Toxicity of zinc, copper, and sediments to early life stages of freshwater mussels in the Powell River, Virginia

by

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(ABSTRACT)

The decline in mussel fauna of the Powell River has been attributed to extensive mining activities in headwater streams of Wise and Lee counties, Virginia. Surface mining causes erosion, sedimentation, and contamination of water with toxic substances from coal washing and waste piles. Historical water quality data of the Powell River have documented concentrations of metals at levels determined to be toxic to molluscs, specifically zinc (Zn) and copper (Cu). Acute toxicity tests with Zn, Cu, and mixtures of these two metals were conducted with glochidia and juvenile freshwater mussels. Effects of varying conditions such as water source, temperature, length of exposure, species, and lifestage were determined. Additionally, the effects of Powell River sediment on survival and growth of juvenile mussels were evaluated.

The Cumberland moccasin shell (*Medionidus conradicus*) was the most sensitive species tested, with 24-hr and 48-hr LC50 values for glochidia ranging from 423 to 725 μg Zn/L. Glochidia of the pheasantshell (*Actinonaias pectorosa*) exhibited LC50 values from 274 to 2886 μg Zn/L, depending on test conditions. Similar results were obtained for glochidia of the rainbow (*Villosa iris*), with LC50 values ranging from 577 to 4123 μg Zn/L. Juveniles were more sensitive, with 48-hr LC50 values ranging from 360 to 1885 μg Zn/L for *A. pectorosa*, and 339 to 1122 μg Zn/L for *V. iris*, depending on test conditions. Juvenile mussels were affected by Zn at lower concentrations as evidenced by valve gaping and a swollen and nonresponsive foot. Copper was 5 to 15 times more toxic than Zn, with 48-hr LC50 values ranging from 52 to 156 μg Cu/L, and EC50 values ranging from 25 to 115 μg Cu/L for juveniles of *A. pectorosa*. Copper appeared to exert a different toxic mode of action, as evidenced by closed valves and

reduced siphoning. In general, sensitivities of early life stages of mussels to Zn and Cu increased with higher temperature, soft water, and length of exposure. At certain concentrations, Zn seemingly had an antagonistic effect (less than additive) when mixed wtih Cu. This effect was evidenced by reduced mortality of juveniles in Cu solutions when Zn was added at concentrations of about 400 to 800 μg/L. However, this antagonistic effect was not reflected in the percent of juveniles affected, which increased continuously with increasing metal concentrations.

Glass beads were found unsuitable as a control substratum for use in sediment testing with juvenile mussels. Results of sediment tests indicated that sediment in some areas of the Powell River may be toxic to juvenile mussels, and that toxicity may be linked to water quality. After 10 days, survival of juveniles in sediment collected downstream of a coal processing plant was significantly lower than survival in sediment upstream of the plant ($p = 0.01$). Further, survival in sediments with dechlorinated tap water was significantly higher than survival in sediment with river water ($p = 0.0002$). After 20 and 30 days, survival was similar among sites and water types. High metal concentrations in the river water appeared to contribute to toxicity, because juveniles in tap water displayed consistently better growth, and initially better survival than juveniles in river water and sediment. This toxicity was not apparent in sediments collected from the same sites less than two months later, suggesting the character of the sediments may change as new suspended sediment is deposited.

The USEPA water quality criteria for Zn (adjusting for water hardness) are 174 μg/L (acute) and 158 μg/L (chronic), whereas copper criteria are set at 28 μg/L (acute) and 18 μg/L (chronic). Powell River water samples collected during 1991 contained concentrations of Zn and Cu exceeding these criteria, as well as concentrations shown to have adverse effects on mussel populations. Results suggest that some metals are introduced into the river system in runoff, whereas Cu is being introduced as an episodic event. Intensive monitoring of water quality is needed to identify specific sources of metal pollution. If levels of heavy metal concentrations remain high, then the declining mussel populations of the Powell River will not recover, and endangered species may be extirpated from Virginia.

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Chapter One

Introduction

The Cumberland Plateau region of the southeastern U.S. is noted for the highest diversity of freshwater mussel species in the world. The Powell River in southwest Virginia is included in this region (Figure 1). In Virginia, there are currently 14 federally endangered mussel species, and 21 additional species have been proposed for state protection. In a qualitative survey of the mussel fauna, Ortmann (1918) recorded as many as 42 species in the Powell River. It is believed that additional species were present but undetected due to his sampling method (Wolcott 1990). By the mid-1970's, the number of mussel species had declined to 36 (Dennis 1981) or 38 (Tennessee Valley Authority 1979) species. Twelve species reported by Ortmann were not found in the Powell River in a survey conducted by Dennis (1981). Surveys performed in 1983 and 1988 by Jenkinson and Ahlstedt (1988) identified only 23 species. However, a survey of the Virginia section of the Powell River in 1988-89 found at least 28 species present, including six endangered species (Wolcott 1990).

In general, mussel diversity has declined in the Powell River. Previous studies also have documented a decline in mussel densities between 1973 and 1988 (Tennessee Valley Authority

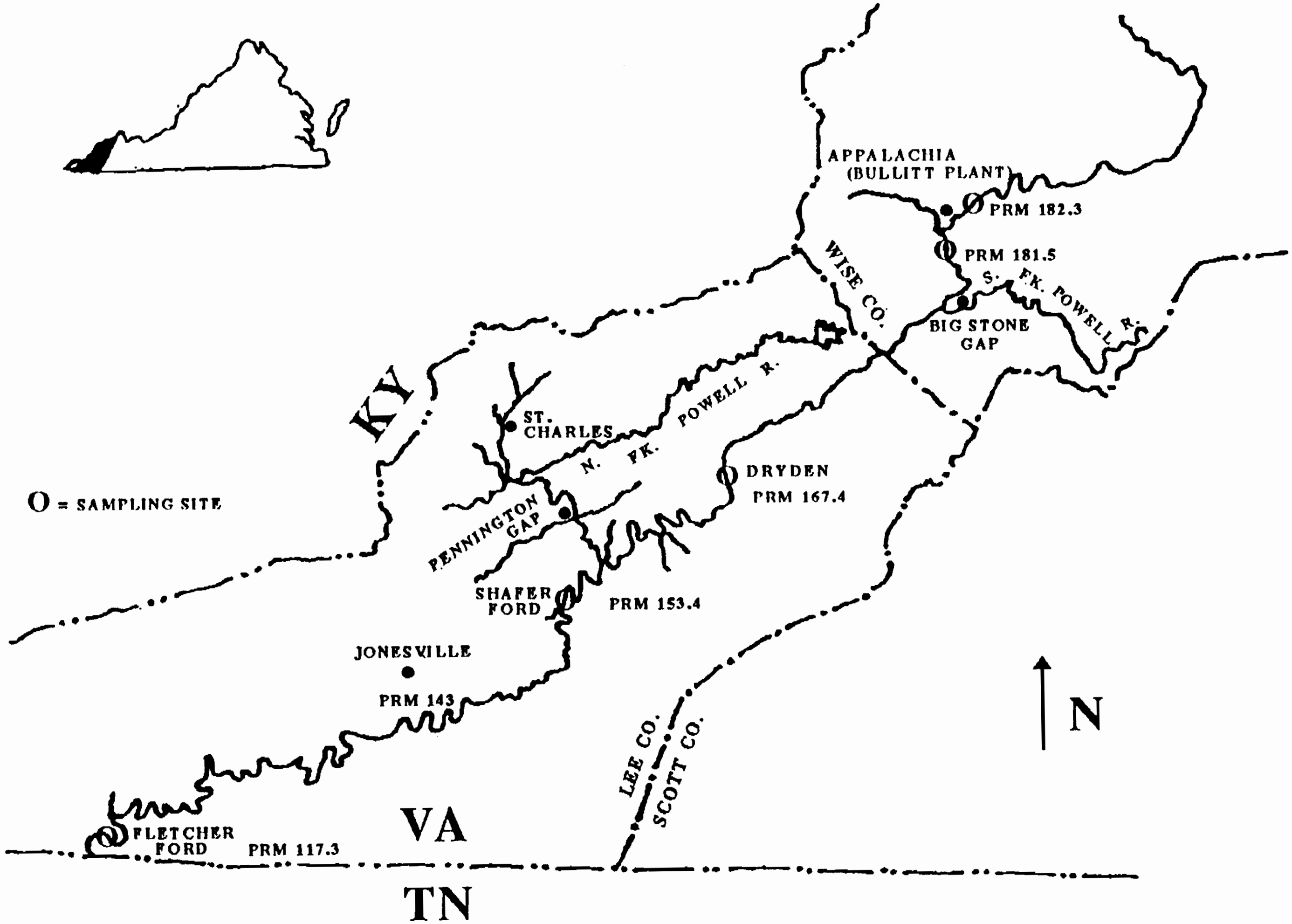


Figure 1. Map of the Powell River, Virginia, with sampling sites identified.

1979, Jenkinson and Ahlstedt 1988, Wolcott 1990). The decline in mussel fauna of the Powell River, Virginia, has been attributed to extensive mining activities in the upper watershed (Neves et al. 1980, Dennis 1981, Wollitz 1985, Biggins 1989, Wolcott 1990).

Surface mining in the Powell River drainage began in 1944, reached a peak in 1976, and is still fairly intensive (Hibbard 1990). Impacts due to surface mining include erosion and sedimentation, stream diversion and channnelization, acid drainage, and contamination of water with toxic substances from coal washing and waste (gob) piles (Starnes 1983). Some headwater streams with acid mine drainage problems have the acidity buffered further downstream by alkaline minerals, although high sulfate levels remain throughout the river (Tennessee Valley Authority 1980). Coal waste deposition or "black water" also was identified as a major problem (Tennessee Valley Authority 1980). However, a recent study by Wolcott (1990) failed to correlate increased sedimentation and coal waste deposition in the Powell with decreased mussel abundances at specific sites. Neves et al. (1980) identified impacted areas that may have been attributed to pH and heavy metal pollution. Water quality above Dryden (no mussels found above this site) differed substantially from downstream areas. The toxic effects of these coal-related contaminants are largely unknown, but they may play a significant role in the decline of mollusks in the river.

Coal Mining Impacts

In simplistic terms, surface mining for coal involves removal of the top soil which is set aside for reclamation. The overburden rock is then removed and placed into an open pit or an adjacent cut where coal has been previously removed (spoil piles). The removal of the overburden reduces its density, exposes it to weathering and thus dissolution and migration of trace elements into the environment. At this point the coal is removed and undergoes a cleaning (coal-washing) process before transportation. The coal-washing processes involve separation of the mineral-rich particles (high density) from the organic-rich particles (low density) through the use of liquid medium with a specific gravity to optimize separation. The resultant cleaning water, which now has an elevated trace-element content, is released into settling ponds and eventually into nearby streams (U.S. National Committee for Geochemistry 1980). Another source of pollution from coal mining is the storage piles of coal awaiting transportation. Precipitation percolates through the piles, leaching out trace metals which eventually enter nearby streams or contaminate the groundwater.

Pyrite is an iron sulfate mineral ($FeSo_4$) commonly associated with coal and composes much of the overburden. Upon mining, this material is crushed and deposited into spoil piles where it is exposed to weathering. In the presence of water and oxygen, pyrite is oxidized to form sulfuric acid and iron salts (ferrous sulfate) which can leach into nearby streams and aquifers. These iron salts may be further oxidized and hydrolyzed as water flows through more basic conditions such as a natural stream and precipitate out as "yellow boy". Ferrous sulfate in the spoil pile can also be further oxidized to form ferric sulfate which can then rapidly oxidize additional pyritic material to form more ferrous sulfate and sulfuric acid (Connell et al. 1976). If the overburden contains sufficient limestone, the acid is neutralized, but other harmful toxic elements remain (Ahmad 1973).

In a review of the literature on trace elements in coal preparation wastes, Wewerka et al. (1976) observed that although acidic effluents had the highest contaminant concentrations, non-acid waste runoff also contained elevated concentrations. They identified large gaps in the data and concluded that a comprehensive assessment of leachate potential of coal preparation wastes could not be made. However, numerous potentially toxic trace elements (e.g., aluminum, zinc, copper, nickel) were known to be present in coal and so have the potential to leach out into the environment (Wewerka et al. 1976).

Attempts have been made to identify the trace elements in coal which have the potential of contaminating the environment. It is the very nature of coal to contain almost every naturally occurring element. However, the chemical composition is site and source specific; i.e., different types of coal (lignite, subbituminous, bituminous and anthracite) will contain different proportions of identical elements (U.S. National Committee for Geochemistry 1980). Also, leachate concentrations are dependent on local geochemical and climatic conditions. The U.S. National Committee for Geochemistry (1980) identified arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) as elements of greatest concern due to their highly toxic effect on most biological systems. They also identified chromium (Cr), vanadium (V), copper (Cu), zinc (Zn), nickel (Ni) and fluorine (F) as potentially toxic and of moderate concern.

Not all elements contained in coal leach out at the same rate. Williams et al. (1977) conducted a laboratory study analyzing leachate from coal preparation waste. Iron concentrations in the leachate represented only a small percentage of the total iron in the waste. Other elements such as cobalt (Co) and nickel (Ni), which made up a small percentage of the waste, were found to be highly leachable. Aluminum (Al), another major constituent of the waste, was not leached very readily. They did not determine whether these leaching rates would be maintained under field conditions.

Before surface mining in the Appalachia region increased dramatically in the mid-1970's, few studies investigated the adverse effect on water quality other than low pH resulting from acid mine drainage. Curtis (1972) was one of the first to document that chemical pollution occurred even when acid drainage was absent. Streams draining surface mined lands in eastern Kentucky exhibited elevated levels of compounds and elements such as sulfate (SO_4), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), Al and Zn following the onset of mining. Some elements peaked quickly after the cessation of mining and showed signs of recovery within a year. Other elements continued to increase in concentration at least 2 years following mining cessation. The highest concentrations of chemicals were associated with late summer rains, so it was recommended that water quality data be analyzed at this time to identify possible pollution problems.

In the New River basin, Tennessee, three undisturbed watersheds were compared to three similar watersheds which had previously been mined or were currently being mined (Minear and Tschantz 1976). High levels of suspended solids, Ca, Mg, SO_4, and Mn were observed in the disturbed watersheds; in one case, long after completion of mining activity.

Other studies also indicated that recovery of streams impacted by surface mining may require 15 to 20 years (Becker et al. 1986, Woods et al. 1986).

The effects of runoff from coal pile storage adversely affected water quality of a nearby creek in western Massachusetts (Tan and Coler 1986). Sulfate levels increased five fold below the coal pile. Aluminum, Mn, and Fe concentrations were correlated with the pH levels (increased with decreasing pH), and Zn reached potentially toxic levels.

Few studies have focused on the effects of coal mining activities on freshwater mussel populations. Wolcott (1990) studied the effects of coal waste deposition on the distribution of mussels in the Powell River, and she found no correlation between the distribution of mussels and percent of coal in the sediment. Another study completed on the Cumberland River drainage in Kentucky determined that mussel densities decreased directly below the confluence with a tributary impacted by mining activities (Anderson 1989). A high concentration of metals (Al, Fe, Mn, Zn) in the lower part of the drainage was associated with the most heavily mined areas. Again, alkaline drainage dominated, so low pH was not a major problem. Although a causative agent was not identified, there was a clear correlation between recent strip mining activities and declining mussel populations (Anderson 1989).

Swift (1985) attempted to correlate possible toxic contaminants with leachate from spoil piles, coal-washing activities, storage piles and the degraded water quality of Georges Creek in western Maryland. The runoff from a coal storage pile was highly contaminated with metals; highest concentrations occurring during summer low flows, especially of Al, As, Zn, and Hg. In another study, the freshwater mussel *Elliptio complanata* accumulated metals from contaminated sediments in an area impacted by mining (Tessier et al. 1984), but the toxicological effects of these metal accumulations were not determined.

Overall, many trace elements present in coal are subject to leaching into the environment, thereby adversely affecting water quality. The elements being leached and the extent of leaching into waterways depend on a variety of factors. Among those are differences in climate (amount of precipitation), types of coal being mined, overburden makeup, general geology and hydrology of the area, and percent of area disturbed by mining. It is apparent that any assessment of water quality changes due to mining needs to be site-specific. It is also apparent that recovery of water quality and biota affected by abandoned (unreclaimed) surface mines may take in excess of 20 years (Minear and Tschantz 1976, Becker et al. 1986, Woods et al. 1986).

Extensive mining has taken place in the Powell River drainage since the 1970's, and many mines have been abandoned without any reclamation efforts. The Division of Mined Land Reclamation has a program for the reclamation of abandoned mines. High priority is assigned to those sites posing a danger to public health and property, with aquatic resources receiving low priority. At the present rate of reclamation in Virginia, it will take in excess of 55 years just to reclaim the high priority sites (Spangler 1989).

Objectives

This project, funded by the U.S. Fish and Wildlife Service, was initiated to identify possible contaminants in the Powell River and assess their effects on freshwater mussels.

Specific objectives of this study were as follows:

Objective 1 - select suspected contaminants from coal mining and processing operations and determine their LC50 and EC50 values using glochidia and juvenile mussels (Chapter 2).

Objective 2 - determine the toxicity of sediments in the Powell River to juvenile freshwater mussels (Chapter 3).

Objective 3 - determine the adequacy of USEPA water quality criteria to protect freshwater mussels (Chapter 4).

Chapter Two describes the acute toxicity tests conducted with glochidia and juvenile mussels, and discusses the results in relation to water quality in the Powell River. Sediment toxicity tests with juvenile mussels are presented in Chapter Three, whereas Chapter Four compares results of this and other studies with current water quality criteria and regulations and provides recommendations to better protect mollusks.

Chapter Two - Acute Toxicity Tests

Introduction

Water quality criteria set for the protection of aquatic life are determined by the U.S. Environmental Protection Agency (USEPA), based in part on toxicity test results. Aquatic toxicity tests expose organisms to a treated and untreated water solution to determine the effect of a chemical (or an effluent). Effective end-points generally include mortality, reduced growth and reproduction, and immobilization. Acute toxicity tests use a range of chemical concentrations to establish the LC50; i.e., the lethal concentration for 50% of the test organisms over a specified exposure period, usually 24 to 96 hours. The chemical concentration which affects, but is not necessarily lethal, to 50% of test organisms is termed the effective concentration (EC50). Effective end-points generally include reduced growth and reproduction, immobilization, and abnormal behavior, among others. Chronic toxicity tests are designed to expose all life stages of an organism to a range of chemical concentrations. Results are used to determine the effect and no-effect concentrations of a contaminant. From these data, the threshold concentration can be estimated and expressed as the maximum acceptable toxicant concentration (MATC) (Petrocelli 1985). However, complete life cycle tests for some organisms are impractical, as they may require years to complete one test and are prohibitively expensive. Therefore, a trend has developed for shorter-term tests. In order to maximize results, the most sensitive early life stages have been used. Estimates of the MATC derived from early life stage toxicity results closely approximate values determined from chronic tests (McKim 1985). When chronic toxicity and MATC data are not available, an application factor is commonly used with the LC50 value in an attempt to ensure safe concentrations.

Information on the toxic effects of metals to freshwater mussels, especially to early life stages, is lacking. Mussels have been used as biomonitoring organisms of toxic contaminants because they are sedentary, occupy a low trophic level, and are sensitive to water quality degradation (Havlik and Marking 1987). Mussels are long-lived and so can reflect historical water quality disturbances in their shell content. Metals that have been monitored in mussel shells include cadmium, copper, lead, manganese, mercury, strontium, iron, magnesium, zinc, barium, sodium, silver, and nickel (Imlay 1982).

Foster and Bates (1978) used caged mussels to monitor industrial effluent in the Muskingum River, Ohio. Even though copper concentrations in the effluent met state-mandated regulations, mussels bioaccumulated copper to lethal levels up to 21 km below the effluent outfall, and mussels up to 53 km downstream were partially affected. This study was used to point out the inadequacy of state effluent limitations to protect aquatic species. Another *in situ* toxicity test using caged mussels (*Elliptio dilatata*) was conducted in the Cumberland River drainage, Kentucky (Anderson 1989). Mortality rates of mussels placed in areas downstream of mining activities were higher than those of mussels placed upstream of mined areas. In the North Anna River, Virginia, the presence of mussel beds was considered an indication of biological recovery from an area impacted by acid mine drainage (Simmons and Reed 1973).

An excellent literature review of the effects of contaminants on freshwater mussels was completed by Havlik and Marking (1987). Unfortunately, most studies dealt with the ability of mussels to take up and store the contaminants in various tissues and shell material, but few addressed the direct toxic effects of the contaminants. It is interesting that manganese was accumulated at very high concentrations without any apparent adverse effects. This was observed even when water concentrations of Mn were barely detectable (Havlik and Marking 1987). However, recent toxicity tests with juvenile mussels (*Anodonta imbecillis* = *A. imbecilis*) indicated that they were sensitive to Mn (LC50 = 36.2 mg/L) (Wade et al. 1989). Copper was lethal at concentrations as low as 25 ug/L under long-term exposures (Havlik and Marking 1987). Other metals reported to be toxic at various concentrations include Cd, Cr, As, and Zn. All of these tests were conducted with adult mussels. Havlik and Marking (1987) identified the need for more qualitative and quantitative analyses of contaminant effects on mussels in conjunction with additional stress factors (high temperature, low dissolved oxygen, low pH). Extensive monitoring programs to identify contaminants and the need for regulatory agencies to control sources of these contaminants were recommended to protect mussel populations.

The toxicity of coal-related contaminants has not been adequately investigated. Anderson (1989) and Wolcott (1990) used freshwater mussels to study the effects of suspected coal mining pollution, but specific contaminants were not identified. Birge (1978) and Birge et al. (1978) used various fish and amphibian species to evaluate the toxicity of metals found in coal combustion waste and fly ash leachate. Twenty-two coal elements were identified as possibly hazardous to aquatic ecosytems (Birge 1978). Embryo-larval toxicity tests were performed using goldfish (*Carassius auratus*), rainbow trout (*Oncorhynchus mykiss*), and the narrow-mouthed toad (*Gastrophyrne carolinensis*). Based on an average of the LC50's for all species, the elements were ranked into groups. Those identified as being highly toxic (LC50 <1 mg/L) were silver (As), Hg, Cd, Al, Co, As, Cr, Pb, Ni and tin (Sn). Several elements were identified as moderately toxic (LC50 1-5 mg/L); Zn, vanadium (V), Cu, germanium (Ge), thallium (Tl), strontium (Sr), antimony (Sb), Mn and selenium (Se) were assigned to this group. An analysis of fly ash leachate revealed concentrations of Hg, Al, Cd, Ni and Zn high enough to adversely affect aquatic species. The interactions of several metal mixtures (Hg/Cd, Hg/Cu, Hg/Se, Hg/Zn) were analyzed by Birge et al. (1978). Results indicated that the toxicity of metal combinations was highly dependent on exposure concentration; i.e., effects may be antagonistic (less than additive) at low concentrations and synergistic (more than additive) at high concentrations. Aluminum was identified as the major active toxicant in a study investigating the toxicity of coal pile leachate (Tan and Coler 1986). Iron, Mn, and Zn also added to the toxicity. Using the asian clam (*Corbicula fluminea*), Doherty et al. (1987) found that increasing concentrations of Cd and Zn (from 0.1 - 0.9 mg/L) resulted in increasing duration of valve closure. Although valve closure may minimize exposure and uptake of metals in the short term, chronic exposures could inhibit feeding, growth or reproduction.

The combined effects of low alkalinity, low pH and metal toxicity were investigated in a study using several invertebrate species (Mackie 1989). Two species of freshwater clams, *Pisidium casertanum* and *P. compressum*, and one species of gastropod, *Amnicola limosa*, were used in the bioassays. For the metals tested (Cd, Al and Pb), results indicated that toxicity may increase or decrease with decreasing pH, depending on species and metal tested. Cadmium was the most toxic metal tested on clams, with an LC50 ranging from 0.36 mg/L to 2.08 mg/L. The decrease in toxicity at low pH may be explained by the mussel's avoidance reaction; i.e., closure of valves to reduce exposure and thus uptake (Mackie 1989). Chronic and acute metal toxicity tests were run on several species of freshwater snails by Nebeker et al. (1986) under soft water and low alkalinity conditions. The no-observed effect levels (NOEL, mortality not significantly different from that of control groups) were 0.124 mg/L (Ni), 0.006 mg/L (Cu), and 0.570 mg/L (Zn). Acute test results (LC50) were as follows: 0.008 - 0.015 mg/L (Cu), 0.237 - 0.239 mg/L (Ni), and 1.274 mg/L (Zn) (Nebeker et al. 1986).

Goudreau (1988) conducted bioassays with glochidia of *Villosa nebulosa* (= *V. iris*) to assess the impact of monochloramine and unionized ammonia, the major toxicants in sewage treatment plant effluent. Glochidia were among the most sensitive organisms tested for these toxicants (Goudreau 1988). Wade et al. (1989) used 6-8 day old juveniles of *Anodonta imbecilis* to determine the short-term (9-day) toxicity of manganese, three pesticides, and a paper mill effluent. Results indicated that Mn is toxic to juveniles (LC50 = 36.2 mg/L) even though it was thought to be non-toxic to adults (Havlik and Marking 1987). Both glochidia and juveniles of five mussel species were used in a study carried out by Jacobson (1990) to determine LC50 and EC50 concentrations of copper. Copper concentrations as low as 16-42 ug/L were toxic to glochidia and juveniles in 24- to 48-hr toxicity tests, and it is probable that longer-term tests would reveal toxicity at even lower concentrations. Acute toxicity of several metals (Cd, Cr, Cu, Hg, Ni, and Zn) was tested with juveniles of *Anodonta imbecilis* by Keller and Zam (1991). In soft water, cadmium was the most toxic (96-hr LC50 = 0.009 mg/L), followed by chromium (0.039 mg/L), copper (0.086 mg/L), mercury (0.147 mg/L), nickel (0.190 mg/L), and zinc (0.268 mg/L). Except for mercury, the metal toxicity was significantly reduced in moderately hard water.

The Powell River drainage basin has many abandoned and active mines that may be leaching toxic concentrations of metals into adjacent streams. Several studies have identified Al and Zn as common leachates from coal piles or mined areas (Curtis 1972, Swift 1985, Tan and Coler 1986, Anderson 1989). Historical water quality data (STORET) of the Powell River document concentrations of certain metals at levels that are toxic to molluscs (Nebeker et al. 1986, Havlik and Marking 1987, Wade et al. 1989, Jacobson 1990) and other aquatic species (Birge 1978, Birge et al. 1978, Tan and Coler 1986). Specifically, Zn concentrations have been recorded as high as 260 μg/L, and have increased from 30 to 160 μg/L in just two days, suggesting episodic metal pollution. Unfortunately, water sampling efforts have been limited to once or twice a year per sampling site. Thus, the chances of recording these pulse events are slim. STORET data also have recorded Cu concentrations of 30 μg/L, which have been reported as high enough to affect young mussels (Jacobson 1990). Aluminum was not listed in the STORET database for the Powell River. However, research has shown Al to be nontoxic at alkaline pH, which is prevalent in the Powell River. Thus Zn and Cu were chosen for the toxicity tests to be conducted on mussels indigenous to the Powell River. Information on the toxicity of these metals may indicate reasons for the decline in mussel populations of the Powell River. It is imperative that current water quality criteria be evaluated for their adequacy to protect freshwater mussel populations. Use of the most sensitive life stages (larval and juvenile) should provide the best means to evaluate USEPA criteria for acceptable water quality.

Materials and Methods

Acute Tests with Glochidia

Three mussel species were used in toxicity testing; rainbow (*Villosa iris*), pheasantshell (*Actinonaias pectorosa*), and the Cumberland moccasinshell (*Medionidus conradicus*). Gravid mussels were collected from the Clinch River, Tazewell and Russell counties, and Copper Creek (a Clinch River tributary), Scott County, Virginia. Mussels were held in a recirculating 100-L tank or 285-L Living Stream (Frigid Units Inc., Toledo, OH) until needed. Temperature was maintained at or below 18°C to discourage premature release of the glochidia.

Depending on availability, glochidia from one to three adult mussels were pooled for each test. The glochidia were removed from the adult by gently prying open the shell and inserting a sterile, 22 gauge 3.8 cm hypodermic needle into the marsupium. Water was used to gently force the glochidia through holes made by the needle and into a petri dish. This method was used as an alternative to sacrificing the adult. Mussels were later returned to one of the collection sites. Once the glochidia were collected from the adult(s), a viability test was performed. This test consisted of removing a sample of glochidia to another dish and adding saturated NaCl solution. It has been shown that healthy viable glochidia snap shut when exposed to NaCl solution (Zale and Neves 1982). Glochidia were considered viable when a minimum of 90% of the test glochidia closed valves in response to the salt solution.

A stock solution of Zn was prepared by adding 1 g of $ZnSO_4.7H_2O$ to 1 L of distilled deionized water. Test solutions were then prepared by the addition of appropriate amounts of stock solution to the dilution water to obtain the desired metal concentration. Samples of test solutions were preserved with concentrated nitric acid (HNO_3) at the concentration of 2 ml/1000 ml, and analyzed for metal content by the Virginia Tech Soils Testing Laboratory. Metal analysis was conducted by inductively coupled, argon plasma emission spectroscopy (Jarrell-Ash ICAP 9000). Dissolved oxygen, pH, alkalinity, and hardness also were determined (APHA 1989). Conductivity was measured with a conductivity pen (Nester Instruments). Powell River water and Blacksburg dechlorinated tap water (source, New River) were used as dilution waters. Stored Powell River water was kept refrigerated (4°C) and then filtered through Fisherbrand filter paper of medium porosity (5-10 μm particle retention) to remove organisms which may prey on young mussels or otherwise interfere with testing. Tap water was run through an activated charcoal filter and aerated prior to testing to remove chlorine and increase the dissolved oxygen concentration to at least 6 mg/L. Prior to any toxicity tests, tap water was analyzed for total residual chlorine content ($<$ 0.02 mg/L) before use as dilution water.

Test containers consisted of covered, 6-well polystyrene tissue culture plates (Falcon 3046). Three replicates of each test concentration were randomly assigned a well, and 12 ml were added to each well. A pasteur pipet was then used to transfer approximately 50 to 100 glochidia to each well. The tray was then covered and placed in an incubator (Fisher Model 307). The incubator had been previously set at the desired temperature (12° or 20°C) and photoperiod (16:8 hour light-dark). Test duration was confined to 24-hr or 48-hr because high mortality of glochidia occurs thereafter (Goudreau 1988, Jacobson 1990).

At the end of the exposure period, individual trays were removed and examined under a dissecting microscope. The number of open and closed valves was counted. A saturated NaCl solution was then added and the count of open valves repeated. As discussed previously, healthy glochidia responded by snapping shut. Those glochidia which were closed before the addition of NaCl solution and those that remained open following NaCl addition were recorded as dead. A glochidium with closed valves cannot attach to a host fish and thus will not survive (functionally dead). Those that responded by snapping shut were counted as live. An average of the three replicates was used in a Probit and Spearman-Karber analysis to estimate the LC50 value (Finney 1971, Buikema et al. 1982). Toxicant solutions were pooled from three replicates and later analyzed for Zn concentration. The mean Zn concentration at the start and finish of each test was used in the statistical analysis.

Acute Toxicity Tests with Juveniles

Juvenile mussels were obtained by infesting host fish with glochidia in the laboratory. Rock bass (*Ambloplites rupestris*), a host fish for the rainbow, were collected by electrofishing in the New River, Montgomery County, Virginia. Ten to thirteen centimeter largemouth bass (*Micropterus salmoides*), host fish for the pheasantshell, were purchased from Zett's Tri-State Fish Hatchery in Inwood, West Virginia. Fish were held in a 285-L flow-through Living Stream until needed.

Glochidia were removed from 1 to 3 gravid mussels (depending on availability) and tested for viability, as discussed previously. Two methods were used to infest the host fish with glochidia. The first method followed the procedure of Bruenderman (1989), with the exception of using MS-222 as the anesthetic. Several fish were placed in a bucket of water. Each fish to be infested was placed in another bucket containing MS-222. Once anesthetized, the fish was held in one hand and the right operculum held open. A pipet was filled with glochidia from the petri dish and emptied over the gills of the fish. The operculum was held closed approximately 30 seconds to give the glochidia time to attach to the gills. The fish was then placed in another bucket containing clean water and allowed to recover from the anesthetic. This procedure was repeated until all fish had been infested. Only one half of the gills were infested in an attempt to avoid too heavy an infestation and thus stress to the fish. The second method used was similar to that described by Jacobson (1990). The fish to be infested were dipped briefly into a bucket containing MS-222 to calm them down. They were then transferred into a 20-L bucket approximately two-thirds full with water, aerated by three airstones.

Glochidia were then added to the bucket and left for 6 to 10 minutes. Following infestation, fish were then transferred to 38-L aquaria held in a walk-in environmental chamber set at 20°C. Small rockbass were fed mealworms every 2 to 3 days. The larger rockbass and largemouth bass were fed small goldfish or minnows every 3 to 4 days. Once juveniles began to drop from the host fish (about 3 to 4 weeks), feeding was cut back to every 4 to 6 days to decrease the amount of debris in the aquaria. The bottoms of the tanks were siphoned, and contents were sieved through 100 μm mesh. This siphonate was then examined under a dissecting microscope, and juveniles were removed by pipet or capillary tube to a clean dish of water. The juveniles were held in a 20°C incubator prior to testing. If held for several days, the juveniles were periodically checked, dead ones removed, and healthy ones transferred to a clean dish of water. Juveniles were not fed before or during acute toxicity testing.

Test solutions of Zn and Cu (as $CuSO_4.5H_2O$) were made with the desired dilution water (Powell River or dechlorinated tap). The 6-well tissue culture plates were randomly assigned a toxicant concentration and replicate (A, B, or C). A sterile pipet was used to transfer 12 ml of test solution to the appropriate well. Just prior to starting a test, stored juveniles were examined under the dissecting microscope. Only juveniles exhibiting foot movement were judged alive and healthy for testing. Juveniles which had been collected over several days (depending on the number available) were combined into one petri dish. This was done to ensure random selection of juveniles to each replicate. Twenty juveniles were then transferred by capillary tube to each well, and the covered trays were placed in an incubator set at the desired temperature (12° or 20°C) and a 16:8 hour light-dark photoperiod. Exposure times were 48 and 96 hr.

At the end of 48 hr, the juveniles were removed from the incubator and examined under a dissecting microscope. For each replicate, the numbers of live, dead, and affected juveniles were counted. Juveniles were considered alive if any movement was evident, either by siphon, foot, or cilia. Normal, healthy juveniles were observed siphoning with open valves and would immediately close their valves upon tactile stimulation. Evidence of 'gaping' (open valves with a swollen and nonresponsive extended foot) was used as an effective criterion for affected individuals in Zn tests. In Cu test solutions, juveniles were considered affected when valves were shut and little movement observed. For Cu and Zn mixtures, juveniles exhibiting either effective criteria were counted as affected. Dead juveniles, if present, were removed from each well. These trays were then returned to the incubator for another 48 hr, bringing the total exposure time to 96 hr. At the end of 96 hr, the trays were examined again under the microscope, and numbers of live, dead, and affected juveniles were recorded. The numbers of juveniles recorded as dead after 48 and 96 hr were combined.

A vital stain procedure was tested to elaborate differentiation of live from affected juveniles according to methods described by Crippen and Perrier (1974) and Jacobson (1990). However, staining of juveniles following initial tests with Zn did not distinguish the three categories with any confidence. The degree of staining seemed to blend from light to dark without any clearly defined end points. Therefore, staining was discontinued after the second test.

Pooled replicate samples from each toxicant concentration were preserved with HNO_3 and later analyzed for Zn (or Cu) concentration. An average of these values then was used for statistical analysis. The LC50's for tests completed before March 1991, were analyzed with the beginning test metal concentration only. After this date, samples from three replicates pooled at the end of the test were used. If the test extended beyond 48 hr, aged solution from trays with no juveniles were sampled for metal analysis. The total number of live and dead juveniles was used to determine the LC50 estimates, and the number of live and affected juveniles was used to estimate the EC50 values. Probit analysis and Spearman Karber method were used to calculate these values (Finney 1971, Buikema et al. 1982). A two-way analysis of variance (ANOVA) evaluated the interaction between Zn and Cu.

Results

Glochidia

Values for 24-hr LC50s ranged from 392 μg Zn/L for *Actinonaias pectorosa* to 4123 μg Zn/L for *Villosa iris*. Values for 48-hr LC50s ranged from 274 μg Zn/L for *Actinonaias pectorosa* to 1775 μg Zn/L for *Villosa iris* (Tables 1, 2). The raw data obtained from these tests are listed in Appendix A, and all water quality data are in Appendix B.

Sensitivities within and between species varied widely. *Medionidus conradicus* was the most sensitive species tested; 24-hr LC50 values ranged from 423 to 549 μg Zn/L for river water at 20°C (Table 3). Glochidia of *A. pectorosa* exhibited a 24-hr LC50 of 1154 μg Zn/L under the same test conditions (Table 1), and *V. iris* exhibited 24-hr LC50 values ranging from 1004 to 1764 μg Zn/L (Table 2).

All three species were more sensitive to Zn at higher temperature (20°C). The pheasantshell had LC50 values of 2886 and 1154 μg Zn/L at 12 and 20 °C, respectively. The LC50 values for *V. iris* increased from 1764 μg Zn/L at 20°C to 4123 μg Zn/L at 12°C, a 2.3 fold increase in toxicity. A similar trend was exhibited by *M. conradicus*, with LC50 values of 492 μg Zn/L at 20°C and 726 μg Zn/L at 12°C.

The effect of water hardness on Zn tolerance was evident in Powell River water (140 to 160 mg/L as $CaCO_3$) and the dechlorinated laboratory tap water (about 50 mg/L) (Figures 2, 3). Two tests run concurrently with Powell River water and tap water using *V. iris* resulted in LC50 values of 1042 and 577 μg Zn/L, respectively. This represented a 1.8 fold increase in toxicity. Similar tests with *A. pectorosa* resulted in LC50 values of 664 and 274 μg Zn/L, respectively; a 2.4 fold increase in toxicity.

Greater exposure time increased Zn toxicity 1.5 times, on average. For example, concurrent tests run with glochidia of *A. pectorosa* and *V. iris* resulted in 24-hr LC50 values of 1154

Table 1. Results of acute toxicity tests with zinc and glochidia of *Actinonaias pectorosa*.

Diluent	Temp. (°C)	Hardness (mg/L)	Chemistry (date)	Laboratory Held (days)	Test Duration (hr)	LC50[A] (95% Confidence limits) Probit
Tap (New RIver)	12	50	4-19-91	11	24	720.8 (700.0 - 741.9)
Tap	12	50	4-19-91	11	48	525.0 (508.6 - 541.9)[B]
Tap	20	50	4-16-91	8	24	392.3 (379.2 - 405.8)
Tap	20	50	4-16-91	8	48	273.5 (70.2 - 1487.5)
River (Powell)	12	150	3-18-91	49	24	2009.4 (1942.2 - 2078.8)[B]
River	12	160	4-23-91	15	24	2886.3 (1761.2 - 4954.5)
River	12	150	3-18-91	49	48	1134.4 (1099.6 - 1170.4)[B]
River	12	160	4-19-91	11	48	1049.1 (1012.9 - 1086.9)
River	20	160	4-25-91	17	24	1153.8 (147.2 - 13414.5)
River	20	160	4-16-91	8	48	664.5 (643.8 - 685.9)[B]
River	20	160	4-25-91	17	48	739.2 (719.1 - 759.0)

[A] Units of μg Zn/L

[B] Spearman-Karber values (LC50 and 95% confidence limits) are presented when Probit values could not be calculated using the test data.

Table 2. Results of acute toxicity tests with zinc and glochidia of *Villosa iris*.

Diluent	Temp. (°C)	Hardness (mg/L)	Chemistry (date)	Laboratory Held (days)	Test Duration (hr)	LC50[A] (95% Confidence limits) Probit
Tap (New River)	12	40	3-09-91	33	24	1908.0 (1361.1 - 2748.3)
Tap	12	50	3-14-91	38	24	1765.2 (1094.5 - 2782.8)
Tap	12	40	3-09-91	33	48	1172.5 (1133.6 - 1212.7)[B]
Tap	12	50	3-14-91	38	48	1202.0 (1163.9 - 1241.4)[B]
Tap	20	40	6-30-91	26	24	1620.4 (1287.8 - 2075.4)
Tap	20	50	7-10-91	8	24	739.2 (426.6 - 1366.3)
Tap	20	40	6-30-91	26	48	1154.7 (906.8 - 1470.3)
Tap	20	50	7-10-91	8	48	576.9 (459.1 - 738.4)
River (Powell)	12	140	3-09-91	33	24	3087.3 (1717.6 - 6272.0)
River	12	160	4-23-91	15	24	4122.6 (3871.4 - 4390.1)[B]
River	12	140	3-09-91	33	48	1665.9 (1345.9 - 2039.4)
River	12	140	3-14-91	38	48	1774.9 (1002.6 - 3155.6)
River	20	160	4-25-91	17	24	1764.0 (1211.6 - 2518.9)
River	20	160	6-30-91	26	24	1004.3 (805.1 - 1225.6)
River	20	140	7-10-91	8	24	1330.7 (671.6 - 2438.8)
River	20	160	4-25-91	17	48	1230.0 (1187.1 - 1274.4)[B]
River	20	160	6-30-91	26	48	835.9 (410.4 - 1510.9)
River	20	140	7-10-91	8	48	1042.2 (997.4 - 1089.0)[B]

[A] Units of μg Zn/L

[B] Spearman-Karber values (LC50 and 95% confidence limits) are presented when Probit values could not be calculated using the test data.

Table 3. Results of acute toxicity tests with zinc and glochidia of *Medionidus conradicus*.

Diluent	Temp. (°C)	Hardness (mg/L)	Chemistry (date)	Laboratory Held (days)	Test Duration (hr)	LC50[A] (95% Confidence limits) Probit
Tap (New River)	12	40	3-05-91	35	48	725.5 (366.8 - 1523.2)
Tap	20	60	7-04-91	24-30	48	491.8 (470.1 - 514.4)[B]
River (Powell)	20	160	6-30-91	20-26	24	422.8 (228.2 - 795.4)
River	20	140	7-10-91	8	24	549.2 (376.8 - 790.4)

[A] Units of μg Zn/L

[B] Spearman-Karber values (LC50 and 95% confidence limits) are presented when Probit values could not be calculated using the test data.

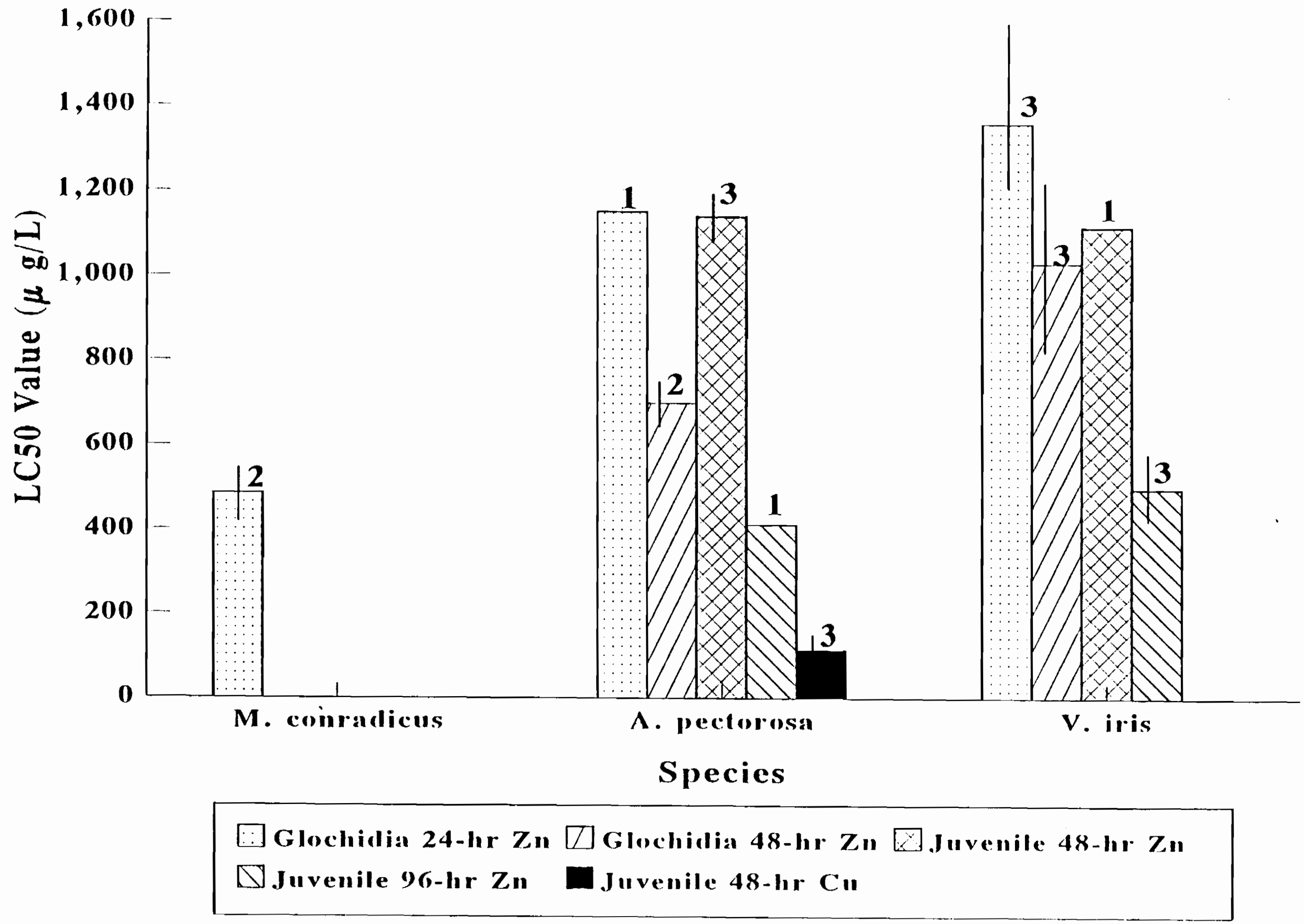


Figure 2. Comparison of acute toxicity tests with Zn and Cu and early life stages of mussels in river water at 20°C. (Numbers represent the number of tests performed and lines represent the range of LC50 values obtained.)

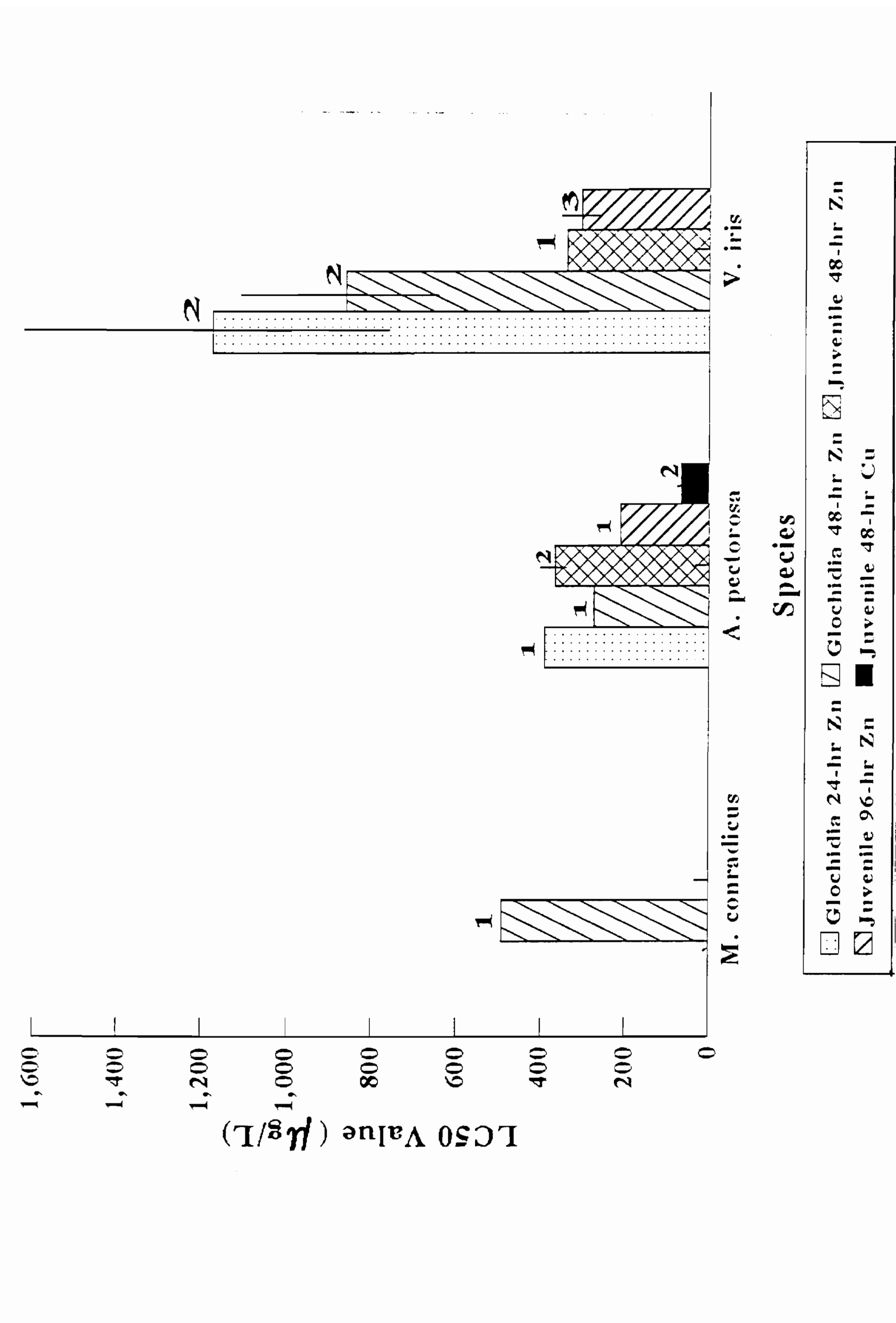


Figure 3. Comparison of acute toxicity tests with Zn and Cu and early life stages of mussels in tap water at 20°C. (Numbers represent the number of tests performed and lines represent the range of LC50 values obtained.)

and 1764 μg Zn/L, respectively, and 48-hr LC50 values of 739 and 1230 μg Zn/L, respectively (Tables 1 and 2).

The length of time gravid females were held in the laboratory after field collection produced no consistent effect on the tolerance of glochidia to Zn. For example, tests run under similar conditions with glochidia of *V. iris* held 8 and 26 days, resulted in LC50 values of 739 and 1620 μg Zn/L, respectively (Table 2). Another series of tests with *V. iris* held 8, 17, and 26 days resulted in LC50 values of 1331, 1764, and 1004 μg Zn/L, respectively. The only direct comparison tested glochidia from the same female *A. pectorosa* held 8 days (LC50 664 μg Zn/L), and then tested again under similar conditions after 17 days (LC50 739 μg/L Zn) (Table 1).

Juveniles

Values for 48-hr LC50's ranged from 360 to 1885 μg Zn/L for *A. pectorosa* (Table 4) and 339 to 1122 μg Zn/L for *V. iris* (Table 5). The 96-hr LC50 values ranged from 211 to 457 μg Zn/L for *A. pectorosa* and 236 to 877 μg Zn/L for *V. iris*. EC50 values were lower, ranging from 158 to 1222 μg Zn/L for *A. pectorosa*, and 209 to 1026 μg Zn/L for *V. iris* (Tables 4, 5). The raw data used for the statistical analysis are presented in Appendix A, and water chemistry is listed in Appendix B.

Zinc was 3.3 times more toxic to juvenile mussels in soft (tap) water than in hard (river) water (Figures 2, 3). Juveniles of *A. pectorosa* exhibited LC50 values of 360, 612, and 1186 μg Zn/L when tested with New River tap water (40 mg/L as $CaCO_3$), EPA reconstituted water (100 mg/L), and Powell River water (160 mg/L), respectively. Juveniles of *V. iris* had LC50 values of 339 and 1122 μg Zn/L when tested with tap water and Powell River water, respectively.

Higher water temperature decreased the tolerance of juvenile mussels to Zn. The mean 96-hr LC50 values for *V. iris* were 865 and 500 μg Zn/L, at 12 and 20°C, respectively, a 42%

Table 4. Results of acute toxicity tests with zinc and juveniles of *Actinonaias pectorosa*.

Diluent	Temp. (°C)	Hardness (mg/L)	Chemistry (date)	Age (days)	Test Duration (hr)	LC50[A] (95% Confidence Limits) Probit	EC50[A] (95% Confidence Limits) Probit
Tap (New River)	12	40	5-10-91	3-4	48	410.8 (373.2 - 452.4)	235.7 (216.0 - 258.4)
					96	274.2 (255.7 - 299.9)	183.4 (173.7 - 194.4)
Tap	20	40	4-30-91	1-4	48	360.5 (329.3 - 394.4)	247.4 (223.8 - 273.5)[B]
					96	211.3 (189.1 - 236.1)[B]	157.5 (64.4 - 615.2)
Tap	20	40	5-10-91	3-4	48	370.5 (338.1 - 404.8)	201.0 (184.6 - 219.7)
River (Powell)	12	160	5-06-91	2-4	48	1885.0 (1704.7 - 2084.4)[B]	1222.3 (1094.0 - 1363.0)
River	20	160	4-30-91	1-4	48	1060.1 (980.8 - 1153.9)	663.8 (576.7 - 764.1)[B]
					96	412.9 (116.2 - 1577.2)	-
River	20	160	5-06-91	2-4	48	1186.2 (1086.1 - 1295.7)	662.8 (174.5 - 3359.2)
River	20	160	5-21-91	7-11	48	1181.7 (1091.3 - 1279.7)[B]	726.2 (658.7 - 800.0)
EPA[C]	12	100	5-13-91	1-5	48	799.4 (390.6 - 2210.4)	374.4 (232.9 - 587.7)
					96	457.2 (422.9 - 494.8)	201.3 (182.7 - 221.7)
EPA[C]	20	100	5-13-91	1-5	48	611.7 (568.6 - 666.5)	298.7 (271.7 - 327.1)
					96[D]	364.5 (198.3 - 655.5)	291.6 (270.2 - 314.7)[B]

[A] Units of μg Zn/L
[B] Spearman-Karber values (LC50 and 95% confidence limits) are presented when Probit values could not be calculated using the test data.
[C] Reconstituted laboratory water.
[D] Control mortality for this test was 10%.

Table 5. Results of acute toxicity tests with zinc and juveniles of *Villosa iris*.

Diluent	Temp. (°C)	Hardness (mg/L)	Chemistry (date)	Age (days)	Test Duration (hr)	LC50[A] (95% Confidence Limits) Probit	EC50[A] (95% Confidence Limits) Probit
Tap (New River)	20	50	12-07-90	6-7	48	-	228.7 (207.0 - 251.5)
					96	236.2 (211.8 - 263.4)[B]	208.9 (186.8 - 233.6)[B]
Tap	20	50	2-19-91	0-4	48	-	298.3 (274.7 - 323.9)[B]
					96	353.8 (327.9 - 384.2)	295.0 (271.6 - 320.5)[B]
Tap	20	50	2-19-91	4-6	48	-	279.3 (256.1 - 304.7)
					96	325.4 (300.3 - 352.7)[B]	252.6 (231.5 - 275.5)
Tap	20	50	8-02-91	1-7	48	339.3 (316.1 - 364.2)[B]	280.0 (261.6 - 299.7)[B]
River (Powell)	12	140	2-14-91	5	96	856.4 (829.2 - 884.3)[B]	434.3 (434.3 - 434.3)[B]
River	12	140	2-14-91	5	48	-	744.1 (689.8 - 802.7)[B]
					96	866.9 (848.0 - 886.1)[B]	471.4 (445.1 - 499.3)[B]
River	12	140	2-15-91	6	48	-	669.8 (614.2 - 733.6)
					96	861.2 (831.8 - 891.7)[B]	484.3 (442.1 - 522.7)
River	12	140	2-15-91	4-6	48	-	826.8 (786.9 - 868.8)[B]
					96	876.6 (876.6 - 876.6)[B]	530.1 (485.0 - 574.0)
River	20	140	2-03-91	2	48	-	571.9 (539.6 - 606.1)[B]
					96	577.5 (546.2 - 610.7)[B]	515.5 (476.7 - 557.4)[B]
River	20	140	2-06-91	4	48	-	498.3 (452.6 - 548.5)[B]
					96	418.0 (371.8 - 470.0)[B]	288.3 (255.6 - 324.2)
River	20	140	2-09-91	2	48	-	498.0 (457.5 - 542.0)[B]
					96	505.5 (463.1 - 551.8)[B]	471.3 (426.7 - 520.7)[B]
River	20	160	8-02-91	1-7	48	1121.6 (1076.7 - 1168.4)[B]	1025.7 (950.4 - 1106.9)[B]

[A] Units of μg Zn/L

[B] Spearman-Karber values (LC50 and 95% confidence limits) are presented when Probit values could not be calculated using the test data.

increase in toxicity. However, mean EC50 values decreased only 11% with higher temperature, from 480 to 425 μg Zn/L, at 12 and 20°C, respectively (Table 5). Two tests run concurrently with *A. pectorosa* at 12 and 20°C, resulted in 48-hr LC50 values of 1885 and 1186 μg Zn/L respectively, a 37% increase in toxicity. The EC50 values were reduced by 46%, from 1222 to 663 μg Zn/L, respectively (Table 4).

Juveniles of *A. pectorosa* exhibited 33 to 61% lower LC50 values as exposure time increased from 48 to 96 hours. EC50 values were more variable, decreasing from 2 to 46% for *A. pectorosa*, and 1 to 42% for *V. iris* (LC50 data not determined for *V. iris*).

Copper was 5.7 to 15.7 times more toxic to *A. pectorosa* than Zn, with 48-hr LC50 values ranging from 52 to 156 μg Cu/L, and EC50 values ranging from 25 to 115 μg Cu/L (Table 6). Variation among tests run with Powell River water was high, with LC50 values ranging from 76 to 156 μg Cu/L. As with Zn, Cu toxicity increased in soft water. For example, tests run concurrently with soft and hard water (New River tap and Powell River water, respectively) resulted in LC50 values of 53 and 112 μg Cu/L, respectively.

Copper and Zinc Mixtures

At certain concentrations, Zn appears to have an antagonistic effect (less than additive) when mixed with Cu. Three tests were conducted for 48 hours with several concentrations of Cu and Zn mixtures (Tables 7, 8). There was significant interaction between Cu and Zn, as determined by ANOVA (p = .023, .0001, .0001 for tests 1, 2, and 3, respectively). Test one suggests an interaction at the highest Cu concentration; percent survival increased as Zn concentrations increased (Table 7). The second test, also run with Powell River water, clearly showed this trend. When Cu concentration was low (5 μg/L), survival began to decrease as Zn concentrations exceeded 800 μg/L. At the intermediate Cu level of 77 μg/L, survival was low (73%) when Zn was absent (background level 10 μg/L). However, at 415 μg/L Zn, survival increased to 100%. At higher Zn levels (860 and 1670 μg/L), and constant Cu levels (77

Table 6. Results of acute toxicity tests with copper and juveniles of *Actinonaias pectorosa*.

Diluent	Temp. (°C)	Hardness (mg/L)	Chemistry (date)	Age (days)	Test Duration (hr)	LC50[A] (95% Confidence Limits) Probit	EC50[A] (95% Confidence Limits) Probit
Tap (New River)	20	50	7-17-91	5-8	48	62.9 (56.3 - 70.1)	41.6 (37.2 - 46.2)
Tap	20	40	7-25-91	8-10	48	51.6 (43.4 - 61.3)[B]	25.5 (20.5 - 31.6)[B]
River (Powell)	20	160	5-21-91	7-11	48	155.8 (144.0 - 169.7)	114.9 (108.4 - 126.2)
River	20	140	7-17-91	5-8	48	111.6 (45.4 - 295.1)	43.9 (39.3 - 48.5)
River	20	150	7-21-91	8-10	48	75.6 (61.8 - 92.5)[B]	39.3 (31.8 - 48.4)[B]

[A] Units of μg Cu/L

[B] Spearman-Karber values (LC50 and 95% confidence limits) are presented when Probit values could not be calculated using the test data.

Table 7. Results of a preliminary copper-zinc mixture toxicity test with juveniles of *Actinonaias pectorosa*.

Conditions	Target Conc.[A] Cu / Zn	Measured Conc. Cu / Zn	% Survival[B]
TEST # 1	0 / 0	57.3 / 8.4[C]	98.3
5-29-91			
River Water	20 / 0	72.7 / 8.4	90.0
(Powell)	20 / 100	72.7 / 118.2	95.0
48-hr duration	20 / 200	72.7 / 231.9	88.3
	20 / 400	72.7 / 452.5	91.7
	40 / 0	88.5 / 8.4	98.3
	40 / 100	88.5 / 118.2	96.7
	40 / 200	88.5 / 231.9	93.3
	40 / 400	88.5 / 452.5	85.0
	80 / 0	129.0 / 8.4	95.0
	80 / 100	129.0 / 118.2	93.3
	80 / 200	129.0 / 231.9	95.0
	80 / 400	129.0 / 452.5	96.7
	160 / 0	207.4 / 8.4	56.7
	160 / 100	207.4 / 118.2	78.3
	160 / 200	207.4 / 231.9	75.0
	160 / 400	207.4 / 452.5	91.7

[A] Concentrations are in μg/L.
[B] Average of three replicates.
[C] Background concentrations.

Table 8. Results of copper-zinc mixture toxicity tests on juveniles of *Actinonaias pectorosa* comparing two water sources.

Conditions	Target Conc.[A] Cu / Zn	Actual Conc.[A] Cu / Zn	% Survival[B]	% Affected
TEST # 2	0 / 0	5.2 / 9.8[C]	98.3	1.7
7-21-91	0 / 400	5.2 / 414.2	100.0	3.3
River Water	0 / 800	5.2 / 861.2	95.0	43.3
(Powell)	0 / 1600	5.2 / 1670.0	0.0	100.0
48-hr duration				
	80 / 0	76.9 / 9.8	73.3	78.3
	80 / 400	76.9 / 414.2	100.0	98.3
	80 / 800	76.9 / 861.2	55.0	100.0
	80 / 1600	76.9 / 1670.0	1.7	100.0
	160 / 0	148.4 / 9.8	16.7	100.0
	160 / 400	148.4 / 414.2	81.7	100.0
	160 / 800	148.4 / 861.2	30.0	100.0
	160 / 1600	148.4 / 1670.0	1.7	100.0
	320 / 0	282.5 / 9.8	0.0	100.0
	320 / 400	282.5 / 414.2	16.7	100.0
	320 / 800	282.5 / 861.2	6.7	100.0
	320 / 1600	282.5 / 1670.0	0.0	100.0
TEST # 3	0 / 0	3.4 / 12.4[C]	98.3	1.7
7-25-91	0 / 200	3.4 / 236.8	100.0	88.3
Tap Water	0 / 400	3.4 / 464.1	11.7	100.0
(New River)	0 / 800	3.4 / 927.2	0.0	100.0
48-hr duration				
	40 / 0	34.2 / 12.4	86.7	40.0
	40 / 400	34.2 / 464.1	56.7	100.0
	40 / 400	34.2 / 464.1	48.3	100.0
	40 / 800	34.2 / 927.2	0.0	100.0
	80 / 0	69.8 / 12.4	50.0	98.3
	80 / 200	69.8 / 236.8	90.0	95.0
	80 / 400	69.8 / 464.1	33.3	100.0
	80 / 800	69.8 / 927.2	0.0	100.0
	160 / 0	141.6 / 12.4	0.0	100.0
	160 / 200	141.6 / 236.8	53.3	100.0
	160 / 400	141.6 / 464.1	37.0	100.0
	160 / 800	141.6 / 927.2	3.3	100.0

[A] Concentrations are in $\mu g/L$.
[B] Average of three replicates.
[C] Background concentrations.

$\mu g/L$), survival decreased to 55 and <5%, respectively (Table 8). This trend of higher survival at Zn concentrations around 400 $\mu g/L$, and to a lesser extent at 860 $\mu g/L$, was repeated for the higher Cu concentrations of 148 and 282 $\mu g/L$. This test was repeated using tap water (source, New River) and lower metal concentrations. The same trend was evident at Zn concentrations between 200 and 400 $\mu g/L$ (Table 8). This trend of reduced toxicity of Cu when mixed with Zn is not reflected in the percent of juveniles affected. This number increased continuously with increasing metal concentration.

Discussion

Test Procedure

Glochidia have rarely been used in toxicity tests (Varanka 1977, 1978, Goodreau 1988, Jacobson 1990), and standard testing procedures have not been approved, although a proposed guide has recently been submitted to the USEPA (Johnson 1990). Procedures used for my study basically followed those of Jacobson (1990). However, as an alternative to sacrificing the adult mussel, glochidia were removed by gently forcing water through holes made in the marsupium by a syringe. Most adults held in the laboratory after removing the glochidia appeared healthy when returned to the stream; however, their long term survival is unknown. Goudreau (1988) reported a high percentage of glochidia with closed valves when removed by a syringe, but that problem was not encountered in my study.

When tested at high Zn concentrations (>2 mg/L), glochidial response to saturated NaCl solution slowed dramatically. There was variability in the count of open valves following NaCl addition, depending on how rapidly the count was made. For this study, counts were made immediately after NaCl addition. These glochidia may have exhibited a physiological response to the salt but were, in fact, dead. If these glochidia were not dead, their encystment ability would most likely be impaired due to the slowed response. In addition, the adductor muscles were noticably swollen, further evidence of their affected condition. There was also a delayed response to NaCl solution at 12°C, but glochidia appeared healthy and would 'snap' valves closed as opposed to gradual closing at the highest Zn concentrations.

Sublethal concentrations of Zn appeared to "sensitize" glochidia. The slightest movements of the test trays or tactile stimulation would cause the glochidia to close their valves. This was not observed in controls. Variability within replicates was high at these "sensitized" levels (personal observation). Control mortality was >10% in approximately one third of the tests conducted with *M. conradicus*, and appeared to be linked to fungus or to a ciliated protozoan (personal observation). It is also possible this species is especially sensitive to handling stress.

Control mortality was a problem in tests with juvenile mussels. Except for one test, all control mortalities >10% occurred at 20°C. The lower test temperature of 12°C may have inhibited fungal growth. In addition, test conditions were static, without renewal, and these tests may not be appropriate for juvenile mussels for prolonged exposures. Wade et al. (1989) reported low control mortality in their toxicity tests with juvenile mussels. Their methodology consisted of static, renewal tests, and the juveniles were supplied with algae and silt. Keller and Zam (1991) used laboratory reconstituted water for their tests with juveniles, which may decrease problems with fungus and parasites in natural river water. In both cases, *in vitro* transformed juveniles were used in testing (Isom and Hudson 1982), whereas juveniles used in my study were derived from fish encysted in the laboratory. Collection of the juveniles involved siphoning the bottom of the aquaria, which included fish excrement and food particles. This debris then had to be sorted under a microscope to remove the juveniles. It is likely that various micro-organisms were collected with the juveniles, causing higher stress. Although Jacobson (1990) did not report excessive control mortalities for juveniles collected by this method, he discontinued feeding encysted fish prior to glochidial transformation, thus decreasing the amount of debris accumulated on the aquaria bottoms. In addition, the juvenile tests he conducted were limited to 24 hr, whereas tests conducted in my study extended to 48- and 96-hr, with a concomitant increase in cases of control mortality $>10\%$.

Effects of Zn on Freshwater Mussels

In general, freshwater mussel glochidia and juveniles in my tests were about equally sensitive to Zn. Glochidia of *V. iris* were somewhat more resistant than glochidia or juveniles of the other species in soft water (Figures 2 and 3). Sensitivities to Zn decreased with lower temperature and in hard water.

The only other data on acute toxicity of Zn to glochidia was compiled by Cherry et al. (1991). Glochidia of five freshwater mussel species exposed to Zn in Clinch River water (170 mg/L hardness) yielded 48-hr LC50 values ranging from 212 to 695 μg/L (Table 9). *Medionidus conradicus* exhibited a somewhat higher 48-hr LC50 value than would be expected based on 24-hr tests of my study; *V. iris* and *A. pectorosa* had lower LC50 values than those obtained by me. The results obtained by Cherry et al. (1991) are based on a single test; thus, variability is not reflected in their point estimates. Further, unknown components of the Powell and Clinch River waters may be affecting results, e. g., the presence of other contaminants or complexing agents.

Keller and Zam (1991) obtained LC50 values for juveniles of the unionid mussel *Anodonta imbecilis* ranging from 268 to 588 μg Zn/L. These values are comparable to those obtained in my study for soft water. However, 48-hr LC50 values in hard water for *A. imbecilis* were considerably lower than those exhibited by *V. iris* or *A. pectorosa*. The hard water used by Keller and Zam was reconstituted laboratory water, which may have differed significantly from the natural river water used in my tests. It is likely that the river water contained algae, which the juveniles could feed on, or which may have complexed with Zn. Algae and natural water source have been shown to reduce Cu toxicity (Borgmann and Charlton 1984), and other studies have shown reduced toxicity when organisms are fed (Biesinger and Christensen

Table 9. Sensitivities of various freshwater organisms to zinc exposure.

Species	Hardness (mg/L)	Temp. (°C)	Effect[A]	Value (μg/L)	Reference
Mollusks					
Medionidus conradicus	40	12	48-hr LC50	726	present study
glochidia	60	20	48-hr LC50	492	
	140-160	20	24-hr LC50	423-549	
Actinonaias pectorosa	50	12	24-hr LC50	721	
glochidia	50	12	48-hr LC50	525	
	50	20	24-hr LC50	392	
	50	20	48-hr LC50	274	
	150-160	12	24-hr LC50	2009-2886	
	150-160	12	48-hr LC-50	1049-1134	
	160	20	24-hr LC50	1154	
	160	20	48-hr LC50	664-739	
Actinonaias pectorosa	40	12	48-hr LC50	411	
juvenile	40	12	48-hr EC50	236	
	40	12	96-hr LC50	274	
	40	12	96-hr EC50	183	
	40	20	48-hr LC50	360-370	
	40	20	48-hr EC50	201-247	
	40	20	96-hr LC50	211	
	40	20	96-hr EC50	158	
	100	12	48-hr LC50	799	
	100	12	48-hr EC50	374	
	100	12	96-hr LC50	457	
	100	12	96-hr EC50	201	
	100	20	48-hr LC50	612	
	100	20	48-hr EC50	299	
	100	20	96-hr LC50	364	
	100	20	96-hr EC50	292	
	160	12	48-hr LC50	1885	
	160	12	48-hr EC50	1222	
	160	20	48-hr LC50	1060-1186	
	160	20	48-hr EC50	663-726	
	160	20	96-hr LC50	413	
Villosa iris	40-50	12	24-hr LC50	1765-1908	
glochidia	40-50	12	48-hr LC50	1172-1202	
	40-50	20	24-hr LC50	739-1620	
	40-50	20	48-hr LC50	577-1155	
	140-160	12	24-hr LC50	3087-4123	
	140	12	48-hr LC50	1666-1775	
	140-160	20	24-hr LC50	1004-1764	
	140-160	20	48-hr LC50	836-1230	

Table 9. (Continued)

Species	Hardness (mg/L)	Temp. (°C)	Effect[A]	Value (µg/L)	Reference
Villosa iris	50	20	48-hr LC50	339	present study
juvenile	50	20	48-hr EC50	280	
	50	20	96-hr LC50	236-354	
	50	20	96-hr EC50	209-295	
	140	12	96-hr LC50	856-877	
	140	12	96-hr EC50	434-530	
	160	20	48-hr LC50	1122	
	160	20	48-hr EC50	1026	
	140	20	96-hr LC50	418-578	
	140	20	96-hr EC50	288-516	
Anodonta imbecilis	Soft	23±3	48-hr LC50	355	Keller and Zam 1991
juvenile	Soft	23±3	96-hr LC50	268	
	Hard	23±3	48-hr LC50	588	
	Hard	23±3	96-hr LC50	438	
Medionidus conradicus glochidia	170	20	48-hr LC50	570	Cherry et al. 1991
Lampsilis fasciola glochidia	170	20	48-hr LC50	695	
Actinonaias pectorosa glochidia	170	20	48-hr LC50	309	
Villosa nebulosa (iris) glochidia	170	20	48-hr LC50	656	
Ptychobranchus fasciolaris glochidia	170	20	48-hr LC50	212	
Corbicula sp.	55-89	17-25	30-d LOEC	57	
adult	55-89	17-25	30-d NOEC	<20	
Corbicula fluminea adult	64	-	96-hr LC50	6040	Rodgers et al. 1980
Physa gyrina	30	15±1	96-hr LC50	1274	Nebeker et al. 1986
adult	30	15±1	30-d NOEC	570	
Crustaceans					
Daphnia magna adult	60±10	20-24	48-hr LC50	920	Hall et al. 1986
Daphnia magna	130	20	36-hr LC50	861	Attar and Maly 1982
neonates	130	20	48-hr LC50	799	
	130	20	96-hr LC50	68	
Daphnia magna	45	18±1	48-hr LC50	280 (fed)	Biesinger and Christensen 1972
neonates	45	18±1	48-hr LC50	100 (unfed)	
	45	18±1	21-d repro impair	70	
Ceriodaphnia dubia neonates	182±10	25±1	48-hr LC50	105-153	Cherry et al. 1991

Table 9. (Continued)

Species	Hardness (mg/L)	Temp. (°C)	Effect[A]	Value (μg/L)	Reference
Fish					
Pimephales promelas late juv-early adult	60±10	20-24	96-hr LC50	21	Hall et al. 1986
Pimephales promelas adult	100	20-23	24-hr LC50	6880	Benson and Birge 1985
	100	20-23	96-hr LC50	6090	
	250	20-23	24-hr LC50	7620	
	250	20-23	96-hr LC50	7450	
Pimephales promelas juvenile	46	25±1	96-hr LC50	600	Benoit and Holcombe 1978
Pimephales promelas juvenile	203	23	96-hr TLm	12000	Brungs 1969
	203	23	96-hr TLm	9200	
Pimephales promelas eggs	174-198	20±1	48-hr TLm	2470-2630	Pickering and Vigor 1965
	174-198	20±1	96-hr TLm	1820-1850	
Pimephales promelas fry	174-198	20±1	48-hr TLm	950	
	174-198	20±1	96-hr TLm	870	
Salvelinus fontinalis	45	-	96-hr LC50	2000	Holcombe et al. 1979

[A] LC50 - lethal concentration to 50% of test population
LOEC - lowest observable effect concentration
NOEC - no observable effect concentration
TLm - median tolerance limit; $\simeq$LC50

1972, Lewis and Weber 1985, Adams and Heidolph 1985, Belanger et al. 1989). However, by 96-hr, the LC50 values obtained for *Anodonta imbecilis* (Keller and Zam 1991) were comparable to those obtained in my study.

Water hardness is a significant factor in the variation of Zn toxicity to freshwater organisms (USEPA 1987), and criteria are adjusted based on this trait. The effect of increasing toxicity of Zn in soft water was verified for freshwater mussels in my study, and by Keller and Zam (1991) and Jacobson (1990). This trend also has been demonstrated for *Daphnia magna* (USEPA 1987), fathead minnow (*Pimephales promelas*) (Benson and Birge 1985), and rainbow trout (*Oncorhynchus mykiss*) (Lloyd 1960). Zinc toxicity also has been shown to increase with increasing water temperature for mussels (present study, Jacobson 1990, Cherry et al. 1991) and other organisms (Lloyd 1960, Cairns et al. 1978, Smith and Heath 1979). However, this trend has not been studied extensively, and is not used in calculating water quality criteria.

The high variability of Zn test results has also been reported by Attar and Maly (1982) and J. Farris (personal communication). Most of the samples analyzed for metal content, in field and laboratory studies were based on total metal content. It is well established that not all metal present is biologically available. Generally, the free ionic form (Zn^{2+}, Cu^{2+}) is considered the most toxic, along with some hydroxides (Andrew et al. 1977, Borgmann and Charlton 1984, USEPA 1985, USEPA 1987). Andrew et al. (1977) suggested that toxicity based on total or dissolved Cu measurements would vary, depending on strengths and concentrations of complexing agents of water. The type and amount of suspended solids also affects Zn toxicity (Hall et al. 1986).

In comparison to the standard test organisms used by the USEPA to develop water quality criteria for Zn (Table 9), larval and juvenile mussels are seemingly as sensitive as daphnids and more sensitive than fish. Thus, based on acute toxicity data, it seems that freshwater mussels would be protected by information obtained from use of cladocerans. However, water quality criteria are also based on chronic tests. Long term, or chronic exposures to contaminants take into account sublethal effects such as reduced growth and reproductive impairment. A three week exposure to 70 μg Zn/L significantly decreased the number of young produced by *Daphnia magna* (Biesinger and Christensen 1972) compared to the acute 48-hr LC50 of 100 μg/L. Fathead minnow eggs were affected by Zn at concentrations as low as 145 μg/L as compared to the acute 96-hr LC50 value of 600 μg Zn/L (Benoit and Holcombe 1978), and spawning was virtually blocked at concentrations of only 180 μg Zn/L as compared to the 96-hr TLm (Median Tolerance Limit$\simeq$LC50) value of 12000 μg Zn/L (Brungs 1969). A 12 week exposure to 360 μg Zn/L significantly affected brook trout (*Salvelinus fontinalis*) eggs and fry as compared to the controls, whereas the acute exposure of 96 hours required 2000 μg Zn/L for 50% mortality. As yet, no chronic studies assessed on the effects of Zn on juvenile freshwater mussels, but it is expected that Zn levels lower than the acute values determined in my study would adversely affect mussel populations.

Effects of Cu on Freshwater Mussels

Juveniles of *A. pectorosa* were more sensitive to Cu than Zn by at least four orders of magnitude in Powell River water. One half the juveniles exposed to 76 μg Cu/L in Powell River water died after 48 hours, and concentrations as low as 39 μg/L affected 50% of the juveniles exposed. Increased sensitivity in soft water was indicated, and this has been documented by Jacobson (1990) for glochidia and juvenile mussels. This seems to be a general trend for many metals and is incorporated into the USEPA's water quality criteria for Cu (USEPA 1985).

Jacobson (1990) obtained Cu values similar to those of my study for juveniles of *Anodonta grandis* and *V. iris*, whereas Keller and Zam (1991) found that *Anodonta imbecilis* exhibited lower sensitivity to Cu in both soft and moderately hard reconstituted water (Table 10). Studies on the toxicity of Cu to glochidia have shown them to be more sensitive than juvenile mussels. Fifty percent of the glochidia exposed for 48 hours were killed by Cu levels as low as 17 μg/L for two species, and <40 μg/L for three additional species (Jacobson 1990, Cherry et al. 1991), present in the Powell River. This high sensitivity of glochidia and juveniles ranks them among the most sensitive species tested for acute effects (Table 10), and the present national criteria for Cu may not be adequately protective of young mussels.

As with Zn, chronic or sublethal effects of Cu on freshwater mussels have rarely been studied. Farris et al. (1991) investigated the effects of Cu on growth and cellulolytic enzyme activity in freshwater mussels and asian clam. The cellulase activity of *V. iris* was impaired at Cu exposures of 11 μg Cu/L. Various studies have been completed on chronic effects of Cu on the life cycle of the fathead minnow. Complete blockage of spawning occurred at 18 μg/L (Horning and Neiheisel 1979) and 33 μg/L (Mount 1968), and fry survival was reduced at Cu concentrations of 113 to 119 μg/L (Horning and Neiheisel 1979, Scudder et al. 1988). Pickering et al. (1977) suggested that Cu affects sexually mature fish only during spawning, as duration of prespawning exposure was not significant. Sublethal concentrations of Cu (31 μg/L) also have been shown to reduce food consumption of bluegill (*Lepomis macrochirus*), suggesting that growth may be affected (Sandheinrich and Atchison 1989).

It was apparent during testing that juvenile mussels responded differently to Cu than Zn. Juvenile mussels responded to Cu by valve closure and reduced siphoning activity (personal observation, Jacobson 1990). Harrison et al. (1984) also noted this response for veligers and juveniles of *Corbicula fluminea*. However, my observations during testing with Zn noted that juvenile mussels responded by gaped valves and developed a swollen and extended foot. This would indicate a different toxic mode of action for the two metals, as has been suggested previously (Dixon and Sprague 1981, Leland and Kuwabara 1985). Rainbow trout, acclimated to sublethal concentrations of Cu, exhibited increased tolerance to Cu as reflected in acute toxicity tests (Dixon and Sprague 1981). However, this increased tolerance was not reflected in exposures to Zn. In a similar study, fathead minnows increased their tolerance to Cu and Cd following acclimation, but tolerance to Zn was not affected (Benson and Birge 1985). In both studies, tolerance was lost within 7 days after transfer to clean water. It seems reasonable to expect that if Zn and Cu had similar modes of action, then increased tolerance to one would be reflected by increased tolerance to the other.

Table 10. Sensitivities of various freshwater organisms to copper exposure.

Species	Hardness (mg/L)	Temp. (°C)	Effect[A]	Value (μg/L)	Reference
Mollusks					
Actinonaias pectorosa	40-50	20	48-hr LC50	52-63	present study
juvenile	40-50	20	48-hr EC50	26-42	
	140-160	20	48-hr LC50	76-156	
	140-160	20	48-hr EC50	39-115	
Anodonta imbecilis	Soft	23±3	48-hr LC50	171	Keller and Zam 1991
juvenile	Soft	23±3	96-hr LC50	86	
	Hard	23±3	48-hr LC50	388	
	Hard	23±3	96-hr LC50	199	
Medionidus conradicus	160	10	24-hr LC50	66	Cherry et al. 1991
glochidia	150-170	20	24-hr LC50	34-74	
	178	10	48-hr LC50	79	
	162	20	48-hr LC50	16	
Lampsilis fasciola	160-170	20	24-hr LC50	26-49	
glochidia	178	10	48-hr LC50	51	
	162	20	48-hr LC50	30	
Actinonaias pectorosa	170	20	24-hr LC50	56	
glochidia	178	10	48-hr LC50	172	
	162	20	48-hr LC50	30	
Villosa nebulosa (iris)	150-186	20	24-hr LC50	41-79	
glochidia	178	10	48-hr LC50	98	
	162	20	48-hr LC50	37	
Villosa nebulosa (iris)	152-159	-	14-30-d LOEC	11	
adult	152-159	-	14-30-d NOEC	3	
Lasmigona costata	178	10	48-hr LC50	86	
glochidia	162	20	48-hr LC50	124	
Ptychobranchus fasciolaris	178	10	48-hr LC50	201	
glochidia	162	20	48-hr LC50	17	
Corbicula sp.	108-162		14-30-d LOEC	20	
adult	108-162		14-30-d NOEC	5	
Actinonaias pectorosa	140	10	24-hr LC50	132	Jacobson 1990
glochidia	150	15	24-hr LC50	93	
	170	20	24-hr LC50	67	
	140	25	24-hr LC50	42	
	170	20	48-hr LC50	51	
Anodonta grandis	170	10	24-hr LC50	347	
glochidia	50	20	24-hr LC50	46	
Anodonta grandis	70	20	24-hr LC50	44	
Lampsilis fasciola	75	20	24-hr LC50	46	
glochidia	160	20	24-hr LC50	26	
	170	20	48-hr LC50	40	

Table 10. (Continued)

Species	Hardness (mg/L)	Temp. (°C)	Effect[A]	Value (μg/L)	Reference
Medionidus conradicus	150	20	24-hr LC50	81	Jacobson 1990
glochidia	160	20	24-hr LC50	41	
	185	20	24-hr LC50	37-69	
	170	20	48-hr LC50	16	
Villosa nebulosa (iris)	50-55	20	24-hr LC50	38-71	
glochidia	150-190	20	24-hr LC50	37-80	
	160	25	24-hr LC50	46	
	150-170	20	48-hr LC50	46-66	
Villosa nebulosa (iris)	190	20	24-hr LC50	27-29	
juvenile	190	20	24-hr LC50	83	
Corbicula fluminea	64	-	24-hr LC50	590	Rodgers et al. 1980
adult	64	-	96-hr LC50	40	
Corbicula fluminea veliger	17	19±1	24-hr LC50	28	Harrison 1984
Corbicula fluminea juvenile	17	19±1	24-hr LC50	600	
Corbicula fluminea	17	19±1	96-hr LC50	>2600	
adult	17	19±1	ILC50	<10	
Goniobasis livescens	154	14-16	96-hr LC50	390	Paulson et al. 1983
Juga plicifera	20	15±1	96-hr LC50	15	Nebeker et al. 1986
adult	20	15±1	30-d NOEC	6	
Lithoglyphus virens	20	15±1	96-hr LC50	8	
adult	20	15±1	30-d NOEC	<8	
Crustaceans					
Daphnia magna	45	18±1	48-hr LC50	60 (fed)	Biesinger and Christensen 1972
neonates	45	18±1	48-hr LC50	9.8 (unfed)	
	45	18±1	21-d repro impair	22	
Ceriodaphnia dubia	157	-	7-d LOEC	30	Cherry et al. 1991
neonates	157	-	7-d NOEC	20	
Ceriodaphnia dubia	94	25±1	48-hr LC50	35	Belanger et al. 1989
neonates	94	25±1	7-d NOEC	6.3	
	170	25±1	48-hr LC50	79	
	170	25±1	7-d NOEC	<20.1	
Ceriodaphnia dubia	47	24-25	48-hr LC50	17	Carlson et al. 1986
neonates	20-44	24-25	48-hr LC50	18	

Table 10. (Continued)

Species	Hardness (mg/L)	Temp. (°C)	Effect[A]	Value (μg/L)	Reference
Fish					
Pimephales promelas adult	200	-	96-hr TLm	430	Mount 1968
Pimephales promelas adult	100	20-23	24-hr LC50	270	Benson and Birge 1985
	100	20-23	96-hr LC50	210	
	250	20-23	24-hr LC50	720	
	250	20-23	96-hr LC50	390	
Pimephales promelas	47	24-25	96-hr LC50	85	Carlson et al. 1986
	20-44	24-25	96-hr LC50	95	
Pimephales notatus	200	25	96-hr LC50	220-270	Horning and Neiheisel 1979

[A] LC50 - lethal concentration to 50% of test population
TLm - median tolerance limit; ≃LC50
NOEC - no observable effect concentration
LOEC - lowest observable effect concentration
ILC50 - incipient lethal conentration, ie., concentration lethal to 50% of test population exposed for periods sufficiently long that acute action has ceased

Effects of Zn and Cu Mixtures

Reduced toxicity of Cu in the presence of Zn was noted for *Corbicula fluminea* (Rodgers et al. 1980). The only other study on metal mixture effects on unionid mussels was conducted by Keller and Zam (1991) with *Anodonta imbecilis.* Acute LC50 values were reduced when Zn was mixed with Ni or Cd, and Cu was mixed with Cd, suggesting increased toxicity of metal mixtures. Rainbow trout were exposed to an equally toxic ratio of Zn and Cu in hard and soft waters (Lloyd 1961). The author suggested the metals had similar joint action in hard water and in soft water at low metal concentration, but the toxic effect was greater than the predicted value at higher concentrations in soft water. *Daphnia magna* was less sensitive to equally toxic mixtures of Zn and Cd than to individual metals (Attar and Maly 1982), whereas a trimetal mix of Zn, Cu, and Cd resulted in toxicity to fathead minnows greater than would be predicted by a single metal. In chronic testing of metal mixtures, the toxic effects attributable to Zn were approximately equal, effects of Cu appeared to increase, while that attributable to Cd decreased (Eaton 1973). Results of a comprehensive study on the effects of metal mixtures (As, Cd, Cu, Cr, Hg, and Pb) suggested that interactions of metals may exert different responses on an acute and chronic basis and between different classes of organisms (Spehar and Fiandt 1986). Further, when these metals were mixed at individual no effect concentrations (as determined by USEPA), they elicited adverse effects to the three species tested (*Ceriodaphnia dubia*, fathead minnow, and rainbow trout). Biesinger et al. (1986) also found that mixtures of Cd, Zn, and Hg caused effects on the reproduction of *Daphnia magna* at concentrations which did not cause effects when metals were tested individually.

Generally, Zn is thought to exert simple additive joint action when mixed with other metals (Alabaster and Lloyd 1984), but as demonstrated above, this may depend on species, water quality, and concentrations (or ratios) of metals used in testing. Therefore, although Zn reduced acute Cu toxicity, variable conditions in the river (identity and concentrations of metals present, water quality, life stage of organism exposed, and duration of exposure) may change that trend. Also, although organisms may be protected by water quality criteria for individual metals, occurrences of metal mixtures in natural systems may exert adverse effects.

Water Quality of the Powell River

During the course of this study, water samples were collected from the Powell River and were analyzed for various metals, including Zn, Cu, and Al (Table 11). A trend of decreasing concentration progressing downstream was not evident. Such a trend would have suggested that metals were leaching from coal mining areas mainly in the upper Powell, and were diluted as they progress downstream. Aluminum levels can be quite high in the river (Table 11). If the pH becomes too acidic (< 6), due to acid mine drainage or containment pond spillage, then Al could become very toxic to aquatic life (Lewis 1989). Zinc concentrations were generally low (<21 μg/L), but one sample above the coal processing plant in Appalachia, Virginia, had 160 μg Zn/L. Copper concentrations were high in late spring, and seemed to be highest at Dryden, Virginia. Based on the laboratory toxicity data of several studies, these Cu concentrations exceeded LC50 levels determined for larval and juvenile mussels. Further, these high Cu concentrations occurred when many mussel species are known to release glochidia (Neves and Widlak 1988). Two mussel species of the Powell River, *Medionidus conradicus* and *Ptychobranchus fasciolaris*, exhibited 48-hr LC50 values as low as 17 μg Cu/L, and several other species yielded LC50 values below 50 μg Cu/L (Jacobson 1990, Cherry et al. 1991). From April to June, Cu levels in the Powell River exceeded 17 μg/L in all 11 samples collected, and exceeded 48 μg/L in 6 samples.

There are two USEPA water quality criteria for Zn. The acute value is based on a one hour average concentration, not to be exceeded more than once every three years on average and is calculated by the following equation:

$$\text{Final Acute Value} = e^{(0.8473[\ln(\text{hardness})] + 0.8604)}$$

Table 11. Measured metal concentrations ($\mu g/L$) in water sampled from four sites in the Powell River, Virginia, in 1991.

Metal/Date	Above Bullitt PRM 182.3	Below Bullitt PRM 181.5	Dryden PRM 167.4	Fletcher PRM 117.3
Zn				
1-18-91	-	-	10.2	-
4-12-91	8.6	6.1	10.2	-
5-11-91	9.8	20.6	10.1	5.4
6-18-91	13.4	11.0	20.4	6.3
7-05-91	8.0	7.0	4.7	5.7
9-05-91	<4.0	4.9	8.6	<4.0
12-04-91	160.1	4.9	10.2	13.3
Cu				
4-12-91	89.7	24.5	100.2	-
5-11-91	48.3	49.7	152.4	51.7
6-18-91	32.1	18.6	37.8	27.8
7-05-91	<2.0	<2.0	<2.0	<2.0
9-05-91	<2.0	<2.0	<2.0	<2.0
12-04-91	50.4	<2.0	<2.0	<2.0
Al				
1-18-91	-	-	73.8	-
4-12-91	150.6	103.4	221.4	-
5-11-91	95.8	845.5	101.6	44.3
6-18-91	288.2	200.7	457.4	159.8
7-05-91	334.9	288.2	165.7	183.2
9-05-91	87.9	69.5	234.8	118.5
12-04-91	751.5	796.1	898.1	2548

Powell River flow rates for Jonesville station (PRM 144.6) taken at 8 AM are as follows:

1-18-91 613 cfs
4-12-91 398 cfs
5-11-91 224 cfs
6-18-91 1411 cfs
7-05-91 207 cfs
9-05-91 148 cfs
12-4-91 5017 cfs

Adjusting for the average water hardness of the Powell River (160 mg/L), this value is 174 μg/L. The chronic value is based on a four day average concentration which should not be exceeded more than once every three years on average and is calculated by the following equation:

$$\text{Final Chronic Value} = e^{(0.8473[\ln(\text{hardness})]+0.7614)}$$

The chronic value for Zn was calculated to be 158 μg/L. Historical water quality data from the Powell River have recorded Zn levels in excess of the criterion on several occasions (STORET), and one occurrence of 160 μg Zn/L was recorded in my study. These measurements, however, have been very sporadic, and the true extent of these excesses is not known. Results of my study indicate the acute value may be protective of mussel glochidia, although results of another study indicate at least one species of mussel (*Ptychobranchus fasciolaris*) exhibited acute sensitivity near the criterion (Table 9). Chronic (sublethal) effects of Zn exposure have not been determined for glochidia or juvenile mussels and thus the adequacy of the chronic criterion cannot be evaluated.

The water quality criteria for Cu, based on USEPA guidelines are based on the following equations:

$$\text{Final Acute Value} = e^{(0.9422[\ln(\text{hardness})]-1.464)}$$

$$\text{Final Chronic Value} = e^{(0.8545[\ln(\text{hardness})]-1.465)}$$

Adjusting for hardness, the acute value for Cu in the Powell River is 28 μg/L, and the chronic value is 18 μg/L. These values may not be protective of freshwater mussels, as demonstrated by several previous studies (Jacobson 1990, Cherry et al. 1991, Farris et al. 1991). However, laboratory tests conducted with juvenile mussels may not reflect actual exposures in rivers. In rivers, juvenile mussels inhabit coarse gravel sediment (Isely 1911, Neves and Widlak 1987), with flowing water providing a continuous supply of food and oxygen and removing wastes. Most laboratory acute testing is conducted in small containers with static water and no substratum. Additionally, effects of metals may be indirect. For example, fish may avoid areas of metal pollution (Sprague 1964, Sprague 1968, Cherry and Cairns 1982, Hartwell et al.

1988, Hartwell et al. 1989). If host fish species are unavailable for glochidia to encyst, recruitment in mussel populations cannot occur.

Although the USEPA develops guidelines for water quality criteria, it is state responsibility to enforce them; thus, the Virginia State Water Control Board (VSWCB) has regulatory responsibility for point source pollution in Virginia. However, the VSWCB has delegated that authority to the Division of Mined Land Reclamation (DMLR), Office of Surface Mining, in areas of coal mining, such as the upper Powell River watershed. The coal processing plant located in Appalachia, Virginia, is under DMLR jurisdiction. The Bullitt plant is required by the DMLR to monitor its own effluent for pH, Fe, Mg, and suspended solids, but not for other metals. Further, many chemicals are used in the coal preparation process, but none are monitored in the water exiting the settling ponds. Several fish kills have been reported following spills from these containment ponds and other mines (Osborne 1986, Coafield Progress 1990). Occasionally, grease and oil (such as Solcenic oil) used for mine equipment is discharged into the river; the effects upon mussel populations of these and other chemicals used by the mining industry have not been evaluated.

I did not identify point sources responsible for the high metal concentrations recorded in the Powell River. More intensive water quality monitoring needs to be conducted to isolate the sources. Water samples should be taken prior to and during rain events to determine whether metals are leaching from abandoned mine sites, as well as from areas currently being mined. Although the DLMR currently has a program for the reclamation of abandoned mines, protection of aquatic resources receives low priority. If it can be established that coal mining activities are responsible for metal concentrations exceeding USEPA criteria (and concentrations shown to adversely affect mussel populations), perhaps more emphasis can be placed on reclamation efforts.

The question of the adequacy of USEPA criteria for the protection of freshwater mussels in the Powell River is a moot point if there is no regulation or enforcement of criteria. If these high metal concentrations continue, it seems doubtful that the declining mussel populations of the Powell River will recover, and more mussel species will be extirpated.

Chapter Three - Sediment Toxicity Tests

Introduction

Sedimentation is the most visible impact on streams draining land which has been surface or strip mined for coal. Siltation effects on fauna following coal mining have been widely studied (Charles 1966, Branson and Batch 1972, Matter et al. 1978, Vaughan et al. 1978, Vaughan 1979, Matter and Ney 1981, Mettee and O'Neil 1985, Dick et al. 1986, Anderson 1989). Generally, increased siltation resulting from mining has been correlated with reduced or eliminated benthic invertebrate populations and benthic fish, such as darters. In laboratory experiments, Imlay (1972) found that freshwater mussels covered with various amounts and types of sediment often were unable to burrow upward and were virtually smothered. Mussel populations reduced or eliminated from areas affected by coal mining in the Cumberland River drainage, Tennessee, were attributed to siltation and an unidentified toxic component of alkaline mine drainage (Anderson 1989). Wolcott (1990) also found fewer mussels in coal mining areas of the Powell River, Virginia, but was unable to correlate low mussel abundance with a causative agent.

Along with increased sedimentation, water quality parameters such as alkalinity, conductivity, hardness, and occasionally SO_4, Ca, Mg, Mn, and Fe were measured and found to increase with mining activities. However, heavy metals have rarely been studied. High metal content in water and sediment has been found near unreclaimed coal mines (Anderson 1989), and lakes formed by abandoned coal mines in the mid-western U.S. had high metal content in bottom sediment (Brugam et al. 1988). Dick et al. (1986) found that metal concentration of suspended sediment exiting sedimentation ponds was not significantly different from that entering the ponds. In fact, metal concentration in the suspended sediment tended to increase at the outfall as the heavier particles settled to the bottom.

It is generally accepted that sediments serve as a sink for trace metals, especially smaller-sized particles. Metals may be adsorbed on particulate surfaces (such as clays, humic acids or metal hydroxides), bound to carbonate minerals, complexed with Fe or Mn oxyhydroxides, or bound with organic matter or sulfides (Tessier and Campbell 1987). Thus, metals leaching from exposed coal piles and spoil piles presumably adsorb to particulate matter and are either stored in the bottom sediment or transported in the streams by suspended sediment. However, the concept that there is little interaction with contaminants once bound to sediments has come under question (Reynoldson 1987), and it is now believed that there can be significant biological interaction (e.g., the microbial methylation of Hg). Chadwick et al. (1986) found that insect populations required nearly 10 years to fully recolonize a stream following major improvements in mine wastewater treatment, suggesting that metals in the sediment were affecting insects long after water quality was improved. A three-year study in the Yellow Creek drainage in Kentucky, demonstrated that sediment may act as a reservoir for contaminants which do not show up in the water column (Westerman 1989). Sediments below a coal-washing plant contained high levels of metals such as Cu, Zn, and Fe, whereas the water concentrations were much lower.

A variety of chemicals is used in coal preparation plants, such as those used in density separation for coal cleaning and flocculants or surfactants used in dewatering processes. The final waste slurry contains fine particles of coal, shale, and clay plus water contaminated with a variety of dissolved materials and chemicals. Eventually the waste is piped to settling ponds where the contaminants presumably settle out, and "clean" water is released into a nearby stream.

The bioavailability of these trace metals is poorly understood and thus difficult to determine (Tessier et al. 1984, O'Donnell et al. 1985, Reynoldson 1987, Tessier and Campbell 1987). Willford et al. (1987) suggested the most direct and meaningful approach to estimating bioavailability of metals in sediment was to measure bioaccumulation. However, the long-term effects of metals accumulated in tissues are unknown. Mathis and Cummings (1973) found that annelids and mussels closely reflected the concentration of metals found in bottom sediments. Generally, metal concentrations were highest in the sediment and lowest in the water column. Another study suggested chironomids in benthic samples could be used as an index of Cu and Zn pollution in streams (Winner et al. 1980). Bivalves and gastropods were rare or absent in these streams but were common in similar streams without metal pollution, suggesting their greater sensitivity. Recently, more emphasis has been placed on an integrative approach to determining both numerical (e.g., chemical concentration) and biological effects of sediments (Chapman 1989, Chapman et al. 1991, Burton 1991).

Sediment toxicity tests have been used since the late 1970's, mainly to assess the effects of marine dredge material on benthic organisms (Burton 1991). In the 1980's, research increased on developing appropriate tests with sediment and freshwater organisms. LeBlanc and Suprenant (1985) developed sediment tests using several species, including ones associated with the water column (e.g., *Daphnia magna* and eggs of the fathead minnow) as well as the midge (*Paratanytarsus parthenogenica*), which resides within the sediment. Acute effects (96-hr) were not apparent, but impairment effects such as daphnid reproduction and fish growth were good indicators of sublethal effects. This toxicity was correlated to a hazard index which is based on sediment chemical parameters, including metal concentration.

Freshwater bivalves have rarely been used in sediment testing, generally because of their ability to avoid exposure by closing their valves. Wolcott (1990) used juveniles of *V. iris* to test coal silt and Powell River silt, but results were inconclusive. Larval and juvenile mussels are some of the most sensitive organisms tested with metals in water (Cherry et al. 1991). Juvenile mussels are benthic organisms and a more realistic exposure to contaminants would be in the sediment.

Historical data in STORET on metal concentrations of Powell River sediments reveal concentrations of As high enough to be categorized as moderately to heavily polluted in five out of the six samples, based on classification by Bowden (in LeBlanc and Suprenant 1985). Additionally, Cu, Zn, Ni, and Pb have been found at levels high enough to categorize the sediment as moderately to highly polluted. Thus, the objective of this chapter was to determine the toxicity of sediments in the Powell River to juvenile mussels, the most sensitive life stage.

Materials and Methods

Juveniles of two species of mussels, rainbow (*Villosa iris*) and pheasantshell (*Actinonaias pectorosa*), were used to evaluate the effects of Powell River sediment. Juveniles were obtained by infesting host fish with glochidia as described previously (Chapter Two, Juvenile Acute Toxicity Tests).

Sediment was collected from the Powell River, Virginia, at five sites between river mile 117.3 (Fletcher Ford) and 182.3 (Appalachia). Sediment was collected to obtain depositional layers from storm runoff and particulate fractions $\leq$ 200 μm to allow separation of juveniles from sediment during the test. Several methods of collection were attempted to facilitate these objectives and will be elaborated under individual test descriptions.

Once collected, all sediment samples were immediately chilled for transport to the laboratory and then stored at 4°C. Tests were started within two weeks of sample collection. Prior to testing, all sediment samples were seived through a 202 μm mesh using water from the same site. Subsamples of 10 to 20 ml of sediment were placed into 100 x 80 mm pyrex storage dishes, and topped with 250 ml of test water (river or dechlorinated tap water). Test containers were placed in an incubator set at 20°C, and sediments were allowed to settle overnight. Juveniles were examined under the microscope to verify relative health as exhibited by foot movement or siphoning. Selected juveniles were enumerated and separated to ensure randomness in testing. The length (maximum distance between anterior and posterior shell margins) and height (maximum distance between dorsal and ventral shell margins) of each juvenile mussel were measured using an ocular micrometer and recorded prior to transfer to the test dish. The addition of test organisms to all treatments was randomized among treatments and replicates of treatments. Aeration was provided by air pumps (Wisper 500) and aquarium tubing fitted with pipet tips to ensure adequate dissolved oxygen; a 16:8 hour light-dark photoperiod was maintained. Juveniles were fed 4 to 5 drops of a concentrated (approximately 3.2 X 10^7 cells/ml) tri-algal culture (*Chlamydomonas, Chlorella, Ankistrodesmus*) every two days in conjunction with the water renewal. Due to problems with the tri-algal culture, juveniles were not fed during the last 20 days of sediment test 5.

Water in the dishes was changed every two days. Approximately 150 to 200 ml water were decanted off the top of the sediment and this water was checked for juveniles. The amount of water decanted varied somewhat due to evaporation. This water was then replaced with 200 ml of fresh water, and the dish was returned to the incubator. Water quality parameters (DO, pH, hardness, and alkalinity) were monitored throughout the tests (APHA 1989), and conductivity was measured with a conductivity pen (Nester Instruments).

At the end of 10 days, juveniles were examined in each dish. A dish (replicate) was randomly selected, and overlay water was decanted. The sediment in the dish was seived through a 202 μm mesh using fresh test water. Some debris (worm tubes, algae) and the juvenile mussels remained on the seive. These were transferred to a gridded petri dish with water and examined under the microscope. The number of live and dead juveniles was recorded, along with length and height measurements. If the test continued, juveniles were returned to the dish after measurement and returned to the incubator. This procedure was then repeated for each replicate.

Sediment Test One

Test one was intended to examine appropriateness of test design for juveniles of *V. iris* and to compare upstream and downstream sites in the Powell River. River sites at Dryden (PRM 167.4), Shafer Ford (PRM 153.4), and Fletcher Ford (PRM 117.3) were chosen. Acid-washed jars, covered with 202 μm mesh nitex, were set into the river substratum by digging a hole and surrounding the jars with large rocks to stabilize them. This technique had been used successfully in the Powell River (Lisa Wolcott, personal communication); however, jars recovered after 4 weeks had clogged screens and very little sediment. Sediment was then collected by scooping finer fractions deposited immediately behind rocks and areas where river flow was interrupted. Three replicate sediment samples (A, B, C) were collected from each site. A control sediment (known to be uninfluenced by point and nonpoint pollution) was unavailable. Thus, a control of no sediment and dechlorinated tap water was used. River water collected from the same site was added to dishes containing sediment. Additionally, three replicates of river water without sediment were completed for each site in an attempt to distinguish effects caused by sediment from those due to the river water itself. The control consisted of dechlorinated tap water without sediment. Due to limited availablility, only 10 juveniles (3 to 5 days old) of *V. iris* were used per replicate, and replicates were tested 2 days apart (Table 12). Initial growth was most evident in shell height of juveniles. Thus, heights of juveniles were recorded for this trial, which was completed in 10 days. Mean growth for each replicate was the mean height at 10 days minus mean height at day 0.

A two-way analysis of variance (ANOVA) and Duncan's multiple range test were used to determine differences in growth (as measured by increase in height) and survival among sites and the two water sources. An arcsin square root transformation of the percent survival was performed to normalize the variance.

Table 12. Conditions of a preliminary 10-day sediment test with juveniles of *Villosa iris*.

River Site	Condition	Replicate	Number per Replicate	Date Started
Dryden PRM 167.4	sediment with site water	A	10	11-11-90
		B	10	11-13-90
		C	10	11-15-90
	no sediment site water	D	10	11-15-90
		E	10	11-17-90
		F	10	11-17-90
Shafer Ford PRM 153.4	sediment with site water	A	10	11-11-90
		B	10	11-13-90
		C	10	11-15-90
	no sediment site water	D	10	11-15-90
		E	10	11-17-90
		F	10	11-17-90
Fletcher Ford PRM 117.3	sediment with site water	A	10	11-11-90
		B	10	11-13-90
		C	10	11-15-90
	no sediment site water	D	10	11-15-90
		E	10	11-17-90
		F	10	11-17-90
Control	no sediment tap water	A	10	11-11-90
		B	10	11-13-90
		C	10	11-15-90
		D	10	11-15-90
		E	10	11-17-90
		F	10	11-17-90

Sediment Test Two

For this experiment, I evaluated whether the test design was appropriate for a duration greater than 10 days so that delayed mortality and chronic effects (as represented by reduced growth) could be examined in juveniles of *V. iris*. Also, it was desirable to find a substratum suitable for use as a control. On November 8, 1990, clean jars were placed again at Dryden (PRM 167.4) as described for Sediment Test One, but no nitex netting was used. The jars were set as deep as possible behind rocks or other outcrops with the expectation of collecting suspended sediment. Jars collected after 12 days contained little sediment. Three replicates of sediment then were collected as in sampling for test one. Washed glass beads ($\leq$106 μm diameter) were used as a control substrate. Forty juveniles (4 to 5 days old) of *V. iris* were added to each of three replicates of Dryden sediment or glass beads with river water from Dryden. A separate sediment test was set up with midge larvae (*Chironomus riparius*) to compare the response of a standard test organism with that of juvenile mussels. The test was similar to juvenile mussel sediment tests with the exception that ten, 2nd instar (3 days post-hatch) larvae were added to each of three replicates of Dryden sediment using river water. Midges were fed a suspension of 0.06 g/ml Tetramin fish food every two days, and survival was checked after 10 days. Growth and survival of juvenile mussels were measured every 10 days. Tests were terminated after 10 days (midge), 20 days (control mussels in glass beads), and 90 days (mussels in sediment) (Table 13). Measurements of maximum shell length and height were recorded for each juvenile. As judged by results from sediemtn test one, a better measurement of shell growth was the increase in mussel shell area. Thus, the length and height of each shell were multiplied to give a number defined as the size index. Since it was impossible to keep track of individual juveniles within the test dishes, a mean size index (the average of all size indices per dish) was determined for each replicate. Growth was defined as the mean increase in size index.

Table 13. Conditions of sediment test with freshwater organisms comparing river and artificial substratum.

Condition	Species	Replicates	Number per Replicate	Date Started
Dryden sediment 90 day test	*Villosa iris*	A,B,C	40	11-30-90
Glass beads 20 day test	*Villosa iris*	A,B,C	40	11-30-90
Dryden sediment 10 day test	*Chironomus riparius*	A,B,C	40	12-05-90

A Chi-square contingency table was used to determine whether survival was significantly different between sediments. To test for differences in growth, the size index (length X height) was determined for each replicate. The difference in mean size index after 10 and 20 days was considered the amount of growth which occurred. This increase was tested by ANOVA to compare differences in growth between river sediment and glass beads.

Sediment Tests Three and Four

Two additional sediment tests measured the effects of sediments upstream and downstream of a coal processing plant. Separate tests were conducted with site river water (test three) or dechlorinated tap water (test four) to distinguish the effects of water versus sediment. Sediment was collected from above and below a coal preparation plant (Bullitt) located in Appalachia, Virginia. Discharge from the Bullitt Plant is released into Luny Creek, a tributary of the Powell River. On April 12, 1991, glass jars were set out with four replicates per site. The site above Bullitt was located approximately 0.6 km upstream of Luny Creek confluence with the Powell River (PRM 182.3). A downstream site was selected approximately 0.6 km below this confluence (PRM 181.5). Jars were filled with pebbles (16 to 64 mm) found at the site in an attempt to prevent the collected sediment from being flushed out. When the jars were collected a month later, the water was turbid and one replicate was not recovered from the site below Bullitt. Sediment also was hand-collected from Dryden for use as a reference site.

An acute 48-hr Ceriodaphnia test (ASTM 1988) was conducted in Powell River water from each of the above sites and in dechlorinated tap water (source, New River) to test the suitability of water for use in the sediment tests. There was no significant mortality ($\leq$ 10%) in any of the waters tested, so site water (Above Bullitt, Below Bullitt, Dryden) was used. To differentiate potential effects due to the water, a test also was conducted with sediment and dechlorinated tap water. There was insufficient sediment from Dryden to set up a second test.

Forty juveniles of *Actinonaias pectorosa* (6 to 8 days old) were added to each sediment dish. Due to time constraints and availability of juveniles, the sediment tests were started 2 days apart. Tests were run for 30 days, with growth and survival checked every 10 days (Table 14).

Data from the two Bullitt sites were analyzed by a two-way ANOVA and a Duncan's multiple range test using an arcsine square root transformation of the percent survival. Additionally, a one-way ANOVA was used on sediment test three to detect any significant differences in survival of juveniles among the three sites. The differences in mean size indices were compared by ANOVA to test for significant differences among sites and water type.

Sediment Test Five

A fifth sediment test compared responses of two mussel species, *A. pectorosa* and *V. iris* to sediment collected from above and below the Bullitt plant in Appalachia, and from Dryden and Fletcher Ford. As before, jars were filled with pebbles and sunk into the substratum, four replicates per site. The water level and turbidity in the river was high when setting out the jars (6-18-91) and again when collecting them (7-5-91). Two sets of jars from below Bullitt were not recovered, and current and depth made additional collection impossible. Two sets of jars from Fletcher Ford also were lost; however, sediments were collected from the bank of a sand island to replace these. All jars collected at Dryden were cracked, albeit still intact, and broken glass was found where the lost jars had been set out at Fletcher Ford and below Bullitt. Using available sediments, two sets of sediment dishes were set up. Juveniles of *V. iris* (8 to 12 days old) were added to one set, and those of *A. pectorosa* (6 to 10 days old) were added to the second set. Replicates A and B (A only for below Bullitt) were started July 12 and replicates C and D (B only for below Bullitt) were started two days later (Table 14).

Again, an arcsin square root transformation of the percent survival was used to normalize the variance. A two-way ANOVA and Duncan's multiple range test compared differ-

Table 14. Conditions of 30-day Powell River sediment tests comparing response of two mussel species and diluent source.

River Site	Test Water	Species	Replicates	Number per Replicate	Date Started
Test Three					
Above Bullitt PRM 182.3	Site	*A. pectorosa*	A,B,C,D	40	5-20-91
Below Bullitt PRM 181.5	Site	*A. pectorosa*	A,C,D	40	5-20-91
Dryden PRM 167.4	Site	*A. pectorosa*	A,B,C,D	40	5-20-91
Test Four					
Above Bullitt	Tap	*A. pectorosa*	A,B,C,D	40	5-22-91
Below Bullitt	Tap	*A. pectorosa*	A,C,D	40	5-22-91
Test Five					
Above Bullitt	Tap	*A. pectorosa*	A,B C,D	40 40	7-12-91 7-14-91
Above Bullitt	Tap	*V. iris*	A,B C,D	40 40	7-12-91 7-14-91
Below Bullitt	Tap	*A. pectorosa*	A B	40 40	7-12-91 7-14-91
Below Bullitt	Tap	*V. iris*	A B	40 40	7-12-91 7-14-91
Dryden	Tap	*A. pectorosa*	A,B C,D	40 40	7-12-91 7-14-91
Dryden	Tap	*V. iris*	A,B C,D	40 40	7-12-91 7-14-91
Fletcher Ford	Tap	*A. pectorosa*	A,B C,D	40 40	7-12-91 7-14-91
Fletcher Ford	Tap	*V. iris*	A,B C,D	40 40	7-12-91 7-14-91

ences among sites and species responses. The differences among growth and mean size indices were compared by one-way ANOVA to test for significant differences among sites for each species.

Results

Sediment Test One

Survival of juveniles of *V. iris* in sediment and river water was similar at Dryden (92.6%), Shafer Ford (96.4%), and Fletcher Ford (86.7%) (Table 15). Survival among sites without sediment also was similar (93 - 100%), and controls had no mortality. Results of an ANOVA and Duncan's test revealed significantly ($p = 0.0001$) greater growth (increase in shell height) of juveniles in river water with sediment (mean of 88.5 μm) than in river water without sediment (mean of 53.8 μm) or controls (mean of 43.9 μm). The growth of juveniles was similar among water types without sediment (Figure 4). The mean heights of juveniles at 10 days were 401.8 μm (sediment), 378.2 μm (no sediment), and 361.1 μm (controls) (Table 15).

Sediment Test Two

The glass beads used in this test were not a suitable substratum. Survival of juveniles in glass beads was 78% and 49% at 10 and 20 days, respectively, whereas juveniles in river sediment exhibited 95% and 87% survival, respectively (Table 16). At 10 days, juveniles exhibited significantly ($p = 0.0228$) greater growth (as represented by the increase in mean size index or area of shell) in river sediment (73.1 μm^2) than in glass beads (44.8 μm^2). This trend

Table 15. Growth and survival of juveniles of *Villosa iris* in a preliminary 10-day test, with and without sediment.

River Site	% Survival[A]	Mean Height (μm) Day 0	Mean Height (μm) Day 10	Mean Growth[B] (μm)
		With Sediment		
Dryden	92.6	315.0	411.1	96.1
Shafer Ford	96.4	310.0	391.0	81.0
Fletcher Ford	86.7	315.0	403.3	88.3
Control[C]	100	311.7	355.5	43.8
		Without Sediment		
Dryden	100.0	330.0	391.7	61.7
Shafer Ford	93.0	321.6	371.3	49.6
Fletcher Ford	100.0	321.6	371.6	50.0
Control[C]	100.0	321.7	366.7	45.0

[A] Average of three replicates.
[B] Growth = increase in height measurement.
[C] Controls were in dechlorinated tap water, without sediment.

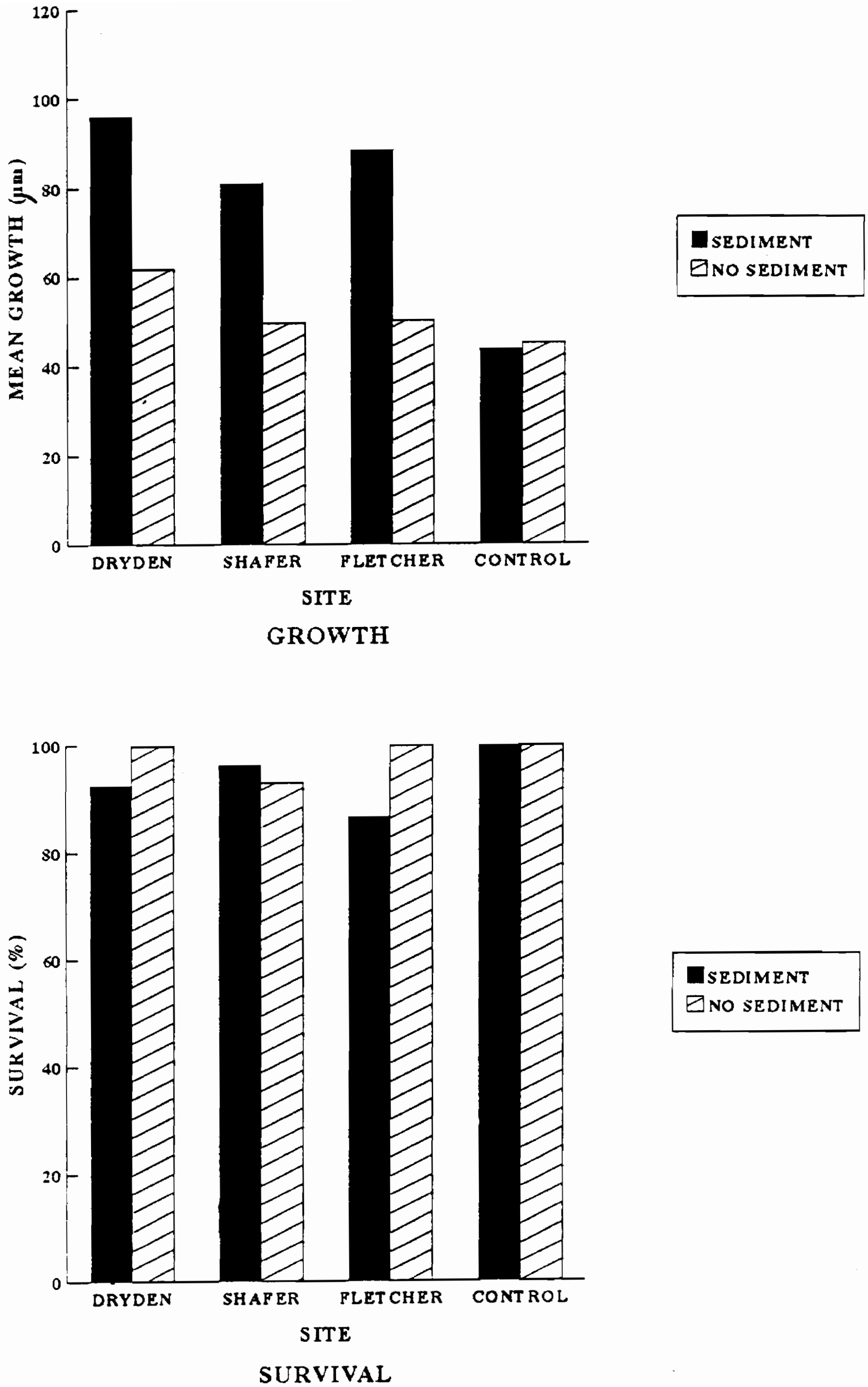


Figure 4. Comparison of 10-day growth and survival of juveniles of *Villosa iris* with and without sediment.

Table 16. Growth and survival of juveniles of *Villosa iris* in river sediment from Dryden and in glass beads.

Day of test	% Survival[A]		Mean Size Index[B] (μm^2)		Mean Growth[C] (μm^2)	
	Dryden	Glass Beads[D]	Dryden	Glass Beads	Dryden	Glass Beads
0	-	-	117.1	111.7	-	-
10	94.8	78.3[E]	190.2	156.5[E]	73.1	44.8[E]
20	87.1	49.2[E]	261.6	173.0[E]	71.4	16.5[E]
30	85.3		351.9		90.3	
40	84.5		489.1		137.2	
50	84.5		599.4		110.3	
60	83.6		734.9		135.5	
70	81.7		938.2		203.3	
80	81.7		1091.0		152.8	
90	81.7		1261.6		170.6	

[A] Average of three replicates.
[B] Size index = length X height.
[C] Growth = increase in size index.
[D] Test with glass beads was terminated after 20 days.
[E] Significantly less than Dryden ($p < .05$).

was continued at 20 days, when growth of juveniles was 71.4 μm^2 and 16.5 μm^2 in river sediment and glass beads, respectively. The mean size index (length X height) of juveniles in river sediment was 261.6 μm^2 at 20 days, as compared to 173.0 μm^2 in glass beads (Table 16). The juveniles in river sediment continued to grow throughout the 90-day test (Figure 5). Most of the mortality occurred in the first 20 days (12.9%), with only 5.4% additional mortality thereafter (Figure 6). The sediment from Dryden was not acutely toxic, as evidenced by the high survival of midge larvae (96.7%).

Sediment Tests Three and Four

Results of a two-way ANOVA indicated that at 10 days, survival (90%) for juveniles of *A. pectorosa* in sediment from AB (above Bullitt) was significantly higher ($p = 0.01$) than survival (80%) in sediment from BB (below Bullitt) (Table 17). Additionally, survival in tap water (94%) was significantly higher ($p = 0.0002$) than survival in river water (77%). However, at 20 and 30 days, survival was similar among sites and between water sources (Figure 7).

When results of survival in river water were analyzed by one-way ANOVA, there was no significant difference among sites. Survival was lowest at BB (69%), highest at AB (84%) and intermediate at Dryden (77%) (Table 17).

The analysis of growth data on sediment test three (river water) indicated significantly ($p = 0.0169$) lower growth of juveniles in Dryden sediment (6.4 μm^2) than in sediment from AB (17.6 μm^2) or BB (19.3 μm^2) by the end of the 30-day test. However, the mean size index was not significantly different among sites ($p = 0.3766$). Analysis of growth data on sediment test four (tap water) indicated no significant difference among the two Bullitt sites in mean size indices or growth (p values >0.48).

Results of a two-way ANOVA indicated that juveniles in sediment with tap water had consistently greater growth than juveniles in sediment with river water (p values <0.02) (Fig-

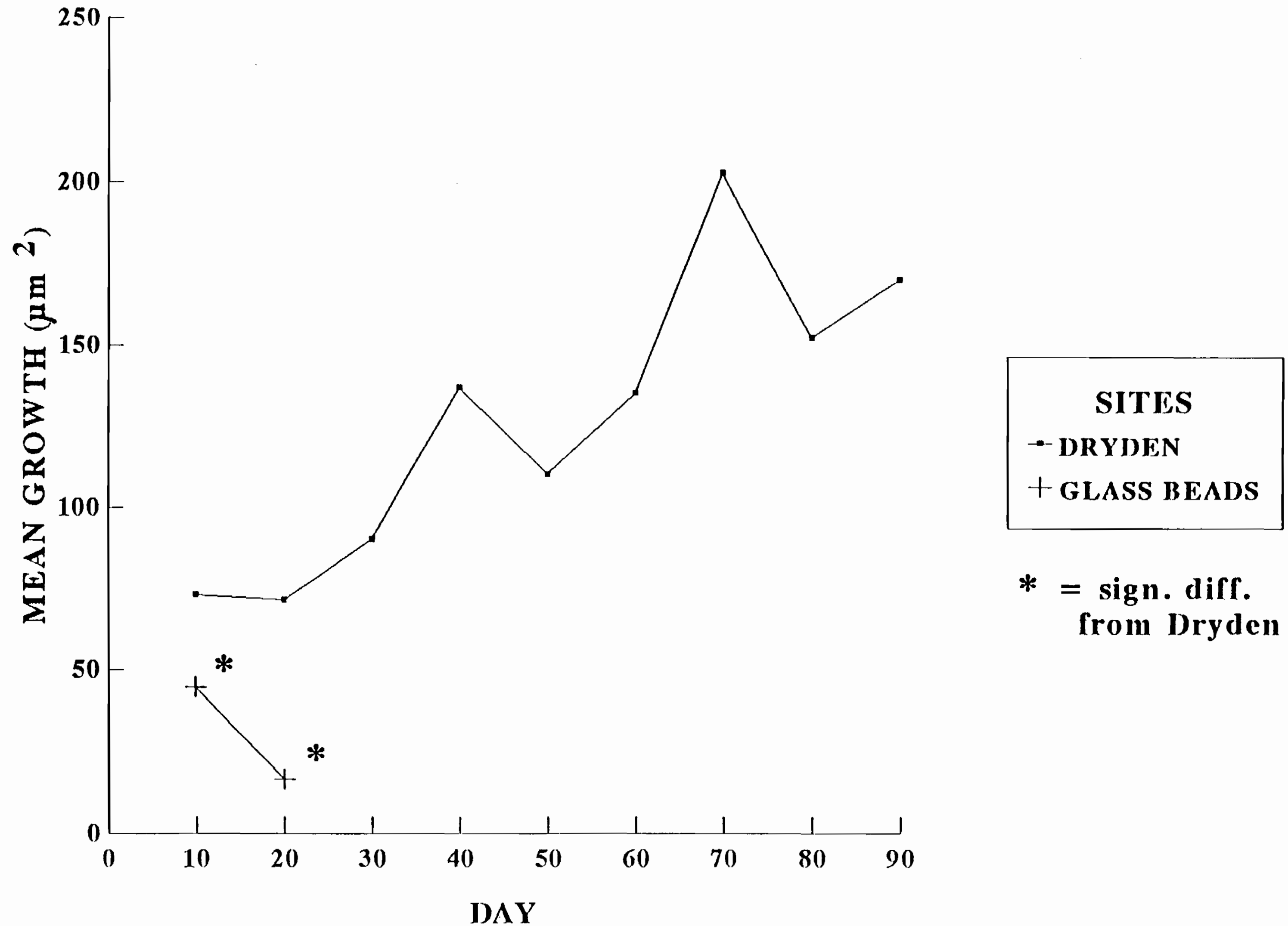


Figure 5. Comparison of growth of juveniles of *Villosa iris* in river sediment and in glass beads.

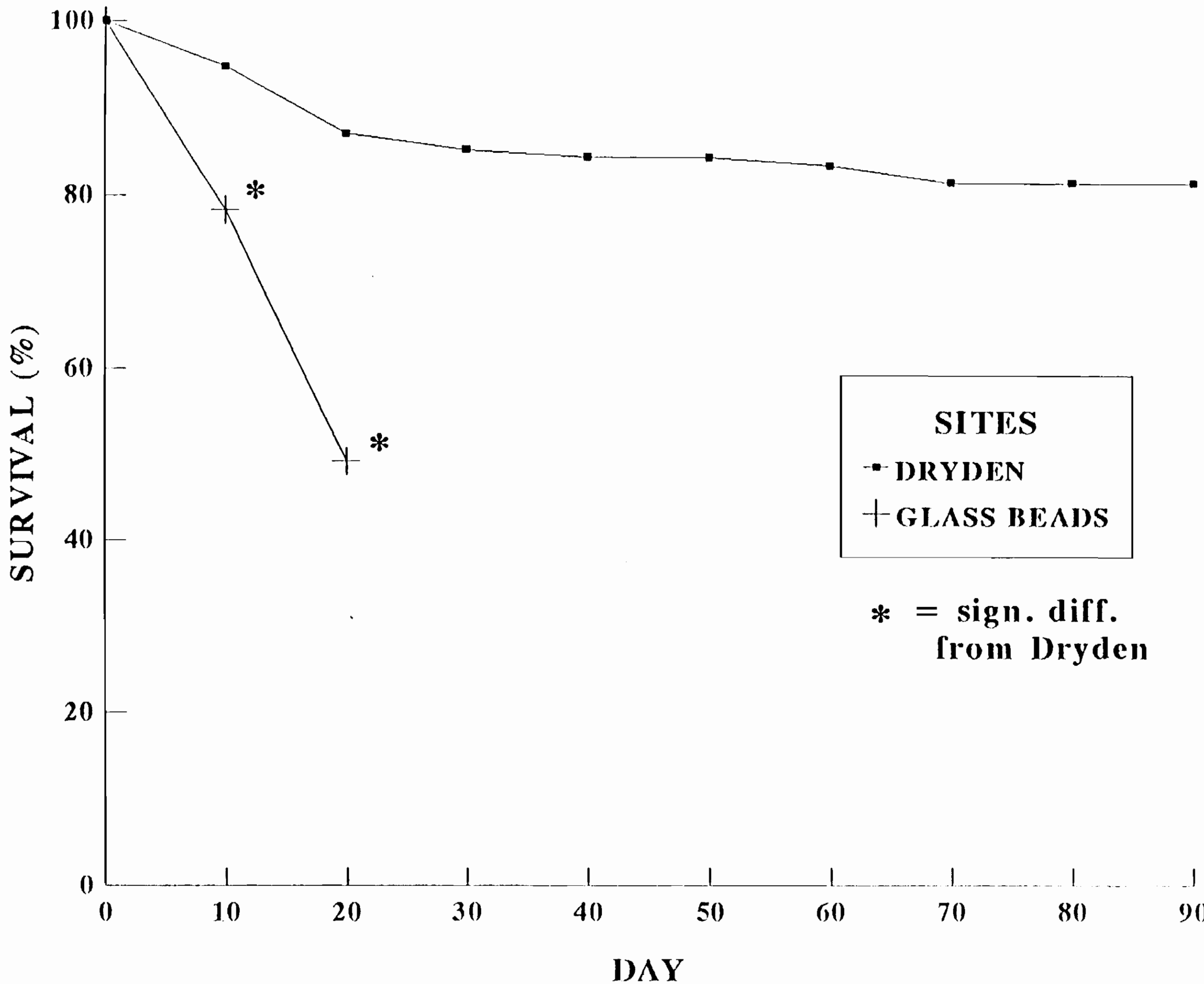
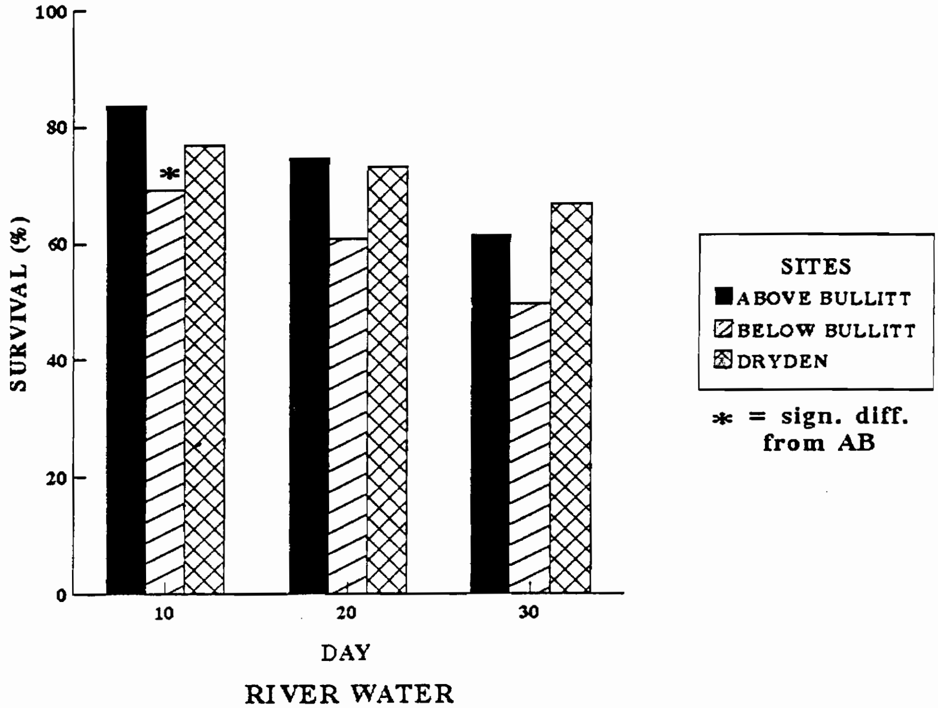


Figure 6. Comparison of survival of juveniles of *Villosa iris* in river sediment and in glass beads.

Table 17. Comparison of growth and survival of juveniles of *Actinonaias pectorosa* in Powell River sediment using river water and tap water.

Day of Test	% Survival[A]			Mean Size Index[B] (μm^2)			Mean Growth[C] (μm^2)		
	AB	BB	Dry	AB	BB	Dry	AB	BB	Dry
Test Three - River Water									
0	-	-	-	94.7	93.2	93.8	-	-	-
10	83.6	69.2[D]	76.9	134.8	134.3	133.6	40.1	41.1	39.8
20	74.7	60.8	73.1	162.5	157.8	162.7	27.7	23.5	29.1
30	61.4	49.6	66.7	180.1	177.1	169.1	17.6	19.3	6.4[E]
	AB	BB		AB	BB		AB	BB	
Test Four - Tap Water									
0	-	-		88.0	89.2		-	-	
10	96.1	91.5		139.2	142.4		51.2	53.2	
20	74.7	70.9		175.1	178.1		35.9	35.6	
30	56.4	50.9		209.3	217.4		34.1	39.4	

[A] Average of 4 replicates for AB (Above Bullitt) and Dry (Dryden), and 3 replicates for BB (Below Bullitt).
[B] Size index = length X height.
[C] Growth = increase in size index.
[D] Significantly less than AB (excluding Dry data, $p < .05$).
[E] Significantly less than AB or BB ($p < .05$).



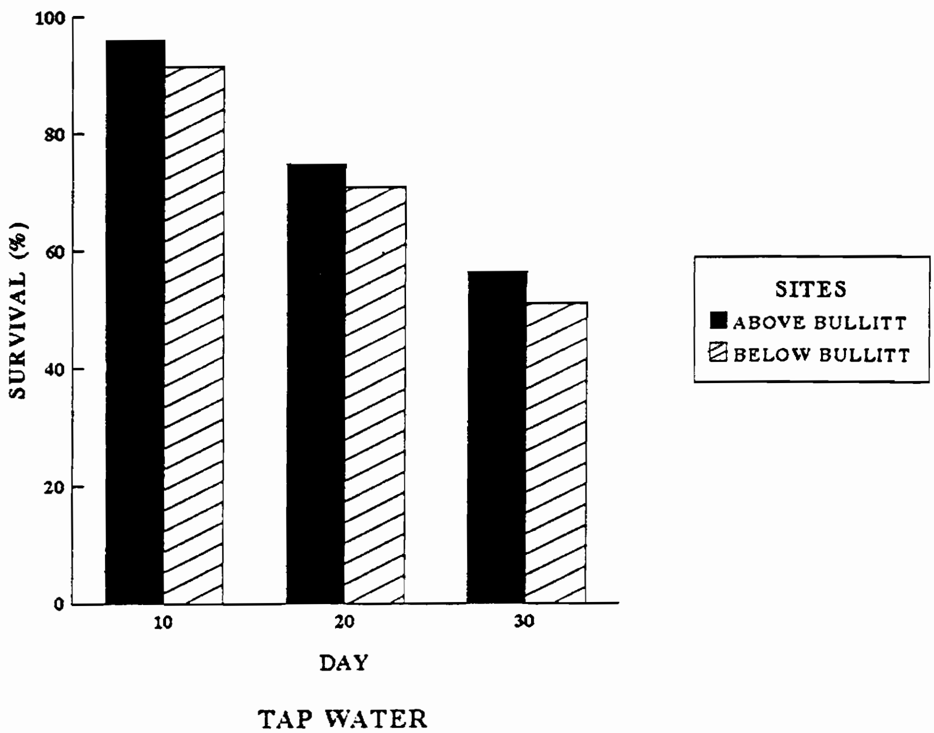


Figure 7. Comparison of 10, 20, and 30-day survival of juveniles of *Actinonaias pectorosa* in river sediment using river and tap water.

ure 8). At 30 days, the mean size index of juveniles in tap water was 212.8 μm^2, as compared to 178.8 μm^2 for juveniles in river water (Table 17).

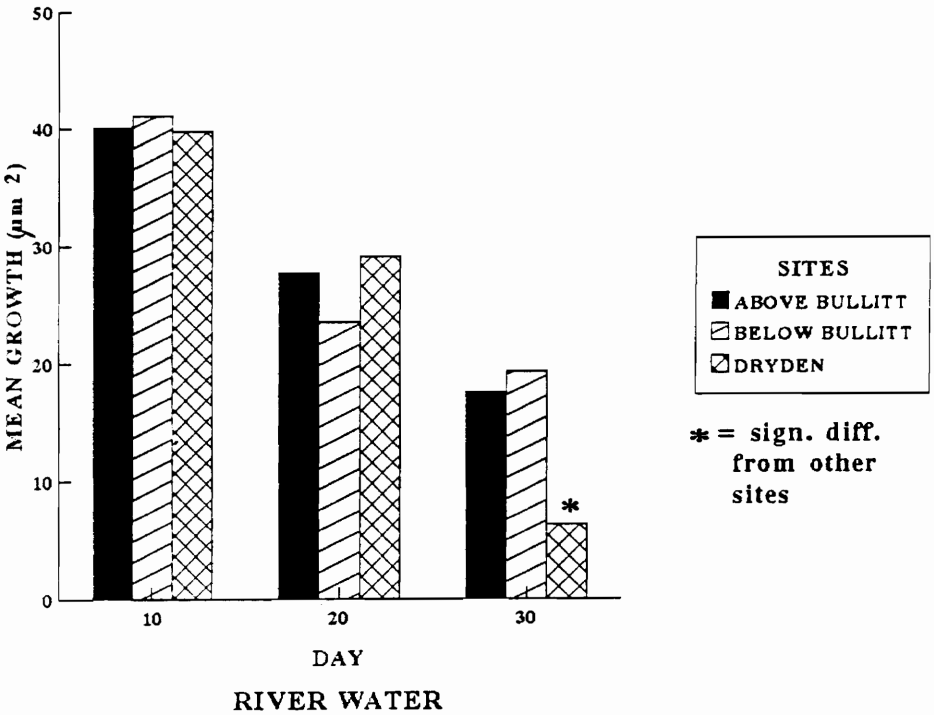
Sediment Test Five

Survival of juveniles was high throughout the 30-day test for all sites and both species, with no significant differences (two-way ANOVA, p values >0.12). After 30 days, survival in river sediment with tap water averaged 93% for *A. pectorosa* and 95% for *V. iris* (Tables 18, 19).

Growth of juveniles of *A. pectorosa* in Powell River sediment was similar for all sites throughout the 30-day test (one-way ANOVA, p values ≥ 0.08) (Figure 9). Juveniles in sediment from BB exhibited the best growth (37.9 μm^2), as compared to Flet (32.6 μm^2), Dry (25.1 μm^2), and AB (20.2 μm^2) (Table 18). This trend also was evident at 10 and 20 days. Growth of juveniles of *V. iris* varied from that of *A. pectorosa*. *Villosa iris* in sediment from BB consistently showed less growth than other sites. However, this was only significant at 10 days ($p = 0.0016$), when growth for each site was 48.5 μm^2 (BB), 61.1 μm^2 (Dry), 61.9 μm^2 (AB), and 68.7 μm^2 (Flet) (Table 19). The difference in growth among sites also was reflected by the mean size index of juveniles. At 30 days, juveniles in sediment from BB (232.5 μm^2) were significantly smaller ($p = 0.0414$) than juveniles in sediment from AB (263.3 μm^2), Flet (297.3 μm^2), or Dry (298.6 μm^2).

Discussion

Collection of depositional sediment using pebble-filled jars in the river bottom worked well, although glass jars are fragile under high flow conditions and require stable placement.



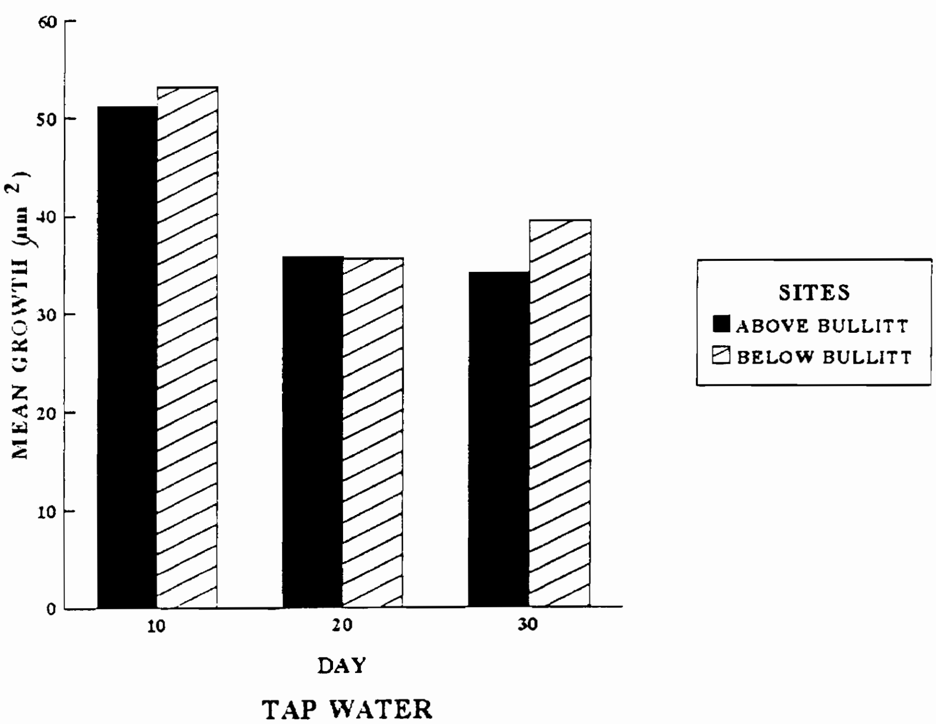


Figure 8. Comparison of 10, 20, and 30-day growth of juveniles of *Actinonaias pectorosa* in river sediment using river and tap water.

Table 18. Growth and survival of juveniles of *Actinonaias pectorosa* in sediment from four sites in the Powell River, Virginia.

Day of Test	% Survival[A]		Mean Size Index[B] (μm^2)		Mean Growth[C] (μm^2)	
	AB	BB	AB	BB	AB	BB
0	-	-	102.9	99.7	-	-
10	96.2	98.7	149.4	148.7	46.5	49.0
20	95.5	93.7	176.2	181.6	26.8	32.9
30	92.9	92.3	196.5	219.5	20.2	37.9
	Dry	Flet	Dry	Flet	Dry	Flet
0	-	-	103.1	103.8	-	-
10	99.4	95.6	151.3	156.1	48.2	52.3
20	97.5	93.1	179.8	183.2	28.5	27.1
30	96.2	89.3	204.9	215.7	25.1	32.6

[A] Average of 4 replicates for AB (Above Bullitt), Dry (Dryden), Flet (Fletcher), and 2 replicates for BB (Below Bullitt).
[B] Size index = length X height.
[C] Growth = increase in size index.

Table 19. Growth and survival of juveniles of *Villosa iris* in sediment from four sites in the Powell River, Virginia.

Day of Test	% Survival[A]		Mean Size Index[B] (μm^2)		Mean Growth[C] (μm^2)	
	AB	BB	AB	BB	AB	BB
0	-	-	113.0	111.8	-	-
10	98.8	98.7	174.9	160.3[D]	61.9	48.5[D]
20	98.1	97.5	220.8	200.0	46.0	39.7
30	96.2	92.4	263.3	232.5[D]	42.4	32.5
	Dry	Flet	Dry	Flet	Dry	Flet
0	-	-	114.4	111.9	-	-
10	98.1	98.1	175.5	180.6	61.1	68.7
20	96.9	97.5	237.8	235.2	62.3	54.6
30	96.3	94.4	298.6	297.3	60.9	62.1

[A] Average of 4 replicates for AB (Above Bullitt), Dry (Dryden), Flet (Fletcher), and 2 replicates for BB (Below Bullitt).
[B] Index = length X height.
[C] Growth = increase in size index.
[D] Significantly less than other sites ($p < .05$).

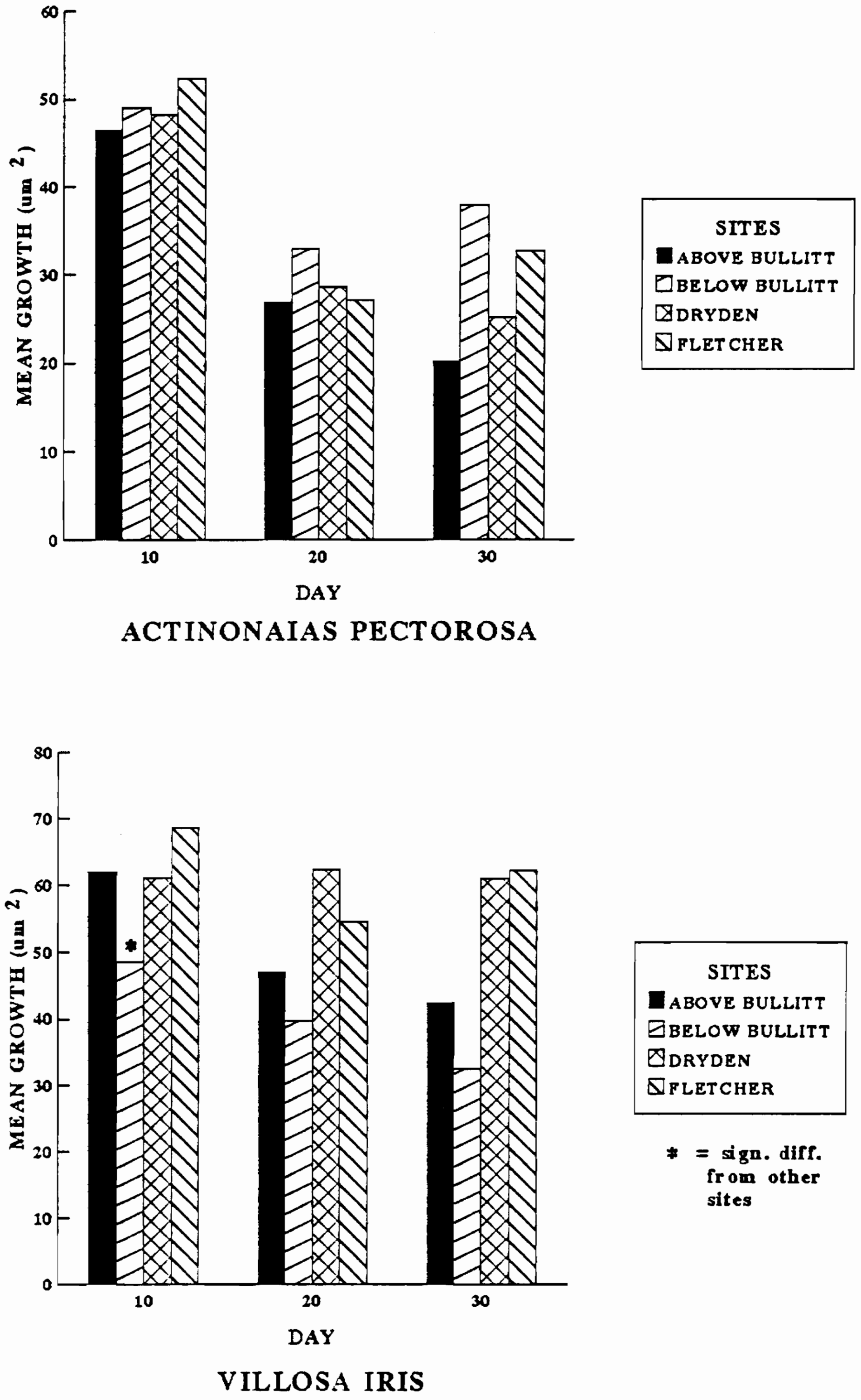


Figure 9. Comparison of 10, 20, and 30-day growth of juveniles of two species of freshwater mussels in sediment from four sites in the Powell River.

Use of the small sediment particle size allowed for easy recovery of juveniles, although some juveniles were lost, possibly in the decanted water or tangled in algal mats and worm tubes. Some transparent mussel shells were recovered during sediment test four. Possibly the shells dissolve rapidly in soft water following mortality of the juvenile mussel. Therefore, it may be necessary to monitor mortality more frequently. Initial seiving of the sediment through a 202 μm screen was necessary to facilitate recovery of juveniles during testing. However, initial seiving of sediment samples to separate particulate fractions required large amounts of water, and it is unknown whether this might have affected any potential toxic properties of the sediment.

There was no evidence of acute sediment toxicity based on results of the preliminary 10-day test (Figure 4). However, evidence of impairment such as reduced growth may require longer exposure. Although juvenile survival was high in both river (with and without sediment) and tap water, growth was noticeably better in the dishes containing sediment. Since tri-algal culture was added as a food source to all dishes, it seems that something (possibly micronutrients) in the sediment was responsible for the increased growth. This also was evident in the second trial, when juveniles in river sediment showed significantly greater growth than juveniles in glass beads (Figure 5). In this case, however, survival also was much lower in the glass beads (Figure 6). Wolcott (unpublished data) also found low survival when testing glass beads as a control substratum for juvenile mussels. In my study, many juveniles were observed on the surface of the glass beads, whereas they were burrowed in the river sediment. Hudsom and Isom (1984) found that addition of silt increased growth and survival of juvenile mussels. Suggested possible mechanisms for silt increasing growth include increased filtration rate, adsorption of organic food onto silt particles, and the use of particles to grind food (Hudsom and Isom 1984). Growth of juveniles held in sediment for 90 days showed an increasing trend; mortality rate stabilized, indicating that chronic or sublethal effects were absent (Figure 5).

Sediments and water collected below the coal processing plant seemed to be acutely toxic to juveniles of *A. pectorosa*, as indicated by the poor survival after 10 days (Figure 7).

However, when dechlorinated tap water was used, survival was high (91 to 96%). Test results suggest that the river water was contributing to toxicity of the sediment. This might have been expected considering the high metal content of the river water collected with the sediment samples; Cu levels were 48.3 and 49.7 $\mu g/L$ above and below the sites, respectively (Table 11). Mortality continued to increased after 20 and 30 days (Figure 7), so that survival was similar among sites and the two water types. Growth of juvenile mussels was also better in tap water; however, juveniles in sediments with tap water and river water showed a continual decline in growth rates throughout the 30-day test (Figure 8). This is in contrast to the growth exhibited by juveniles of *V. iris* in Dryden sediment during trial two. Reduced growth is an indication of an impairment effect of the sediments. In addition, I observed what appeared to be dark 'pits' or eroded areas in the mussel shells. These were most evident in mussel shells tested in sediment using river water, although the pits were observed in all dishes to some extent. These 'pits' were not observed on mussel shells in the previous tests. The toxic component of the sediment may have required sufficient time to affect the juveniles, and this illustrates the need for longer exposure times to evaluate chronic effects on aquatic organisms. Although sediment was collected upstream of the coal processing plant, this location was not unimpacted. The upper part of the Powell River is heavily impacted by surface coal mining and domestic wastes.

The acutely toxic component of the suspended sediment collected during April was not present in the suspended sediment collected during June and July, as reflected by the high survival of both species thoughout the 30-day test. Differences in growth response were apparent for the two species. Juveniles of *V. iris* consistently exhibited the poorest growth in sediment from below the Bullitt plant, whereas those of *A. pectorosa* tended to show the best growth in the same sediment (Figure 9). Growth of juveniles in sediment collected from above the plant was generally low for both species. Growth of *A. pectorosa* was reduced compared to that observed in the same site and water of the previous test, although the difference was only slight in the BB sediment. Also, growth rates for juveniles declined during the test, most noticeably for the upper sites (AB and BB). During the 90-day test with *V. iris* in Dryden sediment, growth rate had exhibited an increasing trend. Hudsom and Isom (1984) had noted that growth of juvenile *Anodonta imbecilis* had shown an increasing trend after an initially slow start.

Dryden sediment was to be used as a reference site since good growth and survival had been demonstrated previously. However, the poor growth and survival of juveniles in Dryden sediment during trial three and reduced growth in trial five indicate the character of the sediment changes as new suspended sediment is deposited.

I am unaware of any other sediment tests conducted with juvenile freshwater mussels; thus, comparative information is lacking. Tests conducted with *Corbicula fluminea* have shown growth to be a sensitive indicator of Zn (Belanger et al. 1986) and Cu (Belanger et al. 1990) pollution. Concentrations as low as 50 μg/L Zn and 16 μg/L Cu resulted in reduced growth in *C. fluminea* from tests conducted in artificial streams and natural river systems. Marine sediment samples were tested with five species of marine organisms including amphipods, a polychaete, sea urchin and a mussel (Long et al. 1990). Embryos of the mussel (*Mytilus edulis*) were highly sensitive to samples contaminated with a wide variety of metals and organic compounds; however, tolerance levels were not identified for individual contaminants.

There have been a number of studies evaluating the effects of metal-contaminated sediments, most commonly with midges, mayflies, amphipods, and daphnids. In a study comparing responses of five taxa to contaminated sediments, the midge (*Chironomus tentans*) was found to be the least sensitive, and *Daphnia magna* the most sensitive (Nebeker et al. 1984b). However, the midge is the most common species used to evaluate effects in sediment tests. Some studies have substituted food particles (Cerophyll) for substratum (Nebeker et al. 1984a, Kosalwat and Knight 1987). There is some question as to how results based on metal concentrations found in the food alone would compare to effects in a natural sediment. Midge larvae avoided metal-contaminated sediment when exposed in the laboratory, and characteristics such as sediment texture and total organic carbon were not significant (Wentzel et al. 1977a). In another set of experiments using the same sediment, Wentzel et al. (1977b) found a correlation between metal concentrations in the sediment and midge larval length. Adult emergence from the midge larvae also was reduced and delayed in the most heavily polluted sediments (Wentzel et al. 1978). Metal concentrations in the sediments exhibiting these effects were quite high, ranging from 213 to 1030 mg/kg Cd, 4385 to 17300 mg/kg Zn, and 799 to 1640 mg/kg Cr. Larval growth of midges was the most sensitive indicator of Cu pollution in food substatum, with reductions found at Cu concentrations of 900 mg/kg dry weight (Kosalwat and Knight 1987). Chronic tests conducted with Cu contaminated sediments (concentrations not reported) resulted in 100% mortality or significantly reduced reproduction by *Daphnia magna* and *Chironomus tentans* (Nebeker et al. 1988). Acute tests conducted with four species of benthic invertebrates in sediments spiked with Cu resulted in LC50 values ranging from 681 (48-hr for *Daphnia magna*) to 2296 mg/kg dry weight (10-d for *Chironomus tentans*) (Cairns et al. 1984).

These values are all much higher than historical concentrations of Cu and Zn found in the Powell River sediments, which ranged from 12 to 33 mg/kg and 52 to 120 mg/kg dry weight, respectively (STORET). Although metal concentrations in sediments were not measured in my study, my results suggest that sediments in the Powell River have the potential to adversely affect juvenile mussels either through direct toxic action or impairment effects such as reduced growth. Additionally, poor water quality may contribute to toxic effects. Juvenile freshwater mussels are among the most sensitive species tested with Cu in water (Cherry et al. 1991). Trace metals such as copper and zinc are transferred from the water column to sediments though sorption on inorganic solids and by living and non-living organic matter (Nienke and Lee 1982, Moore and Ramamoorthy 1984), and the majority of these metals are found in the smaller size fractions of the sediment (O'Donnell et al 1985). Although the bioavailability of metals in sediments is not well understood, it seems probable that juveniles also would be sensitive to metals associated with sediment which they may be ingesting.

The USEPA is evaluating the use of sediment testing in the NPDES permitting process (Burton 1991). However, current regulations regarding water quality in mined land areas in Virginia do not require monitoring for trace metals. Analysis of river sediment and water samples only once per year will not record episodic events such as has been suggested by the results of my study. Such events may release contaminants such as Cu and Zn into the water column and contaminate suspended or deposited sediments to cause significant harm to the river biota. Filter feeding organisms such as mussels are especially vulnerable as they are exposed to contaminants in the water column and adsorbed to suspended particles which they ingest. Control over sedimentation (and associated contaminants) resulting from abandoned mines, currently mined areas, and possibly reclaimed mines will be difficult. Specific sources of the high levels of Cu (and other metals) need to be identified before any plan of action can be recommended. Until this is done, it is doubtful that recovery of mussel populations in the Powell River and other drainages affected by significant mining activity can occur.

Chapter Four - Summary and Recommendations

Glochidia and juvenile freshwater mussels were about equally sensitive to Zn, with 48-hr LC50 values as low as 274 μg Zn/L in soft water. Glochidia appeared to become "sensitized" to Zn exposure at concentrations at or below estimated LC50 values. This was evidenced by valve closure in response to slight tactile stimulation or vibration, and was not observed in control organisms. Concentrations as low as 158 μg Zn/L (in tap water) and 288 μg Zn/L (in Powell River water) affected 50% of the juveniles after 96 hours, as evidenced by a 'gaping' response (open valves with a swollen and nonresponsive extended foot).

Copper was 5.7 to 15.7 times more toxic than Zn, with concentrations as low as 52 to 76 μg Cu/L causing 50% mortality of juveniles exposed for 48 hours. Juvenile mussels exposed to 39 μg Cu/L in Powell River water exhibited reduced siphoning activity. There seemed to be a different toxic mode of action for the two metals. Juvenile mussels responded to Cu by closed valves and reduced siphoning (personal observation, Jacobson 1990), whereas Zn affected juveniles by causing gaped valves and a swollen and extended foot. In general, sensitivities of freshwater mussels to Zn and Cu increased with higher temperature, soft water, and time of exposure. Depending on water hardness, certain levels of Zn reduced the acute (lethal) toxicity of Cu to juvenile mussels. However, sublethal effects were not reduced, and variable field conditions, including the presence of other metals, may change the amelioration of the acute toxicity of Cu.

There are few comparable studies testing sensitivities of glochidia and juvenile freshwater mussels to metals. Glochidia and juveniles of the species tested in my study exhibited about the same sensitivity as those ranked as the six most sensitive species used by the USEPA to determine Zn water quality criteria (Table 20). The sensitivity of *A. pectorosa* to Cu is also comparable to the most sensitive species tested (Table 21). Differences in values between studies are attributed to source of dilution water, species, and duration of exposure. The wide variation in responses among mussel species suggests other mussels native to the Powell River may be more sensitive to Zn, Cu and other contaminants. Endangered and threatened species are likely to have the greatest sensitivity to contaminants, while being the least available for testing. Thus water quality criteria may fail to take into account the most sensitive species. The current USEPA water quality criteria for Zn (adjusted for Powell River water hardness) are 174 μg/L (acute) and 158 μg/L (chronic). Results from my study indicate the acute value may be protective of mussel glochidia, although results of another study indicate at least one species of mussel exhibited acute sensitivity near the criterion (Table 21). Chronic (sublethal) effects of Zn exposure have not been determined for glochidia or juvenile mussels, and thus the adequacy of the chronic criterion cannot be evaluated. The current USEPA water quality criteria for Cu (adjusted for Powell River water hardness) are 28 μg/L (acute) and 18 μg/L (chronic). My results and those of recent studies (Jacobson 1990, Cherry et al. 1991, Farris et al. 1991) suggest these criteria may not be protective of young freshwater mussels.

Water samples collected from the Powell River and analyzed for various metals, including Zn, Cu, and Al, displayed a wide range in ambient metal concentrations. Copper concentrations occurred at levels found to adversely affect glochidia and juvenile mussels, and both Cu and Zn were detected at concentrations which exceeded USEPA water quality criteria. This is especially significant in light of the fact these levels occurred at a time when many mussels are known to release glochidia (Neves and Widlak 1988, personal observations).

Table 20. A comparison of species most sensitive to acute zinc exposure.

Species	Life Stage	Value (μg/L)	Conditions	Reference
Ceriodaphnia reticulata	-	50.7	SMAV (Species Mean Acute Value) adjusted to hardness of 50 mg/L	USEPA 1987
Morone saxatilis (striped bass)	63 days	119.4		
Ceriodaphnia dubia	-	174.1		
Agosla chrysogasta (longfin dace)	-	227.8		
Daphnia pulex	-	252.9		
Daphnia magna	-	355.5		
Ceriodaphnia dubia	neonates	125	48-hr LC50 180 mg/L hardness	Cherry et al. 1991
Ptychobranchus fasciolaris	glochidia	212	48-hr LC50 20°C 170 mg/L hardness	
Actinonaias pectorosa	glochidia	309		
Medionidus conradicus	glochidia	570		
Villosa nebulosa (iris)	glochidia	656		
Isonychia bicolor mayfly	-	425	96-hr LC50 170 mg/L hardness	
Medionidus conradicus	glochidia	492	60 mg/L hardness	present study (all tests 48-hr LC50 and 20°C)
Actinonaias pectorosa	glochidia	274	50 mg/L	
A. pectorosa	glochidia	664-739	160 mg/L	
Villosa iris	glochidia	577-1155	40-50 mg/L	
V. iris	glochidia	836-1230	140-160 mg/L	
A. pectorosa	juvenile	360-370	40 mg/L	
A. pectorosa	juvenile	1060-1186	160 mg/L	
V. iris	juvenile	339	50 mg/L	
V. iris	juvenile	1122	160 mg/L	

Table 21. A comparison of species most sensitive to acute copper exposure.

Species	Life Stage	Value (μg/L)	Conditions	Reference
Daphnia pulicaria	-	9.3	SMAV	USEPA 1985
Ptychochellus oregonensis	-	16.7	Species Mean Acute Value)	
Ceriodaphnia reticulata	-	18.8	adjusted to	
Daphnia magna	-	21.2	hardness of	
Gammarus pseudolimnaeus	-	22.1	50 mg/L	
Medionidus conradicus	glochidia	16	48-hr LC50	Cherry et al. 1991
Ptychobranchus fasciolaris	glochidia	17	162 mg/L hardness 20°C	
Actinonaias pectorosa	glochidia	30		
Lampsilis fasciola	glochidia	30		
Villosa nebulosa (iris)	glochidia	37		
Ceriodaphnia dubia	neonates	88		
Isonychia bicolor mayfly	-	52	96-hr LC50 170 mg/L hardness	
Medionidus conradicus	glochidia	16	170 mg/L hardness	Jacobson 1990
Lampsilis fasciola	glochidia	40		(all tests 48-hr LC50
A. pectorosa	glochidia	51		and 20°C)
Villosa nebulosa (iris)	glochidia	46-66	150-170 mg/L	
A. pectorosa	juvenile	52-63	48-hr LC50	present study
A. pectorosa	juvenile	26-42	48-hr EC50 40-50 mg/L hardness	
A. pectorosa	juvenile	76-156	48-hr LC50	
A. pectorosa	juvenile	38-115	48-hr EC50 140-160 mg/L hardness	

Results of regression analysis indicate a significant correlation between river flow and Al concentration at all sites sampled, with the exception of below Bullitt (Table 22). Zinc was positively correlated with river flow at two sites sampled, above Bullitt and Fletcher Ford. Positive correlation with river flow suggests these metals may be associated with increased runoff (nonpoint source), at least at certain sites. However, Cu concentrations were not correlated with river flow at any site sampled, suggesting it is entering the river system as an episodic event (point source) and is not associated with runoff.

Glass beads were found to be unsuitable as a control substratum for use in sediment testing with juvenile mussels, whereas I was able to maintain apparently healthy juveniles in collected river sediment for at least 90 days (test termination). Juvenile mussels displayed significantly greater growth in river sediment as compared to water only. There was no clear trend of decreased acute toxicity of Powell River sediment to juvenile freshwater mussels as distance from the mining activities increased. The only evidence of acute sediment toxicity was observed in sediments collected near a coal processing plant in Appalachia, Virginia. High metal concentrations in the river water appeared to contribute to toxicity. This toxicity was not apparent in sediments collected from the same sites less than two months later, indicating the character of the sediment changes as new suspended sediment is deposited. Growth responses differed between the two species used in the sediment tests. Juveniles of *V. iris* consistently exhibited the poorest growth in sediment from below the Bullitt plant, whereas those of *A. pectorosa* tended to show the best growth in the same sediment. Growth rates for both species declined during the test, most noticeable for the upper sites (nearest the coal mining activities), whereas growth rates for *V. iris* showed an increasing trend during a 90-d test in Dryden sediment. The USEPA is currently evaluating the use of sediment testing in the NPDES permitting process (Burton 1991). Considering their high sensitivity, and to ensure their protection, it is recommended that sediment toxicity evaluations include juvenile bivalves.

The Division of Mined Land Reclamation has regulatory responsibility for water quality in areas of coal mining activities in Virginia. However, only pH, Fe, Mn, and total suspended

Table 22. Correlation between river flow rates and metal concentrations at four sites in the Powell River, Virginia.

Site	Cu p-value	Cu R^2	Zn p-value	Zn R^2	Al p-value	Al R^2
Above Bullitt	0.678	0.05	<0.001[A]	0.96	0.007[A]	0.87
Below Bullitt	0.492	0.12	0.552	0.10	0.288	0.27
Dryden	0.463	0.14	0.683	0.04	0.001[A]	0.91
Fletcher Ford	0.610	0.10	0.018[A]	0.88	0.006[A]	0.94

[A] Significantly ($p<0.05$) correlated with river flow.

solids are monitored. More intensive monitoring is necessary to identify specific sources responsible for high metal concentrations in the Powell River. In an intensive sampling program conducted in the upper Cumberland River basin, Layzer and Anderson (1992) concluded "there is a clear relationship between the presence of strip mines, higher concentrations of metals downstream, and the extirpation of mussels." It is likely that more than nine species of freshwater mussels have been extirpated from the Powell River. There are currently seven species of freshwater mussels native to the Powell River on the federally endangered list and an additional twelve species listed as state endangered or protected. The Virginia State Water Control Board recently set a site-specific water quality criterion for Cu in the Clinch River at 19.5 μg/L (acute) and 12.4 μg/L (chronic). This new criterion was developed from research which determined that freshwater mussels were especially susceptible to Cu (Cherry et al. 1991). The species used in toxicity testing (acute and chronic) are also native to the Powell River. Further, water hardness, an important factor in developing site-specific criteria for the Clinch River, is comparable to the Powell River. It seems logical therefore, to apply the same Cu criteria to the Powell River because of similar water quality traits and the presence of a similar mussel fauna.

There may be other factors contributing to the decline of freshwater mussels in the Powell River. Lack of prey organisms, specifically crayfish, are believed responsible for low abundance of fish species in the Powell River (Alan Temple, personal communication, Fisheries Academy, Kearneysville, West Virginia). High siltation due to surface mining and nonpoint runoff is believed to degrade crayfish habitat. The reduced abundance of potential fish hosts for mussel glochidia also could be a factor in the apparent lack of recruitment in mussel populations of the lower Powell River. Increased sedimentation, which has been linked with reduced fish populations, was reported even in streams draining reclaimed surface and strip coal mines (Matter and Ney 1981). Gas and oil exploration may also be contributing contaminants to the Powell River. Raw sewage is commonly dumped into the river, especially near the headwaters (personal observation). Municipal wastes from the Pennington Gap Sewage Treatment Plant are not in compliance with state and federal water quality standards and pollute the North Fork Powell. Perturbations due to agricultural practices include habitat degradation from animals invading stream areas containing mussel beds, increased siltation, and chemical pollution from spills and runoff. It may be a combination of several factors causing a decline in mussel populations. Thus the entire watershed needs to be evaluated for potential impacts on aquatic organisms.

If it is established that coal mining activities or runoff from mined lands are responsible for metal concentrations exceeding USEPA criteria (and concentrations shown to have adverse effects on mussel populations), more emphasis needs to be placed on reclamation efforts and better enforcement of water quality regulations in mined areas. If high metal concentrations continue in the Powell River, the decline in mussel populations of the Powell River will continue. Unless water quality problems are identified and corrected, federally endangered and state protected species will likely be extirpated from the Powell River in Virginia.

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Appendix A. Raw Data for Acute Toxicity Tests

APPENDIX A. RAW DATA FOR ACUTE TOXICITY TESTS.

Glochidia tests with zinc. An asterisk signifies the data were used to determine the LC50 values.

Test Description	Target Value (µg/L)	Actual Value (µg/L)	# Exposed	# Affected	% Response	LC50 Value (µg Zn/L) (95% confidence limits) Spearman-Karber
Actinonaias pectorosa	0	34.1	435	5	1.1	
24-hr; 12°C	100	147.9	427	8	1.9	
tap water	200	246.4	455	12	2.6	
4/19/91	* 400	500.8	413	27	6.5	
	* 800	986.0	475	429	90.3	718.7
	* 1600	1989.0	484	484	100.0	(701.3 - 736.5)
A. pectorosa	0	33.8	644	6	0.9	
48-hr; 12°C	* 100	150.4	572	13	2.3	
tap water	* 200	246.4	544	12	2.2	
4/19/91	* 400	509.2	491	196	39.9	
	* 800	991.0	491	491	100.0	525.0
	1600	1967.0	430	430	100.0	(508.6 - 541.8)
Villosa iris	0	32.6	347	1	0.3	
24-hr; 12°C	100	153.0	401	9	2.2	
tap water	* 200	249.6	288	1	0.3	
3/09/91	* 400	519.5	341	11	3.2	
	* 800	973.3	390	37	9.5	
	* 1600	1968.0	285	118	41.4	
	* 3200	3912.5	314	299	95.2	1968.7
	* 6400	7602.5	351	351	100.0	(1874.5 - 2067.6)
V. iris	0	38.2	568	8	1.4	
48-hr; 12°C	100	161.6	498	14	2.8	
tap water	* 200	250.2	464	6	1.3	
3/09/91	* 400	521.9	420	17	4.0	
	* 800	991.2	370	86	23.2	
	* 1600	1997.5	335	330	98.5	
	* 3200	3972.0	356	356	100.0	1172.5
	6400	7772.5	372	372	100.0	(1133.6 - 1212.7)

V. iris		0	45.4	375	14	3.7	
24-hr; 12°C	*	200	292.0	418	12	2.9	
tap water	*	400	529.3	363	23	6.3	
3/14/91	*	800	1049.0	388	77	19.8	
	*	1600	2023.0	343	135	39.4	
	*	3200	3961.0	456	428	93.8	1875.1
	*	6400	7502.0	455	455	100.0	(1784.6 - 1970.3)
V. iris		0	45.4	446	24	4.9	
48-hr; 12°C		200	278.3	459	17	3.7	
tap water	*	400	539.6	417	21	5.0	
3/14/91	*	800	1049.0	444	118	26.6	
	*	1600	1979.0	438	422	96.3	
	*	3200	3946.0	373	373	100.0	1202.0
		6400	7509.0	436	436	100.0	(1163.9 - 1241.4)
Medionidus conradicus		0	27.0	236	22	9.3	
48-hr; 12°C	*	100	139.3	378	34	9.0	
tap water	*	200	265.0	360	51	14.2	
3/05/91	*	400	535.5	263	40	15.2	
	*	800	1001.6	334	183	54.8	
	*	1600	1958.5	304	297	97.7	774.1
	*	3200	3962.0	284	284	100.0	(732.2 - 818.4)
A. pectorosa		0	23.2	414	10	2.4	
24-hr; 20°C		50	90.4	405	14	3.4	
tap water		100	123.7	507	38	7.5	
4/16/91	*	200	226.8	470	22	4.7	
	*	400	471.1	423	299	70.7	390.0
	*	800	951.3	379	379	100.0	(377.0 - 403.5)
A. pectorosa		0	23.1	479	31	6.5	
48-hr; 20°C	*	50	83.8	317	17	5.4	
tap water	*	100	123.2	371	36	9.7	
4/16/91	*	200	225.5	377	46	12.2	
	*	400	469.5	432	404	93.5	293.4
	*	800	954.0	481	481	100.0	(283.8 - 303.2)

V. iris	0	14.7	361	6	1.7	
24-hr; 20°C	100	127.6	456	21	4.6	
tap water	200	250.7	465	22	4.7	
6/30/91	* 400	510.4	487	17	3.5	
	* 800	1015.5	437	77	17.6	
	* 1600	1991.5	352	205	58.2	
	* 3200	4032.5	375	370	98.7	1658.2
	* 6400	7763.5	294	294	100.0	(1585.0 - 1734.9)
V. iris	0	19.0	447	14	3.1	
48-hr; 20°C	100	128.4	473	25	5.3	
tap water	200	249.7	494	23	4.6	
6/30/91	* 400	504.7	373	21	5.6	
	* 800	1012.0	438	148	33.8	
	* 1600	1985.0	378	340	89.9	
	* 3200	3983.5	358	358	100.0	1159.2
	6400	7774.0	381	381	100.0	(1113.6 - 1206.7)
V. iris	0	20.1	446	12	2.7	
24-hr; 20°C	* 200	267.7	414	18	4.3	
tap water	* 400	508.6	406	63	15.5	
7/10/91	* 800	1042.0	377	287	76.1	750.4
	* 1600	2004.5	357	357	100.0	(720.9 - 781.1)
V. iris	0	18.7	469	21	4.5	
48-hr; 20°C	50	89.2	381	16	4.2	
tap water	100	141.6	369	18	4.9	
7/10/91	* 200	273.9	310	19	6.1	
	* 400	508.2	312	103	33.0	
	* 800	1037.0	272	254	93.4	586.9
	* 1600	1984.5	327	327	100.0	(562.0 - 613.0)
M. conradicus	0	21.8	428	24	5.6	
48-hr; 20°C	100	139.0	334	19	5.7	
tap water	* 200	253.9	290	18	6.2	
7/04/91	* 400	509.0	271	130	48.0	491.8
	* 800	996.7	330	330	100.0	(470.1 - 514.4)

A. pectorosa	0	45.4	705	10	1.4	
24-hr; 12°C	* 250	311.6	525	7	1.3	
river water	* 500	629.2	537	17	3.2	
3/18/91	* 1000	1242.5	417	36	8.6	
	* 2000	2372.0	568	356	62.7	
	* 4000	4632.0	397	397	100.0	
	8000	-	553	549	99.3	2009.4
	16000	-	582	568	97.6	(1942.2 - 2078.9)
A. pectorosa	0	34.9	565	12	2.1	
48-hr; 12°C	* 250	307.0	560	22	3.9	
river water	* 500	627.0	522	26	5.0	
3/18/91	* 1000	1219.5	622	321	51.6	
	* 2000	2370.5	458	458	100.0	
	4000	4678.0	666	594	89.2	
	8000	-	683	628	91.9	1134.5
	16000	-	673	661	98.2	(1099.6 - 1170.4)
A. pectorosa	0	18.6	522	4	0.8	
48-hr; 12°C	140	147.7	511	6	1.2	
river water	280	313.4	418	17	4.1	
4/19/91	* 560	625.8	464	21	4.5	
	* 1120	1297.5	349	263	75.4	1041.3
	* 2240	2636.0	420	420	100.0	(1005.2 - 1078.7)
A. pectorosa	0	10.5	508	2	0.4	
24-hr; 12°C	* 250	259.3	400	4	1.0	
river water	* 500	558.5	379	16	4.2	
4/23/91	* 1000	1114.5	468	28	6.0	
	* 2000	2304.0	367	165	45.0	
	* 4000	6430.5	374	272	72.7	2881.4
	* 8000	8817.5	378	378	100.0	(2718.4 - 3054.3)

V. iris	0	14.6	425	6	1.4	
24-hr; 12°C	100	125.8	417	7	1.7	
river water	200	260.8	321	6	1.9	
3/09/91	* 400	482.8	340	4	1.2	
	* 800	947.2	310	25	8.1	
	* 1600	1921.0	267	50	18.7	
	* 3200	3608.0	407	199	48.9	3073.4
	* 6400	7363.5	282	282	100.0	(2922.1 - 3232.4)
V. iris	0	18.4	423	8	1.9	
48-hr; 12°C	100	130.4	494	4	0.8	
river water	200	259.6	413	4	1.0	
3/09/91	* 400	502.6	382	6	1.6	
	* 800	963.4	340	32	9.4	
	* 1600	1964.5	343	241	70.3	
	* 3200	3581.0	403	366	90.8	1648.6
	* 6400	7217.0	390	390	100.0	(1579.1 - 1721.3)
V. iris	0	21.8	551	16	2.9	
48-hr; 12°C	* 250	298.9	487	26	5.3	
river water	* 500	621.6	427	40	9.4	
3/14/91	* 1000	1217.5	424	94	22.2	
	* 2000	2320.5	355	286	80.6	
	* 4000	4524.5	477	363	76.1	1740.6
	* 8000	8535.0	424	424	100.0	(1654.1 - 1831.6)
V. iris	0	22.0	276	7	2.5	
24-hr; 12°C	* 250	267.4	174	3	1.7	
river water	* 500	562.8	179	5	2.8	
4/23/91	* 1000	1124.0	367	33	9.0	
	* 2000	2292.2	136	17	12.5	
	* 4000	4079.5	196	56	28.6	4122.6
	* 8000	8640.0	254	254	100.0	(3871.4 - 4390.1)
A. pectorosa	0	12.2	547	6	1.1	
48-hr; 20°C	* 70	78.6	560	5	0.9	
river water	* 140	147.0	448	5	1.1	
4/16/91	* 280	300.2	413	1	0.2	
	* 560	620.1	497	186	37.4	664.5
	* 1120	1251.5	580	580	100.0	(643.8 - 685.9)

A. pectorosa	0	14.9	396	2	0.5	
24-hr; 20°C	200	236.0	438	1	0.2	
river water	* 400	468.5	380	11	2.9	
4/25/91	* 800	910.9	458	86	18.8	
	* 1600	1883.0	433	408	94.2	
	* 3200	3373.5	450	450	100.0	1172.2
	6400	6457.5	446	446	100.0	(1136.8 - 1208.7)
A. pectorosa	0	16.8	649	20	3.1	
48-hr; 20°C	200	235.0	479	7	1.5	
river water	* 400	474.0	441	14	3.2	
4/25/91	* 800	917.2	533	435	81.6	
	* 1600	1854.5	371	371	100.0	
	3200	3321.0	590	590	100.0	732.0
	6400	6367.5	409	409	100.0	(713.9 - 750.4)
V. iris	0	19.2	234	0	0.0	
24-hr; 20°C	200	237.4	306	4	1.3	
river water	* 400	469.9	243	4	1.6	
4/25/91	* 800	908.6	266	10	3.8	
	* 1600	1828.5	226	121	53.5	
	* 3200	3405.5	258	241	93.4	1761.7
	* 6400	6470.5	289	289	100.0	(1674.7 - 1853.3)
V. iris	0	19.5	221	4	1.8	
48-hr; 20°C	* 200	236.2	295	6	2.0	
river water	* 400	460.0	229	11	4.8	
4/25/91	* 800	910.3	301	22	7.3	
	* 1600	1847.0	273	255	93.4	
	* 3200	3416.0	224	224	100.0	1230.0
	6400	6212.0	194	194	100.0	(1187.1 - 1274.4)

V. iris	0	22.9	437	22	5.0	
24-hr; 20°C	100	148.6	375	12	3.2	
river water	* 200	229.2	276	11	4.0	
6/30/91	* 400	461.0	283	17	6.0	
	* 800	913.4	351	163	46.4	
	* 1600	1632.0	352	279	79.3	
	* 3200	2957.5	448	415	92.6	1008.7
	* 6400	5975.0	366	366	100.0	(959.1 - 1060.9)
V. iris	0	12.8	554	20	3.6	
48-hr; 20°C	100	115.9	588	24	4.1	
river water	* 200	224.6	548	20	3.6	
6/30/91	* 400	462.0	420	28	6.7	
	* 800	917.2	501	253	50.5	
	* 1600	1670.0	424	406	95.8	
	* 3200	2838.5	439	439	100.0	854.7
	6400	5976.0	402	402	100.0	(824.5 - 885.9)
V. iris	0	10.0	505	14	2.8	
24-hr; 20°C	100	113.4	366	6	1.6	
river water	* 200	219.1	383	9	2.3	
7/10/91	* 400	450.2	378	23	6.1	
	* 800	895.2	298	31	10.4	
	* 1600	1772.0	373	243	65.1	
	* 3200	2860.0	316	304	96.2	1386.9
	* 6400	5678.0	309	309	100.0	(1328.0 - 1448.3)
V. iris	0	12.4	430	21	4.9	
48-hr; 20°C	100	114.1	378	30	7.9	
river water	* 200	226.2	356	24	6.7	
7/10/91	* 400	446.5	334	40	12.0	
	* 800	897.1	333	65	19.5	
	* 1600	1743.5	444	387	87.2	1042.2
	* 3200	2706.0	355	355	100.0	(997.4 - 1089.0)
M. conradicus	0	15.2	645	31	4.8	
24-hr; 20°C	* 100	122.8	469	35	7.5	
river water	* 200	224.5	568	46	8.1	
6/30/91	* 400	455.9	485	227	46.8	
	* 800	907.4	486	461	94.8	

	* 1600	1644.0	576	576	100.0	
	3200	2931.0	483	461	95.4	434.7
	6400	5585.0	474	474	100.0	(418.0 - 452.2)
M. conradicus	0	13.2	178	13	7.3	
24-hr; 20°C	* 100	117.2	124	10	8.1	
river water	* 200	222.0	130	13	10.0	
7/10/91	* 400	447.5	126	38	30.2	
	* 800	900.7	135	97	71.8	
	* 1600	1855.0	128	124	96.9	571.6
	* 3200	3081.5	128	128	100.0	(521.5 - 626.5)

Juvenile tests with zinc. An asterisk signifies the data were used to determine the LC50 or EC50 value.

Test Description	Target Value (µg/L)	Actual Value (µg/L)	# Exposed	% Affected	% Dead	Spearman-Karber Value (95% confidence limits) (µg Zn/L)
A. pectorosa	0	16.8	60	0.0	0.0	413.8 LC50
48-hr; 12°C	* 100	136.9	60	5.0	0.0	(376.2 - 455.2)
tap water	* 200	241.3	60	53.0	8.3	
5/10/91	* 400	486.0	60	98.3	65.0	240.2 EC50
	* 800	962.8	60	100.0	100.0	(219.6 - 262.7)
A. pectorosa	0	29.8	60	1.7	0.0	290.3 LC50
96-hr; 12°C	* 100	158.6	60	20.0	1.7	(270.5 - 311.5)
tap water	* 200	251.6	60	96.7	33.3	185.6 EC50
5/10/91	* 400	498.0	60	100.0	100.0	(175.9 - 195.9)
A. pectorosa	0	24.6	60	8.3	8.3	362.6 LC50
48-hr; 20°C	50	74.4	60	10.0	10.0	(331.8 - 396.2)
tap water	* 100	125.6	60	13.3	13.3	
4/30/91	* 200	250.0	60	36.7	13.3	
	* 400	477.2	60	100.0	80.0	
	* 800	962.5	60	100.0	100.0	247.4 EC50
	1600	1930.0	60	100.0	100.0	(223.8 - 273.5)
A. pectorosa	0	25.4	60	8.3	8.3	211.3 LC50
96-hr; 20°C	* 50	77.0	60	10.0	10.0	(189.1 - 236.0)
tap water	* 100	125.3	60	18.3	18.3	
4/30/91	* 200	254.2	60	90.0	51.7	
	* 400	484.6	60	100.0	100.0	
	800	969.8	60	100.0	100.0	163.0 EC50
	1600	1937.5	60	100.0	100.0	(149.4 - 177.8)
A. pectorosa	0	15.8	60	3.3	3.3	367.4 LC50
48-hr; 20°C	50	70.3	60	6.7	6.7	(339.3 - 397.9)
tap water	* 100	137.8	60	13.3	10.0	
5/10/91	* 200	238.4	60	68.3	6.7	
	* 400	492.8	60	100.0	83.3	206.1 EC50
	* 800	958.8	60	100.0	100.0	(188.5 - 225.4)

V. iris	0	17.9	60	3.3	3.3	
48-hr; 20°C	25	47.1	58	5.2	5.2	
tap water	50	72.5	60	6.7	6.7	
12/07/90	* 100	123.8	60	5.2	5.2	
	* 200	257.9	60	56.7	1.7	228.4 EC50
	* 400	472.6	60	100.0	91.7	(207.8 - 251.1)
V. iris	0	17.9	60	8.3	8.3	236.2 LC50
96-hr; 20°C	25	47.1	58	22.4	20.7	(211.8 - 263.4)
tap water	50	72.5	60	18.3	18.3	
12/07/90	* 100	123.8	60	23.3	23.3	
	* 200	257.9	60	53.3	35.0	208.9 EC50
	* 400	472.6	60	100.0	100.0	(186.8 - 233.6)
V. iris	0	24.0	60	1.7	1.7	
48-hr; 20°C	25	67.6	60	0.0	0.0	
tap water	50	81.5	60	1.7	0.0	
2/19/91	* 100	149.2	60	0.0	0.0	
	* 200	284.9	60	43.3	1.7	298.3 EC50
	* 400	549.3	60	100.0	76.7	(274.7 - 323.9)
V. iris	0	24.0	60	5.0	5.0	358.8 LC50
96-hr; 20°C	25	67.6	60	1.7	0.0	(334.2 - 385.2)
tap water	50	81.5	60	5.0	5.0	
2/19/91	* 100	149.2	60	0.0	0.0	
	* 200	284.9	60	45.0	18.3	295.0 EC50
	* 400	549.3	60	100.0	96.7	(271.6 - 320.5)
V. iris	0	24.0	60	0.0	0.0	
48-hr; 20°C	25	67.6	60	3.3	3.3	
tap water	50	81.5	60	0.0	0.0	
2/19/91	* 100	149.2	60	3.3	3.3	
	* 200	284.9	60	48.3	3.3	282.5 EC50
	* 400	549.3	60	100.0	76.7	(258.7 - 308.6)

V. iris	0	24.0	60	3.3	3.3	325.4 LC50
96-hr; 20°C	25	67.6	60	5.0	5.0	(300.2 - 352.7)
tap water	50	81.5	60	1.7	1.7	
2/19/91	* 100	149.2	60	5.0	5.0	
	* 200	284.9	60	63.3	25.0	253.5 EC50
	* 400	549.3	60	100.0	100.0	(232.2 - 276.8)
V. iris	0	5.6	48	0.0	0.0	339.3 LC50
48-hr; 20°C	* 100	101.4	48	0.0	0.0	(316.1 - 364.2)
tap water	* 200	219.7	48	12.5	0.0	
8/02/91	* 400	427.1	48	100.0	85.4	280.0 EC50
	* 800	893.6	48	100.0	100.0	(261.6 - 299.7)
A. pectorosa	0	9.8	60	6.7	6.7	1885.0 LC50
48-hr; 12°C	* 400	432.4	60	1.7	1.7	(1704.7-2084.4)
river water	* 800	872.0	60	23.3	15.0	
5/06/91	* 1600	1838.5	60	78.3	23.3	
	* 3200	3362.5	60	100.0	100.0	
	6400	-	60	100.0	100.0	1224.4 EC50
	12800	-	60	100.0	100.0	(1099.5 - 1363.6)
V. iris	0	4.0	60	0.0	0.0	856.4 LC50
96-hr; 12°C	* 70	80.0	60	0.0	0.0	(829.2 - 884.3)
river water	* 140	148.6	60	0.0	0.0	
2/14/91	* 280	307.1	60	0.0	0.0	
	* 560	614.3	60	100.0	3.3	434.3 EC50
	* 1120	1251.0	60	100.0	100.0	(434.3 - 434.3)
V. iris	0	4.0	60	0.0	0.0	
48-hr; 12°C	70	80.0	60	0.0	0.0	
river water	140	148.6	60	1.7	1.7	
2/14/91	* 280	307.1	60	0.0	0.0	
	* 560	614.3	60	23.3	0.0	744.1 EC50
	* 1120	1251.0	60	100.0	6.7	(698.8 - 802.7)

V. iris	0	4.0	60	0.0	0.0	866.9 LC50
96-hr; 12°C	* 70	80.0	60	0.0	0.0	(848.0 - 886.1)
river water	* 140	148.6	60	1.7	1.7	
2/14/91	* 280	307.1	60	0.0	0.0	
	* 560	614.3	60	88.3	0.0	471.4 EC50
	* 1120	1251.0	60	100.0	100.0	(445.1 - 499.3)
V. iris	0	4.0	60	0.0	0.0	
48-hr; 12°C	* 70	80.0	60	0.0	0.0	
river water	* 140	148.6	60	0.0	0.0	
2/15/91	* 280	307.1	60	1.7	1.7	
	* 560	614.3	60	33.3	0.0	685.5 EC50
	* 1120	1251.0	60	100.0	1.7	(628.0 - 748.3)
V. iris	0	4.0	60	0.0	0.0	861.2 LC50
96-hr; 12°C	70	80.0	60	0.0	0.0	(831.8 - 891.7)
river water	* 140	148.6	60	1.7	0.0	
2/15/91	280	307.1	60	1.7	1.7	
	* 560	614.3	60	86.7	1.7	471.5 EC50
	* 1120	1251.0	60	100.0	100.0	(441.8 - 503.2)
V. iris	0	4.0	60	0.0	0.0	
48-hr; 12°C	* 70	80.0	60	0.0	0.0	
river water	* 140	148.6	60	0.0	0.0	
2/15/91	* 280	307.1	60	0.0	0.0	
	* 560	614.3	60	8.3	0.0	826.8 EC50
	* 1120	1251.0	60	100.0	5.0	(786.8 - 868.8)
V. iris	0	4.0	60	0.0	0.0	876.6 LC50
96-hr; 12°C	* 70	80.0	60	0.0	0.0	(876.6 - 876.6)
river water	* 140	148.6	60	0.0	0.0	
2/15/91	* 280	307.1	60	1.7	0.0	
	* 560	614.3	60	71.7	0.0	523.7 EC50
	* 1120	1251.0	60	100.0	100.0	(481.5 - 569.6)

A. pectorosa	0	5.9	60	5.0	5.0	1081.6 LC50
48-hr; 20°C	* 100	83.8	60	6.7	6.7	(1016.0 - 1151.4)
river water	* 200	186.1	60	10.0	10.0	
4/30/91	* 400	379.6	60	23.3	20.0	
	* 800	819.4	60	35.0	13.3	
	* 1600	1622.0	60	100.0	96.7	663.8 EC50
	* 3200	2936.0	60	100.0	100.0	(576.7 - 764.0)
A. pectorosa	0	5.9	60	6.7	6.7	427.6 LC50
96-hr; 20°C	* 100	82.8	60	13.3	11.7	(368.0 - 496.9)
river water	* 200	184.9	60	20.0	20.0	
4/30/91	* 400	374.6	60	26.7	25.0	
	* 800	829.4	60	93.3	78.3	
	* 1600	1643.0	60	100.0	100.0	
	3200	2936.0	60	100.0	100.0	
A. pectorosa	0	9.8	60	6.7	5.0	1205.4 LC50
48-hr; 20°C	* 200	212.0	60	6.7	3.3	(1135.7 - 1279.4)
river water	* 400	428.0	60	23.3	18.3	
5/06/91	* 800	855.2	60	55.0	11.7	
	* 1600	1799.5	60	100.0	93.3	
	* 3200	2697.5	60	100.0	100.0	678.0 EC504
	6400	-	60	100.0	100.0	(597.6 - 769.2)
A. pectorosa	0	5.5	60	5.0	5.0	1181.7 LC50
48-hr; 20°C	50	81.0	60	1.7	1.7	(1091.3 - 1279.7)
river water	100	116.4	60	1.7	1.7	
5/21/91	* 200	247.2	60	0.0	0.0	
	* 400	501.8	60	16.7	5.0	
	* 800	1006.2	60	80.0	20.0	
	* 1600	1954.0	60	100.0	100.0	724.2 EC50
	3200	-	60	100.0	100.0	(657.7 - 797.4)
V. iris	0	4.1	60	0.0	0.0	
48-hr; 20°C	50	58.6	60	1.7	1.7	
river water	100	107.5	60	0.0	0.0	
2/03/91	* 200	229.5	60	0.0	0.0	
	* 400	445.5	60	13.3	3.3	571.9 EC50
	* 800	877.9	60	100.0	58.3	(539.6 - 606.1)

V. iris	0	4.1	60	3.3	3.3	577.5 LC50
96-hr; 20°C	50	58.6	60	3.3	3.3	(546.2 - 610.7)
river water	100	107.5	60	1.7	1.7	
2/03/91	* 200	229.5	60	0.0	0.0	
	* 400	445.5	59	28.8	11.9	515.5 EC50
	* 800	877.9	60	100.0	100.0	(476.7 - 557.4)
V. iris	0	4.1	60	5.0	3.3	
48-hr; 20°C	* 50	58.6	60	0.0	0.0	
river water	* 100	107.5	60	3.3	3.3	
2/06/91	* 200	229.5	60	8.3	1.7	
	* 400	445.5	60	23.3	8.3	498.3 EC50
	* 800	877.9	60	98.3	56.7	(452.6 - 548.5)
V. iris	0	4.1	60	8.3	8.3	418.0 LC50
96-hr; 20°C	50	58.6	60	5.0	3.3	(371.8 - 470.0)
river water	* 100	107.5	59	6.8	6.8	
2/06/91	* 200	229.5	60	26.7	20.0	
	* 400	445.5	60	78.3	31.7	291.5 EC50
	* 800	877.9	60	100.0	100.0	(259.5 - 327.5)
V. iris	0	4.0	60	0.0	0.0	
48-hr; 20°C	* 50	54.2	60	0.0	0.0	
river water	* 100	107.8	60	3.3	1.7	
2/09/91	* 200	216.6	60	8.3	0.0	
	* 400	421.2	60	15.0	1.7	498.0 EC50
	* 800	847.7	60	100.0	31.7	(457.5 - 542.0)
V. iris	0	4.0	60	1.7	1.7	505.5 LC50
96-hr; 20°C	* 50	52.2	60	1.7	1.7	(463.1 - 551.8)
river water	* 100	104.6	60	5.0	3.3	
2/09/91	* 200	215.4	60	11.7	8.3	
	* 400	429.1	60	20.0	13.3	471.3 EC50
	* 800	865.0	60	100.0	100.0	(426.6 - 520.7)

V. iris	0	4.0	48	0.0	0.0	1121.6 LC50
48-hr; 20°C	200	172.7	48	6.2	4.2	(1076.7 - 1168.4)
river water	* 400	398.2	48	0.0	0.0	
8/02/91	* 800	802.0	48	16.7	4.2	
	* 1600	1665.0	48	100.0	100.0	1025.7 EC50
	3200	-	48	100.0	100.0	(950.4 - 1106.9)
A. pectorosa	0	25.2	60	1.7	0.0	818.8 LC50
48-hr; 12°C	* 50	79.6	60	1.7	1.7	(742.8 - 902.6)
reconstituted	* 100	118.7	60	3.3	3.3	
EPA water	* 200	264.9	60	11.7	1.7	
5/13/91	* 400	474.3	60	71.7	6.7	
	* 800	914.3	60	98.3	61.7	
	* 1600	1929.0	60	100.0	100.0	383.9 EC50
	3200	-	60	100.0	100.0	(347.8 - 423.7)
A. pectorosa	0	24.0	60	1.7	1.7	462.0 LC50
96-hr; 12°C	* 50	79.6	60	1.7	1.7	(425.3 - 501.8)
reconstituted	* 100	118.7	60	5.0	5.0	
EPA water	* 200	259.0	60	75.0	1.7	
5/13/91	* 400	475.8	60	100.0	55.0	201.2 EC50
	* 800	918.2	60	100.0	100.0	(184.9 - 218.9)
A. pectorosa	0	22.2	60	5.0	5.0	628.0 LC50
48-hr; 20°C	50	78.4	60	0.0	0.0	(596.7 - 660.9)
reconstituted	* 100	119.0	60	0.0	0.0	
EPA water	* 200	253.4	60	33.3	1.7	
5/13/91	* 400	473.6	60	90.0	8.3	
	* 800	906.7	60	100.0	98.3	
	* 1600	1901.0	60	100.0	100.0	293.3 EC50
	3200	-	60	100.0	100.0	(266.4 - 323.0)
A. pectorosa	0	24.4	60	10.0	10.0	367.6 LC50
96-hr; 20°C	* 50	78.4	60	0.0	0.0	(336.6 - 401.4)
reconstituted	* 100	120.9	60	1.7	1.7	
EPA water	* 200	251.4	60	23.3	10.0	
5/13/91	* 400	474.4	60	100.0	78.3	291.6 EC50
	* 800	919.4	60	100.0	100.0	(270.2 - 314.7)

Juvenile tests with copper. An asterisk signifies the data were used to determine LC50 or EC50 value.

Test Description	Target Value (µg/L)	Actual Value (µg/L)	# Exposed	% Affected	% Dead	Spearman-Karber Value (95% confidence limits) (µg Zn/L)
A. pectorosa	0	3.6	59	1.7	1.7	62.7 LC50
48-hr; 20°C	* 20	16.2	60	3.3	1.7	(56.1 - 70.1)
tap water	* 40	39.2	60	38.3	18.3	
7/17/91	* 80	69.6	60	90.0	48.3	
	* 160	128.0	60	100.0	96.7	40.9 EC50
	* 320	273.1	60	100.0	100.0	(36.8 - 45.5)
A. pectorosa	0	2.3	60	1.7	1.7	51.6 LC50
48-hr; 20°C	* 40	36.8	60	38.3	13.3	(43.4 - 61.3)
tap water	* 80	63.8	60	96.7	50.0	25.4 EC50
7/25/91	* 160	128.0	60	100.0	100.0	(20.5 - 31.6)
A. pectorosa	0	36.5	60	0.0	0.0	159.6 LC50
48-hr; 20°C	10	50.8	60	0.0	0.0	(147.3 - 172.9)
river water	20	65.6	60	3.3	3.3	
5/21/91	* 40	83.8	60	5.0	3.3	
	* 80	102.4	60	23.3	13.3	
	* 160	185.5	60	100.0	65.0	124.4 EC50
	* 320	332.7	60	100.0	100.0	(119.0 - 130.0)
A. pectorosa	0	2.9	60	0.0	0.0	113.4 LC50
48-hr; 20°C	* 20	17.3	60	1.7	1.7	(100.7 - 127.8)
river water	* 40	41.4	60	40.0	8.3	
7/17/91	* 80	82.2	60	96.7	18.3	
	* 160	158.8	60	100.0	71.7	43.1 EC504
	* 320	322.5	59	100.0	100.0	(38.8 - 47.9)
A. pectorosa	* 0	5.8	60	1.7	1.7	75.6 LC50
48-hr; 20°C	* 80	76.4	60	60.0	26.7	(61.8 - 92.5)
river water	* 160	149.9	60	100.0	83.3	39.3 EC50
7/21/91	* 320	297.0	60	100.0	100.0	(31.8 - 48.4)

Appendix B. Water Quality

APPENDIX B. WATER QUALITY.

Water quality data from acute exposures of glochidia to zinc referenced in Appendix A.

Date	Diluent Source	Zn Target (μg/L)	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
3-05-91	Tap	0	8.2	7.66	113	31.0	40
		100	8.7	7.57	115	32.5	40
		200	8.5	7.50	114	32.0	40
		400	8.4	7.48	114	32.5	40
		800	8.5	7.44	116	32.0	40
		1600	8.3	7.36	118	31.5	40
		3200	8.3	7.32	123	32.0	50
3-09-91	Tap	0	8.5	7.97	113	33.0	40
		100	8.3	7.94	115	32.0	50
		200	8.3	7.88	115	33.0	50
		400	8.3	7.76	115	32.0	40
		800	8.2	7.74	118	32.0	40
		1600	8.2	7.66	119	32.0	50
		3200	8.2	7.59	124	32.0	50
		6400	8.2	7.52	135	32.0	50
3-09-91	River	0	9.1	8.32	380	88.0	140
		100	9.2	8.25	380	87.0	140
		200	9.3	8.22	380	88.0	140
		400	9.1	8.18	380	88.0	140
		800	9.0	8.13	380	87.0	140
		1600	9.0	8.11	380	87.0	140
		3200	9.0	8.02	380	86.0	140
		6400	9.0	7.90	390	84.0	140
3-14-91	Tap	0	8.0	7.27	120	33.0	50
		200	8.1	7.25	119	33.0	40
		400	8.1	7.25	119	33.0	50
		800	8.1	7.22	120	32.0	40
		1600	8.1	7.15	122	32.0	50
		3200	8.1	7.10	131	33.0	50
		6400	8.1	7.06	139	32.0	60
3-14-91	River	0	10.2	8.10	390	89.0	140
		250	9.7	8.00	390	87.0	140
		500	9.7	7.97	390	87.0	140
		1000	9.6	7.95	390	87.0	140
		2000	9.6	7.88	390	87.0	150
		4000	9.4	7.77	390	85.0	140
		8000	9.3	7.67	400	83.0	150

3-18-91	River	0	9.9	8.07	390	88.0	150
		250	9.9	8.05	385	87.0	140
		500	9.9	8.05	385	86.0	140
		1000	9.9	8.02	390	86.0	130
		2000	9.6	7.90	390	86.0	150
		4000	9.7	7.98	390	84.0	150
		8000	9.3	7.88	400	82.0	150
		16000	8.9	7.65	410	78.0	150
4-16-91	Tap	0	8.4	8.39	110	38.5	50
		50	8.4	8.35	110	38.0	50
		100	8.2	8.25	110	37.5	50
		200	8.3	8.22	112	38.0	50
		400	8.3	8.25	114	38.0	50
		800	8.3	8.17	117	39.0	50
4-16-91	River	0	9.0	8.91	470	114.5	160
		70	9.0	8.88	460	114.0	160
		140	8.7	8.88	460	113.0	160
		280	8.9	8.86	460	114.0	160
		560	8.8	8.81	460	114.0	150
		1120	8.8	8.78	470	113.0	160
4-19-91	Tap	0	9.5	8.30	120	38.5	50
		100	8.7	8.18	120	38.5	40
		200	8.3	8.23	120	39.5	50
		400	8.7	8.18	122	39.0	40
		800	8.5	8.23	123	40.0	50
		1600	8.5	8.17	125	40.0	50
4-19-91	River	0	9.1	8.88	460	121.0	160
		140	9.0	8.81	460	116.5	160
		280	8.2	8.84	460	114.5	160
		560	8.4	8.84	460	114.0	160
		1120	8.4	8.79	470	114.0	160
		2240	9.2	8.64	470	116.0	160
4-23-91	River	0	8.8	8.32	460	106.0	160
		250	8.5	8.30	460	106.5	160
		500	8.6	8.25	460	106.0	160
		1000	8.7	8.20	460	106.0	160
		2000	8.5	8.11	460	105.0	160
		4000	8.5	8.04	460	104.0	160
		8000	8.4	7.83	470	102.0	160
4-25-91	River	0	9.5	8.29	460	107.0	160
		200	9.3	8.29	460	107.5	160
		400	9.3	8.30	460	107.5	160
		800	9.2	8.30	460	107.5	160
		1600	9.2	8.25	460	106.5	160
		3200	9.2	8.16	460	106.0	160
		6400	9.0	8.04	470	104.5	160

6-30-91	Tap	0	7.2	7.04	173	7.0	40
		100	7.3	7.01	175	7.0	50
		200	7.2	7.03	173	7.0	40
		400	7.3	7.03	174	7.0	50
		800	7.3	7.01	176	7.0	50
		1600	7.3	7.01	179	7.0	50
		3200	7.4	6.99	183	7.5	50
		6400	7.4	6.97	194	7.5	50
6-30-91	River	0	8.9	8.37	370	131.0	160
		100	7.8	8.31	370	131.0	160
		200	7.8	8.31	370	130.0	150
		400	7.7	8.33	370	130.0	150
		800	7.8	8.28	370	130.0	160
		1600	7.8	8.23	370	128.5	160
		3200	7.7	8.16	370	127.0	160
		6400	7.7	8.07	370	126.0	150
7-04-91	Tap	0	7.3	8.05	140	38.0	70
		100	7.3	7.96	140	38.0	50
		200	7.3	7.92	140	38.0	50
		400	7.3	7.87	140	38.0	50
		800	7.3	7.82	142	38.0	50
		1600	7.3	7.70	142	37.5	60
		3200	7.3	7.58	148	37.0	50
		6400	7.3	7.45	157	37.0	50
7-10-91	Tap	0	8.8	7.54	140	39.0	50
		50	8.2	7.58	140	38.0	40
		100	7.9	7.66	144	40.0	50
		200	7.9	7.56	145	40.0	50
		400	7.3	7.54	145	40.0	50
		800	8.3	7.51	146	39.0	50
		1600	8.7	7.52	149	39.0	40
		3200	9.3	7.44	152	38.0	70
		6400	9.1	7.47	162	37.5	60
7-10-91	River	0	7.4	8.41	360	121.0	140
		100	7.8	8.24	360	121.0	140
		200	8.0	8.20	360	121.0	150
		400	7.7	8.26	360	120.5	140
		800	7.8	8.17	365	121.0	150
		1600	7.8	8.10	370	121.0	140
		3200	7.8	7.96	370	119.0	150
		6400	7.8	7.92	370	118.0	160

Water quality data from acute exposures of juvenile mussels to zinc referenced in Appendix A.

Date	Diluent Source	Zn Target (μg/L)	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
12-07-90	Tap	0	7.9	7.50	138	35.0	50
		400	7.3	7.59	134	37.0	50
2-03-91	River	0	8.3	8.27	390	87.0	140
		50	9.0	8.09	390	87.0	140
		100	9.1	8.09	380	87.5	140
		200	9.1	8.08	385	86.0	140
		400	9.3	8.06	380	86.5	140
		800	9.2	7.97	385	86.0	140
2-06-91	River	0	8.9	8.16	390	87.5	140
2-09-91	River	0	7.8	8.37	390	88.0	-
		800	7.2	8.36	375	86.0	140
2-14-91	River	0	8.2	8.27	380	86.0	140
		1120	8.8	8.04	380	85.5	140
2-15-91	River	0	8.2	8.27	380	86.0	140
		1120	8.8	8.04	380	85.5	140
2-19-91	Tap	0	7.9	8.02	127	34.0	50
		25	8.2	7.80	129	35.0	50
		50	8.2	7.78	131	35.0	50
		100	8.2	7.74	131	35.5	60
		200	8.2	7.76	130	34.5	50
		400	8.2	7.79	130	35.0	40
4-30-91	Tap	0	7.7	7.92	122	35.0	40
		50	7.5	7.88	120	34.0	40
		100	7.5	7.88	120	34.0	50
		200	7.5	7.81	120	34.0	50
		400	7.5	7.81	121	34.0	40
		800	7.6	7.74	123	34.0	50
		1600	7.5	7.67	125	33.5	50
4-30-91	River	0	8.0	8.41	460	107.0	160
		100	8.0	8.39	460	107.0	160
		200	8.0	8.39	460	107.0	160
		400	8.0	8.37	460	107.0	160
		800	8.0	8.36	460	107.0	160
		1600	8.0	8.30	460	106.5	160
		3200	8.0	8.18	460	105.0	160

5-06-91	River	0	7.7	8.46	460	108.0	160
		200	7.6	8.39	460	106.0	160
		400	7.6	8.39	460	106.5	160
		800	7.6	8.36	460	106.0	160
		1600	7.6	8.30	460	106.0	160
		3200	7.6	8.20	460	104.5	160
		6400	7.6	8.01	460	102.0	160
		12800	7.6	7.62	460	93.0	160
5-06-91	River	0	7.7	8.46	460	108.0	160
		200	7.6	8.39	460	106.0	160
		400	7.6	8.39	460	106.5	160
		800	7.6	8.36	460	106.0	160
		1600	7.6	8.30	460	106.0	160
		3200	7.6	8.20	460	104.5	160
		6400	7.6	8.01	460	102.0	160
		12800	7.6	7.62	460	93.0	160
5-10-91	Tap	0	7.7	8.01	121	35.0	40
		50	7.6	7.95	122	35.0	40
		100	7.6	7.94	121	35.0	50
		200	7.6	7.90	121	34.5	50
		400	7.6	7.86	122	34.5	40
		800	7.6	7.81	123	34.0	50
		1600	7.6	7.75	126	34.0	40
5-13-91	EPA	0	7.1	8.20	330	59.0	100
		50	7.2	8.09	330	58.0	90
		100	7.2	8.16	330	58.0	90
		200	7.2	8.16	330	58.0	90
		400	7.2	8.13	330	58.0	100
		800	7.2	8.08	330	57.0	100
		1600	7.2	7.95	330	57.0	100
		3200	7.2	7.88	330	57.0	100
5-13-91	EPA	0	7.1	8.20	330	59.0	100
		50	7.2	8.09	330	58.0	90
		100	7.2	8.16	330	58.0	90
		200	7.2	8.16	330	58.0	90
		400	7.2	8.13	330	58.0	100
		800	7.2	8.08	330	57.0	100
		1600	7.2	7.95	330	57.0	100
		3200	7.2	7.88	330	57.0	100
5-21-91	River	0	8.5	8.50	350	112.0	160
		50	7.9	8.44	350	110.0	140
		100	8.3	8.46	350	110.0	140
		200	8.3	8.44	350	110.0	140
		400	8.4	8.43	350	110.0	140
		800	8.4	8.43	350	110.0	140
		1600	8.3	8.34	350	110.0	140
		3200	8.4	8.23	350	109.0	140

8-02-91	Tap	0	7.0	7.84	137	38.0	50
		100	7.0	7.87	137	38.0	50
		200	7.0	7.80	137	38.5	50
		400	7.0	7.78	137	39.0	50
		800	7.1	7.66	138	38.0	50
		1600	7.1	7.56	141	38.0	50
8-02-91	River	0	8.2	8.24	370	124.0	160
		200	8.4	8.07	370	121.0	140
		400	8.2	8.04	370	121.0	150
		800	8.2	8.04	370	120.5	150
		1600	8.2	7.99	370	120.0	150
		3200	7.9	7.92	370	118.0	150

Water quality data from acute exposures of juvenile mussels to copper referenced in Appendix A.

Date	Diluent Source	Cu Target (μg/L)	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
5-21-91	River	0	8.5	8.50	350	112.0	160
		10	8.8	8.43	350	110.5	140
		20	8.8	8.43	350	110.5	140
		40	8.7	8.39	350	110.0	140
		80	8.8	8.39	350	110.0	140
		160	8.4	8.46	350	110.0	140
		320	8.7	8.36	350	110.0	140
7-17-91	Tap	0	7.3	7.94	135	38.0	50
		20	7.4	7.98	135	37.0	50
		40	7.5	8.01	134	37.0	50
		80	7.5	7.99	134	37.0	50
		160	7.6	8.01	134	37.0	50
		320	7.6	7.96	135	37.0	50
7-17-91	River	0	8.3	8.34	365	123.0	140
		20	8.0	8.29	365	122.0	150
		40	8.0	8.31	365	122.0	150
		80	8.0	8.29	360	122.0	160
		160	8.0	8.29	360	121.0	140
		320	8.1	8.26	360	122.0	140
7-21-91	River	0	7.5	8.29	360	121.0	150
		80	8.0	8.15	360	121.0	150
		160	7.8	8.29	360	122.0	170
		320	8.0	8.27	360	123.0	150
7-25-91	Tap	0	7.0	7.87	129	36.5	40
		40	7.2	7.84	129	36.0	50
		80	7.2	7.93	129	37.0	50
		160	7.1	7.87	129	37.0	50

Water quality data from acute exposure of juvenile mussels to copper-zinc mixtures referenced in Chapter Two.

Date/ Diluent	Cu Target (μg/L)	Zn Target (μg/L)	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
5/29/91	0	0	7.7	8.36	350	110.0	140
River	20	0	7.7	8.27	350	110.0	140
	20	100	7.9	8.29	350	110.0	140
	20	200	7.9	8.27	350	110.0	140
	20	400	8.0	8.22	350	110.0	140
	40	0	7.9	8.31	350	112.0	140
	40	100	7.3	8.29	350	110.0	150
	40	200	7.3	8.26	350	110.0	140
	40	400	7.3	8.22	350	110.0	140
	80	0	7.4	8.26	350	110.0	140
	80	100	7.3	8.33	350	110.0	150
	80	200	7.3	8.34	350	112.0	140
	80	400	7.3	8.33	350	112.0	140
	160	0	7.1	8.33	350	110.0	140
	160	100	7.1	8.29	350	109.0	140
	160	200	7.2	8.31	350	110.0	150
	160	400	7.2	8.27	350	110.0	140
7/21/91	0	0	7.5	8.29	360	121.0	150
River	0	400	8.2	8.11	360	122.0	150
	0	800	8.2	8.06	360	121.0	140
	0	1600	8.1	7.99	360	120.0	150
	80	0	8.0	8.15	360	121.0	150
	80	400	8.2	8.10	360	121.0	150
	80	800	7.5	8.18	360	121.0	150
	80	1600	7.4	8.15	360	120.5	140
	160	0	7.8	8.29	360	122.0	170
	160	400	7.8	8.25	360	122.0	140
	160	800	7.8	8.20	360	122.0	150
	160	1600	7.8	8.20	360	121.5	150
	320	0	8.0	8.27	360	123.0	150
	320	400	8.1	8.23	370	123.0	150
	320	800	8.0	8.20	370	123.5	150
	320	1600	8.0	8.17	370	122.0	160
7/25/91	0	0	7.0	7.87	129	36.5	40
Tap	0	200	7.2	7.79	129	36.5	50
	0	400	7.1	7.82	129	36.5	50
	0	800	7.1	7.69	131	36.5	50
	40	0	7.2	7.84	129	36.0	50
	40	200	7.1	7.79	129	36.5	50
	40	400	7.1	7.77	130	36.5	50
	40	800	7.1	7.63	130	36.0	50
	80	0	7.2	7.93	129	37.0	50
	80	200	7.2	7.82	129	36.5	40
	80	400	7.2	7.84	130	37.0	50
	80	800	7.2	7.74	132	37.0	50
	160	0	7.1	7.87	129	37.0	50
	160	200	7.2	7.87	130	37.0	50
	160	400	7.2	7.86	131	36.5	50
	160	800	7.2	7.82	132	36.5	50

Water quality data from sediment test one referenced in Chapter Three.

Site	Date	Species[A]	Source[B]	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
DRYDEN	11-11-90	V	IN	8.6	8.32	450	151.5	180
DRYDEN	11-13-90	V	IN	7.6	7.65	440	139.0	180
DRYDEN	11-13-90	V	A	8.4	8.30	500	165.0	220
DRYDEN	11-15-90	V	IN	-	7.90	510	139.0	180
DRYDEN	11-15-90	V	A	-	8.14	610	155.0	220
DRYDEN	11-15-90	V	B	-	8.26	680	180.0	220
DRYDEN	11-17-90	V	IN	8.2	7.91	510	141.0	170
DRYDEN	11-17-90	V	A	7.9	8.41	610	164.5	220
DRYDEN	11-17-90	V	B	7.8	8.41	680	181.0	240
DRYDEN	11-17-90	V	C	7.7	8.38	585	155.0	200
DRYDEN	11-17-90	V	D	8.1	8.38	580	162.0	200
DRYDEN	11-19-90	V	IN	8.9	8.40	480	122.0	160
DRYDEN	11-19-90	V	A	7.6	8.62	585	164.0	200
DRYDEN	11-19-90	V	B	7.8	8.62	590	161.0	210
DRYDEN	11-19-90	V	C	7.8	8.64	570	156.0	210
DRYDEN	11-19-90	V	D	8.0	8.67	580	168.0	200
DRYDEN	11-19-90	V	E	7.9	8.67	580	165.0	200
DRYDEN	11-19-90	V	F	7.9	8.64	545	152.0	200
DRYDEN	11-21-90	V	IN	8.0	8.15	490	124.5	180
DRYDEN	11-21-90	V	A	7.8	8.64	580	144.5	220
DRYDEN	11-21-90	V	B	8.1	8.62	610	149.0	220
DRYDEN	11-21-90	V	C	7.9	8.62	560	137.0	200
DRYDEN	11-21-90	V	D	7.9	8.64	570	144.0	210
DRYDEN	11-21-90	V	E	7.9	8.61	550	141.0	190
DRYDEN	11-21-90	V	F	8.2	8.56	540	141.0	190
DRYDEN	11-23-90	V	IN	7.5	8.44	490	124.0	170
DRYDEN	11-23-90	V	B	7.7	8.59	590	142.0	210
DRYDEN	11-23-90	V	C	7.6	8.61	550	135.5	190
DRYDEN	11-23-90	V	D	7.8	8.66	540	139.0	190
DRYDEN	11-23-90	V	E	7.9	8.69	560	145.5	190
DRYDEN	11-23-90	V	F	7.7	8.66	520	134.0	190
DRYDEN	11-25-90	V	IN	7.7	8.40	490	125.0	180
DRYDEN	11-25-90	V	C	7.8	8.67	550	132.5	190
DRYDEN	11-25-90	V	D	8.0	8.71	550	139.0	210
DRYDEN	11-25-90	V	E	7.9	8.69	550	141.0	190
DRYDEN	11-25-90	V	F	7.8	8.66	520	133.0	180
DRYDEN	11-27-90	V	E	8.0	8.64	550	140.0	180
DRYDEN	11-27-90	V	F	7.9	8.66	520	134.0	180
TRASH	11-11-90	V	IN	9.2	8.39	390	124.5	160
TRASH	11-13-90	V	IN	9.3	7.95	390	118.5	160
TRASH	11-13-90	V	A	8.2	8.23	445	150.0	210
TRASH	11-15-90	V	IN	-	8.03	450	117.0	150
TRASH	11-15-90	V	A	-	8.17	550	145.0	200
TRASH	11-15-90	V	B	-	8.17	585	149.0	200
TRASH	11-17-90	V	IN	9.9	8.05	450	119.0	160
TRASH	11-17-90	V	A	7.9	8.40	560	151.0	200
TRASH	11-17-90	V	B	7.7	8.31	530	135.0	180
TRASH	11-17-90	V	C	7.7	8.38	565	155.0	210
TRASH	11-17-90	V	D	7.8	8.41	510	137.0	170

TRASH	11-19-90	V	IN	9.4	8.56	445	122.0	160
TRASH	11-19-90	V	A	8.0	8.62	530	146.0	190
TRASH	11-19-90	V	B	7.8	8.57	545	143.0	190
TRASH	11-19-90	V	C	7.8	8.59	520	140.0	180
TRASH	11-19-90	V	D	7.9	8.59	510	138.0	190
TRASH	11-19-90	V	E	7.9	8.59	540	148.0	190
TRASH	11-19-90	V	F	8.1	8.52	520	144.0	180
TRASH	11-21-90	V	IN	9.2	8.39	445	120.5	200
TRASH	11-21-90	V	A	7.8	8.62	540	144.0	210
TRASH	11-21-90	V	B	7.9	8.59	535	140.0	190
TRASH	11-21-90	V	C	7.9	8.67	540	142.5	180
TRASH	11-21-90	V	D	8.0	8.64	510	140.5	190
TRASH	11-21-90	V	E	7.9	8.64	510	138.0	180
TRASH	11-21-90	V	F	8.1	8.61	500	136.0	180
TRASH	11-23-90	V	IN	8.6	8.57	445	121.0	160
TRASH	11-23-90	V	B	7.8	8.61	500	130.0	180
TRASH	11-23-90	V	C	8.7	8.83	500	131.5	170
TRASH	11-23-90	V	D	7.9	8.69	490	132.5	170
TRASH	11-23-90	V	E	7.9	8.66	520	141.0	180
TRASH	11-23-90	V	F	7.8	8.59	490	131.0	170
TRASH	11-25-90	V	IN	8.7	8.52	450	121.0	150
TRASH	11-25-90	V	C	8.6	8.76	510	135.5	180
TRASH	11-25-90	V	D	8.0	8.71	530	142.5	180
TRASH	11-25-90	V	E	7.9	8.67	500	130.5	170
TRASH	11-25-90	V	F	8.0	8.61	490	131.5	180
TRASH	11-27-90	V	E	8.1	8.66	520	140.0	180
TRASH	11-27-90	V	F	8.1	8.67	540	147.0	200
FLETCHER	11-11-90	V	IN	9.0	8.48	330	131.0	160
FLETCHER	11-13-90	V	IN	8.9	7.90	335	132.0	160
FLETCHER	11-13-90	V	A	8.2	8.30	440	176.0	230
FLETCHER	11-15-90	V	IN	-	8.10	380	133.0	160
FLETCHER	11-15-90	V	A	-	8.31	490	164.0	220
FLETCHER	11-15-90	V	B	-	8.21	510	190.0	210
FLETCHER	11-17-90	V	IN	9.7	8.21	380	133.5	170
FLETCHER	11-17-90	V	A	7.8	8.43	440	150.0	200
FLETCHER	11-17-90	V	B	7.6	8.40	480	175.0	220
FLETCHER	11-17-90	V	C	8.8	8.57	430	153.0	190
FLETCHER	11-17-90	V	D	7.9	8.38	410	147.0	200
FLETCHER	11-19-90	V	IN	9.1	8.54	370	131.0	170
FLETCHER	11-19-90	V	A	8.0	8.66	450	160.0	210
FLETCHER	11-19-90	V	B	7.8	8.59	440	160.0	200
FLETCHER	11-19-90	V	C	9.2	8.72	370	113.0	160
FLETCHER	11-19-90	V	D	8.0	8.64	420	153.0	180
FLETCHER	11-19-90	V	E	8.1	8.64	410	152.0	200
FLETCHER	11-19-90	V	F	8.1	8.66	405	149.0	180
FLETCHER	11-21-90	V	IN	9.4	8.47	370	130.0	160
FLETCHER	11-21-90	V	A	8.0	8.67	440	150.0	210
FLETCHER	11-21-90	V	B	7.8	8.64	495	169.0	230
FLETCHER	11-21-90	V	C	8.9	8.89	370	118.0	150
FLETCHER	11-21-90	V	D	8.0	8.67	420	149.0	170
FLETCHER	11-21-90	V	E	8.2	8.69	440	159.5	200
FLETCHER	11-21-90	V	F	8.0	8.56	400	142.0	180
FLETCHER	11-23-90	V	IN	8.8	8.62	370	131.0	180
FLETCHER	11-23-90	V	B	7.8	8.49	460	148.0	210
FLETCHER	11-23-90	V	C	7.7	8.69	430	153.0	190

FLETCHER	11-23-90	V	D	7.9	8.76	420	150.0	190
FLETCHER	11-23-90	V	E	7.8	8.72	410	149.0	190
FLETCHER	11-23-90	V	F	8.0	8.66	410	147.0	190
FLETCHER	11-25-90	V	IN	9.1	8.62	370	130.0	170
FLETCHER	11-25-90	V	C	7.7	8.67	420	153.0	190
FLETCHER	11-25-90	V	D	8.0	8.74	420	149.0	180
FLETCHER	11-25-90	V	E	8.2	8.74	450	162.5	200
FLETCHER	11-25-90	V	F	7.9	8.71	410	148.0	180
FLETCHER	11-27-90	V	E	8.2	8.67	440	158.0	210
FLETCHER	11-27-90	V	F	8.0	8.67	410	147.0	190
CONTROL	11-13-90	V	IN	7.0	7.71	125	34.0	60
CONTROL	11-13-90	V	A	8.2	7.69	140	40.0	80
CONTROL	11-15-90	V	IN	-	6.97	126	34.0	50
CONTROL	11-15-90	V	A	-	7.65	153	39.0	60
CONTROL	11-15-90	V	B	-	7.71	169	44.0	60
CONTROL	11-17-90	V	IN	5.3	7.71	133	35.0	40
CONTROL	11-17-90	V	A	7.9	7.74	156	42.0	60
CONTROL	11-17-90	V	B	8.1	7.91	176	46.5	60
CONTROL	11-17-90	V	C	7.9	7.84	151	39.5	60
CONTROL	11-17-90	V	D	7.9	7.88	153	40.5	60
CONTROL	11-19-90	V	IN	6.9	8.12	139	38.0	50
CONTROL	11-19-90	V	A	7.9	8.18	153	44.0	50
CONTROL	11-19-90	V	B	7.9	8.20	174	48.0	60
CONTROL	11-19-90	V	C	7.9	8.15	160	46.0	50
CONTROL	11-19-90	V	D	7.9	8.12	150	43.0	50
CONTROL	11-19-90	V	E	8.0	8.12	152	43.0	60
CONTROL	11-19-90	V	F	8.1	8.18	150	44.0	50
CONTROL	11-21-90	V	IN	8.0	7.79	139	139.0	50
CONTROL	11-21-90	V	A	8.1	8.07	156	43.0	50
CONTROL	11-21-90	V	B	8.1	8.08	180	50.0	60
CONTROL	11-21-90	V	C	8.0	8.15	184	51.5	60
CONTROL	11-21-90	V	D	7.9	8.01	154	42.0	60
CONTROL	11-21-90	V	E	8.0	8.15	157	43.5	60
CONTROL	11-21-90	V	F	8.3	8.13	168	48.0	50
CONTROL	11-23-90	V	IN	7.9	7.78	139	36.5	50
CONTROL	11-23-90	V	B	7.9	8.18	168	47.0	60
CONTROL	11-23-90	V	C	7.9	8.27	180	49.0	60
CONTROL	11-23-90	V	D	8.0	8.18	165	45.5	60
CONTROL	11-23-90	V	E	8.2	8.17	175	49.5	60
CONTROL	11-23-90	V	F	8.1	8.23	188	53.0	60
CONTROL	11-25-90	V	IN	8.0	7.93	131	37.0	40
CONTROL	11-25-90	V	C	8.2	8.37	177	50.0	60
CONTROL	11-25-90	V	D	8.0	8.20	173	47.0	60
CONTROL	11-25-90	V	E	8.0	8.18	157	43.5	60
CONTROL	11-25-90	V	F	8.3	8.25	200	56.5	70
CONTROL	11-27-90	V	E	8.2	8.20	161	50.0	60
CONTROL	11-27-90	V	F	8.0	8.12	154	43.0	60

[A] V = *Villosa iris*

[B] IN = fresh water added to replicates; A - F = water removed from replicates after 2 days in dishes.

Water quality data from sediment test two referenced in Chapter Three.

Site	Date	Species[A]	Source[B]	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
DRYDEN	11-30-90	V	IN	8.0	8.59	550	138.0	180
DRYDEN	12-02-90	V	IN	8.1	-	550	135.5	190
DRYDEN	12-02-90	V	A	8.0	8.64	630	146.5	190
DRYDEN	12-02-90	V	B	7.8	8.67	620	150.5	210
DRYDEN	12-02-90	V	C	8.1	8.71	715	171.5	240
DRYDEN	12-04-90	V	IN	8.7	8.50	540	144.0	190
DRYDEN	12-04-90	V	A	7.9	8.64	610	144.0	220
DRYDEN	12-04-90	V	B	7.8	8.66	630	149.0	220
DRYDEN	12-04-90	V	C	7.6	8.62	620	146.0	220
DRYDEN	12-06-90	V	IN	-	8.52	540	133.0	190
DRYDEN	12-06-90	V	A	-	8.64	645	155.0	230
DRYDEN	12-06-90	V	B	-	8.61	655	157.0	220
DRYDEN	12-06-90	V	C	-	8.49	605	142.5	200
DRYDEN	12-08-90	V	IN	8.8	8.17	570	145.5	200
DRYDEN	12-08-90	V	A	7.8	8.38	620	146.5	220
DRYDEN	12-08-90	V	B	8.0	8.38	695	154.5	240
DRYDEN	12-08-90	V	C	7.7	8.31	655	142.5	230
DRYDEN	12-10-90	V	IN	7.6	8.34	560	137.0	200
DRYDEN	12-10-90	V	A	6.8	8.36	650	147.5	220
DRYDEN	12-10-90	V	B	6.8	8.36	690	155.0	250
DRYDEN	12-10-90	V	C	6.5	8.26	650	145.5	230
DRYDEN	12-12-90	V	IN	8.9	8.33	540	132.0	190
DRYDEN	12-12-90	V	A	7.6	8.40	620	144.0	210
DRYDEN	12-12-90	V	B	7.8	8.41	640	147.0	230
DRYDEN	12-12-90	V	C	7.9	8.41	640	144.0	220
DRYDEN	12-14-90	V	IN	7.9	8.07	350	89.5	130
DRYDEN	12-14-90	V	A	7.9	8.40	600	139.0	210
DRYDEN	12-14-90	V	B	8.0	8.40	620	143.0	210
DRYDEN	12-14-90	V	C	7.8	8.40	600	141.0	220
DRYDEN	12-16-90	V	IN	8.4	8.09	350	88.5	130
DRYDEN	12-16-90	V	A	7.8	8.38	470	113.0	170
DRYDEN	12-16-90	V	B	7.9	8.43	450	111.0	160
DRYDEN	12-16-90	V	C	8.0	8.43	480	117.5	170
DRYDEN	12-18-90	V	IN	8.4	8.24	350	89.0	120
DRYDEN	12-18-90	V	A	7.9	8.38	460	112.0	170
DRYDEN	12-18-90	V	B	7.8	8.38	410	103.0	270
DRYDEN	12-18-90	V	C	7.5	8.31	410	103.0	150
DRYDEN	12-20-90	V	IN	8.6	8.22	355	90.0	150
DRYDEN	12-20-90	V	A	8.0	8.34	425	106.0	160
DRYDEN	12-20-90	V	B	7.9	8.33	420	106.0	150
DRYDEN	12-20-90	V	C	7.8	8.31	430	108.0	160
DRYDEN	12-22-90	V	IN	9.5	8.21	350	90.0	130
DRYDEN	12-22-90	V	A	7.9	8.38	430	110.0	150
DRYDEN	12-22-90	V	B	7.7	8.34	410	102.0	150
DRYDEN	12-22-90	V	C	7.9	8.40	480	120.0	180
DRYDEN	12-24-90	V	IN	9.4	8.19	350	88.0	120
DRYDEN	12-24-90	V	A	7.5	8.34	410	104.0	150
DRYDEN	12-24-90	V	B	8.0	8.38	480	121.0	170
DRYDEN	12-24-90	V	C	7.5	8.34	400	100.0	150
DRYDEN	12-26-90	V	IN	9.9	8.21	350	90.0	140

DRYDEN	12-26-90	V	A	7.7	8.34	420	104.0	160
DRYDEN	12-26-90	V	B	7.9	8.38	450	114.5	160
DRYDEN	12-26-90	V	C	7.7	8.38	410	102.0	140
DRYDEN	12-28-90	V	IN	10.2	8.15	350	89.5	130
DRYDEN	12-28-90	V	A	7.9	8.40	420	104.0	150
DRYDEN	12-28-90	V	B	8.2	8.45	460	116.0	160
DRYDEN	12-28-90	V	C	7.9	8.43	410	101.0	150
DRYDEN	12-30-90	V	IN	8.7	8.24	350	89.0	140
DRYDEN	12-30-90	V	A	7.9	8.38	460	115.5	170
DRYDEN	12-30-90	V	B	7.8	8.43	430	108.0	150
DRYDEN	12-30-90	V	C	7.6	8.38	420	104.0	160
DRYDEN	01-01-91	V	IN	9.6	8.19	350	89.0	140
DRYDEN	01-01-91	V	A	7.9	8.42	420	105.0	150
DRYDEN	01-01-91	V	B	8.0	8.40	450	113.5	170
DRYDEN	01-01-91	V	C	7.9	8.42	420	102.0	150
DRYDEN	01-03-91	V	IN	10.0	8.22	350	89.0	130
DRYDEN	01-03-91	V	A	7.9	8.42	410	102.5	150
DRYDEN	01-03-91	V	B	8.0	8.45	450	112.0	160
DRYDEN	01-03-91	V	C	7.9	8.38	410	100.0	140
DRYDEN	01-05-91	V	IN	10.4	8.19	350	87.5	120
DRYDEN	01-05-91	V	A	8.0	8.42	430	108.0	160
DRYDEN	01-05-91	V	B	8.0	8.45	430	107.5	150
DRYDEN	01-05-91	V	C	7.9	8.43	410	100.5	150
DRYDEN	01-07-91	V	IN	10.4	8.13	350	85.0	120
DRYDEN	01-07-91	V	A	8.0	8.45	460	116.0	160
DRYDEN	01-07-91	V	B	7.8	8.43	420	106.0	150
DRYDEN	01-07-91	V	C	7.8	8.38	425	107.0	160
DRYDEN	01-09-91	V	IN	8.6	8.29	350	89.5	130
DRYDEN	01-09-91	V	A	7.8	8.42	400	101.0	140
DRYDEN	01-09-91	V	B	8.2	8.42	460	116.0	160
DRYDEN	01-09-91	V	C	7.9	8.38	400	102.0	150
DRYDEN	01-11-91	V	IN	8.6	8.26	350	89.0	120
DRYDEN	01-11-91	V	A	7.9	8.42	570	149.0	210
DRYDEN	01-11-91	V	B	7.8	8.42	430	110.0	150
DRYDEN	01-11-91	V	C	7.8	8.42	430	107.0	160
DRYDEN	01-13-91	V	IN	9.9	8.21	355	88.0	130
DRYDEN	01-13-91	V	A	7.9	8.43	470	121.5	170
DRYDEN	01-13-91	V	B	8.3	8.47	480	123.5	180
DRYDEN	01-13-91	V	C	7.8	8.36	440	111.0	160
DRYDEN	01-15-91	V	IN	10.0	8.21	350	87.0	130
DRYDEN	01-15-91	V	A	8.0	8.43	460	117.5	160
DRYDEN	01-15-91	V	B	7.9	8.43	430	110.0	150
DRYDEN	01-15-91	V	C	7.9	8.40	420	104.0	150
DRYDEN	01-17-91	V	IN	8.7	8.21	350	88.0	120
DRYDEN	01-17-91	V	A	7.9	8.35	460	116.0	170
DRYDEN	01-17-91	V	B	7.9	8.38	420	105.0	150
DRYDEN	01-17-91	V	C	8.1	8.42	460	116.5	160
DRYDEN	01-19-91	V	IN	8.7	8.33	350	89.0	130
DRYDEN	01-19-91	V	A	7.7	8.47	430	108.0	150
DRYDEN	01-19-91	V	B	7.9	8.49	450	113.5	160
DRYDEN	01-19-91	V	C	7.5	8.40	430	107.5	150
DRYDEN	01-21-91	V	IN	9.5	8.12	380	86.0	140
DRYDEN	01-21-91	V	A	7.8	8.45	430	109.0	150
DRYDEN	01-21-91	V	B	7.8	8.47	450	114.5	160
DRYDEN	01-21-91	V	C	7.7	8.45	430	116.5	150
DRYDEN	01-23-91	V	IN	8.2	8.08	390	89.0	140

DRYDEN	01-23-91	V	A	7.9	8.41	470	108.0	170
DRYDEN	01-23-91	V	B	7.8	8.39	450	-	160
DRYDEN	01-23-91	V	C	7.9	8.41	500	114.0	180
DRYDEN	01-27-91	V	IN	9.3	8.15	400	90.0	140
DRYDEN	01-27-91	V	A	7.9	8.46	450	101.5	150
DRYDEN	01-27-91	V	B	8.3	8.46	520	122.0	190
DRYDEN	01-27-91	V	C	8.0	8.46	460	104.0	160
DRYDEN	01-29-91	V	IN	8.5	8.20	380	87.0	140
DRYDEN	01-29-91	V	A	8.0	8.46	540	122.0	190
DRYDEN	01-29-91	V	B	8.0	8.44	490	113.0	180
DRYDEN	01-29-91	V	C	7.8	8.39	470	106.0	170
DRYDEN	01-31-91	V	IN	9.6	8.02	390	87.0	140
DRYDEN	01-31-91	V	A	8.1	8.44	475	108.0	160
DRYDEN	01-31-91	V	B	8.2	8.44	490	113.0	190
DRYDEN	01-31-91	V	C	8.0	8.41	470	107.0	170
DRYDEN	02-08-91	V	IN	9.4	8.23	390	88.0	140
DRYDEN	02-08-91	V	A	7.8	8.41	450	104.0	160
DRYDEN	02-08-91	V	B	7.8	8.39	460	106.0	180
DRYDEN	02-08-91	V	C	7.7	8.37	450	101.0	170
DRYDEN	02-10-91	V	IN	8.2	8.18	385	86.0	140
DRYDEN	02-10-91	V	A	8.2	8.55	540	125.0	200
DRYDEN	02-10-91	V	B	7.9	8.50	480	111.5	180
DRYDEN	02-10-91	V	C	7.8	8.51	500	113.0	180
DRYDEN	02-12-91	V	IN	9.8	8.20	385	87.5	130
DRYDEN	02-12-91	V	A	8.2	8.43	495	115.5	180
DRYDEN	02-12-91	V	B	7.9	8.44	470	105.0	170
DRYDEN	02-12-91	V	C	7.8	8.43	450	100.0	170
DRYDEN	02-18-91	V	IN	9.0	8.22	390	86.5	140
DRYDEN	02-18-91	V	A	8.8	8.43	430	102.0	160
DRYDEN	02-18-91	V	B	9.0	8.46	480	112.0	180
DRYDEN	02-18-91	V	C	9.0	8.48	430	98.0	150
DRYDEN	02-28-91	V	A	7.7	8.55	540	122.0	210
DRYDEN	02-28-91	V	B	7.7	8.53	520	122.0	200
DRYDEN	02-28-91	V	C	8.0	8.55	490	111.0	180
BEADS	12-02-90	V	A	8.2	8.76	710	174.0	190
BEADS	12-02-90	V	B	8.0	8.74	670	163.0	180
BEADS	12-02-90	V	C	8.1	8.70	670	164.5	180
BEADS	12-04-90	V	A	8.0	8.74	695	177.5	230
BEADS	12-04-90	V	B	7.9	8.78	625	159.0	210
BEADS	12-04-90	V	C	7.9	8.76	650	165.0	210
BEADS	12-06-90	V	A	-	8.67	635	162.0	220
BEADS	12-06-90	V	B	-	8.66	670	172.0	220
BEADS	12-06-90	V	C	-	8.64	710	184.0	240
BEADS	12-08-90	V	A	7.9	8.50	670	170.0	230
BEADS	12-08-90	V	B	8.0	8.52	730	185.0	250
BEADS	12-08-90	V	C	7.8	8.47	640	161.5	220
BEADS	12-10-90	V	A	7.6	8.45	700	179.0	240
BEADS	12-10-90	V	B	7.9	8.50	770	193.0	260
BEADS	12-10-90	V	C	7.6	8.50	670	171.0	220
BEADS	12-12-90	V	A	7.9	8.48	670	170.0	230
BEADS	12-12-90	V	B	8.0	8.48	680	171.0	240
BEADS	12-12-90	V	C	7.8	8.47	630	160.0	220
BEADS	12-14-90	V	A	8.0	8.47	680	168.5	240
BEADS	12-14-90	V	B	8.0	8.52	640	156.5	220

BEADS	12-14-90	V	C	8.1	8.43	690	170.0	260
BEADS	12-16-90	V	A	8.1	8.45	480	124.0	180
BEADS	12-16-90	V	B	8.1	8.48	520	134.0	190
BEADS	12-16-90	V	C	7.9	8.45	460	117.0	160
BEADS	12-18-90	V	A	7.8	8.40	420	111.0	150
BEADS	12-18-90	V	B	8.0	8.45	480	124.0	180
BEADS	12-18-90	V	C	7.9	8.41	430	111.0	150
BEADS	12-20-90	V	A	8.0	8.38	430	111.0	150
BEADS	12-20-90	V	B	8.1	8.43	480	126.5	180
BEADS	12-20-90	V	C	8.1	8.36	470	122.0	180

[A] V = *Villosa iris*.
[B] IN = fresh water added to replicates; A - C = water removed from replicates after 2 days in dishes.

Water quality data from sediment test three referenced in Chapter Three.

Site	Date	Species[A]	Source[B]	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
DRYDEN	05-20-91	A	IN	5.3	8.22	480	131.0	160
DRYDEN	05-22-91	A	IN	7.0	8.23	470	126.0	170
DRYDEN	05-22-91	A	A	7.6	8.57	530	136.0	180
DRYDEN	05-22-91	A	B	7.5	8.58	620	174.0	230
DRYDEN	05-22-91	A	C	7.3	8.46	540	145.0	190
DRYDEN	05-22-91	A	D	7.7	8.62	540	152.0	200
DRYDEN	05-24-91	A	IN	8.1	8.44	450	113.0	160
DRYDEN	05-24-91	A	A	7.6	8.58	530	135.0	190
DRYDEN	05-24-91	A	B	7.4	8.55	560	153.0	190
DRYDEN	05-24-91	A	C	7.6	8.57	530	136.0	200
DRYDEN	05-24-91	A	D	7.6	8.57	550	149.0	200
DRYDEN	05-28-91	A	IN	8.1	8.33	450	110.0	140
DRYDEN	05-28-91	A	A	7.6	8.33	510	123.0	170
DRYDEN	05-28-91	A	B	7.6	8.36	560	136.0	190
DRYDEN	05-28-91	A	C	7.5	8.36	550	128.0	180
DRYDEN	05-28-91	A	D	7.5	8.34	560	136.0	190
DRYDEN	05-30-91	A	IN	6.9	8.36	450	114.0	140
DRYDEN	05-30-91	A	A	7.5	8.50	680	172.0	230
DRYDEN	05-30-91	A	B	6.2	8.38	590	145.0	210
DRYDEN	05-30-91	A	C	7.3	8.45	560	132.0	190
DRYDEN	05-30-91	A	D	7.3	8.48	570	141.0	190
DRYDEN	06-03-91	A	IN	9.0	8.14	450	111.0	150
DRYDEN	06-03-91	A	A	7.7	8.21	520	129.0	180
DRYDEN	06-03-91	A	B	7.6	8.21	530	127.0	190
DRYDEN	06-03-91	A	C	7.4	8.14	520	125.0	180
DRYDEN	06-03-91	A	D	7.7	8.21	560	136.0	190
DRYDEN	06-07-91	A	IN	8.6	8.01	440	110.0	150
DRYDEN	06-07-91	A	A	8.0	8.13	520	127.0	180
DRYDEN	06-07-91	A	B	8.1	8.14	520	124.0	170
DRYDEN	06-07-91	A	C	8.0	8.14	520	126.0	180
DRYDEN	06-07-91	A	D	8.0	8.16	520	125.0	170
DRYDEN	06-09-91	A	IN	8.5	8.42	450	113.0	140
DRYDEN	06-09-91	A	A	8.0	8.47	530	131.5	180
DRYDEN	06-09-91	A	B	8.0	8.49	540	133.5	190
DRYDEN	06-09-91	A	C	7.9	8.44	510	124.5	160
DRYDEN	06-09-91	A	D	7.8	8.47	520	130.0	180
DRYDEN	06-13-91	A	IN	8.8	8.30	440	111.0	140
DRYDEN	06-13-91	A	A	7.9	8.44	520	130.0	170
DRYDEN	06-13-91	A	B	7.9	8.46	510	129.0	160
DRYDEN	06-13-91	A	C	7.9	8.44	505	124.5	170
DRYDEN	06-13-91	A	D	7.9	8.42	510	125.0	180
DRYDEN	06-17-91	A	IN	8.7	8.28	440	112.0	150
DRYDEN	06-17-91	A	A	7.5	8.46	500	130.0	170
DRYDEN	06-17-91	A	B	7.7	8.51	580	149.0	210
DRYDEN	06-17-91	A	C	7.6	8.44	500	127.5	170
DRYDEN	06-17-91	A	D	7.7	8.44	520	131.0	180
DRYDEN	06-19-91	A	A	7.8	8.43	560	132.0	170
DRYDEN	06-19-91	A	B	7.8	8.47	600	139.5	190
DRYDEN	06-19-91	A	C	7.8	8.45	600	140.0	190
DRYDEN	06-19-91	A	D	7.6	8.40	550	129.0	170

ABOVE B	05-20-91	A	IN	6.5	8.32	560	144.0	210
ABOVE B	05-22-91	A	IN	7.1	8.39	580	155.0	220
ABOVE B	05-22-91	A	A	7.1	8.39	630	160.0	240
ABOVE B	05-22-91	A	B	7.6	8.58	610	158.0	210
ABOVE B	05-22-91	A	C	7.6	8.74	1100	242.0	420
ABOVE B	05-22-91	A	D	7.6	8.55	630	144.0	220
ABOVE B	05-24-91	A	IN	7.5	8.08	550	125.0	150
ABOVE B	05-24-91	A	A	7.6	8.55	630	151.0	240
ABOVE B	05-24-91	A	B	7.4	8.53	640	160.0	220
ABOVE B	05-24-91	A	C	7.7	8.64	760	177.0	280
ABOVE B	05-24-91	A	D	7.6	8.55	670	148.0	240
ABOVE B	05-28-91	A	IN	7.8	8.26	530	110.5	170
ABOVE B	05-28-91	A	A	7.7	8.33	670	133.0	230
ABOVE B	05-28-91	A	B	7.6	8.36	640	138.0	230
ABOVE B	05-28-91	A	C	7.5	8.33	640	135.0	220
ABOVE B	05-28-91	A	D	7.5	8.31	630	127.0	220
ABOVE B	05-30-91	A	IN	8.4	8.40	525	112.0	180
ABOVE B	05-30-91	A	A	7.2	8.48	630	130.0	220
ABOVE B	05-30-91	A	B	7.5	8.50	650	140.0	220
ABOVE B	05-30-91	A	C	7.3	8.50	650	142.0	240
ABOVE B	05-30-91	A	D	7.6	8.47	720	148.0	240
ABOVE B	06-03-91	A	IN	9.2	7.97	530	112.0	180
ABOVE B	06-03-91	A	A	7.6	8.12	600	130.0	230
ABOVE B	06-03-91	A	B	7.6	8.24	640	136.0	230
ABOVE B	06-03-91	A	C	7.7	8.19	620	135.0	210
ABOVE B	06-03-91	A	D	7.4	8.15	610	129.0	220
ABOVE B	06-07-91	A	IN	8.7	7.99	530	120.0	170
ABOVE B	06-07-91	A	A	8.0	8.13	635	134.0	220
ABOVE B	06-07-91	A	B	8.1	8.16	620	129.0	220
ABOVE B	06-07-91	A	C	8.0	8.16	640	133.0	220
ABOVE B	06-07-91	A	D	8.0	8.18	610	127.0	220
ABOVE B	06-09-91	A	IN	8.3	8.47	520	113.0	190
ABOVE B	06-09-91	A	A	7.9	8.53	630	140.5	200
ABOVE B	06-09-91	A	B	7.9	8.51	620	135.0	200
ABOVE B	06-09-91	A	C	7.9	8.49	640	141.0	210
ABOVE B	06-09-91	A	D	7.9	8.49	630	140.0	210
ABOVE B	06-13-91	A	IN	9.5	8.26	510	113.5	180
ABOVE B	06-13-91	A	A	7.8	8.39	600	137.5	220
ABOVE B	06-13-91	A	B	7.6	8.37	590	128.0	210
ABOVE B	06-13-91	A	C	7.6	8.35	580	126.5	190
ABOVE B	06-13-91	A	D	7.6	8.35	585	130.0	220
ABOVE B	06-17-91	A	IN	8.4	8.32	520	112.0	180
ABOVE B	06-17-91	A	A	7.5	8.39	660	141.0	240
ABOVE B	06-17-91	A	B	7.6	8.44	600	127.0	210
ABOVE B	06-17-91	A	C	7.7	8.44	610	128.0	210
ABOVE B	06-17-91	A	D	7.6	8.42	640	129.5	220
ABOVE B	06-19-91	A	A	7.8	8.49	670	132.0	210
ABOVE B	06-19-91	A	B	7.7	8.47	710	137.0	230
ABOVE B	06-19-91	A	C	7.7	8.49	650	128.0	200
ABOVE B	06-19-91	A	D	7.8	8.45	650	128.0	220
BELOW B	05-20-91	A	IN	6.4	8.58	610	207.0	190
BELOW B	05-22-91	A	IN	7.0	8.55	640	234.0	210
BELOW B	05-22-91	A	A	7.4	8.65	690	213.0	230
BELOW B	05-22-91	A	C	7.4	8.71	640	238.0	200
BELOW B	05-22-91	A	D	7.5	8.72	710	224.0	-

BELOW B	05-24-91	A	IN	7.8	8.34	550	134.0	190
BELOW B	05-24-91	A	A	7.6	8.71	710	221.0	240
BELOW B	05-24-91	A	C	7.7	8.72	710	252.0	250
BELOW B	05-24-91	A	D	7.6	8.69	700	241.0	210
BELOW B	05-28-91	A	IN	8.3	8.15	540	129.0	170
BELOW B	05-28-91	A	A	7.5	8.36	670	143.0	220
BELOW B	05-28-91	A	C	7.4	8.36	650	147.0	190
BELOW B	05-28-91	A	D	7.3	8.34	660	150.5	200
BELOW B	05-30-91	A	IN	7.3	8.50	550	130.0	160
BELOW B	05-30-91	A	A	7.1	8.53	700	160.0	230
BELOW B	05-30-91	A	C	7.1	8.53	690	157.0	210
BELOW B	05-30-91	A	D	7.2	8.52	660	150.0	200
BELOW B	06-03-91	A	IN	9.1	7.86	540	128.0	160
BELOW B	06-03-91	A	A	7.6	8.24	670	158.5	210
BELOW B	06-03-91	A	C	7.3	8.17	670	152.0	200
BELOW B	06-03-91	A	D	7.3	8.22	640	147.5	200
BELOW B	06-07-91	A	IN	8.5	7.95	525	115.0	170
BELOW B	06-07-91	A	A	7.8	8.18	630	151.0	190
BELOW B	06-07-91	A	C	7.9	8.20	630	149.0	200
BELOW B	06-07-91	A	D	7.7	8.14	650	157.0	200
BELOW B	06-09-91	A	IN	8.0	8.25	540	128.0	160
BELOW B	06-09-91	A	A	7.8	8.47	630	152.0	220
BELOW B	06-09-91	A	C	8.0	8.46	720	172.0	240
BELOW B	06-09-91	A	D	7.9	8.49	640	152.0	210
BELOW B	06-13-91	A	IN	9.9	8.16	530	129.0	160
BELOW B	06-13-91	A	A	7.7	8.47	610	155.0	200
BELOW B	06-13-91	A	C	7.8	8.47	625	155.0	200
BELOW B	06-13-91	A	D	7.8	8.49	610	153.0	200
BELOW B	06-17-91	A	IN	9.0	8.14	540	130.0	160
BELOW B	06-17-91	A	A	7.3	8.46	600	150.0	200
BELOW B	06-17-91	A	C	7.5	8.53	625	159.5	190
BELOW B	06-17-91	A	D	7.6	8.51	625	159.0	200
BELOW B	06-19-91	A	A	7.6	8.54	740	169.5	220
BELOW B	06-19-91	A	C	7.6	8.52	680	160.0	190
BELOW B	06-19-91	A	D	7.4	8.52	680	155.0	190

[A] A = *Actinonaias pectorosa*.
[B] IN = fresh water added to replicates; A - D = water removed from replicates after 2 days in dishes.

Water quality data from sediment test four referenced in Chapter Three.

Site	Date	Species[A]	Source[B]	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
TAP	05-22-91	A	IN	7.5	7.80	117	34.5	40
TAP	05-24-91	A	IN	7.6	8.02	119	34.0	40
TAP	05-28-91	A	IN	7.6	7.77	122	34.0	40
TAP	06-01-91	A	IN	7.5	6.67	121	34.0	40
TAP	06-05-91	A	IN	7.9	7.59	113	30.0	40
TAP	06-07-91	A	IN	8.0	7.57	121	33.0	40
TAP	06-11-91	A	IN	7.6	7.16	119	34.5	50
TAP	06-13-91	A	IN	7.9	7.91	123	35.0	50
TAP	06-17-91	A	IN	7.7	7.80	124	35.5	40
ABOVE B	05-24-91	A	A	7.6	8.43	280	111.0	140
ABOVE B	05-24-91	A	B	6.6	8.23	260	113.0	120
ABOVE B	05-24-91	A	C	7.5	8.48	280	119.0	120
ABOVE B	05-24-91	A	D	7.4	8.37	260	96.0	120
ABOVE B	05-28-91	A	A	7.5	8.06	240	77.0	120
ABOVE B	05-28-91	A	B	7.5	8.05	250	75.0	100
ABOVE B	05-28-91	A	C	7.4	8.08	220	82.0	120
ABOVE B	05-28-91	A	D	7.5	7.98	250	60.0	100
ABOVE B	06-01-91	A	A	7.4	7.79	210	55.0	80
ABOVE B	06-01-91	A	B	7.2	7.95	200	67.5	90
ABOVE B	06-01-91	A	C	7.3	7.93	210	64.5	90
ABOVE B	06-01-91	A	D	7.4	7.83	198	54.0	90
ABOVE B	06-05-91	A	A	7.8	7.83	184	55.0	70
ABOVE B	06-05-91	A	B	7.7	7.85	186	56.0	70
ABOVE B	06-05-91	A	C	7.6	7.97	200	58.0	70
ABOVE B	06-05-91	A	D	7.7	7.95	182	54.0	70
ABOVE B	06-07-91	A	A	7.8	7.73	176	55.0	80
ABOVE B	06-07-91	A	B	7.9	7.78	190	54.0	70
ABOVE B	06-07-91	A	C	7.8	7.78	193	57.0	80
ABOVE B	06-07-91	A	D	7.7	7.71	178	54.0	70
ABOVE B	06-11-91	A	A	7.6	8.09	169	56.0	70
ABOVE B	06-11-91	A	B	7.6	7.95	171	54.0	70
ABOVE B	06-11-91	A	C	7.6	8.09	170	53.0	-
ABOVE B	06-11-91	A	D	7.7	8.05	160	49.0	60
ABOVE B	06-13-91	A	A	7.8	8.05	166	53.0	60
ABOVE B	06-13-91	A	B	7.6	8.00	168	49.5	70
ABOVE B	06-13-91	A	C	7.8	8.11	169	52.0	70
ABOVE B	06-13-91	A	D	7.9	8.09	164	47.5	70
ABOVE B	06-17-91	A	A	7.6	8.04	177	56.0	70
ABOVE B	06-17-91	A	B	7.5	7.97	164	48.0	70
ABOVE B	06-17-91	A	C	7.5	8.02	180	55.0	70
ABOVE B	06-17-91	A	D	7.6	8.04	164	48.0	60
ABOVE B	06-21-91	A	A	7.7	8.03	185	53.5	70
ABOVE B	06-21-91	A	B	7.7	8.02	166	44.0	60
ABOVE B	06-21-91	A	C	7.6	8.12	191	53.0	70
ABOVE B	06-21-91	A	D	7.7	8.05	175	47.0	60
BELOW B	05-24-91	A	A	7.5	8.44	330	138.0	140
BELOW B	05-24-91	A	C	7.3	8.41	320	145.0	140
BELOW B	05-24-91	A	D	7.2	8.37	300	137.0	120

BELOW B	05-28-91	A	A	7.4	8.19	320	101.0	150
BELOW B	05-28-91	A	C	7.4	8.22	260	95.0	130
BELOW B	05-28-91	A	D	7.4	8.20	270	94.0	120
BELOW B	06-01-91	A	A	7.2	8.21	370	115.0	190
BELOW B	06-01-91	A	C	7.2	8.10	310	106.0	150
BELOW B	06-01-91	A	D	7.3	8.15	300	97.0	140
BELOW B	06-05-91	A	A	7.8	8.32	290	103.0	130
BELOW B	06-05-91	A	C	7.8	8.37	260	89.0	120
BELOW B	06-05-91	A	D	7.5	8.27	250	86.0	110
BELOW B	06-07-91	A	A	7.6	7.95	280	99.0	140
BELOW B	06-07-91	A	C	7.8	7.95	260	87.5	120
BELOW B	06-07-91	A	D	7.9	7.97	250	86.5	110
BELOW B	06-11-91	A	A	6.7	8.14	260	100.0	120
BELOW B	06-11-91	A	C	7.6	8.25	240	88.5	110
BELOW B	06-11-91	A	D	6.7	8.07	220	81.0	100
BELOW B	06-13-91	A	A	7.6	8.26	240	87.0	100
BELOW B	06-13-91	A	C	7.8	8.25	210	74.0	100
BELOW B	06-13-91	A	D	7.6	8.19	198	67.5	100
BELOW B	06-17-91	A	A	7.4	8.30	230	86.0	110
BELOW B	06-17-91	A	C	7.5	8.23	210	73.0	90
BELOW B	06-17-91	A	D	7.4	8.20	200	70.0	80
BELOW B	06-21-91	A	A	7.7	8.26	230	76.0	90
BELOW B	06-21-91	A	C	7.6	8.21	220	68.0	90
BELOW B	06-21-91	A	D	7.5	8.16	205	65.0	80

[A] A = *Actinonaias pectorosa*.
[B] IN = fresh water added to replicates; A - D = water removed from replicates after 2 days in dishes.

Water quality data from sediment test five referenced in Chapter Three.

Site	Date	Species[A]	Source[B]	DO (mg/L)	pH	Cond. (μmhos)	Alkalinity (mg/L)	Hardness (mg/L)
TAP	07-12-91	AV	IN	7.8	8.03	154	43.0	60
TAP	07-14-91	AV	IN	7.6	7.82	137	37.5	40
TAP	07-16-91	AV	IN	8.0	7.75	149	37.0	40
TAP	07-18-91	AV	IN	7.6	7.74	138	37.5	50
TAP	07-20-91	AV	IN	7.7	7.75	136	38.0	40
TAP	07-22-91	AV	IN	7.6	7.91	138	39.0	50
TAP	07-24-91	AV	IN	7.3	7.72	129	36.5	50
TAP	07-26-91	AV	IN	7.2	7.74	128	35.5	30
TAP	07-28-91	AV	IN	7.8	7.94	131	37.0	50
TAP	07-30-91	AV	IN	7.9	7.89	131	37.0	50
TAP	08-01-91	AV	IN	7.6	7.81	133	37.0	40
TAP	08-03-91	AV	IN	7.3	7.87	138	39.0	50
TAP	08-05-91	AV	IN	7.7	7.92	140	39.5	50
TAP	08-07-91	AV	IN	7.8	8.00	150	40.5	50
TAP	08-09-91	AV	IN	7.5	7.91	141	39.5	50
TAP	08-11-91	AV	IN	8.1	7.89	144	39.5	50
ABOVE B	07-14-91	V	A	7.6	8.27	330	95.0	140
ABOVE B	07-14-91	V	B	7.7	8.33	[illegible]0	104.0	140
ABOVE B	07-14-91	A	A	7.2	8.29	330	95.0	140
ABOVE B	07-14-91	A	B	7.7	8.38	360	108.0	140
ABOVE B	07-16-91	V	C	7.7	8.29	310	100.0	130
ABOVE B	07-16-91	V	D	7.8	8.31	330	96.0	130
ABOVE B	07-16-91	A	C	7.5	8.29	290	99.5	120
ABOVE B	07-16-91	A	D	7.8	8.33	320	100.0	140
ABOVE B	07-18-91	V	A	7.8	8.17	245	77.5	110
ABOVE B	07-18-91	V	B	7.7	8.18	235	77.0	100
ABOVE B	07-18-91	A	A	7.7	8.18	260	84.0	110
ABOVE B	07-18-91	A	B	7.8	8.18	260	85.0	110
ABOVE B	07-20-91	V	C	7.8	8.15	220	74.0	100
ABOVE B	07-20-91	V	D	7.7	8.15	230	76.0	100
ABOVE B	07-20-91	A	C	7.5	8.11	225	76.0	100
ABOVE B	07-20-91	A	D	7.6	8.15	240	82.0	100
ABOVE B	07-22-91	V	A	7.6	8.08	220	72.5	90
ABOVE B	07-22-91	V	B	7.6	7.98	210	69.0	80
ABOVE B	07-22-91	A	A	7.6	8.13	220	68.5	100
ABOVE B	07-22-91	A	B	7.8	8.08	220	71.0	90
ABOVE B	07-24-91	V	C	7.7	8.05	210	68.0	90
ABOVE B	07-24-91	V	D	7.7	8.10	199	62.0	80
ABOVE B	07-24-91	A	C	7.7	8.11	210	66.0	80
ABOVE B	07-24-91	A	D	7.7	8.20	220	71.5	100
ABOVE B	07-26-91	V	A	7.8	7.84	177	54.0	90
ABOVE B	07-26-91	V	B	7.8	7.86	184	56.4	70
ABOVE B	07-26-91	A	A	7.7	7.98	184	57.0	80
ABOVE B	07-26-91	A	B	7.8	8.04	189	59.0	80
ABOVE B	07-28-91	V	C	7.9	7.98	183	56.5	70
ABOVE B	07-28-91	V	D	7.9	8.06	183	56.0	70
ABOVE B	07-28-91	A	C	8.0	7.99	181	55.0	70
ABOVE B	07-28-91	A	D	7.8	8.03	192	60.0	80
ABOVE B	07-30-91	V	A	8.0	7.91	189	57.5	70

ABOVE B	07-30-91	V	B	8.0	7.96	191	57.5	80
ABOVE B	07-30-91	A	A	7.9	7.96	183	56.0	80
ABOVE B	07-30-91	A	B	7.8	7.93	187	57.0	70
ABOVE B	08-01-91	V	C	7.7	8.06	178	53.0	70
ABOVE B	08-01-91	V	D	7.8	8.13	189	57.0	70
ABOVE B	08-01-91	A	C	7.7	8.10	181	55.0	60
ABOVE B	08-01-91	A	D	7.7	8.13	188	59.0	80
ABOVE B	08-03-91	V	A	7.8	7.87	177	58.5	60
ABOVE B	08-03-91	V	B	7.8	7.90	186	56.5	70
ABOVE B	08-03-91	A	A	7.6	7.95	171	51.0	60
ABOVE B	08-03-91	A	B	7.7	7.97	175	52.5	60
ABOVE B	08-05-91	V	C	7.8	7.92	169	50.0	60
ABOVE B	08-05-91	V	D	7.9	7.95	169	50.0	60
ABOVE B	08-05-91	A	C	7.7	8.02	176	52.0	70
ABOVE B	08-05-91	A	D	7.7	8.06	189	57.0	60
ABOVE B	08-07-91	V	A	7.9	7.95	180	53.0	60
ABOVE B	08-07-91	V	B	7.9	8.02	190	54.0	70
ABOVE B	08-07-91	A	A	7.7	7.86	180	52.0	60
ABOVE B	08-07-91	A	B	7.8	7.90	180	53.0	70
ABOVE B	08-09-91	V	C	7.9	7.97	179	51.0	70
ABOVE B	08-09-91	V	D	7.9	7.98	173	49.0	70
ABOVE B	08-09-91	A	C	7.8	8.05	175	51.0	60
ABOVE B	08-09-91	A	D	7.8	8.09	188	56.0	70
ABOVE B	08-11-91	V	A	8.0	8.01	181	51.0	60
ABOVE B	08-11-91	V	B	7.9	7.99	195	54.5	70
ABOVE B	08-11-91	A	A	7.9	7.99	177	50.0	60
ABOVE B	08-11-91	A	B	7.9	7.97	182	52.0	70
ABOVE B	08-13-91	V	C	7.8	7.85	190	52.0	60
ABOVE B	08-13-91	V	D	7.8	7.90	190	51.0	60
ABOVE B	08-13-91	A	C	7.8	7.87	180	48.0	60
ABOVE B	08-13-91	A	D	7.5	7.89	190	52.0	70
BELOW B	07-14-91	V	A	7.7	8.31	320	94.0	130
BELOW B	07-14-91	A	A	7.7	8.29	320	97.0	140
BELOW B	07-16-91	V	B	7.8	8.36	340	102.5	140
BELOW B	07-16-91	A	B	7.4	8.27	320	99.0	130
BELOW B	07-18-91	V	A	7.8	8.15	250	80.0	100
BELOW B	07-18-91	A	A	7.6	8.15	220	74.0	100
BELOW B	07-20-91	V	B	7.6	8.20	250	84.5	100
BELOW B	07-20-91	A	B	7.6	8.15	230	77.0	100
BELOW B	07-22-91	V	A	7.8	8.08	220	71.0	90
BELOW B	07-22-91	A	A	7.6	8.10	210	69.5	90
BELOW B	07-24-91	V	B	7.6	8.11	200	71.0	90
BELOW B	07-24-91	A	B	7.9	8.15	210	68.5	80
BELOW B	07-26-91	V	A	7.9	8.01	197	62.0	80
BELOW B	07-26-91	A	A	7.7	8.01	187	58.0	70
BELOW B	07-28-91	V	B	8.0	8.06	197	66.5	80
BELOW B	07-28-91	A	B	7.8	8.04	190	59.0	70
BELOW B	07-30-91	V	A	7.9	7.91	183	56.0	70
BELOW B	07-30-91	A	A	7.8	7.88	185	56.5	70
BELOW B	08-01-91	V	B	7.6	8.10	190	59.0	80
BELOW B	08-01-91	A	B	7.8	8.12	192	59.5	70
BELOW B	08-03-91	V	A	7.9	7.87	178	54.0	60
BELOW B	08-03-91	A	A	7.7	7.92	176	52.0	70
BELOW B	08-05-91	V	B	7.8	7.97	170	50.5	60
BELOW B	08-05-91	A	B	7.8	8.01	181	53.5	80

BELOW B	08-07-91	V	A	7.9	8.05	190	54.0	70
BELOW B	08-07-91	A	A	8.0	7.97	190	55.0	60
BELOW B	08-09-91	V	B	8.0	8.02	182	53.0	60
BELOW B	08-09-91	A	B	7.8	8.07	184	54.5	60
BELOW B	08-11-91	V	A	7.9	8.02	191	54.0	70
BELOW B	08-11-91	A	A	7.9	7.97	181	50.5	60
BELOW B	08-13-91	V	B	7.5	7.94	210	58.5	80
BELOW B	08-13-91	A	B	7.8	8.06	200	56.5	70
DRYDEN	07-14-91	V	A	7.6	8.20	250	73.0	100
DRYDEN	07-14-91	V	B	7.5	8.19	250	75.0	110
DRYDEN	07-14-91	A	A	7.4	8.22	270	77.0	100
DRYDEN	07-14-91	A	B	7.5	8.24	250	74.0	110
DRYDEN	07-16-91	V	C	7.8	8.13	220	-	80
DRYDEN	07-16-91	V	D	7.8	8.13	220	65.5	80
DRYDEN	07-16-91	A	C	7.8	8.12	230	62.0	90
DRYDEN	07-16-91	A	D	7.6	8.20	230	71.0	80
DRYDEN	07-18-91	V	A	7.6	7.94	194	57.0	80
DRYDEN	07-18-91	V	B	7.5	7.94	195	58.0	80
DRYDEN	07-18-91	A	A	7.3	7.99	210	66.0	80
DRYDEN	07-18-91	A	B	7.6	8.05	199	59.0	80
DRYDEN	07-20-91	V	C	7.7	7.94	162	45.0	70
DRYDEN	07-20-91	V	D	7.7	7.98	175	56.5	60
DRYDEN	07-20-91	A	C	7.6	7.91	163	46.0	70
DRYDEN	07-20-91	A	D	7.6	7.96	177	52.5	70
DRYDEN	07-22-91	V	A	7.5	8.05	210	61.0	80
DRYDEN	07-22-91	V	B	8.0	7.87	195	55.0	70
DRYDEN	07-22-91	A	A	7.8	7.86	200	59.0	80
DRYDEN	07-22-91	A	B	7.7	8.03	186	54.5	80
DRYDEN	07-24-91	V	C	7.8	7.82	160	43.5	60
DRYDEN	07-24-91	V	D	7.7	7.96	166	46.5	70
DRYDEN	07-24-91	A	C	7.8	7.84	159	44.0	60
DRYDEN	07-24-91	A	D	7.7	7.98	174	49.5	70
DRYDEN	07-26-91	V	A	7.1	7.86	174	51.5	70
DRYDEN	07-26-91	V	B	7.9	7.94	176	50.5	60
DRYDEN	07-26-91	A	A	7.7	7.94	171	50.0	70
DRYDEN	07-26-91	A	B	7.7	7.91	162	46.5	60
DRYDEN	07-28-91	V	C	7.9	7.88	152	42.0	60
DRYDEN	07-28-91	V	D	7.9	7.88	154	43.0	60
DRYDEN	07-28-91	A	C	7.9	7.84	151	41.5	60
DRYDEN	07-28-91	A	D	7.8	7.91	154	43.0	50
DRYDEN	07-30-91	V	A	7.8	7.77	169	48.0	70
DRYDEN	07-30-91	V	B	7.9	7.84	167	48.5	60
DRYDEN	07-30-91	A	A	7.9	7.89	176	51.0	70
DRYDEN	07-30-91	A	B	7.9	7.89	173	50.0	70
DRYDEN	08-01-91	V	C	7.8	7.94	152	42.0	60
DRYDEN	08-01-91	V	D	7.8	8.01	165	46.5	60
DRYDEN	08-01-91	A	C	7.7	8.03	159	44.0	60
DRYDEN	08-01-91	A	D	7.7	8.00	162	45.5	60
DRYDEN	08-03-91	V	A	7.7	7.85	169	48.5	60
DRYDEN	08-03-91	V	B	7.8	7.82	187	54.0	70
DRYDEN	08-03-91	A	A	7.2	7.84	167	48.5	60
DRYDEN	08-03-91	A	B	7.8	7.89	170	49.0	60
DRYDEN	08-05-91	V	C	7.9	7.92	159	44.0	70
DRYDEN	08-05-91	V	D	7.8	7.94	168	47.0	60
DRYDEN	08-05-91	A	C	7.7	7.89	157	43.0	60

DRYDEN	08-05-91	A	D	7.7	7.90	156	43.5	50
DRYDEN	08-07-91	V	A	7.9	7.95	170	47.0	60
DRYDEN	08-07-91	V	B	7.9	7.98	180	51.0	70
DRYDEN	08-07-91	A	A	7.7	7.97	170	50.0	60
DRYDEN	08-07-91	A	B	7.8	7.90	160	45.0	60
DRYDEN	08-09-91	V	C	7.9	7.93	160	44.0	50
DRYDEN	08-09-91	V	D	7.8	7.91	166	46.0	60
DRYDEN	08-09-91	A	C	7.9	7.95	160	44.0	70
DRYDEN	08-09-91	A	D	7.9	7.95	157	43.0	60
DRYDEN	08-11-91	V	A	7.9	7.92	191	53.0	70
DRYDEN	08-11-91	V	B	7.9	7.90	176	48.0	60
DRYDEN	08-11-91	A	A	7.9	7.90	179	49.5	60
DRYDEN	08-11-91	A	B	8.0	7.90	166	46.0	60
DRYDEN	08-13-91	V	C	7.7	7.89	170	46.0	60
DRYDEN	08-13-91	V	D	7.4	7.90	180	50.5	60
DRYDEN	08-13-91	A	C	7.7	7.58	170	44.0	60
DRYDEN	08-13-91	A	D	7.7	7.87	170	46.0	60
FLETCHER	07-14-91	V	A	7.6	8.40	310	120.0	130
FLETCHER	07-14-91	V	B	7.6	8.29	270	98.0	110
FLETCHER	07-14-91	A	A	7.4	8.38	290	111.0	130
FLETCHER	07-14-91	A	B	7.5	8.31	260	90.0	100
FLETCHER	07-16-91	V	C	7.8	8.33	290	99.0	120
FLETCHER	07-16-91	V	D	7.8	8.36	290	104.0	120
FLETCHER	07-16-91	A	C	7.6	8.24	270	94.0	120
FLETCHER	07-16-91	A	D	7.8	8.33	280	107.5	120
FLETCHER	07-18-91	V	A	7.6	8.15	240	80.5	90
FLETCHER	07-18-91	V	B	7.5	8.10	220	71.5	100
FLETCHER	07-18-91	A	A	7.7	8.13	230	73.5	90
FLETCHER	07-18-91	A	B	7.7	8.10	200	66.0	90
FLETCHER	07-20-91	V	C	7.7	8.18	240	83.0	110
FLETCHER	07-20-91	V	D	7.7	8.15	220	73.0	100
FLETCHER	07-20-91	A	C	7.7	8.15	220	77.0	90
FLETCHER	07-20-91	A	D	7.6	8.15	230	78.0	90
FLETCHER	07-22-91	V	A	7.9	8.03	210	65.0	80
FLETCHER	07-22-91	V	B	7.7	8.08	200	61.0	80
FLETCHER	07-22-91	A	A	8.0	8.01	200	59.0	80
FLETCHER	07-22-91	A	B	7.9	7.98	188	57.0	50
FLETCHER	07-24-91	V	C	7.7	8.20	240	80.5	90
FLETCHER	07-24-91	V	D	7.7	8.06	198	61.0	90
FLETCHER	07-24-91	A	C	7.8	8.17	210	68.0	90
FLETCHER	07-24-91	A	D	7.7	8.15	210	65.0	80
FLETCHER	07-26-91	V	A	7.8	7.96	171	49.0	70
FLETCHER	07-26-91	V	B	7.8	7.94	170	49.5	60
FLETCHER	07-26-91	A	A	7.9	7.93	158	44.0	60
FLETCHER	07-26-91	A	B	7.7	7.94	176	52.0	70
FLETCHER	07-28-91	V	C	7.8	8.04	199	64.0	80
FLETCHER	07-28-91	V	D	7.9	8.01	176	52.0	70
FLETCHER	07-28-91	A	C	7.8	8.03	195	62.0	80
FLETCHER	07-28-91	A	D	7.8	7.98	176	52.0	70
FLETCHER	07-30-91	V	A	7.9	7.74	181	50.5	70
FLETCHER	07-30-91	V	B	8.0	7.82	165	47.0	60
FLETCHER	07-30-91	A	A	7.9	7.82	160	44.0	50
FLETCHER	07-30-91	A	B	7.9	7.84	176	50.0	70
FLETCHER	08-01-91	V	C	7.8	8.13	196	62.0	70
FLETCHER	08-01-91	V	D	7.7	8.06	179	52.0	70

FLETCHER	08-01-91	A	C	7.5	8.17	190	58.0	70
FLETCHER	08-01-91	A	D	7.6	8.00	184	53.0	70
FLETCHER	08-03-91	V	A	7.7	7.87	157	43.5	50
FLETCHER	08-03-91	V	B	7.7	7.85	167	47.0	60
FLETCHER	08-03-91	A	A	7.6	7.92	157	43.0	50
FLETCHER	08-03-91	A	B	7.8	7.95	167	47.0	60
FLETCHER	08-05-91	V	C	7.8	8.01	169	50.5	60
FLETCHER	08-05-91	V	D	7.8	7.97	169	48.5	70
FLETCHER	08-05-91	A	C	7.7	7.94	172	49.5	60
FLETCHER	08-05-91	A	D	7.7	7.90	164	46.5	60
FLETCHER	08-07-91	V	A	7.9	7.91	160	45.0	60
FLETCHER	08-07-91	V	B	7.9	7.98	160	46.0	60
FLETCHER	08-07-91	A	A	7.7	7.90	160	43.5	60
FLETCHER	08-07-91	A	B	7.9	7.93	160	45.5	60
FLETCHER	08-09-91	V	C	7.8	7.98	178	52.0	60
FLETCHER	08-09-91	V	D	7.9	7.97	170	47.5	60
FLETCHER	08-09-91	A	C	7.7	7.97	164	47.0	60
FLETCHER	08-09-91	A	D	7.7	7.95	162	45.0	60
FLETCHER	08-11-91	V	A	7.8	7.92	167	45.0	60
FLETCHER	08-11-91	V	B	7.9	7.90	167	45.0	60
FLETCHER	08-11-91	A	A	7.8	7.94	167	45.5	60
FLETCHER	08-11-91	A	B	7.6	7.89	168	47.0	60
FLETCHER	08-13-91	V	C	7.6	7.97	190	51.5	60
FLETCHER	08-13-91	V	D	7.9	7.68	170	46.0	60
FLETCHER	08-13-91	A	C	7.7	7.94	165	46.5	50
FLETCHER	08-13-91	A	D	7.7	7.75	170	47.0	60

[A] A = *Actinonaias pectorosa*; V = *Villosa iris*.
[B] IN = fresh water added to replicates; A - D = water removed from replicates after 2 days in dishes.

Vita

Mary T. McCann was born November 4, 1955, in Portland, Maine. She graduated from Catherine McAuley High School in Portland, Maine in 1974 and received an A.A.S. degree in Marine Biology and Oceanography at Southern Maine Vocational Technical Institute in 1978. In 1981, she received a B.S. degree in Fisheries Biology at the University of Massachusetts at Amherst. After working eight years for an environmental consulting firm in New Hampshire and Iowa, she became a candidate for the Master of Science degree in Fisheries and Wildlife Sciences (Fisheries Science) at Virginia Polytechnic Institute and State University, Blacksburg, Virginia.