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# Metal accumulation in sediment, Asian clams (*Corbicula fluminea*), and periphyton at selected freshwater mussel preserves in the Clinch and Powell Rivers, Virginia.

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**Abstract**

An ecotoxicological assessment was carried out in four preserves managed by The Nature Conservancy (TNC) and a suspected acid mine drainage (AMD) site in the Clinch and Powell Rivers, Virginia, during summer-fall 2000. Parameters evaluated included benthic macroinvertebrate sampling, *in situ* toxicity testing with Asian clams, acute toxicity testing with *Ceriodaphnia dubia*, analysis of ten trace metals of interstitial water found in sediment, bioaccumulation of the metals by Asian clams and periphyton, and standard water quality analysis of the water column and sediment interstitial water. From the literature, molluscan population data were summarized at the study locations during 1979, 1983 and 1994, and a literature review was carried out for acute/chronic toxicity thresholds of sensitive test species and unionids to the trace metal data.

The benthic macroinvertebrate community data were least diverse and had the poorest relevant community indices at Russell Creek while values were most diverse at Semones Island and then Fletcher Ford. Macroinvertebrate diversity and index values at Grays and Pendleton Islands were slightly poorer but not substantially lower than Semones Island and Fletcher Ford. Molluscan richness and density values were highest at Pendleton Island and similar but lower at the other sites. *In situ* Asian clam growth was highest at Russell Creek and lowest at Semones and Pendleton Islands. Asian clam mortality in the *in situ* tests was minimal to none.

Water quality (conductivity, dissolved oxygen, pH, alkalinity, hardness) values were similar for most parameters at the four TNC preserves and Russell Creek. At the Russell Creek site, however, conductivity (1,080 umhos/cm) was two to three times higher. Sediment interstitial water had lower conductivity, dissolved oxygen and pH than the water column samples between all sites. Total concentrations of trace metals in sediment interstitial water was highest for aluminum (Al), followed by iron (Fe) and manganese (Mn). Concentrations were lowest for cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni) and selenium (Se), and were intermediate for lead (Pb) and zinc (Zn). The highest concentrations were 3,108 ugFe/L, 2,660 ugAl/L in the river sites and 3,034 ugMn/L at the Russell Creek site.

Six (Al, Fe, Mn, Cu, Pb, Zn) of the ten metals were highly bioaccumulated in periphyton and clam samples, and the uptake was substantially higher in periphyton. For example, the highest Al concentration was 1,200 mg/kg in clam tissue at Fletcher Ford while in periphyton, the concentrations ranged from 7,607 to 14,333 mg/kg at all sites. Iron was the highest metal accumulated in periphyton ranging from 12,642 to 25,885 mg/kg across all sites, while in clams it ranged from 444 to 653 mg/kg. The Al and Fe uptake values appeared to be excessively concentrated in the periphyton.

The ecotoxicological ramifications of the metal uptake in periphyton and clams is unknown, but the burden of metal uptake in periphyton should have some ecotoxicological significance. The relevance of the metal accumulation data suggests that the greater the metal concentrations measured in clam and periphyton tissue, the greater the decline in mussel richness and density. As Pendleton Island had the highest metal uptake in periphyton and clams, it likewise had the greatest observed drop in unionid diversity and density over the past 1.5 decades.

Finally, the sediment interstitial trace metal data were compared to acute/chronic toxicity thresholds from the literature. We found a concern at some sampling sites where interstitial water containing Al, Fe and Se exceeded chronically toxic thresholds of sensitive test species reported in the literature. In addition, the US EPA recommended cladoceran test species, *Ceriodaphnia*, was found to be less sensitive than several unionid and other species tested in the Clinch River watershed from two studies carried out a decade ago. The current US EPA Water Quality Criteria thresholds currently in place may not be protective enough for more sensitive unionids that inhabit the riverine sediments and the interstitial water spaces.

## Introduction

The upper Tennessee River system, including the Clinch, Holston and Powell Rivers in southwestern Virginia, contains aquatic habitat richer in unionid mussel species than any other area of the United States. Of the approximately 73 species found in Virginia, 34 are either endangered or threatened, and most of those listed species reside in the tributaries of the upper Tennessee River drainage (Neves 1991). The lower Powell River system provides habitat for a number of mussel species including the endangered *Quadrula intermedia* (Yeager and Saylor 1995), *Elliptio crassidens*, *Fusconaia cor*, and others (Neves 1991). However, all mussel fauna in the upper Powell River have been eliminated above Dryden, Virginia, at ~ mile 165 (Neves et al. 1980). Wolcott (1990) attributed the decline in mussel populations in the Powell to coal waste and metal loading; however, she was not able to correlate mussel population distributions with coal content in sediments. In the Clinch River, a toxic fly-ash spill from the coal fired power plant at Carbo, VA, in 1967 and 1970 eliminated most of the aquatic fauna for about 19 river kilometers. Although fish have recolonized this area, the mussels have not. Other factors potentially affecting mussel populations in the Clinch and Powell Rivers include increased sedimentation and siltation due to poor land use practices and deforestation (Neves 1991).

The purpose of the present study was to utilize Asian clams (*Corbicula fluminea*) as surrogates for endangered freshwater mussels to determine their growth and metal accumulation potential in four preserves managed by The Nature Conservancy (TNC) and an additional site thought to be impacted by acid mine drainage (AMD). The Asian clam is an efficient bioaccumulator of metals (Cherry et al. 1980), and has been shown to be more sensitive to metal contamination than most mollusks (Cherry et al. 1991; Farris et al. 1991). In studies performed at the Clinch River Power Plant in southwestern Virginia, site specific chronic water quality criterion values for copper were calculated based on testing with 15 different benthic invertebrate species found in the Clinch River. *Corbicula,* with a criterion value of 12.4 μg Cu/L, was observed to be the most sensitive of the 15 species and had similar values to three native freshwater mussels: *Elliptio* sp., *Medionidus* sp., and *Villosa* sp., which had criterion values of 15.5, 15.5, and 15.9 μg Cu/L, respectively (Farris et al., 1991). These data suggested that *Corbicula* would be an effective surrogate species to evaluate metal impacts on freshwater mussels. By analyzing clam, periphyton, and interstitial water samples for concentrations of a number of different metals, we hoped to gain insight into the potential for metal contamination as a causative agent in the recent mussel population declines at these sites.

## Methods

### *Sampling stations*

The sites selected for this study included four preserves managed by TNC: Grays Island, Semones Island, Pendleton Island, and Fletcher Ford. The remaining site was at the mouth of Russell Creek, a tributary to the Clinch River. The site at Pendleton Island, in the Clinch River, has historically had the richest mussel fauna of any site in the upper Tennessee River basin, but is reported to have experienced population declines in the past 10-15 years. Grays and Semones Islands in the Clinch River, and Fletcher Ford, of the Powell River, have also experienced mussel population declines in the past ~20 years. Russell Creek is thought to be degraded by acid mine drainage, and is suspected of introducing metals into the Clinch River.

Portions of maps were made available for each site and are found in Appendix I. In the first map, all sites were included. The second map included Russell Creek at the confluence with the Clinch River. Map 3 is for Greys Island and Semones Island. Pendleton Island is in map 4 while Fletcher Ford in map 5 is shown in the Powell River. Benthic macroinvertebrate and periphyton sampling was carried out throughout the upper area of each site in pools and riffles. Asian clam *in situ* bags were placed along the right bank facing downstream in areas that were deep enough to prevent exposure during drought conditions.

Eight trips were made from August 22, 2000 to October 8, 2000 to carry out the field research. On the initial trip with Dr. Braven Beaty, we visited all the sampling sites. The next trip was by D. S. Cherry and his team on 8/24/00 to reconnaissance the sampling sites more thoroughly by wading through each site in the river to evaluate the available habitat to be studied thereafter. The interstitial water samplers were removed from each site on 8/30/00, replaced with other bottles, and then removed on 9/8/00. Other trips included setting out the *in situ* clam bioassays on 9/8/00 and then removing them on 10/8/00. Periphyton analysis from rocks and branches were collected on 9/15/00. Benthic macroinvertebrate samples were taken during 9/18/00 at two sites and then on 9/20/00 at the remaining sites. The water levels in the river sites were low, and ranged from 3.0 to 3.8 ft gauge height at the Cleveland, VA gauge site. The riverine sites were workable with flow ranging from above ankle to at knee/thigh height at the sampling sites.

*Benthic macroinvertebrate sampling*

Benthic macroinvertebrate surveys were conducted according to the US Environmental Protection Agency (U.S. EPA/444 (4-89-001)) Rapid Bioassessment Protocols (RBP) (Plafkin et al., 1989). Riffle, run, pool and shoreline rooted areas were thoroughly sampled for 20 min per site using dip nets with an 800-μm mesh. Two replicate samples were collected per site. Organisms were identified to the lowest practical taxonomic level (usually genus) using standard keys (Merritt and Cummins, 1996; Pennak 1989). A number of community indices were calculated included total taxonomic richness, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, percent EPT abundance, Shannon-Wiener diversity index, Simpson's index of diversity, Family level Biotic Index (FBI; Hilsenhoff, 1988), and percent composition of various functional feeding groups, i.e., shredders, scrapers, collector-filterers, collector-gatherers, and predators. Index values for replicate samples were combined to obtain mean index values per station (i.e. mean taxon richness etc.).

*In situ toxicity testing with Asian clams*

For *in situ* toxicity tests, Asian clams were collected from the New River near Ripplemead, Virginia, using clam rakes. Clams were held in Living Streams® (Toledo, OH) at the Ecosystem Simulation Laboratory, Virginia Tech, Blacksburg, VA, until use. Individual clams were given one of five distinctive marks and measured for width to the nearest 0.01 mm using Vernier calipers prior to placement into bags for testing (Belanger et al., 1990). Testing procedures consisted of tying five mesh bags, each bag containing five clams, to stakes at each sampling station. Bags were 18-cm wide by 36-cm long with a mesh size of ~0.5 $cm^2$, and they were placed on the sediment surface and braced with surrounding rocks. Each bag had five individually marked clam bags and each station contained five bags or 25 clams total. At the end of 30 days, clam bags were collected from each testing station and transported on ice to the laboratory. Clams were counted as dead or alive; clams found with valves separated, or that were easily opened, were considered dead. Surviving clams were measured again for width, and growth was calculated by subtracting beginning- from ending- width.

*Metals analysis*

At the conclusion of the *in situ* toxicity test, whole clam tissue (viscoral maps) samples from each station were prepared for metal analysis according to US EPA protocol (US EPA, 1991). Prepared samples were analyzed for 10 metals [aluminum (Al), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), cadmium (Cd), nickel (Ni), chromium (Cr), lead (Pb) and selenium (Se)] at the Virginia Tech Inductively Coupled Plasma (ICP) Spectrometry Soil Testing Lab.

In addition, interstitial water and periphyton samples were collected from each sampling station for analysis of the same ten metals. Interstitial water samples were collected using interstitial water samplers, consisting of a 250 ml Nalgene bottle with a screw-on cap. The center of the cap was cut out and replaced with a fine (45 micron) mesh and the bottle was filled with deionized water. Bottles then were placed cap-down into the sediments at each site. Bottles remained in the sediments for seven days to allow ion exchange between the deionized water inside the bottle and the interstitial water in the sediments. This technique provides a measurement of metal levels thought to be bioavailable in the sediments (Bufflap and Allen, 1995) . Filtered (0.45 micron filter) and unfiltered samples were analyzed separately for metals to obtain dissolved and total concentrations, respectively. Periphyton also was collected at each station by scraping a 9- by 9-cm area from submerged rocks. Samples were prepared for metals analysis according to US EPA (1991) methods, and all water, sediment and periphyton samples were analyzed for metals at the Virginia Tech ICP Lab.

*Acute toxicity of interstitial water*

Interstitial water samples from the stations were tested for 48-h acute toxicity to the cladoceran, *Ceriodaphnia dubia*. Test organisms, raised by Virginia Tech personnel, were cultured in filtered water from a non-toxic reference stream near Newport, Virginia (Sinking Creek). Analysis of the culture water indicated that metal concentrations were low to undetectable (1.6 μg Al/L, 14.3 μg Fe/L, and Cu and Zn were below detection limits). Average pH, conductivity, alkalinity, and hardness for culture/diluent water was 8.01 ± 0.10, 225 ± 5.48 μmhos/cm, 131 ± 9.45 mg/L as $CaCO_3$, and 122.6 ± 4.16 mg/L as $CaCO_3$, respectively.

Prior to testing, organisms were fed a diet of *Selenastrum capricornutum* and a Yeast-Cereal Leaves-Trout Chow (YCT) mixture at a rate of 0.18 ml each per 30-ml water, daily. Five organisms were placed into each of four replicate 50-ml beakers containing water collected from each station for 48 hours. Sinking Creek water was used as a control group. At the end of the test period, percent survival for each station was recorded.

*Statistical analysis*

To determine the relationship between observed trends in metal accumulation in periphyton and clam tissues and mussel population declines at the sites in question, several bivariate correlation analyses were conducted. Two mussel indices were chosen as dependent variables: change in mussel richness (from 1979/1983 to 1994), and change in mussel density over the same time period. These values were calculated by subtracting the richness or density values for 1994 from those for the earlier date. Metal concentrations in periphyton and clam tissues were used as independent variables to compare with the mussel parameters. Correlation coefficients (r) were calculated using JMP-IN® software (Sall and Lehman, 1996).

## Results

*Benthic macroinvertebrate sampling*

The benthic macroinvertebrate community found at Russell Creek was the least diverse and had the poorest values for most relevant community indices including, for example: taxon richness, EPT richness, FBI value, Simpson's Index, etc. (Table 1). Comparing the other four relatively unimpacted sites, the benthic communities were basically similar with slight variations in the selected indices. The most diverse invertebrate community appeared to be that at Semones Island, which had the highest EPT richness (8.0) and Simpson's Index (0.89), the second highest total taxon richness (22.0) and the second lowest FBI value (3.40; the lower the FBI, the more sensitive the community). Fletcher Ford was the next highest in terms of community diversity with the highest total richness (22.5) and lowest FBI (3.23) and second highest EPT richness (7.5) and the third highest Simpson's Index (0.84). Grays and Pendleton Islands had slightly poorer diversity than Semones Island and Fletcher Ford but their index values were in most cases not substantially lower. Noted exceptions included a total taxon richness of only 15 for Pendleton Island compared to values ranging from 17 to 22.5 for the other three stations, and a 4.45 FBI value at Grays Island compared to values ranging from 3.23 to 3.53 for the other three sites. A total of 21 benthic macroinvertebrate parameters were analyzed, and the results are included in the Appendix.

Table 1. Selected benthic macroinvertebrate population, tolerance and diversity indices for the five sampling stations.

| Site | Taxon Richness | EPT Richness | FBI | Simpson's Index |
|---|---|---|---|---|
| Fletcher Ford | 22.5 | 7.5 | 3.23 | 0.84 |
| Grays Island | 17.5 | 6.5 | 4.45 | 0.82 |
| Semones Island | 22.0 | 8.0 | 3.40 | 0.89 |
| Pendleton Island | 15.5 | 6.5 | 3.53 | 0.85 |
| Russell Creek | 12.0 | 3.0 | 5.46 | 0.66 |

*Molluscan data*

Over the 30-day experimental period, growth of transplanted Asian clams was minimal, ranging from 0.07 mm to 0.23 mm (Table 2). Note that under optimal conditions, clams may grow as much as a full millimeter in 30 days. The slight growth observed in this study was likely due to the fact that water temperatures were beginning to drop when clams were placed into the sites in early September 2000. Clams grow poorly when temperatures drop below 15 °C. Therefore it is difficult to make finite conclusions based upon clam growth data.

Mussel data gathered from the literature for the four main stations also are shown in Table 2. A document prepared by Ahlstedt and Tuberville (1997) provides richness and density data for three of the sites (Fletcher Ford, Semones Island and Pendleton Island) for several different years. Using these data, we calculated changes in mussel richness and density from the earliest date (either 1979 or 1983) to the most recent 1994 date (Table 2). The largest decreases in both richness and density were observed at Pendleton Island (-8 and –13.4, respectively) followed by Fletcher Ford (-6 and –4.2, respectively), then Semones Island (-4 and –1.2, respectively). Ahlstedt (1984) and Jenkinson and Ahlstedt (1988) provide data for Grays Island, which had 14.6 different kinds of mussels on the average in 1984.

Table 2. Selected molluscan population and diversity indices for the five sampling stations.

| Site | Clam Growth (mm) | Mussel Richness | | | Mussel Density/m$^2$ | | |
|---|---|---|---|---|---|---|---|
| | | '79 | '83 | '94 | '79 | '83 | '94 |
| Fletcher Ford | 0.12 | 16 | - | 10 | 11.14 | - | 6.95 |
| Grays Island | 0.14 | - | 14.6 | - | n/a | n/a | n/a |
| Semones Island | 0.07 | - | 14 | 10 | - | 7.7 | 6.5 |
| Pendleton Island | 0.08 | 21 | - | 13 | 24.6 | - | 11.2 |
| Russell Creek | 0.23 | n/a | n/a | n/a | n/a | n/a | n/a |

*Water quality*

Selected water quality parameters were measured at each sampling site from the water column and sediment interstitial water collected from dialysis bottles placed in the sediment (Table 3). Conductivity was highest (1,080 µmhos/cm) in Russell Creek where mining activities were either occurring or had happened previously. Conductivity at the other sites ranged from 380 to 410 µmhos/cm. In the interstitial water, conductivity of distilled water placed initially in the dialysis bottles was basically zero, and it was replaced with interstitial water of 170-310 µmhos/cm.

Dissolved oxygen was high in the water column (8.1-9.3 mg/L) and substantially lower in the interstitial water (3.4-7.9 mg/L) (Table 3). The pH was above neutral and normally ranged from 7.20 to 8.20. The pH of the interstitial water tended to be lower than that in the water column. Alkalinity and hardness were substantially higher in Russell Creek water (548 and 532 mg/L as $CaCO_3$) and usually ranged from 284-296 mg/L (alkalinity) and 308-396 mg/L (hardness).

Table 3. Water quality characteristics of the sampling sites from water taken in the river water column and from dialysis bottles within the sediment from two sampling efforts of 8/30/00 and (in parenthesis) 9/8/00.

| Sampling Site | Conductivity (µmhos/cm) | $DO_2$ (mg/L) | pH (S.U) | Alkalinity (mg/L) | Hardness (mg/L) |
|---|---|---|---|---|---|
| Russell Creek (RC) | 1,080 -- | 8.2 | 7.90 | 548 | 532 |
| RC Interstitial water | 170 (310) | 4.7 | 7.32 (7.51) | -- | -- |
| Fletcher Ford (FC) | 410 -- | 8.1 | 7.87 | 296 | 320 |
| FC Interstitial water | 250 (230) | 7.9 | 7.20 (7.19) | -- | -- |
| Pendleton Island (PI) | 380 -- | 9.0 | 8.12 | 284 | 308 |
| PI Interstitial water | 200 (310) | 3.6 | 7.41 (7.23) | -- | -- |
| Semones Island (SI) | 400 -- | 9.3 | 8.25 | 288 | 336 |
| SI Interstitial water | 230 (100) | 3.4 | 7.34 (6.79) | -- | -- |
| Gray's Island (GI)* | 380 -- | 9.2 | 8.20 | 285 | 330 |
| GI Interstitial water* | -- (310) | -- | -- (7.10) | -- | -- |

* SI and GI sites were in close proximity to each other and access to the GI site was not available on 8/24-30/00.

*Metals in interstitial water*

In the analysis of total and dissolved metals, no clear trend was observed in total metal concentrations measured from interstitial water samplers (Tables 4). For total metals, each station had the highest concentration of at least one metal except Semones Island. The highest concentrations observed were for total Al (ranging from 653 to 2,660 µg/L), Mn (442 to 3034 µg/L) and Fe (536 to 3108 µg/L). These three elements probably are so high because they are a large part of various minerals that make up sediments, and some fine particles may have been able to pass through the mesh of the interstitial water sampler. Total concentrations of the acutely toxic metals Cu, Zn and Pb were much lower, generally being found in the range of less than 10 µg/L to less than 100 µg/L.

Table 4. Total concentrations (µg/L) of various metals in interstitial water samples from the five sampling stations. Total concentrations of Cd were below detection limit for all stations. Detection limits in µg/L were <1.4 for Cu, <3.4 for Cr, <0.9 for Cd, <2.5 for Pb, <7.3 for Ni, and <13.7 for Se.

| Metal | Fletcher Ford | Grays Island | Semones Island | Pendleton Island | Russell Creek |
|---|---|---|---|---|---|
| Al | 2,253.0 | 2,660.0 | 653.0 | 1,300.0 | 1,106.0 |
| Mn | 941.0 | 1,591.0 | 442.3 | 741.0 | 3,034.0 |
| Fe | 795.0 | 943.0 | 1,150.0 | 3,108.0 | 536.0 |
| Cu | 9.4 | 8.9 | bdl | bdl | 1.8 |
| Zn | 66.6 | 62.5 | 10.9 | 28.9 | 4.2 |
| Ni | 20.1 | 18.1 | 10.0 | 9.8 | 22.3 |
| Pb | 98.7 | 110.2 | bdl | 87.7 | 75.0 |
| Cr | 7.7 | 10.4 | bdl | 3.2 | 54.0 |
| Se | 31.8 | 42.8 | bdl | 38.7 | bdl |

Only five metals were found in detectable concentrations in the dissolved form (Al, Fe, Zn, Ni, Se; Table 5). As with total concentrations, no clear trend was observed with one station consistently having the highest concentrations of dissolved metals in interstitial water. While dissolved Al and Fe were found at all stations in concentrations ranging form ~43 to ~65 µg/L (Al) and ~13 to ~72 µg/L (Fe), dissolved Zn was only found at Fletcher Ford while Ni and Se were each found only at three sites.

Table 5. Dissolved concentrations (µg/L) of various metals in interstitial water samples from the five sampling stations. Dissolved concentrations of Mn, Cu, Pb, Cr, and Cd were below detection limit for all stations.

| Metal | Fletcher Ford | Grays Island | Semones Island | Pendleton Island | Russell Creek |
|---|---|---|---|---|---|
| Al | 65.6 | 43.3 | 46.8 | 44.6 | 47.0 |
| Fe | 47.2 | 18.0 | 71.9 | 12.8 | 29.1 |
| Zn | 7.3 | bdl | bdl | bdl | bdl |
| Ni | bdl | 9.1 | 7.8 | bdl | 10.4 |
| Se | 21.9 | 20.3 | bdl | 18.9 | bdl |

*Metals in periphyton*

The three metals found in the highest concentrations in periphyton tissues were Fe, Mn, and Al. Russell Creek had the highest concentrations of these metals in periphyton (25,885 mg/kg, 28,222 mg/kg and 8,411 mg/kg, respectively), reflective of the abandoned mined land influence upon this tributary (Table 6). The other four stations also had high Fe, Mn and Al concentrations, ranging from 12,642 mg/kg to 23,125 mg/kg for Fe, 4439 mg/kg to 19,237 mg/kg for Mn, and 7,607 mg/kg to 14,333 mg/kg for Al.

The highest concentrations of the most toxic metals analyzed in periphyton (Cu, Zn, Pb) were found in Russell Creek which receives input from AMD. Of the four preserves, Pendleton Island had the highest concentrations of all metals measured in periphyton except for Mn and Fe, which are less toxic than the other metals, and Ni, which was below detection limits at all four sites. Of the acutely toxic metals, Pb was found in the highest concentrations, ranging from below detection limits (Semones Island) to 538. 9 mg/kg (Pendleton Island). Zinc was relatively low at Fletcher Ford, Grays Island and Semones island, ranging from ~ 24 mg/kg to ~ 31.5 mg/kg, but was much higher at Pendleton Island (229 mg/kg). A similar trend was observed with Cu where concentrations in periphyton were below detection limits at Fletcher Ford and Grays Island, and only 2.66 mg/kg at Semones Island, but was much higher at Pendleton Island (25 mg/kg). Three other metals, Cr, Cd, and Se, were below detection limits at all five stations.

Table 6. Pooled concentrations of (mg/kg)various metals in periphyton tissue samples from the five sampling stations. Concentrations of Cr, Cd, and Se were below detection limit for all stations (<19, 5, and 78 mg/kg, respectively).

| Metal | Fletcher Ford | Grays Island | Semones Island | Pendleton Island | Russell Creek |
|---|---|---|---|---|---|
| Cu | bdl | bdl | 2.7 | 25.0 | 491.4 |
| Zn | 23.9 | 31.7 | 31.5 | 229.2 | 2571.4 |
| Al | 7607.1 | 10413.5 | 13287.5 | 14333.3 | 8411.4 |
| Mn | 5476.2 | 4439.4 | 19237.5 | 11847.2 | 28222.9 |
| Fe | 12642.8 | 18826.9 | 23125.0 | 21152.8 | 25885.7 |
| Ni | bdl | bdl | bdl | bdl | 155.4 |
| Pb | 140.5 | 513.5 | bdl | 538.9 | 1539.4 |
| Cd | bcl | bdl | bdl | bdl | bdl |
| Cr | bdl | bdl | bdl | bdl | bel |
| Se | bdl | bdl | bdl | bdl | bdl |

*Metals in clam tissues*

The most concentrated metals found in clam tissues were Al (233 to 1200 mg/kg) followed by Fe (444 to 653 mg/kg) and Zn (397 to 623 mg/kg) (Table 7). In contrast to the periphyton data, Russell Creek did not have the highest overall concentrations of acutely toxic metals in clam tissues. Clam tissue concentrations of Cu, Zn, and Pb were highest at Pendleton Island, while either Fletcher Ford or Russell Creek had the next highest concentrations for these metals. Pendleton Island also had the highest concentrations of Ni and Fe in clam tissues and the second highest concentrations of Mn and Al, while Cr, Cd, and Se were below detection limits at all sites. Unfortunately there are no data in the literature that are available to accurately compare or predict clam tissue bioaccumulation thresholds with acute/chronic survivorship responses.

Table 7. Median concentrations (mg/kg) of metals in clam tissue samples from the five sampling stations. Concentrations of Cr, Cd, and Se were below detection limit for all stations (<19, 5, and 78 mg/kg, respectively).

| Metal | Fletcher Ford | Grays Island | Semones Island | Pendleton Island | Russell Creek |
|---|---|---|---|---|---|
| Cu | 66.7 | 40.0 | 35.0 | 87.8 | 63.4 |
| Zn | 507.6 | 397.7 | 592.0 | 623.3 | 558.3 |
| Al | 1200.9 | 236.9 | 230.0 | 576.7 | 233.7 |
| Mn | 30.5 | 7.7 | 20.0 | 62.2 | 71.4 |
| Fe | 528.6 | 444.6 | 518.0 | 653.3 | 605.1 |
| Ni | bdl | bdl | bdl | 88.9 | bdl |
| Pb | 198.1 | 64.6 | 152.0 | 263.3 | 118.3 |
| Cd | bdl | bdl | bdl | bdl | bdl |
| Cr | bdl | bdl | bdl | bdl | bdl |
| Se | bdl | bdl | bdl | bdl | bdl |

*Acute toxicity of interstitial water*

No acute toxicity was observed to *Ceriodaphnia dubia* with 90 to 100 % of test organisms surviving in all samples at the end of the 48-hour test. In the Russell Creek sample 90% survival (18 of 20 organisms) was observed while 95% survival occurred in the Fletcher Ford sample. All of the other sites had 100% survival.

*Relevance of metal accumulation data*

The change in mussel density for the three sites where data are available is negatively correlated with Pb in periphyton ($r = 1$) and Mn ($r = 1$) in clam tissues, while the decrease in mussel richness is negatively correlated with Cu and Pb in clam tissues ($r = 0.993$, and 0.995, respectively) (Figs. 1 and 2). These data suggest that the greater the metal concentrations measured in clam and periphyton tissues, the larger the decrease in either mussel density or richness that has been observed. Thus, Pendleton Island in general had the highest concentrations of metals in both periphyton and clams, and this site likewise has seen the greatest drop in freshwater mussel diversity and density.

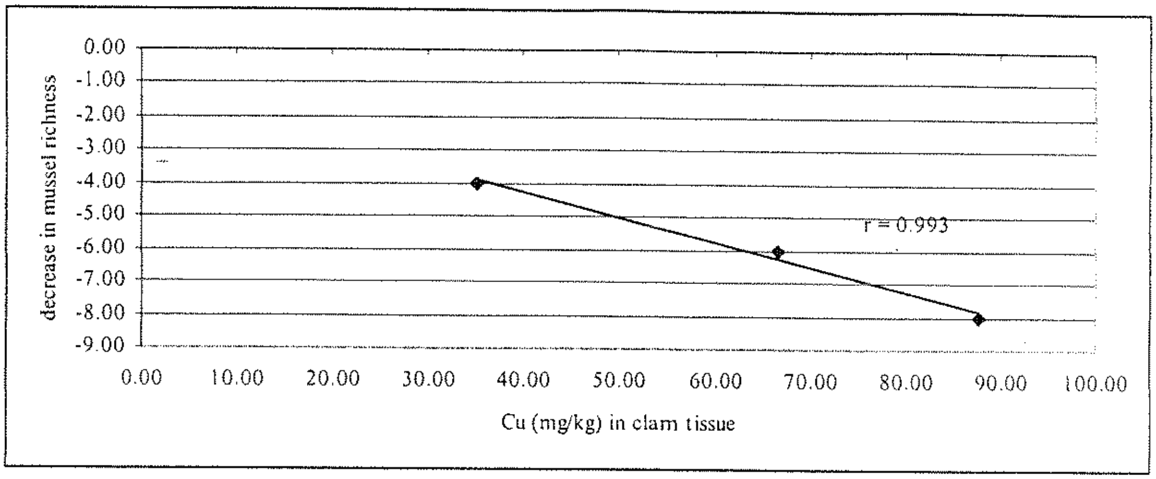


Figure 1. Relationship between Cu concentration in transplanted clam tissues and the decrease in freshwater mussel richness from 1979/1983 to 1994.

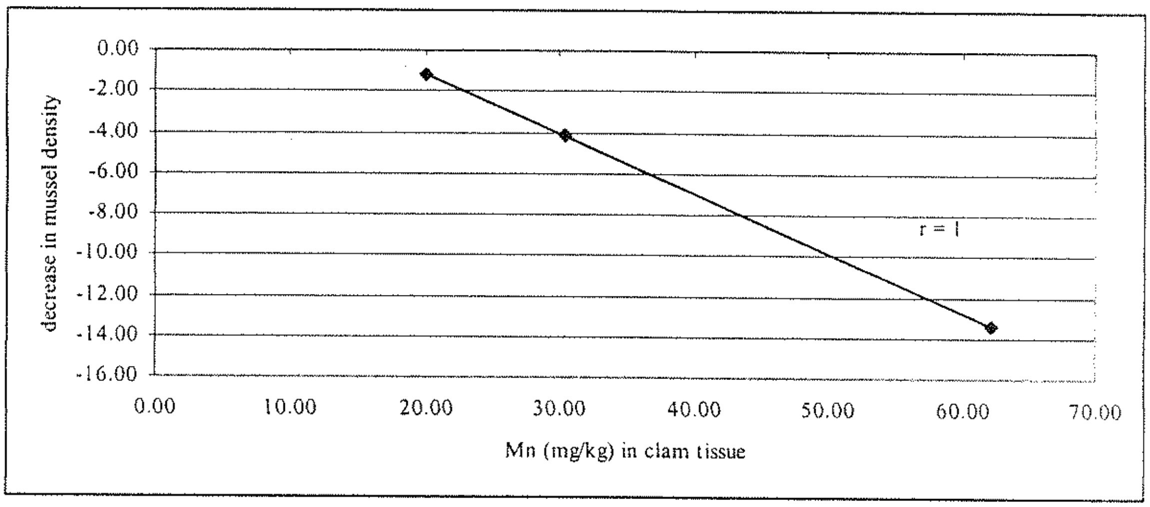


Figure 2. Relationship between Mn concentration in transplanted clam tissues and the decrease in freshwater mussel density from 1979/1983 to 1994.

Exposure to Cu and Zn has been shown to cause various effects in the Asian clam including reduced endo- and exocellulase (the enzyme used to break down food particles) activity (Farris et al., 1994) and even loss of shell size (Belanger et al., 1986, 1990). Concentrations of Cu and Zn in organism collected from the reference area for use in testing generally were ~45 and 200-300 mg/kg, respectively, and similar values have been reported by other researchers from Virginia Tech (Belanger et al., 1986, 1990). Body metal burdens associated with decreases in clam length and weight have been reported as low as ~200 mg/kg for Cu and ~600 mg/kg for Zn (Belanger et al., 1986, 1990). Similar body burdens of Zn were observed at Pendleton Island in this study after 30 days in situ exposure. While these high concentrations of Zn may cause overt effects such as decreased body size, the lower but still elevated concentrations of other metals found at sites like Pendleton Island may be having more subtle effects of which we are unaware.

These correlations are by no means to be interpreted as evidence for a causal relationship between metal accumulation and the decline in freshwater mussel populations in the Clinch and Powell Rivers. Rather, they are preliminary data that indicate that bivalves placed in enclosures at sites where mussel declines have been the greatest accumulated more metals than those placed where population declines have not been as great. More extensive research should be conducted to determine if there is a causal relationship between these data trends and also to determine the sources of these metals in the environment. In addition, clam uptake studies should be carried out for longer than 30 days, and these studies should be conducted in the Spring and Summer months rather than in late summer/early fall.

Bivalves grow more rapidly in the spring when water temperatures rise from 15-24°C, and their growth may stall at maximally high summer conditions. If the fall season is a rapidly declining one in water temperatures, growth will not be as active as in the spring. Our clam in situ survival/growth studies occurred in latter summer to early fall and not in the more desirable spring-early summer season of growth due to funding commitments. This researcher (Cherry) has found Asian clams to grow and produce over a longer spring spawning season than in the fall after a decade of studies in the New River.

*Literature review of trace metal toxicity*

The acute and toxic thresholds of metals for the most sensitive species to metals, along with thresholds for unionids where available, are found in Table 8. From the literature review, the most toxic trace metals were Cd, followed by Cu, and then Se and Pb. Toxic thresholds for unionids ranged from 26 to 102 ug Cu/L for mussels where the most information was collected. One should note that several species of unionids were more acutely and chronically sensitive to copper than the US EPA (*Ceriodaphnia*) recommended test species. Thereafter, for other sensitive organisms to metals, cladocerans were most sensitive. Cadmium was the next toxic metal with $LC_{50}$'s as low as 9.9 ug/L and chronic values down to 0.152 ug/L. Selenium was ranked third in toxic impact to aquatic organisms with acutely toxic values for *D. magna* to 220 ug/L while chronic levels were 4.9 to 10.0 mg/L.

The metals in the intermediate range were Zn, Pb, Cr and Ni. For example, acute LC50's for Zn were 70 to 354 ug/L (acute) and 40 to 140 ug/L for chronic thresholds. For Pb, it was 124 to 1,910 ug/L (acute) and 12.3 to 128.1 ug/L for chronic thresholds. Chromium acute and chronic values ranged from 2,000 to 66 ug/L, while for Ni, they were 1000 ug/L (acute) and 100 to 250 ug/L (chronic).

The three metals of lowest toxicity relative to WQC impacts were Fe, Al and Mn (Table 8). Acute and chronic toxicity values for two cladocerans ranged from 1,160 (Fe) to 2,880 ug/L (Al) to a range of 160-220 chronic thresholds for Fe, and 1,600-1,900 ug/L for Al. For Mn, acute and chronic toxic thresholds were much higher than those for Fe and Al. For example, acute/chronic thresholds for Mn were a whole dimension higher at 40,000 ug/L (acute) and 28,000 ug/L (chronic) thresholds. No US EPA WQC levels exist for Mn. The lowest WQC levels were for Cd followed by Se, Cr and Cu.

Relative to the acute/chronic threshold data found in Table 8, correlations on trends were analyzed for the interstitial water concentrations found in Table 4. The total concentration of Al in the interstitial water was lower than acutely toxic concentrations found in the literature; however, chronic toxicity values of 1,600-1,900 ug/L were lower than interstitial water values at Fletcher Ford and Grays Island. Iron in interstitial water (3,108 ug/L) exceeded acute toxic levels of *Ceriodaphnia* while chronically toxic thresholds of 160 to 220 ug/L were also lower in the interstitial water while acute/chronic toxicity levels in the literature were one to two orders of magnitude higher. There appears to be a concern at some of the sampling sites where interstitial water containing Al and Fe exceed chronically toxic thresholds reported in the literature.

Table 8. Toxic thresholds from the literature of the metals analyzed in µg/L at each station.

| Sensitive Species | Acute Toxicity $LC_{50}$ | Chronic Toxicity Species Chronic Conc | Citation | US EPA WQC Acute | US EPA WQC Chronic |
|---|---|---|---|---|---|
| | | **Copper** | | 18 | 12 |
| *Lampsilis fasciola* | 39 | 15.5 | Cherry et al (1991), Farris et al (1991) | | |
| *Elliptio dilatata* | 26-73 | | | | |
| *Villosa nebulosa* | 41-79 | | " " | | |
| *Medionidus conradicus* * | 41 | 15.5 | " " | | |
| | 18-74 | >28,1 | | | |
| *Pleurobema oviforme* | -- | >28,1 | " " | | |
| *Isonychia bicolor* | 52 | | " " | | |
| *Villosa iris* | 54 | 15.9 | " " | | |
| *Actinonaias pectolosa* | 58-102 | | " " | | |
| *Corbicula fluminea* | 455 | 12.4 | " " | | |
| *Ceriodaphnia dubia* | 88 | 21.3 | " " | | |
| | | **Zinc** | | 320 | 47 |
| *Corbicula fluminea* | 2,945 | -- | Cherry et al 1991 | | |
| *Lasmigona costata* | 10,340 | -- | Cherry et al 1991 | | |
| *Leptoxis praerosa* | 1,210-4,970 | -- | Cherry et al 1991 | | |
| *Isonychia bicolor* | 346-522 | -- | | | |
| *Ceriodaphnia dubia* | 70-354, | 40-140 | Belanger & Cherry (1990) | | |
| *Ceriodaphnia dubia* | 105-153 | | Cherry et al (1991) Schubauer Bergan et al (1993) | | |
| | | **Cadmium** | | 3.9 | 1.1 |
| *Daphnia magna* | 9.9-63.0 | 0.152-0.437 | Chapman et al (manuscript) | | |
| | | **Chromium** | | 16 | 11 |
| *Daphnia magna* | 2,000 | 66 | Biesinger & Christenson (1972), Chapman (manuscript) | | |
| *Pimephales promelas* | 5,070 | 1,020-1,990 | Pickering & Henderson (1966), Pickering (1980) | | |
| | | **Lead** | | 82 | 3.2 |
| *Daphnia magna* | 612-1,910 | 12.3-128.1 | Chapman (manuscript) | | |
| *Gammmarus pseudelimnaeus* | 124-140 | | Spehar et al 1978, Call et al 1983 | | |
| | | **Nickel** | | 1,800 | 96 |
| *Daphnia magna* | 1,000 | 100-250 | Munzinger & Monicelli (1991), Haley & Kurnas (1993) | | |
| | | **Selenium** | | 20 | 5 |
| *16 fish species* | -- | 10.0 | Cumbie & Van Horn, (1978) | | |
| *Lepomis macrochirus* | -- | 4.9 | Lemly (1993) | | |
| *Daphnia magna* | 220-430 | 5.0 | Ingersoll et al (1990) | | |
| | | **Aluminum** | | 87 | 750 |
| *Ceriodaphnia dubia* | 2,880 | 1,600-1,900 | Soucek et al 2001, McCawley et al 1986 | | |
| | | **Iron** | | 1,000 | 1,000 |
| *Ceriodaphnia dubia* | 1,160 | 160-220 | Soucek et al 2001, Milan & Farris 1998 | | |
| | | **Manganese** | - | - | |
| *Daphnia magna* | 40,000 | 28,000 | Bowmer et al 1998 | -- | -- |

* mussel glochidia

For the more toxic trace metals, Cu concentrations in the interstitial water were less than all acute and chronic toxicity values available in the literature (Tables 4 and 8). The same trend occurred for Zn, Cr, Pb and Ni. Selenium concentrations, however, ranged from 31.8 to 42.8 ug/L at three sites and chronic Se values in the literature were much lower (4.9-10.0 ug/L). The more recent concerns of Se as a chronically limiting metal has been promoted over the past decade and WQC limits at 5 ug/L are being questioned as not being protective enough by recent scientific findings (Lemly 1992, 1993). From this literature review, the interstitial water levels of Al, Fe and Se are of concern because they exceed chronic toxicity levels of sensitive test species found in the literature.

In addition, the most sensitive test organism by the US EPA is generally considered to be the cladoceran, *Ceriodaphnia dubia*, which we used as the comparative benchmark species in Table 8. More noteworthy, two earlier studies (Cherry et al 1991, Farris et al 1991) found *Ceriodaphnia* to rank sixth and seventh in acute and chronic toxicity thresholds compared to 16 and 14 test species tested from the Clinch River watershed. The other US EPA endorsed test species, fathead minnow (*Pimephales promelas*), was ranked 14th lowest in sensitivity to both acute and chronic thresholds. At least three species of native unionids and a mayfly were more sensitive to copper than *Ceriodaphnia* which more than suggests that the US EPA test species are not protective enough for maintaining ecological integrity, especially in the Clinch River watershed.

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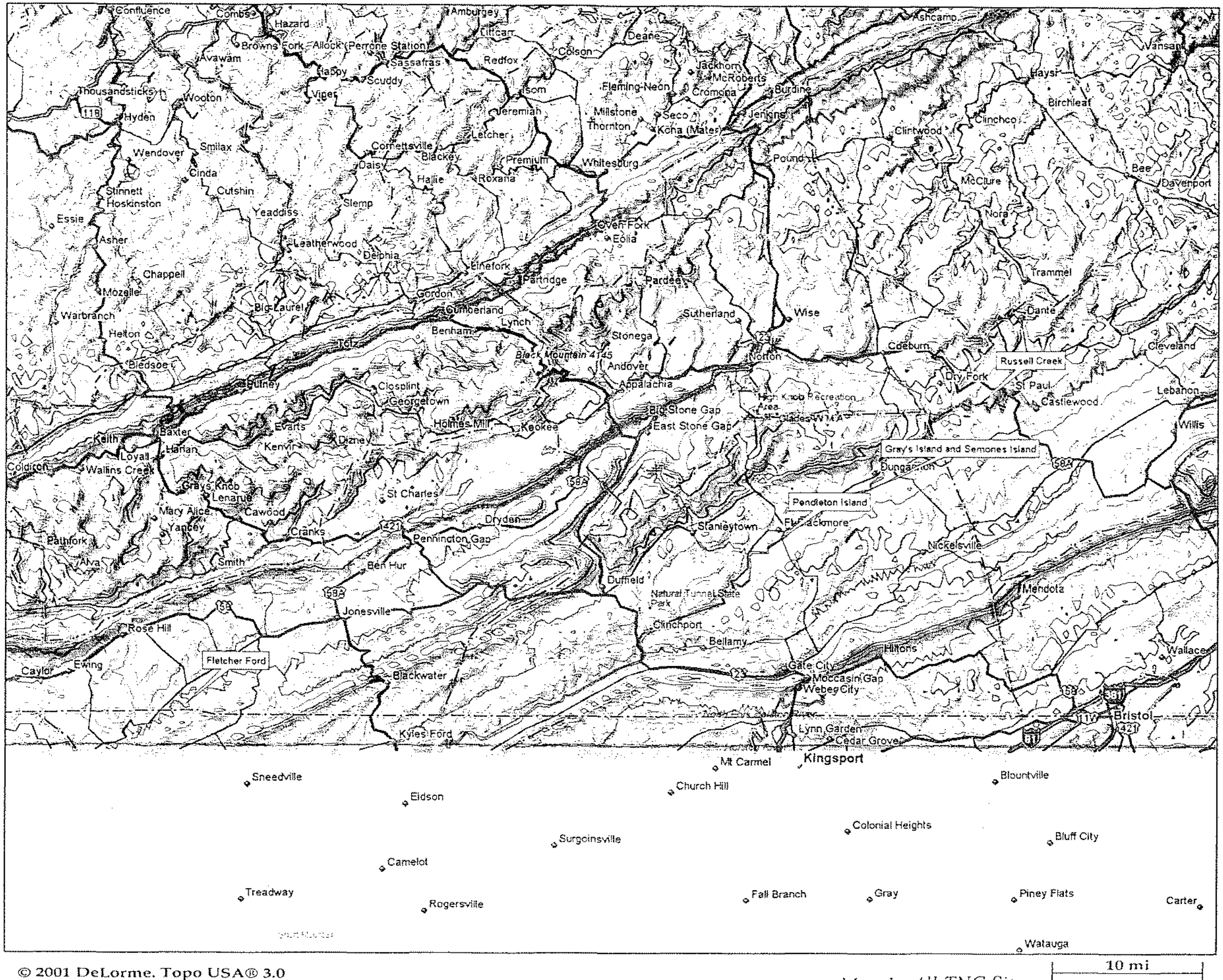
## APPENDIX I: Maps of the Sampling Stations

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Scale: 1 : 550,000 Zoom Level: 8-5 Datum: WGS84 Map Rotation: 0° Magnetic Declination: 5.6°W

*Map 1. All TNC Sites*


*Map 2. Russell Creek*

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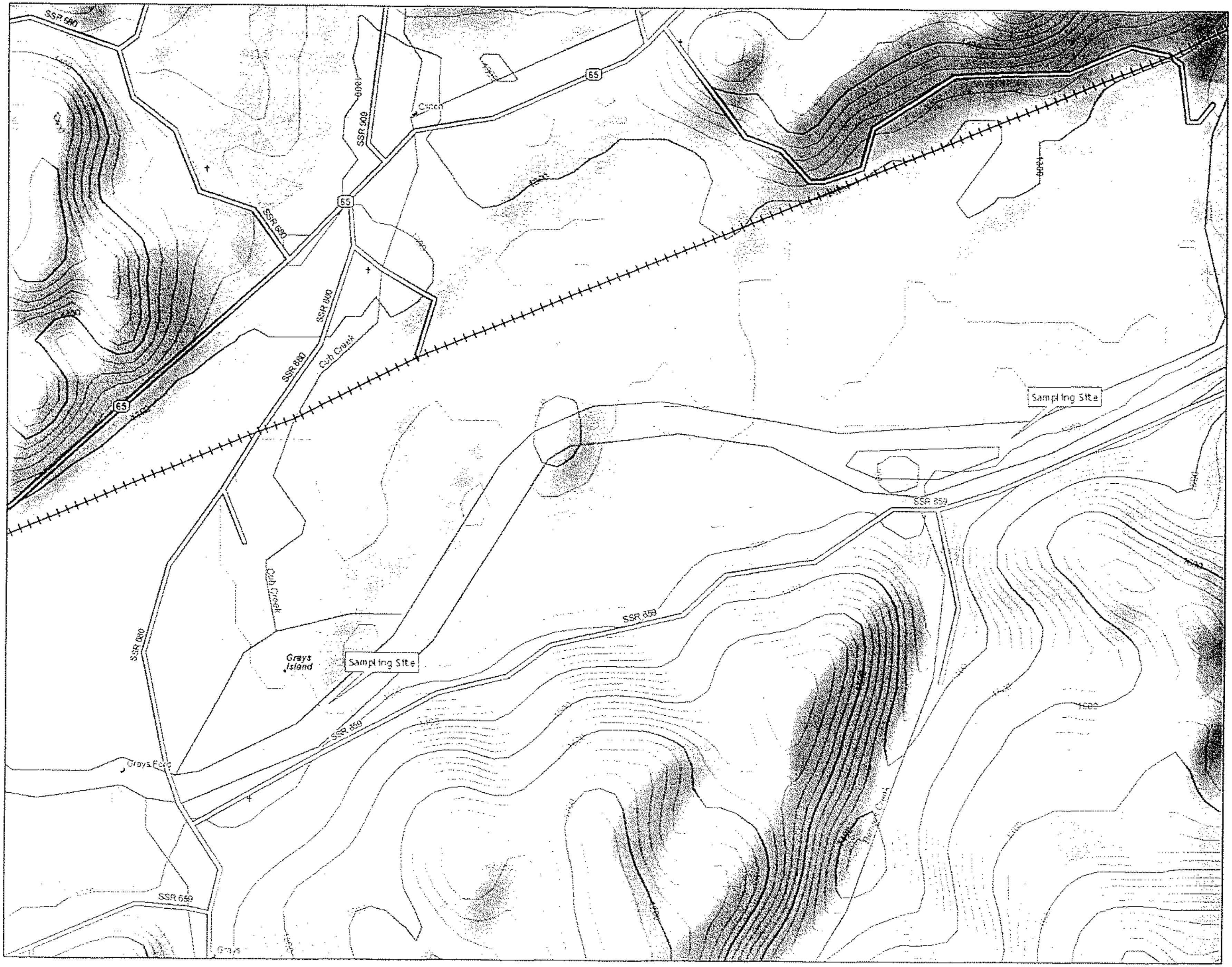
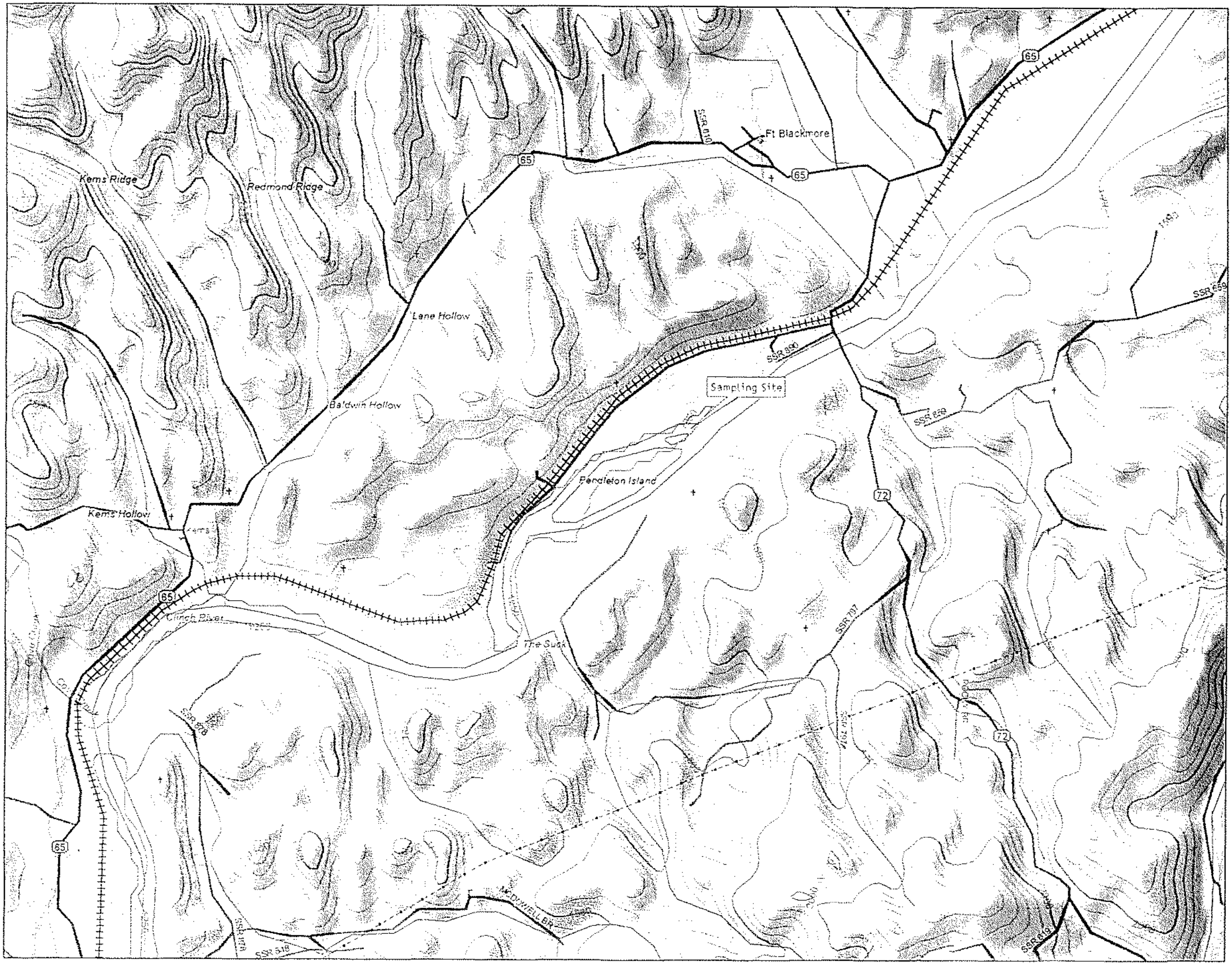
Scale: 1 : 12,800 Zoom Level: 14-0 Datum: WGS84 Map Rotation: 0° Magnetic Declination: 6.0°W




© 2001 DeLorme. Topo USA® 3.0
Scale: 1 : 11,200 Zoom Level: 14-2 Datum: WGS84 Map Rotation: 0° Magnetic Declination: 5.8°W *Map 3. Gray's Island and Semones Island*

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Scale: 1 : 25,000 Zoom Level: 13-0 Datum: WGS84 Map Rotation: 0° Magnetic Declination: 5.7°W *Map 4. Pendleton Island*



Scale: 1 : 6,400 Zoom Level: 15-0 Datum: WGS84 Map Rotation: 0° Magnetic Declination: 5.1°W

*Map 5. Fletcher Ford*

## APPENDIX II: Summary of Benthic Macroinvertebrate Parameters

Appendix Table 1. Summary of 21 benthic macroinvertebrate parameters evaluated for five sampling stations in the Clinch and Powell Rivers.

| Parameter | Fletcher Ford | Russell Creek | Gray's Island | Semone's Island | Pendleton Island |
|---|---|---|---|---|---|
| Taxa Richness | 22.50 | 12.00 | 17.50 | 22.00 | 15.50 |
| Caddisfly Abundance | 16.00 | 50.50 | 8.00 | 28.50 | 15.00 |
| Percent Caddisfly | 8.70 | 50.71 | 3.61 | 15.24 | 8.03 |
| Stonefly Abundance | 2.50 | 0.00 | 0.50 | 2.00 | 4.00 |
| Percent StoneFly | 1.30 | 0.00 | 0.22 | 1.06 | 2.06 |
| Mayfly Abundance | 28.00 | 1.50 | 81.00 | 39.00 | 45.50 |
| Percent Mayfly | 15.30 | 1.43 | 38.64 | 21.06 | 23.52 |
| EPT Abundance | 46.50 | 52.00 | 89.50 | 69.50 | 64.50 |
| Percent EPT | 25.40 | 52.14 | 42.47 | 37.36 | 33.61 |
| EPT Richness | 7.50 | 3.00 | 6.50 | 8.00 | 6.50 |
| Midge/EPT Ratio | 0.09 | 0.65 | 0.05 | 0.03 | 0.11 |
| HBI | 3.42 | 6.16 | 4.56 | 3.49 | 3.58 |
| FBI | 3.23 | 5.46 | 4.45 | 3.40 | 3.53 |
| Shannon-Wiener | 1.02 | 0.67 | 0.94 | 1.08 | 0.98 |
| Simpsons | 0.84 | 0.66 | 0.82 | 0.89 | 0.85 |
| %Collector/Filterers | 19.15 | 55.87 | 17.78 | 34.36 | 35.50 |
| %Collector/Gatherers | 9.53 | 36.35 | 27.75 | 10.81 | 7.62 |
| % Collectors | 28.68 | 92.22 | 45.53 | 45.16 | 43.12 |
| %Scrapers | 60.96 | 0.48 | 44.61 | 48.92 | 46.30 |
| %Shredders | 0.27 | 0.95 | 0.00 | 1.06 | 0.00 |
| %Predators | 10.10 | 5.79 | 9.86 | 4.86 | 10.58 |