Contaminant Impacts to Early Life Stages of the Robust Redhorse (*Moxostoma robustum*) in the Lower Oconee River

Final Report

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Preface

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This is a final report describing a study conducted for the Species at Risk Program of the USGS Biological Resources Division. It consists of two manuscripts; Part I - Sediment-quality Assessment of the Lower Oconee River and Part II - Impacts and Toxic Thresholds of Sediment-associated Contaminants to Robust Redhorse (*Moxostoma robustum*) in the Lower Oconee River.

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Zn & Mn ← highest threat to RRH

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EXECUTIVE SUMMARY

The robust redhorse (*Moxostoma robustum*) was described by the naturalist Edward Cope in 1870, but disappeared from the scientific record until single specimens were collected during the 1980s from the Savannah and PeeDee Rivers in Georgia and South Carolina, and a viable population was discovered in the Oconee River, Georgia, in 1991. The Oconee River population is confined to a 120 km reach between Milledgeville and Dublin. State and Federal biologists determined that the population consists of between 600 and1000 individuals ranging from 8 to 26 years old. Robust redhorse (RRH) have been observed spawning at one location in this reach, but survival through the early-life stages is apparently low. There are five major tributaries entering this stretch of river that drain urban and agricultural watersheds. Several carry municipal and industrial effluents that are permitted to contain Cd, Cu and Zn under the National Pollution Discharge Elimination System of the Clean Water Act. Agriculture (primarily silviculture and row crops) is the largest land use, but there are numerous quarries producing kaolinite clay.

In 1995, the State of Georgia and the U. S. Fish and Wildlife Service initiated an artificial propagation program as part of a larger effort to reestablish this species throughout its former range in Georgia and the Carolinas and avoid its listing under the Endangered Species Act. Sedimentation associated with deforestation is believed to be one cause for the historical decline of the species. The impact of contaminants, particularly those permitted for discharge to the system, needed to be evaluated as another potential stress. In aquatic systems the majority of contaminants are bound to fine sediment particles. Therefore, the effects of sedimentation and contaminants are often intertwined. Robust redhorse spawn in gravel. Siltation of fine sediment may reduce habitat quality within the gravel by causing entrapment, reducing concentrations of dissolved oxygen and providing exposure to contaminants bound to the fine sediment.

The objectives of this study were to identify sediment-associated contaminants in the lower Oconee River, determine their sources and evaluate their potential to threaten survival and growth of RRH early-life stages. To accomplish this goal, the project was divided into two tasks. The first was to conduct a sediment-quality assessment using sediment and porewater toxicity tests with a surrogate species in conjunction with analytical analyses of contaminant levels. The second was to establish the toxic thresholds of suspected stressors to early-life stages of the RRH and assess survival and development in exposures to collected sediments and pore waters.

Sediments and pore waters were toxic to freshwater amphipods and RRH early-life stages. Reduced amphipod growth reflected sediment Zn concentrations with significant effects observed in exposures to sediments collected below the confluence of Buffalo Creek. Elevated Zn levels below Buffalo Creek were most likely the result of chronic low-level additions of Zn contained in permitted municipal and industrial effluents. Pore waters were also toxic to amphipods, but Mn appeared to be the primary stressor. Toxic concentrations of Mn developed in pore waters due to microbial reduction during decomposition of organic material. A burst of decomposition appears to be an artifact of sediment collection, homogenization and storage. There was no relationship

Sediment-quality Assessment of the Lower Oconee River

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The lower Oconee River in central Georgia is habitat for the robust redhorse (*Moxostoma robustum*), a fish species that has been listed by the U. S. Fish and Wildlife Service as a "Species at Risk". The robust redhorse (RRH) was discovered spawning in an area of gravel bars downstream of the Sinclair Dam at Milledgeville. However, recruitment to the population is low despite successful spawning (Jennings et al. 1996, 1998). Robust redhorse are benthic fish that are lithophilic spawners, depositing eggs into course gravel substrates (Jennings et al. 1996). Factors causing reproductive failure and lack of recruitment are currently being investigated, including impacts from sediment-associated contaminants. Effluents containing heavy metals are permitted in discharges released into several major tributaries of the river where RRH have been collected. Manufacturing plants, municipal waste treatment facilities and kaolin quarries are permitted to discharge effluents containing Cd, Cu and Zn under the National Pollution Discharge Elimination System (NPDES) of the Clean Water Act (Public Law 95-217). In addition to point sources, non-point source pollution may also be contributing toxic substances. The lower Oconee River watershed is primarily agricultural (silviculture and row crops), but several small cities (Milledgeville and Sandersville) and numerous kaolin quarries are also present.

The Oconee River is often turbid. Fine sediments may impact the survival of RRH early-life stages by clogging gravel bars used for spawning (Dilts 1999) and by providing a route of exposure to sediment-associated contaminants. Sediments are repositories for contaminants released to the environment (Lee and Jones, 1984: Salomons et al., 1987). Fine particle sizes within the sediment matrix tend to accumulate the majority of contaminants by virtue of the chemical and physical characteristics inherent to their large surface areas (O'Conner 1990, Horowitz 1991, de Groot 1995). Depositional sediments are predominated by fine particle sizes, and thereby serve well as records of recent contamination. Quality assessments of depositional sediments can provide useful information concerning stresses to the habitat.

Sediment toxicity is an essential component of sediment-quality assessments. Toxicity generally reflects the bioavailability of sediment-associated contaminants better than concentrations of contaminants in whole sediments (Anderson et al. 1984). Contaminant concentrations are often poorly correlated to biological responses because of sorption/desorption factors controlling their availability (Allen 1996). Concentrations of contaminants in sediment pore waters can be more reflective of bioavailable proportions than whole-sediment levels (Anderson et al. 1984), and pore water evaluations are currently being used in conjunction with sediment exposures to evaluate sediment quality (Carr et al. 1996, Winger and Lasier 1998, Carr et al. 2000, Winger et al. 2000).

The objectives of this study were to evaluate sediment quality in the lower Oconee River and to identify potential sources of contaminants. Sediments, pore waters and surface waters from multiple sites were collected during the fall of 1998 for assessments of toxicity and chemistry.

between Mn concentrations in sediment and sediment toxicity to amphipods.

Significant reductions in sediment-metal concentrations occurred between fall 1998 (amphipod tests) and spring 1999 (RRH tests), presumably due to the flushing by high water levels that occurred during spring. Sediments collected in the spring contained concentrations of Zn that were 50 to 90 percent less than those contained in sediments collected from the same locations the previous fall. Five out of six sediments collected below Buffalo Creek during fall contained Zn concentrations above the level established as a threshold of adverse effects to aquatic organisms, but Zn concentrations in spring-collected sediments were well below this level.

Manganese concentrations were also reduced by 40 to 60 percent in spring-collected sediments, but were still elevated above the toxic threshold for RRH. Sediments collected below Bluff Creek and Buffalo Creek during spring were toxic to RRH, as were pore waters extracted from the collected sediments. Manganese concentrations in Bluff Creek and Buffalo Creek sediments were above the threshold effect level established for freshwater sediments, and Mn in laboratory-extracted pore waters also exceeded toxic levels. Manganese toxicity is dependent on the development of reducing conditions within the sediment. Under these conditions, Mn sequestered within mineral and oxide forms is converted to the Mn^{2+} ion which is more soluble and toxic to many aquatic organisms. Although produced rapidly under reducing conditions, Mn^{2+} can persist for some time in oxic environments.

Manganese was the most likely cause of toxicity to RRH in sediment exposures. Soluble Mn^{2+} may have diffused from anoxic sediments into the oxygenated overlying water to produce toxic concentrations at the sediment/water interface. The RRH life stage most affected in sediment exposures was the immobile egg/embryo stage which remained in contact with the sediment surface through hatching and several days of development in the yolk-sac stage. The difference in responses between amphipods and RRH to sediment Mn may be related to the mobility of the amphipods and their ability to avoid Mn^{2+} emanating from the sediments.

In the lower Oconee River, Zn contained in permitted municipal and industrial effluents accumulates in sediments during low-flow conditions. As a result, Zn concentrations can reach levels that elicit toxic responses in aquatic organisms. Sediment-associated Zn does not appear to pose a risk to the early-life stages of the RRH if concentrations are routinely diluted by high seasonal flows prior to the RRH spawning season. Flow regimes in this reach of the Oconee River are regulated by releases from Sinclair Dam. Periodic high-volume releases during low-flow periods may provide a management option to reduce concentrations of Zn and other contaminants in the sediments and thereby reduce sediment toxicity.

Sediment exposures to RRH suggested that Mn toxicity may be another possible threat posed by sedimentation. The greatest source of Mn to aquatic systems is from soil erosion. Manganese may be contained in industrial effluents, but water quality criteria for Mn are only now being established. Under anoxic sediment conditions produced by restricted infiltration of surface water or by decomposition of egg masses, Mn can be reduced to its toxic form which can diffuse into nearby oxic environments and remain toxic for sufficient time to reduce survival and effect development. Through this mechanism, sediment-associated Mn may contribute to reduced survival of RRH early-life stages. Management options that reduce the amount of sedimentation to RRH spawning areas would also reduce the potential for toxicity from Mn.

Methods

Robust redhorse have been observed spawning in the lower Oconee River adjacent to the Avant Kaolin Mine (approximately 25 km downstream of Milledgeville, GA), and ripe individuals have been collected downstream to below the Beaverdam Wildlife Management Area (BWMA) located approximately 25 km upstream of Dublin, GA. Twelve sites within this reach were selected for study providing an assessment of potential impacts from major tributaries (Figure 1-1). Depositional sediments were collected at the Avant Mine, above and below the five major tributaries (Bluff Creek, Black Creek, Buffalo Creek, Commissioners Creek and Big Sandy Creek) and below BWMA. Approximately 20 L of surficial sediments (0-3 cm deep) were collected at each site with a shovel and placed in plastic containers for transport to the laboratory. Sediments were stored at 15 °C for 2 to 4 d before testing was initiated and for 12 d there after for porewater extractions. At each site, an in-situ sample of pore water was collected using a 60-cc syringe attached to a fused-glass airstone by airline tubing (Winger and Lasier 1991). The porewater samplers were inserted to a depth of approximately 3 cm, and sediments were packed around the tubing at the point of insertion to avoid sampling surface water. Grab samples of surface water were collected in 20-L carboys from three sites at the time of sediment collection: Avant Mine (SW-1), above Commissioners Creek (SW-8) and below BWMA (SW-12). These waters were transferred to the laboratory, stored in the dark with constant aeration and maintained at 23 °C throughout the testing period.

Sediment toxicity was evaluated with the freshwater amphipod, *Hyalella azteca*, in 28-d solid-phase toxicity tests (USEPA 1994a). Sediments were homogenized in the laboratory by stirring and distributed to test chambers for the solid-phase exposures. Test chambers consisted of 300-mL glass beakers with a screened notch at the top. Sediment (100 mL) was added to each chamber followed by 175 mL of overlying water. For each sediment, 5 replicate test chambers, each containing 10 7-d old *H. azteca*, were prepared and randomly distributed in a test exposure system that automatically renewed 70 % of the overlying water twice daily (Zumwalt et al. 1994). The overlying water was a moderately-hard reconstituted water (MRW) prepared with reagent-grade chemicals and deionized water (Smith et al. 1997). A commercial coarse-grained sand equilibrated with the MRW was used as the control sediment. A 1-mL food supplement of yeast-cerophyll-trout chow (USEPA 1994b) was added to each test chamber daily. Sediment exposures were conducted at room temperature (23 °C) with a 16 h light:8 h dark photoperiod. Dissolved oxygen (DO), conductivity and pH were measured in the overlying water three times per week and alkalinity, hardness and ammonia at the beginning and end of the test. Overlying-water samples (20-mL aliquots from each replicate composited by site) were collected before the morning renewal from within 1 cm of the sediment surface. Endpoints for sediment exposures were survival and growth (total length). Total length was determined by measuring the animal's image from a slide projector with a flexible ruler calibrated to the appropriate magnification with a micrometer.

Pore waters were evaluated in 96-h, static-renewal exposures using *H. azteca*. Fresh porewater samples were extracted daily (Winger and Lasier 1991) from the remaining sediments. Ten porewater extractors were inserted into each sediment and approximately 300 mL of pore water extracted. Pore waters were aerated for 15 min prior to use which provided near-saturation levels of DO and sufficient time for the solution temperature to rise to the exposure temperature

of 23 °C. The five replicates of each test solution consisted of a 30-mL plastic cup containing 10 7-d old animals, 20 mL of solution and a 2.3 cm^2 piece of Nitex® netting. The test endpoint was *H. azteca* survival. Test animals were transferred daily to freshly-prepared exposures. Surface waters from the river were used as reference waters. Pore water extracted from the control sediment (coarse sand) was used as a control pore water and MRW was an additional control. The control pore water had a pH of 8.6, conductivity of 662 µS/cm, hardness of 49 mg/L $CaCO_3$, alkalinity of 206 mg/L $CaCO_3$, 118 mg/L Na, 6.7 mg/L K, 17.2 mg/L Ca and 1.5 mg/L Mg. MRW had a pH of 8.2, conductivity of 360 µS/cm, hardness of 69 mg/L $CaCO_3$ and alkalinity of 68 mg/L $CaCO_3$.

Metals were measured in sediments, pore waters, surface waters and control waters by a contract laboratory. Outside laboratories also determined concentrations of organic contaminants in a subset of the sediment samples and dissolved organic carbon (DOC) in aqueous samples. After the homogenization of sediments at test initiation, three subsamples of each sediment were placed in glass containers and held at 4 °C for inorganic and organic chemical analyses and for physical characterizations (organic content, particle-size distribution, acid volatile sulfides). Sediments from two sites were split at the time of collection to provide field duplicates. Duplicate samples were also submitted from two porewater samples and deionized water was submitted as a blank.

Cation concentrations were determined by inductively coupled plasma-mass spectrometry. Sediment samples were prepared for metal analyses by digestion in concentrated nitric acid (HNO_3) followed by microwave radiation. Organochlorines and polynuclear aromatic hydrocarbons were measured in sediments from sites 1, 3, 7 and 11 by accelerated solvent extraction and gel permeation chromatography. Dissolved organic carbon concentrations in test waters were determined using a carbon analyzer. Detection limits for metals in waters, pore waters and sediment digestates were less than 1 µg/L, with the exceptions of K and Ca, which were 13 µg/L. Detection limits for the organic compounds in sediments were 10 µg/Kg. The detection limit of DOC in water samples was 0.1 mg/L. Limits of quantification were set at three times the detection limits. Precision and accuracy of analyses were within acceptable limits based on spike recoveries and contract-laboratory splits. Differences in sediment cation concentrations of field splits were <12%. Differences in porewater splits averaged <5% for Na, Ca, Mg and Mn, 9.5% for Zn and 20% for K and Cu. Cation concentrations in the blank sample were below detection limits with the exceptions of Na, Cu and Zn, which were 100 µg/L, 1 µg/L and 3 µg/L, respectively.

Sediment organic content was measured by loss on ignition at 430 °C for 4 h (Davies 1974). Particle-size distributions were determined using the micro-pipette method of Miller and Miller (1987) and acid-volatile sulfide (AVS) concentrations were established by the diffusion method of Brouwer and Murphy (1994). Simultaneously-extracted metals (SEM) from AVS digestates were analyzed by contract laboratory using inductively coupled plasma-mass spectrometry. Redox potential was measured in each sediment at the initiation of testing (24 h after homogenization) and after 7 d and 12 d of storage at a depth of 15 cm using a redox electrode.

Surface water and aerated porewater samples were collected for cation analyses at test initiation, after 48 h and after 12 d. The control pore water and MRW were sampled for these analyses only at test initiation. Prior to analytical analyses, samples were filtered through a 0.2

μm nylon filter and acidified with 1% (v:v) ultra-pure HNO_3.

Basic chemistries of aqueous samples included DO, pH, conductivity, alkalinity, and ammonia. Dissolved oxygen, pH, conductivity and ammonia were measured with the appropriate meters and electrodes. Alkalinity was determined by colorimetric titration with H_2SO_4 (APHA 1992). Water hardness values were calculated from measured Ca and Mg concentrations (Hem 1985).

Statistically significant ($p \leq 0.05$) differences in *H. azteca* survival and growth between sampling sites and controls for sediment and porewater exposures were determined using analysis of variance and Dunnett's one-tailed means comparison (SAS 1988). Differences in surface water cation concentrations were determined using Tukey's studentized range test (SAS 1988). Relationships between toxicity, sediment and porewater characteristics were identified by performing Pearson product-moment correlation analyses (SAS 1988). In addition to whole-sediment concentrations, sediment-cation concentrations were also normalized by physical characteristics (% clay, % silt, % clay + % silt, % organic matter) for correlation analyses. Normalization of contaminant concentrations to the amount of fine material in the sediments was used for comparisons of sediments with different particle-size distributions (O'Conner 1990, Horowitz 1991, de Groot 1995). Correlations with $p \leq 0.05$ were considered significant.

Results and Discussion

Pore waters from the lower Oconee River were acutely toxic to *H. azteca* and sediments from several sites demonstrated chronic toxicity (Table 1-1). Compared to controls, survival in pore water was significantly reduced at all sites, and in sediment exposures, growth was significantly less at sites 1 and 8 - 12. Survival in sediment and surface-water exposures was not significantly less than in controls. Toxicity appeared to be related to the availability of sediment-associated metals.

Sediments collected for this study exhibited elevated concentrations of several metals (Table 1-2); no organic contaminants (organochlorines or polynuclear aromatic hydrocarbons) were detected. Concentrations of Mn in all but one sediment exceeded the probable effects level of 1200 mg/Kg established by Ingersoll et al. (1996), nine sediments exceeded the threshold effects level (TEL) for Cr (36 mg/Kg), five sediments exceeded the TEL for Zn (98 mg/Kg) and three exceeded the TEL for Cu (28 mg/Kg). Whole-sediment concentrations of Hg from sediments collected at sites 2, 3, 9, 10 and 12 exceeded levels considered high (0.3 mg/Kg) by O'Conner (1990).

Metal concentrations in whole sediments tended to reflect the chronic toxicity exhibited by *H. azteca* (Table 1-2). Whole-sediment Zn concentrations were elevated at sites below the confluence of Buffalo Creek. Zinc is an element contained in municipal and industrial effluents currently permitted for discharge into that tributary. Sediment from site 1 also significantly reduced amphipod growth (Table 1-1). The highest concentration of Cr was measured at this site (Table 1-2), which exceeded the TEL for this element (Ingersoll et al. 1996). Other trace metals measured in these sediments, but not included in Table 1-2, were As, Cd, Mo, Pb and Se. Arsenic ranged from 1.6 to 4.2 mg/Kg; Cd ranged from non-detectable to 0.26 mg/Kg; Mo ranged from 0.17 to 0.41 mg/Kg; Pb ranged from 7 to 22 mg/Kg; and Se ranged from 0.4 to 1.8 mg/Kg.

Depositional sediments collected for testing were composed primarily of sand particles (>53 μm) with fine particles (silt and clay) comprising between 14 and 41 % (Table 1-3). The

amount of organic material in the sediments varied between 0.7 and 3.4 %. Similar to results of Ankley et al. (1991), there was no obvious relationship between organic material and AVS. Half of the sediments had no measurable AVS. Acid-volatile sulfide concentrations in the remaining six sediments were low (0.18 to 1.62 μmol/g), yet SEM concentrations ranged from 3.75 to 18.77 μmol/g resulting in SEM/AVS ratios well above 1 (Table 1-3). SEM/AVS ratios above 1 have been associated with sediment toxicity and reflect metal concentrations that exceed the binding capacity of available sulfide (Di Toro et al. 1990, Di Torro et al. 1992, Ankley et al. 1994); however, other binding phases can also control metal availability (Ankley et al.1993). The lack of acute toxicity from Oconee River sediments with SEM/AVS ratios greater than 1 suggests that the toxicity of metals was regulated by factors other than sulfide concentration.

Manganese and Fe oxides also have the ability to sorb metals, particularly Cu, Pb and Zn and release them upon chemical reduction (Jenne and Zachara 1984, Forstner 1990). Under anoxic conditions, microbial communities within sediments reduce Mn and Fe oxides during organic decomposition, resulting in the release of reduced Mn and Fe species as well as metals sorbed to the oxides (Boyd 1995). Reduced Fe quickly oxidizes and precipitates under oxic conditions at pH levels above 7 (Vuori 1995), but reduced Mn persists in oxic solutions with pH below 8.5 for much longer periods of time (Patrick and Henderson 1981, Davison 1982, Hirst and Aston 1983, Lee and Jones 1984, Stokes et al. 1988, Luther 1995). Despite collecting surficial and presumably oxic sediments, redox potentials in stored sediment samples became highly reducing in all sediments except the sample collected from site 1 (Table 1-3). No obvious chemical or physical characteristic could be identified as a reason for this sediment to remain oxic. However, we presume that the clay portion of the sediment was a purer form of kaolinite than encountered downstream from the quarry.

To facilitate comparisons among sites and with sediments from other systems, whole-sediment cation concentrations were normalized to the proportion of fine material in the sediment (O'Conner 1990, Horowitz 1991, de Groot 1995). Normalized concentrations of Cu, Hg and Zn have been considered high when they exceed 87, 0.51 and 280 mg/Kg, respectively (O'Conner 1990). Concentrations of these metals in sediments from several sites in the Oconee River were elevated with respect to these threshold values (Figure 1-2). Inputs from Buffalo Creek were obvious in the adjusted concentrations of Zn. Concentrations of Zn normalized to the fine materials were significantly correlated (negatively) to mean total length of *H. azteca*. Normalized Hg concentrations in Oconee River sediments were high (>0.5 mg/Kg) at most of the sites, particularly those below Commissioners Creek. Copper tended to be highest at sites in the middle of the study reach with the greatest concentrations above Buffalo Creek and Commissioners Creek. The source of Cu to sediments above these tributaries is unknown.

Surface-water cation concentrations increased downstream in the Oconee River (Table 1-4). Concentrations of Na, Ca and Zn were significantly greater in samples collected at the lowest site (SW-12) compared to the uppermost site (SW-1). Concentrations of K, Mg and Cu also increased downstream, but not significantly. Concentrations of DOC in surface waters were at or below the limit of quantification. The chemical characteristics of surface waters and pore waters were generally within acceptable limits for *H. azteca* exposures. Basic porewater chemistries after aeration indicated near saturated DO, pH values around 8.5, conductivities between 100 and 725 μS/cm with hardness and alkalinity measurements ranging from 20 to almost 400 mg/L (Table 1-5). Surface waters were categorized as soft with pH near 8, hardness between 10 and 30

mg/L $CaCO_3$ and alkalinity around 25 mg/L $CaCO_3$. The control pore water was similar to those extracted from river sediments with the exception of lower hardness. Pore waters from sites 11 and 12 had alkalinities from 350 to 400, which may have contributed to their toxicity (Lasier et al. 1997). Ammonia concentrations were highest in pore waters from sites 7 and 12, but were below levels known to cause toxicity to *H. azteca* (Ankley et al. 1995).

Manganese appears to be responsible for most of the toxicity elicited in exposures to pore waters. Concentrations in pore waters (Table 1-6) were often above the 96-h LC_{50} of 14 mg/L established for *H. azteca* in hard water (Lasier et al. 2000). Concentrations of Mn in surface waters, control pore water and MRW were low or undetected and survival of *H. azteca* was high. Concentrations of Cu and Zn in pore waters (Table 1-6) were generally below levels that would cause acute toxicity (Eisler 1993, 1997). However, concentrations of Zn in pore waters from sites 6, 7 and 12 were 65 to 75 % of the LC_{50} described by Eisenhauer et al. (1999). These levels of Zn in the presence of some Cu may have contributed to the toxicity of those pore waters due to the more-than-additive toxicity of Zn and Cu in mixtures (de March 1988, Eisler 1993, 1997).

Hyalella azteca had limited survival in pore waters from only four sites; 1, 5, 7 and 12 (Table 1-1). The lowest concentrations of Mn in pore waters were measured at sites 1 and 5 (Table 1-6), but these levels were within ranges expected to elicit toxicity. The 96-h LC_{50} for Mn to *H. azteca* is around 3 mg/L in soft water (hardness of 26 mg/L) and 9 mg/L in moderately-hard (80 mg/L) water (Lasier et al. 2000). Survival in pore water from site 1 was 22 % after 96-h exposures to concentrations of Mn between 1.5 and 3.9 mg/L with 20 to 40 mg/L hardness. Survival was 18% in pore water from site 5 that had Mn concentrations between 10 and 17 mg/L and hardness around 80 mg/L. Limited survival occurred in exposures to pore water from sites 7 and 12 in spite of high Mn concentrations; however, pore waters from these sites also had highly-elevated levels of hardness.

Porewater cation concentrations were not correlated with whole-sediment or normalized-sediment cation concentrations with the exception of Zn. Sediment Zn concentrations (whole-sediment and concentrations normalized to clay and silt components) were positively correlated to porewater Zn concentrations, but only in pore waters extracted after 12 d of storage. Concentrations of Zn in pore waters exhibited considerable temporal variability during sediment storage (Table 1-6) which may reflect changing chemical conditions. Decomposition of organic matter during sediment storage (through 12 d after homogenization) was evidenced by the increasing concentrations of porewater DOC (Table 1-6). Thomson et al. (1980) reported continued synthesis of organic material during extended sediment storage at both 4 °C and 25 °C, and that extended storage promoted a more homogenous partitioning of Zn in sediments. The significant correlations between sediment and porewater Zn concentrations determined in this study after 12 d of storage supports their results.

Elevated Mn concentrations were encountered in pore waters extracted in situ as well as in pore waters extracted from collected sediments (Table 1-6). Even though several pore waters contained low to undetectable Mn concentrations when extracted in situ (sites 1, 2, 4, 5, 6), disturbance of those solid-phase sediments during collection, homogenization and storage apparently caused concentrations of porewater Mn to increase. In-situ porewaters from five sites (3, 7,9,10,11) contained Mn at concentrations equal to or above those measured in the laboratory-extracted samples indicating that reducing conditions were present within 2-3 cm of the surface of in-situ sediments, and that the depth of porewater extraction at these sites was

below the oxic-anoxic boundary. Similar to Mn, concentrations of other major porewater cations (Na, K, Ca, Mg) tended to increase after homogenization, but concentrations of Cu and Zn tended to decrease (Table 1-6).

Porewater concentrations of the major cations (including Mn) increased over the 12 d of storage. Decomposition of organic material and its effect on sediment redox potential may have contributed to this trend, but sediment disturbance that triggers this process is unavoidable during sample collection and handling. In this study, sediments were stored at 15°C instead of the recommended 4°C (Mudroch and Bourbonniere 1994, USEPA 1994a) because of limitations on refrigerated space. Although the lower storage temperature is suggested to reduce biological activity within the sediments, storage at 4°C does not prevent surficial sediments from becoming anoxic. Brumbaugh et al. (1994) found that pore waters extracted from surficial sediments maintained at 4°C contained elevated levels of reduced Fe and Mn, demonstrating that sediments became anoxic despite storage at a low temperature. The process of sediment becoming anoxic during storage promotes reduction and release of soluble Mn into the pore water. Unfortunately, assessments that include porewater toxicity may reflect toxic levels of Mn without it being considered the potential toxicant.

Utilization of porewater toxicity in assessments of sediment quality is justified by theories of equilibrium partitioning and contaminant bioavailability (Adams et al. 1985, Knezovich et al. 1987), but toxicities that are elicited are often caused by natural processes not associated with pollution (Ankley et al. 1990, Hoke et al. 1992, Lasier et al. 1997). Manganese appears to be one of these confounding variables associated with porewater toxicity. Sources of elevated Mn in sediments may be anthropogenic, but natural processes may also influence concentrations in toxicity tests. Boucher and Watzin (1999) considered Mn to be the primary toxicant in sediment pore waters from Vermont. Manganese concentrations in Oconee River sediments varied from 963 to 4095 mg/Kg (Table 1-2), similar to ranges observed in other studies of freshwater sediments (Brumbaugh et al.1994, Winger and Lasier 1998). Due to its labile nature in the reduced form and the influence of sediment collection and handling on the redox conditions of collected sediments, Mn may have contributed to observed toxicities in these studies. For example, sediments from the Clark Fork River and Milltown Reservoir that had the highest Mn concentrations (Brumbaugh et al.1994) were the most toxic in solid-phase exposures (Kemble et al. 1994), and Winger and Lasier (1998) noted a negative correlation between porewater survival and whole-sediment Mn concentrations. Based on these findings, concentrations of Mn in porewaters should be considered when assessing potential causes of toxicity along with other chemical characteristics such as NH_3 (Ankley et al. 1995, Borgmann and Borgmann 1997), CO_3 (Hoke et al. 1992, Lasier et al.1997) and H_2S (Bagarinao 1992, Ortiz et al. 1993, Knezovich et al. 1996) that tend to be present in pore waters extracted from anoxic sediments.

Conclusion

Sediments from the lower Oconee River caused reduced growth in freshwater amphipods exposed in 28-d tests. This chronic response may be indicative of metal contamination. Toxicity of sediments collected below the confluence of Buffalo Creek appeared to be caused by Zn, and increased concentrations of Zn in sediments downstream of Buffalo Creek were attributed to municipal and industrial discharges released to the Buffalo Creek watershed. These discharges are permitted to contain Zn under the NPDES program. Concentrations of sediment-associated

Zn below this tributary exceed those known to adversely effect aquatic organisms. Toxicity of sediment collected from site 1 (Avant Mine) may have been caused by Cr; however, the source of this metal to the watershed is unknown.

Porewater toxicity was caused by elevated concentrations of Mn. Water quality criteria for Mn are not currently available, but research is underway to establish them. Although Mn is not typically a problem in surface waters because it is sequestered in insoluble forms, it may contribute to observed porewater toxicity due to the toxic nature of its reduced form. Benthic organisms that inhabit areas that border on anoxic conditions may be at particular risk. Manganese was undetectable in surface water samples, but concentrations in pore waters were often above lethal thresholds of aquatic organisms. The collection and handling of surficial sediments can cause Mn concentrations in pore water to increase due to processes associated with the decomposition of organic matter involving the chemical and microbial reduction of sediment-associated Mn. Porewater toxicity is often confounded by naturally-occurring chemical constituents such as ammonia, alkalinity and hydrogen sulfide, and Mn is another potentially confounding variable that should be considered in evaluating results from porewater toxicity tests.

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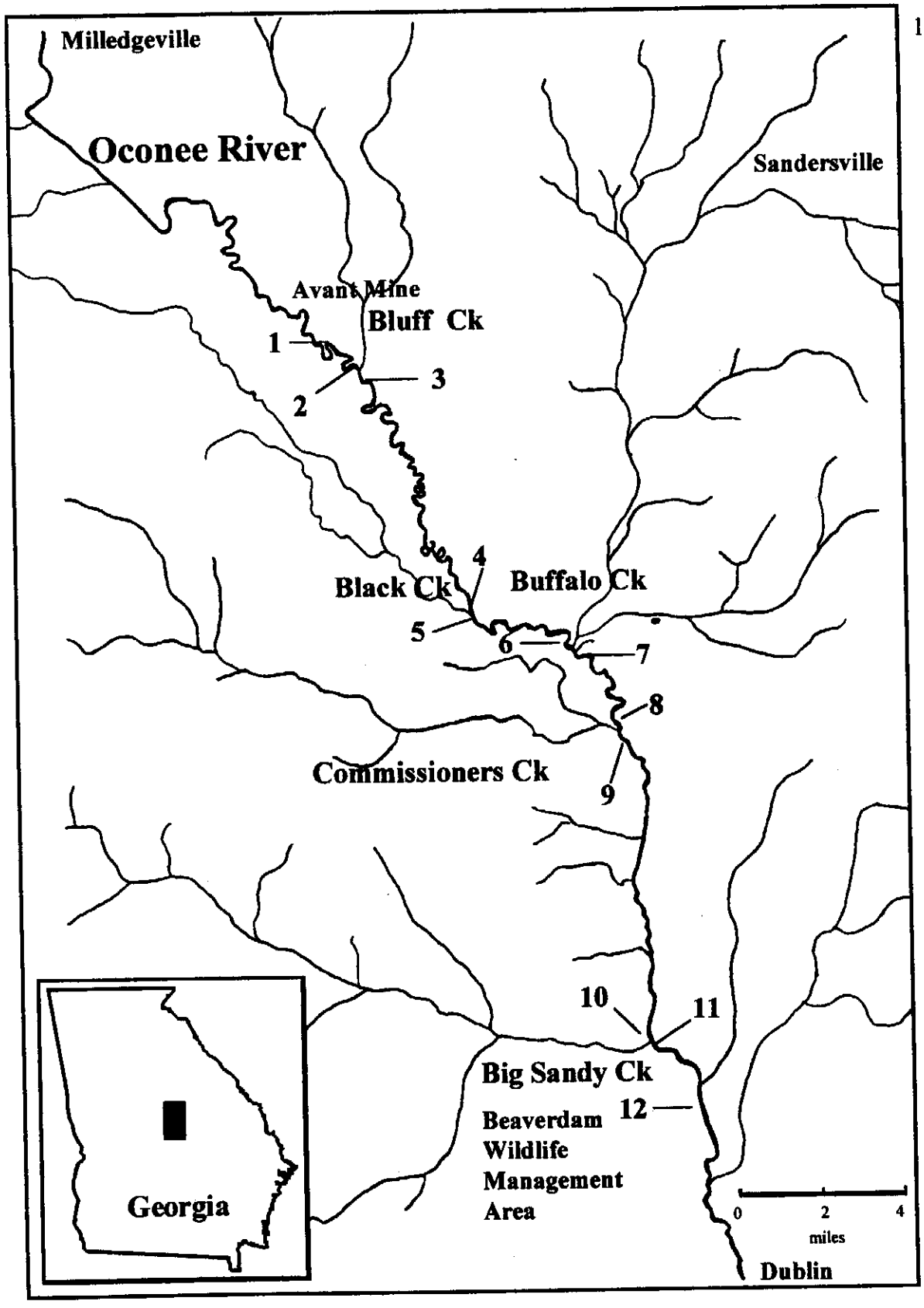

Figure 1-1. Locations (sites 1-12) along the lower Oconee River where sediments and pore waters were collected.

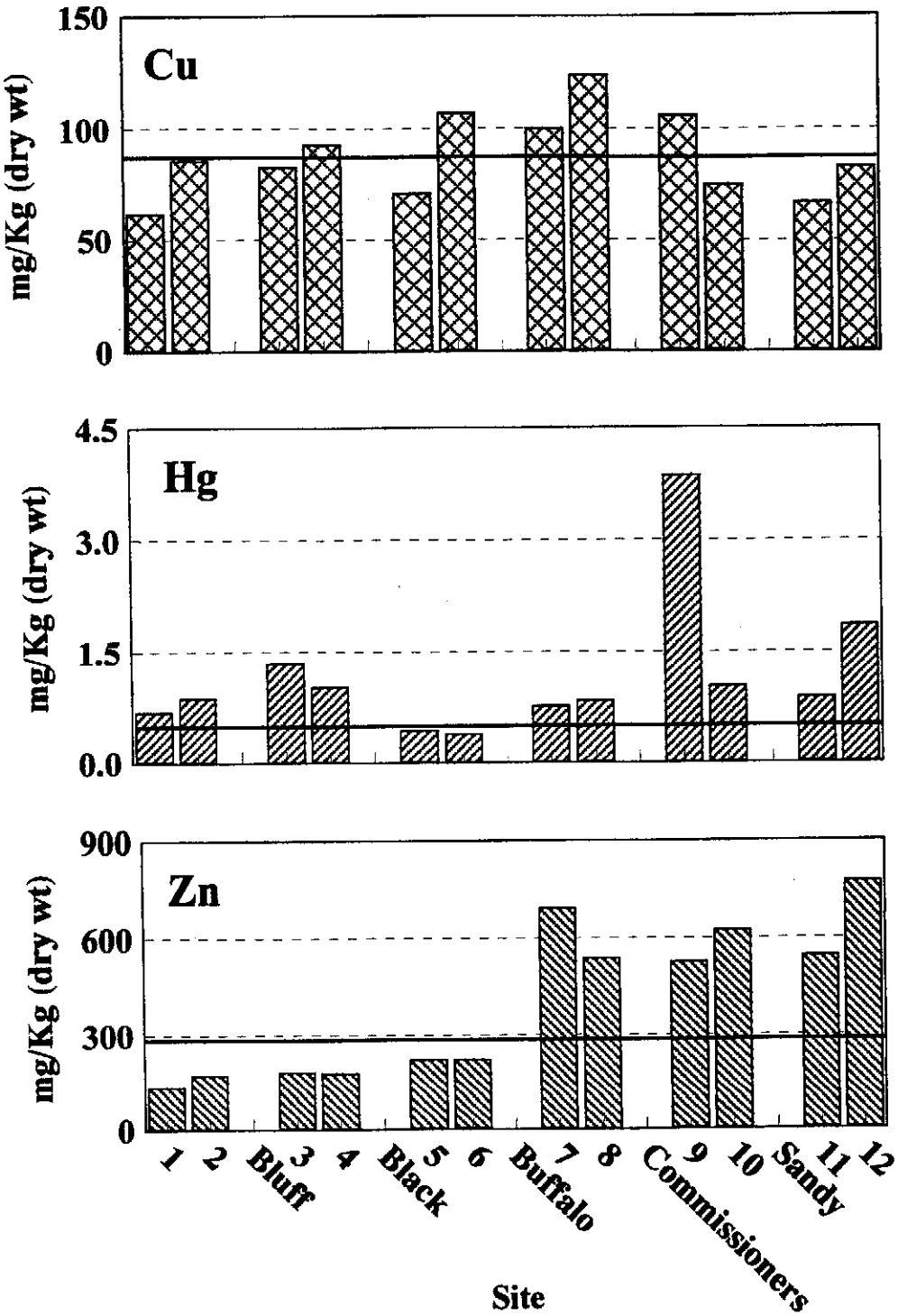

Figure 1-2. Metal concentrations in sediments after adjustment to proportion of fine material. Line indicates "adjusted" levels considered high by O'Conner (1990). Locations of tributary inputs indicated between sampling sites.

Table 1-1. Toxicity of Oconee River surface waters (96-h), pore waters (96-h), and sediments (28-d) to *Hyalella azteca* compared to control water (MRW), control pore water and control sediment.

Site	Surface water (% survival)	Porewater (% survival)	Sediment (% survival)	Sediment (mean length in mm with 95% CI)
Control	98	100	94	4.13 (4.02 - 4.24)
1	94	22*	96	3.86 (3.74 - 3.98)*
2		0*	80	4.53 (4.37 - 4.69)
3		0*	90	3.99 (3.88 - 4.10)
4		0*	98	4.03 (3.90 - 4.16)
5		18*	98	4.09 (3.98 - 4.20)
6		0*	100	4.44 (4.35 - 4.53)
7		4*	100	3.95 (3.81 - 4.09)
8	98	0*	100	3.61 (3.52 - 3.70)*
9		0*	98	3.68 (3.58 - 3.78)*
10		0*	100	3.80 (3.69 - 3.91)*
11		0*	96	3.90 (3.81 - 3.99)*
12	100	2*	94	3.67 (3.55 - 3.79)*

* denotes mean significantly less than control pore water or sediment ($p \leq 0.05$)

Table 1-2. Cation concentrations (mg/Kg dry wt) in depositional sediments collected from the Oconee River.

Site	Na	K	Ca	Mg	Mn	Fe	Cr	Cu	Hg	Zn
1	303	1540	95	2916	1476	49,330	55	24	0.27	53
2	350	2631	37	3876	3350	43,259	47	31	0.32	63
3	659	2397	162	3404	3374	42,796	51	34	0.55	76
4	965	1120	37	1677	1885	23,184	29	14	0.15	22
5	651	733	425	1053	963	17,710	26	10	0.06	16
6	762	2237	290	3459	4095	45,598	49	31	0.11	65
7	574	1863	81	2575	1380	44,361	40	18	0.14	125
8	867	2346	107	3253	2228	40,157	50	26	0.18	113
9	876	1383	146	1961	1335	27,013	38	17	0.62	85
10	267	1729	122	2632	1957	34,428	44	24	0.33	200
11	575	909	425	1504	1764	22,927	30	12	0.16	98
12	255	1664	937	3093	3228	53,870	38	25	0.56	232

Table 1-3. Physical characteristics of depositional sediments collected from the Oconee River. Redox potentials (Eh) were measured on the day of test initiation (Eh1), after 7 d of storage (Eh2) and after 12 d of storage (Eh3). Simultaneously extracted metals (SEM) concentrations are molar sums (dry weight) of copper, cadmium, lead, mercury and zinc. Acid volatile sulfides (AVS) are molar concentrations (dry weight) of cold-acid extractable sediment sulfides.

Site	Sand (%)	Silt (%)	Clay (%)	Organic Content (%)	SEM/AVS	Eh1 (mV)	Eh2 (mV)	Eh3 (mV)
1	61	23	16	1.0	NC*	125	128	149
2	64	26	10	2.1	NC	-133	-212	-208
3	58	28	13	2.7	20.8	-326	-239	-236
4	85	7	8	0.6	NC	-405	-260	-221
5	86	5	9	0.3	NC	-278	-221	-182
6	72	16	13	2.0	NC	-366	-284	-303
7	82	11	7	1.8	3.9	-385	-298	-257
8	79	14	7	1.2	NC	-281	-190	-216
9	84	11	5	0.7	14.2	-303	-121	-105
10	68	22	10	1.5	9.9	-277	-247	-246
11	81	10	8	1.9	14.6	-278	-201	-245
12	70	19	11	3.4	20.0	-252	-210	-253

* NC - SEM/AVS ratio not calculable due to no measurable AVS.

Table 1-4. Mean cation concentrations of surface waters from the lower Oconee River*.

Surface water	Na (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Cu (μg/L)	Zn (μg/L)
SW-1	3.2[a]	1.7[a]	1.6[a]	1.4[a]	3.0[a]	13.3[a]
SW-8	11.3[b]	1.9[a]	4.3[ab]	1.7[a]	4.3[a]	18.3[ab]
SW-12	12.3[b]	2.0[a]	6.2[b]	1.8[a]	5.7[a]	36.3[b]

* Mean concentrations with the same letter superscript are not significantly different ($p \leq 0.05$).

Table 1-5. Chemical characteristics of pore waters and surface waters (SW-1, SW-8, SW-12) from the Oconee River.

Site	pH	Conductivity (µS/cm)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Ammonia (mg/L NH_3)
1	8.0	111	21	28	0.4
2	8.5	302	140	150	1.8
3	8.6	338	148	170	4.0
4	8.6	280	77	134	2.3
5	8.4	187	82	82	0.4
6	8.6	457	253	242	2.5
7	8.6	563	291	288	9.6
8	8.6	455	158	232	3.7
9	8.6	364	146	178	2.8
10	8.6	395	187	200	4.8
11	8.5	629	317	348	5.3
12	8.5	725	377	398	7.1
SW-1	7.9	79	10	24	0
SW-8	8.1	132	17	24	0
SW-12	8.1	127	29	26	0

Table 1-6. Cation and dissolved organic carbon (DOC) concentrations in Oconee River porewater samples extracted in situ (1) and from collected sediments at test initiation (2), 48 h after test initiation (3) and 12 d after test initiation (4).

Site-sample	Na mg/L	K mg/L	Ca mg/L	Mg mg/L	Mn mg/L	Cu µg/L	Zn µg/L	DOC mg/L
1-1	3.3	1.5	2.0	1.6	blq[1]	7	94	18
1-2	5.0	1.5	4.3	2.6	1.5	blq	44	blq
1-3	6.7	2.3	7.3	4.8	3.9	6	85	blq
1-4	4.8	1.6	7.3	5.0	5.7	3	blq	12
2-1	5.6	2.8	10.0	3.8	2.5	6	53	blq
2-2	9.5	5.4	30.4	16.1	13.8	3	blq	12
2-3	10.9	7.6	60.8	32.1	46.5	11	147	18
2-4	11.0	8.1	72.5	43.1	36.7	7	34	51
3-1	12.2	12.1	118.5	49.3	46.3	11	105	56
3-2	10.1	3.9	37.4	13.7	42.1	10	40	23
3-3	6.8	3.6	45.3	17.3	64.9	4	67	27
3-4	4.0	2.1	26.9	11.1	52.9	2	blq	52
4-1	3.1	1.6	1.8	1.4	0.4	3	33	blq
4-2	6.8	3.8	18.1	8.7	16.0	6	blq	blq
4-3	5.5	3.7	24.9	11.9	26.0	3	blq	18
4-4	8.2	5.6	39.1	20.5	30.0	3	blq	35
5-1	5.3	2.5	2.6	2.0	0.3	6	60	blq
5-2	8.5	4.7	18.6	8.8	9.9	4	30	blq
5-3	9.6	3.7	19.2	9.1	16.6	5	blq	blq
5-4	4.2	2.9	25.0	12.3	27.2	3	blq	35
6-1	6.7	0.7	8.4	2.5	4.8	5	65	blq
6-2	11.0	7.0	55.2	28.7	37.0	3	blq	25
6-3	26.9	34.3	76.9	40.0	43.3	5	349	32
6-4	10.8	7.5	62.7	38.5	30.0	22	43	74

[1] blq= below limit of quantification

Table 1-6. continued.

Site-sample	Na mg/L	K mg/L	Ca mg/L	Mg mg/L	Mn mg/L	Cu µg/L	Zn µg/L	DOC mg/L
7-1	19.5	2.0	23.1	3.4	47.0	4	70	blq[1]
7-2	39.6	6.9	83.6	20.6	36.2	10	56	36
7-3	36.5	6.9	95.6	23.9	43.3	9	338	43
7-4	36.5	7.3	111.0	30.7	33.0	6	175	58
8-1	23.3	1.7	23.2	5.6	14.5	10	67	blq
8-2	10.4	4.2	37.5	16.0	28.8	blq	blq	14
8-3	9.8	4.3	45.1	19.0	43.2	4	46	22
8-4	9.0	3.9	50.8	22.7	30.8	4	blq	48
9-1	11.7	2.2	30.4	8.7	48.0	6	116	13
9-2	15.8	4.2	37.5	13.1	40.2	4	31	17
9-3	9.8	2.7	25.2	8.9	28.3	2	blq	14
9-4	14.1	3.9	45.0	16.5	47.6	3	27	40
10-1	14.6	2.4	54.4	26.7	49.7	6	76	25
10-2	15.6	5.8	49.9	15.6	40.7	blq	35	20
10-3	10.2	4.0	41.1	12.9	34.1	3	127	21
10-4	14.6	5.6	67.2	22.8	53.6	5	blq	47
11-1	24.0	8.6	123.3	27.9	41.1	10	54	24
11-2	19.8	5.6	99.8	16.8	36.4	5	33	38
11-3	22.3	6.7	151.4	24.7	50.3	6	40	46
11-4	16.8	4.8	131.9	23.4	38.7	6	90	97
12-1	12.9	13.9	93.6	54.5	18.6	2	38	48
12-2	20.4	7.8	96.5	33.8	40.2	3	32	34
12-3	17.0	7.6	107.4	36.2	42.8	5	81	37
12-4	18.7	7.3	117.7	41.2	53.7	10	304	95

[1] blq=below limit of quantification

Impacts and Toxic Thresholds of Sediment-associated Contaminants to Robust Redhorse (*Moxostoma robustum*) in the Lower Oconee River

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The robust redhorse (*Moxostoma robustum*) is a large benthic, riverine fish that historically occurred in Atlantic-slope watersheds of the southeastern United States (Jenkins and Burkhead 1994). The species was described by Edward Cope in 1870, but disappeared from the scientific record until single specimens were collected during the 1980s from the Savannah and PeeDee Rivers in Georgia and South Carolina, and a viable population was discovered in the Oconee River, Georgia, in 1991 (Robert E. Jenkins, personal communication). The population is limited to under 1000 aging individuals with very little natural recruitment (Evans 1994, 1996, Jennings et al. 1998). The robust redhorse (RRH) is a lithophilic spawner that deposits fertilized eggs into gravel for development of the early-life stages (Jennings et al. 1996). Spawning has been observed near the Avant Kaolin Mine approximately 25 km downstream of Milledgeville, and developing embryos and larvae have been collected in the gravel bars at this location (Jennings et al. (1996). Poor recruitment may indicate reduced survival of embryos and larvae prior to emergence from the gravel (Jennings et al. 1998).

Reduced habitat quality has been suggested as a cause for limited recruitment of the RRH in the Oconee River. Stage height variability, sedimentation and water temperature fluctuations associated with releases from a hydroelectric dam above the spawning area may be involved. Deposition of fine sediment into spawning gravel may be particularly important. Dilts (1999) reported that RRH embryos and larvae were tolerant of <15% fine sediment (particles < 2 mm), but emergence was significantly reduced when fine materials exceeded 25%. He concluded that >50% fine sediment caused mortality through entrapment of developing larvae and reduction of dissolved oxygen. The RRH disturbs the gravel during spawning which tends to flush fine material from the gravel, but siltation can occur after the eggs are deposited. Approximately 33% of the substrate at the RRH spawning site was composed of sediment particles <2 mm (Byron J. Freeman - personal communication). These elevated concentrations of fine sediment may be limiting survival of the RRH during development in the gravel.

Geochemical characteristics of fine sediments may also pose an additional threat to the RRH early-life stages. Most contaminants in aquatic systems are associated with the fine particles of sediments (O'Conner 1990, Horowitz 1991, de Groot 1995), which are easily transported and often in intimate contact with benthic organisms (de Groot 1995). These small size fractions can infiltrate gravel substrates and impact developing fish embryos and larvae. Fine sediments also contain organic particles, which bind contaminants (Salomons 1985, de Groot 1995, Jenne 1995, Chapman et al. 1998) and fuel microbial respiration.

Contaminants bound to fine materials in the sediments from the Oconee River could impact the early-life stages of the RRH. Sediments from this reach of the Oconee River induced chronic toxicity to *Hyalella azteca* (freshwater amphipod) when exposed for 28 d (Lasier et al. 2001). Zinc, identified as the most likely cause for this toxicity, occurs in several effluents permitted by the National Pollution Discharge Elimination System of the Clean Water Act for discharge to this watershed. Early-life stages of the RRH may be exposed to sediment-borne toxicants through their intimate contact with fine sediment during development and concentrations may exceed toxic thresholds. Metals of particular concern include Cd, Cu and Zn

due to their presence in permitted discharges and Mn. Elevated concentrations of Mn in pore waters extracted from Oconee River sediments were responsible for decreased survival of *H. azteca* in 96-h porewater exposures (Lasier et al. 2001). Ammonia is another chemical stressor associated with sediments that may also impact this species.

The objectives of this study were to; 1) determine the toxic thresholds of Cd, Cu, Mn, Zn and NH_3 to early-life stages of the RRH and 2) evaluate the toxicity of sediments and pore waters from the lower Oconee River to early-life stages of the RRH. This research was conducted through two spawning seasons (spring of 1998 and 1999); metal toxicities were determined during both seasons, and sediment and porewater toxicities were assessed during 1999.

Methods

Toxicity tests were conducted in late spring concurrent with RRH artificial propagation activities coordinated by the State of Georgia and the U.S. Fish and Wildlife Service. Fertilized eggs from a single cross were used each year. Eggs were delivered from river-side propagation facilities to our laboratory where they were either used immediately in the initial phases of the study or held for later use in McDonald hatching jars with a recirculating water system containing dechlorinated and filtered (activated charcoal) laboratory water. This water had low conductivity, hardness and alkalinity (150 µS/cm, 30 mg/L $CaCO_3$, 30 mg/L $CaCO_3$, respectively). Flow through the jars was adjusted to slightly roll the eggs, and water temperature was maintained at 23 °C. As embryos hatched (72-96 h), they were released into a 500-L holding tank, where they remained until needed in later phases of the study. At 23 °C, the interval between developmental stages (egg, yolk-sac fry and swim-up fry) was approximately 96 h. Test exposures were initiated at these transitional stages and terminated after 4-, 8-, or 12-d exposures.

A tiered approach used to evaluate toxic effects on the various life stages of the RRH facilitated identification of susceptible life stages and determination of cumulative impacts through successive life stages. Multiple tests were started simultaneously but were terminated consecutively at the transitional intervals between embryo, yolk-sac and swim-up fry, and after 96 h as swim-up fry. At initiation, three identical toxicity tests were started for each matrix (sediments, pore waters and toxicant dilutions) with eggs that had been fertilized within 24 h. As development progressed from embryo to yolk-sac fry (day 4), one block of the test ended, leaving two to progress through the yolk-sac fry stage. As development continued into the swim-up stage with the resorption of the yolk sac (day 8), the second test ended leaving one test to proceed into the swim-up stage which was then terminated after four additional days (day 12). As the first test (embryos) ended on day 4, two tests were started with yolk-sac fry. Yolk-sac fry were supplied from the developing embryos held in the laboratory holding system. One of these tests ended at the transition from yolk-sac fry to swim-up fry (day 8) and one continued for four days as swim-up fry. At the transition to swim-up fry on day 8, an additional test was begun with swim-up fry (also supplied from the original batch of eggs) and ended after 96 h. This tiered design produced six experimental blocks within each matrix being evaluated, each representing a life stage/exposure duration combination. In summary, tests initiated with fertilized eggs represented three of the six blocks. One block was exposed for 4 d, one block was exposed for 8 d and one block was exposed for 12 d. Two blocks started with yolk-sac fry (4- and 8-d exposures) and one block started with swim-up fry (4-d exposure).

Eggs and larvae of RRH were exposed to pore waters and toxicant solutions using static

renewal procedures. The 30-mL plastic test chambers, containing 25 mL of solution, were randomly distributed within a block representing the stage of RRH being tested and the duration of the exposure. All test chambers were maintained at 23 °C in an environmental chamber that also provided a 16 h light/8 h dark photoperiod. Animals were to be transferred to fresh solutions every other day, but high mortality of embryos (treatments and controls) during the first transfer (tests conducted in 1998) dictated transfer on a daily basis thereafter. Decomposing eggs reduced available DO in the test solutions, and probably increased stress on the remaining live individuals. Oxygen saturation remained acceptable (>65% saturation) when surviving individuals were transferred daily to fresh test solutions. A transfer pipette with the tip cut to approximately a 3 mm opening was used to transfer embryos and yolk-sac fry. However as fry became more agile and faster swimmers, it became more efficient to remove dead animals, reduce the water volume to a minimum (1-2 mL) and pour the remaining live animals into the new test chambers containing fresh solutions. Sediment exposures were conducted in an automatic static-renewal system that replaced water twice daily and maintained acceptable water quality for the duration of the tests. Swim-up fry (yolk-sac resorption complete) were fed <24-h old *Artemia* sp. daily. Each test chamber used in porewater and toxicant evaluations received 2.3 mg (dry wt) of *Artemia* sp. and each sediment-exposure chamber received 16 mg.

Endpoints included survival and growth (total length) along with the type and frequency of deformities. However, only larvae from 1999 exposures were included in assessments of deformities. All surviving animals (including deformed individuals) were included in calculations of average survival, but only non-deformed animals were used for calculations of average length. Larval lengths were measured with an ocular micrometer installed in a dissecting microscope. Confidence intervals (95%) for length measurements were calculated using Student's t distribution (Snedecor and Cochran 1980). Survivors were classified into groups representing either no deformity or deformity types that included yolk-sac abnormalities, curvatures of the spine and deformities of the head and mouth region.

Metal concentrations in sediments, porewaters and toxicant solutions were analyzed by contract laboratory using inductively-coupled plasma-mass spectrometry. Dissolved organic carbon (DOC) concentrations in pore waters, surface water and laboratory water were also determined by contract laboratory using a carbon analyzer. Sediment samples were digested with concentrated HNO_3 and microwave radiation prior to metals analyses. Data for analytical quality assurance were produced for sediments from field splits and recovery from standard samples and spiked samples. Quality assurance data for pore waters and toxicant solutions included blanks, laboratory splits and recovery from spiked samples.

Detection limits for metals were below 1 µg/L with the exception of K and Ca which were below 13 µg/L. The detection limit for DOC was 0.1 mg/L. Limits of quantification were set as three times the detection limit. Quality assurance data for solutions analyzed in 1998 indicated concentrations of Cd and Cu in blank samples to be below detection limits but concentrations of Zn in blank samples averaged 22 µg/L $\pm$ 1 sd. Recovery from standard solutions averaged 103% $\pm$ 1 sd for Cd, 100% $\pm$ 2 sd for Cu and 111% $\pm$ 1 sd for Zn. Relative standard deviations (RSD) for Cd, Cu, and Zn in laboratory splits averaged 8.1% $\pm$ 8.4 sd, 7.5% $\pm$ 4.8 sd, and 3.5% $\pm$ 4.4 sd, respectively. Quality assurance results from 1999 analyses indicated cation concentrations in blank samples to be below detection limits with the exception of Zn (11 µg/L) and K (30 µg/L). Analyses of standards averaged recoveries of 101% $\pm$ 6 sd with the exception of Zn which was

130 %. Relative standard deviation (RSD) of cation concentrations among laboratory splits averaged 4.3% ± 3.0 sd in porewater and toxicant solutions. Recovery from standard soil samples averaged 89% ± 18 sd, and recovery from laboratory spikes of sediment digestates averaged 101% ± 6 sd. The average RSD for sediment cations in field splits was 2.8 % ± 2.5 sd with the exception of Na which varied by up to 12%. Blank samples analyzed for DOC contained an average of 0.6 mg/L ± 0.2 sd and recovery from the standard was 103%. Split porewater samples resulted in an average RSD for DOC of 1.7 % ± 1.4 sd.

Basic water chemistry of pore waters, toxicant solutions, control waters and overlying waters in sediment exposures, which included DO, pH, conductivity and alkalinity, was measured at the beginning of exposures. Pore waters were aerated prior to measuring the chemical parameters. Ammonia concentrations were measured with an NH_3 gas-sensing electrode and ion-selective meter. Dissolved oxygen, pH and conductivity were measured with the appropriate meters. Alkalinity was measured using a H_2SO_4 titration (APHA 1992). Hardness was determined for the overlying waters of sediment exposures with EDTA titration (APHA 1992). Hardness was calculated for porewater and toxicant solutions using measured concentrations of Ca and Mg (Hem 1985).

Toxicant Exposures

The toxic thresholds of contaminants (Cd, Cu, Mn, Zn and NH_3) potentially impacting the early-life stages of RRH were established in 1998 and 1999. A laboratory water that simulated the basic water chemistry of Oconee River surface water was used for these tests in 1998. This soft, reconstituted water (SRW) had pH between 7.5 and 7.6, conductivity between 80 and 110 µS/cm, hardness of 16 mg/L $CaCO_3$ and alkalinity of 23 mg/L $CaCO_3$. The formula for this water was 8 mg/L each of $CaSO_4 \cdot 2H_2O$ and $CaCl_2$, 5 mg/L $MgSO_4$, 31 mg/L $NaHCO_3$ and 0.5 mg/L KCl added to deionized water. During the 1998 season, bioassays were conducted in SRW using solutions of Cd, Cu, Zn and NH_3. In 1999, Cd, Cu and Zn were evaluated again along with Mn, and the study design was expanded to include testing in Oconee River surface water (SW). Prior to and during testing, surface water was collected from the most upriver site in 20-L carboys and transported to the laboratory where it was allowed to stand and settle for 3 d. After settling, the supernatant was removed and composited in a 200-L container that served as the water source for the water-renewal system employed in the sediment exposures. This water received constant aeration throughout the duration of testing.

Basic chemical characteristics of SRW and SW were similar, but there were slight differences in concentrations of major cations. Surface water contained 5.4 mg/L Na, 2.2 mg/L K, 2.9 mg/L Ca and 2.0 mg/L Mg, while SRW contained 8.5 mg/L Na, 0.3 mg/L K, 4.8 mg/L Ca and 1.0 mg/L Mg. The concentration of DOC was 3.9 mg/L in SW and 0.5 mg/L in SRW. Trace metals were non-detectable in SRW, but SW contained 44 µg/L Zn and 3 µg/L Cu.

Toxicant solutions were prepared with reagent-grade chemicals added to base waters (SRW or SW) and allowed to equilibrate for 48 h with gentle aeration prior to use in the bioassays. Compounds used to prepare the toxicant solutions were $CuCl_2 \cdot 2H_2O$, $CdCl_2 \cdot 2.5H_2O$, $ZnCl_2$, $MnCl_2 \cdot 4H_2O$ and NH_4Cl. Serial dilutions (0.5x) were accomplished with appropriate volume additions of base water. Each exposure solution containing Zn, Cu, Cd and Mn was sampled at test initiation, acidified with 1% (v:v) ultra-pure HNO_3 and submitted for analysis of metals.

Control treatments for these tests were SRW and SW. In 1998, five dilutions of each toxicant were evaluated with five replicates per dilution and four RRH exposed in each replicate. In 1999, four dilutions of each toxicant were evaluated with four replicates per dilution and five RRH exposed in each replicate.

Median lethal concentrations (LC_{50}) were determined using mortality within the dilution series and the measured toxicant concentration of each solution (Hamilton et al. 1977). No observable effect levels (NOEL) and lowest observable effect levels (LOEL) were established for NH_3 by comparing average lengths within each dilution to those from SRW with analysis of variance and Dunnett's one-tailed means comparison with $p \leq 0.05$ (SAS 1988).

Sediment and porewater exposures

Robust redhorse eggs and fry were exposed to sediments and pore waters collected during spring 1999. Locations for sediment collections were a subset of those used for a sediment quality assessment by Lasier et al. (2001). Four sites were selected (Figure 2-1) that included a site adjacent to the Avant Kaolin Mine within 30 m of the only currently known RRH spawning area and sites below the confluences of Bluff Creek, Buffalo Creek and Big Sandy Creek.

Sediments were collected by scraping the top 3 cm of material from shallow depositional areas with a shovel and contained in 20-L plastic containers. All four sediments were collected in one day, returned to the laboratory and stored at 15°C for 72 h prior to initiation of exposures. Sediments were homogenized 24 h prior to test initiation with portions of each sediment distributed to sediment exposure chambers (24 per sediment). An aliquot of each sediment was taken for whole-sediment metal analyses and physical characterization. The remaining volumes of sediment were used for porewater extractions. Physical characterization consisted of particle-size distribution (Miller and Miller 1987) and organic matter content (Davies 1974).

Sediment toxicity was assessed following procedures outlined by USEPA (1994). Test chambers were 300-mL beakers notched at the top with stainless steel screen covering the notch. Each chamber contained 100 mL of sediment with 175 mL of overlying water. Laboratory conditions during sediment evaluations were maintained at 23°C with a 16 h light/8 h dark photoperiod using wide-spectrum flourescent light. Water quality in the test chambers was maintained using an automatic static-renewal system that replaced approximately 70 percent of the overlying water twice daily (Zumwalt et al.1994). Surface water (SW) collected at Avant Mine was used as the overlying water for these tests. Basic water chemistry (conductivity, pH, alkalinity, hardness and NH_3) of the overlying water in solid-phase exposure chambers was measured at the beginning and end of exposures. Dissolved oxygen in the overlying waters was measured daily. Samples of overlying waters were collected for each sediment using a 60-cc syringe with 10 cm of airline tubing attached. Approximately 10 mL of water were collected about 1 cm above the sediment surface from each replicate and composited for measurements. Samples were collected within 30 min prior to the morning renewal of water and represented the most degraded water quality over the water renewal cycle.

Five sediments were evaluated, four river sediments and a control. A commercial sand was used as the control after it was washed with deionized water and saturated with SRW. The tiered approach described earlier was accommodated within the capacity limitations of the renewal system by using four replicates for each sediment creating an experimental block of 20 test chambers (five sediments with four replicates each). At test initiation, chambers representing

three blocks were placed in the renewal system with chamber positions randomly assigned within each block. An additional 60 chambers containing sediments received 100 mL of SW, were covered with plastic wrap and stored in darkness at 4 °C. As portions of the test terminated, new exposures using these chambers were started. Prior to their use on day 4 (yolk-sac exposures) or day 8 (swim-up exposures), these chambers were warmed to 23 °C and the overlying water was replaced. Solid-phase exposure chambers were stocked with 10 individuals (eggs or larvae).

Bulk sediments were stored in darkness at 15°C for the duration of porewater exposures. Multiple porewater extractors consisting of fused-glass airstones connected by airline tubing to 60-cc syringes (Winger and Lasier 1991) were inserted into the sediments. Pore water was extracted daily, aerated vigorously for 30 minutes and distributed to the test chambers. Surface water (SW) was included as a reference solution within each experimental block of porewater tests. Four replicates were used for each pore water, and each replicate was stocked with five eggs/larvae.

Pore water was collected in situ using similar techniques as those used in the laboratory. In-situ porewater extractors were installed at two sites (Avant Mine and Bluff Creek) one week prior to the initiation of testing. A large fused-glass airstone (15 cm x 3 cm) was inserted into the sediment and connected to a peristaltic pump with roughly 20 m of 7-mm plastic tubing. Approximately 500 mL of pore water was extracted and returned to the laboratory daily where it was tested concurrently with pore waters extracted in the laboratory from collected sediments.

The location of the in-situ sampler at Bluff Creek was in depositional sediments within 2 m from where sediments had been collected for laboratory use. The airstone was inserted horizontally at a depth of approximately 5 cm and covered with surrounding sediment. The in-situ porewater extractor at Avant Mine was installed about 5 m from the depositional area where sediments were collected for laboratory extractions but in gravel similar to those used by RRH for spawning. The airstone was buried in a horizontal position approximately 15 cm deep in substrate composed predominately of gravel 1 to 4 cm in size with a substantial amount of coarse sand. Three substrate samples were collected from this area to characterize the particle-size distribution. These substrate samples were dried at 105°C and sorted through a stack of sieves with decreasing mesh sizes (50-mm, 25-mm, 12.5-mm, 2-mm, 1-mm, 0.25-mm, 0.053-mm).

Aerated porewater samples were collected for chemical analyses at test initiation and on every third day of testing to document chemical changes that occurred after homogenization. All porewater samples taken for analytical analyses were filtered through a nylon filter (0.2 µm) and acidified with 1% (v:v) ultra-pure HNO_3 after aeration.

Survival and growth of RRH exposed to Oconee River sediments and pore waters were statistically compared to those of RRH exposed to control sediment and SW using analysis of variance and Dunnett's one-tailed means comparison (SAS 1988). Differences were considered significant if $p \leq 0.05$

Results and Discussion

Toxicant exposures

The RRH egg was generally the most resistant stage to the metals tested, and toxicity tended to increase with the duration of exposure (Table 2-1). Different sensitivities of eggs were evident between years, but the differences were not consistent among the metals. Cadmium and Cu LC_{50} values for embryos were lower in 1998 than in 1999 but the LC_{50} for Zn in 1998 tests

was over twice the LC_{50} derived in 1999. As previously mentioned, some DO concentrations declined to 10 - 20% saturation during the first 48 h of the 1998 egg exposures necessitating daily transfers into fresh test solutions. However, this added stress in 1998 may have contributed to the differences observed between years. Apart from the initial survival in 1998, RRH tested in 1999 appeared to be more sensitive to Cd and Zn than those in 1998, but equally sensitive to Cu.

Median lethal concentrations of Cd, Cu, Mn and Zn were established for early-life stages of RRH (Table 2-1). Data generated in 1999 with Cd, Zn and, to some degree, Cu were limited due to an insufficient number of dilutions and because the lowest concentrations of the test solutions were too high. As a result, these concentrations often elicited more than 50% mortality. In these cases, the LC_{50} values were identified as less than the measured concentration in the lowest-concentration dilution (Table 2-1). Additional dilutions provided a better coverage of the toxic range of Zn in bioassays initiated with swim-up fry. Confidence intervals could not be calculated around some LC_{50} estimates due to a lack of partial mortality within the dilution series; however, the estimates are still of practical use in assessing toxicity (Stephan 1977).

Toxic effects of Cd were elicited in a range from <22 to 107 µg/L (Table 2-1), but significant variability existed between years and between base waters used for the exposures. Embryos were more tolerant to Cd in solutions prepared with SRW than those prepared with SW, but differences were less for those exposed for longer periods and those exposed initially as yolk-sac and swim-up fry. The early-life stages of RRH were tolerant of Cd concentrations (0.02 to 0.2 µg/L) typically encountered in unpolluted surface waters (Laxen 1984). Lethal concentrations of Cd to RRH were generally less than LC_{50} values (70 to 630 µg/L) reported for fathead minnows *(Pimephales promelas)* by Hall et al. (1986) and Sherman et al. (1987), but greater than lethal concentrations (1 to 7 µg/L) reported for juvenile or younger salmonids (Buckley et al. 1985, Eisler 1985).

Toxicity of Cu to RRH eggs and larvae increased with exposure duration and yolk-sac fry appear to be the most sensitive stage (Table 2-1). The decrease in lethal concentrations (increase in toxicity) of Cu with increasing exposure duration was similar to results summarized by Sorensen (1991). Lethal concentrations of Cu to RRH (18 to 153 µg/L) were within toxic ranges reported for other fish species. In waters with similar chemical characteristics, Welsh et al. (1993) determined an LC_{50} (96-h) for larval fathead minnows of 8 µg/L in water with 0.4 mg/L DOC and an LC_{50} of 60 µg/L in water with 3.4 mg/L DOC. Eisler (1997) reported the Cu 96-h LC_{50} for channel catfish (*Ictalurus punctatus*) fingerlings to be around 55 µg/L. McKim et al. (1978) observed toxic effects of Cu to white sucker (*Catostomus commersoni*) embryos and larvae at 15 and 30 µg/L, respectively.

Robust redhorse eggs were more resistant to Cu than yolk-sac fry in 1999 (Table 2-1), which is similar to observations made by McKim et al. (1978) on the white sucker. Copper was generally less toxic when mixed in SW as opposed to SRW, reflecting the influence of DOC; surface water (SW) contained 3.9 mg/L DOC and SRW had 0.5 mg/L DOC. Organic material in aqueous solutions can reduce the toxicity of Cu by reducing concentrations of toxic Cu species through processes of adsorption and complexation (Meador 1991, Sorensen 1991, Welsh et al. 1993, Erickson et al. 1996). Swim-up fry also appeared to be more tolerant of Cu than yolk-sac fry, although the addition of food (*Artemia* sp.) may have influenced speciation or availability in the same manner.

Manganese was equally toxic to all three early-life stages of RRH and its toxicity

significantly increased with exposure duration (Table 2-1). In 96-h exposures, Mn LC_{50}s ranged from 8.5 to 16 mg/L. Prolonged exposures (8 or 12 d) resulted in LC_{50}s between 2 and 11 mg/L. The Mn^{2+} ion assessed in this bioassay is the predominant form of Mn under reducing conditions in fresh water (Stumm and Morgan 1981) and can be toxic to aquatic organisms at concentrations exceeding 5 mg/L (Hockett and Mount 1996, Stubblefield et al. 1997, Boucher and Watzin 1999, Lasier et al. 2000). Lethal concentrations to RRH eggs and larvae were similar to those reported for early-life stages of other fish species. Stokes et al. (1988) reported that 96-h LC_{50}s of Mn for rainbow trout (*Salmo gairdneri*) ranged between 3.7 and 116 mg/L depending on alkalinity and hardness. Chronic toxicity for early-life stages of brown trout (*Salmo trutta*) occurs at Mn concentrations between 4 and 5 mg/L in waters of similar hardness (Stubblefield et al. 1997).

Zinc concentrations of 0.2 to 1.9 mg/L caused toxic effects to RRH eggs and larvae (Table 2-1). Eggs tested in 1998 were more tolerant of Zn than those tested in 1999. Lethal concentrations determined for the RRH were within ranges described by others (USEPA 1987, Sorensen 1991, Eisler 1993, Hamilton and Buhl 1997). The lowest concentrations of Zn tested in 1999 were 0.8 mg/L in SRW and 0.7 in SW and survival of eggs was 50% in SRW and 20% in SW. Complete mortality occurred in the longer exposures.

Ammonia concentrations up to 0.8 mg/L (un-ionized NH_3) were not acutely toxic to the early-life stages of RRH. Ammonia toxicity to fish is dependent upon the concentration of un-ionized NH_3 present (USEPA 1985), which is controlled by pH and temperature and increases when either parameter increases (Emerson et al. 1975). Un-ionized NH_3 in RRH exposures was approximately 1.54% of the total NH_3 at pH of 7.5 and temperature of 23 °C (Thurston et al. 1979). Survival of embryos in NH_3 exposures averaged 47% and was not significantly different than survival in SRW. Survival in the most concentrated solution (53 mg/L total NH_3) was above 90% for yolk-sac fry and above 60% for swim-up fry. Although survival of swim-up fry in this NH_3 concentration was significantly lower than survival in the control, a median lethal concentration could not be estimated. The LC_{50} of un-ionized NH_3 was greater than 0.8 mg/L for all three early-life stages, but growth was affected when yolk-sac fry were exposed. The NOEL and LOEL concentrations for eggs exposed through the yolk-sac stage (8-d exposures) were 0.4 and 0.8 (mg/L un-ionized NH_3), respectively, and 0.1 and 0.2, respectively, in 12-d exposures. Bioassays initiated with yolk-sac fry resulted in NOEL and LOEL concentrations of 0.4 and 0.8, respectively, for both 4- and 8-d exposures.

The observed tolerance of RRH embryos and larvae to un-ionized NH_3 was similar to reported ranges for other bottom-dwelling fish such as channel catfish and white sucker (Arthur et al. 1987, Nimmo et al. 1989, Bader and Grizzle 1992). Although contrary to the results of Bader and Grizzle (1992), survival in these exposures indicated yolk-sac fry to be more tolerant to NH_3 than swim-up fry. The tolerance of RRH early-life stages to acute concentrations of NH_3 may be an adaptation to temporary chemical conditions during incubation that coincide with decreased mobility of the early-life stages. Concentrations of total NH_3 exceeding 53 mg/L would rarely be found in surface waters. However, RRH eggs have been observed in large clumps within the gravel matrix in which they were spawned (Freeman et al. 1998), and decomposing eggs within these masses could potentially elevate localized NH_3 concentrations. The greatest NH_3 concentrations tested did not significantly contribute to mortality during the egg stage, when the embryo was completely immobile. However, tolerance to NH_3 tended to decrease in the yolk-sac stage (demonstrated by reduced growth) when mobility began to increase and was lowest in the

swim-up stage (demonstrated by reduced survival) when the fry were most capable of avoidance.

Sediment and porewater exposures

Sediments collected from the lower Oconee River were toxic to RRH yolk-sac fry and swim-up fry, but only to those exposed through the transition from egg to yolk-sac fry (Table 2-2). Animals exposed initially in the yolk-sac or swim-up stages were not adversely affected nor were embryos exposed for only 4 d. Toxic elements appear to be contributed from the watersheds of Buffalo Creek and Bluff Creek, with survival and growth of larvae exposed to sediments collected below these tributaries significantly less than controls. Reduced survival in exposures to Avant Mine sediments and reduced growth in exposures to Big Sandy Creek sediments was also evident in tests lasting 8 d.

Sediment toxicity corresponded to sediment concentrations of Mn (Table 2-3). Changes in the chemical conditions within the test sediments along with differences in organism mobility and exposure duration probably affected the observed toxicity. The toxicity of sediment-associated Mn is partially independent of its concentration. The key mechanism to its potential toxicity in sediments and pore waters is the significant difference between rates of Mn^{4+} reduction and Mn^{2+} oxidation. Manganese contained within the sediment matrix can be readily reduced from the Mn^{4+} ion (within MnO_2) to the more-soluble Mn^{2+} ion under anoxic conditions but it takes much longer for the oxidation of Mn^{2+} to Mn^{4+} under oxic conditions (Stumm and Morgan 1981, Davison 1982, Stokes et al. 1988, Luther 1995). Reduction of Mn^{4+} to Mn^{2+} in anoxic sediments is primarily due to microbial decomposition of organic carbon and is regulated by the availability of organic material (Boyd 1995). There are often significant spatial differences (horizontal and vertical) in porewater Mn^{2+} concentrations that reflect the heterogenous distribution of sediment organic material (Shuttleworth et al. 1999). Manganese oxides also tend to bind other toxic metals and release them upon reduction (Meyer et al 1994).

Decomposition of organic material within the test sediments may influence sediment toxicity. Sediments collected from below Bluff Creek and Buffalo Creek contained more organic material than the other two sediments (Table 2-3). Dissolved oxygen concentrations in the overlying waters of Bluff Creek and Buffalo Creek fell to around 60% saturation after 6 and 9 d, respectively, and tended to remain at those levels. Concentrations of DO fell to 56% in the Avant Mine replicates by day 6 but then increased to over 70% for the remaining time. Dissolved oxygen decreased to 72% in Big Sandy Creek replicates on days 7 and 8 but increased after that. The minimum DO measured in the overlying waters of the control sediment (sand with no organic material) was 83% saturation. Although 60% DO saturation is within the acceptable range for sediment exposures (USEPA 1994), the depressions indicate an oxygen demand associated with anoxic and reducing conditions within the sediments. Manganese (Mn^{2+}) diffusing from the sediment into the overlying water may have occurred in sufficient concentrations to elicit a toxic response before being diluted by the next renewal of water. Nagorski and Moore (1999) found the same mechanism was contributing to the presence of reduced (and toxic) arsenic in oxic surface waters. Additional research is needed to determine the diffusion characteristics of sediment-associated Mn, particularly with respect to sediment exposures using periodic renewals of overlying waters.

Sediments were toxic to RRH larvae in 8- and 12-d exposures that included the immobile egg and early yolk-sac fry stages (Table 2-2). The mobility of larvae exposed initially as yolk-sac

fry and swim-up fry apparently enabled them to avoid toxic concentrations of Mn at the sediment surface. Toxicity tests using freshwater amphipods did not implicate Mn as a stressor in sediments collected from these four sites despite equal or greater concentrations of Mn (Lasier et al. 2001). However, the amphipods were mobile and could also avoid toxic concentrations building at the sediment surface. Lack of toxicity in the egg stage exposed for only 4 d may reflect greater tolerance of Mn over shorter exposure durations (Table 2-1) or a lag period necessary for test sediments to become anoxic and begin the Mn reduction process.

Sources of Mn^{2+} to surface waters include industrial effluents, mining discharges, hypolimnetic releases from reservoirs and anaerobic sediments (National Academy of Science 1973, Hannan 1979, Nix and Ingols 1981, Stokes et al 1988, Boyd 1995). At this time there is no water quality criteria established for Mn (USEPA 1999). Manganese was not a monitored element in effluents permitted for discharge to the watershed, and despite possible hypolimnetic releases from the Sinclair Dam upstream and significant numbers of kaolin mines in the watershed, Mn was undetectable in surface waters collected for this study as well as in those collected the previous fall (Lasier et al. 2001). The greatest potential for Mn to cause toxicity in this system is associated with the sediments. Manganese in these sediments ranged from 423 to 1382 mg/Kg, with concentrations at Bluff Creek and Buffalo Creek exceeding the probable effects level (PEL) of 1200 mg/Kg (Ingersoll et al. 1996). Manganese concentrations ranged from 963 to 4095 mg/Kg in sediments collected the previous fall from this section of the Oconee River (Lasier et al. 2001). Brumbaugh et al. (1994) found a range of 68 to 4,460 mg/Kg in depositional sediments from a contaminated river in Montana with their most toxic sediments (Kemble et al 1994) having Mn concentrations exceeding 1200 mg/Kg. Winger and Lasier (1998) reported Mn concentrations in sediments from the lower Mississippi River between 180 and 1550 mg/Kg, with sediment and porewater toxicity observed in most sediments containing over 1200 mg/L Mn. In the lower Savannah River, sediment toxicity was significantly correlated with sediment Mn concentrations which ranged between 160 and 2130 mg/Kg (Winger et al. 2000).

Copper, Pb and Zn were present at low concentrations in Oconee River sediments (Table 2-3), and the influence of these metals on sediment toxicity was probably negligible. Despite the greatest concentrations of Cu, Pb and Zn (occurring in the Bluff Creek and Buffalo Creek sediments) corresponding to toxicity results, other research suggests toxic levels of sediment-associated Cu, Pb and Zn to be at least four to five times greater than levels measured in these sediments. Concentrations of all three metals were below the threshold effect levels established by Ingersoll et al. (1996). Long and Morgan (1990) concluded that concentrations of Cu, Pb and Zn generally need to be above 70, 35 and 120 mg/Kg, respectively, in the sediment to elicit toxic effects. Even when Cu, Pb and Zn concentrations were normalized to the proportion of fine material (O'Conner 1990, Horowitz 1991, de Groot 1995), levels remained below the effect concentrations reported by Long and Morgan (1990). Concentrations of Cd were at or below the limit of quantification, and Lasier et al. (2001) found no evidence of organochlorine pesticides, polychlorinated biphenyls or polynuclear aromatic hydrocarbons in depositional sediments sampled from these sites seven months earlier.

Sediments were predominately sand (> 53 μm), but Bluff Creek and Buffalo Creek were composed of more fine material (clay and silt) compared to Avant Mine and Big Sandy Creek (Table 2-3). Cation concentrations in all four sediments were substantially lower than those measured in sediments collected seven months earlier from the same sites (Lasier et al. 2001). In

Table 2-4, comparisons between metal concentrations reported by Lasier et al. (2001) and those determined for sediments in this study were provided with sediment concentrations normalized by the amount of fine material (O'Conner 1990, Horowitz 1991, de Groot 1995). Cation concentrations in 1999 were between 23 and 43% of levels reported in the fall of 1998, although calcium was an exception to this trend. This significant reduction may have been caused by increased river flows during the winter months that effectively washed the sediments and reduced concentrations of Fe and Mn oxides and adsorbed cations.

Exposures of RRH to pore waters extracted from Oconee River sediments also demonstrated toxicity with the egg and yolk-sac stages being the most susceptible (Table 2-5). Pore water collected in situ (I) from Avant Mine was not toxic to embryos and larvae, but pore water extracted in the laboratory (L) from Avant Mine sediments as well as pore waters collected from Bluff Creek, Buffalo Creek and Big Sandy Creek were toxic. Survival and growth in exposures to Bluff Creek-I, Bluff Creek-L, Buffalo Creek-L and Big Sandy Creek-L pore waters were significantly lower than those in the control, and growth was significantly reduced in exposures to Avant Mine-L pore water.

Manganese appears to be responsible for the toxicity observed in the porewater exposures (Table 2-6). Concentrations of Mn exceeded toxic levels established in toxicant exposures, but the concentrations of Ca and Mg in the pore waters were also much greater than those in the toxicant solutions. Hardness (Ca and Mg) is known to reduce the toxicity of Mn to fish and other aquatic species (Stokes et al. 1988, Stubblefield et al. 1997, Lasier et al, 2000). Manganese was often the most prevalent cation in solution with concentrations ranging from 0.1 to almost 50 mg/L. Porewater toxicity tended to increase with increasing Mn; however the amelioratory effects of Ca and Mg were also evident. Porewater concentrations of Zn were well below lethal levels. Concentrations of Cu in the pore waters were at or below limits of quantification and also nonlethal. Total NH_3 concentrations were generally low, but with pH around 8.5, un-ionized NH_3 concentrations in Buffalo Creek-L and Bluff Creek-L were 0.8 and 0.5 mg/L, respectively (Thurston et al. 1979). While not lethal, these concentrations of un-ionized NH_3 were near LOELs established for RRH growth.

Porewater toxicity can provide valuable information on the bioavailability of sediment contaminants (Anderson et al. 1984), but interpretations must consider a number of confounding variables. Porewater concentrations of alkalinity, NH_3, H_2S and Mn can be above lethal levels for many aquatic organisms (Bagarinao 1992, Hoke et al. 1992, Ortiz et al. 1993, Ankley et al. 1995, Knezovich et al. 1996, Borgmann and Borgmann 1997, Lasier et al. 1997, Boucher and Watzin 1999) independent of anthropogenic contamination. Pore water extracted in the laboratory from collected sediment was more toxic than pore water extracted in situ (Table 2-5), and in-situ extracted pore waters had lower pH, conductivity, hardness and alkalinity than pore waters extracted from collected sediments (Table 2-7). Comparisons of Bluff Creek pore waters extracted in situ and in the laboratory reflect the apparent effects of collecting and handling on depositional sediment. Concentrations of Mn, Na, Mg, Ca, Zn, DOC and NH_3 in laboratory-extracted pore water were often double those measured in in-situ extracted pore water (Tables 2-6, 2-7). Significantly greater concentrations of DOC in pore waters extracted from collected sediments suggest that additional decomposition occurred as a result of sediment disturbance.

Manganese and NH_3 concentrations in these pore waters generally coincided with concentrations of DOC (Tables 2-6, 2-7). Concentrations of porewater DOC, NH_3, Mn and Fe

increase as byproducts of organic decomposition and, in the process, redox potentials are driven downward (Presley and Trefry 1980, Boyd 1995). Teal (1962) noted a burst of respiration when sediment microbes were introduced to new sources of organic material, and Winger and Lasier (1993) observed that redox potentials within sediments often decreased after the sediments were homogenized. Burton (1992) cautioned that sediment collection can significantly alter its physicochemical and biological integrity. Increases in the concentrations of Mn, DOC and NH_3 (and probably Ca, Mg and Zn) in pore water from collected sediment over in-situ concentrations were most likely due to sediment disturbance and the subsequent decomposition of newly-exposed organic surfaces. Increases in porewater contaminant concentrations with sediment handling have also been observed in other sediment evaluations (Burgess and McKinney 1997).

Pore water collected in situ from Avant Mine may have lacked toxicity due to the inadvertent collection of surface water through the predominately gravel substrate. The composition of substrate where in-situ extraction occurred was 68% gravel (2 mm to 50 mm), 31% sand (53 μm to 1 mm) and 1% fine material (clay and silt). Normally, pore waters are quite distinct from the overlying water. However, water chemistry of Avant Mine-I pore water (Table 2-6) was similar to that of SW, with only slightly greater conductivity, alkalinity and pH. There was a reduction in Avant Mine-I cation concentrations at the end of the testing period as well as reductions in several cations in Bluff Creek-I, but DOC concentrations remained similar to previously-extracted samples. Unfortunately, chemical analyses of SW were not conducted on waters collected at that time, and therefore it remains unknown whether decreases in cation concentrations were a result of similar reductions in the surface water or due to reactions occurring in the porewater environment.

Chemistries of laboratory-extracted pore waters did not change substantially over the 12 d testing period. Zinc concentrations decreased over time, but concentrations of major cations and DOC remained fairly constant (Table 2-6). There were no obvious relationships between cation concentrations in sediments (whole-sediment or normalized to fine material) and those in pore waters. Porewater cation concentrations in samples from Bluff Creek, Buffalo Creek and Big Sandy Creek generally decreased between fall 1998 (Lasier et al. 2001) and spring 1999 (Table 2-6), similar to decreases observed in sediment cations. However, cation concentrations in Avant Mine pore water extracted in 1999 increased despite decreases in sediment cations.

RRH Deformities

Three general types of deformities (yolk-sac abnormality, spinal curvature and head/mouth abnormality) were observed in these tests. Abnormalities in yolk-sac development (including supplemental, bulbous and truncated yolk sacs) were the most prevalent type of deformity observed in larvae exposed during the egg stage. The yolk-sac abnormalities significantly altered the larvae's shape and swimming ability. Deformities involving curvatures of the spine (along both lateral and horizontal axes) tended to occur in all three developmental stages as did abnormalities in head and mouth development. Head and mouth irregularities included formation of a very pointed mouth, swelling of the throat area and malformed eyes. Many deformed animals died before the termination of a test (e.g. 12-d exposure). Therefore, deformities observed for each early-life stage in all exposure durations were combined for analysis. There were no obvious differences in the proportions of deformities between base waters (SRW and SW) used to make toxicant solutions. Results from these exposures were also pooled for summary.

Surviving larvae from sediment, porewater and toxicant exposures were often deformed, particularly in exposures initiated with embryos (Table 2-8). Obviously, the implications to RRH recruitment could be severe given that deformed larvae are far less able to survive. The proportions of deformities were greater from exposures to several sediments and pore waters compared to controls. The number of larvae with yolk-sac abnormalities was three to six times greater for eggs exposed to Avant Mine, Bluff Creek and Buffalo Creek sediments than those exposed to control sediment. Exposures of eggs to laboratory-extracted pore waters from Big Sandy Creek and Bluff Creek resulted in increased proportions of yolk-sac abnormalities as well. The incidence of spinal curvatures was also increased in exposures to Avant Mine and Buffalo Creek sediment and in exposures to Buffalo Creek-L porewater that were initiated with eggs. Deformities associated with the head and mouth region were most prevalent in exposures initiated with yolk-sac fry.

Deformities were common in exposures to solutions of Cd, Cu, Mn and Zn (Table 2-8). Abnormal development of the yolk sac was the most prevalent deformity affecting larvae that had been exposed as embryos. Exposures of embryos to Cd and Mn increased the proportion of spinal curvatures observed. Cadmium concentrations have been linked to spinal deformities in other research as well (Sorensen 1991). Curvatures of the spine were also the primary deformity observed in Cd exposures initiated with yolk-sac fry. Observations during the exposures (at daily transfers) revealed a consistent pattern in the condition of dead larvae. Those animals that died in exposures to Cd and Mn were often deformed with spinal curvatures.

Conclusions

Toxicity assessments of sediments and pore waters from the lower Oconee River to RRH early-life stages indicated toxic conditions at several locations. Tributaries (Bluff Creek and Buffalo Creek) to this reach of river receive permitted municipal and industrial effluents containing Cd, Cu and Zn. These metals are of concern because of the potential effects on the reproductive success of the RRH, a "Species at Risk". Bioassays with these metals were conducted to establish the tolerances of RRH early-life stages. Ammonia and Mn were also assessed due to their potential toxicities and confounding influences in sediment and porewater exposures.

Sediments and pore waters collected downstream of Bluff Creek, Buffalo Creek and, to some extent, Big Sandy Creek were toxic to the RRH. Concentrations of Cd, Cu and Zn in the sediments and pore waters were below toxic levels, but Mn concentrations were considered high enough to account for the observed toxicity. Porewater Mn concentrations were often greater than established toxic levels but did not elicit comparable toxicity due to the amelioratory effects of elevated Ca and Mg concentrations. Decomposition of sediment organic material due to collection and handling and the resulting decrease in sediment redox conditions appears to have contributed to the impaired quality of the sediments and pore waters. Organic decomposition tends to increase porewater concentrations of DOC, NH_3 and Mn. Reducing conditions within test sediments may have promoted the diffusion of reduced (and toxic) Mn into the overlying water where it could remain in solution long enough to elicit a response. The importance of this phenomenon may be significant to interpretations of sediment and porewater toxicity determined using currently accepted methods. The occurrence of Mn in freshwater sediments is ubiquitous and factors affecting its toxicity are associated with the decomposition of organic materials within

the sediment.

Toxicity observed in sediment exposures followed the whole-sediment concentrations of Mn but may have been an artifact of unavoidable disturbances involved with collection. However, Mn associated with fine sediments may pose a risk to developing embryos and larvae of RRH if conditions favor its reduction to the Mn^{2+} species. Hence, the threat to RRH early-life stages that is posed by sedimentation includes not only entrapment and reduction of DO, but also Mn toxicity. A synergistic relationship may develop with increasing sedimentation that entraps developing RRH in close proximity to areas with reducing conditions due to low DO and decomposition of egg masses. Manganese associated with fine sediments can be reduced to Mn^{2+}, which can diffuse into areas with healthy embryos and larvae and persist long enough to cause adverse effects. Management strategies that limit soil erosion and sedimentation of fine soil materials into gravel bars used for RRH spawning would simultaneously alleviate the threat of Mn toxicity.

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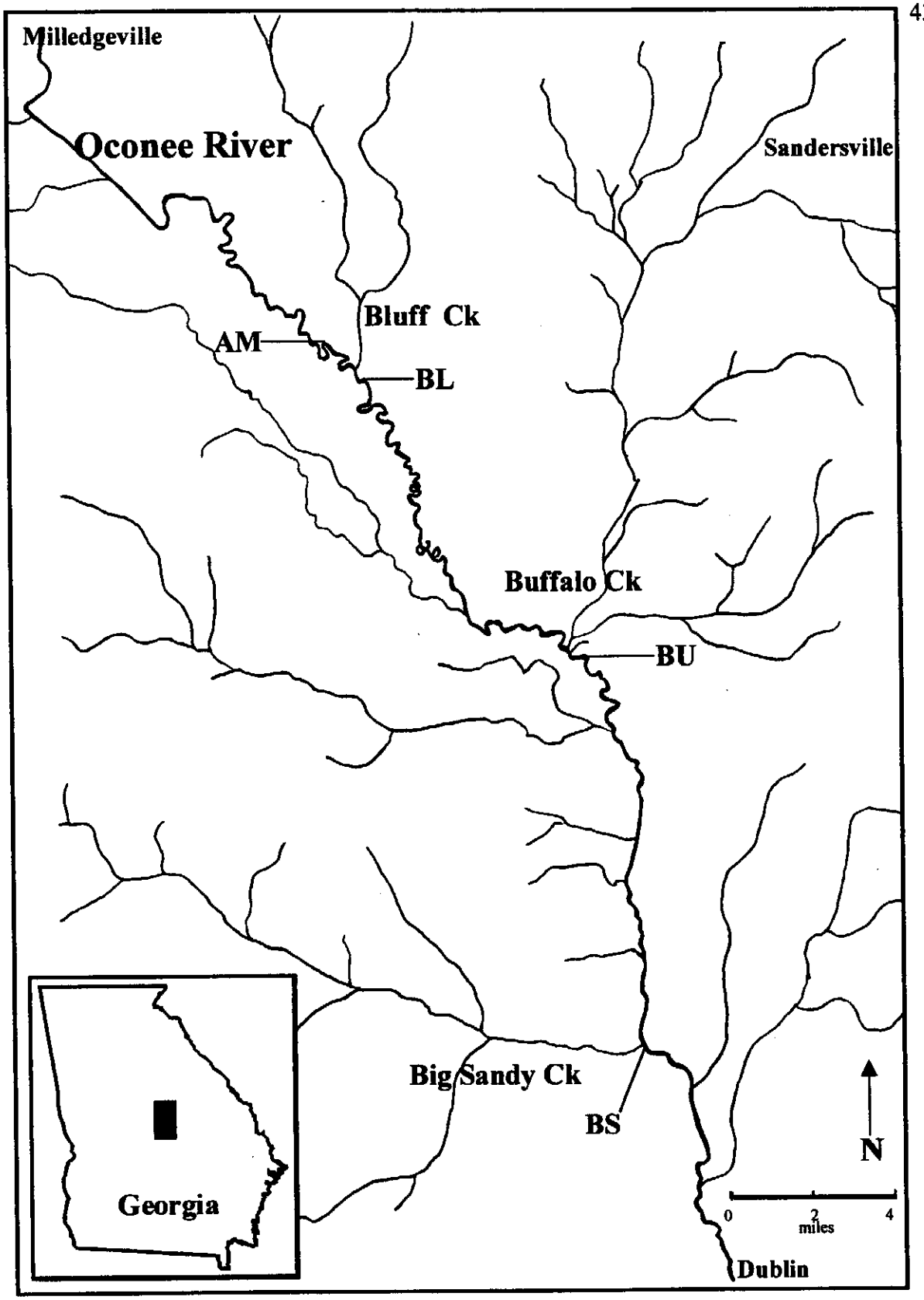

Figure 2-1. Locations (AM, BL, BU, BS) where sediments and pore waters were collected for robust redhorse exposures.

Table 2-1. Median lethal concentrations (LC_{50} with 95% CI when calculable) established for early-life stages of robust redhorse exposed for three durations to Cd, Cu, Mn and Zn during 1998 and 1999 in soft reconstituted water (SRW) and surface water (SW).

Metal	Stage at test initiation	Exposure duration (d)	1998-SRW	1999-SRW	1999-SW
Cd (µg/L)					
	Egg	4	35 (28-43)	107	69 (63-76)
		8	28 (23-32)	85 (69-105)	<22
		12	26 (18-38)	<27	<22
	Yolk-sac	4	60 (53-68)	<27	28
		8	55 (46-65)	<27	<22
	Swim-up	4	52 (42-63)	27	30
Cu (µg/L)					
	Egg	4	45 (40-52)	134 (90-198)	122 (102-145)
		8	23 (20-26)	32	72 (58-90)
		12	18 (17-19)	<23	31
	Yolk-sac	4	58 (51-67)	32	64 (52-78)
		8	38 (35-40)	<23	42 (37-48)
	Swim-up	4	71	65 (61-70)	>153
Mn (mg/L)					
	Egg	4		>16.1	11.4 (7.9-16.4)
		8		8.4 (6.4-11.1)	8.3 (6.8-10.3)
		12		2.5 (1.9-3.4)	3.7 (2.7-5.0)
	Yolk-sac	4		10.1 (8.0-12.8)	12.3
		8		4.8 (4.0-5.8)	4.6 (3.5-6.0)
	Swim-up	4		8.5 (7.2-10.1)	11.0 (9.1-13.4)

Table 2-1. continued.

Metal	Stage at test initiation	Exposure duration (d)	1998-SRW	1999-SRW	1999-SW
Zn (mg/L)					
	Egg	4	1.9	0.8	<0.7
		8	1.0	<0.8	<0.7
		12	0.4 (0.2-0.8)	<0.8	<0.7
	Yolk-sac	4	1.0	<0.8	<0.7
		8	1.0 (0.9-1.1)	<0.8	<0.7
	Swim-up	4	1.2 (0.9-1.5)	0.8 (0.7-1.0)	1.2 (1.1-1.5)

Table 2-2. Percent survival and average length (mm - in parentheses) of robust redhorse early-life stages exposed for three durations to sediments collected from the lower Oconee River. AM - Avant Mine, BL - Bluff Creek, BU - Buffalo Creek, BS - Big Sandy Creek.

Stage at test initiation	Exposure duration (d)	Sediment				Control
		AM	BL	BU	BS	
Egg						
	4	63(8.4)	68(8.9)	68(8.4)	60(8.1)	60(9.0)
	8	25*(12.2)	28*(11.8*)	5*(10.3*)	38(11.4*)	58(12.5)
	12	30(13.5)	10*(13.1*)	25(13.2*)	18(13.3)	55(13.5)
Yolk-sac						
	4	100(13.1)	100(13.0)	95(13.0)	98(13.0)	100(13.1)
	8	98(13.7)	90(13.7)	98(13.7)	85(13.7)	85(13.7)
Swim-up						
	4	95(14.0)	98(13.8)	98(13.9)	100(13.9)	100(14.0)

* denotes a response significantly ($p \leq 0.05$) lower than control.

Table 2-3. Cation concentrations (mg/Kg - dry wt) and physical characteristics (% composition) of sediments collected from the lower Oconee River for robust redhorse exposures. AM - Avant Mine, BL - Bluff Creek, BU - Buffalo Creek, BS - Big Sandy Creek.

	Sediment			
	AM	BL	BU	BS
Cu	6	13	11	6
Pb	5	8	7	4
Zn	13	33	32	29
Na	329	88	299	86
K	346	553	399	325
Mg	833	1141	874	690
Ca	205	182	360	268
Mn	423	1258	1382	519
Fe	8653	9773	9132	6284
Sand	80	63	55	87
Silt	14	25	35	9
Clay	6	12	10	4
Organic content	0.7	2.8	2.5	0.6

Table 2-4. Comparison of cation concentrations (mg/Kg - dry wt normalized to percent fine material in sediments) in depositional sediments collected from four sites in the lower Oconee River during fall, 1998 (Lasier et al. 2001) and spring 1999. AM - Avant Mine, BL - Bluff Creek, BU - Buffalo Creek, BS - Big Sandy Creek.

Sediment	Cu	Zn	Na	K	Ca	Mg	Mn	Fe
AM								
Fall 1998	62	135	778	3948	244	7476	3785	126,487
Spring 1999	28	67	1646	1731	1025	4167	2116	43,265
BL								
Fall 1998	82	185	1607	5846	396	8303	8230	104,381
Spring 1999	34	88	237	1494	493	3083	3399	26,414
BU								
Fall 1998	102	693	3189	10,350	452	14,305	7669	246,452
Spring 1999	24	71	665	887	799	1941	3072	20,294
BS								
Fall 1998	66	547	3193	5050	2364	8353	9801	127,372
Spring 1999	44	226	663	2500	2063	5311	3993	48,336

Table 2-5. Percent survival and average length (mm - in parentheses) of robust redhorse early-life stages exposed for three durations to surface water (SW) and pore waters extracted either in situ (I) or in the laboratory (L). AM - Avant Mine, BL - Bluff Creek, BU - Buffalo Creek, BS - Big Sandy Creek.

Stage at test initiation	Exposure duration (d)	Pore water						SW
		AM - I	AM - L	BL - I	BL - L	BU - L	BS - L	
Egg								
	4	40(10.1)	60(9.3*)	45(10.1)	30(ns)	40(9.1*)	45(ns)	75(10.3)
	8	60(sd)	45(11.7*)	60(12.0*)	30(11.4*)	10*(ns)	45(12.0*)	60(12.8)
	12	55(13.9)	55(13.1*)	0*	5*(ns)	10*(ns)	0*	75(14.0)
Yolk-sac								
	4	100(13.1)	100(12.7*)	90(12.9)	90(12.8*)	70*(12.4*)	75*(12.9)	100(13.1)
	8	90(14.0)	75(14.0)	85(14.0)	20*(13.7)	40*(13.5*)	70(13.8*)	95(14.0)
Swim-up								
	4	90(13.9)	75(14.1)	75(13.8)	40*(13.9)	90(13.9)	90(14.0)	100(14.1)

* denotes a response significantly ($p \leq 0.05$) lower than SW
ns = no surviving non-deformed larvae
sd = sample destroyed

Table 2-6. Cation and dissolved organic carbon (DOC) concentrations (mg/L- except Zn in µg/L) in in-situ (I) and laboratory-extracted (L) pore waters sampled throughout testing. AM - Avant Mine, BL- Bluff Creek, Bu - Buffalo Creek, BS - Big Sandy Creek.

Pore water	Element	Sample Date			
		5/7	5/10	5/13	5/16
AM-I	Na	5.4	5.2	5.3	1.4
	K	2.6	3.7	3.8	1.4
	Mg	2.0	1.8	1.9	0.5
	Ca	2.9	2.5	2.4	0.6
	Mn	0.1	0.2	0.2	0.1
	Zn	36	23	10	1
	DOC	4.9	3.9	4.0	3.9
AM-L	Na	9.4	8.5	8.9	8.3
	K	3.9	3.9	4.1	3.8
	Mg	10.9	12.3	14.3	13.7
	Ca	14.7	16.0	18.4	17.6
	Mn	11.0	15.7	19.2	17.7
	Zn	85	92	62	29
	DOC	7.1	7.1	7.8	8.8
BL-I	Na	4.8	5.1	5.1	3.9
	K	3.1	3.1	2.7	3.5
	Mg	2.2	3.3	4.5	1.8
	Ca	4.6	6.3	9.0	2.9
	Mn	6.2	7.9	10.8	1.9
	Zn	17	20	15	7
	DOC	6.4	7.1	6.6	6.2

Table 2-6. continued.

Pore water	Element	Sample Date			
		5/7	5/10	5/13	5/16
BL-L	Na	7.5	5.9	6.8	6.3
	K	2.5	2.4	2.5	2.4
	Mg	10.4	12.2	13.4	13.6
	Ca	19.2	21.7	23.1	22.7
	Mn	34.3	40.7	43.9	41.8
	Zn	134	89	16	20
	DOC	16.9	16.2	15.9	15.2
BU-L	Na	46.7	44.9	35.4	33.0
	K	3.8	3.7	3.4	3.3
	Mg	11.6	11.8	9.9	9.0
	Ca	45.2	44.3	36.8	32.9
	Mn	47.7	48.8	41.1	34.6
	Zn	24	26	17	22
	DOC	16.8	18.8	22.3	21.5
BS-L	Na	19.2	17.8	13.7	13.3
	K	3.1	3.0	2.8	3.2
	Mg	8.2	8.9	7.0	8.7
	Ca	25.3	27.3	21.3	28.7
	Mn	16.2	22.9	17.2	27.9
	Zn	37	22	17	6
	DOC	17.3	17.7	17.2	17.6

Table 2-7. Basic chemistry of in-situ (I) and laboratory-extracted (L) pore waters. AM - Avant Mine, BL - Bluff Creek, BU - Buffalo Creek, BS - Big Sandy Creek.

Pore water	pH	Conductivity µS/cm	Hardness mg/L as $CaCO_3$	Alkalinity mg/L as $CaCO_3$	Ammonia mg/L as NH_3
AM-I	7.91	169	15.3	38	nd
AM-L	8.43	261	80.8	136	1.6
BL-I	7.94	115	20.3	50	1.4
BL-L	8.51	324	89.6	192	3.8
BU-L	8.48	607	159.4	306	6.2
BS-L	8.53	353	73.3	174	2.4

nd = non-detectable

Table 2-8. Percentages of surviving robust redhorse larvae from toxicity tests of sediments, pore waters (I - in situ, L - laboratory) and toxicant solutions classified as non-deformed (A) or within three types of deformities (B = yolk-sac abnormalities, C = spinal curvatures, D = head/mouth abnormalities). AM- Avant Mine, BL - Bluff Creek, BU - Buffalo Creek, BS - Big Sandy Creek.

Stage at test initiation	Sediment	Deformity			
		A	B	C	D
Egg	Sand (control)	82	8	8	2
	AM	63	25	12	
	BL	48	48	3	
	BU	39	46	15	
	BS	100			
Yolk-sac	Sand (control)	88		11	1
	AM	95			5
	BL	93		3	4
	BU	97			3
	BS	99			1
Swim-up	Sand (control)	100			
	AM	97			3
	BL	100			
	BU	95		2	3
	BS	98			2

Stage at test initiation	Pore water	Deformity			
		A	B	C	D
Egg	SW (control)	95		5	
	AM-I	99		1	
	AM-L	92	1	3	4
	BL-I	90	5	4	1
	BL-L	54	26	7	13
	BU-L	84	1	14	1
	BS-L	79	17	3	1
Yolk-sac	SW (control)	87		8	5
	AM-I	84	1	3	12
	AM-L	83		4	13
	BL-I	81	2	2	8
	BL-L	75	1	1	11
	BU-L	89		1	10
	BS-L	82		2	16

Table 2-8. continued.

Stage at test initiation	Toxicant solution	Deformity				Stage at test initiation	Pore water	Deformity			
		A	B	C	D			A	B	C	D
Egg	SRW/SW (controls)	97		1	2	Swim-up	SW (control)	95			5
	Cd	36	36	28			AM-I	94			6
	Cu	73	19	7	1		AM-L	97		3	
	Mn	46	28	19	7		BL-I	89		4	7
	Zn	3	89	7			BL-L	92		4	4
Yolk-sac	SRW/SW (controls)	76	1	6	17		BU-L	97		3	
	Cd	36	7	57			BS-L	97		3	
	Cu	81		11	8						
	Mn	83		9	8						
	Zn										
Swim-up	SRW/SW (controls)	100									
	Cd	96		4							
	Cu	99		1							
	Mn	99		1							
	Zn	100									