Evaluating the Feasibility of Rearing Juvenile Freshwater Mussels in a Flow-Through Pond System at White Sulphur Springs National Fish Hatchery

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by

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(ABSTRACT)

A flow-through pond at White Sulphur Springs National Fish Hatchery was evaluated as culture environment for juvenile freshwater mussels of *Villosa iris* and *Lampsilis fasciola*. Survival did not differ significantly ($p = 0.1910$) over 93 d for *V. iris* cultured with silt (mean 49.8% ± SD 14.5) and without (mean 32.9% ± SD 11.7). Survival differed significantly ($p < 0.0001$) between juveniles of *V. iris* (mean 49.8% ± SD 14.5 at age 93 d) and *L. fasciola* (mean 6.3% ± SD 4.5 at age 86 d). This may indicate that the pond failed to meet requirements of *L. fasciola*, or may have resulted from microhabitat variables. Growth did not differ significantly between species ($p = 0.1315$). *Villosa iris* reached a mean length of 1.81 mm ± SD 0.67, and *L. fasciola* 1.78 mm ± SD 0.78. Water quality parameters remained within suitable ranges, and planktonic algal densities were between 2850 - 6892 cells/mL. Survival of *V. iris* and growth of both species compares favorably to previous culture attempts.

Juveniles of *V. iris* and *L. fasciola* were exposed to ammonium chloride solutions for 96 h in static renewal conditions at 12°C and 20°C. Calculating LC_{50} values with the Trimmed Spearman-Karber method, juveniles of *L. fasciola* (mean 96 h LC_{50} of 0.26 mg/L NH_3-N) were significantly more tolerant of unionized ammonia than juveniles of *V. iris* (mean 96 h LC_{50} of 0.11 mg/L NH_3-N). The only organisms with reported LC_{50} values lower than those seen for *V. iris* juveniles were *Ceriodaphnia dubia* and *Hyella azteca*.

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Chapter 1: General Introduction and Justifications

The freshwater mussel fauna (Unionidae) of North America is the most diverse in the world, with 297 recognized species and sub-species (Turgeon *et al.*1988). Living as benthic filter feeders, freshwater mussels occur in habitats ranging from headwater streams and large rivers to ponds and lakes. Ecologically, mussels are important as a food source for vertebrate mammals, including muskrats, otters, and raccoons, and for some fish. They also play a significant role in filtering the water column, removing phytoplankton and particulate organic matter (Amyot and Downing 1991, Nalepa *et al.* 1991). Unionids sometimes constitute up to 90% of the total biomass of benthic communities (Ökland 1963).

Humans utilized North America's freshwater mussels prior to European colonization, and the use of mussels continues into modern times. Native Americans used mussels for making tools and decorative objects and as a supplemental food source (Parmalee *et al.*1982, Williams *et al.* 1993). In the first half of the 20th century, the shells of some mussel species were harvested to manufacture pearl buttons (Matteson 1955). Presently, portions of some species' shells are used as seed pearls in the cultured pearl industry (Williams *et al.* 1993). Mussels are also recognized for their potential in biomonitoring. As sedentary filter feeders, they are exposed to large volumes of water and are unable to rapidly move away from pollution. Therefore, declines in mussel populations can indicate water quality problems (Matteson 1955, Neves 1993, National Native Mussel Conservation Committee 1998). Additionally, they can bioaccumulate heavy metals in their shells, and, because they are long lived, analysis of shell material can provide a history of metal levels in the water (Metcalfe-Smith 1994).

Threats to Freshwater Mussels

In recent decades, freshwater mussel populations have undergone tremendous declines. Over 70% of North America's mussel species are considered to be either endangered, threatened or of special concern (Williams *et al.* 1993). Poor water quality, habitat destruction, and competition from exotic species are the primary factors that have led to their decline. Since their introduction to North America in the early 1900's, Asian

clams (*Corbicula fluminea*) have colonized almost every major river system in the United States and are believed to compete with juvenile mussels in situations where resources are limited (Neves 1993, Parmalee and Bogan 1998). Zebra mussels (*Dreissena polymorpha*) first came to the Great Lakes region from Europe in the 1980's and have spread rapidly throughout many major river systems where native freshwater mussels occur. Zebra mussels tend to dominate the sites where they are found and have the potential to eliminate most other members of the benthic community (Neves 1993, Trdan and Hoeh 1993, National Native Mussel Conservation Committee 1998, Parmalee and Bogan 1998).

Direct destruction of unionid habitat can occur as a result of dredging, channelization, and the impoundment of flowing waters (Williams *et al.* 1993). The construction of dams is particularly disruptive of mussel populations in that not only is the physical habitat above and below the dam altered, but water quality and flow characteristics are also affected. Dams result in the creation of static, deep waters in the impounded area, alternations of low flow and scouring of the river channel, and changes in temperature, oxygen content, and substrate composition (Parmalee *et al.*1982, Layzer *et al.*1993). Besides the temperature fluctuations produced by dams, clearing of riparian vegetation can result in elevated water temperatures. Mussel populations can be negatively affected because changes in temperature may result in physiological stress, disruption of reproductive cycles, and altered water chemistry (Matteson 1955, Young and Williams 1984, Gordon and Layzer 1989, Layzer *et al.*1993). Sedimentation of waterways resulting from poor land use practices, such as over-clearing in agriculture or clear cutting, also impacts mussel habitat (Layzer *et al.*1993, Michaelson and Neves 1995). The deposition of fine silt can smother mussels or impair their ability to filter feed (Imlay 1972, Box and Mossa 1999). Muddy water is filtered less efficiently and also causes mussels to close their valves, thus reducing the time spent feeding (Bailey 1989, Rogers 1999).

Water pollution also poses a significant threat to mussels. Agricultural and mining runoff, industrial discharges, and wastewater treatment effluents can both alter water chemistry and contain toxicants. Inflow of these pollutants can result in alterations in dissolved oxygen content, pH, alkalinity and hardness levels and may carry toxic

substances such as potassium, zinc, copper, chlorine, cadmium, and arsenic (Michaelson and Neves 1995, Layzer *et al.*1993).

A number of factors make freshwater mussels especially vulnerable to these anthropogenic disturbances. Their sedentary nature and inability to travel efficiently in a directional manner prevents them from actively or rapidly seeking new habitats to avoid unfavorable conditions (Matteson 1955). Mussel exposure to water quality problems is also maximized by their filter feeding mechanism, which requires them to pass large quantities of water through their gills as they feed. Finally, unionids' complex life cycle also contributes to their vulnerability to degraded environmental conditions.

Life Cycle

Freshwater mussels are predominantly dioecious, although hermaphroditic species do occur (Lefevre and Curtis 1912, Zale and Neves 1982, Gordon and Layzer 1989). Males release sperm into the water, and females then take the sperm in through their inhalent siphons as they filter feed. Fertilized eggs develop into larval glochidia in the female's marsupial gill and then are released into the water column. In a single season, a female mussel may produce from 75,000 to 3.5 million glochidia (Neves 1993). Mussel species may be either long-term (bradytictic) or short-term (tachytictic) brooders. In long-term brooders, eggs are generally fertilized in the summer, glochidia are held in the marsupial chamber over the winter, and released in the spring. In short-term brooders, eggs are fertilized in the spring, and the glochidia are released that summer (Weiss and Layzer 1993). The release of glochidia is triggered by environmental cues, such as flow levels and water temperature (Michaelson and Neves 1995).

In order to develop into juvenile mussels, the larval glochidia must attach parasitically to a fish host. Glochidia will attach to either the fish host's fins or gills, depending on the species of mussel. Generally, the glochidial infestation will not harm the fish host, but occasionally gill infestations may be so heavy that the fish's respiration is impaired. The mussel-host fish relationship is species-specific, with a particular species of mussel only able to develop on certain species of fish. Mussel species differ in the extent to which their use of hosts is restricted, with some mussels able to transform on

a variety of fish, while others may have only one or two potential hosts (Zale and Neves 1982, Gordon and Layzer 1989).

Once attached to a suitable fish host, the glochidia become encysted within the tissue of the fish and obtain nutritive and sera factors from the fish blood which enable it to transform into a juvenile mussel (O'Connell 1991). The process of metamorphosis may last from 7 to 30 days, and the duration is dependent on both the species and environmental factors, especially temperature (Neves 1991). Once metamorphosis has occurred, juvenile mussels drop from the host fish and begin the benthic phase of life in the substrate. Reproductive maturity is reached in three to five years (Neves 1991).

The large number of glochidia produced by a female mussel each year represents a tremendous reproductive potential. The fact that mussels evolved such a high fecundity to sustain their populations indicates that most glochidia will not make it to adulthood. Many biologists have pointed out that significant mortality probably occurs at both the glochidial and juvenile stages. First, contact between the glochidia and an appropriate host fish is an improbable event. As a result, this is generally believed to be the life stage at which the greatest levels of mortality occur (Young and Williams 1984, Neves *et al*. 1985, Neves 1993). Secondly, when the juveniles drop from the host fish, they will only survive if they land in suitable habitats. Juveniles are also especially susceptible to predation by fish. (Kat 1982, Amyot and Downing 1991).

Anthropogenic influences may be especially hard on the glochidial and juvenile phases. For one thing, it is generally believed that they both are particularly susceptible to contaminants (Neves 1993). Furthermore, at the glochidial stage, attachment to a suitable host fish becomes even less likely if host fish populations are diminished as a result of water problems or other human influences (Michaelson and Neves 1995). Dams that block fish passage could also both inhibit the occurrence of appropriate host fish and also could curtail the distribution of juveniles (Layzer *et al*.1993).

When field surveys of mussel populations are conducted, juvenile mussels are rarely found, even among aggregations of adults (Kat 1982, Yeager *et al*.1994, Michaelson and Neves 1995). In some cases, this may simply be because their small size makes them difficult to locate (Matteson 1955, Gordon and Layzer 1989, Michaelson and Neves 1995). Juveniles may occupy different habitats than adults. For example, in a

river where adults were found in freely flowing reaches, juveniles were found in depositional areas behind large rocks and along the banks (Yeager *et al*.1994). Other researchers have found juveniles to have a greater tendency than adults to remain below the sediment, endo-benthic (Amyot and Downing 1991). As a result of these habitat variations, some researchers have attributed the infrequency with which juveniles are found to a lack of knowledge of juveniles' habitat and distribution (D'Eliscu 1972, Kat 1982, Gordon and Layzer 1989, Amyot and Downing 1991).

Biologists have also speculated that the difficulty of locating juveniles in field studies reflects current population declines. Decreased recruitment might be a major problem facing remaining reproducing mussel populations, with juveniles' sensitivity to environmental degradation interfering with their recruitment (Amyot and Downing 1991, Yeager *et al*.1994). Juveniles' thin shells may be less able than adults' shells to protect them from contaminants. Additionally, their close relationship with the sediment, including pedal feeding and filtering of interstitial water, may increase their exposure to sediment-bound or settled-out toxicants (Yeager *et al*.1994).

Some researchers have also suggested that the sensitivity of juvenile mussels may result in population dynamics that include weak or missing year classes and where the majority of recruitment occurs during benign periods (Kat 1982, Amyot and Downing 1991, Michaelson and Neves 1995). If this is the case, it is difficult to know whether these patterns of irregular recruitment have always been characteristic of mussel populations, or if they are indicative of declining populations.

Project Goal and Objectives:

Goal: To evaluate the facilities at White Sulphur Springs National Fish Hatchery for their potential use in rearing juvenile mussels.

Objectives:

1. To establish appropriate conditions and techniques for grow-out of juvenile mussels in a raceway receiving hatchery effluent water and in a flow-through pond system receiving well water and spring water.

2. To determine whether the addition of fine silt to the substrate affects the growth or survival of juvenile mussels held in the pond system.
3. To determine whether growth and survival differ between two species held in the pond system.
4. To evaluate the tolerance of juvenile mussels to total and unionized ammonia.

Study Species

The rainbow mussel (*Villosa iris*) and wavy-rayed lampmussel (*Lampsilis fasciola*) were chosen as study species for a number of reasons. Both are suitable species to work with because they are fairly common, with stable populations. Both are long-term brooders, and gravid specimens can be obtained almost year round. Additionally, both species have host fish in the centrarchid family which are suitable for use in laboratory culture. Largemouth bass and smallmouth bass (*Micropterus salmoides* and *M. dolomieu*) serve as hosts for *L. fasciola,* and rock bass (*Ambloplites rupestris*) serves as a host for *V. iris*. Centrarchids are good candidates for laboratory culture work because they are relatively easy to hold in captivity, have been shown to transform large numbers of juvenile mussels, and can usually be easily collected from the wild or purchased from fish hatcheries.

Justification for Objectives 1, 2, and 3:

Recovery plans for endangered mussel species recommend propagation and reintroduction as a means to conserve diminishing populations (National Native Mussel Conservation Committee 1998). Once mussel populations decline, recolonization rates are slow, even if environmental conditions improve (Ahlstedt 1979). Reintroduction can restore species into their historical range and augment existing populations. The enhancement of existing populations may allow denser populations to achieve greater reproductive success as increased proximity of male and female mussels may increase fertilization rates (Downing *et al.*1993, National Native Mussel Conservation Committee 1998).

Artificial culture of juvenile mussels in the laboratory may enable some of obstacles present in the natural life cycle to be circumvented. For example, contact between the glochidia and an appropriate host fish can be ensured. Additionally, once habitat requirements for juveniles are understood, newly metamorphosized juveniles can be guaranteed suitable habitat in the laboratory and can also eventually be reintroduced to the most suitable habitat available. Cultured juveniles may also be raised under controlled conditions and released into natural habitats once they have reached a developmental stage when their vulnerability to either predation or environmental conditions is diminished (Hudson and Isom 1984, Buddensiek 1995).

In the recent decades, a fair measure of success has been obtained in culturing juveniles in the laboratory. Hudson and Isom (1984) first successfully raised juvenile mussels from artificially infested host fish. Subsequent studies have attempted to improve culture techniques and to determine the best conditions and techniques for obtaining viable glochidia, for transforming juveniles on host fish and in artificial media, and for raising juveniles. In order to understand optimal conditions for rearing juveniles, there is a need for further study of juvenile nutritional requirements and suitable habitat, including substrate, flow, and water quality.

Hatchery raceways and ponds have been suggested as potential locations for propagation efforts (National Native Mussel Conservation Committee 1998). Raceways and ponds would be less labor intensive than captive laboratory facilities if mussels can be released and simply allowed to grow rather than requiring the maintenance of laboratory conditions. Additionally, raceways and ponds may provide better habitat than laboratory facilities. Ideally, conditions in which juveniles are raised should mimic environmental conditions in natural habitats (Rogers 1999). Outdoor hatchery raceways and ponds with natural (as opposed to concrete) substrate represent semi-natural conditions. Such hatchery facilities are colonized by invertebrates, algae, and bacteria, and also accumulate organic detritus and allochthonous debris. Thus, these facilities develop an aquatic community that simulates the community that would occur in a river or stream. Researchers studying the feeding habits of juvenile mussels have noted that natural environments may provide nutrients, vitamins, or sediment-associated bacteria not provided in laboratory conditions (Gatenby *et al*.1996). In the semi-natural

conditions of a raceway or pond, some of the benefits of a natural environment may be achieved. In laboratories, juvenile mussels typically go through a survival bottleneck at approximately 6 weeks (R.J. Neves, Ph.D., VPI and SU, pers. comm. 2000). In a 1998 – 1999 juvenile growth study conducted at Buller Hatchery in Marion, Virginia, however, no such bottleneck was observed (Hanlon 2000).

An earthen-bottomed California raceway and two gravel-bottomed ponds are located at White Sulfur Springs National Fish Hatchery in West Virginia. The hatchery facilities are spring fed and, as a result, water quality is excellent. The purpose of this study was to evaluate the growth and survival of juvenile mussels released into the raceway and ponds and to compare the results to previous laboratory culture studies in order to evaluate the use hatchery facilities for growing freshwater mussels. In addition, conditions within the facilities and modifications needed to allow survival were documented.

Justification for Objective 4:

Mussels held in raceways receiving hatchery effluent may encounter elevated nutrient levels. Nutrients will be present, particularly in the form of ammonia, as a result of fish waste products, feed residue, and the fertilization regime used to promote algal growth. Natural processes within the raceway serve to regulate ammonia levels. In the presence of oxygen, ammonia is oxidized to nitrate by bacteria. Bacteria in the *Nitrosomonas* group oxidize NH_4^+ to $N0_2^-$, and *Nitrobacter* oxidize NO_2^- to NO_3^- (Summers 1998). Concentrations of all of these nitrogenous compounds are also regulated by uptake by photosynthesizing algae.

Nonetheless, elevated ammonia levels are anticipated as a result of the high densities of rainbow trout. Ammonia in its ionized form (NH_4^+) is relatively benign to most forms of aquatic life. In its unionized form, however, significant toxicity can occur (US EPA 1984). In an aqueous environment, the proportions of the compound that will occur in each ionization state have been shown to depend on pH and temperature (Stephens 1975, US EPA 1984).

Beyond the potential for ammonia to occur in a raceway environment, it is also a fairly common compound in surface waters. Ammonia is a common chemical

intermediate in a number of industrial processes, including refrigeration, metals extraction, effluent treatment, and detergent production. Fertilizers, however, are the greatest source of ammonia, with the manufacture of fertilizer constituting 85% of the world's ammonia production (Summers 1998). In the period between 1970 and 1981, fertilizer application rates increased 68% (Neves 1993). The correlation between nitrogenous compounds and declining mussel populations is in need of further study (Bauer 1988).

Freshwater mussels, particularly juveniles, have been found to exhibit greater sensitivity to environmental contaminants than other aquatic organisms, including organisms typically used in bioassays to set water quality standards. Many studies have found mussels to exhibit extreme sensitivity to metal concentrations (Keller and Zam 1991, Jacobson *et al*.1993). Conversely, some researchers have found bivalve species to be relatively tolerant to some organic pesticides (Johnson *et al.*1993, Keller 1993, Keller and Ruessler 1997).

Currently, research documenting the effects of ammonia on unionid mussels is limited. Epifanio and Srna (1975) found adult hard clams and oysters to be extremely tolerant of ammonia and ammonium, as compared to other marine species that had been studied. A few studies have shown glochidia and juvenile freshwater mussels to be as or more sensitive than common bioassay test species (Goudreau 1993, Scheller 1997, Summers 1998). Anderson *et al.* (1978) observed a 50% decrease in ciliary response of *Elliptio complanata* at 0.06 mg/L unionized ammonia.

The National Strategy for the Conservation of Native Freshwater Mussels (1995) recommends that bioassays should be conducted to evaluate the sensitivity of all life stages of mussels relative to the sensitivities of standard bioassay organisms. This knowledge base is needed so that water quality standards will ensure protection of the most sensitive mussel species (National Native Mussel Conservation Committee 1998). Conducting toxicity tests to evaluate the sensitivity of juvenile mussels to total and unionized ammonia will help to determine whether raceways used for holding fish are potentially suitable environments for growing juvenile mussels. Additionally, if significant toxic effects are observed, another factor contributing to the declines of mussel populations may be documented.

Chapter 2: Evaluation of a Flow-Through Pond System at White Sulphur Springs National Fish Hatchery for Rearing Juvenile Mussels

ABSTRACT

A flow-through pond system at White Sulphur Springs National Fish Hatchery in West Virginia was evaluated for its suitability as a culture environment for juvenile freshwater mussels. Newly metamorphosized juveniles of *Villosa iris* and *Lampsilis fasciola* were released in the flow-through pond and their growth and survival were evaluated. Throughout the study, water quality parameters remained within ranges suitable for juvenile survival. Planktonic algal densities in the pond system ranged from 2850 to 6892 cells/mL. A total of 37 algal genera were identified, comprised primarily of green algae (Chlorophyta), diatoms (Bacillariophyceae), and blue-green algae (Cyanopokaryota). *V.iris* were cultured with and without the addition of fine silt (<200 μm). Survival of juveniles age 93 d did not differ significantly ($p = 0.1910$) between *V. iris* cultured with silt (mean 49.8% ± SD 14.5) and without silt (mean 32.9% ± SD 11.7). Over the entire culture period, survival differed significantly between the two species ($p < 0.0001$), with *L. fasciola* (juveniles age 86 d) having a mean survival of 6.3% ± SD 4.5. It is not known whether this difference indicates a failure of the culture environment to meet the habitat requirements of *L. fasciola*, or if microhabitat variables were responsible for the lower survival among *L. fasciola*. Growth did not differ significantly between either of the treatments ($p = 0.7913$) or species ($p = 0.1315$), with *V.iris* reaching a mean length of 1.81 mm ± SD 0.67 (with silt) and 1.78 mm ± SD 0.68 (without silt) at 93 d, and *L. fasciola* reaching a mean length of 1.78 mm ± SD 0.78 at 86 d. Survival of *V. iris* and growth of both species compares favorably with previous juvenile mussel culture attempts. Furthermore, given that survival as high as 66.4% at 93 d was seen in one container for *V. iris*, it appears that the potential exists to successfully culture juveniles.

INTRODUCTION

Recovery plans for endangered mussel species often recommend propagation and reintroduction as a means to conserve diminishing populations (National Native Mussel

Conservation Committee 1998). Once mussel populations decline, recolonization rates are slow, even if environmental conditions improve (Ahlstedt 1979). Reintroduction can both restore species into their historical range and to augment existing populations. Fish hatchery ponds are potential locations for artificial propagation and grow-out of juvenile mussels (National Native Mussel Conservation Committee 1998). Ponds may be less labor intensive than captive laboratory facilities if mussels can be released and simply allowed to grow rather than requiring the maintenance of laboratory conditions. Furthermore, the conditions in which juveniles are raised should mimic environmental conditions in natural habitats (Rogers 1999). Outdoor ponds are colonized by invertebrates, algae, bacteria, protozoans, and other microorganisms and also accumulate organic detritus. Thus, these facilities develop an aquatic community that simulates the community that would occur in a local river or stream. Researchers studying the feeding habits of juvenile mussels note that natural environments may provide nutrients, vitamins, or sediment-associated bacteria not provided in laboratory conditions (Gatenby *et al.*1996). In the semi-natural conditions of a raceway or pond, some of the benefits of a natural environment may be achieved.

The purpose of this study was to evaluate the growth and survival of juvenile mussels released into a flow-through pond system located at White Sulphur Springs National Fish Hatchery (WSSNFH) in West Virginia and to compare the results to previous laboratory culture studies in order to assess the potential use of hatchery facilities for growing freshwater mussels. Conditions within the facilities and necessary modifications were documented. As the facilities are developed, the availability of a grow-out environment into which newly transformed juveniles could be immediately released would be beneficial. Therefore, juvenile mussels in this study were released in the flow-through pond system without a preliminary period of laboratory culture to determine whether this would be a suitable technique for this system.

Algae as a Food Source

Algae constitute an important component of diet of freshwater mussels. In laboratory settings, live algae are ingested and serve as a suitable diet for captively reared juvenile mussels (Hudson and Isom 1984, Gatenby *et al*. 1996, Gatenby *et al.* 1997,

O'Beirn *et al.* 1998, Steg 1998, Beck 2000). The value of an algal species as food source is dependent on a number of factors, including ingestibility, digestibility, and nutritional content. Ingestibility is a function of size and shape, with features such as elongation or the presence of spines likely to inhibit ingestion (B. Parker, Ph.D., VPI and SU, pers. comm. 2001). When fed algae, adult and juvenile bivalves exhibit selectivity correlating with particle size (Gatenby 1994, Beck 2001). Estimates of cell size ranges potentially ingestible by juveniles are variable.

Some studies suggest that pedal-feeding juveniles may take in particle sizes comparable to those ingested by adults (2 – 70 μm), while other researcher suggests that smaller particle sizes (<25 μm) are more suitable (Gatenby 1994, Beck 2001). Notably, unicellular algae are generally smaller and therefore more likely to be ingested, especially by small juveniles, than colonial or filamentous forms. Digestibility is a function of chemical constituents of the algae and structure components, such as cell wall thickness (Gatenby 1994). Although the nutritional requirements of juvenile mussels are not fully characterized, key compounds of importance to a wide array of aquatic organisms are likely to be important to juvenile mussels. These include sterols, steroids, and carotenoids (Campbell 1969, Gatenby 1994). Additionally, algal species containing oils rich in polyunsaturated fatty acids promote growth in juvenile mussels (Gatenby *et al.* 1997).

Particle size and cell density influence the rate at which filter feeding bivalves remove algae from the water column (Paterson 1984, Paterson 1986). In studies of the freshwater bivalve *E. complanata*, filtration efficiency peaked at densities of 11,000 – 15,000 cells/mL and declined at higher concentrations (Paterson 1984). Target densities of 10,000+ cells/mL are suggested as adequate nutrition for cultured mussels. A second objective of this study was to characterize the species composition and density of the algae community at WSSNFH to assess how well this biotic component of the system served to meet juveniles' nutritional requirements.

Supplemental Silt

An additional objective of this study was to determine whether the addition of fine silt to the substrate affects the growth or survival of juvenile mussels held in the

pond system. The presence of fine silt in juvenile culture environments is preferable to no substrate at all (Hudson and Isom 1984, Yeager *et al.* 1994, Gatenby *et al.* 1996). Comparisons of silt with other substrate types and comparisons of silts with differing organic content are less conclusive (Steg 1998, Rogers 1999). Suggested benefits of silt include the possibility that sediment associated bacteria or organic matter serve as a food source for juveniles, or that bacteria enhance enzymatic activity, thus aiding in digestion (Gatenby *et al*. 1996, O'Beirn *et al.*1998). Silt particles could also play a physical role, either serving as a substrate on which to collect food or being ingested as an internal grinding substrate for other particles, such as algae with tough cell walls (Gatenby 1994). Some evidence suggests, however, that the accumulation of fine silt in culture environments may be negatively correlated with survival (Hanlon 2000). Negative effects may include the filling of interstitial spaces inhabited by juveniles and possible clogging of filtering mechanisms (Hanlon 2000).

In recent work at the Virginia Tech Aquaculture Center, juvenile mussels reared in fine silt collected from Buller Hatchery in Marion, Virginia have shown unusually good growth and survival (J. Jones, VPI and SU, pers. comm. 2001). Because the function of silt in juvenile culture is not understood, it is unknown what characteristics of this silt may be most important. For example, if resident bacteria serve as a food source, the associated biotic community is the key characteristic. If, on the other hand, the silt plays a role as an internal grinding substrate, suitable size for ingestion and appropriate rigidity would be key characteristics. Given the range of hypotheses as to the role of silt, this study at WSSNFH did not aim at elucidating the mechanism by which silt is involved in juvenile nutrition, nor resolving conflicting reports of positive or negative effects of fine silt. Rather, based on the observed success with Buller Hatchery silt, this same silt was supplied to juvenile containers in the pond at WSSNFH, in an attempt to maximize the likelihood of providing a successful culture environment. However, the addition of silt collected from an off-site location represented an extra labor component. If nutritional and habitat requirements could be met by allowing the containers to receive the colonizing biota, algae, and fine particulates from within the WSSNFH pond, the additional step adding fine silt could be bypassed. Therefore, a comparison was made

between juveniles raised in containers with and without fine silt added to the gravel substrate.

Comparison of Two Species

The final objective was to determine whether growth and survival differ between two species held in the flow-through pond system. Habitat requirements of juvenile mussels are not well documented (D'Eliscu 1972, Kat 1982, Gordon and Layzer 1989, Amyot and Downing 1991). Propagation and culture studies illuminate some of these needs. As the field of mussel culture develops, it can be assumed that differences between species' habitat requirements will emerge. In order for a facility to be useful for conservation propagation and culture, it should provide (or be able to be modified to provide) suitable habitat for multiple species. Therefore, to evaluate whether the current set-up within the WSSNFH flow-through pond system is suitable for multiple species, a comparison was made between the growth and survival of two species held in the same conditions.

METHODS

Establishing Appropriate Conditions for Growing Juvenile Mussels

In cooperation with the US Fish and Wildlife Service, a flow-through pond system located at the White Sulphur Springs National Fish Hatchery in White Sulphur Springs, West Virginia was used for this study (Ponds B1 and B2, Figure 2.1.). Both ponds were approximately 11 m wide by 84 m long and were lined with hyphalon pond liners. Pond B2 (hereafter denoted Flow-Through Pond) was used to hold mussels, while Pond B1 (hereafter denoted Algae Pond) was maintained to foster unicellular algae growth. The Algae Pond had a bottom layer of quarried cobble (stone sizes 2.5 – 7.6 cm), while the bottom of the Flow-Through Pond consisted of a dredged creek rock material. In order to supplement the nutrient base and natural community of the pond system, burlap bags containing leaf detritus were added to the Flow-Through Pond prior to this study. Fifty bags at an average weight of 6.8 kg/bag were added in the fall of both

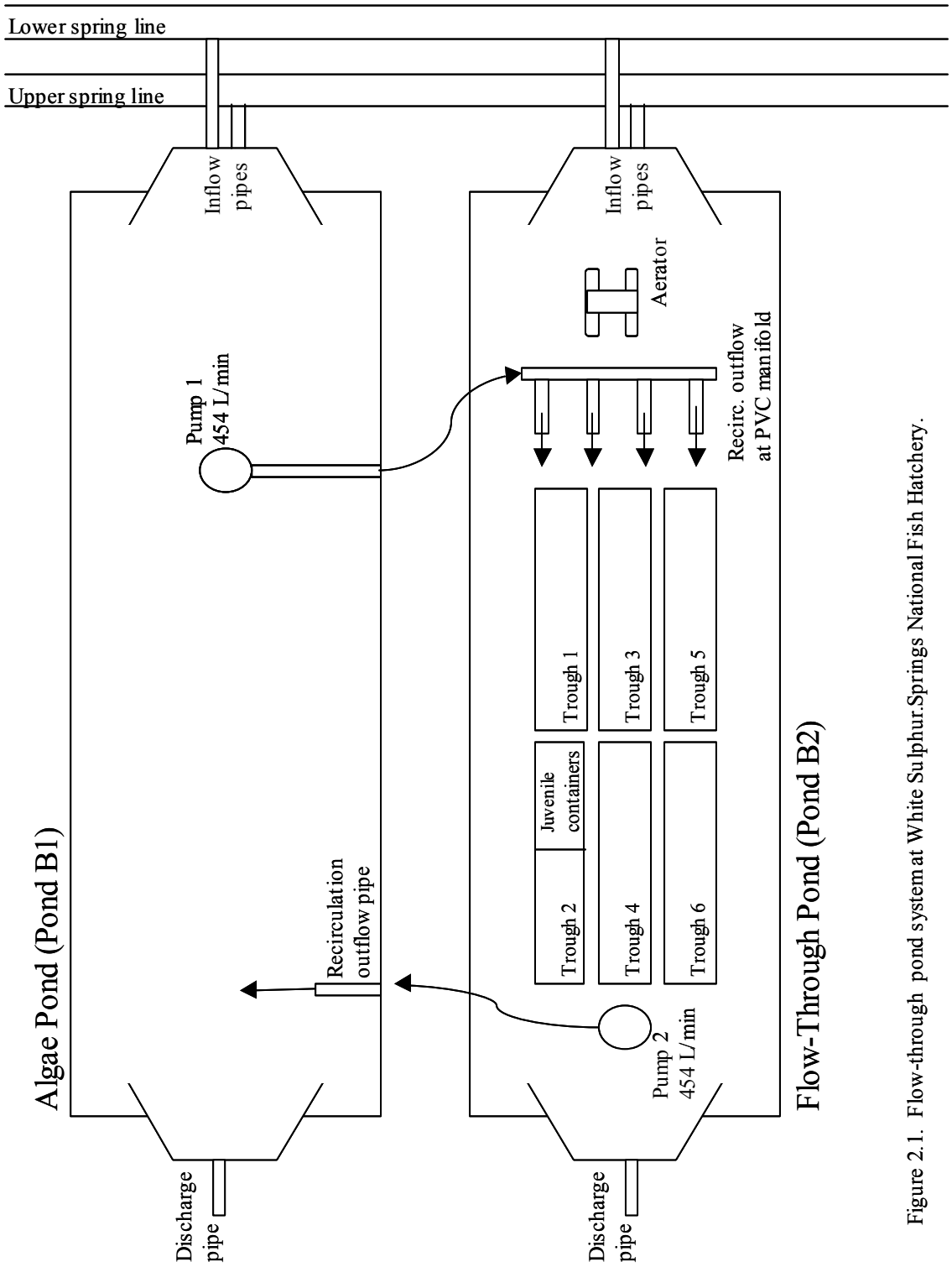


Figure 2.1. Flow-through pond system at White Sulphur Springs National Fish Hatchery.

1999 and 2000, October 1- November 15. Six elevated cinderblock troughs were constructed in the Flow-Through Pond. The troughs were approximately 8.5 m long by 1.5 m wide by 40 cm deep, lined with plastic liner and filled with pea gravel substratum (stone sizes 0.6 – 2.5 cm). To confine juvenile mussels within the pond, rectangular plastic containers (20 cm x 40 cm x 15 cm deep) were placed in two rows in the upper third of trough 2, on the surface of the gravel substrate, at a depth approximately 40 cm above the bottom of the pond and 40 cm below the water surface. Conditions throughout the upper third of the compartment were considered to be uniform. Treatments were assigned randomly to each container within a row.

The ponds were supplied initially with 12ºC water from two springs (an upper and lower spring) and a well located on the hatchery premises. Waterlines ran from both springs and the well to a flume house where water can be stored in an elevated tower. Water pipes ran from the spring lines to inflow pipes at the upper end of each pond. At the lower end of each pond, outlet structures with discharge pipes ran into a tributary of the Greenbrier River. Water was not supplied continually to the ponds, nor was water released. Rather, the ponds were filled and then water was recirculated between the two ponds. Water was added only as needed to maintain water levels and to lower water temperatures when temperatures exceeded 25ºC. Recirculating the water allowed a retention time adequate for solar radiation to warm the water to temperatures greater than 15ºC, which is the temperature necessary to support growth in juvenile mussels (Hanlon 2000) and also warm enough to foster algae production. Retention times were also adequate for algae species to complete their life cycles and reproduce. Using a recirculating system also eliminated any concerns that juvenile mussels would be inadvertently released into the wild.

Water was recirculated between the two ponds using two ½ horsepower submersible pumps (Peabody Barnes model 35E54) which pumped approximately 454 L/ minute. From Pump 2 in the Flow-Through Pond, water was pumped through a 5 cm diameter waterline to an outflow pipe at the lower end of the Algae Pond. From Pump 1 in the Algae Pond, water was pumped through 5 cm diameter waterline into a 5 cm diameter PVC manifold at the upper end of the Flow-Through Pond. Water flow was directed through four PVC outflow pipes from this manifold. The outflow from these pipes

provided a source of flow to the elevated troughs in which the mussel containers were located. A paddle-wheel aerator (Aqua and Co., Model Eolo 2) was placed at the upper end of the Flow-Through Pond to enhance dissolved oxygen and flow.

When filling of the ponds was necessary, spring water was fed from the upper spring into the Flow-Through Pond. When the upper spring was dry, combined well water and lower spring water stored in the flume house tower was pumped into the ponds. Backflow through the upper spring line from the ponds into the flume house (and possible contamination of the flume house) was a concern if the pump were to fail. However, backflow could not occur through the lower spring line. Therefore, the lower spring line from the flume house tower was used to fill the Flow-Through Pond (the Algae Pond could not be filled with this line because the valve from lower spring line to the Algae Pond was broken). To further ensure against contamination of the flume house, the valve from the upper spring line to the Algae Pond was never opened, and on no occasion was pumping done while hatchery staff were not on the property to monitor the function of the pump.

Maximum depth and water volume were desired in the Algae Pond in order to promote the growth and proliferation of unicellular algae to serve as food for mussels held in the Flow-Through Pond. Therefore, the stop boards at the lower end of the Algae Pond were used to maintain nearly the maximum depth allowed by the edges of the liner. At the outlet structure, a depth of 1.4 m was maintained, which resulted in a depth of 0.7 m at the upper end and a volume of approximately 819,000 L. Stop boards at the outlet structure of the Flow-Through Pond maintained a depth of approximately 1.1 m, which resulted in a depth of 0.4 m at the upper end and a volume of approximately 545,000 L. Given the pumping rate and volume of the ponds, the time to fully exchange water between the two ponds was approximately one day.

Monitoring Habitat Parameters and Water Quality

Discharge in the Flow-Through Pond was calculated from the pumping rate and the cross-sectional area of the pond. Water velocity was measured with a pygmy flow meter. Measurements were taken on cross-sections of the raised mussel troughs immediately below the PVC manifold outlet into trough 1, at the midpoint and end of

trough 1, and at the top of trough 2, adjacent to the juvenile mussel containers. A Hobo™ (Onset Corporation) temperature logger was placed in the Flow-Through Pond adjacent to the juvenile mussel holding containers to provide a continuous record of water temperature.

At two week intervals, water chemistry was monitored for hardness, alkalinity, pH, dissolved oxygen, orthophosphate, nitrate, nitrite, and ammonia. Samples were taken at a depth of approximately 20 cm, adjacent to the juvenile holding containers. The nutrient parameters (orthophosphate, nitrate, nitrite, and ammonia) were monitored in samples taken both the Flow-Through and Algae Ponds, thus allowing correlation between differences in water chemistry and algae communities between the two ponds. Dissolved oxygen was measured with a YSI model 85 D.O. meter, and pH was measured with an Orion model 290A pH meter. Hardness and alkalinity were measured using Hach™ (Hach Company) test kits. Phosphorus, nitrate, nitrite, ammonia, and ammonium were analyzed using procedures from *Standard Methods for the Examination of Water and Wastewater* (Eaton *et al.* 1995). Ammonia was measured with an Orion ion-selective probe and an Orion model 290A pH meter. By measuring pH and temperature at the time of water sampling, the proportions of ammonia occurring as unionized ammonia (NH_3-N) and ionized ammonium (NH_4+-N) were determined using the formulas of Emerson *et. al* (1975). Nitrite and nitrate concentrations were measured by spectrophotometric analysis using the cadmium reduction and sulfanilamide method. Orthophosphate was measured with spectrophotometric analysis using the ascorbic acid-molybdate method.

Algae Production and Monitoring

The dominant genera and density of algae in the water column of the two ponds were monitored every two weeks. At locations 1 – 2 m from each pond's edge and depths of approximately 20 cm, 1 L water samples were collected in Nalgene bottles and immediately preserved with 10 mL of acid Lugol's fixative (Saraceni and Ruggio 1969). According to the Utermohl technique, a 100 mL settling chamber was then used to concentrate the algae in the water sample. The algae was allowed to settle onto a slide for 48 hours, and then was identified and ennumerated using an inverted microscope

(Vollenweider 1969). Genera were identified according to a dichotomous key ( ) and counted by transect, and a conversion formula calibrated to the microscope was used to calculate the cell density in the sample from the number of cells on the transects. Filamentous algae observed in the pond was also collected into small plastic bags and refrigerated until examined and identified with a standard binocular microscope at 400X magnification.

A fertilization regime was also applied to ponds in order to foster the growth of unicellular algae as a food source for mussels. Initial water chemical analyses of the total inorganic nitrogen and soluable reactive phosphate-phosphorus showed an approximately 4:1 ratio of N:P. An N:P ratio of 15:1 was deemed ideal to promote the growth of unicellular green algae and diatoms and to discourage blue-green algae (Redfield 1958, Hillebrand and Sommer 1999). In order to achieve this ratio, nitrogen was added as ammonium nitrate. According to the amounts of nitrogen and phosphorus initially present and the calculated volume of the two ponds (approximately 1360 x 10^3 L), 1375 grams of ammonium nitrate was needed to raise the N:P ratio to 15:1. Fertilization occured once per retention time, with additional supplementation to replenish nutrients taken up by algae and other biota in the system. According to water supply records for the ponds, approximately 1200 x 10^3 L were added each month, such that total retention time of the water in the ponds was approximately 1.1 months. Therefore, fertilization was initially scheduled to occur once a month. A target density of 10,000 cells/mL was desired, and fertilization frequency was adjusted according to observed densities and measured nutrient levels.

Comparison of Growth and Survival of *Villosa iris* Reared with and without Fine Silt

Ten gravid adult female *V. iris* were collected from Indian Creek, Tazewell County, Virginia and transported to the Virginia Tech Aquaculture Center in a cooler with river water. At the laboratory, they were held in temperature-controlled water in a Living Stream and were fed daily with the green algae *Neochloris* and *Scenedesmus* spp. from cultures grown in 250 L Kalwall clear plastic tubes.

Rock bass (*Ambloplites rupestris*) approximately 7 - 15 cm in length were collected from Tom's Creek in Montgomery County, Virginia. At the Virginia Tech

Aquaculture Center, fish were held in static 95 L glass aquaria supplied with airstones and a 1:1 mixture of well water and dechlorinated Blacksburg municipal water. Fish were allowed to acclimate for several days before being infested with *V. iris* glochidia.

Infestation of host fish was conducted according to procedures outlined by Zale and Neves (1982), except that the gravid female mussels were not sacrificed. Glochidia were obtained from the gravid mussels by puncturing the marsupial gill with a water-filled hypodermic needle and flushing the gill with water, causing it to rupture. Glochidia were collected in a Petri dish, and viability was confirmed by adding a few grains of salt to a sample of the glochidia to observe closure of the valves. Rock bass were infested by placing them in an aerated bucket with enough water to cover their dorsal fins and adding glochidia to the bucket. Periodically, the water was agitated to keep the glochidia in suspension. The gills of a few fish were checked for infestation approximately every two min. Once infestation was observed, fish were returned to their holding tanks. One week after infestation, feeding of the fish was suspended, so that solid waste products would not interfere with the collection of juvenile mussels from the bottom of the tank.

Starting 10 days after infestation, the bottom of the tanks were siphoned daily through a 130 μm mesh screen to collect juvenile mussels. This continued until juveniles were no longer found. The juvenile mussels were counted and held in static conditions in rectangular containers (40 cm x 20 cm wide x 15 cm deep) containing 1:1 mixture of well water and dechlorinated Blacksburg municipal water until they were transported to the hatchery. During this holding period, water in the juveniles containers underwent a daily 50% renewal with fresh water. At each renewal, 500 mL of *Neochloris* and *Scenedesmus* also were added.

Once a total of 3000 juveniles were collected for this study (7 - 10 days old), and the lengths 10 juveniles were measured (anterior to posterior distance) using an ocular micrometer, juveniles were then randomly distributed into 5 cm x 12 cm x 9 cm plastic containers of approximately 500 juveniles/ container, and transferred to the hatchery.

At the hatchery, juveniles were acclimated to the hatchery's water by gradually replacing their water with water from the flow-through pond system in 20% increments over the course of several hours. Juveniles were then transferred to their randomly assigned plastic containers with one of two substrate treatments; approximately 1500

juveniles per treatment. The first treatment consisted of a 1:1 mixture of quarried limestone sand (sieved to a particle size between 1000 - 3000 μm) and sediment collected from the Little River, Tazewell County, Virginia (sieved to particle size 800 – 1500 μm) to a depth of 0.5 mm. Mixed particle sizes were used to increase the availability of interstitial space for juvenile movement and feeding.

The second treatment consisted of the same substrate mix and depth as treament one, but was supplemented with a thin layer of fine silt (particle size < 200 μm) collected from Buller Hatchery, Marion, Virginia. Sieving the silt to < 200 μm minimized the risk of introducing potential predators. Care was taken to preserve the microbial composition of the silt by storing it in aerated water after it was collected and prior to introduction to juvenile containers. Silt was supplied to all containers receiving treatment 2 at each sampling event.

In order to transfer juveniles to the treatment containers in the Flow-Through Pond, 60 cm long, 12 cm diameter PVC tubes were placed upright in the plastic container, so that one end of the tube sat on the container's bottom, while the other end stood above the water surface. The small transport containers of juveniles were then poured and rinsed into the tubes, so that they would settle in the static water column to the bottom of the plastic containers. Once adequate time had been allowed for settling, the PVC tubes were gently removed.

All of the containers were sampled at two week intervals for juvenile survival and growth over the summer growing season. Sampling occurred at two week intervals. Containers were subsampled, so that all containers were sampled at least three times by the end of the season, and, generally, containers were not sampled at two sampling events in a row. On the final sampling date, all containers were sampled. All live juveniles were counted in a container, and a subsample of 10 juveniles measured using an ocular micrometer. In order to collect the juveniles, the substrate was sieved with a screen fine enough to separate the juveniles. Once juveniles reached a size too large for this separation technique, an elutriator was used to separate them from the substrate (Hanlon 2000).

Growth and survival were compared between the two substrate treatments. Growth data were compared with a general linear model multivariate analysis of variance

test (SAS statistical software). Because growth was measured for sub-samples of 10 out of 500 juveniles/container, growth data were considered to be independent. Survival data were compared as binomial proportions (based on the proportion surviving). Survival data were considered right-censored, because observed live individuals were known to have survived to a given sampling event, while individuals considered dead may possibly have been alive and unaccounted for, as a result of escapement or error in handling. The survival proportions were arc-sine transformed to achieve a normal distribution. At each sampling event, survival for a given container was not independent of previous survival rates. Therefore, survival was compared with a mixed procedure multivariate analysis of variance test intended to model and account for the covariance structure of the data (SAS statistical software). Relative declines in survival between sampling events also were calculated as $[(\text{Survival}_{t1} - \text{Survival}_{t2})/ \text{Survival}_{t1}] \times 100$ (Ricker 1975).

Comparison of Growth and Survival of *Villosa iris* and *Lampsilis fasciola*

Five gravid female *L. fasciola* were collected from the Clinch River in Russell County, Virginia and held at the Virginia Tech Aquaculture Center in the same conditions described for *V. iris*. Largemouth bass (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*), approximately 15 – 30 cm in length, were collected from the New River in Montgomery County. Fish were held in a 850 L recirculating system held at 22°C and fitted with a UV filter, a trickle filter, and.a solids filter, and supplied with airstones and a 1:1 mixture of well water and dechlorinated municipal water. Fish were allowed to acclimate for several days before infestation with *L. fasciola* glochidia.

Juvenile *L. fasciola* were produced according to the procedures outlined above for *V. iris*, using largemouth bass and smallmouth bass. Once a total of 1500 juveniles had been collected, all juveniles were combined (1 - 5 days old) and a sample of 10 juveniles was measured using an ocular micrometer. The juveniles were then randomly distributed into 5 cm x 12 cm x 9 cm deep, containers of approximately 500 juveniles/ container, and were transferred to the hatchery.

At WSSNFH, the juveniles were acclimated and introduced to their randomly assigned containers as described for *V. iris*. All of the plastic containers contained a 1:1

mixture of quarried limestone sand (sieved to a particle size between 1000 µm - 3000 µm) and sediment collected from the Little River, Tazewell County, Virginia (sieved to particle size 800 µm - 1500 µm) to a depth of 0.5 mm, supplemented with a thin layer of fine silt (particle size < 200 µm) collected from Buller Hatchery, Marion (the same as treatment 2 above).

The same sampling schedule and methods were employed for *L. fasciola* as for *V. iris*. Growth and survival were compared between *V. iris* reared with supplemental silt and *L. fasciola* reared with supplemental silt. Because juveniles of *L. fasciola* were released at an earlier date than those of *V. iris*, sampling event 1 for *V.iris* does not coincide with sampling event 1 for *L. fasciola.* Rather, the sampling events for *V. iris* are set back by one sampling interval, with the first *V. iris* sampling occurring on the same date as the second sampling for *L. fasciola*. Therefore, growth and survival could not compared by date. The two age classes (1 - 6 d and 7 – 10 d) were considered close enough in age to be compared by sampling event.

Growth data were compared with a general linear model multivariate analysis of variance test (SAS statistical software). Growth data also were compared using a paired t-test on the mean lengths at each sampling time using (SAS statistical software). Survival data were compared as binomial proportions (based on the proportion surviving) and survival data were considered right-censored. The survival proportions were arc-sine transformed to achieve a normal distribution, and survival was compared with a mixed procedure multivariate analysis of variance test intended to model and account for the covariance structure of the data (SAS statistical software). Relative declines in survival between sampling events also were calculated (Ricker 1975).

RESULTS

Flow

Using cross-sectional area of the Flow-Through Pond (65,270cm^2) and the volume pumped per minute (908.6 L), the discharge rate across the pond was approximated at 1.39 mL/min/cm^2, assuming uniform flow throughout the cross-section of the pond. Flow in the area of the juvenile mussel containers probably was greater than in other parts of the cross-section, because the containers were located in the raised

troughs in-line with the PVC water outlets. However, the flow experienced by the juveniles was probably somewhat diminished because of their confinement in the containers. Direct flow velocity measurements with a pygmy flow meter were 4.8 cm/s immediately below the PVC manifold outlet into trough 1 and 4.6 cm/s at the midpoint of trough 1. Flow was too low to be detected by the flow meter at the bottom of trough 1 or at the top of trough 2, adjacent to the juvenile mussel containers.

Temperature

Mean daily temperature in the Flow Through Pond ranged from 18.7°C to 26.8°C (Figure 2.2.). Mean daily temperatures observed for the intervals between sampling events ranged from 20.8°C (8/30 – 9/14) to 25.6°C (8/2 – 8/15) (Table 2.1). The minimum recorded temperature was 16.8°C and the maximum was 31.3°C (Table 2.1). Daily mean, minimum, and maximum temperatures and temperature flux are summarized in Appendix A.

Algae Production

Thirty-seven genera of algae were identified and enumerated in water samples from the Flow-Through Pond and five from the Algae Pond (Table 2.2.). Complete data on cell counts in each sample are summarized in Appendix B. The observed genera included unicellular, colonial, and filamentous forms, and species that inhabit both planktonic and benthic habitats. Although all cells were collected as plankton in water column samples, some species had become dislodged from benthic habitats. Six groups of algae were represented; 17 genera of green algae, 12 diatoms, 5 blue-green algae, 1 golden algae, 1 cryptophyte, and 1 euglenoid. Green algae comprised 58.4 – 79.6% of the cells observed in the samples (Table 2.3.). Proportions of blue-green algae and diatoms were fairly similar, with diatoms ranging from 4.3 - 19.8% of the cells observed and blue-greens ranging from 2.7 - 18.4% (Table 2.3.). The proportion of cells accounted for by each group did not differ significantly between the Flow-Through Pond and Algae

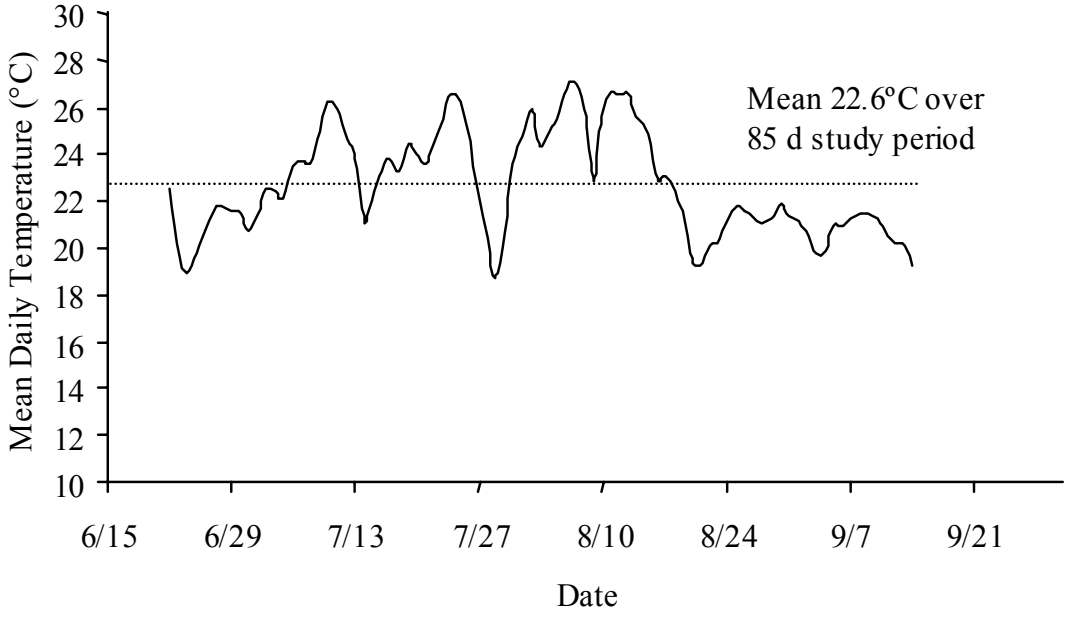


Figure 2.2. Mean daily temperatures (ºC) present in hatchery Flow-Through Pond between June 22, 2001 – September 14, 2001.

Table 2.1. Summary of temperature data recorded in Flow-Through Pond between each sampling event and over the 85-day study period.

Time Period	Number of Days	Mean Temperature (°C) ± SD	Min. Recorded Temp. (°C)	Mean Daily Min. Temp. (°C) ± SD	Max. Recorded Temp. (°C)	Mean Daily Max. Temp. (°C) ± SD	Mean Daily Flux (°C) ± SD
6/22 - 7/4	13	21.1 ± 2.1	16.8	18.9 ± 1.2	29.1	23.8 ± 1.4	4.9 ± 1.4
7/5 - 7/18	14	23.7 ± 2.4	17.9	21.1 ± 1.8	29.5	26.8 ± 1.6	5.7 ± 1.5
7/19 - 8/1	14	23.5 ± 2.8	18.3	21.5 ± 2.0	29.9	26.0 ± 3.0	4.5 ± 2.2
8/2 - 8/15	14	25.6 ± 2.3	18.3	22.9 ± 1.9	30.3	28.8 ± 1.1	5.9 ± 1.8
8/16 - 8/29	14	21.2 ± 1.8	16.8	19.2 ± 1.3	26.0	23.5 ± 1.4	4.3 ± 0.9
8/30- 9/14	16	20.8 ± 1.6	17.9	18.9 ± 0.8	24.8	23.0 ± 1.3	4.1 ± 1.4
6/22 - 9/14	85	22.6 ± 2.8	16.8	19.5 ± 4.7	30.3	24.1 ± 5.9	4.7 ± 1.8

Table 2.2. List of genera of algae observed in water samples taken from the hatchery Flow-Through and Algae Ponds, July 5 - September 15, 2001.

Algae	Growth Form	Habitat	Algae	Growth Form	Habitat
Blue Green Algae (Cyanoprokaryota)			Green Algae (Chlorophyta)		
Aphanocapsa	Colonial	Planktonic	*Chlamydomonas*	Unicellular	Planktonic
Chroococcus	Colonial	Benthic	*Chlorella*	Unicellular	Planktonic
Lyngbya	Filamentous	Benthic or Planktonic	*Chlorococcum*	Unicellular	Benthic or Planktonic
Oscillatoria	Filamentous	Benthic or Planktonic	*Chodatella*	Unicellular	Planktonic
Spirulina	Filamentous	Planktonic	*Closterium*	Unicellular	Benthic or Planktonic
			Koliella	Unicellular	Planktonic
Diatoms (Bacillariophyceae)			*Monocilia*	Filamentous	Benthic
			Mougeotia	Filamentous	Benthic
Cocconeis	Unicellular	Benthic	*Oedogonium*	Filamentous	Benthic
Cyclotella	Unicellular	Planktonic	*Oocystis*	Unicellular	Planktonic
Cymbella	Unicellular	Benthic	*Pediastrum*	Unicellular	Planktonic
Diatoma	Unicellular	Benthic	*Protoderma*	Colonial	Benthic
Fragilaria	Colonial	Planktonic	*Schroederia*	Colonial	Planktonic
Gomphonema	Unicellular	Benthic	*Selenstrum*	Colonial	Planktonic
Meridion	Unicellular	Benthic	*Scenedesmus*	Colonial	Planktonic
Nitzschia	Unicellular	Benthic	*Sphaeroplea*	Filamentous	Benthic
Pennate diatom	Unicellular	Benthic	*Ulothrix*	Filamentous	Benthic
Pinnularia	Unicellular	Benthic			
Synedra	Unicellular	Planktonic	Euglenoids (Euglenophyta)		
Tabellaria	Pseudo-colonial	Planktonic	*Euglena*	Unicellular	Planktonic
Golden Algae (Chrysophyceae)					
Cromulina	Unicellular	Planktonic			
Cryptophytes (Cryptophyta)					
Chroomonas	Unicellular	Planktonic			

Table 2.3. Summary of the algae community in the Flow-Through Pond and Algae Pond on each sample date.

	7/5/01		7/19/01	8/1/01		8/16/01		8/29/01		9/14/01	
	Flow-Through Pond	Algae Pond	Flow-Through Pond	Flow-Through Pond	Algae Pond	Flow-Through Pond	Algae Pond	Flow-Through Pond	Algae Pond	Flow-Through Pond	Algae Pond
% Diatoms	16.5	10.3	12.8	14.7	4.3	12.3	19.8	9.9	7.3	7.6	11.5
% Greens	71.5	75.1	77.2	64.7	78.1	72.2	58.4	72.2	79.6	73.2	68.2
% Bluegreens	3.2	13.0	2.7	18.4	15.8	7.9	10.8	16.9	7.6	15.4	14.8
% Other	8.2	1.6	7.3	2.2	1.8	7.6	11.0	1.1	5.5	3.8	5.5
Diversity (# genera)	24	21	17	20	16	20	18	20	20	18	18
% Ingestible by Juveniles	88.4	81.7	84.9	72.9	65.4	82.2	85.3	80.8	88.8	81.7	81.1
Density (cells/mL)	6892	4382	3854	4339	4305	3284	3710	3605	2850	3599	3157

Pond (2-sample t-tests for differences in proportion of diatoms, p = 0.5630; for green algae, p = 0.9916; for blue-green algae, p= 0.6433) (Table 2.4.). Algal diversity in a single sample ranged from 16 – 24 genera (Table 2.3.), with a mean of 19.8 in each sample from the Flow-Through Pond and 18.6 in each sample from the Algae Pond, which also did not differ significantly (2-sample t-test assuming equal variance, p = 0.3812) (Table 2.4.). Of 10 genera dominating all samples, 7 were unicellular, 2 were colonial, and 1 was filamentous. Five groups were represented in these top 10 genera: 5 greens, 2 diatoms, 1 cryptophyte, 1 golden, and 1 blue-green. The top 10 genera made up between 75.1% and 91.9% of the individual samples (Figure 2.3.).

All observed genera were classified as potentially ingestible by juveniles (J), ingestible by adults (A), or unlikely to be ingested by either (X) (Appendix B.). Ingestibility was subjectively evaluated based on size, shape, cellular make-up, and growth form. The ingestible proportion of cells in each sample was then calculated. Potentially ingestible proportions ranged from 65.4 – 88.8% (Table 2.3.). Calculated cell densities ranged from 2850 cells/mL - 6892 cells/mL (Table 2.3. and Figure 2.3.), with a mean of 4262 cells/mL in the Flow-Through Pond samples, and 3681 cells/mL in Algae Pond. Mean cell density did not differ significantly between the two environments (2-sample t-test assuming unequal variance, p = 0.3789) (Table 2.4.).

After July 19, 2001, because algae densities were observed to be lower than desired, frequency of fertilization application was increased from once a month to every other week. After August 1, 2001, because algae densities still did not increase and measured nutrient levels remained approximately the same, fertilization frequency was increased to once every week.

Large quantities of macroscopic filamentous algae colonized the Algae Pond. The onset of this bloom was approximately July 7th, 2001, and it persisted throughout the study period. Filamentous algae also occurred in the Flow-Through Pond, but was not nearly as prolific or widespread. The dominant filamentous forms were identified as *Sphaeroplea* and *Cladophora* (?*C. glomerata*). In the process of conducting algal cell counts, other microscopic biota were observed in the plankton samples; notably, large numbers of small, colorless bacterial cells, as well as protozoans and the aquatic fungus *Alternaria*.

Table 2.4. Comparison of the algae community between the Flow-Through and Algae Ponds at the hatchery. Values are means for samples collected between July 5 and September 14, 2001. No significant differences were found as analyzed by 2 sample t-tests.

	Flow-Through Pond Mean ± SD	Algae Pond Mean ± SD	p-value
% Diatoms	12.3 ± 5.8	10.6 ± 2.4	0.5630
% Greens	71.8 ± 8.7	71.9 ± 2.4	0.9916
% Bluegreens	10.8 ± 3.3	12.4 ± 6.6	0.6433
% Other	5.0 ± 3.8	5.1 ± 3.1	—
Diversity (# genera)	19.8 ± 1.9	18.6 ± 1.5	0.3812
% Ingestible by Juveniles	81.8 ± 9.0	80.5 ± 1.8	—
Density (cells/mL)	4262 ± 679	3681 ± 233	0.3789

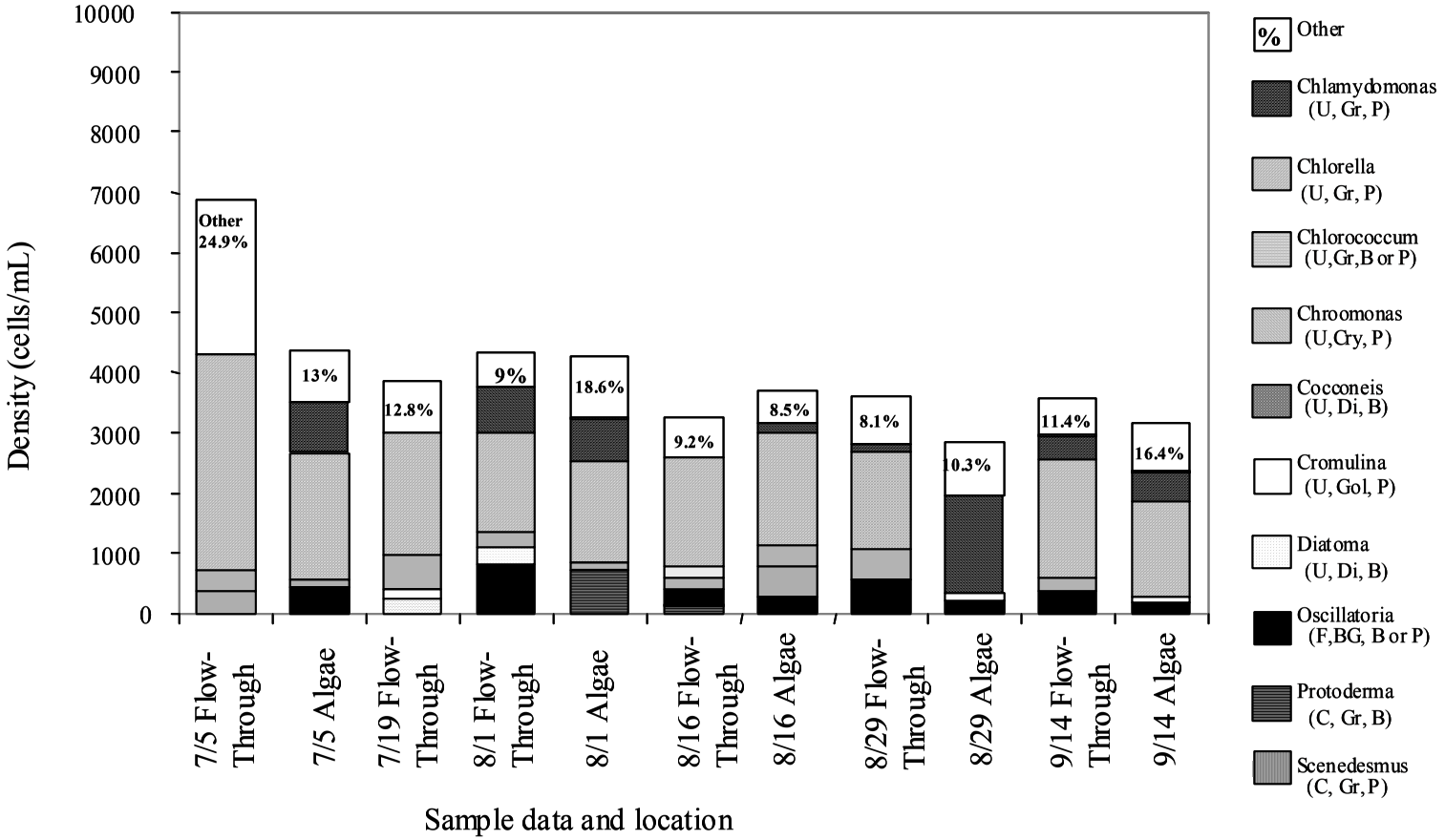


Figure 2.3. Proportions of dominant genera in phytoplankton samples.

Water Quality

Water chemical parameters remained within acceptable ranges throughout the study period (Table 2.5). Values of dissolved oxygen, hardness, alkalinity, and pH were relatively stable over the course of the sampling dates (Appendix 2.3). In the Flow-Through Pond, dissolved oxygen averaged 8.92 mg/L, hardness 305.7 mg/L, alkalinity 71.4 mg/L, and pH 8.49.

Orthophosphate levels in the Flow-Through Pond were low throughout the study period, with 5 of the 8 samples having undetactable levels and a maximum value of 0.052 mg PO_4-P /L (Appendix 2.3). Total ammonia in the Flow-Through Pond ranged from 0.052 to 0.212 mg (NH_3-N +NH_4-N)/L, with a mean of 0.087 (± 0.054) mg (NH_3-N +NH_4-N)//L (Table 2.5). The maximum level of 0.212 mg (NH_3-N +NH_4-N)//L was measured just after fertilization and represents an area where fertilizer was disproportionately concentrated. Calculated levels of unionized ammonia ranged from 0.0029 to 0.060 mg NH_3-N /L (Table 2.5). Nitrate concentrations in the Flow-Through Pond ranged from 0.005 to 0.135 mg NO_3-N/L, with a mean of 0.035 (± 0.043) mg NO_3-N /L. The highest level again occurring in the post-fertilization sample. Nitrite levels were only detectable on two sampling dates, at concentrations of 0.001 and 0.03 mg NO_2-N /L.

On the sampling dates that nutrient parameters were measured in both the Mussel Pond (B2) and Algae Pond (B1), total ammonia was higher in Mussel Pond (B2) on five of the six sampling dates, nitrate levels were higher in Mussel Pond (B2) on all five sampling dates, and, on the two sampling dates that orthophosphate was detectable in the Mussel Pond (B2), it was either lower or un in the Algae Pond (B1) (Appendix 2.3.).

Comparison of Growth and Survival of *Villosa iris* with and without Fine Silt

At the end of the study period, mean survival for *V. iris* (age 93 d) cultured with silt was 49.8% (± 14.5) and without silt was 32.9% (± 11.7) (Table 2.6). Note that increases in mean survival between sampling events do not represent actual increases in the number of surviving individuals, but are an artifact of the sampling design, with

Table 2.5. Water chemical values obtained between June 7 - October 12, 2001 in the Flow-Through Pond (except where nutrient parameters are listed for both ponds).

	Hardness (mg/L) (n = 7)	Alkalinity (mg/L) (n = 7)	D.O. (mg/L) (n = 7)	Total Ammonia [mg (NH_3-N + NH_4-N)/L]		Unionized Ammonia (mg NH_3-N/L) (n = 7)
				Algae Pond (n = 6)	Flow-Through Pond (n = 8)	
Mean	305.7	71.4	8.92	0.058	0.087	0.015
±SD	79.8	15.7	0.52	0.018	0.054	0.020
Range	200 - 420	60 - 100	8.44 - 10.09	0.033 - 0.089	0.052 - 0.21	0.002 - 0.060

	pH (n = 7)	Nitrate (mg NO_3-N /L)		Nitrite (mg NO_2-N/L) (n = 8)	Orthophosphate (mg PO_4-P/L)	
		Algae Pond (n = 5)	Flow-Through Pond (n = 8)		Algae Pond (n = 7)	Flow-Through Pond (n = 8)
Mean	8.48	0.025	0.035	0.016	0.033*	0.031
±SD	0.25	0.015	0.043	0.021	0.016	n/a**
Range	8.18 - 8.65	0.010 - 0.043	0.005 - 0.14	0.001 - 0.03	0.014 - 0.052	n/a**

* mean of samples in which orthophosphate was detectable

** SD and range n/a, orthophosphate was only detectable in one sample

Table 2.6. Mean percent survival (± SD) and range among replicate containers for *Villosa iris* juveniles cultured with and without fine silt over 85 d.

Treatment		% Survival at Mean Age (d)					
		9	21	35	49	63	93
With Silt	Mean	100	80.8	87.0	63.5	64.9	49.8*
	(± SD)	—	—	(11.0)	(24.2)	—	(14.5)
	Range	—	—	79.2 - 94.8	45.5 - 79.6	—	39.7 - 66.4
Without Silt	Mean	100	66.33	66	49.7	47.6	32.9*
	(± SD)	—	—	(1.8)	—	(4.9)	(11.7)
	Range	—	—	64.7 - 67.3	—	44.1 - 51.1	25.1 - 46.4

* Not significantly different (t-test, p = 0.1910)

different containers being sampled on subsequent dates. Also, on some sampling dates, range and SD are not available because only one container of a given treatment was sampled. On all 5 sampling dates, mean survival of juveniles was higher in containers with the silt treatment (Figure 2.4.). Juveniles exhibited similar survival trends for both treatments, with survival rates alternately declining and remaining fairly stable between sampling events. For juveniles cultured without silt, the greatest declines in survival (33.7%) occurred between 6/22 – 7/4 (juveniles aged from 9 d to 21 d) (Table 2.7.). For juveniles cultured with silt, the greatest declines in survival (27.0%) occurred between 7/19 – 8/1 (juveniles aged from 21 d to 35 d) (Table 2.7.).

Survival over the culture period, using the mixed multivariate ANOVA procedure, showed a significant difference in survival between the two treatments ($p = 0.0102$), with juveniles provided with supplemental silt exhibiting better survival. However, the mixed procedure was unable to model the covariance structure of the survival data because of the small size (29 observations) and imbalance of the data set. Therefore, the analysis procedure treated survival proportions for each container over the course of the experiment as independent data points, which gave undue weight to lowered survival in the no-silt containers which persisted over time. Additionally, no statistical difference in survival between treatments was found when comparing survival for all containers on the last sampling date using a 2 sample t-test assuming equal variance ($p = 0.1910$). As analyzed over the entire culture period with the general linear model procedure, differences in survival between containers within each treatment were not significant ($p = 0.2756$).

At the end of the study period, shell lengths of *V.iris* cultured with supplemental silt ranged from 0.75 - 3.21 mm, with a mean length of 1.81 (± 0.67) mm (Table 2.8). Shell lengths of *V.iris* cultured without silt ranged from 0.94 - 3.33 mm, with a mean length of 1.78 (± 0.68) mm (Table 2.8.). Similar mean shell lengths were seen for the two treatments at all sampling dates (Figure 2.5). For juveniles cultured without silt, the greatest relative growth rate occurred between 6/22 – 7/4 (juveniles aged from 9 d to 21 d), with mean length increasing by 39.4% (Table 2.7.). For juveniles cultured with silt, the greatest relative growth rate occurred between 7/5 – 7/18 (juveniles aged from 21 d to 34 d), with mean length increasing by 50.2% (Table 2.7.). No statistical difference in

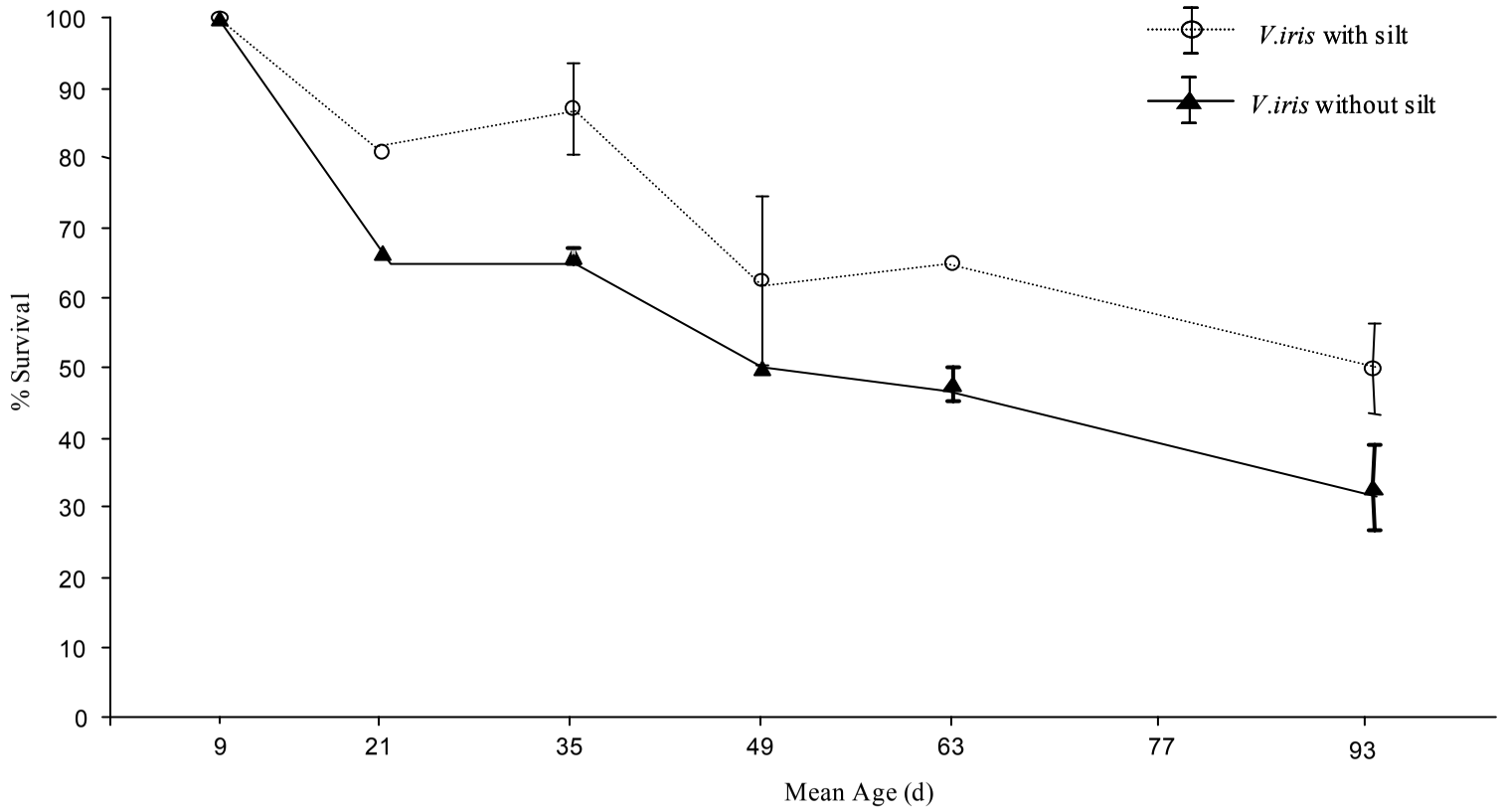


Figure 2.4. Mean survival (±SD) for *Villosa iris* juveniles cultured with and without fine silt over 85 d.

Table 2.7. Relative growth rates and declines in survival between sampling events as compared with the temperature regime for each interval.

Time Interval	Number of Days	Mean Temp. (°C)	Mean Daily Maximum Temp. (°C)	Relative Decline in % Survival Over the Time Interval			Relative Growth Rate Over the Time Interval		
				L. fasciola, with silt	*V.iris*, with silt	*V.iris*, no silt	*L. fasciola*, with silt	*V.iris*, with silt	*V.iris*, no silt
6/7 - 6/21	15	—	—	52.8%	—	—	99.9%	—	—
6/22 - 7/4	13	21.1	23.8	64.6%	19.2%	33.7%	14.9%	27.9%	39.4%
7/5 - 7/18	14	23.7	26.8	38.9%	+ 7.67%	0.5%	30.8%	50.2%	32.5%
7/19 - 8/1	14	23.5	26.0	24.5%	27.0%	24.7%	31.2%	17.4%	26.6%
8/2 - 8/15	14	25.6	28.8	5.2%	+ 2.2%	4.2%	46.6%	36.0%	31.6%
8/16 - 8/29	14	21.2	23.5	13.7%	—	—	12.6%	—	—
8/16 - 9/14	30	21	23.3	—	23.3%	30.9%	—	42.0%	39.0%

Table 2.8. Mean (± SD) and range of shell lengths for *Villosa iris* juveniles cultured with and without fine silt over 85 d.

Treatment		Length (mm) at Mean Age (d)					
		9	21	35	49	63	93
With Silt	Mean	0.42	0.53	0.80	0.94	1.28	1.81
	(± SD)	(0.061)	(0.10)	(0.15)	(0.23)	(0.31)	(0.67)
	Range	0.28 - 0.50	0.38 - 0.69	0.50 - 1.06	0.56 - 1.44	0.88 - 1.44	0.75 - 3.21
Without Silt	Mean	0.42	0.58	0.77	0.98	1.28	1.78
	(± SD)	(0.061)	(0.11)	(0.15)	(0.20)	(0.29)	(0.68)
	Range	0.28 - 0.50	0.38 - 0.75	0.50 - 1.00	0.75 - 1.31	0.75 - 2.00	0.94 - 3.33

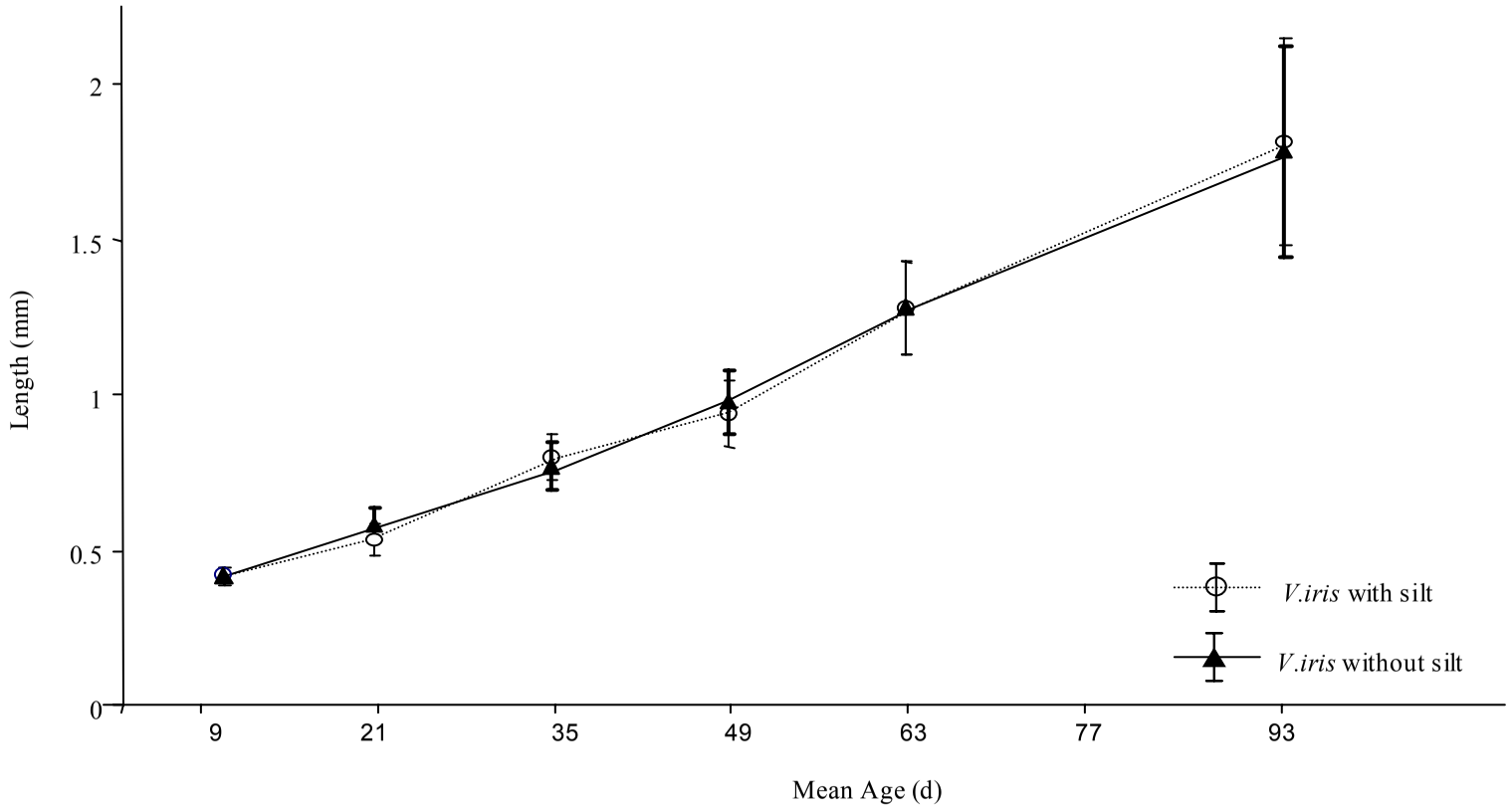


Figure 2.5. Mean shell length (±SD) for *Villosa iris* juveniles cultured with and without fine silt over 85 d.

growth occurred between the two treatments ($p = 0.7913$), using the multivariate ANOVA general linear models procedure.

Comparison of Growth and Survival of *Villosa iris* and *Lampsilis fasciola*

At the end of the experiment, mean survival for *V. iris* (mean age 93 d), was 49.8% (± 14.5), while mean survival for *L. fasciola* (mean age 86 d) was 6.3% (± 4.5) (Table 2.9.). On all five sampling events, mean survival was higher for *V. iris* than for *L. fasciola* (Figure 2.6.). Comparisons were made by sampling event rather than by date or age, because *L. fasciola* were released earlier and at a slightly younger age range than *V. iris*. In contrast to the pattern seen for *V. iris*, with survival alternately declining and remaining stable between sampling events, survival declined sharply for *L. fasciola* over the first two sampling dates and then stabilized. For *L. fasciola*, the greatest declines in survival (64.6%) occurred between 6/22 – 7/4 (juveniles aged from 16 d to 30 d) (Table 2.7.). Comparison of survival over the course of the culture period using the mixed multivariate ANOVA procedure showed a significant difference in survival between the two species ($p < 0.0001$). Differences in survival among containers within each treatment also were significant ($p = 0.0125$), indicating that microhabitat differences between containers had significant effects on survival.

At the end of the experiment, shell lengths of *V.iris* ranged from 0.75 - 3.21 mm, with a mean length of 1.81 (± 0.67) mm; and shell lengths of *L. fasciola* ranged from 0.75 - 3.59 mm, with a mean length of 1.78 (± 0.78) mm (Table 2.10.). For *L. fasciola*, the greatest relative growth rate occurred between 6/7– 6/21 (juveniles aged from 4 d to 16 d), with mean length increasing by 99.9% (Table 2.7.). Upon initial release in the Flow-Through Pond, *V. iris* juveniles were larger and slightly older (0.42 mm, mean age 9 d) than *L. fasciola* (0.27 mm, mean age 4 d). Greater mean shell lengths continued in *V. iris* for four of the five sampling events (Figure 2.7.). However, length differences were not large, and the multivariate ANOVA did not show a statistical difference between the two species ($p = 0.1315$).

The length frequency distributions of *V. iris* and *L. fasciola* after approximately 12 wk of culture show that juvenile lengths are highly variable (Figure 2.8.). As no difference was evident between the growth of *V. iris* cultured with and without silt, length

Table 2.9. Mean percent survival (± SD) and range among replicate containers for juveniles of *Villosa iris* and *Lampsilis fasciola* cultured over 85 d.

Treatment		Sampling Event 1	2	3	4	5	6	7
V. iris	Mean Age (d)	9	21	35	49	63	—	93
	Mean Survival (%)	100	80.8	87.0	63.5	64.9	—	49.8
	(± SD)	—	—	(11.0)	(24.2)	—	—	(14.5)
	% Survival Range	—	—	79.2 - 94.8	45.5 - 79.6	—	—	39.7 - 66.4
L. fasciola	Mean Age (d)	4	16	30	44	58	72	86
	Mean Survival (%)	100	47.2	16.7	10.2	7.7	7.3	6.3
	(± SD)	—	(27.9)	(10.5)	—	(7.5)	—	(3.5)
	% Survival Range	—	27.5 - 66.9	9.2 - 24.1	—	2.4 - 13.0	—	2.4 - 9.2

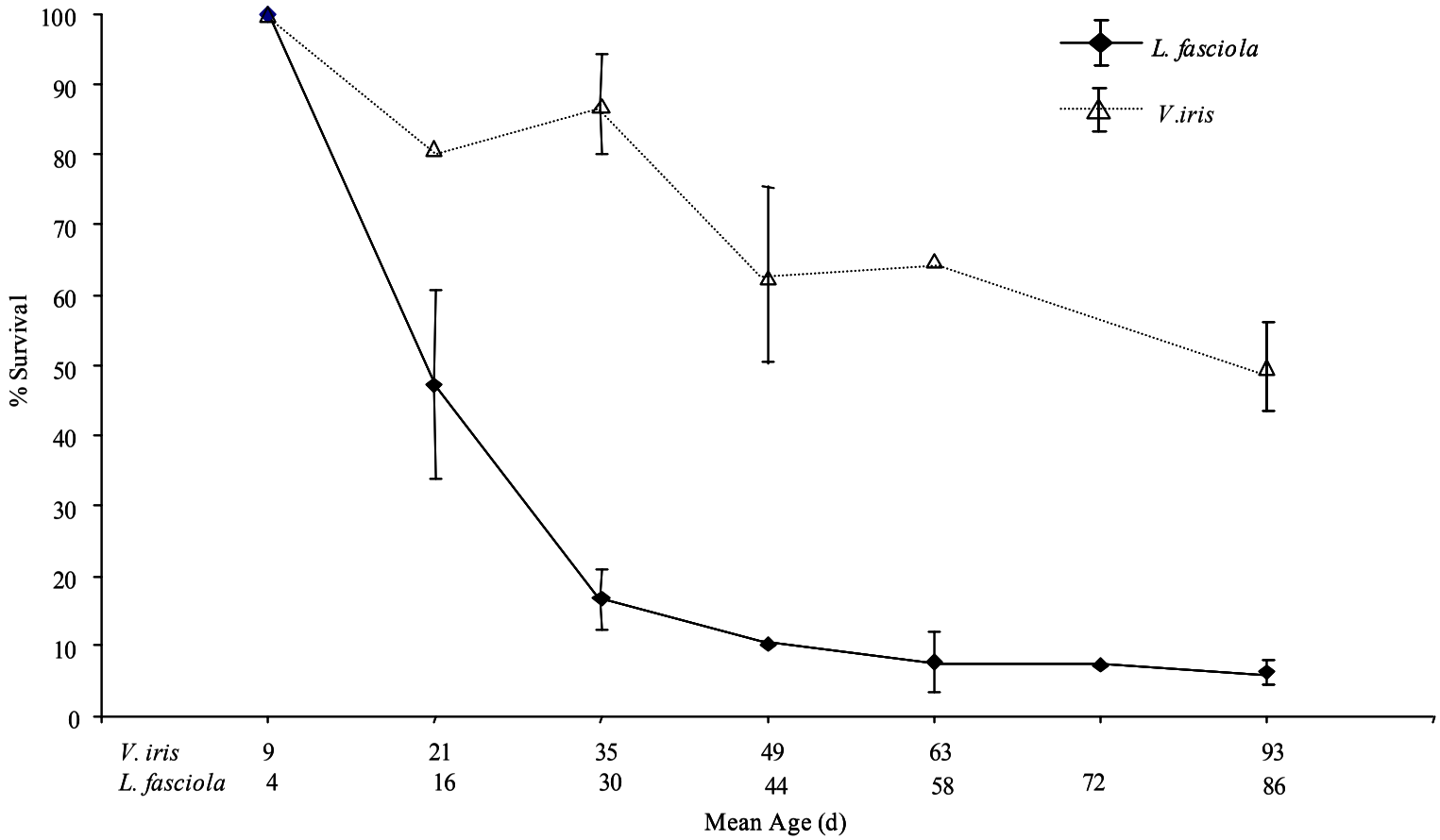


Figure 2.6. Mean survival (±SD) for juveniles of *Villosa iris* and *Lampsilis fasciola* over 85 d.

Table 2.10. Mean (± SD) and range of shell lengths for juveniles of *Villosa iris* and *Lampsilis fasciola* cultured over 85 d.

Treatment		Sampling Event 1	2	3	4	5	6	7
V. iris	Mean Age (d)	9	21	35	49	63	—	93
	Mean Length (mm)	0.42	0.53	0.80	0.94	1.28	—	1.81
	(± SD)	(0.061)	(0.10)	(0.15)	(0.23)	(0.31)	—	(0.67)
	Length Range (mm)	0.28 - 0.50	0.38 - 0.69	0.50 - 1.06	0.56 - 1.44	0.88 - 1.44	—	0.75 - 3.21
L. fasciola	Mean Age (d)	4	16	30	44	58	72	86
	Mean Length (mm)	0.27	0.55	0.63	0.82	1.08	1.58	1.78
	(± SD)	(0.052)	(0.10)	(0.10)	(0.24)	(0.21)	(0.43)	(0.78)
	Length Range (mm)	0.19 - 0.38	0.38 - 0.70	0.44 - 0.75	0.50 - 1.25	0.75 - 1.31	1.00 - 2.44	0.75 - 3.59

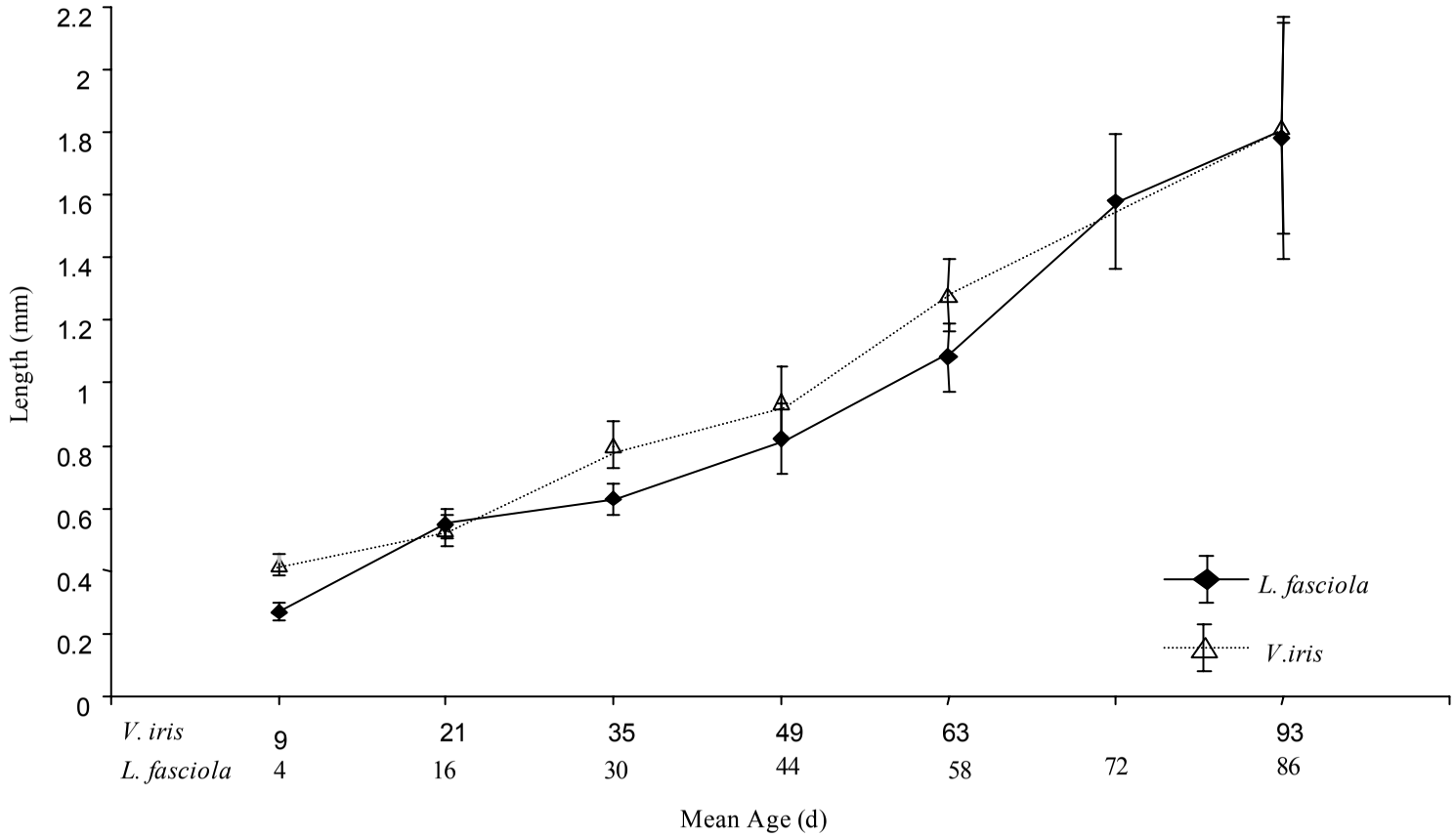


Figure 2.7. Mean shell lengths (±SD) for juveniles of *Villosa iris* and *Lampsilis fasciola* over 85 d.

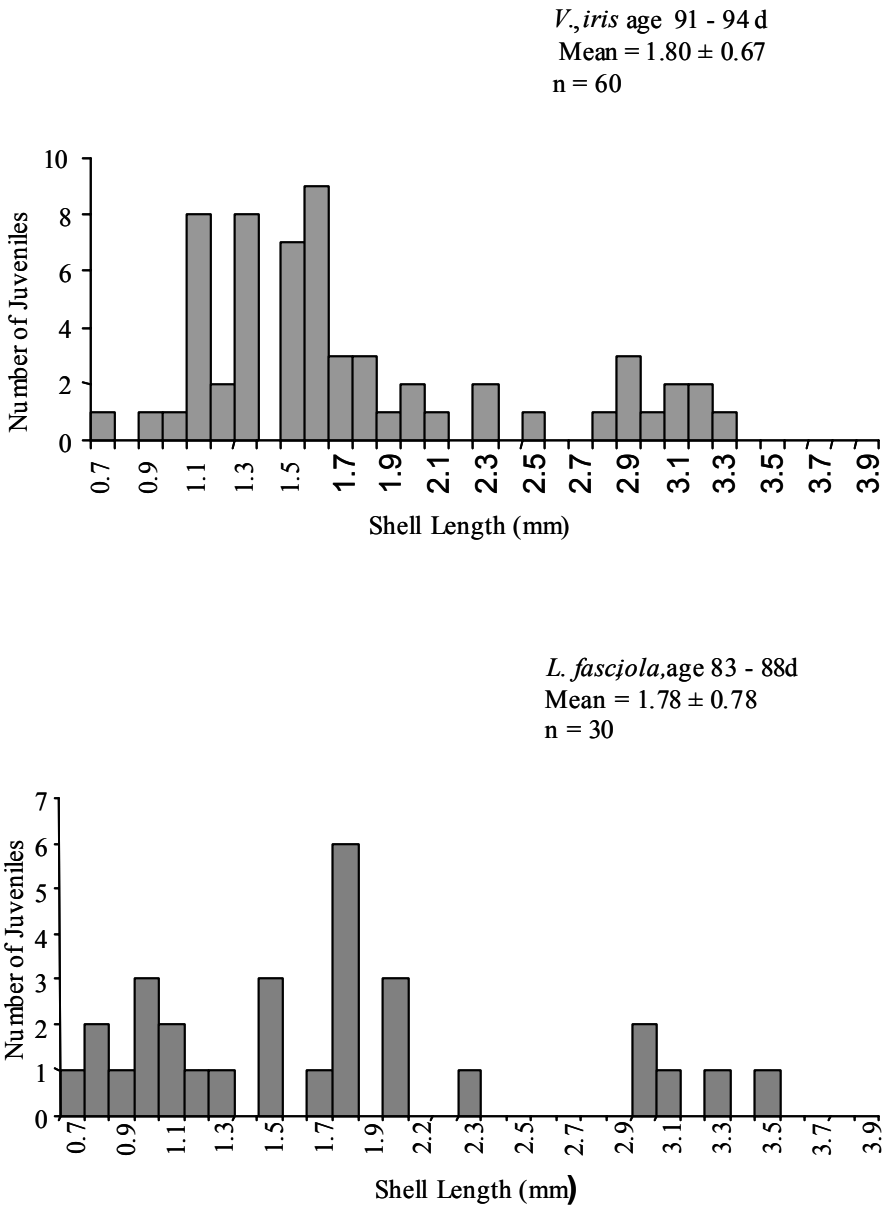


Figure 2.8. Length frequency distribution of juveniles of *Villosa iris* and *Lampsilis fasciola* sampled from the hatchery Flow-Through Pond after 85 d of culture.

data on the final sampling date for the two treatments were combined to view the length frequency distribution of *V. iris* juveniles. These graphs suggest a bi-modal distribution of lengths, but larger sample sizes would be needed for corroboration.

DISCUSSION

Flow and Mussel Survival and Growth

Flow is important to juvenile mussels both to flush wastes and to bring nutritional material to the sediment-water interface (Yeager *et al.* 1994, Hanlon 2000). Previous researchers have found exposure to increased flow or water agitation to be correlated with increased growth (Steg 1998, Hanlon 2000), and others have found a certain degree of constant flow to be beneficial to bivalve survival (Hudson and Isom 1984). In this study, recirculated water was directed through the PVC outlet manifold to provide a steady flow, and juveniles were held in containers of 20 cm x 40 cm (rather than smaller containers) in order to minimize the extent to which flow was inhibited by the sides of the container. The calculated cross-sectional discharge rate of 1.39 mL/ min/ cm^2 is lower than the 5 mL/ min/ cm^2 cited as appropriate for culturing other bivalves and found suitable by Steg (1998). The inability to get a measurable flow velocity reading at the location of the juvenile containers also demonstrates that the Flow-Through Pond environment was relatively static compared to the natural stream and river systems inhabited by *V. iris* and *L. fasciola*. Flow requirements for adult and juvenile freshwater mussels are not fully characterized. Juvenile mussels inhabit different environments than adults and are often found in depositional areas with low flow, such as behind boulders. If this characterization of habitat use by juveniles is accurate, the low flow conditions in the Flow-Through Pond may not be problematic. As culture requirements of different mussel species become better understood, it is likely that juveniles of different species will exhibit different flow requirements and the relatively static Flow-Through Pond environment may be less suitable for some. Placement of containers closer to flow outlets or further concentration of water flow with control structures could address different flow requirements.

Temperature

The temperature regime in the Flow-Through Pond was well above the minimum threshold of 15°C required for juvenile mussel growth (Hanlon 2000) and was also suitable for unicellular algae production. Juvenile mussel growth increases at higher temperatures as a result of higher metabolic rates (Hanlon 2000). Additionally, uptake of planktonic algae increases at higher temperatures (Stuart *et al*. 1999). While increased growth rates have clearly been correlated with higher temperatures (Beaty 1999, Hanlon 2000), the relationship between temperature and mortality is less clear. Some researchers have reported mortality increases as temperature increases (Buddensiek 1995, Beaty 1999). Temperatures around 20°C are generally recommended as most suitable for juvenile growth and survival (Steg 1998). In my study, the mean temperature of 22.6°C was close to this reported ideal, but maximum daily temperatures exceeded 25°C on 41 days of the 85 d study period.

Comparison of survival and growth rates between each sampling event with the temperature regime occurring at that time period may illuminate whether maximum temperatures in the Flow-Through Pond were high enough to be problematic (Table 2.1). The highest mean and daily maximum temperatures occurred during late July and the first half of August. Declines in survival for *V. iris* occurred intermittently over the entire study period, without any apparent relationship to temperature. For *L. fasciola*, survival declined most sharply over the first two sampling periods, before the highest mid-summer temperatures were reached. Reduction in relative growth rate over the warmest mid-summer time period was not seen for either species or treatment. Based on these observations, elevated temperatures did not have deleterious effects on growth or survival.

In an attempt to regulate temperatures, additions of 12°C spring or well water were made when the hatchery personnel observed temperatures greater than 25°C. However, the summer season was extremely busy and short-staffed at the hatchery, and it is likely that elevated temperatures went unnoticed on many days. If temperatures greater than 25°C are ultimately found to be problematic, a regular schedule for checking temperature and adding spring and well water should be implemented.

Previous work with juveniles of *L. fasciola* showed juvenile growth to follow increasing temperatures (Hanlon 2000). For my study, the relationship between growth and temperature is less clear, as growth rates do not decrease after mid-August when temperatures decline (Table 2.1.). However, in Hanlon's study of *L. fasciola* (2000), it was not until the second growing season that the temperature-growth relationship became especially clear, so these juveniles at WSSNFH may exhibit a similar trend if their growth is followed for a second season.

Algae as a Food Source

In spite of the failure to reach target algal densities, the growth of juveniles compared favorably to that seen in other freshwater mussel culture studies (see Growth, below). Additionally, the juvenile mussels exhibited green and brown coloration of their guts, indicating that they were ingesting chlorophyll-containing algae and possibly other food particles. As discussed previously, the relative importance of various food sources in the natural diet of juvenile mussels is unknown. It has recently been shown that saltwater bivalves feed on a full array of naturally occurring particles including bacteria, protozoans, and phytoplankton (Baldwin & Newell 1991). In the WSSNFH Flow-Through Pond, other biota and particles may have provided adequate nutrition to counter the deficient phytoplankton densities.

Another possibility for why these juveniles grew well in spite of the failure to reach the target density of 10,000 algal cells/mL is that the densities achieved were in fact sufficient to support growth. The freshwater mussel *E. complanata* exhibits maximum ingestion efficiency at algal densities between 11,000 – 15000 cells/mL (Paterson 1984) and some marine bivalves at densities from 75,000 – 100,000 cells/mL for (Rajesh *et al*. 2001). Perhaps, however, such high densities are not necessary for freshwater mussels to attain adequate nourishment. Estimates of phytoplankton concentrations in natural systems are extremely variable. Densities of less than 5,000 cells/mL are widely reported, such as in the Kentucky river where researchers found an annual mean density of 1162 cells/mL (Stephenson and White 1995); in the Arkansas river where the maximum annual density found was 533 cells/mL (Wilhm *et al*. 1977); in the Nottoway River in Virginia with a single sample density of 2850 cells/mL (W. Henley, VPI and SU,

pers. comm. 2001); in a Kenyan river, with an annual mean of 283 cells/mL (Mwashote and Shimbira 1994); in the Danube with an annual maximum of 3770 cells/mL (Stoyneva and Dragonov 1991), and in an Argentinian river with an annual maximum of 387 cells/mL (Boltovskoy *et al.* 1995). Additionally, wide ranges of phytoplankton densities are reported within a given river system, often varying seasonally. For example, density estimates from the Mississippi River range from 5,000 – 55,000 cells/mL (Gale and Lowe 1971); in the Ohio River from 31,000 – 104,000 cells/mL (Wehr & Thorp 1997) and 10,000 – 100,000 cells/ mL (Paterson *et al*. 1999); and in the James River, Virginia from 100 – 100,000 cells/mL (Davis *et al.* 1997). These widely variable findings demonstrate that it is uncertain how commonly bivalves in the wild encounter phytoplankton densities resulting in maximum ingestion rates and demonstrate that mussels in natural habitats may live on densities similar to those in this study.

Another possibility for why these juveniles grew well in spite of the failure to reach target algal densities is that the genera of algae that did occur were of high suitability as sources of food. Polyunsaturated fatty acids (PUFA) promote growth in juvenile freshwater mussels (Gatenby *et al.* 1996). Diatoms generally are rich in PUFA (Pohl and Zurheide 1979) and were fairly prevalent in samples. Twelve genera of diatoms were observed, and diatoms made up a mean of 12.3% of the cells observed in the Flow-Through Pond samples, and 10.6% of the cells in the Algae Pond samples (Table 2.4.) Two diatom genera (*Diatoma* and *Cocconeis*) were also among the top 10 dominant genera in all samples. The Cryptophyceae also produce some PUFA, and cryptophyte genus (*Chroomonas*) was among the top 10 dominant genera. The Chlorophyceae and Euglenoids, however, lack substantial amounts of PUFA and blue-green algae generally don't supply any (Pohl and Zurheide 1979). Other nutrients of potential importance to juvenile mussel nutrition include sterols and steroids, which occur in all the groups of algae observed, except the blue-green algae. Carotenoids, which have been shown to influence marine mussel growth, also will occur in all of the algae, including the blue-green genera (Campbell 1969, Gatenby 1994). Another factor that may have contributed to the nutritional value of the algae, in spite of lower than targeted cell densities, was the diversity of observed genera. Researchers have noted that mixed algae diets are preferential to unicellular diets for rearing marine bivalves (Epifanio 1979,

Romberger and Epifanio 1981), and greater algae diversity has been correlated with increased growth (Shpigel *et al*. 1993).

Juvenile mussels ingest a number of the algae genera observed in the flow-through pond system. *Clamydomonas spp*. and *Scendedesmus spp.*, both of which were in the top 10 dominant genera, have been suggested for freshwater mussel culture (Foe and Knight 1986, Lauritsen 1986). In laboratory feeding studies, *Chlamydomonas reinhardtii* was ingested and found partially digested in gut squashes (Gatenby *et al.* 1996). *Chlorella spp.* constituted the dominant genus in all samples, making up from 38.3 - 56.8% of cells observed. *Chlorella vulgaris* has been found partially digested in gut squashes of juveniles of *V. iris* (Gatenby *et al.* 1996). *Chlorella spp*. and the observed diatom genus *Fragilaria* were also found in guts of 3 - 14 day old laboratory-reared juveniles of *V. iris* (Yeager *et al.* 1994). A number of the genera observed in the flow-through pond system (notably *Chlorella, Chlorococcum, Cyclotella, Diatoma, Scenedesmus*) have also been found in the guts of adult freshwater mussels (Parker *et al.* 1998).

Water Quality and Habitat Parameters

Hardness levels in the Flow-Through Pond, with a mean of 305 mg/L, should have been adequate for juvenile growth (Steg 1998). Values for water pH were high, averaging 8.48, but are consistent with previous successful mussel culture studies that documented pH levels of 8.4 and 8.5 (Steg 1998). Dissolved oxygen levels in the Flow-Through Pond were well above 5 mg/L threshold recommended for unionids (Havlik and Marking 1987). Nitrite levels were below levels reported to be acutely toxic (Eddy and Williams 1994).

Orthophosphate levels of 0.13 – 0.35 mg PO_4-P/L and above have been reported to be problematic (Lemly 1998). The values measured in the Flow-Through and Algae ponds were far below these harmful levels. Low levels of orthophosphate are problematic for juvenile culture only in as much as algae production is limited. Although initial levels of orthophosphate were relatively low, they were comparable to levels (mean 0.15 mg/L) recorded in habitat that supports mussel populations (Strayer 1999). Therefore, fertilization was planned to balance the nitrogen to existing orthophosphate

levels, and phosphate-based fertilizer was never added. Addition of phosphate should be avoided if it occurs naturally, as it is easy to over-supplement phosphorus (B. Parker, Ph.D., VPI and SU, pers. comm. 2001). The decline in orthophosphate from an initial measurable level to a series of samples where orthophosphate was below the limit is thought to correspond with uptake by filamentous algae (see discussion of Filamentous Algae below). Increases to detectable orthophosphate values toward the end of the study period are also hypothesized to be related to both the removal of large quantities of the filamentous algae and to increased fertilization rates, which may have established a nutrient balance that discouraged the uptake of all available orthophosphate. On the final sample date, orthophosphate was undetectable, but the fertilization and algae removal had been terminated almost a month before.

Estimates of potentially harmful environmental nitrate levels range from 0.8 to 2.13 mg NO_3-N/L (Lemly 1998). All recorded nitrate values were below these levels. Nitrate levels did not exhibit the same pattern of decline from the initial sample. However, it is believed that the filamentous bloom affected nitrate levels. On all sample dates on which nitrates were measured in both the Flow-Through and Algae Ponds, concentrations were lower in the Algae Pond, which was where the majority of the filamentous algae occurred (Appendix 2.3). However, complete exchange of water occurs between the two ponds once every 25 hours, so it is uncertain whether water is retained in the filamentous-dominated Algae Pond long enough for observed differences in nutrient levels between the two ponds to be biologically meaningful. Fertilization is known to have increased nitrate concentrations initially, as on the August 16th water sampling date, when elevated levels were measured in samples taken shortly after fertilization (Appendix 2.3.). However, nitrate levels remained less than 0.05 mg NO_3-N /L for all other samples. The inabililty to sustain elevated nitrate levels in either pond, in spite of increased frequency of fertilization with ammonium nitrate, is further evidence that the filamentous algae removed the nitrate.

Excluding the elevated total ammonia measurement taken just after fertilization, mean total ammonia in the Flow-Through Pond was 0.069 (± 0.019) mg (NH_3-N + NH_4-N)/L, which is higher than environmental levels measured in mussel habitats in the Clinch, Powell, and Holston River systems, where total ammonia concentrations typically

are less than the 0.04 mg (NH_3-N + NH_4-N)//L detectable limit (VA DEQ 2001). The highest recorded level of total ammonia occurred at the start of the study period, and consistently higher levels were seen in the Flow-Through Pond than in the Algae Pond. Also, increased fertilization never raised total ammonia levels above 0.08 mg (NH_3-N + NH_4-N)//L (except the August 16th sample taken just after fertilization) (Appendix 2.3.), and it is believed that concentrations were limited by uptake by filamentous algae.

A number of harmful effects have been documented in bivalves exposed to ammonia solutions (see Chapter 4). Ammonia toxicity is attributed largely to the fraction of ammonia occurring in the unionized form (US EPA 1984). Levels of unionized ammonia reported to be acutely toxic to young bivalves range from 0.10 to 2 mg NH_3-N /L (Goudreau 1993, Stickney 1994, Scheller 1997, Summers 1998). Reduced ciliary action in freshwater bivalves is reported at concentrations as low as 0.06 mg NH_3-N/L of unionized ammonia (Anderson *et al.* 1978). Acute toxicity trials conducted in conjunction with this study (Chapter 4) produced 96-h LC50 values for juvenile *L. fasciola* and *V. iris* between 0.10 - 0.28 mg NH_3-N /L unionized ammonia. Only one calculated unionized ammonia level (0.06 mg NH_3-N /L on August 16th) approached these acutely toxic levels. However, this sample was collected shortly after fertilization, and it likely represents an area where fertilizer was disproportionately concentrated before it had mixed.

Levels of unionized ammonia safe for long-term exposure of juvenile mussels were estimated at 0.01 mg NH_3-N/L, based on acute toxicity values and a projected acute:chronic ratio using a surrogate species (see Chapter 4). In addition to the elevated post-fertilization value, unionized ammonia levels in the Flow-Through Pond were above this threshold for two of six remaining samples. Levels of unionized ammonia above 0.01mg NH_3-N/L are common in laboratory culture environments. For example, Steg (1998) reported mean unionized ammonia values of 0.029 and 0.016 mg NH_3-N /L, and Hanlon (2000) reported laboratory values ranging from 0.0115 – 0.0687 mg NH_3-N/L unionized ammonia. Therefore, levels above 0.01 mg NH_3-N/L do not preclude growth and survival, but they may cause stress. It is noteworthy that a comparison of water quality parameters at the Buller Hatchery (where extremely good survival and growth of juvenile mussels have been achieved) with parameters in a laboratory culture

environment shows that lab values of unionized ammonia were consistently higher by almost an order of magnitude (Hanlon 2000, Appendix I). The Buller Hatchery values for unionized ammonia ranged from 0.0006 – 0.0101, with mean of 0.004 (± 0.016) NH_3-N mg/L, and only 1 value out of 10 above 0.01 mg NH_3-N/L, while laboratory values of unionized ammonia ranged from 0.0115 – 0.0687, with a mean of 0.038 (± 0.016) mg NH_3-N/L.

It is not possible to determine whether unionized ammonia levels affected the reared juveniles. Although the occurrence of three out of seven values above 0.01 mg NH_3-N/L suggests that this estimated safe threshold may have been commonly exceeded, the 0.01 mg NH_3-N/L level is only an estimate, and the calculated levels were within the range of this estimate. Furthermore, this estimated safe level was based on acute studies with juveniles younger than those held in the WSSNFH Flow-Through Pond. Levels of total ammonia in the flow-through pond system are not exceptionally high; rather, the high pH coupled with high temperatures are responsible for large proportions of the ammonia occurring in the unionized form (Emerson *et al.* 1975). However, if further studies indicate that juvenile mussels, or mussels of particular species, experience cumulative effects of low levels of unionized ammonia, further consideration of the stress posed by unionized ammonia in the Flow-Through Pond will be warranted. Lowering temperatures would be a feasible way to limit the fraction of unionized ammonia.

Filamentous Algae

The proliferation of filamentous algae in the Algae Pond is believed to have influenced both nutrient levels and phytoplankton densities. Phytoplankton densities did not reach the target density of 10,000 cells/mL. The highest observed density found in the Flow-Through Pond was 6892 cells/mL, and mean densities for the two ponds were below 5,000 cells/ mL. Once established, filamentous algae often out-compete unicellular algae for nutrients (B. Parker, Ph.D., VPI and SU, pers. comm. 2001). As discussed previously, the onset of the filamentous algae bloom coincided with a decline in orthophosphate and total ammonia levels. Nutrients also tended to be lower in the Algae Pond, where the filamentous algae was much more widespread, than in the Flow-

Through Pond (Appendix C). Additionally, there were no higher aquatic plants present in either pond to take up nutrients. However, because the filamentous bloom occurred early in the study period, and only one set of samples had been analyzed before the onset of the bloom, it is difficult to conclude that the filamentous bloom is responsible for the lowered phytoplankton densities or nutrient parameters. Nonetheless, given the coincidence of these patterns, coupled with the failure of more frequent fertilization to result in a prolonged increase in nutrient levels, it seems likely that filamentous algae took up the nutrients, thereby limited the densities of phytoplankton cells. Given that the juvenile mussels were exhibiting good growth and coloration of their guts, indicating that they were ingesting food particles, fertilization frequency was never increased above once per week to avoid nutrient levels harmful to mussels upon initial fertilization.

While the filamentous algae were extremely prolific throughout the Algae Pond, very little grew in the Flow-Through Pond. The only differences in habitat were that the Algae Pond had greater depth (mean 0.92 m vs. 0.61 m) and was more static than the Flow-Through Pond. In the Flow-Through Pond, the filamentous algae began colonizing above the PVC manifold and aerator, in the location where no flow was directed. Also, although the filamentous algae occurred throughout the Algae Pond, it did not occur around the pipe where water flowed into the pond. Based on these observations, flowing water is less favorable to these filamentous forms.

Given the dominance of the filamentous algae in the Algae Pond, it is questionable whether there is a benefit to using both ponds in the flow-through pond system. The intention of using both ponds was to increase volume and bolster algae production. In fact, it is believed that the filamentous algae actually hindered the production of unicellular algae. No statistical differences in phytoplankton community characteristics between the two ponds were found, so it is not likely that the Algae Pond provides better or different conditions for algae from what is available in the Flow-Through Pond. During the course of the study period, large quantities of the filamentous algae were manually removed, resulting in an estimated of 75% being cleared from the Algae Pond. Nonetheless, the remaining algae seemed to be enough to deplete nutrient levels over the course of each sampling period. In order for the flow-through pond system to serve to support a denser algae community, a control method for the

filamentous algae must be found. Perhaps flow could be increased in the Algae Pond with a PVC water outlet manifold similar to that in the Flow-Through Pond. Another option may be the introduction of fish, such as grass carp, that would consume the algae. However, the density of fish required to control the algae would have to be considered, as high fish densities could elevate ammonia levels. This increased ammonia might be taken up by phytoplankton, but an empirical trial would be needed to determine what ammonia levels would result. If the filamentous algae cannot be brought under control, it may be better to recirculate water within the Flow-Through Pond for future growing seasons.

Growth and Survival

Comparison of Villosa iris and Lampsilis fasciola

The two species exhibited very different survival rates. Overall, survival among *V. iris* was good, while survival among *L. fasciola* was poor. However, this one study is not sufficient to conclude that the WSSNFH Flow-Through Pond is not a suitable culture environment for *L. fasciola*. There are a number of factors that may have contributed to the low survival of *L. fasciola*. Significantly different survival rates have been seen among broods produced from different female mussels (Hanlon 2000). The maturity of glochidia at the time of extraction is another factor that can influence robustness of juveniles [J Jones Virginia Polytechnic Institute and State University (VPI and SU), pers. comm. 2001]. The use of multiple adult females to produce the juveniles used in this study should counter variability among broods, and most of the *L. fasciola* used were displaying their mantle flaps, indicating full maturation of glochidia. Nonetheless, the influence of such factors demonstrates the potential variability of survival among juveniles, regardless of culture conditions. It is also possible that conditions during the infestation period had a negative effect on this group of *L. fasciola.* For example, although a filtered recirculating system was used in to maintain good water quality, there may have been an undetected harmful condition that occurred in the initial hours or days post-metamorphosis and weakened this batch of *L. fasciola*.

It is also possible that specific habitat requirements of *L. fasciola* were not met in the Flow-Through Pond, thus lowering their survival. Possibilities would be that *L. fasciola* have higher flow requirements or different nutritional requirements than *V. iris*. According to the toxicity tests conducted in conjunction with this study (see Chapter 4), juveniles of *L. fasciola* are more tolerant of unionized ammonia than are *V. iris*, so unionized ammonia levels should not have been the responsible habitat factor.

Shell lengths of the two species did not differ significantly. The fact that surviving juveniles of *L. fasciola* grew as well as those of *V. iris* indicates that the habitat and nutritional requirements of those individuals were met, making it seem improbable that the Flow-Through Pond inherently fails to meet the needs of this species. The good growth of the survivors also seems to lessen the likelihood of a scenario in which the *L. fasciola* entered the system in a weakened state, as a poor-quality batch.

The juveniles of *L. fasciola* were released two weeks before the juveniles of *V. iris*, and one possibility to explain their poor survival is that they experienced adverse conditions during the first weeks in the Flow-Through Pond. A continuous temperature record isn't available for the first two weeks that the juveniles of *L. fasciola* were in the pond (due to a malfunction of the temperature logger); however, periodic temperature measurements indicate that the 15°C mean temperature threshold was met, so it is unlikely that temperature was a problem. Because the juveniles of *L. fasciola* were released earlier in the season, another possible problem is that certain biotic components of the system may not have had time to become established to provide an adequate nutritional base. The fact, however, that the first phytoplankton sample showed the greatest density doesn't support this idea of less food availability during these first few weeks.

Flatworms, which are a known predator of juvenile mussels (Sickel 1998), were observed in some of the juveniles' containers when sampling. Flatworms were never very abundant (no more than 10 were ever counted in a single container), but their numbers peaked during the first month of the study period. Thus, predation was a likely threat initially faced by the juveniles of *L. fasciola*. Furthermore, because the juveniles of *L. fasciola* were released at a slightly younger age and smaller size than those of *V. iris*, they would have been more susceptible to predation.

Also, according to the mixed multivariate ANOVA procedure, differences in survival between containers of each species were found to be significant. This difference between containers is probably attributable to microhabitat conditions. For example, large numbers of flatworms were found in some containers, while few were found in others. Chironomids, which enmesh juveniles in their cases, thus immobilizing and ultimately killing them, also colonized the containers at noticeably different rates. Another microhabitat factor that differed among containers was the presence of clumping algae propagules. In conclusion, although a clear difference in survival was seen between the two species, it is likely that unique circumstances affecting this batch of juveniles either during the infestation period or during the first weeks of culture at the hatchery resulted in their low survival. It is possible, however, that their habitat requirements differ from those of *V. iris* and were not met in the Flow-Through Pond system.

Comparison of *Villosa iris* Cultured with and without Fine Silt

For both treatments, survival and growth of the *V. iris* compares favorably to those of other culture studies (Table 2.11.). Over the entire culture period, juveniles provided with fine silt exhibited higher survival rates than those cultured without silt. However, a limitation of this analysis was that survival data were treated as independent data points because covariance could not be modeled. The potential benefits of silt are not fully understood, but may have include nutritional value or usefulness as physical substrate. However, growth was adequate and did not differ between the two treatments, suggesting that juveniles in both treatments were doing equally well. In laboratory culture, when an adequate food source or substrate for rearing juveniles of *V.iris* is not provided, juveniles cease to grow beyond a mean length of approximately 0.45 mm (Gatenby 1994). Juveniles in both treatments exceeded this length by age 21 d, indicating that the nutritional needs and habitat requirements of juveniles in both treatments were being met. Furthermore, comparison of survival rates between the two treatments on the final sampling date did not show a statistical difference between the two treatments.

Table 2.11. Summary of the most successful growth and survival rates reported in juvenile freshwater mussel culture studies.

Species	Age at conclusion of study (days)	Mean % Survival	Mean Length (mm)	Source
V.iris	91 - 94	49.8	1.81	This study
V.iris	91 - 94	32.9	1.78	This study
L. fasciola	83 - 88	6.3	1.78	This study
L. fasciola	122	50	1.76	Hanlon 2000
L. fasciola	200	41.2	1.59	Hanlon 2000
L. fasciola	90	82.0	2.23	Hanlon 2000
L. fasciola	166	74.6	2.28	Hanlon 2000
V. iris	115	27.5	2.10	Beaty 1999
L. fasciola	105	47.3	2.13	Steg 1998
V. iris	154	26.8	2.7	O'Beirn *et al* . 1998
U. imbecilis	91	7.0	18.9	Starkey *et al* . 1998
L. cardium	120	3.5	18.2	Westbrook and Layzer 1998
V. iris	140	30.0	1.80	Gatenby *et al.* 1997

This study was originally designed as a GBRD, with the silt and no-silt treatments to be compared for both *L. fasciola* and *V. iris*. However, failure to produce enough *L. fasciola* to complete the design prevented inclusion of this species in the experiment. As a result, the two treatments were only compared for *V. iris*. Considering the low survival of *L. fasciola*, all of which were provided with fine silt, it is likely that the inclusion of *L. fasciola* would have affected the survival comparison between the silt/ no-silt treatments. Without the completion of the study design to increase statistical power, and considering the lack of difference for the final sampling date and the equivalent growth rates, I don't think there is enough evidence to conclude that supplemental silt had benefits to survival. Given that the addition of silt represents an added labor component and prohibits a self-contained operation where all materials for culture could be maintained on-site, I would suggest further culture attempts using the quarried-sand/ river-sediment mixed-particle size substrate without the addition of supplemental silt. Furthermore, over the course of the culture period, some silt and organic matter settled out of the water column into the no-silt treatment containers. I believe the good growth and fairly good survival of juveniles in the no-silt treatment suggest that sufficient nutritional and substrate materials may be provided from within the Flow-Through Pond.

Suitability of the Flow-Through Pond System at WSSNFH As a Culture Environment for Juvenile Freshwater Mussels

Overall, I believe that the Flow-Through Pond at the hatchery is a promising potential environment for future culture of juvenile freshwater mussels. Mean survival and length data from the most successful culture attempts of previous researchers are summarized in Table 2.11. For both species in this study, mean shell lengths compare favorably to those of previous findings. Two studies at Buller Hatchery (Hanlon 2000) show mean lengths less than those observed in this study, and those lengths were obtained for older juveniles. Hanlon's most successful study resulted in a mean shell length of 2.23 mm at 90 days, which surpasses the growth of juveniles in this study. However, it is noteworthy that the largest individuals in this study (3.59 mm) were nearly

as large as the largest juveniles seen by Hanlon (4.13 mm), and the difference in mean length is largely accounted for by the fact that a narrower size range was observed by Hanlon (2.71 – 4.13 mm at 90 d) than in this study (0.75 – 3.59 mm at 83 – 94 d). In a laboratory culture environment, Steg observed a similar range of sizes (1.4 - 4.5 mm at 105 d) to that seen in this study. This great variation in lengths seems to suggest differential exploitation of resources within the container environment, and could ultimately translate to lower long-term survival rates among highly variable batches of released juveniles, as it is known that accumulated energy stores are vital in ensuring over-winter survival.

Failure to reach 0.48 mm in 6 weeks (42 d) has been cited as a benchmark below which juvenile mussels are unfit for over-winter survival (Hanlon 2000, Beck 2001). Even the smaller juveniles in this study easily surpassed this benchmark. Among juveniles of *V. iris* at 34 d, the smallest measured shell length was 0.50 mm and mean shell lengths were 0.77 mm (no silt) and 0.80 mm (silt). Among *L. fasciola* at 44 d, the smallest measured shell length was 0.50 mm and mean shell length was 0.80 mm. Thus, individuals in this study achieved lengths adequate for survival upon release in the wild, and larger individuals grew to lengths comparable to the largest sizes documented for these species in previous studies.

Survival rates in this study also bode well for the use of the Flow-Through Pond at the hatchery as a culture environment. Although the mean survival rates do not reach those achieved by at Buller Hatchery (Hanlon 2000), they do compare favorably to other survival rates (Table 2.11). Given that survival as high as 66.4% at 90 days was seen in one container, it appears that the potential exists to successfully recruit juveniles, especially if a greater understanding of the microhabitat factors that lead to variability in survival can be achieved.

RECOMMENDATIONS FOR FUTURE JUVENILE MUSSEL CULTURE IN THE FLOW-THROUGH POND

- Culture trials should be undertaken with additional species.
- A means to control blooms of filamentous algae must be found. Possibilities include increasing flow or introducing herbivorous fish.
- Effects of flow rate on survival and growth of juveniles should be undertaken by experimenting with the placement of containers in relation to the PVC water outlet manifold.
- The temperature regime of the Flow-Through Pond should be monitored from early spring onward to determine when mean water temperatures reach 15°C. Juvenile mussels should be released as soon as this temperature threshold is met, in order to allow maximum growth by the end of the summer growing season.
- Although it may not be feasible to control the microhabitat factors that result in variability in survival rates among containers, any studies which would illuminate the role and importance of these factors would be useful.
- Different methods of analyzing the nutritional content of the water would be of interest. This would include analysis of chlorophyll content or nutrient analysis of all organics extracted from water samples.
- Further investigation of the potential beneficial effects of silt is merited. This would include additional comparison of juveniles cultured with and without silt and, if benefits are demonstrated, further analysis of the silt's components, including organic content, mineral make up, and microbial communities.

Chapter 3: An Evaluation of a California Raceway at White Sulphur Springs National Fish Hatchery for its Potential Use in Rearing Juvenile Mussels

ABSTRACT

A California raceway at White Sulphur Springs National Fish Hatchery in West Virginia was evaluated for its suitability to culture juvenile freshwater mussels. Juveniles of *Actinonaias ligamentina*, *Lampsils fasciola*, and *Villosa iris* were released in the raceway, and their growth and survival were evaluated. Throughout the study, water quality parameters remained within ranges suitable for juvenile survival. Planktonic algae densities in the raceway ranged from 1107 to 6298 cells/ mL. A total of 40 genera were identified, and algal diversity in a single sample ranged from 13 - 27 genera. For juveniles of all three mussel species, survival decreased sharply within the first two weeks after release and eventually declined to less than 5%. Shell lengths of all species showed little growth. A sub-optimum temperature regime seemingly prevented successful culture of juveniles in the California raceway.

INTRODUCTION

For the reasons described in Chapter 2, the facilities at the hatchery were investigated as a potential site for the culture of juvenile freshwater mussels. Prior to the studies undertaken in the flow-through pond system during the summer of 2001, culture of juvenile mussels was attempted in a California raceway during the summer, fall, and winter of 2000. The California raceway receives effluent water from raceways used for rainbow trout culture. One goal of the project was to attempt to utilize the nutrients in the effluent to support an algal community that could be used a food source by mussels held in the raceway. Thus, nutritional requirements would be met, and the algae and mussels would serve as biofilters, lowering the nutrient levels in the hatchery effluent before it was released into an adjacent stream. The growth and survival of juvenile mussels released into the raceway was evaluated to assess the suitability of the raceway for rearing juveniles. Two sub-objectives of the project were to determine whether the age

of *Actinonaias ligamentina* upon release in the raceway affects growth and survival and to compare growth and survival between different species. As with the study in the flow-through pond system, conditions within the facility and modifications to the raceway were documented.

METHODS

Establishing Appropriate Conditions for Growing Juvenile Mussels in the Raceway

In cooperation with the US Fish and Wildlife Service, a California raceway located at the White Sulphur Springs National Fish Hatchery in White Sulphur Springs, West Virginia was used for this study. The raceway is earthen-bottomed and divided into 3 compartments. Each compartment is approximately 5.5 m wide by 20.7 m long and shallow (maximum depth of 39 cm at the center with gently sloped sides). Hatchery water is supplied from a spring located on the premises. The spring water is fed into indoor raceways used for holding rainbow trout, and then it enters the outdoor California raceway (Figure 3.1.) The 2nd and 3rd compartments of the raceway were excavated so that a uniform depth of approximately 39 cm was created across the raceway. In the 3rd raceway compartment, a fine river gravel / limestone sand mix was added to a depth of 10 cm. This substrate allowed partial burial of the containers used for holding juveniles to maximize the flow passing through them. The limestone content of the gravel supplemented the hardness of the water, which is required by mussels for shell growth (Steg 1998).

Using the raceway stop boards, the 1st compartment of the raceway was impounded and the volume of flow was reduced to maximize water depth and retention time in this compartment. I hoped to sustain a retention time adequate to elevate the water temperatures to greater than 15ºC and to allow algae species to grow. A fertilization regime also was applied to this pool to create a balance of nutrients that would foster the growth of unicellular algae as a food source for mussels held downstream in the raceway. Two additional measures were taken to increase temperatures in the raceway: on August 1st, flow was decreased to the lowest level possible (95 L/ minute), and, on August 12th, a solar swimming pool cover was placed on the first raceway compartment.

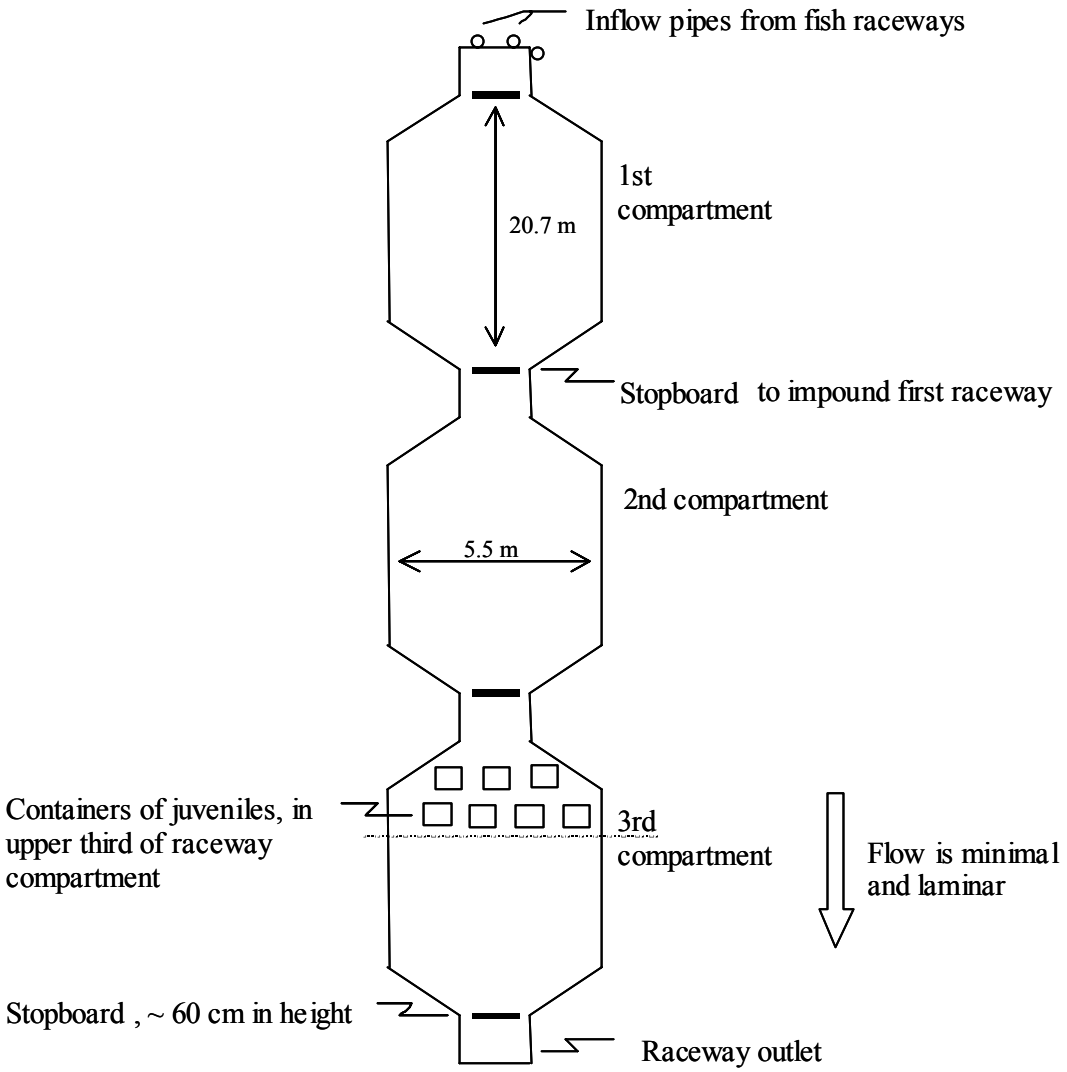


Figure 3.1. California Raceway at the hatchery.

Every two weeks, water chemistry analyses were conducted for hardness, alkalinity, pH, dissolved oxygen, orthophosphate, nitrate, nitrite, and ammonia, using the methods described in Chapter 2. Water samples were taken at three locations in the raceway: at the inflow to the 1st raceway compartment, at the outlet of the 1st raceway compartment (the algae impoundment), and at the outlet of the 3rd raceway compartment. Comparison of water quality at these three locations allowed an assessment of the extent to which the algae and mussels altered water quality and served as biological filters by taking up nutrients. For phytoplankton enumeration, water samples from all three raceway compartments were immediately preserved with 10 mL of acid Lugol's fixative (Saraceni and Ruggio 1969). According to the Utermohl technique, a 100 mL settling chamber was then used to concentrate the algae in the water sample. The algae was allowed to settle onto a slide for 48 hours, and then was identified and ennumerated using an inverted microscope (Vollenweider 1969). Genera were identified according to a dichotomous key ( ) and counted by transect, and a conversion formula calibrated to the microscope was used to calculate the cell density in the sample from the number of cells on the transects. Filamentous algae observed in the pond was also collected into small plastic bags and refrigerated until examined and identified with a standard binocular microscope at 400X magnification. Hobo™ (Onset Corporation) temperature loggers placed 1) where water entered the 1st raceway compartment from the fish raceways and 2) at the outlet of the 3rd raceway compartment provided a continuous record of water temperature.

Evaluation of Growth and Survival of Different Species Released in the Raceway and Comparison of *Actinonaias ligamentina* Released at Two Different Ages

Juveniles of the species *Villosa iris* and *Lampsilis fasciola* were propagated at the Virginia Tech Aquaculture Center as described in Chapter 2. Methods for propagation of juvenile *Actinonais ligamentina* were the same as for the other two species, except that the 10 gravid adult mussels were obtained from the Greenbrier River in West Virginia,

and the largemouth bass used as host fish were purchased from a hatchery as approximately 10 cm long fingerlings.

Juveniles were transferred to the hatchery as described in Chapter 2. At the hatchery, juveniles were confined in plastic containers (200 juveniles/ container) located in the upper third of the third compartment of the raceway (Figure 3.1). Conditions throughout the upper third of the compartment were assumed to be uniform. All containers held a substrate of 0.5 cm of limestone sand (particle size between 1000 μm – 3000 μm).

In June of 2000, a total of 3,800 juveniles of *A. ligamentina* were released in the raceway. Half of these were released at an average age of 1.5 weeks, while the other half were cultured in the greenhouses at the Virginia Tech Aquaculture Center until they reached an average age of 3.5 weeks. The cultured juveniles were held in large batches in plastic containers in a recirculating aquaculture system and were fed daily with *Neochloris* and *Scenedesmus* spp. from algal cultures. In July of 2000, a total of 1,200 *L. fasciola* were released in the raceway, at an average age of 1.5 weeks. In December of 2000, 2000 *V. iris* were released at an average age of 1 week.

In order to sample growth and survival, juveniles were collected by separating them from the substrate with a series of sieves, in the same manner described in Chapter 2. When a container was sampled, all located live juveniles were counted and a subsample of 10 juveniles was measured. The sampling schedule for the containers was adjusted as additional species were placed in the raceway and as survival declined to levels where sampling was no longer worthwhile (once survival dropped below 5%, a container was no longer sampled). Sampling frequency for a given treatment or species ranged from every 2.5 to 3 weeks, with sampling of containers staggered so that all containers were sampled at least twice, and no container was sampled at two sampling events in a row.

RESULTS

Water Quality and Temperature

All water quality parameters remained within ranges suitable for juvenile survival. No substantial differences were observed between the raceway compartments,

with similar values observed for all three compartments on each sampling date (Table 3.1.). Daily mean temperatures in the California raceway ranged from 9°C - 18°C (Figure 3.2). Retention time in the first two compartments served to raise water temperatures between 1 to 5°C by the time water reached the third compartment where juveniles were held, but mean daily temperatures generally remained below 15 °C. Neither the reduction of flow to 95 L/ minute nor the installation of the solar pool cover served to appreciably increase water temperatures.

Algae Production

Algae were identified and enumerated in water samples collected on 7 sampling dates. A total of 40 genera were identified, with a diversity of between 13 and 27 genera observed in each sample. Complete data on cell counts in each sample are summarized in Appendix D. Calculated cell densities in the planktonic algae samples ranged from 1107 to 6298 cells/ mL. Consistent differences in cell densities or community structure were not observed between the three compartments. Mean cell densities for a given sampling date (averaged for the three compartments) remained well below the target density of 10,000 cells/ mL (Figure 3.3). The genera predominant throughout all samples consisted of 2 blue-green algae (*Anabaena* and *Oscillatoria*), 3 green algae (*Chlorella, Chlorococcum,* and *Scenedesmus*), 5 diatoms (*Cyclotella*, *Navicula*, pennate diatoms, *Synedra*, and *Triceratium*), and 1 cryptophyte (*Chroomonas*).

Growth and Survival

For all species, survival decreased sharply by the first sampling event and eventually declined to less than 5% (Table 3.2. and Figure 3.4). Survival trends did not differ between the *A. ligamentina* released at different ages. No live juveniles of *V. iris* from the December release were relocated at any time. Growth measurements were terminated after the first sampling periods, because shell lengths of all species were indistinguishable from the shell lengths at the start of the experiment. Live individuals continued to pass through the 300μm sieve, so it is unlikely that any individuals exceeded 0.35 or 0.4 mm in length. Qualitative observations of the juveniles also indicated

Table 3.1. Range of water quality parameters recorded in the California Raceway (N = 8 - 12 for different parameters).

Parameter	Observed Range
pH	7.6 - 8.4
Dissolved Oxygen (mg/L)	7.6 - 16.4
Hardness (mg/L)	120 - 360
Alkalinity (mg/L)	60 - 120
Nitrate (mg NO_3-N /L)	0.06 - 0.11
Nitrite (mg NO_2-N/L)	(undetectable - 0.084)
Orthophosphate (mg PO_4-P/L)	0.013 - 0.09
Total Ammonia [mg (NH_3-N + NH_4-N/L]	0.139 - 0.375
Unionized Ammonia (mg NH_3-N/L)	0.0007 - 0.013

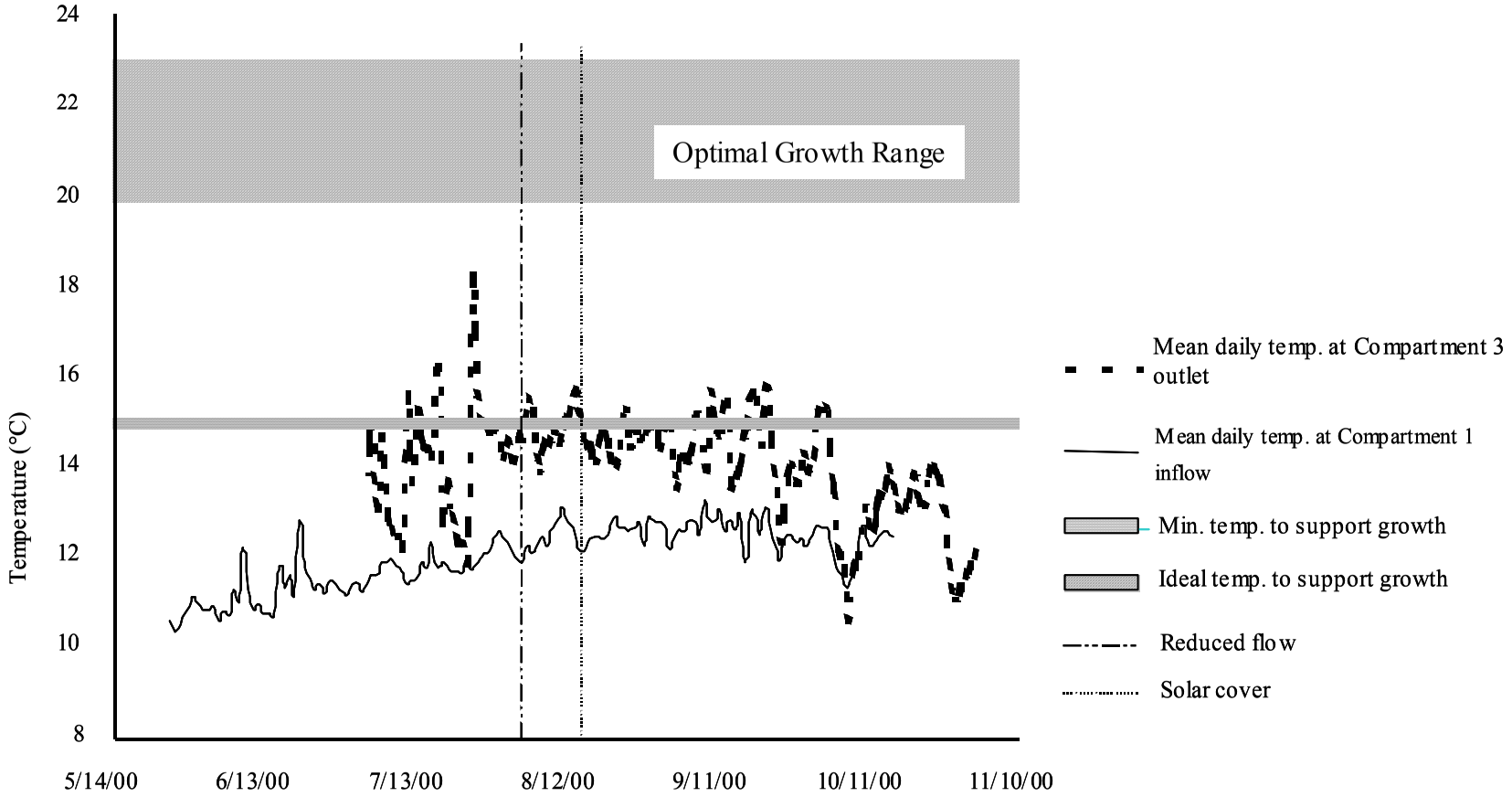


Figure 3.2. Thermograph of mean daily temperature in the California Raceway as compared to suitable temperatures for rearing juvenile mussels.

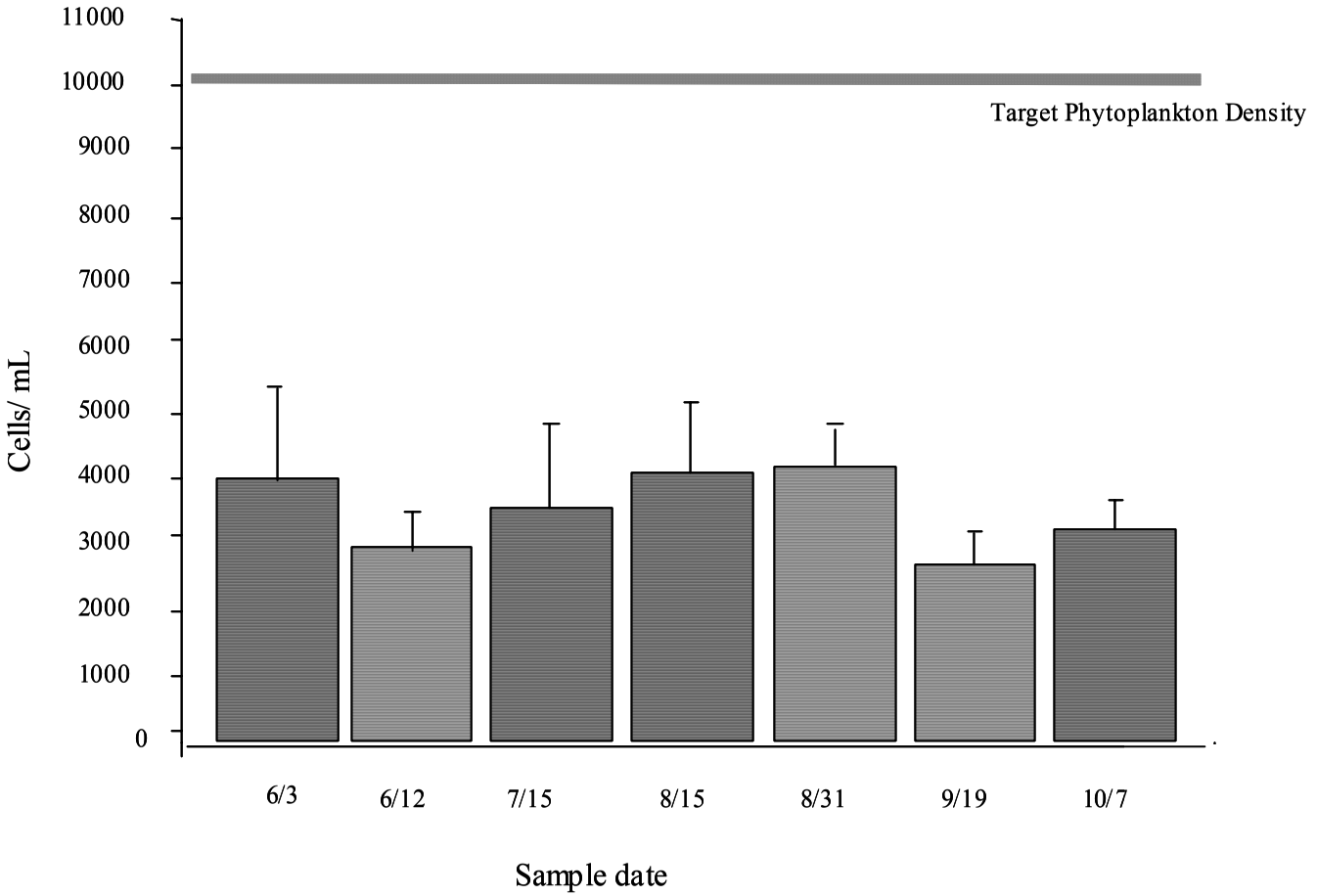


Figure 3.3. Mean densities (± SD) of phytoplankton in the three California Raceway compartments for each sample date.

Table 3.2. Mean survival (%) for juvenile mussels released in the California Raceway.

Species	Age at sampling (weeks)			
	Mean Survival (%)			
A. ligamentina , released at	4.5 wk	7.5 wk	10.5 wk	—
age 1.5 wk	14.5%	18.5%	1.0%	—
A. ligamentina , released at	—	7 wk	9 wk	12 wk
age 4.5 wk	—	14.8%	9.5%	0%
L. fasciola, released at	3.5 wk	7 wk	10 wk	12.5 wk
age 1 wk	4.5%	3.5%	11.0%	3.7%

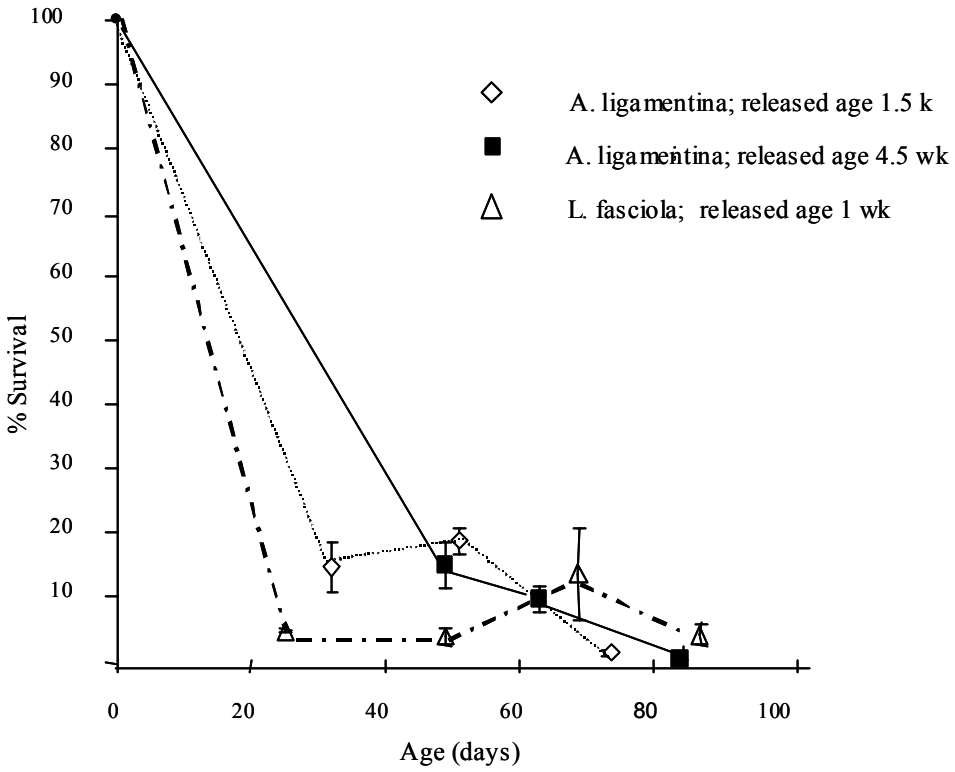


Figure 3.4. Mean survival (±SD) of juvenile mussels released in the California Raceway.

that their condition was poor. Rarely were sampled individuals seen to be active or to have gut coloration.

DISCUSSION

The fact that algal densities and water chemistry measurements did not differ appreciably between the three raceway compartments suggests that the algae and mussels were not affecting nutrient levels. Algal densities were insufficient to take up substantial amounts of nutrients. The failure of phytoplankton to reach target densities was probably related to several factors. First, the temperature regime was less than optimal. Second, retention time in the raceway was less than 24 h, even with flow reduced to the lowest level possible. A retention time of at least 3 – 4 days would have been required to allow algae to complete their life cycles and become established (B. Parker, Ph.D., VPI and SU, pers. comm. 2001). Furthermore, nutrients supplied by fertilization were quickly flushed through the system. Third, filamentous algae became established in the raceway, most likely out-competing the unicellular forms.

Although no phytoplankton samples reached the target density of 10,000 cells/mL, it is unlikely that this was the primary factor responsible for limiting growth and survival. Algal density and diversity were comparable to levels found in the flow-through pond system during the 2001 study, and relatively good growth and survival were achieved in the pond study. Furthermore, the algal genera observed in the raceways, including diatoms and other groups, are thought to have nutritional value for juvenile mussels.

Despite initial concerns that fish waste and feed residue would elevate nutrients to detrimental levels, water chemistry parameters all remained within a suitable range for juvenile survival. Total ammonia levels were higher than values typically recorded in rivers that support mussel communities. (Data on documented environmental ammonia levels is presented in Chapter 4). However, because of low temperatures, fractions occurring as unionized ammonia were low, with only one out of 36 calculated NH_3-N values exceeding the 0.01mg/L estimated safe environmental threshold for juvenile mussels (Chapter 4). It is noteworthy, however, that if the desired higher temperature regime (20 – 25°C) is used to project unionized ammonia levels, 8 of the 36 values would

have exceeded the estimated safe environmental threshold, and further consideration of the stress posed by unionized ammonia would be warranted. Under the existing conditions, however, it is unlikely that any of the measured water quality parameters were responsible for limiting growth or survival.

It is probable that the sub-optimum temperature regime was the primary factor inhibiting successful culture of juveniles in the California raceway. Other researchers have documented little growth in juvenile mussels when mean temperatures are below 15°C, and comparisons of survival between juvenile released in colder months and warmer months have shown lower long-term survival for the colder season releases (Beaty 1999, Hanlon 2000). It has been suggested that the first 30 days post-metamorphosis are a crucial period in which juveniles must obtain nourishment and put on growth if they are to ultimately exhibit good survival (Hanlon 2000). It is likely that the juveniles released in the California raceway responded to the low temperatures with reduced activity and slowed metabolism, and as a result were unable to adequately feed and grow. In assessing the feasibility of potential culture locations for juvenile freshwater mussels, the environment's temperature regime is an important consideration.

Chapter 4: Sensitivity of Juveniles of Two Species of Freshwater Mussels to Total and Unionized Ammonia

ABSTRACT

The sensitivities of <5 d old juvenile *Villosa iris* and *Lampsils fasciola* to total and unionized ammonia were assessed over a 96 h test period, in static-renewal conditions. Juveniles were exposed to five concentrations of ammonium chloride at 12°C and 20°C, and mortality and water chemistry parameters were monitored at 24 h intervals. LC_{50} values at 24, 48, 72, and 96 h were calculated using the Trimmed Spearman-Karber method. At 96 h, significant differences in sensitivity between the two temperatures were not observed for either species. Differences in tolerance, however, occurred between the two species, with *L. fasciola* (mean 96 h LC_{50} of 0.26 mg/L NH_3-N) being more tolerant of unionized ammonia than *V. iris* (mean 96 h LC_{50} of 0.11 mg/L NH_3-N). LC_{50}'s of these two species were comparable to values reported for organisms typically used to set water quality standards. The only organisms with reported LC_{50} values lower than those seen for *V. iris* juveniles were *Ceriodaphnia dubia* and *Hyella azteca*. Based on documented levels of ammonia in some rivers from anthropogenic sources, ammonia may limit freshwater mussel populations at affected sites. Results of these bioassays were used to assess the suitability of ammonia levels in facilities at White Sulphur Springs National Fish Hatchery for captive culture of juvenile mussels.

INTRODUCTION

Freshwater mussel populations have undergone serious declines in recent decades, with over 70% of North America's mussel species considered either endangered, threatened, or of special concern (Williams *et al.* 1993). Declines are attributable to many factors including habitat alteration, competition with exotic species, such as the Asian clam (*Corbicula fluminea*) and zebra mussel (*Dreissena polymorpha*), siltation, and degraded water quality from point sources such as industrial sites and non-point

sources such as agriculture. The extent to which each of these factors contributes to the loss of mussel fauna is often uncertain and varies on a site by site basis (Keller and Zam 1991).

Existing documentation of mussel sensitivities to various contaminants is incomplete. Potentially harmful pollutants include heavy metals, pesticides, complex industrial effluents, and organic waste (Keller and Zam 1991, Jacobsen *et al.* 1993, Johnson *et al.* 1993, Keller 1993, Keller and Ruessler 1997). Ammonia is one contaminant of concern that lacks sufficient data to facilitate conservation efforts (Wade *et al.* 1992). These data are essential in order for regulators to set water quality standards protective of mussels and to identify sites where ammonia levels may limit survival or reproduction. Freshwater bivalves are not currently used as a bioassay organism to set water quality criteria for ammonia or to regulate sewage treatment plant discharges (US EPA 1984, Goudreau *et al.* 1993). Furthermore, mussel sensitivities have not been adequately characterized to determine whether standard test species are sensitive enough to effect water quality standards protective of mussel taxa. Additionally, because captive propagation of mussels has frequently been recommended as a conservation strategy (National Native Mussel Conservation Committee 1998), knowledge of their water quality requirements is necessary both in maintaining suitable conditions in captive culture systems as well as in choosing sites with appropriate water quality for the ultimate release of cultured juveniles.

Ammonia occurs naturally in freshwater environments, with concentrations in unpolluted surface waters ranging from 0.05 – 0.4 mg/L (Goudreau *et al.* 1993). Ammonia enters freshwater systems as a product of organic decomposition and a waste product of aquatic organisms (Huey and Beitinger 1980, Goudreau *et al.* 1993). In the presence of oxygen, nitrifying bacteria convert ammonia to nitrite and nitrate. Aqueous ammonia occurs in two forms, ionized (NH_4^+-N) and unionized (NH_3-N). The proportion occurring in each form is dependent on temperature and pH (Emerson *et al.* 1975). Ammonia in its ionized form generally is relatively benign. The unionized form, however, can have substantial toxic effects to aquatic organisms (US EPA 1984, Arthur *et al.* 1987).

Anthropogenic activity can elevate ammonia concentrations in freshwater environments. Sources of ammonia include sewage treatment plants, food processing plants, oil shale retorting, coal gasification, and industrial effluents (Huey and Beitinger 1980, Kumar and Krishnamoorthi 1983, Chetty and Indira 1995, Scheller 1997). Ammonia is a common chemical intermediate in a number of industrial processes, including refrigeration, metals extraction, and detergent production (Summers 1998). Agriculture also accounts for ammonia inputs, with sources including animal feed-lot runoff, by-products from the manufacture of fertilizer, and surface runoff of fertilizer (Summers 1998). Fertilizer manufacturing constitutes 85% of the world's ammonia production (Summers 1998), and fertilizer application rates have increased tremendously in recent decades, as in the period between 1970 and 1981, during which application rates increase by 68% (Neves 1993). Aside from direct ammonia inputs, any factors causing a disruption of bacterial populations may also trigger in situ increases (Huey and Beitinger 1980).

Ammonia levels also can be elevated in aquaculture settings, such as ponds, recirculating systems, or laboratory holding tanks, as a result of excretion and bacterial action on waste and uneaten food (Huey and Beitinger 1980, Rouse *et al.* 1995). Therefore, ammonia levels are a concern in systems used to propagate juvenile mussels for conservation purposes, particularly in instances where the culture environment is shared with high densities of fish. One instance would be in holding tanks where juveniles undergo transformation on high densities of host fish. Other instances may occur at existing fish hatcheries adapting facilities for mussel culture projects. For example, the Virginia Tech Department of Fisheries and Wildlife Sciences is currently collaborating with the US Fish and Wildlife Service to raise juvenile freshwater mussels at White Sulphur Springs National Fish Hatchery, in White Sulphur Springs, West Virginia. One objective of the project has been to evaluate the potential for raising juvenile mussels in effluent water used to hold rainbow trout. Elevated ammonia concentrations were anticipated in the effluent water as a result of fish waste and feed. Uncertainty as to whether these ammonia levels would be prohibitive for the survival of juveniles was the impetus to undertake this study of their sensitivity to ammonia.

Toxicity of ammonia to fish has been fairly well studied. Toxic effects include gill damage (Adams and Bealing 1994), changes in feeding behavior and excretion rate, lowered blood pH resulting in reduced oxygen carrying capacity (Scheller 1997), and increased oxygen consumption by tissues (Rouse *et al.* 1995). A number of water quality characteristics have been shown to influence the toxicity of ammonia, including dissolved oxygen content, carbon dioxide levels, hardness, and salinity (Kumar and Krishnamoorthi 1983, Adams and Bealing 1994, Summers 1998, Hickey and Martin 1999). Additionally, beyond the role of pH and temperature in determining the fraction of unionized ammonia, these two parameters can also influence the toxicity of unionized ammonia (Kumar and Krishnamoorthi 1983, Adams and Bealing 1994, Summers 1998).

In considering ammonia's impact to freshwater systems, the toxicity of ammonia to invertebrates has been named as an area of research where data are particularly lacking (Adams and Bealing 1994). Ammonia toxicity appears to vary widely among invertebrate taxa (Williams *et al.* 1986, Arthur *et al.* 1987, Besser *et al.* 1998). Uncertainty exists as to whether the pattern of toxicity resulting almost exclusively from the unionized form holds among invertebrates (Williams *et al.* 1986) and as to mechanisms responsible for toxicity (Scheller 1997, Besser *et al.* 1998).

A number of harmful effects have been documented in freshwater and marine bivalves exposed to ammonia solutions, including reduction in the amount of time valves are held open for respiration and feeding (Epifanio and Srna 1975); impaired secretion of the byssus thread (Reddy and Menon 1979); reduced ciliary action (Anderson *et al.* 1978, US EPA 1984); depleted lipid, glycogen and other carbohydrate stores, and altered metabolism (Chetty and Indira 1995); as well as acute lethal toxicity (Goudreau *et al.* 1993, Scheller 1997, Summers 1998).

Some studies have demonstrated relationships between temperature and the toxicity of unionized ammonia. In fish, increased toxicity of unionized ammonia has been documented at lower temperatures (Burrows 1964, Erickson 1985, Summers 1998). This pattern has been reported at temperatures below 10ºC (Arthur *et al.* 1987, Summers 1998), and especially at spawning time (Adams and Bealing 1994). Other studies on fish report no observed relationship between temperature and unionized ammonia toxicity (Thurston 1980, Erickson 1985, Arthur *et al.* 1987). Contrary to the pattern of increased

toxicity at lower temperatures in fish, a review of toxicity studies on Asian clams, *Corbicula* spp., showed that temperatures above 20ºC typically result in toxicants (including unionized ammonia) acting at lower levels, possibly because of increased metabolism promoting uptake of the biocide (Doherty and Cherry 1988). Among invertebrates in general, temperature relationships with unionized ammonia are not widely known (Shubauer-Berigan *et al.* 1995, Hickey and Martin 1999). It has been speculated that the absence of temperature-related sensitivity response in the fingernail clam *Sphaerium novaezelandiae* indicates that mechanism of toxicity in this bivalve may differ from the mode of action in fish, as may be the case for other invertebrates in which sensitivity to unionized ammonia is not affected by temperature (Hickey and Martin 1999). Such varying observations indicate the need for study of ammonia toxicity to invertebrates over a range of temperatures (Hickey and Martin 1999).

The first objective of this study was to evaluate and compare the sensitivity of juveniles of two freshwater mussel species to unionized and ionized ammonia. An organism's early life stages are often the most sensitive to toxicants (ASTM 1980a, Buikema *et al.* 1982), and the success of glochidia and juveniles is critical to the survival of mussel species (Johnson *et al.* 1993). Additionally, conducting toxicity tests with adult mussels can be impractical, because they can close their valves for extended time periods, thus avoiding the toxicant (Goudreau *et al.* 1993). For these reasons, and because data were to be applied to juvenile mussel culture work, bioassays were conducted with juvenile mussels. The two species selected for testing, wavy-rayed lampmussel *Lampsilis fasciola,* and rainbow mussel *Villosa iris,* are widely distributed species in Virginia and also were being used to evaluate facilities at White Sulphur Springs National Fish Hatchery for their potential use in captive culture of mussels. The juveniles' sensitivity was also evaluated at two temperatures. Sensitivity at 12ºC versus at 20ºC was of interest because preliminary culture work at White Sulphur Springs had been done at temperatures averaging less than 15ºC, but future culture work was planned for temperatures above 20ºC, because lower temperatures were insufficient to support mussel growth. Furthermore, as noted above, previous researchers have cited the need for investigation of temperature effects on ammonia toxicity to invertebrates (Hickey and Martin 1999).

The second objective of this research was to evaluate how the sensitivity of juvenile freshwater mussels compares with the sensitivity of other aquatic organisms, including other mollusks and bivalves, known sensitive species, and standard organisms used in toxicity testing. The acute bioassay results were also used to estimate safe environmental levels of NH_3-N for juveniles of these two species. The final objective of this research was to compare both the estimated safe levels and acutely toxic levels of NH_3-N with regulatory standards, with existing data on ambient levels in three Virginia basins, and with concentrations reported at sewage treatment plant outfalls.

METHODS

Juvenile Mussel Bioassays

Gravid adult female *V. iris* were collected from Indian Creek, Tazewell County, Virginia and *L. fasciola* were collected from the Clinch River, Russell County, Virginia. Glochidia were flushed from the gills using a water filled syringe, as described in Zale and Neves (1982), except that the gravid mussels were not sacrificed. Largemouth bass (*Micropterus salmoides*) and smallmouth bass (*Micropterus dolomieu*) collected by electrofishing from the New River in Montgomery County, Virginia, and rock bass (*Ambolplites rupestris*) collected from Tom's Creek in Montgomery County, Virginia, served as host fish for *L. fasciola* and *V. iris*, respectively. Host fish were exposed to the glochidia in water-filled, aerated buckets. Once infestation of the host fish gills was observed, fish were transferred to tanks where they were held until glochidia transformed into juvenile mussels and excysted. The small size and fragility of juvenile mussels (approximately 0.2 mm) makes handling and recovery difficult. Therefore, the procedures used in our study to facilitate handling are documented in detail.

Upon transformation, juveniles were siphoned from the host fish tanks and transferred to plastic containers of a 1: 1 mixture of dechlorinated Blacksburg municipal water combined with well water. Containers of juveniles were placed in an insulated cooler in a walk-in environmental chamber, with controlled temperature and photoperiod. Containers of juveniles remained in the insulated cooler for 24 h, and holding water temperature was gradually equilibrated with chamber temperature. The municipal-well

water mixture was replaced with spring-water from White Sulphur Springs in 20% increments in order to acclimate the juveniles to the dilution water (ASTM 1980a).

Dilution and control water for the toxicant exposures was obtained from an on-site spring at White Sulphur Springs National Fish Hatchery and gravity filtered through a 30 μm seive to remove potential predators. A primary ammonia stock solution of 1000mg/L was prepared from reagent grade ammonium chloride (NH_4Cl) that was oven dried at 105ºC for several hours. This primary stock was sealed in an air-tight glass container and refrigerated for use over a two month period. Dilution concentrations were prepared from the primary stock at the outset of each 96 h trial, and were then refrigerated in air-tight containers and warmed in a warm water bath to ambient test temperatures prior to each daily solution renewal.

The maximum exposure concentration for both species was approximately 0.6 mg NH_3-N/L, and a 50% dilution series was used. Five exposure concentrations were tested, with four replicates at each concentration. In order to prepare a 0.6 mg NH_3 -N/L solution, the pH of the dilution water and temperature of the environmental chamber were used to calculate the expected fraction of NH_3 -N in a NH_4Cl solution using the formulas of Emerson *et al.* (1975).

Tests were conducted under static-renewal conditions in the environmental control chamber (Johnson 1990). A light:dark photoperiod of 16 h:8 h was maintained through all trials (ASTM 1980a, Johnson 1990). For both species, temperature was set at 12 ± 1°C for one 96 h test and at 20 ± 1°C for a second test. Fifty mL beakers containing 40 mL of toxicant or control solution were used as test chambers. Ten juvenile mussels were pipetted into each beaker (ASTM 1980a, Johnson *et al.* 1993). To avoid the generation of waste products and to simplify the protocol, juveniles were not fed during the duration of the test (Keller and Zam 1991, Johnson *et al.* 1993, Scheller 1997). Beakers were covered with plastic wrap to prevent evaporation and arranged according to random number assignments (ASTM 1980b). Solutions were renewed every 24 h.

Water quality and ammonia concentrations were monitored throughout each 96 h test. Ammonia concentrations were measured with an Orion ion-selective probe and an Orion model 290A pH meter in 30 mL aliquots of solution decanted either from the newly prepared toxicant solutions or decanted from test beakers (as described below) at

the end of each 24 h period. The Orion model 290A meter also was used to measure pH directly in the test beakers. Dissolved oxygen, conductivity, and temperature also were measured within the test beakers, using a YSI model 85 D.O. and conductivity meter. Alkalinity and hardness were measured in decanted solutions with HACH chemicals and prescribed methods, and nitrite levels were determined in decanted solutions with a La Motte test kit.

Temperature, pH and ammonia were measured in two replicates of all concentrations and the control at the start of each 24 h interval (fresh solutions) and end of each 24 h interval (old solutions). Dissolved oxygen and conductivity measurements were also taken at the start and end of each 24 h interval. For the first trial with *V. iris*, dissolved oxygen and conductivity were measured at the start and end of the 96 h trial in two replicates of all concentrations, and at the start and end of each 24 h interval in two replicates of the control, the lowest, the median, and the highest concentrations. As these parameters proved to remain within an acceptable range, for subsequent trials, the 24 h interval measurements were limited to the highest and lowest toxicant concentrations. Hardness, alkalinity and nitrites were measured in two replicates of all concentrations at the start and end of each 96 h trial.

Juveniles were examined for mortality at the 24 h, 48 h, 72 h and 96 h time points. After water quality measurements were made in the test beakers, the majority of solution was carefully decanted off, as not to pour out the juveniles, through a 130 micron Nitex sieve. Any juveniles accidentally poured from the beakers were caught in the sieve and were rinsed back into the test beakers using newly prepared toxicant solution of the appropriate concentration. Each beaker was placed under a bottom-lit dissecting microscope and juveniles were observed at 40 X magnification, which allowed internal anatomy to be viewed through their transparent valves. Juveniles were considered dead if they exhibited gaping shells or lack of pedal or internal movement after two minutes of observation (Wade *et al.* 1992, Jacobson *et al.* 1993, Warren 1996, Scheller 1997, Summers 1998).

Data were analyzed using the Environmental Protection Agency (EPA)'s CT-TOX program, and LC_{50} values and 95% confidence intervals were calculated using the Trimmed Spearman-Karber method (Hamilton *et al.* 1977). Using mortality observations

and ammonia concentration measurements for each 24 h interval, 24 h, 48 h, 72 h, and 96 h LC_{50} values were calculated, with the exception of the 20°C *L. fasciola* trial, when mortality numbers were too low to calculate an LC_{50} value until the 72 h interval. Calculations were based on mean values of total or unionized ammonia for a given interval. Differences in LC_{50} values were considered statistically significant ($p = 0.05$) if 95% confidence intervals did not overlap. Tests were considered valid only if control mortality did not exceed 20% (Summers 1998).

Relative Sensitivities

An extensive review of ammonia toxicity to fish and some aquatic invertebrates is provided by the US EPA document Water Quality Criteria for Ammonia (1984). The data compiled by the EPA include LC_{50} values that are derived from tests performed by multiple researchers, using different life stages and sizes, and conducted over a variety of temperatures and other water quality parameters. Subsequent to this EPA report, the toxicity of ammonia to aquatic invertebrates has received increasing study, and LC_{50}'s for a number of invertebrate species have been published. A review of this literature provided a comparison for the LC_{50}'s of juveniles of the two tested mussel species.

Estimated Safe Levels, Regulatory Standards, and Environmental Ammonia

One approach to estimate safe environmental levels of a contaminant from acute LC_{50} data was developed by the Pennsylvania Department of Environmental Protection in the 1980's (D. Cherry, Ph.D., VPI and SU, pers. comm. 2001). For the species of interest, the ratio of the concentration producing acute effects to the concentration producing chronic effects is calculated. This ratio is scaled to an application factor of either 0.1, 0.01, or 0.001, according to which value it most closely matches, and the application factor is then multiplied by the acute LC_{50} to predict a safe environmental level, or no effect concentration. Presently, however, there are no data on chronic effect levels of ammonia to juvenile freshwater mussels. Therefore, a surrogate species, the fingernail clam *Musculium transversum*, was chosen to calculate an expected acute: chronic ratio for freshwater unionids. *M. transversum* was an appropriate surrogate because it is a

filter-feeding freshwater bivalve with similar ecology to unionid mussels; however, a drawback to this surrogate species is that previous toxicity studies have shown *M. transversum* to be less sensitive to toxicants than unionid mussels. For *M. transversum*, chronic effect levels of 0.09 and 0.16 mg/L NH_3-N and acute effect levels of 0.93 and 1.29 mg/L NH_3-N were reported by Zischke & Arthur (1987). The ratio of mean chronic levels (0.125 mg/L) to mean acute levels (1.11 mg/L) produces a value of 0.1126, suggesting an application factor of 0.1 to be applied to acutely toxic concentrations.

The US EPA provides ammonia criteria scaled for specific pH and temperature intervals. Limits are given for a 1 h and a 96 h average concentrations of unionized ammonia not to be exceeded more than once every three years. The Virginia Department of Environmental Quality (VA DEQ) also sets ammonia standards according to pH and temperature, but provides chronic as well as acute standards, defined as 30 d and 1 h average concentrations of unionized ammonia not to be exceeded more than once every three years. Standards appropriate for the pH and temperature ranges used in this study were compared to the LC_{50}'s and estimated safe environmental concentrations for the two tested mussel species.

The VA DEQ maintains a network of ambient water quality monitoring stations located out of the vicinity of known point sources of pollution, in order to obtain typical background levels of the monitored parameters and pollutants. Samples were collected monthly. Total ammonia is measured as nitrogen using the automated phenate method on a Technicon autoanalyzer, with a detection limit of 0.04 mg/L. Temperature and pH were recorded at each sampling event, such that the unionized fraction of ammonia (NH_3-N) can be calculated.

Ambient ammonia data collected over the past five years in the Clinch, Powell, Holston, and North Fork Shenandoah drainages were reviewed to assess the suitability of unionized ammonia levels for freshwater mussels, according to sensitivity of juvenile mussels in this study. The Clinch, Powell, and Holston river basins in southwest Virginia and eastern Tennessee were chosen, because they harbor a diverse assemblage of freshwater mussels, including a number of rare and threatened species, and represent some of the best habitat still available for freshwater mussels. The North Fork of the Shenandoah River in the central Blue Ridge of Virginia hosts a less diverse group of

species, but does hold several species of state and federal concern, and is of interest because of the prevalence of agriculture and poultry farming in the watershed.

Permitted wastewater treatment plants (WTP's) also report total ammonia concentrations in their effluent to VA DEQ on a monthly basis, to verify compliance with established maximum limits. The most recent data available were obtained for the Cleveland WTP on the Clinch, the Jonesville WTP on the Powell, and the Saltville WTP on the North Fork Holston (VA DEQ 2001c). These sites were chosen on the basis of their importance as freshwater mussel habitat. The outfall at Cleveland is significant due to its proximity to a parcel of land recently purchased by The Nature Conservancy and intended as a protected site for mussel populations. The outfall at Jonesville is of interest because it is upstream of Fletcher Ford and Fletcher Cliffs, where a number of federally listed mussel species occur. Finally, WTP data from Saltville on the North Fork Holston River are significant to mussel conservation because this river reach is under consideration for restoration and reintroduction of mussels after long-term industrial inputs eliminated much of the aquatic fauna in the area.

WTP data are reported for total ammonia. Unionized ammonia levels were calculated with pH and temperature ranges obtained for the same time period from the VA DEQ ambient monitoring station closest to the outfall (VA DEQ 2001a), except in the case of the Saltville outfall, where only six data points (all from the summer of 2001) were available for the South, Middle and North Fork of the Holston, thus presenting very little range. Therefore, surrogate temperature and pH data from the Clinch River are applied to the Saltville total ammonia data.

In order to project a potential range of the unionized ammonia in the WTP effluent, the minimum recorded temperature and pH and the maximum temperature and pH values were used in the formulas of Emerson et al. (1975) to project the least and greatest fractions of NH_3-N that might have occurred under documented environmental conditions. For example, considering the pH and temperature ranges for the Clinch River at Cleveland (Table 4.9.), at 2.24°C and pH 7.37, 2.3 % of total ammonia is in its unionized form, while at 25.5° C and pH 8.61, 19.3% of total ammonia is in its unionized form (Emerson et al. 1975). Applying these least and greatest fractions to the mean reported total ammonia for a given outfall resulted in a potential range of unionized

ammonia concentrations. Applying these fractions to the minimum and maximum reported values for total ammonia represented an extreme case scenario in which either the minimum value for total ammonia would co-occur with the lowest temperature and pH, or, conversely, the maximum value would co-occur with the highest temperature and pH. Applying the greatest possible fraction of NH_3-N to the maximum reported total ammonia value provided a worst-case scenario for unionized ammonia.

RESULTS

Juvenile Mussel Bioassays

For both the 12ºC and 20ºC toxicity trials, *V. iris* and *L. fasciola* had concentration-dependent increases in the toxicity of total and unionized ammonia. All trials had acceptable control survival of greater than 80% (Table 4.1.). At each 24 h time point, *V. iris* increased in sensitivity to total ammonia as temperature increased from 12ºC to 20ºC, but had similar LC_{50} values for unionized ammonia (Table 4.2.). This same pattern occurred for *L. fasciola* at 96 h, but at 72 h, *L. fasciola* had decreased sensitive to both total and unionized ammonia at the higher temperature (Table 4.2.). Except for the anomalous pattern seen for *L. fasciola* at 72 h, these data indicate that the ammonia toxicity to juveniles of these mussels species is attributable to the concentration of unionized ammonia present.

Water quality parameters remained within acceptable ranges for all trials and were fairly consistent within each trial (Table 4.3. and Appendix F). Detailed comparisons of replicates prior to and after solution renewals are summarized in Appendix G. Ammonia concentrations and pH measurements exhibited congruity between replicates of each concentration and remained relatively stable over the course of each 96 h trial, prior to and after solution renewals. Detailed comparisons of replicates prior to and after solution renewals are summarized in Appendices E and G. Dissolved oxygen concentrations in the 20°C trials tended to be lower than in the 12°C trials, but were not recorded below 7.30 mg/L and, therefore, were well above the 5mg/L threshold recommended for unionids (Havlick and Marking 1987). Nitrites were not measured at levels. Although dilution water was obtained from the same spring for all trials, water was collected on separate occasions, and hardness and alkalinity were substantially

Table 4.1. Summary of water chemical and mortality data recorded over the duration of each 96 h trial and used to calculate LC_{50} values for total and unionized ammonia.

Species, Temp.	Conc. Level	Mean Temperature ± SD	Mean pH ± SD	Mean Total Ammonia (mg/L) ± SD	Mean Unionized Ammonia (mg/L) ± SD	% Mortality at 24-h	% Mortality at 48-h	% Mortality at 72-h	% Mortality at 96-h
Villosa	Control	12.3 ± 0.25	7.41 ± 0.08	0.00 ± 0.0	0.00 ± 0.0	2.5	5	5	7.5
iris,	1	12.5 ± 0.31	7.32 ± 0.10	11.5 ± 1.0	0.054 ± 0.01	5	15	20	32.5
12 ± 1°C	2	12.5 ± 0.32	7.37 ± 0.10	20.7 ± 1.2	0.11 ± 0.02	15	25	42.5	50
	3	12.6 ± 0.22	7.30 ± 0.11	42.0 ± 1.9	0.19 ± 0.03	62.5	77.5	87.5	95
	4	12.7 ± 0.22	7.26 ± 0.11	82.6 ± 2.9	0.34 ± 0.05	72.5	90	90	95
	5	12.5 ± 0.22	7.17 ± 0.15	162 ± 1.6	0.54 ± 0.11	72.5	82.5	97.5	97.5
Villosa	Control	20.5 ± 0.27	7.45 ± 0.11	0.00 ± 0.0	0.00 ± 0.0	2.5	5	10	12.5
iris,	1	20.6 ± 0.22	7.45 ± 0.12	5.05 ± 0.14	0.058 ± 0.004	2.5	20	20	20
20 ± 1°C	2	20.6 ± 0.17	7.40 ± 0.15	9.62 ± 0.33	0.099 ± 0.02	0	15	32.5	37.5
	3	20.6 ± 0.10	7.44 ± 0.12	19.0 ± 0.72	0.21 ± 0.04	20	57.5	85	95
	4	20.6 ± 0.19	7.39 ± 0.12	38.0 ± 0.51	0.38 ± 0.06	62.5	92.5	97.5	100
	5	20.6 ± 0.19	7.31 ± 0.11	75.5 ± 1.4	0.64 ± 0.09	85	92.5	95	100
Lampsilis	Control	12.7 ± 0.39	7.86 ± 0.28	0.00 ± 0.0	0 ± 0.0	0	2.5	5	5
fasciola ,	1	12.7 ± 0.40	7.88 ± 0.16	3.11 ± 0.21	0.053 ± 0.003	2.5	5	7.5	7.5
12 ± 1°C	2	12.7 ± 0.40	7.88 ± 0.11	5.70 ± 0.30	0.098 ± 0.005	5	5	7.5	10
	3	12.7 ± 0.50	7.87 ± 0.12	12.3 ± 0.37	0.21 ± 0.01	10	12.5	20	22.5
	4	12.4 ± 0.53	7.78 ± 0.12	24.4 ± 0.35	0.33 ± 0.02	62.5	100	100	100
	5	12.6 ± 0.49	7.72 ± 0.13	46.6 ± 1.9	0.55 ± 0.03	95	100	100	100
Lampsilis	Control	20.5 ± 0.54	7.99 ± 0.18	0.00 ± 0.0	0.00 ± 0.0	0	10	10	17.5
fasciola ,	1	20.6 ± 0.49	7.91 ± 0.15	1.51 ± 0.31	0.049 ± 0.004	5	7.5	12.5	12.5
20 ± 1°C	2	20.5 ± 0.40	8.01 ± 0.12	2.51 ± 0.36	0.10 ± 0.003	5	7.5	15	30
	3	20.6 ± 0.30	7.99 ± 0.13	4.92 ± 0.66	0.19 ± 0.008	5	17.5	30	50
	4	20.5 ± 0.35	7.95 ± 0.12	10.3 ± 2.0	0.37 ± 0.02	12.5	15	42.5	45
	5	20.7 ± 0.17	7.91 ± 0.13	19.7 ± 2.1	0.64 ± 0.04	15	22.5	60	97.5

Table 4.2. The 24 h, 48 h, and 96 h LC_{50} values calculated with the Trimmed Spearman-Karber method for juveniles of two freshwater mussel species, *Villosa iris* and *Lampsilis fasciola*, exposed to ammonium chloride at 12 ± 1°C and 20 ± 1°C. Ninety-five percent confidence limits given in brackets.

Species	Temp. (°C)	24-h LC_{50} [95% C.I.]		48-h LC_{50} [95% C.I.]		72-h LC_{50} [95% C.I.]		96-h LC_{50} [95% C.I.]	
		Total Ammonia (mg/L)	Unionized Ammonia (mg/L)	Total Ammonia (mg/L)	Unionized Ammonia (mg/L)	Total Ammonia (mg/L)	Unionized Ammonia (mg/L)	Total Ammonia (mg/L)	Unionized Ammonia (mg/L)
V. iris, juveniles age < 5 d	12 ± 1	36.8 [27.7 - 48.9]	0.22 [0.17 - 0.28]	29.6 [24.7 - 35.6]	0.17 [0.14 - 0.20]	22.8 [19.4 - 26.9]	0.12 [0.10 - 0.14]	20.6 [16.6 - 25.6]	0.10 [0.08 - 0.13]
	20 ± 1	33.4 [28.0 - 40.0]	0.32 [0.27 - 0.37]	18.2 [15.6 - 21.3]	0.18 [0.15 - 0.21]	12.5 [10.8 - 14.6]	0.14 [0.12 - 0.17]	11.4 [10.1 - 12.9]	0.12 [0.11 - 0.14]
fasciola, juveniles age <5 d	12 ± 1	21.1 [18.3 - 24.3]	0.32 [0.28 - 0.36]	16.1 [14.9 - 17.3]	0.23 [0.21 - 0.24]	15.4 [14.1 - 16.9]	0.23 [0.21 - 0.24]	14.9 [13.4 - 16.6]	0.23 [0.21 - 0.25]
	20 ± 1	n/a*	n/a*	n/a*	n/a*	16.5 [11.4 - 25.1]	0.54 [0.39 - 0.74]	7.74 [6.47 - 9.26]	0.28 [0.23 - 0.33]

* For the 20°C trials with *L. fasciola*, 24 h and 48 h LC_{50} values were not calculable because mortality did not reach 50% by 48 h in any of the toxicant concentrations.

Table 4.3. Water quality parameters recorded over the duration of each 96 h trial.

Species, Temperature	Concentration Level	Dissolved Oxygen (mg/L) Min. - Max.	Hardness (mg/L) Min. - Max.	Alkalinity (mg/L) Min. -Max.	Conductivity (µmhos/cm) Min. - Max.
Villosa iris,	Control	9.08 - 10.08	80*	30 - 35	120 - 180
12 ± 1°C	1	9.05 - 10.68	80*	35*	200 - 220
	2	9.59 - 9.73	80*	35*	290 - 310
	3	9.20 - 10.56	80*	30 - 35	470 - 490
	4	9.24 - 9.53	80*	35*	800 - 840
	5	9.32 - 11.48	80*	30 - 35	1360 - 1500
Villosa iris,	Control	7.44 - 8.32	100*	35*	130 - 150
20 ± 1°C	1	7.61 - 8.29	100*	35*	160 - 180
	2	7.30 - 8.50	80 - 100	35*	210 -220
	3	7.23 - 8.67	80 - 100	35*	290 - 310
	4	7.42 - 8.31	80 - 100	35*	450 - 470
	5	7.27 - 8.81	100*	35*	780 - 810
Lampsilis fasciola,	Control	7.44 - 10.33	260*	65 - 70	400 - 470
12 ± 1°C	1	8.04 - 10.40	260*	65 - 70	440 - 500
	2	8.50 - 10.34	260*	65*	470 - 480
	3	8.24 - 10.20	240 - 260	65*	520 - 560
	4	8.31 - 8.41	240 - 260	65*	640 - 660
	5	8.30 - 10.22	240 - 260	65 - 70	770 - 820
Lampsilis fasciola,	Control	8.05 - 8.74	240 - 260	65 - 70	340 - 430
20 ± 1°C	1	8.02 - 9.41	260*	70 - 73	410 - 440
	2	8.00 - 8.73	240 - 260	65 - 75	430 - 460
	3	8.07 - 9.45	240 - 260	70 - 75	430 - 470
	4	7.87 - 8.80	260*	65 - 70	480
	5	7.98 - 9.33	240 - 260	65 - 70	540 - 570

* Range n/a, all measurements of the parameter were the same

higher for the trials with *L. fasciola* (Table 4.3.). Hardness ranged from 80 – 100 mg/L for the *V. iris* trials and from 240 – 260 for the *L. fasciola* trials. Levels of pH also were higher in the dilution water collected for the *L. fasciola* trials (Table 4.1.), while the test solutions pH for all trials tended to decrease with increasing ammonia concentration. Conductivity increased with ammonia concentrations and reached it highest recorded level of 1500 µmhos/cm in the 12°C trial with *V. iris*. Levels at which specific conductivity becomes harmful to juvenile mussels have not been documented. However, based on studies with *Ceriodaphnia dubia*, 2900 µmhos/cm is recommended as a the maximum level safe for long term exposure of aquatic life, and 6000 µmhos/cm is given as the maximum limit not to be exceeded for 48 h acute exposures (D. Cherry, Ph.D. and A. Kennedy, V.P.I and S.U., pers. comm. 2001). Therefore, it is unlikely that the 1500 µmhos/cm condition was problematic.

Juveniles of *L. fasciola* were significantly more tolerant (mean 96 h LC_{50} of 0.24 mg/L NH_3-N) of unionized ammonia than those of *V. iris* (mean 96 h LC_{50} of 0.11 mg/L NH_3-N) (Table 4.2.). At 24 h, a difference in species sensitivity was not yet evident, with 95% confidence intervals of the two LC_{50} values overlapping. At 48 h, the difference in sensitivity between the two species was displayed. For the 20°C trial with *L. fasciola,* 24 h and 48 h LC_{50} values could not be generated because a level of mortality sufficient to calculate an LC_{50} did not occur until the 72 h time point. At 72 h, the two species showed significantly different sensitivities, with *V. iris* emerging as the more sensitive species. A significant difference in the sensitivity of *L fasciola* between the two temperatures also was seen at the 72 h time point, with greater sensitivity exhibited at 12°C (72 h LC_{50} 0.23) than at 20°C (72 h LC_{50} 0.54) (Table 4.2.). The fact that *L. fasciola* had a slower rate of response in this trial is in accordance with the higher LC_{50} values and greater resistance to ammonia toxicity ultimately seen for this species. However, this slower response was only observed in the 20°C trial. At 96 h, neither species exhibited a substantial difference in sensitivity to unionized ammonia between the two temperatures. However, the LC_{50} values were slightly higher at 20°C for both species. Coupled with the fact that *L. fasciola* was less sensitive at 20°C at 72 h, this suggests that these species may exhibit some decreased sensitivity at higher temperatures.

Table 4.4. Range of reported LC_{50} values for standard test species, known sensitive species, and other aquatic invertebrates. Cited LC_{50} ranges include values reported for tests conducted over a variety of temperatures, pH values, and other water quality parameters, as well as for multiple life stages and sizes, except where life stage is specified.

Species	Duration (h)	Range of Reported LC_{50} (mg/L NH_3-N)	Reference
Common test species and known sensitive species			
Cladoceran,*Ceriodaphnia* spp. (*C. dubia* & *C. acanthina*)	48 or 96†	0.07 - 0.63	US EPA 1984, Scheller 1997
Cladoceran, *Daphnia magna*	48 or 96†	0.44 - 2.28	US EPA 1984
Fathead minnow, *Pimephales promelas*	96	0.37 - 2.83	US EPA 1984
Rainbow trout, *Oncorhynchus mykiss*	96	0.13 - 1.04	US EPA 1984, Arthur et al.1987
Freshwater bivalves			
Fingernail clam, *Musculium transversum*	48 or 96†	0.77 - 1.29	US EPA 1984, Arthur et al.1987
Fingernail clam (adults), *Sphaerium novaezelandia*	96	0.49	Hickey and Martin 1999
Asian clam (adults), *Corbicula fluminea*	96	0.71 - 0.88	Scheller 1997
Asian clam (juveniles), *Corbicula fluminea*	96	0.09 - 0.28	Scheller 1997
Freshwater mussel (juveniles), Utterbackia imbecillis	96	0.13 - 0.77	Summers1998*
Freshwater mussel Giant floater (adult), *Pyganadon grandis*	96	0.44- 0.54	Scheller 1997
Rainbow mussel (glochidia), *Villosa iris*	24	0.11- 0.28	Goudreau et al. 1993, Scheller 1997
Rainbow mussel (juveniles), *Villosa iris*	96	0.38 - 0.62	Scheller 1997
Rainbow mussel (juveniles), *Villosa iris*	96	0.10 - 0.11	This study
Wavyrayed lampmussel (juveniles), *Lampsilis fasciola*	96	0.23 - 0.28	This study
Marine bivalves			
Eastern oyster (adults and juveniles), *Crassostrea virginica*	96	8.3 - 37	Epifanio and Srna 1975, US EPA 1984
Hard clam (adults and juveniles), *Mercenaria mercenaria*	96	3.2 - 7.2	Epifanio and Srna 1975, US EPA 1984
Other mollusks			
Snail, *Potamopyrgus antipodarum*	96	0.31 - 0.36	Hickey and Martin 1999
Snail, *Pleurocera unicale unicale*	96	0.742	Goudreau et al. 1993
Snail, *Heliosoma trivolis*	96	2.04 - 2.76	US EPA 1984, Arthur et al.1987
Snail, *Physa gyrina*	96	1.59 - 2.49	US EPA 1984, Arthur et al.1987
Other invertebrates and aquatic insects			
Mayfly, *Ephemerella grandis*	96	3.86 - 5.88	US EPA 1984
Mayfly, *Callibaetis skokianus*	96	3.15 - 4.82	Arthur et al. 1987
Stonefly, *Arcynopteryx paralleia*	48 or 96†	2.00 - 2.06	US EPA 1984
Caddisfly (larvae), *Philartcus quaeris*	96	10.07 - 10.2	US EPA 1984, Arthur et al.1987
Amphipod, *Hyalella azteca*	96	0.04 - 9.2	Hickey and Martin 1999, Whiteman et al.1996
Crayfish, *Oronectes nais*	96	3.15	Goudreau et al. 1993
Crayfish, *Oronectes immunis*	96	14.72 - 33.84	US EPA 1984, Arthur et al.1987
Crayfish, *Cherax quadricans*	96	0.98	Meade and Watts 1995

* unionized ammonia LC_{50} values recalculated from LC_{50}'s reported as total ammonia and reported pH & temperature ranges

† US EPA Water Quality Criteria for Ammonia (1984) does not distinguish between 96 h and 48 h tests for invertebrates.

Relative Sensitivities

Comparison of the 96 h LC_{50} values calculated for juvenile *L. fasciola* and *V. iris* to the range of reported LC_{50}'s for a number of aquatic organisms shows these two mussel species to be among the most sensitive to unionized ammonia (Table 4.4.). Salmonids, especially rainbow trout, emerge as one of the most sensitive groups studied. Marine bivalves, crayfish, and aquatic insects tend to be relatively insensitive to ammonia. LC_{50} values comparable to those observed for *V. iris* and *L. fasciola* are seen among some of the freshwater bivalves and snails. The only organisms with reported LC_{50} values lower than *L. fasciola* juveniles were rainbow trout *Oncorhynchus mykiss*, juvenile Asian clams *Corbicula fluminea*, juvenile mussels of *Utterbackia imbecillis*, glochidia of *V. iris*, the amphipod *Hyalella azteca*, and the cladoceran *Ceriodaphnia dubia*. The only organisms with reported LC_{50} values lower than those seen for *V. iris* juveniles were *C. dubia* and *H. azteca* (Table 4.4.).

Estimated Safe Levels, Regulatory Standards, and Environmental Ammonia

Application of the 0.1 factor to the mean 96 h LC_{50} values found in this study yield estimated safe environmental levels of 0.011 mg/L NH_3-N and 0.026 mg/L NH_3-N for juveniles of *V. iris* and *L. fasciola*, respectively. Comparison of the US EPA acute 1 h limits with 24 h LC_{50}'s (the shortest interval for which LC_{50}'s were calculated) shows the LC_{50} values to be between 2.15 and 3.45 times higher than the concentration limits (Table 4.5.). Comparison of the VA DEQ acute 1 h limit with 24 h LC_{50}'s indicates that the 1 h limits would be protective against a 24 h exposure, with the LC_{50} values being between 1.77 and 2.83 times higher than the concentration limits (Table 4.6.). The VA DEQ chronic, 30 d limits provide protection against 96 h toxicity, with 96 h LC_{50} values being between 3.83 and 9.02 times higher than the 30 d concentration limits (Table 4.6.).

For ambient water quality monitoring stations in the Clinch-Powell, Holston, and North Fork Shenandoah basins, ammonia levels were below levels (<0.04 mg/L) in the majority of samples (Table 4.7.) (VA DEQ 2001a). For those samples in which ammonia

4.5. Comparison between US EPA standards for unionized ammonia and juvenile mussel LC_{50} values.

Test species	24 h LC_{50} value (mg/L NH_3-N) [for temp °C, mean pH]	1 h limit* (mg/L NH_3) [for temp °C, pH]	against 24 h acute toxicity	96 h LC_{50} value (mg/L NH_3-N) [for temp °C, mean pH]	96-h limit* (mg/L NH_3-N) [for temp °C, pH]	against 96 h acute toxicity
Villosa iris	0.22 [12 ± 1°C, pH 7.38]	0.075 [10°C, pH 7.5]	yes	0.10 [12 ± 1°C, pH 7.31]	0.0109 [10°C, pH 7.5]	yes
	0.32 [20 ± 1°C, pH 7.38]	0.149 [20°C, pH 7.5]	yes	0.12 [20 ± 1°C, pH 7.41]	0.0153 [20°C, pH 7.5]	yes
Lampsilis fasciola	0.32 [12 ± 1°C, pH 7.86]	0.0929 [10°C, pH 7.75]	yes	0.23 [12 ± 1°C, pH 7.83]	0.0181 [10°C, pH 7.75]	yes
	0.54†† [20 ± 1°C, pH 7.94]	0.214 [20°C, pH 8.0]	yes	0.28 [20 ± 1°C, pH 7.96]	0.0288 [20°C, pH 8.0]	yes

* avg. concentration not to be exceeded more than once every 3 years

† factor of 0.1 applied to acute 96 h LC_{50} value

†† 72 h LC_{50} value; 24 h value not calculable

Table 4.6. Comparison between VA DEQ standards for unionized ammonia and juvenile mussel sensitivities.

Test species	24 h LC_{50} value (mg/L NH_3-N) [for temp °C, mean pH]	1 h limit* (mg/L NH_3) [for temp °C, pH]	Protective against 24 h acute toxicity	96 h LC_{50} value (mg/L NH_3-N) [for temp °C, mean pH]	Estimated safe environmental level† (mg/L NH_3-N)	30 d limit* (mg/L NH_3-N) [for temp °C, pH]	Protective against 96 h acute toxicity	Acceptable as safe environmental level
Villosa iris	0.22 [12 ± 1°C, pH 7.38]	0.0904 [10°C, pH 7.5]	yes	0.10 [12 ± 1°C, pH 7.31]	0.01	0.0155 [10°C, pH 7.5]	yes	no
	0.32 [20 ± 1°C, pH 7.38]	0.181 [20°C, pH 7.5]	yes	0.12 [20 ± 1°C, pH 7.41]	0.012	0.0313 [20°C, pH 7.5]	yes	no
Lampsilis fasciola	0.32 [12 ± 1°C, pH 7.86]	0.113 [10°C, pH 7.75]	yes	0.23 [12 ± 1°C, pH 7.83]	0.023	0.0255 [10°C, pH 7.75]	yes	no
	0.54†† [20 ± 1°C, pH 7.94]	0.259 [20°C, pH 8.0]	yes	0.28 [20 ± 1°C, pH 7.96]	0.028	0.0590 [20°C, pH 8.0]	yes	no

* avg. concentration not to be exceeded more than once every 3 years

† factor of 0.1 applied to acute 96 h LC_{50} value

†† 72 h LC_{50} value; 24 h value not calculable

Table 4.7. Ammonia data for ambient monitoring stations located in three Virginia basins

	River Basin		
	Clinch/Powell	Holston	North Fork Shenandoah
Sampling Period	1/1996 - 3/2001	2/1996 - 3/2001	1/1996 - 12/2000
Location and Number of Sampling Stations	Total - 25	Total - 18	Total - 6
	Clinch River - 5 Copper Creek - 1 Guest River - 2 Little River - 1 N.F. Clinch - 1 S.F. Powell - 4 Powell River - 8 N.F. Powell - 3	M.F. Holston - 5 N.F. Holston - 5 S.F. Holston - 2 Plum Creek - 2 Wolf Creek - 2 Big Moccasin Crk - 1 Beaverdam Creek - 1	N.F. Shenandoah - 6
Number of Samples	643	716	355
% Samples with Ammonia Undetectable (<0.04 mg/L)	93.6%	88.0%	85.6%
Number of Stations with Ammonia Undetectable in all Samples	14	4	0
Stations with Greatest % Detectable Samples	Guest River 1 - 33% detectable	M.F. Holston - 92 % detectable	N.F. Shenandoah - 22% detectable
	Guest River 2 - 27% detectable	Wolf Creek - 26% detectable	
For samples with Detectable Ammonia Levels:			
Mean Total Ammonia (mg/L) ± SD	0.11 ± 0.096	0.12 ± 0.077	0.087 ± 0.070
Maximum Total Ammonia (mg/L)	0.49	0.42	0.47
Mean Unionized Ammonia (mg/L) ± SD	0.0019 ± 0.0021	0.0021 ± 0.0019	0.0078 ± 0.015
Maximum Unionized Ammonia (mg/L)	0.012	0.0091	0.055

Table 4.8. Calculated levels of unionized ammonia in ambient water samples in which ammonia was detectable. Data collected between January 1996 and March 2001 by VA DEQ.

Level of unionized ammonia (mg/L NH_3-N)	Clinch/ Powell Basin		Holston Basin		North Fork of Shenandoah	
	# of measurements	% of total measurements	# of measurements	% of total measurements	# of measurements	% of total measurements
<0.001	12	29.3	26	30.2	11	22.0
0.001 - 0.003	18	43.9	32	37.2	17	34.0
0.003 - 0.005	4	9.8	19	22.1	8	16.0
0.005 - 0.01	0	0.0	4	4.7	5	10.0
0.01 - 0.03	1	2.4	0	0.0	3	6.0
0.03 - 0.05	0	0.0	0	0.0	2	4.0
0.05 - 0.1	0	0.0	0	0.0	2	4.0
NH_3-N level unknown*	6	14.6	5	5.8	2	4.0
Total # of detectible ammonia	41		86		50	

* NH_3-N level was not calculable from ammonia data if corresponding temperature or pH measurements were not available.

ammonia was measurable, unionized ammonia levels were calculated using the formulas of Emerson et al. (1975) and corresponding temperature and pH data. Mean concentrations of unionized ammonia in measurable samples ranged from 0.0019 mg/L Clinch/ Powell Basin to 0.0078 mg/L in the North Fork Shenandoah (Table 4.7.). Comparing levels of unionized ammonia between basins, only one sample from the Clinch/ Powell basin was above the 0.01mg/L NH_3-N estimated safe level for juvenile *V. iris,* no samples from the Holston Basin were above this level, and seven (14%) of the samples from the North Fork Shenandoah were above this level (Table 4.8.).

Unionized ammonia levels in the Clinch River also were documented in a 1985 - 1986 study comparing unionized ammonia levels downstream of sewage treatment outfalls with levels at reference sites above the outfalls (Goudreau 1993). Measurements taken at reference sites 0.1 km above Cleveland and Richland STP's ranged from 0.001 to 0.02 mg/L NH_3-N. Of 22 total reference site measurements, all but one were below the 0.01 mg/L estimated safe environmental threshold.

For the data obtained for permitted WTP plants on the Clinch, Powell, and North Fork Holston, ammonia levels were reported as undetectable for 14.6% of the samples from Cleveland, 6.2% from Jonesville, and 8.8% from Saltville (VA DEQ 2001c). (It is noteworthy that the facilities use different methods and upgrades of measuring technology have occurred over the time periods assessed, detection limits for these data are variable with detection limits for some data points as high as 1 mg/L.) For the WTP samples in which ammonia was detectable, the mean total ammonia concentrations were calculated for each outfall over three time periods, and ranged from 0.57 – 5.42 mg/L (Table 4.9.). Considering all date ranges for the three WTP outfalls, only two of the nine minimum unionized ammonia concentrations projected from mean total ammonia values were above the 0.01mg/L threshold estimated to be a safe environmental level for juvenile *V. iris* (Table 4.9.). However, all nine of the maximum projected values are not only well above safe environmental levels, but are even above or equal to 0.10 – 0.12 mg/L, which are the 96 h LC_{50}'s for *V. iris* juveniles. Five of these values also are above the 0.23 and 0.28 mg/L 96 h LC_{50}'s for *L. fasciola* (Table 4.9.). In considering the worst

Table 4.9. Total ammonia concentrations reported to VA DEQ from three permitted WTP outfall locations in potential freshwater mussel habitat. Projected unionized ammonia ranges calculated from temperature and pH ranges recorded at the ambient monitoring station closest to the outfall site.

			Total Ammonia (mg/L)				Unionized Ammonia (mg/L)	
Location	Date Range	# of Samples	Min. - Max.	Mean	pH Range	Temperature Range (°C)	Range Calculated from Mean Tot. Amm.	Range Calculated from Min. & Max. Tot. Amm.
Cleveland, Clinch River	1/96 - 12/97	41	0.02 - 5.2	1.61	7.37 - 8.61	2.24 - 25.5	0.0037 - 0.31	$4.6X10^{-5}$ - 1.0
	1/98 - 12/99	29	1.0 - 9.3	1.53			0.0036 - 0.30	0.0023 - 1.8
	1/00 - 6/01	5	0.21 - 1.0	0.57			0.0013 - 0.11	$4.9X10^{-4}$ - 0.19
Jonesville, Powell River	3/99 - 12/99	8	1.6 - 9.3	5.41	7.39 - 8.66	2.30 - 24.5	0.013 - 1.1	0.0039 - 1.9
	1/00 - 12/00	12	0.58 - 8.2	3.92			0.0096 - 0.78	0.0014 - 1.64
	1/01 - 6/01	6	2.5 - 7.2	4.95			0.012 - 0.95	0.0061 - 1.44
Saltville, N.F. Holston	6/94 - 12/94	7	0.14 - 4.4	0.83	7.37 - 8.61*	2.24 - 25.5*	0.0019 - 0.16	$3.3X10^{-4}$ - 0.85
	1/95 - 12/95	11	0.19 - 6.45	1.56			0.0036 - 0.30	$4.4X10^{-4}$ - 1.25
	1/96 - 3/97	13	0.10 - 3.4	1.04			0.0024 - 0.20	$2.3X10^{-4}$ - 0.66

* Temperature and pH data taken from Clinch River, due to small number of samples available from Holston.

case NH_3 –N levels calculated from the maximum reported total ammonia values, all but one of the projected values (exception of 0.19mg/L) are above even the 24 h LC_{50}'s for both species of juvenile mussels, and six of the nine values are above 1 mg/L, which is approximately 10 times the LC_{50}'s found for juvenile *V. iris*.

Previously collected data from sites below WTP's in Table 4.9. are hypothetical values (projected from total ammonia measurements and pH and temperature ranges measured close to the site at different sampling times), it is useful to review the previously documented NH_3 -N levels as a comparison. For 22 monthly measurements taken in 1985 and 1986, NH_3 -N levels in the effluent plume 0.1 km below the outfalls at Cleveland and Richlands (Clinch River) ranged from 0.006 to 0.812 mg/L (Goudreau 1993). This maximum recorded value demonstrates that NH_3 -N levels approaching 1 mg/L occur in the environment. Eight of the 22 NH_3 -N values were above the 0.10 mg/L 96 h LC_{50} found for *V. iris* juveniles in this study, and only two of the 22 measurements were below the 0.01 mg/L estimated safe environmental threshold.

Captive Culture

At White Sulphur Springs National Fish Hatchery, where grow-out of juvenile mussels was attempted in effluent water from rainbow trout, total ammonia levels ranged from 0.139 mg/L to 0.293 mg/L. Temperature ranged from 9 to 16°C and pH from 7.25 to 8.29. Calculated unionized ammonia values ranged from 0.00070 mg/L to 0.013 mg/L. Of 36 calculated values for unionized ammonia, however, only the maximum value of 0.013 mg/L exceeded the 0.010 mg/L estimated safe level for *V.iris* juveniles.

DISCUSSION

Juvenile Mussel Bioassays

It is possible that variations in water chemistry parameters between the trials with *L. fasciola* and *V. iris* may have contributed to the differences in sensitivity seen for the two species. Hardness of the dilution water was substantially different for the two trials, ranging from 80 – 100 mg/L for the *V. iris* trials and from 240 – 260 for the *L. fasciola* trials. Either hardness range is suitable for juvenile survival, although higher hardness

levels (≥ 250mg/L) have been recommended for long term culture in order to provide adequate calcium for shell growth (Steg 1998). Documentation of whether increasing hardness or alkalinity affects ammonia toxicity is inconclusive. Substantially decreased ammonia toxicity at higher hardness has been reported in the amphipod *Hyalella azteca* (Ankely *et al.* 1995), while other researchers have found no effects of hardness on ammonia toxicity in the oligochaete *Lumbriculus variegatus* and midge *Chironomus tentans* (Shubauer-Berigan *et al*. 1995), or in other species (Hickey & Martin 1999). One mechanism by which higher hardness could ameliorate ammonia toxicity is by increasing the ionic strength of the toxicant solutions. Although pH and temperature are the primary factors determining the fraction of ammonia that will occur as NH_3-N, ionic strength also can play a role when total dissolved solids levels reach 200 – 300 mg/L, with higher ionic strength resulting in decreased proportions of NH_3-N (Emerson *et al.* 1975). It is possible, then, that in the trials with *L. fasciola*, hardness levels ranging from 240 – 260 mg/L may have increased the ionic strength of the solutions, thus decreasing the amount of unionized ammonia and subsequent toxicity. The differing pH levels for the two trials may also have had an effect on the two species' sensitivity. The lower pH occurring for the *L. fasciola* trials and at the higher ammonia concentrations within both trials resulted in lesser fractions of unionized ammonia, but this was taken into account when calculating LC_{50} values. However, as with hardness and alkalinity, current data on whether pH effects the toxicity of unionized ammonia are inconclusive. Some researchers report lower toxicity of unionized ammonia toxicity with increasing pH, while in other cases no relationship is observed (Adams and Bealing 1994, Hickey and Martin 1999). In our study, juveniles of *L. fasciola* were significantly more tolerant of unionized ammonia than those of *V. iris;* however, if higher hardness, alkalinity, or pH levels prove to buffer the toxic action of total or unionized ammonia, the difference in sensitivity seen between the two species may be attributable to the differences in these water quality characteristics.

Relative Sensitivities

Of the standard test organisms, neither *P. promelas* nor *D. magna* are reported to exhibit as great a sensitivity to unionized ammonia as the two tested species. As a result,

neither is adequate for use as a surrogate for freshwater mussels in setting water quality standards for ammonia or mixed effluents containing ammonia. Some of the reported LC_{50} values for the standard test daphnid, *C. dubia*, indicate that this organism may have greater sensitivity to ammonia than the juvenile mussels. However, only the lower values in the range of reported LC_{50}'s are below those calculated for the juvenile mussels, while the upper end of the LC_{50} range suggests a lower sensitivity for this species. Further comparative study of *C. dubia* with additional mussel species and under a range of test conditions would be needed to assess whether this species is an appropriate surrogate for ammonia toxicity to juvenile freshwater mussels. Based on existing data showing overlapping LC_{50} ranges, ammonia standards based on the sensitivity of this daphnid would be only marginally protective of juvenile mussels.

Estimated Safe Levels and Comparison with Standards and Environmental Ammonia

Comparison of the US EPA and VA DEQ water quality criteria for ammonia with our calculated LC_{50} values for *V. iris* and *L. fasciola* indicates that current standards should be adequate to protect juveniles of these two mussel species from acute exposures. However, it is noteworthy that the VA DEQ chronic, 30 d limits are above the ammonia levels estimated to be safe for long-term exposure, and thus are not satisfactory for long term protection (Table 4.6.).

The high proportion of ambient samples from the Clinch, Powell, Holston, and North Fork Shenandoah for which ammonia was un, as well as the fact that the large majority of calculated unionized ammonia levels were well below the estimated safe environmental level, suggest that ambient ammonia conditions are within suitable bounds in these important mussel habitats in Virginia. Among the samples where ammonia was and unionized ammonia was calculable, the substantially higher proportion of samples from the N.F. Shenandoah with unionized levels above the estimated safe environmental threshold is notable (14 % in N.F.S. versus 0 and 1% in the Holston and Clinch-Powell basins, respectively). This difference suggests that further investigation of the influence of land-use on ambient ammonia is warranted. The hypothetical maximum $NH_3 - N$ values calculated for STP effluent samples demonstrate that these outfalls have the potential to result in unionized ammonia levels that are not only above estimated safe

environmental levels, but would also be acutely toxic to juveniles of these two mussel species.

While the ambient water column measurements indicate relatively good conditions, two caveats bear consideration. One concern is that variability in ammonia levels may not be captured in monthly measurements. Agricultural discharges, for example, have been noted to be highly variable, as in New Zealand streams receiving dairy-shed discharges, where streams with median concentrations of 2 mg/L total ammonia have spiked up to 100 mg/L (Hickey and Martin 1999). Second, measurements of total and unionized ammonia in interstitial pore waters frequently exceed concentrations in overlying water and are strongly influenced by organic loading from the environment (Whiteman et al. 1996, Hickey and Martin 1999). Total and unionized ammonia concentrations in sediment pore water from the Mississippi River frequently exceed water quality standards for ammonia (Hickey and Martin 1999). Pore water ammonia has been found to be responsible for decreased richness and density among benthic macroinvertebrates in freshwater portions of the Potomac and Anacostia rivers near Washington D.C., with total ammonia measurements ranging from 5.5 – 34.2 mg/L (Schlekat et al. 1994). Other reported total ammonia concentrations for pore water are as high as 40 – 80 mg/L (Whiteman et al. 1996). Studies of some benthic invertebrates have indicated that it is valid to extrapolate the toxic response to ammonia found in water-only tests, such as our study, to an interstitial environment (Whiteman et al. 1996). Interstitial pore water concentrations of ammonia may be especially relevant to juvenile mussels, because they do not yet filter overlying water but pedal-feed in interstitial spaces. Pore water ammonia concentrations in the Clinch-Powell, Holston, and North Fork Shenandoah rivers have not been documented, but it is an area needing investigation.

Captive Culture

At White Sulphur Springs National Fish Hatchery, given that only one of 36 calculated unionized ammonia values exceeded the estimated safe environmental threshold for *V. iris* juveniles, it is unlikely that ammonia toxicity had a substantial role in inhibiting the growth or survival of the juveniles held in these facilities. If, however, the higher temperatures (20 – 25°C) intended for future grow-out efforts were used in the

calculations to project what unionized ammonia levels would have been under these increased temperatures, unionized ammonia values ranged from 0.0016 mg/L to 0.029 mg/L, and, of the 36 calculated values for unionized ammonia, eight values exceeded the 0.01mg/L estimated safe environmental threshold. It is possible, then, that ammonia levels would have proved stressful to juvenile mussels if this effluent water were to be used for grow-out at the desired temperature range.

Implications

While ambient ammonia levels appear to be largely within acceptable bounds for juvenile mussels, differences in the proportion of ammonia measurements and in calculated NH_3-N values between sampling sites and drainages indicates that in choosing potential reintroduction or protection sites, ambient ammonia levels should be a consideration. The maximum values for NH_3-N below WTP effluent sites clearly are potentially harmful to freshwater mussels. Therefore, in regulating and monitoring effluent discharges, mussel populations and habitat should be considered. Finally, in comparing the sensitivity of these two species of juvenile mussels to regulatory standards and environmental ammonia levels, it should also be noted that *L. fasciola* and *V. iris* are among the most common species in Virginia, and rarer species would be expected to be more sensitive.

LITERATURE CITED

Adams, N. and D. Bealing. 1994. Organic pollution: biochemical oxygen demand and ammonia. Pages 264 – 285 *in* P. Calow, editor. Handbook of ecotoxicology, Vol. 2. Blackwell Scientific Publications, Oxford.

Ahlstedt, S.A. 1979. Recent mollusc transplants in the North Fork Holston River in southwest Virginia. Bulletin of the American Malacological Union 1979: 21 – 23.

ASTM (American Society for Testing and Materials). 1980a. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. No. E 729-80. Philadelphia, Pennsylvania.

ASTM (American Society for Testing and Materials). 1980b. Standard practice for conducting acute toxicity tests with larvae of four species of bivalve mollusks. No. E 724-80. Philadelphia, Pennsylvania.

Amyot, J.P. and J.A. Downing. 1991. Endo- and epibenthic distribution of the unionid mollusc *Elliptio complanata*. Journal of the North American Benthological Society 10: 280 – 285.

Anderson, K.B., R.E. Sparks, and A.A. Paparo. 1978. Rapid assessment of water quality, using the fingernail clam, *Musculium transversum*. Water Resources Center Research Report No. 133, Water Resources Center, University of Illinois, Urbana, Illinois.

Ankely, G.T., M.K. Schubauer- Berigan, and P.D. Monson. 1995. Influence of pH and hardness on toxicity of ammonia to the amphipod *Hyalella azteca*. Canadian Journal of Fisheries and Aquatic Sciences 52: 2078 – 2083.

Arthur, J.W., C.W. West, K.N. Allen, and S.F. Hedtke. 1987. Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bulletin of Environmental Contamination and Toxicology 38: 324 – 331.

Bailey, R.C. 1989. Habitat selection by a freshwater mussel: an experimental test. Malacologia 31: 205 – 210.

Baldwin, B.S. and R.I.E. Newell. 1991. Omnivorous feeding by planktotrophic larvae of the eastern oyster *Crassostrea virginica.* Marine Ecology Progress Series 78: 285 – 301.

Bauer, G. 1988. Threats to the freshwater pearl mussel *Margaritifera margaritifera* L. in central Europe. Biological Conservation 45(4): 239 – 254.

Beaty, B.B. 1999. Development of juvenile culture techniques and testing of potential biomarkers of environmental stress in freshwater mussels (Bivalvia: Unionidae). Doctoral dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Beck, K.M. 2001. Development of an algal diet for rearing juvenile freshwater mussels. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Besser, J.M., C.G. Ingersoll, E.N. Leonard, and D.R. Mount. 1998. Effect of zeolite on toxicity of ammonia in freshwater sediments: implications for toxicity identification evaluation procedures. Environmental Toxicology and Chemistry 17 (11): 2310 – 2317.

Boltovskoy, D., I. Izaguirre, and N.Correa. 1995. Feeding selectivity of *Corbicula fluminea* (Bivalvia) on natural phytoplankton. Hydrobiologia 312: 171 – 182.

Box, J.B. and J. Mossa. 1999. Sediment, land use, and freshwater mussels: prospects and problems. Journal of the North American Benthological Society 18 (1): 99 – 117.

Buddensiek, V. 1995. The culture of freshwater pearl mussels *Margaritifera margaritifera* L. in cages: a contribution to conservation programmes and the knowledge of habitat requirements. Biological Conservation 74: 33 – 40.

Buikema, A.L. Jr., B.R. Niederlehner, and J. Cairns, Jr. 1982. Biological monitoring, part IV: toxicity testing. Water Research 16: 239 – 262.

Campbell, S.A. 1969. Seasonal cycles in the carotenoid content in *Mytilus edulis*. Marine Biology 4: 227 – 232.

Chetty A.N. and K. Indira. 1995. Adaptive changes in glucose metabolism of a bivalve to ambient ammonia stress. Bulletin of Environmental Contamination and Toxicology 54: 83 – 89.

Davis, L.N., K.A. Phillips, and H.G. Marshall. 1997. Seasonal abundance of autotrophic picoplankton in the Pagan River, a nutrient enriched subestuary of the James River, Virginia. Virginia Journal of Science 48: 212 – 217.

D'Eliscu, P.N. 1972. Observations of the glochidium, metamorphosis, and juveniles of *Anodonta californiensis* Lea, 1857. The Veliger 15: 57 – 58.

Doherty, F.G. and D.S. Cherry. 1988. Tolerance of the asiatic clam *Corbicula* spp. to lethal levels of toxic stressors – a review. Environmental Pollution 51: 269 – 313.

Downing, J.A., Y. Rochon, M. Perusse, and H. Harvey. 1993. Spatial aggregation, body size, and reproductive success in the freshwater mussel *Elliptio complanata,* Journal of the North American Benthological Society 12 (2): 148 – 156.

Eaton, A., L. Clesceri, and A. Greenberg. 1995. Standard methods for the examination of water and wastewater, 19th Edition. The American Public Health Association, Washington, D.C.

Eddy, F.B., and E.M. Williams. 1994. Freshwater fish and nitrite. Pages 117 – 143 *in* G. Howells . editor. Water quality for freshwater fish. Gordan and Breach Science, Yverdon, Switzerland.

Emerson, K., R.C. Russo, R.E. Lund, and R.V. Thurston. 1975. Aqueous ammonia equilibrium calculations: effects of pH and temperature. Journal of the Fisheries Research Board of Canada 32: 2379 – 2383.

Epifanio, C.E. 1979. Growth in bivalve molluscs: nutritional effects of two or more species of algae in diets fed to the American oyster *Crassostrea virginica* (Gmelin) and the hard clam *Mercenaria mercenaria* (L.). Aquaculture 18: 1 – 12.

Epifanio, C.E. and R.F. Srna. 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to *Mercenaria mercenaria* and *Crassostrea virginica*. Marine Biology 33: 241 – 246.

Erickson, R.J. 1985. An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms. Water Research 19 (8): 1047 – 1058.

Foe, C. and A. Knight. 1986. A thermal energy budget for *Corbicula fluminea.* American Malacological Bulletin, Special Edition No. 2: 143 – 150.

Gale, W.F. and R.L. Lowe. 1971. Phytoplankton ingestion by the fingernail clam, *Sphaerium transversum* (Say), in pool 19, Mississippi River. Ecology 52 (3):507 – 513.

Gatenby, C.M. 1984. Development of a diet for rearing juvenile freshwater mussels. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Gatenby, C.M., R.J. Neves, and B.C.Parker. 1997. Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment. American Malacological Bulletin 14: 57 – 66.

Gatenby, C.M., R.J. Neves, and B.C.Parker. 1996. Influence of sediment and algal food on cultured juvenile freshwater mussels. Journal of the North American Benthological Society 12 (2): 148 – 156.

Gordon, M.E. and J.B. Layzer. 1989. Mussels (Bivalvia: Unionidae) of the Cumberland River: a review of life histories and ecological relationships. US Department of Interior, Fish and Wildlife Service, Biological Report 89 (15). Washington, D.C.

Goudreau, S. E., R.J. Neves, and R.J. Sheehan. 1993. Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. Hydrobiologia 252: 211 – 230.

Goudreau, S.E. 1993. Effects of wastewater treatment plant effluents on mollusks and fish of the Clinch River in Tazewell County, Virginia. Master's thesis, Virginia Polytechnic and State University, Blacksburg,Virginia.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science Technology 11(7): 714-719; Correction 12(4):417 (1978).

Hanlon, S.D. 2000. Release of juveniles into a fish hatchery raceway: a comparison of techniques. Master's thesis, Virginia Polytechnic and State University, Blacksburg, Virginia.

Havlik, M.E. and L.L Marking. 1987. Effects of contaminants on naiad mollusks (Unionidae). US Department of Interior, Fish and Wildlife Service: Resource Publication 164. Washington, D.C.

Hickey, C.W. and M.L. Martin. 1999. Chronic toxicity of ammonia to the freshwater bivalve *Sphaerium novaezelandiae*. Archives of Environmental Contamination and Toxicology 36: 38 – 46.

Hillebrand, H. and U. Sommer. 1999. The nutrient stiochiometry of benthic microalgal growth: Redfield proportions are optimal. Limnology and Oceanography 44 (2): 440 – 446.

Hudson, R.G. and B.G. Isom. 1984. Rearing juveniles of freshwater mussels (Unionidae) in a laboratory setting. The Nautilus 98 (4): 129 – 138.

Huey, D.W. and T.L. Beitinger. 1980. Toxicity of nitrite to larvae of the salamander *Ambystoma texanum*. Bulletin of Environmental Contamination and Toxicology 25: 909 – 912.

Imlay, M.J. 1972. Greater adaptability of freshwater mussels to natural rather than to artificial displacement. The Nautilus 86 (4): 76 – 79.

Jacobson, P.J., J.L. Farris, D.S. Cherry, and R.J.Neves. 1993. Juvenile freshwater mussel (Bivalvia: Unionidae) responses to acute toxicity testing with copper. Environmental Toxicology and Chemistry 12: 879 –883.

Johnson, I.C. 1990. Proposed guide for conducting acute toxicity tests with early life stages of freshwater mussels. Final Report, EPA Contract No. 68-024278. US Environmental Protection Agency, Washington, D.C.

Johnson, I.C., A.E. Keller, and S.G. Zam. 1993. A method for conducting acute toxicity tests with early life stages of juvenile mussels. Pages 381 – 396 *in* W.G. Landis, J.S. Hughes, and M.A.Lewis, editors. Environmental toxicology and risk assessment. American Society for Testing and Materials (ASTM), Standard Technical Publication 1179. Philadelphia, Pennsylvania.

Kat, P.W. 1982. Effects of population density and substratum type on growth and migration of *Elliptio complanata* (Bivalvia: Unionidae). Malacological Review 15: 119 – 127.

Keller, A.E. 1993. Acute toxicity of several pesticides, organic compounds, and a wastewater effluent to the freshwater mussel, *Anadonta imbecillis*, *Ceriodaphnia dubia*, and *Pimephales promelas*. Bulletin of Environmental Contamination and Toxicology 51: 696 – 702.

Keller, A.E. and D.S. Ruessler. 1997. The toxicity of malathion to unionid mussels: relationship to expected environmental concentrations. Environmental Toxicology and Chemistry. 16: 1028 – 1033.

Keller, A.E. and S.G. Zam. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. Environmental Toxicology and Chemistry 10: 539 – 546.

Kumar, N.J. and K.P. Krishnamoorthi. 1983. Evaluation of toxicity of ammoniacal fertiliser effluents. Environmental Pollution (Series A) 30: 77 – 86.

Layzer, J.B., M.E. Gordon, and R.M. Anderson. 1993. Mussels: the forgotten fauna of regulated rivers. A case study of the Caney Fork River. Regulated Rivers: Research and Management 8: 63 – 71.

Lefevre, G. and W.C. Curtis. 1908. Experiments in the artificial propagation of freshwater mussels. Bulletin of the Bureau of Fisheries 29: 615 – 626.

Lemly, D.A. 1998. Bacterial growth on stream insects: potential for use in bioassessment. Journal of the North American Benthological Society 17 (2): 228 – 238.

Matteson, M.R. 1955. Studies on the natural history of the Unionidae. American Midland Naturalist 53: 126 – 145.

Meade, M.E. and S.A. Watts. 1995. Toxicity of ammonia, nitrite, and nitrate to juvenile Austrailian crayfish, *Cherax quadricarinatus.* Journal of Shellfish Research 14 (2): 341 – 346.

Metcalfe-Smith, J.L. 1994. Influence of species and sex on metal residues in freshwater mussels (family Unionidae) from the St. Lawrence River, with implications for biomonitoring programs. Environmental Toxicology and Chemistry 13: 1433 1443.

Michaelson, D.L. and R.J. Neves. 1995. Life history and habitat of the endangered dwarf wedgemussel *Alasmidonta heterodon* (Bivalvia: Unionidae). Journal of the North American Benthological Society 14 (2): 324 – 340.

Mwashote, B.M. and W. Shimbira. 1994. Some limnological characteristics of the lower Sondu-Miriu River, Kenya. Recent trends in research on Lake Victoria fisheries. Proceedings of the second EEC regional seminar on recent trends in research on Lake Victoria fisheries. Kenya Marine and Fisheries Science Research Institute, Nairobi, Kenya.

Nalepa, T.F., W.S. Gardener, and J.M. Malcyk. 1991. Phosphorus cycling by mussels (Unionidae: Bivalvia) in Lake St.Clair. Hydrobiologia 219: 239 – 250.

National Native Mussel Conservation Committee. 1998. National strategy for the conservation of native freshwater mussels. Journal of Shellfish Research 17 (5): 1419 – 1428.

Neves, R.J. 1991. Mollusks. Pages 251 – 320 *in* K. Terwilliger, coordinator. Virginia's endangered species. McDonald and Woodward Publishing Co., Blacksburg, Virginia.

Neves, R.J. 1993. A state-of-the-unionids address. Pages 1 - 10 *in* K.S. Cummins, A.C. Buchanan, and L.M Koch, editors. Conservation and management of freshwater mussels. proceedings of a UMRCC symposium. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Neves, R.J., L.R. Weaver, and A.V. Zale. 1985. An evaluation of host fish suitability for glochidia of *Villosa vanuxemi* and *V. nebulosa* (Pelecypoda: Unionidae). American Midland Naturalist 113 (1): 13 – 19.

O'Beirn, F.X., R.J. Neves, and M.B. Steg. 1998. Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. American Malacological Bulletin 14: 165 – 171.

O'Connell, M.T. 1991. Immunological responses of fishes to glochidia of freshwater mussels. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Ökland, J. 1963. Notes on the population density, age distribution, growth, and habitat of *Anodonta piscinalis* Nilss. (Moll., Lammellibr.) in a eutrophic Norwegian lake. Nytt Magasin for Zoologi 11: 19 – 43.

Parker, B.C., M.A. Patterson, and R.J. Neves. 1998. Feeding interactions between native freshwater mussels (Bivalvia: Unionidae) and zebra mussels (Dreissena polymorpha) in the Ohio River. American Malacological Bulletin 14: 173 – 179.

Parmalee, P.W. and A.E. Bogan. 1998. The freshwater mussels of Tennessee. The University of Tennessee Press, Knoxville, Tennessee.

Paterson, C.G. 1984. A technique for determining apparent selective filtration in the freshwater bivalve *Elliptio complanata* (Lightfoot) in an old reservoir in New Brunswick, Canada. Freshwater Invertebrate Biology 4: 201 – 207.

Paterson, C.G. 1986. Particle-size selectivity in the freshwater bivalve *Elliptio complanata* (Lightfoot). Veliger 29 (2): 235 – 237.

Patterson, M.A., B.C. Parker, and R.J. Neves. 1999. Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding. American Malacological Bulletin 15: 47 – 50.

Pohl, P., and F. Zurheide. 1979. Fatty acids and lipids of marine algae and the control of their biosynthesis by environmental factors. Pages 473 – 523 *in* H.A. Hoppe *et al.*, editors, Marine Algae Pharmaceutical Science. Walter de. Gruyter, New York, New York.

Prescott, G.W. 1978. How to know the freshwater algae. William C. Brown/ McGraw Hill Co. Dubuque, Iowa.

Rajesh, K.V., K.S. Mohamed, and V. Kripa. 2001. Influence of algal cell concentration, salinity and body size on the filtration and ingestion rates of cultivable Indian bivalve. Indian Journal of Marine Sciences 30(2): 87 – 92.

Reddy, N.A. and N.R. Menon. 1979. Effects of ammonia and ammonium on tolerance and byssogenesis in *Perna viridis*. Marine Ecology – Progress Series 1(4): 315 – 322.

Redfield, A.C. 1958. The biological control of the chemical factors in the environment. American Scientist 46: 205 – 221.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries Research Board of Canada Bulletin 191. Department of the Environment Fisheries and Marine Service, Ottawa.

Rogers, S.O. 1999. Population biology of the Tan Riffleshell (*Epioblasma florentina walkeri*) and the effects of substratum and light on juvenile mussel propagation. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Romberger, H.P. and C.E. Epifanio. 1981. Comparative effects of diets consisting of one or two algal species upon assimilation efficiencies and growth of juvenile oyster, *Crassostrea virginica* (Gmelin). Aquaculture 25: 77 – 87.

Rouse D.B., R.J. Kastner, and K.S. Reddy. 1995. Toxicity of ammonia and nitrite to hatchling redclaw crayfish, *Charax quadricarinatus*. Freshwater Crayfish 10: 298 – 303.

Saraceni, C. and D. Ruggiu. 1969. Techniques for sampling water and phytoplankton. Pages 5 - 7 *in* R.A. Vollenweider, editor. A manual for measuring primary production in aquatic environments. Blackwell Scientific Publications, Oxford.

Scheller, J. L. 1997. The effect of die-offs of asian clams (*Corbicula fluminea*) on native freshwater mussels (Unionidae). Master's thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Schlekat, C.E., B.L. McGee, D.M. Boward, E. Reinharz, D.J. Velinsky, and T.L. Wade. 1994. Tidal river sediments in the Washington, D.C. area III. Biological effects associated with sediment contamination. Estuaries 17(2): 334 – 344.

Schubauer-Berigan, M.K., P.D. Monson, C.W. West, and G.T. Ankley. 1995. Influence of pH on the toxicity of ammonia to *Chironomus tentans* and *Lumbriculus variegatus*. Environmental Toxicology and Chemistry 14: 713 – 717.

Shpigel, M., J. Loo, B. Soohoo, R. Fridman, and H. Gordin. 1993. Use of effluent water from fish-ponds as a food source for the Pacific oyster, *Crassostrea gigas* Thunberg. Aquaculture and Fisheries Management 24: 529 – 543.

Sickel, J.B. 1998. Gluttonous feeding behavior in the rhabdocoel, *Macrostomum* sp., induced by larvae of the asiatic clam, *Corbicula fluminea.* Journal of Freshwater Ecology 13(1): 135 – 137.

Steg, M.B. 1998. Identification of host fishes and experimental culture of juveniles for selected freshwater mussel species in Virginia. Master's thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Stephens, C.E. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-660 / 3 - 75 - 009. US Environmental Protection Agency, Corvallis, Oregon

Stevenson, R.J. and K.D. White. 1995. A comparison of natural and human determinants of phytoplankton communities in the Kentucky River basin, USA. Hydrobiologia 297: 201 – 216.

Stickney, R.R. 1994. Principles of aquaculture. John Wiley and Sons, New York, New York.

Stoyneva, M.P. and S.J. Dragnov. 1991. Green algae in the phytoplankton of the Danube – species composition, distribution, cell numbers and biomass. Archiv fur Protistenkunde 139: 243 – 260.

Strayer, D.L. 1999. Use of flow refuges by unionid mussels in rivers. Journal of the North American Benthological Society 18(4): 468 – 476.

Stuart, K.R., A.G. Eversole, and D.E. Brune. 1999. Effect of flow rate and temperature on algal uptake by freshwater mussels. Pages 219 – 224 *in* P.D. Johnson and R.S. Butler, editors. Proceedings of the first Freshwater Mollusk Conservation Society symposium. Columbus, Ohio.

Summers, J.M. 1998. Response of artificially and naturally transformed juvenile mussels, *Utterbackia imbecillis*, to environmental contaminants. Master's thesis, Clemson University, Clemson, South Carolina.

Thurston, R.V. 1980. Some factors affecting the toxicity of ammonia to fishes. Pages 118 – 137 *in* W.R. Swain and V.R. Shannon, editors. Proceedings of the third USA-USSR symposium on the effects of pollutants upon aquatic ecosystems. EPA-600/9-80-034. US Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota.

Trdan, R.J. amd W.R. Hoeh. 1982. Eurytopic host use by two congeneric species of freshwater mussel (Pelecypoda: Unionidae: Anodonta). American Midland Naturalist 108: 381 – 388.

Turgeon, D.D., J.F. Quinn, A.E. Bogan, E.V. Coan, F.G. Hochberg, W.K. Emerson, W.G. Lyons, P.M. Mikkelson, R.J. Neves, G. Rosenberg, B. Roth, M. Vecchione, C.F.E. Roper, A. Schelteman, F.G. Thompson, and J.D. Williams. 1998. Common and scientific names of aquatic invertebrates from the United States and

Canada: mollusks. American Fisheries Society Special Publication 26, Bethesda, MD.

US EPA (United States Environmental Protection Agency). 1984. Ambient water quality criteria for ammonia. EPA-440/5-85-001. US Environmental Protection Agency, Washington, D.C.

VA DEQ (Virginia Department of Environmental Quality). 2001a. Water quality monitoring homepage. <www.deq.state.va.us:4100/webapp/wqm.homepage> (September 22, 2001).

VA DEQ (Virginia Department of Environmental Quality). 2001b. Ambient water quality data for pH, temperature, and total ammonia (parameter 610), North Fork of Shenandoah River, 1996 - 2001. Harrisonburg, Virginia.

VA DEQ (Virginia Department of Environmental Quality). 2001c. Data from Archive Table DMRARC, parameter 312 for permits VA0026808, Saltville, 1994 – 1997; VA0024015, Jonesville, 1999 – 2001; VA0021016, Cleveland, 1996 – 2000. Abingdon, Virginia.

Vollenweider, R.A. 1969. A manual on methods for measuring primary production in aquatic environments. Blackwell Scientific Publications, Oxford and Edinburgh.

Wade, D.C., R.G. Hudson, and A.D. McKinney. 1992. Comparative response of *Ceriodaphnia dubia* and juvenile *Anodonta imbecilis* to selected complex industrial whole effluents. Pages 109 – 112 *in* S.K. Cummings, A.C. Buchanan, and L.M. Koch, editors. Conservation and management of freshwater mussels. Proceedings of a UMRCC symposium. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Warren, L.W. 1996. The use of juvenile mussels, *Utterbackia imbecillis* Say (Bivalvia: Unionidae), as a standardized toxicity testing organism. Doctoral Dissertation, Clemson University, Clemson, South Carolina.

Wehr, J.D. and J.H. Thorp. 1997. Effects of navigation dams, tributaries, and littoral zones on phytoplankton communities in the Ohio River. Canadian Journal of Fisheries and Aquatic Science 54: 378 – 395.

Weiss, J.L. and J.B. Layzer. 1993. Seasonal and spatial variation in glochidial infections of fish in the Barren River, Kentucky. Pages 72 – 75 *in* K.S. Cummins, A.C. Buchanan, and L.M Koch, editors. Conservation and management of freshwater mussels. Proceedings of a UMRCC symposium. Upper Mississippi River Conservation Committee, Rock Island, Illinois.

Whiteman, F.W., G.T. Ankely, M.D. Kahl, D.M. Rau, and M.D. Balcer. 1996. Evaluation of interstitial porewater as a route of exposure for ammonia in sediment tests with benthic macroinvertebrates. Environmental Toxicology and Chemistry. 15(5):794 – 801.

Wildridge, P.J., R.G. Werner, F.G. Doherty, E.F. Neuhauser. 1998. Acute toxicity of potassium to the adult zebra mussel *Dreissena polymorpha*. Archives of Environmental Contamination and Toxicology 34: 265 – 270.

Wilhm, J., T. Dorris, J.R. Seyfer, and N. McClintock. 1977. Seasonal variation in planktonic populations in the Arkansas River near the confluence of Red Rock Creek. Southwest Naturalist 22(4): 411 – 420.

Williams J.D., M.L. Warren, K.S. Cummings, J.L. Harris, and R.J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18: 6- 22.

Williams, K.A., D.W.J. Green, and D. Pascoe. 1986. Studies on the acute toxicity of pollutants to freshwater macroinvertebrates, 3. Ammonia. Archiv fur Hydrobiologie 106 (1): 61 – 70.

Yeager, M.M., D.S. Cherry, and R.J. Neves. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). Journal of the North American Benthological Society 13 (2): 217 – 222.

Young, M. and J. Williams. 1984. The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* L. in Scotland I. Field Studies. Archiv fur Hydrobiologie 99 (4): 405 – 422.

Zale, A.V. and Neves, R.J. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Canadian Journal of Zoology 60: 2535 – 2542.

Zischke J.A. and J.W. Arthur. 1987. Effects of elevated ammonia levels on the Fingernail Clam, *Musculium transversum*, in outdoor experimental streams. Archives of Environmental Contamination and Toxicology 16: 225 – 231.

Appendix A. Daily temperature parameters recorded in hatchery Flow-Through Pond over the 85-d study period.

Date	Daily Mean Temp. (°C)	Daily Min. Temp. (°C)	Daily Max. Temp. (°C)	Daily Temp. Flux (°C)	Date	Daily Mean Temp. (°C)	Daily Min. Temp. (°C)	Daily Max. Temp. (°C)	Daily Temp. Flux (°C)
6/22/01	22.49	20.19	24.31	4.12	8/4/01	24.92	22.86	28.31	5.45
6/23/01	19.65	18.28	21.33	3.05	8/5/01	25.54	22.86	29.10	6.24
6/24/01	18.92	16.76	21.33	4.57	8/6/01	26.72	24.01	30.31	6.30
6/25/01	19.76	17.14	22.86	5.72	8/7/01	27.09	24.40	30.31	5.91
6/26/01	20.57	17.90	23.63	5.73	8/8/01	25.94	22.09	28.70	6.61
6/27/01	21.61	19.04	24.79	5.75	8/9/01	22.80	18.28	27.91	9.63
6/28/01	21.84	19.42	24.79	5.37	8/10/01	25.66	24.01	27.91	3.90
6/29/01	21.59	19.42	24.01	4.59	8/11/01	26.66	24.79	29.10	4.31
6/30/01	21.46	18.28	25.56	7.28	8/12/01	26.57	24.79	28.70	3.91
7/1/01	20.73	19.04	22.86	3.82	8/13/01	26.54	24.79	29.10	4.31
7/2/01	21.45	19.04	24.79	5.75	8/14/01	25.63	23.63	27.91	4.28
7/3/01	22.47	19.81	25.56	5.75	8/15/01	24.78	22.48	27.91	5.43
7/4/01	22.41	21.33	23.24	1.91	8/16/01	22.84	21.33	25.95	4.62
7/5/01	22.13	20.19	25.17	4.98	8/17/01	23.04	21.33	25.17	3.84
7/6/01	23.51	20.57	27.12	6.55	8/18/01	22.35	20.19	24.40	4.21
7/7/01	23.67	20.19	27.12	6.93	8/19/01	21.62	19.42	23.63	4.21
7/8/01	23.58	21.71	25.56	3.85	8/20/01	19.38	18.28	20.57	2.29
7/9/01	24.99	22.48	28.31	5.83	8/21/01	19.19	16.76	22.09	5.33
7/10/01	26.23	24.01	28.70	4.69	8/22/01	20.06	17.52	23.24	5.72
7/11/01	25.99	24.01	28.31	4.30	8/23/01	20.35	18.28	22.48	4.20
7/12/01	24.63	22.09	27.12	5.03	8/24/01	21.14	19.42	23.63	4.21
7/13/01	24.00	20.95	29.50	8.55	8/25/01	21.75	19.42	24.40	4.98
7/14/01	21.13	17.90	24.01	6.11	8/26/01	21.56	19.42	24.01	4.59
7/15/01	22.01	18.66	25.56	6.90	8/27/01	21.19	19.42	23.24	3.82
7/16/01	23.23	19.81	27.12	7.31	8/28/01	21.00	19.42	22.48	3.06
7/17/01	23.84	21.33	26.34	5.01	8/29/01	21.25	19.04	24.01	4.97
7/18/01	23.29	22.09	25.17	3.08	8/30/01	21.86	19.81	24.79	4.98
7/19/01	24.41	22.09	27.52	5.43	8/31/01	21.33	20.19	22.48	2.29
7/20/01	24.01	22.86	25.56	2.70	9/1/01	21.13	19.81	23.24	3.43
7/21/01	23.54	20.57	27.12	6.55	9/2/01	20.73	18.66	23.24	4.58
7/22/01	24.40	20.95	28.31	7.36	9/3/01	19.74	19.04	20.57	1.53
7/23/01	25.50	22.09	29.10	7.01	9/4/01	19.84	17.90	22.48	4.58
7/24/01	26.47	23.63	29.90	6.27	9/5/01	20.95	18.66	24.01	5.35
7/25/01	26.18	24.79	27.52	2.73	9/6/01	20.86	18.66	23.63	4.97
7/26/01	24.50	23.63	25.56	1.93	9/7/01	21.25	19.04	24.01	4.97
7/27/01	22.41	21.71	23.63	1.92	9/8/01	21.51	19.42	24.01	4.59
7/28/01	20.36	19.04	21.71	2.67	9/9/01	21.46	18.66	24.40	5.74
7/29/01	18.71	18.28	19.04	0.76	9/10/01	21.20	20.19	22.09	1.90
7/30/01	20.67	18.28	24.40	6.12	9/11/01	20.39	18.28	23.24	4.96
7/31/01	23.59	20.95	26.73	5.78	9/12/01	20.25	17.90	23.24	5.34
8/1/01	24.71	22.48	27.91	5.43	9/13/01	20.14	18.28	22.86	4.58
8/2/01	25.91	22.09	31.12	9.03	9/14/01	19.20	18.28	20.19	1.91
8/3/01	24.26	19.81	27.12	7.31					
					Mean ± SD	22.95 2.04	20.58 2.00	25.73 2.53	5.15 1.88

Appendix B. Complete cell counts for all genera of algae observed in phytoplankton samples taken in hatchery Flow-Through and Algae Ponds between 7/5/01 and 9/14/ 01.

Sample date					7/5/01				7/19/01		8/1/01			
Sample location					Flow-Through		Algae		Flow-Through		Flow-Through		Algae	
Algae (genus)	Form	Group	Habit	Ingest-ible	# of cells	% of total	# of cells	% of total	# of cells	% of total	# of cells	% of total	# of cells	% of total
Aphanocapsa	C	BG	P	X	0	0	0	0	0	0	0	0	80	15.8
Chlamydomonas	U	Gr	P	J, A	2	0.4	99	19.5	5	1.1	70	17.4	84	16.6
Chlorella	U	Gr	P	J, A	275	52.3	242	47.7	234	53.4	154	38.3	197	38.9
Chlorococcum	U	Gr	B, P	J, A	26	4.9	16	3.2	62	14.2	24	6.0	16	3.2
Chodatella	U	Gr	P	J, A	1	0.2	0	0	0	0	0	0	0	0
Chroococcus	C	BG	B	X	0	0	0	0	0	0	0	0	0	0
Chroomonas	U	Cry	P	J, A	20	3.8	7	1.4	13	3.0	7	1.7	7	1.4
Closterium	U	Gr	B, P	A	0	0	0	0	0	0	0	0	0	0
Cocconeis	U	Di	B	J, A	6	1.1	16	3.2	8	1.8	5	1.2	8	1.6
Cromulina	U	Gol	P	J, A	22	4.2	0	0	19	4.3	1	0.2	1	0.2
Cyclotella	U	Di	P	J, A	2	0.4	3	0.6	0	0	3	0.7	0	0
Cymbella	U	Di	B	J, A	0	0	0	0	0	0	0	0	0	0
Diatoma	U	Di	B	X	16	3.0	6	1.2	28	6.4	27	6.7	7	1.4
Euglena	U	Eug	P	J, A	1	0.2	1	0.2	0	0	1	0.2	1	0.2
Fragilaria	C	Di	P	X	5	1.0	4	0.8	0	0	3	0.7	0	0
Gomphonema	U	Di	B	J, A	0	0	0	0	0	0	0	0	0	0
Koliella	U	Gr	P	J, A	0	0	0	0	0	0	0	0	0	0
Lyngbya	F	BG	B, P	X	17	3.2	16	3.2	12	2.7	0	0	0	0
Meridion	U	Di	B	J, A	10	1.9	6	1.2	4	0.9	6	1.5	0	0
Monocilia	F	Gr	B	X	0	0	14	2.8	0	0	0	0	1	0.2
Mougeotia	F	Gr	B	X	0	0	0	0	0	0	0	0	0	0
Nitzschia	U	Di	B	J, A	0	0	0	0	0	0	0	0	0	0
Oedogonium	F	Gr	B	X	0	0	0	0	0	0	0	0	0	0
Oocystis	U	Gr	P	J, A	1	0.2	4	0.8	2	0.5	7	1.7	3	0.6
Oscillatoria	F	BG	B, P	X	0	0	50	9.9	0	0	74	18.4	0	0
Pediastrum	U	Gr	P	J, A	35	6.7	0	0	0	0	0	0	0	0
Pennate diatom	U	Di	B	J, A	13	2.5	13	2.6	12	2.7	8	2.0	2	0.4
Pinnularia	U	Di	B	A	7	1.3	2	0.4	1	0.2	2	0.5	0	0
Protoderma	C	Gr	B	X	0	0	0	0	0	0	0	0	85	16.8
Schroederia	C	Gr	P	A	0	0	0	0	0	0	1	0.2	2	0.4
Selenstrum	C	Gr	P	A	0	0	0	0	0	0	0	0	0	0
Scenedesmus	C	Gr	P	J, A	28	5.3	5	1.0	13	3.0	4	1.0	7	1.4
Sphaeroplea	F	Gr	B	X	3	0.6	0	0	0	0	0	0	0	0
Spirulina	F	BG	P	J, A	0	0	0	0	0	0	0	0	0	0
Synedra	U	Di	P	J, A	23	4.4	2	0.4	0	0	3	0.7	5	1.0
Tabellaria	F, U	Di	P	X	5	1.0	0	0	0	0	2	0.5	0	0
Ulothrix	F	Gr	B	X	8	1.5	0	0	22	5.0	0	0	0	0
UnID'd diatom	U	Di	B, P	?	0	0	0	0	3	0.7	0	0	0	0
UnID'd green	U	Gr	B, P	?	0	0	1	0.2	0	0	0	0	0	0
TOTAL CELLS					526		507		438		402		506	
# SPP OF CELLS					24		21		17		20		16	
CELLS/ ML					6892		4382		3854		4339		4305	
% CELLS INGESTIBLE BY JUV.S					88.4		81.7		84.9		72.9		65.4	

Form is classified as either unicellular (U), colonial (C), or filamentous (F). Habit is classified as planktonic (P) or benthic (B), or both (P,B).

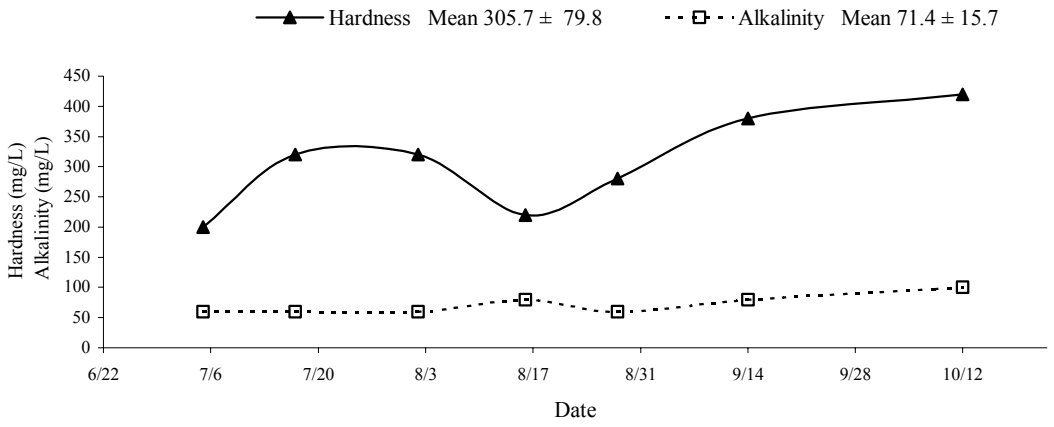
Group is classified as Cyanoprokaryota [bluegreen] (BG), Chlorophyta [green] (Gr), Euglenophyta [euglenoids] (Eug), Bacillariophyceae [diatom] (Di), Chrysophyceae [golden brown] (Gol), or Cryptophyta (Cry).

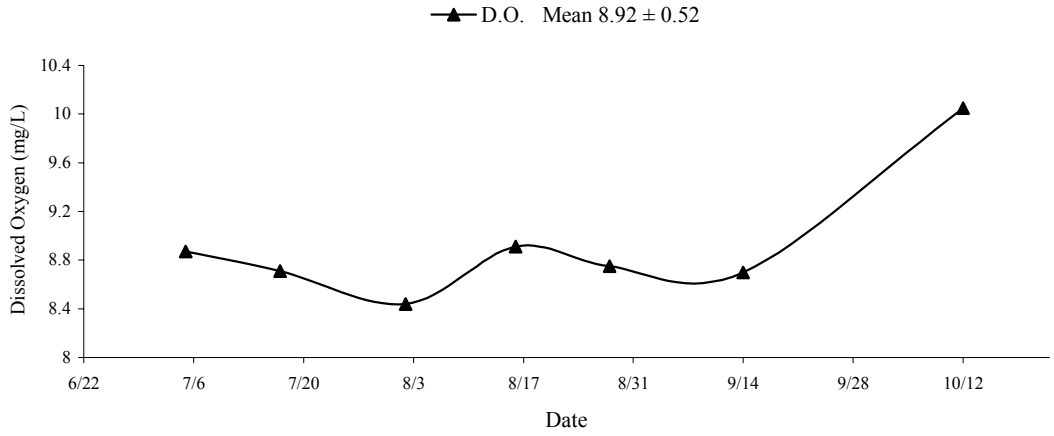
Ingestibility is subjectively evaluated based on a size, shape, cellular make-up, and growth form. Algae was classified as potentially ingestible by juveniles (J), ingestible by adults (A), or unlikely to be ingested by either (X).

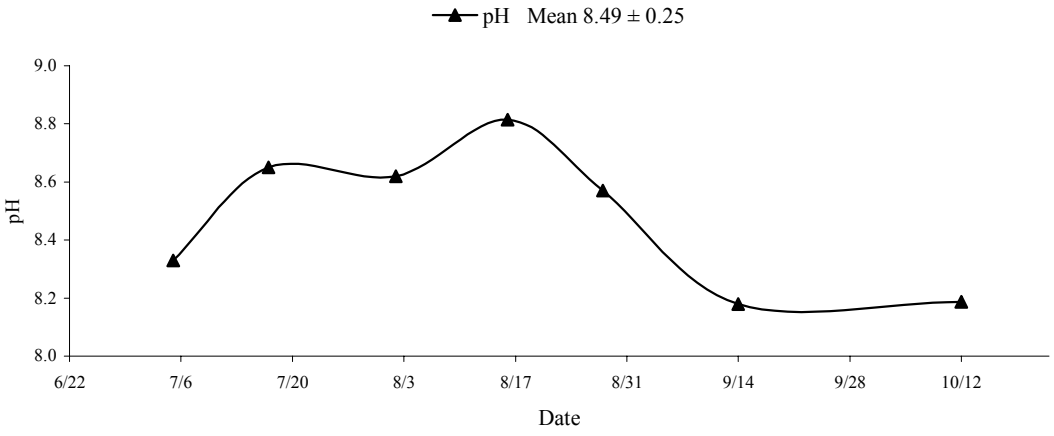
Appendix B. Continued. Complete cell counts for all genera of algae observed in phytoplankton samples taken in hatchery Flow-Through and Algae Ponds between 7/5/01 and 9/14/ 01.

Sample date	8/16/01				8/29/01				9/14/01			
Sample location	Flow-Through		Algae		Flow-Through		Algae		Flow-Through		Algae	
Algae (genus)	# of cells	% of total	# of cells	% of total	# of cells	% of total	# of cells	% of total	# of cells	% of total	# of cells	% of total
Aphanocapsa	0	0	0	0	0	0	0	0	0	0	0	0
Chlamydomonas	11	2.9	18	4.1	16	2.9	34	10.3	47	11.2	57	15.6
Chlorella	210	55.1	222	51.0	294	52.8	187	56.8	231	54.9	184	50.4
Chlorococcum	22	5.8	8	1.8	77	13.8	13	4.0	27	6.4	6	1.6
Chodatella	0	0	0	0	0	0	0	0	0	0	0	0
Chroococcus	0	0	0	0	0	0	0	0	23	5.5	9	2.5
Chroomonas	25	6.6	41	9.4	6	1.1	4	1.2	6	1.4	5	1.4
Closterium	2	0.5	0	0.0	0	0	0	0	0	0	0	0
Cocconeis	11	2.9	56	12.9	11	2.0	7	2.1	2	0.5	11	3.0
Cromulina	3	0.8	4	0.9	0	0	14	4.3	10	2.4	12	3.3
Cyclotella	3	0.8	0	0	1	0.2	1	0.3	3	0.7	4	1.1
Cymbella	0	0	0	0	1	0.2	0	0	0	0	0	0
Diatoma	13	3.4	10	2.3	11	2.0	5	1.5	8	1.9	10	2.7
Euglena	1	0.3	3	0.7	0	0	0	0	0	0	3	0.8
Fragilaria	0	0	4	0.9	2	0.4	1	0.3	0	0	0	0
Gomphonema	3	0.8	0	0	0	0	0	0	0	0	0	0
Koliella	0	0	0	0	0	0	0	0	0	0	2	0.5
Lyngbya	0	0	12	2.8	0	0	0	0	0	0	25	6.8
Meridion	6	1.6	7	1.6	13	2.3	1	0.3	7	1.7	4	1.1
Monocilia	0	0	0	0	0	0	0	0	0	0	0	0
Mougeotia	0	0	0	0	3	0.5	2	0.6	0	0	0	0
Nitzschia	0	0.0	0	0	0	0	0	0	0	0	0	0
Oedogonium	7	1.8	0	0	0	0	0	0	0	0	0	0
Oocystis	2	0.5	2	0.5	4	0.7	2	0.6	1	0.2	0	0
Oscillatoria	30	7.9	35	8.0	89	16.0	25	7.6	42	10.0	20	5.5
Pediastrum	0	0	0	0	0	0	16	4.9	0	0	0	0
Pennate diatom	10	2.6	1	0.2	9	1.6	4	1.2	4	1.0	3	0.8
Pinnularia	1	0.3	3	0.7	2	0.4	2	0.6	2	0.5	5	1.4
Protoderma	15	3.9	0	0	0	0	0	0	0	0	0	0
Schroederia	0	0	0	0	0	0	0	0	0	0	0	0
Selenstrum	0	0	0	0	0	0	2	0.6	2	0.5	0	0
Scenedesmus	6	1.6	4	0.9	8	1.4	6	1.8	0	0	0	0
Sphaeroplea	0	0	0	0	0	0	0	0	0	0	0	0
Spirulina	0	0	0	0	5	0.9	0	0	0	0	0	0
Synedra	0	0	5	1.1	5	0.9	3	0.9	6	1.4	5	1.4
Tabellaria	0	0	0	0	0	0	0	0	0	0	0	0
Ulothrix	0	0	0	0	0	0	0	0	0	0	0	0
UnID'd diatom	0	0	0	0	0	0	0	0	0	0	0	0
UnID'd green	0	0	0	0	0	0	0	0	0	0	0	0
	381		435		557		329		421		365	
	20		18		20		20		18		18	
	3284		3710		3605		2850		3599		3157	
	82.2		85.3		80.8		88.8		81.7		81.1	

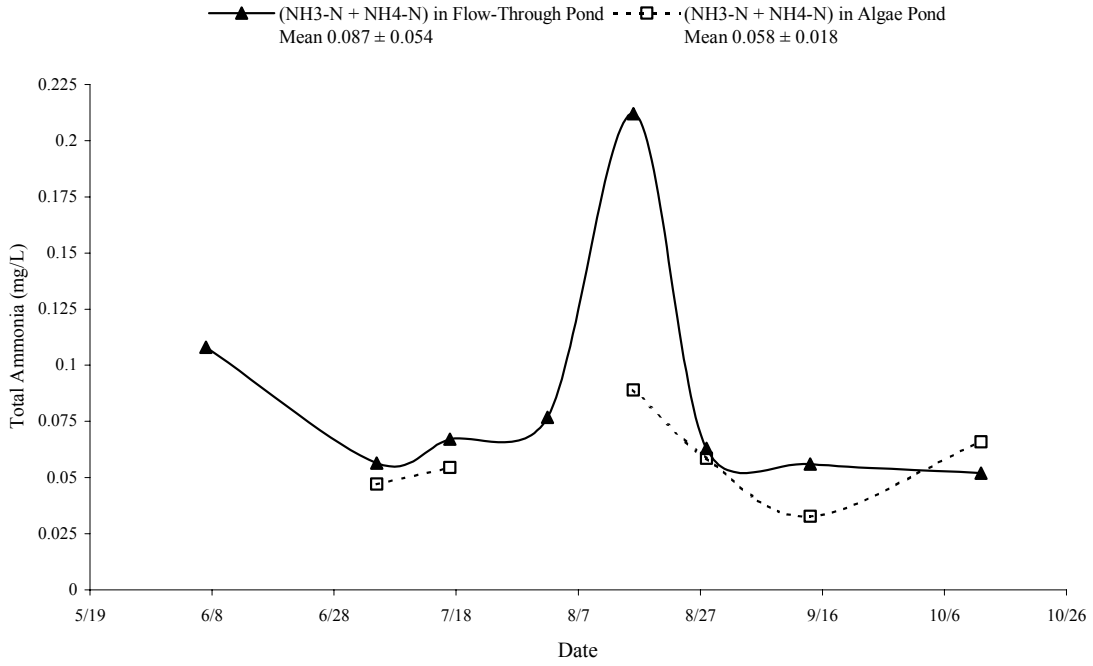
Appendix C.1. Hardness, alkalinity, dissolved oxygen, and pH measured periodically in the hatchery Flow-Through Pond.

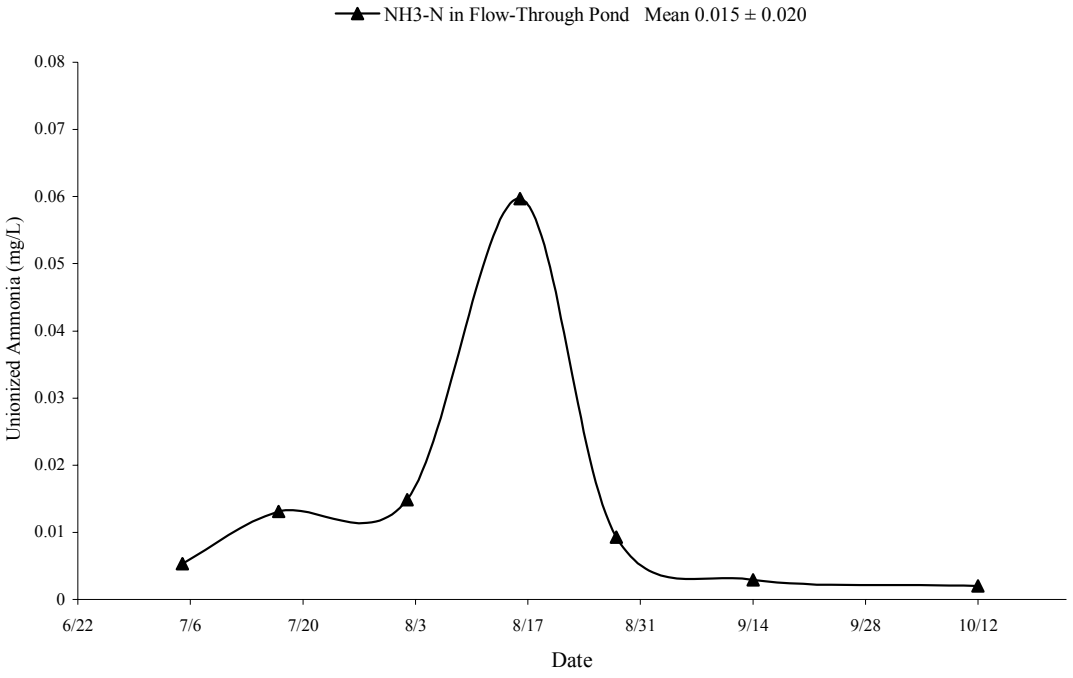




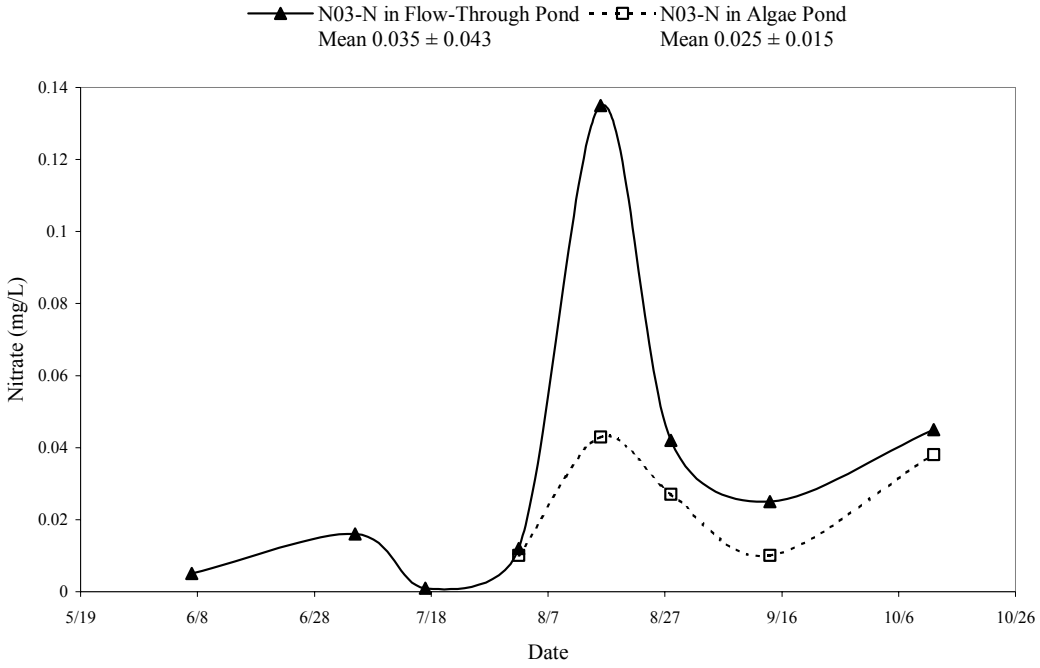


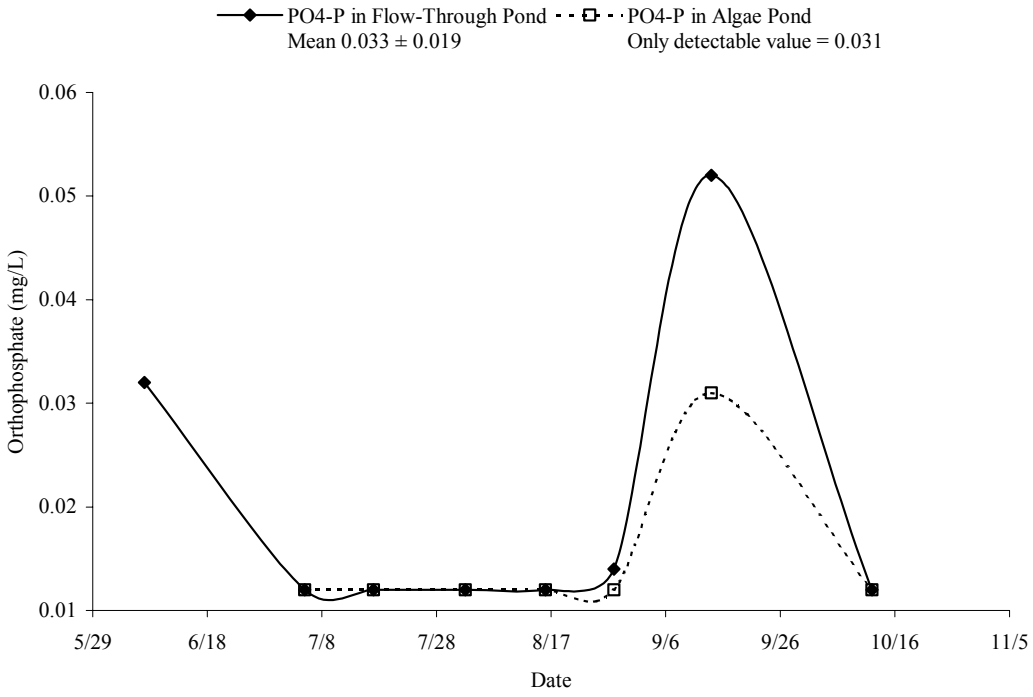
Appendix C.2. Continued. Total and Unionized Ammonia measured periodically in the hatchery Flow-Through and Algae Ponds.





Appendix C.3. Continued. Nitrate and Orthophosphate measured periodically in the hatchery Flow-Through and Algae Ponds.





Appendix C.4. Continued. Summary of water chemical values obtained throughout the 85-d study period. Values are for the Flow-Through Pond, except for the nutrient parameters for which values are listed for both ponds.

Date	Hardness†† (mg/L)	Alkalinity (mg/L)	D.O. (mg/L)	pH	Total Ammonia [mg (NH_3-N + NH_4-N)/L]	
					Algae Pond	Flow-Through Pond
6/7/2001	—	—	—	—	—	0.108**
7/5/2001	200	60	8.87	8.33	0.047	0.056
7/17/2001	320	60	8.71	8.65	0.055	0.067
8/2/2001	320	60	8.44	8.62	—	0.077
8/16/2001	220	80	8.91	8.82	0.089	0.212
8/28/2001	280	60	8.75	8.57	0.059	0.063
9/14/2001	380	80	8.70	8.18	0.033	0.056
10/12/2001	420	100	10.05	8.19	0.066**	0.052**

Date	Nitrate (mg NO_3-N/L)		Nitrite (mg NO_2-N/L)	Orthophosphate (mg PO_4-P/L)		Unionized Ammonia (mg NH_3-N/L)
	Algae Pond	Flow-Through Pond		Algae Pond	Flow-Through Pond	
6/7/2001	—	0.005*	0.001*	—	0.032†	—
7/5/2001	—	0.016	undet.	undet.	undet.	0.005
7/17/2001	—	undet.	undet.	undet.	undet.	0.013
8/2/2001	0.010	0.012	undet.	undet.	undet.	0.015
8/16/2001	0.043	0.135	undet.	undet.	undet.	0.060
8/28/2001	0.027	0.042	undet.	undet.	0.014	0.009
9/14/2001	0.010	0.025	undet.	0.031	0.052	0.003
10/12/2001	0.038*	0.045*	0.03*	undet.†	undet.†	0.002

* value measured by automated hydrazine reduction method at Seitz Water Quality Lab, VPI & SU

** value measured by automated phenate method at Seitz Water Quality Lab, VPI & SU

† value measured by automated ascorbic acid method at Seitz Water Quality Lab, VPI & SU

†† Sulphate ($SO_{4)}$ is the primary element contributing to hardness, followed by moderate levels of calcium (Ca) and lower levels of magnesium (Mg)

Appendix D. Complete cell counts for all genera of algae observed in phytoplanktonic samples taken in California Raceway between 6/3/00 and 10/7/ 00.

Sample date					6/3/00					
Sample location:										
Section of raceway					Compartment 1		Compartment 2		Compartment 3	
Algae				**Ingest-**	**# of**	**% of**	**# of**	**% of**	**# of**	**% of**
(genus)	**Form**	**Group**	**Habit**	**ible**	**cells**	**total**	**cells**	**total**	**cells**	**total**
Anabaena	C	BG	B, P	X	0	0.0	0	0.0	0	0.0
Ankistrodesmus	U	G	P	J, A	1	0.1	8	1.5	1	0.1
Chlamydomonas	U	G	P	J, A	12	1.3	4	0.7	14	1.7
Chlorella	U	G	P	J, A	594	66.4	315	57.0	510	63.5
Chlorococcum	U	G	B, P	J, A	47	8.3	23	4.2	86	1.1
Chroomonas	U	Cry	P	J, A	7	0.8	7	1.3	8	1.0
Cromulina	U	Gol	P	J, A	2	0.2	0	0.0	0	0.0
Cyclotella	U	Di	P	J, A	1	0.1	0	0.0	4	0.5
Cymbella	U	Di	B	J, A	0	0.0	0	0.0	0	0.0
Desmococcus	C	G	B	X	0	0.0	0	0.0	0	0.0
Diatoma	U	Di	B	J, A	2	0.2	0	0.0	0	0.0
Eudorina	C	G	P	A	0	0.0	0	0.0	30	3.7
Euglena	U	Eug	P	J, A	0	0.0	0	0.0	1	0.1
Golenkiniopsis	U	G	P	A	0	0.0	0	0.0	1	0.1
Gomphonema	U	Di	B	J, A	1	0.1	0	0.0	0	0.0
Gyrodinium	U	Dino	P	J, A	0	0.0	0	0.0	0	0.0
Lyngbya	F	BG	B, P	X	0	0.0	0	0.0	0	0.0
Mallomonas	U	Gol	P	J, A	0	0.0	1	0.2	0	0.0
Monocilia	F	G	B	X	0	0.0	0	0.0	0	0.0
Melosira	F	Di	P	X	1	0.1	4	0.7	0	0.0
Navicula	U	Di	B	J, A	45	5.0	52	9.4	33	4.1
Nitzschia	U	D	B	J, A	0	0.0	0	0.0	0	0.0
Oedogonium	F	G	B	X	0	0.0	0	0.0	0	0.0
Oscillatoria	F	BG	B, P	X	76	8.5	75	13.6	38	4.7
Pediastrum	U	G	P	J, A	0	0.0	0	0.0	0	0.0
Pennate diatom	U	Di	B	J, A	23	2.6	19	3.4	49	6.1
Peridinium	U	Dino	P	J, A	0	0.0	0	0.0	0	0.0
Phacus	U	Eug	P	J, A	0	0.0	0	0.0	0	0.0
Pinnularia	U	Di	B	A	3	0.3	3	0.5	0	0.0
Selenstrum	C	G	P	A	5	0.6	0	0.0	2	0.3
Scenedesmus	C	G	P	J, A	0	0.0	0	0.0	0	0.0
Sphaeroplea	F	G	B	X	0	0.0	0	0.0	0	0.0
Spirulina	F	BG	P	J, A	0	0.0	0	0.0	0	0.0
Spirogyra	F	G	B	X	0	0.0	0	0.0	0	0.0
Staurastrum	U	G	P	J, A	0	0.0	0	0.0	0	0.0
Stigeoclonium	F	G	B	X	0	0.0	0	0.0	0	0.0
Synedra	U	Di	P	J, A	34	3.8	21	3.8	10	1.3
Tabellaria	F, U	Di	P	X	1	0.1	0	0.0	0	0.0
Triangular diatom/ Triceratium	U	Di	B	J, A	0	0.0	0	0.0	0	0.0
Ulothrix	F	G	B	X	22	2.5	20	3.6	0	0.0
Unidentified green					0	0.0	0	0.0	0	0.0
Zygospore					0	0.0	0	0.0	0	0.0
TOTAL CELLS					894		553		803	
# SPP OF CELLS					18		13		14	
CELLS/ ML					5704		1178		5124	

Form is classified as either unicellular (U), colonial (C), or filamentous (F). Habit is classified as planktonic (P) or benthic (B), or both.

Group is classified as Cyanoprokaryota [bluegreen] (BG), Chlorophyta [green] (Gr), Euglenophyta [euglenoids] (Eug), Bacillariophyceae [diatom] (Di), Chrysophyceae [golden brown] (Gol), or Cryptophyta (Cry).

Ingestibility is subjectively evaluated based on a size, shape, cellular make-up, and growth form. Algae was classified as potentially ingestible by juveniles (J), ingestible by adults (A), or unlikely to be ingested by either (X).

Appendix D. Continued. Complete cell counts for all genera of algae observed in phytoplankton samples taken in California Raceway between 6/3/00 and 10/7/ 00.

Sample date	6/12/00						7/15/00					
Sample location:												
Section of raceway	Compartment 1		Compartment 2		Compartment 3		Compartment 1		Compartment 2		Compartment 3	
Algae (genus)	**# of cells**	**% of total**	**# of cells**	**% of total**	**# of cells**	**% of total**	**# of cells**	**% of total**	**# of cells**	**% of total**	**# of cells**	**% of total**
Anabaena	31	4.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Ankistrodesmus	0	0.0	4	0.5	1	0.2	2	0.1	30	4.2	0	0.0
Chlamydomonas	16	2.5	0	0.0	5	1.1	6	0.4	5	0.7	0	0.0
Chlorella	280	4.3	340	42.2	274	57.3	610	40.1	477	66.8	390	39.5
Chlorococcum	95	1.5	46	5.7	28	5.9	10	0.7	9	1.3	74	7.5
Chroomonas	19	2.9	35	4.3	6	1.3	38	2.5	7	1.0	20	2.0
Cromulina	0.28	0.0	0	0.0	0	0.0	4	0.3	0	0.0	1	0.1
Cyclotella	0	0.0	8	1.0	3	0.6	10	0.7	5	0.7	8	0.8
Cymbella	14	2.2	1	0.1	1	0.2	0	0.0	0	0.0	0	0.0
Desmococcus	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Diatoma	5	0.8	10	1.2	9	1.9	0	0.0	1	0.1	7	0.7
Eudorina	13	2.0	16	2.0	0	0.0	0	0.0	0	0.0	0	0.0
Euglena	4	0.6	1	0.1	0	0.0	4	0.3	3	0.4	0	0.0
Golenkiniopsis	0	0.0	1	0.1	0	0.0	0	0.0	2	0.3	0	0.0
Gonphonema	0	0.0	6	0.7	0	0.0	0	0.0	0	0.0	0	0.0
Gyrodinium	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Lyngbya	21	3.3	35	4.3	0	0.0	0	0.0	0	0.0	0	0.0
Mallomonas	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1
Monocilia	1	0.2	9	1.1	0	0.0	0	0.0	0	0.0	0	0.0
Melosira	2	0.3	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0
Navicula	57	8.8	94	11.7	45	9.4	31	2.0	47	6.6	26	2.6
Nitzschia	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Oedogonium	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Oscillatoria	34	5.3	60	7.4	77	16.1	122	8.0	105	14.7	71	7.2
Pediastrum	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Pennate diatom	3	0.5	15	1.9	10	2.1	17	1.1	3	0.4	26	2.6
Peridinium	10	1.6	3	0.4	0	0.0	4	0.3	0	0.0	2	0.2
Phacus	0	0.0	1	0.1	1	0.2	0	0.0	0	0.0	0	0.0
Pinnularia	4	0.6	7	0.9	4	0.8	13	0.9	2	0.3	5	0.5
Selenstrum	4	0.6	1	0.1	2	0.4	5	0.3	6	0.8	6	0.6
Scenedesmus	25	3.6	78	9.7	0	0.0	576	37.9	0	0.0	345	35.0
Sphaeroplea	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0
Spirulina	1	0.2	1	0.1	1	0.2	2	0.1	0	0.0	0	0.0
Spirogyra	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Staurastrum	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Stigeoclonium	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Synedra	2	0.3	20	2.5	11	2.3	10	0.7	2	0.3	5	0.5
Tabellaria	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Triangular diatom/ Triseratium	1	0.2	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0
Ulothrix	0	0.0	10	1.2	0	0.0	56	3.7	0	0.0	0	0.0
Unidentified green	4	0.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Zygospore	0	0.0	0	0.0	0	0.0	2	0.1	0	0.0	0	0.0
TOTAL CELLS	647.28		806		478		1522		704		987	
# SPP OF CELLS	24		27		16		19		16		15	
CELLS/ ML	4129		1717		3050		3234		1107		6298	

Appendix D. Continued. Complete cell counts for all genera of algae observed in phytoplankton samples taken in California Raceway between 6/3/00 and 10/7/ 00.

Sample date	8/1	8/15/00						8/1	8/31/01					
Sample location:														
Section of raceway		Compartment 1		Compartment 2		Compartment 3			Compartment 1		Compartment 2		Compartment 3	
Algae		**# of**	**% of**	**# of**	**% of**	**# of**	**% of**		**# of**	**% of**	**# of**	**% of**	**# of**	**% of**
(genus)		**cells**	**total**	**cells**	**total**	**cells**	**total**		**cells**	**total**	**cells**	**total**	**cells**	**total**
Anabaena		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Ankistrodesmus		0	0.0	0	0.0	3	0.4		0	0.0	6	0.5	1	0.1
Chlamydomonas		4	0.5	8	1.0	10	1.3		6	0.8	20	1.6	17	2.3
Chlorella		320	36.4	325	40.8	339	43.3		200	25.1	330	26.9	202	26.9
Chlorococcum		88	0.1	86	10.8	7	0.9		18	2.3	212	17.3	43	5.7
Chroomonas		12	1.4	10	1.3	7	0.9		48	6.0	37	3.0	0	0.0
Cromulina		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Cyclotella		8	0.9	8	1.0	19	2.4		25	3.1	30	2.5	26	3.5
Cymbella		0	0.0	0	0.0	0	0.0		0	0.0	7	0.6	10	1.3
Desmococcus		8	0.9	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Diatoma		0	0.0	4	0.5	0	0.0		0	0.0	8	0.7	0	0.0
Eudorina		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Euglena		7	0.8	10	1.3	0	0.0		0	0.0	5	0.4	1	0.1
Golenkiniopsis		0	0.0	0	0.0	0	0.0		0	0.0	2	0.2	0	0.0
Gonphonema		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Gyrodinium		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Lyngbya		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Mallomonas		0	0.0	0	0.0	0	0.0		5	0.6	1	0.1	0	0.0
Monocilia		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Melosira		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Navicula		22	2.5	46	5.8	25	3.2		21	2.6	57	4.7	12	1.6
Nitzschia		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Oedogonium		4	0.5	0	0.0	0	0.0		22	2.8	0	0.0	0	0.0
Oscillatoria		201	22.8	46	5.8	170	21.7		97	12.2	194	15.8	401	53.5
Pediastrum		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Pennate diatom		0	0.0	15	1.9	0	0.0		8	1.0	81	6.6	6	0.8
Peridinium		1	0.1	0	0.0	0	0.0		1	0.1	6	0.5	1	0.1
Phacus		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Pinnularia		3	0.3	3	3.8	3	0.4		5	0.6	2	0.2	0	0.0
Selenstrum		5	0.6	5	0.6	4	0.5		1	0.1	1	0.1	0	0.0
Scenedesmus		138	15.7	163	20.5	136	17.4		329	41.3	118	9.6	22	2.9
Sphaeroplea		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Spirulina		0	0.0	0	0.0	0	0.0		0	0.0	3	0.3	0	0.0
Spirogyra		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Staurastrum		0	0.0	0	0.0	0	0.0		0	0.0	2	0.2	3	0.4
Stigeoclonium		0	0.0	0	0.0	0	0.0		0	0.0	24	2.0	0	0.0
Synedra		2	0.2	6	0.8	0	0.0		4	0.5	4	0.3	0	0.0
Tabellaria		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Triangular diatom/ Triceratium		0	0.0	0	0.0	0	0.0		0	0.0	3	0.3	0	0.0
Ulothrix		57	6.5	61	7.7	60	7.7		7	0.9	72	5.9	5	0.7
Unidentified green		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
Zygospore		0	0.0	0	0.0	0	0.0		0	0.0	0	0.0	0	0.0
TOTAL CELLS		880		796		783			797		1225		750	
# SPP OF CELLS		16		16		13			16		24		14	
CELLS/ ML		5616		1696		4997			5086		2610		4786	

Flows reduced

Solar cover installed

Appendix D. Continued. Complete cell counts for all genera of algae observed in phytoplankton samples taken in California Raceway between 6/3/00 and 10/7/ 00.

Sample date	9/19/01						10/7/00					
Sample location:												
Section of raceway	Compartment 1		Compartment 2		Compartment 3		Compartment 1		Compartment 2		Compartment 3	
Algae	**# of**	**% of**	**# of**	**% of**	**# of**	**% of**	**# of**	**% of**	**# of**	**% of**	**# of**	**% of**
(genus)	**cells**	**total**	**cells**	**total**	**cells**	**total**	**cells**	**total**	**cells**	**total**	**cells**	**total**
Anabaena	125	11.3	0	0.0	60	6.4	0	0.0	0	0.0	0	0.0
Ankistrodesmus	2	0.2	0	0.0	3	0.3	0	0.0	1	0.1	0	0.0
Chlamydomonas	5	0.5	7	1.2	12	1.3	13	2.8	25	2.2	20	3.0
Chlorella	375	33.9	292	49.4	255	27.3	193	41.5	500	43.1	257	38.4
Chlorococcum	142	12.8	4	0.7	236	25.2	10	2.2	171	14.7	56	8.4
Chroomonas	37	3.3	16	2.7	19	2.0	16	3.4	12	1.0	7	1.0
Cromulina	0	0.0	0	0.0	0	0.0	0	0.0	2	0.2	1	0.1
Cyclotella	12	1.1	14	2.4	11	1.2	18	3.9	18	1.6	9	1.3
Cymbella	1	0.1	0	0.0	5	0.5	4	0.9	0	0.0	1	0.1
Desmococcus	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Diatoma	22	2.0	0	0.0	14	1.5	0	0.0	24	2.1	9	1.3
Eudorina	0	0.0	0	0.0	0	0.0	0	0.0	16	1.4	0	0.0
Euglena	8	0.7	0	0.0	2	0.2	2	0.4	1	0.1	3	0.4
Golenkiniopsis	1	0.1	0	0.0	2	0.2	0	0.0	2	0.2	2	0.3
Gonphonema	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Gyrodinium	0	0.0	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0
Lyngbya	15	1.4	0	0.0	1	0.1	0	0.0	0	0.0	5	0.7
Mallomonas	1	0.1	1	0.2	1	0.1	2	0.4	2	0.2	4	0.6
Monocilia	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Melosira	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Navicula	55	5.0	25	4.2	40	4.3	16	3.4	56	4.8	32	4.8
Nitzschia	0	0.0	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0
Oedogonium	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Oscillatoria	93	8.4	114	19.3	79	8.4	68	14.6	55	4.7	92	13.8
Pediastrum	0	0.0	0	0.0	0	0.0	16	3.4	0	0.0	0	0.0
Pennate diatom	59	5.3	1	0.2	52	5.6	3	0.6	82	7.1	16	2.4
Peridinium	7	0.6	1	0.2	0	0.0	0	0.0	4	0.3	2	0.3
Phacus	0	0.0	0	0.0	2	0.2	0	0.0	0	0.0	0	0.0
Pinnularia	0	0.0	1	0.2	0	0.0	2	0.4	4	0.3	0	0.0
Selenstrum	0	0.0	0	0.0	5	0.5	0	0.0	1	0.1	2	0.3
Scenedesmus	90	8.1	104	17.6	96	10.3	65	14.0	76	6.5	130	19.4
Sphaeroplea	0	0.0	0	0.0	2	0.2	0	0.0	0	0.0	0	0.0
Spirulina	5	0.5	0	0.0	4	0.4	0	0.0	1	0.1	0	0.0
Spirogyra	0	0.0	0	0.0	1	0.1	0	0.0	1	0.1	0	0.0
Staurastrum	0	0.0	0	0.0	0	0.0	2	0.4	0	0.0	0	0.0
Stigeoclonium	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Synedra	25	2.3	11	1.9	6	0.6	31	6.7	75	6.5	13	1.9
Tabellaria	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Triangular diatom/ Triceratium	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1
Ulothrix	25	2.3	0	0.0	25	2.7	4	0.9	32	2.8	7	1.0
Unidentified green	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Zygospore	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
TOTAL CELLS	1106		591		935		465		1161		669	
# SPP OF CELLS	22		13		26		17		23		21	
CELLS/ ML	2356		3771		1992		2967		2474		4269	

Appendix E.1.1. Results of bioassay used to calculate 24 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.45	12.4	10	0
Control B	0.00	0.00	7.40	12.4	10	1
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	0
1 A	10.3	0.054	7.36	12.6	10	0
1 B	10.8	0.057	7.37	12.6	10	0
1 C	—	—	—	—	10	1
1 D	—	—	—	—	10	1
2 A	19.8	0.13	7.45	12.7	10	2
2 B	20.1	0.13	7.45	12.8	10	2
2 C	—	—	—	—	10	1
2 D	—	—	—	—	10	1
3 A	40.9	0.24	7.41	12.6	10	6
3 B	41.2	0.24	7.42	12.7	10	7
3 C	—	—	—	—	10	5
3 D	—	—	—	—	10	7
4 A	80.7	0.43	7.37	12.8	10	6
4 B	80.7	0.43	7.36	12.8	10	8
4 C	—	—	—	—	10	7
4 D	—	—	—	—	10	8
5 A	163	0.72	7.29	12.6	10	7
5 B	164	0.70	7.28	12.6	10	7
5 C	—	—	—	—	10	8
5 D	—	—	—	—	10	7

Appendix E.1.2. Results of bioassay used to calculate 48 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.43	12.3	10	0
Control B	0.00	0.00	7.39	12.5	10	1
Control C	—	—	—	—	10	1
Control D	—	—	—	—	10	0
1 A	10.5	0.054	7.36	12.7	10	2
1 B	10.9	0.057	7.36	12.6	10	1
1 C	—	—	—	—	10	2
1 D	—	—	—	—	10	1
2 A	19.8	0.12	7.44	12.8	10	3
2 B	19.9	0.12	7.43	12.7	10	4
2 C	—	—	—	—	10	1
2 D	—	—	—	—	10	2
3 A	40.7	0.21	7.36	12.6	10	8
3 B	40.9	0.22	7.37	12.7	10	7
3 C	—	—	—	—	10	7
3 D	—	—	—	—	10	9
4 A	80.4	0.38	7.32	12.8	10	9
4 B	80.6	0.38	7.31	12.8	10	10
4 C	—	—	—	—	10	8
4 D	—	—	—	—	10	9
5 A	162	0.64	7.24	12.6	10	8
5 B	163	0.64	7.24	12.6	10	9
5 C	—	—	—	—	10	8
5 D	—	—	—	—	10	8

Appendix E.1.3. Results of bioassay used to calculate 72 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.40	12.2	10	0
Control B	0.00	0.00	7.36	12.3	10	1
Control C	—	—	—	—	10	1
Control D	—	—	—	—	10	0
1 A	11.0	0.057	7.36	12.6	10	3
1 B	11.3	0.058	7.36	12.6	10	1
1 C	—	—	—	—	10	3
1 D	—	—	—	—	10	1
2 A	20.3	0.12	7.40	12.5	10	6
2 B	20.4	0.11	7.39	12.5	10	5
2 C	—	—	—	—	10	2
2 D	—	—	—	—	10	4
3 A	41.3	0.20	7.33	12.6	10	9
3 B	19.2	0.20	7.32	12.6	10	9
3 C	—	—	—	—	10	8
3 D	—	—	—	—	10	9
4 A	81.4	0.36	7.28	12.7	10	9
4 B	81.5	0.36	7.27	12.7	10	10
4 C	—	—	—	—	10	8
4 D	—	—	—	—	10	9
5 A	161.7	0.61	7.21	12.6	10	9
5 B	162.5	0.62	7.22	12.5	10	10
5 C	—	—	—	—	10	10
5 D	—	—	—	—	10	10

Appendix E.1.4. Results of bioassay used to calculate 96 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.44	12.2	10	1
Control B	0.00	0.00	7.37	12.3	10	1
Control C	—	—	—	—	10	1
Control D	—	—	—	—	10	0
1 A	11.4	0.053	7.32	12.5	10	4
1 B	11.6	0.055	7.32	12.5	10	3
1 C	—	—	—	—	10	3
1 D	—	—	—	—	10	3
2 A	20.7	0.11	7.38	12.5	10	7
2 B	20.8	0.11	7.37	12.5	10	7
2 C	—	—	—	—	10	2
2 D	—	—	—	—	10	4
3 A	41.9	0.19	7.30	12.6	10	10
3 B	42.1	0.19	7.30	12.6	10	9
3 C	—	—	—	—	10	9
3 D	—	—	—	—	10	10
4 A	82.6	0.34	7.26	12.7	10	9
4 B	82.6	0.34	7.26	12.6	10	10
4 C	—	—	—	—	10	10
4 D	—	—	—	—	10	9
5 A	162	0.54	7.17	12.5	10	9
5 B	163	0.55	7.18	12.5	10	10
5 C	—	—	—	—	10	10
5 D	—	—	—	—	10	10

Appendix E.2.1. Results of bioassay used to calculate 24 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.47	20.2	10	0
Control B	0.00	0.00	7.45	20.4	10	0
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	1
1 A	5.17	0.054	7.42	20.4	10	0
1 B	5.20	0.056	7.42	20.7	10	0
1 C	—	—	—	—	10	1
1 D	—	—	—	—	10	0
2 A	9.91	0.093	7.37	20.4	10	0
2 B	9.95	0.094	7.37	20.4	10	0
2 C	—	—	—	—	10	0
2 D	—	—	—	—	10	0
3 A	19.3	0.19	7.38	20.6	10	2
3 B	19.9	0.21	7.40	20.6	10	3
3 C	—	—	—	—	10	1
3 D	—	—	—	—	10	2
4 A	37.9	0.36	7.37	20.7	10	4
4 B	37.9	0.37	7.37	20.8	10	7
4 C	—	—	—	—	10	6
4 D	—	—	—	—	10	8
5 A	75.9	0.57	7.27	20.6	10	10
5 B	76.5	0.59	7.27	20.6	10	7
5 C	—	—	—	—	10	9
5 D	—	—	—	—	10	8

Appendix E.2.2. Results of bioassay used to calculate 48 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.45	20.5	10	0
Control B	0.00	0.00	7.47	20.5	10	0
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	2
1 A	5.11	0.58	7.45	20.5	10	3
1 B	5.13	0.59	7.45	20.6	10	1
1 C	—	—	—	—	10	2
1 D	—	—	—	—	10	2
2 A	9.90	0.09	7.35	20.5	10	2
2 B	9.76	0.09	7.35	20.5	10	1
2 C	—	—	—	—	10	1
2 D	—	—	—	—	10	2
3 A	19.4	0.20	7.39	20.6	10	5
3 B	19.7	0.20	7.39	20.5	10	8
3 C	—	—	—	—	10	4
3 D	—	—	—	—	10	6
4 A	38.3	0.36	7.36	20.6	10	8
4 B	38.2	0.36	7.36	20.5	10	9
4 C	—	—	—	—	10	10
4 D	—	—	—	—	10	10
5 A	76.2	0.60	7.28	20.5	10	10
5 B	76.5	0.60	7.29	20.5	10	10
5 C	—	—	—	—	10	9
5 D	—	—	—	—	10	8

Appendix E.2.3. Results of bioassay used to calculate 72 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.48	20.5	10	2
Control B	0.00	0.00	7.49	20.5	10	0
Control C	—	—	—	—	10	1
Control D	—	—	—	—	10	1
1 A	11.0	0.059	7.45	20.5	10	3
1 B	11.3	0.062	7.46	20.6	10	1
1 C	—	—	—	—	10	2
1 D	—	—	—	—	10	2
2 A	9.7	0.11	7.40	20.5	10	3
2 B	9.6	0.11	7.41	20.6	10	3
2 C	—	—	—	—	10	5
2 D	—	—	—	—	10	2
3 A	19.1	0.23	7.46	20.6	10	8
3 B	19.2	0.23	7.45	20.5	10	9
3 C	—	—	—	—	10	8
3 D	—	—	—	—	10	9
4 A	38.2	0.41	7.40	20.6	10	10
4 B	38.0	0.40	7.40	20.5	10	9
4 C	—	—	—	—	10	10
4 D	—	—	—	—	10	10
5 A	75.8	0.68	7.33	20.5	10	10
5 B	76.1	0.67	7.32	20.6	10	10
5 C	—	—	—	—	10	9
5 D	—	—	—	—	10	9

Appendix E.2.4. Results of bioassay used to calculate 96 h LC_{50} values of total and unionized ammonia for *Villosa iris* at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, pH, and temperature were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.43	20.5	10	2
Control B	0.00	0.00	7.47	20.6	10	0
Control C	—	—	—	—	10	1
Control D	—	—	—	—	10	2
1 A	11.0	0.057	7.44	20.6	10	3
1 B	11.3	0.059	7.46	20.6	10	1
1 C	—	—	—	—	10	2
1 D	—	—	—	—	10	2
2 A	9.6	0.098	7.40	20.5	10	4
2 B	9.6	0.10	7.41	20.6	10	4
2 C	—	—	—	—	10	5
2 D	—	—	—	—	10	2
3 A	18.9	0.21	7.44	20.6	10	10
3 B	19.2	0.21	7.44	20.6	10	9
3 C	—	—	—	—	10	9
3 D	—	—	—	—	10	10
4 A	38.1	0.38	7.39	20.6	10	10
4 B	37.9	0.38	7.38	20.6	10	10
4 C	—	—	—	—	10	10
4 D	—	—	—	—	10	10
5 A	75.5	0.64	7.32	20.6	10	10
5 B	75.6	0.64	7.31	20.6	10	10
5 C	—	—	—	—	10	10
5 D	—	—	—	—	10	10

Appendix E.3.1. Results of bioassay used to calculate 24 h LC_{50} values of total and unionized ammonia for *Lampsilis fasciola* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, temperature, and pH were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.87	12.4	10	0
Control B	0.00	0.00	7.89	12.5	10	0
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	0
1 A	3.21	0.058	7.91	12.5	10	1
1 B	3.12	0.056	7.92	12.4	10	0
1 C	—	—	—	—	10	0
1 D	—	—	—	—	10	0
2 A	5.77	0.10	7.90	12.4	10	0
2 B	5.76	0.11	7.92	12.5	10	2
2 C	—	—	—	—	10	0
2 D	—	—	—	—	10	0
3 A	12.1	0.20	7.87	12.4	10	2
3 B	12.2	0.20	7.87	12.5	10	0
3 C	—	—	—	—	10	0
3 D	—	—	—	—	10	2
4 A	24.3	0.37	7.83	12.4	10	6
4 B	24.4	0.37	7.83	12.4	10	9
4 C	—	—	—	—	10	2
4 D	—	—	—	—	10	8
5 A	46.7	0.61	7.77	12.6	10	10
5 B	47.1	0.60	7.76	12.5	10	8
5 C	—	—	—	—	10	10
5 D	—	—	—	—	10	10

Appendix E.3.2. Results of bioassay used to calculate 48 h LC_{50} values of total and unionized ammonia for *Lampsilis fasciola* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, temperature, and pH were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.87	12.4	10	0
Control B	0.00	0.00	7.89	12.5	10	0
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	1
1 A	3.19	0.050	7.85	12.5	10	1
1 B	3.18	0.050	7.85	12.5	10	1
1 C	—	—	—	—	10	0
1 D	—	—	—	—	10	0
2 A	5.74	0.091	7.86	12.5	10	0
2 B	5.86	0.095	7.87	12.5	10	2
2 C	—	—	—	—	10	0
2 D	—	—	—	—	10	0
3 A	12.0	0.18	7.82	12.4	10	2
3 B	12.2	0.18	7.83	12.5	10	1
3 C	—	—	—	—	10	0
3 D	—	—	—	—	10	2
4 A	24.2	0.33	7.78	12.4	10	10
4 B	24.6	0.33	7.78	12.4	10	10
4 C	—	—	—	—	10	10
4 D	—	—	—	—	10	10
5 A	46.3	0.55	7.73	12.6	10	10
5 B	46.8	0.54	7.71	12.6	10	10
5 C	—	—	—	—	10	10
5 D	—	—	—	—	10	10

Appendix E.3.3. Results of bioassay used to calculate 72 h LC_{50} values of total and unionized ammonia for *Lampsilis fasciola* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, temperature, and pH were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.77	12.7	10	0
Control B	0.00	0.00	7.89	12.7	10	0
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	2
1 A	3.17	0.053	7.87	12.8	10	1
1 B	3.15	0.052	7.87	12.7	10	1
1 C	—	—	—	—	10	1
1 D	—	—	—	—	10	0
2 A	5.58	0.091	7.86	12.7	10	0
2 B	5.77	0.096	7.87	12.7	10	3
2 C	—	—	—	—	10	0
2 D	—	—	—	—	10	0
3 A	12.1	0.19	7.84	12.7	10	4
3 B	12.2	0.19	7.84	12.7	10	1
3 C	—	—	—	—	10	1
3 D	—	—	—	—	10	2
4 A	24.2	0.33	7.78	12.4	10	10
4 B	24.6	0.33	7.78	12.4	10	10
4 C	—	—	—	—	10	10
4 D	—	—	—	—	10	10
5 A	46.3	0.55	7.73	12.6	10	10
5 B	46.8	0.54	7.71	12.6	10	10
5 C	—	—	—	—	10	10
5 D	—	—	—	—	10	10

Appendix E.3.4. Results of bioassay used to calculate 96 h LC_{50} values of total and unionized ammonia for *Lampsilis fasciola* at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, temperature, and pH were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.81	12.7	10	0
Control B	0.00	0.00	7.90	12.7	10	0
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	2
1 A	3.12	0.054	7.88	12.7	10	1
1 B	3.09	0.053	7.88	12.7	10	1
1 C	—	—	—	—	10	1
1 D	—	—	—	—	10	0
2 A	5.62	0.096	7.88	12.7	10	0
2 B	5.78	0.10	7.89	12.6	10	3
2 C	—	—	—	—	10	1
2 D	—	—	—	—	10	0
3 A	12.3	0.20	7.87	12.6	10	4
3 B	12.4	0.21	7.87	12.7	10	1
3 C	—	—	—	—	10	2
3 D	—	—	—	—	10	2
4 A	24.2	0.33	7.78	12.4	10	10
4 B	24.6	0.33	7.78	12.4	10	10
4 C	—	—	—	—	10	10
4 D	—	—	—	—	10	10
5 A	46.3	0.55	7.73	12.6	10	10
5 B	46.8	0.54	7.71	12.6	10	10
5 C	—	—	—	—	10	10
5 D	—	—	—	—	10	10

Appendix E.4.1. Results of bioassay used to calculate 72 h LC_{50} values of total and unionized ammonia for *Lampsilis fasciola* at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, temperature, and pH were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.95	20.4	10	0
Control B	0.00	0.00	7.97	20.5	10	1
Control C	—	—	—	—	10	0
Control D	—	—	—	—	10	3
1 A	1.55	0.049	7.89	20.6	10	1
1 B	1.72	0.055	7.90	20.6	10	2
1 C	—	—	—	—	10	1
1 D	—	—	—	—	10	1
2 A	2.50	0.097	7.99	20.6	10	1
2 B	2.57	0.10	8.00	20.6	10	2
2 C	—	—	—	—	10	2
2 D	—	—	—	—	10	1
3 A	4.91	0.18	7.97	20.6	10	3
3 B	5.18	0.19	7.97	20.5	10	2
3 C	—	—	—	—	10	2
3 D	—	—	—	—	10	5
4 A	10.6	0.37	7.94	20.4	10	5
4 B	10.9	0.38	7.94	20.7	10	5
4 C	—	—	—	—	10	2
4 D	—	—	—	—	10	5
5 A	19.5	0.63	7.90	20.7	10	5
5 B	19.8	0.62	7.89	20.6	10	4
5 C	—	—	—	—	10	6
5 D	—	—	—	—	10	9

Appendix E.4.2. Results of bioassay used to calculate 96 h LC_{50} values of total and unionized ammonia for *Lampsilis fasciola* at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24 h period. Ammonia, temperature, and pH were measured in 2 of the 4 replicates at each concentration. Toxicant concentrations increase from 1 A, B, C, and D (lowest concentration) to 5 A, B, C, and D (highest concentration). Dashes indicate replicates in which water chemistry measurements were not taken.

Replicate	Mean Total Ammonia (mg/L)	Mean Unionized Ammonia (mg/L)	Mean pH	Mean Temperature (°C)	No. Exposed	No. Dead
Control A	0.00	0.00	7.98	20.4	10	2
Control B	0.00	0.00	8.00	20.5	10	1
Control C	—	—	—	—	10	1
Control D	—	—	—	—	10	3
1 A	1.44	0.047	7.91	20.6	10	1
1 B	1.57	0.051	7.91	20.6	10	2
1 C	—	—	—	—	10	1
1 D	—	—	—	—	10	1
2 A	2.47	0.099	8.01	20.5	10	2
2 B	2.54	0.11	8.02	20.6	10	3
2 C	—	—	—	—	10	4
2 D	—	—	—	—	10	3
3 A	4.80	0.19	7.99	20.6	10	5
3 B	5.03	0.19	7.99	20.5	10	4
3 C	—	—	—	—	10	6
3 D	—	—	—	—	10	5
4 A	10.2	0.36	7.95	20.6	10	6
4 B	10.5	0.37	7.96	20.5	10	5
4 C	—	—	—	—	10	2
4 D	—	—	—	—	10	5
5 A	19.6	0.64	7.91	20.7	10	10
5 B	19.9	0.64	7.90	20.6	10	10
5 C	—	—	—	—	10	9
5 D	—	—	—	—	10	10

Appendix F.1. Water quality measured during the exposure of *Villosa iris* to ammonium chloride solutions at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24-h period throughout the 96-h bioassay. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration). Minimum - maximum range indicates the lowest and highest recorded values. Measurements were taken in two of the four replicates at each concentration. (N = 4 for each concentration level, except for D.O. in concentration levels: control, 1, 3 and 5, where N = 16).

	Dissolved Oxygen (mg/L)		Hardness (mg/L)	Alkalinity (mg/L)		Conductivity (µmhos/cm)		Nitrites (mg/L)
Replicate	Mean	Min. - Max.	Mean*	Mean	Min. - Max.	Mean	Min. - Max.	Mean†
Control A	9.76	9.08 - 10.05	80	32.5	30 - 35	136.25	120 - 180	< 0.05
Control B	9.65	9.08 - 10.08	80	35	35 - 35	128.75	120 - 150	< 0.05
1 A	9.81	9.05 - 10.68	80	35	35 - 35	212.5	200 - 220	< 0.05
1 B	9.80	9.05 - 10.68	80	35	35 - 35	215	200 - 220	< 0.05
2 A	9.72	9.71 - 9.73	80	35	35 - 35	300	290 - 310	< 0.05
2 B	9.65	9.59 - 9.71	80	35	35 - 35	300	290 - 310	< 0.05
3 A	9.79	9.20 - 10.56	80	35	35 - 35	476.25	470 - 480	< 0.05
3 B	9.77	9.27 - 10.56	80	32.5	30 - 35	476.25	470 - 490	< 0.05
4 A	9.39	9.24 - 9.53	80	35	35 - 35	810	800 - 830	< 0.05
4 B	9.41	9.29 - 9.53	80	35	35 - 35	812	800 - 840	< 0.05
5 A	9.92	9.32 - 11.48	80	32.5	30 - 35	1432.5	1360 - 1480	< 0.05
5 B	9.93	9.32 - 11.48	80	35	35 - 35	1443.8	1360 - 1500	< 0.05

* Range n/a, all measurements were 80 mg/L

† Range n/a, all measurements were <0.05 mg/L

Appendix F.2. Water quality measured during the exposure of *Villosa iris* to ammonium chloride solutions at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24-h period throughout the 96-h bioassay. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration). Minimum - maximum range indicates the lowest and highest recorded values. Measurements were taken in two of the four replicates at each concentration. (N = 4 for each concentration level, except for D.O. in concentration levels: control, 1, 3 and 5, where N = 16).

	Dissolved Oxygen (mg/L)		Hardness (mg/L)		Alkalinity (mg/L)	Conductivity (µmhos/cm)		Nitrites (mg/L)
Replicate	Mean	Min. - Max.	Mean	Min. - Max.	Min. - Max.	Mean	Min. - Max.	Mean†
Control A	8.03	7.44 - 8.32	100	100 - 100	35	138	130 - 150	< 0.05
Control B	7.95	7.44 - 8.32	100	100 - 100	35	134	130 - 140	< 0.05
1 A	7.96	7.61 - 8.29	100	100 - 100	35	171	160 - 180	< 0.05
1 B	8.01	7.68 - 8.29	100	100 - 100	35	171	160 - 180	< 0.05
2 A	7.99	7.30 - 8.50	100	100 - 100	35	214	210 - 220	< 0.05
2 B	8.07	7.53 - 8.50	90	80 - 100	35	214	210 - 220	< 0.05
3 A	8.02	7.36 - 8.67	100	100 - 100	35	300	290 - 310	< 0.05
3 B	8.00	7.23 - 8.67	90	80 - 100	35	300	290 - 310	< 0.05
4 A	8.00	7.54 - 8.31	90	80 - 100	35	462	450 - 470	< 0.05
4 B	7.96	7.42 - 8.31	100	100 - 100	35	462	450 - 470	< 0.05
5 A	7.99	7.35 - 8.81	100	100 - 100	35	786	780 - 810	< 0.05
5 B	7.92	7.27 - 8.81	100	100 - 100	35	789	780 - 810	< 0.05

* Range n/a, all measurements were 35 mg/L

† Range n/a, all measurements were <0.05 mg/L

Appendix F.3. Water quality measured during the exposure of *Lampsilis fasciola* to ammonium chloride solutions at 12 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24-h period throughout the 96-h bioassay. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration). Minimum - maximum range indicates the lowest and highest recorded values. Measurements were taken in two of the four replicates at each concentration. (N = 4 for each concentration level, except for D.O. in concentration levels: control, 1, 3 and 5, where N = 16).

	Dissolved Oxygen (mg/L)		Hardness (mg/L)		Alkalinity (mg/L)		Conductivity (µmhos/cm)		Nitrites (mg/L)
Replicate	Mean	Min. - Max.	Mean	Min. - Max.	Mean	Min. - Max.	Mean	Min. - Max.	Mean†
Control A	9.44	7.44 - 10.26	260	260 - 260	65	65 - 65	432	400 - 470	< 0.05
Control B	9.44	7.44 - 10.33	260	260 - 260	67.5	65 - 70	442	420 - 470	< 0.05
1 A	9.64	8.04 - 10.27	260	260 - 260	65	65 - 65	463.75	440 - 500	< 0.05
1 B	9.67	8.04 - 10.40	260	260 - 260	67.5	65 - 70	466.25	450 - 500	< 0.05
2 A	9.42	8.50 - 10.34	260	260 - 260	65	65 - 65	475	470 - 480	< 0.05
2 B	9.42	8.50 - 10.34	260	260 - 260	65	65 - 65	475	470 - 480	< 0.05
3 A	9.48	8.24 - 10.20	250	240 - 260	65	65 - 65	532	520 - 560	< 0.05
3 B	9.45	8.24 - 10.00	260	260 - 260	65	65 - 65	534	520 - 560	< 0.05
4 A	8.86	8.31 - 8.41	250	240 - 260	65	65 - 65	650	640 - 660	< 0.05
4 B	8.86	8.31 - 8.41	260	260 - 260	65	65 - 65	650	640 - 660	< 0.05
5 A	9.39	8.30 - 10.19	260	260 - 260	65	65 - 65	805	770 - 820	< 0.05
5 B	9.39	8.30 - 10.22	240	240 - 240	67.5	65 - 70	805	770 - 820	< 0.05

† Range n/a, all measurements were <0.05 mg/L

Appendix F.4. Water quality measured during the exposure of *Lampsilis fasciola* to ammonium chloride solutions at 20 ± 1°C. Mean values represent averages of measurements taken at the start and end of each 24-h period throughout the 96-h bioassay. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration). Minimum - maximum range indicates the lowest and highest recorded values. Measurements were taken in two of the four replicates at each concentration. (N = 4 for each concentration level, except for D.O. in concentration levels: control, 1, 3 and 5, where N = 16).

	Dissolved Oxygen (mg/L)		Hardness (mg/L)		Alkalinity (mg/L)		Conductivity (µmhos/cm)		Nitrites (mg/L)
Replicate	Mean	Min. - Max.	Mean	Min. - Max.	Mean	Min. - Max.	Mean	Min. - Max.	Mean†
Control A	8.43	8.11 - 8.74	260	260 - 260	67.5	65 - 70	398	340 - 420	< 0.05
Control B	8.43	8.05 - 8.74	250	240 - 260	70	70 - 70	404	340 - 430	< 0.05
1 A	8.60	8.02 - 9.41	260	260 - 260	72.5	70 - 75	430	410 - 440	< 0.05
1 B	8.59	8.16 - 9.41	260	260 - 260	72.5	70 - 75	431.25	411 - 440	< 0.05
2 A	8.37	8.00 - 8.73	240	240 - 240	65	65 - 65	435	430 - 440	< 0.05
2 B	8.44	8.14 - 8.73	260	260 - 260	72.5	70 - 75	445	430 - 460	< 0.05
3 A	8.51	8.07 - 9.45	260	260 - 260	70	70 - 70	455	440 - 470	< 0.05
3 B	8.54	8.10 - 9.45	250	240 - 260	72.5	70 - 75	460	430 - 470	< 0.05
4 A	8.41	8.01 - 8.80	260	260 - 260	67.5	65 - 70	480	480 - 480	< 0.05
4 B	8.34	7.87 - 8.80	260	260 - 260	65	65 - 65	480	480 - 480	< 0.05
5 A	8.50	8.15 - 9.33	250	240 - 260	67.5	65 - 70	555	540 - 570	< 0.05
5 B	8.46	7.98 - 9.33	260	260 - 260	70	70 - 70	558.75	550 - 570	< 0.05

† Range n/a, all measurements were <0.05 mg/L

Appendix G.1. Water quality parameters measured during the 96 h exposure of *V. iris* to ammonium chloride solutions at 12 ± 1°C. Ammonia, pH, temperature, and conductivity were measured at the start (S) and end (E) of each 24 h interval. Alkalinity, hardness, and nitrites were measured at the start (S) and end (E) of the 96 h bioassay. Measurements were taken in two replicates of each toxicant concentration. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration).

		Total Ammonia (mg/L)		pH		Temp. (°C)		Conductivity (µmho/cm)		Dissolved Oxygen (mg/L)		Alkalinity (mg/L)		Hardness (mg/L)		Nitrites (mg/L)	
Rep.	Time	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E
Cont.	24-h	0.0	0.0	7.46	7.44	12.6	12.1	130	140	9.08	10.26	35	—	100	—	<0.05	—
A	48-h	0.0	0.0	7.39	7.42	12.5	12.0	120	130	9.42	9.98	—	—	—	—	—	—
	72-h	0.0	0.0	—	7.36	—	12.1	130	130	9.72	10.05	—	—	—	—	—	—
	96-h	0.0	0.0	7.49	7.53	12.3	12.1	130	180	9.81	9.77	—	35	—	100	—	<0.05
Cont.	24-h	0.0	0.0	7.46	7.33	12.6	12.2	130	120	9.08	9.95	35	—	100	—	<0.05	—
B	48-h	0.0	0.0	7.39	7.39	12.5	12.7	120	150	9.42	9.66	—	—	—	—	—	—
	72-h	0.0	0.0	—	7.29	—	12.0	130	130	9.72	10.08	—	—	—	—	—	—
	96-h	0.0	0.0	7.49	7.25	12.3	12.0	130	130	9.81	9.51	—	35	—	100	—	<0.05
1 A	24-h	10.2	10.3	7.40	7.33	12.8	12.4	220	210	9.69	9.87	35	—	100	—	<0.05	—
	48-h	10.4	10.9	7.36	7.35	12.8	12.6	210	210	10.27	9.52	—	—	—	—	—	—
	72-h	11.4	12.8	7.39	7.33	12.8	12.1	220	210	9.05	9.98	—	—	—	—	—	—
	96-h	12.5	12.5	7.08	7.33	12.4	12.0	200	200	10.68	9.36	—	35	—	100	—	<0.05
1 B	24-h	10.2	11.4	7.40	7.33	12.8	12.3	220	210	9.69	9.87	35	—	100	—	<0.05	—
	48-h	10.4	11.5	7.36	7.37	12.8	12.6	210	220	10.27	9.52	—	—	—	—	—	—
	72-h	11.4	12.9	7.39	7.30	12.8	12.2	220	220	9.05	9.98	—	—	—	—	—	—
	96-h	12.5	12.6	7.08	7.36	12.4	11.9	200	220	10.68	9.36	—	35	—	100	—	<0.05
2 A	24-h	19.8	19.8	7.54	7.36	12.8	12.6	310	—	9.71	—	35	—	100	—	<0.05	—
	48-h	19.9	19.8	7.36	7.50	12.8	12.8	300	—	—	—	—	—	—	—	—	—
	72-h	19.7	22.5	7.30	7.35	12.1	12.1	300	—	—	—	—	—	—	—	—	—
	96-h	21.9	22.0	7.21	7.39	12.7	12.1	290	300	—	9.73	—	35	—	100	—	<0.05
2 B	24-h	19.8	20.3	7.54	7.37	12.8	12.7	310	—	9.71	—	35	—	100	—	<0.05	—
	48-h	19.9	19.7	7.36	7.45	12.8	12.6	300	—	—	—	—	—	—	—	—	—
	72-h	19.7	22.8	7.30	7.32	12.1	12.1	300	—	—	—	—	—	—	—	—	—
	96-h	21.9	22.3	7.21	7.39	12.7	12.1	290	300	—	9.59	—	35	—	80	—	<0.05
3 A	24-h	40.5	41.2	7.48	7.35	12.8	12.4	480	480	9.65	9.87	35	—	100	—	<0.05	—
	48-h	40.9	40.1	7.24	7.38	12.7	12.6	480	480	10.56	9.55	—	—	—	—	—	—
	72-h	40.1	44.7	7.23	7.30	12.8	12.1	470	480	9.27	9.99	—	—	—	—	—	—
	96-h	43.6	43.7	7.13	7.32	12.7	12.3	470	470	10.21	9.20	—	35	—	100	—	<0.05
3 B	24-h	40.5	41.9	7.48	7.33	12.8	12.5	480	470	9.65	9.81	35	—	100	—	<0.05	—
	48-h	40.9	40.4	7.24	7.38	12.7	12.6	480	470	10.56	9.52	—	—	—	—	—	—
	72-h	40.1	44.8	7.23	7.27	12.8	12.3	470	480	9.27	9.83	—	—	—	—	—	—
	96-h	43.6	44.9	7.13	7.32	12.7	12.3	470	490	10.21	9.34	—	35	—	80	—	<0.05
4 A	24-h	79.4	82.0	7.40	7.33	12.8	12.7	810	—	9.53	—	35	—	100	—	<0.05	—
	48-h	80.7	79.6	7.16	7.38	12.7	12.9	810	—	—	—	—	—	—	—	—	—
	72-h	80.5	86.3	7.13	7.27	12.8	12.3	800	—	—	—	—	—	—	—	—	—
	96-h	85.6	86.4	7.11	7.32	12.7	12.3	800	830	—	9.24	—	35	—	80	—	<0.05
4 B	24-h	79.4	82.0	7.40	7.33	12.8	12.8	810	—	9.53	—	35	—	100	—	<0.05	—
	48-h	80.7	80.2	7.16	7.37	12.7	12.7	810	—	—	—	—	—	—	—	—	—
	72-h	80.5	86.3	7.13	7.24	12.8	12.2	800	—	—	—	—	—	—	—	—	—
	96-h	85.6	86.0	7.11	7.31	12.7	12.4	800	840	—	9.29	—	35	—	100	—	<0.05
5 A	24-h	162	163	7.30	7.28	12.6	12.6	1460	1400	9.52	9.83	35	—	100	—	<0.05	—
	48-h	163	160	7.12	7.28	12.7	12.6	1440	1440	10.52	9.53	—	—	—	—	—	—
	72-h	160	162	7.11	7.19	12.7	12.2	1440	1480	9.32	9.90	—	—	—	—	—	—
	96-h	163	161	6.84	7.24	12.4	12.2	1360	1440	11.48	9.52	—	35	—	100	—	<0.05
5 B	24-h	162	165	7.30	7.26	12.6	12.5	1460	1410	9.52	9.84	35	—	100	—	<0.05	—

Appendix G.2. Water quality parameters measured during the 96 h exposure of *V. iris* to ammonium chloride solutions at 20 ± 1°C. Ammonia, pH, temperature, and conductivity were measured at the start (S) and end (E) of each 24 h interval. Alkalinity, hardness, and nitrites were measured at the start (S) and end (E) of the 96 h bioassay. Measurements were taken in two replicates of each toxicant concentration. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration).

		Total Ammonia (mg/L)		pH		Temp. (°C)		Conductivity (µmho/cm)		Dissolved Oxygen (mg/L)		Alkalinity (mg/L)		Hardness (mg/L)		Nitrites (mg/L)	
Rep.	Time	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E
Cont.	24-h	0.0	0.0	7.37	7.57	20.6	19.8	140	140	8.27	8.24	35	—	80	—	<0.05	—
A.	48-h	0.0	0.0	7.47	7.38	20.9	20.6	140	140	7.44	7.90	—	—	—	—	—	—
	72-h	0.0	0.0	7.41	7.65	20.6	20.6	130	130	8.32	8.09	—	—	—	—	—	—
	96-h	0.0	0.0	7.31	7.32	20.7	20.4	130	130	8.24	7.76	—	30	—	80	—	<0.05
Cont.	24-h	0.0	0.0	7.37	7.53	20.6	20.1	140	140	8.27	7.84	35	—	80	—	<0.05	—
B	48-h	0.0	0.0	7.47	7.51	20.9	20.5	140	130	7.44	7.85	—	—	—	—	—	—
	72-h	0.0	0.0	7.41	7.67	20.6	20.5	130	130	8.32	8.10	—	—	—	—	—	—
	96-h	0.0	0.0	7.31	7.47	20.7	20.5	130	130	8.24	7.50	—	35	—	80	—	<0.05
1 A	24-h	5.20	5.14	7.32	7.51	20.6	20.1	170	180	8.16	7.61	35	—	80	—	<0.05	—
	48-h	5.09	5.01	7.40	7.56	20.7	20.4	170	180	8.04	8.00	—	—	—	—	—	—
	72-h	4.83	4.89	7.30	7.58	20.7	20.5	160	170	8.29	7.74	—	—	—	—	—	—
	96-h	5.05	5.08	7.38	7.48	21.0	20.5	170	170	8.11	7.71	—	35	—	80	—	<0.05
1 B	24-h	5.20	5.19	7.32	7.53	20.6	20.7	170	180	8.16	7.72	35	—	80	—	<0.05	—
	48-h	5.09	5.05	7.40	7.55	20.7	20.5	170	180	8.04	7.92	—	—	—	—	—	—
	72-h	4.83	4.85	7.30	7.68	20.7	20.4	160	170	8.29	8.16	—	—	—	—	—	—
	96-h	5.05	5.23	7.38	7.50	21.0	20.5	170	170	8.11	7.68	—	35	—	80	—	<0.05
2 A	24-h	9.97	9.84	7.22	7.51	20.5	20.3	220	—	8.17	—	35	—	80	—	<0.05	—
	48-h	9.90	9.87	7.16	7.51	20.6	20.4	210	—	8.50	—	—	—	—	—	—	—
	72-h	9.06	9.76	7.39	7.58	20.9	20.5	210	—	—	—	—	—	—	—	—	—
	96-h	9.45	9.30	7.39	7.40	20.6	20.5	210	220	—	7.30	—	35	—	80	—	<0.05
2 B	24-h	9.97	9.92	7.22	7.52	20.5	20.3	220	—	8.17	—	35	—	80	—	<0.05	—
	48-h	9.90	9.25	7.16	7.50	20.6	20.5	210	—	8.50	—	—	—	—	—	—	—
	72-h	9.06	9.76	7.39	7.65	20.9	20.5	210	—	—	—	—	—	—	—	—	—
	96-h	9.45	9.37	7.39	7.48	20.6	20.7	210	220	—	7.53	—	35	—	80	—	<0.05
3 A	24-h	19.4	19.1	7.28	7.48	20.6	20.6	300	310	8.11	—	35	—	80	—	<0.05	—
	48-h	19.9	19.2	7.29	7.52	20.5	20.5	290	310	8.24	—	—	—	—	—	—	—
	72-h	17.9	18.8	7.53	7.62	20.7	20.4	310	300	7.73	—	—	—	—	—	—	—
	96-h	18.6	18.2	7.36	7.40	20.7	20.6	290	290	8.67	7.36	—	35	—	80	—	<0.05
3 B	24-h	19.4	20.4	7.28	7.52	20.6	20.5	300	310	8.11	—	35	—	80	—	<0.05	—
	48-h	19.9	19.0	7.29	7.47	20.5	20.5	290	310	8.24	—	—	—	—	—	—	—
	72-h	17.9	18.6	7.53	7.63	20.7	20.4	310	290	7.73	—	—	—	—	—	—	—
	96-h	18.6	18.6	7.36	7.40	20.7	20.6	290	300	8.67	7.23	—	30	—	80	—	<0.05
4 A	24-h	38.0	37.7	7.26	—	20.9	20.5	470	—	8.16	—	35	—	80	—	<0.05	—
	48-h	39.1	38.3	7.25	7.46	20.3	20.5	460	—	8.31	—	—	—	—	—	—	—
	72-h	38.0	38.0	7.37	7.59	20.6	20.5	460	—	—	—	—	—	—	—	—	—
	96-h	38.1	37.5	7.29	7.40	20.7	20.7	450	470	—	7.54	—	35	—	80	—	<0.05
4 B	24-h	38.0	37.8	7.26	7.48	20.9	20.6	470	—	8.16	—	35	—	80	—	<0.05	—
	48-h	39.1	37.8	7.25	7.45	20.3	20.3	460	—	8.31	—	—	—	—	—	—	—
	72-h	38.0	37.5	7.37	7.59	20.6	20.5	460	—	—	—	—	—	—	—	—	—
	96-h	38.1	37.2	7.29	7.39	20.7	20.7	450	470	—	7.42	—	35	—	80	—	<0.05
5 A	24-h	76.6	75.1	7.17	7.36	20.6	20.5	780	810	8.25	7.35	35	—	80	—	<0.05	—
	48-h	76.2	76.9	7.18	7.42	20.3	20.5	780	780	8.30	7.67	—	—	—	—	—	—
	72-h	74.2	76.0	7.32	7.50	20.9	20.4	790	790	8.02	8.12	—	—	—	—	—	—
	96-h	76.0	73.0	7.21	7.37	20.8	20.6	780	780	8.81	7.40	—	30	—	80	—	<0.05
5 B	24-h	76.6	76.3	7.17	7.38	20.6	20.6	780	810	8.25	7.30	35	—	80	—	<0.05	—

Appendix G.3. Water quality parameters measured during the 96 h exposure of *L. fasciola* to ammonium chloride solutions at 12 ± 1°C. Ammonia, pH, temperature, and conductivity were measured at the start (S) and end (E) of each 24 h interval. Alkalinity, hardness, and nitrites were measured at the start (S) and end (E) of the 96 h bioassay. Measurements were taken in two replicates of each toxicant concentration. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration).

		Total Ammonia (mg/L)		pH		Temp. (°C)		Conductivity (µmho/cm)		Dissolved Oxygen (mg/L)		Alkalinity (mg/L)		Hardness (mg/L)		Nitrites (mg/L)	
Rep.	Time	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E
Cont.	24-h	0.0	0.0	7.81	7.92	12.4	12.4	470	450	8.94	10.24	65	—	260	—	<0.05	—
A	48-h	0.0	0.0	7.73	8.36	12.0	13.0	—	420	7.44	9.71	—	—	—	—	—	—
	72-h	0.0	0.0	7.78	7.00	13.0	13.2	—	420	—	10.05	—	—	—	—	—	—
	96-h	0.0	0.0	7.87	8.01	12.6	12.6	—	400	—	10.26	—	65	—	260	—	<0.05
Cont.	24-h	0.0	0.0	7.81	7.97	12.4	12.5	470	460	8.94	10.33	65	—	260	—	<0.05	—
B	48-h	0.0	0.0	7.73	7.98	12.0	13.0	—	430	7.44	9.81	—	—	—	—	—	—
	72-h	0.0	0.0	7.78	8.06	13.0	13.3	—	430	—	9.97	—	—	—	—	—	—
	96-h	0.0	0.0	7.87	8.03	12.6	12.6	—	420	—	10.16	—	70	—	260	—	<0.05
1 A	24-h	3.08	3.34	7.85	7.96	12.5	12.5	500	470	9.54	10.27	65	—	260	—	<0.05	—
	48-h	3.25	3.09	7.70	7.88	12.1	13.0	470	440	8.04	9.87	—	—	—	—	—	—
	72-h	3.11	3.14	7.74	8.05	13.1	13.3	460	450	—	9.95	—	—	—	—	—	—
	96-h	3.24	2.69	7.89	7.98	12.6	12.6	470	450	—	10.17	—	65	—	260	—	<0.05
1 B	24-h	3.08	3.15	7.85	7.98	12.5	12.3	500	480	9.54	10.40	70	—	260	—	<0.05	—
	48-h	3.25	3.23	7.70	7.87	12.1	13.0	470	450	8.04	9.85	—	—	—	—	—	—
	72-h	3.11	3.09	7.74	8.05	13.1	13.3	460	450	—	9.92	—	—	—	—	—	—
	96-h	3.24	2.55	7.89	7.99	12.6	12.4	470	450	—	10.25	—	65	—	260	—	<0.05
2 A	24-h	5.77	5.76	7.88	7.93	12.4	12.4	480	—	9.42	—	65	—	260	—	<0.05	—
	48-h	5.80	5.62	7.73	7.88	12.0	13.0	—	—	8.50	—	—	—	—	—	—	—
	72-h	5.12	5.40	7.70	8.04	13.1	13.1	—	—	—	—	—	—	—	—	—	—
	96-h	6.00	5.51	7.90	7.99	12.7	12.5	—	470	—	10.34	—	65	—	260	—	<0.05
2 B	24-h	5.77	5.75	7.88	7.96	12.4	12.6	480	—	9.42	—	65	—	260	—	<0.05	—
	48-h	5.80	6.10	7.73	7.90	12.0	13.0	—	—	8.50	—	—	—	—	—	—	—
	72-h	5.12	6.10	7.70	8.05	13.1	13.0	—	—	—	—	—	—	—	—	—	—
	96-h	6.00	5.62	7.90	7.98	12.7	12.3	—	470	—	10.34	—	65	—	260	—	<0.05
3 A	24-h	12.4	11.7	7.79	7.96	12.4	12.4	530	560	9.34	—	65	—	240	—	<0.05	—
	48-h	12.2	11.7	7.71	7.84	11.7	13.0	—	520	8.24	9.75	—	—	—	—	—	—
	72-h	12.0	12.4	7.71	8.02	13.1	13.3	—	530	—	9.86	—	—	—	—	—	—
	96-h	12.9	12.7	7.97	7.97	12.6	12.6	—	520	—	10.20	—	65	—	260	—	<0.05
3 B	24-h	12.4	12.0	7.79	7.95	12.4	12.6	530	560	9.34	—	65	—	260	—	<0.05	—
	48-h	12.2	12.3	7.71	7.87	11.7	13.2	—	530	8.24	9.87	—	—	—	—	—	—
	72-h	12.0	12.5	7.71	8.03	13.1	13.4	—	530	—	9.80	—	—	—	—	—	—
	96-h	12.9	12.5	7.97	7.97	12.6	12.6	—	520	—	10.00	—	65	—	260	—	<0.05
4 A	24-h	24.5	24.0	7.75	7.91	12.4	12.4	660	—	9.41	—	65	—	240	—	<0.05	—
	48-h	24.3	24.1	7.63	7.84	11.7	13.2	—	—	8.31	—	—	—	—	—	—	—
	72-h	24.1	24.4	7.65	7.91	12.9	12.0	—	—	—	—	—	—	—	—	—	—
	96-h	—	—	—	—	—	—	—	640	—	—	—	65	—	260	—	<0.05
4 B	24-h	24.5	24.3	7.75	7.92	12.4	12.4	660	—	9.41	—	65	—	260	—	<0.05	—
	48-h	24.3	25.1	7.63	7.81	11.7	13.2	—	—	8.31	—	—	—	—	—	—	—
	72-h	24.1	25.0	7.65	7.90	12.9	12.0	—	—	—	—	—	—	—	—	—	—
	96-h	—	—	—	—	—	—	—	640	—	—	—	65	—	260	—	<0.05
5 A	24-h	48.1	45.2	7.66	7.87	12.6	12.6	820	820	9.36	10.19	65	—	260	—	<0.05	—
	48-h	46.4	45.5	7.61	7.77	12.0	13.2	810	770	8.30	9.69	—	—	—	—	—	—
	72-h	49.3	43.4	7.55	7.90	13.1	12.1	—	—	—	—	—	—	—	—	—	—
	96-h	—	—	—	—	—	—	—	—	—	—	—	65	—	260	—	<0.05
5 B	24-h	48.1	46.0	7.66	7.86	12.6	12.4	820	820	9.36	10.22	65	—	240	—	<0.05	—

Appendix G.4. Water quality parameters measured during the 96 h exposure of *L. fasciola* to ammonium chloride solutions at 20 ± 1°C. Ammonia, pH, temperature, and conductivity were measured at the start (S) and end (E) of each 24 h interval. Alkalinity, hardness, and nitrites were measured at the start (S) and end (E) of the 96 h bioassay. Measurements were taken in two replicates of each toxicant concentration. Toxicant concentrations increase from 1 A and B (lowest concentration) to 5 A and B (highest concentration).

		Total Ammonia (mg/L)		pH		Temp. (°C)		Conductivity (µmho/cm)		Dissolved Oxygen (mg/L)		Alkalinity (mg/L)		Hardness (mg/L)		Nitrites (mg/L)	
Rep.	Time	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S	E
Cont.	24-h	0.0	0.0	7.74	8.00	19.5	20.9	340	420	8.74	8.37	70	—	260	—	<0.05	—
A	48-h	0.0	0.0	7.84	8.01	21.3	20.5	—	390	—	8.25	—	—	—	—	—	—
	72-h	0.0	0.0	7.95	8.18	20.4	19.7	—	420	—	8.70	—	—	—	—	—	—
	96-h	0.0	0.0	7.87	8.28	20.4	20.6	—	420	—	8.11	—	65	—	260	—	<0.05
Cont.	24-h	0.0	0.0	7.74	8.03	19.5	20.8	340	420	8.74	8.42	70	—	240	—	<0.05	—
B	48-h	0.0	0.0	7.84	8.04	21.3	20.6	—	420	—	8.44	—	—	—	—	—	—
	72-h	0.0	0.0	7.95	8.20	20.4	20.5	—	410	—	8.50	—	—	—	—	—	—
	96-h	0.0	0.0	7.87	8.33	20.4	20.8	—	430	—	8.05	—	70	—	260	—	<0.05
1 A	24-h	1.89	1.55	7.74	8.03	19.6	20.7	410	440	8.82	8.37	75	—	260	—	<0.05	—
	48-h	1.74	1.32	7.73	7.90	21.4	20.6	440	430	8.47	8.41	—	—	—	—	—	—
	72-h	1.36	1.46	7.78	8.19	20.4	20.8	430	430	8.76	8.51	—	—	—	—	—	—
	96-h	1.23	0.984	7.84	8.05	20.6	20.8	430	430	9.41	8.02	—	70	—	260	—	<0.05
1 B	24-h	1.89	1.99	7.74	8.02	19.6	20.7	410	440	8.82	8.26	75	—	260	—	<0.05	—
	48-h	1.74	1.65	7.73	8.05	21.4	20.6	440	430	8.47	8.26	—	—	—	—	—	—
	72-h	1.36	1.69	7.78	8.09	20.4	20.7	430	430	8.76	8.61	—	—	—	—	—	—
	96-h	1.23	0.988	7.84	8.02	20.6	20.9	430	440	9.41	8.16	—	70	—	260	—	<0.05
2 A	24-h	2.89	2.61	7.90	8.02	19.8	20.6	430	—	8.73	—	65	—	240	—	<0.05	—
	48-h	2.79	2.04	7.97	8.04	21.2	20.5	—	—	—	—	—	—	—	—	—	—
	72-h	2.00	2.66	7.87	8.16	20.5	20.2	—	—	—	—	—	—	—	—	—	—
	96-h	2.72	2.07	7.90	8.20	20.4	20.8	—	440	—	8.00	—	65	—	240	—	<0.05
2 B	24-h	2.89	3.01	7.90	8.02	19.8	20.6	430	—	8.73	—	70	—	260	—	<0.05	—
	48-h	2.79	2.21	7.97	8.05	21.2	20.4	—	—	—	—	—	—	—	—	—	—
	72-h	2.00	2.51	7.87	8.18	20.5	20.8	—	—	—	—	—	—	—	—	—	—
	96-h	2.72	2.21	7.90	8.25	20.4	20.8	—	460	—	8.14	—	75	—	260	—	<0.05
3 A	24-h	5.77	4.78	7.84	8.00	20.2	20.6	450	460	8.76	8.26	70	—	260	—	<0.05	—
	48-h	5.29	4.48	7.88	7.98	21.0	20.6	470	450	8.52	8.07	—	—	—	—	—	—
	72-h	4.08	5.06	7.92	8.18	20.6	20.7	460	—	8.58	8.34	—	—	—	—	—	—
	96-h	4.95	4.00	7.87	8.20	20.4	20.8	—	440	9.45	8.13	—	70	—	260	—	<0.05
3 B	24-h	5.77	5.97	7.84	7.99	20.2	20.6	450	460	8.76	8.22	70	—	260	—	<0.05	—
	48-h	5.29	4.42	7.88	8.03	21.0	20.7	470	460	8.52	8.27	—	—	—	—	—	—
	72-h	4.08	5.55	7.92	8.17	20.6	19.9	460	—	8.58	8.40	—	—	—	—	—	—
	96-h	4.95	4.17	7.87	8.19	20.4	20.9	—	460	9.45	8.10	—	75	—	240	—	<0.05
4 A	24-h	13.5	11.6	7.82	7.96	20.1	20.6	480	—	8.80	—	70	—	260	—	<0.05	—
	48-h	10.9	8.89	7.84	8.04	21.1	20.4	—	—	—	—	—	—	—	—	—	—
	72-h	8.28	10.1	7.89	8.11	20.4	20.7	—	—	—	—	—	—	—	—	—	—
	96-h	10.1	8.02	7.84	8.12	20.4	20.9	—	480	—	8.01	—	65	—	260	—	<0.05
4 B	24-h	13.5	13.8	7.82	7.97	20.1	20.6	480	—	8.80	—	65	—	260	—	<0.05	—
	48-h	10.9	8.82	7.84	8.02	21.1	20.4	—	—	—	—	—	—	—	—	—	—
	72-h	8.28	10.1	7.89	8.13	20.4	19.9	—	—	—	—	—	—	—	—	—	—
	96-h	10.1	8.22	7.84	8.14	20.4	20.9	—	480	—	7.87	—	65	—	260	—	<0.05
5 A	24-h	17.3	20.50	7.75	7.94	20.4	20.7	560	540	8.74	8.15	70	—	260	—	<0.05	—
	48-h	22.6	19.2	7.78	8.04	20.7	20.7	570	560	8.54	8.17	—	—	—	—	—	—
	72-h	17.8	19.8	7.83	8.06	20.7	20.9	560	560	8.57	8.33	—	—	—	—	—	—
	96-h	22.5	17.10	7.79	8.10	20.4	20.8	550	540	9.33	8.17	—	65	—	240	—	<0.05
5 B	24-h	17.3	21.3	7.75	7.95	20.4	20.8	560	570	8.74	8.14	70	—	260	—	<0.05	—

VITA

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