Water and Sediment Quality Survey of Threatened and Endangered Freshwater Mussel Habitat in the Ochlockonee River Basin

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Abstract

The U.S. Fish and Wildlife Service (Service) Freshwater Mussels Recovery Plan (USFWS 2003) called for the evaluation of freshwater mussel habitat in the Ochlockonee River of Florida and Georgia to identify areas with factors potentially limiting to mussel life history. Identification of these areas will allow for the implementation of measures to maximize recovery efforts and ensure adequate water quality for these imperiled species. To meet the recovery plan criteria, a coordinated effort among the Service's Environmental Contaminants Program, the U.S. Geological Survey's Patuxent Wildlife Research Center, and the Service's Fisheries Resources Office was conducted. The combined effort of these programs allowed for performance of water chemical analyses, porewater and whole sediment chemical analyses, and porewater and whole sediment toxicity testing. These analyses allowed for the identification of impaired sites and determination of differences between sites that currently support mussels and those that have ceased to support populations. The results further provided an evaluation of potential risk to aquatic species which was used as a preliminary risk estimation for freshwater mussels. The preliminary risk estimation based on degraded water and sediment quality was used for identification and ranking of mussel habitat in need of restoration. Cumulatively, sites posing potential risk to freshwater mussel species achieved an estimated potential risk score of five or greater (below the dam, Little River, and ~3 miles SSE of Cairo, GA). These sites corresponded well with areas that no longer support their historic freshwater mussel populations. Factors contributing to the high risk estimation for these sites were sediment toxicity (porewater and whole sediment), sediment lead, sediment manganese, sediment ammonia, and low dissolved oxygen.

Key Words: Water quality, sediment quality, Ochlockonee River, freshwater mussels

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Contents

Abstract …….*ii*

Acknowledgments …….*iii*

List of Tables…….…….*v*

List of Figures ……...…….v*i*

Introduction……..……1

Materials and Methods……...12

Water Chemistry…….……12

Sediment Quality……....14

Evaluation of Potential Risk and Land Use Analysis ……18

Results and Discussion……..19

Water Chemistry……19

Sediment Quality……28

Evaluation of Potential Risk and Land Use Analysis ……40

Conclusions……56

Water Chemistry……56

Sediment Quality……56

Evaluation of Potential Risk and Land Use Analysis ……57

Recommendations……..59

Literature Cited……..60

Tables

Table 1. Mean flow conditions (ft^3/sec) for each sampling event for sites upstream (Upstream Flow) of Lake Talquin and sites downstream (Downstream Flow) of Lake Talquin, according to U.S. Geological Survey gaging stations…….....19

Table 2. Percent survival and growth (expressed as length) of *Hyalella azteca* exposed to pore water and solid-phase sediments collected from the Ochlockonee River, Florida in August 2004, and EC50s at 5 and 15 minute exposures of sediment extract to *Vibrio fischeri* (Microtox). Values in parentheses are standard deviations and values with an asterisk indicate a significant difference (p = 0.05) to the Control……...……29

Table 3. Chemistry of porewater extracted from sediment collected from the Ochlockonee River, August 2004…….…….36

Table 4. Chemistry of overlying water at the start (September 3, 2004) and the end (October 1, 2004) of the 28-day exposures of *Hyalella azteca* to solid-phase sediment collected from the Ochlocknee River, Florida.……....37

Table 5. Physical characteristics of the sediments and acid volatile sulfides (AVS), simultaneously extracted metals (SEM) and the SEM/AVS ratio for sediments collected from the Ochlockonee River, Florida, in August 2004. ……..……38

Table 6. Concentrations of trace elements in porewaters from sediments collected from the Ochlockonee River in August 2004. ……...39

Table 7. Individual parameters providing the cumulative potential risk score estimated for freshwater mussel species in the Ochlockonee River, Florida based on sampling conducted in the summer of 2004. Water quality standard violations, toxicity test differences from the control or exceeded sediment quality guidelines are listed with respective parameter for each incidence. Each reported incidence represented one risk point assessed. Sites with (*) had no species of threatened or endangered species during 2002-2004 surveys.……...…45

Table 8. National Pollutant Discharge Elimination System (NPDES) permitted discharges in sub-drainages of sites with high estimated risk for freshwater mussels resulting from water and sediment quality measures in the Ochlockonee River watershed of Florida and Georgia according to the Florida Department of Environmental Protection and the Georgia Environmental Protection Department of Natural Resources…….…….51

Table 9. Land use composition in percentages for the Ochlockonee River watershed in northwest Florida and southwest Georgia according to the Florida Department of Environmental Protection, 2001. ……..……..54

Figures

Figure 1. The location of the Ochlockonee River flowing south from Georgia to Florida's Gulf of Mexico Coast……..…….....5

Figure 2. The location of surveys (circles) and located freshwater mussel species (triangles) federally listed as threatened and endangered in the Ochlockonee River prior to 1990……...……6

Figure 3. The location of surveys (circles) and located freshwater mussel species (triangles) federally listed as threatened and endangered in the Ochlockonee River from 1990-1999……7

Figure 4. The location of surveys (circles) and located freshwater mussel species (triangles) federally listed as threatened and endangered in the Ochlockonee River from 2000 to 2004……8

Figure 5. Species richness (# of different species) for federally listed threatened and endangered species of freshwater mussels in the Ochlockonee River prior to 1990……....9

Figure 6. Species richness (# of different species) for federally listed threatened and endangered species of freshwater mussels in the Ochlockonee River from 1990 to 1999.....10

Figure 7. Species richness (# of different species) for federally listed threatened and endangered species of freshwater mussels in the Ochlockonee River from 2000 to 2004.....11

Figure 8. The location of U. S. Fish and Wildlife Service water quality sampling sites (white circles) on the Ochlockonee River 2004.....13

Figure 9. Measured total ammonia concentrations (mg/L NH3+NH4) for water samples taken from the Ochlockonee River compared to the Augspurger *et al*. (2003) recommended range for criteria continuous concentration (CCC, black lower end of range, white higher end of range) and the state of Florida ammonia water quality standard (calculated total ammonia for maximum system pH recorded of 7.3 at 25°C)…….……..…22

Figure 10. Hydrogen ion concentration (pH in standard units) range at selected sites in the Ochlockonee River, summer 2004 compared to the U. S. Environmental Protection Agency criterion range (1976, 6.5-9 SU) and the Florida state water quality standard range (2004, 6-8.5)…….……...22

Figure 11. Calculated free un-ionized ammonia concentrations (mg/L NH3 calculated for maximum pH of 7.3) for water samples taken from the Ochlockonee River compared to the calculated free un-ionized ammonia concentration calculated for Augspurger et al. (2003) recommended criteria continuous concentration (CCC, black-lower end of range, white-higher end of range), and the state of Florida un-ionized ammonia water quality standard……23

Figure 12. Water alkalinity (measured as mg $CaCO_3$/L) for water samples taken from the Ochlockonee River……...……23

Figure 13. Free copper concentrations (ug/L, lower limit of detection 2 ug/L, non-detects reported as 1.0 ug/L) for water samples taken from the Ochlockonee River compared to U. S. Environmental Protection Agency chronic criterion (2002, $Cu \leq e^{(0.8545[\ln H]-1.465)}$) and the Florida state water quality standard (2004, $Cu \leq e^{(0.8545[\ln H]-1.702)}$). Both were calculated for the overall mean water hardness of 23.9 mg/L $CaCO_3$…….……24

Figure 14. Total water hardness (measured as mg $CaCO_3$/L) for water samples taken from the Ochlockonee River……..……..…….....24

Figure 15. Dissolved oxygen concentrations (mg/L) at selected sites in the Ochlockonee River, summer 2004 compared to the U. S. Environmental Protection Agency chronic criterion (1984, 5 mg/L) and the Florida state water quality standard (2004, 5 mg/L).....25

Figure 16. Water temperature (°Celsius) at selected sites in the Ochlockonee River, summer 2004, compared to the overall summer season mean (Mn, 27.4°C) as would be used to determine Florida thermal water quality standards violations (in excess of mean background temperature plus 5°C – intended for thermal point source discharges)…..…….25

Figure 17. Dissolved oxygen percent saturation in water at selected sites in the Ochlockonee River, summer 2004…….…26

Figure 18. Turbidity (NTUs) at selected sites in the Ochlockonee River, summer 2004 compared to the overall summer season mean (Mn, 12.3 NTUs) as would be used to determine Florida water quality standards violations (≤ 29 above natural background conditions of 12.3 = 41.3)……..….....26

Figure 19. Chlorophyll a (ug/L) at selected sites in the Ochlockonee River, summer 2004 compared to the overall summer season mean (Mn, ug/L) in light of the Florida water quality standards for nutrients (nutrient concentrations in a waterbody shall not be altered to cause an imbalance in natural populations of aquatic flora or fauna, nor shall they result in violations of other standards)…….27

Figure 20. Specific conductance (uS/cm^3@25°C) at selected sites in the Ochlockonee River, summer 2004, compared to the overall summer season mean (Mn, 75.9°C) as would be used to determine Florida water quality standards violations (in excess of mean background plus 50% or 1275 uS/cm^3, whichever is greater; 75.9 + (0.5*75.9 or 37.95) = 113.9)…….27

Figure 21. Overall ambient water quality risk estimation for the parameters free copper, un-ionized ammonia, chlorine, dissolved oxygen, temperature, pH, chlorophyll concentration turbidity, conductivity, alkalinity, and hardness. 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 (ammonia federal criterion based on recommended safe exposure range proposed by Augspurger *et al.* 2003)……...……41

Figure 22. Overall sediment toxicity risk estimation from porewater and solid phase exposures of *Hyalella azteca* (acute and chronic assays) and solid-phase exposures for *Vibrio fischeri* (5-minutes and 15-minutes). A score of one was assigned for significant differences from the control for each test (unless qualified in discussion as for *H. azteca* chronic) and scores for all tests were summed by site……...…...…42

Figure 23. Overall sediment quality risk estimation by comparison of analytical data to reference values such as those of McDonald and others (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……..…43

Figure 24. Cumulative potential risk score estimated for freshwater mussel species in the Ochlockonee River, Florida based on sampling conducted the summer of 2004. Water quality standards violations, toxicity test differences from the control or exceeded sediment analyte guidelines each represented one risk point assessed to be summed for each incidence of each parameter by site…….....44

Figure 25. Ochlockonee River segments listed as impaired by sub-watershed (red shaded polygons) according to the Florida Department of Environmental Protection (2001) and the Georgia Environmental Protection Department of Natural Resources (2002)…….....48

Figure 26. National Pollutant Discharge Elimination System (NPDES) permitted discharges (circle in circle) in sub-drainages of sites (red hexagon) with high estimated risk for freshwater mussels resulting from water and sediment quality measures in the Ochlockonee River watershed of Florida and Georgia according to the Florida Department of Environmental Protection and the Georgia Environmental Protection Department of Natural Resources……...…….50

Figure 27. Land-use composition in the Ochlockonee River watershed in northwest Florida and southwest Georgia based on Landsat TM imagery 1988-1990. Figure reproduced with permission from the Florida Department of Environmental Protection…….....……...55

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 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, reported suspects have included 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 are based largely on anecdotal evidence of casual mechanisms (Newton *et al.* 2003). Although not sufficiently documented, 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, rangewide 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).

Unionid mussel losses support the description by Newton (2003) that their life history makes them important sentinels for tracking 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 (ecological toxicology) 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 streams and rivers were known to have rich mussel populations. The reduction and fragmentation of the freshwater mussel populations in these systems have resulted in species vulnerability to extinction. The eastern Gulf Slope streams 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 form a dominant drainage area in the eastern Gulf Coastal Plain.

Within the eastern Gulf Slope drainage, the Ochlockonee River Basin stretches from Georgia southward to the eastern Florida panhandle (Figure 1). The Ochlockonee drainage provides important habitat for three federally listed endangered and one federally listed threatened freshwater mussels, including the Ochlockonee moccasinshell, oval pigtoe, shinyrayed pocketbook, and purple bankclimber. Like many unionids, however, they have suffered declines in recent years as shown in wide-based surveys (Figures 2-4). Much of these declines were observed after 1990 in the Little River tributary and more recently in the upper Ochlockonee River in southeastern Georgia. Additionally, species richness (number of federally listed threatened or endangered species) of imperiled taxa may have simultaneously declined in the Ochlockonee River Basin (Figures 5-7). However, the high variability in sampling results over the years places uncertainty about the issues of species richness.

It is the goal of the Service to restore viable populations of the Ochlockonee moccasinshell, oval pigtoe, shinyrayed pocketbook, and purple bankclimber within their historical ranges. This effort 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 Ochlockonee River drainage survey was to determine water and sediment quality differences between sites that currently support freshwater mussels and those that have ceased to support historic populations. A 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.

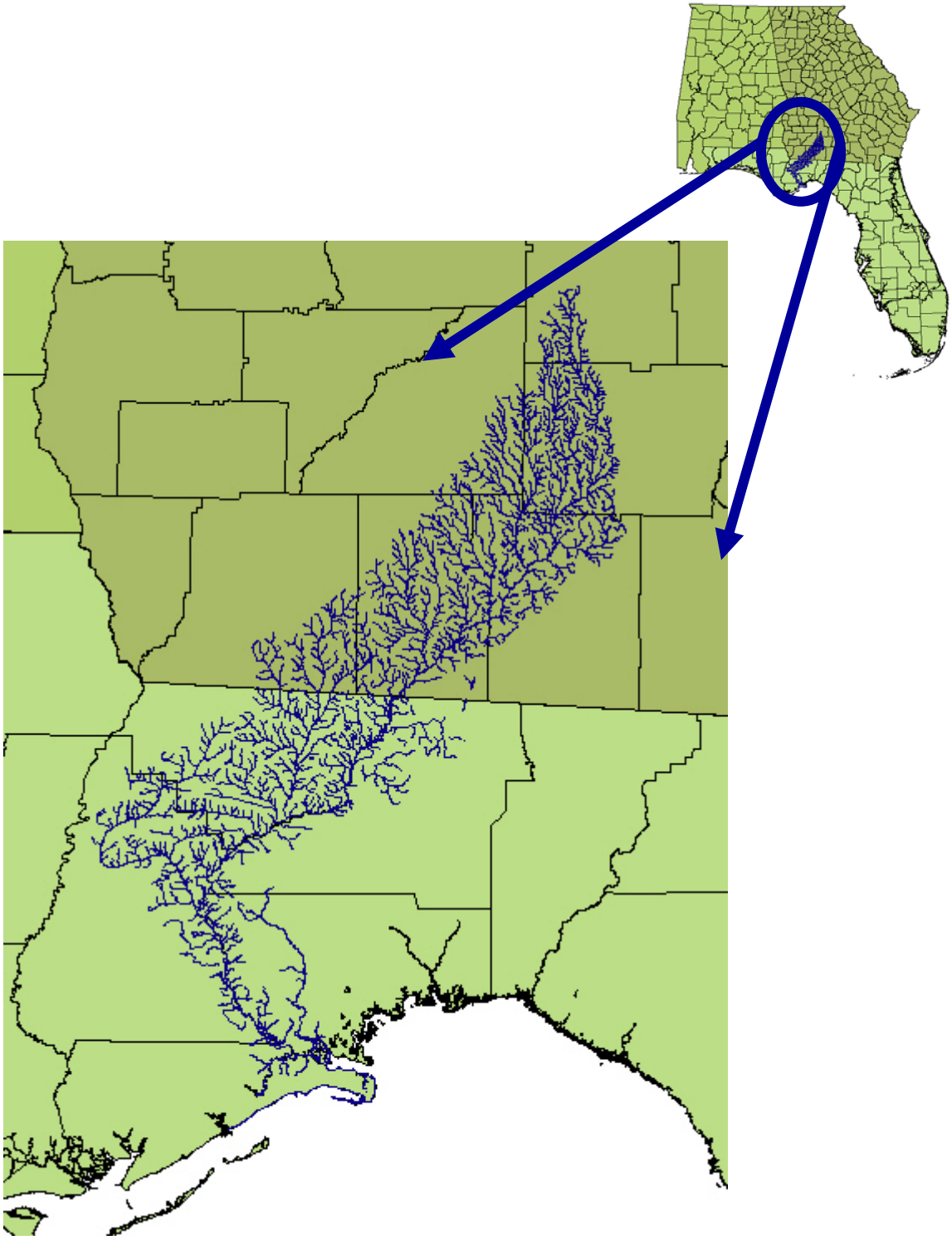


Figure 1. The location of the Ochlockonee River flowing south from Georgia to Florida's Gulf of Mexico Coast.

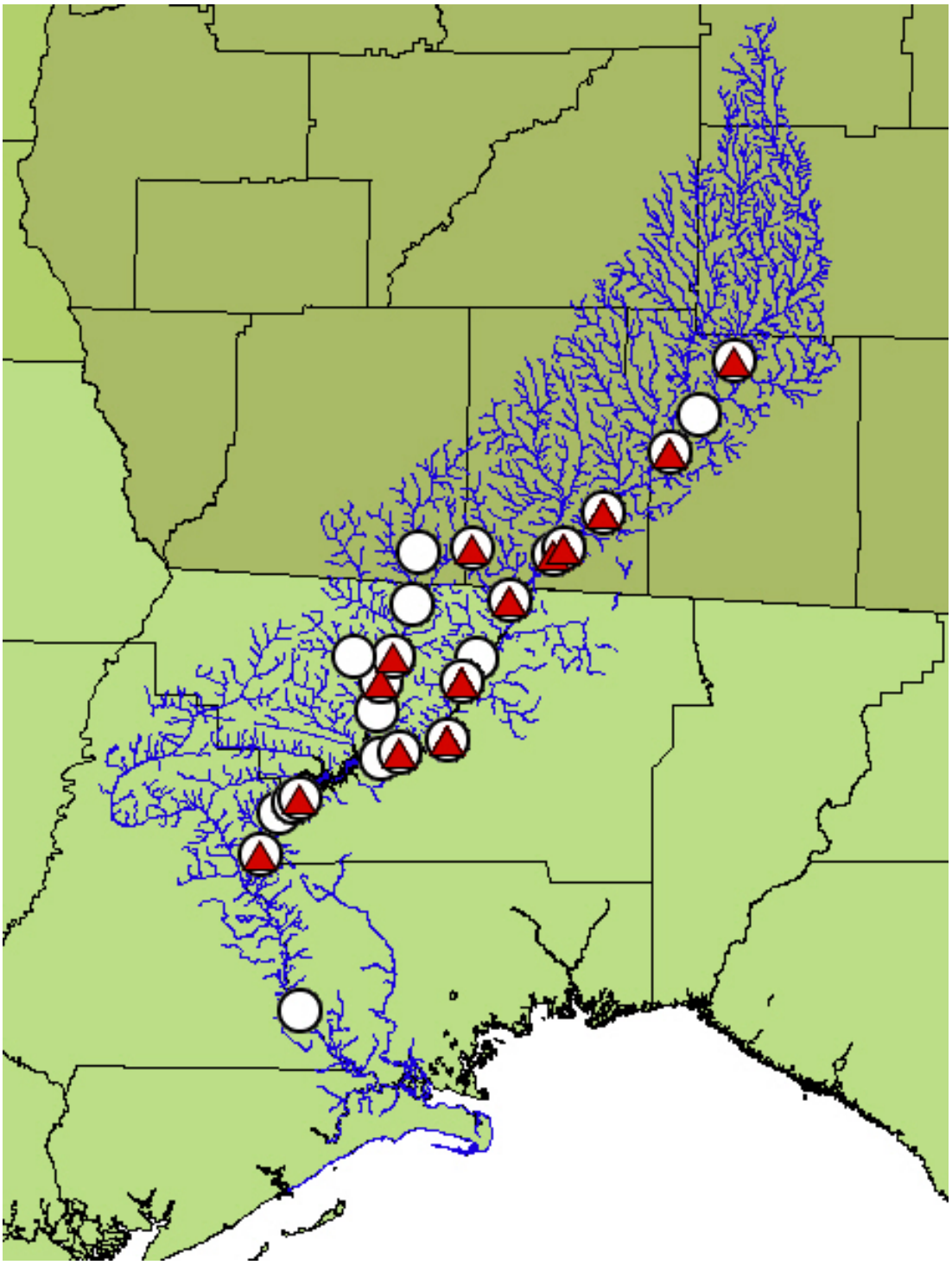


Figure 2. The location of surveys (circles) and located freshwater mussel species (triangles) federally listed as threatened and endangered in the Ochlockonee River prior to 1990 (US FWS freshwater mussel database).

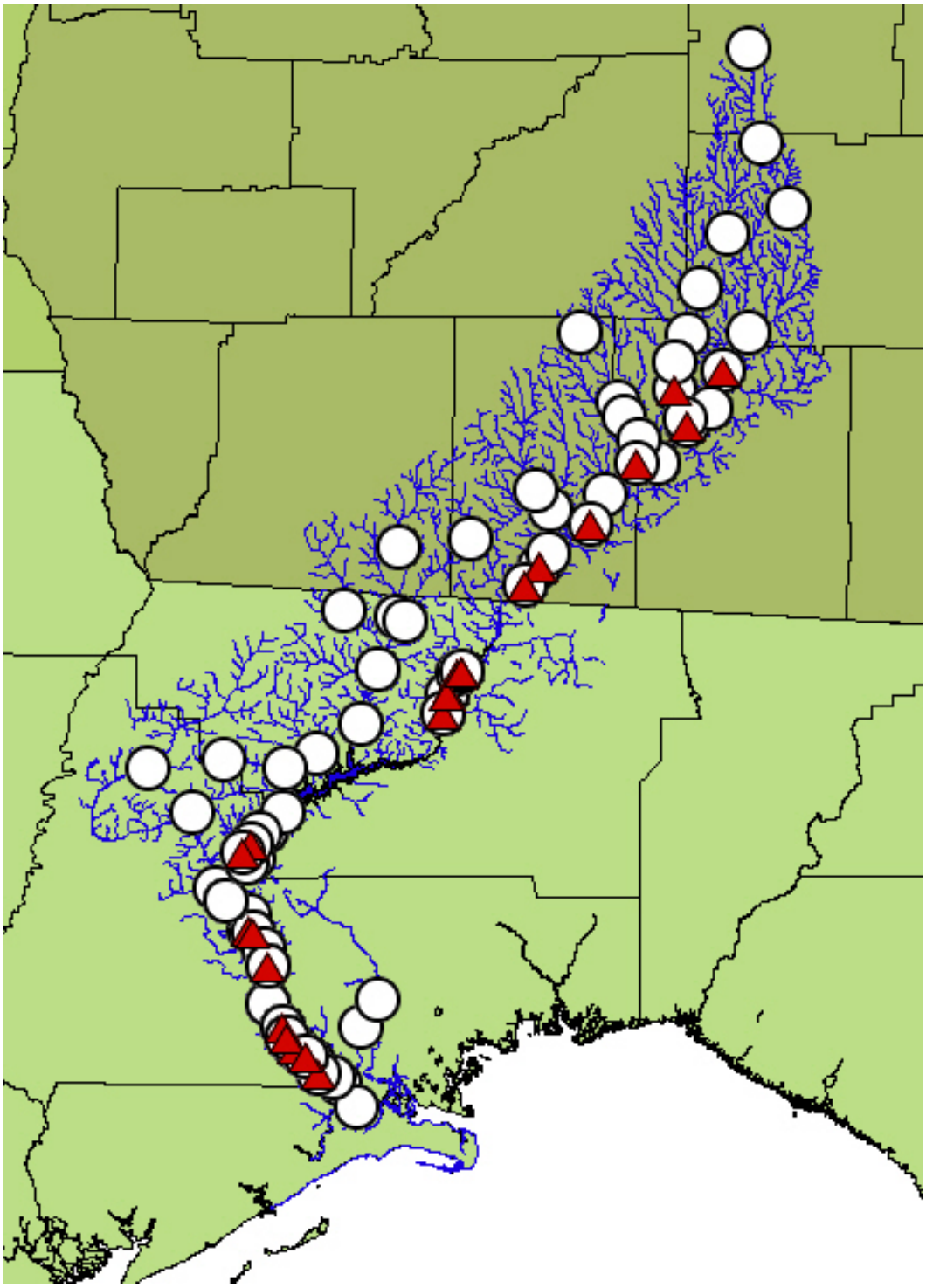
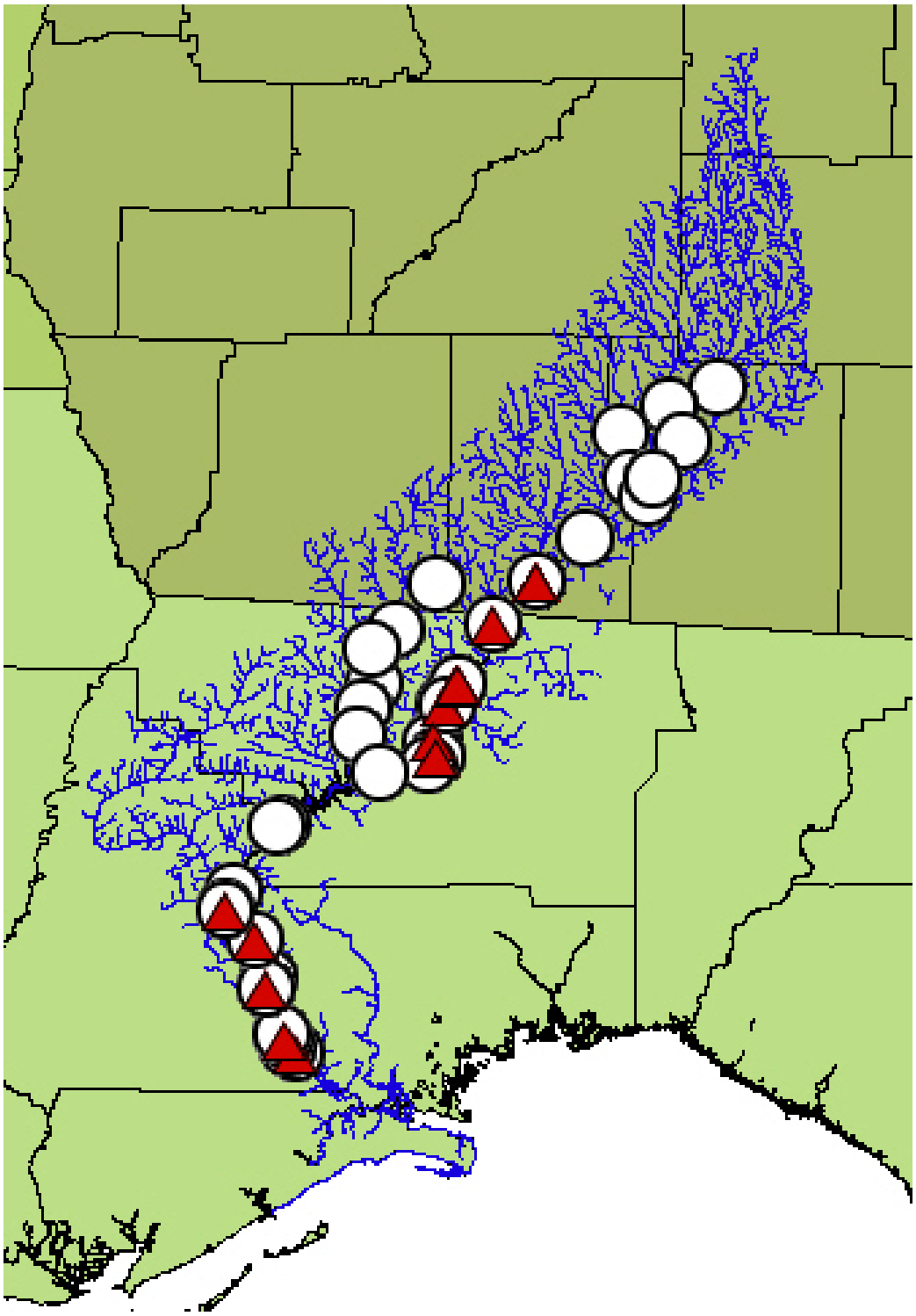


Figure 3. The location of surveys (circles) and located freshwater mussel species (triangles) federally listed as threatened and endangered in the Ochlockonee River from 1990-1999 (US FWS freshwater mussel database).



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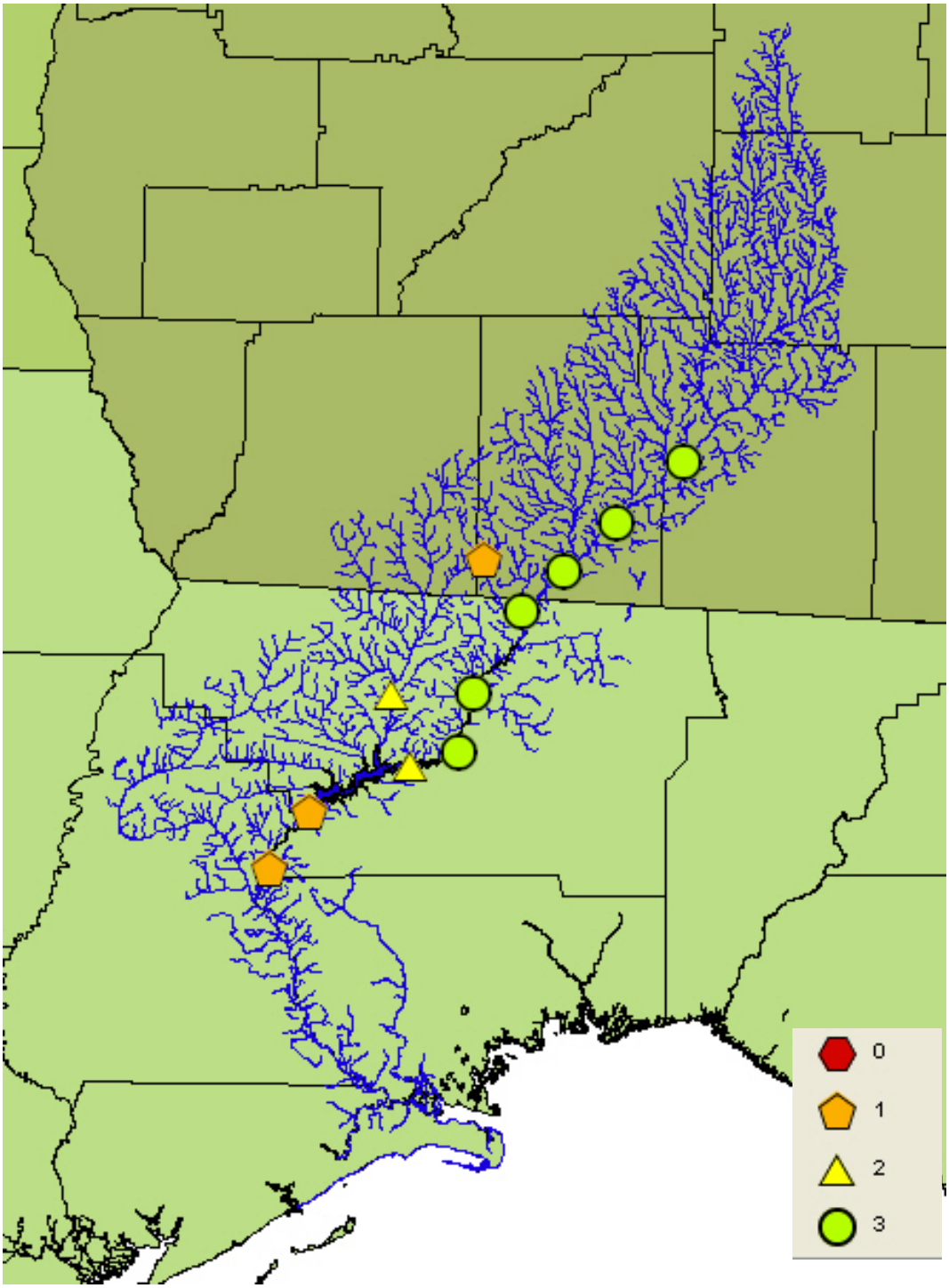

Figure 5. Species richness (# of different species) for federally listed threatened and endangered species of freshwater mussels in the Ochlockonee River prior to 1990 (US FWS freshwater mussel database).

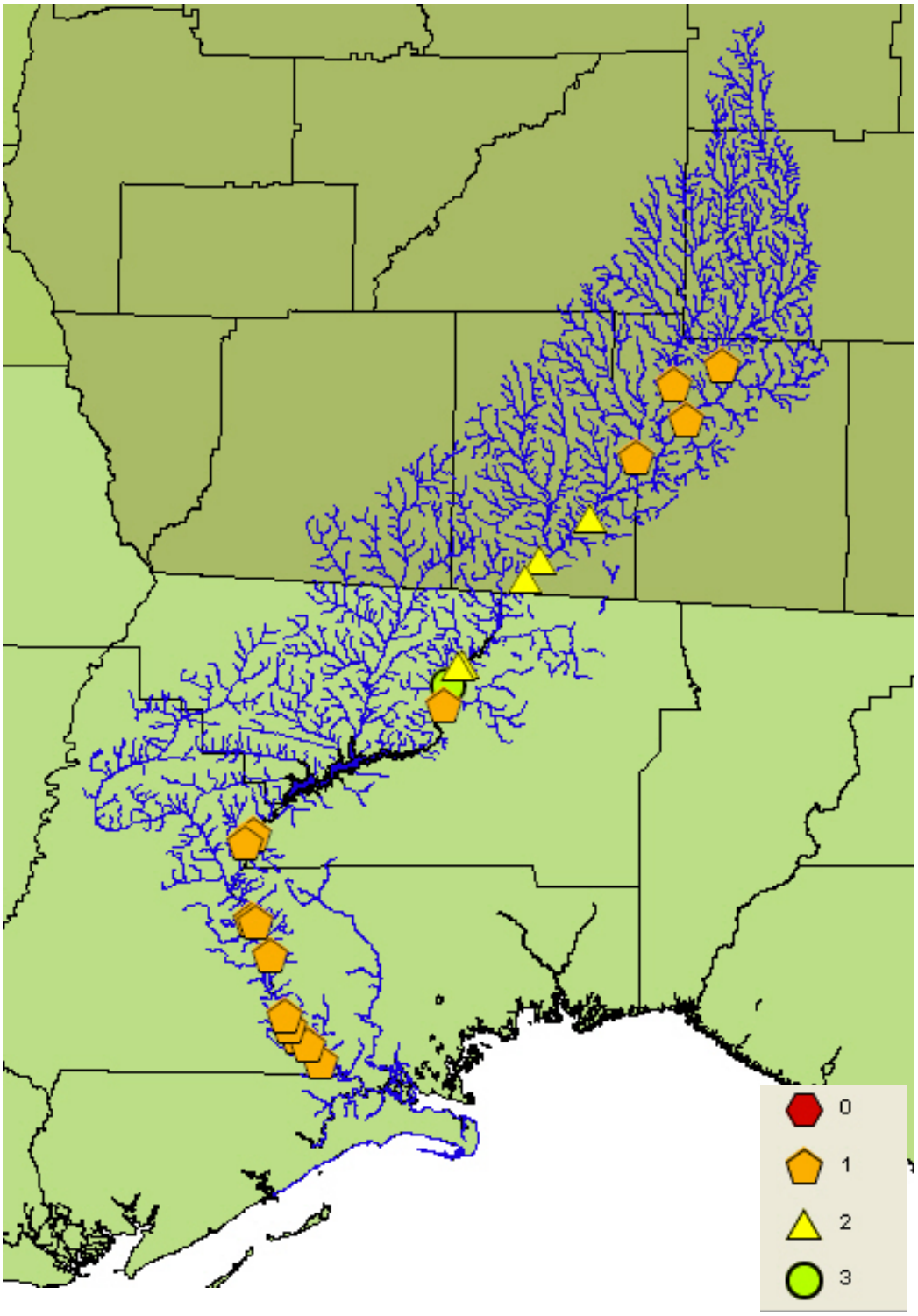

Figure 6. Species richness (# of different species) for federally listed threatened and endangered species of freshwater mussels in the Ochlockonee River from 1990 to 1999 (US FWS freshwater mussel database).

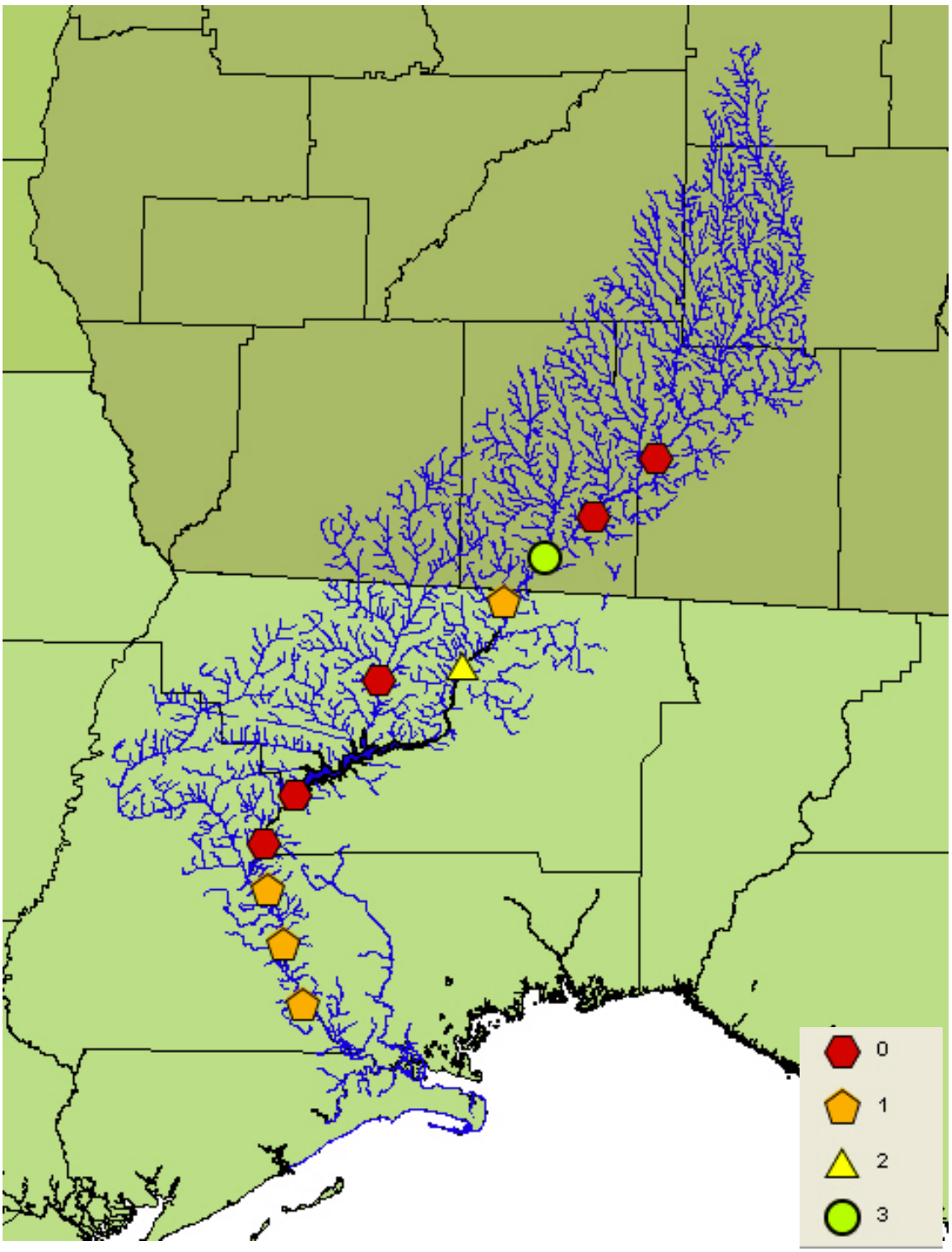

Figure 7. Species richness (# of different species) for federally listed threatened and endangered species of freshwater mussels in the Ochlockonee River from 2000 to 2004 (US FWS freshwater mussel database).

Methods

Water Quality

Water quality data were recorded for 12 sites on the Ochlockonee River in southern Georgia and the Florida panhandle: 6 upstream of Lake Talquin and 6 downstream from Lake Talquin (Figure 8). Sites were selected to correspond with historic mussel survey locations used for the Ochlockonee River. To obtain the required data and provide an evaluation of estimated risk, water column grab samples were taken twice during each of three different water discharge regimes. These discharge regimes included high water, low water and mean water conditions. High water was intended to represent the worst case scenario for nonpoint source pollution due to high volumes of precipitation runoff. Low water was intended to represent the worst case scenario for point source discharges because less water would dilute the effluent. Mean water conditions were intended to provide an alternate condition and provide a comparative baseline to assess unforeseen inputs.

Ambient water quality parameters included free copper (ug/L), un-ionized ammonia (mg/L), chlorine (ug/L), dissolved oxygen (mg/L), temperature (°C), pH (SU), chlorophyll a concentration (ug/L) as calculated from fluorescence, turbidity (NTU), specific conductance (uS/cm@25°C), alkalinity (mg $CaCO_3$/L), and hardness (mg $CaCO_3$/L). 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 total residual chlorine grab samples were analyzed in the field with a HACH DR/2400 portable lab spectrophotometer procured for this project. The remainder of the analyses were performed on aliquots from 1-liter grab samples taken at each site. Total copper and ammonia were measured with the HACH DR/2400 portable lab spectrophotometer, and alkalinity and hardness were measured with a HACH digital titrator Model 16900 at the US FWS Panama City Field

Office. Protocols for sample collection, preservation, and holding times followed American Public Health Association (1998) or HACH Company guidance.

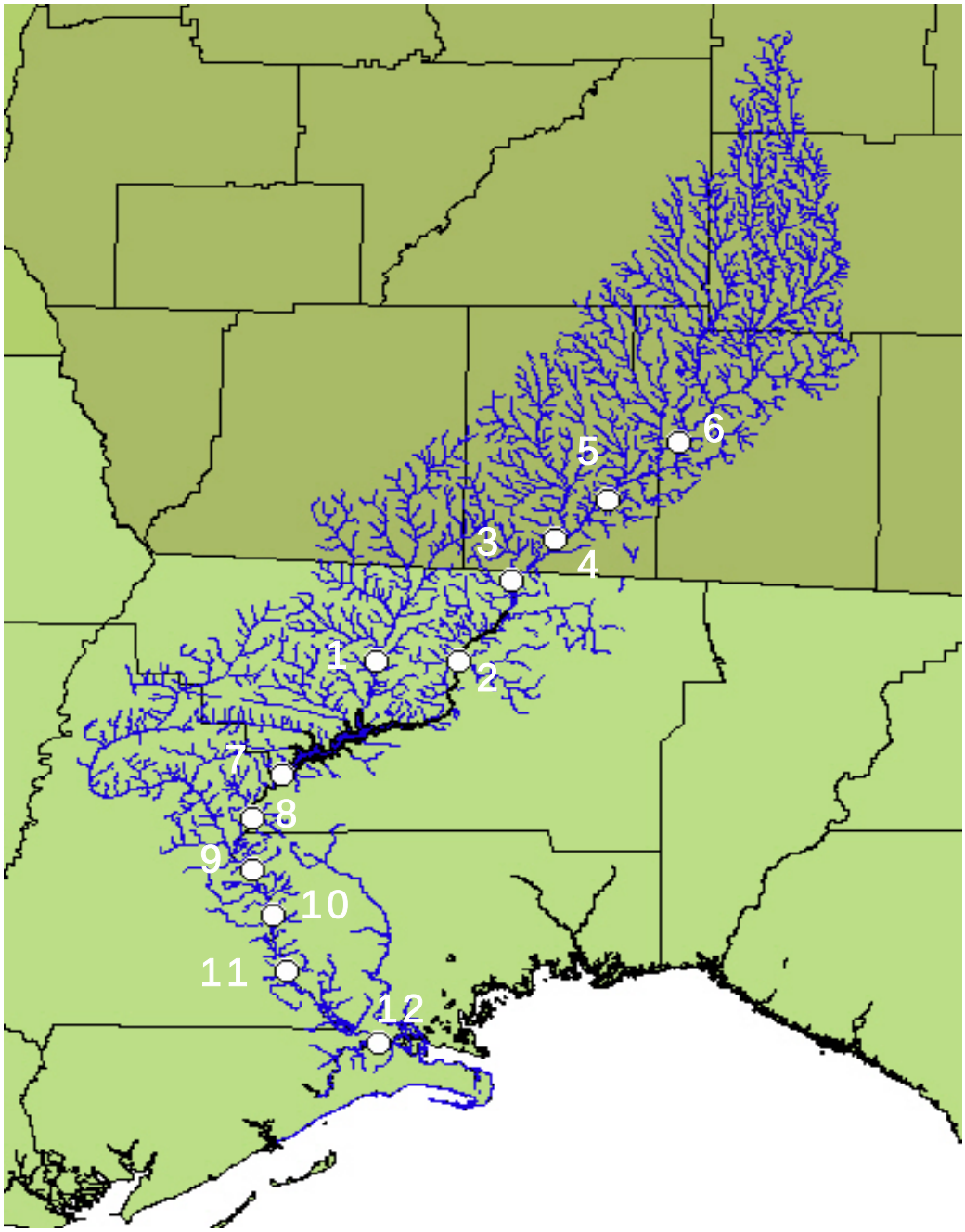

Figure 8. The location of U. S. Fish and Wildlife Service water quality sampling sites (white circles) on the Ochlockonee River 2004.

Sediment Quality

Laboratory sediment toxicity tests were performed on samples collected from 12 sites (same as water quality sites, Figure 9) along the Ochlockonee River during August 2004. Field sampling sites were representative of areas that would be expected to provide suitable habitat for threatened and endangered freshwater mussel species. Multiple sediment samples, collected at each site using a post-hole digger (10-15 cm depth), were composited and homogenized. From each site, the sediment used for laboratory assessment consisted of a 4-L aliquot from the composite sample. Sediment samples were held on ice while in the field and then placed on blue ice for transport and shipment to the U.S. Geological Survey Patuxent Wildlife Research Center's laboratory in Athens, Georgia where they were stored less than 72-hours in the dark at 4°C pending analyses. Assessments in the laboratory included toxicity testing, chemical analyses and sediment characterization. Toxicity testing with *Hyalella azteca* (Crustacea, Amphipoda) followed established protocols (U.S. Environmental Protection Agency 1994; 2000) and included the 28-d chronic toxicity tests (static renewal) on solid-phase sediments, as well as acute 96-h static tests on sediment porewaters. Toxicity of dichloromethane extracts of the sediments were determined by exposing *Vibrio fischeri* (photo-luminescent bacteria) for 5 and 15 minute exposures following procedures described for the basic test by Microbics Corporation (1992). Sediment characterization included: percent moisture, percent organic matter, acid volatile sulfides/simultaneously extracted metals, and particle size analysis in addition to analyses of trace elements from the sediment and porewater.

Prior to analysis in the laboratory, sediment samples were re-homogenized and aliquots were removed for measurements of particle size distribution, acid-volatile sulfides (AVS), simultaneously extracted metals (SEM), percent moisture, percent organic matter, and Microtox bioassays. Five replicates of each field-collected sediment sample, the laboratory-control sediment (commercial sand that was washed and held in test water for two weeks prior to use) and a laboratory reference sample (sediments collected from Lake Buford in northern Georgia) were prepared for solid-phase toxicity testing. For each replicate, 100 ml of bulk sediment were transferred into a 300-ml tall-form beaker with a stainless-steel mesh-

covered notch. The sediment in each beaker was then covered with 175 ml of reconstituted water (described below).

Sediment Toxicity Tests

Solid-phase sediments were tested under static renewal procedures (U.S. Environmental Protection Agency 1994; 2000), with two renewals (70% replacement of overlying water with each renewal) daily (Zumwalt et al. 1994). Deionized water reconstituted to a hardness of 100 mg/l, alkalinity of 70 mg/l, pH of 8 and conductivity of 350 uS/cm was used as the overlying and renewal water. Ten 7-day old *H. azteca* were placed into each of the 5 replicate solid-phase test chambers. The animals were fed 1.5 ml (1.8 g solid/l) of YCT (yeast, Cerophyl, trout chow) daily during the 28-d exposure period. Temperature during the test was maintained at 23 $\pm$ 1 °C and the photoperiod was 16 h light:8 h dark. Survival and growth (length) were the test endpoints. Overlying water chemistry was measured in the exposure chambers at the start and end of the test and included: dissolved oxygen, temperature, pH, alkalinity, hardness, conductivity and ammonia. Temperature, dissolved oxygen, pH and conductivity were monitored at periodic intervals (generally every other day) during the tests. Dissolved oxygen, temperature, pH, conductivity and ammonia were measured with the appropriate meters and electrodes, and alkalinity and hardness were determined by titration.

For the Microtox bioassay organic extractions of the sediments were performed using dichloromethane. The moisture content of each sediment sample was determined for later calculation of dry weight of sediment used in the extraction process. Approximately 5 g of wet sediment were mixed with sodium sulfate (15 g) and then 30 ml of dichloromethane was added and thoroughly mixed. This mixture was centrifuged and the extract decanted into a flask. This extraction process (additional 30 ml of dichloromethane, mixing, and centrifugation) was repeated two more times with the resulting supernatant extract being transferred to the flask. The combined extracts in the flask were concentrated using steam to a volume of approximately 2 ml. Acetone (5 ml) was then mixed with the extract and then re-concentrated to 1 ml. This step was repeated again. Dimethylsulfoxide (DMSO) was

added to make a final volume of 10 ml. A negative control was prepared using DMSO, the test carrier solvent. A positive control was prepared by adding phenol (50 mg/ml) to an extraction blank.

The Microtox bioassays were conducted using the Microtox Toxicity Analyzer System following protocols described by Microbics Corporation (1992). Freeze-dried *V. fischeri* bacteria and reagents were obtained from Microbics (now marketed by Strategic Diagnostic, Inc., Newark, DE). Sediment extracts were tested following the procedures described under "Condensed Protocol for Basic Test, Using Organic Solvent Sample Solubilization" (Microbics Corporation 1992). The amount of light lost was proportional to the toxicity of the test sample. The concentration of the extract that inhibited luminescence of *V. fischeri* by 50% after a 5- and 15-minute exposure represented the EC50 values as mg equivalent sediment wet weight. The wet-weight EC50 values were converted to EC50 dry-weight values for reporting purposes and comparison with other data. The tests were performed in triplicate.

Sediment Porewater Toxicity

Porewater samples for toxicity testing and chemical analyses (porewater samples submitted for analytical analyses were filtered through a 0.45 micron filter) were extracted from the remaining sediment from each sample using a vacuum extractor consisting of a fused glass air stone, airline tubing and a 60-cc syringe (Winger and Lasier 1991; Winger et al. 1998). Approximately 300 ml of porewater were obtained from each sediment sample, aerated for 15 min (dissolved oxygen concentrations exceeded saturation levels during the test), and then 20 ml were transferred to each of 5 replicate 30-ml plastic cups. Ten 7-day old *H. azteca* and a 1-cm^2 piece of Nitex® netting (275 um) were placed into each cup. *Hyalella azteca* were exposed to porewater for 96 h under static conditions and were not fed during the test. The test exposures were maintained at 23 $\pm$ 1°C and 16 h light:8 h dark photoperiod. The endpoint of the porewater test was survival. Basic water chemistry (temperature, dissolved oxygen, pH, alkalinity, hardness, conductivity and ammonia) was measured in porewater after aeration at the start of the test.

Sediment Analytes

Concentrations of Al, As, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, S, Pb and Zn were measured in the sediment after digestion with hydrochloric acid and nitric acid using a Perkin-Elmer Plasma II sequential inductively coupled plasma emission spectrometer. Arsenic and Se in sediments were measured using a Perkin-Elmer Zeeman Model 3030 graphite furnace after digestion with nitric acid and hydrogen peroxide.

Acid volatile sulfides (AVS) were determined following methods described by Brouwer and Murphy (1994). Cations in pore water and metals included in the simultaneously extracted metals (SEM: Cu, Cd, Sn, Hg, Pb, Zn) obtained during the AVS determinations were measured using a Perkin Elmer Elan 6000 ICP-MS (inductively coupled plasma emissions mass spectrometer). Anions (chloride, sulfate, and nitrate) in pore water were determined using an ion chromatograph. Sediment particle size distribution was determined using procedures described by Miller and Miller (1987). Percent moisture was measured as the difference between original wet weight and that after drying for 24 hr at 105 °C. Percent organic matter was derived by loss on ignition at 450 °C for 4 hr (Davies 1974).

Statistical Analyses on Sediment Toxicity Tests

Statistical analyses were conducted using Statistical Analysis Systems (SAS 1990). Comparisons among sites and the control were conducted for survival in porewater and survival and length from solid-phase sediments of *H. azteca*. These test endpoints were compared to water and sediment chemistry and metal concentrations in pore water and solid-phase sediments; analysis of variance using General Linear Models and Dunnett's one-tailed test for significant differences in length and survival in comparison to the control. Similar analyses were performed on the Microtox data. EC50 values from sediment extract testing using Microtox bioassays were compared with test endpoints from the *H. azteca* tests and trace element analyses from porewater and sediments. Analyses were performed on log-transformed data.

Evaluation of Potential Risk and Land Use Analysis

To identify potential sources of stressors, water and sediment quality results were compared to permitted point source discharges (via National Pollution Discharge Elimination System Permits), general land use characterizations provided by the respective state water management authorities, and the current and historic presence/absence data for freshwater mussels in the Ochlockonee River drainage. Additionally, data were evaluated relative to State of Florida water quality standards for the protection of aquatic life, Environmental Protection Agency water quality criteria for aquatic life protection, and to information generated by ongoing multi-regional and multi-agency efforts to better estimate toxicity thresholds for these taxa. However, information is currently insufficient to assess risk to these species from water quality in the targeted watersheds. Therefore, Geographic Information System (GIS) layers of relative water quality and estimated species risk per location were developed to provide a comparative visual tool to better assess watershed conditions. This assessment will allow for ranking of sites for protection, restoration, additional studies, and eventual re-introduction of listed species.

Results and Discussion

Water Quality

Water quality data were recorded at 12 sites on the Ochlockonee River, but not under all flow conditions intended. Three of the six intended sampling events were accomplished and represented two high flow events and one low flow event (Table 1). Extensive rainfall precluded sampling under low flow conditions again or under mean flow conditions.

Table 1. Mean (monthly) flow conditions (ft^3/sec) for each sampling event for sites upstream (Upstream Flow) of Lake Talquin and sites downstream (Downstream Flow) of Lake Talquin separately, according to U.S. Geological Survey gaging stations.

Sampling Event	Date	Upstream Flow	Downstream Flow
High Flow Event 1	June 2004	950	2,300
High Flow Event 2	July 2004	750	3,300
Low Flow Event 1	August 2004	410	1,200

Total ammonia concentrations in the Ochlockonee River system were relatively low (Figure 9) in comparison to the recommended range for a criteria continuous concentration (CCC) by Augspurger (2003) and the state of Florida ammonia water quality standard. For both of the above reference values the free ammonia concentration was maintained as the total ammonia concentration was adjusted for the recorded maximum system pH of 7.3 and 25°C. Based on the recommended criteria and the water quality standard, the risk from ammonia exposure was expected to be low in ambient water. This was supported after examining the influence of the system's low pH (Figure 10) via the calculation of un-ionized ammonia concentrations (Figure 11). This calculation is important because most ammonia toxicity results from the un-ionized form (Augspurger et al. 2003. Newton et al. 2003). Total ammonia is the sum of un-ionized (NH^3) ammonia and ammonium or ionized ammonia (NH^4+). How much of each nitrogen species (NH^3 or NH^4+) is present in a sample depends

on the sample's hydrogen ion concentration or pH and temperature. The lower the aqueous pH, the larger the proportion of the ammonia species that are ionized (NH^4+) and the higher the pH the larger the proportion of ammonium or un-ionized ammonia (NH^3). The low pH condition was a direct reflection of the low buffering capacity or alkalinity that the system possessed (Figure 12).

Free copper concentrations were also quite low in the Ochlockonee River system (Figure 13). It is particularly noteworthy that only one violation of the state standard or EPA water quality criterion was found despite very low calculated standard and criterion values due to low system hardness (Figure 14). Higher water hardness, a measure of the concentration of divalent (2+) cations, acts to naturally chelate or bind ionic metals and reduce their toxicity. Waters, such as those in the Ochlockonee River have low ionic strength and possess very little chelation ability due to their low water hardness. As a result of low copper chelation in waters with low hardness, more free copper is estimated to be biologically available or toxic. Higher water hardness would have resulted in the calculation of a higher copper (metals) state standard or EPA criterion that would have estimated the same concentration of biologically available copper.

The low pH and low buffering capacity of this system did create a situation where metals toxicity could be present. Converse to ammonia, the lower the system pH, the greater the potential for copper toxicity. Lower pH in an aqueous solution shifts copper from the inert elemental form to the free ion (biologically available cupric ion, Cu^{2+}) form often associated with toxicity. When low pH is combined with a soft water system, the risk of metals toxicity is even higher. Taken together, metal concentrations in this system warrant future monitoring and consideration in permitting discharges to the system.

Dissolved oxygen concentrations were consistently below desired levels, state standards, and the EPA criterion in the reach of the Ochlockonee River below Lake Talquin, but also at upstream sites to a lesser extent (Figure 15). It is noteworthy these measures were taken during the summer months with assumed annually high water temperatures (Figure 16). The higher the water temperature, the less gas that can be dissolved in the water. In other words,

a water sample at 20°C is 100% saturated with dissolved oxygen at a concentration of approximately 9.1 mg/L, but a water sample at 30°C is 100% saturated with less dissolved oxygen at a concentration of approximately 7.5 mg/L. However, despite warmer temperatures, less than half of the oxygen that could be dissolved in waters from the site just below the dam was found in those samples (Figure 17). Because percent saturation with oxygen takes into account temperature in the estimation of potential oxygen content, it is particularly useful as an indicator of high oxygen demand from chemical or biological sources. The resulting low oxygen levels have been implicated in causing stress to, and limiting the success of, invertebrate and fish species (Nebeker *et al*., 1992; EPA, 1986; Nebeker, 1972; Dahlberg *et al*., 1968; Sprague,1963). Additionally, dams alone via their alteration of flow regimes have been cited as the cause of extirpation for 30%-60% of the native mussel species in select U.S. rivers (Williams et al. 1992; Layzer et al. 1993).

Turbidity was found to exceed the state water quality standard on one occasion in the Little River tributary just above Lake Talquin (Figure 18), but the lack of clarity did not appear to result from algal production (Figure 19). However, the river segments downstream of Lake Talquin did have approximately double the chlorophyll a concentrations (algal growth indicator) as those sites above the lake. Specific conductance, a measure of total dissolved solids normalized for temperature effects, was low in the system overall and was not expected to have been different than historic background levels due to the lack of driving factors for such changes (Figure 20).

The grab samples to be analyzed for total residual chlorine in the field to prevent volatilization and misrepresentative readings were not successfully analyzed. The HACH DR/2400 portable lab spectrophotometer procedure could not account for tannins in the system and consistently produced nonsensical results. This factor could not be overcome within the means of the project (Personal Communication from Technical Assistance at Hach Co. 2004). As a result no chlorine data were obtained.

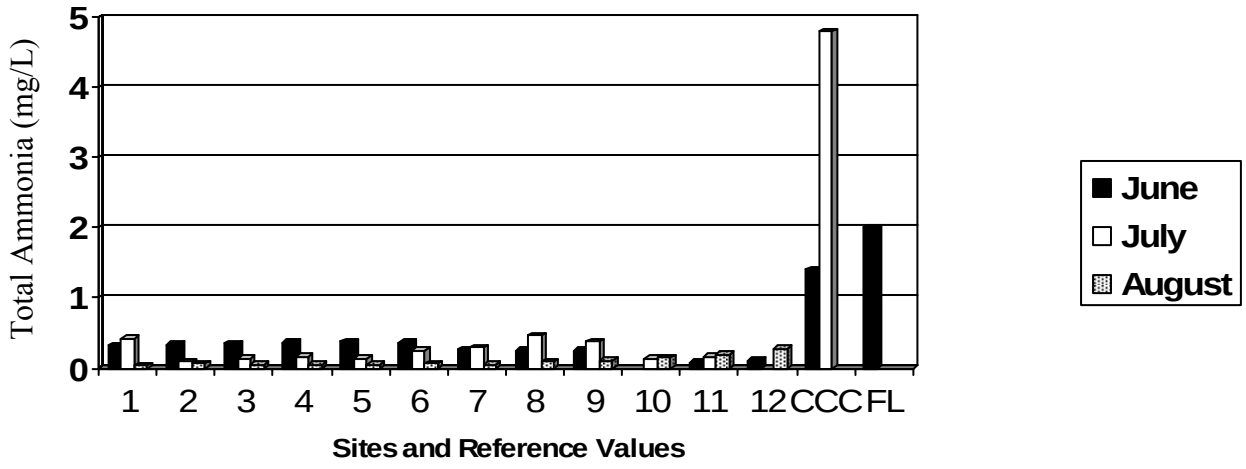

Figure 9. Measured total ammonia concentrations (mg/L NH3+NH4) for water samples taken from the Ochlockonee River compared to the Augspurger *et al.* (2003) recommended range for criteria continuous concentration (CCC, black lower end of range, white higher end of range) and the State of Florida ammonia water quality standard. For the recommended CCC and state standard, the free ammonia concentration was maintained as the total ammonia concentration was adjusted for the recorded maximum system pH of 7.3 and 25°C.

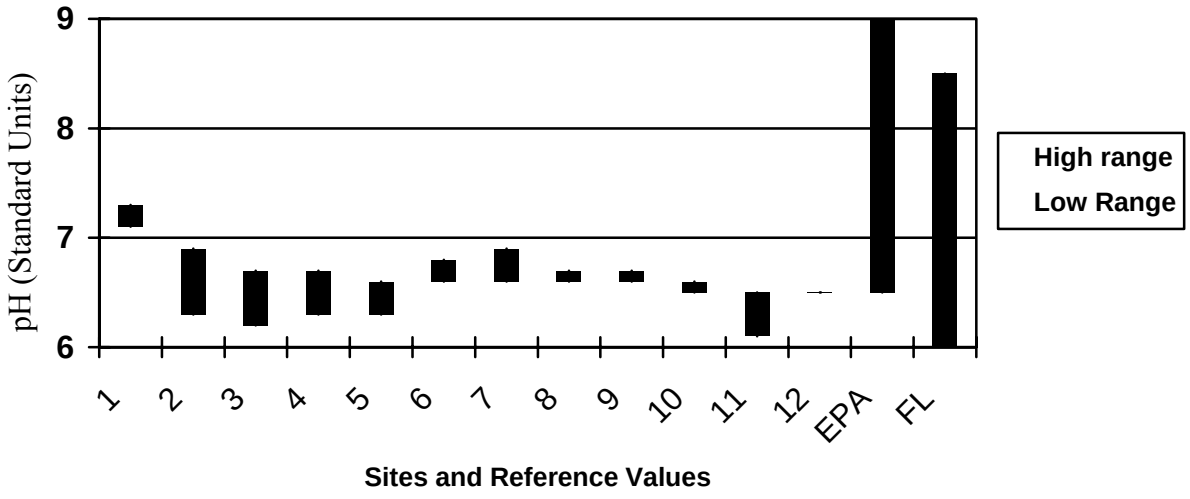

Figure 10. Hydrogen ion concentration (pH in standard units) range at selected sites in the Ochlockonee River, summer 2004 compared to the U. S. Environmental Protection Agency criterion range (1976, 6.5-9 SU) and the Florida state water quality standard range (F.A.C. 2004, 6-8.5).

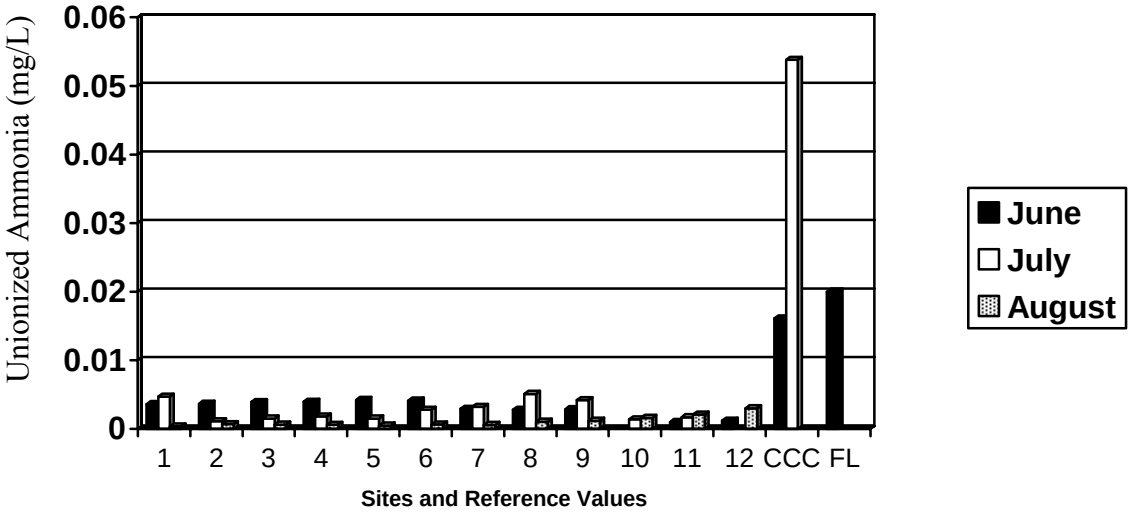

Figure 11. Calculated free un-ionized ammonia concentrations (mg/L NH3 calculated for maximum pH of 7.3) for water samples taken from the Ochlockonee River compared to the calculated free un-ionized ammonia concentration calculated for Augspurger et al. (2003) recommended criteria continuous concentration (CCC, calculated for pH 7.3 and 25°C, black-lower end of range, white-higher end of range) and the State of Florida un-ionized ammonia water quality standard (F.A.C. 2004).

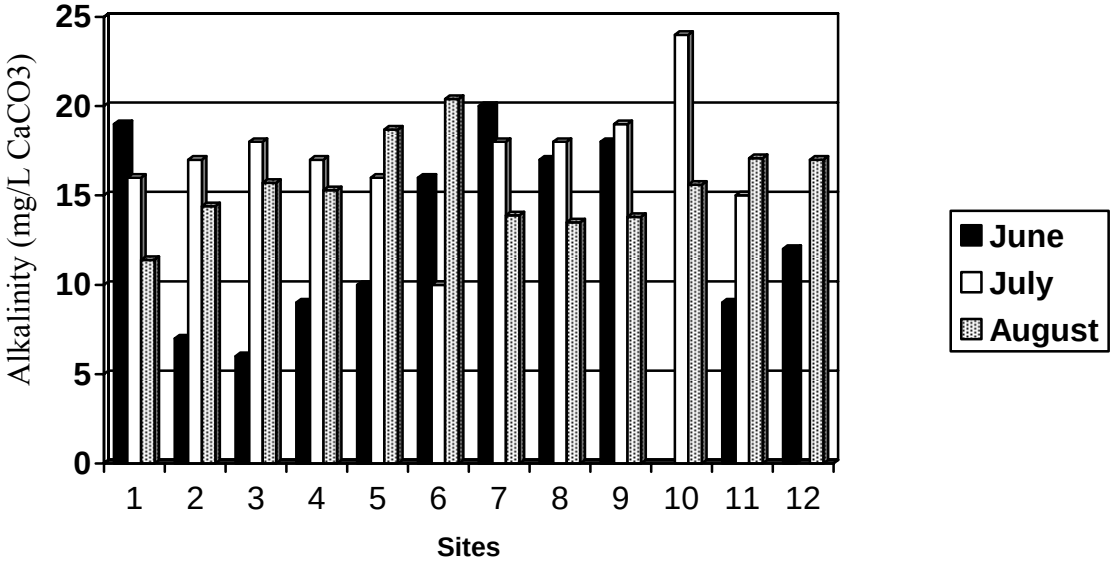

Figure 12. Water alkalinity (measured as mg $CaCO_3$/L) for water samples taken from the Ochlockonee River.

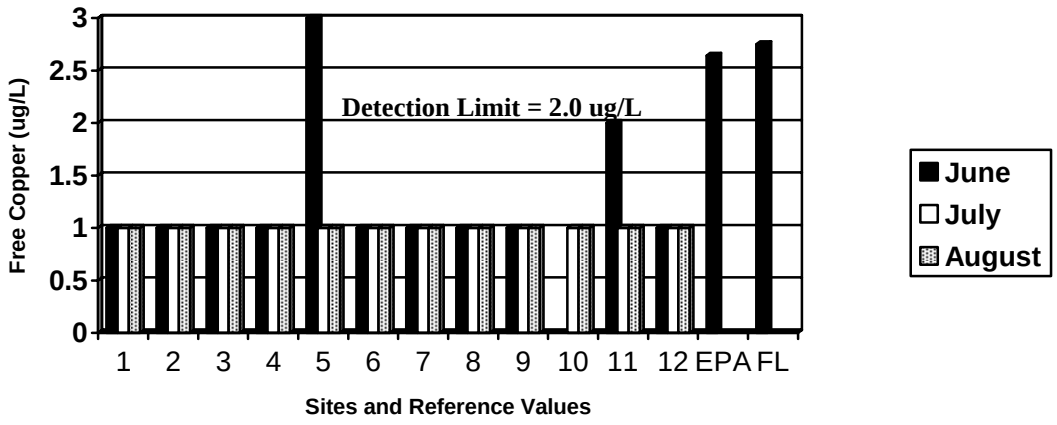

Figure 13. Free copper concentrations (ug/L, lower limit of detection 2 ug/L, non-detects reported as 1.0 ug/L) for water samples taken from the Ochlockonee River compared to U. S. Environmental Protection Agency chronic criterion (2002, $Cu \leq e^{(0.8545[\ln H]-1.465)}$) and the Florida state water quality standard (F.A.C. 2004, $Cu \leq e^{(0.8545[\ln H]-1.702)}$). Both were calculated for the overall mean water hardness of 23.9 mg/L $CaCO_3$.

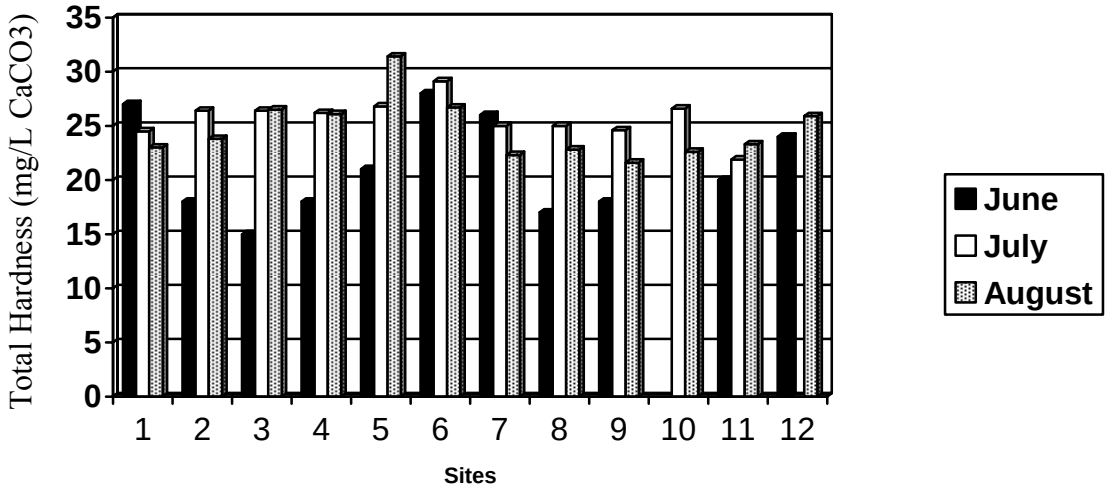

Figure 14. Total water hardness (measured as mg $CaCO_3$/L) for water samples taken from the Ochlockonee River.

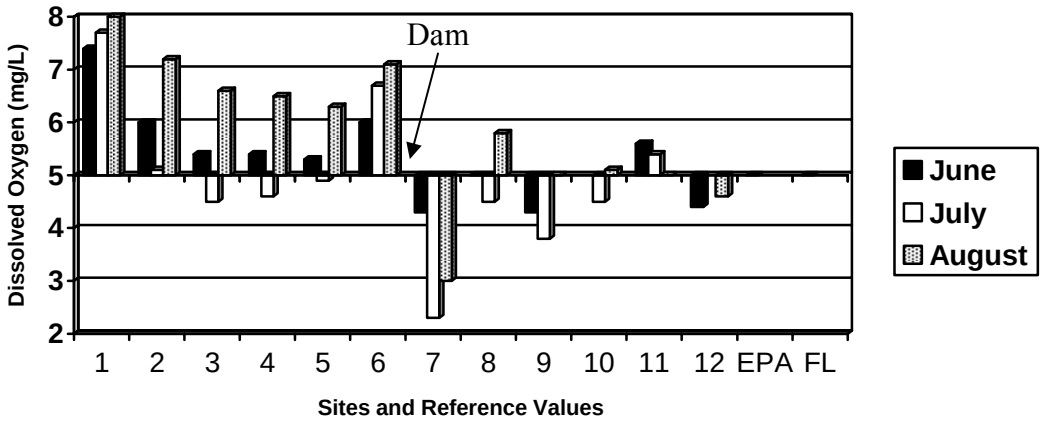

Figure 15. Dissolved oxygen concentrations (mg/L oxygen) at selected sites in the Ochlockonee River compared to the U. S. Environmental Protection Agency chronic criterion (1984, 5 mg/L) and the Florida state water quality standard (F.A.C. 2004, 5 mg/L).

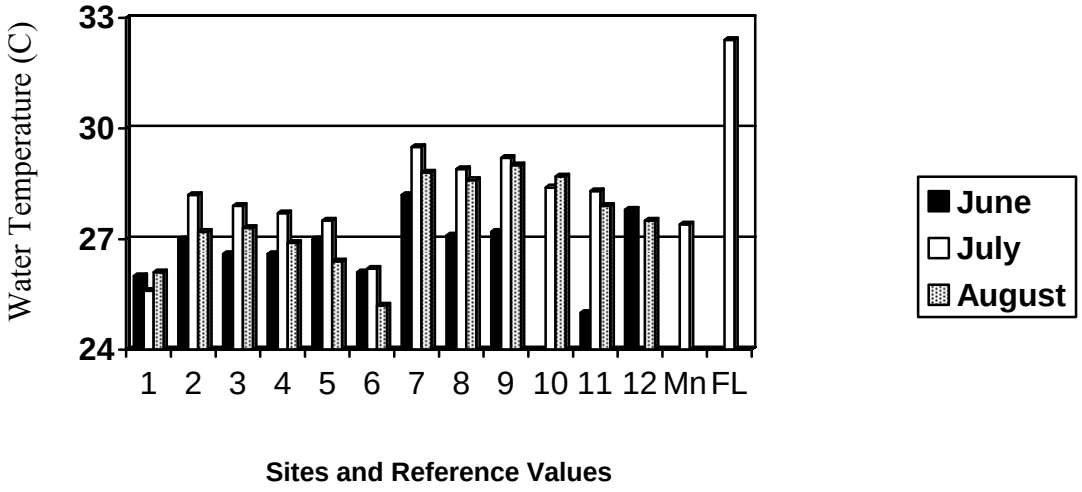

Figure 16. Water temperature (°Celsius) at selected sites in the Ochlockonee River compared to the overall summer season mean (Mn, 27.4°C) as would be used to determine Florida thermal water quality standards violations (F.A.C. 2004, in excess of mean background temperature plus 5°C, intended for thermal point source discharges).

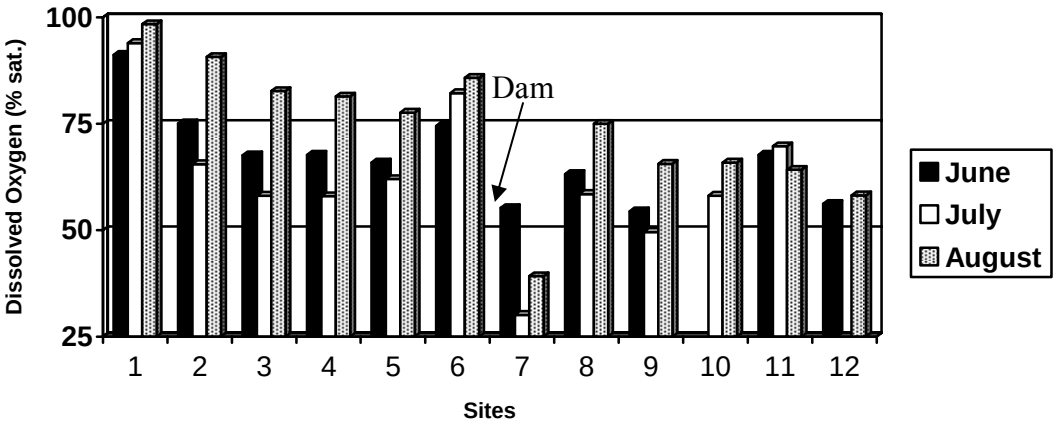

Figure 17. Dissolved oxygen percent saturation in water at selected sites in the Ochlockonee River.

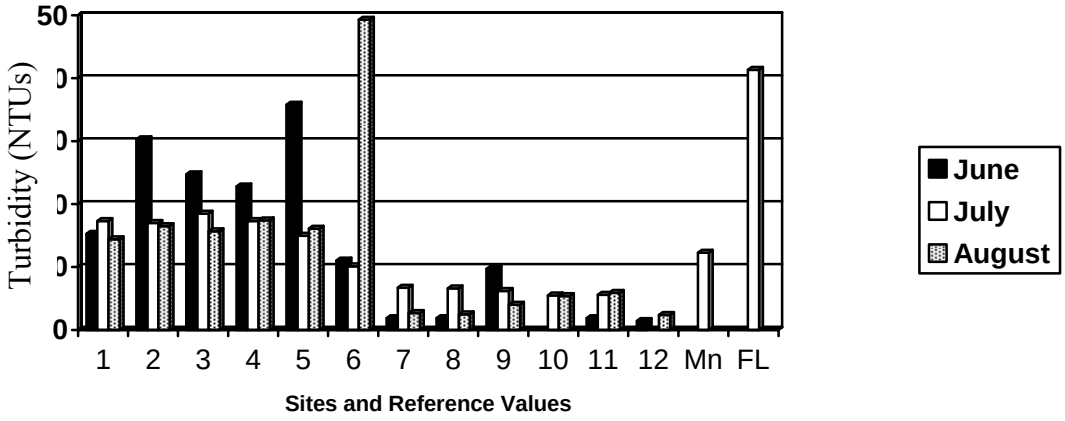

Figure 18. Turbidity (NTUs) at selected sites in the Ochlockonee River compared to the overall summer season mean (Mn, 12.3 NTUs) as would be used to determine Florida water quality standards violations (F.A.C. 2004, ≥ 29 above natural background conditions of 12.3 = 41.3).

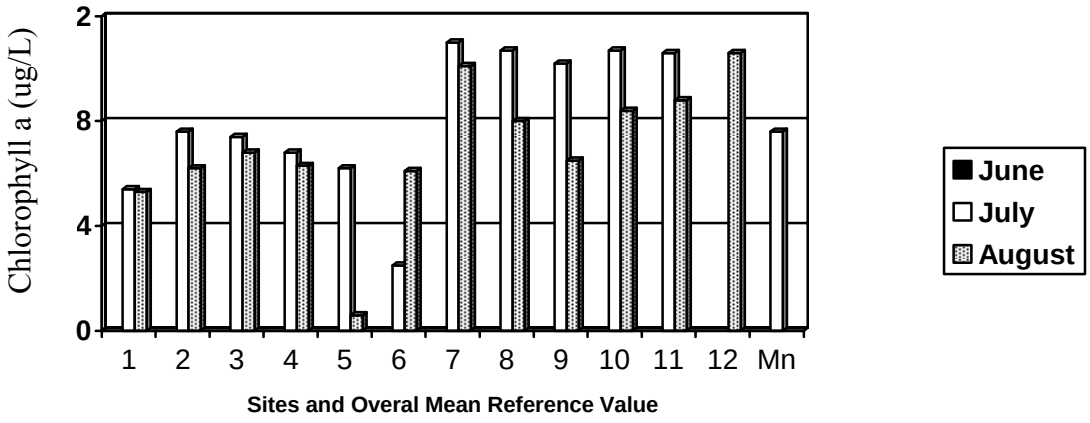

Figure 19. Chlorophyll a concentrations (ug/L) at selected sites in the Ochlockonee River compared to the overall summer season mean (Mn, ug/L) in light of the Florida water quality standards for nutrients (F.A.C. 2004, nutrient concentrations in a waterbody shall not be altered to cause an imbalance in natural populations of aquatic flora or fauna, nor shall they result in violations of other standards).

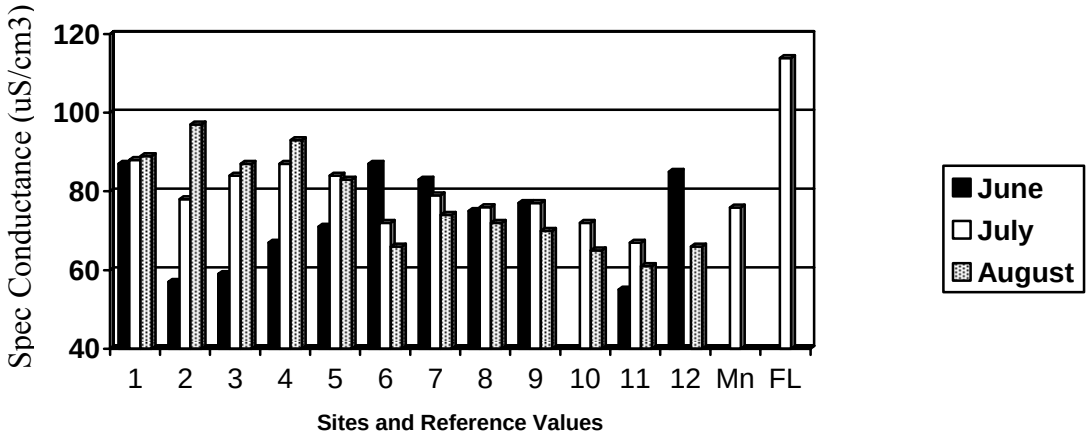

Figure 20. Specific conductance (uS/cm^3@25°C) at selected sites in the Ochlockonee River compared to the overall summer season mean (Mn, 75.9°C) as would be used to determine Florida water quality standards violations (F.A.C. 2004, in excess of mean background plus 50% or 1275 uS/cm^3, whichever is greater; 75.9 + (0.5*75.9 or 37.95) = 113.9).

Sediment Quality

Sediment Toxicity Tests

Survival of *Hyalella azteca* exposed to porewater was significantly reduced compared to the control in sediments from three locations (Stations 1, 5, and 11) on the Ochlockonee River (Table 2). *H. azteca* survival in exposures to the solid-phase sediments was not significantly reduced from the control at any of the sites. Length (measure of growth) of *H. azteca* was significantly less than the control-sediment exposures in sediments from 10 locations. The large number of sites showing a significant difference for length may be partially explained by the comparatively large size (length) of the animals exposed to the control sediment. If length from exposures to sediment from Station 8 (greatest length of *H. azteca* from the test sediments) was used for comparison as a field reference site, no significant differences in length would be detected. Because of the large size of animals from the control sediments, the significance of the statistical differences with the control may be questioned.

Table 2. Percent survival and growth (expressed as total length, mm) of *Hyalella azteca* exposed to pore water and solid-phase sediments collected from the Ochlockonee River, Florida in August 2004, and EC50s at 5- and 15-minute exposures of sediment extract to *Vibrio fischeri* (Microtox). Values in parentheses are standard deviations and values with an asterisk indicate a significant difference ($p < 0.05$) to the Control. Comparisons were made to laboratory-control sediment (Control, commercial sand that was washed and held in test water for two weeks prior to use), a laboratory reference sample (Reference, sediments collected from Lake Buford in northern Georgia for which the laboratory has extensive data), and reconstituted hard water (Recon) exposures.

	Pore water	Solid-phase sediment		Microtox	
Station	Percent survival	Percent survival	Length (mm)	EC50-5 min (mg)	EC50 -15 min (mg)
0104	42 (25.88)*	92 (8.36)	4.01 (0.26)*	93.27 (11.05)*	75.98 (8.10)*
0204	86 (11.40)	92 (4.47)	4.03 (0.36)*	221.06 (10.62)	278.21 (17.40)
0304	100 (0)	96 (5.47)	4.46 (0.36)	177.79 (38.37)	146.51 (27.31)
0404	94 (9.94)	98 (4.47)	4.57 (0.54)	281.85 (10.89)	294.51 (2.12)
0504	50 (15.81)*	94 (8.94)	4.21 (0.31)*	98.78 (9.17)*	135.44 (11.25)
0604	88 (13.03)	100 (0)	4.11 (0.24)*	185.64 (8.5)	182.03 (8.49)
0704	82 (13.03)	90 (12.24)	4.29 (0.32)*	133.88 (3.87)	137.43 (5.17)
0804	100 (0)	98 (4.47)	4.39 (0.36)*	207.23 (25.21)	181.54 (20.76)
0904	100 (0)	96 (5.47)	4.31 (0.45)*	119.57 (6.69)	105.81 (3.21)
1004	92 (17.88)	96 (5.47)	4.31 (0.31)*	276.77 (6.19)	260.60 (12.23)
1104	76 (8.94)*	92 (8.36)	4.13 (0.32)*	267.74 (51.63)	306.24 (64.08)
1204	80 (12.24)	96 (8.94)	4.26 (0.25)*	170.78 (17.22)	158.36 (18.68)
Reference	90 (10.00)	92 (8.36)	3.41 (0.32)*	114.74 (3.48)	112.73 (4.25)
Control	98 (4.47)	96 (5.47)	4.61 (0.35)	156.14 (28.87)	143.52 (22.27)
Recon	90 (10.00)	-	-	-	-

EC50s from the 5-minute exposures for the Microtox bioassays showed significant differences with the laboratory control at Stations 1 and 5 (Table 2). The 15-minute exposures showed only a significant difference at Station 1. Bear in mind that the lower the mass of sediment causing an EC50, the greater the toxicity of the sediment.

Based on the data from the three sets of toxicity tests, sediments at Stations 1 and 5 (and possibly 11) may be somewhat impaired in terms of supporting aquatic life in general. Station 1 is at the lower end of the Little River north of Lake Talquin and southeast of Quincy, Florida. Station 5 is in southern Georgia southeast of Cairo. The quality of sediment from the other sites does not appear to be limiting aquatic organisms; however, toxicity testing with appropriate mussel surrogates will be essential to verifying that freshwater mussels are not subject to sediment toxicity limitations at these sites.

Sediment Analytes

The general chemistry of porewater (Table 3) from the sediments appeared to be within natural variability (Personal Communication from Parley Winger, USGS 2004). Although ammonia concentrations (both total and un-ionized) were elevated at several locations (Stations 1, 2, 5, 8, 9, 10 and 11), there did not appear to be a direct relationship to survival (no dose-response relationship), although the concentration from Station 1 could have influenced survival at that site. Un-ionized ammonia exceeded the 0.02 mg/l estimated safe level for freshwater mussels at 10 of the 12 sites (Augspurger et al. 2003), but survival of *H. azteca* in porewater did not reflect these elevated levels. This is not surprising considering that *H. azteca* are reported to be far less sensitive to un-ionized ammonia than many freshwater mussel species are expected to be (0.4 mg NH_3/L, DMMP 2001; EPA and ACE 1993). Other water chemical characteristics of the porewater were within acceptable ranges for *H. azteca*.

Water chemistry of overlying water from the solid-phase exposure tests was also within expected ranges (Table 4) and changed little during the toxicity testing. Ammonia was

slightly elevated at a few sites (the same sites that had elevated concentrations in the porewater tests) at the start of the test, but concentrations were well below levels of concern. Unfortunately, the overlying water samples at the end of the test were inadvertently discarded before ammonia could be measured. Ammonia concentrations in the overlying water from the solid-phase exposures generally decline during the course of the test due to the daily replacement of the water (that is what the renewal system is designed to do – maintain acceptable water quality during the test). Consequently, ammonia concentrations in the overlying water at the end of the solid-phase exposures would be expected to be less (probably non-detectable) than those measured at the start of the test.

The physical characteristics of the sediments varied little from site to site (Table 5). Organic matter was generally low (within normal ranges) and the sediments consisted primarily of sand. Acid volatile sulfides (AVS) were low, as were the concentrations of the simultaneously extracted metals (SEM). These small concentrations were most likely responsible for the inordinately high ratios shown for the SEM/AVS. Although SEM/AVS ratios exceeding 1 are considered predictive of metal toxicity (Di Toro et al. 1992), the low concentrations of metals available would not be expected to exceed toxic levels and would also most likely be bound to the high concentrations of dissolved organic matter in the porewater and be unavailable.

Concentrations of trace elements in porewater (Table 6) were also generally below levels that would be expected to have adverse effects and were substantially lower than established water quality criteria (U.S. EPA 2002). The only exception was lead (Pb) which had concentrations at Stations 2, 3, 4 and 7 that exceeded the 2.5 µg/l continuous criteria concentration. Concentrations of Pb exceeded aquatic effects levels of 1-5 µg/l (Eisler 1988) at 7 of the 12 test sites, but survival of *H. azteca* in porewater did not correspond to these levels and toxicity of lead to freshwater mussels is not well known. In addition, the manganese (Mn) concentration in porewater from Station 1 was within the acutely toxic range or 3-13 mg/l for *H. azteca*, depending on water hardness (Lasier et al. 2000) and may have contributed to the reduced survival shown for this site. Similarly, concentrations of trace elements in the solid-phase sediments were generally low and considerably less than

consensus-based sediment quality guidelines (MacDonald et al. 2000); except for Pb at Station 7 (site just below the Lake Talquin Dam) that exceeded the Threshold Effects Level (TEL) of 35.8 mg/kg. Lake Talquin is a man-made lake, that was formed in 1927 when the Jackson Bluff Dam was constructed on the Ochlockonee River to produce hydroelectric power.

Concentrations of Pb were also somewhat elevated at Stations 3 (upstream of the dam and south of Cairo, GA) and 11 (on the lower river near the bay). Although concentrations of trace elements (metals) in porewaters and sediments from the Ochlockonee River appear to be below effects levels, concentrations that could have long-term chronic effects are not known. Sublethal effects of metals on freshwater mussels have been observed at less than half of measured lethal concentrations (Naimo 1995).

Table 3. Chemistry of porewater extracted from sediment collected from the Ochlockonee River, August 2004.

Station	Temp °C	DO mg/l	pH	Cond µS/cm	Alk mg/l	Hard mg/l	Tot Amm[a] mg/l	UnNH3 mg/l	Anions (mg/l)			Dissolved carbon (mg/l)		
									Chloride	Nitrate	Sulfate	Inorganic	Organic	Total
0104	23	6.5	7.61	632	308	204	16.0	0.31	8.22	1.16	ND	83.08	11.14	94.22
0204	23	6.8	8.30	515	260	156	6.0	0.54	6.98	bdl	0.34	69.01	4.64	73.65
0304	23	6.6	8.00	165	70	24	1.4	0.06	10.52	bdl	0.42	18.26	15.61	33.87
0404	23	7.5	7.77	141	72	18	1.7	0.05	8.37	1.52	6.99	9.79	8.03	17.82
0504	23	5.6	6.98	163	78	19	7.6	0.04	10.29	0.33	1.06	14.97	15.4	30.37
0604	23	7.5	7.49	256	130	85	1.9	0.03	8.47	bdl	0.84	31.00	8.52	39.52
0704	23	8.1	7.25	294	62	38	0.7	bdl	9.93	10.81	18.77	14.55	7.59	21.14
0804	23	7.1	7.71	265	108	61	6.6	0.16	8.90	bdl	3.65	29.88	8.87	38.75
0904	23	7.5	8.02	300	138	63	8.4	0.39	11.40	bdl	0.97	36.11	9.92	46.03
1004	23	5.8	7.67	279	130	56	14.8	0.36	8.32	bdl	0.36	30.13	17.94	48.07
1104	23	7.3	8.14	385	194	121	6.1	0.36	6.06	bdl	0.35	52.25	4.47	56.72
1204	23	7.7	8.05	338	196	101	1.5	0.07	11.05	bdl	2.29	38.41	5.58	43.99
Reference	23	8.4	8.13	225	110	49	bdl	bdl	2.23	0.25	0.57	33.86	9.52	43.38
Control	23	8.9	8.13	413	84	86	bdl	bdl	39.02	2.65	61.55	20.50	2.27	22.77
Recon	23	8.2	8.14	404	78	85	bdl	bdl	38.95	1.96	65.97	17.18	0.34	17.52

bdl – Not detected within the limits of the methods used.
[a] total ammonia

Table 4. Chemistry of overlying water at the start (September 3, 2004) and the end (October 1, 2004) of the 28-day exposures of *Hyalella azteca* to solid-phase sediment collected from the Ochlocknee River, Florida.

Station	Start								End							
	Temp °C	DO mg/l	pH	Cond µS/cm	Alk mg/l	Hard mg/l	Tot Amm[b] mg/l	UnNH3 mg/l	Temp °C	DO mg/l	pH	Cond µS/cm	Alk mg/l	Hard mg/l	Tot Amm[b] mg/l	NH3 mg/l
0104	22.9	7.2	7.75	404	80	44	1.3	0.03	21.3	7.3	7.78	428	88	116	[a]	[a]
0204	22.9	6.8	7.81	399	84	142	0.5	0.01	21.3	7.3	7.86	437	88	128		
0304	22.9	7.1	7.72	367	58	116	0.2	0.00	21.3	7.6	7.76	421	78	128		
0404	22.9	7.4	7.82	396	72	120	0.0	0.00	21.3	7.6	7.79	436	80	124		
0504	22.9	7.5	7.67	373	62	116	0.7	0.01	21.3	7.0	7.73	434	80	124		
0604	22.9	7.3	7.72	399	72	124	0.0	0.00	21.3	6.9	7.77	450	88	132		
0704	22.9	7.9	7.71	392	70	124	0.0	0.00	21.3	6.9	7.74	431	80	124		
0804	22.9	7.4	7.69	381	70	124	0.4	0.00	21.3	6.9	7.70	434	80	128		
0904	22.9	7.7	7.66	381	70	124	0.9	0.02	21.3	6.8	7.69	437	80	132		
1004	22.9	7.1	7.58	375	70	120	1.6	0.03	21.3	6.9	7.70	434	80	128		
1104	22.9	7.6	7.63	389	78	128	0.7	0.01	21.3	6.8	7.71	443	88	132		
1204	22.9	7.2	7.82	409	88	140	0.0	0.00	21.3	7.1	7.78	445	88	140		
Reference	22.9	7.5	7.70	360	64	112	1.1	0.02	21.3	6.8	7.50	409	68	112		
Control	22.9	8.4	7.97	400	78	128	0.0	0.00	21.3	7.3	7.76	433	84	124		

[a] water samples were inadvertently discarded before ammonia could be measured.
[b] total ammonia

Table 5. Physical characteristics of the sediments and acid volatile sulfides (AVS), simultaneously extracted metals (SEM) and the SEM/AVS ratio for sediments collected from the Ochlockonee River, Florida, in August 2004.

	Sediment characteristics (%)						Acid volatile sulfides (µmol/g)		
Station	Moisture	Total organic	Course organic	Sand	Silt	Clay	AVS	SEM	SEM/AVS
0104	31.6	1.8	0.7	91.2	5.1	3.7	0.08	0.17	2.09
0204	23.8	0.8	0.3	92.1	4.9	2.9	0.05	0.09	1.95
0304	27.1	2.4	1.2	89.5	5.3	5.2	0.06	0.33	5.86
0404	19.1	0.1	0.0	98.8	0.9	0.3	bdl	0.03	0.00
0504	31.6	2.7	1.8	94.4	4.0	1.6	0.03	0.09	2.62
0604	28.9	1.3	0.8	89.5	5.9	4.6	0.14	0.11	0.76
0704	20.4	0.5	0.2	94.9	2.2	2.9	0.00	0.07	16.64
0804	25.7	0.8	0.5	97.5	1.8	0.7	0.02	0.05	2.77
0904	24.9	1.8	0.7	91.8	4.8	3.4	0.02	0.08	5.46
1004	32.1	2.1	1.5	93.6	4.1	2.3	0.06	0.10	1.60
1104	40.2	7.2	4.7	93.7	3.2	3.1	0.07	0.25	3.61
1204	21.4	0.5	1.0	91.5	2.7	5.8	0.00	0.07	14.21
Reference	49.9	7.8	5.7	76.0	19.1	4.9	0.05	0.50	9.26
Control	28.7	0.1	0.1	98.3	1.6	0.1	0.00	0.03	96.30

bdl – Not detected within the limits of the methods used.

Table 6. Concentrations of trace elements in porewaters from sediments collected from the Ochlockonee River in August 2004.

Station	Ag (µg/l)	As (µg/l)	Ca (mg/l)	Cd (µg/l)	Cu (µg/l)	Cr (µg/l)	Fe (mg/l)	K (mg/l)	Hg (µg/l)	Mg (mg/l)	Mn (µg/l)	Na (mg/l)	Ni (µg/l)	Pb (µg/l)	Se (µg/l)	Zn (µg/l)
0104	0.74	2.64	60.37	0.06	1.42	bdl	3.07	3.54	bdl	13.09	8.45	19.34	1.92	0.38	0.30	23.66
0204	0.73	3.81	61.64	0.01	2.12	bdl	0.18	3.69	0.06	5.59	2.53	18.56	10.91	10.79	0.60	88.64
0304	0.13	2.16	6.53	bdl	2.96	0.91	0.42	2.57	bdl	1.98	0.35	19.04	1.24	6.51	0.89	49.24
0404	1.06	0.70	4.29	0.01	2.30	0.61	0.48	2.79	bdl	1.70	0.01	15.62	8.93	13.90	0.74	85.84
0504	2.76	2.25	5.41	0.02	1.29	0.17	0.22	2.75	bdl	1.40	0.26	13.68	1.15	1.21	1.12	22.69
0604	9.72	8.57	24.49	0.03	0.41	0.58	0.21	3.44	bdl	5.77	1.04	14.79	0.69	0.39	0.30	76.75
0704	5.70	0.83	12.19	0.04	1.90	1.45	0.40	1.36	0.34	1.82	0.04	17.31	0.73	6.43	0.31	21.11
0804	4.80	4.26	16.89	bdl	0.81	bdl	0.11	2.42	bdl	4.47	1.35	14.03	0.26	0.37	0.16	14.44
0904	0.80	2.28	19.05	bdl	0.60	bdl	0.10	2.21	bdl	3.82	0.56	22.19	0.15	0.51	0.82	19.72
1094	1.10	3.74	17.57	bdl	0.66	0.28	0.19	2.32	bdl	2.89	1.77	15.51	0.71	0.36	0.52	28.94
1104	1.66	1.73	35.74	0.00	0.30	bdl	0.07	0.93	bdl	7.74	0.32	14.89	1.13	2.34	1.02	39.64
1204	9.70	5.37	27.65	0.08	0.71	0.94	0.14	2.14	bdl	7.77	0.14	21.04	0.99	1.19	0.51	8.26
Reference	1.07	0.93	13.68	0.03	1.94	0.31	5.25	1.85	bdl	3.31	3.56	14.72	1.72	0.22	3.72	69.72
Control	0.79	0.32	25.41	0.22	0.90	bdl	0.05	4.70	bdl	5.60	0.10	30.87	1.09	0.33	11.50	13.80
Recon	bdl	0.06	24.96	bdl	1.30	bdl	0.05	2.97	bdl	5.51	0.00	27.42	bdl	0.67	13.22	10.43

bdl – Not detected within the limits of the methods used.

Evaluation of Potential Risk and Land Use Analysis

An estimation of risk associated with water and sediment quality was performed for the individual habitat integrity categories of water chemical quality, sediment toxicity, and sediment chemical quality. One point was assigned each instance where an exceedance of state water quality standards or federal water quality criteria (designed to be protective of aquatic life) was observed. The points scored were then summed by site to achieve an assessment of water quality risk for aquatic life in the Ochlockonee River (Figure 21). Differences from the control conditions were also assigned an estimated risk level for sediment toxicity, with one point being assigned for each instance of toxicity (Figure 22). The number of occasions when sediment (or porewater) quality guidelines were exceeded also generated a score of one per instance at each site and the total was used as a relative sediment quality assessment (Figure 23). Finally, the total estimated risk represented by the total of points assigned above in all three categories was presented spatially in a cumulative risk assessment for overall habitat water quality (Figure 24). A summary of parameters leading to the cumulative ranking is provided in Table 7.

The relative risk levels were designed for comparison to species occurrence data and species richness data (Figures 2-7). Several coincident locations of high estimated risk to aquatic life and the recent absence of federally protected species (Sites 1, 5, and 7) were noteworthy. Water chemical quality related risk stemmed for low dissolved oxygen most frequently, but one water quality standard violation was observed for turbidity (Site 6) and free copper (Site 5). The turbidity standard violation occurred in the uppermost range of the sampling scheme. Also found in the upper Ochlockonee River was the site where the free copper concentration violated the water quality standard. However, the measured concentration was merely 3.0 ug/L free copper. This concentration was quite low and unlikely to elicit a toxic response. The reason the violation occurred was due to the very low system hardness, the variable used in the calculation of the free copper standard.

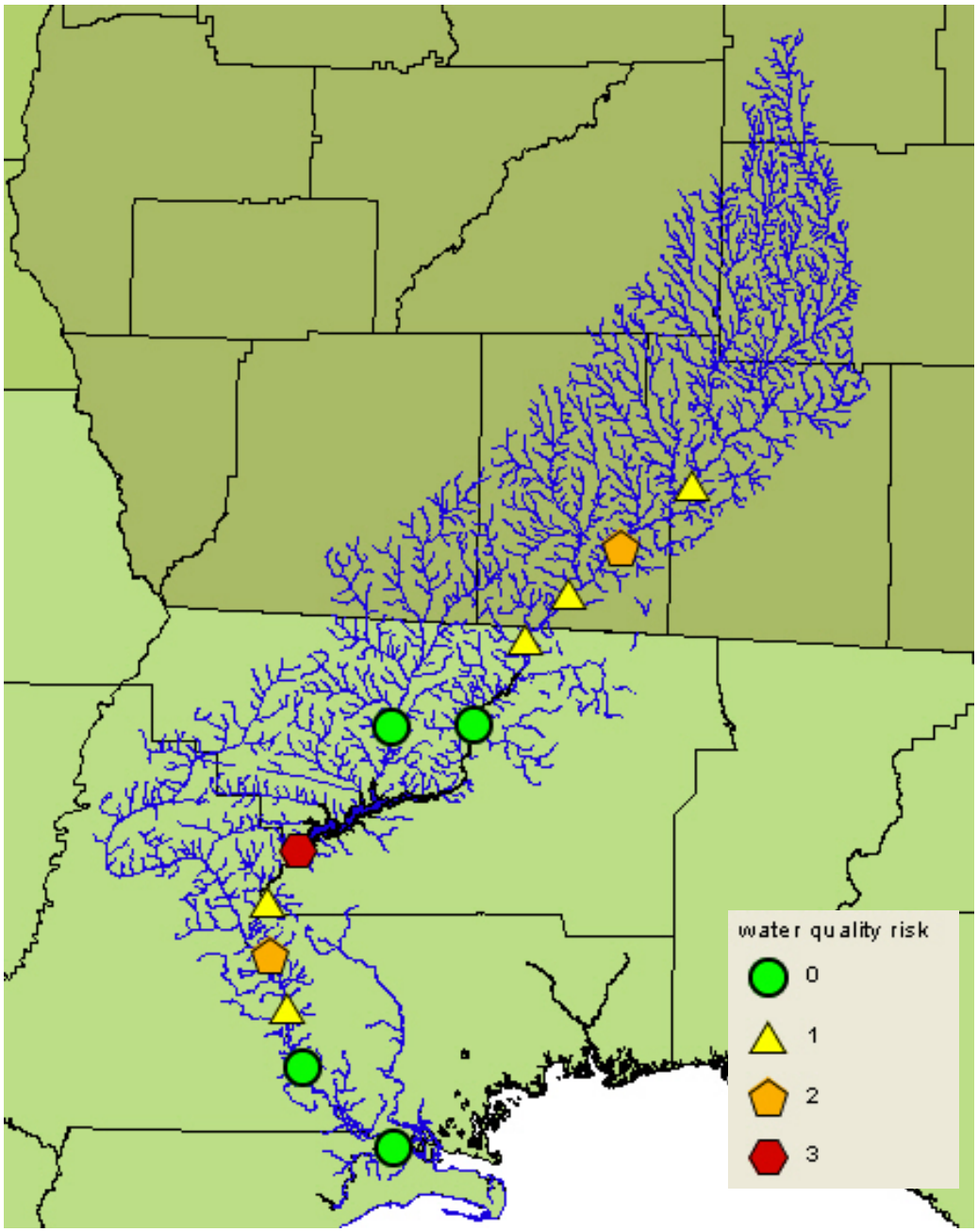

Figure 21. Overall ambient water quality-associated potential risk for the parameters free copper, un-ionized ammonia, chlorine, dissolved oxygen, temperature, pH, chlorophyll concentration turbidity, conductivity. Each violation of a state water quality standard or federal water quality criterion constituted an individual score of one to be summed with scores for all measures of all parameters by site (ammonia federal criterion based on recommended safe exposure range proposed by Augspurger *et al.* 2003).

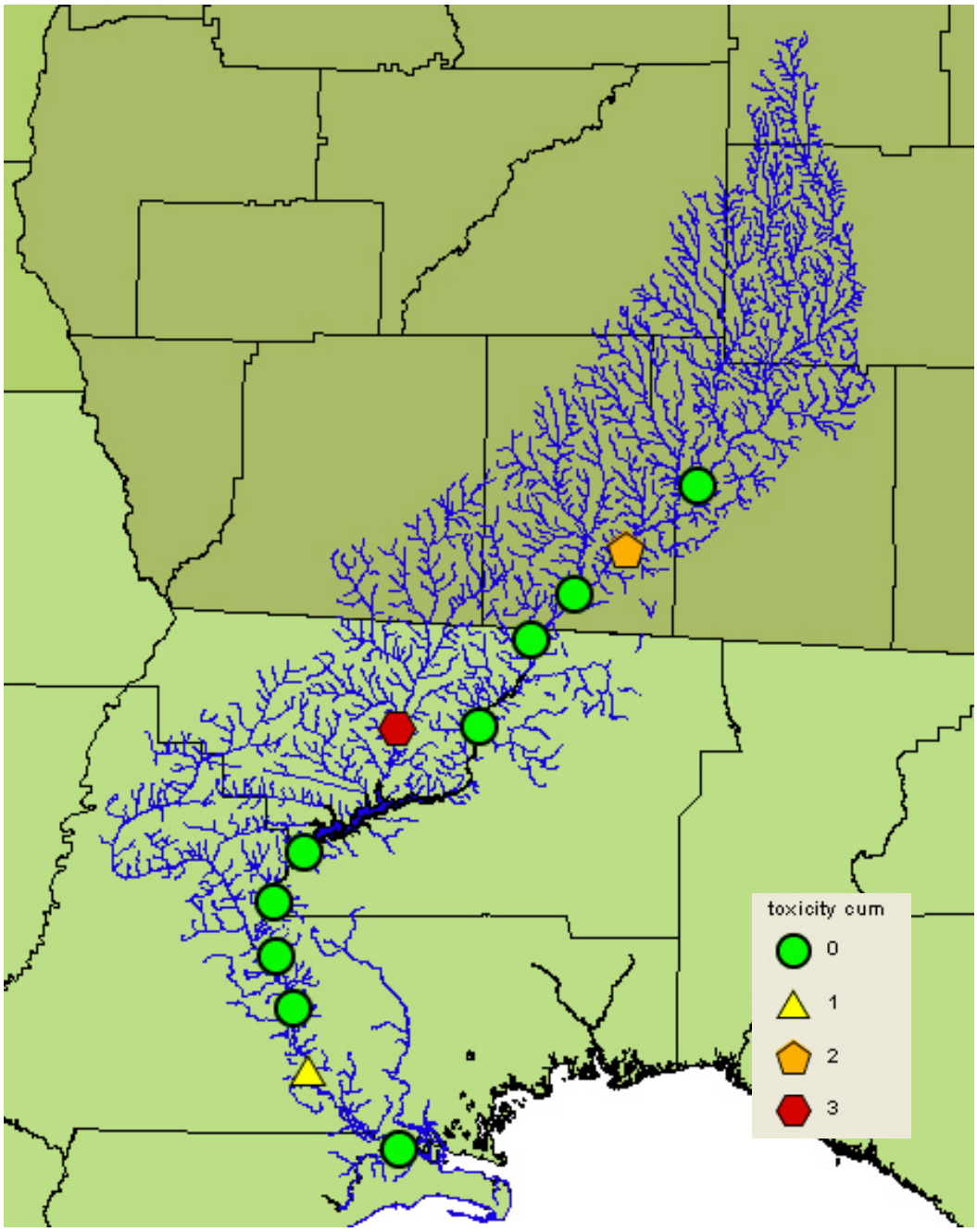

Figure 22. Overall sediment toxicity-associated potential risk estimated from pore and solid phase exposures of *Hyalella azteca* (acute and chronic assays) and solid-phase exposures for *Vibrio fischeri* (5 minutes and 15 minutes). A score of one was assigned for significant differences from the control for each test (unless qualified in discussion as for *H. azteca* chronic) and scores for all tests were summed by site.

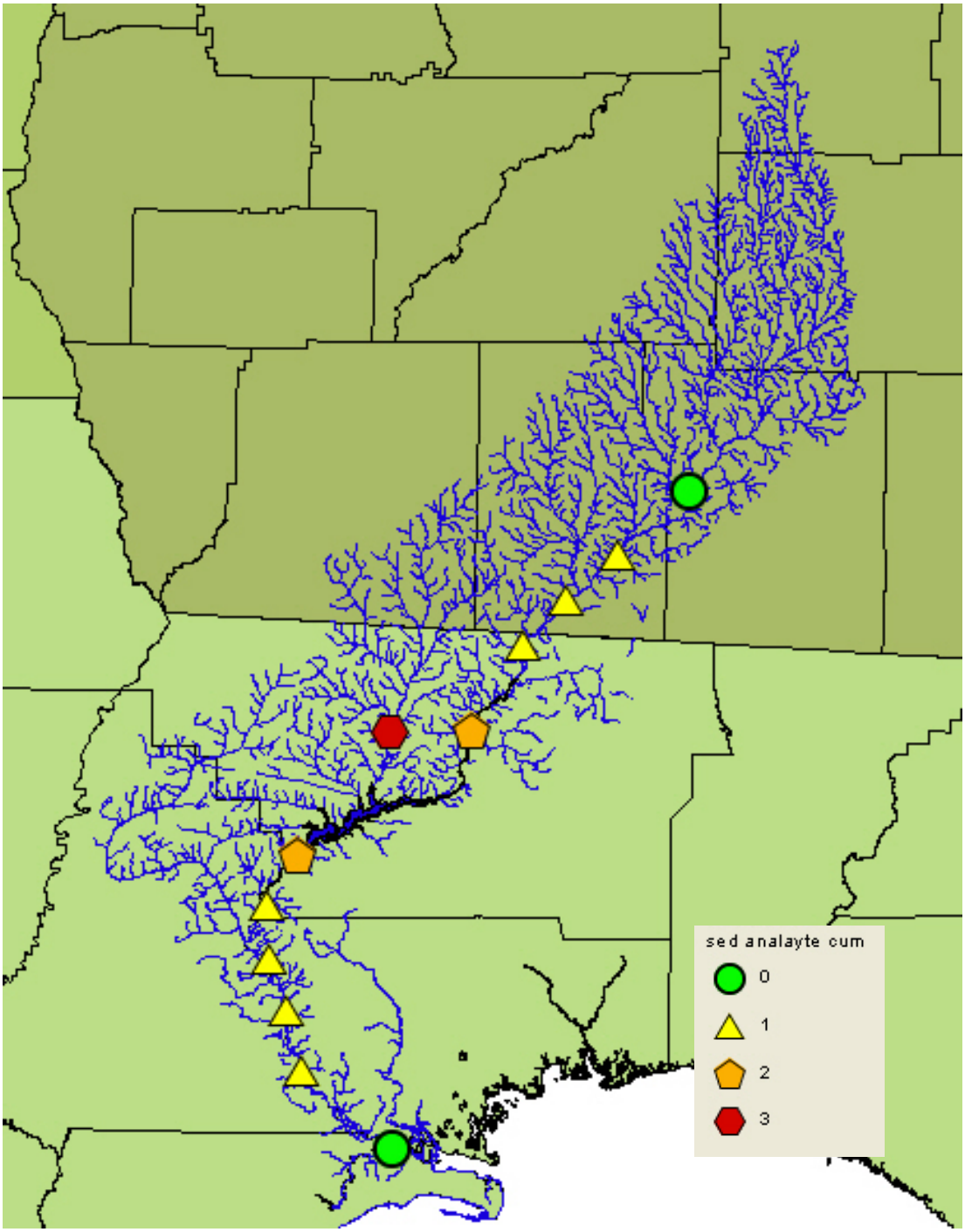

Figure 23. Overall sediment quality-associated potential risk estimated by comparison of analytical data to reference values such as those of MacDonald *et al.* (2000). Each exceedance of the sediment quality guidelines constituted an individual score of one to be summed with scores for all measures of all parameters by site.

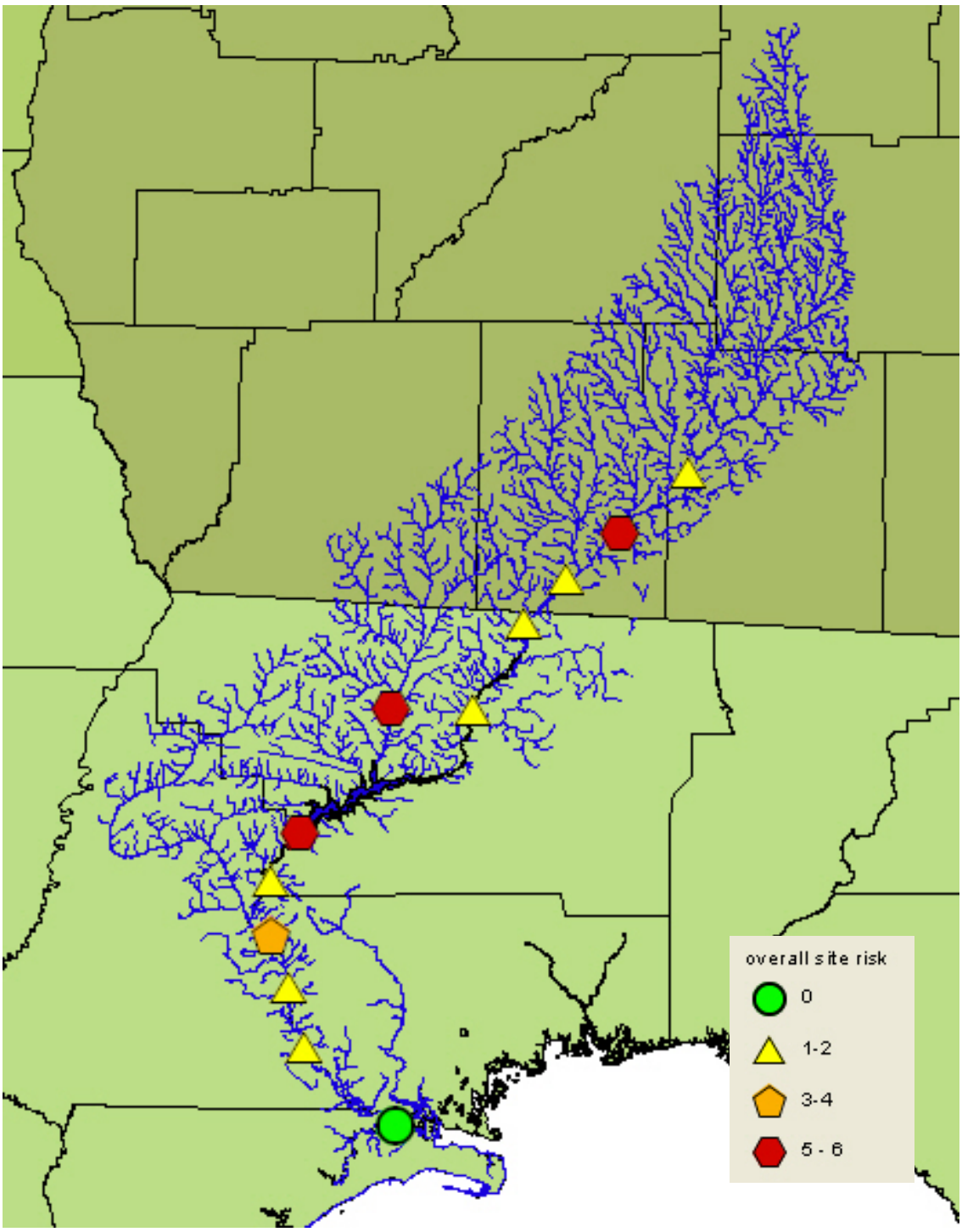

Figure 24. Cumulative risk score estimated for freshwater mussel species in the Ochlockonee River, Florida based on sampling conducted during the summer of 2004. Water quality standards violations, toxicity test differences from the control, or exceeded sediment analyte guidelines each represented one risk point assessed to be summed for each incidence of each parameter by site.

Table 7. Individual parameters providing the cumulative potential risk score estimated for freshwater mussel species in the Ochlockonee River, Florida based on sampling conducted the summer of 2004. Water quality standard violations, toxicity test differences from the control, or exceeded sediment analyte guidelines are listed with respective parameters for each incidence. Each reported incidence represented one risk point assessed. Sites with "*" had no federally listed threatened or endangered freshwater mussel species during 2002-2004 surveys.

Site	Water Quality Violation	Toxicity Test	Guidance Exceeded
1*		*H. azteca* porewater	Total Ammonia
		5 minute *V. fischeri*	Un-ionized Ammonia
		15 minute *V. fischeri*	Manganese
5*	Dissolved Oxygen	*H. azteca* porewater	Total Ammonia
	Free Copper	5 minute *V. fischeri*	
7*	Dissolved Oxygen		Lead (porewater)
	Dissolved Oxygen		Lead (whole sediment)
	Dissolved Oxygen		
9	Dissolved Oxygen		Total Ammonia
	Dissolved Oxygen		
2*			Un-ionized Ammonia
			Lead (porewater)
3	Dissolved Oxygen		Lead (porewater)
4	Dissolved Oxygen		Lead (porewater)
8*	Dissolved Oxygen		Total Ammonia
10	Dissolved Oxygen		Total Ammonia
11		*H. azteca* porewater	Total Ammonia
6*	Turbidity		
12	None		

Estimated potential risk from sediment toxicity was attributed to three sites (Sites 1, 5 and 11). Toxicity associated with the sediments from Sites 1 and 5 was shown three and two times, respectively, compared to only once for sediments from Site 11. The absence of protected freshwater mussels corresponded with measured sediment toxicity at Sites 1 and 5, but not Site 11. All three of these sites showed porewater from their respective sediment samples to be acutely toxic to *Hyalella azteca*. Sites 1 and 5 both showed toxicity via the 5-minute *Vibrio fischeri* (Microtox) bioluminescence assay. And Site 1 also showed toxicity in the 15-minute *V. fischeri* test. The significantly reduced growth observed for the whole sediment, chronic *H. azteca* test was believed to be more a reflection of high control performance rather than an inhibition of growth from Ochlockonee River sediments as discussed in the toxicity test results section. Taken altogether, Sites 1 and 5 (and to a lesser extent Site 11) demonstrated toxicity that would limit the success of aquatic life in general and may have implications for freshwater mussel species in the Ochlockonee River.

Sediment chemical analytes posed potential risk to aquatic species at numerous sites according to sediment quality guidelines (Eisler 1988, Lasier *et al.* 2000, MacDonald *et al.* 2000, U.S. EPA 2002), but only the few listed above demonstrated resulting toxicity. Analytes of concern included total ammonia (6 sites), lead in porewater (4 sites) or whole sediments (1 site), un-ionized ammonia (2 sites), and manganese (1 site). Supporting the toxicity test results, the site with the higher number of these analytes was Site 1 where the greatest toxicity was observed. Total ammonia was the only analyte revealed to contribute to the toxicity measured in sediments from Sites 5 or 11. Notable lead levels were reported for whole sediments and porewater from the site just below the dam (Site 7), which also possessed low dissolved oxygen.

Cumulatively, sites posing potential risk to aquatic life achieved an estimated risk score of five or greater (below the dam, Little River, and ~3 miles SSE of Cairo, GA). These sites corresponded well with reported mussel loss areas. Conversely, sites with cumulative scores below four were found in apparently less impacted areas. However, two sites where protected mussels have not recently been found had cumulative risk scores of 2 (second site below Lake Talquin) or 1 (uppermost northern sampling point NW of Thomasville, GA),

respectively. This may have resulted from a number of factors including, but not restricted to: 1) the limiting factors for the mussels were missed by the survey and therefore risk was underestimated; 2) factors contributing to the decline of the mussels were not chemical in nature at the site; 3) the factors at nearby sites limited the success of the mussels; 4) the mussels' absence was related to an apparent decline that may more accurately be described as a decline in detection probability (Bailey et al. 2004); or 5) differences in sampling efforts over the various time periods.

Water quality issues in the Ochlockonee River system have been reported that prevent river segments from meeting their designated use criteria or water quality standards (FDEP 2001, GDNR 2002). Failure of stream and river segments to meet designated uses has prompted their listing on the Clean Water Act mandated Impaired Waters List (303d-list, Figure 29). Such listings in Georgia's upper Ochlockonee River basin, where the use designation is "fishing", has frequently been noted for violations of the dissolved oxygen and fecal coliform bacteria standards, but has also included fish consumption advisories. In nearly every instance the cause of the impairment has been attributed to nonpoint source pollution. The State of Georgia is addressing these issues through the implementation of their watershed protection strategy (GDNR 2002). Parameters leading to impairment in Georgia have also prevented the Ochlockonee River in Florida from meeting its 'fishable-swimmable" designation at a number of locations (FDEP 2001). Parameters necessitating listing on the impaired water list included low dissolved oxygen, nutrients, turbidity, total suspended solids (TSS), bacteria, fish consumption, and in one case biological integrity (macroinvertebrate indices) and in another case iron. Like the sub-watersheds in Georgia, the cause of water quality impairments has been reported to stem largely from nonpoint sources.

Although numerous instances of water quality violation have been reported by the respective state agencies, low dissolved oxygen may be the more important parameter listed above that could impact aquatic life. In the case of freshwater mussels, the low dissolved oxygen may interfere with respiration or the presence of needed fish hosts for reproduction, although further investigation is needed. It was less clear what additional threat the other parameters would pose mussel species.

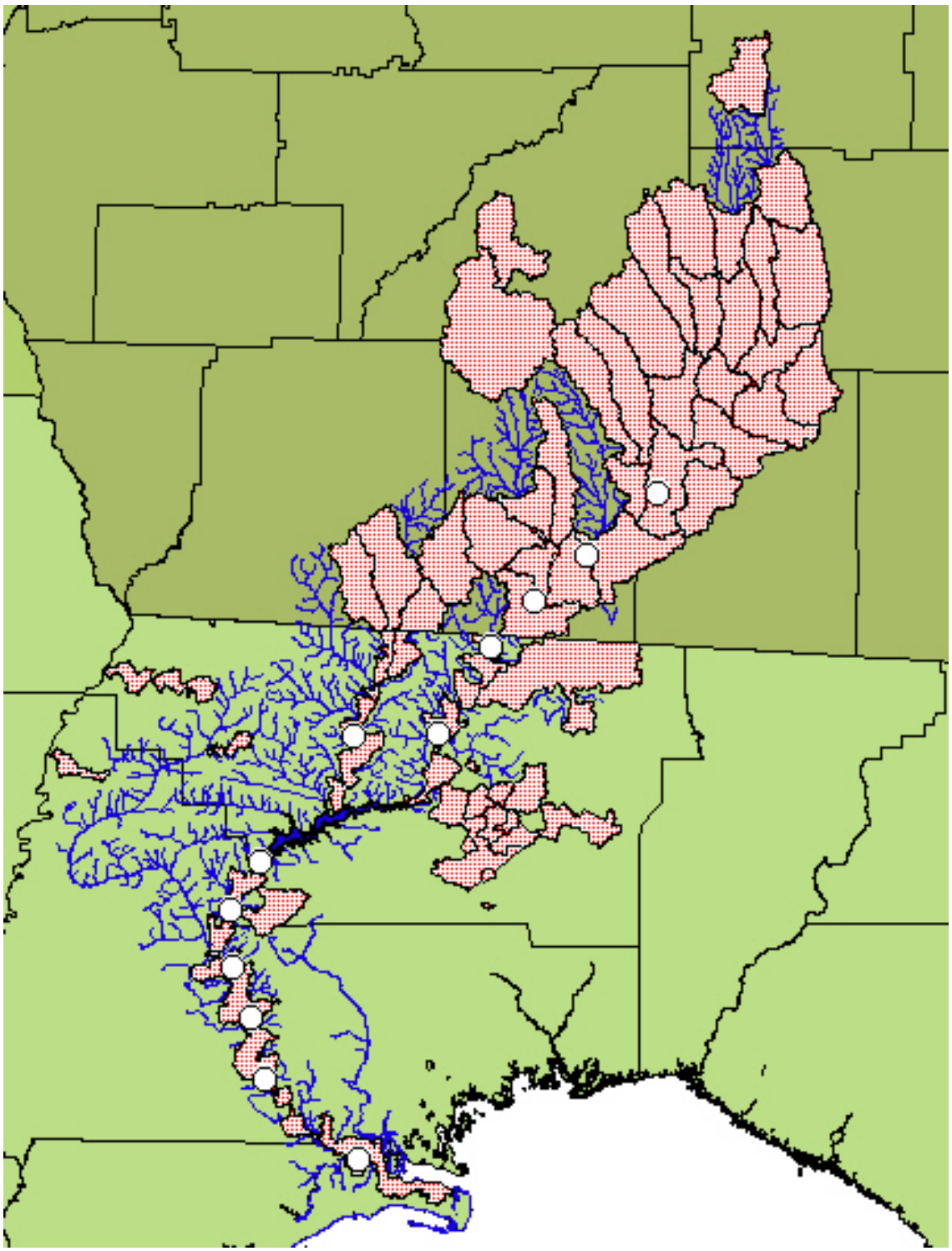

Figure 25. Ochlockonee River segments listed as impaired by sub-watershed (red shaded polygons) according to the Florida Department of Environmental Protection (2001) and the Georgia Environmental Protection Department of Natural Resources (2002).

In addition to the frequently cited nonpoint contribution to the quality of the system's water and sediment, point sources discharges permitted by the respective states via the National Pollutant Discharge Elimination System (NPDES) exist in the watershed. Two reaches of the Ochlockonee River where high risk was estimated for mussels, possessed a number of point source discharges in their sub-drainages (Figure 30). The identities of these dischargers are presented in Table 8.

Twenty NPDES sites were listed for the Little River. Most were associated with business within the City of Quincy. Half of these locations were exempt from the permit requirements by the State of Florida due to their low expected discharge. The permitted discharges included domestic wastewater treatment plants, petroleum wholesalers, plant nurseries, mining and manufacturing. Specific details for all permits in the Ochlockonee watershed in Florida, including NPDES, landfills, and hazardous wastes can be obtained from the Florida Department of Environmental Protection.

Six permitted discharges were found upstream of the northern-most sites on the upper Ochlockonee River in Georgia. Dischargers to the river segment (or its tributaries) included several domestic wastewater treatment plants, a meat processing plant, mining operation to produce absorbents, and a state hospital. It was worth mentioning that the discharges were all upstream of Site 6 (the uppermost site sampled) but the overall risk ranking for that site was considerably lower than Site 5 further downstream. Further information on all permits within the drainages can be obtained from the Georgia Department of Natural Resources.

The third high risk site was located immediately below the dam on Lake Talquin. Lake Talquin is a man-made lake formed in 1927 when the Jackson Bluff Dam was constructed on the Ochlockonee River to produce hydroelectric power. Water from Lake Talquin is released through the dam via an underwater intake and discharge. The design of the discharge may be related to the low dissolved oxygen levels recorded at the site. The State of Florida refers to Lake Talquin as more of a recreational feature than source of electric power for the City of Tallahassee (FDEP 2001). The renewal date for the federal permit is 2022.

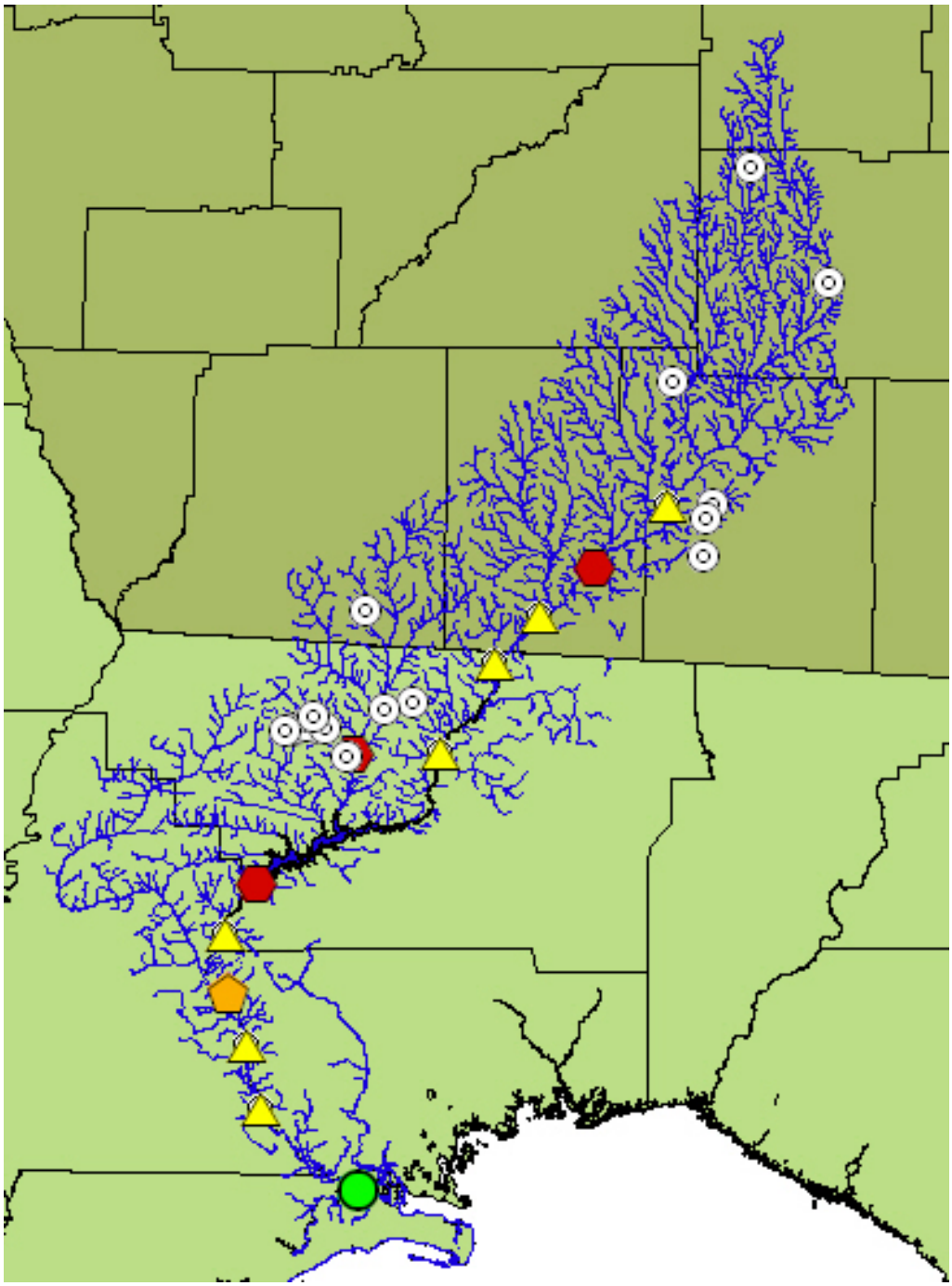

Figure 26.National Pollutant Discharge Elimination System (NPDES) permitted discharges (circle in circle) in sub-drainages of sites (red hexagon) with high estimated risk for freshwater mussels resulting from water and sediment quality measures in the Ochlockonee River watershed of Florida and Georgia according to the Florida Department of Environmental Protection and the Georgia Environmental Protection Department of Natural Resources.

Table 8. National Pollutant Discharge Elimination System (NPDES) permitted discharges in sub-drainages of sites with high estimated risk for freshwater mussels resulting from water and sediment quality measures in the Ochlockonee River watershed of Florida and Georgia according to the Florida Department of Environmental Protection and the Georgia Environmental Protection Department of Natural Resources.

State ID	Facility Name	Address	City
Yellow River (Site 1)			
FLA271888	HOLINESS COMMUNITY CHURCH *	403 WEST JEFFERSON ST.	QUINCY
FLA272001	DETAIL DELITE *	605 EAST JEFFERSON ST	QUINCY
FLA273961	KELLEYS JR #9 *	1958 WEST JEFFERSON ST	QUINCY
FLA275395	DERRICKS AUTO TINTING *	14 SOUTH 10TH STREEET	QUINCY
FLA275409	THE SHINE SHOP *	1209 W. CRAWFORD STREET	QUINCY
FLA275417	RPS INC *	442 SOUTH VIRGINIA STREET	QUINCY
FLA276243	SPOTLIGHT DETAIL & FUN & GAMES-CAR WASH *	214 W CRAWFORD ST	QUINCY
FLA276316	CHARLIE BUSH CAR WASH *	605 EAST JEFFERSON ST	QUINCY
FLA276324	JR AND FAMILY DETAIL SHOP *	703 EAST JEFFERSON ST	QUINCY
FLG910790	MAY'S NURSERY	SALEM ROAD	HAVANA
FLA291633	TOP QUALITY AUTO DETAILING *	353 SOUTH SHELFER ST	QUINCY
FLG910836	QUINCY CLUSTER - SYSTEM 3 (LOLLEYS & SOUTH)	U.S. 90 AND WARD STREET	QUINCY
FLG910837	QUINCY CLUSTER - SYSTEM 1 (QUINCY CITGO)	502 W. JEFFERSON STREET	QUINCY
FLG910838	QUINCY CLUSTER - SYSTEM 2 (BEAN & NEELY BP)	503 W. JEFFERSON STREET	QUINCY
FLG110191	FLORIDA ROCK - QUINCY PLANT	BOSTIC ROAD (CR 274)	QUINCY

Table 8 continued. National Pollutant Discharge Elimination System (NPDES) permitted discharges in sub-drainages of sites with high estimated risk for freshwater mussels resulting from water and sediment quality measures in the Ochlockonee River watershed of Florida and Georgia according to the Florida Department of Environmental Protection and the Georgia Environmental Protection Department of Natural Resources.

State ID	Facility Name	Address	City
Yellow River (Site 1 - continued)			
FL0029033	QUINCY, CITY OF (STP)	300 N G.F.& A DRIVE	QUINCY
FLA010079	RENTZ MHP*	ROUTE 6	QUINCY
FLA010084	NICHOLSON FARMHOUSE RESTAURANT*	STATE ROAD 12	QUINCY
FL0002577	ENGELHARD CORP - QUINCY PLANT	SR 65	QUINCY
GA0001678	ENGELHARD CORPORATION	P.O. BOX 220	ATTAPULGUS
Upper Ochlockonee in Georgia (Sites 5 and 6)			
GA0022021	DHR SOUTHWEST STATE HOSP.	GRACEWOOD DRIVE	THOMASVILLE
GA0024082	THOMASVILLE WPCP	P.O. BOX 1540	THOMASVILLE
GA0032409	WAVERLY MINERAL PRODUCTS	P.O. BOX 106	MEIGS
GA0001279	SUNNYLAND, INC.	603 CASSIDY ROAD	THOMASVILLE
GA0024660	MOULTRIE WPCP	P.O. BOX 580	MOULTRIE
GA0021717	DOERUN WPCP	P.O. BOX 37	DOERUN

* State exempted from permit requirements.

General land use characterizations provided by the respective state water management authorities have shown important differences between sub-drainages (Table 9, Figure 31). The upper Ochlockonee River in Georgia, for example, possesses considerably more agriculture and rangeland (36% and 13%, respectively) than the upper river in Florida (9% and 3%, respectively) or lower river in Florida (0.4% and 2%, respectively). Agriculture and rangeland have often been associated with loadings that contribute to violations of dissolved oxygen and fecal coliform bacteria standards (FDEP 2001, GDNR 2002). The upper Ochlockonee River in Georgia also contains a large percent coverage of upland forest (41%), as does the Florida upper river (68%) and the lower river in Florida below Lake Talquin (51%). The upper river in Florida has more urban development (8%), compared to either Georgia (2%) or the lower river in Florida (1%). Additionally, the lower river has far more wetland coverage (43%) than either of the other two sub-drainages (GA 8% and FL 8%). In fact, considering only the upland forest, wetland and open water categories, they constitute over 95% of the sub-drainage area of the lower river in Florida.

Taken together, potential driving factors for nonpoint source contributions in the sub-drainages can be approximated based on the land use analyses provided by the states. The estimated important nonpoint contributors for Georgia's upper river area would include primarily agriculture and rangeland, but the importance of barren lands has not been overlooked by the respective state agencies (FDEP 2001, GDNR 2002). The upper Ochlockonee River in Florida was shown to possess the highest urban development, as well as noteworthy amounts of agriculture and rangeland. The single candidate for the lower river in Florida may have been rangeland; however, the contributions of the management of the extensive upland forest could not be omitted.

The above summary was based on land-use analyses on the landscape-level in which the Ochlockonee River watershed was viewed as only three sub-watersheds. The percent contribution to water and sediment quality limitations could be refined by improving on the scale (large-scale analyses, smaller size sub-watershed land use definitions). The estimated high risk areas should be subjected to such analyses. Analyses of larger scale should include important spatial issues such as distance of a particular land-use within a sub-drainage from

the concern point, cumulative contributions between specific sub-watersheds, and potentially historic land-uses from which recovery may be taking place (Paul Lang USFWS, Pers. Comm.).

Table 9. Land use composition in percentages for the Ochlockonee River watershed in northwest Florida and southwest Georgia according to the Florida Department of Environmental Protection, 2001.

Land Use Type	Upper River GA	Upper River FL	Lower River FL
Urban	1.6	7.74	0.9
Agriculture	35.6	9.32	0.41
Rangelands	12.9	2.46	2.27
Upland Forest	40.7	67.75	51.27
Water	0.5	4.14	2.01
Wetlands	7.8	7.74	43.0
Barren Lands	0.45	0.02	0.02
Transportation	0.45	0.83	0.13
Total	100	100	100

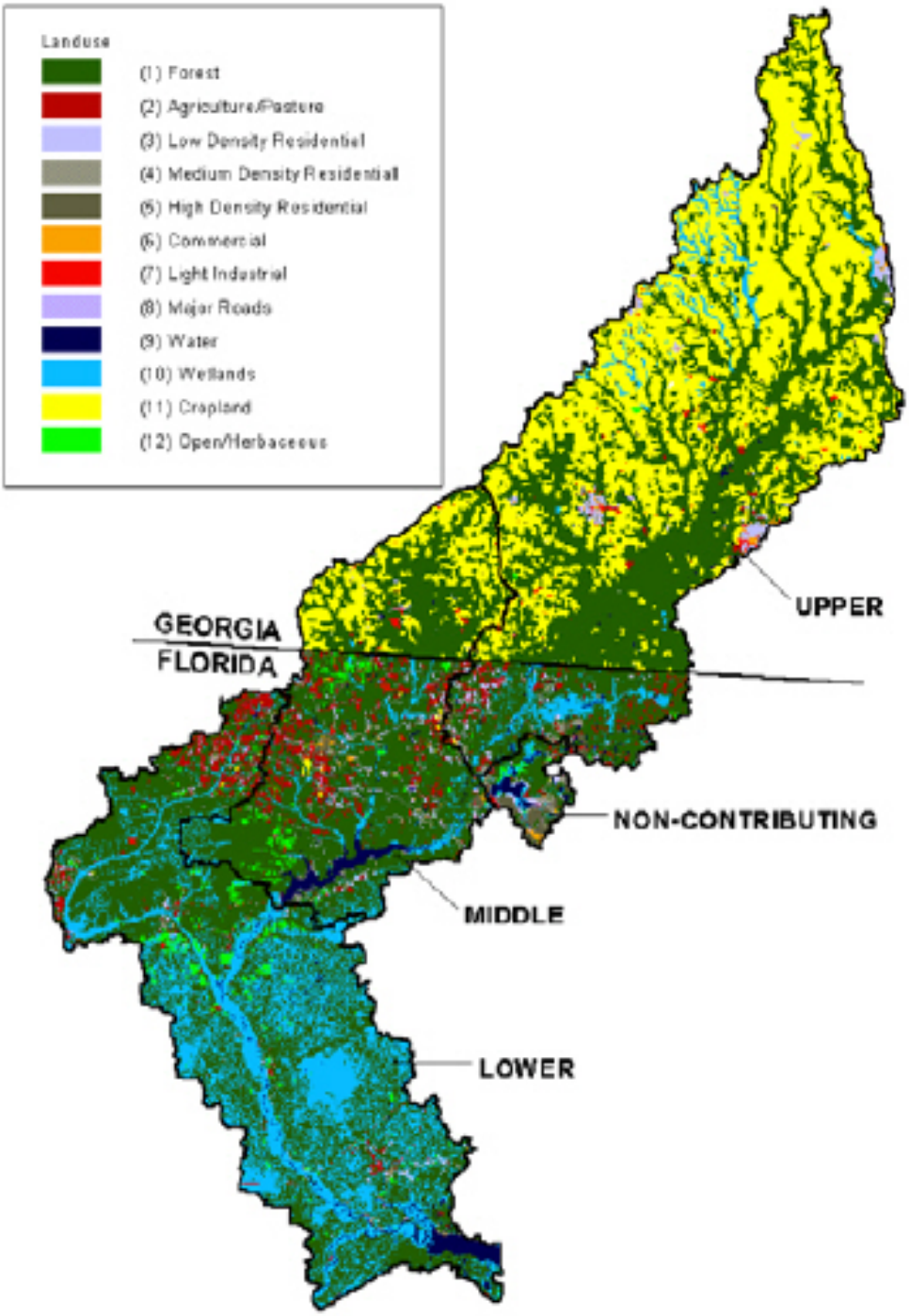

Figure 27. Land-use composition in the Ochlockonee River watershed in northwest Florida and southwest Georgia based on Landsat TM imagery 1988-1990. Figure reproduced with permission from the Florida Department of Environmental Protection.

Conclusions

Water Quality

Water chemical quality related risk to aquatic species was from low dissolved oxygen most frequently, but instances were observed for turbidity and free copper violations. As a result, the coincidence of mussel absence and water quality violations most often occurred with violations of the dissolved oxygen water quality criterion. During this study low dissolved oxygen was the chemical parameter reported that was potentially most limiting to the aquatic life and potentially to the recovery of freshwater mussel species in the Ochlockonee River system, although further investigation is warranted. The turbidity standard violation occurred in the uppermost range of the sampling scheme and was observed on one of the three sampling occasions. The free copper violation was not thought to be important because the measured concentration was quite low. Nevertheless, the potential for metals toxicity in this system is quite high as a result of the low hardness and alkalinity, as well as the low system pH characteristic of blackwater rivers and streams.

Proximate areas of concern have been identified for water quality impairment, including the area immediately below Lake Talquin's dam. However, a more systematic survey of the sub-watersheds where risk from water quality parameters, such as low dissolved oxygen was observed is needed. At least some of the tributaries to the points of concern may also be limiting to freshwater mussel success to a degree that needs to be defined. Additionally, the extent of the reach that was identified as limiting needs to be revealed through the more systematic approach to better delineate areas for improvement versus areas where recovery efforts would be more suitably employed.

Sediment Quality

In general, based on the data collected the sediment quality from the Ochlockonee River appeared to be fairly good, with the exception of Stations 1 and 5 (and maybe 11). Specific factors responsible for the reduced *H. azteca* survival and low *V. fischeri* EC50 values shown

for these sites are not known. Ammonia and Mn concentrations may be important at Station 1. Ammonia and Pb at various sites in this river were elevated, but there was not a clear linear correspondence between dose and effect (test endpoints). Sites with the highest concentrations did not show a corresponding toxic response. The concentrations of trace metals shown in the porewater and in the solid-phase sediment from the Ochlockonee River did not appear to be at levels that would impact freshwater mussels, although Pb may be of some concern. However, toxicity testing with proper freshwater mussel surrogates will be required to confirm this expectation. From a trace element perspective, sediment quality in the Ochlockonee River appears to be within acceptable limits for aquatic life. However, as in the case of water chemistry, sediment toxicity and sediment chemistry needs to be better delineated for tributaries and the river reach extent to further mussel recovery efforts.

Evaluation of Potential Risk and Land Use Analysis

An estimation of risk associated with water and sediment quality was performed for the individual habitat integrity categories of water chemical quality, sediment toxicity, and sediment chemical quality. Cumulatively, sites posing potential risk to freshwater mussel species achieved an estimated potential risk score of five or greater (below the dam, Little River, and ~3 miles SSE of Cairo, GA). These sites corresponded well with areas that no longer support their historic freshwater mussel populations. Conversely, sites with cumulative scores below four were generally found in apparently less impacted areas. However, two sites where protected mussels have not recently been found had cumulative risk scores of 2 (second site below Lake Talquin) or 1 (uppermost northern sampling point NW of Thomasville, GA), respectively. This may have resulted from a number of factors including, but not restricted to: 1) the limiting factors for the mussels were missed by the survey and therefore risk was underestimated; 2) factors contributing to the decline of the mussels were not chemical in nature at the site; 3) the factors at nearby sites limited the success of the mussels; 4) the mussels' absence was related to an apparent decline that may more accurately be described as a decline in detection probability (Bailey et al. 2004); or 5) inherent differences in sampling success were attributed to varying sampling efforts over the various time periods.

Water quality issues in the Ochlockonee River system have been reported to prevent river segments from meeting their respective designated use criteria or water quality standards (FDEP 2001, GDNR 2002). Such listings have frequently been attributed to nonpoint source pollution. Potential driving factors for nonpoint source contributions in the sub-drainages were approximated based on the land use analyses provided by the states. The estimated important nonpoint contributors for Georgia's upper river area would include primarily agriculture and rangeland, but the importance of barren lands has not been overlooked by the respective state agencies (FDEP 2001, GDNR 2002). The upper Ochlockonee River in Florida was shown to possess the highest urban development, as well as noteworthy coverages of agriculture and rangeland. The single candidate for the lower river in Florida may have been rangeland, however the contributions of the management of the extensive upland forest could not be omitted.

In addition to the frequently cited nonpoint contribution to the quality of the system's water and sediment, point source discharges permitted by the respective states via the National Pollutant Discharge Elimination System (NPDES) exist in the watershed. Two reaches of the Ochlockonee River where high risk was estimated for mussels possessed a number of point source discharges in their sub-drainages and the third involved the release of water through the Jackson Bluff Dam.

The land-use analyses were based on a landscape-level view in which the Ochlockonee River watershed was classified as only three sub-watersheds. The percent contribution to water and sediment quality limitations could be refined by improving on the scale (large scale, smaller size sub-watershed land use definitions). The estimated high risk areas should be subjected to such analyses. Analyses of larger scale should include important spatial issues such as distance of a particular land-use within a sub-drainage from the concern point, cumulative contributions between specific sub-watersheds, and potentially historic land-uses from which recovery may be taking place.

Recommendations

The following recommendations are offered for consideration.

1) Conduct a more systematic survey on a larger scale (more resolution) for the sub-watersheds where potential risk from water chemical parameters was observed.

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

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

4) Subject potential high risk areas to land-use analyses of larger scale 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, extent of intact riparian buffer, cumulative contributions between specific sub-watersheds, and potentially historic land-uses from which recovery may be taking place.

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

6) Examine system sedimentation relative to mussel occurrence data.

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