An Ecotoxicological Evaluation of the North Fork Holston River below Saltville, Virginia and Identification of Potential Stressors to Freshwater Mussels (Bivalvia: Unionidae)

by

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ABSTRACT

Mercury contamination of the North Fork Holston River below Saltville, Virginia has nearly extirpated most mussel populations. Because natural recovery of these populations has not occurred, this research combined field and laboratory assessments to determine the extent of ecological impairment in the river. *In situ* 60-day Asian clam (*Corbicula fluminea*) growth studies in 2005 showed a positive correlation (p=0.03) between low clam growth and sediment mercury levels. Because of severe low flow conditions of the NFHR in late 2005 conductivity dissipation from a point source brine discharge downstream rarely reached background level (~345 µS/cm) and was observed as high as 690 µS/cm 640 m below the discharge site. In addition, conductivity doubled in the river section adjacent to the remediated Ponds 5 and 6 (rm 81.6 and 80.4). Such low flow conditions (mean flow < 50 ft^3/sec) occur in the NFHR approximately every five years. This low flow situation also evidenced a thick white flocculent or floc observed to accumulate at the base of the two remediated ponds. Analysis of the flocculent determined it to be high in aluminum (1.9-38 mg/L) and iron (2.0-51.0 mg/L), well above US Environmental Protection Agency Water Quality Criteria limits (0.0087 and 1.0 mg/L, respectively); riverine sediments collected below the accumulated floc also had high levels of calcium (240,000-380,000 mg/kg) and mercury(0.62-1.7 mg/kg). Acute tests with juveniles of *Villosa iris* and <24-hr old *Ceriodaphnia dubia* were used to measure the toxicity of the brine discharge, which had a conductivity of ~ 14,000 µS/cm. Results of these tests indicated *C. dubia* to be more sensitive than *V. iris*; however, chronic toxicity test results were similar for *V. iris* and *C. dubia*. The Lowest Observed Adverse Effect Concentration (LOAEC) for mussel survivorship after 28 days was 10,000 µS/cm, while the LOAEC for growth was 5,000 µS/cm. LOAECs for the *C. dubia* 7-day chronic were 25 % (survivorship) and 12.5 % (reproduction), while mean

conductivity at these two concentrations was 4,054 and 2,211 μS/cm, respectively. Toxicity tests conducted with Pond 6 dyke cut discharges resulted in similar lethal concentrations for *C. dubia* and *V. iris*. Forty-eight hour LC50s of these discharges ranged from 12.07-15.95 % for *C. dubia,* and 17.36-18.95 % for *V. iris*. Dyke cut discharges also exhibited exceedingly high alkaline pH (11.5-12.2), which caused 100 % mortality to *C. dubia* in 15 min. The Pond 5 and 6 dyke discharges are the likely source for the flocculent accumulation at the base of the two remediated pond areas. The combined effect of mercury, aluminum and iron, along with periodic fluxes of high conductivity and alkaline pH during low flow conditions may contribute to low mussel recruitment downstream of Saltville, VA.

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Chapter 1: Introduction

The North Fork Holston River (NFHR) has been the receiving system for a number of industrial constituents, such as mercury, ammonia, aluminum and iron, for more than a century (Turnage 1994). The abundant natural salt and limestone deposits in the region left the area prone to commercial mining. Production of salt in the region has been estimated to have begun sometime in the 1760s (McDonald 1984). In 1895, a British chemical corporation, The Mathieson Alkali Works, began producing salt products such as soda ash and bicarbonate of soda. In the 1950's, Mathieson Alkali Works merged with Olin Corporation to become the Olin-Mathieson Chemical Company (later known only as Olin Corp). The primary products produced by the plant were soda ash, bicarbonate of soda and carbon dioxide. The effluent discharged by the plant was an ammonia-soda waste, which was mainly comprised of calcium and soda chlorides. In addition to the production of soda ash, Olin Corp. took advantage of the rich salt deposits underground to produce chlorine. Electrolysis is the primary process by which chlorine is manufactured (the Deacon process was exercised prior to electrolysis) from brine. Although several electrolysis methods exist in modern production, mercury cell electrolysis was the first method of this type to be used in industry.

In 1953, at the start of chlorine production at the Olin facility in Saltville, this was the only means by which brine could be converted into chlorine. Unfortunately, little was known about the toxicity of mercury in 1953, both to humans and the environment, and mercury waste was diverted to the large holding ponds, primarily muck pond # 5, which was located on the edge of the river. The chlorine production building was located on the river's edge as well. It has been said that mercury waste on the floors was often washed out of the building using a hose, and allowed to run directly into the river (Harry Haynes, personal communication). It is estimated that approximately 34 kg of mercury were being released daily in the holding ponds (Seivard et al.1993). Mercury pollution in the North Fork Holston River has been recognized by Olin Corp., the U.S. Environmental Protection Agency (US EPA) and Virginia Water Control Board. Unable to meet revised state water quality control standards, Olin Corp. began closing down production in 1970.

On February 22, 1972, Olin closed the last of its major plants, the chlorine plant. At the time of its shutdown, the mercury-cell chlor-alkali plant was producing 250 tons of chlorine per day (Turnage 1994).

Five years after closing the chlorine production plant, it is estimated 100 g of mercury were still seeping into the river from the holding pond daily (Carter 1977). At that time, the holding ponds covered approximately 122 ha of surface area and contained an estimated 10,000 metric tons of mercury. An investigation of the Olin Corp. during the 1960s resulted in a fishing ban in Virginia and Tennessee. In 1970, both fishing bans were reduced to ban only consumption of fish due to the mercury presence in the river. Continuous investigations of the NFHR by federal agencies, including The Food and Drug Administration reported fish downstream of the ponds had mercury levels twice the acceptable limit for consumption.

In the past 30 years, several attempts have been made to alleviate the potential threats posed to people and aquatic fauna residing in or downstream of Saltville. In the 1980's, Olin Corp. was forced to temporarily divert a 1000-ft section of the river around the former chlorine plant site in order to remove contaminated sediment from the river bed (YMA 1990; Dye 1999.) Additional recovery efforts were made by the US EPA Superfund Program and Olin Corporation. Remediation of the contamination sites included a geo-membrane cap in addition to a soil cap on Pond 5, and a soil cap only for Pond 6. Construction of diversion dyke cuts on the caped surfaces prevents saturation of excess surface water and the addition of a Waste Water Treatment Plant (WWTP) at the upper portion of the Pond 5 area treats diverted water from the ground table of both ponds before discharging into the river. The WWTP removes mercury waste and neutralizes pH to adhere to National Water Quality Criteria (US EPA 2002).

Ecological impairment of the river has been a concern for more than a decade. In fact, harmful effects on aquatic biota below Saltville were documented in the early 1900s (Adams 1915). Adams (1915) noted that the river at and below the alkali facility "the refuse flowing into the river has covered all the rocks and the bed of the stream with a whitish coating." In 1924, the dam wall of one of the alkali works holding ponds broke, releasing large amounts of a chemical sludge, which covered the surface of the river for miles (Turnage 1994). Jenkins and Burkhead (1984) reported fish kills extending into

Tennessee by the 1940s. Some ecological improvement has been noted during monitoring efforts over the past several decades. Several studies have revealed mercury levels in fish tissue to be reduced substantially (Hildebrand et al 1980; YMA 1990; Dye 1999), while benthic macroinvertebrate sampling efforts downstream have also shown increased faunal diversity (Hill et al 1980). Earlier reports (Hill et al.1974, Feeman 1986) reported that the aquatic fauna have steadily recovered, despite continued erosion and seepage of chemical deposits from the covered holding ponds, into the river.

This, however, does not mean there is no longer a mercury problem affecting the NFHR below Saltville, VA. Mercury analysis of sediments collected downstream still show elevated mercury levels. In studies conducted by Hildebrand et al (1980a, b), mercury binding is correlated with sediment particle size. According to Hildebrand et al. (1980a, b), mercury is most likely to bind to sediments of smaller particle size, such as clay or silt and therefore, mercury levels are expected to be elevated in depositional areas. Recent sediment and interstitial water analysis concur with this assertion. This is a key element in assessing the risk of mercury to aquatic biota, especially those that inhabit these depositional zones such as burrowing bivalves. Seivard et al. (1993) conducted a riverine study which quantified tissue mercury concentrations in resident Asian clams (*Corbicula fluminea)*, in order to measure the geographic distribution of mercury in the NFHR. These clams are often used as a bioassessment indicator of heavy metals for several reasons including:

- Sensitivity to an array of contaminants.
- Sessile nature. Bivalves are unable to migrate away from disturbances as a fish would.
- High rate of filtration. Bivalves either siphon feed (clams) or suspension feed (mussels: Unionidae) and therefore filter large quantities of water daily making them vulnerable to contaminants in the water column.

Results of the Seivard et al. (1993) study reported that both total mercury and methylmercury levels were significantly higher in clams collected downstream compared to those collected at the upstream reference site.

Historically, Ortmann (1918) reported 42 species of unionid mussels present in the NFHR above and below Saltville, VA. This number is not surprising as Saltville lies in the Cumberland Plateau, an area of worldwide recognition for mussel diversity. However, Hill (1974) reported only one species downstream of Saltville, and 11 species recorded upstream. A study by Henley and Neves (1999) reported that there was no evidence of a mussel assemblage for at least 20 river miles below Saltville, VA, as only isolated individuals were found.

Mercury contamination in the NFHR is an obvious concern and has been the topic of most research conducted in the river since the close of the Olin Corp. However, after shutdown of the company, the valuable salt deposits being mined underground were also no longer needed. With the removal of any salvageable mining equipment, the pressure gradients in the mines have forced the brine water to the surface, creating several large salt ponds in Saltville, VA. These ponds, which are located near the downtown area, are still expanding. In order to prevent flooding in downtown Saltville, one large convergence pond was added. This pond, which is located directly in front of the town grocery store, acts as an additional holding resource. Brine is discharged from this pond into the NFHR through 50 cm in diameter culvert on a continuous basis. The discharge enters the river ~ 1.6 km above the town waste water treatment plant (WWTP) discharge. Conductivity measurements from the salt ponds can range from 900 µS/cm (collected in August 2005) to 26,800 µS /cm (unpublished data).

Conductivity of the actual discharge also varies in range but has been as high as 16,000 µs/cm (unpublished data, 2005). Although studies have been conducted, and dissipation usually occurs rather quickly (40 m) during normal flow conditions, it was imperative that this dissipation nature be defined during severe low flow conditions as observed in 2005. Due to the continuous flow of the discharge, it is possible that the influxes of salt from the discharge may also contribute to a reduction in biotic diversity downstream of Saltville, VA.

The NFHR has undergone numerous deleterious influences over the past century. Although the industrial revolution brought prosperity to the region, even if for a short while, it left behind a legacy that continues to affect the environment. The goal of this project was to examine these historical impacts on the NFHR and investigate potential current stressors that are impeding recovery of the NFHR.

1.2 Study Area

The NFHR is located in southwestern, Virginia and flows 216 km in a southwestern direction through Bland, Smyth, Washington and Scott counties to the confluence with the South Fork Holston River near Kingsport, Tennessee. This system has been characterized as a moderately hard-water stream. Hill et al. (1974) described the river has having a high riffle-pool ratio and substrate that was consisted of sand, gravel and cobble. Average annual discharge of the river from 1908 to 2006 was estimated at 91.0 m^3/sec. The river is part of a riverine system (mainstem Holston, Powell and Clinch Rivers) in the Cumberland Plateau Region which historically supported high aquatic biodiversity.

Anthropogenic influences have caused major degradation of this system. The river is mostly surrounded by agriculture, and urban development has been minimal (Henley and Neves 1999); however, industrial impacts in Saltville (~77 km downstream of headwaters) have caused ~ 128 km of the river to be contaminated (Sheehan et al. 1989, Seivard et al. 1993).

1.3 Research Objectives

The primary objective of this study was to fulfill the Ecotoxicological Evaluation component in the Freshwater Mussel Injury Study Plan for the North Fork and Mainstem Holston Rivers, Virginia (April 2004). The overall goal was to access the residual mercury levels remaining in sediments and interstitial water below the closed chlor-alkali plant.

Additional objectives of this project were to characterize chemical makeup of the brine discharge, and access conductivity dissipation in the river during low to intermediate flow. The final objective of this research project was to examine potential seepage occurring around the holding Ponds 5 and 6. Although these two former muck ponds have been remediated due to the US EPA Superfund program, it has been reported (US EPA 2002) that mercury and alkaline pH are continuing to affect the river in the immediate area below the ponds.

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Chapter 2

An Investigation of Remnant Mercury Contamination in the North Fork Holston River, Saltville, VA

Abstract

The North Fork Holston River (NFHR) has historically had one of the most diverse aquatic faunas worldwide and was known for diverse unionid populations (Unionidae). In recent decades, drastic reductions in mussel diversity, abundance and recruitment have been documented by researchers. Unionid declines have been blamed on anthropogenic influences dating back to the early 1900's in and around Saltville, Virginia, and mercury contaminated wastewater from a now closed chlor-alkali plant in Saltville beginning in the 1950s. The objective of this research was to evaluate any residual mercury within sediments and site interstitial water downstream of the closed plant and to identify any existing sources of mercury input. Mercury-contaminated sediments and interstitial water were found downstream of the closed chlorine-alkali plant with the highest sediment concentration of 2.82 mg/kg total Hg found at river mile (rm) 80 and the highest interstitial water value at rm 30.4 with 2.1 μg/L. Total mercury concentrations in Asian clam (*Corbicula fluminea*) tissues after 60-d *in situ* testing ranged from 0.016 to 0.13 mg/kg, while naturalized clams had mercury concentrations of 0.094 and 0.11 mg/kg. Although chronic toxicity was not observed in Asian clam growth and survival tests nor in testing with cladocerans, mercury contamination is still a persistent problem at sites in the NFHR below the closed plant, with positive correlations between mean growth of clams and sediment mercury concentrations.

2.1 Introduction

The major tributaries of the Tennessee River, which include the Powell, Clinch and Holston rivers, provide suitable habitat for some of Virginia's rarest freshwater unionids (Bivalvia: Unionidae) (Woodward and Hoffman 1991). All three rivers have received notable attention in recent years due to declining unionid populations and have been the site of numerous mussel surveys and relocation efforts. Of major concern is the North Fork Holston River (NFHR) which is known worldwide for having a very diverse aquatic fauna that includes many species of unionids. While a study by Ortmann (1918) reported more than 42 species of freshwater mussels in the NFHR above and below the town of Saltville, Virginia, current mussel surveys conducted in the NFHR have shown a dramatic decrease in mussel populations. The highest rates of decline occurred below a closed chlorine-alkali plant located in Saltville, while upstream populations remain relatively healthy (Stansbery 1972; Hill *et al.* 1980; YMA 1990; Dye 1999; Henley and Neves 1999).

Commercial mining began in Saltville in 1895, when Mathieson Chemical Corp. began extracting salt and limestone to produce soda ash. In 1950, Mathieson, which merged with the Olin Corporation in 1954, began using mercury electrodes during chlorine production to increase productivity (Carter 1977; YMA 1990; Henley 2000). Resulting mercury waste was diverted into several holding ponds adjacent to the NFHR. During normal production, it is estimated that 34 kg of mercury were being released daily into the ponds as a result of this process (Seivard *et al.* 1993). The Olin-Mathieson Corp. terminated production of the Saltville plant in February 1972 due to the increasing difficulty in meeting the heightened water quality criteria of the 1970's. The 44.9 ha holding pond site (Ponds 5 and 6) was placed on the National Priorities List (NPL) in September 1983, with final remediation and upgrades to the holding ponds completed in 2002 (US EPA 2002a).

Residual mercury that has bioaccumulated in sediments below Saltville has been hypothesized to be responsible for the limited recovery of unionid populations in the lower NFHR. Hildebrand *et al.* (1980) and others reported that the holding ponds are a potential source of mercury contamination (Lindsey and Dimmick 1983; Bailey 1974; US EPA 2002a). Prior to pond remediation, Carter (1977) reported contamination from the holding ponds at an estimated rate of 100 g of mercury daily, while Lindsey and Dimmick (1983) estimated 0.23 kg of elemental Hg entered the river per day. Turner and Lindberg (1978) estimated soluble mercury leaching from the ponds in amounts approaching 39 kg/yr. As part of the remediation efforts, a wastewater treatment facility was built in 1995. Discharges originating from the holding ponds (5 and 6) are diverted to the facility, treated, and discharged into the river adjacent to Pond 5. In 2001, the treatment plant released 87,071 kl of waste-water over a period of 105 (nonconsecutive) days, with an average mercury concentration of 0.8 µg/L (US EPA, 2002a).

Mercury-contaminated waters, a growing concern for researchers and environmental regulators, have been increasing since the 1970's. Mercury has a high potential for bioaccumulation and becomes more concentrated as it passes through food chains (Panda *et al.* 1992; Stein *et al.* 1996; Zagury *et al.* 2006). Although the potential damage to aquatic biota is obvious, most regulatory action is implemented over a concern for human health. Specifically, this concern focuses on the consumption of methylmercury in contaminated aquatic organisms such as fish. For the protection of aquatic life, the US EPA has established the Criteria Maximum Concentration (CMC) or acute standard for mercury as 1.4 µg/L, and the Criterion Continuous Concentration (CCC) or maximum chronic standard as 0.77 µg/L. The criterion for human health consumption is 0.051µg/L (US EPA 2006). Virginia and Tennessee have implemented modified fishing bans on the contaminated section of the NFHR. The ban allows for sport fishing, but human consumption of fish remains prohibited.

The objective of this research was to assess residual mercury effects upon aquatic biota in the NFHR and to locate potential residual mercury contamination below the two remediated holding ponds that contain an accumulation of salts and mercury waste. The scope of this research spanned over 50 river miles, beginning at the holding ponds (rm 84.1) and continuing downstream ~54 river miles to the furthest sampling site at rm 30.4.

2.2 Materials and Methods

2.2.1 Study Sites

Samples were collected in 2005 at nine sites in the North Fork Holston River (NFHR) above and below Saltville, Virginia. Two sampling stations were located approximately ten river miles (rm) upstream of Saltville (rm 95.3 and 95.0). One site was located within town limits, below the capped Olin landfill (rm 84.1) and the remaining six were located downstream of the closed Olin Corporation facility from rm 80.4, with Sites 4a, b, and c directly below the toe of Pond 6, to the furthest site sampled just below Mendota, Virginia, at rm 30.4 (Fig. 2.1).

2.2.2 Water Quality and Sediment Quality

Water column samples were collected using acid-washed 1-L Nalgene® bottles from the nine study locations during each sampling event and selected water-quality parameters were analyzed. Following collection, samples were brought to the laboratory and stored at 4°C for no longer than 24 h for water chemistry analysis. Conductivity measurements were made using a Yellow Springs Instruments, Inc. ® (YSI) Model 30 salinity/conductivity/temperature meter (Dayton, OH, USA). The pH was measured using an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter with an Accumet gel-filled combination electrode (accuracy < ± 0.05 pH at 25°C). A YSI Model 55 meter was used to measure dissolved oxygen (mg/L). Alkalinity and hardness (mg/L $CaCO_3$) were measured by titration as described in APHA *et al*. (1995). In addition, mercury analysis was conducted on site interstitial water (SIW) or porewater and sediments collected at each of the nine sampling sites. Unfiltered SIW was preserved (trace metal grade H_2SO_4) with nitric acid (pH < 2), and shipped to Severn-Trent Laboratories (STL), Savannah, GA within 24 hrs of collection. Mercury analysis performed at STL followed US EPA protocol for analysis of mercury in liquid waste (SW846 7470A, 1986).

Sediment was collected for mercury analysis at each sampling station in July 2005. An acid-washed polyurethane cup was used to scoop sediments from the top layer (< 2 cm) of substrate, which were then placed into sterile plastic bags and transported on ice back to the laboratory. Prior to being shipped to STL for analysis, sediments were divided into replicates by being transferred into sterile Whirlpak® bags. Mercury analysis for site sediments was conducted using the Manual Cold Vapor technique following US EPA protocol for the analysis of solid and semi-solid waste (SW846 7471A, 1986).

2.2.3 Chronic Interstitial Water Toxicity Tests with *Ceriodaphnia dubia*

The SIW was collected from depositional zones at each sampling station using diffusion samplers (peepers). Peepers were constructed from 250-ml amber glass jars with 32-mm diameter holes cut out of the plastic lids. Amber glass was used to prevent photolysis of PAH compounds (Miller and Olejnik, 2001). Prior to deployment, peepers were completely filled with distilled water and 105-µm nylon mesh was inserted under the lid so that it remained secure. Peepers were placed lid side down approximately 10 cm into the sediment. They were allowed to equilibrate for a minimum of 21 days (Webster *et al*. 1998; Vroblesky *et al*. 2002). At collection, SIW was transferred into 1-L Nalgene® containers, and transported back to the laboratory on ice, where they were refrigerated at 4º C.

A *Ceriodaphnia dubia* seven-day chronic toxicity test was conducted with SIW following US EPA protocols (US EPA 2002b). For this initial round of chronic testing, 100% site interstitial water was used to evaluate potential survival and reproductive impairment at each of the study sites. Tests were conducted with 10 replicates per site concentration, with mortality and reproduction observed and recorded daily. SIW was changed daily, and each replicate was fed a mixture of 0.4ml/50ml 1:1, *Selenastrum capricornutum*: Yeast-Cerophyll-Trout chow (YCT). Statistical differences in survivorship and reproduction were determined using SAS® (SAS 2003).

2.2.4 10-day Sediment Toxicity Tests with *Daphnia magna*

Sediment samples were collected from depositional areas from nine study locations in the NFHR and transported to Virginia Tech for testing. Sediments were collected in 4-L freezer bags, placed in coolers and chilled to 4° C. Toxicity tests were conducted according to ASTM (1995) and Nebeker *et al*. (1984) protocols. Test chambers consisted of 250-ml beakers with approximately 100 ml of site sediment (v/v) and 150 ml of filtered reference water. Reference water was collected from Sinking Creek (SC), Newport, VA, vacuum filtered through 0.45µm Whatman® glass microfibre filters, then aerated overnight to ensure adequate dissolved oxygen (DO_2).

There were five replicates with three, 5-day old *Daphnia magna* in each. *D. magna* have been cultured in the Aquatic Ecotoxicology Laboratory at Virginia Tech for 20+ years. Organisms used for testing were raised in filtered Sinking Creek water and fed 5ml/L of *S. capricornutum* and 5ml/L YCT daily until they were five days old.

Sediments were added to beakers, followed by overlaying reference water, and then placed in an incubator to allow the sediments to settle out overnight. Prior to loading the daphnids, dissolved oxygen was checked to ensure adequate saturation (>4.0 mg/L).

Due to low dissolved oxygen levels, beakers were gently aerated for the duration of the test. *D. magna* were randomly selected and placed one at a time into the beakers. Mortality and reproduction were checked and organisms were fed 1 ml of *Selenastrum*/ YCT mixture after each 24-hr interval. Due to the test being aerated, overlaying water was changed over every other day. Water chemistry was analyzed on in-water at the start of the test, and on out-water for each changeover. Parameters measured for in-water included temperature, conductivity, DO_2, pH, alkalinity and hardness. Out-water was measured for conductivity, DO_2 and pH. Sediment tests ran for 10 days and were maintained at 25± 2° C.

2.2.5 In Situ Asian Clam Toxicity Tests

2.2.5.1 Growth and Survival Study

Asian clams (*Corbicula fluminea*) were used for *in situ* survival and growth studies at the nine sampling stations. The Asian clam was chosen as a surrogate bivalve for freshwater mussels (Bivalvia: Unionidae), which is the organism of concern for this project (Hull *et al*. 2002). Clams were collected from a minimally disturbed site in the New River near Ripplemead, VA, and maintained in 570-L Living Streams® (Frigid Units, Toledo, OH) until used. For each clam, width was determined using ProMax® digital calipers and marked on the exterior shell using a file. For this study, clams measuring between 9 and 11 mm were used. Five clams were placed into each of 18 x 36 cm mesh bags (~0.5 cm^2 mesh size), with five replicate bags at each sampling station. After 60 days, clams were retrieved and returned to the laboratory where mean survival and final growth were measured. Mortality was determined if clams were found gaping or easily opened, or failed to close when the visceral mass was probed. Survival along with changes in growth and average growth for each replicate and for each site were determined. Statistical analyses were conducted using SAS® Statistical Analysis Software (SAS Institute 2003).

2.2.5.2 Bioaccumulation Studies

In situ bioaccumulation studies with *C. fluminea* were conducted at the nine study sites during the same period of time as the growth study. Asian clams used for the bioaccumulation study were collected from a minimally disturbed site in the Clinch

River, VA near Pounding Mill. Twenty five clams were placed *in situ* at each site for 60 days. At test initiation, approximately 20 clams were preserved and frozen as a reference for mercury analysis. After 60 days, clams were removed from the NFHR sites, survival was determined and the visceral mass of living clams was removed. For each site and the reference clams, >5g of tissue was sent for mercury analysis to STL, Savannah, GA.

2.3 Results

2.3.1 Water and Sediment Quality

Mean water chemistry values and standard deviations are summarized in Table 1 for each of the nine sampling sites in the river. Differences in measurements were minimal between the reference sites and Site 3. Lowest values for pH and conductivity were recorded at the upstream Sites (1, 2 and 3). Mean conductivity measurements were 301, 302.5 and 284.5 µS/cm for Sites 1, 2 and 3, respectively. Values for pH were similar from June though August for the three upstream sites, with averages of 8.0, 7.9 and 8.0.

Water chemistry values were variable for Sites 4 through 7. Highest conductivity was observed at Sites 4a, 4b and 4c, with mean values ranging from 736.3-759.8 µS/cm, twice as high as those at the upstream sites (Table 2.1). Mean pH was highest at Site 6 (8.5), while mean pH at all other downstream sites was consistently 8.2-8.3. Dissolved oxygen measurements were high at each sampling station due to the lotic nature of the system.

SIW had an increase in mercury levels below the former Olin facility. Water collected just below the remediated holding ponds (Sites 4a, 4b and 4c) averaged 0.17, 0.39 and 0.41 µg/L mercury, respectively. Mercury levels at Sites 5 and 6 were lower, 0.18 and 0.16 µg/L, respectively. However, the furthest downstream location at rm 30.4 (Site 7) which was 52.4 river miles downstream of the closed Olin facility, had the highest mercury concentration of 2.1 µg/L, 1.5 times higher than the CMC for mercury (Fig. 2).

Analytical results of sediments indicated a moderate increase in mercury from Sites 1 through 5. Upstream reference locations had mercury levels of 0.01 and 0.03 mg/kg. Site 3, below the former Olin landfill, was slightly higher at 0.11 mg/kg. Sites directly below the remediated holding ponds (4a, 4b and 4c) had mercury concentrations of 0.64, 0.84 and 1.60 mg/kg, respectively. Site 5 sediments had a considerable spike in mercury (2.82 mg/kg), while downstream Sites 6 and 7 were comparable to the Site 4 locations, at 0.57 and 0.36 mg/kg (Fig. 2.2). No current criteria exist for acceptable sediment mercury levels.

2.3.2 Chronic Interstitial Water Toxicity Tests with *Ceriodaphnia dubia*

Chronic toxicity to *C. dubia* was measured in a 7-day static test with SIW collected from each sampling site in the NFHR. Test survivorship was not statistically significant (p= 0.2600, NPAR1WAY), as all sites had 90-100% survivorship, except for Sites 4c and 5, which both had 80% survivorship of test organisms (Fig. 2.3). Reproduction, however, was statistically different from the control (p=0.0003, NPAR1WAY). Mean neonate production was highest at Site 1 (53.4 neonates) and lowest at Site 4c (rm 80.4) with 32.9 neonates.

2.3.3 10-day Sediment Toxicity Tests with *Daphnia magna*

Chronic sediment toxicity tests were conducted on Sites 4a, 4b and 4c during June 2005, utilizing *D. magna* and with sediments from Sites 1-7 in July 2005. In the June 2005 study, no significant impairment was observed in daphnid survivorship (p=0.1153, α=0.05 ANOVA). Site 4a had the highest survivorship (93.3 %) while Site 4c had the lowest at 80%. Reproduction was determined to be significantly different (p=<0.0001, α=0.05 ANOVA) from the control. Mean neonate production was highest at Site 4b (103.8), closely followed by Site 4a with 102.0. Site 4c was significantly lower, with a mean of 47.0 neonates.

Tests conducted in July 2005 yielded no significant impairment to daphnid survivorship (p=0.1208, NPAR1WAY) compared to the control (100 % survivorship). Survivorship was highest (100%) at Sites 5 and 7, and lowest at Site 1 (73.3 %). The second reference site (Site 2) and Site 3 had minimal mortality with 86.7 % survivorship. Mean reproduction was highest at Site 7 with 140.8 neonates. Reproduction was variable at all other sites, with means ranging from 133.2 (Site 5) to 66.6 at Site 3. Overall, reproduction was lowest at the upstream sites, compared to those downstream, and significantly different at Sites 2 and 3, than the control, Site 1, and downstream sites (Tukeys HSD, α=0.05).

2.3.4 In Situ Asian Clam Toxicity Tests

2.3.4.1 Growth and Survival Studies

Clam survivorship at the upstream reference sites (1 and 2) was 88 and 96 %, respectively. Sites within Saltville (4a, 4b, 4c and 5) had the lowest survivorship at 76, 84, 80 and 80%, respectively (Table 2.2). Asian clam survivorship was high at the downstream sites, with 100% survival at Site 6 and
96% at Site 7.

Change in clam growth was distributed significantly different than normal at the nine sampling sites (p=0.0001, Shapiro-Wilks test for normality). Site 2 clams yielded the highest change in growth, with a mean of 1.90 mm, followed by Site 1 with 1.49 mm (Table 2.2). Clam growth was lowest at Sites 3 and 5 with 0.43 and 0.10 mm, respectively. Although Site 4a clams had a mean growth of 1.24 mm, clams at Sites 4b and 4c had a significantly lower average growth of 0.70 mm (p=<0.0001, NPAR1WAY).

Mean *Corbicula* growth was positively correlated with sediment Hg concentrations (r=0.70, p=0.03) for data collected at the nine sampled sites (Fig. 2.4). The correlation between mean clam growth and sediment Hg increased when Sites 1, 2 and 3 were excluded from the analysis (JMPIN Software 2005).

2.3.4.2 Bioaccumulation

Total mercury levels in clam tissues were found to be significantly higher in clams placed downstream of Saltville, VA, than those upstream (p=<0.0001, t-test). Mercury was lowest at Site 3 (0.016 mg/kg) followed by Site 2 with 0.018 mg/kg. Site 6 clams had the highest mercury tissue concentration at 0.13 mg/kg, while clams at Sites 4a, 4b and 4c had levels of 0.11, 0.12, and 0.11 mg/kg, respectively. Sites 5 and 7 had mercury levels of 0.086 and 0.096 mg/kg (Table 2.2). Native clams collected from Sites 5 and 7 had mercury levels similar to those placed *in situ* for 60 days with mercury concentrations of 0.094 and 0.11 mg/kg. Reference clam tissue had a mercury concentration of 0.007 mg/kg.

2.4 Discussion

The North Fork Holston River is not the only river in the Upper Tennessee River system that has been experiencing declines in native unionid faunas. According to research by Dennis (1987, 1989), mussel declines have occurred in both the Clinch and

Powell river systems. No specific factor has been determined to be contributing to this extirpation; rather multiple stressors have been suggested as the cause of declining biodiversity in these systems which include active mining, abandoned mined lands and industrial inputs (Goudreau *et al*. 1993; Soucek *et al*. 2000; Cherry *et al*. 2002; Hull *et al*. 2002). However, anthropogenic influences have been well documented in the NFHR (Bailey 1974; Carter 1977; Lindsey and Dimmick 1983; Henley and Neves 1999). The NFHR has undergone drastic physiochemical changes during the past century. Industry that dominated the region and provided economic stability has contributed significantly to the biotic impairment in the river. Inputs such as mercury, calcium carbonate, alkaline pH coupled with influxes of salinity and high conductivity have created an unstable environment for organisms such as freshwater mussels (Bivalvia: Unionidae).

In addition to chemical impacts, the NFHR has also undergone major physical alterations. In 1982, sediments were dredged in a 305 meter section below the closed Olin facility, in an attempt to remove mercury laden sediments during remediation (US EPA 2002a). This process not only removed sediments from the streambed, but also required the river to be diverted and channelized during this period of remediation. Although populations of mussels were once abundant in this system, years of such anthropogenic influences have caused a severe decline, almost to the point of complete extirpation to river mile 59.9 (Henley and Neves 1999).

The fate of metals such as mercury can be very difficult to predict in surface waters. Toxicity and movement of metals between sediments, interstitial water and the water column is dependent on numerous chemical and physical reactions (Burton 1992). Custer *et al*. (2007) examined the effects of mercury on cavity-nesting birds and found varying concentrations of mercury in Carson River sediments over time and at different sampling sites. At no sampled sites in the NFHR were similar levels of mercury found in sediments and interstitial water. For instance, sediment-mercury levels were highest at Site 5 (2.8 mg/kg), while interstitial water was only 0.18 μg/L. However, mercury was highest (2.1 μg/L) in interstitial water ~ 50.4 river miles downstream at Site 7.

Certain bioassessment techniques used for this project proved to be more beneficial and more predictive than others in determining the overall health and water quality of the river. Toxicity tests with the two cladoceran species (*Ceriodaphnia dubia* and *Daphnia magna*) were unreliable in predicting the effect of mercury in the river, even though mercury analysis of the interstitial water and sediments used in the two respective tests indicated elevated levels up to 2.1 μg/L for interstitial water and 2.8 mg/kg for sediments. It is probable that the level of mercury in the interstitial water and in sediments was too minimal to cause an adverse effect. Acute laboratory studies by Spehar and Fiandt (1986) reported a median LC50 value (lethal concentration) of mercury to *C. dubia* of 8.8 μg/L, substantially higher than the USEPA CMC of 1.4 μg/L and approximately four times higher than levels determined at Site 7 (2.1 ug/L). In a study by Stewart and Konetsky (1998), it was suggested that 7-d exposure of *C. dubia* to water or sediments containing mercury may not be long enough to show an adverse effect on reproduction. Other researchers advocate using caution when determining sediment toxicity based on daphnid responses in sediment bioassays (Giesy and Hoke 1990; Giesy *et al*. 1990; Ankley *et al*. 1991). One complication in sediment bioassays is the dilution of contaminants in the sediments when overlaying water is added and renewed (Giesy *et al*. 1990). The use of interstitial water as an overlaying water may provide a more realistic outcome and prevent dilution of toxicity.

Results of the *in situ* Asian clam studies may be a more predictive measure of the toxicity potential in the NFHR. Asian clam survival and growth have been shown in numerous *in situ* studies to be good predictors of water quality (Doherty 1990; Belanger *et al*. 1990; Soucek *et al*. 2001; Hull *et al*. 2002; Kennedy *et al*. 2003). Growth and survivorship studies on Asian clams placed in the river for 60 days were negatively correlated with sediment mercury levels in the river for ~ 54 river miles downstream (Fig. 2.4), supporting the assumption that mercury-laden sediments could be having a direct effect on unionid populations in the NFHR.

Bioaccumulation of mercury in *Corbicula* showed a moderate increase in clam tissues after 60 days *in situ*. These mercury levels were proportional to those obtained from native clams at two NFHR Sites (5 and 7). Brown *et al*. (2005) examined bioaccumulated mercury levels in relic mussel shells and found residual mercury in shells of several species collected at various sites (rm 79.9, 68.6 and 56.0) downstream of Saltville to be higher (23-4,647 μg/kg) than shells collected upstream at river miles 96.0 and 85.0 (5-31μg/kg). In fact, mussel shells from the Saltville-contaminated sites were not only significantly higher than the upstream reference sites but had bioaccumulated mercury to levels that were 2-3 orders of magnitude higher.

2.5 Summary and Conclusions

The data collected from this study support the conclusion that remnant mercury concentrations still persist in the North Fork Holston River below the closed chlor-alkali plant located in Saltville at rm 82.8 as of 2005, when this research was conducted. Mercury was observed in sediments, site interstitial water and clam tissues ~54 river miles down to the lowest sampled site at rm 30.4, supporting the hypothesis that mercury is continuing to enter the river from the remediated holding/settling ponds. Although acute toxicity was not evident, chronic effects suggest that long-term exposure to mercury may be responsible for the lack of unionid recovery below Saltville, Virginia. Future research should focus on determining the total amount of elemental mercury entering the North Fork Holston River and the long term effects that this contamination will have on the invertebrate and vertebrate communities downstream of Saltville.

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Table 2.1. Average water chemistry measurements from sites in the North Fork Holston River (NFHR) taken during June through August, 2005 (± SD).

Site Name/Location (River Mile (RM) and GPS Coordinates)	Temp. (°C)	DO[2] (mg/L)	pH (su)	Cond. µS/cm	Alk. (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Site 1/ Upstream Reference (RM 95.3; 36°56.064N, 081°38.960W)	24.0 ± 2.2	8.0 ± 0.4	8	301± 27.8	147 ± 18.4	146 ± 8.5
Site 2/Upstream Reference (RM 95.3; 36°56.064N, 081°38.960W)	24.2 ± 2.0	7.8 ± 0.4	7.9	302.5 ± 24.8	137 ± 9.9	146 ± 8.5
Site 3 (RM 84.1; 36°53.598N, 081°45.377W)	24.3 ± 2.0	7.7 ± 1.1	8	284.5 ± 39.1	128 ± 11.3	130 ± 28.3
Site 4A (RM 80.4; 36°52.647N, 081°48.321W)	23.9 ± 2.4	8.8 ± 0.9	8.3	736.3 ± 231.3	119 ± 12.7	217 ± 80.6
Site 4B (RM 80.4; 36°52.647N, 081°48.321W)	23.6 ± 2.5	8.8 ± 0.9	8.2	750.3 ± 235.3	124 ± 5.7	222 ± 73.5
Site 4C (RM 80.4; 36°52.647N, 081°48.321W)	23.9 ± 2.4	8.6 ± 0.5	8.3	759.8 ± 233.6	124 ± 5.7	222 ± 73.5
Site 5 (RM 80.0; 36°52.430N, 081°48.622W)	25.0 ± 1.7	8.5 ± 0.8	8.3	751.3 ± 231.4	120 ± 14.1	229 ± 69.3
Site 6 (RM 55.9; 36°46.415N, 082°06.984W)	23.5 ± 2.7	9.5 ± 0.6	8.5	641.0 ± 196.8	125 ± 7.1	180 ± 0.0
Site 7 (RM 30.4; 36°39.879N, 082°22.969W)	25.1 ± 0.8	8.1 ± 1.0	8.3	671.3 ± 323.7	120 ± 0.0	174 ± 48.1

Table 2.2. Percent survival, mean growth (mm ± SD), and tissue mercury concentrations (mg/kg) for Asian clam (*Corbicula fluminea*) *in situ* toxicity tests upstream and downstream of the closed Olin facility, Saltville, VA in 2005.

Site	**Survival (%)**	**Mean Growth (mm)***	**Mean Tissue Hg levels (mg/kg)**
1	88	$1.49^{a\,b} \pm 0.39$	0.021
2	96	$1.90^{a} \pm 0.40$	0.018
3	88	$0.43^{d\,e} \pm 0.55$	0.016
4a	76	$1.24^{a\,b\,c} \pm 1.15$	0.11
4b	84	$0.70^{c\,d\,e} \pm 1.11$	0.12
4c	80	$0.70^{c\,d\,e} \pm 0.29$	0.11
5	80	$0.10^{e} \pm 0.08$	0.086
6	100	$1.40^{a\,b\,c} \pm 0.23$	0.13
7	96	$1.02^{a\,b\,c} \pm 0.94$	0.096

* Means with the same letter are not significantly different (Tukeys HSD, α=0.05).

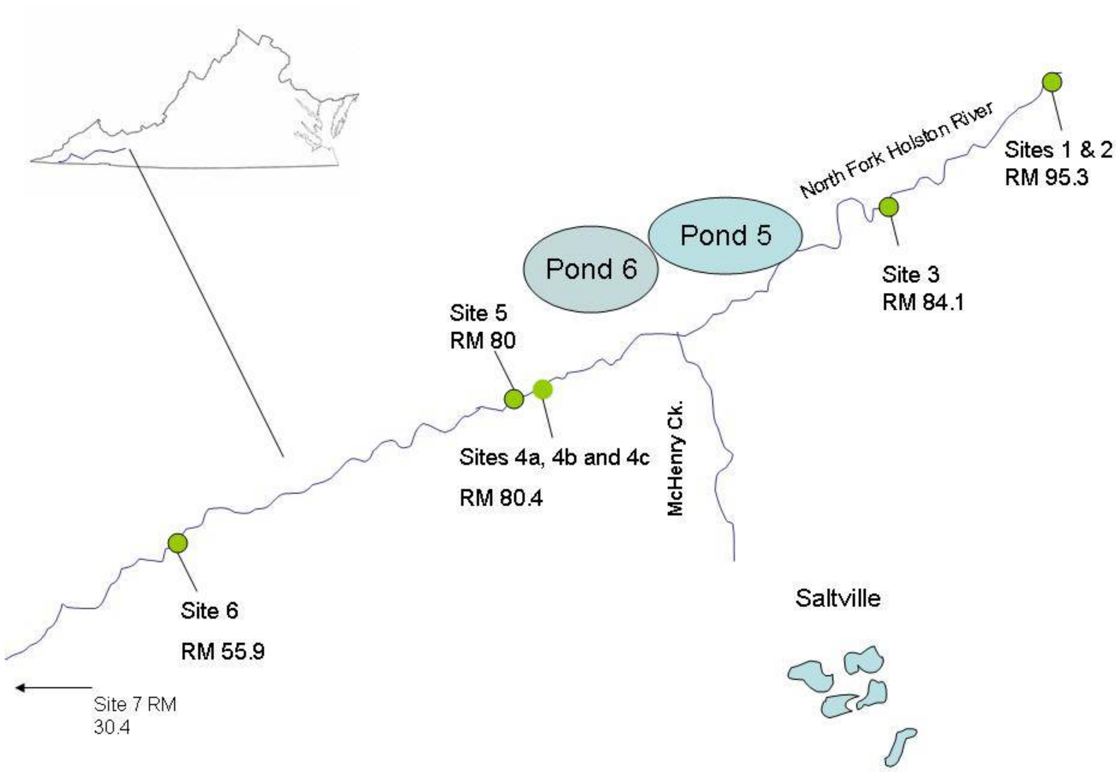

Figure 2.1. North Fork Holston River (NFHR) sampling sites in Smyth and Washington Counties, VA (not to scale) at specific river miles (rm).

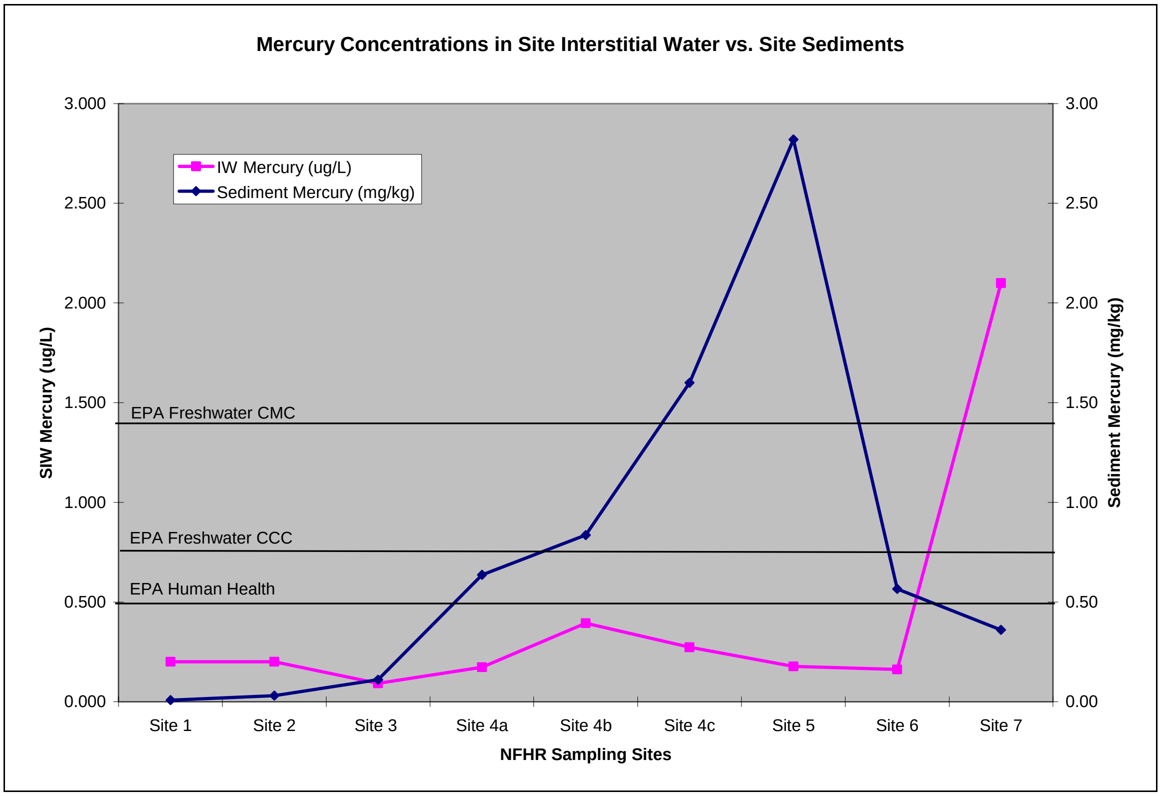

Figure 2.2. Mercury analysis of Site Interstitial Water (SIW) (µg/L) and site sediments (mg/kg) vs. US EPA National Water Quality Criteria for Mercury (US EPA 2006) in the North Fork Holston River above/below Saltville, VA.

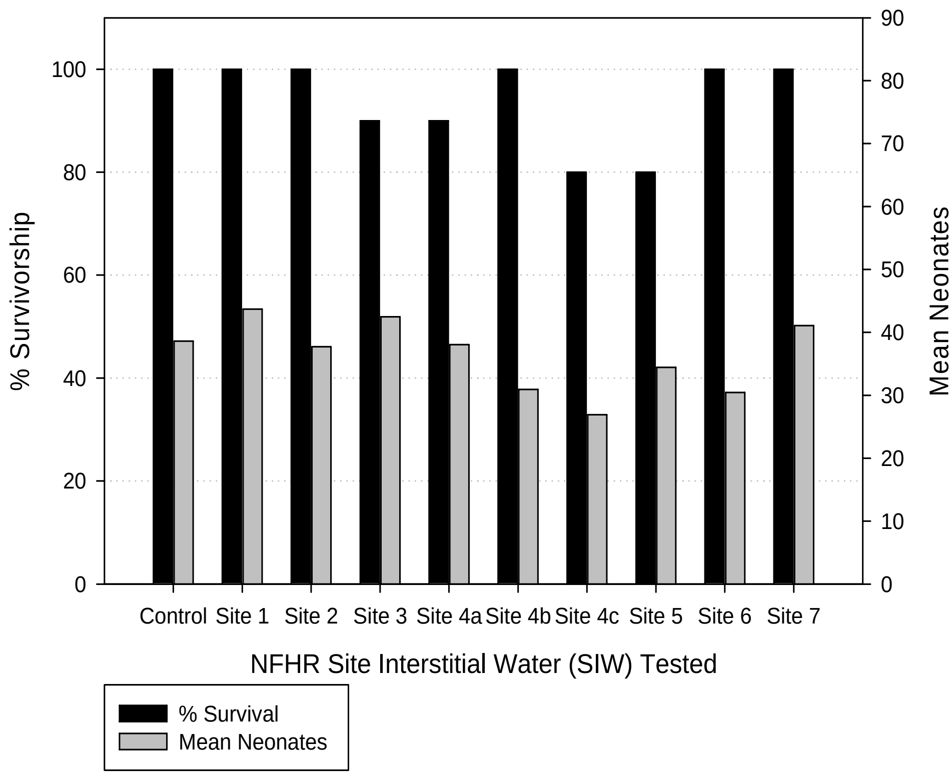

Figure 2.3. *Ceriodaphnia dubia* chronic toxicity test with site interstitial water (SIW), comparing mean neonate production and % survivorship among NFHR sites.

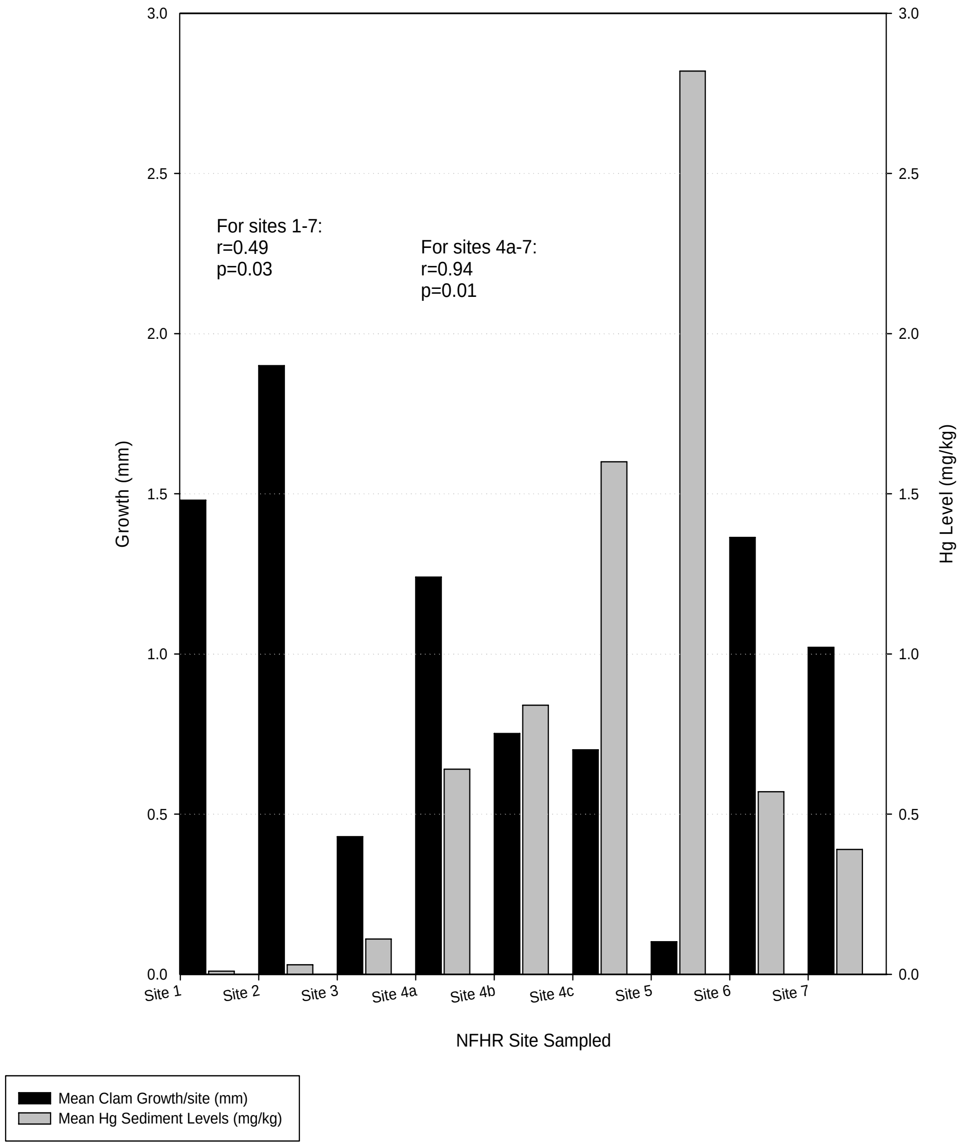

Figure 2.4. Sediment Hg levels vs. Asian clam (*Corbicula fluminea*) mean growth (mm) after 60-d *in situ*.

Chapter 3

Comparison of Laboratory Toxicity Tests from a Point-Source Brine Discharge Using *Ceriodaphnia dubia* and a Juvenile Mussel (*Villosa iris*)

Abstract

The North Fork Holston River (NFHR) in southwestern Virginia has been the subject of many studies, focusing primarily on the extirpation of freshwater mussels species (Bivalvia: Unionidae) due to mercury contamination. Related to that contamination is a point-source discharge, high in conductivity and salt constituents that continuously discharges into the river just below Saltville, VA. Acute and chronic toxicity tests with the brine discharge were conducted using a daphnid, *Ceriodaphnia dubia,* and juveniles of the rainbow mussel, *Villosa iris,* to assess the potential toxicity of the discharge to freshwater mussels and other biota. Acute toxicity of the brine discharge to *V. iris* was minimal with a 96-hr LC50 of 85.2 %, whereas *C. dubia* was more sensitive, with a 48-hr LC50 of 50%. Both organisms were more sensitive, however, to the reference toxicant, NaCl, with LC50 values of 2.2 (*C. dubia*) and 3.2 (*V. iris*) g/L. Chronic toxicity results were similar for *V. iris* and *C. dubia*; Lowest Observable Adverse Effect Concentration (LOAEC) for mussel survivorship after 28 days of exposure was 10,000 µS/cm, while the LOAEC for growth was 5,000 µS/cm. LOAECs for *C. dubia* 7-day survivorship and reproduction were 25 % and 12.5 % brine, respectively, while conductivity averages were 4,054 and 2,211 µS/cm, respectively. The water flea (*C. dubia*) was found to be substantially more sensitive to the discharge in the 7-day chronic reproductive impairment test than the 2-mo old juvenile mussel that was evaluated for growth impairment for 28 days.

3.1 Introduction

The alarming reduction in biodiversity observed in recent years throughout the Tennessee River system has led to numerous field assessments in an attempt to identify causes of current extirpations of vertebrates and invertebrates. Of particular interest is the North Fork of the Holston River (NFHR), renowned for its historic biodiversity, particularly freshwater mussels or unionids (Ortmann 1918, Stansbery 1973). Numerous anthropogenic influences have been documented in the NFHR, including sedimentation, channelization, and contamination from point and non-point sources (Bailey 1974, Hill et al. 1980, Carter 1977, Lindsey and Dimmick 1983). One possible source of contamination is a brine discharge that continuously discharges into the NFHR just below Saltville, VA.

Although the potential instream effects from the brine discharge are compounded by other influences in the NFHR, the discharge itself contributes high (4,500 -13,500 mg/L) total dissolved solids (TDS), which is currently a pollutant of concern for the Virginia Department of Environmental Quality (VA DEQ) and the United States Environmental Protection Agency (US EPA). Currently there are no national water quality criteria for TDS (US EPA 1999), but many states have begun establishing Total Maximum Daily Loads (TMDLs) to regulate the permitted discharge of effluents high in TDS. The TDS is a measure of cationic and anionic constituents dissolved in the water, but it is often expressed in conjunction with specific conductivity (µS/cm). Specific conductivity measures how easily water can conduct electricity, which is dependent upon dissolved anions and cations. Therefore, the relation of TDS and conductivity are not always constant, but a reliable estimate of TDS can be derived by the conductivity measurement using a basic formula (Kennedy et al. 2005), whereby TDS (mg/L) = Conductivity (SC)-50 x 0.74.

High conductivity (~ 3,700 µS/cm) values have been positively correlated with *in situ* Asian clam (*Corbicula fluminea*) growth and benthic impairment in field assessments (Kennedy et al. 2003). Low fecundity in laboratory chronic tests with *Ceriodaphnia dubia* also was highly correlated with elevated specific conductivity, with a Lowest

Observable Adverse Effect Concentration (LOAEC) for reproduction at approximately 3,700 µS/cm and a chronic value of 3,286 µS/cm (Kennedy et al. 2003, 2005).

Conductivity in the brine discharge at Saltville has been measured as high as 18,000 µS/cm (Cherry unpublished data), although the normal average is ~10,000 µS/cm. Dissipation usually occurs quickly under normal flow conditions, but during summer lower flows, elevated conductivity has been observed for many (~ 52.4) river miles downstream of the point-source discharge, with the potential to affect biota.

The Town of Saltville, appropriately named the Salt Capital of the Confederacy, has a long, colorful history relating to the presence of salt deposits in the area (Turnage 1994). Brine originates as the overflow from a series of salt ponds located in Saltville that were developed to contain the outflow of high saline groundwater from a series of underground salt mines. The overflow from the ponds is channeled through an underground culvert approximately 1.5 km to an outflow site in the NFHR, approximately 1.6 km above the old Olin Corporation settling pond # 5.

US EPA recommended standard test organisms for Whole Effluent Toxicity (WET) testing, such as *C. dubia,* may not be sufficient in predicting in-stream effects of the discharge to resident biota (Chapman 2000). It was therefore necessary to use more than one organism to test the brine discharge. The purpose of this study was to assess potential toxicity with laboratory testing of the brine discharge, using two aquatic organisms. More specifically, our objective was to determine whether the high conductivity and related TDS were factors contributing to the inhibition of survival and mussel recruitment downstream of Saltville, VA, by utilizing juvenile mussels in these toxicity tests.

3.2 Materials and Methods

3.2.1 Brine Discharge

The point-source, brine discharge was collected directly from the culvert just before it entered the NFHR. The culvert measured approximately 50 cm in diameter and discharged into a small channel that flowed along the left bank of the river. Flow measurements for the discharge ranged from 0.40 to 0.57 m/sec. The brine discharge was collected in clean acid-washed, 20-L carboys, and transported in coolers on ice (<4° C) to the laboratory at Virginia Tech. For toxicity testing, carboys of brine were collected on three occasions so that no single carboy was used for more than two changeovers, as outlined by US EPA WET testing protocols (US EPA 2002a, b). An unfiltered sample of the brine discharge was sent to Severn-Trent Laboratories, Savannah, GA, for analysis of trace metal composition as well as inorganic constituents. Analytical methodology followed protocols outlined by the US EPA (SW 8846 1986, MCAWW 1983).

3.2.2 Test Organisms

Juveniles of *Villosa iris* were obtained from the Freshwater Mollusk Conservation Center (FMCC) at Virginia Tech (Blacksburg, VA, USA), where they had been reared from glochidia (Zale and Neves 1982) to the juvenile stage of development. Juvenile mussels were maintained in circulating troughs with sediment (150 µm) and fed a daily diet of *Neochloris oleoabdundans* (3 X 10^7 cell/L) until they were ~ 2.5 mo of age.

Ceriodaphnia dubia were cultured at the Virginia Tech Aquatic Ecotoxicology Laboratory according to US EPA standard methods (2002a). Moderately-hard, synthetic water (EPA^{100}) was used as the culture medium. Cultures were fed a daily mixture of the green algae, *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), and Yeast-Cereal Leaves-Trout Chow (YCT). Neonates less than 24 hr old were removed from individual culturing beakers and placed in a 250-ml beaker with EPA^{100} until test initiation.

3.2.3 Acute Toxicity Tests

3.2.3.1 Villosa iris

A 96-hr acute toxicity test was conducted using juveniles of the rainbow mussel to determine toxicity of the point-source brine discharge. A subsample of approximately 50 juvenile mussels (~2.5 mo) were pipetted into a glass Petri dish, and observed under magnification (x 40) for viability. Mussels were observed for ~ 10 min for pedal movement. Live mussels were pipetted into the appropriate test concentration. Static acute toxicity tests followed ASTM protocols (2005) for conducting such tests with freshwater mussels. Test concentrations were based on a 0.5 serial dilution, with filtered Sinking Creek water (Sinking Creek, Newport, VA) as the diluent. Sinking Creek water has been used a reference and culturing water in our laboratory for 25 yr. Each test concentration had four replicates with five mussels each. Juvenile mussels were not fed during the first 48 hr of testing, a standard practice in acute testing; however, mussels were fed at 48 hr with 0.40ml/50ml of *Neochloris oleoabundans*. Tests were conducted in a Fisher Isotemp® Low Temperature Incubator (25± 1° C, Fisher Scientific, Pittsburg, PA, USA). After 48 hr, organisms were gently pipetted into a Petri dish and observed for survivorship under magnification. A mussel was considered dead if it was gaped and failed to close after gentle prodding, or ciliated activity was not observed. Results were recorded, and living organisms were gently placed back into their respective beakers. This process was repeated at 72 hr and at test termination (96 hr). General water chemistry was analyzed at the beginning of the test (in-water) for temperature (° C), dissolved oxygen (DO_2 mg/L), conductivity (µs/cm) and pH. Alkalinity and hardness (mg/L $CaCO_3$) were measured by titration following protocols outlined in APHA et al. (1995). Temperature, DO_2, conductivity, and pH also were analyzed at the end of the test (out-water). Lethal concentration (LC50) values were generated using US EPA Trimmed Spearman Karber software (US EPA 1993).

In addition to the brine acute toxicity test, a 48-hr acute reference toxicant test (NaCl) with *V. iris* was conducted simultaneously. Reference toxicity tests are used as a quality assurance/quality control (QA/QC) component in US EPA whole effluent toxicity (WET) testing to assess health of test organisms (US EPA 2002a, b). Although WET testing does not currently apply to freshwater mussels, recent research advocates the application of reference testing with freshwater mussel bioassays (Valenti et al. 2006). Test concentrations of NaCl were based on those used in *C. dubia* reference testing, ranging from 0.50-8.0 g NaCl/L.

3.2.3.2 Ceriodaphnia dubia

Static, non-renewal acute toxicity tests were conducted with *C. dubia* during spring 2006, following US EPA protocols (2002a). Both brine discharge and NaCl were tested simultaneously. Tests were conducted in 50-ml glass beakers, filled with ~ 40 ml of test solution and maintained in the incubator at 25 ± 1° C. Moderately-hard, synthetic water (EPA^{100}) was used as the diluent. Test organisms were checked for survivorship at 24 hr and at test termination after 48 hr. Results were recorded, and Trimmed Spearman-Karber (US EPA 1993) LC50 values were calculated. The methodology for NaCl reference tests was similar to that outlined above. A 0.5 serial dilution was used to prepare test concentrations, which ranged from 0.5 to 8.0 g NaCl/L.

3.2.4 Chronic Toxicity Tests

3.2.4.1 Villosa iris

Sensitivity of juveniles of *V. iris* to the brine discharge was tested over a 28-day period in March, 2006. Mussels were obtained from the FMCC at VA Tech and test procedures followed protocols outlined in ASTM (2005). Test chambers were modeled after simulated lotic microcosms as described in Valenti et al. (2005). The simulated microcosms consisted of five glass vials (outside diameter x height= 25 x 15 mm) placed into a glass Petri dish resting on two inverted glass, 50-ml beakers, housed in a 1-L glass beaker. Each vial contained ~ 2 ml of fine sediments (< 200 µm) as an additional food source and substrate. Each 1-L beaker represented a concentration, while each glass vial (test chamber) was a replicate (Fig. 3.1). Tests were conducted in a temperature-controlled room (21 ± 2° C), and the beakers aerated using a glass pipette which also created a circular motion to simulate flow.

As with juveniles used in the acute toxicity tests, a subsample of organisms was placed onto a Petri dish and observed under magnification (x 10) for viability. Initial measurements of shell length were taken using an ocular micrometer and converted to millimeters, and then five mussels were individually pipetted into their respective vials. Treatment concentrations were based on conductivity, not a straight serial dilution. The brine discharge at 100% strength had a conductivity of 14,000 µS/cm, a level much higher than that which was acutely toxic; therefore, the highest concentration of the brine effluent was diluted to a conductivity of 10,000 µS/cm. Lower concentrations were established by diluting the brine to conductivities of 5,000, 2,500, 1,200 and 625 µS/cm. Diluent used for this test was unfiltered NFHR water, collected from an upstream location. Organisms were fed a daily mixture (3ml/L) of *Nannochloropsis* and Shellfish Diet (Instant Algae®). Water was renewed every third day by siphoning and replacing 90 % of the test water. Water chemistry was analyzed for the renewed water (in-water) and out-water after each changeover. Parameters included conductivity (µS/cm), DO_2 (mg/L), pH (su) and temperature (°C). After 28 days, organisms were removed and assessed for survivorship and growth. Survivorship was determined by assessing pedal movement or ciliated activity. Statistical analysis was performed using TOXSTAT® Statistical Software (Gulley 1996).

3.2.4.2 Ceriodaphnia dubia

A chronic toxicity test with *C. dubia* was used to evaluate the potential toxicity of the brine point-source discharge originating from the salt ponds in Saltville, VA. Chronic tests followed US EPA protocols (US EPA 2002b), with five concentrations and a control. Concentrations were based on a 0.5 dilution series, with 100 % salt-brine discharge being the highest concentration. Moderately-hard, synthetic lab water (EPA^{100}) was used as the diluent and control water. Ten replicates with one individual each (50 ml beakers) were used per concentration. The test was conducted in a Fisher Isotemp® Low Temperature incubator (25 ± 1° C, Fisher Scientific, Pittsburg, PA, USA). Water renewal was conducted daily, and survivorship and reproduction of organisms were recorded. Organisms were fed a daily mixture of *P. subcapitata* and YCT (0.40ml/50 ml). Conductivity (µS/cm), DO_2 (mg/L), pH (su) and temperature (° C) were measured for in-water and out-water after each changeover, while alkalinity and hardness were measured by titration (APHA 1995) on the highest concentration and control for each batch of renewal water. No Observable Adverse Effect Concentration (NOAEC) and Lowest Observable Adverse Effect Concentration (LOAEC) were calculated for survivorship and reproduction, using TOXSTAT® Statistical Software (Gulley 1996).

3.3 Results

3.3.1 Brine Discharge Analysis

An unfiltered sample of the brine discharge was analyzed at Severn-Trent Laboratories, Savannah, GA in March, 2006 to determine ionic makeup of the discharge. Chloride was the most prevalent constituent at 4,600 mg/L, followed by sodium (3, 200 mg/L, Table 3.1). Although no WQC exists for sodium, chloride was substantially higher than the acute (CMC) and chronic (CCC) limits (830 and 260 mg/L). Calcium, potassium and magnesium also were high, with levels of 400, 7.6 and 40 mg/L, respectively. Specific conductivity and TDS also were high, at 17,000 µS/cm and 9,000 mg/L.

3.3.2 Acute Toxicity Testing

3.3.2.1 Villosa iris

No mortality was observed in the control or concentrations of 6.25-50 % for the duration of the brine acute toxicity test with *V. iris* (Table 3.2). After the initial 48 hrs, minimal mortality (45 %) was observed in the 100 % concentration. At the 72-hr interval, mortality increased to 65 %, and remained until test termination at 96 hr. The 72 and 96-hr LC50 values were the same at 85.2 % brine.

The NaCl reference test with *V. iris* was conducted using juvenile mussels. The test ran for 48 hr, after which mussels were observed for survivorship. All organisms were alive in the control, 0.5, and 1.0 g NaCl/L concentrations. One mussel was dead in the 2.0 g/L concentration, and 15 were dead in the 4.0 g/L concentration (25 % survivorship). All mussels died in the highest concentration, 8.0 g/L (conductivity of 14,280 μS/cm). The calculated 48-hr LC50 value was 3.25 g NaCl/L (Table 3.2).

3.3.2.2 Ceriodaphnia dubia

No mortality was observed in the control or concentrations of 6.25-50 % brine after the first 24 hr of exposure. All organisms in the 100 % concentration were dead at 24 hr. After 48 hr, all organisms were alive in the control and concentrations of 6.25-25 % brine effluent. Fifty percent mortality occurred at the 50 % concentration, resulting in a 48-hr LC50 value of 50 % brine (Table 3.2).

After 24 hr, no mortality was observed in the control or 0.5, 1.0 and 2.0 g/L concentrations of the NaCl reference acute test. All daphnids were dead in the two highest concentrations of 4.0 and 8.0 g/L (Table 3.2). After 48 hr, no mortality was observed in the control, 0.5, or 1.0 g/L concentrations; however, some mortality (25 %) occurred in the 2.0 g/L concentration, resulting in a 48-hr LC50 value of 2.22 g NaCl/L.

3.3.3 Chronic Toxicity Testing

3.3.3.1 V. iris

Chronic toxicity of the brine discharge to *V. iris* was tested over a 28-day period. No mortality was observed in the control, and only minimal mortality was observed in the 625-5,000 μS/cm concentrations (Table 3.3). All organisms in the 10,000 μS/cm concentration were dead at the termination of the test. The NOAEC for survivorship was 5,000 μS/cm while the LOAEC was 10,000 μS/cm.

Growth was the more sensitive endpoint for *V. iris,* as the NOAEC and LOAEC were 2,500 and 5,000 μS/cm, respectively. Growth was highest in the controls and fluctuated between a low of 0.172 mm at 625 µS/cm and 0.204 mm at 1,200 µS/cm, and then declined consistently at 2,500 µS/cm and thereafter in the 5,000 and 10,000 µS/cm concentrations (Table 3.3). Change in growth values was significantly different between test concentrations of 10,000 and 5,000 µS/cm compared to 2,500 µS/cm and lower, (p= 0.0005, NPAR1Way ANOVA).

3.3.3.2 C. dubia

Survivorship impairment was observed in the two highest concentrations (50 and 100 %) of brine discharge after the initial 24 hr of testing. All daphnids died in the 100 % concentration, while there was 70 % mortality in the 50 % concentration after 24 hr. All daphnids died in the 50 % concentration after 48 hr. No mortality occurred in any other test concentration or in the control. The LOAEC for survivorship was 50 %, with an NOAEC of 25 % (Table 3.4). Conductivity ranged from 14,300 µS/cm in the 100% concentration to 1,281 µS/cm in the 6.25 % concentration. Correlation analysis using simple linear regression indicated a strong relationship between conductivity and daphnid survivorship (p=0.02, SigmaStat); as conductivity increased, survivorship decreased.

Reproduction was the more sensitive endpoint in this test, with an NOAEC of 12.5 % and LOAEC of 25 %. Mean neonate production ranged from 24.8 in the 6.25 % concentration to12.6 neonates in the 25 % concentration, and 18.0 in the control (Table 3.4). Linear regression of conductivity and mean fecundity identified a significant positive correlation between the two variables (p=0.03, SigmaStat).

3.4 Discussion

The brine discharge presented an excellent opportunity to evaluate a high conductivity/high TDS discharge into a freshwater system without the interference of low pH and high metals, found in mining effluents which are more common sources of high conductivity and TDS. Kennedy et al. (2003, 2004 and 2005) studied the effects of a coal mining discharge high in conductivity (~8,000 µS/cm) and TDS (~6,000 mg/L) on *Ceriodaphnia dubia* and *Isonychia bicolor*. Reproductive impairment to *C. dubia* occurred at 3,700 µS/cm (2,701 mg/L), while 7-d tests with *I. bicolor* resulted in an LOAEC for survivorship of 1,562 µS/cm (1,119 mg/L).

Several other researchers have examined the legitimacy in using standard test organisms such as *C. dubia* in whole effluent testing to predict toxicity to resident species

(Diamond and Daley 2000, Chapman et al. 2000, Kennedy et al. 2004). Overall test results indicate that such laboratory tests fail to correspond with biological assessments in the field, unless the effluent being tested comprised > 80% of stream flow under designed conditions (Diamond and Daley 2000). For the purpose of our testing, surrogate test organisms were chosen to represent freshwater mussels, the taxon of particular concern in the NFHR.

Results of the acute toxicity tests indicated that the brine was substantially more toxic to *C. dubia* than to *V. iris*. After 48 hr, total mortality was observed in the 100% concentration of the daphnid test; however, 100 % mortality did not occur in the mussel test even after 96 hr of exposure. Reference acute toxicity tests with these two species indicated that *V. iris* also was more tolerant than *Ceriodaphnia*, suggesting that the mussel may be less predictive of toxicological consequences than *C. dubia*.

Chronic test results were similar for both *V. iris* and *C. dubia* (Table 3.5). After 28 days of exposure to brine at the highest conductivity of 10,000 µS/cm, all mussels were dead. Only minimal mortality (8 %) was observed in the 5,000 µS/cm test concentration. It would have been more conclusive to have a concentration equivalent to 75% brine, whereby conductivity would have fallen between 5,000 and 10,000 µS/cm, in order to fine-tune a survivorship threshold of mussels to conductivity. Growth was the more sensitive endpoint for the test, with an NOAEC much lower at 2,500 µS/cm or 25 % brine.

Both *V. iris* and *C. dubia* were substantially more sensitive to NaCl in the reference toxicity tests than the brine discharge. After 48 hr of exposure, 100 % mortality of *V. iris* occurred in the highest concentration (8 g NaCl/L) where conductivity ranged from ~ 14,000-17,000 µS/cm, as well as in the test concentrations of 2 (5 %) and 4 (75 %) g/L (Table 3.2). However, mortality for *V. iris* was minimal in the brine test at concentrations with similar conductivity values. In the brine test, no 48-hr LC50 was generated for *V. iris*, while the NaCl test resulted in a 48-hr LC50 of 3.2 g/L. *Ceriodaphnia* were somewhat more sensitive to the NaCl than *V. iris* with a 48-hr LC50 of 2.2 g NaCl/L. Conversely, previous studies with *V. iris* glochidia and *C. dubia* indicate that the mussel is a more sensitive test organism than the daphnid (Cherry et al. 2002). Several studies have reported the glochidial stage of several unionid species, including *V. iris,* to be more sensitive than juveniles to various toxicants (Keller and Ruessler 1997, Augspurger et al. 2003, Valenti et al. 2005).

The varying degree of toxicity between NaCl and the brine discharge is possibly due to the difference of pure sodium chloride vs. the brine discharge with multiple constituents. The brine discharge is made up of various ions of sodium, chloride, sulfate and calcium, not all of which are at acutely toxic levels. Sodium and chloride were the most prevalent ions in the discharge, with high levels of 3,200 and 4,600 mg/L (Table 3.1). Although no WQC currently exist for sodium, the chloride level was five times higher than the recommended limit (US EPA 1999). Calcium, potassium and manganese also were at high levels, but no WQC exist for these ions either. The TDS for this sample was 9,000 mg/L while conductivity was 17,000 µS/cm.

3.5 Summary and Conclusions

The results of this study indicated that the brine discharging into the NFHR below Saltville, VA could be a potential cause of biotic impairment downstream. Acute toxicity to juvenile mussels was minimal, with no significant mortality after the initial 48 hr; however, an LC50 value (85.2 %) was generated after 72 hr. *Ceriodaphnia* were more acutely sensitive, with an LC50 value of 50 % after 48 hr. Chronic tests with *V. iris* were more conclusive, indicating growth impairment in the 5,000 µS/cm concentration after 28 days. Results of the *C. dubia* chronic test were similar after only 7 days of exposure, with growth and reproduction being impaired at a conductivity of ~ 4,000 µS/cm. Linear regression of conductivity and *C. dubia* fecundity was positively correlated ($p=0.03$, SigmaStat), while differences in growth for *V. iris* were significantly different at concentrations of 10,000 and 5,000 µS/cm from lower test concentrations ($p=0.0005$, NPAR1Way ANOVA).

Although results of this research indicate that the brine discharge may be having a chronic effect on unionids in the NFHR, further laboratory tests and field studies must be conducted to substantiate this claim. In addition, toxicity tests with mussel glochidia may be useful in predicting the toxicological effects that the brine discharge is having on the earlier, more sensitive life stage. Toxicity tests with *Isonychia bicolor* or another mayfly such as *Maccaffertium spp*. may also be beneficial in assessing the effects of the discharge on the benthic community downstream.

3.6 Acknowledgments

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Table 3.1. Ion concentrations from an unfiltered sample of the brine discharge, collected in March 2006 with chronic and acute WQC for the protection of aquatic life.

Brine Discharge			US EPA WQC [a]	
Parameter	Result (mg/L)	Reporting Limit (mg/L)	Acute (CMC) (mg/L)	Chronic (CCC) (mg/L)
Aluminum	0.18	0.2	0.750	0.087
Calcium	400	0.5	N/A	N/A
Chromium	0.01	0.01	0.016	0.011
Copper	0.02	0.02	0.095	0.053
Iron	0.23	0.05	N/A	1.0
Potassium	7.6	1.0	N/A	N/A
Magnesium	30.0	0.5	N/A	N/A
Manganese	0.029	0.01	N/A	N/A
Lead	0.005	0.005	0.65	0.0025
Zinc	0.004	0.02	0.12	0.12
Mercury	0.0002	0.0002	0.0014	0.00077
Sodium	3,200	100	N/A	N/A
Chloride	4,600	100	860	230
Nitrogen/Nitrate	0.66	0.05	N/A	N/A
Phosphorus	0.10	0.10	N/A	N/A
Sulfate	810	200	N/A	N/A
TDS	9,000	5.0	N/A	N/A
Conductivity	17,000 µS/cm	50 µS/cm	N/A	N/A

[a] US EPA 1999

Table 3.2. Forty-eight hour acute toxicity for *Ceriodaphnia dubia* and juveniles of *Villosa iris* to a laboratory reference toxicant (NaCl) and brine discharge (BD).

Test Solution	**Test Organism**	**48-hr LC50**	**Maximum Conc. Tested**	**Conductivity µS/cm (in-out)**	**pH (su) (in-out)**	**Max. Conc. Survival % in hours**			
						24	**48**	**72**	**96**
NaCl	*C. dubia*	2.2 g/L	8 g/L	14,650-16,580	7.80-8.15	0	--	--	--
NaCl	*V. iris*	3.2 g/L	8 g/L	14,280-17,430	7.83-8.03	100	0	--	--
BD	*C. dubia*	50.0 %	100 %	9,870-9,790	8.12-8.25	0	--	--	--
BD	*V. iris*	>100 %[a]	100 %	9,870-11,290	8.12-8.26	100	55	35	35

[a] 72-hr LC50= 85.2 %

Table 3.3. Twenty-eight day brine toxicity test results with *Villosa iris*. Values represent mean change in growth over 28 days.

Concentration/ Conductivity (µS/cm)	Chamber	Initial Length (mm)	Final Length (mm)	Change in Length (mm)	Mean Growth per Conc.	% Survival
Control	A	1.26	1.46	0.20		
	B	1.22	1.44	0.22		
	C	1.24	1.48	0.24		
	D	1.10	1.30	0.20		
	E	1.22	1.48	0.26	0.224	100
625	A	1.20	1.40	0.20		
	B	1.14	1.36	0.22		
	C	1.20	1.38	0.18		
	D	1.16	1.44	0.28		
	E	1.18	1.34	0.16	0.172	96.0
1,250	A	1.10	1.30	0.20		
	B	1.14	1.32	0.18		
	C	1.18	1.44	0.26		
	D	1.12	1.32	0.20		
	E	1.24	1.42	0.18	0.204	96.0
2,500	A	1.24	1.42	0.18		
	B	1.16	1.36	0.20		
	C	1.10	1.26	0.16		
	D	1.10	1.32	0.22		
	E	1.28	1.44	0.17	0.185	96.0
5,000	A	1.18	1.24	0.06		
	B	1.24	1.28	0.04		
	C	1.16	1.22	0.06		
	D	1.20	1.32	0.12		
	E	1.14	1.20	0.06	0.068[a]	92.0
10,000	A	1.18	1.20	0.02		
	B	1.06	1.06	0.00		
	C	1.12	1.14	0.02		
	D	1.22	1.24	0.02		
	E	1.16	1.16	0.00	0.012[a]	0[a]

[a] denotes significant differences-Wilcoxon's Rank Sum Test w/Bonferroni Adjustment.

Table 3.4. *Ceriodaphnia dubia* 7-day chronic toxicity data with the brine discharge.

Test Concentration	Mean Conductivity	Percent Survivorship			Mean Neonates	NOAEC		LOAEC	
	(µS/cm)	24 hr	48 hr	7 days		Surv.	Reprod.	Surv.	Reprod.
Control	273	100	100	100	18.0				
6.25	1,281	100	100	100	24.8				
12.5	2,211	100	100	100	23.7		12.5 %		
25	4,054	100	100	100	12.6[a]	25 %			25 %
50	7,455	30	0	0[a]	0.0[a]			50 %	
100	14,300	0	0	0[a]	0.0[a]				

[a] denotes significant differences from the control.

Table 3.5. Comparison of chronic NOAEC and LOAEC endpoint values for reproduction (*Ceriodaphnia dubia*) and growth (*Villosa iris*).

***C. dubia* Chronic**				***V. iris* Chronic**		
Test Conc. (%)	**Mean Cond. (μS/cm)**	**Reproduction**		**Test Conc./ Cond. (μS/cm)**	**Growth (mm)**	
		NOAEC	**LOAEC**		**NOAEC**	**LOAEC**
0	273			**0**		
6.25	1,281			**625**		
12.5	2,211	**12.5 %**		**1,250**		
25	4,054		**25 %**	**2,500**	**2,500**	
50	7,455			**5,000**		**5,000**
100	14,300			**10,000**		

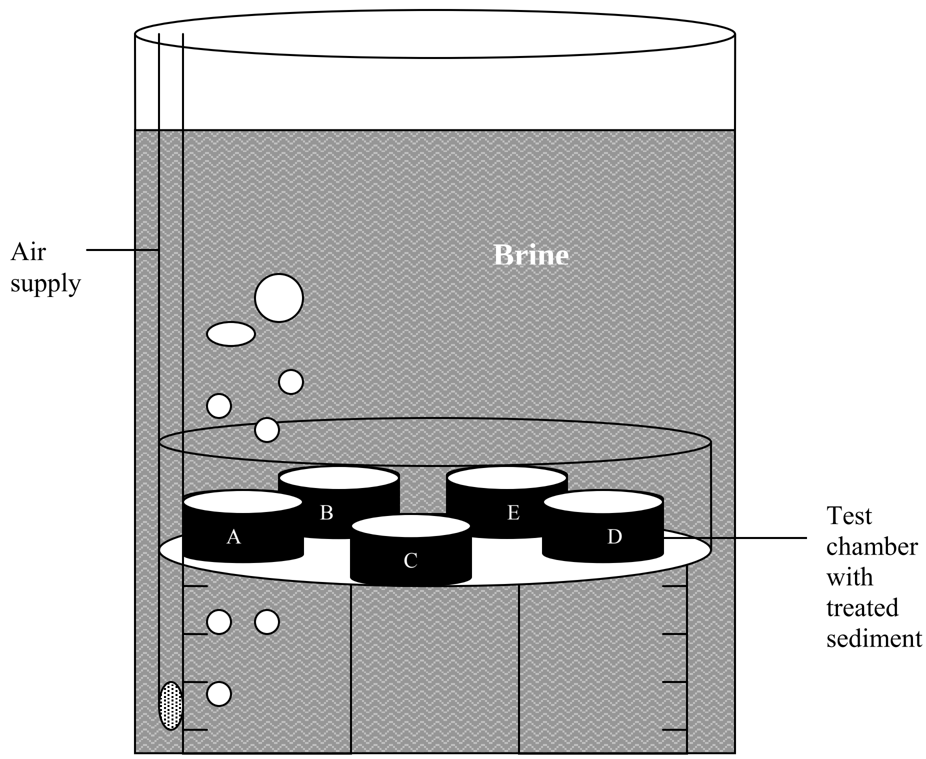

Figure 3.1. Testing apparatus for juvenile mussels in the 28-day brine chronic toxicity test.

<u>Chapter 4</u>

Evaluation of Conductivity Dissipation in the North Fork Holston River Downstream of a Point Source Brine Discharge during Severe Low-Flow Conditions.

Abstract

Previous studies of the North Fork Holston River (NFHR) attribute the decline of freshwater mussels (Unionidae) to mercury contamination from a chlor-alkali plant operated by Olin Corporation, formerly located in Saltville, Virginia. However, it is necessary to consider all potential sources of contamination. An additional source of pollution maybe a point-source brine discharge, high in chlorides and sodium. During recent sampling, conductivity ranged from 5,900-10,930 μS/cm, but has been measured as high as 18,000 μS/cm during previous sampling events. The discharge enters the NFHR at river mile (rm) 82.3, and conductivity dissipates rapidly downstream during normal flow conditions; however, severely low flow conditions in 2005 impaired dissipation downstream. Conductivity in the immediate mixing zone (5-m) diminished to 1,160-2,360 μS/cm, while continued dissipation downstream varied. As the drought conditions persisted through September into October 2005, conductivity in the river (500-690 μS/cm) remained above background level (~345 μS/cm) 0.5 mi downstream. It was also apparent that Olin ponds 5 and 6 were contributing to a conductivity spike in the river, observed at rm 81.6 and 80.4. Benthic macroinvertebrate surveys were inconclusive in determining a direct effect of conductivity influxes on aquatic biota. All benthic metrices were statistically significant according to site, except for Taxa Richness ($p=0.056$). EPT abundance was not a sufficient parameter in determining stress due to the increase in hydropsychid caddisflies, which were abundant at the two sites below the discharge (Sites 3 and 4). Mayflies and stoneflies were present at upstream and downstream sites, but caddisflies were the predominant EPT taxa (~90 %) at these sites. EPT abundance minus Hydropsychidae was the most accurate parameter measured. When hydropsychids were removed from analysis, EPT abundance was significantly higher upstream at Sites 1 and 2 (255.75 and 297.25, respectively) and lowest at downstream Sites 4 and 5 (36.0 and 23.75). Overall, the results suggest that the brine discharge may be an additional stressor of concern in the NFHR, especially under low flow conditions. Although the discharge itself may not be the primary cause of toxicity affecting mussel recruitment downstream, a potential synergistic effect between the

saline discharge and mercury residuals in the river may increase the toxicological potential.

4.1 Introduction

In order to protect or remediate a watershed, it is important to understand the anthropogenic influences affecting that system. In most watersheds, common sources of point and nonpoint pollution have been identified and are usually associated with common land-use practices such as agriculture, coal-mining and industry. However, less obvious sources of contamination also can cause deleterious effects on aquatic systems and must be identified and assessed before biotic systems can be managed.

The North Fork Holston River in southwestern Virginia has been the subject of numerous studies, which were mainly concerned with mercury contamination from a chlor-alkali plant operated in Saltville, VA for nearly a century (Stansbery 1972, Hill et al. 1980, YMA 1990, Dye 1999, Henley and Neves 1999, Brown et al. 2005). The primary focus of these studies examined the deteriorating freshwater mussel (Unionidae) populations downstream. Freshwater mussels were once prevalent in the NFHR basin, but have been decreasing in numbers since industrial development began in the late 1800's.

Echols et al. (2007) identified potential toxicity resulting from residual mercury contamination in the river below the town of Saltville. A second contributing factor may be the high conductivity/high TDS brine discharge originating from salt ponds within the town limits. Saline discharges into freshwater systems like in the NFHR are rare in this country, and therefore, toxicological effects have not been well studied. Other countries, such as Australia, are dealing with increasing salinity issues (Williams 1987). Numerous research endeavors have examined tolerances of freshwater organisms to increased salinity (Berezina 2003, Kefford et al. 2003, Kefford and Nugegoda 2005, Nielson et al. 2003 and Zalizniak et al. 2006). Researchers in the United States, however, have been more focused on effluents associated with coal mining (Kennedy et al. 2003, Meyer et al. 1985, Masnado et al. 1995, Chapman 2000), industry and irrigation drain waters (Dickerson et al. 1996), known to be high in dissolved solids and conductivity, both pollutants of concern. Kennedy et al. (2003, 2004 and 2005) studied the effects of a coal mining discharge with high conductivity and TDS on freshwater biota in the Leading Creek Watershed, Ohio.

The objectives of this research were to develop an understanding of how the brine discharge dissipates in the river and how influxes of high conductivity affect benthic macroinvertebrate fauna downstream. Fortunately, the year 2005 presented a unique opportunity to examine these effects during a worst-case-scenario situation, whereby river discharge was at one of the lowest levels observed in the NFHR over the past 86 years of United States Geological Survey (USGS) streamflow data. In addition to studying conductivity dissipation in the river, analytical characterization of the brine discharge was conducted to better understand what constituents were contributing to the high conductivity.

4.2 Materials and Methods

4.2.1 Site Selection

4.2.1.1 Conductivity Dissipation

Initial sampling locations for conductivity measurements were based upon dissipation sampling records from 2002 by Dr. Don Cherry (Cherry unpublished data). Measurements for conductivity were taken ~ 5 m above the salt discharge, directly from the discharge, and downstream in increasing increments (5 m, 10 m, 20 m, 40 m, etc.) to 640 m below the discharge. As drought-like conditions persisted through the fall of 2005, an additional six sites were added. The furthest downstream site was located near Weber City, Scott County, VA at approximately river mile 13.5, 68.8 river miles below Saltville.

4.2.1.2 Benthic Macroinvertebrate Surveys

Benthic macroinvertebrates were sampled at seven locations in the North Fork Holston River (Table 4.1). Sampling locations were based upon conductivity measurements and sampling feasibility. One reference location was included upstream of Saltville, VA at river mile (rm) 85, approximately 10 m downstream of the United States Geological Society (USGS) gauging station. The second sampling station (Site 2) was located at rm 84.1. Site 3 was located above the remediated Olin Pond 5 (Superfund site) at rm 82.3, which is approximately half a mile downstream of the brine discharge (rm 82.8). Site 4 was located at rm 81.6, at Henrytown Rd, where the road used to extend across the river between Ponds 5 and 6. Site 5 was located at rm 80.0, while Sites 6 and

7 were located a substantial distance downstream at river miles 55.9 and 30.4, respectively.

4.2.2 Salt/Conductivity Dissipation

From September 8 to November 9, 2005, eight conductivity sampling trips were carried out in the NFHR at Saltville and downstream to Weber City, Scott Co, VA. In some cases, conductivity measurements were taken on both sides of the river where accessible. From a preliminary investigation in 2002, before this project developed, D. Cherry conducted a river conductivity reconnaissance from the Saltville brine pond discharge to just above Olin Pond 5 area (rm 82.3) and found the conductivity to dissipate to a background level of 325 μS/cm when the brine discharge was 6070 μS/cm. This background experience was used as a marker for the 2005 effort, which began on 9/8/2005. Dissipation was determined simply by walking along the edge of the river, below the discharge, with the conductivity meter and obtaining measurements at various intervals (5, 10, 20 m, etc.) downstream.

4.2.3 Benthic Macroinvertebrate Surveys

Benthic macroinvertebrate surveys were conducted at seven sites in the NFHR on 10/16/2005 (Table 4.1). Sampling adhered to US EPA Rapid Bioassessment Protocols (Barbour et al. 1999). Riffle, run, pool and shoreline habitats were sampled and four replicates were collected at each site using 800 μm mesh dipnets. Specimens collected were transferred into 1-liter plastic jars and preserved in 95 % ethyl alcohol. Sample jars were labeled and transported back to the laboratory for processing.

Samples were sorted in metal pans by separating the insects from sediment and other debris. Each sample was sorted completely until no organism was observed in a five-minute period. Organisms were identified to lowest practical taxonomic level and community indices determined using the RBP tier III approach and standard taxonomic keys (Pennak 1989, Merritt and Cummins 1996). Indices included total taxonomic abundance, total taxonomic richness, and Ephemeroptera-Plecoptera-Trichoptera (EPT) abundance and richness. Statistical differences among sites were calculated using SAS® (SAS Inc. 2003).

4.2.4 Analytical Characterization of the Brine Discharge

Samples of the brine discharge were submitted to Severn-Trent Laboratories in Savannah, GA and North Canton, OH to determine ionic composition. Samples were unfiltered with the exception of one which was filtered using a disposable Corning® filter system (pore size 0.45 µm). The discharge was analyzed for dissolved metals and general chemical parameters following methods outlined by the US EPA (1983, 1986).

4.2.5 Review of River-Flow Data

In 2005, severe low flow conditions were observed in the NFHR resulting from low precipitation during spring and summer months. As a result of the low flow conditions, conductivity levels below the brine discharge failed to decrease substantially downstream as observed in previous years. Because of this condition, it was important to examine flow data collected by the US Geological Survey (USGS) over the past 80 years to determine the frequency of such drought conditions. Flow data were obtained from the USGS National Water Information System (http://www.waterdata.usgs.gov/va). These data were then compiled into spreadsheets and years were then compared to determine where the conditions observed in 2005 fell in regards to low-flow.

4.3 Results

4.3.1 Conductivity Dissipation

Conductivity dissipation was evaluated five times during September 2005 (Table 4.2). During the first reconnaissance effort on 9/8/05, conductivity was taken at 10 sites in the river plus the discharge. Background conductivity was measured at 340 µS/cm and the conductivity in the discharge was 10,930 µS/cm. Dissipation occurred rather quickly as conductivity was 2,360 µS/cm 5 m into the mixing zone area ; however, conductivity was not observed to background level at any site downstream during this sampling event. Levels were measured as low as 1,189 µS/cm, at 320 m below the discharge; however, levels jumped to 1,575 µS/cm at the lowest sampling site, ~ 2 mi downriver. The NFHR flow was 68.8 ft^3/sec.

A second effort to monitor dissipation was carried out the following week (9/13/05). Background conductivity was similar as the previous week (315 µS/cm), but the conductivity of the discharge was much lower (6,030 µS/cm) (Table 4.2). Although not measured, the flow of the discharge was noticeably lower than observed during the previous week. This difference was also noticeable in the river, as conductivity measurements were substantially lower. In addition to the sites sampled on 9/8/05, three additional sites were added, including a site at ~ 52 river miles downstream. In the initial mixing zone area (5 m below discharge), conductivity was 1,160 µS/cm, and 749 µS/cm 10 m downstream. Conductivity reached background level 640 m downstream (323 µS/cm). However, conductivity increased to 624 µS/cm at rm 81.6, where the two remediated holding ponds (5 and 6) converge. This spike in conductivity was also observed in the first sampling. Conductivity remained elevated (975 µS/cm) downstream to the last sampling site at rm 30.4. The flow average in the NFHR on 9/13/05 was 61.7 ft^3/sec.

On 9/20 and 9/22/05, two additional reconnaissance sampling efforts were completed. The river flow at the USGS gauge station was 40 and 35 ft^3/sec respectively on these two sampling dates. Upstream conductivity was 347-348 µS/cm while the brine discharge was 5,900 to 8,100 µS/cm (Table 4.2). Flow (and depth) from the center of the discharge pipe was 0.40 m/sec (6 in) on 9/20/05 and substantially higher at 0.52 m/sec (7 in) on 9/22/05. In the initial mixing zone area (5-m), conductivity varied from 795 to 1,372 µS/cm on 9/20 and 9/22/05 respectively. Conductivity dissipation downstream declined to 795 µS/cm in the 10-m zone and eventually to 384-384 µS/cm 640 m downstream. During the greater discharge of 9/22/05, conductivity was substantially higher in the 10-m zone (1,040 µS/cm), to 413-423 µS/cm 640 m downstream.

On 9/24/05, conductivity dissipation was measured from the brine discharge to Weber City, VA at rm 13.5 (Table 4.2). The brine discharge was 9,050 µS/cm with a flow of 0.57 m/s and 7.5 inch depth at the center of the pipe. Conductivity values were higher at the mixing zone (1,424 µS/cm), 10 m downstream (1,180 µS/cm) and at the next five sampling sites downstream (839-706 µS/cm) than measured previously. River flow at the gauge oscillated from 33-35 ft^3/sec on 9/23-24/05, the lowest seen to date.

At Site 3, just above remediated Olin Pond 5 (rm 82.3), conductivity ranged from 502-620 µS/cm and at Site 4 it increased to 1,026-1,136 µS/cm (Table 4.2). Again at Site 4a near the toe of Olin Pond 6, conductivity increased another 200 units to 1,316-1,332 µS/cm and then dissipated slightly at Site 5 (1,228-1,303 µS/cm). At the downstream Sites, 6 and 7, conductivity was 1,530-1,425 µS/cm. At the furthest downstream site, conductivity declined substantially to 710 µS/cm at Weber City, VA.

Conductivity was only measured once in October, on 10/11/05. Two days prior to sampling, the river flow rose to 120 ft^3/sec but had declined to 55 ft^3/sec by the day of sampling. This increase in river flow was substantially greater than that of the previous sampling efforts. Conductivity was 309 µS/cm upstream and 9,310 µS/cm at the outfall pipe (Table 4.2). Brine flow/volume was about 35% greater than on 9/24/05. Conductivity dissipated rapidly from 1,309 µS/cm in the 5-m mixing area to 620 µS/cm at the 640-m sampling site downstream. Conductivity oscillated to 1,136 µS/cm at rm 81.6, again indicating influence from the two remediated holding pond areas.

Dissipation was measured twice during November 2005. Conductivity dissipation was checked on 11/1/05 when river flow was 43 ft^3/sec, after being 54 ft^3/sec four days earlier and continued to remain at that low level over the next three days. Background conductivity was 325 µS/cm, and only 5,950 µS/cm at the brine discharge (Table 4.2). Conductivity dissipation declined to 790 µS/cm at 640 m and then to 537 µS/cm just above the Pond 5 area. At Site 4, conductivity increased ~150 µS/cm and then escalated to 1,049 µS/cm at Site 4a at the toe of Pond 6. Conductivity then declined steadily thereafter to 1,010 µS/cm at Site 5. Conductivity at site 7 was 755 µS/cm and declined somewhat to 723 µS/cm at rm 13.5, near Weber City.

On 11/9/05, conductivity dissipation was calculated when the river was 30 ft^3/sec, the lowest flow rate seen so far in this drought that was initiated more than two months prior. Conductivity above the discharge was 348 µS/cm (Table 4.2). The discharge conductivity returned to a higher level, as seen in September (9,230 µS/cm). Dissipation of conductivity down the river occurred from 1,475 µS/cm in the initial mixing zone to 690 µS/cm at the site above Pond 5. At Site 4, conductivity increased to 1,080 µS/cm and then to 1,310 µS/cm at Site 4a just at the toe of Pond 6. Thereafter, conductivity was similar at Site 5 (1,297 µS/cm) and thereafter with some unusual high and low measurements occurring between Site 5 (1,293 µS/cm) and site 6 (1,511 µS/cm).

It was apparent that conductivity pulses were happening down the river between sites from the brine discharge, through Ponds 5 and 6, and thereafter to Site 7. At Site 6, a pulse of conductivity increased to 1,511 µS/cm then declined to 1,280 µS/cm at Site 7 several miles (~25 rm) downstream (Table 4.2).

4.3.2 Benthic Macroinvertebrate Surveys

Total taxonomic abundance was highest at Site 5 (rm 80.0) with a mean of 610.8, followed by Site 4 at rm 81.6 (531.3). Sites 1 and 2, located above the brine influence had mean abundance values of 385 and 399, respectively (Table 4.3). Site 3, the first site below the brine discharge had a similar mean abundance of 380, while Sites 6 and 7 located at rm 55.9 and 30.4, had much lower means of 244.8 and 199.3.

Total taxonomic richness was highest at Sites 6 and 7 (20.0 and 28.3), but lowest at Site 1 with a mean of 13.5 different taxa (Table 4.3). Sites 2 and 3 averages were the same at 15.8. Sites 4 and 5 were somewhat lower with values of 15.0 and 14.8.

Mean caddisfly abundance was highest at Sites 3 (366) and 4 (309) (Table 4.3). The upstream reference site (Site 1) had an average of 94.3 caddisflies, while Sites 2 and 5 had 123.8 and 175.8, respectively. Caddisfly abundance was substantially less at Sites 6 and 7. Site 7 had a mean abundance of 62.3, while Site 6 had the lowest caddisfly abundance with a mean of 51.0.

Ephemeroptera-Plecoptera-Trichoptera (EPT) abundance and richness were also calculated for each site (Table 4.3). EPT abundance was highest at Sites 2, 3 and 4 with means ranging from 401.5 at Site 4 to 334.5 (Site 3). Site 1 had a mean EPT abundance of 322.8, while the downstream Sites (5, 6 and 7) were significantly lower than the upstream sites with mean EPT abundance of 196.5 (Site 5), 67.5 (Site 6) and 100.5 (Site 7). Mean EPT richness results were considerably different than EPT abundance with Site 7 having the most diversity of EPT taxa (12.5). Sites 1 and 2, which were above the brine discharge influence, had EPT richness means of 7.0 and 6.0, respectively. Site 3, the first site below the brine discharge had similar EPT richness values, with a mean of 6.5. Site 5 had a mean value of 5.0. Consequently, Site 6, which was the worst site in regards to EPT abundance, had the second highest mean EPT richness (8.75). Site 4 had the lowest mean for this parameter at 3.75.

Differences in mean EPT abundance and EPT richness at the seven sampling sites can be explained by the large quantity of hydropsychid caddisflies collected at Sites 3 and 4 (Table 4.4). When Hydropsychidae were removed from EPT abundance, means for each site dropped dramatically. Site 4, which initially had the highest mean of 401.5 for EPT abundance, dropped to 36.0 when Hydropsychidae were removed. Site 3 also had a dramatic decrease in mean EPT (52.0 vs. 334.3); however, it's ranking amongst the other sites remained relativity the same. Sites 1 and 2 had the most EPT abundance after hydropsychids were removed, with mean EPT values of 255.8 and 297.3, respectively. Means stayed relatively high compared to the downstream sites due to the presence of mayflies at these two upstream sites. Mayfly abundance was highest at Site 2 (243.0) followed by Site 1 (224.5). Mayfly abundance at downstream sites ranged from 7.0 (Site 6) to 35.5 (Site 4). Except for Sites 1 and 2, caddisflies were the most dominant order of insects in the EPT group (Figure 4.1). All parameters were determined to be statistically different by site (Table 4.3), with the exception of total taxonomic richness (NPAR1Way, α=0.05).

4.3.3 Analytical Characterization of the Brine Discharge

Samples of the brine discharge were analyzed for general chemistry and metals content during fall 2005 and spring 2006. Since prior analysis of the brine had not yet been conducted prior to fall 2005, both unfiltered and filtered samples were analyzed for comparison. Filtering did not make a difference, as results were very similar for both samples (Table 4.5). Sodium, calcium, magnesium and chlorides were the most prevalent constituents in the discharge. Not surprisingly, sodium (Na) had the highest concentration with 1,600 mg/L in the filtered sample and 1,640 mg/L unfiltered. Calcium (Ca) levels were also high, but quite similar for both the filtered (219 mg/L) and unfiltered (220 mg/L) samples. Magnesium (Mg) was 26.2 mg/L (filtered) and 26.4 mg/L (unfiltered), while chlorides were 29.3 and 29.7 mg/L, respectively. Analysis conducted at STL Savannah, GA also included TDS, which were slightly higher in the filtered sample (4,800 mg/L) than the unfiltered one (4,500 mg/L). Analysis was also conducted for aluminum (Al), iron (Fe), lead (Pb), selenium (Se), arsenic (As) and mercury (Hg), all of which were undetectable, except for iron, which was 0.27mg/L in the unfiltered sample.

Results of brine analysis in spring, 2006 were similar but slightly higher than those of 2005 (Table 4.6). The average Na level in the discharge was 2,233 mg/L, somewhat higher than that in the fall (1,600 mg/L). Calcium was also higher with an average of 320 mg/L, and the average Mg level was 28 mg/L. Chlorides were substantially higher during the spring collections of the discharge with an average of

4,600 mg/L. Total dissolved solids (TDS) were also measured once during spring 2006 and were much higher than in the fall with a concentration at 9,000 mg/L. The discharge was also analyzed for trace metals Al, Fe, Pb, Se, As and Hg; however, Fe was the only detectable metal and averaged 0.27 mg/L.

4.3.4 Review of River-Flow Data

The US Geological Survey (USGS) has documented the flow of the NFHR for 86 years, providing a sufficient database of flow data necessary to characterize the frequency of such low flow events as observed in 2005. Daily flow data were averaged for the four lowest flow months of 2005, August-November (Table 4.7). September flow was the lowest observed (30.0 ft^3/sec) while August had the highest flow average at 53.9 ft^3/sec. The overall four-month average flow was 43.8 ft^3/sec. This average was used to compare to previous years of flow data.

The same low-flow months (Aug-Nov 2005) were averaged using the last 50 years of data (1955-2005). Flow was considered to be severely low if the average was $\leq$ 50 ft^3/sec. Ten years fell within this category. In addition, four other years (1955, 1960, 1987 and 1997) were as low as or lower than 2005 (Fig. 4.2). The lowest average flow was in 1955 (36.8 ft^3/sec).

4.4 Discussion

The brine discharge into the North Fork Holston River, Saltville, VA, presented an unique opportunity to examine the effects of a saline discharge into a freshwater river. Such discharges are rare in the United States, and thus not well understood. Although saline discharges are rare, related pollutants such as TDS and specific conductance (conductivity) are a growing concern for the United States Protection Agency (US EPA). As a result, 15 states have begun implementation of water quality standards to regulate discharged amounts of these pollutants.

The emphasis of this research focused on the dissipation of the brine discharge in the NFHR during severely rare low-flow conditions. The results suggest that during such low-flow events, as observed in 2005, conductivity below the discharge fails to dissipate as rapidly as during normal flow regimes, causing concern for the aquatic biota in the river. During the eight reconnaissance efforts in 2005, conductivity was elevated downstream of the brine discharge well above background levels, which averaged 315 μS/cm (Table 4.2). This elevated conductivity was observed as low as river mile (rm) 13.5, near Weber City, VA. Although conductivity varied in the discharge itself (range of 5,900-10,930 μS/cm), dissipation in the initial 5-m mixing zone was consistent, whereby conductivity usually dropped by 4-5%. The one exception was on 9/20/05 where conductivity in the immediate mixing zone dropped to 795 μS/cm when the discharge conductivity was also at its lowest (5,900 μS/cm). Flow, however, did not seem to be a factor because it was not increased on this date, and was consistently 27 ft^3/sec from 9/13/05 to 9/20/05.

Conductivity was measured at close to background levels at the 640-m sampling location on three of the sampling dates; however, as the drought persisted into late September through November, conductivity remained from 502-690 μS/cm. At the sampling sites near the remediated holding ponds (5 and 6), it was evident that conductivity spikes were occurring. Each time these sites were sampled, conductivity doubled over that measured at the previous site (640 m). Conductivity remained elevated downstream thereafter at each of the remaining sampling locations.

Benthic macroinvertebrate surveys conducted for this study, however, were inconclusive in determining any possible effects of the high conductivity and salinity on benthic fauna (Fig. 4.3). Total and EPT abundance values were highest around the ponds 5 and 6 area, where conductivity was observed to be increasing. Total taxa richness, however, was consistent at all the sites near Saltville, VA, but increased substantially downstream at sites 6 and 7. EPT richness, the metric that gauges benthic diversity, was lowest at site 4, where EPT abundance was highest. This was explained by the vast number of hydropsychid caddisflies collected, which compartmentalize contaminants (Cain et al. 2001). The two genera of hydropsychids identified were *Cheumatopsyche* and *Hydropsyche,* both of which are considered to be facultative, or moderately tolerant for the mid-Atlantic region (Barbour et al. 1999). In a study by Williams and Williams (1998), it was reported that after four hours of exposure to a 35% salinity increase, a species of *Hydropsyche* showed signs of impairment (animals had ceased mobility) but were not considered dead. In addition, Blinn and Ruiter (2006) reported that two hydropsychid species (*Cheumatopsyche enonis* and *Hydropsyche occidentalis*) had highest Stream Condition Index (SCI) scores in streams with high salinities. Kennedy et al. (2003) also observed skewed EPT indices due to a high number of hydropsychids. Other EPT taxa, such as stoneflies and mayflies, were not abundant at the sites downstream of the discharge (Fig. 4.1).

Total chloride levels of 2,970 mg/L in the fall and 4,600 mg/L in the spring, were well above both the US EPA acute and chronic Water Quality Criteria (WQC). The Virginia Department of Environmental Quality (VA DEQ) recently drafted a Total Maximum Daily Load (TMDL) for the Upper North Fork Holston River (VA DEQ 2006). The TMDL rationale, which is widely based upon benthic macroinvertebrate data, determined the pollutant of primary concern as chlorides. In addition to the chlorides being discharged in the brine, six National Pollutant Discharge Elimination System (NPDES) permits exist for this upper portion of the NFHR, in Saltville, VA (VA DEQ 2006). Each additional discharge is permitted to release chlorides (230 mg/L-376mg/L) in their respective effluents. These discharges are the Saltville Waste-water Treatment Plant (SWWTP), two family treatment units, and the Saltville Gas and Storage Facility which discharge in the same vicinity of the remediated pond area, where conductivity was observed to be doubling in this study. One more additional source contributing to the conductivity spike in this area is the WWTP facility operated by Olin, which treats runoff from ponds 5 and 6.

The declining mussel population in the NFHR has led to increased attention of the health of this river. The majority of this work has focused on the mercury contamination originating from the former chlor-alkali plant in Saltville, VA; however results of this study indicate that the brine discharge could be another potential source of stress in the river. In addition, the synergistic effect of salinity on mercury toxicity could be another factor inhibiting mussel recruitment in the NFHR near Saltville, VA. A study by Modassir (2000) studied such an effect using the Mangrove clam (*Polymesoda erosa*). Although this is an estuarine bivalve, results reported showed an increase in mortality as salinity increased. Furthermore, Wright (1977) reported that salinity changes may affect the chemical form and interaction of metals in seawater, increasing bioavailability.

4.5 Summary and Conclusions

Over the past 20 years, increased attention has been given to the North Fork Holston River (NFHR) and other aquatic systems in southwestern, VA, due to the severe decline of unionid populations that were once abundant in the region. Because of this problem, it is important to document all potential factors that could be inhibiting reestablishment of healthy mussel assemblages.

The data presented in this study were inconclusive in determining benthic macroinvertebrate impairment. The best benthic parameters used were EPT-Hydropsychidae abundance and EPT richness, which eliminated the tolerant Hydropsychidae caddisflies. Once these organisms were removed from the database, results indicated that EPT abundance and richness were significantly affected at sites directly below the brine discharge. Although mussel surveys were not included in this study, the chemical composition of the discharge (high chloride levels) and the influx of high conductivity during the severe low-flow riverine situation could be a contributing factor to low mussel recruitment in the NFHR near Saltville, VA. In order to fully substantiate this possibility, long-term chronic toxicity tests would need to be conducted in the laboratory with freshwater mussels. In addition to the influxes of conductivity and related constituents originating from the discharge, the synergistic effect that salinity has on mercury could also be a potential issue affecting mussel recruitment in the NFHR near Saltville, VA.

4.6 Acknowledgments

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Table 4.1. North Fork Holston River (NFHR) sampling locations for benthic macroinvertebrates and conductivity measurements, with corresponding river miles.

NFHR Sampling Site	NFHR River Mile	Parameter(s) Sampled
Site 1	85.0	Benthic only
Site 2	84.1	Benthic only
Above Discharge	82.7	Conductivity only
Brine Discharge	82.8	Conductivity only
5-m mixing zone	82.8	Conductivity only
10 m	82.8	Conductivity only
20 m	82.8	Conductivity only
40 m	82.8	Conductivity only
80 m	82.8	Conductivity only
160 m	82.7	Conductivity only
320 m	82.6	Conductivity only
640 m	82.5	Conductivity only
Site 3	82.3	Benthic and Conductivity
Site 4	81.6	Benthic and Conductivity
Site 4a	80.4	Conductivity only
Site 5	80.0	Benthic and Conductivity
Site 6	55.9	Benthic and Conductivity
Site 7	30.4	Benthic and Conductivity
Weber City	13.5	Conductivity only

Table 4.2. Conductivity (µS /cm) dissipation below the brine discharge in the North Fork Holston River (NFHR) during eight sampling events in fall 2005.

NFHR	**Conductivity µS/cm**							
Sampling Site	**9/8/2005**	**9/13/2005**	**9/20/2005**	**9/22/2005**	**9/24/2005**	**10/11/2005**	**11/1/2005**	**11/9/2005**
Above Discharge	340	315	347	348	349	309	325	348
Brine Discharge	10,930	6,030	5,900	8,100	9,050	9,310	5,950	9,030
5 m (mixing zone)	2,360	1,160	795	1,372	1,424	1,309	1,242	1,475
10 m	1,942	749	699	1,040	1,180	1,234	1,033	1,329
20 m	1,755	665	660	775	839	958	816	1,005
40 m	902	540	630	755	804	934	790	988
80 m	1,189	488	565	760	832	903	744	923
160 m	1,190	449	481	623	779	843	661	881
320 m	1,189	412	392	603	706	747	602	789
640 m	—	323	389	413	502	602	537	690
Site 4	—	624	745	857	1,026	952	697	1,080
Site 4a	1,575	944	976	1,113	1,316	1,135	1,049	1,310
Site 5	1,572	920	966	1,113	1,228	1,092	1,010	1,293
Site 6	—	975	778	826	1,474	1,094	697	1,511
Site 7	—	—	—	856	1,425	1,023	722	1,380
Weber City, VA	—	—	—	—	710	723	711	704

Table 4.3. Mean benthic macroinvertebrate parameters with NPAR1Way ANOVA scores and statistical differences between means.

NFHR Sampling Site	Total Abundance	Taxa Richness	Caddisfly Abundance	EPT Abundance	EPT Richness
Site 1	385 $^{a\,b}$	13.5 ^{b}	94.3 $^{b\,c}$	322.8 $^{a\,b}$	7.0 $^{b\,c}$
Site 2	399 $^{a\,b}$	15.8 ^{b}	123.8 $^{b\,c}$	368.3 $^{a\,b}$	6.0 $^{b\,c}$
Site 3	380 $^{a\,b}$	15.8 ^{b}	366 $^{a\,b}$	334.3 $^{a\,b}$	6.5 $^{b\,c}$
Site 4	531.3 $^{a\,b}$	15.0 ^{b}	309 ^{a}	401.5 $^{a\,b}$	3.75 ^{c}
Site 5	610.8 ^{a}	14.8 ^{b}	175.8 $^{a\,b\,c}$	196.5 ^{a}	5.0 $^{b\,c}$
Site 6	244.8 $^{a\,b}$	20.0 $^{a\,b}$	51.0 ^{c}	67.5 ^{b}	8.75 $^{a\,b}$
Site 7	199.3 ^{b}	28.3 ^{a}	62.3 ^{c}	100.5 $^{a\,b}$	12.5 ^{a}
NPAR1Way	*p=0.035*	*p=0.056*	*p=0.0070*	*p=0.0060*	*p=0.0083*

* Means with different letters indicate statistical difference (Tukeys HSD, α=0.05).

Table 4.4. Comparative Ephemeroptera-Plecoptera-Tricoptera (EPT) abundance means with and without Hydropsychidae caddisflies.

NFHR Sampling Site	EPT Abundance w/Hydropsychidae Caddisflies	EPT Abundance w/o Hydropsychidae Caddisflies
Site 1	322.8 [a b]	255.75 [a]
Site 2	368.3 [a b]	297.25 [a]
Site 3	334.3 [a b]	52.0 [b]
Site 4	401.5 [a]	36.0 [b]
Site 5	196.5 [a b]	23.75 [b]
Site 6	67.5 [b]	38.25 [b]
Site 7	100.5 [a b]	62.75 [b]

* Means with different letters indicate statistical significance (Tukeys HSD, $\alpha=0.05$)

Table 4.5. Analytical results of the brine discharge (mg/L) from October 2005 comparing filtered vs. unfiltered samples.

Filtered Brine Discharge			Unfiltered Brine Discharge		
Parameter	**Level (mg/L)**	**Detection Limit (mg/L)**	**Parameter**	**Level (mg/L)**	**Detection Limit (mg/L)**
Aluminum	ND	0.2	Aluminum	ND	0.2
Arsenic	ND	0.01	Arsenic	ND	0.01
Calcium	219	5	Calcium	220	5
Iron	ND	0.01	Iron	0.276	0.01
Magnesium	26.2	5	Magnesium	26.4	5
Sodium	1,600	25	Sodium	1,640	25
Lead	ND	0.003	Lead	ND	0.003
Selenium	ND	0.005	Selenium	ND	0.005
Mercury	ND	0.0002	Mercury	ND	0.0002
Chloride	2,930	20.0	Chloride	2,970	20.0
Sulfate	468	20.0	Sulfate	473	20.0
Total Dissolved Solids	4,800	40.0	Total Dissolved Solids	4,500	40.0

Table 4.6. Spring 2006 analytical results (mg/L) for the brine discharge.

Parameter Analyzed	**Average Level (mg/L)**	**Detection Limit (mg/L)**
Aluminum	ND	0.2
Arsenic	ND	0.01
Calcium	320	5
Iron	0.27	0.01
Magnesium	26.2	5
Sodium	2,233	25
Lead	ND	0.003
Selenium	ND	0.005
Mercury	ND	0.0002
Chloride	4,600	20.0
Sulfate	810	20.0
Total Dissolved Solids	9,000	40.0

Table 4.7. Average daily flow (ft^3/sec) data for the North Fork Holston River during low-flow months in 2005. Flow data were obtained from the US Geological Survey (USGS) website database for real-time data (http://www.waterdata.usgs.gov/va).

DAY	AUG	SEPT	OCT	NOV
1	64	41	30	31
2	60	40	28	31
3	56	38	27	31
4	53	36	26	32
5	51	34	26	30
6	51	32	27	31
7	52	30	32	29
8	58	30	58	30
9	64	29	103	30
10	75	29	64	33
11	82	28	44	31
12	63	28	35	30
13	54	27	32	31
14	49	27	30	32
15	46	27	27	34
16	46	27	26	41
17	49	27	25	46
18	57	27	25	47
19	56	27	25	49
20	57	27	25	42
21	72	25	26	42
22	63	25	39	63
23	52	25	44	67
24	47	25	51	70
25	45	25	41	56
26	42	26	39	48
27	40	30	39	44
28	41	37	40	43
29	41	39	36	171
30	43	33	33	341
31	42		32	
Total Avg.	53.9	30.0	36.6	54.5

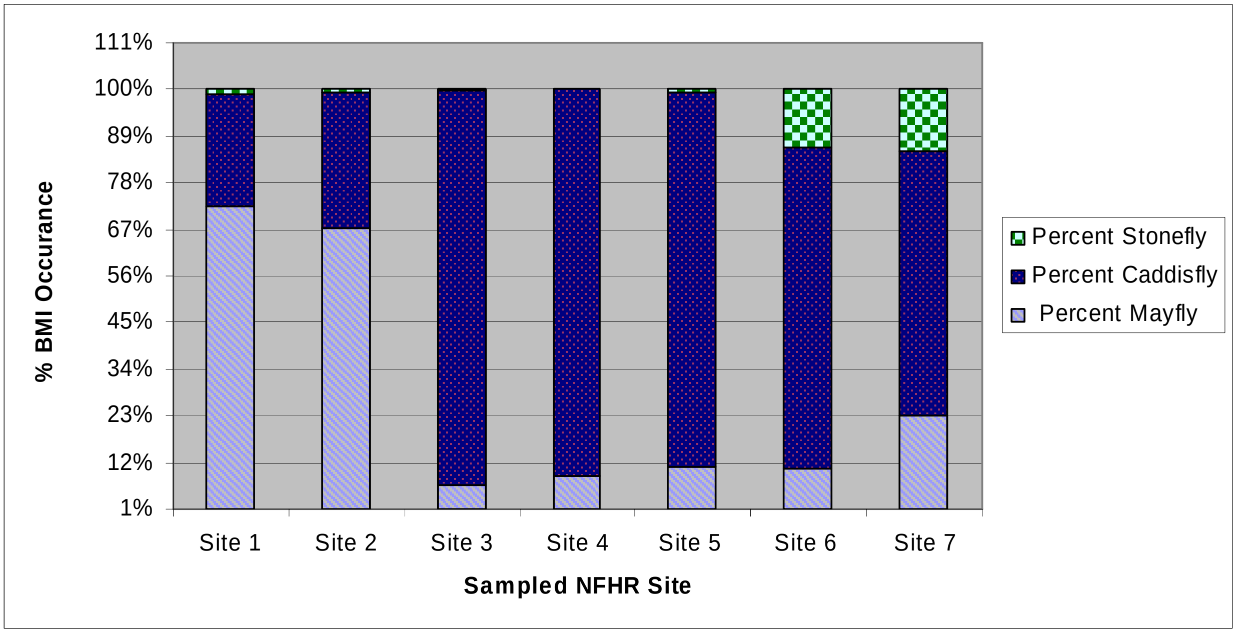

Figure 4.1. Percent benthic macroinvertebrate (BMI) occurrence at each site for the Ephemeroptera (Mayfly)-Plecoptera (Stonefly)-Trichoptera (Caddisfly) groups. Each group contributes to the overall 100% for each site.

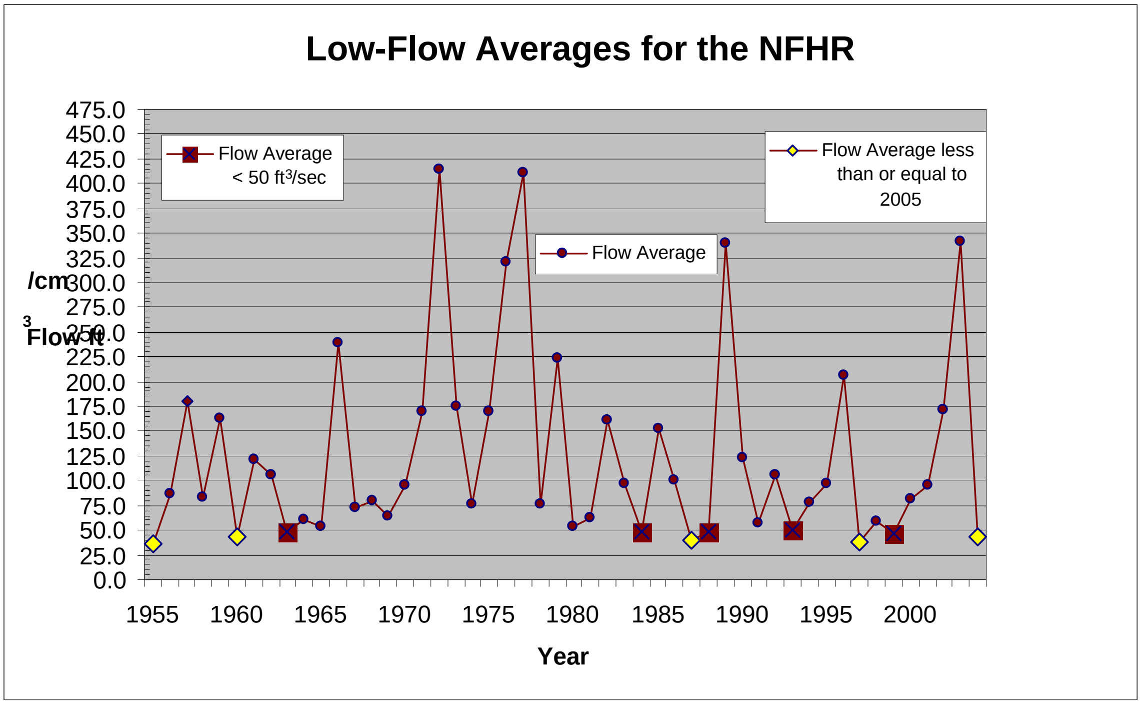

Figure 4.2. Fifty-year (1955-2005) flow averages for the North Fork Holston River (NFHR).

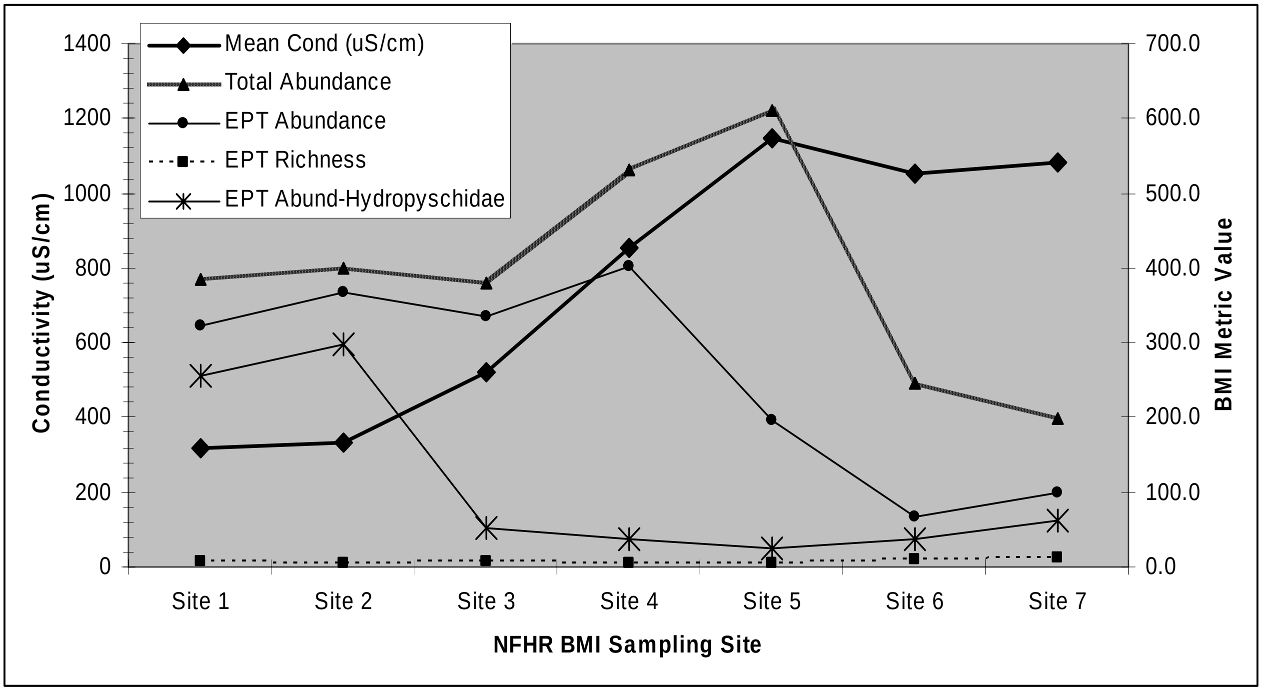

Figure 4.3. Mean conductivity (µS/cm) and selected benthic macroinvertebrate (BMI) metrices for all North Fork Holston River (NFHR) sites.

Chapter 5

Field and Laboratory Assessments of the North Fork Holston River below Two US EPA Superfund Sites, Saltville, Virginia

Abstract

An evaluation of the North Fork Holston River below Saltville, VA in spring 2006 revealed a thick white flocculent accumulating in the river below two remediated holding ponds, formerly used by the Olin Chemical Corporation. Ponds 5 and 6 were used by the chemical company to contain alkaline and mercury waste, and have been remediated as part of the US EPA Superfund Program. The flocculent accumulated at the base of diversion dyke cuts from the ponds, which were constructed to re-route subsurface water to the river. The flocculent was high in Al (1.9 – 38 mg/L) and Fe (2.0-51 mg/L) and may be related to the diversion dyke cut discharges from Ponds 5 and 6. Toxicity testing of Pond 6 dyke cut discharge water was acutely toxic to both *Ceriodaphnia dubia* and *Villosa iris.* Forty-eight hour lethal concentration (LC50) values for *C. dubia* ranged from 12.07-15.95 %. Juvenile *Villosa* were somewhat more tolerant, with LC50 values of 18.95 and 17.36%. Timed toxicity tests with daphnids were conducted due to the extremely alkaline pH of these discharges (11.50-12.25) and low LC50 values from the acute tests. In 15 min, 100 % mortality occurred in the East and West diversion discharges of Pond 6. The ecological integrity of the river to recover its mussel assemblages downstream may be compromised by the discharge and accumulation of flocculent, especially during low-flow river drought conditions which have occurred on 10 occasions over the past 50 years.

5.1 Introduction

In April, 2004, the United States Department of the Interior (US DOI) in a joint effort with the State of Tennessee and Tennessee Valley Authority (TVA) drafted a Freshwater Mussel Injury Study Plan for the North Fork and mainstem Holston rivers, Virginia. In this draft proposal, a comprehensive evaluation of these riverine systems would be conducted to examine freshwater mussel (Bivalvia: Unionidae) assemblages in the North Fork Holston River (NFHR) and determine possible ecotoxicological effects preventing the re-establishment of unionid communities below Saltville, VA. This effort came after several decades of research aimed at determining the extent of unionid extirpation in the NFHR following mercury pollution (Stansbery 1972, Hill et al. 1980, YMA 1990, Dye 1999 and Henley and Neves 1999).

Recent studies have shown that mercury may still pose a threat to aquatic biota in the NFHR. Echols et al. (2007) investigated remnant mercury contamination in the NFHR below Saltville (rm 82.3) and found mercury in sediments and interstitial water as far downstream as river mile 30.4, below Mendota, VA (~ 52.1 rm). Brown et al. (2005) examined mussel shells from the NFHR above and below Saltville, and found residual mercury to be 2-3 orders of magnitude higher (23-4,647 μg/L) in shells downstream at rm 79.9, 68.6 and 56.0, than those that were collected upstream at river miles 96.0 and 85.0 (5-31 μg/L).

The initial source of the mercury pollution was a chlor-alkali plant operated by the Olin Chemical Corporation (Olin Corp). Production at the Saltville facility ceased in 1972 (Turnage 1994), but remnant mercury in river sediments has remained a concern. Early investigations estimated that 100 g/day of Hg was continuing to seep from two of the company's large holding ponds into the NFHR (Carter, 1977, Lindsey and Dimmick 1983, US DOI 2004). The two holding ponds of concern, formerly known as Muck Ponds 5 and 6, were used for containment of ammonia soda ash wastes. Pond 5, which was constructed in the 1920s, began receiving mercury-contaminated waste-water from the mercury-cell chlorine plant in 1951 which continued until 1971 (US EPA 2002a).

Pond 6 is also reported to have received mercury waste-water, but not to the extent of Pond 5.

The two pond areas were remediated by the United States Protection Agency (US EPA) as part of the Superfund Program, and are periodically monitored by the program every five years (US EPA 2002a). In accordance with the remediation plan, Olin Corp. also installed a waste-water treatment plant (WWTP) to treat ground water from Pond 5 (minimizes mercury) and Pond 6 (pH adjustment). The pond areas, which cover ~ 49 hectares of land adjacent to the NFHR, have been capped and diversion dyke cuts were constructed to carry runoff from a neighboring mountain (Little Mountain) to the river. This precaution was taken to reduce the surface water flow on the now dry ponds.

The environmental fate of the NFHR has been a concern of researchers for many years. Although the majority of work in recent years has focused on the mercury contamination caused by the Olin Corp. facility, researchers in the early 1900s also noted environmental impacts originating from Olin's parent company, Mathieson Alkali Corp. Adams (1915) reported that the river at and below the alkali facility "had refuse flowing into the river which has covered all the rocks and the bed of the stream with a whitish coating." Recent accounts of such observations have also been made. During low-flow periods in the NFHR, a white flocculent can be observed accumulating at the base of the muck pond areas and at times, such as in 1987, this flocculent was observed covering the width of the river for several miles downstream (Stanley Haynes, personal communication).

The focus of this research was to study the area of the NFHR closest to the remediated Ponds 5 and 6 areas. The primary objective of this study was to identify possible sources of contamination originating from the now dry ponds, including seeps and discharges, both permitted (WWTP) and unregulated (diversion dyke cuts) using field and laboratory assessments.

5.2 Materials and Methods

5.2.1 Study Sites

Eight study sites were selected to assess the effects that the two remediated (now dry) Ponds 5 and 6, were having on the NFHR. Four sample sites were located in the direct vicinity of the remediated area and included P5R, P5L, P6R and P6L. As part of the remediation effort, diversion dyke cuts were installed to divert runoff from Little Mountain (located behind the two holding ponds) and reduce surface water on the dry ponds (Fig.5.1). The dyke cuts then drain via a down chute (~ 8 m) into the NFHR. In addition, a waste-water treatment plant (WWTP) was established in 1994 to treat the ground water/leachate from Pond 5. Site P5R was located below the Olin WWTP, Pond 5 Dyke Cut and Western Diversion Ditch discharges (Fig. 1), while P5L was located directly across from P5R in the river, on the left descending bank. Sites were included on right and left banks around the ponds so that direct associations could be linked to pond influence. Site P6R was located in the river below the Pond 6 Eastern Diversion Dyke Cut. As with the Pond 5 sites, a site was also included on the opposite river bank (P6L). Three additional sites were included downstream (NFHR 5, 6 and 7) with an additional upstream reference. These sites were established based upon results of Echols et al. (2007), in order to determine whether pollutants, such as mercury, entering the river were detectable downstream.

5.2.2 Site Interstitial Water Collection

Site Interstitial Water (SIW) was collected from depositional zones at each sampling station using diffusion samplers (peepers). Peepers were constructed using 250-ml amber glass jars with 32-mm diameter holes cut out of the plastic lids. Amber glass was used to prevent photolysis of PAH compounds (Miller and Olejnik, 2001). Prior to deployment, peepers were completely filled with distilled water and 105 μm nylon mesh was inserted under the lid-so that it remained secure. Peepers were placed lid side down approximately 10 cm into the sediment. They were allowed to equilibrate for a minimum of 21 days (Webster *et al*. 1998, Vroblesky *et al*. 2002). At collection, SIW was transferred into 1-L Nalgene® containers, then transported back to the laboratory on ice where they were acidified (< 2 pH) using nitric acid (20/20) and shipped to Severn-Trent Laboratories (STL), Savannah, GA for analysis.

5.2.3 Dissolved Ion and Solids Analysis

Water and sediment samples were collected from the NFHR and analyzed at STL, Savannah, GA. Water samples included SIW and riverine samples from below the Pond 5 and 6 dyke cut discharges where a thick white floc or flocculent was observed accumulating in spring 2006. The flocculent was collected by Dr. Don Cherry while observing riffle entering into pool areas that contained floc and then collected the sample at the natural point of floc disturbance as it entered the pool. Sediment samples from each of the sampling sites in the NFHR as well as river sediments below where the flocculent was accumulating were also analyzed. Samples were analyzed for Al, Fe and Hg following procedures outlined in US EPA (1986) protocol for inductively coupled plasma (ICP) spectrometry.

5.2.4 *In Situ* Toxicity Testing

5.2.4.1 Growth and Survivorship

The Asian clam (*Corbicula fluminea*) was used for *in situ* survival and growth studies in the NFHR, as they have been a successful indicator of toxicity in previous studies (Soucek et al. 2001, Echols et al. 2007a). Clams were collected using clam rakes from the New River, Ripplemead, Virginia, and kept in Living Streams® (Frigid Units, Toledo, OH). Clams were measured for initial width using ProMax® digital calipers and marked on the exterior shell using a file. For this study, clams measuring between 9 and 11 mm were used. Five clams were placed into 18 x 36 cm mesh bags (~0.5 cm^2 mesh size) with five replicate bags per sampling station. After 60 days, clams were retrieved and returned to the laboratory to measure mean survival and final growth were measured. Mortality was declared if clams were found gaping or easily opened, or failed to close when the visceral mass was probed. Survival, change in growth, and average growth for each replicate/site were determined. Statistical analyses were conducted using SAS® (SAS Institute 2003).

5.2.4.2 Bioaccumulation Study

Bioaccumulation studies with *C. fluminea* were also conducted at the eight study locations in the NFHR. Asian clams used for the bioaccumulation study were collected from an undisturbed site in the Clinch River near Pounding Mill, VA. Fifty clams were placed *in situ* at each site for 60 days. At test initiation, approximately 20 clams were preserved and frozen as a reference for mercury analysis. After 60 days, clams were removed from the river, survival assessed and the visceral mass of living clams were removed. For each site and the reference clams, 10 g of tissue was sent to STL, Savannah, GA, to be analyzed for aluminum and iron using ICP spectrometry and total mercury by the manual cold vapor technique (US EPA 1986).

5.2.5 Water Column Toxicity Testing

Acute toxicity of dyke cut runoff from Pond 6 and WWTP discharge from Ponds 5 and 6 was determined with < 24 hr *Ceriodaphnia dubia* and using 48 h static, non-renewal tests (US EPA 2002b). In addition, juveniles of the freshwater mussel, *Villosa iris*, were also tested in acute toxicity tests with Pond 6 dyke cut discharge. The *C. dubia* used for this testing were cultured at VA Tech following US EPA (2002b) protocol. The *V. iris* were obtained from the Freshwater Mollusk Conservation Center (FMCC) at VA Tech and were ~ 2.5 mo old at test initiation. Tests were conducted at 25 ± 1° C using 50-ml glass beakers as test chambers. Test concentrations followed a 0.5 serial dilution with 100 % being the highest concentration. Diluent used for this test was moderately hard synthetic water (EPA[100]). The lethal concentration (LC50) for each test was determined using the Spearman-Karber Method (US EPA 1994).

Timed response tests were also conducted using *C. dubia*, whereby neonates (< 24 h) were placed into 100 % Pond 6 dyke cut discharges (P6E and 6W), and checked at one, three, 10 and 15 min of exposure. Two replicates were used for each sample tested, each with five daphnids. Behavioral and survivorship responses were recorded for each time interval.

5.3 Results

5.3.1 Dissolved Ion and Solids Analysis

Water samples were collected in the river below the pond dyke discharges as well as through pore-water extraction (SIW) and analyzed at STL, Savannah, GA. During low-flow conditions of early 2006, white precipitate was observed accumulating below the Pond 5 Western Diversion Ditch (P5WDD) and Pond 6 Eastern Diversion Dyke Cut (Fig. 5.1). Water samples from these two areas were collected and analyzed for ionic content. Flocculent collected below the P5WDD had high concentrations of Al, Fe and Hg (Table 5.1). During March, 2006 Al was 1.9 mg/L, Fe was 2.0 mg/L and Hg was 0.00023 mg/L. During the June 2006 analysis, Al had doubled (3.4 mg/L) and Fe had increased (3.1 mg/L). The mercury level (0.0019 mg/L) was eight times higher than in March. Results of the July analysis showed no substantial increases from that in June.

Mercury levels were actually lower and consistent to levels measured in March (0.00021 mg/L). In March 2006, sediments from the river below where the flocculent had accumulated, were analyzed for Al, Fe and Hg levels as well. All three of these metals were substantially higher in the sediments than in the water column. Aluminum and Fe were 5,000 and 7,600 mg/kg, respectively, while Hg was 1.7 mg/kg. Calcium was also measured at 240,000 mg/kg.

Analytical results were substantially higher for P6 samples, which were collected below P6 Eastern Diversion Dyke Cut, especially during the June and July analysis. Aluminum, Fe and Hg levels in March were consistent with those from the P5 samples; values were Al (4.0 mg/L), Fe (2.3 mg/L) and Hg (0.00042 mg/L) (Table 5.1). These metal concentrations were much higher in June. Aluminum was drastically higher (28 mg/L), and greatly exceeded the national water quality criteria (WQC) of 0.087 mg/L (US EPA 1999). Iron was 13.5 x higher in June than in March, at 31 mg/L, approximately 31 x higher than the WQC (1 mg/L). The most significant result was for Hg which was 0.0076 mg/L (0.00042 mg/L in March), an approximate 18-fold increase. Aluminum and Fe remained high in July 2006 (38 and 51 mg/L); however, Hg levels declined to 0.00018 mg/L. Sediments collected in March from the riverbed, below the accumulated flocculent were also high in Al (3,400 mg/kg), Fe (2,900 mg/kg) and Hg (0.62 mg/kg). Calcium at this site was also high at 380,000 mg/kg.

Site interstitial water analyzed in 2006 had moderately high levels of mercury at all downstream sites below Ponds 5 and 6 (Table 5.2). As expected, P5 and P6 peepers had the highest Hg concentrations (0.0034 and 0.0042 mg/L). These two sites were also analyzed for Al and Fe, which were 20 and 35 mg/L, respectively at P5, and 18 and 30 mg/L respectively, at P6. Downstream at NFHR-5, mercury levels were as high as P5 (0.0034 mg/L). Upstream at NFHR-1, mercury was 0.00020 mg/L and 0.00080 mg/L at NFHR-7, the furthest downstream site.

Sediments were collected from each site in the NFHR during fall 2006 with subsequent analysis at STL Savannah, GA to determine Al, Fe and Hg concentrations in the sediments (Fig. 5.2). Results of this analysis confirmed the presence of Fe and Al at all sampled sites, including the upstream reference location (NFHR-1). The level of Al in sediments was highest (6,700 mg/kg) at NFHR-5 and similar at P6L and P6R (6,600 and

6,300 mg/kg). Surprisingly, Al levels were the same for NFHR-1 (reference) and P5L, equaling 5,500 mg/kg. Site P5R had slightly lower Al results (5,100 mg/kg). Lowest Al levels were at downstream sites NFHR-6 and 7 (4,200 and 3,500 mg/kg).

Iron concentrations were highest at P6L (31,000 mg/kg), nearly double that of P6R (16,000 mg/kg) which was on the right bank nearest to Pond 6 dyke influence (Fig. 5.2). Of note is that Fe levels upstream at NFHR-1 were the second highest (28,000 mg/kg) among sites. Iron levels gradually decreased downstream at NFHR Sites 5, 6 and 7, although NFHR-7 did not have the lowest measured Fe content. Site P5R was lowest for Fe with 5,800 mg/kg. This was substantially lower than P5L on the opposite bank and half the level determined ~50 river miles downstream at NFHR-7 (10,000 mg/kg).

Mercury levels were also analyzed because Hg is the primary pollutant of concern for the NFHR. Results of the sediment analysis determined Hg content to be higher at all sites below the Ponds 5 and 6 influence (Fig. 5.2). The reference site (NFHR-1) had a minimal trace of mercury (0.021 mg/kg). Site P6L had the highest mercury content (3.9 mg/kg), followed by P6R (2.5 mg/kg). Site P5L again had higher results than the right bank site (P5R). Mercury at P5L was 1.9 mg/kg, while P5R mercury was only 0.079 mg/kg, considerably lower. Mercury concentrations were much lower at NFHR-6 and 7 (0.66 and 0.17 mg/kg).

5.3.2 *In Situ* Toxicity Testing

5.3.2.1 Growth and Survivorship

Asian clam survivorship was highest (100 %) at NFHR 1, the reference location (Table 5.3). No other site had 100% survivorship. Impacted riverine sites in the Pond 5 and 6 vicinity had varying survivorship impairment. Two sets of clams were placed on the right bank by Pond 5; one bag was placed in the river below the Pond 5 Dyke Cut (P5R-DC), while another set was placed downstream below the Pond 5 Western Diversion Ditch (P5R-DV). Clams at the P5R-DC site had 68 % survivorship, while all clams at P5R-DV were dead after 60 days *in situ* (Fig. 5.3). Clams placed on the left bank at Pond 5 (P5L) were not retrieved as the bags were most likely vandalized and were not found at the end of the *in situ* testing period. Only one set of clams was placed on the right bank near the Pond 6 input (P6R) and survivorship was somewhat higher

(80%) than that of P5R. Statistical analysis determined mean survivorship among sites to be significantly different ($p<0.0001$, ANOVA).

Mean growth (mm) was highest (2.06 mm) at the upstream reference site (NFHR-1) (Table 5.3). Site P6L had the second highest mean growth (1.97 mm) followed by NFHR5 with 1.85 mm. Sites NFHR6 and NFHR7 had significantly lower growth (0.97 and 0.60 mm, respectively) than the upstream sites closer to the pond discharges, except for P5R-DV in which complete mortality occurred soon after being placed in the river. Growth was determined to be significantly different among sites ($p<0.0001$).

5.3.2.2 Bioaccumulation

Asian clams were placed *in situ* for 60 days in summer 2006. Subsequent analysis was conducted at STL Savannah, GA for Al, Fe and total Hg content in clam tissues. Results indicate relative low Hg, but high Al and Fe levels (Table 5.4). NFHR-1 had the highest Al (125.3 mg/kg) and Fe (191.3 mg/kg) levels in tissue analysis but the lowest detected Hg level (0.014 mg/kg). Site P6L was also substantially higher for Al (93.7 mg/kg) and Fe (144.3 mg/kg) than the other sites located in the Pond 5 and 6 areas and further downstream. Mean Hg levels at this site, however, were consistent with all other sites below the Pond 5 influence. In general, bioaccumulation of Hg was minimal at all sites with the highest detectable level being at site P6R (0.086 mg/kg).

5.3.3 Water Column Toxicity Testing

Acute toxicity tests were used to determine the toxicological potential of the dyke cut discharges from Pond 6. *Ceriodaphnia* were more sensitive to the discharges than juvenile *V. iris*. Forty-eight hour lethal concentration (LC50s) of the Pond 6 East Diversion Dyke Cut (P6E) were determined twice for *C. dubia* and once for *V. iris* (Fig. 5.4). For the *C. dubia tests*, 100% mortality was observed as low as the 25 % test concentration after 24 h, and minimal (15 and 30 %) mortality occurred in the 12 % concentration after 48 h of exposure. The 48-h LC50 values for these two tests were 14.36 and 15.95 %. The pH in the 100 % concentration at test initiation for both tests was extremely high at 12.05 and 12.13. Conductivity (µS/cm), alkalinity and hardness (mg/L $CaCO_3$) were also high in the 100 % concentration for these tests. Conductivity at test initiation was 2,841 and 3,776 µS/cm, but dropped dramatically by the 48-h test termination (517 and 1,055 µS/cm). Alkalinity was 572 and 858 mg/L $CaCO_3$, while hardness was 654 and 944 mg/L $CaCO_3$. Results of the acute toxicity test with *V. iris* were similar, with a slightly higher LC50 value (18.95 %). Mortality was only checked at the 48-h interval, to minimize stress on the organisms. At this time, 100 % mortality was observed in the 50 and 100% concentrations, while 90 % mortality had occurred in the 25 % concentration. No mortality was observed in any of the lower concentrations of 12.5 % and below. Water chemistry for this test was similar to that of the *C. dubia* tests, with high conductivity, pH, alkalinity and hardness in the 100% concentration at test initiation, and conductivity reduced at test termination.

Discharge from the Pond 6 Western Diversion Dyke cut (P6W) was more toxic to *C. dubia* than the P6E discharge (Table 5.5). Acute toxicity was measured once using *C. dubia* and once using *V. iris* with 48 hr LC50s of 12.07 and 17.36 %, respectively. In the *C. dubia* test, 100 % mortality occurred down to the 25 % concentration after the first 24 h, and 55 % mortality occurred in the 12.5 % concentration at 48 h. All *V. iris* were dead in the 25, 50 and 100 % concentrations after 48 hr and minimal mortality occurred in the 12.5 and 6.25 % concentrations (5 %). No mortality occurred in the control. Water chemistry for both of these tests was similar. Conductivity was highest at test initiation (4,320 and 3,210 μS/cm) and declined substantially by 48 hr (2,123 and 1,567 μS/cm). The pH for these tests was 12.20 and 12.30, while alkalinity and hardness were the same for both tests, at 854 and 1,160 mg/L $CaCO_3$, respectively.

Acute toxicity was also measured using *C. dubia* and *V. iris* with the Olin WWTP discharge. After 48 hr of exposure, only minimal (20 %) mortality occurred in the 100 % concentration of the *C. dubia* test, and no mortality occurred in the *V. iris* test. Therefore, no LC50 values were generated for either test.

Due to the significant toxicity and extremely elevated pH of the P6E and P6W discharges, timed toxicity tests were conducted using *C. dubia*. The two discharges had relatively similar results during this experiment (Fig.5.5). After the first minute of exposure, all organisms were observed swimming in a normal manner. At the three minute interval, organisms in both the P6E and P6W samples were fluttering on the bottom of the beaker and no longer swimming in the water column, but no mortality had occurred. After 10 minutes, 60% mortality had occurred in the P6E test, while 90 %

mortality occurred in P6W. At 15 minutes, all organisms were dead in both P6E and P6W (Fig.5.5).

5.4 Discussion

Data from this research project indicate a continuing source of contamination coming from the remediated Ponds 5 and 6, by means of unregulated dyke cut discharges and possible seepage. The original focus of this research considered this possibility, but it was the large amounts of thick, white flocculent observed at the base of the diversion discharges which substantiated this hypothesis. The white precipitate was accumulating on the right bank of the river, adjacent to the pond areas due to the severely reduced flow of the river during late 2005-spring 2006. During late summer-winter 2005, flow in the NFHR averaged 43.8 ft^3/sec (Echols et al. In-Prep). Analysis confirmed that the flocculent had extremely high levels of Al (1.9 – 38 mg/L) and Fe (2.0-51 mg/L), well above the US EPA (1999)WQC limits of 0.0087 (Al) and 1 mg/L (Fe). River sediments from the area where this accumulation occurred also had extremely high levels of Al, Fe, and Ca. Aluminum ranged from 3,400 to 5,000 mg/kg, while Fe was 2,900-7,600 mg/kg. Because of these high levels, it became necessary to examine the diversion dyke cut discharges where the flocculent was accumulating. Laboratory toxicity testing was the most informative method used, whereas *in situ* testing was inconclusive.

Asian clam studies conducted in 2006 were not as conclusive in determining quality of the river-water sites adjacent to the Ponds 5 and 6 areas as in previous research (Echols et al. 2007). Survivorship impairment occurred to some extent at all sites downstream of Saltville, with percent survival ranging from 96 % at NFHR-6 (rm 55.9) to 0 % at P5R-DV. Site P5R-DC also had survivorship impairment (32 % of clams were dead); however, growth impairment at this site was not significantly different from the reference location, indicating that toxicity occurred, but perhaps was more intermittent. Interestingly enough, growth impairment was most significant at the furthest downstream sites from Saltville, at NFHR-6 and 7. Mean growth of clams at these sites was 0.96 and 0.60 mm, respectively. These are much lower measurements than those of P5R-DC clams (Table 5.3).

The bioaccumulation of mercury in Asian clam tissues was lower than expected based upon previous studies (Echols et al. 2007), but levels were still substantially higher at sites proximate to the remediated pond areas (0.069-0.086 mg/kg) and further downstream than at the upstream reference site (0.014 mg/kg). The bioaccumulation of Al and Fe was much greater in the clam tissues than Hg. The upstream reference site, NFHR-1, had the highest Al sediment levels (125.3 mg/kg), so it was difficult to compare downstream sites to the reference. However, control clams collected from the Clinch River, VA were also analyzed and results were substantially higher for the *in situ* placed clams compared to the control. Aluminum levels were 42.33 and 45.67 mg/kg at sampling sites located adjacent to Pond 5 and 93.67 and 36.67 mg/kg for clams placed at the base of Pond 6. Aluminum levels at Pond 6 sites seemed reversed as the 93.67 mg/kg level was actually obtained from clams which were placed on the left bank (P6L), across the river from the Pond 6 area. Iron concentrations corresponded with the results of the Al analysis. Levels of Fe were highest upstream at the reference location but fluctuated downstream. Site P6L had the highest tissue concentration of Fe downstream (144.33 mg/kg), more than double that of P6R. In addition to these data, sediment analytical results also indicated that P6L concentrations of these metals were higher than the right bank samples.

Toxicity testing was the most predictive measure in this study. The P6 East and West dyke cut discharges were acutely toxic to *C. dubia* and *V. iris*, with LC50 values ranging from 12.1-18.95 %. Because of these very low LC50s, it was necessary to estimate how quickly mortality was occurring in the 100% concentration of both discharges. *Ceriodaphnia* used for these "rate of toxicity" tests began showing an adverse effect after three minutes of exposure. At three minutes, organisms were no longer swimming in the water column, but rather, were fluttering on the bottom of the beaker. Some mortality was observed at 10 min, and 100% mortality occurred in both tests by 15 min.

Conductivities from the 100% acute test concentration ranged from 2,841-4,320 µS/cm, but declined significantly during the 48-hr testing period (517-1,567 µS/cm). Furthermore, the pH of the discharges ranged from 11.5 – 12.2, well above the US EPA (1999) WQC limits. Previous studies on toxicity of pH, has primarily focused on acidic pH but alkaline pH can be toxic as well. Belanger and Cherry (1990) reported a 48-hr LC50 for *C. dubia* at pH of 10.3, significantly lower than the pH observed in this study.

These results were supported by Polonsky and Clements (1999) which reported acute toxicity to *C. dubia* at a pH of 10.6. Unfortunately, studies involving unionids and alkaline pH have not been done, and, therefore, the results of this study cannot be well substantiated.

5.5 Summary and Conclusions

The results of this research clearly indicate a continuing source of contamination from the remediated/capped holding ponds especially during 5-year low-flow drought conditions, the ecological integrity and assimilative capacity of the river is compromised, and therefore, unable to sustain healthy, mussel populations downstream. This condition can especially occur during periods of low-flow conditions during a drought, which has occurred 10 times over the past 50 years. In addition to mercury entering the river, high levels of Al, Fe and extremely alkaline pH may also be contributing factors to this riverine impairment of mussels in the NFHR below Saltville, VA.

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Table 5.1. Trace element concentrations in water and sediment samples from the North Fork Holston River in 2006. Water and sediments were collected on the right descending banks below the Pond 5 and Pond 6 diversion dyke cuts, where a flocculent was observed in the river.

WATER [a]						
Month Sampled	**Pond 5 Results (mg/L)**			**Pond 6 Results (mg/L)**		
	Al	**Fe**	**Hg**	**Al**	**Fe**	**Hg**
March 2006	1.9	2.0	0.00023	4.0	2.3	0.00042
June 2006	3.4	3.1	0.0019	28.0	31.0	0.0076
July 2006	3.7	4.6	0.00021	38.0	51.0	0.00018

SEDIMENT [B]								
Month Sampled	**Pond 5 Results (mg/Kg)**				**Pond 6 Results (mg/Kg)**			
	Al	**Fe**	**Hg**	**Ca**	**Al**	**Fe**	**Hg**	**Ca**
March 2006	5,000	7,600	1.7	240,000	3,400	2,900	0.62	380,000

[a] Minimal detection limits (MDL) for Al, Fe and Hg in water are 0.03, 0.027 and 0.00008 mg/L, respectively.
[b] MDLs for Al, Fe, Hg and Ca in sediment are 7.4,6.9, 0.071 and 79 mg/Kg, respectively.

Table 5.2. Results of mercury analysis of Site Interstitial Water (SIW) from the North Fork Holston River with additional analyses for aluminum and iron from sites located in the river below Ponds 5 and 6.

Site Name	Mercury (mg/L) [a]	Aluminum (mg/L) [b]	Iron (mg/L) [c]
NFHR-1	0.0002		
Pond 5	0.0034	20.0	35.0
Pond 6	0.0042	18.0	30.0
NFHR-5	0.0034		
NFHR-6	0.00090		
NFHR-7	0.00080		

[a] MDL for Hg= 0.00008 mg/L
[b] MDL for Al= 0.03 mg/L
[c] MDL for Fe= 0.027 mg/L

Table 5.3**.** Mean Asian clam (*Corbicula fluminea*) survivorship (%) and growth (mm) after 60 days *in situ*.

Sampled Site	**Mean Survivorship (%)**	**Mean Growth (mm)**
NFHR-1	100[a]	2.06 ± 0.41 [a]
P5R-DC	68 [b]	1.46 ± 1.10 [a b]
P5R-DV	0 [c]	0.01 ± 0.02 [c]
P6R	80 [a b]	1.27 ± 2.11 [a b]
P6L	92 [a b]	1.97 ± 1.10 [a]
NFHR-5	92 [a b]	1.85 ± 0.89 [a]
NFHR-6	96 [a]	0.97 ± 0.44 [b]
NFHR-7	84 [a b]	0.60 ± 0.34 [b c]

***** Means with different letters denote statistical difference.

Table 5.4. Select trace metal levels (mg/kg) in Asian clam (*Corbicula fluminea*) tissues after 60 days *in situ* in the North Fork Holston River.

Site Name	**Hg (mg/Kg)**	**Al (mg/Kg)**	**Fe (mg/Kg)**
Clinch River Control	0.016	14.67	36.33
NFHR-1	0.014	125.33	191.33
P5L	0.078	42.33	79.00
P5R	0.069	45.67	72.33
P6L	0.071	93.67	144.33
P6R	0.086	36.67	66.00
NFHR-5	0.060	47.00	127.33
NFHR-6	0.084	58.67	107.67
NFHR-7	0.053	61.00	95.67

Table 5.5. Survival of *Ceriodaphnia dubia* at several test concentrations of Pond 6 Western Dyke discharge water, with unusual fluctuations in conductivity and pH over 48 hr.

Test Conc. %	**Conductivity (µS/cm)**		**pH**		**Survival %**
	0	**48 hr**	**0**	**48 hr**	
0	292	332	7.78	8.08	100
6.25	378	347	10.27	8.30	100
12.5	469	396	10.80	8.30	45
			48 hr LC50 = 12.07 %*		
25	927	395	11.40	9.26	0
50	2050	533	11.82	9.55	0
100	4320	2123	12.20	11.74	0

*Estimated mean conductivity at LC_{50} = ~ 430 µS/cm.

Table 5.6. Survival of juveniles of *Villosa iris* at several test concentrations of Pond 6 Western Dyke discharge water, with unusual fluctuations in conductivity and pH over 48 hr.

Test Conc. %	**Conductivity (µS/cm)**		**pH**		**Survival %**
	0	**48 hr**	**0**	**48 hr**	
0	203	235	7.86	8.08	100
6.25	205	203	9.69	8.32	95
12.5	215	204	10.50	8.33	95
			48 hr LC50 = 17.36 %		
25	522	241	11.41	8.88	0
50	1,414	415	11.94	9.83	0
100	3,210	1,567	12.30	11.62	0

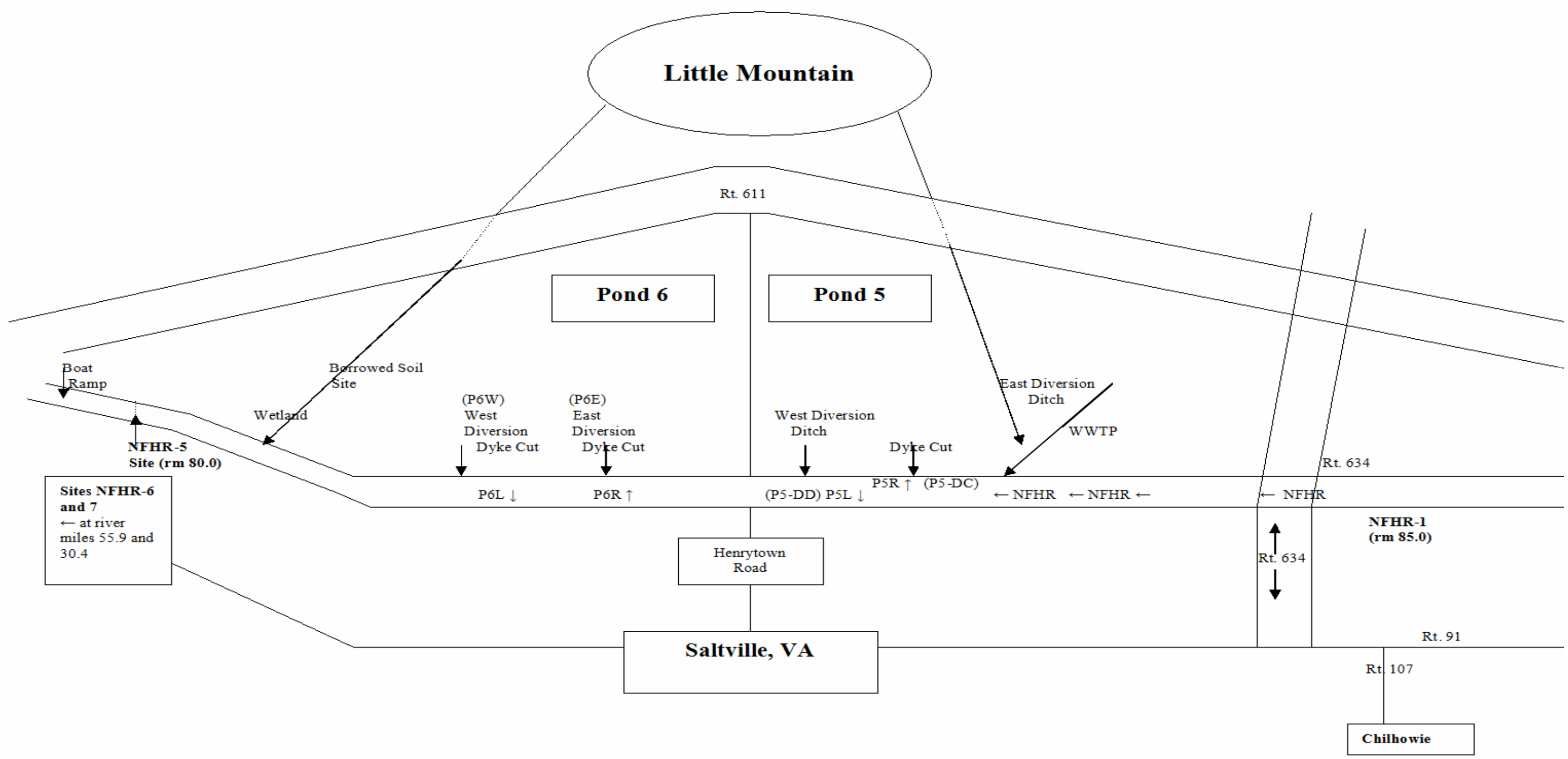
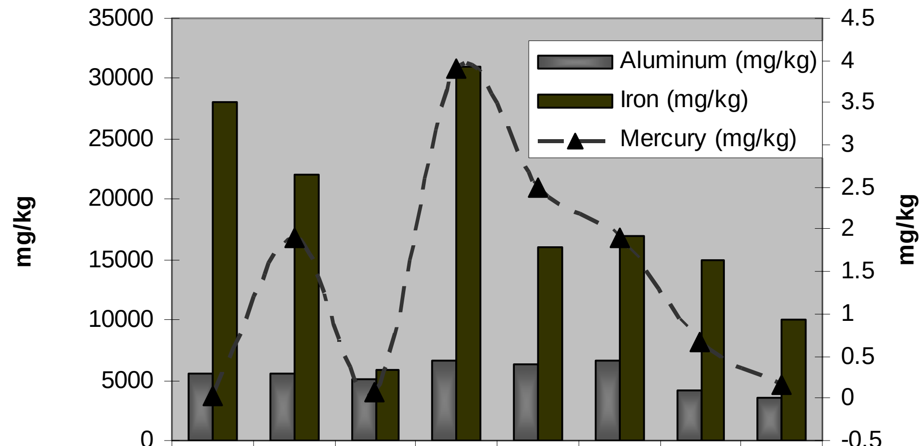


Figure 5.1. Schematic of the Ponds 5 and 6 remediated areas and diversion dyke cuts as they enter the adjacent river.



	NFHR-1	P5L	P5R	P6L	P6R	NFHR-5	NFHR-6	NFHR-7
Aluminum (mg/kg)	5500	5500	5100	6600	6300	6700	4200	3500
Iron (mg/kg)	28000	22000	5800	31000	16000	17000	15000	10000
Mercury (mg/kg)	0.021	1.9	0.079	3.9	2.5	1.9	0.66	0.17

Figure 5.2. Aluminum, iron and mercury levels (mg/kg) in North Fork Holston River (NFHR) sediments collected in fall 2006.

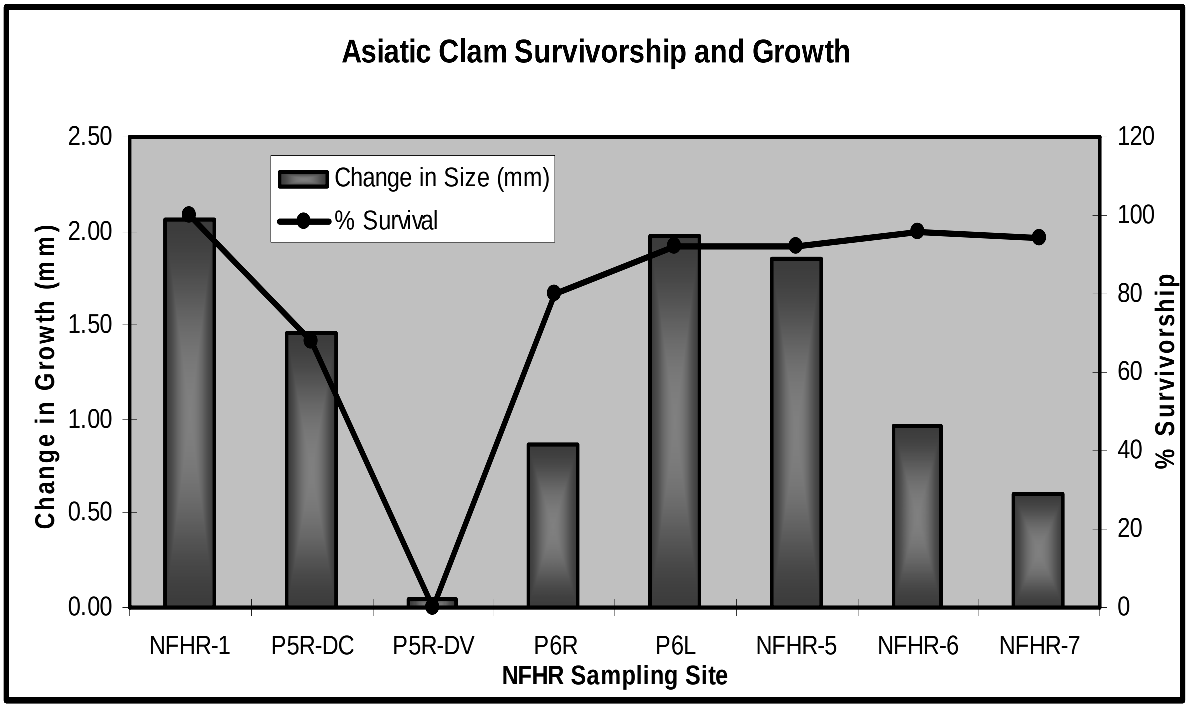


Figure 5.3. Change in Asian clam (*C. fluminea*) growth (mm) vs. clam survivorship after 60 days.

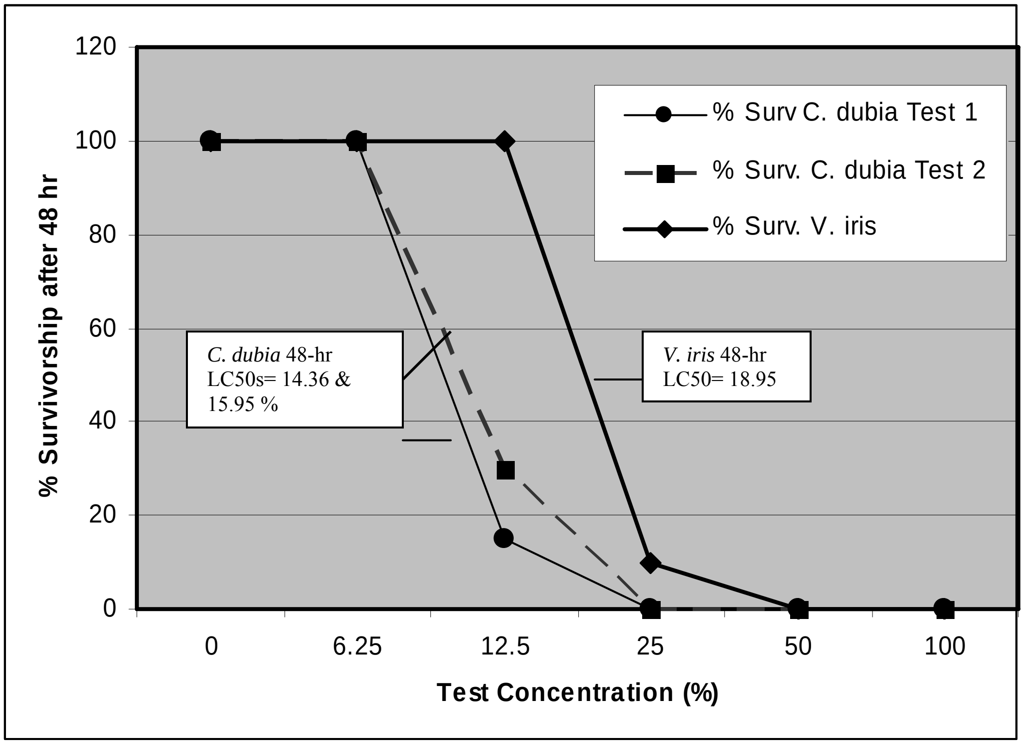


Figure 5.4 Pond 6 Eastern Dyke Cut discharge acute toxicity test results for *Ceriodaphnia dubia* and *Villosa iris*. Lines represent percent (%) survivorship after 48 hr of exposure in each test concentration.

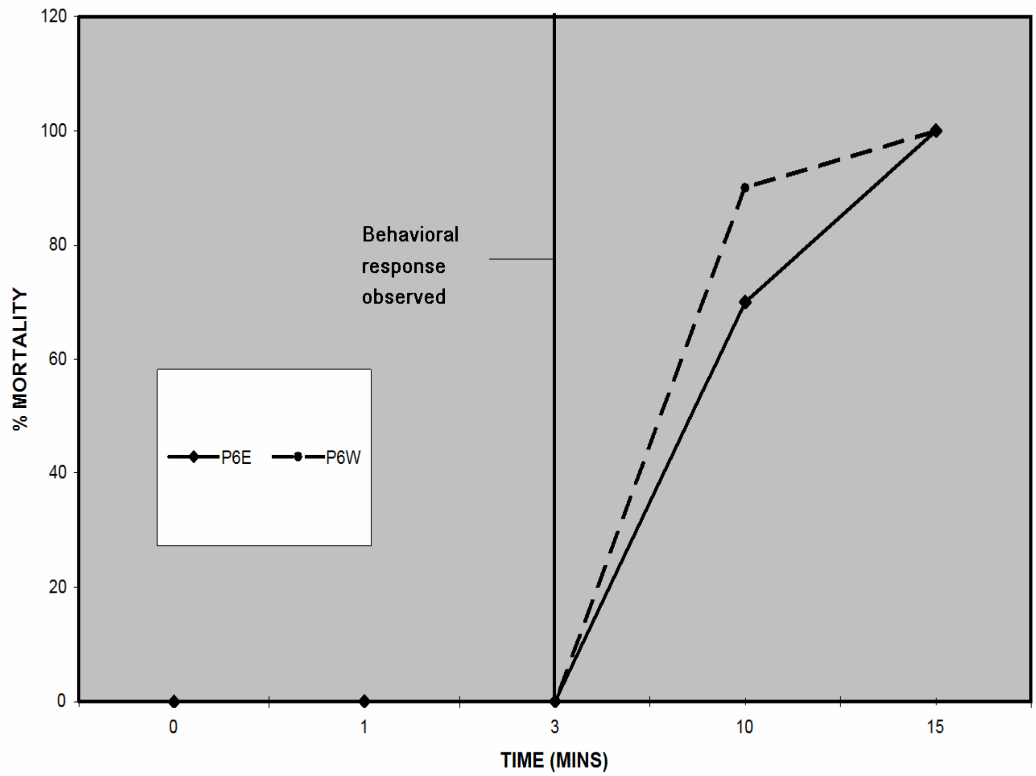


Figure 5.5. Timed response of *Ceriodaphnia dubia* to Pond 6 Eastern and Western Dyke Cut discharges.

CURRICULUM VITA

Brandi Shontia Echols

1.0 BACKGROUND/EDUCATION

1.1 Birthdate/Birthplace: November 30, 1979; Montgomery County, Virginia

1.2 Present Position: Graduate Research Assistant/Laboratory Manager, Cherry Laboratory, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061

1.3 Present Address:

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1.4 Education

1993-1997: Blacksburg High School Graduate.

1999-2001: Virginia Western Community College, Roanoke, Virginia, Associate Degree- Administration of Justice.

2000: Emergency Medical Technician License, Roanoke, Virginia.

2001-2003: Roanoke College, Salem Virginia, Bachelor of Arts, Criminal Justice major and Biology minor.

2004-Present: Graduate student, Aquatic Ecotoxicology, Dept. of Biological Sciences, Virginia Tech. Masters Degree to be completed Spring 2007.

1.5 Positions Held

2002-2003: Undergraduate Researcher, Dr. D.S. Cherry's Laboratory, Department of Biology, Virginia Tech, Blacksburg, VA.

2003: Student Researcher/ Assistant, Dr. Todd C. Peppers, Department of Public Affairs, Roanoke College, Salem, VA.

2003-2004: Substitute Teacher, Montgomery County Public Schools, Montgomery County, VA.

2003-2004: Laboratory Technician, Aquatic Ecotoxicology Lab, Roanoke College Department of Biology, Salem, VA.

2004: Laboratory Technician, Aquatic Ecotoxicology Lab, Virginia Tech, Blacksburg VA.

2005: Graduate Teaching Assistant, Department of Biological Sciences, Virginia Tech, Blacksburg, VA.

2005: Biology Graduate Student Association Service Chair person, Department of Biological Sciences, Virginia Tech, Blacksburg, VA.

2004-Present: Laboratory Manager, Virginia Tech, Cherry Laboratory, Department of Biological Sciences, Virginia Tech, Blacksburg, VA.

2005-Present: Graduate Research Assistant, Department of Biological Sciences, Virginia Tech, Blacksburg, VA.

2.0 RESEARCH SUMMARY

2.1 Undergraduate

Research conducted in Dr. Cherry's Aquatic Ecotoxicology Laboratory consisted of toxicity testing and field work for the evaluation or assessment of habitat destruction due to both active and abandoned coal-mining impacts in the upper-most portion of the Powell River Watershed, Southwestern, Virginia., including the North Fork of the Powell River and accompanying tributaries. Field assessments included benthic macroinvertebrate sampling, water grab sampling, sediment collection, habitat assessment (US EPA Rapid Bioassessment Protocols 1999), and *in situ* Asian clam (*Corbicula fluminea*) survival/growth studies. Laboratory testing consisted of acute and chronic toxicity testing, following US EPA protocols (2002), with two species of cladocerans *Ceriodaphnia dubia and Daphnia magna*.

2.2 Master of Science

Determination of ecotoxicological influences upon freshwater mussel (Unionidae) populations in the North Fork Holston River, below Saltville, VA. Evaluation of site interstitial water and sediments below two holding ponds (now USEPA Superfund Sites) from a closed alkaline-chlorine plant. Evaluation of a point-source brine discharge and dissipation during severe low flow conditions. Utilization of two freshwater mussel species (*Epioblasma capsaeformis* and *Villosa* *iris*) in laboratory toxicity test organisms to determine possible effects of residual mercury in site interstitial water and sediments from the North Fork Holston River.

Thesis Title: An Ecotoxicological Evaluation of the North Fork Holston River below Saltville, VA and Identification of Potential Stressors to Freshwater Bivalves: (Unionidae).

Evaluation of the Asian clam (*Corbicula fluminea*) as a surrogate toxicity test organism for freshwater mussels (Bivalvia: Unionidae). This work included the establishment of culturing techniques to facilitate the healthy release of pediveligers (juvenile clams) from parent for use in toxicity tests. Preliminary toxicity tests were also conducted to determine sensitivity of juvenile clams compared to juvenile freshwater mussels and other standard US EPA toxicity test organisms.

Establishment of laboratory mayfly toxicity tests with mayflies collected from natural habitats. Determination of suitable temperature acclimation time frames to minimize organism impairment during field to laboratory adjustment.

Evaluation of a closed landfill discharge on the biota of an unnamed tributary of the Roanoke River.

Evaluation of benthic macroinvertebrates assemblages in an intermittent tributary to support a strontium variance for the Ohio Paperboard Corporation located in Baltimore, OH.

3.0 EXPERTISE

3.1 Benthic Macroinvertebrates

Benthic macroinvertebrate sampling/identification and taxonomy (2002-present).

Qualitative and Quantitative methods and Utilization of Biological Integrity metrics such as the ICI (Ohio EPA 1987) and the SCI (Tetra Tech 2002).

Conducted benthic macroinvertebrate surveys in the following watersheds: Leading Creek, Ohio; Black Creek, Virginia; Pigeon Creek, Virginia; tributaries of the North Fork Powell River watershed; Virginia, Holston River, Saltville, VA; Roanoke River, Roanoke, VA; Masons Creek, Roanoke, VA; Tom’s Creek, Blacksburg, VA; Paw-Paw Creek, Baltimore, OH; Zellerbach Tributary, Baltimore, OH. Surveys were conducted for Cumberland Coal Company (CCC), Ohio Paperboard, United States Geological Survey (USGS) and US Army Corps of Engineers.

3.2 Toxicity Testing

Conducted acute and chronic toxicity tests with freshwater species, *Ceriodaphnia dubia, Daphnia magna, Pimephales promelas, Isonychia bicolor, Ameletus sp.* and the Asian clam *Corbicula fluminea*; sediment tests with *Daphnia magna, Chironomus tentans* and two freshwater mussel species, *Villosa iris* and *Epioblasma capsaeformis*; *in situ* chronic survival/growth impairment tests with *Corbicula fluminea* as well as chronic bioaccumulation studies with *C. fluminea*.

3.3 Laboratory Culturing

Maintained cultures of *Ceriodaphnia dubia, Daphnia magna, Chironomus tentans*, *Corbicula fluminea* and algal cultures including, *Selenastrum capricornutum*, *Neochloris oleoabdundans* and *Chlorella* sp.

3.4 Field Sampling

-Conducted *in situ* Asian clam chronic survival/growth rate studies in subwatersheds of the Powell River, Roanoke River, North Fork Holston River and Black and Ely Creeks.

-Conducted habitat assessments using US EPA Rapid Bioassessment Protocols (RBPs) in Virginia and Ohio Environmental Protection Agency's Qualitative Evaluation Index (QHEI) in Ohio.

-Conducted Porewater (interstitial water) collection in the North Fork Holston River using Porewater samplers (peepers, Hesslein, 1976).

4.0 ASSOCIATIONS

4.1 Memberships

Virginia Academy of Science (VAS)
North American Benthological Society (NABS)
Society of Environmental Toxicology and Chemistry (SETAC)
Virginia Natural History Society
American Fisheries Society (AFS)

5.0 GRANTS

6.0 PUBLICATIONS

6.1 Published Abstracts

1. B.S. Echols, R.J. Currie, D.S. Cherry, and J. Uerz. 2003. An Evaluation of the Most Predictive Bioassessment Indicators in the Identification of Active and Abandoned Mine Land Impacts in Subwatersheds of the Powell River, Southwestern Virginia. Virginia Journal of Science. 54(2) p. 62.

2. B.S. Echols, R.J. Currie and D.S. Cherry. 2005. A Preliminary Comparison of Ecotoxicological Ratings from Tributaries in the North Fork of the Powell River Watershed. Virginia Journal of Science. 56 (1) p 29.

3. B.S. Echols, R.J. Currie and D.S. Cherry. 2005. A Comparison of Ecotoxicological Ratings from Acid Mine-Influence Tributaries in the Powell River Watershed, Virginia. Society of Environmental Toxicology and Chemistry (SETAC) Abstract Book 26. pp. 374-375.

6.2 Papers in Review

1. B.S. Echols, R.J. Currie and D.S. Cherry. In-Review. An Investigation of Remnant Mercury Contamination in the North Fork Holston River, Saltville, VA. Submitted to Archiv Envir Contam Toxicol

2. B.S. Echols, R.J. Currie, D.S. Cherry, R.J. Neves and J.J Schmerfeld. In-Review. Comparison of Laboratory Toxicity Tests from a Point-Source Brine Discharge Using *Ceriodaphnia dubia* and a Juvenile Mussel (*Villosa iris*). Submitted to Envir Monit Assess

6.3 Papers in Preparation

1. B.S. Echols, R.J. Currie and D.S. Cherry. Field and Laboratory Assessments of the North Fork Holston River below two US EPA Superfund Sites, Saltville, Virginia. In-Prep

2. B.S. Echols, D.S. Cherry, and R.J. Currie. Evaluation of Conductivity Dissipation in the North Fork Holston River Downstream of a Point Source Brine Discharge during Severe Low-Flow Conditions. In-Prep

3. B.S. Echols, R. J. Currie and D.S. Cherry. Evaluation of Bioassessment Indicators in the Identification of Active and Abandoned Mine Land Impacts in Subwatersheds of the Powell River, VA. In Prep

4. R.J. Currie, B.S. Echols and D.S. Cherry. An Integrative Bioassessment of Abandoned Mine Land and Active Mining Influences to Upper Sub-basins in the North Fork and Powell Rivers, Southwest Virginia. In Prep

7.0 PRESENTATIONS

7.1 Posters

1. B.S. Echols, R.J. Currie, D.S. Cherry, and J. Uerz. An Evaluation of the Most Predictive Bioassessment Indicators in the Identification of Active and Abandoned Mine Land Impacts in Subwatersheds of the Powell River,

Southwestern Virginia. Meeting of the Virginia Academy of Science, Charlottesville, VA, May 2003.

2. B.S. Echols, R.J. Currie and D.S. Cherry. A Preliminary Comparison of Ecotoxicological Ratings from Tributaries in the North Fork of the Powell River Watershed. Meeting of the Virginia Academy of Science, Harrisonburg, VA, May 2005.

3. B.S. Echols, R.J. Currie and D.S. Cherry. A Comparison of Ecotoxicological Ratings from Acid Mine-Influenced Tributaries in the Powell River Watershed, Virginia. SETAC 26th Annual Meeting, Baltimore, MD, November 17th, 2005.

4. R.J. Currie, R.D. Baxter, B.S. Echols, A.E. Alexander and D. S. Cherry. Preliminary Assessment of the Asiatic Clam (*Corbicula fluminea*) as a Standard Toxicity Test Organism. SETAC 26th Annual Meeting, Baltimore, MD, November 17th, 2005.

7.2 Invited Talks

1. B.S. Echols, J. Griggs and A. Lahey. Fundamentals of Biomonitoring. Presented at Town of Blacksburg Development Committee Hearing, Blacksburg, VA. December 9, 2004.

2. B.S. Echols and D.S. Cherry. Ecotoxicological Influences upon Residual Mercury in Interstitial Water and Sediments in the North Fork Holston River, Saltville, Virginia. Presented at Olin Corporation/ US Fish and Wildlife Service Quarterly Meeting, Saltville, VA. February 8, 2006.

3. D.S. Cherry, B.S Echols and R.J. Currie. Research Objectives Proposed for Olin Corporation Ponds 5/6 in the North Fork Holston River, Spring 2006-2007. Presented at Olin Corporation/ US Fish and Wildlife Service Quarterly Meeting, Blacksburg, VA. March 13, 2006.

4. B.S. Echols, D.S. Cherry and R.J. Currie. North Fork Holston River Ecotoxicological Evaluation 2006. Presented at Olin Corporation/ US Fish and Wildlife Service Quarterly Meeting, Saltville, VA. December 5, 2006.

5. B.S. Echols. An Aquatic Ecotoxicological Evaluation of the North Fork Holston River, Saltville, VA. Presented at Roanoke College, Salem, VA,. December 6, 2006.

8.0 TECHNICAL REPORTS

1. Integrative Bioassessment of Abandoned Mine Land and Active Mining Influences to Sub-Watersheds and the North Fork of the Powell River: Final

Report. D. S. Cherry, R.J. Currie, B.S. Echols, Department of Biology, Virginia Tech. June 2004.

2. Acute Toxicity Evaluation of 003 Effluent from Celanese Acetate, Celco Plant, Virginia: Quarterly Report. D.S. Cherry, R. J. Currie and B. S. Echols, Department of Biology, Virginia Tech. August 2005 and December 2005.

3. Toxicity Test Laboratory Performance Evaluation (DMR-QA 24 WET) for Acute and/or Chronic Tests. D.S. Cherry, R.J. Currie, T.W. Valenti, B. A. Locke, M.L. Simon and B.S. Echols, Department of Biology, Virginia Tech. August 2004.

4. Toxicity Test Laboratory Performance Evaluation (DMR-QA 25 WET) for Acute and/or Chronic Tests. D.S. Cherry, R.J. Currie and B.S. Echols, Department of Biology, Virginia Tech. August 2005.

5. Development of Alternative Strontium Limits for Ohio Paperboard Corporation Using the Invertebrate Community Index and Best Professional Judgment in Zellerbach Tributary, Baltimore, Ohio. D.S. Cherry, R.J. Currie, and B.S. Echols, Department of Biology, Virginia Tech. February 2006.

6. Evaluation of Potential Acute Toxicity of 003 Effluent from International Business Machines Corporation (IBM), Poughkeepsie, New York: Quarterly Report. D.S. Cherry, Brandi S. Echols, and R.J. Currie, Department of Biology, Virginia Tech. February, May and August, 2006.

7. Five-Year Status of Asian Clam Invasion, Fouling and Control Measures at the Celco Plant, Narrows, VA. Annual Report. D.S. Cherry, R.J. Currie, and B.S. Echols. August 2006.

9.0 REFERENCES

1. Dr. Donald S. Cherry, Virginia Polytechnic Institute and State University, Department of Biology, Blacksburg, VA 24061. (540) 231-6766, dcherry@vt.edu.

2. Dr. Rebecca J. Currie, Roanoke College, Department of Biology, Salem, Virginia. (540) 375-2034, currie@roanoke.edu.

3. Dr. Richard J. Neves, Virginia Polytechnic Institute and State University, Department of Fish and Wildlife Services, Blacksburg, VA 24061. (540) 231-5927, mussel@vt.edu.

4. Dr. Todd C. Peppers, Roanoke College, Department of Public Affairs, Salem, Virginia. (540) 375-241, peppers@roanoke.edu.