

THE EFFECT OF DIEOFFS OF ASIAN CLAMS (CORBICULA FLUMINEA) ON
NATIVE FRESHWATER MUSSELS (UNIONIDAE)

by

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(ABSTRACT)

There is a great deal of concern about the declining freshwater mussel fauna of North America. Although deteriorating water quality and habitat degradation may account for much of the decline, it has been suggested that the exotic Asian clam, *Corbicula fluminea*, may be having an effect on native unionids. Negative impacts may result directly from competition or indirectly, because of *Corbicula* population crashes that release ammonia and reduce dissolved oxygen in the sediment.

Laboratory tests were conducted to determine the relative sensitivity of native mussel and Asian clam life stages to unionized ammonia, and mussel glochidia were the most sensitive (24-hr LC50 of 0.11 mg/L NH₃-N). Juvenile and adult mussels were similarly sensitive, with average 96-hr LC50's of 0.49 and 0.52 mg/L NH₃-N, respectively. Adult *C. fluminea* were the least sensitive, having an average LC50 of 0.80 mg/L NH₃-N. The EPA standard test organism, *Ceriodaphnia dubia*, had one of the lowest LC50's (0.07 mg/L NH₃-N) of the five species, and the fathead minnow, *Pimephales promelas*, had the highest (1.18 mg/L). The differing sensitivities of the various life stages are important when trying to determine the impact of an Asian clam dieoff. If a dieoff occurs at a time of year when the more sensitive life stages, such as glochidia are present, then the impact on mussel recruitment may be greater.

Two miniature artificial stream tests were used to determine the effect of clam density on dieoff rate, ammonia production and dissolved oxygen levels. Only clams at the highest density of 10,000/m² experienced 100% mortality. Unionized ammonia levels exceeded 4.0 mg/L, and dissolved oxygen levels dropped below 1.0 mg/L during the dieoff. The amount of unionized ammonia produced was twofold greater than the concentration that produced an LC50 in adult *C. fluminea* and ~40 times greater than the LC50 for *V. iris* glochidia. Factors thought to have contributed to the *C. fluminea* dieoff were flow rate, low dissolved oxygen levels, temperature and perhaps ammonia. A complete dieoff did not occur until flow was stopped and dissolved oxygen concentrations began to drop. One-hundred percent mortality occurred in 38 days for the first test, and 21 days in the second test. Higher water temperatures in the first test (26±°C) compared to the second test (average = 21.7°C) are thought to have resulted in the faster dieoff.

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CHAPTER 1 - INTRODUCTION

The Asian clam (*Corbicula fluminea*) is an exotic species that was first discovered in the United States in 1938 (Sinclair and Isom, 1963). Since then the clam has spread throughout the southern U.S. The Asian clam has proven to be very prolific, occurring in densities greater than 10,000/m² in some areas (Cherry et al., 1986; McMahon and Williams, 1986). This species is found in both lentic and lotic habitats and inhabits a variety of substrates.

The prognosis for the native freshwater unionids of the United States is not as encouraging. North America boasts one of the most diverse assemblages of freshwater mussels in the world, with approximately 300 species of freshwater bivalves found north of Mexico (Bogan, 1996). However, many areas have experienced a drastic decline in unionid populations (Anderson et al., 1991; Dennis, 1987). Currently it is believed that at least 35 of the taxa are extinct, 52 are endangered, five are threatened and 70 are candidates for being listed as threatened or endangered (Bogan, 1996). In the western basin of Lake Erie the mean number of native mussels collected decreased from 10/m² in 1961 to 4/m² in 1982, and the number of stations where mussels were found decreased from 16 out of 17 to only 6 during the same period (Nalepa et al., 1991).

Virginia has one of the most diverse freshwater mussel faunas of the United States consisting of approximately 73 species. Of these species 28 are considered endangered, six are threatened and seven are of special concern (Neves, 1991). In the Clinch River of Virginia and Tennessee that contains many of the species found in Virginia, the species *Epioblasma capsaeformis* that was once a dominant member of the mussel community has nearly disappeared (Dennis, 1987).

Pressure on the unionid community comes from several areas. Earlier this century, mussels were harvested and their shells were used in the button industry. Mussels are currently being harvested and nacre from their shells is used to culture pearls (Sinclair and Isom, 1963). Native mussels are also being severely affected by the destruction and loss of aquatic habitat due to human activities such as dam construction, increased urban development, logging and use of streams as livestock watering sites. Decreases in water quality of many freshwater lakes and streams due to anthropogenic pollution, including point and nonpoint discharges, are taking their toll.

Another concern being raised is that the Asian clam may be out-competing the unionids. Often, a decline in native mussels coincides with an increase in the density of Asian clams (Gardner et al., 1976; Anderson et al., 1991). Sickel (1973) noted that, "where *Corbicula* were most dense there were no unionids, even though the habitat

appeared suitable". Unionid growth has been shown to decrease as the density of Asian clams increase (Belanger, 1990). It can be difficult to determine if the Asian clam is actually out-competing the native mussels or if they are simply opportunistic invaders of habitats where the native unionid populations have been reduced by anthropogenic effects. In the relatively unaltered Buffalo River in Arkansas, *C. fluminea* are abundant only in certain patches and apparently have no negative effect on the native unionids in the system. In the Arkansas River, however, where dredging occurs to maintain navigation channels, *C. fluminea* is the most abundant benthic organism (Kraemer, 1979). Clark (1988) showed that in Atlantic coastal rivers extinction of some species of unionids coincided with population increases of Asian clams, even in undisturbed systems.

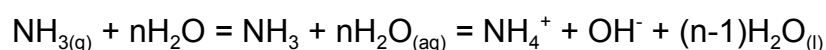
There are many parameters that may give Asian clams a competitive advantage over freshwater mussels. *Corbicula fluminea* may have a higher rate of filtration than the native unionids. Reported rates of filtration for *C. fluminea* have ranged from 11-1370 ml/hr/clam (Lauritsen, 1986; and Buttner and Hiedenger, 1981) compared to rates of 60-490 ml/hr reported for Unionidae by Mattice et al. (1973). This activity may give Asian clams an advantage when competing for food.

The Asian clam possesses a simplified life cycle that does not require a fish host to incubate its larvae as the native unionids do. This eliminates the problem of reduced transformation success that occurs when the fish host is rare or absent from the system. Native unionids are dioecious, and fertilization takes place when females siphon sperm from the water column. Low population densities may limit unionid reproductive success by reducing the chances of females being fertilized, while having little effect on the hermaphroditic Asian clams. Gametes of both sexes were present year-round in *C. fluminea* allowing the possibility of self-fertilization (Kennedy and Heukelem, 1985). Juvenile *C. fluminea* released during the spring grow rapidly, reaching sexual maturity by the following fall, allowing them to contribute to the fall spawn (Britton and Morton, 1982). Unionids, in contrast, require 3 to 9 years to reach sexual maturity (Zale and Neves, 1982). *Corbicula fluminea*'s simplified life cycle, short time to sexual maturity and its hermaphroditic nature are all advantages that may allow it to outcompete the native mussels.

It is possible that adult *C. fluminea* are having a negative impact on juvenile mussels that occur in the same area. Adult clams in feeding chambers have been observed to intake juvenile mussels by siphoning and trapping the juveniles in the mucous at the pedal gape during pedal feeding. It has also been suggested that the activity of adult clams may cause the resuspension of recently excysted juvenile clams (Yeager, 1994).

Another possible negative impact of *C. fluminea* on native unionids is the toxic effect of the ammonia produced during massive dieoffs of the Asian clam. Sinclair and Isom (1973) cited several occasions when massive dieoffs of *C. fluminea* occurred in the Tennessee River (1963). Other instances of massive mortalities of the Asian clam were reported in the Ohio and Cumberland rivers (Sickel, 1986). Often the cause of these massive dieoffs is unclear, although several reasons have been proposed. Silt loads occurring during spring flooding have been linked to annual mortalities of *C. fluminea* in the Ohio River (Bickel, 1966). A *C. fluminea* dieoff, occurring in the Tennessee River that left native mussels unaffected, was attributed to a viral infection specific to the Asian clam (Sickel, 1986). Other suggested causes of dieoffs include: high and low temperature extremes, pollution, and interspecific and intraspecific competition (Sickel, 1986).

In an aquatic environment, the unionized ammonia ion is in equilibrium with the ammonium and hydroxide ions. This equilibrium can be expressed as:



The amount of total ammonia in a solution includes the unionized species of ammonia represented as NH_3 and the ionized species NH_4^+ . The unionized species of ammonia is the component that accounts for most of the toxicity to aquatic organisms. Unionized ammonia is lipid soluble and lacks a charge, allowing it to easily pass across the gill membrane unlike the ionized form (Redner and Stickney, 1979). There are several factors that affect the percent of total ammonia present as the unionized species. Temperature and pH have the greatest influence on the NH_3 concentration. As temperature and pH increase, the concentration of unionized ammonia increases as well. Ionic strength is another factor that plays a role in NH_3 concentration, with unionized ammonia decreasing with increasing ionic strength. However, even in water with 200-300 mg/L of dissolved solids, the reduction in unionized ammonia is negligible (Emerson et al., 1975). Decomposition of organic matter is not the only source of ammonia in aquatic systems. Other sources include the fixation of nitrogen in water and sediments, and dissolved particulate matter from surface runoff. Human inputs of fertilizers, sewage treatment plants, cattle feedlots and food processing facilities also contribute to the ammonia levels in streams (Ecological Analysts, Inc., 1981).

Unionized ammonia can produce acute and chronic mortality of aquatic organisms as well as a variety of sublethal effects. In the coho salmon, *Oncorhynchus kisutch*, ammonia was found to lower the pH of the blood, reducing the oxygen carrying capacity of hemoglobin. It is assumed that if the pH drop is severe enough, it could result in suffocation of fish (Sousa and Meade, 1977). Sublethal levels of ammonia were found to interfere with the reproduction of *Channa punctatus*, decreasing the ovarian weight

and the number and diameter of mature oocytes (Dey and Bhattacharya, 1989). Redner and Stickney (1979) showed that the fish, *Tilapia aurea*, was able to acclimate to ammonia, surviving much higher ammonia concentrations after being exposed to low levels of ammonia for 35 days than fish that had not been acclimated. Bluegill can detect and avoid an ammonia gradient at 22°C and an unionized ammonia level of 0.04 mg/L NH₃ - N, although the avoidance response may have been inhibited at lower temperatures (Lubinski et al., 1978). Exposure of fish to ammonia has also been demonstrated to change feeding behavior, predator-prey behavior and urine excretion rates (Lloyd and Orr, 1969; Olson and Fromm, 1971; Woltering et al., 1978 and Hedtke et al., 1980).

While the effects of ammonia exposure to fish have been well documented, the effect on invertebrates such as mussels and clams has not. Due to restricted mobility, clams and mussels cannot avoid areas of high ammonia concentration as fish can. However, they can avoid exposure by closing their valves. Epifania and Srna (1975) noted that the marine clam, *Mercenaria mercenaria*, and the oyster, *Crassostrea virginica*, rarely opened their valves when exposed to high ammonia concentrations. The secretion of byssus threads was found to decrease as ammonia concentrations increased for the green mussel, *Perna viridis* (Reddy and Menon, 1979). Metabolic changes can also occur in mussels exposed to sublethal levels of ammonia. Ammonia has also been found to reduce and inhibit the beating of cilia in the fingernail clam, *Musculium transversum*, the Asian clam, *Corbicula fluminea*, and the mussel, *Elliptio complanata*. In this study adult fingernail clams were more sensitive than juveniles, the mussel and the Asian clam (EPA 440/5-85-001, 1984). Chetty and Indira (1994) found that the bivalve *Lamellidens marginalis* increased its use of total lipids, phospholipids and cholesterol to provide extra energy to combat exposure stress. Depletion of carbohydrates to meet extra energy needs and a drop in glycogen levels indicating a mobilization of energy through glycolysis also occurred when *L. marginalis* was exposed to ammonia (Chetty and Indira, 1995).

Asian clam dieoffs are likely to occur during summer months when river flow and dissolved oxygen (D.O.) levels are low and ambient temperature is high, stressing the resident organisms. Dieoffs occurring during this time could be particularly devastating because the high temperatures would increase the concentration of toxic unionized ammonia present. Low flow rates would allow ammonia to persist in a temporary containment area for a longer period. Low D.O. levels could increase the toxicity of ammonia released from the dying clams.

The sedentary lifestyle of unionids makes them particularly vulnerable to this type of localized phenomenon. While other species such as fish may be able to actively avoid a contaminated area, the only defense mussels have is to close their shells until water

quality improves. Whether this is a successful maneuver depends on how high the concentrations of ammonia reach and how long they persist. Juvenile and glochidial unionids do not have a thick shell to protect them, and as a result, may be more vulnerable to ammonia toxicity than their adult counterparts.

The purpose of this research is to determine whether levels of ammonia produced from the decomposition of the soft body of the Asian clam after a population dieoff are high enough to have a negative effect on native unionids present. The first objective of this study was to determine the sensitivity of unionids and the Asian clam to ammonia by comparing LC50's generated from water-only laboratory toxicity tests. The glochidia, juvenile and adult mussels were compared to determine which life stage was most sensitive. The juvenile and adult *Corbicula fluminea* were compared to each other and the glochidia, juvenile, and adult mussels to determine which was the most sensitive. The second objective was to compare the sensitivity of *C. fluminea* to ammonia in water only and sediment tests. The third objective was to determine the effect of density on mortality rate, amount of ammonia produced and reduction of dissolved oxygen resulting from an Asian clam dieoff.

CHAPTER 2 - WATER COLUMN AND SEDIMENT TESTING

Introduction

Female freshwater mussels brood their larvae called glochidia in the outer gills which undergo extensive morphological changes to create the brood chambers called marsupia (Tankersley and Dimock, 1993). When glochidia are mature they are released into the water column and must encyst on a fish host in order to mature into juvenile mussels. After completion of the parasitic lifestage, juvenile mussels fall off the fish host and continue to mature to adulthood in the sediment. Mussel mortality is highest when glochidia fail to encyst on the proper fish host or after encystment when juveniles land in an inhospitable habitat (Neves and Widlak, 1987).

Dieoffs of *C. fluminea* may impact native freshwater mussels in several ways. Ammonia levels may be high enough to kill the adults residing in the area of the dieoff. This event removes them from the area and prevents them from reproducing and contributing to the recruitment of mussels in other areas and the establishment of mussels in new areas. Although ammonia concentrations may not be high enough to kill the adults, they may have an impact on the earlier life stages. Early life stages are generally considered to be more sensitive than their adult counterparts to pollution (McKim, 1984). Juvenile mussels in the area may be killed, eliminating one age class from the area and perhaps affecting future recruitment of mussels.

Ammonia concentrations may also have a lethal effect on the glochidia if a dieoff occurs during glochidial release impacting recruitment throughout the stream. Glochidia may also be at risk during the brooding period when they are being held in the adult marsupia. Mussels are divided into two groups called bradytictic and tachytictic. Bradytictic mussels from the subfamilies Anodontinae and Lampsilinae are long-term brooders that spawn in the summer and brood glochidia until release the following spring. Some glochidia from the subfamily Lampsilinae were found throughout the year peaking in June and July (Neves and Widlak, 1988). Tachytictic mussels are short-term brooders that spawn in the spring and release their glochidia in the summer (Neves and Widlak, 1988). Long-term brooders would be at the greatest risk of having their brooding glochidia affected by a dieoff due to the long brooding period that includes the summer months. Short-term brooders and mussels from Lampsilinae may be at greater risk of having their released glochidia affected because they are released during the summer months.

Ammonia produced during dieoffs could also have an effect if the dieoff occur during the spawning stage of the mussels. Spawning is highly synchronous and occurs midsummer for many species (Zale and Neves, 1982). No toxicity testing has been done on unionid sperm but it could be a very sensitive stage. Because spawning is highly synchronous, any dieoff occurring during this critical stage could have an impact on the reproductive success of mussels that season.

Dieoffs could also have an impact on mussel recruitment by killing or damaging the fish that serve as hosts for the glochidia. Fish are mobile organisms and may avoid the dieoff area if they can detect the ammonia. If they avoid the area, then the mussel glochidia will be less likely to encounter their appropriate fish host. To determine the impact of a *C. fluminea* dieoff, it is necessary to know how sensitive the various unionid life stages are to ammonia. Very little work has been done to determine the impact of toxicants on freshwater mussels, especially at the juvenile and glochidia life stages (Keller and Zam 1991; Jacobson, 1990; and Warren et al, 1995;). Even less has been done specifically with ammonia. Work to develop standardized testing methods, for these early life stages, is still in progress.

The Asian clam and freshwater unionids are benthic organisms, and except for brief periods as glochidia and during encystment on their fish host, spend their lives in contact with sediment. Ammonia has been identified as a possible sediment contaminant in many areas (Ankley et al., 1990, Schubauer-Berigan and Ankley, 1991). In the Upper Mississippi River, total ammonia and unionized ammonia concentrations were significantly higher in the pore-water than the surface water and increased as sediment depth increased (Frazier et al., 1996). When a *C. fluminea* dieoff occurs, ammonia will be released into the sediment as the soft body of the dead clams decays.

The ammonia will eventually dissipate into the water column and be washed downstream. However, ammonia may persist in the sediment, and concentrations of ammonia may be higher in the pore-water than the overlying water. Even in areas where adult mussels were abundant and no dieoff had occurred, ammonia concentrations were higher in the interstitial than in overlying water (Buddensiek et al., 1993).

Asian clam dieoffs may affect their own population size and recruitment as well. It will reduce the number of adults found in the area of the dieoff or may eliminate them entirely. These adults may no longer be able to contribute to recruitment. Dieoffs could also kill the juveniles already in the area impacting recruitment. The objectives of this phase of the study were to 1) determine the relative sensitivity of the glochidia, juvenile and adult mussels to ammonia 2) determine the relative sensitivity of juvenile and adult *C. fluminea* and 3) compare the sensitivity of *C. fluminea*, juveniles and adults, to ammonia in sediment and water only tests.

Materials and Methods

Water Column Testing

The water used for the water column tests was obtained from Sinking Creek at Newport, VA. Water was filtered and aerated prior to being used in the test. Sinking Creek water was dosed with ammonium chloride. A control of Sinking Creek water also was used. Ammonia concentration, pH, temperature, conductivity, alkalinity and hardness of the overlying water were measured. Tests were aerated to maintain D.O. saturation where necessary. The concentration of unionized ammonia was determined using the pH, temperature and ammonia concentrations measured in the test (Thurston et al., 1979). Tests were considered successful if control survival was equal to or greater than 80%. All LC50's were calculated using the Spearman-Kärber method.

Villosa iris glochidia were obtained from gravid adults collected from the Clinch River at Pounding Mill, VA. Gravid adults were held in an incubator at 4°C in order to prevent the premature release of glochidia. Before testing began, adults were removed from the incubator and were allowed to gradually come to room temperature. Glochidia were rinsed from the gills of the gravid adults using a syringe. The viability of the glochidia was checked by taking a subsample of the glochidia and adding a drop of salt water to their container. If greater than 95% of the glochidia snapped shut, the glochidia were considered viable and used in the test. If glochidia were viable they were used for toxicity testing within two hours.

Glochidia of *V. iris* were exposed to ammonia for 24 hours in 12 well culture plates. Each well contained 5 mL of solution with concentrations of 3.12, 6.25, 12.5, 25, 50 and 100 mg/L total ammonia-N. Three replicates were used per concentration, with approximately 40 glochidia per replicate. The test was conducted in an incubator at 20°C. At the end of the 24-hour exposure period, a drop of salt solution was added to each test chamber. The glochidia that closed in response to the salt solution were considered viable. The glochidia that failed to close in response to the addition of the salt solution, and those that were closed prior to the addition of the solution, were considered dead for the purposes of calculating an LC50 (Jacobson, 1990). Water chemistry was done at the beginning of the test, and the initial ammonia concentrations were used to calculate the LC50.

Villosa iris juveniles were obtained by encysting rock bass, *Ambloplites rupestris*, or smallmouth bass, *Micropterus dolomieu*, with glochidia from adult mussels collected in the Clinch River at Pounding Mill, VA. Glochidia were tested for viability before encystment. Three tests were conducted with the juvenile mussels. The ages of the mussels used in the three tests were <72 hours, 5 days and 9 days old. Juvenile Asian clams were obtained from gravid adults collected in the Clinch and New rivers, VA. Adults were placed into the collection chamber and water was circulated through to induce the clams to release juveniles (Figure 2.1). Three tests were conducted with juvenile clams. One test was conducted with clams <48 hours old, and two others with clams one week old. Juvenile clam and mussel tests were conducted in 12-well culture plates. Each well was considered to be one replicate and contained 5 mL of test solution. Four replicates were used for each concentration, and 5 juveniles were placed in each replicate. A 0.5 serial dilution was used for both Asian clam and native mussel tests. Concentrations for the three juvenile mussel tests ranged from 0.62 - 40 mg/L total ammonia - N. Concentrations for the three juvenile clam tests ranged from 0.25 - 25 mg/L total ammonia. Tests were conducted in an incubator at 25±1°C. The juveniles were exposed for 96 hours, and mortality was recorded every 24 hours. Juveniles were considered dead if no pedal locomotion, heartbeat or cilia movement was observed. Water chemistry was done at the beginning of the test, and the initial ammonia values were used to calculate the LC50's.

Adult *C. fluminea* were obtained from the Clinch and New rivers, Virginia. Two tests were conducted with the adult clams. Adults were exposed in 1-L glass beakers. A stir plate and stir bar were used to achieve constant movement of the water in the test chambers. A piece of styrofoam was placed between the test chamber and the stir plate to prevent heat generated by the stir plate from warming the test water. Two replicates were used per concentration, with 10 clams per replicate. The first test was conducted using a 0.30 serial dilution with concentrations of 10, 30, 100, 300, and 1000 mg/L total ammonia. The second test was conducted using a 0.5 serial dilution

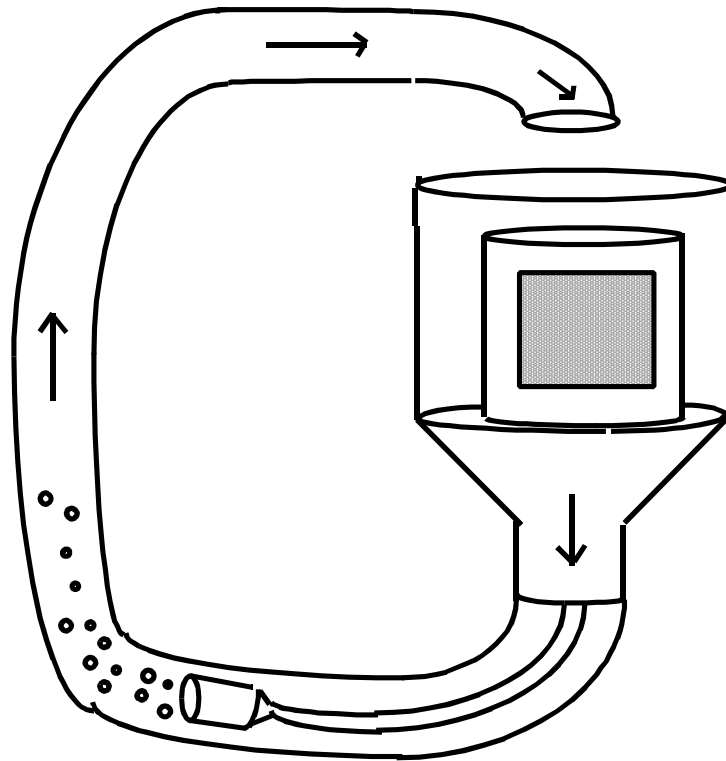


Figure 2.1 : Juvenile Clam Collection Chamber. Water circulates through the system as indicated by the arrow. Gravid adult clams are placed in the large funnel and when juveniles are released they circulate through the system and are trapped in the screened cup.

with concentrations of 6.25, 12.5, 25, 50, and 100 mg/L total ammonia. Both tests were conducted at $25\pm 2^{\circ}\text{C}$. The clams were exposed for 96 hours, and mortality was checked daily and dead clams were removed. Water chemistry was done daily, and initial and final ammonia concentrations were averaged to calculate the LC50.

Adult *Pyganodon grandis* were obtained from the Zetz's fish hatchery in Inwood, WV. Two tests were conducted with the adult mussels. Adult *P. grandis* were exposed in 16-L polycarbonate containers each holding 10 L of water. Concentrations of 10, 30, 100, 300 and 1000 ppm total ammonia-N and a control were used. The 30, 100, 300 and 1000 concentrations were mixed directly from dried ammonium chloride. The 10 mg/L solution was made from a 1000 ppm stock solution of ammonium chloride in distilled water. Two replicates were used per concentration, with 10 mussels per replicate. The average size of the mussels used in the test was 53.3 mm and they ranged from 36.3-79.1 mm. Tests were conducted at $25\pm 2^{\circ}\text{C}$. The mussels were exposed for 96 hours, mortality checked daily, and dead mussels were removed. Water chemistry was done daily, and initial and final ammonia concentrations were averaged to calculate the LC50's.

Tests with the water flea, *Ceriodaphnia dubia*, and the fathead minnow, *Pimephales promelas*, were conducted according to EPA standard testing procedures (Weber, 1991). Both the *Ceriodaphnia* and the minnows were obtained from in-lab cultures. One test was conducted with each species. Both species were exposed for 48 hours at $25\pm 1^{\circ}\text{C}$. Serial dilutions of 0.5 were used for both tests. The total ammonia concentrations for the *Ceriodaphnia* test were 12.5, 25, 50, 100 and 200 mg/L. The concentrations for the fathead minnow test were 1.56, 3.12, 6.26, 12 and 25 mg/L. Water chemistry was done at the beginning and ending of the test, and initial and final ammonia values were averaged to calculate the LC50's.

Sediment Testing

The sediment used in this test was collected from Sinking Creek at Eggleston, VA. Sediment was collected from depositional areas and sieved through a 2-mm sieve. Sediment was stored prior to use at 4°C . Water was collected from Sinking Creek at Newport, VA and aerated prior to use. Sinking Creek sediment was dosed with ammonium chloride based on sediment dry weight in a 0.5 serial dilution. Stock solutions for each concentration were made using dried ammonium chloride added to distilled water. A 10-mL aliquot of the appropriate stock solution was added to the measured sediment in the test chamber and mixed for 1 minute. All sediment tests were conducted using a 4:1 water to sediment ratio. Temperature, pH, D.O. and conductivity of the overlying water were measured daily. Ammonia concentration was measured at the beginning, midpoint and end of the test. LC50's were calculated

based on the average ammonia concentration measured in the overlying water. Tests were aerated when necessary to maintain D.O. at saturation. The concentration of unionized ammonia was determined using the pH, temperature and ammonia concentrations measured in the test (Thurston, 1979). All LC50's were calculated using the Spearman - Karber method.

Juvenile *C. fluminea* used in the sediment tests were obtained from gravid adults collected in the Clinch and New rivers, VA. Five tests were conducted. Three tests were run using 1 week old clams, one test using 3 week old individuals and another with clams 8 weeks old. Juvenile clam sediment tests were run in 500-mL glass beakers. One-hundred milliliters of wet sediment was measured for each replicate and placed in the test chamber. After being dosed with ammonium chloride, water was gently poured into the test chamber, to avoid disturbing the sediment, until it reached the 500-ml mark. The juveniles were placed in tubes constructed from PVC uplift tubing and 105 u nitex screen that prevented the escape of the juveniles but allowed flow of water into the tubes. One tube was placed into the test container and inserted into the sediment. Four replicates were used per concentration, and 5 juveniles were used per replicate. Juveniles were exposed for 96 hours. At the end of the 96-hour period, tubes were removed from the beaker, juveniles were extracted and mortality was recorded.

Adult *C. fluminea* were collected from the Clinch and New rivers, VA. Two tests were conducted with the adults. Adult clam sediment tests were conducted in 1-L glass beakers. Two-hundred milliliters of wet sediment was measured and placed into each test chamber. After the sediment was dosed with ammonium chloride, water was gently poured into the test container until it reached the 1- L mark. Two replicates were used per concentration, with 10 clams per replicate, and exposure lasted 96 hours. Mortality was checked daily, and dead clams were removed.

Results

Water Column Testing

Villosa iris glochidia was one of the organisms most sensitive to ammonia, with a 24-hour LC50 of 3.29 mg/L total ammonia and 0.11 mg/L NH_3 - N (Table 2.1). Raw data used in the analysis are presented in Appendix A. Control survival for the glochidia was high at 97.4%. The juvenile mussel, *V. iris*, and the adult mussel, *Pyganodon grandis*, showed similar sensitivity to unionized ammonia with overlapping 95% confidence intervals and average 96-hr LC50's approximately 5 times higher than for the *V. iris* glochidia (Figure 2.2). The *V. iris* 96-hour LC50's for the <72-hour, 5-day

Table 2.1 : The 96-hour LC50 values for water-only exposure to ammonium chloride of the various life stages of the freshwater mussels, *Villosa iris* and *Pyganodon grandis*.

Species	Stage	Age	LC50 Total Ammonia mg/L (95% C.I.)	LC50 NH ₃ mg/L (95% C.I.)
<i>V. iris</i> *	Glochidia	<24hrs	3.29 (2.90 - 3.73)	0.11 (0.10 - 0.12)
<i>V. iris</i>	Juvenile	5 days	7.81 (7.00 - 8.70)	0.62 (0.57- 0.67)
<i>V. iris</i>	Juvenile	<72hrs	7.07 (no limits)	0.56 (no limits)
<i>V. iris</i>	Juvenile	9 days	5.52 (4.36 - 6.98)	0.38 (0.33 - 0.46)
<i>P. grandis</i>	Adult		25.13 (11.47 - 55.05)	0.44 (0.20 - 0.97)
<i>P. grandis</i>	Adult		18.84 (9.72 - 36.52)	0.54 (0.31 - 0.76)

*24-hour LC50.

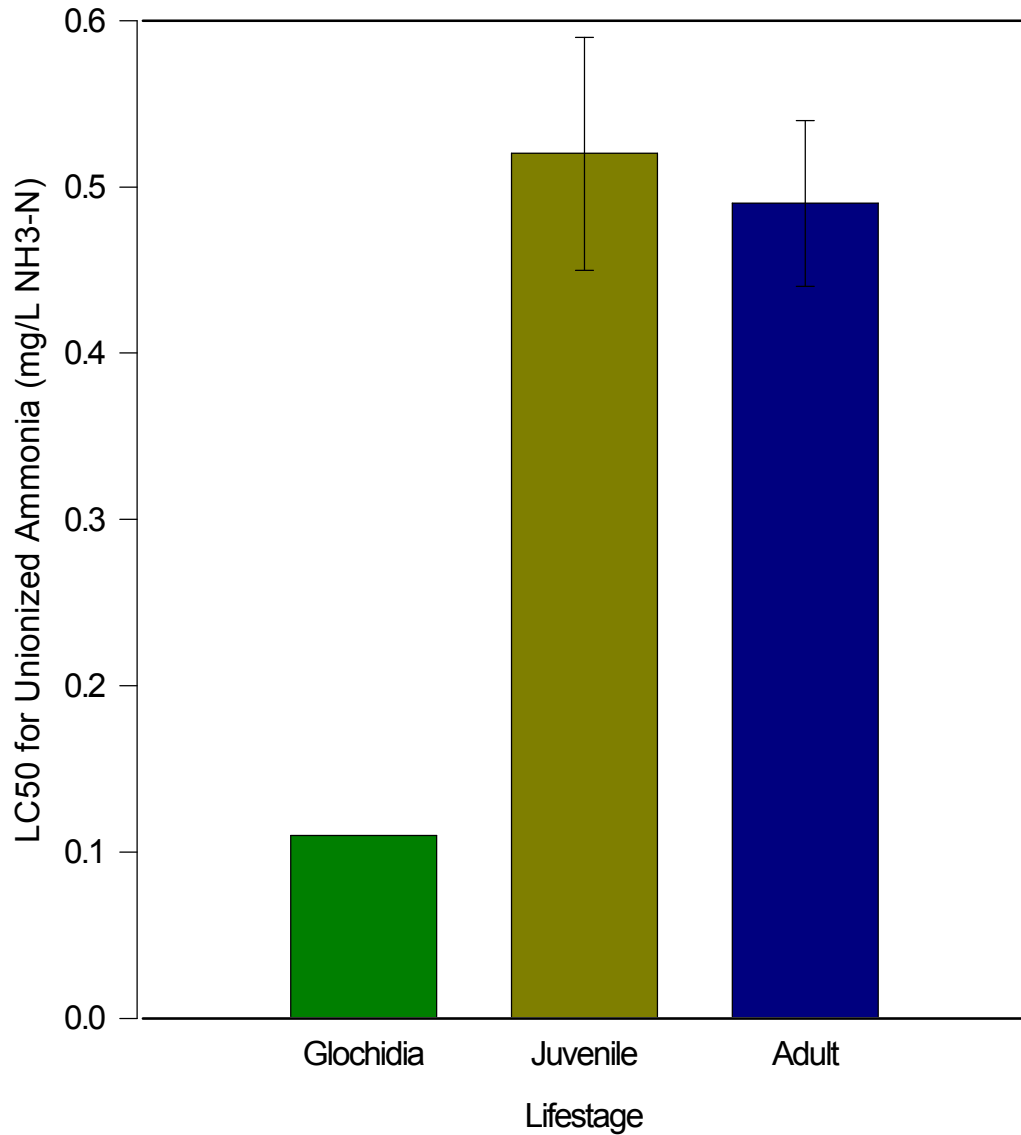


Figure 2.2 : A comparison of the average LC50's of the various mussel life stages. The 24-hour LC50 for glochidia (n=1), and the 96-hour LC50 for juveniles (n=3) and adults (n=2).

and 9-day old mussels were 7.07, 7.81 and 5.52 mg/L total ammonia and 0.56, 0.62 and 0.38 mg/L NH_3 -N, respectively. Control survival of the juvenile mussels ranged from 80-100%. The 96-hour LC50's for the two adult *P. grandis* tests were 25.13 and 18.84 mg/L total ammonia and 0.44 and 0.54 mg/L NH_3 -N (Figure 2.3). Control survival for the two tests was 95 and 100%.

Juvenile Asian clams were approximately four times more sensitive to unionized ammonia than the adults. The 96-hour LC50's for the one test with 48-hour old juvenile *C. fluminea*, and the two with one-week old clams were 2.25, 1.00 and 1.78 mg/L total ammonia and 0.28, 0.09 and 0.18 mg/L NH_3 -N, respectively (Table 2.2). Control survival for all three tests was 100%. The 96-hour LC50's for the adult *C. fluminea* tests were 13.96 and 14.55 mg/L total ammonia and 0.88 and 0.71 mg/L NH_3 -N. Control survival for both adult *C. fluminea* tests was 100%. Juvenile unionid *V. iris* were about 2 times less sensitive to unionized ammonia than juvenile *C. fluminea*. However, the opposite trend was seen for the adults. The average 96-hour LC50 for *P. grandis* was almost 40% lower than that for *C. fluminea*, although the broad 95% confidence intervals of *P. grandis* overlapped those of the Asian clam (Tables 2.1 and 2.2).

Of the two EPA standard tests organisms used, *Ceriodaphnia dubia* was much more sensitive to unionized ammonia than *Pimephales promelas* (FHM), with 48-hour LC50's of 0.07 and 1.19 mg/L and control survival of 95 and 100%, respectively. In fact *C. dubia* had the lowest LC50 of all organisms tested, although the 95% C.I. did overlap the intervals of the *V. iris* glochidia and juvenile *C. fluminea*, indicating similar sensitivity of these organisms (Table 2.3). In contrast, the FHM was the least sensitive organism tested, and its 95% confidence intervals did not overlap with any of the other organisms tested.

Sediment Testing

Juvenile *C. fluminea* tested in sediment dosed with ammonium chloride were 10 times more sensitive to unionized ammonia than similarly exposed adults (Figure 2.4). The 96-hour LC50's for the three tests using one-week old *C. fluminea* ranged from 1.31 - 6.32 mg/L total ammonia and 0.19 - 0.41 mg/L NH_3 -N. The LC50's for the 3- and 8-week old *C. fluminea* were 1.09 and 0.76 mg/L total ammonia and 0.17 and 0.52 mg/L NH_3 -N. Raw data used in the analysis are presented in Appendix B. Control survival for the one- and three-week old *C. fluminea* tests conducted on 6/29/95, was 37 and 51%. These tests were run simultaneously in the same test containers but different tubes. Control survival for replicate A was 0 and 20% and for replicate B, 86.7 and 76.1%. Survival for the water reference was 80 and 83%. Because control survival was low in only one replicate and survival was acceptable in the water reference, the results from the two tests were still used. Control survival for the remaining tests ranged from 83 -

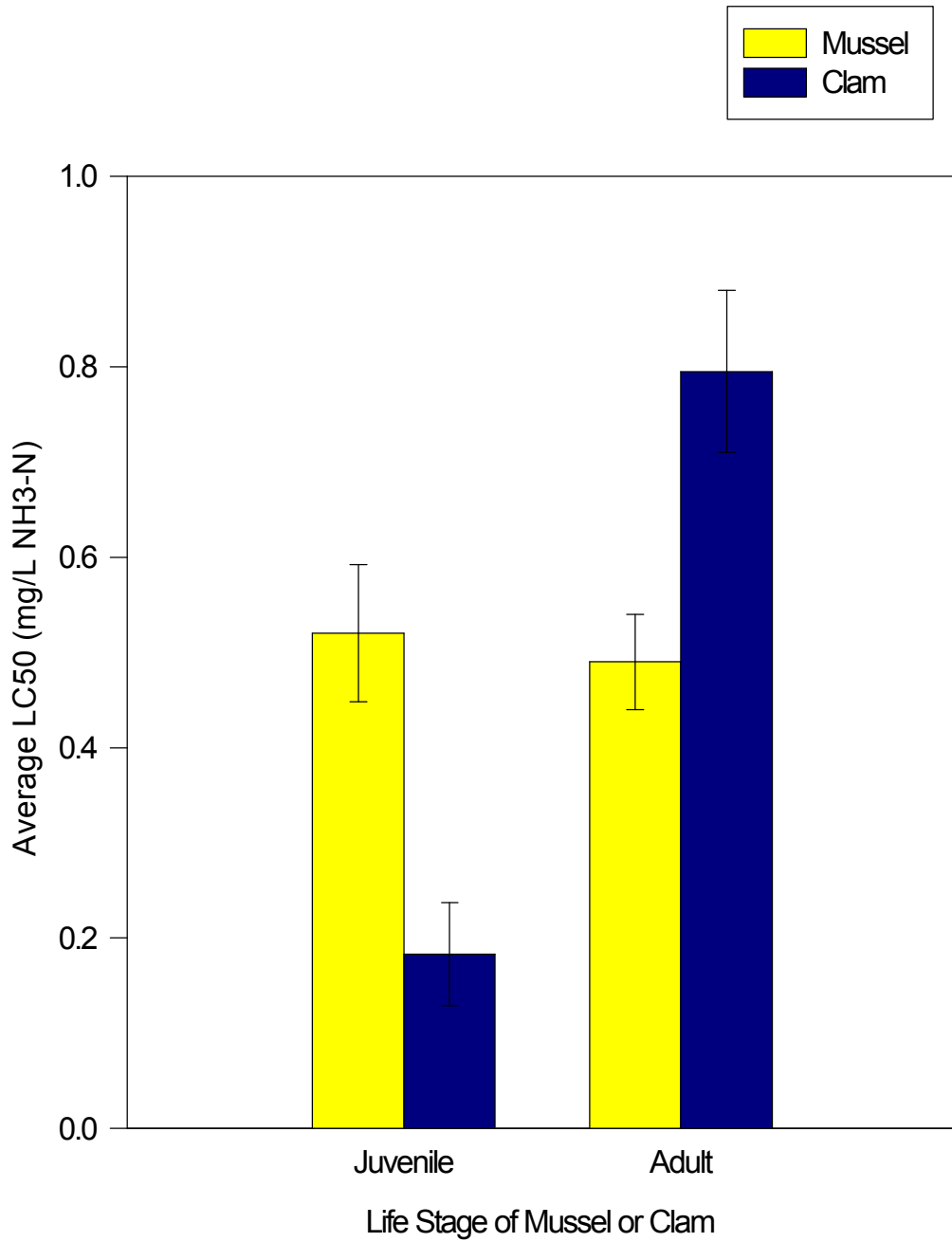


Figure 2.3 : A comparison of average 96 - hour LC50's for juvenile *V. iris* (n=3), juvenile clam (n=3), the adult *A. grandis* (n=2) and adult *Corbicula* (n=2).

Table 2.2 : The 96-hour LC50 values for water only exposure of juvenile and adult *Corbicula fluminea* to ammonium chloride.

Stage	Age	LC50 Total Ammonia mg/L (95% C.I.)	LC50 NH ₃ -N mg/L (95% C.I.)
Juvenile	<48hrs	2.25 (1.83 - 2.76)	0.28 (0.23 - 0.35)
Juvenile	1 week	1.00 (0.82 - 1.24)	0.09 (0.07 - 0.11)
Juvenile	1 week	1.78 (1.50 - 2.12)	0.18 (0.15 - 0.21)
Adult		13.96 (11.98 - 16.26)	0.88 (0.78 - 0.99)
Adult		14.55 (no limits)	0.71 (no limits)

Table 2.3 : Results of the 48-hour exposure of the EPA standard test organisms, *Ceriodaphnia dubia* and *Pimephales promelas* (fathead minnow), to ammonium chloride.

Name	Age	LC50 Total Ammonia mg/L (95% C.I.)	LC50 NH ₃ -N mg/L (95% C.I.)
<i>C. dubia</i>	<24 hrs	14.52 (11.26 - 18.72)	0.07 (0.05 - 0.11)
<i>P. promelas</i>	< 24 hrs	8.96 (8.15 - 9.87)	1.18 (1.08

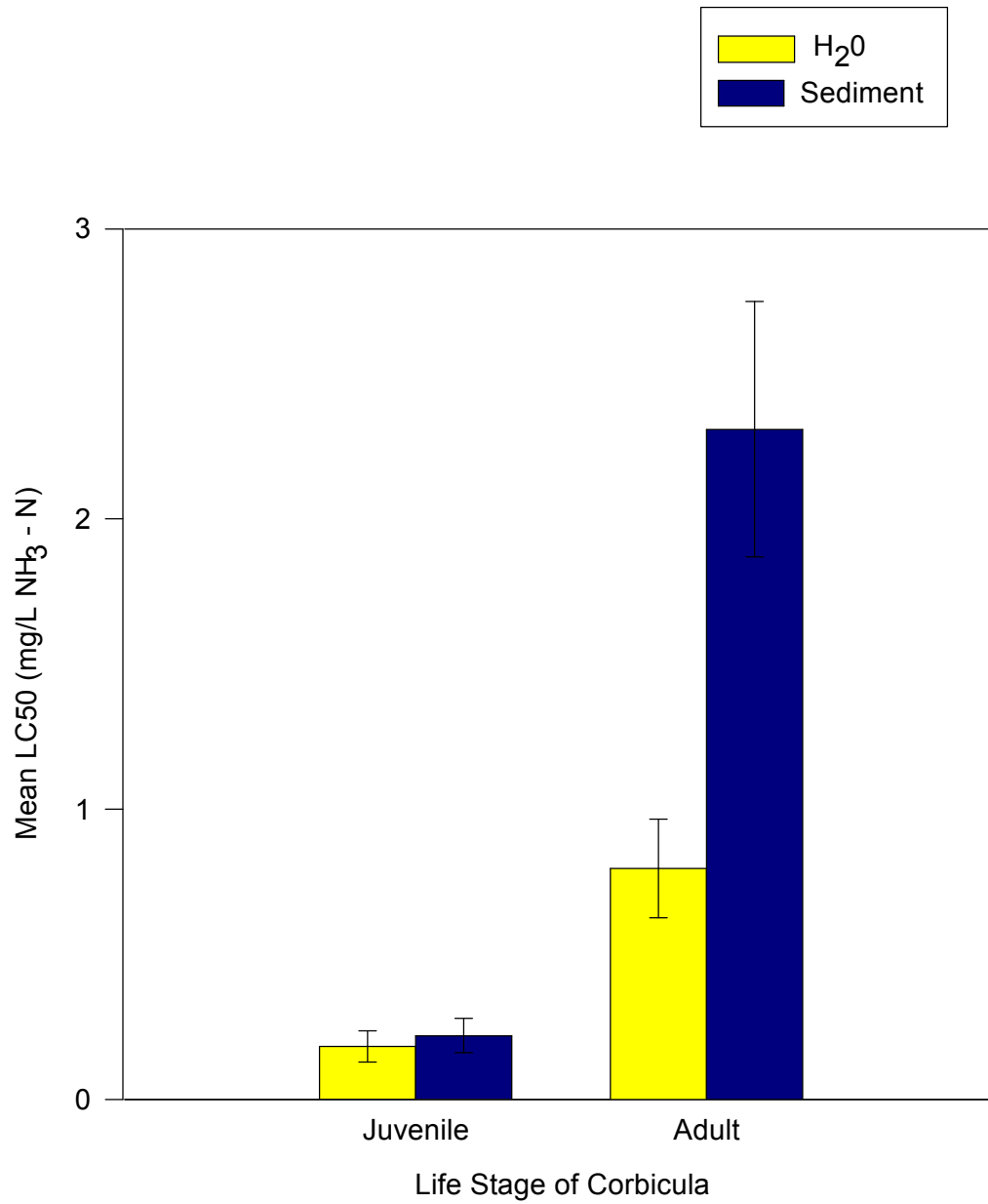


Figure 2.4 : A comparison of mean 96-hour LC50's for juvenile and adult *Corbicula* tested in sediment and water only.

100%. Juvenile *C. fluminea* had similar sensitivity to unionized ammonia when tested with sediment or with water only, having overlapping standard errors (Figure 2.3) and overlapping 95% confidence intervals (Table 2.2 and 2.4). The 96-hour LC50's for the two adult *C. fluminea* tests were 28.04 and 32.04 mg/L total ammonia and 1.87 and 2.75 mg/L NH₃-N. Control survival for both tests was 100%. Adult *C. fluminea* showed differing sensitivity depending on exposure method. Adult *C. fluminea* were almost 3 times more sensitive to ammonia when tested in water only than when tested in sediment.

Table 2.4 : *Corbicula fluminea* 96 - hour sediment test LC50's for the exposure of juveniles and adults to ammonium chloride.

Stage	Age	LC50 Total Ammonia mg/L (95% C.I.)	LC50 NH ₃ mg/L (95% C.I.)
Juvenile	1week	6.32 (5.45-7.32)	0.41 (0.37-0.47)
Juvenile	1week	4.61 (3.65-5.83)	0.29 (0.24-0.36)
Juvenile	1week	1.31 (1.04-1.66)	0.19 (0.16-0.24)
Juvenile	3weeks	1.09 (0.83-1.43)	0.17 (0.14-0.21)
Juvenile	8weeks	0.76 (0.57-1.01)	0.52 (0.40-0.70)
Adult		28.04 (22.23-35.36)	1.87 (1.57-2.24)
Adult		32.04 (24.25-43.28)	2.75 (2.17-3.48)

Discussion

Water Column Testing

As expected the glochidial life stage of the freshwater mussel was much more sensitive to the effects of unionized ammonia than adult mussels. Similarly, glochidia of *V. iris* were found to be much more sensitive to copper than adults (Jacobson et al., 1997). Although a dieoff may not produce ammonia levels high enough to affect adults, it may effect reproductive success if the dieoff occurs during glochidial release. The LC50 of 0.11 mg/L of NH_3 -N for *V. iris* glochidia is lower than that found by Goudreau et al. (1993) of 0.284 mg/L and may indicate natural variation in mussel populations.

One surprising result was the similar sensitivity of the adult and juvenile mussels to unionized ammonia. Different species were used which makes direct comparisons difficult. Perhaps *Pyganodon grandis* is in general a more sensitive species than *Villosa iris*, and this would have been seen if the juvenile and adult of both species could have been tested. It may be that the thick shell of the adults does not give an advantage in survival, but this seems unlikely as adults in the higher concentrations of ammonia were observed to close up. Although dead adults were removed from the test chambers daily and test chambers were aerated to prevent the D.O. from sagging, the rotting body mass of dead mussels may have had an effect on water quality that increased the mortality of mussels at the higher concentrations of ammonia.

As expected, juvenile *C. fluminea* were more sensitive to unionized ammonia than the adults. It was observed that like adult *Pyganodon grandis*, adult *C. fluminea* also closed their shells at high ammonia concentrations. This avoidance mechanism may allow them to survive longer at higher concentrations than juveniles. Adult *C. fluminea* have also been observed to decrease the amount of time their valves are open and increase the amount of time with valves closed as concentrations of Cd and Zn increased (Doherty et al., 1987). It is not known whether juveniles close their shells in response to high concentrations of ammonia, but it has been observed that juvenile *C. fluminea* could remain closed for 24 hours when exposed to copper (Harrison et al., 1984). In addition the smaller juvenile clam's and mussel's filtration rates and gill areas are relatively higher than the adult, increasing the potential of being exposed to contaminants (Muncaster et al., 1990). The higher filtration and metabolic rate of juveniles would reduce the amount of time the juveniles could remain closed compared to the adults. In tests to determine the effectiveness of ammonia as a biocide for *C. fluminea*, it was found that sensitivity to ammonia was size dependent. The LT50 and LT100 (lethal time) decreased as the size of the

clams decreased (Belanger et al., 1991). Juvenile *C. fluminea* also were found to be much more sensitive to copper exposure than their adult counterparts (Harrison et al., 1984). The greater sensitivity of juveniles to unionized ammonia implies that any dieoff of the adult population would have an effect on the juveniles in the area of the dieoff as well.

The lower sensitivity of the juvenile mussel, *V. iris*, to unionized ammonia than the juvenile Asian clam may provide a competitive advantage for the mussels found in areas of elevated ammonia concentrations. When comparing adults, however, the Asian clam has the advantage over *A. grandis*. It was found that during a seven-day exposure to 5 mg of total ammonia that two species of mussels, *Amblema p. plicata* and *Utterbackia imbecillis*, had higher percent mortality than *C. fluminea*, and two mussel species, *Cyrtornais tampicoensis* and *Toxolasma texasensis*, had lower mortality (Horne and McIntosh, 1979). Obviously, generalizations about the greater sensitivity of mussels compared to the Asian clam must be made carefully, and testing of individual species may be necessary.

The relatively high sensitivity of *C. dubia* to unionized ammonia indicated that this may be an appropriate organism for setting water quality criteria standards for ammonia. However, given that the FHM was the least sensitive of all species tested it would be inappropriate to use this species as a means of assessing the effects of ammonia on the environment. This same pattern of sensitivity was seen when juvenile mussels were exposed to metals. *Ceriodaphnia dubia* showed similar sensitivity to metal exposure as the juvenile mussel, *Utterbackia imbecillis*, while the fish (bluegill) were not as sensitive (Keller and Zam, 1991).

The EPA document Ambient Water Quality Criteria for Ammonia (1984) has a listing of LC50's from the literature for various organisms. The listing for fish, especially salmonids, is rather extensive but there are few listings for invertebrates. The only bivalve listed is the clam, *Musculium transversum*, with LC50's comparable to adult *C. fluminea* (Table 2.5). The values given for the fathead minnow agree with the LC50 obtained in my research. The most sensitive species listed were the salmonids with LC50's comparable to juvenile *V. iris*. The mayfly, amphipod and isopod were all relatively insensitive to unionized ammonia with LC50's higher than any of the organisms tested. None of the organisms listed in the EPA document were as sensitive as the *V. iris* glochidia.

The water quality criteria given by the EPA is dependant upon the pH, temperature and the presence or absence of salmonids or other sensitive coldwater species. The one-hour average concentrations for ammonia which are not to be exceeded more than once every three years on the average, range from 0.0075 - 0.21

Table 2.5 : Range of 48 or 96 - hour LC50 values obtained from the EPA document Ambient Water Quality Criteria for Ammonia (EPA 440/5 - 85 - 001, 1984).

Common Name	Species Name	Life Stage or Size	LC50 (mg/L NH ₃ -N)
Clam	Musculium transversum	Adult	0.82 - 0.90
Snail	Physa gyrina	Adult	1.30 - 2.05
Fathead Minnow	Pimephales promelas	0.03-0.5 g	0.60 - 1.54
Coho Salmon	Oncorhynchus kisutch	Juvenile	0.22 - 0.72
Chinook Salmon	Oncorhynchus tshawytscha	15 - 18 g	0.33 - 0.39
Rainbow Trout	Oncorhynchus mykiss	0.61-1.47g	0.32 - 0.84
Cladoceran	Daphnia magna	< 24 hr old	0.45 - 2.28
Mayfly	Ephemerella grandis	10 - 11mm	3.17 - 4.83
Amphipod	Crangonyx pseudogracillis	Adult	1.33 - 4.63
Isopod	Asellus racovitzal	12mm	2.42
Crayfish	Orconectes lamunis	Adult	18.74

mg/L NH₃-N when salmonids or other sensitive coldwater species are present, and from 0.0075 - 0.30 mg/L NH₃-N when they are absent. Although these levels exceed the concentrations required to produce toxicity in *V. iris* glochidia and juvenile *C. fluminea*, these levels are only allowed to be exceeded for a short period of time. Hence, they may still be protective. The four-day average concentrations for ammonia are 0.0005 - 0.029 mg/L NH₃-N when salmonids are present, and 0.0006 - 0.041 mg/L NH₃-N when they are absent. These values are lower than any concentrations that produced acute toxicity in mussels and clams and should be protective of them. Site specific-criteria can also be developed based on only the species present in the system. Anyone wanting to develop a site specific criterion in an area containing mussels should consider the effects on all life stages in order to protect the species.

Sediment Testing

As expected, juvenile Asian clams showed the same trend of increased sensitivity when compared to adults, as was seen in the water column tests. *Corbicula fluminea* are benthic organisms, and sediment tests more closely mimic the environment in which they are normally found. By testing benthic organisms, such as *C. fluminea*, in tests with water and no sediment, an additional stress factor may be added. This may account for the greater sensitivity of adult *C. fluminea* tested in water-only compared to adults tested in sediment. In a comparison of spiked-sediment and water-only tests for the oligochaete, *Lumbriculus variegatus*, and the midge, *Chironomus tentans*, similar LC50's were found for both test methods, with the organisms being slightly less sensitive in the sediment tests (Whiteman et al, 1996). In contrast to adults, juveniles showed very similar sensitivities to unionized ammonia in the water-only and the sediment tests. This result may be an artifact of the testing procedure. The juveniles were held in the PVC uplift tube which was placed down in the sediment. While the juveniles were in contact with the interstitial water, they had no direct contact with the sediment and this may account for the similar results of the two testing methods.

In summary, differences are seen in sensitivity to unionized ammonia at different life stages for both the Asian clam and freshwater mussels. The type of testing method used such as water-only and sediment tests will also affect the sensitivity of the organism to ammonia. The most sensitive life stages are the early ones such as the glochidia and the juvenile, although some overlap occurs depending on the species. Further testing on different species as well as brooding glochidia and mussel sperm will be necessary to fully assess the impact of ammonia on freshwater unionids. The usefulness of this type of research is not limited to determining the impact of Asian clam dieoffs on clams and mussels. It can also be used to determine

possible impacts of ammonia input from all sources, including wastewater treatment plants and fertilizer runoff from agricultural lands.

CHAPTER 3 : ASIAN CLAM DIEOFFS IN MINIATURE ARTIFICIAL STREAMS

Introduction

There may be a variety of factors impacting the occurrence and severity of Asian clam dieoffs. These include *C. fluminea* density, dissolved oxygen concentrations, temperature, pH, pollutants and sedimentation. *Corbicula fluminea* density may be one of the primary contributing factors. In areas with high densities of clams, demand for oxygen and food will be higher. Increases in the production of waste products will also be seen. The amount of ammonia produced as the result of a *C. fluminea* dieoff should increase as the density of *C. fluminea* increases.

The density of *C. fluminea* varies greatly from area to area and over time. Factors affecting density may include food availability, sediment and water quality, streambed stability, time of year, and length of time the species has been present in the system. Payne et al. (1989) reported that an abundance of larger *C. fluminea* was associated with more stable streambed, and populations of mostly small clams were thought to be due to flooding. In the Altamaha River in Georgia, *C. fluminea* populations were found to reach maximum densities in late summer or fall, and minimum densities in winter and spring (Gardner et al., 1976). However, other reports have given peak densities in the winter and lower densities during the summer (Aldridge and McMahon, 1978 and Miller and Payne, 1993). In the Mechums River in Charlottesville, Virginia, 173 clams/m² were collected in June and 1459 clams/m² in December. Mean *C. fluminea* densities in the Ohio River were reported to range from 66.8 - 1352.8/m² (Miller and Payne, 1993). In a power plant discharge canal in Texas, peak clam densities of 16,938/m² and 7,656/m² were seen in early spring and fall corresponding with the accumulation of recently released juveniles (Williams and McMahon, 1986). Perhaps one of the most impressive changes in clam density over time was reported in the New River, Virginia. Clam density increased from 2,529/m² in July of 1981 to 269,105/m² by September of 1981 (Cherry et al., 1986).

Low D.O. and high water temperature may also be important contributing factors to *C. fluminea* dieoffs. During the summer, water temperatures can be very high especially in areas with little shading. In addition high temperatures reduce the saturation level of oxygen. Owen and Cahoon (1991) found that at temperatures of 30°C and anoxia, 50% mortality of clams occurred in 15 hours. In a study using

immature clams (8.00-11.00mm) increasing temperature from 16 - 30°C resulted in increased respiration rate, increased filtration rate, decreased assimilation efficiency and decreased activity levels (Foe and Knight, 1986). McMahon and Williams (1986) reported the complete elimination of *C. fluminea* from a power station discharge canal when average water temperatures exceeded 36°C. In the Vermillion River, Louisiana, clam density and growth were found to be related to dissolved oxygen concentration and the presence of sewage treatment plant discharges with unionized ammonia concentrations from 0.46 - 0.91 mg/L NH₃ (Belanger, 1991).

The objective of this study was to create dieoffs of Asian clams in artificial streams to determine how the density of clams affects the rate at which a dieoff occurs, the amount of ammonia produced, and the dissolved oxygen levels. The results from the artificial stream dieoffs were compared to the LC50's generated in laboratory water column and sediment tests to determine if enough ammonia was produced to negatively affect any mussels present.

Materials and Methods

Miniature Artificial Stream Asian Clam Dieoffs

System Description

Miniature artificial streams were made of vinyl gutters and allowed one way flow of water through the stream. The streams were 47.7 cm long, 11.4 cm wide and 6.4 cm deep. They had a surface area of 0.05 m² and contained approximately 1 L of sediment and 2 L of water. Water drained through hoses made from airline tubing into the streams from a headbox.

Test Description

Streams were filled with sediment and had water flowing through them at a rate of 30mL/min. Blacksburg tapwater dechlorinated with sodium thiosulfate was used for the tests. Sediment was collected from depositional areas of Sinking Creek, Eggleston, VA. Rocks and debris larger than ~ 5 cm were removed manually from the sediment. Sediment was stored at 4°C prior to use. One liter of sediment was placed into each of the streams after which flow was initiated. After the streams had filled, adult clams were placed into each stream at the appropriate densities. Control, low, medium and high densities streams containing 0, 2,000, 5,000 and 10,000 clams/m² were used. Since the surface area of the artificial streams was 0.05 m² the actual number of clams in each stream was 100, 250 and 500 clams in the low,

medium and high density streams, respectively. There were two replicates for each density. Adult *Corbicula fluminea* ranging in size from 5 - 25 mm were used in the test. In order to assure an even size distribution of clams at each density, clams were divided into three size classes of small (5.0 - 10.0 mm), medium (10.1 - 15.00 mm) and large (15.1 - 25.0 mm). Equal percentages of each class were placed in each stream. Two separate tests were run each consisting of three different phases. During the first phase, flow to the streams was 30 mL/min, while flow was stopped in the second. After a dieoff had occurred and ammonia levels had begun to drop, flow was reinitiated for the third phase. The test ended when ammonia concentrations in the streams experiencing a dieoff returned to levels near that of the controls. During the static period, water removed for ammonia analysis was replaced as needed. The water added was dosed with ammonia so that the concentration matched that of the stream to which it was added.

At test termination *C. fluminea* were removed from the streams, and dead and living clams were separated, counted and frozen. Shell length was measured using digital calipers, and the median lengths of dead and living clams were tested for significance using a Mann-Whitney Rank Sum Test. A p-value of less than 0.05 was considered to be significant. Temperature, pH, D.O., conductivity, and mortality were recorded daily. Ammonia measurements were made every other day until a dieoff occurred, and then they were made daily.

A repeated measures analysis was done to determine if there were any significant differences in unionized ammonia concentration, mortality, D.O., pH and conductivity 1) at different densities; 2) over time; 3) at different densities over time, and 4) at different temperatures. A p-value of less than 0.05 was considered to be significant. If the variable measured was not significantly different at different temperatures, then temperature was removed from the analysis. Data from the first, second, and third phases of each test were analyzed separately.

Test I

The first artificial stream dieoff lasted 64 days and ran from January 19, 1996 to March 23, 1996. Temperature was not controlled and fluctuated with the laboratory temperature, ranging from 16.3 to 24.1°C (average of 21.7°C). Clams were collected from the New River and the Clinch River, VA. The size distribution of the clams was 47% small, 37% medium and 16% large. The first phase of the test lasted from day 0 to day 13, while the second static phase lasted from day 14 until day 42. The third phase of the test started when flow was reinitiated (day 42) and continued until test termination (day 64).

Test II

The second experiment lasted 34 days and ran from July 24, 1996 to August 27, 1996. Water temperature during the test was controlled using aquarium heaters to maintain the temperature at $26\pm 2^{\circ}\text{C}$. The size distribution for clams in this test was 27% small, 59% medium and 14% large. The first phase of the test lasted from day 0 to 14, and the second static phase from day 14 until day 24. The third phase started on day 24 and continued until test termination on day 34.

Results

Test I

Clams at the highest density ($10,000/\text{m}^2$) experienced a 100% dieoff (Figure 3.1). The average mortality at the end of the experiment in the low and medium density streams was 35.5% and 38.6%, respectively. Complete mortality and water chemistry data is presented in Appendix C. Mortality gradually increased over the first 14 days of the test at all three densities. During this period mortality was not significantly different ($\alpha = 0.05$) at different densities, but was significantly different over time and at different densities over time (Table 3.1). The gradual increase in mortality continued during the second phase of the test until day 34. After day 34 mortality in the high density streams increased sharply, and on day 38 all clams were dead. Mortality in the low and medium density streams continued to increase gradually until test termination. Significant differences in mortality were observed at different densities for the second and third phases. Mortality was also significantly different over time and at different densities over time.

Ammonia concentrations were low for the three clam densities and the control for the first phase of the test, ranging from 0.00004 - 0.0015 mg/L NH_3 -N (Figure 3.2). No significant differences were found in unionized ammonia concentrations at different densities, over time or at different densities over time. After flow was terminated, unionized ammonia levels remained low until day 25 when a gradual increase in ammonia levels occurred in the high density streams. On day 34 a sharp increase in ammonia levels occurred in the high density streams as mortality began to sharply increase. A peak unionized ammonia concentration of 4.06 mg/L was measured in the $10,000$ clams/ m^2 stream four days after mortality reached 100%. Unionized ammonia concentrations were significantly different at

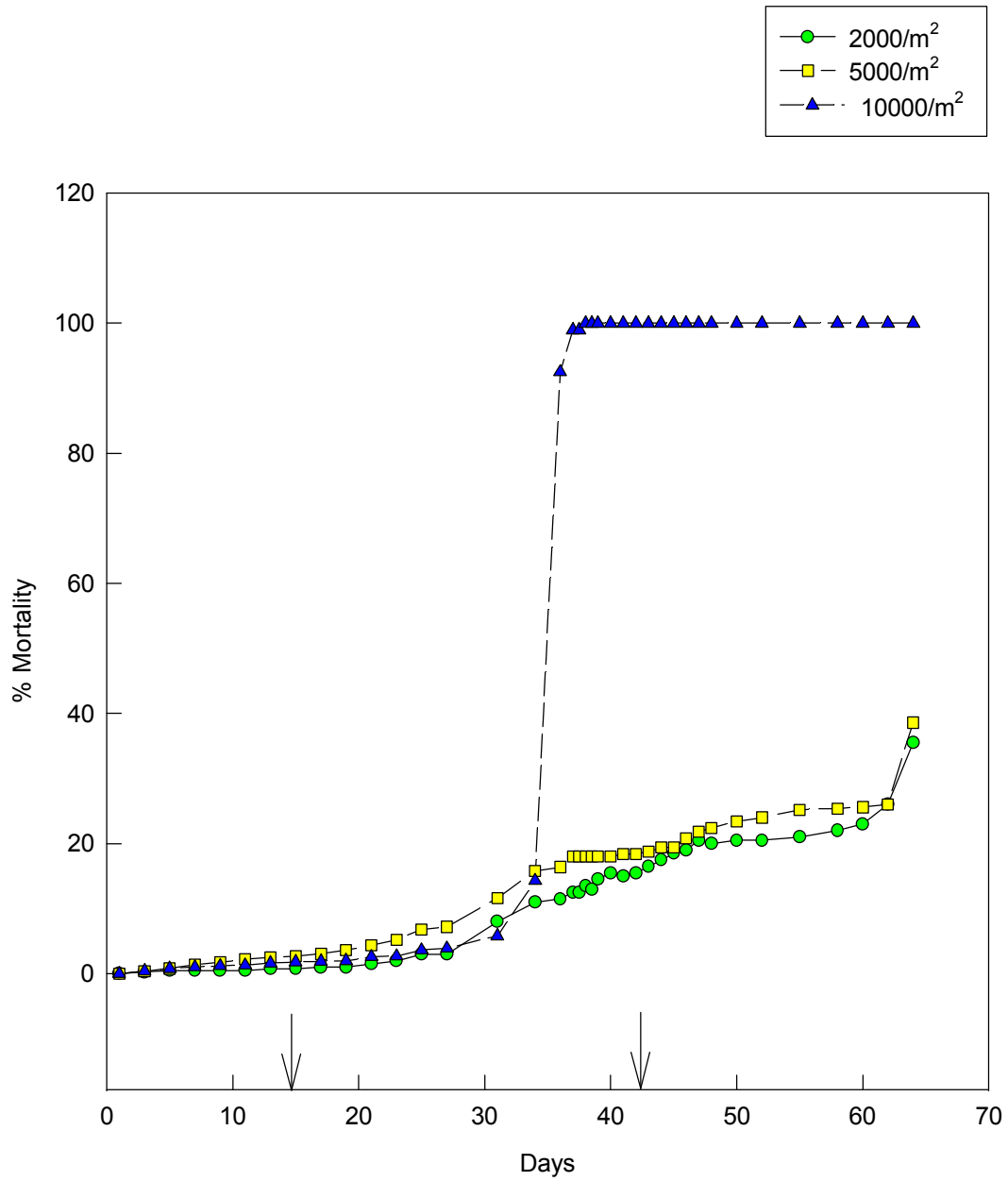


Figure 3.1 : Miniature Artificial Stream Test I
 Percent Mortality at Different Clam Densities
 (arrows indicate start of second and third test phases)

Table 3.1 : Results (p-values) of the repeated measures analysis of test I to determine significant differences at different densities, over time and at different densities over time for unionized ammonia concentration, mortality, D.O., pH, and conductivity. (significance is $p < 0.05$)

Variable	Phase 1	Phase 2	Phase 3
NH₃-N			
Density	0.7603	0.0066	0.0105
Time	0.3317	0.0001	0.0007
Time*Density	0.8207	0.0001	0.0001
Temperature	-	0.0026	-
Mortality			
Density	0.9220	0.0031	0.0005
Time	0.0001	0.0001	0.0001
Time*Density	0.0001	0.0001	0.0001
Temperature	-	-	0.0001
D.O.			
Density	0.1112	0.0852	0.0016
Time	0.8899	0.0085	0.0352
Time*Density	0.7525	0.0001	0.0001
Temperature	-	0.0002	0.0029
pH			
Density	0.7359	0.6534	0.1404
Time	0.0482	0.0001	0.0001
Time*Density	0.9176	0.0001	0.1596
Temperature	-	-	0.0305
Conductivity			
Density	0.8894	0.0031	0.1032
Time	0.0001	0.0001	0.0001
Time*Density	0.9155	0.0001	0.0276
Temperature	-	-	0.0001

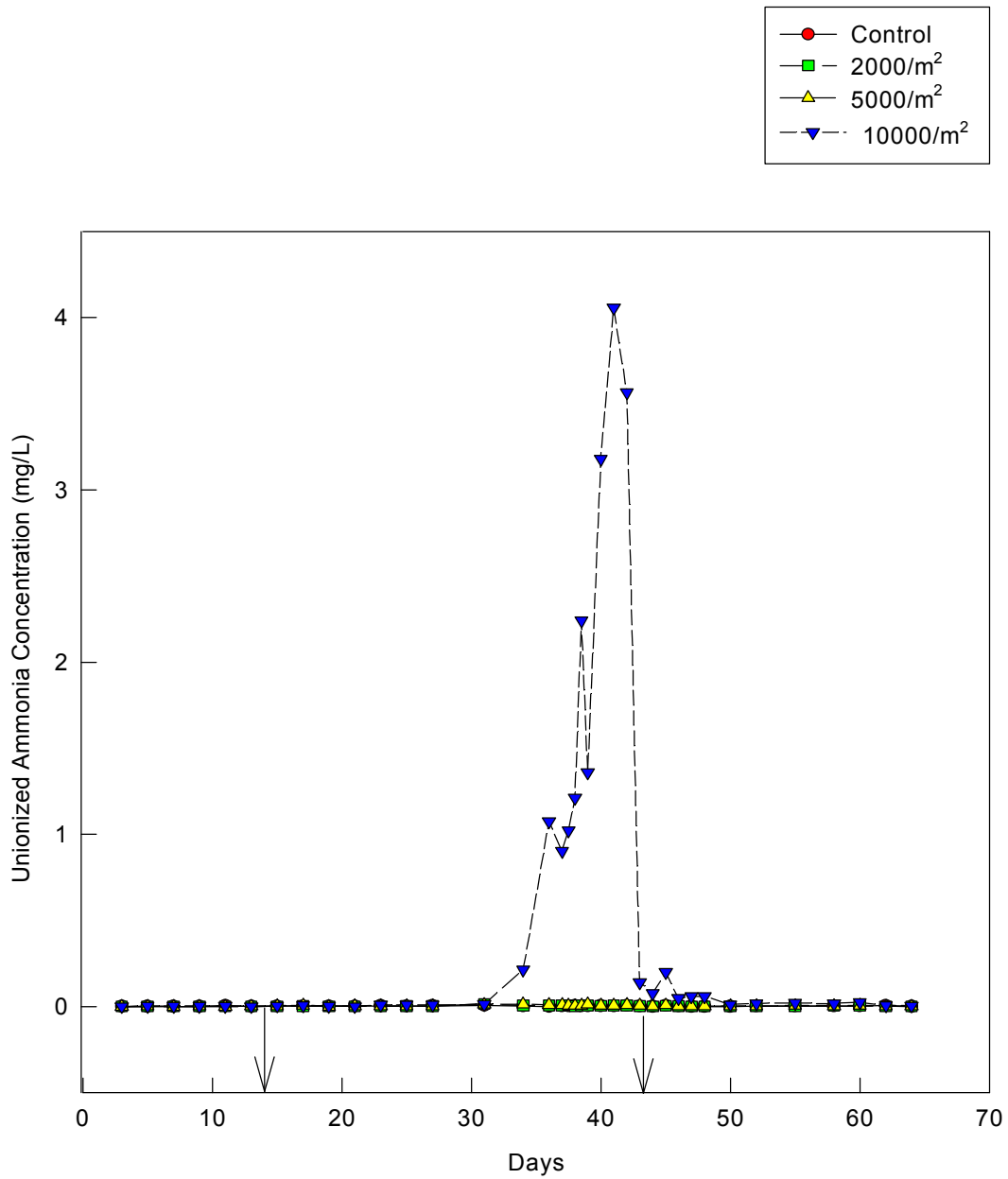


Figure 3.2 : Miniature Artificial Stream Test I
 Changes in NH₃ over Time at Different Clam Densities
 (arrows indicate start of second and third test phases)

different densities. Ammonia levels remained low in the control, low and medium density streams; the highest reading was 0.015 for one of the low density streams. Unionized ammonia concentrations quickly dropped after flow was reinitiated. However, unionized ammonia levels were still significantly different at different densities, over time and at different densities over time.

Dissolved oxygen levels were lowest for the high density streams and highest for the control and low density streams (Figure 3.3). The D.O. levels remained above 60% saturation throughout the test for the streams having 0, 2,000 and 5,000 clams/m². Dissolved oxygen concentrations for the streams containing 10,000 clams/m² ranged from 6.6 - 7.3 mg/L for the first 13 days of the test, and D.O. concentrations were significantly different at different densities, over time, and at different densities over time (Table 3.1). After flow was stopped, D.O. levels began dropping in the high density streams. The lowest D.O. concentrations of 0.53 - 0.77 mg/L coincided with 100% mortality. Dissolved oxygen concentrations were not significantly different at different densities but were significantly different over time and at different densities over time. D.O. levels increased after flow was reinitiated and ranged from 2.6 - 7.2 mg/L. Dissolved oxygen concentrations were still significantly different during the third phase of the test at different densities, over time and at different densities over time.

During the first phase of the test, the pH ranged from 7.43-8.04 and was significantly different at different densities and at different densities over time, but not over time (Figure 3.4). During the second phase of the test, pH was generally lower in the high density streams than in the control, low and medium density stream. No significant differences in the pH at different densities were observed, but there was a significant difference over time and at different densities over time. The pH values ranged from 7.47-8.08 in the high density streams and from 7.74-8.57 in the control, low and medium density streams. The pH values for the third phase of the test, when flow was reinitiated, ranged from 7.50-8.34. The pH was not significantly different at different densities or at different densities over time, but was significantly different over time.

During the first phase of the test, no significant differences in conductivity at different densities or at different densities over time were observed, but the difference in conductivity over time was significant. Conductivity for this period ranged from 190-320 umhos (Figure 3.5). After flow was terminated, conductivity gradually increased for all densities until day 34. On day 34 a sharp increase in conductivity was observed in the high density streams, corresponding with the peak in ammonia concentration. Conductivity levels peaked at 1600 umhos in the high density stream and ranged from 300-640 in the control, low and medium density

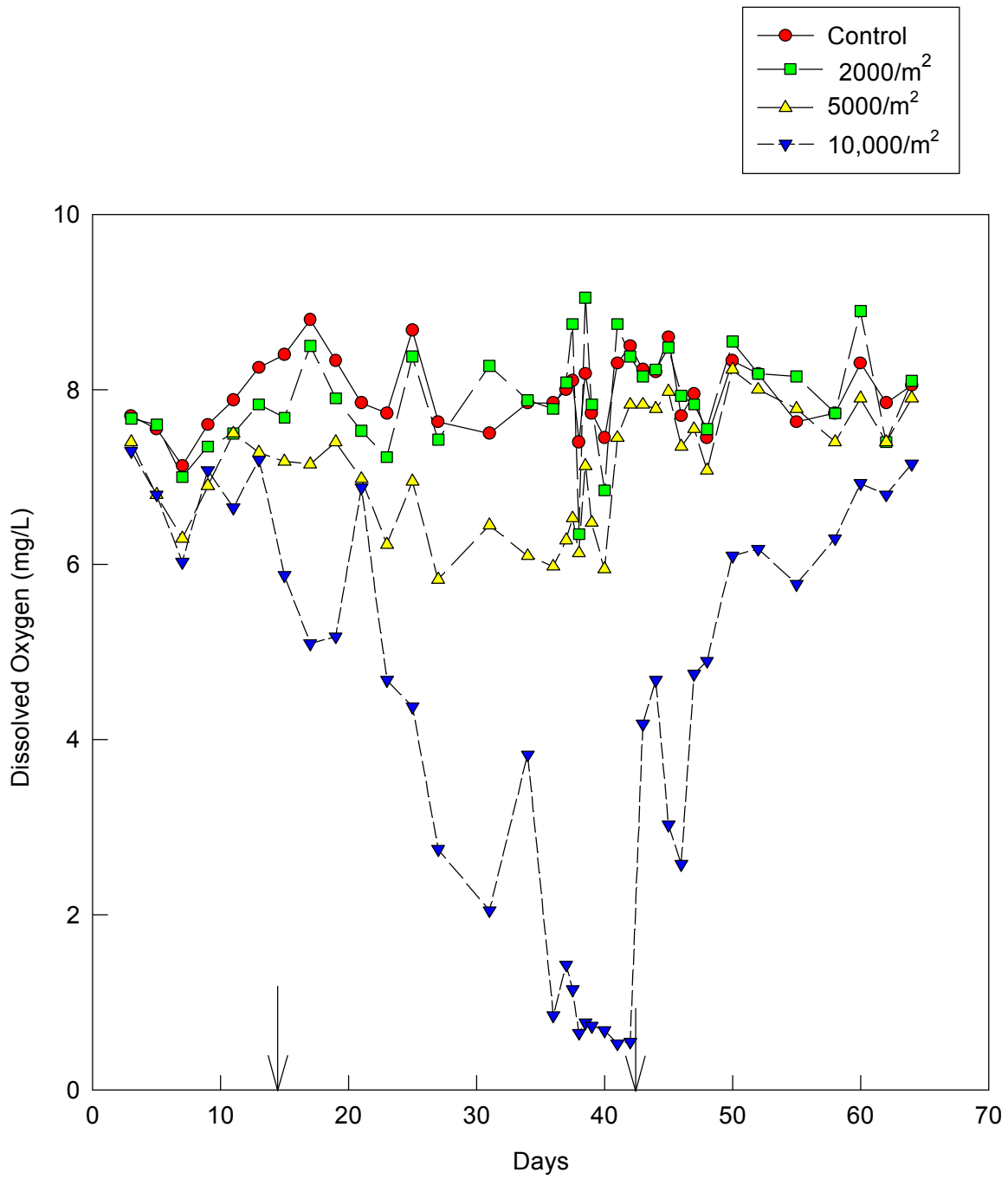


Figure 3.3 : Miniature Artificial Stream Test I
 Changes in D.O. over Time at Different Clam Densities
 (arrows indicate the start of the second and third test phases)

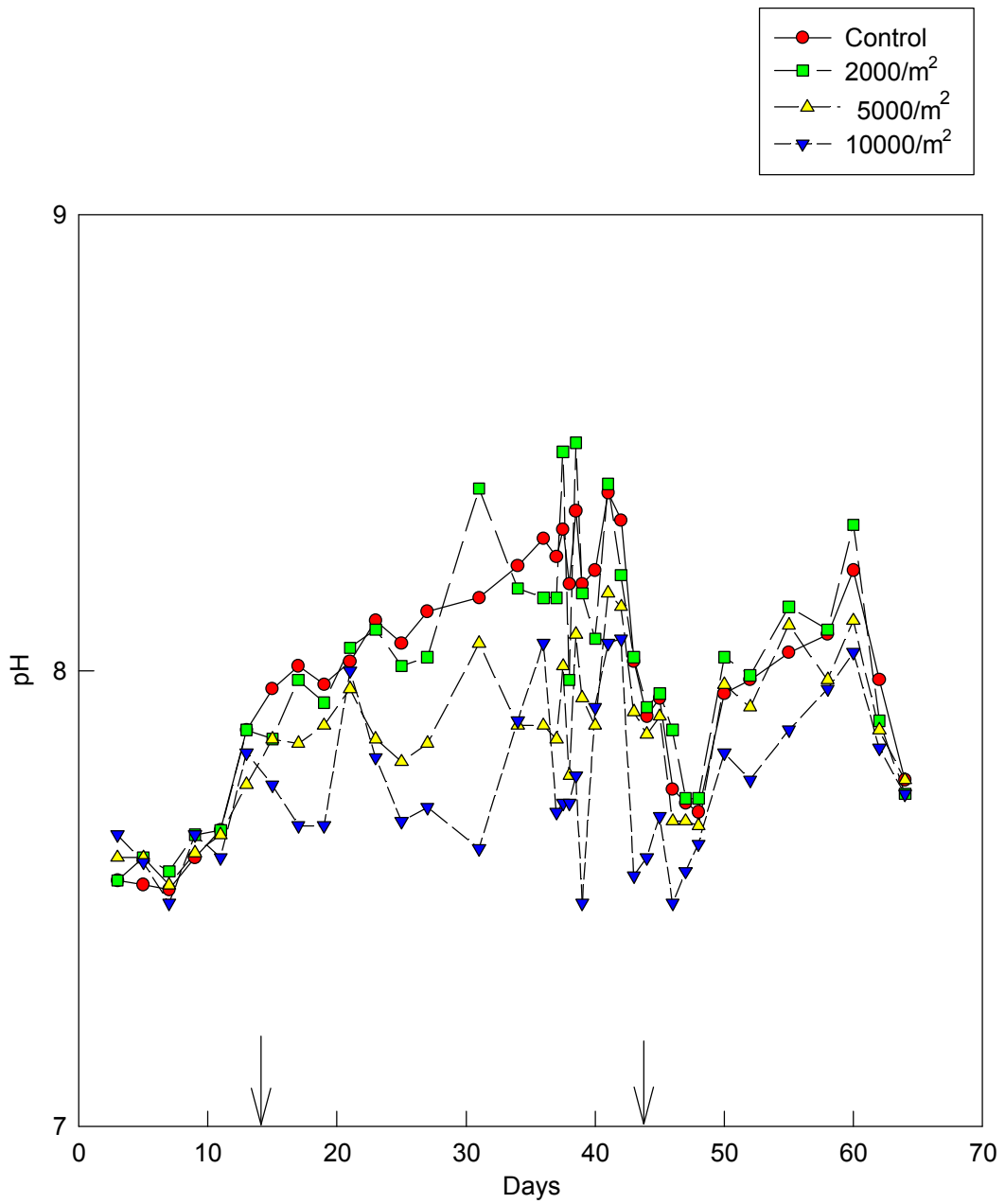


Figure 3.4 : Miniature Artificial Stream Test I
 Changes in pH over Time at Different Clam Densities
 (arrows indicate start of second and third test phases)

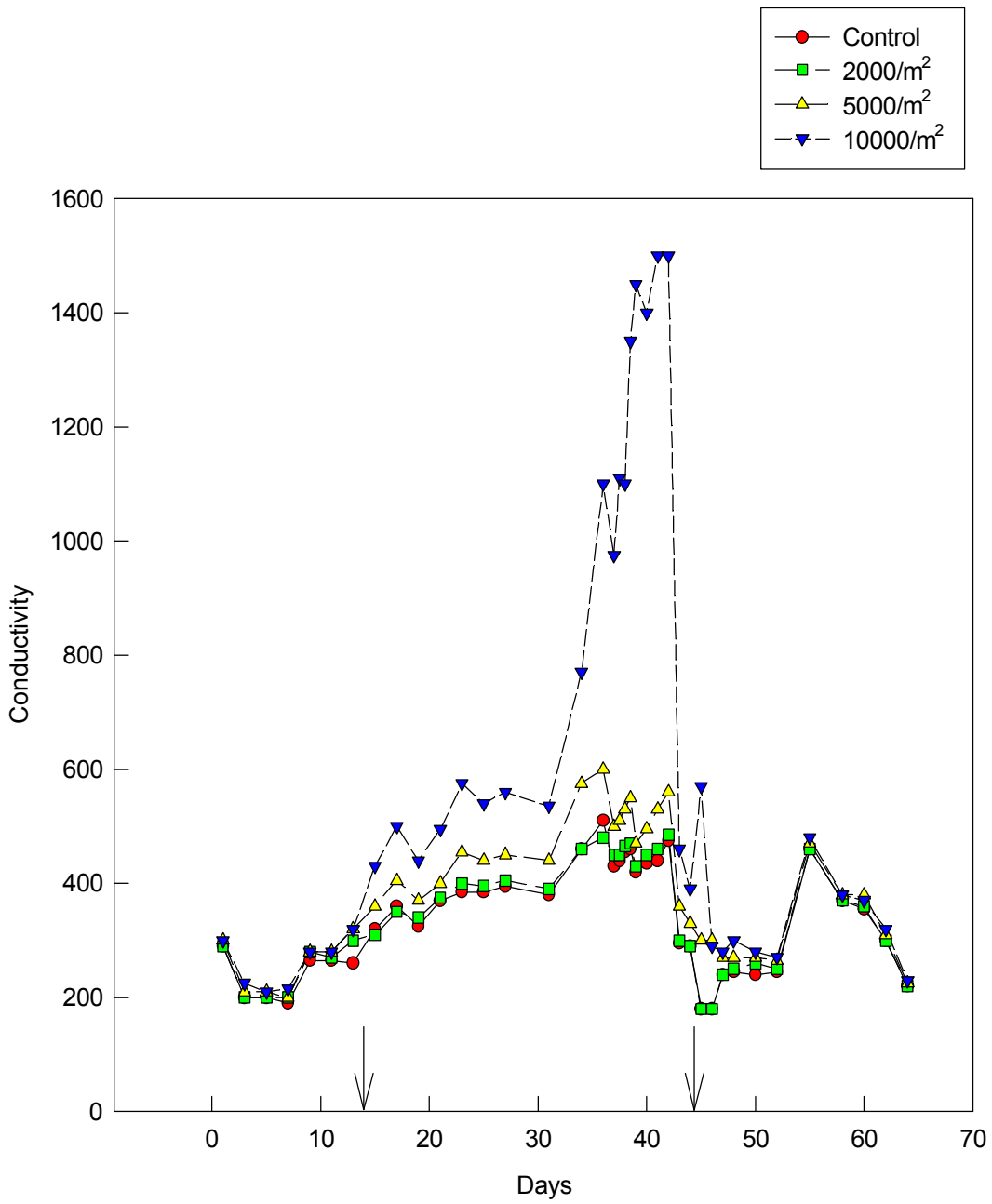


Figure 3.5 : Miniature Artificial Stream Test I
 Changes in Conductivity over Time at Different Clam Densities
 (arrows indicate start of second and third test phases)

streams. Significant differences in conductivity were found at different densities, over time, and at different densities over time during this period. After flow was reinitiated in the streams conductivity dropped quickly from 1600 to 460 umhos in 24 hours. Conductivity for the third phase of the test ranged from 180-480 umhos. Differences in conductivity were not significant at different densities but were significant over time and at different densities over time.

Temperature varied widely over time but was similar between densities and replicates. Temperatures for the first phase of the test ranged from 19.2-22.1°C, and no significant differences were observed in unionized ammonia concentration, percent mortality, D.O., pH or conductivity at different temperatures. During the second phase of the test, temperatures ranged from 16.0-24.1°C, and mortality and unionized ammonia concentrations were significantly different. Temperatures during the third phase of the test ranged from 17.1-22.9°C. Mortality, D.O., pH, and conductivity were significantly different at different temperatures during this period.

The Mann-Whitney Rank Sum Test showed significant differences in the size of clams that survived until the end of the test, and those that died at low and medium density (Table 3.2). The median size of the surviving clams ranged from 12.3-12.9 mm and the median size of the dead clams ranged from 8.2- 8.5 mm.

Table 3.2 : Mann-Whitney Rank Sum Tests comparing the size of clams alive and dead at the end of test I.

Group	Number of Clams	Median Value (mm)	p Value
2000A Alive	48	12.7	<0.0001
2000A Dead	43	8.4	
2000B Alive	65	12.8	<0.0001
2000B Dead	31	8.5	
5000A Alive	182	12.3	<0.0001
5000A Dead	66	8.2	
5000B Alive	128	12.7	<0.0001
5000B Dead	126	8.3	

Test II

Clams at the highest density experienced a 100% dieoff (Figure 3.6). The average mortality, at the end of the experiment in the low and medium density streams, was 30.5% and 11.4%, respectively. Mortality gradually increased over the first 14 days of the experiment for all three densities. During this period mortality was not significantly different at different densities or at different densities over time, but the difference over time was significant (Table 3.3). After the flow was terminated, a sharp increase in mortality was seen for the clams at the highest density, and at day 21 all the clams were dead. Mortality in the low and medium density streams continued to increase gradually until test termination. During the second and third phases of the test, mortality was significantly different at different densities, over time and at different densities over time.

During the first 14 days of the test, unionized ammonia levels were low for the three densities and the control, ranging from 0.002 - 0.044 mg/L NH₃-N (Figure 3.7). No significant differences were found at different densities or at different densities over time, but unionized ammonia concentration was significantly different over time (Table 3.3). After flow was terminated, unionized ammonia levels increased in the high density streams reaching a peak concentration of 5.04 mg/L four days after mortality reached 100%. Ammonia levels remained low in the control, low, and medium density streams with the highest reading being 0.022 mg/L in one replicate of the low density streams. Ammonia concentrations were significantly different at different densities, over time, and at different densities over time. Ammonia levels dropped quickly when flow was reinitiated in the high density streams. Unionized ammonia levels were not significantly different at different densities or over time, but were significantly different at different densities over time.

Dissolved oxygen levels were lowest for the 10,000 clams/m² streams, and highest for the control streams throughout the test (Figure 3.8). The D.O. levels remained above 60% saturation for the streams having 0, 2,000 and 5,000 clams/m². Dissolved oxygen concentrations for the high density streams ranged from 2.0-5.2 mg/L for the first 14 days and from 0.8 - 3.5 mg/L during the static phase. Significant differences in D.O. levels were observed at different densities and over time, but not at different densities over time (Table 3.3). During the static phase, D.O. levels were not significantly different at different densities but were significantly different over time and at different densities over time. The lowest D.O. concentrations coincided with 100% mortality. Dissolved oxygen levels increased after flow was reinitiated ranging from 1.6 - 5.5 mg/L, and were significantly different at different densities, over time, and at different densities over time.

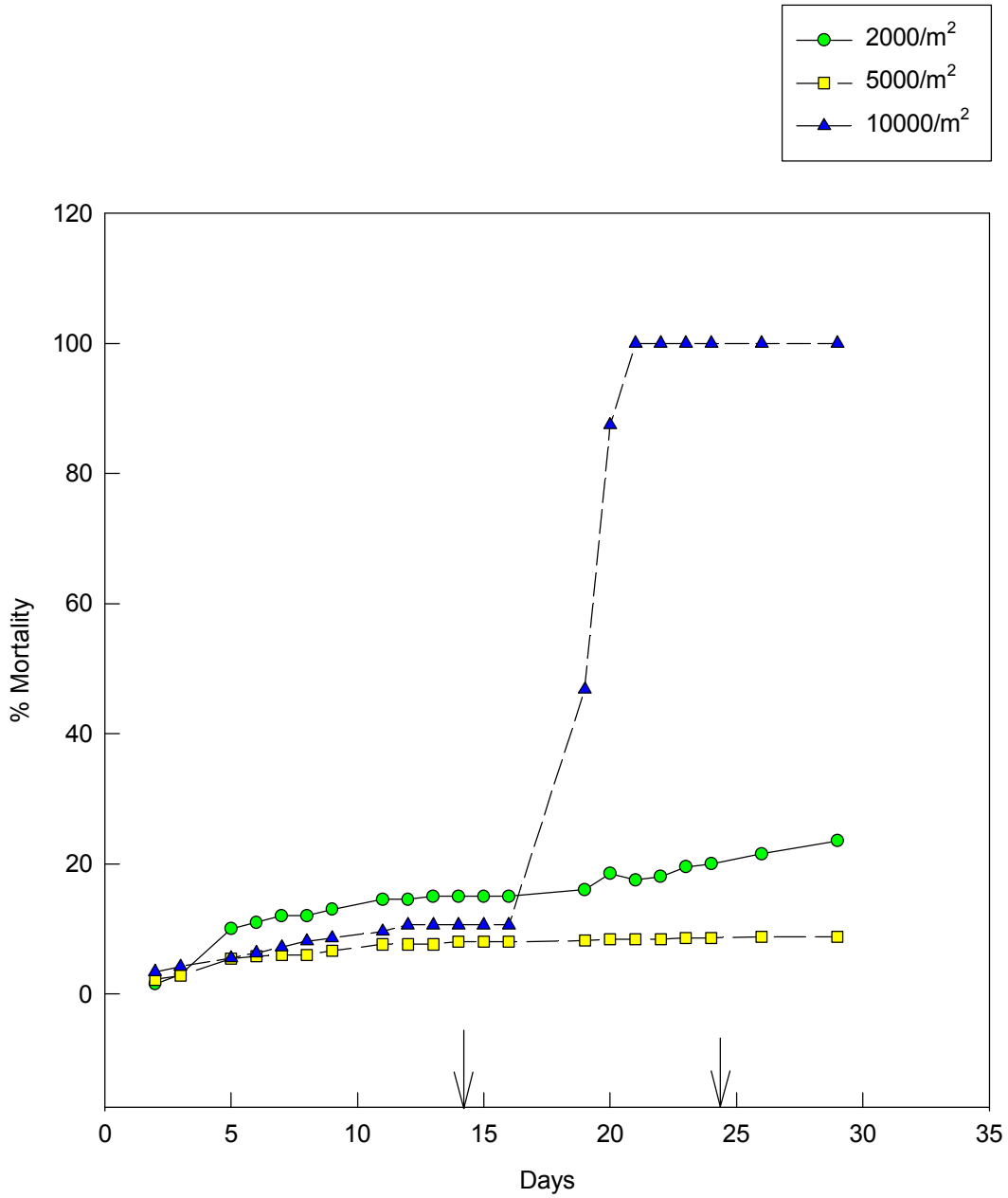


Figure 3.6 : Miniature Artificial Stream Test II
 Percent Mortality at Different Clam Densities
 (arrows indicate start of second and third test phases)

Table 3.3 :Results (p values) of the repeated measures analysis of test II to determine significant differences at different densities, over time and at different densities over time for unionized ammonia concentration, mortality, D.O., pH, and conductivity.(significance is $p < 0.05$)

Variable	Phase 1	Phase 2	Phase 3
NH₃-N			
Density	0.4590	0.0063	0.0902
Time	0.0313	0.0001	0.0549
Time*Density	0.1238	0.0001	0.0205
Temperature	0.0095	-	-
Mortality			
Density	0.0857	0.0041	0.0002
Time	0.0001	0.0001	0.0001
Time*Density	0.0001	0.0001	0.0001
Temperature	0.0114	-	-
D.O.			
Density	0.0050	0.2321	0.0163
Time	0.0022	0.0001	0.0001
Time*Density	0.4989	0.0001	0.0008
Temperature	-	-	-
pH			
Density	0.0101	0.3186	0.0834
Time	0.7204	0.0110	0.0969
Time*Density	0.5926	0.0686	0.0364
Temperature	0.0002	-	-
Conductivity			
Density	0.8031	0.0008	0.0265
Time	0.0086	0.0001	0.0001
Time*Density	0.9985	0.0001	0.0004
Temperature	-	-	-

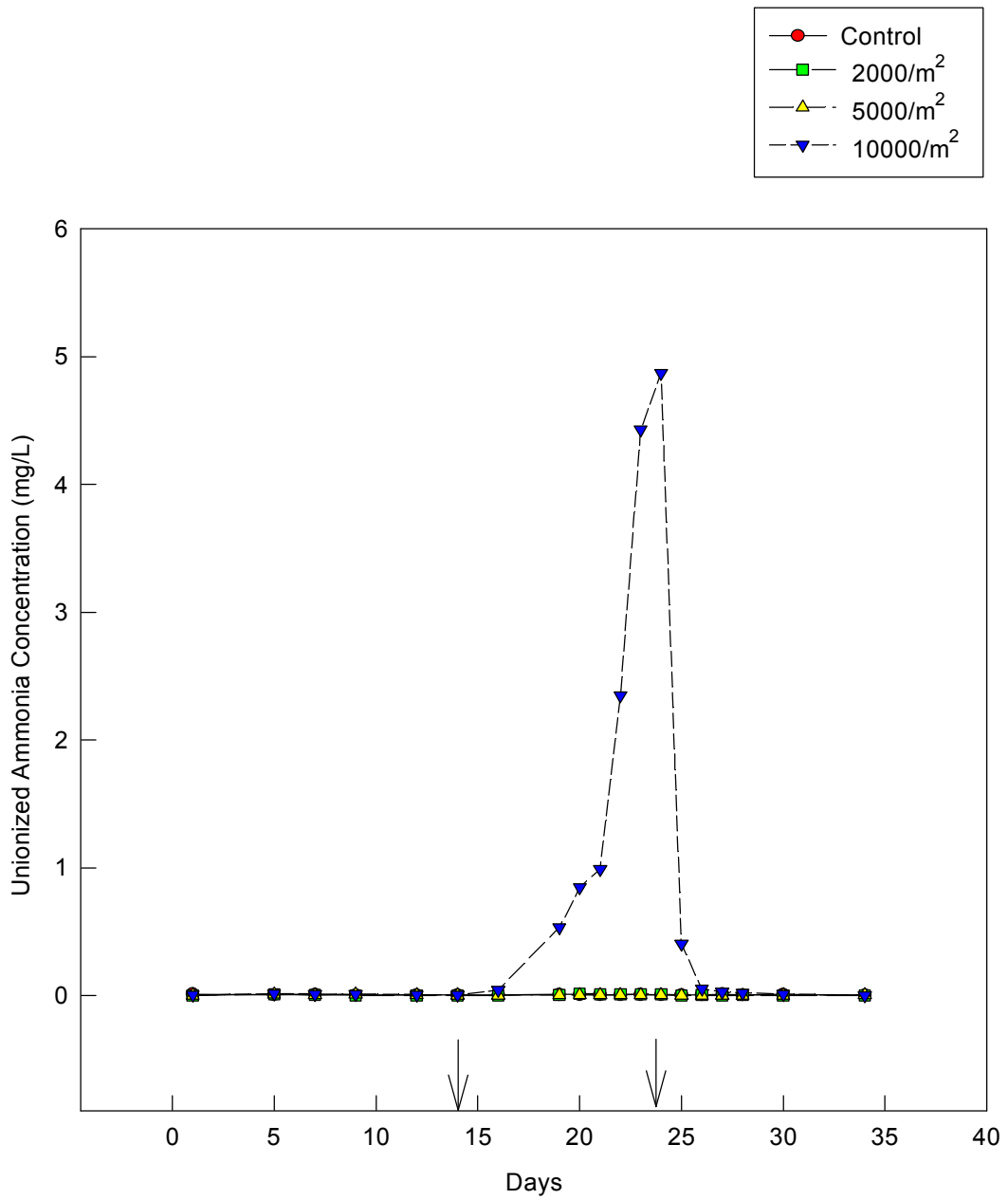


Figure 3.7 : Miniature Artificial Stream Test II
 Changes in NH₃ over Time at Different Clam Densities
 (arrows indicate start of second and third test phases)

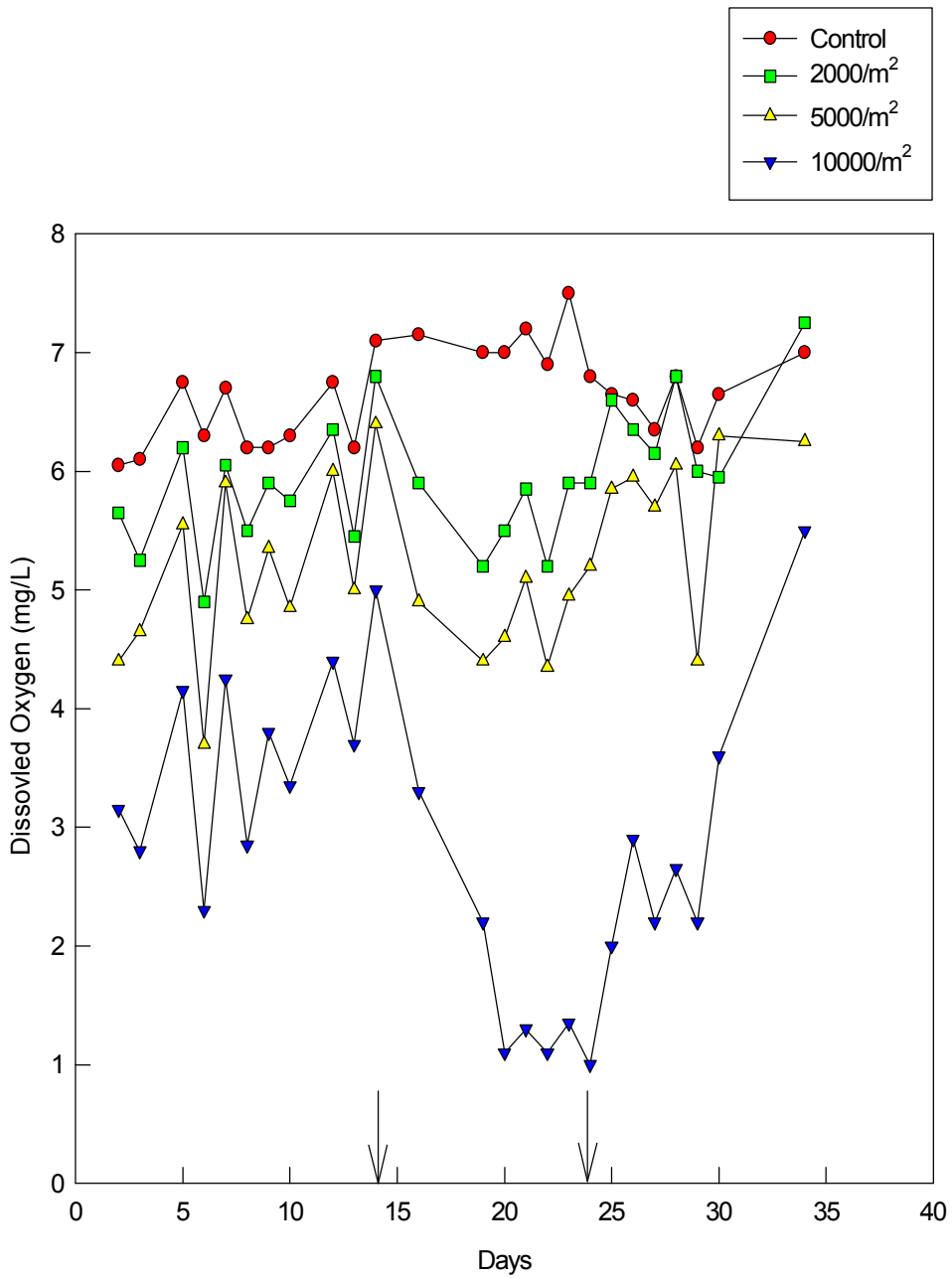


Figure 3.8 : Miniature Artificial Stream Test II
 Changes in D.O. over Time at Different Clam Densities
 (arrows indicate start of second and third test phases)

The pH was generally lowest in the high density streams and highest in the control streams (Figure 3.9). The pH in the control, low, medium and high density streams ranged from 7.77-8.25, 7.57-7.98, 7.44-7.96, and 7.39-8.14, respectively. Significant differences in the pH occurred at different densities during the first phase of the test but not during the second or third phase. The pH levels were significantly different over time during the static phase of the test but not the first or third phases. A significant difference in pH at different densities over time occurred only during the third phase .

During the first phase of the test, conductivity ranged from 230-330 umhos and conductivity was significantly different over time (Table 3.3). After flow was terminated, conductivity rose quickly in the high density streams and more gradually in the control, low and medium density streams (Figure 3.10). Conductivity peaked at 1400 umhos in the high density streams and ranged from 260-580 umhos in the other streams. Significant differences at different densities, over time and at different densities over time were observed. After flow was reinitiated, conductivity quickly dropped from 1300 to 550 umhos in a 24-hours in the high density streams. Conductivity for the third phase of the test ranged from 200-550 umhos and was significantly different at different densities, over time and at different densities over time.

Temperature during the test stayed within the preset limits of $26 \pm 2^{\circ}\text{C}$, except on the first day when the temperature ranged from $23.3\text{-}24.0^{\circ}\text{C}$. Unionized ammonia concentration, mortality and pH were significantly different at different temperatures during the first phase of the test. No significant differences in unionized ammonia concentration, mortality, D.O., pH or conductivity were observed at different temperatures during the second and third phases.

The Mann-Whitney rank sum tests showed no significant difference in the size of clams that were dead at test termination and those that survived. The median size of surviving clams ranged from 12.2-12.5 mm, and the median size of dead clams ranged from 11.8-12.4 mm (Table 3.4).

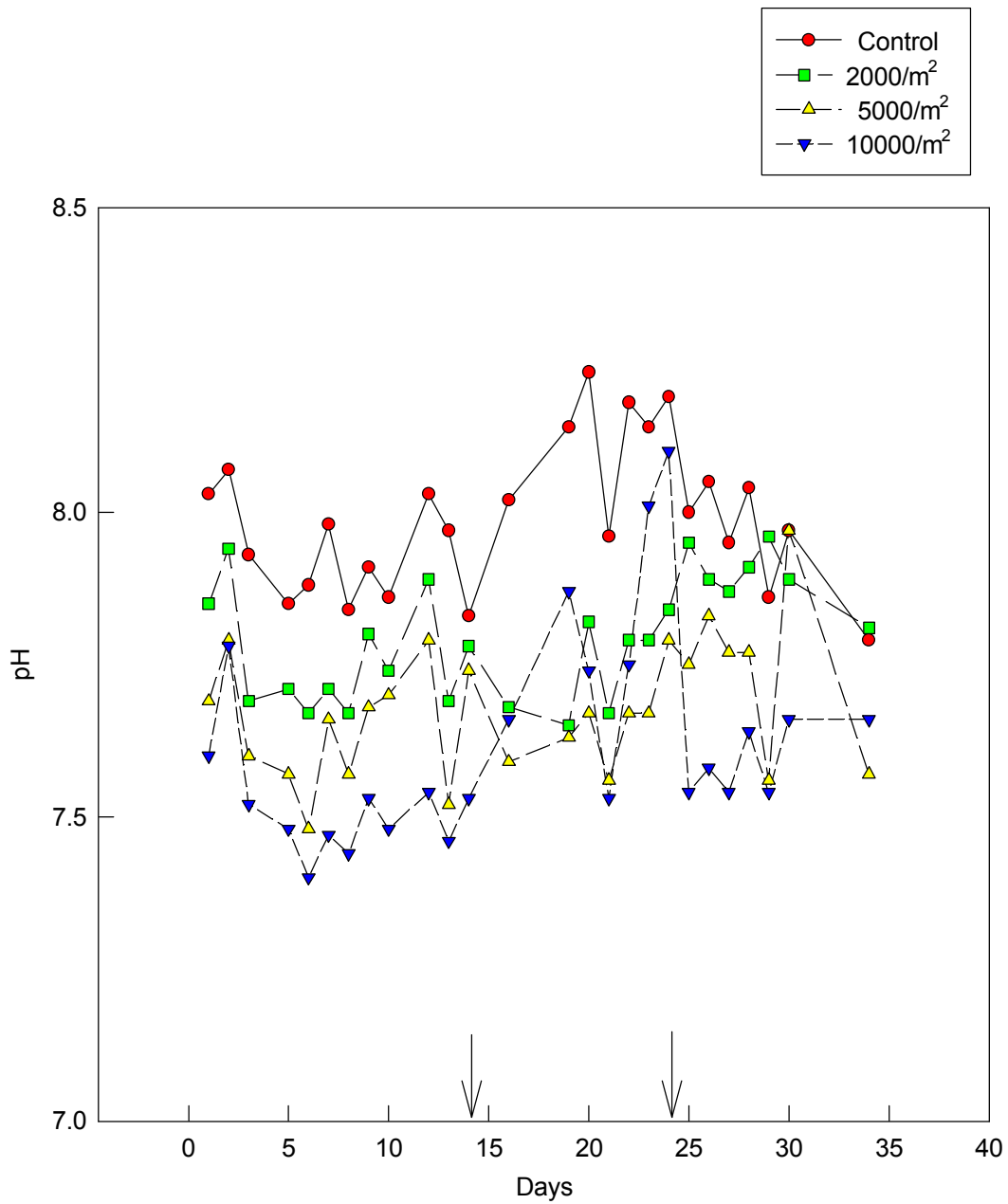


Figure 3.9 : Miniature Artificial Stream Test II
 Changes in pH over Time at Different Clam Densities
 (arrows indicate start of second and third test phases)

Table 3.4 : Mann-Whitney Rank Sum Tests comparing the size of

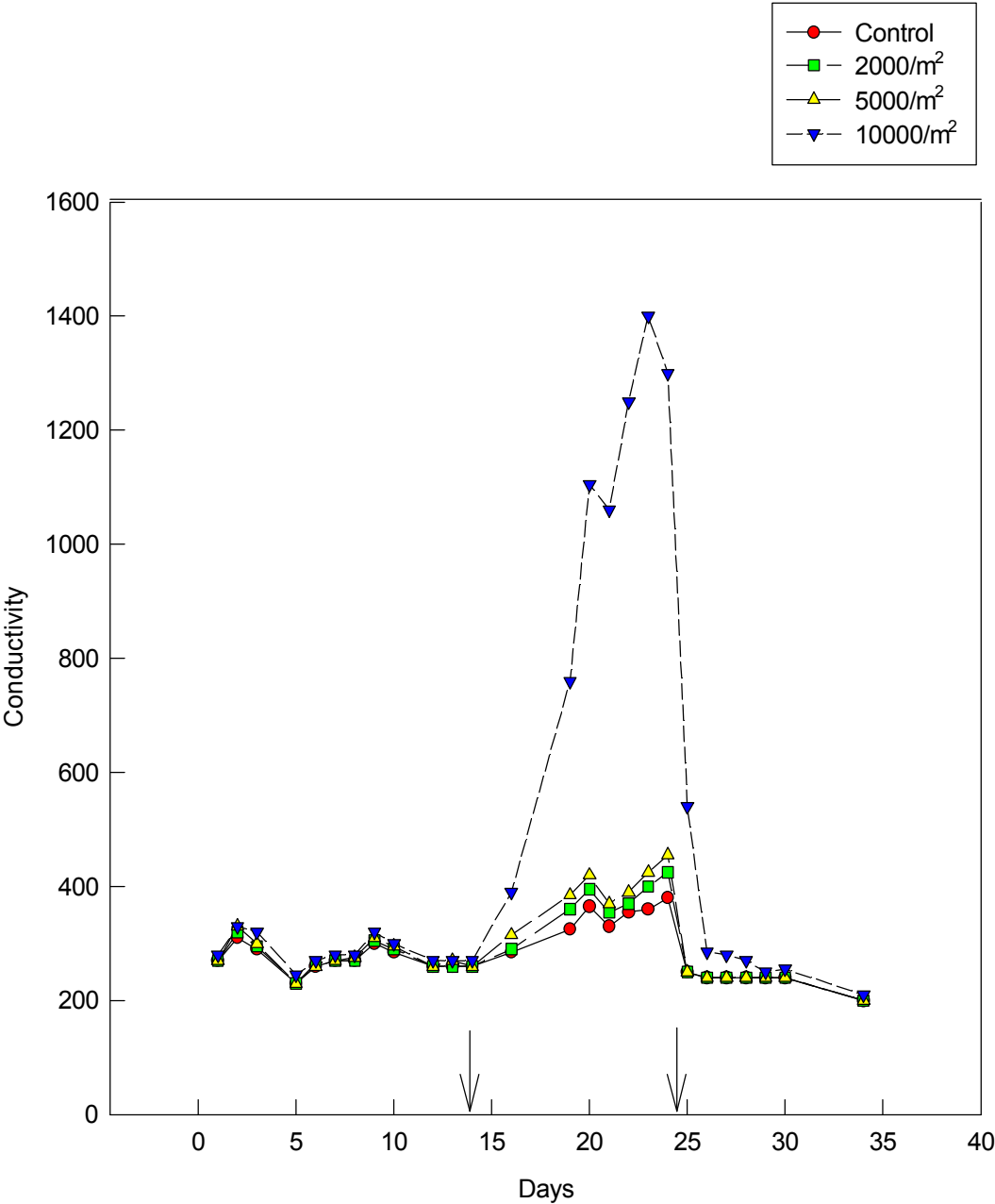


Figure 3.10 : Miniature Artificial Stream Test II
 Changes in Conductivity over Time at Different Clam Densities
 (arrows indicate start of second and third test phases)

Table 3.4 : Mann-Whitney Rank Sum Tests comparing the size of clams alive and dead at the end of test II.

Group	Number of Clams	Median Value (mm)	p Value
2000A Alive	78	12.5	0.2371
2000A Dead	28	11.8	
2000B Alive	60	12.5	0.4724
2000B Dead	33	12.4	
5000A Alive	213	12.4	0.8562
5000B Dead	25	12.3	
5000B Alive	215	12.2	0.6426
5000B Dead	32	12.2	

Discussion

The rate of Asian clam dieoffs, the amount of ammonia produced, and the dissolved oxygen levels were all affected by density. One-hundred percent mortality only occurred in the high density streams, and ammonia concentrations were higher and D.O. levels lower. Sickel (1986) stated that the most probable cause of mass mortalities of *C. fluminea* is high population levels, especially during the summer months when high temperatures increase metabolic rates and energy demand. However, there is nothing in the literature indicating the possible effects on other organisms resulting from these dieoffs. In areas where density or biomass is high, the consumption of oxygen and excretion of ammonia will be high. In northern German lowland waters, areas with adult mussels (higher biomass) had higher concentrations of ammonia and lower dissolved oxygen levels than areas with juvenile mussels (lower biomass) (Buddensiek, 1993).

Ammonia concentrations resulting from population crashes in the high density streams would have been high enough to produce mortality in most aquatic organisms. Unionized ammonia concentrations reached 4.71 mg/L in test I and 5.04 mg/L in test II, which is 10 times the LC50 for adult *P. grandis* and over 40 times that for *V. iris* glochidia. This result indicates that dieoffs could negatively affect native mussels, especially if the dieoff occurs during glochidia release. Unionized ammonia concentrations for the control, low and medium density streams ranged from 0.00015-0.022 mg/L, which is below levels that produced toxicity in any of the organisms tested. Mussels may not be the only organisms affected by *C. fluminea* dieoffs. Peak unionized ammonia concentrations were approximately three and five times greater than the LC50's for the snail, *Physa gyrina*, and the clam, *Musculum transversum*, respectively (Table 2.5). Ammonia levels were also greater than the LC50 concentrations for several species of fish including the coho salmon, *Oncorhynchus kisutch*, and the rainbow trout, *Oncorhynchus mykiss*. Less sensitive species such as the amphipod, *Crangonyx pseudogracillis*, the mayfly, *Ephemerella grandis*, and the crayfish, *Orconectes lamunis*, may not have been affected by the high ammonia levels. Mobile organisms such as fish and insects that have the ability to detect and avoid ammonia may not be at as great a risk as sedentary mussels.

Flow rate could be another factor affecting the impact of *C. fluminea* dieoffs. When conditions are static or low flow, ammonia will persist longer and at higher concentrations, increasing the impact on organisms present. Low flow also contributes to sagging dissolved oxygen levels. High flow may also reduce the impact of *C. fluminea* dieoffs by washing away dead clam bodies. As the clams decay, gases are produced that can cause clams to float and be washed away from the dieoff site. Several reports of floating clam bodies have been made (Sinclair and Isom, 1963; Sickel and Heyn, 1980; and Sickel, 1986).

Low dissolved oxygen concentrations resulting from a *C. fluminea* dieoff could also negatively affect freshwater mussels. Imlay (1971) found that riffle species of mussels require 2.5 mg/L of dissolved oxygen at summer temperatures and 6.0 mg/L for normal growth. Dissolved oxygen concentrations dropped below 2.5 mg/L during the dieoff and remained below this mark for nine days during Test I and seven days during Test II. Although D.O. levels rose quickly after flow was reinitiated, levels remained well below saturation, ranging from 1.20 - 7.20 mg/L in test I and 1.60 - 5.50 mg/L in test II. Five freshwater mussel species from the Blanco River of central Texas were exposed to low dissolved oxygen concentrations that ranged from 0 - 0.5 mg/L for seven days, and survival ranged from 0 to 75 percent (Horne and McIntosh, 1979). Dissolved oxygen levels ranged from 0.45 - 0.60 mg/L in test I and from 0.80 - 1.2 mg/L in test II, which may be low enough to produce mortality in some mussel species. Juvenile mussels may be at greater risk than adults due to their higher

metabolic rates and they are pedally feeding in the sediment. Mortality was 100% for two species of juvenile mussels, *Utterbackia imbecillis* and *Pyganodon cataracta*, within 24 hours of exposure to anoxic conditions of 0.1 mg/L O₂ (Dimock and Wright, 1993). Tankersley and Dimock (1993) noted that short-term exposure to low dissolved oxygen concentrations may result in the premature release of brooding glochidia, further impacting mussel recruitment.

Flow rate, low dissolved oxygen, temperature and ammonia concentrations may have contributed to the *C. fluminea* dieoffs. Mortality was gradual during the period when flow was 30mL/min, and a complete dieoff did not occur until flow was shut off. Constant flow reduces the build up of toxins including ammonia and keeps dissolved oxygen levels high, reducing stress on resident organisms. Once flow was reinitiated, ammonia concentrations dissipated quickly and dissolved oxygen levels increased. This implies that in natural systems with high flow rates, dieoffs may be less likely to occur and ammonia concentrations will be lower.

Low dissolved oxygen concentrations may be another factor implicated in *C. fluminea* population crashes. Mass mortality of *C. fluminea* in the lower Tennessee and Cumberland rivers below Kentucky and Barkley dams coincided with near anoxic conditions (Sickel and Heyn, 1980). However, *C. fluminea* are able to survive low oxygen concentrations for several days. In exposures to low D.O. concentrations of 0 - 0.5 mg/L for 7 days, *C. fluminea* had an 89% survival rate (Horne and McIntosh, 1979). Dissolved oxygen levels remained above 0.5 mg/L prior to the dieoff, indicating that although low D.O. may have contributed to the dieoff, it may not be the only causal factor.

A slight increase in unionized ammonia occurred after flow was stopped and reached a maximum concentration of 0.016 mg/L prior to the dieoffs. Belanger et al. (1991) exposed adult clams in artificial streams to an unionized ammonia concentration of 0.74mg/L at 24°C, producing 100% mortality of clams in 13 days. The 96-hour LC50's for adult *C. fluminea* was 0.71-0.88 and 1.87-2.75 mg/L NH₃-N for water column and sediment tests, respectively. Given that unionized ammonia concentrations prior to the dieoff were well below levels that produced mortality in artificial stream and laboratory toxicity tests, it seems unlikely that ammonia concentration alone produced mortality. However, a combination of low dissolved oxygen levels and ammonia may have produced an additive effect contributing to mortality. In the Vermillion River, Louisiana, reduced *C. fluminea* density and growth were associated with low dissolved oxygen levels and sewage treatment plant effluent containing ammonia (Belanger, 1991). Reduction of species richness in Gowrie Creek, southeast Queensland, Australia, was attributed to reduced dissolved oxygen concentrations and possible ammonia toxicity (Cosser, 1991).

The *C. fluminea* dieoff occurred much faster in test II (21 days) than in test I (38 days). Temperature may have affected the dieoff rate. The average temperature of test I was 21.7°C, and the temperature of test II was 26+/-2°C. Higher temperature can stress organisms in several ways. An increase in *C. fluminea*'s excretion rate was reported as temperature increased (Lauritsen and Mozley, 1989). Higher excretion rates would increase ammonia concentrations in clam beds and would be more severe in areas with high clam densities. McMahon (1979) found that *C. fluminea* increase O₂ uptake as temperature increases up to 25°C, and at 30°C uptake is depressed. While metabolic rates increase as temperature rises, oxygen saturation levels decrease. The combination of decreased oxygen saturation and increased oxygen consumption may reduce dissolved oxygen enough to produce mortality. Dissolved oxygen concentrations were lower in Test II ranging, from 2.0 - 5.2 mg/L during the first 14 days of the test, compared to 4.4 - 7.5 mg/L in test I. In an experiment where channel catfish ponds were stocked with *C. fluminea*, high mortality was attributed to high temperatures and low dissolved oxygen levels (Buttner, 1986). The amount of total ammonia present as the unionized species increases with temperature, and the toxicity of unionized ammonia increases. Adult *C. fluminea* exposed to 0.74mg/L NH₃ at 20°C had an LT50 (lethal time until 50% mortality) of 8.1 days while those exposed to 0.6mg/L NH₃ at 30°C had and LT50 of 6.2 days (Belanger et al., 1991). A combination of these temperature effects may account for the difference in the amount of time required to produce a dieoff.

Although pH was generally lower in the high density streams than the control, low or medium density streams, the difference was only significant during phase I of test II. The pH levels remained well within the range found in unpolluted streams and should not have contributed to mortality. Conductivity was significantly different at different densities during the static phase of test I and II and was generally higher in the high density streams than the control, low and medium density streams. Concentration of ions due to water evaporation and production of ionized ammonia contributed to increased conductivity during the static phase. Peak conductivity corresponded with peak ammonia concentrations.

In Test I, small clams were more likely to die than larger clams. Belanger et al. (1991) also found that smaller clams were more sensitive to ammonia. However, in Test II no difference was seen in the size of the clams dead at the end of the test and those alive. This difference in test response may be due to the fewer number of small clams used in Test II. In Test I, 47% of the clams used were from the 5.0-10.0mm size class, while in Test II only 27% were from this size class. The absence of the more sensitive smaller clams in Test II may account for the higher median size of the dead clams as compared to Test I. More larger clams were used in test II, which increased the biomass in each stream and may have contributed to the earlier dieoff.

It may also account for the slightly higher peak ammonia concentrations. The greater the clam biomass in each stream the more dissolved oxygen consumed and the more ammonia produced when a dieoff occurs. Therefore, a tradeoff may be occurring in areas with high densities of *C. fluminea*. The lower sensitivity of larger clams may allow some to survive and repopulate the area after a dieoff. However, the presence of many large clams in an area may increase the likelihood of a dieoff due to the increased biomass.

In summary, density of clams affected the rate of dieoff, the amount of ammonia produced, and the depression of dissolved oxygen levels. One-hundred percent mortality only occurred in the streams containing 10,000 clams/m². The biomass present determines the amount of ammonia that can be produced, and under static conditions unionized ammonia reached levels many times higher than that which produced mussel mortality. Dissolved oxygen levels were below 1.0 mg/L during the dieoffs and could also be a threat to native mussels found in a dieoff area. Flow rate, dissolved oxygen, temperature and perhaps ammonia concentration are thought to be factors contributing to *C. fluminea* population crashes. As with any artificial stream experiment, caution should be taken when trying to apply results to the "real world". Although there have been reports of clam densities in excess of 10,000/m² (Cherry et al., 1986 and McMahon and Williams, 1986), they are rare and usually occur soon after larval release. Conditions of no flow and 10,000 adult clams/m² are probably seldom found in a real stream environment. Massive *C. fluminea* dieoffs have been reported and this research provides some clues into understanding these occurrences. Further research, including creating dieoffs in situ and monitoring of naturally occurring dieoffs, will be necessary in order to completely understand this phenomenon and its impact on freshwater mussels.

Literature Cited

Aldridge, D.W. and McMahon, R.F. 1978. Growth, Fecundity and Bioenergetics in a Natural Population of the Asiatic Freshwater Clam, *C. fluminea manilenis* Philippi, from North Central Texas. *Journal of Molluscan Studies*. 44:49-70.

Anderson, R.M. ;Layzer, J.B. and Gorden, M.E. 1991. Recent Catastrophic Decline of Mussels(*Bivalvia: Unionidae*) in the Little South Fork Cumberland River, Kentucky. *Brimleyana*. 17:1-8.

Ankley, G.T.; Katko, A.; and Arthur, J.W. 1990. Identification of Ammonia as an Important Sediment - Associated Toxicant in the Lower Fox River and Green Bay, Wisconsin. *Environmental Toxicology and Chemistry*. 9:313-322.

Belanger, S.E. 1991. The Effect of Dissolved Oxygen, Sediment, and Sewage Treatment Plant Discharges upon Growth, Survival and Density of Asiatic Clams. *Hydrobiologia*. 281:113-126.

Belanger, S.E.; Cherry, D.S., Farris, J.L.; Sappington, K.G.; and Cairns, J. 1991. Sensitivity of the Asiatic Clam to Various Biocidal Control Agents. *Research and Technology*. 79-87.

Belanger, T.V. 1990. Growth Rates of the Asiatic Clam, *Corbicula fluminea*, in the Upper and Middle St. Johns River, Florida. *The Nautilus*. 104(1):4-9.

Bogan, A.E. 1996. Decline and Decimation : The Extirpation of the Unionid Freshwater Bivalves of North America. *Journal of Shellfish Research*. 15(2):484.

Bickel, D. 1966. Ecology of *Corbicula manilensis* Philippi in the Ohio River at Louisville, Kentucky. *Sterkiana* 23:19-24.

Britton, J.C. and Morton, B. 1982. A Dissection Guide, Field and Laboratory Manual for the Introduced Bivalve *Corbicula fluminea*.

Buddensiek, E.H.; Engel, H.; Fleischauer-Rossing S.; and Wachtler, K. 1993. Studies on the Chemistry of Interstitial Water Taken from Defined Horizons in the Fine Sediments of Bivalve Habitats in Several Northern German Lowland Waters II: Microhabitats of *Margaritifera margaritifera* L., *Unio Crassus* (Philipsson) and *Unio tumidus* Philipsson. *Archive Hydrobiologia*. 127(2):151-166.

Buttner, J.K. 1986. Corbicula as a Biological Filter and Polyculture Organism in Catfish Rearing Ponds. *Progressive Fish-Culturist*. 48:136-139.

Buttner, J.K. and Heidinger, R.C. 1981. Rate of Filtration in the Asiatic Clam, *Corbicula fluminea*. *Transactions of the Illinois State Academy of Science*. 74(3-4):13-18.

Chetty, A.N. and Indira, K. 1994. Alterations in the Tissue Lipid Profiles of *Lamellidens marginalis* Under Ambient Ammonia Stress. *Bulletin of Environmental Contamination and Toxicology*. 53:693-698.

Chetty, A.N. and Indira, K. 1995. Adaptive Changes in the Glucose Metabolism of a Bivalve to Ambient Ammonia Stress. *Bulletin of Environmental Contamination and Toxicology*. 54:83-89.

Cherry, D.S.; Roy, R.L.; Lechleitner, R.A.; Dunhardt, P.A.; Peters, G.T. and Cairns Jr., J. 1986. *C. fluminea* fouling and Control Measures at the Celco Plant, Virginia. *American Malacological Bulletin, Special Edition No. 2*. 69-81.

Clark, A.H. 1988. Aspects of Corbiculid-Unionid Sympatry in the United States. *Malacology Data Net*. 2(3/4):57-99.

Cosser, P.R. 1988. Macroinvertebrate Community Structure and Chemistry of an organically Polluted Creek in Southeast Queensland Australia. *Australian Journal of Marine and Freshwater Research*. 39(5):671-684.

Dennis, S.D. 1987. An Unexpected Decline in Populations of the Freshwater Mussel, *Dysnomia*(=*Epioblasma*) *capsaeformis*, in the Clinch River of Virginia and Tennessee. *Virginia Journal of Science*. 48(4):281-288.

Dey, S. and Bhattacharya, S. 1989. Ovarian Damage to *Channa punctatus* and Chronic Exposure to Low Concentrations of Elsan, Mercury, and Ammonia. *Ecotoxicology and Environmental Safety*. 17: 247-257.

Dimock, R.V. and Wright, A.H. 1993. Sensitivity of Juvenile Freshwater Mussels to Hypoxia, Thermal and Acid Stress. *The Journal of the Elisha Mitchell Scientific Society*. 109(4):183-192.

Doherty, F.G.; Cherry, D.S. and Cairns Jr., J. 1987. Valve Closure Response of the Asiatic Clam *Corbicula fluminea* Exposed to Cadmium and Zinc. *Hydrobiologia*. 153:159-167.

Ecological Analysts, Inc. 1981. The Sources, Chemistry, Fate, and Effects of Ammonia in Aquatic Environments.

Emerson, K.; Russo, R.C.; Lund, R.E.; and Thurston, R.V. 1975. Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature. Journal of the Fisheries Research Board of Canada. 32:2379-2383.

Epifano, L.E. and Srna, R.F. 1975. Toxicity of Ammonia, Nitrite Ion, and Orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. Marine Biology. 33:241-246.

Foe, C. and Knight, A. 1986. A Thermal Energy Budget for Juvenile *Corbicula fluminea*. American Malacological Bulletin, Special Edition No. 2 : 143-150.

Frazier, B.E.; Naimo, T.J.; and Sandheinrich, M.B. 1996. Temporal and Vertical Distribution of Total Ammonia Nitrogen and Unionized Ammonia Nitrogen in Sediment Pore Water from the Upper Mississippi River. Environmental Toxicology and Chemistry. 15(2):92-99.

Gardner, J.A. et al. 1976. The Invasion of the Asiatic Clam (*C. fluminea manilensis* Philippi) in the Altamaha River, Georgia. The Nautilus. 90:117-25.

Goudrea, S.E.; Neves, R.J. and Sheehan, R.J. 1993. Effects of Wastewater Treatment Plant Effluents on Freshwater Mollusks in the Upper Clinch River, Virginia, USA. Hydrobiologia. 252:211-230.

Harrison, F.L.; Knezovich, J.P.; and Rice, D.W. 1984. The Toxicity of Copper to the Adult and Early Life Stages of the Freshwater Clam, *C. fluminea manilensis*. Archives of Environmental Contamination and Toxicology. 13:85-92.

Hedtke, J.L. and Norris, L.A. 1980. Effect of Ammonium Chloride on Predatory Consumption Rates of Brook Trout (*Salvelinus fontinalis*) on Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in Laboratory Streams. Bulletin of Environmental Contamination and Toxicology. 24:81-89.

Hornbach, D.J. 1992. Life History Traits of a Riverine Population of the Asian Clam *Corbicula fluminea*. American Midland Naturalist. 127:248-257.

Horne, F.R. and McIntosh, S. 1979. Factors Influencing Distribution of Mussels in the Blanco River of Central Texas. The Nautilus. 94(4):120-133.

Imlay, M.J. 1971. Bioassay Tests with Naiads. In Jorgensen and Sharp. pp 38-41.

Jacobson, P.J. 1990. Sensitivity of Early Life Stages of Freshwater Mussels (Bivalvia : Unionidae) to Copper. Master Thesis. Virginia Tech. Blacksburg, VA.

Keller, A.E. and Zam, S.G. 1991. The Acute Toxicity of Selected Metals to the Freshwater Mussel, *Anodonta imbecilis*. *Environmental Toxicology and Chemistry*. 10:539-546.

Kennedy, V.S. and Van Huekelem, L. 1985. Gametogenesis and Larval Production in a Population of the Introduced Asiatic Clam, *C. fluminea* sp. (Bivalvia : Corbiculidae), in Maryland. *Biological Bulletin*. 168:50-60.

Kraemer, L..R. 1979. *C. fluminea* (Bivalvia:Sphaeriacea) vs. Indigenous Mussels (Bivalvia:Unionacea) in U.S. Rivers: A Hard Case for Interspecific Competition? *American Zoologist*. 19:1085-1096.

Lauritsen, D.D. 1986. Filter Feeding in *Corbicula fluminea* and its Effect on Seston Removal. *Journal of the North American Benthological Society*. 5(3):165-172.

Lauritsen, D.D. and Mozley S.C. 1989. Nutrient Excretion by the Asiatic Clam *Corbicula fluminea*. *Journal of the North American Benthological Society*. 8(2):134-139.

Lloyd, R. and Orr, L.D. 1969. The diuretic response by rainbow trout to sub-lethal concentrations of ammonia. *Water Resources*. 3:335-344.

Lubinski, K.S., Cairns Jr., J. and Dickson, K.L. 1978. Quantifying the effects of ammonia on the swimming behavior of bluegills. *Trace Substances in Environmental Health*. 12:508-514.

McMahon, R.F. 1979. Response to Temperature and Hypoxia in the Oxygen Consumption of the Introduced Asiatic Freshwater Clam *Corbicula fluminea* (Muller). *Comparative Biochemistry and Physiology*. 63A:383-388.

McMahon R.F. and Williams, C.J. 1986. Growth, Life Cycle, Upper Thermal Limit and Downstream Colonization Rates in a Natural Population of the Freshwater Bivalve Molluscs, *Corbicula fluminea* (Muller) Receiving Thermal Effluents. *American Malacological Bulletin, Special Edition No. 2* : 231-239.

- Miller, A.C. and Payne, B.S. 1993. Qualitative Versus Quantitative Sampling to Evaluate Population and Community Characteristics at a Large-River and Mussel Bed. *American Midland Naturalist*. 130:133-145.
- Muncaster, B.W.; Herbert, P.D.N.; and Lazar R. 1990. Biological and Physical Factors Affecting the Body Burden of Organic Contaminants in Freshwater Mussels. *Archives of Environmental Contamination and Toxicology*. 19:25-34.
- Nalepa, T.F., Manny, B.A., Roth, J.C., Mozley, S.C., and Schloesser, D.W. 1991. The Long - Term Decline in Freshwater Mussels (Bivalvia:Unionidae) of the Western Basin of Lake Erie. *The Journal of Great Lakes Research*. 17(2):214-219.
- Neves, R.J. Virginia's Endangered Species, Proceedings of a Symposium, ed. by Karen Terwilliger. Macdonald and Woodward Publishing, Blacksburg. pgs 251-320.
- Neves, R.J. and Widlak, J.C. 1987. Habitat Ecology of Juvenile Freshwater Mussels (Bivalvia : Unionidae) in a Headwater Stream in Virginia. *American Malacological Bulletin*. 5(1):1-7.
- Neves, R.J. and Widlak, J.C. 1988. Occurrence of the Glochidia in Stream Drift and on Fishes of the Upper North Fork Holston River, Virginia. *The American Midland Naturalist*. 119(1):111-120.
- Olson, K.R. and Fromm, P.O. 1971. Excretion of Urea by Two Teleosts Exposed to Different Concentrations of Ambient Ammonia. *Comparative Biochemistry and Physiology*. 40A:999-1007.
- Owen, D.A. and Cahoon, L.B. 1991. An Investigation into the Use of Exotic and Native Bivalves as Indicators of Eutrophication-Induced Hypoxia. *Journal of the Elisha Mitchell Scientific Society*. 107(2): 71-74.
- Payne, B.S.; Miller, A.C.; Hartfield; P.D. and McMahon, R.F. 1989. Variation in Size and Demography of Lotic Populations of *Corbicula fluminea* (Muller). *The Nautilus*. 103(2): 78-82.
- Reddy, N.A. and Menon, N.R. 1979. Effects of Ammonia and Ammonium on Tolerance and Byssogenesis in *Perna viridis*. *Marine Ecology - Progress Series*. 1(4):315-322.

Schubauer-Berigan, M.K. and Ankely, G.T. 1991. The Contribution of Ammonia, Metals and Nonpolar Organic Compounds to the Toxicity of Sediment Interstitial Water From an Illinois River Tributary. *Environmental Toxicology and Chemistry*. 10:925-939.

Stanczyowska, A.; Lawacz, W. and Mattice, J. 1973. Bivalves as a Factor Affecting Circulation of Matter in the Lake. *International Symposium on Eutrophication and Water Quality Control*. 53-58.

Sickel, J.B. 1973. A New Record of *Corbicula manilensis*(Phillippi) in the Southern Atlantic Slope Region of Georgia. *The Nautilus*. 87(1):11-12.

Sickel, J.B. 1986. *Corbicula* Population Mortalities: Factors Influencing Population Control. *American Malacological Bulletin, Special Edition No. 2*:89-94.

Sickel, J.B. and Heyn, M.W. 1980. Decline of the Asiatic Clam, *Corbicula fluminea*, in the Lower Tennessee and Cumberland Rivers. *Bulletin of the American Malacological Union*. 24-26.

Sinclair, R.M. and Isom, G.B. 1963. Further Studies on the Introduced Asiatic Clam (*Corbicula*) in Tennessee. Tennessee Stream Pollution Control Board, Tennessee Department of Public Health. 1-75.

Sousa, R.J. and Meade, T.L. 1977. The Influence of Ammonia on the Oxygen Delivery System of Coho Salmon Hemoglobin. *Comparative Biochemistry and Physiology*. 58A:23-28.

Tankersley, R.A.; and Dimock, R.V. 1993. The Effect of Larval Brooding on the Respiratory Physiology of the Freshwater Unionid Mussel *Pyganodon cataracta*. *American Midland Naturalist*. 130:146-163.

Thurston, R.V.; Russo, R.C.; and Emerson K. 1979. Aqueous Ammonia Equilibrium - Tabulation of Percent Un-ionized Ammonia. EPA Ecol. Res. Ser. EPA-600/3-79-091. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, MN: 427 p.

Warren, L.W. and Klaine, S.J. 1995. Development of a Field Bioassay with Juvenile Mussels. *Journal of the North American Benthological Society*. 14(2):341-346.

Weber, C.I. 1991. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms(fourth edition). EPA-600/4-90/027. Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC: 293p.

Whiteman, F.W., Ankley, G.T., Kahl, M.D., Rau, D.M., and Balcer, M.D. 1996. Evaluation of Interstitial Water as a Route of Exposure for Ammonia in Sediment Tests With Benthic Macroinvertebrates. *Environmental Toxicology and Chemistry*. 15(5):794-801.

Woltering, D.M.; Zehender, F. and Woker, H. 1978. Predator - Prey Interactions of Fishes Under the Influence of Ammonia. *Transactions of the American Fisheries Society*. 107(3):500-504.

Yeager, M.M. 1994. Abiotic and Biotic Factors Influencing the Decline of Native Unionid Mussels in the Clinch River, Virginia. Ph.D. Dissertation. Virginia Tech. Blacksburg, VA.

Zale, A.V. and Neves, R.J. 1982. Reproductive Biology of Four Freshwater Mussel Species (Mollusca: Unionidae) in Virginia. *Freshwater Invertebrate Biology*. 1(1):17-28.

APPENDIX A - WATER-ONLY TEST RESULTS

24 - hour acute exposure of *V. iris* glochidia to ammonium chloride. 4/26/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	114	3
3.25	0.11	159	56
6.40	0.18	112	110
14	0.40	161	160
28	0.43	134	133
55	0.57	147	147
95	0.80	144	144

96 - hour exposure of juvenile *V. iris* (<72 hours) to ammonium chloride. 1/9/96

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	1
0.63	0.07	20	0
1.25	0.12	20	1
2.50	0.21	20	0
5	0.41	20	10
10	0.79	20	10

96 - hour exposure of juvenile *V. iris* (9 days) to ammonium chloride. 1/13/95

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	4
2.50	0.19	20	1
5	0.41	20	8
10	0.61	20	4
20	0.99	20	20
40	1.52	20	20

96 - hour exposure of juvenile *V. iris* (5 days) to ammonium chloride. 1/23/96

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
1.22	0.11	20	0
3.58	0.28	20	0
6.13	0.55	20	1
9.29	0.69	20	3
18.18	1.14	20	12

96 - hour exposure of adult *P. grandis* to ammonium chloride. 5/5/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
10.0	0.17	20	7
29.2	0.52	20	10
102.5	1.70	20	18
294.7	3.43	20	20
1030	6.90	20	20

96 - hour exposure of adult *P. grandis* to ammonium chloride. 5/13/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	1
9.6	0.30	20	7
29.8	0.82	20	12
99	1.85	20	20
311.1	3.87	20	19
1006.7	7.15	20	19

96 - hour exposure of juvenile *C. fluminea* (1 week) to ammonium chloride. 9/15/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
0.25	0.02	21	1
0.43	0.03	20	0
1.41	0.14	20	14
2.75	0.26	20	20
5.39	0.45	20	20
11.10	0.81	20	20
22.50	1.26	20	20

96 - hour exposure of juvenile *C. fluminea* (< 48 hrs) to ammonium chloride 10/21/95

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	19	0
0.60	0.08	21	0
1.20	0.16	21	6
2.50	0.32	20	7
5	0.59	20	20
10	1.04	19	19

96 - hour exposure of juvenile *C. fluminea* (1 week) to ammonium chloride. 10/26/95

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	19	0
0.38	0.04	21	1
0.75	0.08	20	2
150	0.15	20	4
3	0.28	20	20
6	0.50	20	20

96 - hour exposure of adult *C. fluminea* to ammonium chloride. 9/7/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
10	0.52	20	1
30	1.05	20	20
100	2.99	20	20
300	6.15	20	20
1000	13.2	20	20

96 - hour exposure of adult *C. fluminea* to ammonium chloride. 9/14/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
4.45	0.34	20	0
9.95	0.71	20	0
23.20	1.25	20	14
48.00	2.33	20	18
99.60	2.76	20	20

48 - hour exposure of *Ceriodaphnia dubia* (<24 hrs) to ammonium chloride. 4/28/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	1
11.6	0.05	17	6
23.3	0.16	21	17
46.5	0.35	20	20
93	0.72	20	20
200	0.80	20	20

48 - hour exposure of the fathead minnow (<24 hrs) to ammonium chloride. 10/21/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
1.68	0.24	20	0
3.23	0.46	20	0
6.32	0.84	20	1
12.7	1.67	20	19
25.7	3.26	20	20

APPENDIX B - SEDIMENT TEST DATA

96 - hour exposure of juvenile *C. fluminea* (1 week) to ammonium chloride. 6/29/95

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	35	22
Water Ref.	Water Ref.	20	4
0.21	0.04	32	6
0.46	0.08	37	2
0.91	0.15	37	13
4.43	0.50	40	39
8.87	0.88	40	40
26.01	2.10	40	40

96 - hour exposure of *C. fluminea* (3 weeks) to ammonium chloride. 6/29/95

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	41	20
Water Ref.	Water Ref.	18	2
0.21	0.04	35	10
0.46	0.08	38	2
0.91	0.15	40	18
4.43	0.50	40	39
8.87	0.88	39	39
26.01	2.10	40	40

96 - hour exposure of *C. fluminea* (8 weeks) to ammonium chloride. 7/18/95

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	36	6
0.16	0.01	39	5
0.21	0.02	41	9
0.60	0.04	41	17
1.69	0.11	36	25
4.75	0.31	21	21

96 - hour exposure of juvenile *C. fluminea* (1 week) to ammonium chloride. 10/13/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
0.14	0.01	20	3
0.58	0.04	20	0
1.95	0.13	20	0
5.45	0.35	20	12
12.9	0.73	20	20

96 - hour exposure of juvenile *C. fluminea* (1 week) to ammonium chloride. 10/11/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
0.24	0.02	20	0
0.45	0.04	20	0
1.82	0.15	20	0
4.58	0.33	20	3
11.5	0.65	20	20

96 - hour exposure of adult *C. fluminea* to ammonium chloride. 9/14/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
2.03	0.20	20	0
5.354	0.50	20	0
14.5	1.06	20	2
33.8	0.99	20	13
74.9	3.61	20	19

96 - hour exposure of adult *C. fluminea* to ammonium chloride. 10/5/94

Total Ammonia Concentration (mg/L)	NH ₃ -N Concentration (mg/L)	# Exposed	# Dead
Control	Control	20	0
1.72	0.21	20	0
4.88	0.58	20	1
13.4	1.30	20	1
31.1	2.71	20	10
70.5	5.06	20	17

APPENDIX C - DATA FROM ASIAN CLAM DIEOFFS
 Test 1 : Unionized Ammonia Concentrations (NH₃ - N) mg/L.

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	2.2e-3	1.4e-3	2.0e-3	1.3e-3	2.1e-3	2.2e-3	2.0e-3	2.1e-3
3	8.0e-4	3.0e-4	7.9e-5	1.5e-5	4.8e-5	6.2e-4	7.9e-4	9.1e-4
5	8.2e-4	3.6e-4	5.1e-4	6.6e-4	9.6e-4	2.1e-3	2.1e-3	9.4e-4
7	5.0e-4	3.5e-4	4.1e-4	4.1e-4	4.3e-4	8.2e-4	2.0e-3	1.1e-3
9	1.1e-3	5.4e-4	6.6e-4	6.2e-3	1.7e-3	1.1e-3	1.1e-3	7.0e-4
11	9.7e-4	4.2e-4	4.8e-4	7.1e-4	7.7e-4	7.6e-4	1.6e-3	8.8e-4
13	1.3e-3	8.0e-5	BD	1.1e-3	1.8e-3	1.3e-3	8.0e-5	1.3e-3
15	3.3e-3	7.7e-4	4.7e-4	2.1e-3	1.7e-3	4.5e-3	6.1e-3	2.2e-3
17	2.6e-3	9.5e-4	2.1e-3	2.1e-3	6.6e-3	4.7e-3	6.3e-3	7.8e-3
19	3.1e-3	1.0e-3	1.0e-3	1.3e-3	1.4e-3	1.4e-3	8.1e-3	5.3e-3
21	4.3e-3	1.8e-3	1.2e-3	1.5e-3	1.2e-3	2.2e-3	1.0e-3	4.9e-4
23	5.6e-3	1.7e-3	8.7e-4	2.5e-3	3.2e-3	2.8e-3	4.4e-3	0.015
25	4.0e-3	1.3e-3	1.4e-3	3.2e-3	3.7e-3	3.0e-3	8.9e-3	0.011
27	5.4e-3	1.8e-3	1.7e-3	2.7e-3	2.6e-3	4.9e-3	5.6e-3	0.012
31	5.5e-3	3.9e-3	7.1e-3	0.24	0.011	0.011	0.016	9.5e-3
34	1.9e-3	2.5e-3	4.3e-3	6.0e-3	0.011	0.015	0.16	0.27
36	BD	8.7e-5	5.7e-3	5.8e-3	0.011	0.010	0.75	1.39
37	1.7e-3	1.5e-3	6.5e-3	7.3e-3	0.011	8.7e-3	0.89	0.91
37.5	2.9e-3	2.7e-3	7.2e-3	7.9e-3	6.1e-3	4.2e-3	0.89	1.15
38	5.9e-3	3.3e-3	3.5e-3	2.3e-3	6.1e-3	2.6e-3	1.09	1.34
38.5	3.2e-3	9.9e-4	5.5e-3	7.1e-3	4.5e-3	9.5e-3	1.82	2.66
39	3.6e-3	1.4e-3	6.3e-3	5.6e-3	7.8e-3	0.022	1.12	1.60
40	4.6e-3	2.9e-3	4.1e-3	3.8e-3	3.3e-3	9.9-3	2.47	3.89
41	1.2e-3	1.8e-3	6.7e-3	6.4e-3	4.3e-3	0.011	3.41	4.71
42	3.4e-3	5.9e-3	5.5e-3	5.5e-3	5.3e-3	0.013	3.16	3.97

Test I : Concentration of Unionized Ammonia (NH₃- N) mg/L. (cont.)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
43	1.9e-3	1.3e-3	2.1e-3	2.6e-3	3.5e-3	1.1e-3	0.12	0.16
44	1.6e-4	9.1e-5	6.7e-4	1.1e-3	2.1e-3	4.6e-3	0.068	0.086
45	6.4e-3	3.7e-3	5.2e-3	3.8e-3	0.011	4.8e-3	0.058	0.34
46	9.3e-4	1.2e-3	2.1e-3	3.5e-3	1.8e-3	2.0e-3	0.062	0.034
47	2.9e-4	5.8e-4	8.2e-4	9.9e-4	1.7e-3	2.6e-3	0.069	0.047
48	6.9e-4	5.2e-4	1.0e-3	9.8e-4	1.9e-3	2.9e-3	0.053	0.065
50	2.9e-4	5.3e-4	9.9e-4	1.6e-3	1.9e-3	3.1e-3	0.020	3.1e-3
52	9.1e-4	9.7e-4	2.1e-3	1.6e-3	1.8e-3	2.7e-3	0.017	0.018
55	9.0e-4	1.4e-3	3.0e-3	3.5e-3	3.5e-3	5.5e-3	0.015	7.2e-3
58	1.2e-3	2.1e-3	3.7e-3	3.3e-3	3.1e-3	4.1e-3	0.019	0.014
60	7.3e-4	1.0e-3	3.1e-3	2.7e-3	4.7e-3	7.3e-3	0.023	0.023
62	9.3e-3	1.9e-4	4.5e-4	7.4e-4	1.4e-3	1.5e-3	7.2e-3	6.9e-3
64	1.5e-4	2.9e-4	4.8e-4	5.4e-4	1.2e-3	1.1e-3	1.6e-3	8.3e-3

Test I : Percent Mortality of *Corbicula fluminea*

Day	2000A	2000B	5000A	5000B	10000A	10000B
1	0	0	0	0	0	0
3	0	0	0	0	0	0
5	0	1	0.4	1.2	1.2	0.4
7	0	1	0.4	2.4	1.4	0.6
9	0	1	0.4	4	2	0.6
11	0	1	0.4	5.2	2.6	0.6
17	1	1	0.8	6.4	2.6	1.2
19	1	1	0.8	8	3	1.4
21	1	2	0.8	9.6	3	2.2
23	1	3	0.8	12	4	2.4
25	1	5	1.6	12.8	4.6	3.2
27	1	5	1.6	21.2	7.2	3.2
31	6	10	2	26.8	16	4.4
34	8	14	4.8	28	90	13.0
36	8	15	4.8	31	99	95.0
37	8	17	4.8	31.2	100	99.0
38	10	13	4.8	31.2	100	100
39	12	17	4.8	31.2	100	100
40	12	19	4.8	31.2	100	100
41	12	19	5.6	31.2	100	100
42	12	19	5.6	31.2	100	100
43	14	19	6.4	31.2	100	100
44	15	20	7.6	31.2	100	100

Test I : Percent Mortality of *Corbicula fluminea*

Day	2000A	2000B	5000A	5000B	10000A	10000B
45	15	22	7.6	31.2	100	100
46	16	22	9.6	32	100	100
47	19	22	9.6	34	100	100
48	21	22	10.8	34	100	100
50	21	22	12	35	100	100
55	23	22	15	35	100	100
60	26	22	15.2	36	100	100
12	30	22	16	36	100	100
64	41	30	28	50	100	100

Test I : Dissolved Oxygen Concentrations (mg/L).

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	8.15	8.15	8.15	7.90	7.35	7.45	7.50	7.30
2	7.50	7.30	7.20	7.20	7.10	6.60	7.00	6.60
3	7.80	7.60	7.75	7.06	7.00	7.00	7.20	7.50
4	7.40	7.40	7.50	7.30	7.00	6.50	6.80	6.80
5	7.50	7.60	7.60	7.60	7.10	6.50	6.50	7.10
6	7.10	7.10	7.15	6.90	6.50	6.80	6.00	6.85
7	7.10	7.15	7.00	7.00	6.40	6.20	5.40	6.65
8	7.00	6.80	6.90	6.80	6.40	5.90	6.50	6.20
9	7.70	7.50	7.35	7.35	6.60	7.20	7.25	6.90
11	7.80	7.85	7.50	7.50	7.50	7.50	6.50	6.80
12	7.60	7.45	6.90	7.15	6.95	7.00	6.70	6.85
13	8.25	8.25	8.05	7.60	7.55	6.90	7.50	7.50
14	8.00	8.00	7.15	6.60	6.70	6.45	4.40	6.60
15	8.40	8.40	7.70	7.65	7.50	6.85	5.15	6.60
16	8.35	8.30	7.90	8.10	7.25	6.30	7.25	6.85
17	8.80	8.80	8.60	8.40	7.20	7.10	5.20	5.00
18	8.40	8.45	7.80	8.05	7.30	7.40	4.35	4.85
19	8.30	8.35	7.80	8.00	7.50	7.30	4.90	5.45
20	7.80	7.80	7.70	7.60	6.85	6.90	2.20	4.50
21	7.85	7.85	7.55	7.50	7.25	6.70	7.00	6.75
22	7.55	7.70	6.80	7.30	6.20	5.65	2.50	5.15
23	7.70	7.75	7.30	7.15	6.50	5.95	5.45	3.90
25	8.65	8.70	8.35	8.40	7.30	6.60	4.35	4.40
26	8.00	8.15	8.30	8.65	7.10	6.05	4.70	4.00
27	7.50	7.75	7.20	7.65	6.75	4.90	3.00	2.50
31	7.30	7.70	7.65	8.88	7.25	5.65	2.20	1.90

Test I : Dissolved Oxygen Concentrations (mg/L). Cont.

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
32	8.00	8.30	8.15	7.85	7.40	5.80	3.90	3.50
33	7.10	7.55	7.35	7.50	6.55	5.10	1.80	1.70
34	7.80	7.90	7.95	7.80	6.75	5.45	4.05	3.60
35	7.20	7.30	7.00	6.35	5.30	3.20	1.90	1.45
36	7.75	7.95	7.55	8.00	6.40	5.55	1.00	0.70
37	7.90	8.10	7.95	8.20	6.30	6.25	1.30	1.55
37.5	7.80	8.40	8.40	9.10	7.45	5.60	1.30	1.00
38	7.50	7.30	6.40	6.30	4.95	7.30	0.70	0.60
38.5	7.75	8.60	8.75	9.35	7.50	7.00	0.95	0.60
39	7.65	7.80	7.65	8.00	6.90	6.05	0.85	0.60
40	7.50	7.40	6.80	6.90	6.30	5.60	0.85	0.50
41	8.00	8.60	8.40	9.10	8.05	6.85	0.60	0.45
42	8.25	8.75	8.45	8.30	8.25	7.40	0.60	0.50
43	8.20	8.25	8.30	8.00	7.75	7.90	4.85	3.50
44	8.20	8.20	8.35	8.10	7.65	7.90	5.40	3.95
45	8.30	8.90	8.65	8.30	8.00	7.95	4.85	1.20
47	8.05	7.85	7.90	7.75	7.70	7.40	4.60	4.90
48	8.35	7.15	7.90	7.20	7.15	7.00	5.00	4.80
49	8.00	8.35	9.25	8.10	7.80	7.95	4.75	4.90
50	8.25	8.40	8.60	8.50	8.05	8.40	6.00	6.20
51	7.85	7.90	8.45	8.35	7.45	8.0	4.60	5.15
52	8.25	8.10	8.20	8.15	8.0	8.00	6.15	6.20
54	7.15	7.80	7.85	7.85	7.20	7.35	4.10	3.90
55	7.60	7.65	8.10	8.00	7.60	7.95	6.20	5.35
57	7.25	7.35	7.15	7.10	6.70	6.20	5.30	5.40

Test I : Dissolved Oxygen Concentrations (mg/L). Cont.

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
58	7.70	7.75	7.80	7.65	7.30	7.50	6.30	6.30
60	8.05	8.55	8.90	8.90	7.65	8.15	7.00	6.85
62	8.00	7.70	7.45	7.35	7.70	7.10	7.00	6.60
64	8.10	8.00	8.10	7.90	7.90	7.60	7.10	7.20

Test I : pH

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	8.04	7.99	7.92	7.87	7.81	7.82	7.79	7.75
2	7.45	7.39	7.40	7.41	7.43	7.52	7.41	7.48
3	7.54	7.54	7.54	7.55	7.55	7.63	7.58	7.64
4	7.65	7.65	7.62	7.64	7.57	7.54	7.49	7.51
5	7.50	7.56	7.59	7.60	7.58	7.60	7.58	7.57
6	7.54	7.57	7.58	7.58	7.53	7.56	7.46	7.50
7	7.50	7.54	7.56	7.56	7.55	7.51	7.47	7.50
8	7.57	7.56	7.59	7.61	7.57	7.57	7.59	7.54
9	7.59	7.60	7.63	7.64	7.59	7.61	7.64	7.63
10	7.56	7.59	7.58	7.59	7.63	7.59	7.58	7.51
11	7.63	7.68	7.64	7.65	7.63	7.65	7.58	7.60
12	7.59	7.66	7.67	7.68	7.66	7.65	7.65	7.65
13	7.86	7.88	7.92	7.81	7.80	7.77	7.88	7.77
14	7.86	7.89	7.75	7.76	7.79	7.77	7.71	7.84
15	7.94	7.99	7.83	7.86	7.89	7.81	7.69	7.81
16	7.97	7.97	7.84	7.97	7.87	7.76	7.91	7.94
17	7.99	8.02	7.99	7.98	7.83	7.84	7.66	7.65
18	7.87	7.96	7.85	7.89	7.81	7.86	7.54	7.53
19	7.98	7.97	7.88	7.98	7.87	7.88	7.65	7.66
20	7.97	7.99	7.99	8.04	8.01	7.94	7.56	7.70
21	8.01	8.03	8.02	8.07	8.02	7.89	8.01	7.98
22	8.06	8.08	7.96	8.20	7.91	7.82	7.69	7.77
23	8.12	8.10	8.15	8.02	7.88	7.82	7.81	7.80
25	8.05	8.07	7.99	8.03	7.85	7.75	7.65	7.69
26	8.17	8.20	8.25	8.21	7.99	7.82	7.77	7.73
27	8.12	8.13	8.00	8.05	7.92	7.74	7.73	7.67

Test I : pH (cont.)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
30	8.17	8.14	8.11	8.07	7.86	7.70	7.68	7.74
31	8.10	8.22	8.21	8.47	8.20	7.86	7.61	7.60
32	8.16	8.19	8.09	8.03	7.94	7.75	7.67	7.66
33	8.18	8.25	8.14	8.17	8.00	7.82	7.72	7.71
34	8.21	8.25	8.20	8.11	7.95	7.80	7.85	7.94
35	8.17	8.23	8.06	7.97	7.97	7.68	7.94	7.89
36	8.26	8.32	8.12	8.19	7.92	7.83	8.05	8.08
37	8.19	8.30	8.12	8.20	7.83	7.86	7.82	7.52
37.5	8.23	8.39	8.43	8.53	8.13	7.85	7.79	7.62
38	8.16	8.23	8.00	7.97	7.75	7.80	7.76	7.66
38.5	8.26	8.44	8.44	8.57	8.12	8.05	7.74	7.80
39	8.16	8.21	8.09	8.23	7.98	7.89	7.47	7.50
40	8.19	8.25	8.01	8.13	7.92	7.84	7.89	7.94
41	8.32	8.45	8.32	8.50	8.27	8.03	8.04	8.08
42	8.18	8.45	8.20	8.21	8.22	8.05	8.03	8.12
43	7.99	8.05	8.08	7.97	7.88	7.94	7.50	7.60
44	7.87	7.93	7.96	7.87	7.82	7.89	7.61	7.57
45	7.81	8.04	8.01	7.88	7.95	7.85	7.50	7.81
47	7.70	7.71	7.73	7.71	7.66	7.68	7.54	7.58
48	7.67	7.70	7.76	7.67	7.64	7.67	7.59	7.64
49	7.88	8.10	8.33	8.01	7.95	8.06	7.64	7.68
50	7.93	7.96	8.01	8.04	7.91	8.03	7.79	7.84
51	7.90	8.06	8.15	8.15	8.02	8.07	7.68	7.71
52	7.97	8.00	8.00	7.98	7.91	7.92	7.75	7.77
54	7.87	7.98	8.05	8.11	7.93	8.03	7.64	7.62

Test I : pH (cont.)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
55	8.02	8.06	8.14	8.15	8.06	8.14	7.90	7.84
57	8.22	8.18	8.17	8.18	8.06	8.17	8.07	8.00
58	8.08	8.09	8.10	8.09	7.94	8.02	7.93	7.92
60	8.18	8.25	8.34	8.31	8.03	8.19	8.05	8.02
62	7.96	7.99	7.90	7.89	7.93	7.80	7.85	7.80
64	7.77	7.75	7.74	7.71	7.78	7.74	7.74	7.72

Test 1 : Temperature (°C)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	21.2	21.0	20.8	20.8	20.7	20.5	20.5	20.5
2	20.3	20.3	20.2	20.0	19.9	19.4	19.5	19.0
3	19.6	19.9	19.7	19.7	19.6	19.0	19.2	19.4
4	20.9	21.1	21.1	21.1	21.2	20.5	20.5	20.7
5	22.1	22.1	22.0	22.0	21.8	21.5	21.7	21.8
6	20.5	20.7	20.6	20.6	20.2	20.3	20.0	20.3
7	20.9	21.0	21.0	20.9	20.7	20.6	20.5	20.8
8	21.0	20.8	21.0	21.0	20.8	20.5	20.8	20.9
9	21.0	20.7	20.5	20.4	20.3	20.4	20.4	20.4
10	20.5	20.3	19.9	20.0	19.6	19.9	19.9	19.5
11	20.4	20.2	20.2	20.2	20.1	20.1	20.1	20.0
12	20.8	20.5	19.8	20.0	20.2	20.2	20.2	20.1
13	19.2	19.1	19.2	19.1	19.1	19.1	19.2	19.2
14	18.8	18.6	18.8	18.6	18.5	18.5	18.3	18.3
15	18.6	18.5	18.7	18.7	18.5	18.5	18.5	18.5
16	16.6	16.7	16.8	16.7	16.8	16.7	16.0	16.5
17	16.4	16.3	16.3	16.3	16.3	16.3	16.3	16.3
18	17.7	17.4	17.3	17.3	17.2	17.2	17.2	17.2
19	19.4	19.1	19.0	19.0	18.9	18.9	18.8	18.9
20	19.9	19.7	19.6	19.6	19.5	19.4	19.4	19.4
21	20.4	20.3	20.2	20.3	20.3	20.2	20.2	20.2
22	21.0	20.8	20.7	20.8	20.7	20.6	20.5	20.3
23	22.0	21.7	21.6	21.6	21.6	21.6	21.8	21.9
25	18.7	18.3	18.2	18.1	18.2	18.1	18.3	18.4
26	22.2	22.0	21.9	21.9	21.9	21.9	21.9	21.9
27	20.5	20.1	20.0	19.9	19.9	19.7	19.8	19.8

Test 1 : Temperature (°C) cont.

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
30	22.1	21.9	21.7	21.6	21.5	21.5	21.5	21.4
31	22.6	22.2	22.3	22.2	22.0	21.8	21.7	21.7
32	22.4	22.2	22.1	22.1	22.2	22.2	22.2	22.3
33	22.8	22.8	22.7	22.7	22.6	22.5	22.5	22.4
34	22.8	22.8	22.9	22.9	22.9	22.9	23.0	23.0
35	23.5	23.2	23.0	22.9	22.8	22.8	22.8	22.7
36	23.2	23.1	23.1	23.2	23.1	23.1	23.0	23.0
37	22.5	22.4	22.3	22.4	22.3	22.3	22.3	22.3
37.5	23.5	23.4	23.4	23.3	23.3	23.2	23.2	23.1
38	22.9	23.0	22.7	22.6	22.7	22.7	22.6	22.7
38.5	23.9	23.8	23.8	23.9	23.9	23.9	23.8	23.8
39	24.1	24.1	24.1	24.0	24.0	24.0	23.9	23.9
40	23.2	23.1	23.1	23.1	23.1	23.1	23.0	23.0
41	21.0	20.9	20.8	20.9	20.9	20.8	20.8	20.9
42	20.7	20.4	20.3	20.5	20.3	20.4	20.4	20.4
43	22.0	21.7	21.6	21.5	21.6	21.5	21.5	21.5
44	21.0	20.8	20.7	20.6	20.6	20.6	20.5	20.6
45	22.1	21.9	21.8	21.6	21.3	21.5	21.4	21.2
47	22.5	22.4	22.4	22.4	22.4	22.5	22.5	22.5
48	22.7	22.6	22.7	22.6	22.6	22.6	22.6	22.7
49	19.1	19.1	18.9	19.0	19.4	19.1	19.2	19.4
50	18.3	17.9	18.1	18.1	18.2	17.9	18.0	18.0
51	18.7	18.5	18.5	18.5	18.5	18.4	18.4	18.5
52	19.1	19.0	18.9	18.9	18.9	18.8	18.8	18.8
54	21.5	21.3	21.3	21.3	21.3	21.2	20.9	20.8

Test I : Temperature (°C) cont.

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
55	22.2	22.0	22.0	22.0	22.0	21.9	21.9	21.8
57	22.5	21.8	22.0	22.0	21.9	22.0	22.2	22.3
58	23.0	22.5	22.6	22.6	22.6	22.7	22.7	22.8
60	23.2	22.6	22.8	22.8	22.7	22.7	22.8	22.9
62	20.0	19.3	19.5	19.5	19.7	19.6	19.7	19.7
64	17.4	17.5	17.3	17.1	17.2	17.1	17.4	17.2

Test I : Conductivity

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	290	290	290	290	300	300	300	300
2	190	190	200	200	210	220	220	220
3	200	200	200	200	200	220	220	230
4	200	200	200	200	200	220	210	200
5	200	200	200	200	200	220	220	200
6	200	200	200	200	200	200	210	200
7	180	200	200	200	200	200	220	210
8	260	260	260	260	270	260	260	260
9	250	280	280	280	280	280	280	280
10	260	270	270	280	300	280	280	320
11	260	270	270	270	280	280	280	280
12	270	270	270	270	280	280	280	270
13	260	260	300	300	300	340	320	320
14	300	300	280	310	340	340	390	380
15	320	320	280	340	360	360	440	420
16	330	330	300	340	380	390	460	440
17	360	360	330	370	400	410	500	500
18	300	320	310	310	340	350	380	360
19	320	330	330	350	370	370	450	430
20	350	340	340	360	380	380	440	440
21	380	360	370	380	400	400	500	490
22	400	380	400	400	420	420	450	500
23	400	370	400	400	450	460	570	580
25	390	380	390	400	440	440	520	560
26	430	420	430	430	470	490	600	640
27	400	390	400	410	440	460	540	580

Test I : Conductivity (cont.)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
30	420	420	440	440	490	510	630	610
31	350	410	370	410	410	470	530	540
32	420	420	410	430	460	520	600	610
33	440	430	430	440	480	540	630	680
34	460	460	460	460	570	580	710	830
35	460	460	470	460	510	600	820	960
36	530	490	480	480	560	640	1000	1200
37	420	440	460	440	480	520	850	1100
37.5	440	440	460	440	480	540	920	1300
38	460	450	470	460	500	560	1100	1100
38.5	460	460	480	460	520	580	1200	1500
39	420	420	420	440	460	480	1400	1500
40	440	430	450	450	480	510	1200	1600
41	440	440	460	460	510	550	1400	1600
42	490	460	490	480	540	580	1400	1600
43	300	290	300	300	300	300	420	460
44	290	290	290	290	300	300	360	390
45	180	180	180	180	340	200	260	570
47	240	240	240	240	240	240	300	280
48	240	250	250	250	250	250	290	300
49	240	240	250	240	250	250	260	260
50	230	250	260	260	260	260	280	280
51	250	250	250	250	250	250	260	260
52	240	250	250	250	260	260	270	270
54	350	350	350	350	350	350	370	360

Test I : Conductivity (cont.)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
55	460	460	460	460	460	470	480	480
57	410	420	420	430	440	440	460	440
58	370	370	370	370	380	380	380	380
60	350	360	360	360	390	360	370	370
62	300	300	300	300	300	310	320	320
64	220	220	220	220	225	220	225	230

Test II : Unionized Ammonia Concentrations (mg/L)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	0.016	5.5e-3	2.8e-3	1.5e-3	2.4e-3	2.4e-3	4.7e-3	3.2e-3
5	8.1e-3	2.2e-3	8.3e-3	9.3e-3	3.5e-3	0.015	0.011	0.015
7	0.016	2.9e-3	4.3e-3	4.7e-3	2.6e-3	7.7e-3	0.010	0.013
9	4.7e-3	1.9e-3	2.4e-3	2.2e-3	1.9e-3	0.014	0.011	8.6e-3
12	6.6e-3	2.8e-3	3.2e-3	1.6e-3	7.8e-3	6.9e-3	6.8e-3	7.2e-3
14	3.0e-3	1.6e-3	1.5e-3	1.6e-3	1.6e-3	2.9e-3	5.6 e-3	3.5-3
16	3.9e-3	2.5e-3	1.6e-3	2.3e-3	1.1e-3	4.1e-3	0.023	0.064
19	0.012	2.8e-3	2.0e-3	9.4e-3	5.4e-3	3.3e-3	0.26	0.80
20	0.010	6.8e-3	2.8e-3	0.022	3.4e-3	9.3e-3	0.59	1.10
21	3.7e-3	4.7e-3	1.7e-3	0.020	2.4e-3	3.8e-3	0.74	1.24
22	4.2e-3	5.6e-3	2.6e-3	0.018	2.3e-3	4.5e-3	1.94	2.75
23	0.010	4.3e-3	2.3e-3	0.019	2.4e-3	0.011	4.19	4.66
24	5.8e-3	3.9e-3	2.9e-3	0.020	2.8e-3	2.7e-3	4.69	5.04
25	5.2e-3	2.4e-3	1.9e-3	2.2e-3	1.3e-3	1.9e-3	0.43	0.38
26	8.6e-3	3.3e-3	3.7e-3	3.1e-3	2.2e-3	2.7e-3	2.7e-3	0.041
27	0.022	6.2e-4	1.3e-3	1.9e-3	1.1e-3	1.6e-3	0.031	0.025
28	5.3e-3	4.0e-3	1.9e-3	6.1e-3	1.1e-3	2.3e-3	0.015	0.026
30	0.013	3.3e-3	2.5e-3	3.4e-3	1.7e-3	1.9e-3	6.5e-3	0.014
34	2.2e-3	1.9e-3	1.4e-3	1.1e-3	1.1e-3	1.6e-3	2.3e-3	3.0e-3

Test II : % Mortality

Day	2000A	2000B	5000A	5000B	10000A	10000B
2	1	2	1.6	2.8	3.4	3.4
3	3	3	2.8	2.8	4.8	3.6
5	10	10	4.4	6.4	5.6	5.4
6	11	11	4.8	6.8	5.6	7.2
7	13	11	5.2	6.8	6.8	7.2
8	13	11	5.2	6.8	7.8	8.4
9	14	12	5.2	8	8.2	9
11	15	14	6	9.2	10.2	9
12	15	14	6	9.2	10.2	11
13	15	15	6	9.2	10.2	11
14	15	15	6	10	10.2	11
15	15	15	6	10	10.2	11
16	15	15	6	10	10.2	11
19	15	17	6.4	10	12.8	80
20	15	20	6.8	10	80	95
21	15	20	6.8	10	100	100
22	16	20	6.8	10	100	100
23	16	23	7.2	10	100	100
24	16	24	7.2	10	100	100
26	17	26	7.2	10.4	100	100
29	17	30	7.2	10.4	100	100
34	28	33	10	13.2	100	100

Test II : Dissolved Oxygen mg/L

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
2	6	6.1	5.6	5.7	4.2	4.6	3.3	3.0
3	6.1	6.1	5.3	5.2	4.7	4.6	3.6	2.0
5	6.7	6.8	6.3	6.1	5.6	5.5	4.9	3.4
6	6.3	6.3	4.7	5.1	3.5	3.9	2.5	2.1
7	6.7	6.7	6.0	6.1	6.2	5.6	4.6	3.9
8	6.2	6.2	5.5	5.5	5.2	4.3	3.2	2.5
9	6.2	6.2	5.9	5.9	5.6	5.1	4.2	3.4
10	6.3	6.3	5.8	5.7	5.3	4.4	3.8	2.9
12	6.9	6.6	6.5	6.2	6.2	5.8	4.6	4.2
13	6.2	6.2	5.6	5.3	5.2	4.8	3.9	3.5
14	7.1	7.1	6.9	6.7	6.7	6.1	4.8	5.2
16	7.2	7.1	6.5	5.3	5.4	4.4	3.5	3.1
19	7.0	7.0	6.0	4.4	4.2	4.6	3.2	1.2
20	7.0	7.0	6.3	4.7	4.9	4.3	1.3	0.9
21	7.2	7.2	6.5	5.2	5.1	5.1	1.5	1.1
22	6.9	6.9	6.0	4.4	4.9	3.8	1.3	0.9
23	7.5	7.5	6.7	5.1	5.7	4.2	1.6	1.1
24	6.8	6.8	6.7	5.1	5.3	5.1	1.2	0.8
25	6.7	6.6	6.6	6.6	5.7	6.0	2.2	1.8
26	6.6	6.6	6.6	6.1	5.9	6.0	3.0	2.8
27	6.4	6.3	6.4	5.9	5.7	5.7	2.5	1.9
28	6.8	6.8	6.8	6.8	5.8	6.3	3.0	2.3
29	6.0	6.4	6.3	5.7	4.0	4.8	2.8	1.6
30	6.6	6.7	5.4	6.5	6.5	6.1	4.4	2.8
34	6.9	7.1	7.2	7.3	6.2	6.3	5.5	5.5

Test II : pH

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	8.02	8.04	7.84	7.85	7.66	7.72	7.60	7.60
2	8.05	8.10	7.89	7.98	7.79	7.80	7.80	7.76
3	7.91	7.94	7.68	7.71	7.58	7.62	7.59	7.43
5	7.85	7.85	7.70	7.72	7.55	7.59	7.50	7.45
6	7.87	7.89	7.62	7.71	7.44	7.52	7.40	7.39
7	8.05	7.92	7.69	7.72	7.71	7.62	7.49	7.46
8	7.81	7.87	7.66	7.67	7.63	7.52	7.47	7.40
9	7.94	7.89	7.80	7.80	7.69	7.67	7.57	7.49
10	7.85	7.86	7.72	7.75	7.58	7.80	7.50	7.46
12	8.04	8.02	7.90	7.88	7.82	7.76	7.55	7.53
13	7.97	7.97	7.70	7.68	7.53	7.51	7.50	7.42
14	7.82	7.83	7.79	7.77	7.78	7.70	7.46	7.59
16	8.02	8.03	7.78	7.56	7.58	7.59	7.65	7.67
19	8.12	8.14	7.71	7.57	7.46	7.75	7.93	7.80
20	8.24	8.23	7.88	7.74	7.59	7.74	7.87	7.54
21	7.87	8.03	7.64	7.71	7.47	7.63	7.52	7.55
22	8.03	8.29	7.85	7.73	7.64	7.71	7.66	7.82
23	8.13	8.15	7.86	7.72	7.68	7.67	7.95	8.06
24	8.13	8.25	7.90	7.78	7.71	7.86	8.06	8.14
25	8.03	7.98	7.97	7.93	7.64	7.84	7.54	7.55
26	8.11	7.98	7.95	7.83	7.78	7.88	7.58	7.58
27	7.99	7.91	7.93	7.81	7.72	7.81	7.55	7.53
28	8.03	8.04	7.89	7.92	7.65	7.87	7.64	7.65
29	7.80	7.92	7.96	7.95	7.52	7.59	7.52	7.55
30	7.97	7.97	7.81	7.97	7.96	7.96	7.98	7.68
34	7.77	7.81	7.80	7.81	7.51	7.62	7.67	7.65

Test II : Temperature (°C)

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	23.6	23.7	24.0	23.9	23.5	23.6	23.5	23.3
2	24.9	25.1	23.9	25.4	25.2	25.0	24.8	24.8
3	26.8	26.4	26.7	26.8	26.6	26.5	26.4	25.0
5	26.5	27.1	27.0	27.1	26.6	26.8	26.4	26.6
6	26.4	26.7	26.7	26.8	26.7	26.8	26.6	26.3
7	26.4	26.8	26.6	26.8	26.5	26.9	26.3	26.3
8	26.5	26.6	26.5	26.8	26.5	26.4	26.5	26.3
10	26.4	26.6	26.6	26.4	26.3	26.8	26.3	26.1
12	26.6	26.8	26.9	26.6	26.3	26.9	26.6	26.6
13	26.2	26.5	26.4	26.3	25.9	26.4	26.3	25.4
14	26.2	26.3	26.3	25.9	25.9	26.3	26.1	25.5
16	25.2	25.4	25.3	25.2	25.4	25.5	25.4	25.2
19	25.7	25.9	25.7	25.7	26.1	25.9	25.9	25.7
21	25.1	25.4	25.4	25.3	25.5	25.5	25.3	25.5
22	25.1	25.3	25.2	25.3	25.2	25.3	25.5	25.4
23	25.3	25.6	25.7	25.4	25.6	25.2	25.5	25.5
25	26.3	26.6	26.8	26.4	25.8	26.3	25.0	25.0
26	26.2	26.4	26.6	25.9	26.1	25.8	25.8	25.8
27	28.0	27.3	28.8	27.7	27.2	27.9	27.6	27.7
29	25.9	26.2	25.9	24.1	25.5	26.1	25.2	24.2
30	25.9	26.4	24.4	26.1	26.0	26.1	25.4	24.7
34	24.2	24.1	24.1	24.0	24.1	24.0	24.0	24.0

Test II : Conductivity

Day	0A	0B	2000A	2000B	5000A	5000B	10000A	10000B
1	270	270	270	270	270	270	280	280
2	300	320	310	330	330	330	330	330
3	290	290	300	290	300	300	300	300
5	230	230	230	230	230	230	240	240
6	260	260	270	260	260	260	270	270
7	270	270	270	270	270	270	280	280
8	270	270	270	270	270	280	280	280
9	300	300	300	310	310	310	320	320
10	280	290	290	290	290	300	300	300
12	260	260	260	260	260	260	270	270
13	260	260	260	260	270	270	270	270
14	260	260	260	260	260	260	280	280
16	290	280	290	290	300	330	380	380
19	320	330	340	380	370	400	660	660
20	370	360	270	420	380	460	910	910
21	330	330	330	380	360	380	920	920
22	360	350	340	400	370	410	1200	1200
23	360	360	360	440	400	450	1400	1400
24	380	380	370	480	430	480	1300	1300
25	250	250	250	250	250	250	550	550
26	240	240	240	240	240	240	390	290
27	240	240	240	240	240	240	280	280
28	240	240	240	240	240	240	260	260
29	240	240	240	240	240	240	250	250
30	240	240	260	240	240	240	250	250
34	200	200	200	200	200	200	210	210

Curriculum Vita

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April 1997

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Education

M.S. Program in Aquatic Toxicology, Virginia Tech University, Blacksburg, VA 24061. August 1994 to present.

B.S. Biology, Purdue University, West Lafayette, IN. May 1993.

Employment

Graduate Teaching Assistant. Virginia Tech University. Spring 1995 - Spring 1997. Duties include giving prelab lectures and preparing and grading exams, quizzes and papers for general biology lab sections.

Independent Biological Consultant. March 1994 - Present. Performed bioassays and water chemistry. Conducted field sampling and macroinvertebrate identification.

Graduate Research Assistant. Virginia Tech University. Spring 1994 - Fall 1994. Conducted acute and chronic aqueous and sediment bioassays with 11 different test organisms. Conducted artificial stream and field biomonitoring studies. Cultured organisms for laboratory toxicity testing.

Employment, (cont.)

Laboratory Technician. Pitman-Moore. Terre Haute, IN. Fall 1993. Performed inhibition assays to determine efficacy of agricultural pharmaceuticals. Prepared chemical inventory and MSDS.

Research Assistant. Purdue University. West Lafayette, IN. Summer 1992 and 1993. Assisted in lab and field research on soybean and turf grass fungal diseases. Responsible for isolating and transferring fungal cultures and preparing media agar. Maintained plants in field and greenhouse.

Research Assistant. Purdue University. West Lafayette, IN. August 1991-May 1993. Assisted in lab research on fungal infections of stored grain. Performed serial dilutions, whole kernel plating and germination tests on stored corn seed. Identified fungi and recorded data.

Laboratory Technician. Eli Lilly and Company. Clinton, IN. Summer 1990. Performed various analytical assays to determine lipid content, particle size and moisture content of agricultural pharmaceuticals.

Published Abstracts and Presentations

Scheller J.L., Cherry D.S., Yeager M.M., Lynde S.R., and Shepard N.D. 1994. Water and Sediment Toxicity of Freshwater Mussels for Population Crashes of Asiatic Clams. Fifteenth annual meeting, Society of Environmental Toxicology and Chemistry. (Poster Presented).

Cherry D.S., Yeager M.M., Scheller J.L., Lynde S.R., and Shepard N.D. 1994. The Influence of Ammonia Sediment Toxicity From Population Crashes of Asiatic Clams on Freshwater Mussels. Forty-second annual meeting, North American Benthological Society.

Lynde S.R., Scheller J.L., Balfour D.L., Cherry D.S. and Fenster A.F. 1995. Non-target Effects of a Non-oxidizing Molluscicide on Water Column and Sediment Associated Organisms. Sixteenth annual meeting, Society of Environmental Toxicology and Chemistry.

Scheller J.L., Cherry D.S., and Yeager M.M. 1996. The Impact of Ammonia Produced During Dieoffs of the Asian Clam (*C. fluminea fluminea*) on Freshwater Mussels. Seventeenth annual meeting, Society of Environmental Toxicology and Chemistry. (Poster presentation)

Technical Reports

Lynde, S.R.; and Scheller, J.L and Cherry, D.S. 1994. Bentonite Clay: Didecyldimethylammonium Chloride (DDAC): Evaluation in a Chronic (10-day) Static Sediment Toxicity Bioassay with *Chironomus tentans*. Lonza Inc. Fair Lawn, New Jersey. 35pp.

Lynde, S.R.; and Scheller, J.L and Cherry, D.S. 1994. Bentonite Clay :Didecyldimethylammonium Chloride (DDAC): Evaluation in a Chronic (10-day) Static Sediment Toxicity Bioassay with *Daphnia magna*. Lonza Inc. Fair Lawn, New Jersey. 35pp.

Lynde, S.R.; and Scheller, J.L and Cherry, D.S. 1994. Bentonite Clay :Didecyldimethylammonium Chloride (DDAC): Evaluation in a Chronic (10-day) Static Sediment Toxicity Bioassay with *Hexagenia limbata*. Lonza Inc. Fair Lawn, New Jersey. 35pp.

Professional Activity

Sonoco Products Company, Downingtown, Pennsylvania. Benthic macroinvertebrate surveys to determine the impact of chlorine dioxide treated effluent into a stream ecosystem.

Virginia Environmental Endowment, Floyd County, Virginia. Rapid bioassessment of benthic macroinvertebrate community structure in the Little River watershed to assess habitat degradation and instream community impairment due to erosion and sedimentation from agricultural runoff.

Activities and Organizations

President-Purdue Biology Club
Activities Director-Purdue Biology Club
Corridor Representative-Residence Hall Council
Volunteer-Wolf Research Park
Member-Science Forum
Biology Graduate Student Association

Special Recognition

Dean's List
Semester Honors
Phi Beta Kappa
SETAC Travel Award (\$275 dollars, matched by Virginia Tech)

Professional Organizations

Society for Environmental Toxicology and Chemistry
North American Benthological Society
American Society for Testing and Materials

Research Experience

Laboratory skills

Culturing *Chironomus tentans*, *Hyallela azteca*, *Hexagenia limbata*,
Ceriodaphnia dubia, *Daphnia pulex*, and *Daphnia magna*.
Water Chemistry (pH, alkalinity, hardness, ammonia etc.)
Benthic Macro-invertebrate Identification

Freshwater acute and chronic toxicity testing

Ceriodaphnia dubia
Pimephales promelas
Daphnia magna
Pyganodon grandis
Corbicula fluminea

Sediment toxicity testing

Chironomus tentans
Hyallela azteca
Daphnia magna
Corbicula fluminea
Villosa iris
Hexagenia limbata

Field Monitoring

Benthic Macro- invertebrate sampling
Sediment Sampling

Relevant Coursework

Diversity, Ecology & Behavior
Development, Structure & Function
Cell Structure & Function
Statistics
Evolution of Behavior
Environmental Engineering
Biochemistry
Hazard Evaluation
Advanced Ecology
Quantitative Analytical Chemistry

Evolution
Limnology
Fish Environmental Physiology
Genetics
General Chemistry
Organic Chemistry
Aquatic Entomology
Evolution of Behavior
Wildlife Management
Stream Habitat Management