An Evaluation of Adult Freshwater Mussels Held in Captivity at the White Sulphur Springs National Fish Hatchery, West Virginia

by

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ABSTRACT

Due to the increasing need to provide refugia for freshwater mussels impacted by anthropogenic activities and exotic species, facilities should be identified and protocols developed for holding mussels in captivity. White Sulphur Springs National Fish Hatchery (WSSNFH), White Sulphur Springs, WV, has held freshwater mussels for nearly eight years, and has the potential to become an important refugium and propagation facility for conservation of mussels in the Ohio River Basin and elsewhere. The goal of this study was to determine the feasibility of holding adult freshwater mussels in long-term captivity at WSSNFH by evaluating survival, energy reserves, and gametogenesis of captive mussels in a recirculating pond system.

I relocated three mussel species in the summer of 2001 and 10 mussel species in the summer of 2002 to a recirculating pond system (reservoir and raceway) at the hatchery. Water quality parameters of pH, alkalinity, hardness, temperature, and dissolved oxygen; and algal concentrations were measured periodically from summer 2001 to summer 2003. Annual survival rates of 10 species were estimated (August 2002 to August 2003) using the program MARK. Glycogen, protein, and lipid concentrations in mantle tissue of three captive species (*Actinonaias ligamentina*, *Cyclonaias tuberculata*, and *Tritogonia verrucosa*) were compared to those of wild mussels in the New River. Gametogenic activity and synchrony in *A. ligamentina* and *C. tuberculata* were compared between captive and wild mussels.

Water quality parameters, with the exception of temperature, were within desirable ranges for most of the study. Temperatures of > 28° C were observed for several days during

summers 2002 and 2003. Algal concentrations averaged 1903 cells ml^{-1} in the raceway (range: 300 to 4658 cells ml^{-1}), which is comparable to algal concentrations reported for nearby rivers. The overall survival rate for 10 freshwater mussel species held in the raceway for one year was 77%. *Villosa vanuxemensis* had the highest survival rate (96%), and *Lampsilis cardium* had the lowest survival rate (31%).

Although there were fluctuations in glycogen, protein, and lipid levels over 2 yr, there were no overall differences in energy substrates between captive and wild mussels at the end of the study. Captivity did not appear to have a negative affect on gametogenesis. Captive *C. tuberculata* spawned within the expected time frame between January and June, but slightly earlier than their wild counterparts in the New River. Due to the infestation of the gonads of both captive and wild *A. ligamentina* by digenean trematodes, little gametogenesis was observed. However, captive holding did not appear to have an effect on trematode infestation rates.

From these results, I conclude that captive holding conditions in the recirculating pond system at WSSNFH were adequate for long-term holding of a wide range of mussel taxa. I recommend that WSSNFH continue to be used as an adult holding facility. Further research should be conducted to determine food and habitat preferences of freshwater mussel species in captivity so that optimal holding conditions can be provided for each species.

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INTRODUCTION

The greatest global diversity of freshwater mussels (Unionoida) is found in North America, with 297 known taxa (Williams et al. 1993). This diversity reaches its highest levels in the southeastern United States, where the physiography, geology, water chemistry and natural history of the region have led to distinct faunal groups and endemism within particular river basins (Neves et al. 1997). Invasive species and habitat loss due to poor water quality, dam construction, and sedimentation have caused extinctions and decline of over 70% of North American mussel species in the past century (Williams et al. 1993, Richter et al. 1997). Due to the rapid decline of freshwater mussels, there has been a national effort to conserve habitat, propagate juveniles in captivity, and provide refugia for threatened and endangered species. Freshwater mussels have been relocated to alternate stream locations or into captivity for reasons including removal from areas of proposed construction or invasive species, population augmentation, and re-establishment (Cope and Waller 1995, Dunn 1997, Sickel et al. 1997, Dunn et al. 2000, Gatenby 2000, Newton et al. 2001). However, according to Cope and Waller (1995), survival of relocated mussels in numerous studies averaged less than 50%. Low survival of relocated mussels was attributed to differences in habitat conditions between sites of origin and destination. Hence, relocation of mussels to man-made facilities for captive holding presents the challenge of creating habitat that simulates natural conditions.

Conservation professionals have initiated programs to construct captive facilities to provide temporary refugia for endangered mussel species, and for propagation of juveniles for

relocation to the wild (Gatenby 1994, Burress 1995, Dunn and Layzer 1997, Patterson 1998, Hallac and Marsden 2001, Newton et al. 2001). Captive holding of adult mussels and propagation of juveniles has begun at several federal fish hatcheries including Genoa National Fish Hatchery, Genoa, WI; Neosho National Fish Hatchery, Neosho, MO; Warm Springs National Fish Hatchery, Warm Springs, GA; and White Sulphur Springs National Fish Hatchery, White Sulphur Springs, WV. However, holding mussels for long periods in artificial ponds has been largely unsuccessful in the past (Gatenby 2000, Newton et al. 2001). Low survival rates, reduced growth rates, and poor physiological condition of mussels held in captivity for one to three years have been reported (Cope and Waller 1995, Gatenby 2000, Newton et al. 2001). Propagation and captive holding are relatively new and promising tools in mussel conservation, but to use hatcheries as refugia and propagation facilities, we must determine appropriate environmental conditions for long-term holding of mussels.

Abiotic factors such as water quality, substrate, flow, and temperature are important factors to consider when relocating mussels to alternate river sites and into captivity (Cope and Waller 1995). Mussels are most abundant and diverse in flowing water and prefer substrates into which they can burrow (Matteson 1955, Neves and Widlak 1987). Measurements of water velocity over mussel beds commonly range from near 0 cm s^{-1} to 113 cm s^{-1}, with optimum values ranging from 14.0 cm s^{-1} to 32.4 cm s^{-1} (Brunke et al. 2001, Hardison and Layzer 2001). Although species have varying flow requirements, Hardison and Layzer (2001) found a negative correlation between mussel density and velocity, suggesting that there is a preference for lower velocities. However, regardless of flow rates, substrate stability is reported to be a key factor affecting survival (Hamilton and Dorazio 1997, Dunn et al. 2000). Suggested water quality parameters for holding freshwater mussels in captivity are water temperatures less than 28°C, pH

greater than 6.5, DO greater than 6 mg L^{-1}, alkalinity greater than 15 mg L^{-1}, and hardness greater than 50 mg L^{-1} (Gatenby et al. 2000).

Adequate food supply is also an important component of holding freshwater mussels in captivity. Freshwater mussels are filter feeders that rely on detritus, bacteria, and planktonic algae for nutrients (Coker et al. 1920, Paterson 1986, Cahoon and Owen 1996, Gatenby et al. 1996, Nichols and Garling 2000). Size, shape, and nutritional value of algal species are factors in determining suitability as food (Paterson 1984, Paterson 1986, Gatenby et al. 1996, and Parker et al. 1998). Planktonic algae such as Cyanoprokaryota (green algae) and Bacilariophyta (diatoms) have been confirmed as food for freshwater mussels in previous studies (Gatenby et al. 1996, Parker et al. 1998, Nichols and Garling 2000). Algal cell densities in rivers can average 200 cells ml^{-1} in winter months, 4.5 x 10^3 cells ml^{-1} in summer months, and range from 12 cells ml^{-1} to 5 x 10^4 cells ml^{-1} (Gale and Lowe 1971, Lizotte and Simmons 1985, Stevenson and White 1995). According to Paterson (1984), algal densities between 1.1 x 10^4 cells ml^{-1} and 1.5 x 10^4 cells ml^{-1} produced the most efficient filtration rates in *Elliptio complanata*. However, cell densities in an outdoor holding facility at White Sulphur Springs National Fish Hatchery did not exceed 7 x 10^3 cells ml^{-1} (Mummert 2001). In order to achieve concentrations of algal cells suitable for mussels, it is recommended that a dissolved nitrogen (N) to phosphorus (P) ratio of greater than 10:1 be established in the water column (Hillebrand and Sommer 1999, Gatenby et al. 2000).

A variety of captive conditions, from indoor recirculating aquaculture systems to outdoor ponds and raceways have been used to hold freshwater mussels (Isom and Hudson 1982, Dunn and Layzer 1997, Gatenby 2000, Starkey et al. 2000, Tankersley and Butz 2000, Henley et al. 2001). Closed system holding facilities have included aquaria in closed-water recirculating

systems, troughs in recirculating systems, and baskets in a partitioned aquaculture system (Starkey et al. 2000, Tankersley and Butz 2000, and Henley et al. 2001). Mussels have also been held in ponds or raceways, where they were either contained within pocket nets or glass racks, or allowed to burrow into substrate (Burress 1995, Dunn and Layzer 1997, Gatenby 2000). However, mussels held under these conditions had variable survival rates.

Short-term studies showed relatively high survival, while longer studies resulted in low survival rates. Studies conducted from 1 to 6 mo in recirculating aquaculture systems yielded between 98.7% and 100% survival of adult *Elliptio complanata* (Starkey et al. 2000, Tankersley and Butz 2000, and Henley et al. 2001). However, longer studies of up to one year resulted in lower survival of 0 to 89% (Burress 1995, Dunn and Layzer 1997, Gatenby 2000). Mussels held at several facilities, including Leetown Science Center (Leetown, WV), White Sulphur Springs National Fish Hatchery (White Sulphur Springs, WV), and Genoa National Fish Hatchery (Genoa, WI), had even lower survival rates in the second and third years of captivity (Gatenby 2000, Newton et al. 2001). Dunn and Layzer (1997) and Burress (1995) showed that survival rates were variable among species and relocation sites. In addition, previous studies suggest that species of the subfamily Lampsilinae are more sensitive than species of the subfamily Ambleminae to stressful conditions such as relocation or zebra mussel attachment (Haag et al. 1993, Baker and Hornbach 1997, Dunn and Layzer 1997, Newton et al. 2001).

In addition to measuring survival rates, measuring concentrations of energy substrates can help researchers determine whether captive holding conditions are appropriate. Glycogen is an important energy reserve and the main storage carbohydrate in bivalves (deZwaan and Zandee 1972, Hummel et al.1989). Commonly used to assess the physiological condition of freshwater mussels, glycogen content can indicate stressful environmental conditions (Patterson et al. 1997,

Naimo and Monroe 1999, Newton et al. 2001, Hyotylainen et al. 2002). For example, Hallac and Marsden (2001) reported a decrease in glycogen levels of mussels infested with zebra mussels; Infested *Elliptio complanata* had a 50% reduction in glycogen content compared to the non-infested control, but survival rates did not differ. Therefore, glycogen levels measured over time can provide additional information about energy storage and use in captive mussels, indicating physiological condition.

The White Sulphur Springs National Fish Hatchery (WSSNFH), because of its location in the upper Ohio River Basin, has the potential to serve as a regional facility for freshwater mussel propagation and adult refugium. However, it has not yet been fully evaluated for long-term holding of mussels. Beginning in 1995, WSSNFH served as a holding facility for adult mussels collected from the zebra mussel-infested waters of the Ohio River. From 1995 to 1998, 444 mussels of 8 species were scrubbed and quarantined before being brought to WSSNFH (D. Rhine, USFWS, personal communication). Attempts were made to accommodate the mussels in different types of holding containers, but mortality was high, and less than 10% of the relocated mussels survived 3 yr (Gatenby 2000). A recent study by Mummert (2001) evaluated the suitability of WSSNFH for culturing 2 species of juvenile mussels. The rainbow mussel (*Villosa iris*) and wavy-rayed lamp mussel (*Lampsilis fasciola*) were held at WSSNFH in recirculating ponds for 90 days. *Villosa iris* exhibited mean survival rates ranging from 32% to 50%, which is higher than those of previous studies (O'Beirn 1998, Beaty 1999). *Lampsilis fasciola* exhibited a lower mean survival rate of only 6% after 90 days.

The goal of this study was to evaluate the feasibility of holding 10 species of freshwater mussels of 3 subfamilies in a recirculating pond system at WSSNFH for 1 yr. The specific objectives were to: 1) establish suitable abiotic conditions in the recirculating pond system,

including water chemistry, temperature, and water velocity over 2 yr; 2) create suitable algal density and composition over 2 yr; and 3) determine survival rates, incidence of gravidity, and glycogen level of 10 mussel species held in captivity for 1 yr. Ten common species, fatmucket (*Lampsilis siliquoidea*), giant floater (*Pyganodon grandis*), mountain creekshell (*Villosa vanuxemensis*), mucket (*Actinonaias ligamentina*), pistolgrip (*Tritogonia verrucosa*), plain pocketbook (*Lampsilis cardium*), purple wartyback (*Cyclonaias tuberculata*), rainbow (*Villosa iris*), Tennessee clubshell (*Pleurobema oviforme*), and threeridge (*Amblema plicata*) were chosen for this study as surrogates to threatened and endangered species. These species represent the 3 subfamilies of the family Unionidae and consititute a range of reproductive strategies and habitat preferences.

METHODS

This study was conducted at WSSNFH, in Greenbrier County, WV. Two ponds, B2 and B1 at WSSNFH, were used for the study. Pond B2 was modified to function as a raceway in which water was pumped over mussel confinement structures. Pond B1 was modified to function as a reservoir for algal production. Each pond was approximately 11 m wide by 84 m long, with a mean depth of 1.2 m and an approximate volume of 1.1×10^6 L (Figure 1.1). Two one-half horsepower submersible pumps (Peabody Barnes model 35E54) were used to circulate water between the raceway and the reservoir through 10 cm pipes. The 10 cm pipe carrying water into the raceway was branched into a manifold of four 5 cm pipes that forced water over mussel confinement structures (Figure 1.1). Water from two springs fed the ponds when needed, and stop boards at the low end of the ponds regulated pond elevation and outflow. A paddle wheel aerator (Aqua and Co., Model Eolo 2) operated in the raceway, upstream of the manifold in order to enhance dissolved oxygen and water circulation.

Abiotic factors

I recorded dissolved oxygen (mg L^{-1}), alkalinity (mg L^{-1} as $CaCO_3$), hardness (mg L^{-1} as $CaCO_3$), pH, and temperature (°C) in the raceway every 15 to 45 d, from August 2001 to August 2003. Dissolved oxygen was measured using a YSI (Yellow Springs Instruments, OH) meter, alkalinity and hardness were measured using Hach ™ (Hach Company) test kits, and pH was measured with an Orion model 290A meter. Water samples collected in July 2002 and May 2003 were analyzed for nitrate using the cadmium reduction and sulfanilamide method, and for orthophosphate using the ascorbic acid-molybdate method (Eaton et al. 1995). A Hobo ™ (Onset Corporation) temperature logger was used to record temperatures in the raceway throughout the study. Due to equipment failure, some temperature data were lost and had to be estimated using air temperature data, water temperature from previous years, and manual readings during those time periods. At the end of the study, water velocity was recorded (at 0.6 depth) at three locations within each confinement structure in the raceway using a Marsh-McBirney™ flow meter. Target values for abiotic factors were set according to the recommendations made by Gatenby et al. (2000). Flow rates were compared to those measured over mussel beds in the Green, Licking, and Rough rivers in Kentucky and the River Spree in Germany (Brunke et al. 2001, Hardison and Layzer 2001).

Algal density and composition

A fertilization regimen was developed for the 2-pond system using an indoor enrichment study to determine the effects of phosphate, nitrogen, ammonium, and iron on algal growth. Water samples from the raceway were supplemented with varying ratios of $NaH_2PO_4 \cdot H_2O$, NH_4Cl, $NaNO_3$, and $FeCl_2 \cdot 4H20$, and subjected to bright light at 20 º C for approximately 1 mo. At the peak of growth, each sample was poured through a pre-weighed, ashed fiberglass filter,

which was then dried at 100 ºC for 24 h. After drying, the filter was re-weighed and algal growth in weight was determined by subtraction. From these results, a fertilization regime was developed to maximize microalgal production and implemented in the pond. To the recirculating pond system, I added 9 kg of commercial 34-0-0 ammonium nitrate fertilizer (NH_4NO_3) weekly from April to December 2002 and April to August 2003; and 0.7 kg of commercial 0-45-0 triple super-phosphate fertilizer ($Ca(H_2PO_4)_2$) weekly from July to December 2002 and from April to August 2003. In addition to commercial fertilizer, approximately 6 burlap bags of leaf litter were added to the raceway in October of 2000, 2001, and 2002 to increase the availability of particulate organic matter. A target nitrogen to phosphorus ratio was set at greater than 10:1 following the recommendations of Gatenby et al. (2000).

I collected water samples from the raceway and reservoir ponds every 15 to 45 d throughout the study. Samples were fixed with acid Lugol's solution to preserve planktonic algae. To determine density and relative abundance of algal genera present in the water column, samples were concentrated in a 100 mL settling chamber for 48 h. Algae were counted and identified using an inverted microscope. The stage was moved randomly between 20 and 40 times per water sample. At each field of view, all algal cells within the ocular grid were counted. Phytoplankton genera were identified using the dichotomous key of Prescott (1978). Algal density was calculated using the equation from Vollenweider (1969):

$$\text{cells ml}^{-1} = (((\#\text{cells in a grid} * 130)\ \#\text{grids counted}^{-1}) * 98.174)\ 100^{-1}$$

Survival

Approximately 20 individuals each of 10 species of the subfamilies Ambleminae, Lampsilinae, and Anodontinae were collected from various locations in Virginia and West Virginia in August 2002 (Table 1.1). Mussels were transferred initially to the Freshwater

Mollusk Conservation Center at Virginia Tech in Blacksburg, VA for a quarantine period of 30 d. While in quarantine, numbered tags were glued to each mussel, and lengths were recorded. Also, approximately 10 to 25 cm of fly line was glued to each shell to aid in locating burrowed mussels when subsequently placed in the raceway. Ten percent of the mussels of each species were sent to the Lamar Fish Health Center, U.S. Fish and Wildlife Service, Lamar, PA to be analyzed for fish pathogens prior to deployment in the raceway.

Following the 30-day quarantine period and receipt of certification that mussels were pathogen-free, remaining mussels were transported in aerated coolers to WSSNFH. Mussels were placed within the raceway in 4 confinement structures (Figure 1.1), each approximately 1 m wide by 6 m long and 0.5 m deep. Cinder blocks were used to form the perimeter of the confinement structures. A hyphalon liner, covered with approximately 0.3 m of gravel, formed the substratum of each structure.

Mussels were assessed for survival after 7 mo in captivity in March 2003. Glycogen was analyzed in tissue clips of the mantle of 3 individuals of each species, using the phenol-sulfuric acid method (Dubois et al. 1956, Naimo et al. 1998). Gravidity was assessed using reverse pliers to open mussels, and visually determining whether mussels had glochidia in their brooding gills. This process was repeated at 12 mo. All remaining mussels then were returned to their stream of origin.

Data analysis

Due to the inability to recapture all of the captive mussels because they were deeply burrowed at 7 and 12 mo, survival rates were estimated with the Burnham joint live-dead model using MARK (White and Burnham 1999). The log ratio test was used to determine significance in survival rates among species and subfamilies held in the raceway for 1 yr. Glycogen levels of

mussel species were compared within species over time using Wilcoxon rank sums and Kruskal-Wallis tests in SAS (SAS Institute Carey, NC). A significance level of α=0.05 was used for all data analyses. Survival rates are reported $\bar{x} \pm 1$ SE; all other data are reported as $\bar{x} \pm 1$ SE.

RESULTS

Abiotic factors

Water quality parameters in the recirculating pond system at WSSNFH were within the target ranges for most of the 2 yr of this study (Table 1.2). Mean alkalinity was 58.8 ± 28.61 mg L^{-1} as $CaCO_3$, but fell below the target minimum value of 15 mg L^{-1} on one sample date in April of 2003. Recorded temperature data, which had a mean of 14.9 ± 7.3°C, exceeded the target maximum of 28°C on 10 d in the summer of 2002 and 12 d in the summer of 2003. However, mean weekly temperature never exceeded 28°C (Figure 1.2).

Approximately 454 L min^{-1} of water were pumped from the reservoir to the raceway into a volume of approximately 1.1 x 10^6 L. The highest velocities of 5.5 cm s^{-1} and 6.1 cm s^{-1} were recorded closest to each outlet pipe of the manifold (Figure 1.3). Zero flow was recorded at the upstream end of the second row of mussel confinement structures. However, flow increased downstream within some of the second row structures to a high of 1.5 cm s^{-1}. Velocities recorded over the confinement structures, although low, were within the range of flow rates recorded over mussel beds (Brunke et al. 2001).

Algal density and composition

In July 2002, a water sample collected from the source pipe had a nitrate concentration of 0.015 mg L^{-1}, and an orthophosphate concentration of less than 0.0001 mg L^{-1}, resulting in an N:P ratio of 15:1. The nitrate concentration of the fertilized reservoir was 0.207 mg L^{-1}, and the orthophosphate concentration was 0.01 mg L^{-1}, resulting in an N:P ratio of 21:1. In May of

2003, nitrate concentration of the fertilized reservoir was 0.8 mg L^{-1}, and the orthophosphate concentration was 0.06 mg L^{-1}, resulting in a N:P ratio of 13:1. All samples had a N:P ratio greater than the target ratio of 10:1.

Phytoplankton in the reservoir and raceway consisted of 50 genera of 7 phyla (Table 1.3), with mean densities of 1,915 cells ml^{-1} in the reservoir and 1,903 cells ml^{-1} in the raceway. Algal concentrations were lowest in December 2002 at 236 cells ml^{-1}, and increased during the summer months to a high of 4.7 x 10^3 cells ml^{-1} in July 2002 (Table 1.4). Algal diversity reached its peak in July of 2003, when 23 of the 50 identified genera were present in the raceway. Diversity of 20 genera or more also were observed in the raceway in November of 2001, July and September of 2002, and May and August of 2003.

Green, blue-green and diatom genera were numerically dominant in the open water of the reservoir throughout the study (Table 1.4). In addition, filamentous algae of *Sphaeroplea* sp. and *Cladophora* sp., undetected in water samples, colonized the reservoir by mid-July of each year of the study. By July of 2003, the raceway also was colonized with filamentous algae. In an attempt to control filamentous algae, water was re-circulated within the raceway each winter, and the reservoir was drained to remove dried filamentous algae from the pond bed.

Survival and condition

Survival estimates for freshwater mussels held in captivity for 1 yr at WSSNFH, were high, with 7 out of 10 species exhibiting survival greater than 70%. The mean survival estimate for all 10 species combined was 77%. Survival estimates differed significantly ($p<0.0001$) among species (Table 1.5); however, survival rates for the 3 subfamilies were not significantly different ($p<0.25$) (Table 1.5, Figure 1.4). Although there was no subfamily effect on survival, 2 lampsiline species, *L. cardium* ($S=0.31 \pm 0.138$) and *L. siliquoidea* ($S=0.57 \pm 0.111$), had the

lowest overall survival rates. The only anodontine in the study, *Pyganodon grandis*, also showed relatively low survival (S=0.59 ± 0.095). Four species had greater than 90% survival: the three lampsilines, *A. ligamentina* (S=0.91 ± 0.061), *V. iris* (S=0.90 ± 0.067), and *V. vanuxemensis* (S=0.96 ± 0.041), and the amblemine *C. tuberculata* (s=0.95 ± 0.041). Survival decreased in *A. plicata*, *L. cardium*, *L. siliquoidea*, *P. grandis*, *P. oviforme*, and *T. verrucosa* during the summer months, with most mortalities occurring between March 2003 and August 2003 (Figure 1.5). Glycogen levels (mg g^{-1}) were not significantly different over time in most species, but decreased significantly over time in 2 species, *A. ligamentina* (p<0.03) and *T. verrucosa* (p=0.05) (Figure 1.6). However, none of the species with the lowest survival rates exhibited a decrease over time in glycogen levels.

At the time of relocation, 22 gravid mussels were observed of the species *L. cardium*, *L. siliquoidea*, *P. oviforme*, *V. iris*, and *V. vanuxemensis* (Table 1.6). At subsequent dates, 9 of these individuals were still gravid and 6 additional individuals were observed gravid. All of the gravid individuals were bradytictic (long-term) brooders, except for one *P. oviforme* observed in August 2002.

DISCUSSION

Abiotic factors

Water quality parameters remained within target ranges for most of the 2 yr of the study. Some observed values were outside the desired range, but only water temperature exceeded the maximum target value (28° C) on more than one sample date. Despite attempts to reduce water temperature of the ponds during summer months by the periodic addition of 10°C spring water to cool the recirculating ponds, temperature readings exceeded 28° C on several days during summer of 2002 and summer of 2003. While temperatures reaching 34°C had little effect on

survival rates of freshwater mussels relocated to Kentucky Lake, TN (Dunn and Layzer 1997), other studies have cited high temperature as the cause of bivalve mortalities (Hummel et al. 1989, Burress 1995, Berthelin et al. 2000, Gatenby 2000). Survival rates of less than 6% for captive mussels held at the Leetown Science Center for 3 yr were attributed to high summer temperatures (Gatenby 2000). In addition, higher than normal summer temperatures have been the cause of sub-lethal effects such as increased parasitism (Barber et al. 1988), decreased egg production (Bayne et al. 1978), and decreased energy stores and physiological condition (Widdows 1978, Hummel et al. 1989) in marine bivalves. However, except for summer spikes in water temperature, all other water quality parameters were within the target ranges suggested by Gatenby et al. (2000), and appeared to be adequate for mussels held in the 2-pond system at WSSNFH.

Algal density and composition

Fertilization had an effect on both the nutrient level of the pond and planktonic algal concentrations. Due to greater hours of sunlight, nutrient levels of greater than 10:1 N:P ratio, and increased water temperatures in the summer months, algal concentrations reached 4.7×10^3 cells ml^{-1}. Average densities in the reservoir (1915 cells ml^{-1}) and the raceway (1903 cells ml^{-1}) at WSSNFH were high when compared to those in two rivers in the Ohio River Basin, the Kentucky River, KY, and the Kanawha River, WV, (algal densities 952 cells ml^{-1} and 1162 cells ml^{-1}, respectively) (Lizotte and Simmons 1985, Stevenson and White 1995). Cell concentrations at WSSNFH, however, were not as high as in some man-made systems used to contain mussels. Cell densities as high as 2.9×10^5 cells ml^{-1} were reported in an outdoor holding pond (Gatenby 2000), and average cell densities in an indoor partitioned aquaculture system were 1.1×10^5 cells ml^{-1} (Starkey et al. 2000). In contrast, Newton et al. (2001) reported that unfertilized, spring-fed

ponds used to hold mussels averaged chlorophyll *a* concentrations only 7% of those in the Upper Mississippi River. Despite fertilization of the WSSNFH ponds, algal density was lower than those reported at other holding facilities. Lower planktonic algal densities may be due to high densities of filamentous algae, inappropriate fertilization methods, or the displacement of algae-rich water caused by the addition of spring water needed to regulate pond temperature. Overall, algal densities appeared to be adequate to meet the nutritional needs of mussels.

Despite the presence of undesirable filamentous algae in the reservoir for most of my study, there were numerous planktonic genera observed, indicating that mussels had a diverse diet. Diatoms and green algae were two of the dominant algal types. Diatoms and green algae also were dominant in the Ohio River, and gut contents of freshwater mussels collected from the river had similar concentrations to those found in water samples (Parker et al. 1998). A study of the biochemical composition of two green algae species and one diatom species revealed that protein was the most abundant component, followed by carbohydrate and then lipid (Gatenby et al. 2003). While protein is necessary for tissue synthesis and gametogenesis in bivalves (Kreeger 1993), carbohydrates are important energy reserves and necessary for metabolism (de Zwaan 1983), and lipids are essential in gonad maturation during gametogenesis (Gabbott 1976). Polyunsaturated fatty acids (PUFA) and sterols, also reported to be important in bivalve diets (Leonardos and Lucas 2000), are present in green algae and diatoms (Gatenby et al. 2003). Assuming that algal densities met the needs of captive freshwater mussels, algal composition comprised a diverse diet.

Survival and condition

Although survival estimates for freshwater mussels held in captivity for 1 yr were reasonably high ($S = 0.77$), survival variation among species indicated that holding conditions

were suitable for most but not all species. Variability in survival estimates, ranging from 31% to 96% in captive species, was comparable to the results of 1-yr survival studies conducted at other captive holding facilities (Burress 1995, Dunn and Layzer 1997, Gatenby 2000). In evaluating various holding facilities for freshwater mussels, both Burress (1995) and Dunn and Layzer (1997) found that survival varied by species and holding facility. According to the results of these studies, mussels held in hatchery ponds or raceways (Frankfort Fish Hatchery, KY, and Marion Fish Hatchery, VA) in which fish also were being held, had the lowest survival rates. Although conducted at a hatchery, the reservoir and raceway at WSSNFH were conditioned specifically for mussels, with no fish being held simultaneously. Compared to survival rates of less than 50% in numerous relocation studies (Cope and Waller 1995, Dunn et al. 2000), survival estimates in this study indicate that holding conditions at WSSNFH are adequate for most species.

In contrast to previous studies, in which mussels were relocated to captivity or infested with zebra mussels (Haag et al. 1993, Baker and Hornbach 1997, Dunn and Layzer 1997, Newton et al. 2001), survival in my study did not differ among subfamilies. Two species within the subfamily Lampsilinae, *L. cardium* and *L. siliquoidea*, had the lowest survival estimates (S=0.31, 0.57), but three other lampsiline species, *A. ligamentina*, *V. iris*, and *V. vanuxemensis* had high survival rates(S=0.91, 0.90, 0.96). Lampsilines, in previous studies, also exhibited lower glycogen levels over time than other subfamilies (Haag et al. 1993, Baker and Hornbach 2001). Although declining glycogen levels were observed in one lampsiline species (*A. ligamentina*) and one amblemine species (*T. verrucosa*) in my study, glycogen levels did not decline in any of the species with lower survival rates.

No conclusions can be drawn as to why Lampsilis spp. had poor survival, since other lampsilines exhibited high survival under captive conditions. Other factors, such as water chemistry, water velocity, or lower tolerance in these species to high water temperatures, may have been the cause of variable survival rates among species. The three species with the lowest survival estimates; *L. cardium*, *L. siliquoidea*, and *P. grandis*, typically reside in deeper water with slow or negligible current (Cummings and Mayer 1992, Parmalee and Bogan 1998). Due to this preference, it is possible that these species are less tolerant to high or highly variable water temperatures. As mentioned previously, mortalities of captive mussels have been attributed to high summer temperatures (Gatenby 2000). In my study, survival recorded between August 2002 and March 2003 was higher in *L. cardium*, *L. siliquoidea*, and *P. grandis* than that recorded between March 2003 and August 2003 when temperatures exceeded 28°C. In marine bivalves, higher than normal temperatures can lead to increased metabolism and decreased carbohydrate or protein levels (Gabbott and Walker 1971, Gabbott and Bayne 1973). Therefore, decreases in glycogen analyses should have been apparent if high temperatures were spurring increased metabolism, but may have been too infrequent or sample size may have been too small to detect declines in these species. It is also possible that these species were sensitive to chemicals or elements present in the water that were not measured as part of this study. In order to improve captive holding conditions for these species, water chemistry, velocity and temperature preferences should be further investigated.

Conclusions and recommendations

Captive conditions in the raceway at WSSNFH were suitable for most mussel species held for 1 yr. In addition, abiotic factors were within target ranges for most of the study except summer water temperatures. I recommend that additional spring water be added directly to the

raceway in the summer months in order to keep temperatures below 28°C. Algal density and composition appeared to be adequate, but could be increased to promote growth and continued health of freshwater mussels. I recommend that additional fertilizer be added at a N:P ratio of greater than 10:1 to increase algal densities. Fertilization should be increased during the summer months when spring water is being added to cool the system. In addition, alternate fertilization regimes, such as liquid fertilizer or organic fertilizer, should be investigated. Although conditions were not seemingly suitable for all mussel species, most species maintained high survival rates and glycogen levels over 1 yr. In order to determine habitat preferences within confinement structures, a more controlled long-term study in which mussels are placed in areas of the raceway with differing velocities should be conducted. Specifically, more research should be conducted to determine suitable captive conditions for *L. cardium*, *L. siliquoidea*, and *P. grandis* before large numbers are brought into captivity. Until this can be accomplished, I recommend that species be placed in raceway confinement structures that have similar water velocities to those in their habitat of origin. The similarity in survival rates of species in the 3 subfamilies indicates that the raceway at WSSNFH provides adequate holding conditions for a suite of mussel species.

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Table 1.1. Species, sample sizes, and origins of freshwater mussels relocated to WSSNFH in August 2002.

Subfamily / Species	*n*	Origin
Ambleminae		
Amblema plicata	23	Little Kanawha, WV
Cyclonaias tuberculata	24	New River, WV
Pleurobema oviforme	24	North Fork Holston River, VA
Tritogonia verrucosa	23	New River, WV
Anodontinae		
Pyganodon grandis	27	Claytor Lake, VA
Lampsilinae		
Actinonaias ligamentina	22	New River, WV
Lampsilis cardium	19	Little Kanawha, WV
Lampsilis siliquoidea	21	Little Kanawha, WV
Villosa iris	20	North Fork Holston River, VA
Villosa vanuxemensis	22	North Fork Holston River, VA

Table 1.2. Mean (± 1SD), minimum, and maximum observed and target values for water quality parameters at the WSSNFH from August 2001 to August 2003.

		Observed Values			Target Values
Parameters	*n*	Mean	Min	Max	
pH	26	7.71 ± 0.48	6.43	8.72	> 6.5
Temperature (°C)	776	14.9 ± 7.3	0.73	29.4	< 28
Alkalinity (mg L^{-1} as $CaCO_3$)	26	58.8 ± 28.6	10	100	> 15
Hardness (mg L^{-1} as $CaCO_3$)	25	228.2 ± 129.68	70	440	> 50
Dissolved Oxygen (mg L^{-1})	26	10.8 ± 1.06	8.26	12.28	> 6

Table 1.3. Genera of algae observed in water samples from recirculating ponds at WSSNFH from September 2001 through August 2003.

Order / Genus	**Order** / Genus
Blue-Green Algae (Cyanoprokaryota)	**Green Algae (Chlorophyta)**
Chroococcus	*Ankistrodesmus*
Coelospherium	*Carteria*
Gloeocapsa	*Chlamydomonas*
Hyella	*Chlorella*
Oscillatoria	*Chlorococcum*
Spirulina	*Closterium*
Cryptophytes (Cryptophyta)	*Cosmarium*
Chroomonas	*Desmococcus*
Diatoms (Bacilariophyta)	*Eremosphaera*
Cocconeis	*Eudorina*
Cyclotella	*Gloeocystis*
Cymatopleura	*Golenkiniopsis*
Cymbella	*Microspora*
Diatoma	*Monocillia*
Fragilaria	*Oedogonium*
Gonphonema	*Oocystis*
Meridian	*Pandorina*
Navicula	*Pediastrum*
Nitzschia	*Rhizoclonium*
Pinnularia	*Scenesdesmus*
Synedra	*Schroederia*
Tabellaria	*Stephanodiscus*
Euglenoids (Euglenophyta)	*Stichococcus*
Euglena	*Trochiscia*
Trachelomonas	*Ulothrix*
Golden Algae (Chrysophyceae)	**Dinoflagellate (*Dinophyta*)**
Chromulina	*Peridinium*
Mallomonas	

Table 1.4. Summary of ordinal composition, cell density (cells ml^{-1}), and number of algal genera in water samples collected from the raceway (pond B2) at WSSNFH, August 2001 to August 2003, and from the reservoir (pond B1), May 2002 to January 2003 and May 2003 to August 2003. *n*=28 samples collected from the raceway and *n*=18 samples collected from the reservoir.

	Raceway (pond B2)	Reservoir (pond B1)
	Percent Composition	
Green	38.1	42.2
Blue-Green	30.2	28.7
Diatom	26.2	17.9
Cryptophyte	3.9	8.1
Golden	1.0	2.7
Dinoflagellate	0.3	0.3
Euglenoid	0.1	0.1
Other	0.2	0.0
	Cell Density	
Mean	1904	1915
Maximum	4658	3420
Minimum	300	236
	No. of Genera	
Mean	16	15
Maximum	23	18
Minimum	5	12

Table 1.5. Survival and recapture (fraction of stocked individuals found) estimates (± 1 SE) for freshwater mussels after 7 mo (August 2002 to March 2003) and 12 mo (August 2002 to August 2003) in captivity at WSSNFH, calculated using program MARK.

		Survival Rate		Recapture Rate	
Subfamily / Species	*n*	March 2003	August 2003	March 2003	August 2003
Ambleminae		**0.93 ± 0.016**	**0.77 ± 0.026**	**0.82 ± 0.025**	**0.80 ± 0.027**
Amblema plicata	23	0.93 ± 0.016	0.77 ± 0.026	0.82 ± 0.025	0.81 ± 0.027
Cyclonaias tuberculata	24	0.95 ± 0.041	0.95 ± 0.041	0.92 ± 0.056	0.87 ± 0.070
Pleurobema oviforme	24	1.00 ± 0.000	0.88 ± 0.068	0.71 ± 0.090	0.58 ± 0.100
Tritogonia verrucosa	23	1.00 ± 0.000	0.74 ± 0.092	0.78 ± 0.086	0.91 ± 0.059
Anodontinae		**0.96 ± 0.036**	**0.59 ± 0.095**	**0.81 ± 0.075**	**0.73 ± 0.087**
Pyganodon grandis	27	0.96 ± 0.036	0.59 ± 0.095	0.81 ± 0.074	0.73 ± 0.087
Lampsilinae		**0.90 ± 0.030**	**0.75 ± 0.038**	**0.84 ± 0.036**	**0.81 ± 0.039**
Actinonaias ligamentina	22	1.00 ± 0.000	0.91 ± 0.061	1.00 ± 0.000	0.95 ± 0.044
Lampsilis cardium	19	0.68 ± 0.107	0.31 ± 0.138	0.95 ± 0.051	0.92 ± 0.074
Lampsilis siliquoidea	21	0.90 ± 0.064	0.57 ± 0.111	0.81 ± 0.085	1.00 ± 0.000
Villosa iris	20	0.90 ± 0.067	0.90 ± 0.067	0.85 ± 0.080	0.78 ± 0.098
Villosa vanuxemensis	24	0.96 ± 0.041	0.96 ± 0.041	0.62 ± 0.099	0.52 ± 0.104
Pooled Species		0.94 ± 0.011	0.77 ± 0.018		

Table 1.6. Gravid individuals observed at the time of relocation to WSSNFH in August 2002, and previously gravid and newly gravid individuals observed in March 2003 and August 2003. *n* = number of individuals of each species relocated.

Species	*n*	August 2002	March 2003		August 2003	
			prev	new	prev	new
Actinonaias ligamentina	22	-	-	2	-	-
Lampsilis cardium	19	2	1	-	1	-
Lampsilis siliquoidea	21	7	5	2	2	-
Pleurobema oviforme	24	1	-	-	-	-
Villosa iris	20	4	1	-	-	-
Villosa vanuxemensis	22	8	3	1	3	1

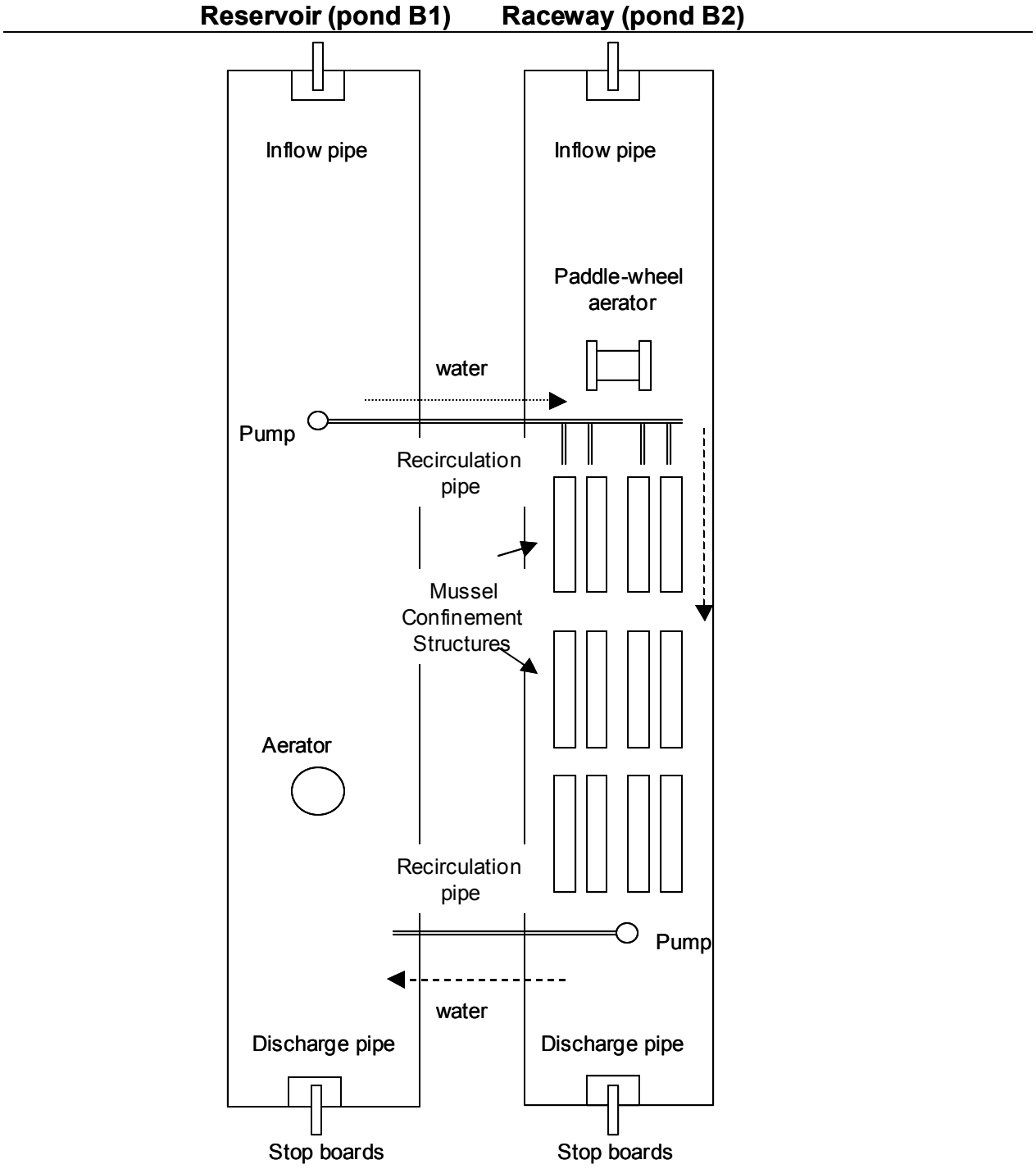

Figure 1.1. Recirculating pond system at WSSNFH. Dimensions of reservoir and raceway were 11m x 84m x 1m.

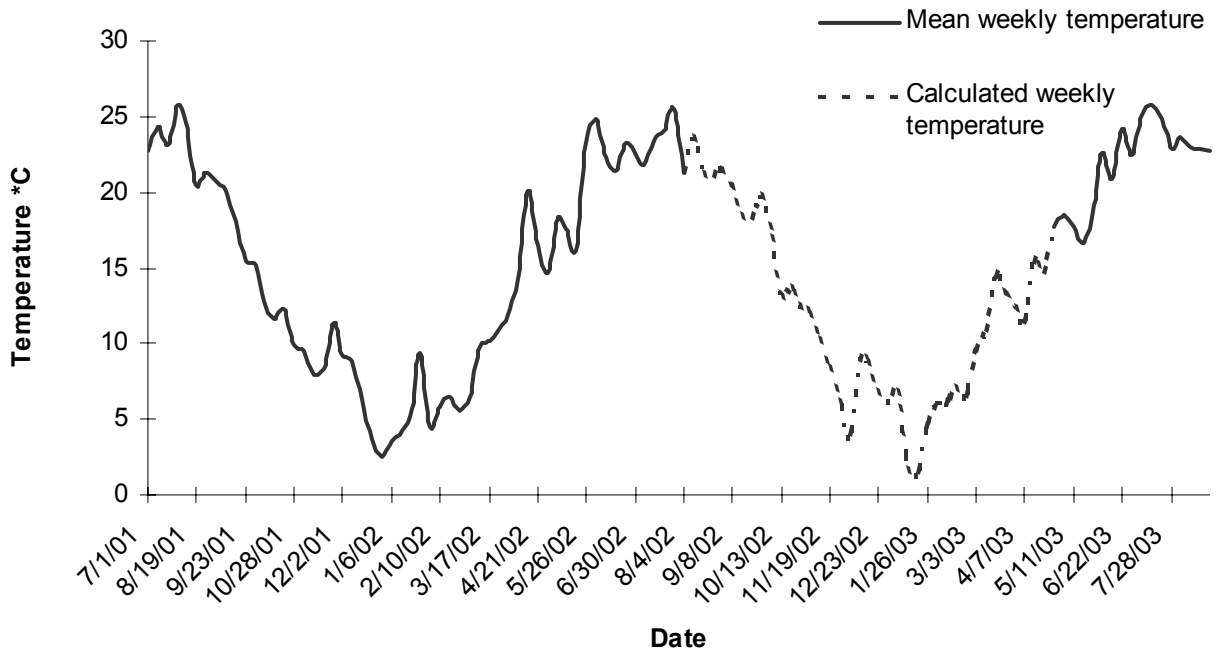

Figure 1.2. Mean weekly water temperature (°C) for the raceway at WSSNFH.

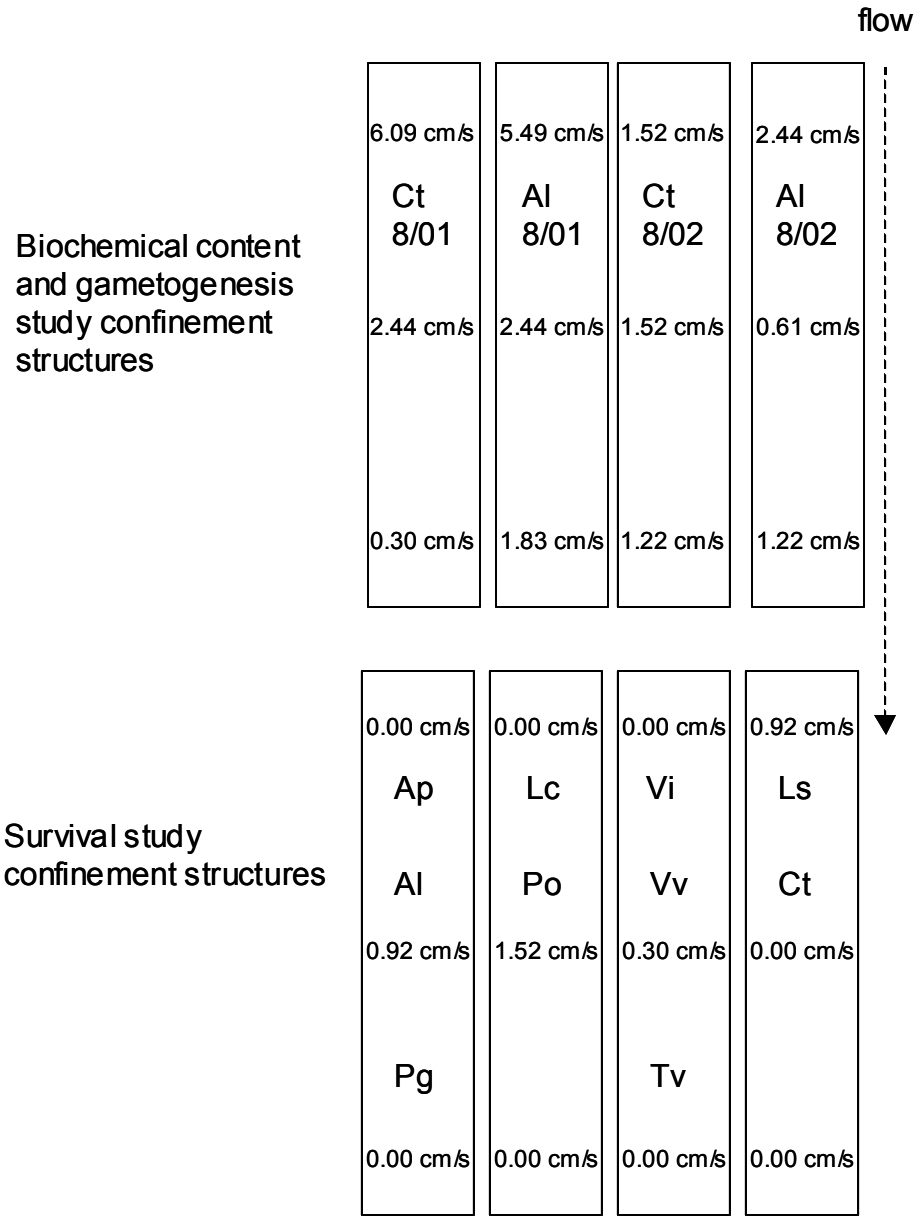

Figure 1.3. Water velocities and locations of freshwater mussels within mussel confinement structures in the raceway at WSSNFH. Species in the study are Al (*Actinonaias ligamentina*), Ap (*Amblema plicata*), Ct (*Cyclonaias tuberculata*), Lc (*Lampsilis cardium*), Ls (*Lampsilis siliquoidea*), Pg (*Pyganodon grandis),* Po (*Pleurobema oviforme*), Tv (*Tritogonia verrucosa*), Vi (*Villosa iris*), and Vv (*Villosa vanuxemensis*). Dimensions of each structure were 1m x 6m x 0.5m.

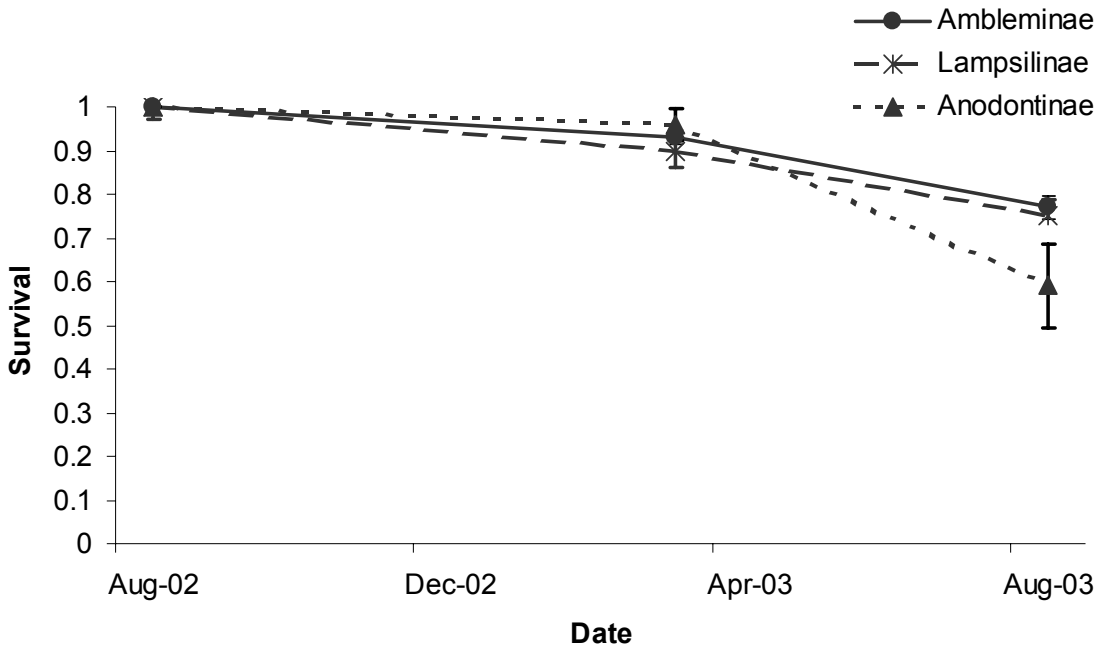

Figure 1.4. Annual survival estimate (± 1 SE) for three subfamilies of freshwater mussels held in captivity at WSSNFH from August 2002 to August 2003.

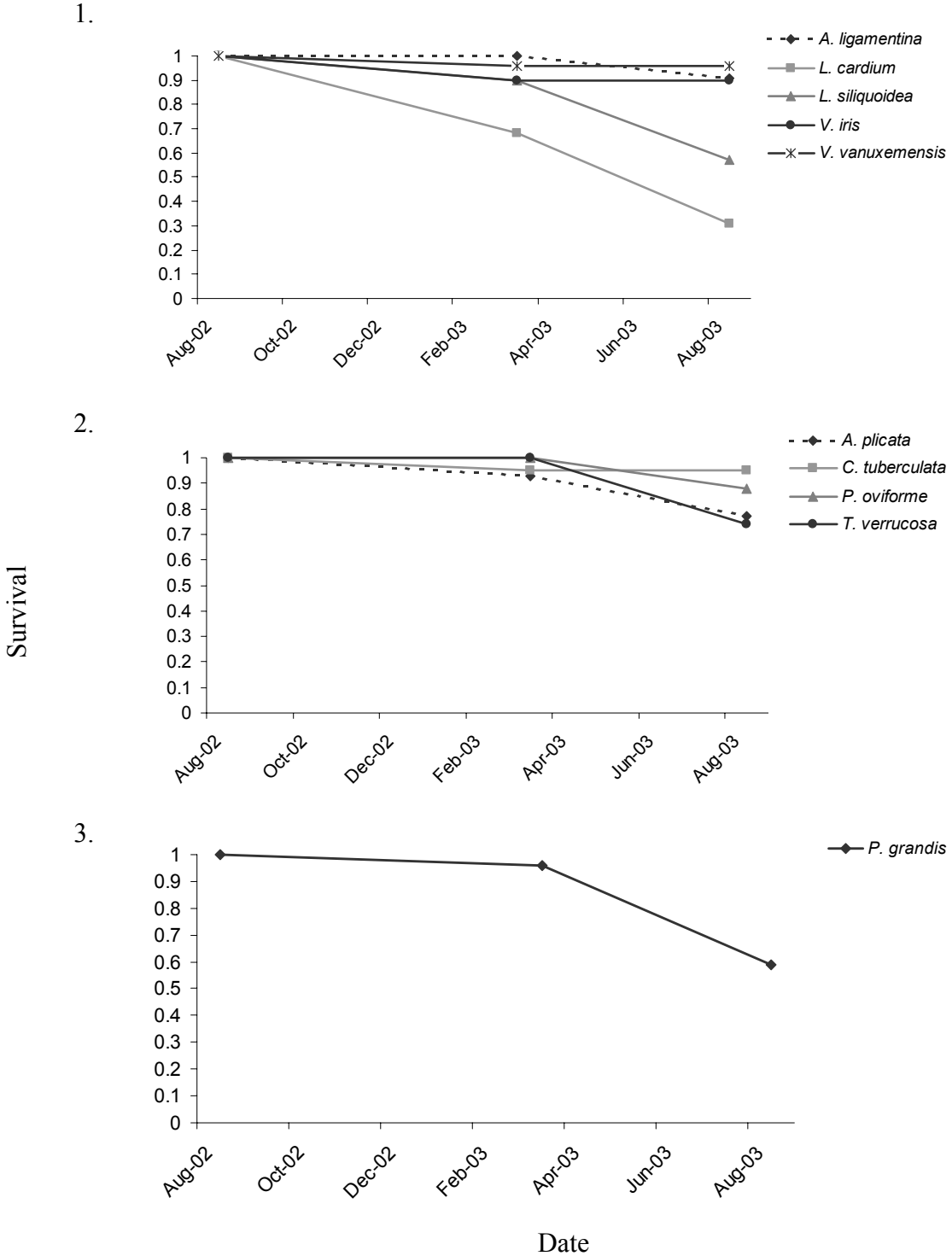

Figure 1.5. Survival estimates of species in subfamilies 1. Lampsilinae 2. Ambleminae and 3. Anodontinae held in captivity at the WSSNFH from August 2002 to August 2003.

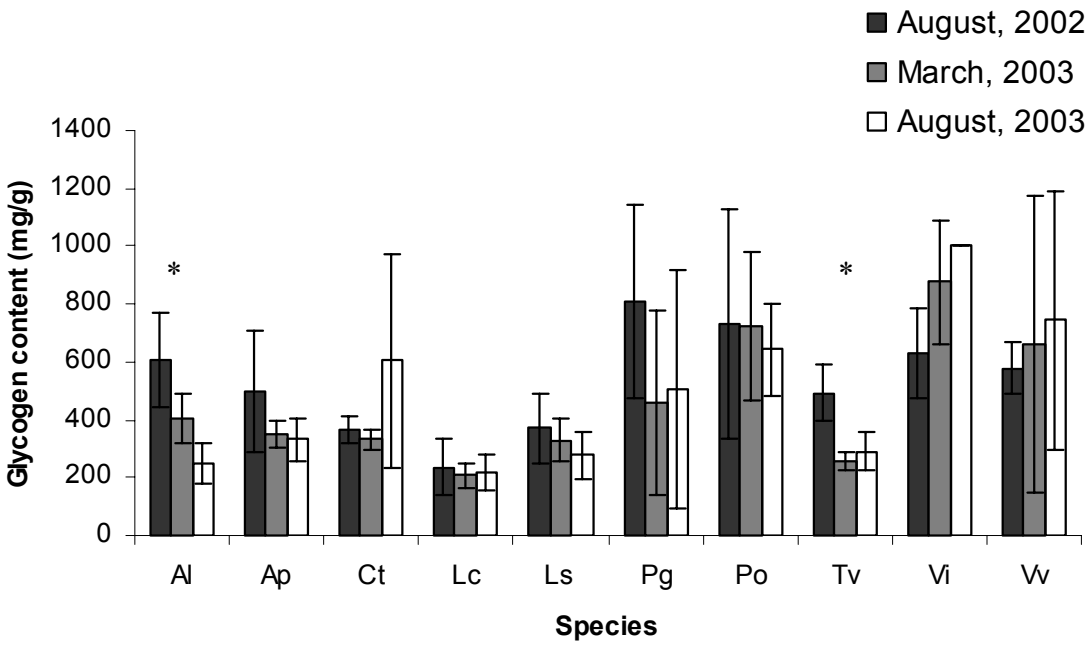

Figure 1.6. Mean (± 1 SD) glycogen content in mg/g dry weight in August 2002, March 2003, and August 2003 for ten mussel species relocated to WSSNFH. Species in the study were Al (*Actinonaias ligamentina*), Ap (*Amblema plicata*), Ct (*Cyclonaias tuberculata*), Lc (*Lampsilis cardium*), Ls (*Lampsilis siliquoidea*), Pg (*Pyganodon grandis),* Po (*Pleurobema oviforme*), Tv (*Tritogonia verrucosa*), Vi (*Villosa iris*), and Vv (*Villosa vanuxemensis*). * Denotes significant differences of p=0.027 in Al and p=0.05 in Tv determined by the non-parametric Kruskall-Wallace test.

Chapter 2: EVALUATION OF THE BIOCHEMICAL COMPOSITION AND GAMETOGENESIS OF FRESHWATER MUSSELS HELD IN CAPTIVITY AT WSSNFH, WEST VIRGINIA

INTRODUCTION

Captive holding has conservation applications for freshwater mussels, serving objectives such as providing refugia for imperiled species and propagating juveniles for reintroduction. However, holding freshwater mussels in captivity for extended periods of time has proven challenging to researchers and managers. Stressful environmental conditions, from poor water quality, unsuitable substrate type, inadequate food availability, and extreme water temperature, can adversely affect physiological processes, and eventually lead to mortality (Imlay 1972, Imlay 1973, Baker and Hornbach 1997, Heinricher and Layzer 1999, Newton et al. 2001). For captive holding to prove successful, mussel condition should be monitored to determine whether environmental conditions are adequate. Observations of gametogenesis and biochemical composition of tissue samples are two approaches that have been used in previous studies to assess condition of freshwater bivalves (Haag et al. 1993, Makela and Oikari 1995, Baker and Hornbach 1997, Patterson et al. 1997, Naimo et al. 1998, Chen et al. 2001, Hallac and Marsden 2001, Hyotylainen et al. 2002, Greseth et al. 2003). More than 85% of the biochemical composition of freshwater mussels is carbohydrate, protein, and lipid (Baker and Hornbach 2001). Drastic or sustained declines in gametogenesis or energy substrates indicate a decrease in physiological condition.

Carbohydrate, protein, and lipid content are commonly measured in bivalves to study the seasonal cycles of energy use and physiological condition (Gabbott and Walker 1971, Bayne 1973, Gabbott 1976, Haag et al. 1993, Kreeger 1993, Makela and Oikari 1995, Baker and

Hornbach 1997, Berthelin et al. 2000, Greseth et al. 2003). Glycogen is the main storage carbohydrate in bivalves (Hummel et al. 1989) and the primary energy reserve (deZwaan and Zandee 1972). In freshwater mussels, it is the first energy substrate to be catabolized (Haag et al. 1993, Baker and Hornbach 2000). Commonly used to assess the physiological condition of mussels, glycogen content can be an indicator of stressful environmental conditions (Patterson et al. 1997, Naimo and Monroe 1999, Newton et al. 2001, Hyotylainen et al. 2002). Hallac and Marsden (2001) reported that glycogen content often decreases in mussels subjected to environmental stress before mortality occurs. They found that zebra mussel-fouled *Elliptio complanata* had a 50% reduction in glycogen content compared to the un-fouled control, but survival did not differ between the groups. Because it is typically the first energy reserve to be used, researchers have relied upon glycogen analyses to detect adverse impacts upon freshwater mussels.

Although glycogen is the main energy reserve in bivalves, proteins and lipids are important energy sources throughout the reproductive cycle, especially in nutritionally stressed individuals (Bayne 1973, Hummel et al. 1989). Several studies have shown an inverse relationship between levels of glycogen and both proteins and lipids (Bayne 1973, Gabott 1976, Berthelin et al. 2000, Baker and Hornbach 2001). Glycogen is utilized as energy by marine bivalves prior to and during the reproductive cycle, while proteins and lipids are conserved. After spawning, proteins and lipids are utilized as energy, while glycogen reserves are recovered (Gabbott 1976, Kreeger 1993, Berthelin et al. 2000). According to Bayne (1973), the marine bivalve *Mytilus* sp. relies on the catabolism of protein and lipids to continue physiological processes during prolonged starvation. These studies of marine bivalves suggest that protein and

lipid content can provide additional information about the condition of freshwater mussels not readily apparent from glycogen analyses alone.

Biochemical studies of marine bivalves are numerous, but few researchers have measured glycogen, protein, and lipid content simultaneously in freshwater mussels. Baker and Hornbach (1999) determined the physiological status and biochemical composition of freshwater mussels infested with zebra mussels. While lipid content was not significantly different, protein and carbohydrate levels were significantly lower in infested versus non-infested mussels. These results provided evidence of a weakened physiological condition in infested mussels. Makela and Oikari (1995) also measured glycogen, protein, and lipid content in captive and wild freshwater mussels. Although they reported no significant differences in glycogen and lipid levels, there were lower protein levels in captive mussels compared with those taken directly from the natural environment. These studies provided evidence that biochemical content of freshwater mussels, including protein and lipid, can be adversely affected by environmental stress and zebra mussel infestation.

While it is best to analyze whole bodies to determine biochemical composition of organisms, individual organ and mussel tissues have often been used (Hagar and Dietz 1986, Makela and Oikari 1995, Naimo and Monroe 1999, Hyotylainen 2002, Greseth et al. 2003). Naimo and Monroe (1999) compared glycogen content in mantle tissue and foot tissue, and concluded that mantle tissue, although more variable than foot tissue, had higher glycogen levels. This result supports the use of mantle tissue for comparison between specimens, if care is taken to sample the tissue consistently.

The occurrence of gametogenesis in freshwater mussels can indicate that there are enough energy reserves to carry out normal physiological processes. Over the past two decades,

gametogenesis has been studied in a number of mussel species (Zale and Neves 1982, Peredo and Parada 1984, Jones et al. 1986, Holland-Bartels and Kammer 1989, Weaver et al. 1991, Jirka and Neves 1992, Haggerty et al. 1995, Makela and Oikari 1995, Heinricher and Layzer 1999, Garner et al. 1999). Evidence of asynchronous gametogenic stage or lack of gametogenesis in marine bivalves has been used to indicate environmental stress (Bayne et al. 1978, Barber 1996). However, only a few studies have compared the gametogenic cycle of relocated populations to natural populations of freshwater mussels (Makela and Oikari 1995, Heinricher and Layzer 1999, Henley 2002). Makela and Oikari (1995) used a gametogenic stage index to report that gametogenesis was delayed in captive mussels compared to the 'natural' population. Stage indices, which have been used to identify the reproductive stage of bivalve gonads, are the most common method of categorizing gametogenesis (Dinimani 1974, Lowe et al. 1982, Peredo and Parada 1984, Holland-Bartels and Kammer 1989, Barber 1996, Heinricher and Layzer 1999, Henley 2002, Choi and Chang 2003). Gametogenic stage analyses can provide information that explains seasonal variation in biochemical composition (Kreeger 1993, Makela and Oikari 1995). Decreased or asynchronous gametogenesis in captive mussels compared to their wild counterparts would indicate that captive conditions have changed natural cycles or are not conducive to normal reproductive activity.

The goal of this study was to determine the feasibility of holding adult freshwater mussels in long-term captivity at White Sulphur Springs National Fish Hatchery (WSSNFH), White Sulphur Springs, WV. Specific objectives were to determine 1) whether captive conditions affect energy storage of glycogen, proteins, and lipids compared to the wild population in the New River; and 2) whether captive conditions affect gametogenic activity and spawning compared to the wild population in the New River. Species chosen for this study were

the mucket (*Actinonaias ligamentina*), purple wartyback (*Cyclonaias tuberculata*), and pistolgrip (*Tritogonia verrucosa*). These species are common in the New River, represent two subfamilies, and differ in reproductive strategies.

METHODS

Relocation

Freshwater mussels were collected from the New River below Bluestone Dam, in Summers County, West Virginia, in June 2001 and June 2002, for relocation to WSSNFH. A total of 110 *A. ligamentina,* 108 *C. tuberculata,* and 54 *T. verrucosa* were collected in June of 2001. An additional 75 *A. ligamentina* and 75 *C. tuberculata* were collected from the New River in June 2002. Mussels initially were transferred to the Freshwater Mollusk Conservation Center at Virginia Polytechnic Institute and State University (VPI & SU) in Blacksburg, VA for a quarantine period of 30 d. While in quarantine, numbered tags were glued to each mussel, and lengths were recorded. Additionally, approximately 25 cm of fly-fishing line was glued to each mussel to aid in subsequently locating burrowed mussels while in captivity. Ten percent of the specimens of each species were sent to the Lamar Fish Health Center, U.S. Fish and Wildlife Service, in Lamar, PA to be examined for pathogens.

Following the 30-day quarantine period and receipt of certification that mussels were free of pathogens, remaining mussels were relocated to a recirculating pond system at WSSNFH in August of each year. Mussels were placed in substrate within concrete block confinement structures in the raceway (pond B2) as described in the methods section of Chapter 1 (Figure 2.1). The water quality parameters of temperature, dissolved oxygen (DO), pH, alkalinity, and hardness were measured in the raceway. Algal cell concentration was measured in both the raceway and reservoir (pond B1) periodically throughout the study. Due to equipment failure,

some temperature data had to be estimated using air temperature data, water temperatures from previous years, and manual readings for those time periods. The Army Corps of Engineers recorded temperatures from the New River weekly at Bluestone Dam. Water velocity within the confinement structures also was measured (Figure 2.1)(Chapter 1).

Sampling

Six individuals each of *A. ligamentina* and *C. tuberculata* were collected every 2-3 mo between June 2001 and June 2003 from the captive population at WSSNFH and the wild population in the New River. Due to the lower initial number of *T. verrucosa* collected in June 2001, tissue samples were collected from this species only during the first year of the study. After the addition of mussels collected from the wild in 2002, 6 *A. ligamentina* and 6 *C. tuberculata* from each of the captive groups (yr 1 and yr 2) were collected every 2 to 3 mo, in addition to the 6 mussels of each species collected from the wild populations in the New River. Mussels sampled for tissue analyses were packed on ice and transported to the Freshwater Mollusk Conservation Center at VPI & SU for processing. For each individual, tag number and shell length were recorded, gills were checked for gravidity, and mantle tissue and visceral mass were removed. Mantle tissue was placed in a -60°C freezer for later biochemical analysis, and gonads were placed in Bouin's fixative for later histological analysis.

Glycogen analysis

Samples of mantle tissue for the three species were lyophilized and pulverized into a powder. Tissue samples of between 2.5 mg and 5 mg were weighed and placed into 2 ml cryovials. Glycogen concentration was analyzed by the phenol-sulphuric acid method (Dubois et al. 1956, Naimo et al. 1998). After the initial weight was recorded, 500 µl of 30% KOH was added to the cryovials which then were incubated at 100°C for 20 min to digest the tissue.

Samples then were cooled, 750 µl of 95% EtOH was added to prevent the precipitation of additional polysaccharides (Naimo et al. 1998), and incubated for an additional 15 min. Following the second incubation, water was added to each sample to bring the volume to 6.6 ml. Aliquots of 2 ml then were placed in a test tube and 100 µl of 80% phenol followed by 5 ml of sulphuric acid were added before incubation at room temperature for 30 min. After the final incubation, optical density of each sample at 490 nm was observed spectrophotometrically (Genesis 8; Thermo Spectronic, Rochester, NY). A standard curve was generated using glycogen standards, prepared with powdered glycogen (Type VII, *Mytilus edulis*, Sigma Chemical Company, St. Louis, MO) and analyzed using identical methods to those used for the tissue samples. Sample values were compared to the standard curve, and the glycogen concentration was determined using the following equation:

mg/g glycogen = (mg/L glycogen / 1000ml) / g tissue weight

Protein analysis

Between 5 and 6.5 mg of lyophilized, pulverized mantle tissue from *A. ligamentina* or *C. tuberculata* was weighed into a test tube. Then, 8 ml of 0.1 N NaOH was added to the sample and a Tekmar Tissuemizer (Tekmar Co., Cincinnati, OH) was used to homogenize and dissolve the tissue (Barber et al. 1988, Kreeger 1993). Proteins then were analyzed using a BCA Protein Assay Reagent Kit (#23225, Pierce, Rockford, IL) based on methods developed by Lowry et al. (1951). Standards were prepared using bovine serum albumin provided in the kit; a standard curve was generated, and sample values were compared to the curve. Protein concentration of the tissue was determined using the following equation:

mg/g protein = ((µg/ml/1000)*8) / g tissue weight

Lipid analysis

Lipid content of lyophilized, pulverized mantle tissue samples was analyzed using a chloroform-methanol method (Folch et al. 1957, Barnes and Blackstock 1973, and Barber et. al 1988). Approximately 50 mg of tissue was weighed into a scintered glass filter funnel; 10 ml of 2:1 chloroform-methanol solution and 2 ml of 80% NaCl solution were added. Samples then were vortexed and centrifuged to separate the sample, leaving the lipid-rich chloroform on the bottom. The top layer was removed, and 5 ml of the remaining chloroform solution was placed in a pre-weighed pan. Chloroform was evaporated from the pan in a convection oven for 10 min and pans were re-weighed. To determine the lipid weight, the pan weight was subtracted from the final weight. Lipid concentration of the tissue was calculated as follows:

mg/g DW lipid = [(lipid weight /aliquot volume) x (total volume/tissue)]/1000

Histology and gametogenic analysis

Gonad samples from river mussels collected between June 2001 and June 2003 were evaluated, but because of expense only gonad samples collected from captive mussels between October 2002 and June 2003 were evaluated. A central section of the gonad of each mussel was excised from the visceral mass using a razor blade, preserved in Bouin's fixative (75% picric acid, 20% formalin, 5% acetic acid), and stored in 95% ethyl alcohol until processing. The tissue was cleared with xylene and embedded in paraffin. Four 5 μm-thin sections were cut from each gonadal mass with a microtome. The sections then were affixed to glass microscope slides, and the gonadal tissue stained with Heidenhain's iron hematoxylin and eosin-orange G according to standard methods (Humason 1979).

The prepared gonadal section slides were magnified 10 X and quantitatively assessed. Gametogenic stage was determined by randomly selecting 16 fields of view per slide. Four

points within each field of view, as described by Chalkley (1943), were assigned a developmental stage on a scale of 0 to 5, using the gamete development index (GDI) adapted from Barber (1996) and Henley (2002). This amounted to 64 observations per slide, and 256 observations per gonad. Developmental stage, or GDI, of each observation site was categorized (Table 2.1): 0, inactive; 1, early active; 2, late active; 3, mature; 4, spawning; 5, spawned and resorbing (Barber 1996, Henley 2002). For each of the 256 points observed per gonad, gamete area fraction (GAF) also was recorded by designating cells as active (1, 2, 3, 4, or 5) or inactive (0) (Bayne et al. 1978, Kreeger 1993, Henley 2002). Each mussel also was assessed for gravidity at the time of collection by determining presence or absence of glochidia within the gills of females.

Data analysis

Biochemical data collected from captive and wild mussels were analyzed using Wilcoxon rank sums two-sample tests and Kruskal-Wallis tests using the SAS software package (SAS Institute, Carey, NC). GDI values, collected from captive and wild mussels during the second year of this study (October 2002 to June 2003), were analyzed using Chi-Square Test of Homogeneity. Values of the GAF observed in captive and wild mussels and water temperature data collected from the New River and the raceway were compared using paired t-tests (JMPIN, SAS Institute, Carey, NC). Due to the small sample size ($n<6$) and confirmation from previous studies that male and female mussels are synchronous spawners (Jirka and Neves 1992, Haggerty et al. 1995, Garner et al. 1999), data were not analyzed by sex. A significance level of $\alpha=0.05$ was used for all data analyses.

RESULTS

Biochemical content

There was little difference in the glycogen levels of captive and wild mussels (Tables 2.2, 2.3, and 2.4). Mantle glycogen levels of wild and captive *A. ligamentina* differed significantly on two of the sample dates (Table 2.2, Figure 2.2). In September 2001, mean glycogen content of mantle tissue in captive mussels relocated in yr 1 was significantly lower ($p<0.02$) than that observed in the wild samples. The other significant difference was in January 2003, when glycogen content of captive samples was significantly higher ($p=0.002$) than that of wild samples. However, sample size of the wild population was very small ($n=2$). Glycogen content among captive and wild *C. tuberculata* differed only once. Mantle glycogen in August 2002 was higher in wild than captive *C. tuberculata* relocated in yr 1 ($p<0.02$) (Table 2.3, Figure 2.2). There were no differences in mantle glycogen contents of captive and wild *T. verrucosa* (Table 2.4, Figure 2.2). At the end of the 23-mo captive period, there was no significant difference in mantle glycogen levels between captive and wild populations of any of the three species.

Mantle glycogen content of *A. ligamentina* and *C. tuberculata* in the New River varied over time. The highest glycogen concentrations observed in both *A. ligamentina* and *C. tuberculata* occurred in January 2002 (Tables 2.2 and 2.3, Figure 2.2). The lowest glycogen content of wild *A. ligamentina* was observed in January 2003. Lower mantle glycogen content also was apparent in *C. tuberculata* in October 2002 and January 2003. Mantle glycogen of *T. verrucosa* varied less over time than for other species.

There were significant differences in protein and lipid content among wild and captive mussels on multiple sample dates. Protein content of wild and captive *A. ligamentina* mantle tissue differed in April 2003 (Table 2.5, Figure 2.3), when protein content of the wild sample

was significantly higher than that of captive mussels relocated in yr 1 (p<0.02). Protein content of *C. tuberculata* mantle tissue differed significantly on 6 of the 10 dates of sample collections (Table 2.6, Figure 2.3). At each of the sample events in which there was a significant difference, protein content was higher in captive mussels. Lipid content of captive *A. ligamentina* was significantly higher (p<0.04) than that of wild *A. ligamentina* in January 2003 (Table 2.7, Figure 2.4). Lipid content of captive *C. tuberculata* mantle tissue was higher (p>0.005) in October of 2002 (Table 2.8, Figure 2.4).

Gametogenesis

Gonad condition and gamete development were examined in *A. ligamentina* (n=117) and *C. tuberculata* (n=116) collected from the New River from June 2001 to June 2003, and WSSNFH from October 2002 to June 2003. Of the *A. ligamentina* examined, 44% were female, 36% were male, and 20% were undifferentiated; for *C. tuberculata*, 39% were female, 59% males, and 2% hermaphrodites.

Active gametogenesis was evident in both captive and wild *C. tuberculata* (Figures 2.5, 2.6, and 2.7). Although multiple stages of gamete development were observed at each sample date, a distinct gametogenic cycle was observed in wild *C. tuberculata*. The highest number of observations categorized as mature and spawning was in May or June of each year, indicating that spawning occurred in late spring. In August, September, and October, a greater percentage of developing gametes were observed, which suggests that gametogenesis was reinitiated during these months. A similar gametogenic cycle was observed from October 2002 to June 2003 in captive mussels. The largest fraction of observations categorized as mature and spawning were observed in June in captive *C. tuberculata*.

The gamete development index (GDI) of wild *C. tuberculata* was significantly different from that of captive samples on three of the four sample dates (Table 2.9). In October of 2002, the GDI of *C. tuberculata* relocated in yr 1 and yr 2 differed significantly from the wild sample ($p<0.0001$ and $p=0.0008$, respectively), and the GDI of relocated samples differed significantly from each other ($p<0.0001$). In January of 2003, there were no significant differences in the GDI among captive and wild mussels. In April of 2003, the GDI of captive *C. tuberculata* relocated in yr 2 again differed from that of both the wild sample and the captive sample relocated in yr 1 ($p<0.0001$). There was no significant difference in the GDI of wild and captive *C. tuberculata* relocated in yr 1. Finally, in June of 2003, GDI of all samples were significantly different from each other ($p<0.0001$).

The fraction of gonad used for reproductive activity (GDI stages 1-5), or gamete area fraction (GAF), was significantly different between captive and wild *C. tuberculata* on two of the four sample dates analyzed (Table 2.10, Figure 2.8). Gamete area fraction differed between wild and captive *C. tuberculata* in January 2003 ($p<0.005$) and April 2003 ($p>0.01$), when there were little or no significant differences in the GDI of wild and captive mussels. The fraction of observations categorized as mature and spawning (GDI stages 2-4) was also different between wild and captive *C. tuberculata* sampled in January 2003 ($p<0.01$) and April 2003 ($p<0.04$) (Table 2.11). The fraction of observations categorized as mature and spawning peaked in the captive samples between January and April, while gametes in the wild were still maturing (Figure 2.9).

Fifty-five percent of all *A. ligamentina* examined had trematode-infested gonads. Of the mussels examined in the captive population, 53% were trematode-infested, whereas 56% of the mussels collected from the wild population were infested. Gametes were commonly absent in

infested gonads, making it impossible to ascertain gender so that infestation rates could be compared between the sexes. The trematode infesting the gonads of *A. ligamentina* was identified as *Digenea* sp. (W. Henley, Virginia Polytechnic Institute and State University, personal communication, August 2003). Fewer than 10% of the infested gonads contained mature gametes. In addition, gonads of non-infested individuals contained very few gonad observations categorized as mature (stage 2, 3, 4) (Figures 2.10, 2.11, and 2.12). The highest number of mature and spawning individuals occurred in June of each year. No significant difference was found in glycogen levels ($p>0.07$), protein levels ($p>0.14$), or lipid levels ($p>0.29$) of infested versus non-infested *A. ligamentina* (Tables 2.12, 2.13, and 2.14).

Water quality

Water quality parameters were within target ranges for most of the 2 yr of the study (Chapter 1). Water temperature surpassed the recommended maximum of 28°C on 10 days in the summer of 2002 and 12 days in 2003. However, temperatures of the raceway and the New River were not significantly different ($p<0.43$)(Figure 2.13). Algal concentration in the raceway had a mean cell density of 1904 cells ml^{-1}, and range of 300 cells ml^{-1} to 4658 cells ml^{-1} (Figure 2.14). A water sample collected from the New River during high water conditions in January of 2003 yielded a cell density of only 240 cells ml^{-1}. The water level of the New River was much higher in the second year of the study than the first. Gauge height of the New River at Hinton, WV, was over 1 m only 38 d from September 2001 to August 2002 verses 207 d from September 2002 to August 2003.

DISCUSSION

Biochemical content

The results of this study indicate that biochemical energy reserves of mussels held at WSSNFH did not appear to be affected by captive holding conditions when compared to mussels from the wild populations. Although there were variations in biochemical content over time, there was no significant difference in glycogen, protein, or lipid levels between captive and wild *A. ligamentina* and *C. tuberculata* after 23 mo in captivity. After an initial decline in mantle glycogen content of *A. ligamentina* in September 2001, glycogen levels were lower in captive mussels only once, in *C. tuberculata* in August 2002. Lower protein concentrations in the mantle tissue of captive compared to wild mussels were recorded only on April 2003 for *A. ligamentina*. At all other times during the study, there was either no difference in energy reserves between captive and wild mussels, or observed glycogen, protein, and lipid concentrations were higher in mantle tissues of captive versus wild mussels. In addition, mussels collected from captive and wild populations in my study were comparable in biochemical composition to that of mussels collected from 'natural' or 'native' populations in previous studies (Naimo and Monroe 1999, Baker and Hornbach 2000, and Greseth 2003). For example, glycogen concentrations of three species (*Lampsilis cardium, Quadrula p. pustulosa,* and *Elliptio dilatata*) collected from Wolf River in Wisconsin (range 388-640 mg g^{-1} dry weight) and *Amblema plicata* collected from the upper Mississippi River (range 243-434 mg g^{-1} dry weight) were similar to mean glycogen concentrations of mussels in my study, ranging from 339-705 mg g^{-1} dry weight (Naimo and Monroe 1999, Greseth et al. 2003). The similarity of key biochemical concentrations to those in previous studies, coupled with the lack of differences in biochemical

content between captive and wild mussels within the study, suggests that captive holding conditions at WSSNFH did not compromise energy reserves.

These results differ from those of previous studies in which glycogen, protein, or lipid levels declined in captive, nutritionally stressed, or zebra mussel-infested mussels (Haag et al. 1993, Makela and Oikari 1995, Patterson et al. 1997, Gatenby 2000, Baker and Hornbach 2000, Newton et al. 2001). Gatenby (2000) reported that compared to controls from the Ohio River glycogen levels of captive mussels decreased 58-77% after 1 yr and 87-93% after 3 yr. Newton et al. (2001) also observed significantly lower glycogen levels in pond-relocated mussels than river-relocated mussels. In contrast, glycogen levels of captive mussels in my study varied over time but were higher at the end than the beginning of the study, with the exception of *T. verrucosa.* Zebra mussel infestation, which presumably causes nutritional stress in freshwater mussels, results in catabolization of first glycogen and then protein stores in freshwater mussels (Haag et al. 1993, Baker and Hornbach 2000). Starvation also results in a decline in glycogen and protein content as well as lipid content of mantle tissue over time (Patterson et al. 1997, Henley 2002). In my study, protein and lipid levels varied little over time, and captive conditions did not result in a decline in these parameters. Compared to the wild population, biochemical composition did not appear to be affected by captive holding at WSSNFH, indicating that mussels were not nutritionally or physiologically stressed.

Seasonal variations in glycogen content have been observed in previous studies of freshwater mussels. *Amblema plicata* and *A. ligamentina* collected from the Mississippi River had their highest glycogen levels in the spring and lowest in late fall, which was attributed to their gametogenic cycle (Baker and Hornbach 2001, Monroe and Newton 2001). In comparison, it was difficult to discern a seasonal pattern in my study. Similar to the results from Mississippi

River studies, *C. tuberculata* and *A. ligamentina* both had slightly higher glycogen levels in the spring and lower levels in the fall, but winter glycogen content in wild mussels was much higher in the first year than in the second year of the study. This trend was not as pronounced in captive mussels. Lack of consistent glycogen levels over the 2 yr of the study in wild mussels may be attributed simply to small sample size, but environmental stochasticity cannot be discounted. As noted in results, the New River had much higher discharge in 2003 than in 2002. High water and flooding can lead to dilution of algae in the water and increased suspended solids (Stevenson and White 1995), decreasing food available to mussels. Lower variation in glycogen levels in captive mussels likely was due to the stable environmental conditions at WSSNFH. Although glycogen content of mussels in this study varied over time, seasonal variation was not consistent from year to year.

Because gametes contain high levels of protein and lipid (Bayne et al. 1978), stores of these substrates in bivalves should vary seasonally with the gametogenic cycle (Gabbott and Walker 1971, Kreeger 1993, Berthelin et al. 2000). I observed little variation over time in protein and lipid concentration of mantle tissue of either captive or wild mussels. These results differ from those of Baker and Hornbach (2000), in which protein concentration varied over time in whole bodies of *A. plicata* and *A. ligamentina* collected from the Mississippi River. Makela and Oikari (1995) also noted a variation in protein content over time in wild *Anodonta anatina*. However, they analyzed only adductor mussel tissue. The only other study, in which lipid and protein were measured over time, reported a decline in protein and lipid content of mantle tissue in nutritionally stressed mussels (Henley 2002). The lack of variation in protein and lipid levels in my study suggests that these substrates are not being catabolized in the mantle tissue to fuel the gametogenic cycle.

Biochemical content differs among tissues. Due to the concurrent gametogenic analyses of gonad tissue in my study, homogenized mantle tissues instead of whole bodies were subsampled for glycogen, protein, and lipid content. Mantle tissue was chosen because it has been analyzed for biochemical content of freshwater mussels in previous studies (Berg et al. 1995, Patterson et al. 1997 Naimo et al. 1998, Henley 2002, Greseth et al. 2003). While glycogen levels in mantle tissue are reportedly higher than in other tissues (Naimo and Monroe 1999, Chen et al. 2001), comparable evaluations of protein and lipid levels among such tissues have not been conducted. My literature search resulted in only four studies in which researchers analyzed glycogen, protein, and lipids concurrently in freshwater mussels. Baker and Hornbach (2000) used whole bodies, Makela and Oikari used adductor mussel tissue, and Henley (2002) and Greseth et al. (2003) used mantle tissue to test for the energy substrates. While Henley (2002) found a decrease in glycogen, protein, and lipid levels in nutritionally stressed mussels, Greseth et al. (2003) found no difference in glycogen, protein, or lipids in emersed mussels compared to controls. In my study, lack of variation in protein and lipid levels over time suggests that these substrates may be stable in mantle tissue, unless the mussel is under severe environmental or nutritional stress. Analyses of the biochemical content of whole bodies, including gonads, would have provided more information on the use and source of energy stores during gametogenesis.

Results of the biochemical analyses indicate that the captive holding conditions at WSSNFH did not negatively affect the energy stores of freshwater mussels. Previous studies reported sharp declines in glycogen, protein, and/or lipid content of mussels held in captivity or starved (Patterson et al. 1997, Gatenby 2000, Monroe and Newton 2001, Henley 2002), whereas captive mussels in my study maintained energy reserves at or above those of wild mussels.

Gametogenesis

Captive conditions did not negatively affect gametogenesis in mussels, when compared to their wild counterparts. After 23 mo in captivity, *C. tuberculata* exhibited gametogenic activity and contained mature gametes. However, *A. ligamentina* contained few developing or mature gametes in captive or wild mussels due to trematode infestation. Few studies have attempted to compare gametogenesis in wild and captive freshwater mussels. However in a starvation study, Henley (2002) observed a decline in the gamete area fraction (GAF) of unfed mussels to almost 0% after 8 mo in captivity, indicating that none of the gonad was being used for gamete production. In addition, Bayne et al. (1978) observed that *Mytilus edulis* held at high temperatures and under nutritional stress for 8 wk had a gamete volume fraction (similar to GAF) almost 50% lower than that of the control specimens. GAF of *C. tuberculata* observed in my study never fell below 50% in captive or wild mussels. Although the GAF of captive and wild *C. tuberculata* differed at two of four sample events, mean GAF of captive mussels was higher in captive mussels at both sample dates. From these results, it appears that captive *C. tuberculata* maintained gametogenic activity at or above that of wild mussels, but because of trematode infestation, no conclusions could be made for *A. ligamentina*.

Spawning in captive *C. tuberculata* appears to have occurred in early spring of 2003. Although it was not possible to pinpoint the exact spawning date, captive *C. tuberculata* had higher GAF and fraction of gonad observations categorized as mature and spawning in January and April 2003 than observed in October 2002 or June 2003. These results were temporally consistent with the gametogenic cycle of wild *C. tuberculata* collected in the previous year, when a higher GAF and lower fraction of mature and spawning observations occurred between February and May 2002. They also are consistent with previous studies in which spawning was

reported to occur in early spring (Jirka and Neves 1992, Haggerty et al. 1995). However, the gametogenic stage of captive mussels was not consistent with their wild counterparts in 2003. Wild *C. tuberculata* had a lower GAF and fraction of observations categorized as mature and spawning in January and April 2003. Gamete area fraction and the fraction of mature and spawning observations increased in June 2003, suggesting that spawning occurred later in wild than captive *C. tuberculata*. From these results, it appears that captive conditions had the affect of hastening the gametogenic cycle compared to mussels from the wild population.

Environmental conditions can affect spawning time in freshwater bivalves. A possible cause of the apparent difference in spawning period is discharge (Jones et al. 1986). Discharge in the New River was much higher during the second year of the study than the first, and could be the cause of the asynchronous spawning between captive and wild *C. tuberculata*. Another possible cause is temperature differences between the New River and the raceway. Hastie and Young (2003) reported that spawning in *Margaritifera margaritifera* occurred over a month later in rivers with lower temperatures. Jirka and Neves (1992) also suggested that spawning times differed among mussel species in the New River and at other locations due to water temperature differences. *Megalonaias nervosa* failed to produce mature gametes and spawn when located below Cordell Hull Dam, TN where temperatures never climbed above 20°C, but experienced normal gametogenesis when relocated to a warmer section of the river (Heinricher and Layzer 1999). Although lower temperature is cited in the literature as the cause of delayed or no spawning, there was no statistical difference in temperature between the raceway and the New River in my study. Beaty (1999) related growth rates of young mussels to degree-days in an artificial stream. Therefore, I recommend that future studies consider a threshold temperature

above which spawning occurs, or compare cumulative degree-days to discern whether effects of location on spawning time are regulated by water temperature.

Unlike *C. tuberculata*, gametogenesis was not occurring normally in captive *A. ligamentina* because the gonads of many specimens were infested with digenean trematodes. However, infestation rates did not appear to be affected by captive holding conditions. Captive and wild *A. ligamentina* both had infestation rates greater than 50%. Digenean trematodes have been found in a variety of mollusks and most commonly affect the digestive tract (Huffman and Fried 1985). Over 90% of the gonadal observations of infested mussels were categorized as inactive in histological thin-sections of gonad tissue of *A. ligamentina*. Jirka and Neves (1997) reported that gametogenesis began in October and culminated in mature gametes the following summer, with spawning occurring in August. From their study, we would expect developing and mature gametes to be present in the gonads from early spring through late summer. However, even gonads of non-infested captive and wild *A. ligamentina* were nearly devoid of mature gametes, and a majority of the GDI observations were categorized as inactive. From these results, it appears that many captive and wild *A. ligamentina* were functionally sterile due to high trematode infestation. The effect of parasitism on the demography of wild mussel populations is yet to be determined.

Parasites can have considerable effects on the biochemistry and physiology of mollusks (Huffman and Fried 1985, Barber et al. 1988). Barber et al. (1988) reported that protozoan parasites had detrimental effects on the biochemical composition of *Crassostrea virginica*, significantly diminishing glycogen, protein, and lipid contents. Similarly, Huffman and Fried (1985) reported that parasitic digenean trematode larvae, like those infesting mussel gonads in my study, depleted glycogen and lipid levels and destroyed the digestive tract of the gastropod

Goniobasis virginica. Although my sample sizes were small, there was no evidence that biochemical content differed between infested and non-infested individuals of captive and wild *A. ligamentina*. From these results, it appears that although infestation of the gonad by digenean trematodes had a negative effect on the gametogenesis of *A. ligamentina*, there was no effect on energy substrates in the mantle tissue.

Gametogenic activity of neither *C. tuberculata* nor *A. ligamentina* appeared to be adversely affected by captive holding conditions. Gametogenesis and spawning of captive *C. tuberculata*, although earlier than wild mussels, occurred within the time frame previously reported. *A. ligamentina* was not experiencing normal gametogenesis, but incidence of trematode infestation was high in both captive and wild mussels.

Conclusions and recommendations

Assuming that gametogenic activity and energy substrate levels are good measures of body condition, *C. tuberculata* maintained adequate physiological condition compared to wild mussels for the 2 yr in captivity at WSSNFH. Based on glycogen levels, *T. verrucosa* seemingly maintained good condition compared to wild mussels in captivity as well. Although the results of the gametogenesis study were inconclusive because of high trematode infestations, energy substrate levels indicate that *A. ligamentina* also maintained good condition in captivity compared to wild mussels. Additional research is needed to assess the extent of trematode infestation of *A. ligamentina* in the New River and its possible long-term effects. Research also is required to determine the communicability of trematode infestation in captivity, before additional *A. ligamentina* are held in captivity with other species. Stable energy reserves and gametogenic activity of captive mussels indicate that water quality, food availability, and water temperature were adequate in the raceway at WSSNFH. However, in order to obtain a more

complete picture of seasonal fluctuations in energy storage, future studies should include a side-by-side study over time of glycogen, protein, and lipid levels in various freshwater mussel tissues, including adductor mussel, mantle, foot, and visceral mass. In conclusion, the use of this facility for holding adult mussels in captivity for extended periods of time is a viable option for species in need of conservation.

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Table 2.1. Classification stages of the gamete development index (GDI) for evaluating gametogenic activity in male and female gonads of freshwater mussels. Table adapted from Barber (1996) and Henley (2002).

Gametogenic Stage	Stage Classification	Description
0	Inactive	No gametogenic activity.
1	Early active	Female gonads contain primary oogonia or oocytes but no free oocytes. Male gonads contain spermatogonia, spermatocytes or spermatids but no spermatozoa.
2	Late active	Some attached and some free oocytes present Spermatocytes, spermatids, and some spermatozoa present.
3	Mature	Mature gametes (oocytes and spermatozoa) present.
4	Spawning	Acini walls broken, some free spaces within acini, numerous mature gametes still present.
5	Spawned and resorbing	Only refractory gametes remain. Phagocytes for resorption of gametes present. Occasional redeveloping primary oogonia and spermatogonia present.

Table 2.2. Mean (± 1 SD) glycogen content in mg/g dry weight of captive *Actinonaias ligamentina* relocated to WSSNFH in August 2001 (year 1) and August 2002 (year 2), and wild *A. ligamentina* from the New River. *P*-values determined by Wilcoxon Two-Sample Test and Kruskal-Wallis Test. Letters represent significant difference. $n = 6$ except where noted.

	Treatment						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Jun '01	655.5 ± 295.2						
Sep '01	646.5 ± 166.3	a	255.1 ± 73.5	b			0.0172
Nov '01	267.9 ± 176.8		396.6 ± 256.0				0.3973
Jan '02	812.6 ± 222.1		619.5 ± 268.1				0.3203
Apr '02	659.5 ± 261.8		444.9 ± 179.5				0.2007
Jun '02	296.3 ± 233.2		426.9 ± 287.7				0.5864
Aug '02	419.0 ± 187.4^{+}		421.9 ± 206.9		347.2 ± 306.3		0.6026
Oct '02	241.2 ± 125.8		361.4 ± 80.0		349.7 ± 165.1^{+}		0.2314
Jan '03	163.9 ± 54.6	a	309.7 ± 129.6	b	655.6 ± 120.5^{+}	c	0.0022
Apr '03	530.0 ± 175.8		429.5 ±118.1		587.7 ± 196.4		0.2811
Jun '03	537.0 ± 188.1		644.2 ±170.9		591.3 ± 156.6		0.6119
All Dates	491.4 ± 278.9		431.0 ± 211.2		506.6 ± 229.4		

+ n=5

Table 2.3. Mean (± 1 SD) glycogen content in mg/g dry weight of captive *Cyclonaias tuberculata* relocated to WSSNFH in August 2001 (year 1) and August 2002 (year 2), and wild *C. tuberculata* from the New River. *P*-values determined by Wilcoxon Two-Sample Test and Kruskal-Wallis Test. Letters represent significant difference. $n = 6$ except where noted.

	Treatment						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Jun '01	747.0 ± 308.2						
Sep '01	709.1 ± 226.4		618.9 ± 193.0				0.4862
Nov '01	551.2 ± 96.7		470.8 ± 154.8				0.2258
Jan '02	1238.3 ± 35.5++		735.5 ± 203.4				0.1094
Apr '02	642.8 ± 238.9+		439.5 ± 68.6+				0.3235
Jun '02	762.0 ± 267.8		460.1 ± 260.1				0.0706
Aug '02	786.0 ± 185.6	a	406.9 ± 96.6	b	504.1 ± 240.8+	a,b	0.0168
Oct '02	406.7 ± 114.7+		686.7 ± 352.0		675.2 ± 224.6		0.1610
Jan '03	517.9 ± 152.6		733.0 ± 217.9		559.6 ± 233.6		0.2291
Apr '03	555.7 ± 186.3		738.3 ± 113.2		795.6 ± 283.7		0.2199
Jun '03	781.9 ± 237.9		907.0 ± 129.8		959.3 ± 159.6		0.2359
All Dates	676.8 ± 254.8		622.7 ± 242.3		705.5 ± 271.1		

+ n=5
++ n=2

Table 2.4. Mean (± 1 SD) glycogen content in mg/g dry weight of captive *Tritogonia verrucosa* relocated to WSSNFH in August 2001, and wild *T. verrucosa* from the New River. *P*-values determined by Wilcoxon Two-Sample Test. $n = 6$ except where noted.

	Treatment		
Sample Date	Wild	Captive	*P*-values
Jun '01	503.6 ± 210.2		
Sep '01	478.2 ± 184.8	560.6 ± 202.0	0.3973
Nov '01	464.9 ± 119.3	276.3 ± 109.9	0.0706
Jan '02	382.3 ± 90.4^{+}	265.9 ± 59.6	0.0616
Apr '02	361.4 ± 115.3^{+}	302.9 ± 85.3^{+}	0.5464
Jun '02	309.3 ± 50.8^{+}	284.8 ± 114.1	0.7898
All Dates	435.0 ± 160.0	339.3 ± 163.8	

+ n=5

Table 2.5. Mean (± 1 SD) protein content in mg/g dry weight of captive *Actinonaias ligamentina* relocated to WSSNFH in August 2001 (year 1) and August 2002 (year 2), and wild *A. ligamentina* from the New River. *P*-values determined by Wilcoxon Two-Sample Test and Kruskal-Wallis Test. Letters represent significant difference. $n = 6$ except where noted.

	Treatment						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Jun '01	459.9 ± 159.8						
Sep '01	467.6 ± 38.1		523.2 ± 30.9				0.0535
Nov '01	409.9 ± 93.3		529.8 ± 69.5^{+}				0.0621
Jan '02	423.1 ± 46.6		407.7 ± 17.1				0.9376
Apr '02	450.2 ± 38.8		467.8 ± 32.9				0.4862
Jun '02	463.4 ± 66.4		443.6 ± 62.8				0.6966
Aug '02	429.1 ± 38.8^{+}	a,b	476.5 ± 70.2	a	373.5 ± 50.9	b	0.0142
Oct '02	535.9 ± 38.0		485.8 ± 45.7		496.3 ± 30.3		0.0622
Jan '03	456.7 ± 107.4		488.6 ± 71.8		555.4 ± 41.8		0.0759
Apr '03	470.8 ± 24.2	a	425.9 ± 14.7	b	424.6 ± 44.7	a,b	0.0189
Jun '03	467.7 ± 30.3		464.7± 22.8		462.5 ± 8.6		0.2501
All Dates	450.9 ± 86.6		457.2 ± 81.9		458.1 ± 89.7		

+ n=5

Table 2.6. Mean (± 1 SD) protein content in mg/g dry weight of captive *Cyclonaias tuberculata* relocated to WSSNFH in August 2001 (year 1) and August 2002 (year 2), and wild *C. tuberculata* from the New River. *P*-values determined by Wilcoxon Two-Sample Test and Kruskal-Wallis Test. Letters represent significant difference. $n = 6$ except where noted.

	Treatment						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Jun '01	525.0 ± 58.3						
Sep '01	543.8 ± 49.2		608.8 ± 17.1				0.0706
Nov '01	533.1 ± 19.3		582.1 ± 46.5				0.0706
Jan '02	525.4 ± 4.8^{++}		564.0 ± 30.6				0.1094
Apr '02	532.0 ± 20.7	a	589.4 ± 28.3^{+}	b			0.0452
Jun '02	502.5 ± 58.8	a	575.2 ± 21.3	b			0.0305
Aug '02	558.8 ± 45.0	a,b	586.1 ± 67.5	a	495.2 ± 35.4^{+}	b	0.0346
Oct '02	602.9 ± 25.6^{+}	a,b	575.1 ± 50.7	a	672.6 ± 81.4	b	0.0371
Jan '03	527.5 ± 15.3	a	568.2 ± 54.7	a,b	645.4 ± 44.2^{+}	b	0.0138
Apr '03	510.8 ± 21.3	b	561.8 ± 25.7	a	531.9 ± 41.7	a,b	0.0423
Jun '03	539.1 ± 38.7		576.0 ± 41.2		586.3 ± 55.9		0.8490
All Dates	534.2 ± 44.3		574.6 ± 42.4		576.7 ± 87.0		

+ n=5
++ n=2

Table 2.7. Mean (± 1 SD) lipid content in mg/g dry weight of captive *Actinonaias ligamentina* relocated to WSSNFH in August 2001 (year 1) and August 2002 (year 2), and wild *A. ligamentina* from the New River. *P*-values determined by Wilcoxon Two-Sample Test and Kruskal-Wallis Test. Letters represent significant difference. $n = 6$ except where noted.

	Treatment						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Sep '01	46.3 ± 7.5		44.9 ± 3.9				0.8146
Nov '01	46.1 ± 6.1		53.8 ± 10.0[+]				0.2009
Jan '02	42.7 ± 8.7		42.7 ± 8.7				1.0000
Apr '02	49.6 ± 4.3		48.8 ± 6.9				0.6966
Jun '02	32.8 ± 10.7		40.6 ± 12.8				0.4862
Aug '02	32.5 ± 5.3[+]		39.2 ± 8.1		42.3 ± 8.2		0.1523
Oct '02	50.8 ± 7.3		39.8 ± 7.1		50.6 ± 10.2		0.0636
Jan '03	41.1 ± 7.6	a	46.2 ± 5.4	a,b	61.8 ± 24.2	b	0.0446
Apr '03	42.1 ± 10.4		41.5 ± 9.1		48.5 ± 13.4		0.8054
Jun '03	41.5 ± 2.6		42.7 ± 2.6		50.8 ± 7.1		0.7961
All Dates	42.6 ± 6.2		43.7 ± 8.6		48.7 ± 15.1		

+ n=5

Table 2.8. Mean (± 1 SD) lipid content in mg/g dry weight of captive *Cyclonaias tuberculata* relocated to WSSNFH in August 2001 (year 1) and August 2002 (year 2), and wild *C. tuberculata* from the New River. *P*-values determined by Wilcoxon Two-Sample Test and Kruskal-Wallis Test. Letters represent significant difference. $n = 6$ except where noted.

	Treatment						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Sep '01	54.2 ± 13.1[+]		49.5 ± 15.1				0.7898
Nov '01	69.8 ± 11.6		107.0 ± 22.5[+]				0.1034
Jan '02	51.0 ± 3.4[++]		56.2 ± 3.8				0.1773
Apr '02	53.4 ± 10.7		68.5 ± 16.7[+]				0.1517
Jun '02	46.3 ± 18.4		66.5 ± 10.5				0.0927
Aug '02	56.9 ± 10.9		56.5 ± 11.5		49.0 ± 12.3[+]		0.2274
Oct '02	52.3 ± 9.6[+]	a	56.1 ± 9.6	a,b	75.3 ± 6.4	b	0.0052
Jan '03	54.6 ± 9.3		60.6 ± 5.7		53.2 ± 8.0[+]		0.6642
Apr '03	50.9 ± 9.6		57.7 ± 7.0		65.7 ± 9.8		0.0516
Jun '03	52.4 ± 6.0		61.0 ± 3.8		60.8 ± 1.9		0.3025
All Dates	54.2 ± 6.2		62.0 ± 17.0		60.3 ± 12.4		

+ n=5
++ n=2

Table 2.9. Chi-Square Test of Homogeneity among gametogenic stages of gonads of wild *Cyclonaias tuberculata* from the New River and captive *C. tuberculata* relocated to WSSNFH in August 2001(year 1) and August 2002 (year 2). P-values of < 0.05 indicate significant difference in gametogenic stages.

	Captive year 1 and Wild		Captive year 2 and Wild		Captive year 1 and year 2	
Sample Date	*p*-value	X^2	*p*-value	X^2	*p*-value	X^2
Oct '02	<0.0001	118.03	0.0008	11.32	<0.0001	64.66
Jan '03	0.4917	0.47	0.1192	2.43	0.2875	1.13
Apr '03	0.0995	2.71	<0.0001	42.98	<0.0001	29.65
Jun '03	<0.0001	45.34	0.0001	14.45	<0.0001	104.90

Table 2.10. Mean (± 1 SD) gamete area fraction (GAF) of wild *Cyclonaias tuberculata* and captive *C. tuberculata* relocated to WSSNFH in August 2001(year 1) and August 2002 (year 2). Letters represent significant difference.

	Treatments						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Jun '01	0.74 ± 0.17						
Sep '01	0.71 ± 0.19						
Nov '01	0.82 ± 0.14						
Feb '02	0.79 ± 0.16						
May '02	0.80 ± 0.09						
Jun '02	0.59 ± 0.24						
Aug '02	0.52 ± 0.17						
Oct '02	0.69 ± 0.11		0.75 ± 0.20		0.81 ± 0.12		0.2195
Jan '03	0.66 ± 0.13	a	0.91 ± 0.08	b	0.75 ± 0.07	a,b	0.0056
Apr '03	0.59 ± 0.25	a	0.87 ± 0.07	b	0.82 ± 0.07	c	0.0180
Jun '03	0.74 ± 0.18		0.80 ± 0.10		0.64 ± 0.27		0.5287

Table 2.11. Mean (± 1 SD) percentage of wild and captive *Cyclonaias tuberculata* relocated to WSSNFH in August 2001 and August 2002. Gonad observations categorized as mature and spawning (including 2: late active, 3: mature, and 4: spawning). Letters represent significant difference.

	Treatment						
Sample date	Wild		Captive year 1		Captive year 2		*P*-values
Jun '01	0.73 ± 0.17						
Sep '01	0.60 ± 0.35						
Nov '01	0.77 ± 0.15						
Feb '02	0.77 ± 0.17						
May '02	0.77 ± 0.09						
Jun '02	0.59 ± 0.24						
Aug '02	0.21 ± 0.25						
Oct '02	0.47 ± 0.24		0.71 ± 0.18		0.70 ± 0.14		0.0793
Jan '03	0.57 ± 0.23	a	0.86 ± 0.08	b	0.73 ± 0.07	c	0.0102
Apr '03	0.59 ± 0.25	a	0.79 ± 0.07	b	0.74 ± 0.20	c	0.0380
Jun '03	0.74 ± 0.18		0.52 ± 0.38		0.60 ± 0.24		0.3417

Table 2.12. Mean (± 1 SD) glycogen content in mg/g dry weight of trematode-infested and non- trematode-infested *Actinonaias ligamentina* sampled from the wild (river) population and captive populations (relocated to WSSNFH in August 2001 and August 2002). *P*-values determined by Wilcoxon Two-Sample Test.

River Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01	756.1 ± 321.7	3	554.8 ± 289.8	3	0.6807
Sep '01	536.4 ± 93.0	3	756.7 ± 155.2	3	0.2474
Nov '01	344.7 ± 213.8	3	187.0 ± 171.4	2	0.7872
Feb '02	1044.0 ± 164.7	2	696.9 ± 140.0	4	0.1661
May '02	300.4 ± 0	1	731.4 ± 216.8	5	0.2943
Jun '02	91.8 ± 0	1	337.2 ± 235.5	5	0.5836
Aug '02	318.1 ± 130.1	3	570.5 ± 173.6	2	0.4353
Oct '02	245.3 ± 60.3	4	233.1 ± 42.9	2	0.8261
Jan '03	199.1 ± 3.4	3	128.7± 51.2	3	0.2474
Apr '03	479.1 ± 171.6	2	555.5 ± 197.8	4	0.5184
Jun '03	540.7 ± 284.1	3	533.2 ± 87.7	3	0.0687

Relocated 8/01 Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01					
Sep '01					
Nov '01					
Feb '02					
May '02					
Jun '02					
Aug '02					
Oct '02	365.0 ± 164.3	2	360.9 ± 49.6	3	1.0000
Jan '03	425.2 ± 0	1	286.6 ± 130.4	5	0.5836
Apr '03	385.6 ± 40.8	2	494.6 ± 141.1	3	0.4353
Jun '03	765.8 ± 142.0	3	522.6 ± 92.2	3	0.2474

Relocated 8/02 Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01					
Sep '01					
Nov '01					
Feb '02					
May '02					
Jun '02					
Aug '02					
Oct '02	460.9 ± 0	1	321.9 ± 176.6	5	0.3486
Jan '03	622.9 ± 150.6	4	704.7 ± 68.6	2	0.7872
Apr '03	679.4 ± 349.1	2	514.9 ± 136.2	3	0.7872
Jun '03	605.9 ± 170.4	5	518.4 ± 0	1	1.0000

Table 2.13. Mean (± 1 SD) protein content in mg/g dry weight of trematode-infested and non-trematode-infested *Actinonaias ligamentina* sampled from the wild (river) population and captive populations (relocated to WSSNFH in August 2001 and August 2002). *P*-values determined by Wilcoxon Two-Sample Test.

River					
Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01	375.2 ± 26.8	3	544.6 ± 203.8	3	0.1413
Sep '01	499.0 ± 11.9	3	436.3 ± 23.0	3	0.1413
Nov '01	396.8 ± 107.6	3	377.7± 83.5	2	0.7872
Feb '02	406.1 ± 27.1	2	431.6 ± 55.5	4	1.0000
May '02	484.5 ± 0	1	443.4 ± 39.1	5	0.5836
Jun '02	458.1 ± 0	1	464.4 ± 74.2	5	1.0000
Aug '02	434.5 ± 48.1	3	421.0 ± 34.3	2	0.7872
Oct '02	521.7± 38.5	4	564.1 ± 19.3	2	0.2994
Jan '03	511.8 ± 97.3	3	401.6 ± 101.5	3	0.4227
Apr '03	500.6± 7.5	2	455.8 ± 8.4	4	0.1661
Jun '03	381.0 ± 24.3	3	381.7 ± 61.3	3	1.0000

Relocated 8/01					
Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01					
Sep '01					
Nov '01					
Feb '02					
May '02					
Jun '02					
Aug '02					
Oct '02	485.5 ± 34.5	2	508.0 ± 41.2	3	0.7872
Jan '03	466.7± 0	1	493.0 ± 79.3	5	1.0000
Apr '03	437.0 ± 23.1	2	423.8 ± 3.8	3	0.7872
Jun '03	445.8 ± 115.6	3	225.3 ± 14.9	3	0.1413

Relocated 8/02					
Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01					
Sep '01					
Nov '01					
Feb '02					
May '02					
Jun '02					
Aug '02					
Oct '02	518.3 ± 0	1	491.9 ± 31.6	5	0.5836
Jan '03	567.5 ± 47.8	4	531.3 ± 12.3	2	0.5184
Apr '03	423.8 ± 22.9	2	425.9 ± 68.7	3	0.7872
Jun '03	408.2 ± 109.8	5	603.3 ± 0	1	0.2943

Table 2.14. Mean (± 1 SD) lipid content in mg/g dry weight of trematode-infested and non-trematode-infested *Actinonaias ligamentina* sampled from the wild (river) population and captive populations (relocated to WSSNFH in August 2001 and August 2002). *P*-values determined by Wilcoxon Two-Sample Test.

River					
Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01	79.2 ± 85.0	3	107.9 ± 68.9	3	0.7244
Sep '01	48.8 ± 10.9	3	43.7 ± 2.1	3	1.0000
Nov '01	50.4 ± 3.1	3	40.5 ± 6.9	2	0.2224
Feb '02	43.9 ± 14.3	2	42.1 ± 7.5	4	1.0000
May '02	57.5 ± 0	1	48.0 ± 2.2	5	0.2943
Jun '02	31.7± 0	1	33.1 ± 11.9	5	1.0000
Aug '02	31.6 ± 7.0	3	33.8 ± 2.6	2	0.7872
Oct '02	48.3 ± 5.0	4	55.8 ± 10.6	2	0.5184
Jan '03	45.0 ± 6.4	3	37.1 ± 7.6	3	0.4227
Apr '03	47.0 ± 9.9	2	39.7 ± 11.1	4	0.8261
Jun '03	42.4 ± 1.2	3	41.2 ± 12.8	3	0.6807

Relocated 8/01					
Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01					
Sep '01					
Nov '01					
Feb '02					
May '02					
Jun '02					
Aug '02					
Oct '02	41.7 ± 8.6	2	41.4 ± 6.8	3	0.7872
Jan '03	55.4 ± 0	1	44.3 ± 3.2	5	0.2943
Apr '03	45.8 ± 5.6	2	38.6 ± 12.6	3	0.7872
Jun '03	42.4 ± 7.0	3	40.5 ± 6.1	3	1.0000

Relocated 8/02					
Sample Date	No Trematodes	*n*	Trematodes	*n*	*p*-values
Jun '01					
Sep '01					
Nov '01					
Feb '02					
May '02					
Jun '02					
Aug '02					
Oct '02	53.6 ± 0	1	50.0 ± 11.3	5	1.0000
Jan '03	54.4 ± 4.0	4	76.5 ± 47.1	2	1.0000
Apr '03	42.2 ± 5.7	2	55.0 ± 17.6	3	0.7872
Jun '03	39.9 ± 6.7	5	41.6 ± 0	1	1.0000

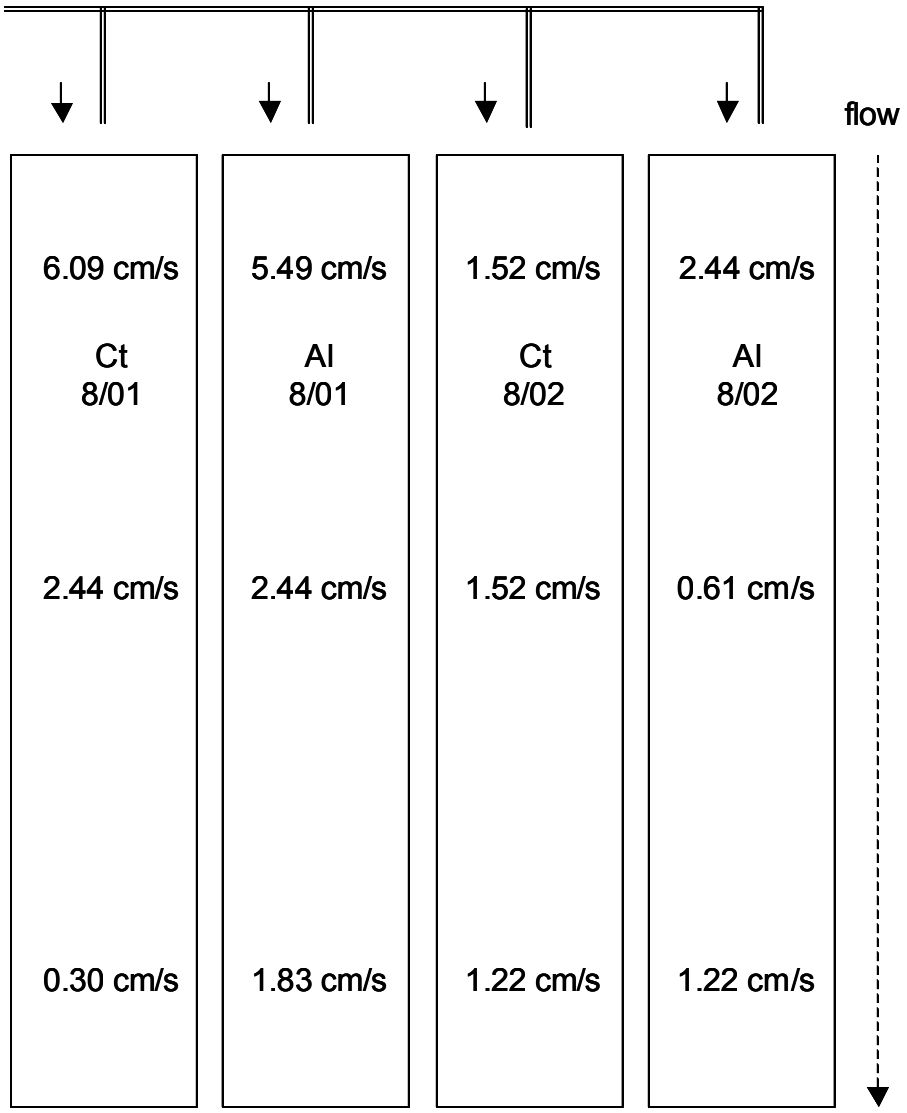

Figure 2.1. Water velocities and locations of Al (*Actinonaias ligamentina*) and Ct (*Cyclonaias tuberculata*) relocated to WSSNFH in August 2001 and August 2002, within mussel confinement structures in the raceway (pond B2) at WSSNFH. Dimensions of each structure were 1m x 6m x 0.5m.

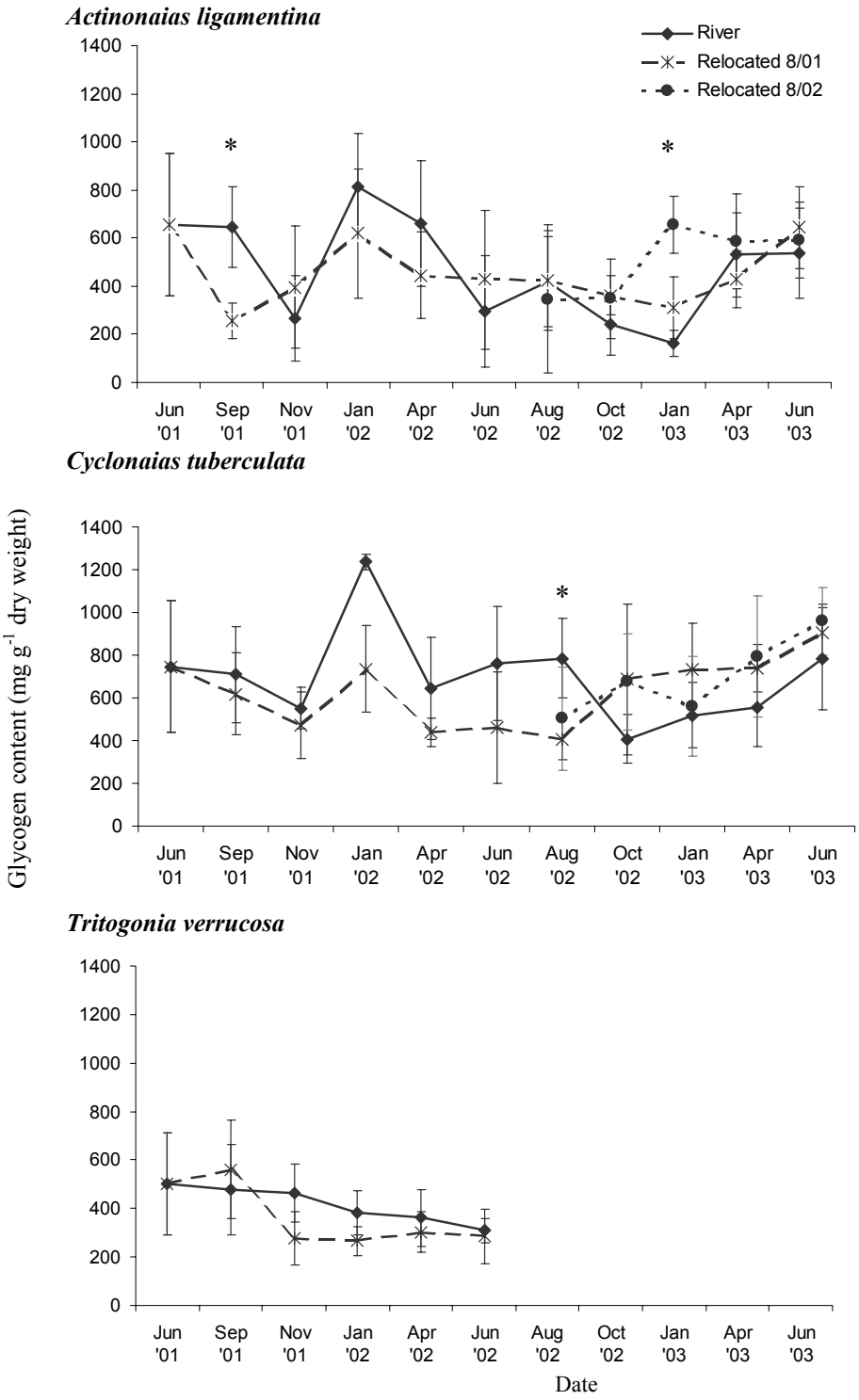

Figure 2.2. Comparison of mean glycogen content (± 1 SD) of three species of mussels sampled from the New River and captive mussels (relocated August 2001 and August 2002) at WSSNFH. * denotes significant difference. *n*=6 (except for *A. ligamentina* when *n*=5 in wild Aug '02, captive year 2 Oct '02, and captive year 2 Jan 2003, for *C. tuberculata* when *n*=5 in wild Apr '02, captive year 1 Apr '02, and captive year 2, and when *n*=2 in captive year 1 Apr '02, and for *T. verrucosa* when *n*=5 in wild January 2002, wild Apr '02, wild Jun '02, and captive Apr '02).

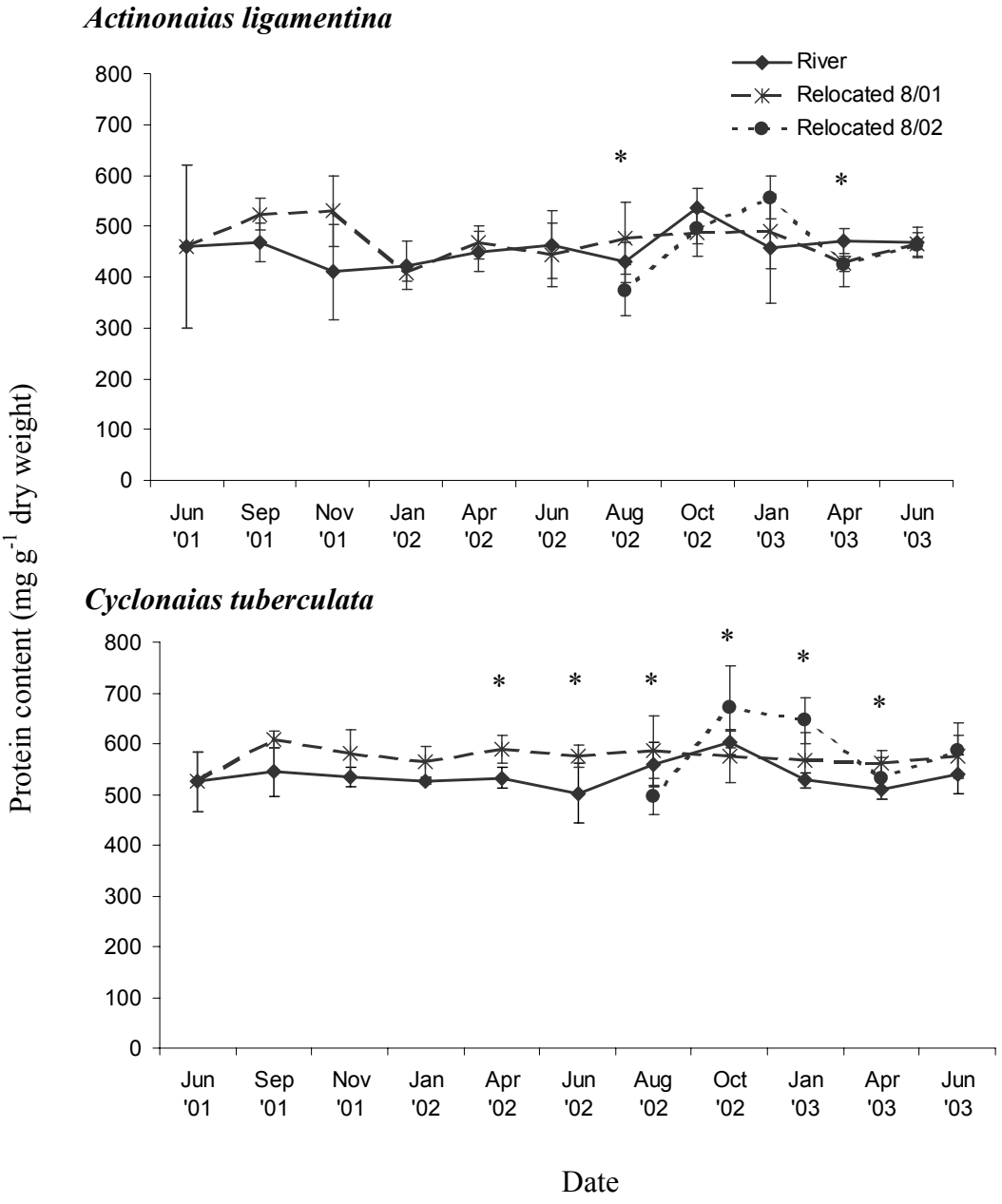

Figure 2.3. Comparison of mean protein content (± 1 SD) of two species of mussels sampled from the New River and captive mussels (relocated August 2001 and August 2002) at WSSNFH. * denotes significant difference. *n*=6 (except for *A. ligamentina* when *n*=5 in wild Aug ’02 and captive year 1 Nov ’01 and for *C. tuberculata* when *n*=5 in wild Oct ’02, captive year 1 Apr ’02, captive year 2 Aug ‘2, and captive year 2 Jan ’03 and when *n*=2 in wild Jan ’02).

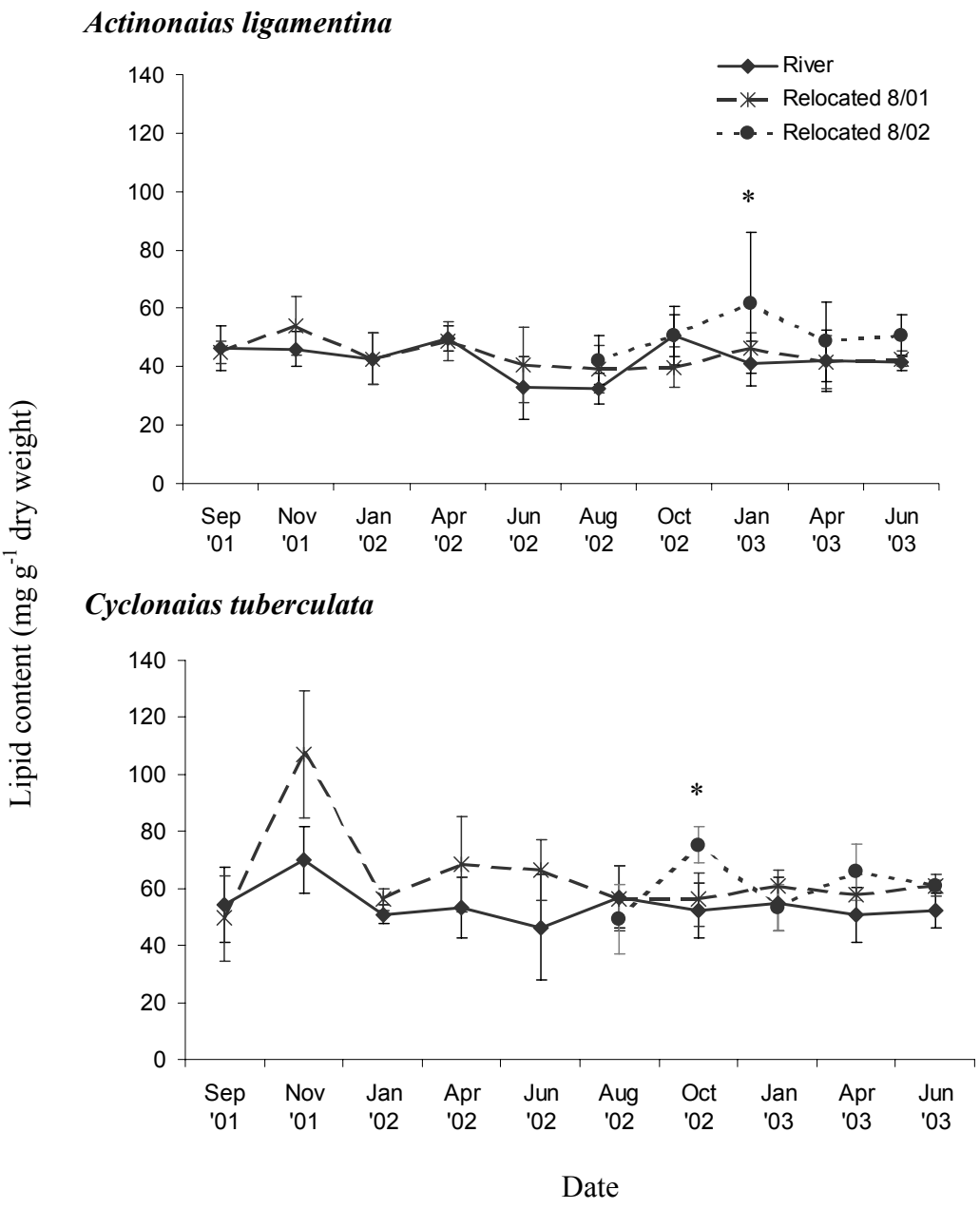

Figure 2.4. Comparison of mean lipid content (± 1 SD) of two species of mussels sampled from the New River and captive mussels (relocated August 2001 and August 2002) at WSSNFH. * denotes significant difference. n=6 (except for *A. ligamentina* when *n*=5 in wild Aug ’02 and captive year 1 Nov ’01 and *C. tuberculata* when *n*=5 in wild Sep ’01, wild Oct ’02, captive year 1 Nov ’01, captive year 1 Apr ’02, captive year 2 Aug ’02, and captive year Jan ’03 and when *n*=2 in wild Jan ’02).

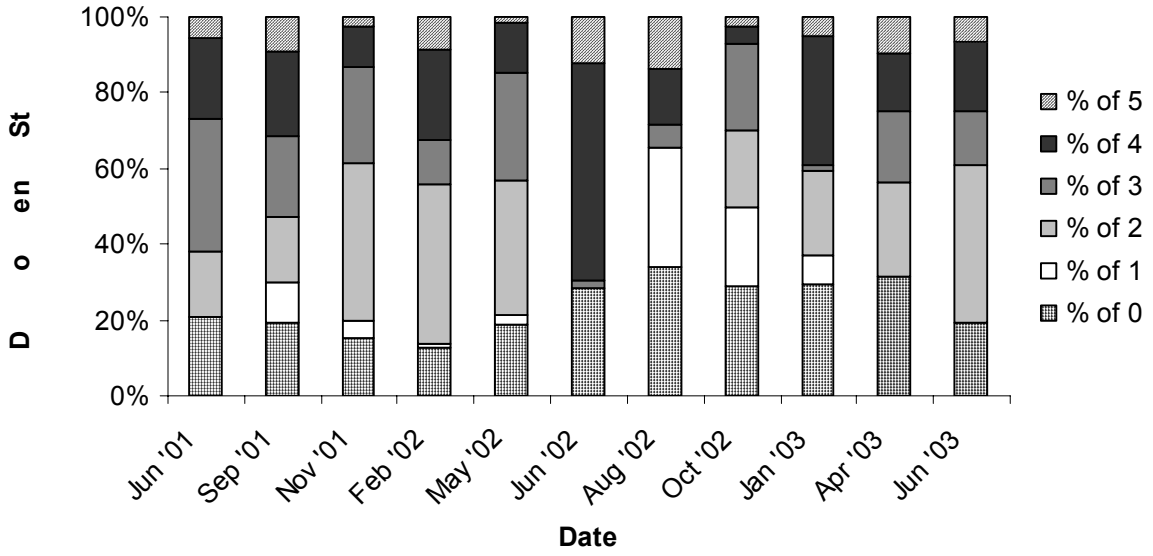

Figure 2.5. Gamete development index of gonads of wild *Cyclonaias tuberculata* (*n*=6) collected from the New River, showing percent of observations identified in each developmental stage. Stages noted in key are 0: inactive, 1: early active, 2: late active, 3: mature, 4: spawning, 5: spawned and resorbing.

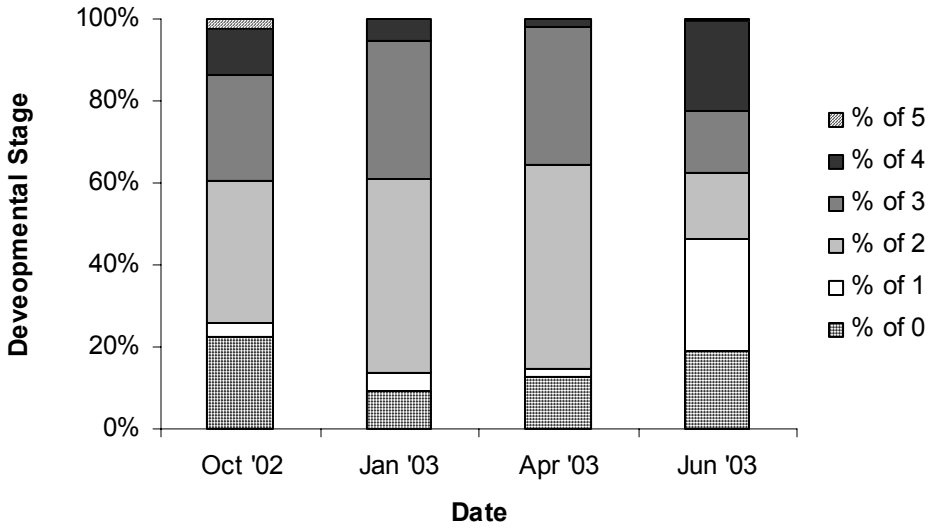

Figure 2.6. Gamete development index of gonads of captive *Cyclonaias tuberculata* (*n*=6) relocated in August 2001 to WSSNFH showing percent of observations identified in each developmental stage. Stages noted in key are 0: inactive, 1: early active, 2: late active, 3: mature, 4: spawning, 5: spawned and resorbing.

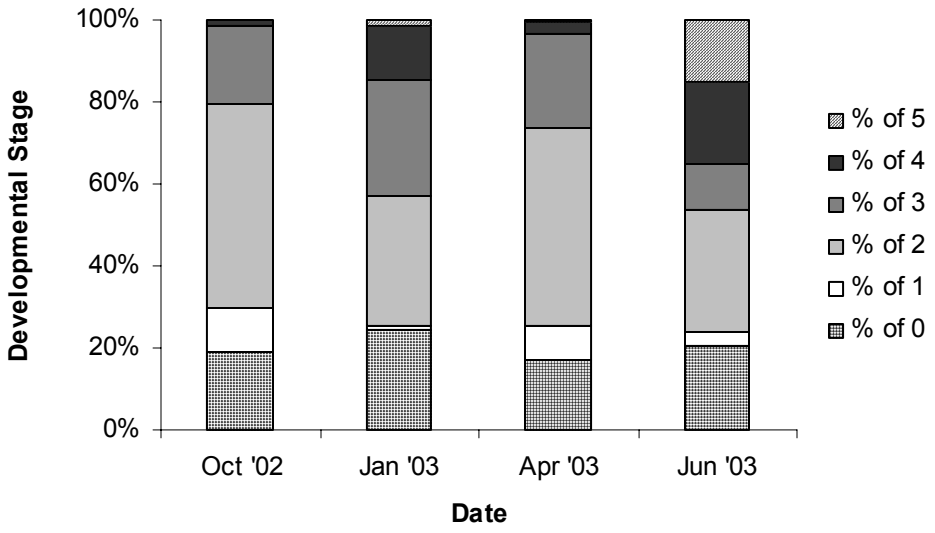

Figure 2.7. Gamete development index of gonads of captive *Cyclonaias tuberculata* (*n*=6) relocated in August 2002 to WSSNFH, showing percent of observations identified in each developmental stage. Stages noted in key are 0: inactive, 1: early active, 2: late active, 3: mature, 4: spawning, 5: spawned and resorbing.

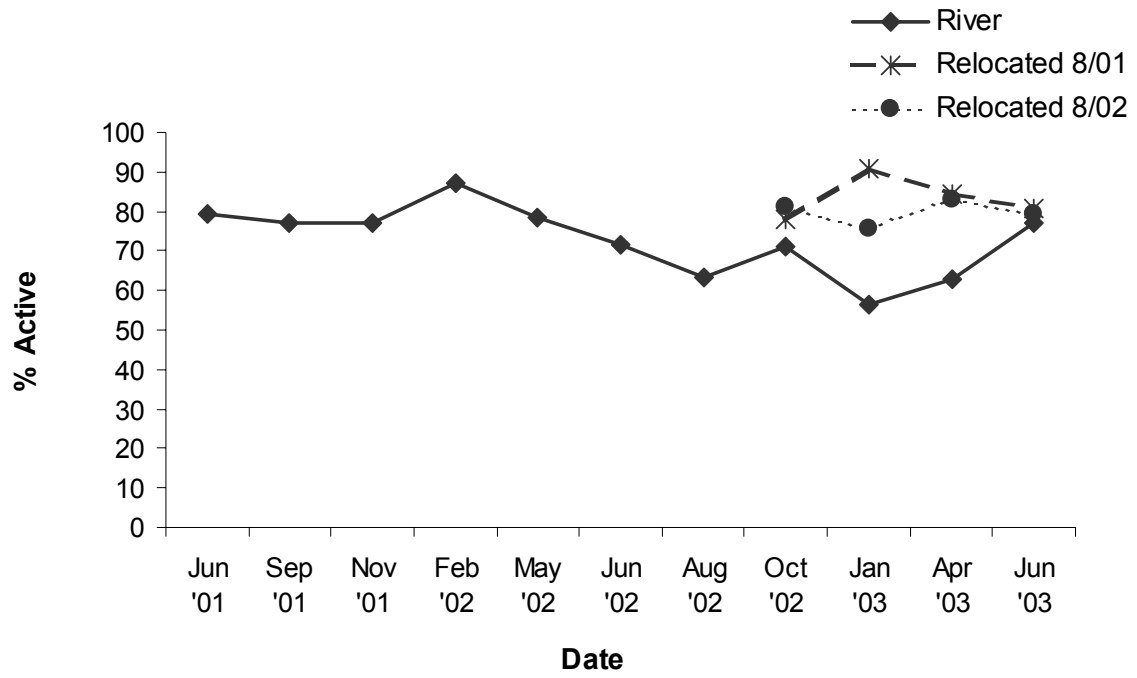

Figure 2.8. Gamete area fraction (GAF) of gonads of *Cyclonaias tuberculata* sampled from the New River and captive *C. tuberculata* (relocated August 2001 and August 2002) at WSSNFH, categorized as active from June 2001 to June 2003.

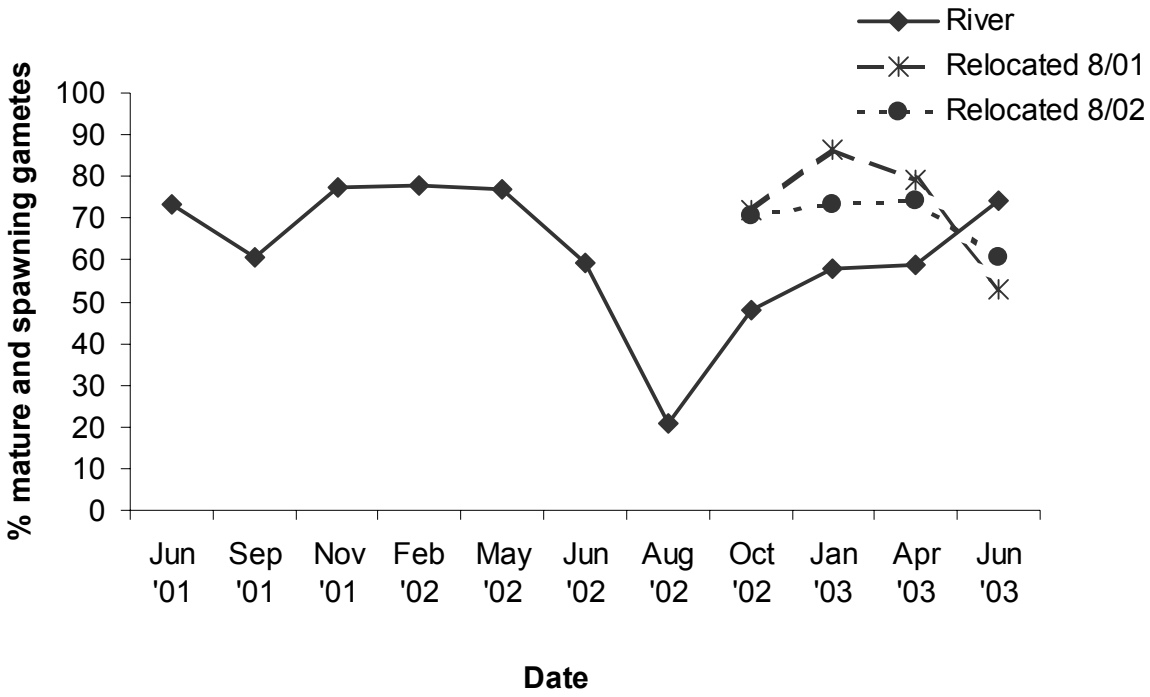

Figure 2.9. Fraction of gonad observations of *Cyclonaias tuberculata* sampled from the New River and captive *C. tuberculata* (relocated to WSSNFH August 2001 and August 2002) at White Sulphur Springs National Fish Hatchery, categorized as mature or spawning (stages 2-4) from June 2001 to June 2003.

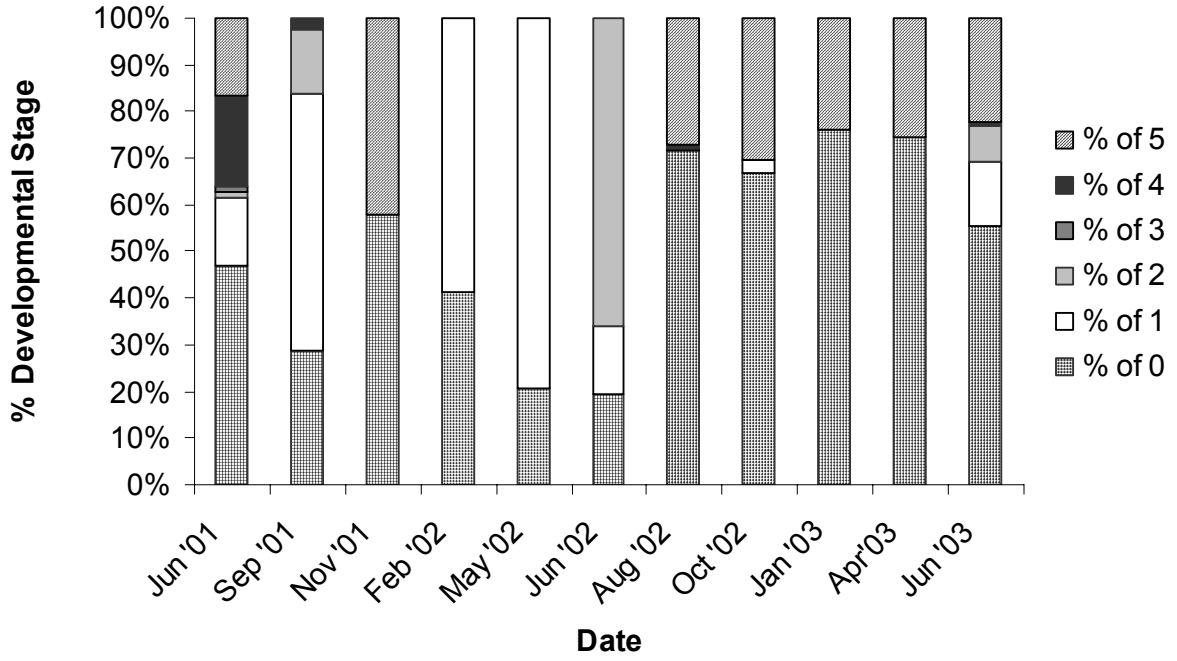

Figure 2.10. Gamete development index of uninfested gonads of wild *Actinonaias ligamentina* from the New River, showing percent of observations identified in each developmental stage. Stages noted in key are 0: inactive, 1: early active, 2: late active, 3: mature, 4: spawning, 5: spawned and resorbing.

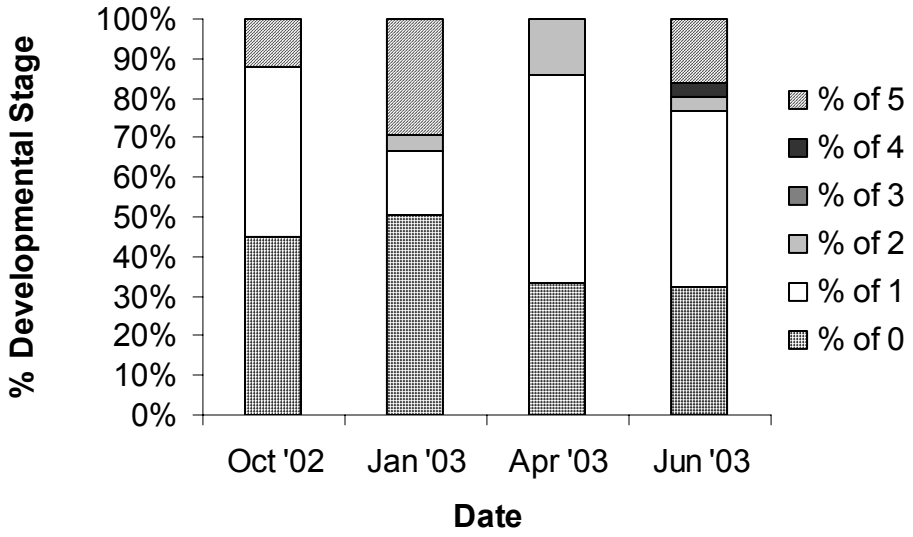

Figure 2.11. Gamete development index of uninfested gonads of captive *Actinonaias ligamentina* relocated in August 2001 to WSSNFH, showing percent of observations identified in each developmental stage. Stages noted in key are 0: inactive, 1: early active, 2: late active, 3: mature, 4: spawning, 5: spawned and resorbing.

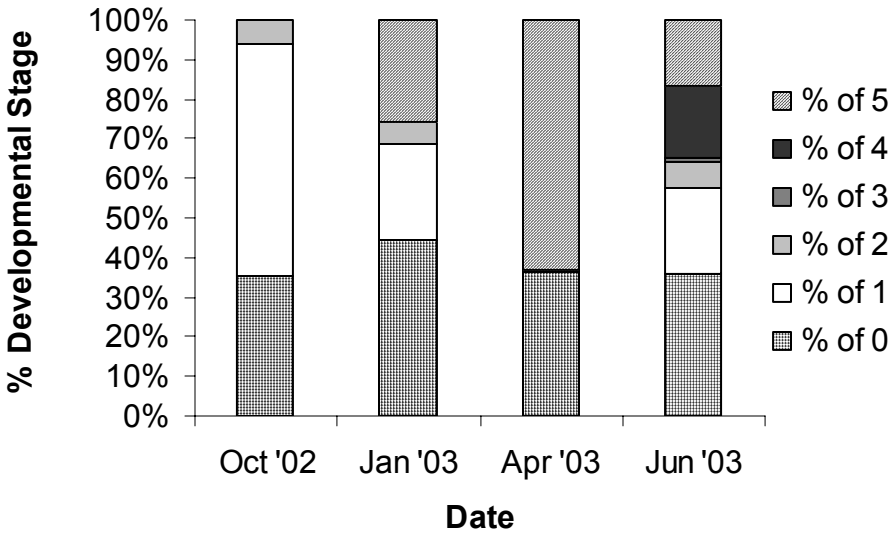

Figure 2.12. Gamete development index of uninfested gonads of captive *Actinonaias ligamentina* relocated in August 2002 to WSSNFH showing percent of observations identified in each developmental stage. Stages noted in key are 0: inactive, 1: early active, 2: late active, 3: mature, 4: spawning, 5: spawned and resorbing.

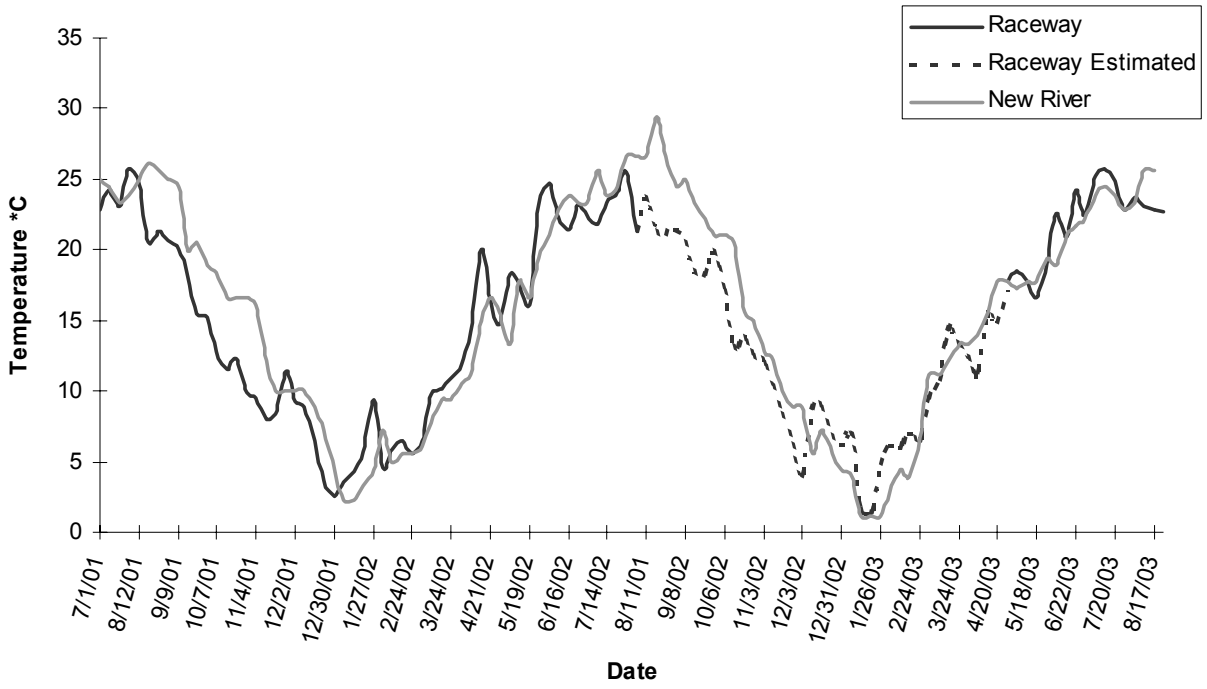

Figure 2.13. Weekly mean temperature (°C) of the raceway (pond B2) at WSSNFH and weekly temperature measurements of the New River at Bluestone Dam, Hinton, WV.

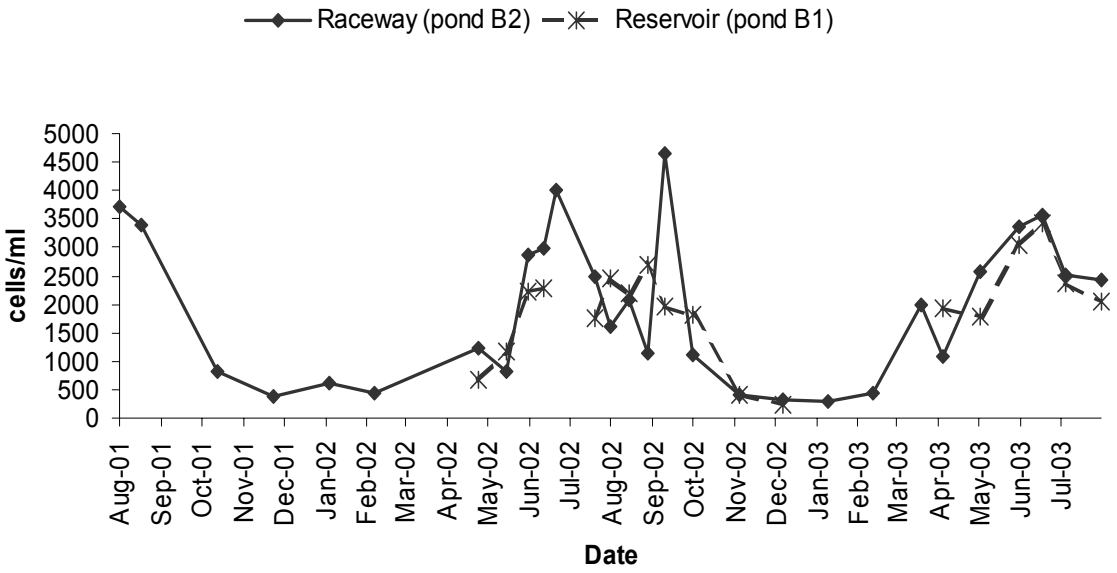

Figure 2.14. Algal cell density in water samples collected from the raceway (pond B1) and reservoir (pond B2), at WSSNFH from August 2001 to August 2003.

Appendix 1. Summary of water chemistry data in the raceway from August 2001 to August 2003.

Date	pH	Alkalinity (mg/L Ca CO_3)	Hardness (mg/L $CaCO_3$)	DO (mg/L)
9/28/01	8.149	80	420	9.88
11/9/01	8.241	100	400	11.14
12/21/01	7.23	--	--	12.02
1/31/02	8.172	120	400	9.6
2/15/02	7.432	60	100	12.28
3/6/02	8.068	100	340	12.03
4/25/02	8.25	80	100	12.12
5/22/02	7.056	80	140	--
6/12/02	8.716	60	240	12.01
6/27/02	7.874	80	320	11.2
7/9/02	7.8	80	320	11.03
7/19/02	7.4	80	440	10.03
8/1/02	7.4	100	--	10.13
8/16/02	8.55	40	260	9.43
9/11/02	7.41	40	290	8.26
10/7/02	7.828	40	290	9.42
10/28/02	7.433	40	210	10.24
12/2/02	6.43	40	150	12.34
1/3/03	7.429	40	140	11.23
2/6/03	7.54	20	170	11.49
3/11/03	7.45	40	70	11.28
4/16/03	--	40	120	11.37
4/30/03	7.562	10	140	10.89
5/29/03	7.72	40	160	9.568
6/28/03	7.734	60	220	11.32
7/29/03	7.63	40	80	10.66
8/27/03	7.852	20	180	10.79
Average ± 1 SD	7.71 ± 0.48	58.8 ± 28.61	228.2 ± 129.68	10.8 ± 1.06

Appendix 2. Cell counts in water samples collected from the raceway, August 2001 to December 2001.

	Algae Characteristics				Sample Date			
Algae (genus)	1	2	3	4	8/29/01	9/14/01	11/9/01	12/21/01
Ankistrodesmus	U	Gr	P	I	0	0	1	0
Carteria	U	Gr	P	I	0	0	0	0
Chlamydomonas	U	Gr	P	I	16	47	23	4
Chlorella	U	Gr	P	I	294	231	24	14
Chlorococcum	U	Gr	B, P	I	77	27	0	0
Chromulina	U	Gol	P	I	0	10	1	1
Chroococcus	C	BG	B	I	23	0	0	0
Chroomonas	U	Cry	P	I	6	6	1	0
Closterium	U	Gr	B, P	I	0	0	0	0
Cocconeis	U	Di	B, P	I	11	2	0	0
Coelospherium	C	BG	P	X	0	0	0	0
Cosmarium	U	Gr	B, P	I	0	0	0	0
Cyclotella	U	Di	P	I	1	3	0	0
Cymatopleura	U	Di	P	I	0	0	0	0
Cymbella	U	Di	B, P	I	1	0	1	0
Desmococcus	C	Gr	P	I	0	0	0	0
Diatoma	U	Di	B, P	I	11	8	26	8
Eremosphaera	U	Gr	P	I	0	0	0	0
Eudorina	C	Gr	P	I	0	0	0	0
Euglena	U	Eug	P	I	0	0	0	0
Fragilaria	C	Di	P	X	2	0	0	0
Gloeocapsa	C	BG	B	I	0	0	0	0
Gloeocystis	C	Gr	P	I	0	0	0	0
Golenkiniopsis	U	Gr	P	I	0	0	0	0
Gonphonema	U	Di	B, P	I	0	0	0	0
Hyella	F	BG	B	X	0	0	0	0
Mallomonas	U	Gol	P	I	0	0	0	0
Meridion	U	Di	B, P	I	13	7	0	0
Microspora	F	Gr	B	X	0	0	0	0
Monocilia	F	Gr	B, P	X	0	0	6	0
Navicula	U	Di	B	I	0	0	24	2
Nitzschia	U	Di	B, P	I	0	0	0	0
Oedogonium	F	Gr	B, P	X	0	0	0	0
Oocystis	U	Gr	P	I	4	1	0	0
Oscillatoria	F	BG	B, P	X	89	42	10	0
Pandorina	C	Gr	P	I	0	0	0	0
Pediastrum	C	Gr	P	I	0	0	0	0
Peridinium	U	Din	P	I	0	0	0	0
Pinnularia	U	Di	B	I	11	6	0	0
Rhizoclonium	F	Gr	B	X	0	0	0	0
Scenesdesmus	C	Gr	P	I	8	0	0	0
Schroederia	C	Gr	P	I	0	0	0	0
Spirulina	F	BG	P	I	5	0	0	0
Stephanodiscus	U	Di	P	I	0	0	0	0
Stichococcus	F	Gr	P	I	0	0	0	0
Synedra	U	Di	P	I	5	6	7	0
Tabellaria	C	Di	P	X	0	0	3	0
Trachelomonas	U	Eug	P	I	0	0	1	0
Trochiscia	U	Gr	P, G	I	0	0	1	0
Ulothrix	F	Gr	B	X	0	0	0	0
Other					3	2	0	0
Total					580	398	129	29
# genera					18	14	14	5
# of Grids counted					20	15	20	10
Cell/ml					3701.2	3386.3	823.2	370.1

1. Form is the classification of unicellar (U), colonial (C), or filamentous (F) algae
2. Group is the classification of Cyanoprokaryota [bluegreen] (BG), Chlorophyta [green] (Gr), Euglenophyta [euglenoids] (Eug), Bacillariophyceae [diatom] (Di), Chrysophyceae [golden brown] (Gol), or Cryptophyta (Cry) algae, Dinophyta [dinoflagellate] (Din),
3. Habitat is the classification of mostly planktonic (P) or mostly benthic (B) algae
4. Ingestibility is the classifcation of ingestible (I) algae and algae not likely to be ingested (X) by adult mussels

Appendix 2(continued). Cell counts in water samples collected from the raceway, January 2002 to September 2002.

	Sample Date									
Algae (genus)	1/31/02	3/6/02	5/22/02	6/12/02	6/28/02	7/9/02	7/19/02	8/16/02	8/28/02	9/11/02
Ankistrodesmus	0	0	0	0	0	0	0	0	0	0
Carteria	0	0	0	0	0	0	0	0	1	2
Chlamydomonas	0	0	1	0	0	0	0	0	2	0
Chlorella	52	95	43	31	53	137	95	59	113	114
Chlorococcum	5	6	0	6	0	3	17	17	0	1
Chromulina	0	0	2	0	0	2	0	0	4	2
Chroococcus	0	0	0	0	0	0	0	0	0	0
Chroomonas	0	0	7	45	8	4	32	37	24	35
Closterium	0	0	0	0	0	1	0	0	0	0
Cocconeis	18	0	2	11	3	0	6	0	1	4
Coelospherium	0	0	0	60	80	25	250	30	0	0
Cosmarium	0	0	3	0	3	1	0	0	0	0
Cyclotella	5	3	0	0	0	9	0	1	0	0
Cymatopleura	0	0	0	0	0	0	0	0	0	0
Cymbella	0	1	7	0	4	3	3	2	7	3
Desmococcus	0	0	0	0	0	0	0	0	0	0
Diatoma	57	27	36	9	28	20	15	14	19	23
Eremosphaera	0	0	0	0	0	0	0	0	0	0
Eudorina	0	0	0	0	0	0	0	89	35	5
Euglena	1	0	0	0	0	0	0	0	0	0
Fragilaria	8	51	25	0	0	4	0	41	0	30
Gloeocapsa	0	0	0	20	128	105	53	0	0	0
Gloeocystis	0	0	0	0	0	0	32	5	0	0
Golenkiniopsis	0	0	0	0	0	0	0	0	0	0
Gonphonema	10	0	9	3	1	2	0	0	0	2
Hyella	0	0	0	0	0	10	0	0	0	0
Mallomonas	0	0	0	0	0	0	0	0	0	0
Meridion	7	2	1	18	5	0	1	2	3	3
Microspora	0	0	0	0	0	0	0	0	0	0
Monocilia	0	0	0	0	0	0	0	0	0	0
Navicula	36	28	43	30	53	31	9	29	11	55
Nitzschia	0	0	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0	0	0
Oocystis	23	2	2	7	5	16	7	8	1	4
Oscillatoria	19	14	0	47	28	70	100	43	30	20
Pandorina	0	8	0	0	0	0	0	0	0	0
Pediastrum	0	0	0	0	0	0	0	0	0	0
Peridinium	0	0	0	3	2	0	1	2	0	14
Pinnularia	0	0	0	3	39	22	6	0	2	3
Rhizoclonium	0	0	0	0	0	0	1	0	0	0
Scenesdesmus	0	0	0	0	0	0	0	0	0	0
Schroederia	0	0	0	0	0	0	0	7	0	2
Spirulina	0	0	0	0	0	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0	0	0	0
Stichococcus	0	0	0	0	1	0	1	0	0	0
Synedra	0	3	7	0	4	3	0	0	1	0
Tabellaria	0	0	0	0	0	0	0	0	0	0
Trachelomonas	2	0	0	0	0	0	0	0	0	0
Trochiscia	1	2	4	0	0	0	0	5	0	1
Ulothrix	0	0	0	0	3	0	0	0	0	0
Other	0	0	0	0	0	0	0	7	0	0
Total	244	242	192	293	448	468	629	391	254	325
# genera	14	13	15	14	18	20	17	17	13	22
Cell/ml	**622.8**	**429.0**	**1225.2**	**831.0**	**2858.8**	**2986.5**	**4013.8**	**2495.1**	**1620.9**	**2073.9**

Appendix 2 (continued). Cell counts in water samples collected from the raceway, September 2002 to March 2003.

	Sample Date						
Algae (genus)	9/25/02	10/7/02	10/28/02	12/2/02	1/3/03	2/6/03	3/11/03
Ankistrodesmus	3	0	0	1	0	0	0
Carteria	8	0	0	0	0	0	0
Chlamydomonas	5	7	0	3	4	0	1
Chlorella	63	228	86	67	30	12	33
Chlorococcum	2	0	2	1	3	1	0
Chromulina	4	3	3	5	2	0	0
Chroococcus	0	4	0	0	0	0	0
Chroomonas	44	8	8	2	3	0	0
Closterium	2	0	0	2	2	7	2
Cocconeis	2	0	1	0	0	0	0
Coelospherium	0	0	5	0	0	0	0
Cosmarium	0	0	0	0	0	0	0
Cyclotella	0	0	0	0	0	0	3
Cymatopleura	0	0	0	0	0	0	1
Cymbella	1	0	1	0	0	0	0
Desmococcus	0	0	0	0	0	0	0
Diatoma	12	43	0	3	24	10	26
Eremosphaera	0	0	0	0	0	0	0
Eudorina	0	0	0	0	0	0	0
Euglena	0	4	0	0	0	1	0
Fragilaria	0	0	0	0	0	0	0
Gloeocapsa	0	15	0	6	0	0	0
Gloeocystis	0	0	0	0	0	0	0
Golenkiniopsis	0	0	0	0	0	0	0
Gonphonema	0	0	0	0	0	0	0
Hyella	0	0	0	0	0	0	0
Mallomonas	0	0	0	0	0	0	0
Meridion	1	1	1	1	0	1	0
Microspora	0	0	0	0	0	0	0
Monocilia	0	0	0	0	0	0	0
Navicula	32	78	24	14	21	11	34
Nitzschia	0	0	0	0	0	0	2
Oedogonium	0	0	0	0	0	0	0
Oocystis	1	3	1	1	1	4	2
Oscillatoria	35	330	36	12	10	45	10
Pandorina	0	0	0	0	0	0	2
Pediastrum	0	0	0	0	0	0	0
Peridinium	3	2	0	0	0	0	0
Pinnularia	0	0	2	4	0	1	4
Rhizoclonium	0	0	0	0	0	0	0
Scenesdesmus	0	0	0	0	0	0	0
Schroederia	0	0	0	0	0	0	0
Spirulina	0	1	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0
Stichococcus	0	0	0	1	0	1	1
Synedra	1	3	0	1	0	0	0
Tabellaria	0	0	0	0	0	0	0
Trachelomonas	1	0	0	0	0	0	0
Trochiscia	1	0	2	2	1	0	1
Ulothrix	1	0	0	0	1	0	0
Other	0	0	0	0	0	0	0
Total	222	730	172	126	102	94	122
# genera	20	15	14	17	12	11	14
Cell/ml	**1133.3**	**4658.4**	**1097.6**	**402.0**	**325.4**	**299.9**	**444.9**

Appendix 2 (continued): Cell counts in water samples collected from the raceway, April 2003 to August 2003

	Sample Date						
Date pond B2	4/16/03	5/2/03	5/30/03	6/27/03	7/15/03	8/1/03	8/27/03
Ankistrodesmus	0	0	0	2	0	1	1
Carteria	0	0	0	0	0	0	0
Chlamydomonas	0	0	7	11	8	6	2
Chlorella	14	11	105	136	126	137	102
Chlorococcum	1	0	6	23	21	7	18
Chromulina	0	1	28	0	8	0	0
Chroococcus	0	0	0	0	24	0	0
Chroomonas	0	0	2	41	7	15	16
Closterium	11	3	7	6	5	4	1
Cocconeis	2	0	0	0	0	0	0
Coelospherium	0	0	0	0	0	0	0
Cosmarium	0	0	0	2	0	0	0
Cyclotella	0	0	0	0	0	0	0
Cymatopleura	0	0	0	0	0	0	0
Cymbella	19	8	26	9	4	9	8
Desmococcus	0	0	0	0	0	0	0
Diatoma	74	43	39	28	12	11	26
Eremosphaera	0	0	0	0	0	0	0
Eudorina	0	0	0	0	0	0	10
Euglena	0	0	0	0	0	0	0
Fragilaria	0	0	0	0	0	0	6
Gloeocapsa	0	0	10	0	0	0	0
Gloeocystis	0	0	0	0	0	0	0
Golenkiniopsis	0	0	0	0	0	0	0
Gonphonema	0	0	0	1	1	2	1
Hyella	0	0	0	0	0	0	0
Mallomonas	0	0	0	0	0	0	10
Meridion	4	0	8	9	4	3	8
Microspora	0	0	8	0	0	0	0
Monocilia	0	0	0	0	0	0	0
Navicula	118	51	90	45	49	43	44
Nitzschia	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0
Oocystis	3	2	5	4	2	3	2
Oscillatoria	130	0	50	200	240	145	120
Pandorina	0	0	0	0	9	0	0
Pediastrum	3	0	0	0	16	0	0
Peridinium	0	0	1	0	0	1	0
Pinnularia	0	5	2	0	2	0	3
Rhizoclonium	0	0	0	0	0	0	0
Scenesdesmus	0	12	0	0	3	0	2
Schroederia	0	0	0	6	6	1	1
Spirulina	0	0	1	0	0	0	0
Stephanodiscus	7	1	0	0	0	0	0
Stichococcus	2	2	4	0	1	1	0
Synedra	0	31	1	0	3	3	0
Tabellaria	0	0	0	0	0	0	0
Trachelomonas	0	0	1	0	0	0	0
Trochiscia	2	1	3	3	6	0	1
Ulothrix	0	0	0	0	0	0	0
Other	0	0	0	0	8	0	2
Total	390	171	404	526	557	392	382
# genera	13	13	21	16	23	17	21
Cell/ml	**1991.0**	**1091.2**	**2578.0**	**3356.6**	**3554.4**	**2501.5**	**2437.7**

Appendix 3. Cell counts in water samples taken from the reservoir, May 2002 to September 2002

	Sample Date							
Algae (genus)	5/22/02	6/12/02	6/28/02	7/9/02	8/16/02	8/28/02	9/11/02	9/25/02
Ankistrodesmus	2	0	0	0	0	0	0	0
Carteria	0	7	0	0	4	1	13	2
Chlamyd	5	0	7	0	2	9	5	9
Chlorella	83	61	170	262	36	77	70	72
Chlorococcum	0	8	10	3	0	6	14	9
Chromulina	31	0	0	3	36	14	17	0
Chroococcus	0	0	0	0	0	0	0	0
Chroomonas	69	105	34	19	0	62	13	32
Closterium	1	0	0	0	0	0	0	0
Cocconeis	0	4	0	0	0	0	0	0
Coelospherium	0	80	0	130	100	40	0	0
Cosmarium	0	2	0	1	0	0	0	0
Cyclotella	0	0	0	1	0	0	0	1
Cymatopleura	0	0	0	0	0	0	0	0
Cymbella	0	0	0	0	0	0	0	2
Desmococcus	0	0	0	0	3	0	0	0
Diatoma	19	27	27	18	11	14	17	46
Eremosphaera	0	0	0	0	0	1	0	0
Eudorina	0	0	0	0	0	25	0	0
Euglena	0	0	0	1	0	0	0	0
Fragilaria	0	0	0	0	0	0	0	6
Gloeocapsa	0	0	0	22	0	0	0	0
Gloeocystis	0	0	0	0	32	20	0	0
Golenkiniopsis	2	0	1	0	0	0	0	0
Gonphonema	0	3	2	2	0	0	0	0
Hyella	0	0	0	0	0	0	0	0
Mallomonas	0	0	0	0	0	0	0	0
Meridian	1	0	14	6	3	6	2	9
Microspora	0	0	0	0	0	0	0	0
Monocillia	0	0	0	0	0	0	0	0
Navicula	24	14	70	9	9	25	29	110
Nitzschia	0	0	0	0	0	0	0	0
Oedogonium	0	0	0	0	0	0	0	0
Oocystis	4	6	5	3	3	1	1	0
Oscillatoria	15	18	2	50	20	80	60	110
Pandorina	0	0	0	0	0	0	102	0
Pediastrum	0	24	0	0	0	0	0	0
Peridinium	3	1	0	0	1	0	1	8
Pinnularia	0	0	0	1	0	0	0	0
Rhizoclonium	0	0	0	0	0	0	0	0
Scenesdesmus	0	0	0	0	0	0	0	0
Schroederia	0	0	0	0	9	1	0	1
Spirulina	0	0	0	0	0	0	0	0
Stephanodiscus	0	0	0	0	0	0	0	0
Stichococcus	0	0	0	0	0	0	0	0
Synedra	2	0	5	5	0	0	0	1
Tabellaria	0	0	0	0	0	0	0	0
Trachelomonas	0	0	0	0	0	1	0	0
Trochiscia	1	0	1	0	0	3	0	4
Ulothrix	0	6	0	0	6	0	0	0
other	0	0	0	0	0	0	0	0
Total	262	366	348	536	275	386	344	422
# of genera	15	15	13	17	15	18	13	16
# cells/ml	**668.8**	**1167.8**	**2220.7**	**2280.3**	**1754.9**	**2463.2**	**2195.2**	**2692.9**

Appendix 3 (continued). Cell counts in water samples taken from the reservoir, October 2002 to May 2003.

	Sample Date				
Algae (genus)	10/7/02	10/28/02	12/2/02	1/3/03	5/2/03
Ankistrodesmus	0	0	1	0	0
Carteria	14	0	0	0	0
Chlamyd	15	0	3	7	172
Chlorella	118	110	69	16	19
Chlorococcum	6	12	0	2	0
Chromulina	13	3	3	3	0
Chroococcus	0	0	3	0	0
Chroomonas	26	12	1	1	2
Closterium	0	0	0	0	0
Cocconeis	0	3	0	1	0
Coelospherium	5	10	0	0	0
Cosmarium	0	0	0	1	0
Cyclotella	0	2	0	0	0
Cymatopleura	0	0	0	0	1
Cymbella	0	1	1	0	2
Desmococcus	0	0	0	0	0
Diatoma	18	0	19	11	18
Eremosphaera	0	0	0	0	0
Eudorina	0	0	0	0	0
Euglena	2	0	0	0	0
Fragilaria	6	2	0	0	0
Gloeocapsa	0	0	0	0	0
Gloeocystis	0	0	0	0	0
Golenkiniopsis	0	0	0	0	0
Gonphonema	0	0	0	0	0
Hyella	0	0	0	0	0
Mallomonas	0	0	0	0	0
Meridian	2	2	0	0	8
Microspora	0	0	0	0	0
Monocillia	0	0	0	0	0
Navicula	39	26	15	15	39
Nitzschia	0	0	0	0	0
Oedogonium	0	0	0	0	0
Oocystis	0	0	1	1	2
Oscillatoria	40	100	10	15	30
Pandorina	0	0	0	0	0
Pediastrum	0	0	0	0	0
Peridinium	2	0	0	0	0
Pinnularia	0	0	0	0	1
Rhizoclonium	0	0	0	0	0
Scenesdesmus	0	0	0	0	0
Schroederia	0	0	0	0	0
Spirulina	0	0	0	0	2
Stephanodiscus	0	0	0	0	0
Stichococcus	0	0	0	0	0
Synedra	0	0	0	0	6
Tabellaria	0	0	0	0	0
Trachelomonas	0	0	1	0	0
Trochiscia	0	1	1	1	2
Ulothrix	0	0	0	0	0
other	0	0	0	0	0
Total	306	284	128	74	304
# of genera	14	15	13	12	14
# cells/ml	**1952.7**	**1812.3**	**408.4**	**236.1**	**1939.9**

Appendix 3 (continued). Cell counts in water samples taken from the reservoir, May 2003 to August 2003.

	Sample Date				
Sample Date B1	5/30/03	6/27/03	7/15/03	8/1/03	8/27/03
Ankistrodesmus	0	0	0	0	0
Carteria	0	0	0	0	0
Chlamyd	16	47	4	2	6
Chlorella	91	120	102	145	98
Chlorococcum	2	14	16	6	10
Chromulina	20	2	8	0	1
Chroococcus	0	0	0	10	0
Chroomonas	6	36	11	32	27
Closterium	7	4	2	1	0
Cocconeis	0	0	0	0	0
Coelospherium	0	0	0	0	0
Cosmarium	0	0	0	0	0
Cyclotella	0	0	0	0	0
Cymatopleura	0	0	0	0	0
Cymbella	6	2	16	4	0
Desmococcus	0	0	0	0	0
Diatoma	20	32	15	13	10
Eremosphaera	0	0	0	0	0
Eudorina	0	0	0	0	0
Euglena	0	0	0	0	0
Fragilaria	0	0	0	0	0
Gloeocapsa	40	0	0	0	0
Gloeocystis	0	0	0	0	0
Golenkiniopsis	0	0	0	0	0
Gonphonema	0	2	1	0	0
Hyella	0	0	0	0	0
Mallomonas	0	0	1	0	7
Meridian	2	8	1	5	4
Microspora	0	0	0	0	0
Monocillia	0	0	0	0	0
Navicula	30	48	41	13	10
Nitzschia	0	0	0	0	0
Oedogonium	0	0	0	0	0
Oocystis	8	0	0	1	0
Oscillatoria	25	132	300	133	145
Pandorina	0	0	0	0	0
Pediastrum	0	16	0	0	0
Peridinium	2	0	2	0	0
Pinnularia	0	0	0	1	0
Rhizoclonium	0	0	0	0	0
Scenesdesmus	0	0	4	0	0
Schroederia	0	15	8	0	1
Spirulina	0	0	0	0	0
Stephanodiscus	0	0	0	0	0
Stichococcus	0	0	0	1	1
Synedra	2	0	4	2	0
Tabellaria	0	0	0	0	0
Trachelomonas	0	0	0	0	0
Trochiscia	3	0	0	1	3
Ulothrix	0	0	0	0	0
other	0	0	0	0	2
Total	280	478	536	370	323
# of genera	16	14	17	16	14
# cells/ml	**1786.8**	**3050.3**	**3420.4**	**2361.1**	**2061.2**

VITA

Julie L. Boyles was born to David J. and Elaine S. Boyles on July 23, 1974 in Montoursville, PA. Her love of nature was fostered by many family hiking, camping and beach trips throughout her childhood. She graduated from Montoursville Area High School in June 1992 and went on to earn a B.S. in Biology at Millersville University of Pennsylvania. While at Millersville University, she competed on the cross-country and track and field teams. She graduated in May 1996 with a double major in Marine Biology and Environmental Science.

Following graduation, Julie worked as an environmental educator for Nature's Classroom while awaiting an assignment with the U.S. Peace Corps. She departed on her Peace Corps adventure to Gabon, Central Africa, in July 1997. While in Gabon, Julie learned to speak French, taught Gabonese farmers to raise tilapia in a sustainable agriculture system, and overcame seemingly insurmountable challenges. After nearly two and a half years, Julie left her Gabonese friends to return to the United States in November of 1999.

Julie worked again as an environmental educator for Nature's Classroom until joining the U.S. Fish and Wildlife Service as a Biological Technician in May 2000. After one year in Abingdon, VA assisting Service biologists with freshwater mussel surveys, Section 7 consultations, and public outreach, she left to begin an M.S. in Fisheries and Wildlife Sciences and research assistantship with Dr. Richard Neves at Virginia Tech. While at Virginia Tech, she held a SCEP (Student Career Employment Program) position with the U.S. Fish and Wildlife Service. Julie has accepted a position at White Sulphur Springs National Fish Hatchery in West Virginia as a Fisheries Biologist.