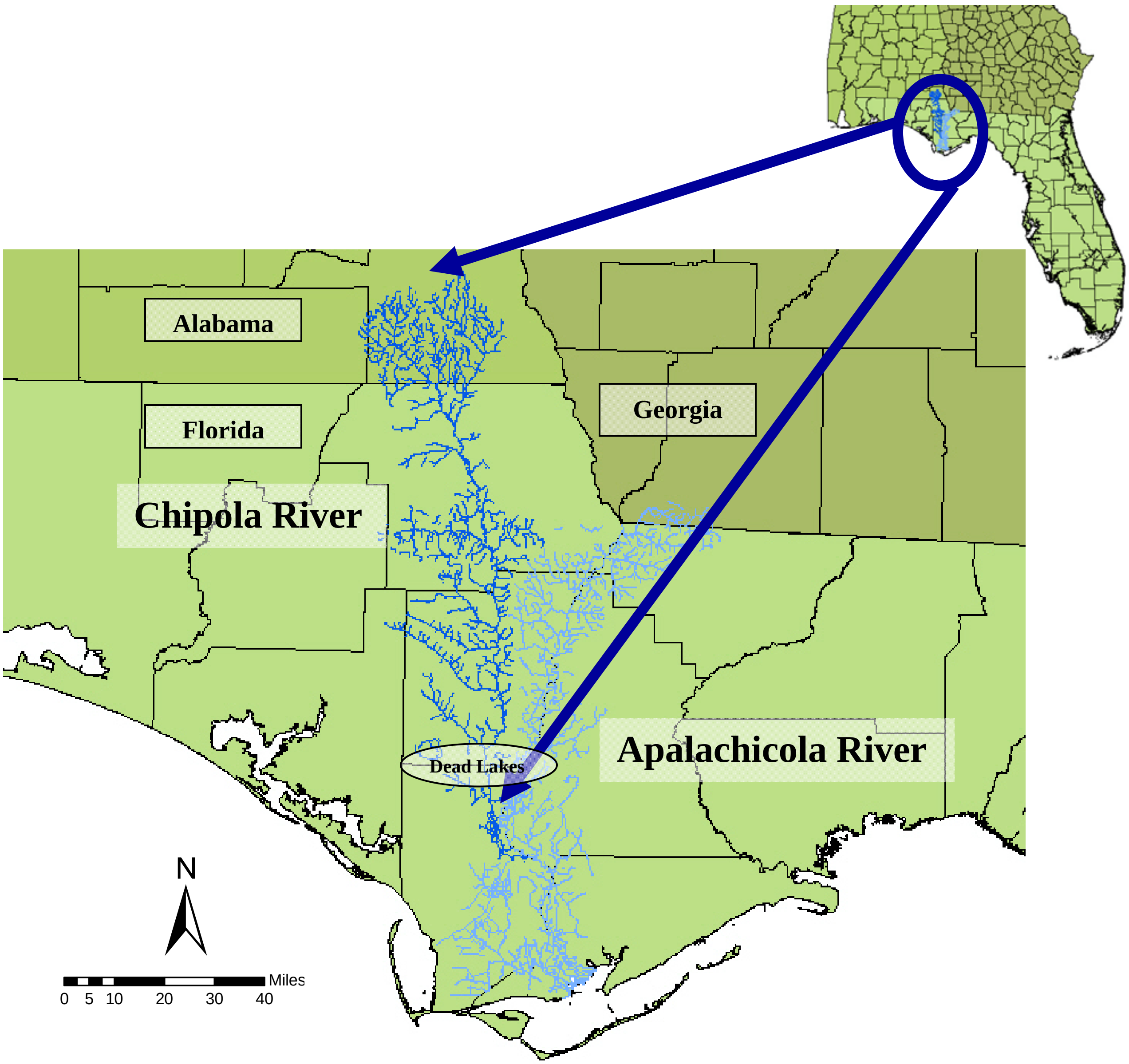

Figure 1. The location of the Chipola River flowing south from Alabama to the Apalachicola River in Florida.

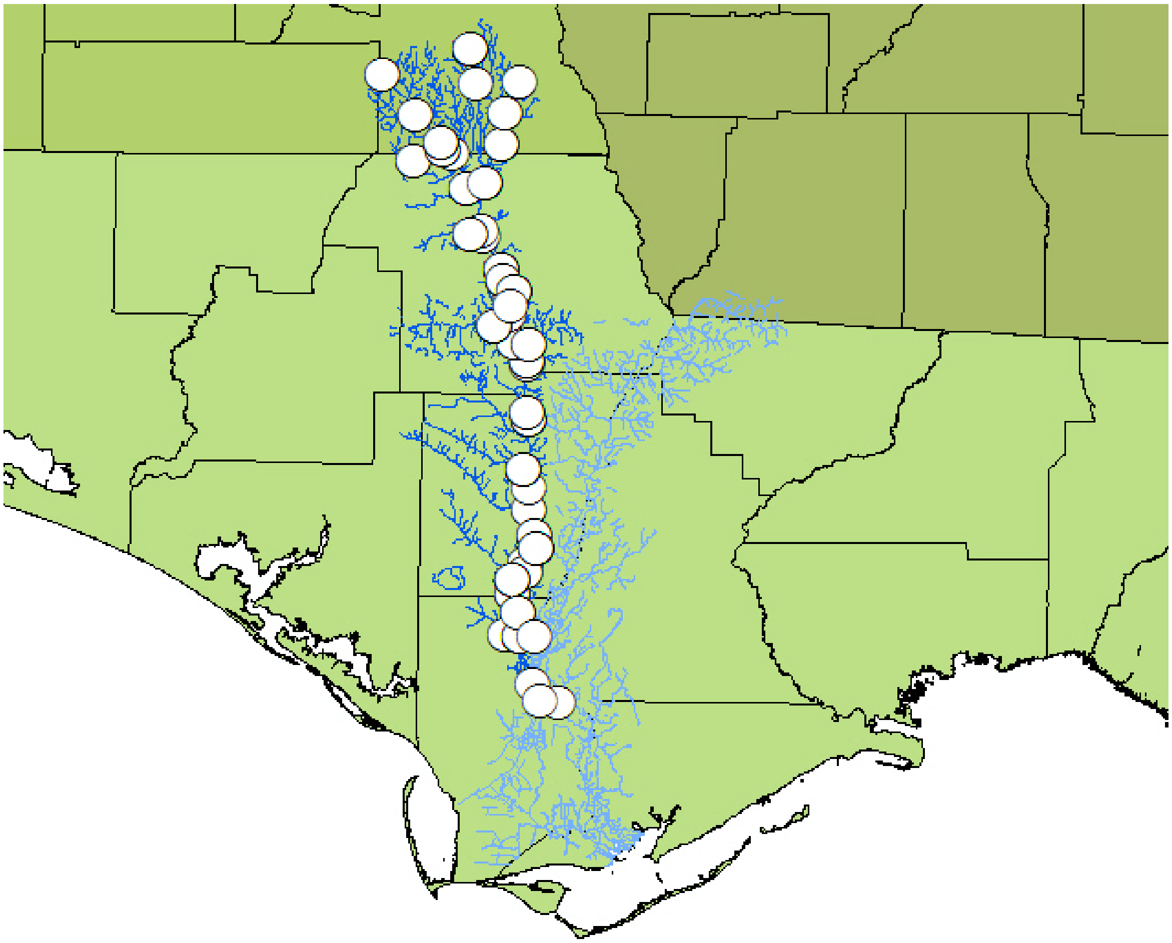

Figure 2. Historic and current mussel survey sites (white circles) in the Chipola River (USFWS freshwater mussel database).

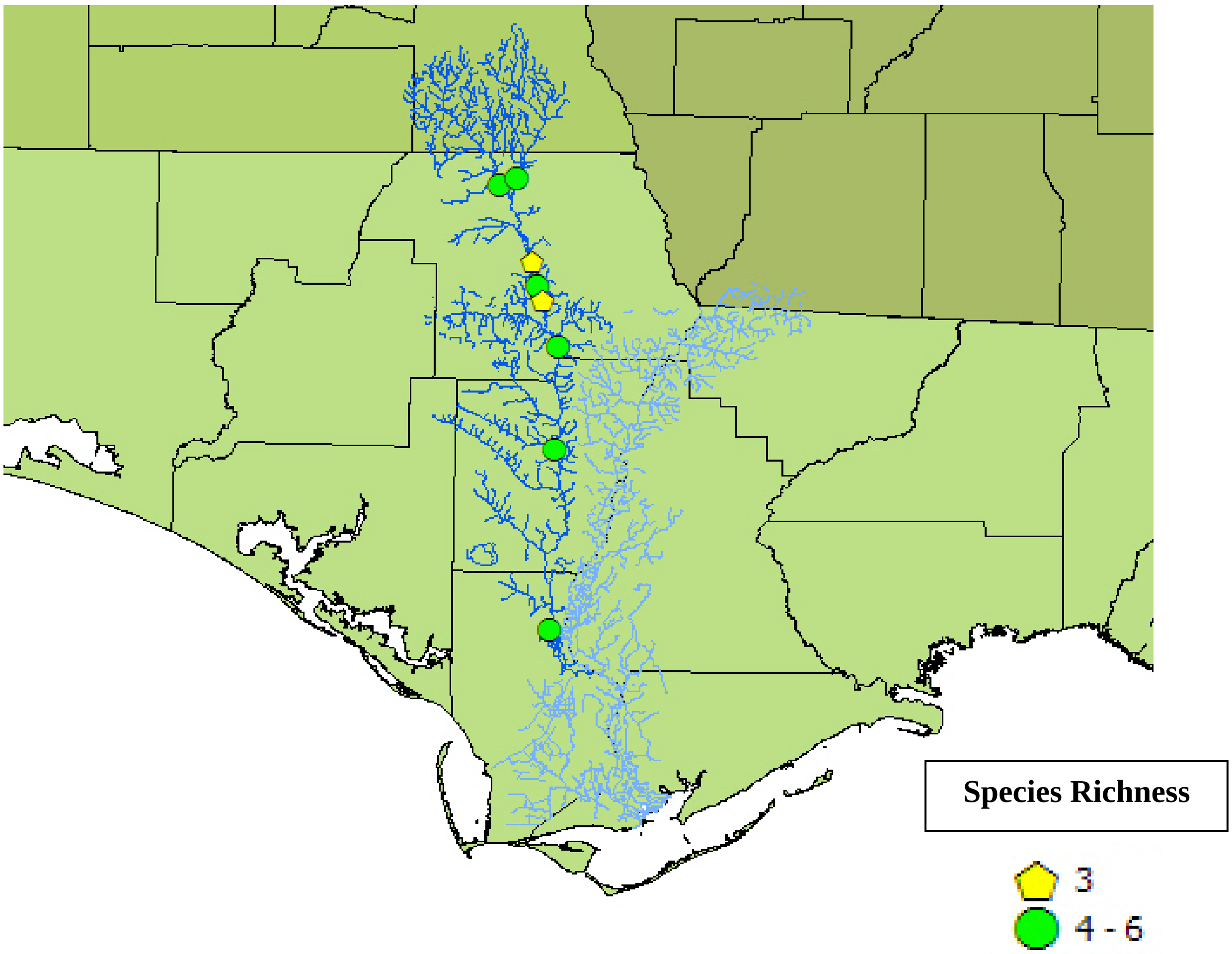

Figure 3. Species richness (number of different species) for federally listed threatened and endangered species of freshwater mussels in the Chipola River prior (including museum records as early as 1915) to 1990 (USFWS freshwater mussel database).

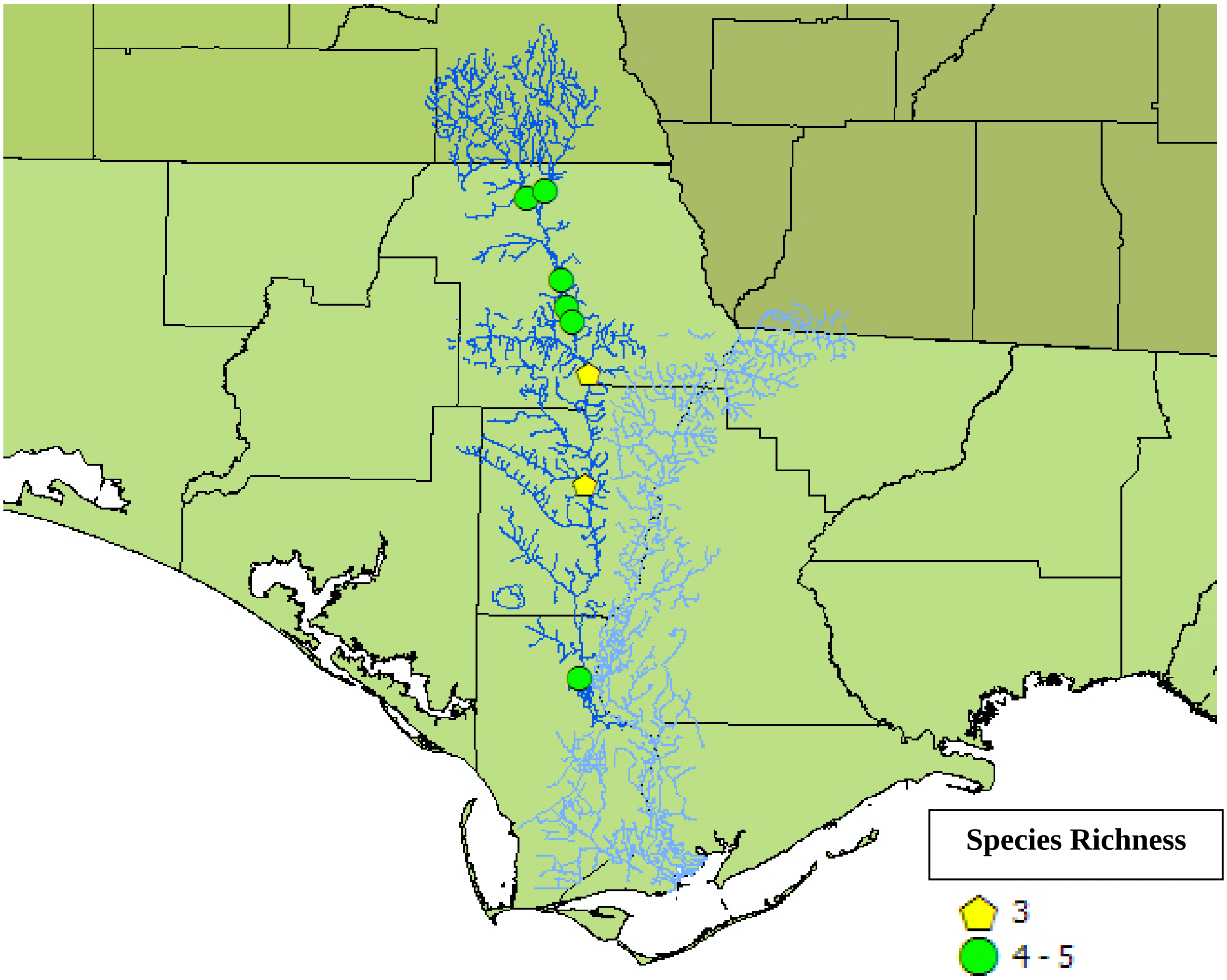

Figure 4. Species richness (number of different species) for federally listed threatened and endangered species of freshwater mussels in the Chipola River from 1990 to the present (USFWS freshwater mussel database).

METHODS AND MATERIALS

Ambient Water Quality

Ambient water quality was characterized for eight sites along the Chipola River distributed from the headwaters to near the mouth of the Chipola River (Figure 5). Sites were selected to correspond with historical mussel survey locations (see Figure 2, 6-13). Water column grab samples were taken from each site during high water, low water, and mean water discharge. Sampling was conducted during 2006 and 2007.

Water quality parameters included dissolved oxygen (mg l^{-1}), temperature (°C), pH (SU), chlorophyll *a* concentration (ug l^{-1}) as calculated from fluorescence, turbidity (NTU), specific conductance (uS cm^{-1}@25°C), alkalinity (mg $CaCO_3$ l^{-1}), and hardness (mg $CaCO_3$ l^{-1}). Dissolved oxygen, temperature, conductivity, pH, chlorophyll concentration and turbidity were monitored in the field using a YSI Model 6600 multiparameter data logger. The instrument included a rapid pulse dissolved oxygen probe, conductivity/temperature probe, fluorescence derived chlorophyll probe, nephlometric turbidity probe, pH probe and calculated salinity and total dissolved solids. Readings were taken 15 to 30 cm from the river bottom at each site. The data were recorded to a YSI 650 multiparameter display system. The remaining analyses were performed on aliquots from 1 L samples taken at each site. Alkalinity and hardness were measured in the laboratory with a HACH digital titrator Model 16900. Protocols for sample collection, preservation, and holding times followed American Public Health Association (1998) or HACH Company guidance.

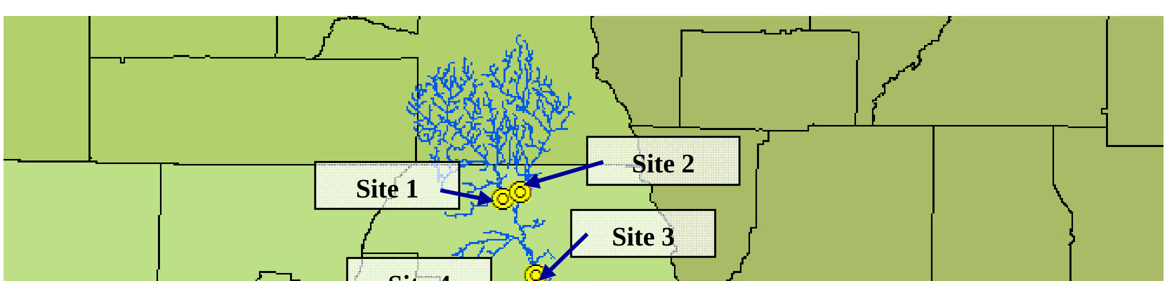

Figure 5. The location of U. S. Fish and Wildlife Service water quality sampling sites (yellow circles) on the Chipola River 2006-2007.

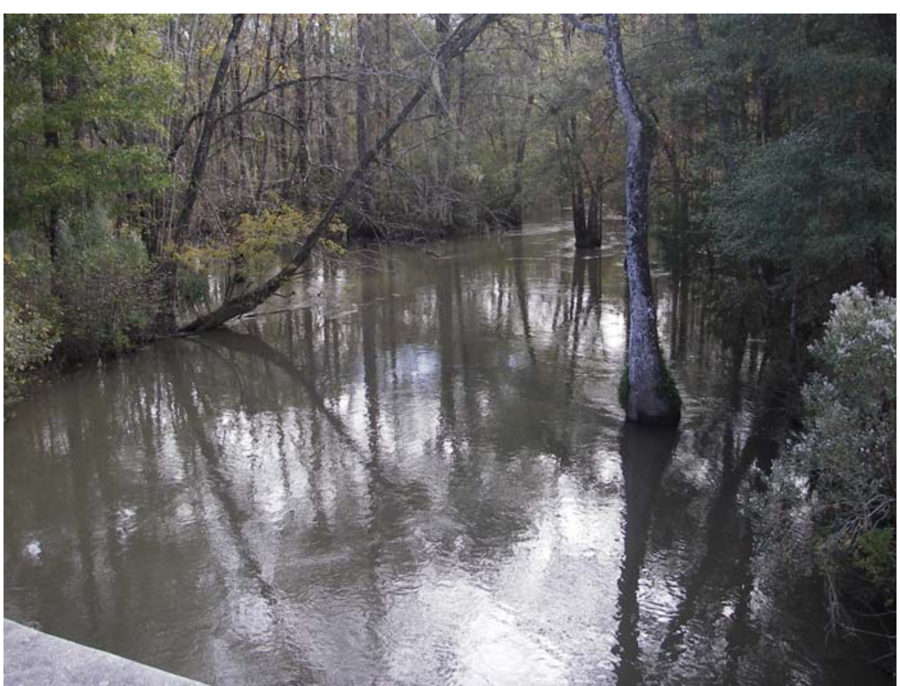

Figure 6. Photograph of the location of U.S. Fish and Wildlife Service water quality sampling Site 1 (in decimal degrees, 30.93645 N, 85.29662 W) on the Chipola River. Photograph taken at the intersection of Highway 2 and the Marshall Creek channel at the west end of the bridge on December 07, 2005.

Figure 7. Photograph of the location of U.S. Fish and Wildlife Service water quality sampling Site 2 (in decimal degrees, 30.94745 N, 85.25791 W) on the Chipola River. Photograph taken at the intersection of Highway 2 and the Cowarts Creek channel at the east end of the bridge on December 07, 2005.

Figure 8. Photograph of the location of U.S. Fish and Wildlife Service water quality sampling Site 3 (in decimal degrees, 30.79313 N, 85.22237 W) on the Chipola River. Photograph taken at the boat ramp on the northwest side of the intersection of Highway 166 and the Chipola River on December 07, 2005.

Figure 9. Photograph of the location of U.S. Fish and Wildlife Service sampling Site 4 (in decimal degrees, 30.74199 N, 85.21065 W) on Chipola River on December 07, 2005.

Figure 10. Photograph of the location of U.S. Fish and Wildlife Service sampling Site 5 (30.71728 N, 85.20040 W) on the Chipola River. Photograph taken at the boat ramp one quarter of a mile south of Interstate 10 at the Chipola River on December 07, 2005.

Figure 11. Photograph of the location of U.S. Fish and Wildlife Service water quality sampling Site 6 (in decimal degrees, 30.62745 N, 85.16521 W) on the Chipola River on December 07, 2005.

Figure 12. Photograph of the location of U.S. Fish and Wildlife Service sampling Site 7 (30.43143 N, 85.17168 W) on the Chipola River. Photograph taken at the boat ramp at the intersection of Highway 20 and the Chipola River on December 07, 2005.

Figure 13. Photograph of the location of U.S. Fish and Wildlife Service w sampling Site 8 (in decimal degrees, 30.08854 N, 85.17914 W) on the Chipola River. Photograph taken at the Land's Landing boat ramp off Highway 71 on December 07, 2005.

Statistical analyses on water quality data were performed using SAS version 9.1.3 (SAS Institute Inc, 2007). Statistically significant differences were accepted at α=0.05. Data were analyzed with parametric Analysis of Variance when assumptions of normality and homogeneity were met. The Tukey-Kramer Honest Significant Difference (HSD) multiple comparison tests (MCT) was used when differences were found. When parametric assumptions were not met, the non-parametric Kruskal-Wallis analysis was used with a ranked Tukey-Kramer HSD MCT when differences were found. Associations were examined via Pearson Correlation Analysis for parametric data and Spearman Correlation Analysis for non-parametric data. Data are presented as means plus one standard deviation.

Sediment Quality

Most anthropogenic compounds (inorganic and organic) entering aquatic systems eventually accumulate in the sediment matrix, which serves not only as a sink for these contaminants, but also as a potential source. Because of these unique characteristics, assessments utilizing the sediment matrix have been shown to provide useful information in the process of categorizing the quality of habitat in aquatic systems (Winger and Lasier 1995). Using the preponderance of evidence approach, examination of multiple lines of evidence provides a robust means of characterizing sediment quality. This evaluation of habitat quality in the Chipola River utilizes sediment chemistry (contaminant residues in the sediment), toxicity elicited through exposure to the sediment in the laboratory, and an evaluation of the in-situ benthic populations. These components provide the foundation for the Sediment Quality Triad that has been shown to provide useful information for the categorization of sediment quality (Chapman 1990).

Sediment samples were collected from the same eight water quality monitoring sites on May 2-3, 2006. Samples were collected with a stainless-steel petite Ponar grab. Sampling equipment was thoroughly rinsed between sites. Three to four grab samples from the top 10-15 cm of bottom sediment were composited in a stainless steel pan where these sediments were homogenized with a stainless steel spoon. Sticks and grass were removed during the homogenization process. Aliquots (500 ml) of the homogenized sediment sample were placed into glass jars, one for metals analyses and another for organic contaminant analyses. Approximately 4 L of sediment from each site were transported to the laboratory for toxicity testing and sediment characterization. Sediment samples were held in the dark at 4°C pending testing and analyses.

In addition to sediment samples, benthic organisms were also collected at each site. Aquatic dip nets were used to collect benthic organisms from all available habitat types at each site shallow enough to wade, typically sites found upstream. However, downstream sites were in the larger river area and therefore were collected by boat via Ponar grabs of bottom sediments or using an aquatic dip net to sample snags and patches of aquatic vegetation. Three samples of at least 100 benthic macroinvertebrate organisms were picked live from white sorting pans using forceps and pipettes. Benthic samples were preserved using a mixture of ethanol, methanol, glyoxal, iodine, propionic acid, and formalin. In the laboratory, benthic organism were identified to the lowest practical taxonomic unit (generally genus) using the following taxonomic keys: Brigham *et al.* 1982; Daigle 1991, 1992; Epler 1996, 2001, 2006; Parrish 1975; Pennak 1978; Pescador and Richard 2004; Pluchino 1984; Thompson 2004. Metrics used in the assessment of benthic community structure were total number of taxa, Sequential Comparison Index (Cairns and Dickson 1971), Shannon Weaver Diversity (Poole 1974), and percentage comprised by the 3 most numerically dominant taxa (Plafkin *et al.* 1989).

In the laboratory, sediments were tested for toxicity and physical and chemical characteristics were determined. Toxicity was assessed following procedures described by Ingersoll *et al.* (1994), except the time of exposure was increased from the described 10-d period to a 28-d exposure period, to provide a more sensitive measure of chronic effects. Prior to testing, each sample was re-homogenized and two 100-ml aliquots were taken: one for physical characterization and acid volatile sulfide and simultaneously extracted metals, and one for metal analyses. From each sediment sample and the laboratory control sediment, five replicate samples were prepared for toxicity testing. The laboratory control sediment consisted of sand conditioned for two weeks in moderately hard water and a mixture of *Selenastrum* (algae) and YCT (yeast, Cerophyl and trout chow). Each replicate consisted of 100 ml of sediment and 175 ml of laboratory reconstituted water placed in a 300 ml high-form beaker with a notch in the lip covered with a stainless steel mesh (250 µm). The reconstituted water was prepared following guidelines given in Ingersoll *et al.* (1994) and consisted of deionized water, calcium sulfate, calcium chloride, magnesium sulfate, sodium bicarbonate, and potassium chloride providing a hardness of 100 mg/l, 70 mg/L alkalinity, 350 µS/cm conductivity, and a pH of 8. The five replicates were randomly positioned on the static-renewal testing system that replaced the overlying water twice daily (Zumwalt *et al.* 1994). Ten 7-d old *Hyalella azteca* (Crustacea: Amphipoda) were placed into each test chamber. Test chambers were maintained at 23 $\pm$ 1°C under wide-spectrum fluorescent lights with a 16-h light and 8-h dark regime. Animals were fed 1.5 ml YCT (1.8 g solids/L) daily. Solid-phase sediments were tested under static-renewal conditions, with two renewals daily (Ingersoll *et. al.* 1994). Test endpoints for the 28-d static- renewal tests on sediments were survival and growth. Growth was determined by measuring the length of a projected image of *H. azteca* using a microscope slide projector calibrated with a stage micrometer. Chemistry of the overlying water was monitored during the test and consisted of temperature, dissolved oxygen, pH, alkalinity, hardness, conductivity, and ammonia. Meters and electrodes were used to measure water chemical parameters, except alkalinity and hardness were measured by titration.

In addition to testing solid-phase sediments, sediment porewaters were also evaluated for toxicity using 96-h static exposures (Winger and Lasier 1995). Porewater was isolated from the sediment by using vacuum extractors (Winger and Lasier 1991). Ten extractors (each consisting of a 60 cc syringe, airline tubing and a fused glass air stone) were inserted into each sediment sample and a vacuum applied by extending and bracing the plunger. Approximately 300 ml of porewater were extracted from each sediment sample. A 20 ml aliquot for analyses of trace elements was filtered through a 0.45 µm filter and acidified with ultra-pure nitric acid. The remaining porewater was aerated for 15 minutes prior to test initiation. Five replicates of each sample were prepared for testing and each consisted of 20 ml of porewater, ten 7-d old *H. azteca* and a 1 cm^2 of Nitex netting (275 µm) in a 30 ml plastic cup. The animals were not fed during the test. The test endpoint for the 96-h static exposures to porewater was survival. The same basic chemistry measured in the overlying water in the solid-phase sediment tests were measured in the porewater after aeration.

Sediments were characterized by measuring percentage of organic content, particle size analyses, acid volatile sulfides (AVS) and the simultaneously extracted metal concentrations (SEM). Organic content was estimated by loss on ignition at 430°C for 4 hours (Davis 1974). Particle size analyses were determined using methods described by Miller and Miller (1987), except that coarse organic material was measured by loss on ignition and subtracted from the total. Acid volatile sulfides (AVS) were measured following procedures described by Brouwer and Murphy (1994). Simultaneously extracted metals (Cd, Cu, Hg, Ni, Pb, Zn) were measured in the AVS digestates after they were passed through a 0.45 µm nylon filter. Trace elements (As, Ba, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Se, Zn) in porewater and the AVS digestates were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Total organic carbon in porewater was determined after acidification with a Leco CR-412 carbon analyzer (St. Joseph, MI, USA) calibrated with calcium carbonate. Chloride and sulfate were measured using an ion chromatograph. Polynuclear aromatic hydrocarbons (PAHs) were measured on sediment samples after extraction with petroleum ether and methylene chloride using gel-permeation chromatography.

Analyses were within acceptable limits for precision and accuracy based on quality assurance data that included blanks, duplicates, spikes, and standard samples. The ICP-MS instrument detection limits for trace elements were as follows (in µg/L): Ag, 0.054; As, 0.183; Ca, 12.78; Cd, 0.018; Cu, 0.129; Cr, 0.918; Fe, 2.89; k, 13.07; Hg, 0.009; Mg, 0.135; Mn. 0.213; Na, 0.033; NI, 0.177; Pb, 0.015; Se, 0.609; Zn, 0.528. The limit of quantitation was established as three times the lower limit of detection. The mean relative standard deviation between duplicate samples of porewater was 8.57($\pm$ 8.59)%, with the high of 31.51 for Sn and the low of 0.00 % for Cr, Se, Ag, and Cd. Blanks were below the instrument detection limits and recovery from spiked samples averaged 72%. For sediments, the mean relative standard deviation between duplicates was 10.74($\pm$ 11.74)%, with a high of 42.85% for Cd and a low of 0.57% for Na. The relative standard deviation for laboratory replicates was 7.6($\pm$ 7.85)%.

Shapiro-Wilks tests for normality were performed on the data and then ANOVA and Dunnett's pair-wise tests were used to evaluate differences ($p < 0.05$) with the controls. Spearman rank correlations among variables and test parameters were determined. All statistical analyses were performed using Statistical Analysis Systems (SAS 1990).

Evaluation of Potential Risk

A risk score estimating the relative threat that ambient conditions may cause sediment-dependent freshwater species was derived with a modification of the approach used by Hemming *et al.* (2006). In this evaluation, a risk score was estimated for each of the following categories: sediment toxicity (porewater and whole sediment), relative *in-situ* benthic macroinvertebrate community health, ambient water quality, sediment metals, pore-water metals, sediment general chemistry, and porewater general chemistry. Each category had multiple occasions to score risk points. For example, sediment toxicity included three separate tests, one acute test for pore-water, one acute test for whole sediment, and one chronic test for whole sediment. Each qualifying occasion received a score of one and was summed with all such occurrences in a category. After all categories were scored, all risk points for all categories were summed by site.

The risk from sediment toxicity for aquatic life was estimated from porewater and solid-phase exposures of *Hyalella azteca* (acute and chronic assays). A score of one was assigned for significant differences from the control for each test and scores for all tests were summed by site. Benthic macroinvertebrate community assemblage data were compared among sites and to reference data to evaluate if important differences were present. If relatively low measures for total number of taxa, Sequential Comparison Index, Shannon Weaver Diversity, and percentage comprised by the 3 most numerically dominant taxa, impairment of the macroinvertebrate community was suspected and the site received individual score of one. Risk to aquatic life was assigned for overall ambient water quality for the parameters dissolved oxygen, temperature, pH, chlorophyll concentration turbidity, and conductivity. Each violation of a State of Florida water quality standard (FAC 2004) or federal water quality criterion (USEPA 2002) constituted an individual score of one. Risk via exposure to whole sediment metals was estimated by comparison of sediment metals analytical data to reference values such as those of MacDonald *et al.* (2000). Each exceedance of the sediment quality guidelines constituted an individual score of one. Similarly, porewater metals risk was estimated by comparison of metal pore-water constituents to State of Florida surface water quality standards or federal water quality criteria. Violations received a score of one each. Sediment quality risk to aquatic life stemming from general sediment chemistry was based on reference values for relative sediment quality. Violations of the guidelines provided by Di Toro *et al.* (1992) received a score of one each. Finally, potential risk to aquatic life associated with sediment porewater chemistry was determined by comparison to State of Florida surface water quality standards or federal water quality criteria or pertinent recommendations thereof (Augspurger *et al.* 2003). Violations received a score of one each.

RESULTS AND DISCUSSION

Ambient Water Quality

According to historic average flows estimated by measures taken by the U.S. Geological Survey gage on the Chipola River at Altha, Florida (USGS 02359000), most water quality sampling (n=6) was conducted at low flow conditions (553 to 670 cm^3/sec), however one sample was taken at both the median flow condition (Sample 2, 1,180 cm^3/sec) and a higher flow condition (Sample 8, 1,910 cm^3/sec). Drought conditions prevented further sampling under median or higher flow conditions.

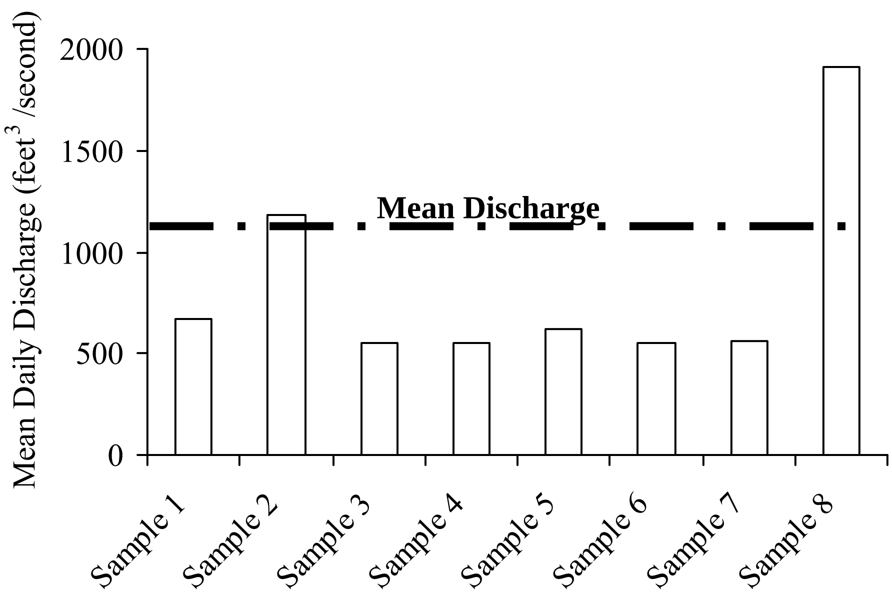

Figure 14. Discharge rates during water quality sampling on the Chipola River during 2006 and 2007 according to the U.S. Geological Survey gage on the Chipola River at Altha, Florida (USGS 02359000). Dashed line at 1,130 ft^3/sec indicates the average discharge based on 72 years of record (1935-2007).

Water quality on the Chipola River was unremarkable during the sampling sessions (Figures 15-22). Although statistically significant differences were observed among the sites for dissolved oxygen (mg l^{-1}), chlorophyll *a* concentration (ug l^{-1}) turbidity (NTU), specific conductance (uS cm^{-1}@25°C), alkalinity (mg $CaCO_3$ l^{-1}), and hardness (mg $CaCO_3$ l^{-1}), none appeared to be ecologically relevant. Differences among sites are depicted in the accompanying figures.

No parameters were observed to be in violation of State of Florida or State of Alabama water quality standards. Temperature ranged from 9.2 to 30.7ºC throughout the year. Specific conductance (uS/cm@25°C) ranged from 76 to 297 over the course of the river. The dissolved oxygen concentration was high ranging from a low of 6.9 mg/l to supersaturation. However, it should be noted that all sampling was conducted during the day and nocturnal dissolved oxygen depressions would have not been recorded.

Hydrogen ion concentration (pH) was measured in the Chipola from 7.1 to 8.3 in standard units during the sampling year. Relative turbidity (range river-wide <3.0 to 31.2 NTUs) seemed to vary more when compared to relative chlorophyll *a* concentration (0.3 to 8.2 ug/l as estimated via fluorescence). Neither turbidity nor chlorophyll a concentration (as estimated by community imbalance) violated water quality standards. Alkalinity was measured to be from 11 to 132 mg $CaCO_3$/l during the study and hardness was very similar ranging from 50 to 131 mg $CaCO_3$/l.

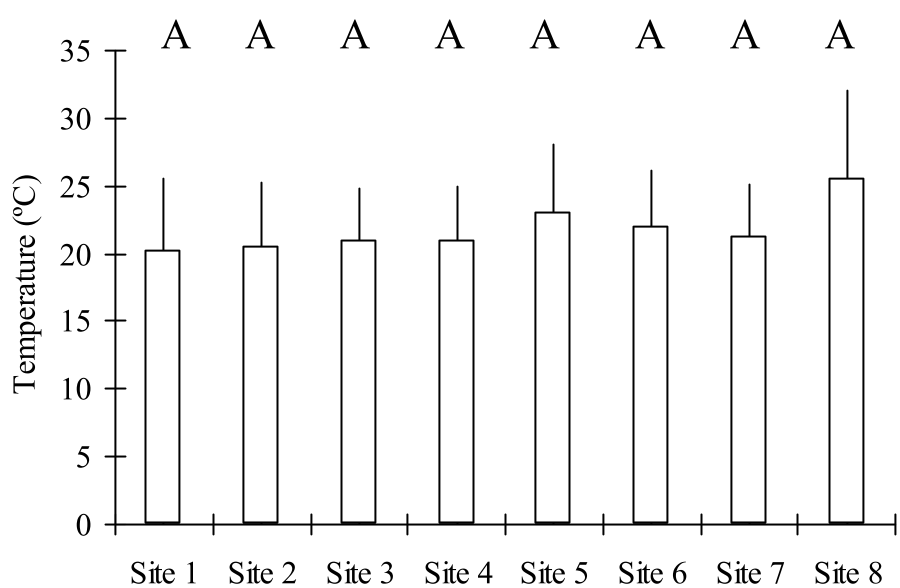

Figure 15. Mean temperature (°C) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

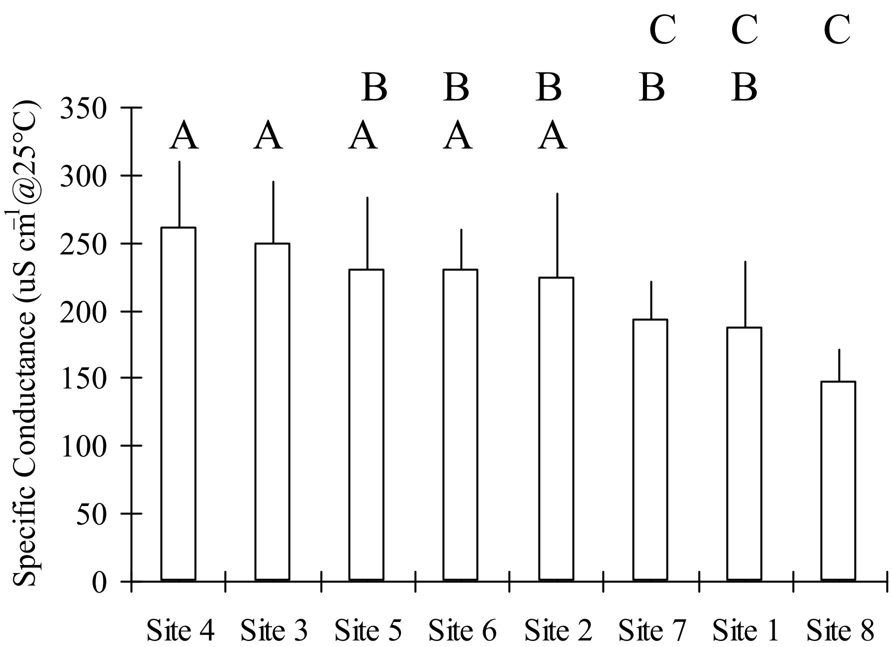

Figure 16. Mean specific conductance (uS/cm@25°C) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

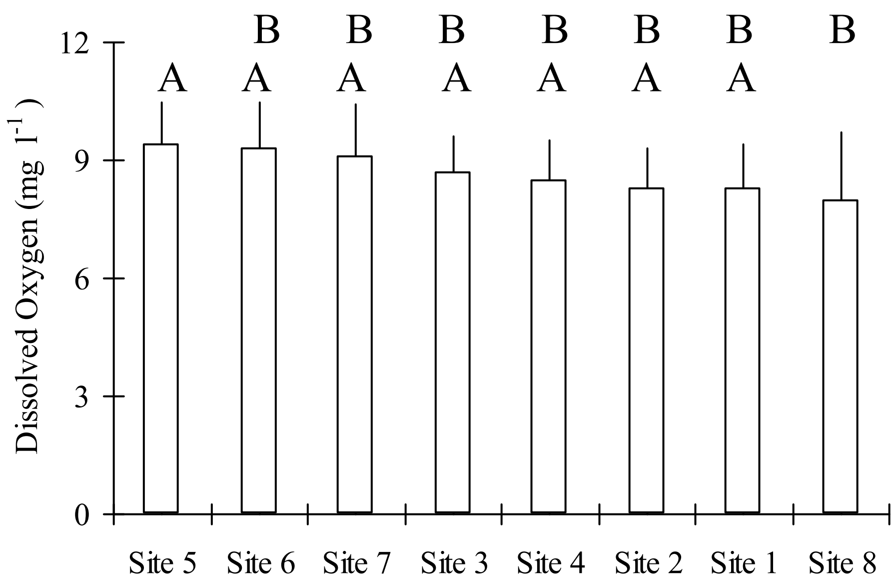

Figure 17. Mean dissolved oxygen (mg/l) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

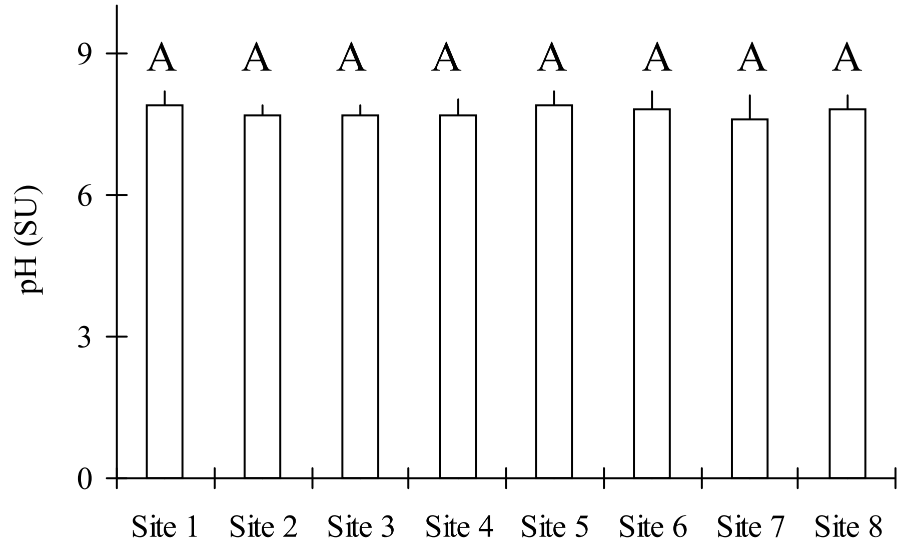

Figure 18. Mean pH (SU) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

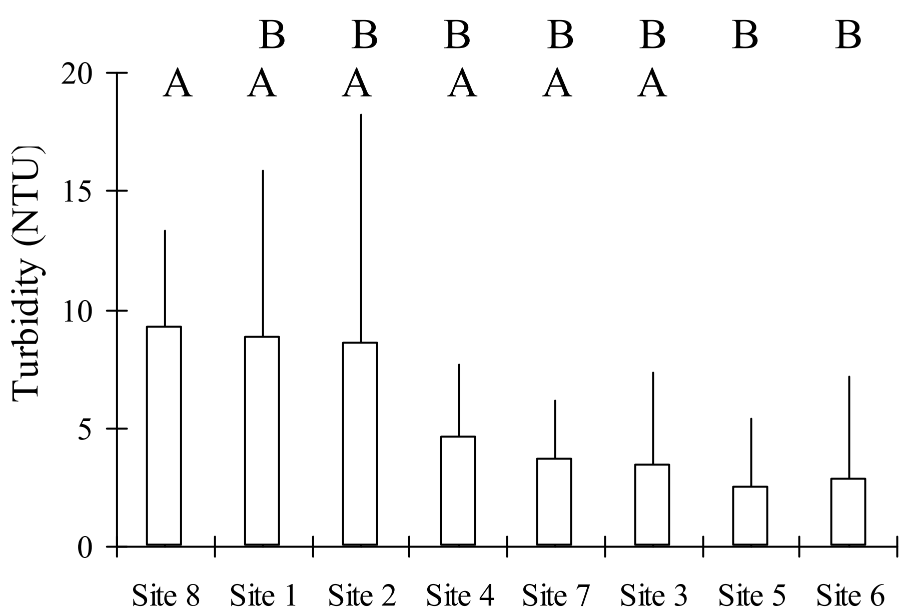

Figure 19. Mean turbidity (NTU) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

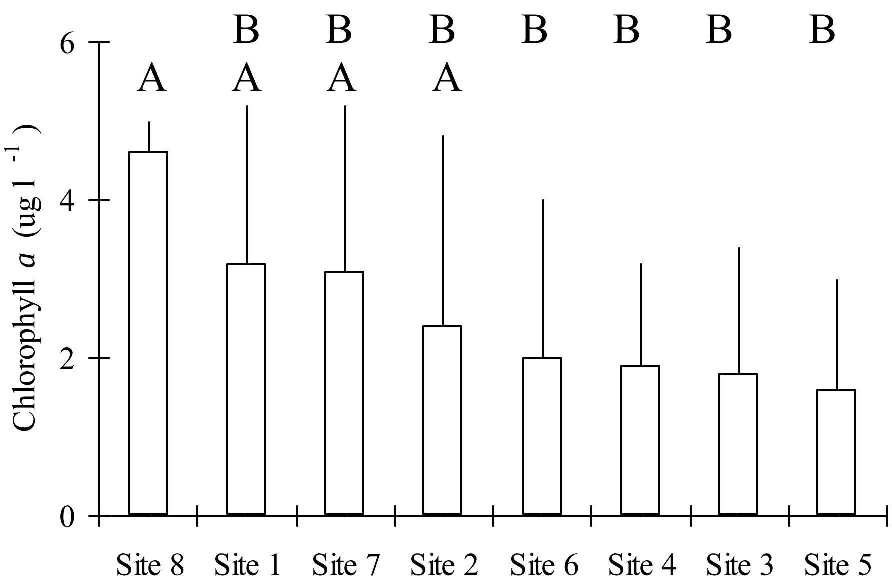

Figure 20. Mean chlorophyll *a* concentration (ug/l, as calculated from fluorescence) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

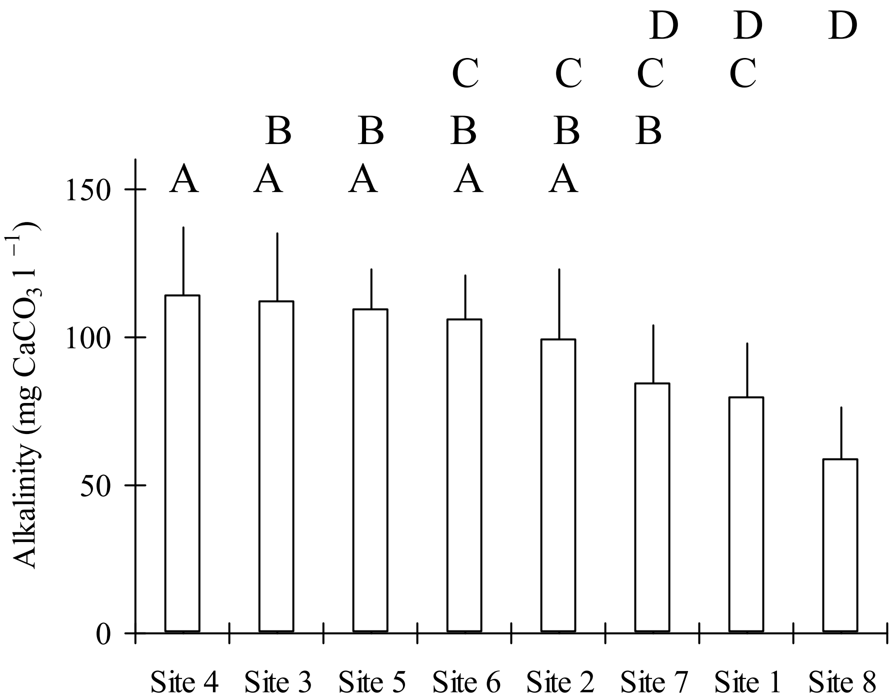

Figure 21. Mean alkalinity (mg $CaCO_3$/l) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

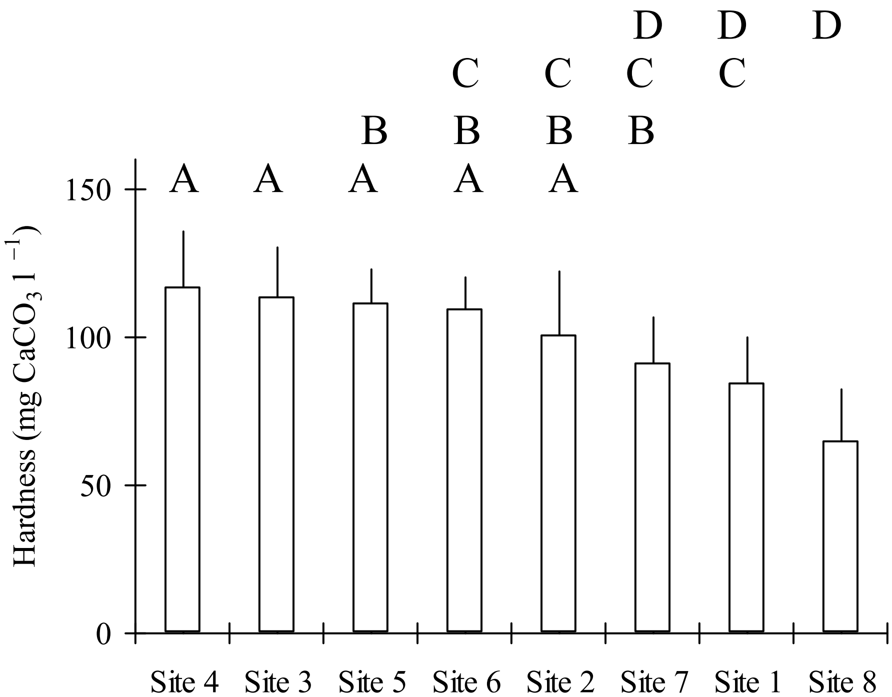

Figure 22. Mean hardness (mg $CaCO_3$/l) and one standard deviation for ambient water quality samples taken at eight sites on the on the Chipola River during 2006 and 2007. Sites sharing the same letter are not different from each other (Tukey MRT).

Almost all water quality parameters showed a significant correlation with the discharge rate as estimated by the U.S. Geological Survey gage on the Chipola River at Altha, Florida (USGS 02359000) with the only exception being pH (Appendix 2). The correlation was likely influenced by the low flow water quality condition being represented by six replicate measures per site, but the median and high flow measures being represented by one sample per site. The survey was designed to capture more median and high flow events to better represent variable associations, however drought conditions prevented this.

Although the associations were statistically valid, the correlation coefficients (showing degree of association, between 0 and 1.0) were not particularly high for specific conductance (r= -0.407), dissolved oxygen concentration (r= 0.304), hardness (r= -

0.388), alkalinity (r= -0.512), or turbidity (r= 0.495). Stronger associations with discharge rate were observed for both temperature (r= -0.727) and chlorophyll *a* concentration (r= 0.632). Other noteworthy significant correlations included those between water clarity and dissolved solids. For example, chlorophyll concentration was associated with specific conductance (r= -0.787), alkalinity (r= -0.831) and hardness (r= -0.857). Similar associations was also observed for turbidity and specific conductance (r= -0.664), turbidity and alkalinity (r= -0.718) and turbidity and hardness (r= -0.614). Another significant association worth noting was between turbidity and chlorophyll *a* concentration (r= 0.704), which may indicate that the water clarity of the Chipola River is driven by both organic and inorganic contributions. However, no association was observed between dissolved oxygen concentration and chlorophyll concentrations that may have explained primary productivity related to the organic component.

Sediment Quality

Survival of *H. azteca* was significantly reduced in exposures to porewater from Sites 1, 4, 7, and 8 compared to laboratory control sediments (Table 1). However, survival and growth from solid-phase exposures were not significantly reduced, although growth was lower at Sites 1, 4, and 8 compared to growth on sediments from the other sites tested. Basic chemistry in porewater and overlying water from the solid-phase tests were generally within acceptable limits; however, ammonia was elevated in porewaters from Sites 3, 4, 5, 6, 7, and 8 (Table 2). This elevation in porewater ammonia at these sites may have toxicological implications for freshwater mussels (Augspurger *et al.* 2003), particularly for juvenile stages that spend more time feeding in pore-waters (Yeager *et al.* 1994; Neves *et al.* 1987; Reid *et al.* 1992). The characteristics of the solid-phase sediments were also within acceptable ranges, except that the ratio of SEM/AVS exceeded the threshold value of 1 at Sites 2 and 4 (Table 3). SEM/AVS ratios greater than 1 suggest that the metal concentrations in the sediments exceed the sulfides and may be biologically available to cause toxicity (Di Toro *et al.* 1992).

Table 1. Toxicity measured as percent survival and growth of *Hyalella azteca* after exposure for 96-h to porewater and 28-d to solid-phase sediments collected from the Chipola River, May 2-3, 2006.

Test parameter	**Site**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Control**
Porewater survival (%)	78*	92	98	58*	98	92	76*	32*	98
Sediment survival (%)	98	98	100	100	96	96	94	98	98
Sediment growth (length in mm, +/- 1 SD)	4.4 ±0.5	4.6 ±0.4	4.6 ±0.6	4.3 ±0.5	4.4 ±0.5	4.8 ±0.4	4.6 ±0.5	4.3 ±0.4	4.4 ±0.6

* Significantly reduced compared to control ($p < 0.05$)

Table 2. Water chemical characteristics of porewater and overlying water from toxicity tests on porewater and solid-phase sediments from samples collected from the Chipola River, May 2-3, 2006.

Parameter	**Site**								
Porewater	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Control**
Temperature (°C)	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7	22.7
Dissolved Oxygen (mg/L)	6.9	8.0	7.8	7.4	7.0	5.7	7.4	7.8	8.0
pH	8.25	8.18	8.22	8.29	8.24	8.16	8.13	8.18	8.26
Conductivity (µS/cm)	536	319	721	492	644	527	486	385	446
Alkalinity (mg/L $CaCO_3$)	280	186	400	292	398	318	282	228	102
Hardness (mg/L $CaCO_3$)	247	187	422	275	344	201	229	134	145
Ammonia (mg/L NH_3)	1.9	2.2	10.3	9.7	7.1	9.4	8.5	6.2	0
Chloride (mg/L)	11.6	2.75	14.66	2.84	3.13	3.34	3.08	1.73	32.3
Nitrate (mg/L)	3.1	0.0	0.0	0.0	0.0	0.0	7.0	0.0	0.0
Sulfate (mg/L)	27.09	1.35	5.47	17.96	24.80	2.66	3.12	24.25	88.36
Organic carbon (mg/L)	9.49	4.24	18.90	6.94	6.94	8.71	7.90	15.38	1.83
Inorganic carbon (mg/L)	61.87	44.00	104.50	71.84	95.66	74.50	68.85	55.36	23.86
Total carbon (mg/L)	71.4	48.2	123.4	78.8	102.3	83.2	76.8	70.7	25.7
Overlying Water	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Control**
Temperature (°C)	23.5	23.5	23.5	23.5	23.5	23.5	23.5	23.5	23.5
Dissolved oxygen (mg/L)	7.18	7.01	6.62	6.77	6.72	6.47	6.42	6.75	6.90
pH (SU)	8.44	7.80	7.83	7.70	7.73	7.71	7.74	7.64	7.65
Conductivity (µS/cm)	312	334	325	314	318	313	320	297	286
Alkalinity (mg/L $CaCO_3$)	90	92	90	86	86	82	88	74	80
Hardness (mg/L $CaCO_3$)	120	110	122	119	118	121	121	120	118
Ammonia (mg/L NH_3)	0	0	0.1	0.1	0	0	0.1	0.1	0

Table 3. Characterization (physical characteristics, acid volatile sulfides, and simultaneously collected metals) of sediments collected from the Chipola River, May 2-3, 2006.

Parameter	**Site**								
Sediment characteristics	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Control**
Moisture (%)	24.0	42.8	40.3	33.6	29.3	27.7	44.5	47.4	23.6
Total organic matter (%)	2.4	5.4	4.7	2.3	1.4	0.6	6.7	4.9	0.0
Course organic matter (%)	1.0	4.0	4.0	2.0	1.0	0.0	6.0	5.0	0.0
Sand (%)	88	90	80	96	95	95	85	49	93
Silt (%)	1	5	10	0	2	3	9	29	7
Clay (%)	11	4	9	4	3	2	6	22	1
AVS (µmol/g)	0.14	0.10	2.51	0.21	0.00	0.00	0.44	2.13	0.00
SEM (µmol/g)	0.10	0.14	0.20	0.87	0.10	0.06	0.26	0.52	0.02
SEM/AVS	0.74	1.36	0.08	4.13	-	-	0.59	0.25	-
Copper (ng/g)	53	23	25	233	11	5	17	73	3
Cadmium (ng/g)	2	9	6	4	5	3	12	5	0
Tin (ng/g)	3	1	1	3	1	1	1	2	1
Mercury (ng/g)	1	1	0	0	0	0	0	0	0
Lead (ng/g)	100	82	192	140	65	31	124	227	9
Zinc (ng/g)	116	132	208	1130	131	90	291	535	35

The highest concentrations of As and, to a lesser extent, Ni in porewater occurred at sites in the middle of the study range (Table 4). Concentrations of As, Cu, Ni, Pb, and Zn were elevated in sediments, especially at Sites 3, 4, 7 and 8 (Table 5). Although these concentrations were elevated over those at other sites on the river, they were not alarmingly high (MacDonald *et al.* 2000, Eisler 1988a, 1988b, 1993, 1997, 1998). The total concentrations of these trace elements could contribute to a reduction in overall habitat quality at those sites where they are elevated, however there were no statistically significant correlations between trace element concentrations and test metrics (survival or growth).

Table 4. Concentrations of trace elements in porewaters from sediments collected May 2-3, 2006 from the Chipola River, Florida.

Site	**Ag (ug/L)**	**As (ug/L)**	**Ca (mg/L)**	**Cd (ug/L)**	**Cu (ug/L)**	**Cr (mg/L)**	**Fe (mg/L)**	**K (mg/L)**
1	0.28	1.9	93.0	bdl	bdl	0.7	0.4	1.8
2	bdl	1.0	70.6	bdl	bdl	0.1	0.3	0.9
3	bdl	3.5	161.6	bdl	0.27	0.7	0.5	4.9
4	bdl	5.5	103.2	bdl	bdl	0.6	2.2	1.9
5	bdl	5.1	121.2	bdl	bdl	bdl	0.4	1.5
6	bdl	5.6	70.0	bdl	bdl	bdl	0.2	1.4
7	bdl	0.6	80.4	bdl	bdl	bdl	0.3	0.8
8	bdl	0.3	45.8	bdl	bdl	bdl	4.0	2.0
Control	bdl	0.3	43.6	bdl	1.99	bdl	0.2	6.3
Site	Hg (ug/L)	Mg (mg/L)	Mn (ug/L)	Na (mg/L)	Ni (ug/L)	Pb (ug/L)	Se (ug/L)	Zn (ug/L)
1	2.48	3.7	5.6	36.8	3.56	0.57	45.0	206.0
2	2.40	2.7	1.9	11.7	2.92	0.57	50.8	121.2
3	0.44	4.5	2.1	27.4	4.52	1.90	44.2	350.0
4	1.38	4.5	8.8	16.9	3.88	0.46	46.6	41.4
5	1.37	10.2	8.1	12.9	3.46	0.30	53.6	97.8
6	bdl	6.3	11.3	13.6	0.96	bdl	54.8	47.2
7	0.21	6.9	3.7	17.4	2.14	1.95	49.0	1374.0
8	1.97	4.9	6.5	33.8	1.84	0.25	50.8	148.4
Control	1.42	8.9	0.1	44.2	1.40	1.41	62.6	270.0

Bdl – indicates that analyte is below detection limits.

Table 5. Concentrations (mg/kg) of trace elements in sediments collected May 2-3, 2006 from the Chipola River, Florida

Site	Ag	As	Ca	Cd	Cu	Cr	Fe	K
1	bdl	0.47	888	0.8	1.49	[1]	2407	31.9
2	bdl	0.61	980	0.56	2.12		1881	35.1
3	bdl	1.19	3446	0.31	2.78		4060	34.1
4	bdl	0.87	704	0.14	4.27		2414	46.6
5	bdl	0.43	1348	0.16	0.98		1562	42.0
6	bdl	0.28	2056	0.21	0.43		1143	28.4
7	bdl	1.04	10821	0.59	2.50		6250	61.1
8	bdl	1.53	1246	0.94	7.75		124729	251.1
Control	bdl	0.19	11.68	0.03	0.20		109	17.4

Site	Hg	Mg	Mn	Na	Ni	Pb	Se	Zn
1	0.02	57.1	62.1	8.92	1.55	7.90	[1]	6.18
2	0.04	83.4	128.0	10.87	2.25	6.18		9.33
3	0.07	131.9	81.9	12.05	2.59	11.1		11.07
4	0.03	185.4	68.4	10.03	2.17	5.5		11.07
5	0.02	577.9	80.3	12.05	1.66	3.9		6.12
6	0.01	1151	120.8	18.37	1.14	1.8		3.25
7	0.07	8816	126.3	46.64	2.83	7.9		15.12
8	0.08	1554	152.5	14.59	3.01	11.5		32.49
Control	0.00	11.0	1.4	14.25	0.08	0.8		0.79

[1]Not available

Bdl – indicates that analyte is below detection limits.

Benthic macroinvertebrate populations were quite similar throughout the study range even though there was a marked increase from upstream to downstream in size (width, depth and discharge) of the river (Table 6). Data are provided in the report appendix. The total number of taxa ranged from 32 to 56, but no longitudinal trend was apparent (Vannote *et al.*, 1980). The highest number of taxa was found at Site 5 (56 taxa) and the lowest at Site 2 (32 taxa). Diversity of the benthic organisms was high throughout the study range and dominance of individual taxa was fairly consistent throughout. There were some shifts in taxa dominance from upstream to downstream (e.g., Ephemeroptera taxa), but this may have been influenced by differences in sampling efficiency. In general, no sites appeared to have diminished benthic populations that may indicate stressful conditions.

Table 6. Relative abundance and associated metrics of *in-situ* populations of benthic macroinvertebrates collected in the Chipola River in Florida, May 2-3, 2006.

Index	**Site**	**1**	**2**	**3**	**4**
Total number of taxa		41	32	36	42
Mean SCI (mean± 1 standard deviation)		18.54±10.27	12.45±6.34	16.79±5.69	20.67±6.38
Shannon Diversity (d)		4.27	3.73	3.92	4.44
% 3 dominant taxa		50	54	55	43
Index	**Site**	**5**	**6**	**7**	**8**
Total number of taxa		56	43	41	42
Mean SCI (mean± 1 standard deviation)		26.51±10.17	22.16±4.8	23.75±2.44	22.14±2.02
Shannon Diversity (d)		5.13	4.61	4.26	4.42
% 3 dominant taxa		30	37	48	44

Risk Estimation

Sediment toxicity testing showed toxicity in the interstitial or pore-waters of the sampled sediments (Figure 23). Acute toxicity was only observed in these porewater tests. Whole sediments did not yield an acutely toxic response, nor did the chronic whole-sediment assay based on growth. No chronic assays were performed on pore-waters.

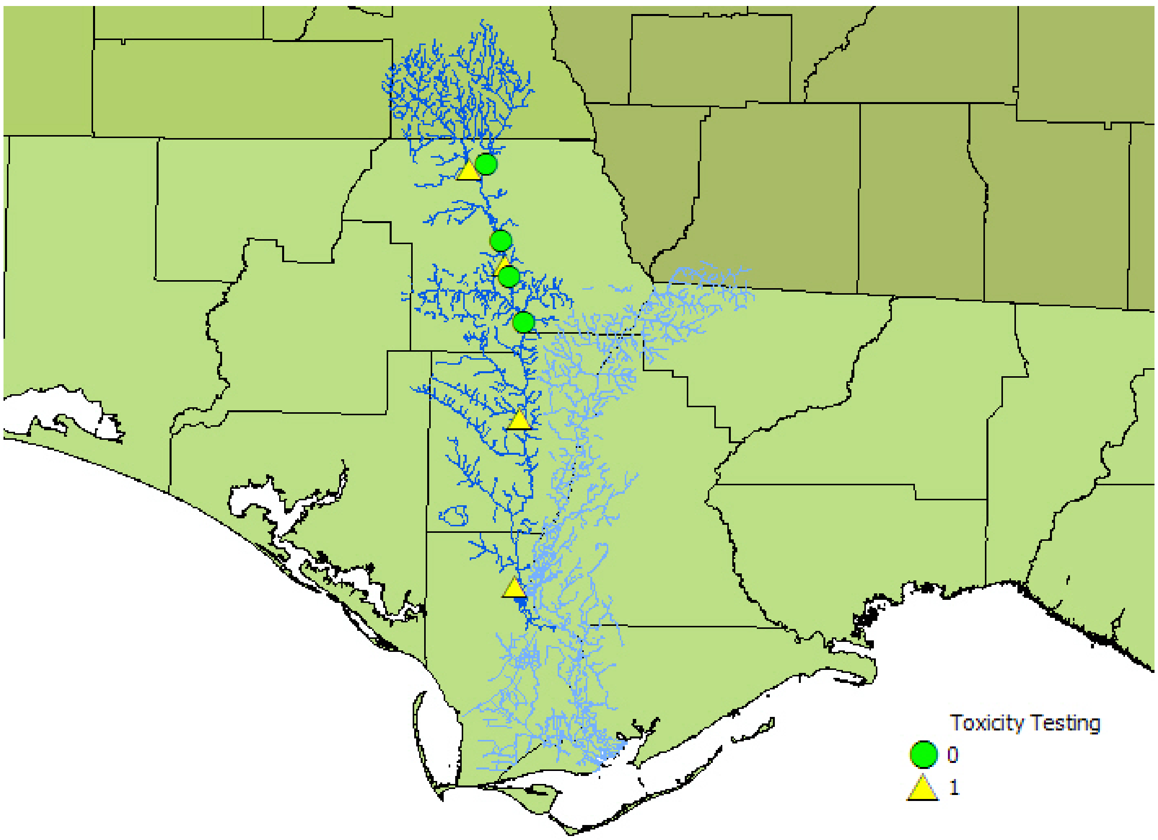

Figure 23. Overall sediment toxicity-associated potential risk estimated from pore and solid phase exposures of *Hyalella azteca* (acute and chronic assays). A score of one was assigned for significant differences from the control for each test and scores for all tests were summed by site.

Benthic macroinvertebrate populations were consistent among the eight sites sampled on the Chipola River. No site was thought to have impaired populations populations and none received a score of one (Figure 24). This remained true despite a marked increase from upstream to downstream in size (width, depth, and discharge) of the river. Diversity of the benthic organisms was high throughout the study reach and dominance of individual taxa was fairly consistent throughout. In general, benthic populations appeared to be unstressed throughout the system.

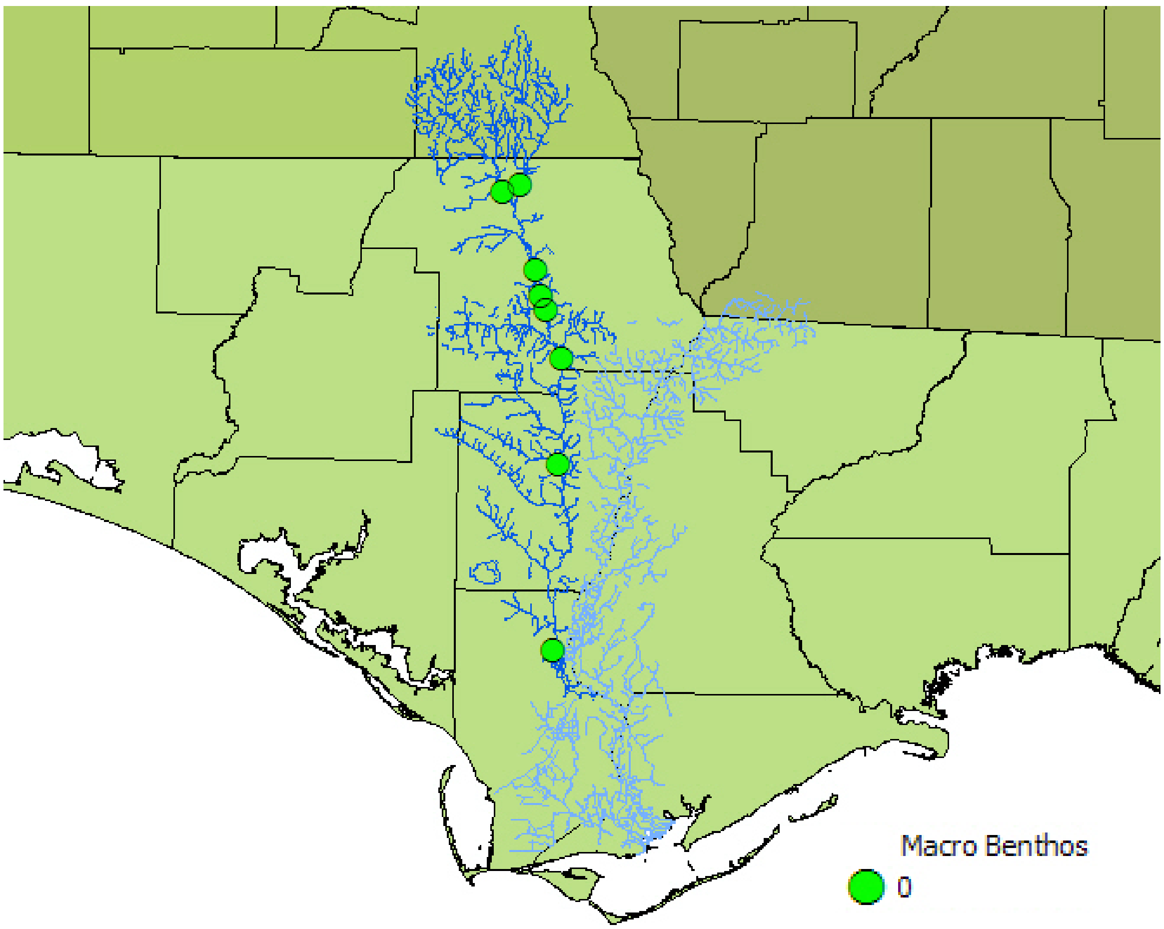

Figure 24. Relative benthic macroinvertebrate community assemblage in comparison to neighboring sites and reference data. Suspected impairment of the macroinvertebrate community received an individual score of one to be summed with scores for all measures of all parameters by site.

Sampling results showed little concern for ambient water quality. Data were compared to the Florida's and Alabama's water quality standards, as well as the ammonia criterion that has been recommended to the USEPA for the protection of freshwater mussels by Augspurger et al (2003). In no case were water quality standards found to be exceeded and all sites received a risk score of zero for ambient water quality (Figure 25). However, the samples represented single point and time measures and cannot be taken to indicate the entire water quality condition of the Chipola River, particularly under more variable flow conditions.

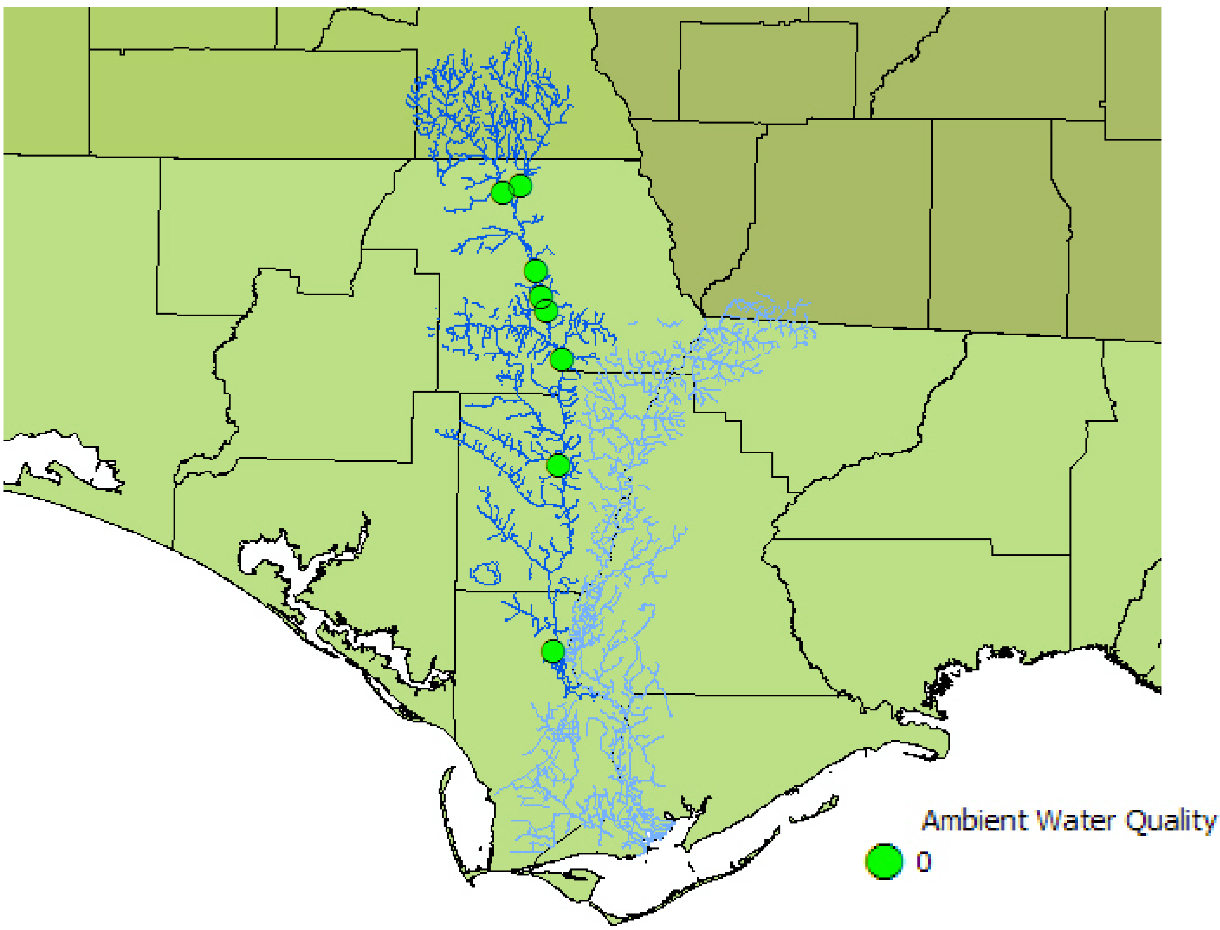

Figure 25. Overall ambient water quality potential risk for the parameters dissolved oxygen, temperature, pH, chlorophyll concentration turbidity, and conductivity. Each violation of a state water quality standard or federal water quality criterion constituted an individual score of one to be summed with scores for all measures of all parameters by site.

Similarly, no sediment sample metal concentrations exceeded sediment quality guidelines for any site evaluated. All sites received a risk score of zero indicating no risk from sediment metals (Figure 26). However, the samples represented single point and time measures and cannot be taken to indicate the entire sediment quality condition where metals are concerned.

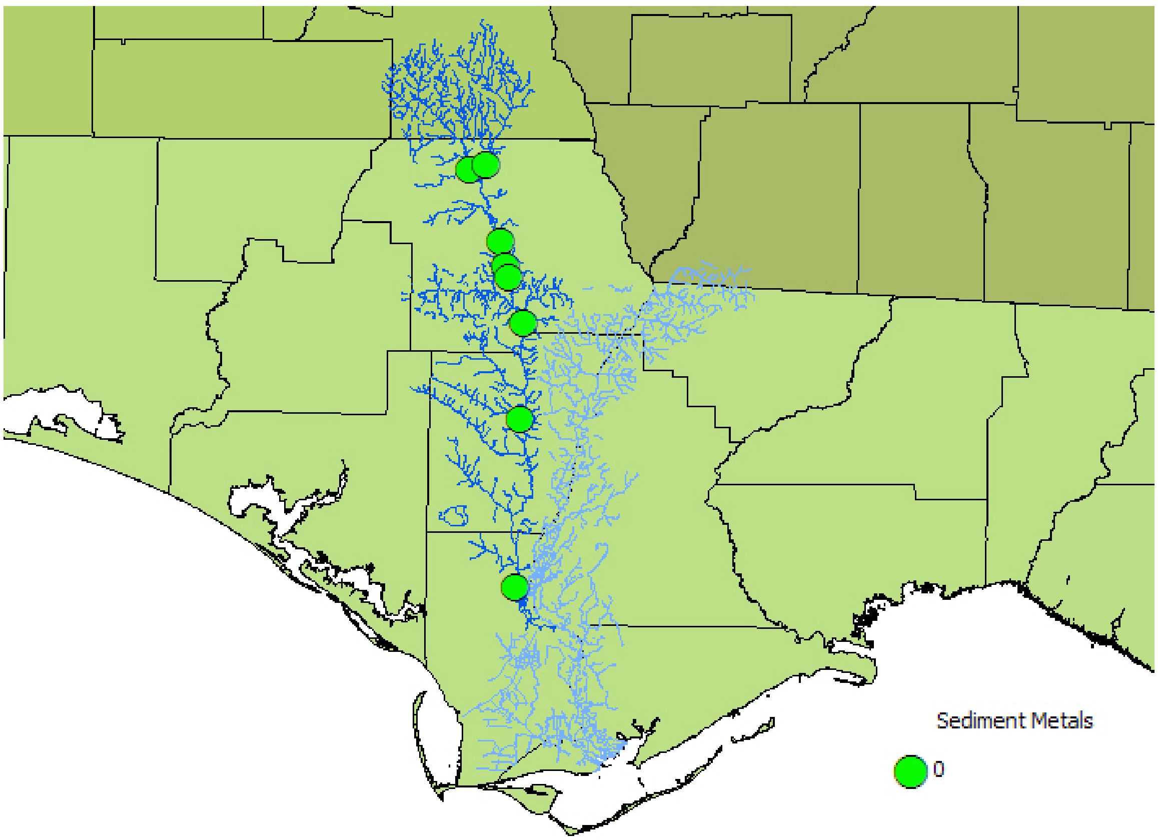

Figure 26. Overall sediment quality potential risk estimated by comparison of sediment metals analytical data to reference values such as those of MacDonald *et al.* (2000). Each exceedance of the sediment quality guidelines constituted an individual score of one to be summed with scores for all measures of all parameters by site.

Metals in sediment and porewaters are not regulated at the state or federal level. For this reason, porewater metal concentrations were compared to Florida's and Alabama's water quality standards (Figure 27). The likelihood of metal exposure may be high for sensitive juvenile stages because juvenile mussels spend more time feeding in the porewater environment (Yeager *et al.* 1994; Neves *et al.* 1987; Reid *et al.* 1992). Porewater metals found to exceed state standards designed to be protective of aquatic life included mercury, selenium, and zinc. While the water quality standard for mercury is a given number set to ≥ 0.012 ug/L, the standard for many metals like selenium and zinc are derived from an algorithm based on water hardness. This estimation of risk based on porewater metal concentrations discounts the potential difference between surface water hardness and porewater hardness. With this being the case, the influence that selenium or zinc may have on freshwater mussels exposed via porewaters needs further investigation.

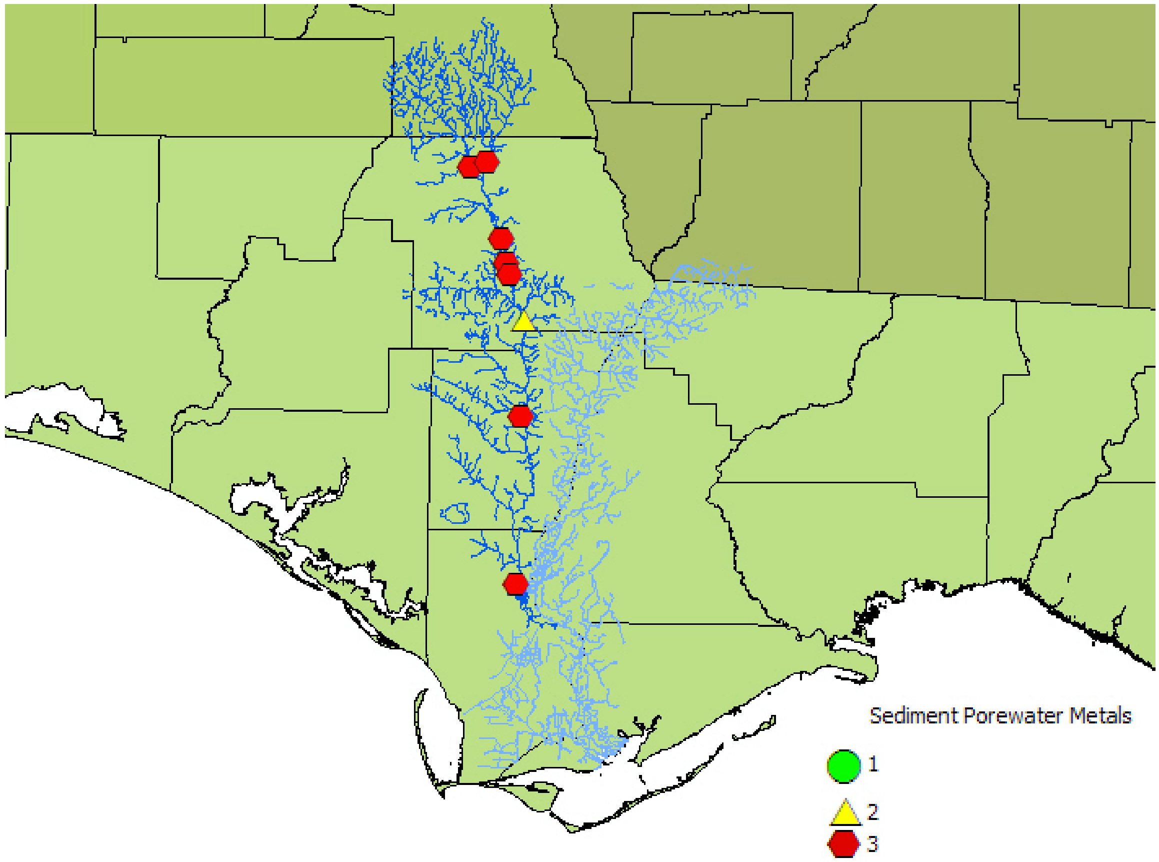

Figure 27. Overall sediment porewater quality potential risk estimated from metal porewater constituents. Each water quality standard violation constituted an individual score of one to be summed with scores for all measures of all parameters by site.

Risk for general sediment characteristics stemmed from the ratio of simultaneously collected metals to (SEM) to acid volatile sulfides (AVS) in the sediment samples. The SEM- AVS ratios were relatively high at Site 2 and particularly Site 4 (Figure 28). A SEM/AVS greater than 1.0 indicates that the metal concentrations in the sediments exceed the sulfides and may be biologically available (Di Toro *et al.* 1992). This is particularly noteworthy with the presence of elevated porewater metals at all sites.

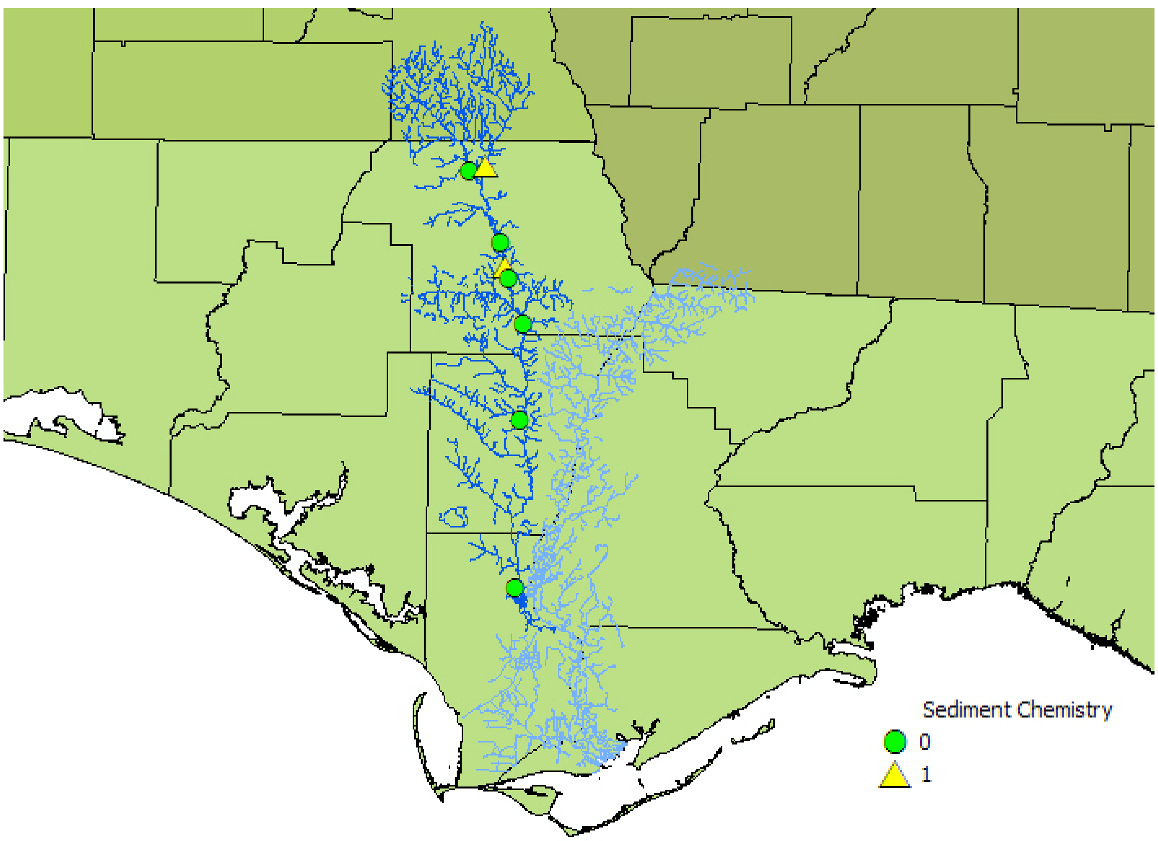

Figure 28. Overall sediment quality risk estimation based on general sediment chemistry. Sediment characteristics that may be associated with a lower sediment quality constituted an individual score of one to be summed with scores for all measures of all parameters by site.

Non-metal porewater chemistry risk was associated with ammonia concentrations (mg/L NH_3) only (Figure 29). Ammonia concentrations were consistently measured to be above the recommended water quality criteria for ammonia as described by Augspurger *et al.* (2003). As with metals in porewaters, there is a lack of regulation of ammonia in porewaters. This risk to freshwater mussels is likely to be particularly pronounced for the juvenile stages that spend more time feeding in porewater environment (Yeager *et al.* 1994; Neves *et al.* 1987; Reid *et al.* 1992).

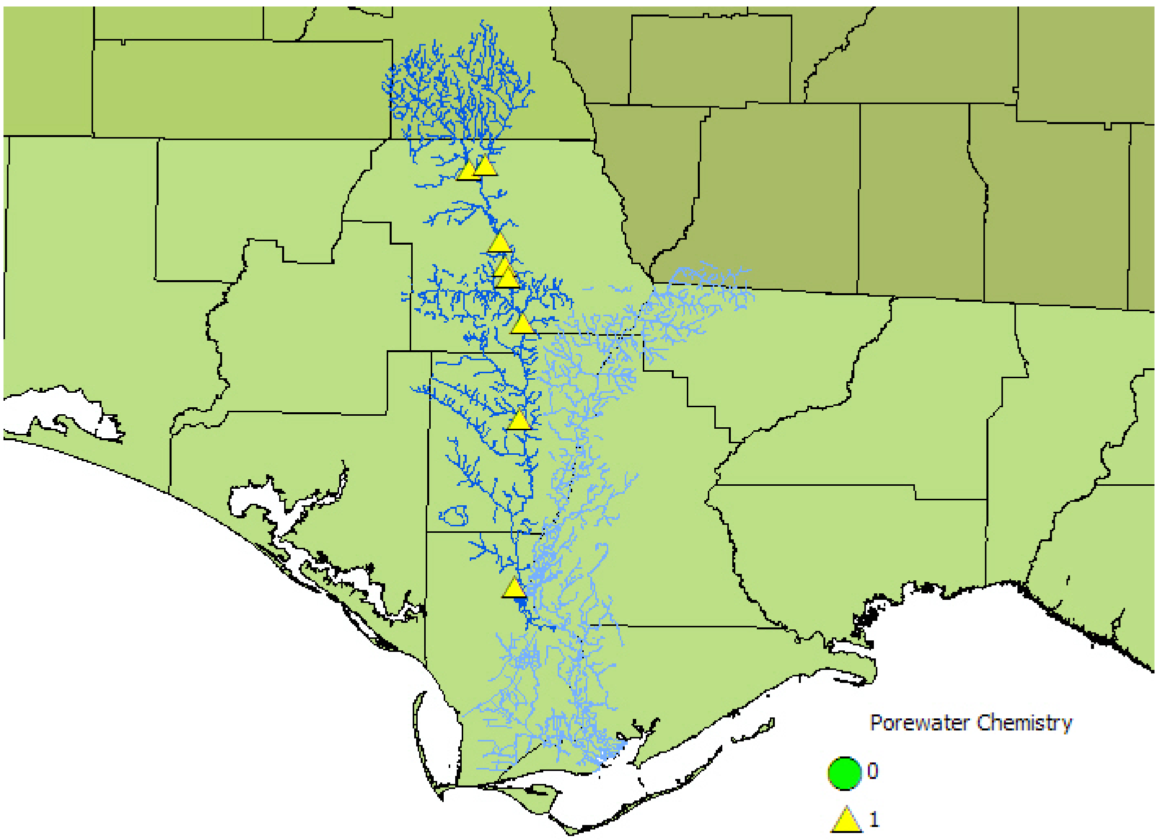

Figure 29. Overall potential risk associated with sediment pore-water chemistry. Each proposed criterion violation constituted an individual score of one.

Combining the 1) data on ambient water chemistry and sediment chemistry, 2) laboratory toxicity, and the 3) *in-situ* benthic assemblages indicated that Site 4 likely has impaired habitat quality, and that Sites 1, 2, 7, and 8 may be impaired (Table 7, Figure 30). Although there is not strong evidence (lack of consensus among data sets), the preponderance of information suggests possible habitat impairment at these sites. Based on the decision matrix, the elevated trace elements (Zn, Se, Hg) in the porewater, irregular basic chemistry in the porewater (NH_3) and solid-phase sediment (SEM/AVS), and the sediment toxicity (acute in porewater) are the major factors leading to these conclusions.

There is a particularly noteworthy co-occurrence of elevated porewater metals and a high SEM/AVS at Site 4 that may help explain the porewater toxicity observed there. At Site 2 these same porewater metal concentrations and elevated SEM/AVS relationship may lead to pore-water toxicity and is cause for further investigation. Although the lack of confirmation from the *in-situ* benthic assemblages tends to somewhat diminish these categorizations of impairment, they may represent a different environmental compartment (more surficial) than those experienced by freshwater mussel species (within sediment and feeding in pore-waters as juveniles). Analytical analyses for organic chemicals associated with the sediments may provide additional insight on the habitat quality in the Chipola River, especially at Sites 1, 2, 7, and 8.

Table 7. Decision matrix summarizing habitat quality based on porewater and sediment toxicity, in-situ benthic community structure, ambient water quality, contaminant concentrations in porewater and sediments, and basic chemistry of porewater, sediment and overlying water in sediment tests. Analyses were performed on water and sediments from the Chipola River, Florida during 2006 and 2007. Scores are summed for a cumulative risk, rank estimation.

Site	Toxicity	Macro-invertebrates	Water Quality	Sediment Metals	Pore-water Metals	Sediment Chemistry	Pore-water Chemistry	Cumulative Rank
1	1	0	0	0	3	0	1	**5**
2	0	0	0	0	3	1	1	**5**
3	0	0	0	0	3	0	1	**4**
4	1	0	0	0	3	1	1	**6**
5	0	0	0	0	3	0	1	**4**
6	0	0	0	0	2	0	1	**3**
7	1	0	0	0	3	0	1	**5**
8	1	0	0	0	3	0	1	**5**

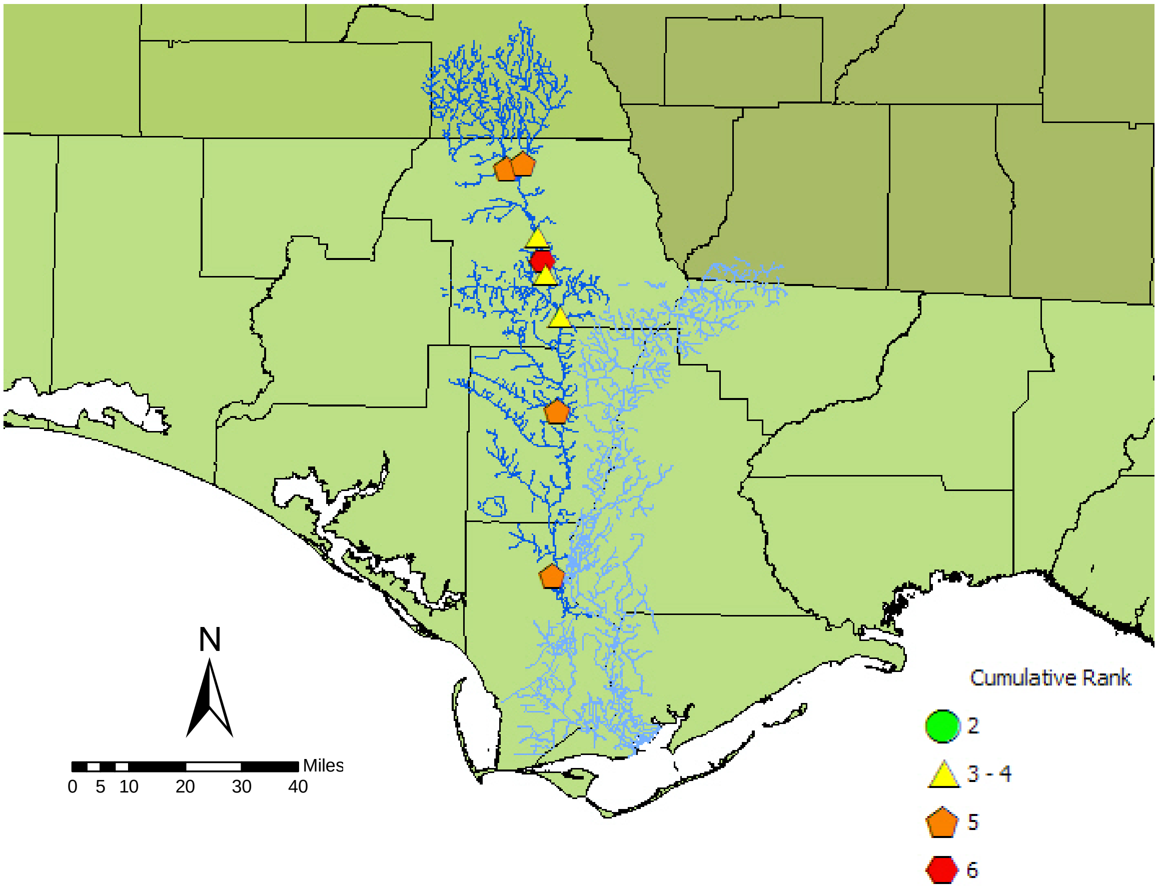

Figure 30. Cumulative risk score estimated for freshwater mussel species in the Chipola River, Florida based on sampling conducted during 2006 and 2007. Water quality standards violations, toxicity test differences from the controls, exceeded sediment analyte guidelines, elevated pore-water contaminants, or abnormal *in-situ* benthic macroinvertebrate assemblages each represented one risk point assessed. All assessed risk points were summed for each incidence of each parameter by site.

CONCLUSIONS

All sites evaluated on the Chipola River during this survey showed at least three parameters that may be associated with risk to sediment-dependent aquatic life such as federally-protected freshwater mussel species. Most sites showed more than three risk parameters. The high risk score of six (Site 4) included pore-water toxicity, porewater metals, altered sediment chemistry (elevated SEM/AVS), and elevated porewater ammonia. The largest driving factor may be elevated metals in the sediment pore-water, where juvenile mussels tend to feed. Although these factors may pose risk to the natural life history of freshwater mussels, related factors such as ambient water quality, whole-sediment metals, and *in-situ* benthic macroinvertebrate communities did not show agreement with the elevated risk assessment at those sites.

The elevated risk areas did not correspond to an apparent decrease in species richness (number of federally listed threatened or endangered species) of imperiled taxa, however abundance numbers and distribution of these species cannot be ascertained from the available database. Non-listed species richness, distribution, and overall abundance of species would be useful comparisons to make with these findings. However, these factors were not considered in this evaluation because of the limiations of the data currently available in the U.S. Fish and Wildlife Service Freshwater Mussel Database. Future plans include the quantification of populations of both federally-protected and other freshwater mussel species in the northeastern Gulf of Mexico drainage.

RECOMMENDATIONS

The following recommendations are offered for consideration.

1) Conduct a more systematic survey on a larger scale for the sub-watersheds where potential risk from sediment toxicity was observed.

2) Conduct a more systematic survey on a larger scale for the sub-watersheds where potential risk from sediment chemical parameters was observed.

3) Subject potential high risk areas to land-use analyses for factors contributing to potential site risk. These analyses should include important spatial issues such as distance of a particular land-use within a sub-drainage from the concern point, point source discharges, unpaved roads, extent of intact riparian buffer, cumulative contributions between specific sub-watersheds, and potentially historic land-uses from which recovery may be taking place.

4) Confirm toxicity test results by conducting ambient water and sediment toxicity testing with appropriate, juvenile freshwater mussel surrogates.

5) Examine system sedimentation relative to mussel occurrence data.

6) Evaluate the relationship among elevated simultaneously extracted metals (SEM) to acid volatile sulfide (AVS) ratios, elevated pore-water metals, and sediment pore-water toxicity.

7) Investigate the influence that different life history strategies may have on the bioassay results. For example, the difference between *in situ* benthic macroinvertebrates use of sediment versus the interstitial existence of juvenile freshwater mussel species.

8) Conduct analytical analyses for organic chemicals associated with sediments to provide additional insight on the habitat quality in the Chipola River.

9) Conduct population studies on freshwater mussel species in the northeastern Gulf of Mexico drainage to provide abundance, diversity and recruitment information.

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Appendix

Appendix 1. Relative abundance and associated metrics of *in-situ* populations of benthic macroinvertebrates collected in the Chipola River,in Florida, May 2-3, 2006.

Taxa	Station							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
OLIGOCHAETA	1		1	4	4	4	1	4
HIRUDINEA								
Gloiobdella elongata				1	1			
Placobdella								1
GASTROPODA								
Amnicola	1							
Campeloma		2	3	1	1	1	2	1
Elimia	14	5	19	2	3	6	2	1
Gyraulus								2
Haitia		1						
Laevopex			1					
Notogillia			1	1	1	2		
Physella								6
Pseudosuccinea								2
Somatogyrus		7	6	4	5	6	1	1
Viviparus							1	
PELYCYPODA[1]		1			1			
Elliptio							1	
Corbicula	2	2	1	1	1			
Sphaerium	1				1	1		1
CRUSTACEA								
Asellus	1	9	3	1	1			1
Hyalella azteca	1	1	6	28	20	23	27	5
Lirceus	3				1			
Palaemonetes paludosus	25	37	30	9	3	8	6	1
Procambarus	1	3	3	1	5	4	6	1

HYDRACARINA								
Arrenurus	1							
Hydrachna						3	1	
Lebertia	1				3	1		1
Limnesia				2				
EPHEMEROPTERA								
Brachycerus				1				
Caenis					1			
Euylophella doris	1						1	
Hexagenia bilineata	11	8	2	3	1		1	1
Isonychia					3			1
Leptophlebia	1							
Maccaffertium	3		1		1	2		
Neoephemera youngi							1	1
Plauditus					1			
Pseudiron							1	1
Pseudocloeon	1	1	1					
Pseudocentroptiloides				1	1			
Serratella					1			
Stenacron	1		1		1	1		
Trichorythodes		1		1	2	2	15	1
ODONATA (Zygoptera)								
Argia	1	1		2	5	6	2	
Calopteryx maculata						1	1	
Enallagma			1			1		1
Hetaerina titia					1			
Ischnura	1			2	1	1		
ODONATA (Anisoptera)								
Erpetogomphus				1				
Boyeria vinosa					1	1	1	1

Didymops						1		
Dromogomphus	1	1	1	2	1	1	2	2
Dythemis		1						
Gomphus	1							
Progomphus						1		
Macromia			1	1	1	1	1	
PLECOPTERA								
Perlesta	1		1		1			1
Perlinella	1							
Neoperla					1			
HEMIPTERA								
Gerris	1	1		1				
Lethocerus			1					
Metrobates					1			
Mesovelia			1					
Microvelia					1	1		
Ranatra australis		1	1		1	1	1	
Ranatra buenoi	4	1	2	1	1	2	4	
Rhagovelia					3			
Trebobates					1			
Trichocorixa		1		1	1			
TRICHOPTERA								
Cernotina						1		
Cheumatopsyche					1		1	1
Chimarra						1		
Hydroptila			1					6
Lype				1				
Nectopsyche							1	1
Oecetis				1				
Oxyethira								

Potamyia flava	1							
Triaenodes						1	1	
COLEOPTERA					1			
Ancyronyx	1							
Bidessonotus							1	
Celina			1	1				
Coptotomus		1			1			1
Cyphon	1							
Dineutus	2	1		2				2
Dubiraphia			1	1	1	2	1	
Dytiscus								
Gyrinus		1	1					18
Gyretes								
Helocharis							1	
Hydochara								
Hydroporus		2	1	1	1		1	
Laccophilus		2						
Laccodytes		1						
Microcylloepus					1	1	1	
Neoporus	1			1				
Ochthebius	1							
Peltodytes		1	1	1			1	1
Rhantus							1	
Scirtes			1			1	1	
Stenelmis	4				2		1	
Tropisternus		1				1		
DIPTERA								
Bezzia		1		6	1	1	1	2
Palpomyia								1
Simulium						1		

Ablabesmyia	1	1		4	1	1	1	1
Chironomus				1				1
Cladopelma			1					
Cladotanytarsus				1	1			
Clinotanypus		2						
Constempelina			1					
Corynoneura	1							
Cricotopus			1	1		1	2	1
Cryptochironomus						1		
Cryptotendipes				1				1
Dicrotendipes				1				
Eukiefferiella	1							
Labrundinia								1
Larsia	1							
Monopelopia				1				
Orthocladius					1			
Paralauterborniella							1	
Phaenopsectra	1	1	1	1				
Polypedilum	1		1		1	1	1	20
Procladius								1
Rheotanytarsus						1		
Stenochironomus					1			
Sublettea					1			
Synorthocladius							1	
Tanytarsus	1		1	2	1	1	2	1
Xenochironomus						1		1
Xylotopus	1							
Total number of taxa	41	32	36	42	56	43	41	42

[1]Unidentified freshwater mussels collected but returned to the river.