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[Article]

Captive Survival and Pearl Culture Potential of the Pink Heelsplitter *Potamilus alatus*

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Abstract.—Pink heelsplitter mussels *Potamilus alatus* were held at two bottom locations and suspended in pocket nets in a pond at the Freshwater Mollusk Conservation Center, Virginia Polytechnic Institute and State University, for 1 year to evaluate survival in captivity. Survival after 1 year differed significantly; the poorest survival (30.0%) was at the bottom of the deep end (2.5 m), and there was no difference in survival) at the shallow end (83.3%; 0.6 m) and in pocket nets (63.3%; 1–1.5 m). Monthly survival was inversely related to water temperature ($R = -0.72$); the lowest monthly survival occurred in summer, resulting in a significant difference among the three locations. Differences in the mean glycogen content of mussels among the three containment locations and wild-sampled pink heelsplitter mussels were significant ($P = 0.001$); the highest value was in mussels at the shallow end and the lowest value was in mussels at the deep end. We used pocket nets to hold surgically implanted mussels and measured glycogen reserves to monitor body condition and assess the potential of the pink heelsplitter mussel to produce nonnucleated and image pearls. Results of the pearl culture experiment in two ponds showed that nonnucleated pearls and image pearls with purple or purplish luster were successfully produced. There was no significant difference in pearl weight in mussels held in the two experimental ponds under different environmental conditions. Similarly, no differences in monthly survival rates of mussels were observed in either pond or among mussels with surgical implants and the no-surgery control mussels. Therefore, pink heelsplitter mussels can be considered a potentially suitable species for producing purple pearls in farm pond environments.

A number of holding systems have been tested for suitability in maintaining mussels in captivity. Burress and Neves (1995) evaluated survival of 15 species of freshwater mussels in captivity in four separate pond sites in Virginia and reported that survival rates varied by species, captivity period, and habitat conditions. Dunn and Layzer (1997) also concluded that survival rates are influenced by captive facilities when comparing 20 species of mussels relocated to various types of holding conditions. In an evaluation of suspended nylon pocket nets and benthic dish racks, Gatenby (2000) reported that mussels held in pocket nets generally had higher survival rates than those in dish racks. Culture methods in commercial aquaculture include hanging culture (suspended nets in water) and bottom culture for marine bivalve production (Wang 1993; Wang and Tian 1998). Freshwater mussels used for pearl culture are usually held in net bags, net cages, or pocket nets that are suspended in ponds, reservoirs, or lakes for pearl production (Hua and Gu 2002). The advantage of hanging culture is that it maximizes the "cubic" water column by using the total water body instead of the "square" area in bottom culture. Moreover, hanging mussels are free of substrate, such that occurrence of disease caused by benthic microbes is minimized.

Biochemical composition of soft tissues in mussels is commonly used to quantify their physiological condition (Haag et al. 1993; Naimo et al. 1998; Gatenby 2000; Newton et al. 2001; Liberty 2004). Glycogen is a primary energy reserve in adult bivalves (De Zwaan and Zandee 1972; Gabbott 1983; Hummel et al. 1989; Leavitt et al. 1990) and is considered an appropriate indicator of condition for this group (Naimo and Monroe 1999; Patterson et al. 1999; Gatenby 2000; Boyles 2004; Liberty 2004). Environmental changes can stress bivalves, evident by reduction in glycogen content (Hummel et al. 1989; Naimo and Monroe 1999). Haag et al. (1993) studied survival and fitness in two native mussels, eastern lampmussel *Lampsilis radiata* and threeridge mussel *Amblema plicata*, fouled by zebra mussels *Dreissena polymorpha* and concluded that low glycogen is the immediate impact to survival and reproduction of these

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species. Similarly, Hallac and Marsden (2001) supported this conclusion by studying glycogen stores in zebra mussel-fouled eastern elliptio *Elliptio complanata*; glycogen content was 50% lower in fouled mussels than in unfouled ones. While many studies of glycogen dynamics have been previously conducted among a suite of bivalves under various stress conditions, glycogen reserves in the pink heelsplitter mussel *Potamilus alatus* have not been studied.

The frequency of natural pearl formation in freshwater mussels is low: approximately 1 in 10,000 mussels may produce a valuable pearl (McGregor and Gordon 1992; Anthony and Downing 2001). Because the pink heelsplitter mussel is one of four mussels in which natural pearls were collected in the Clinch River (Davis 2000), this report generated interest in assessing the feasibility of using this species for pearl formation. The pink heelsplitter mussel is a large species with moderately thick valves such that it has potential to produce pearls fairly quickly. Freshwater pearls vary in color and include white, cream, pink, salmon, lavender, and purple; of these, purple, pink, and lavender are most desirable (USGS 2002) and fetch high prices in the world market. Pearl color is inherent and mainly associated with the nacre color of mussel shells, but it is affected somewhat by habitat location and water quality. Nacre color of pink heelsplitter mussels is rich purple and iridescent, presumably resulting in the likely formation of purple pearls. The limited number of natural purple pearls available in the marketplace is mostly produced from the Chinese triangle sail mussel *Hyriopsis cumingii*, which has a wide variation in nacre color (white, cream, pink, purple, and gold); consequently, purple pearls are infrequent and more difficult to obtain for matched strands of pearls. However, the pink heelsplitter mussel has uniform purple nacre and the presumed capability to produce purple pearls consistently for matched jewelry products.

Pearl formation is a complicated process, involving inorganic and organic chemistry, biochemistry, and crystallography. The mantle tissue secretes mineral aragonites and conchiolins, which are the primary components of nacre. Translucent aragonite gives pearls and shells their lustrous appearance (Hua et al. 2001). Natural pearls are formed through pathological regeneration or hyperplasia and can be triggered by the introduction of a foreign stimulus into the mantle of a mollusk. Epidermal cells in mantle tissue secrete crystalline fluid nacre around the foreign stimulus and subsequent layers of nacre to form a pearl (Hua et al. 2001). To culture pearls, pieces of mantle tissue, or a bead nucleus accompanied by a mantle piece, are inserted into the mantle of a live mussel, triggering the nacre-secreting process. Pearls then are produced in a suitable aquaculture environment over a long-term culture period. Therefore, a suitable captive facility and environment for grafted mussels is essential to recovery from implantation surgery as well as for pearl growth and survival.

The objectives of this study were to assess (1) the survival rate and condition of adult pink heelsplitter mussels held in three captive enclosures in a pond for 1 year and (2) the feasibility of pearl formation using two surgical treatments to compare the pearl formation, survival, and glycogen content of implanted mussels in two different ponds.

Methods

Collection and sites.—Adult pink heelsplitter mussels were collected from a submerged creek channel in the Kentucky Lake portion of the Tennessee River (river kilometer 103.0 measuring from Paducah, Kentucky), Humphreys County, Tennessee, and then quarantined in a 1,000-L isolated recirculating system for 1 month. The experiment was conducted in a 0.1-ha pond at the Freshwater Mollusk Conservation Center (FMCC) at Virginia Polytechnic Institute and State University (hereafter, Virginia Tech), Blacksburg. The pond contained 985 m^3 of water, roughly 1 m deep at the shallow end and 2.5 m at the deep end, and circulation was provided by a 0.5-hp combination paddle wheel and aerator. The second pond was the Duck Pond on the campus of Virginia Tech. The Duck Pond has a surface area of 2.4 ha, is fed by two branches of Stroubles Creek that drain approximately 289 ha, including most of Blacksburg and the Virginia Tech campus.

Experimental design and sampling.—To test the suitability of captive enclosures, 90 pink heelsplitter mussels were randomly assigned to three captive treatments in the FMCC pond for 1 year (October 2003 to October 2004). In bottom treatments, 10 mussels were confined in each of three bottomless plastic containers ($0.50 \times 0.50 \times 0.20$ m) at a water depth of 0.6 m (shallow end) in a sandy–clay substrate, and 10 mussels were placed in each of three plastic buckets ($0.60 \times 0.35 \times 0.20$ m) with a mixed substrate of sand, gravel, and fine sediment at 2.5 m (deep end). In a third treatment, 10 mussels were held in each of three plastic pocket nets (0.60×0.45 m) suspended in the water column (1 m below the water surface). Since sample size was limited to 10 mussels per replicate, dead mussels were replaced with additional live mussels and held in the same pond as the experimental mussels during the 1-year period. Survival was monitored monthly from October 2003 to October 2004. Mussels were defined as dead when valves remained open and did not close when stimulated.

To evaluate pearl formation and growth, implanted pink heelsplitter mussels were held in pocket nets and suspended in the two ponds for 10 months (July 2004 to May 2005). One hundred and twenty mussels were randomly assigned to the three treatments (20 implanted mussels per pond for each operation and 20 no-surgery mussels per pond as the control group). Survival rate and pearl formation were monitored monthly, except from December 2004 to March 2005 because of ice cover on the ponds. Pearl formation was assessed by sampling two pearls from each live mussel and measuring pearl weights. Glycogen (mg glycogen/g dry tissue) was measured to compare the difference between captive mussels in ponds and wild mussels collected concurrently from the source population in Kentucky Lake in both experiments. Three mussels of each treatment and available wild mussels were sampled after the experimental period. Mantle tissue was removed from each sampled mussel, placed in a vial, and frozen at −60°C for glycogen analysis. Vials of mantle tissue were dried in a freeze-dryer (Labconco Corporation, Kansas City, Missouri; Model 79480) for 3 d until the weight of mantle tissues became constant. Samples were treated at −40°C at a vacuum pressure of 110×10^{-3} mm Hg. The dried mantle tissues were minced into small pieces, ground into powder with a mortar and pestle, and stored frozen at −60°C. Glycogen content was determined with the phenol–sulfuric acid method (Dubois et al. 1956; Naimo et al. 1998). Additionally, glycogen content of eight wild male mussels and five female mussels were measured from available specimens during the experimental period.

To quantify environmental conditions, dissolved oxygen (DO) was measured at three locations in the FMCC pond in late June and water temperature was monitored continually at 1-h intervals from October 2003 to October 2004 during the captive survival experiment. Parameters of pH, DO, alkalinity, total ammonia nitrogen (TAN), and algae and organic matter were measured monthly during the implant experiment in both ponds from July 2004 to May 2005. Water temperature was monitored with continuous Hobo temperature loggers (Onset Computer Corporation, Pocasset, Massachusetts). Dissolved oxygen was measured with a DO meter (YSI, Inc., Yellow Springs, Ohio; Model 55/12 FT). Alkalinity was measured with a Hach test kit (Hach Company, Loveland, Colorado), and pH was measured with a pH meter (Model 9024, Apopka, Florida). Total ammonia nitrogen was determined by the Nessler method with a Hach DR/2400 spectrophotometer (Hach Company). Dominant algae were identified during the culture period, and amount of suspended organic matter was determined by ash-free dry weight.

Surgical operation.—The surgeries for nonnucleated implant (NNI) pearls were conducted in two steps: (1) the excision and preparation of pieces of mantle tissue and (2) the transplantation of these pieces into live pink heelsplitter mussels. Mantle tissues at the edge of a sacrificed mussel were excised from the mussel body and separated into two layers. The epidermal strips of the mantle tissue were cut into square pieces of approximately 2×2 mm for transplantation. For the treatment of NNI surgery, 10 pieces of mantle tissue were transplanted into the mantle at the posterior end of a live mussel. A piece of mantle tissue was transplanted into a live mussel by making an incision pocket between the two layers of the recipient mantle (and in tight contact with both mantle tissue layers) so that cell division and multiplication would create a pocket-like pearl sac for depositing nacre. The nacre was initially soft and glue-like and later hardened to become a pearl. Pearls grew gradually as more nacre was deposited. Image pearls (IP), an implanted image covered by pearly nacre, were obtained by inserting a sculpted nucleus into the cavity between the shell and mantle of the implanted mussel, subsequently to be covered by secreted nacre. The inserted IP were oval-shaped (25×35 mm), made from wax, and had a distinct convex surface design. Surgeries were performed with special tools, including needles, hooks, knives, forceps, mussel opener, scissors, sponger, glass board, and mussel holder (Hua et al. 2001). Surgeries were performed in the laboratory at 20°C from 11 to 16 July 2004. Twenty implanted mussels from each surgical procedure were then held in pocket nets and suspended in the two ponds for pearl formation. Mussels were suspended at a depth of 80 cm below the water surface with floats and fixed lines.

Data analyses.—Data were analyzed with JMP (SAS Institute, Inc., Cary, North Carolina, 2001), and the normality of these data, due to small sample size, was tested with a Kolmogorov–Smirnov test (Sall et al. 2001). The rank-based, nonparametric Wilcoxon test was used to test the probability of significant differences among nonnormally distributed data. To test for differences in captive survival of mussels in the FMCC pond after 1 year, analysis of variance (ANOVA) was used for normally distributed data of survival rates and glycogen content of mussels at three locations in the FMCC pond after 1 year. Survival rates were analyzed by honestly significant difference, while glycogen content was analyzed by least significant difference (LSD) to compare differences among variables. To compare pearl formation in the two ponds, the rank-based, nonparametric Wilcoxon test

was used for the nonnormal distribution of survival rates. The ANOVA was used for normally distributed glycogen content of mussels in the two ponds and treatments compared with those of wild mussels. Finally, data were analyzed by LSD to compare differences among variables. A paired *t*-test was used to test for significance differences in pearl growth over time in the two ponds, and ANOVA was used for analysis of water quality, except for pH (Wilcoxon test).

Results

Captive Survival in Pond

In testing the suitability of containment units and locations, survival rates of pink heelsplitter mussels after 1 year differed significantly among treatments ($P = 0.001$, $N = 3$) in the FMCC pond. Survival was lowest in the deep bottom treatment (30.0%), but survival rates at the shallow end (83.3%) and in suspended net pockets (63.3%) did not differ significantly. Mean monthly survival rates (with replacement) at the deep end, shallow end, and in pocket nets were 92.2, 97.1, and 95.9%, respectively, throughout the year. There was no significant difference among the three containment locations for monthly survival rates, except during August 2004 ($P = 0.006$, $N = 3$), when survival was lowest in the deep-end treatment. There was no significant difference in mean survival rate between mussels in the shallow end and in suspended pocket nets (Table 1). Monthly mortality of mussels was positively correlated to water temperature ($R = 0.72$) at both ends of the pond (Figure 1). Dissolved oxygen level was lowest at the bottom of the deep end of the FMCC pond (1.9 mg/L) in late June, while it was 5.8–6.4 mg/L at 0.2 m above the bottom of the deep end and 5.0 mg/L at the shallow end of the FMCC pond, when water temperature was about 24.7°C. Mean glycogen content of mussels among the three containment locations, when compared with wild mussels, was significantly different ($P = 0.001$). Glycogen content of mussels held in the suspended nets and wild reference specimens was lower than those at the shallow end but higher than those at the deep end. There was no significant difference in mean glycogen content between wild specimens and mussels in the suspended nets (Table 1). Additionally, glycogen content differed by sex ($P = 0.001$) in the wild population: it was higher in males than in females (Figure 2).

TABLE 1.—Comparison of mean ± SE survival rates of pink heelsplitter mussels after 1 year (excluding replacements), mean monthly survival rates, and glycogen content of mussels (dry weight basis) at the deep, shallow, and suspended pocket net locations in the Freshwater Mollusk Conservation Center pond, 24 October 2003 to 25 October 2004. Means followed by the same letter are not significantly different at $\alpha = 0.05$.

	Survival rate (%)		
Treatment	1 year	Monthly	Glycogen (mg/g)[a]
Deep	30.0 ± 0.0 z	92.2 ± 2.1	215.5 ± 4.8 z
Shallow	83.3 ± 3.3 y	97.1 ± 1.0	458.6 ± 27.0 y
Suspended	63.3 ± 8.8 y	95.9 ± 1.1	323.1 ± 35.4 x
Wild[b]			330.7 ± 5.2 x

[a] For the deep, shallow and suspended treatments, $n = 3$. For the wild treatment, $n = 2$.
[b] From Kentucky Lake, Tennessee.

Pearl Formation in Ponds

Monthly survival rates of pink heelsplitter mussels implanted for pearls in the Duck Pond (95.5%) and the FMCC pond (97.6%) were not significantly different ($P = 0.051$). Similarly, survival rates of mussels from the two surgeries and the no-surgery control in the Duck Pond ($P = 0.851$) and FMCC pond ($P = 0.915$) did not differ (Table 2).

Water temperatures in the two ponds were recorded hourly throughout the year and reported as mean daily temperature. Overall, seasonal temperature fluctuations in the two ponds were similar; maximum temperatures were 29.0°C at FMCC pond and 25.0°C at Duck Pond in August and the minimum temperature was about 2.0°C in December (Figure 3). Water quality parameters of DO, temperature, pH, alkalinity, TAN, and suspended organic matter showed some differences between the two ponds. Mean values differed significantly in alkalinity, TAN, and organic matter ($P < 0.05$), but not significantly in DO, water temperature, and pH ($P > 0.05$; Table 3).

In the pearl culture experiment, NNI pearls were obtained after 44 d from captive pink heelsplitter mussels in the two ponds. Overall, pearls in the mantle tissue of mussels were growing incrementally during the 10-month period (Figure 4), although variation in pearl weights was high. Statistical analysis showed no significant difference in pearl weight between the two ponds ($P = 0.562$). Image pearls also began to form after 40 d, and the surface pearl layer grew over time with a purple nacre coating the nucleus surface (Figure 5).

Comparison of glycogen content in pink heelsplitter mussels that experienced the two surgical operations for pearl production and the no-surgery control in both ponds showed highly significant differences ($P < 0.0001$) between captive and wild specimens. The lowest glycogen content occurred in mussels at the FMCC pond (262.6 ± 43.4 mg/g dry weight), but there was no significant difference between the wild mussels (868.2 ± 38.9 mg/g dry weight) and mussels held in

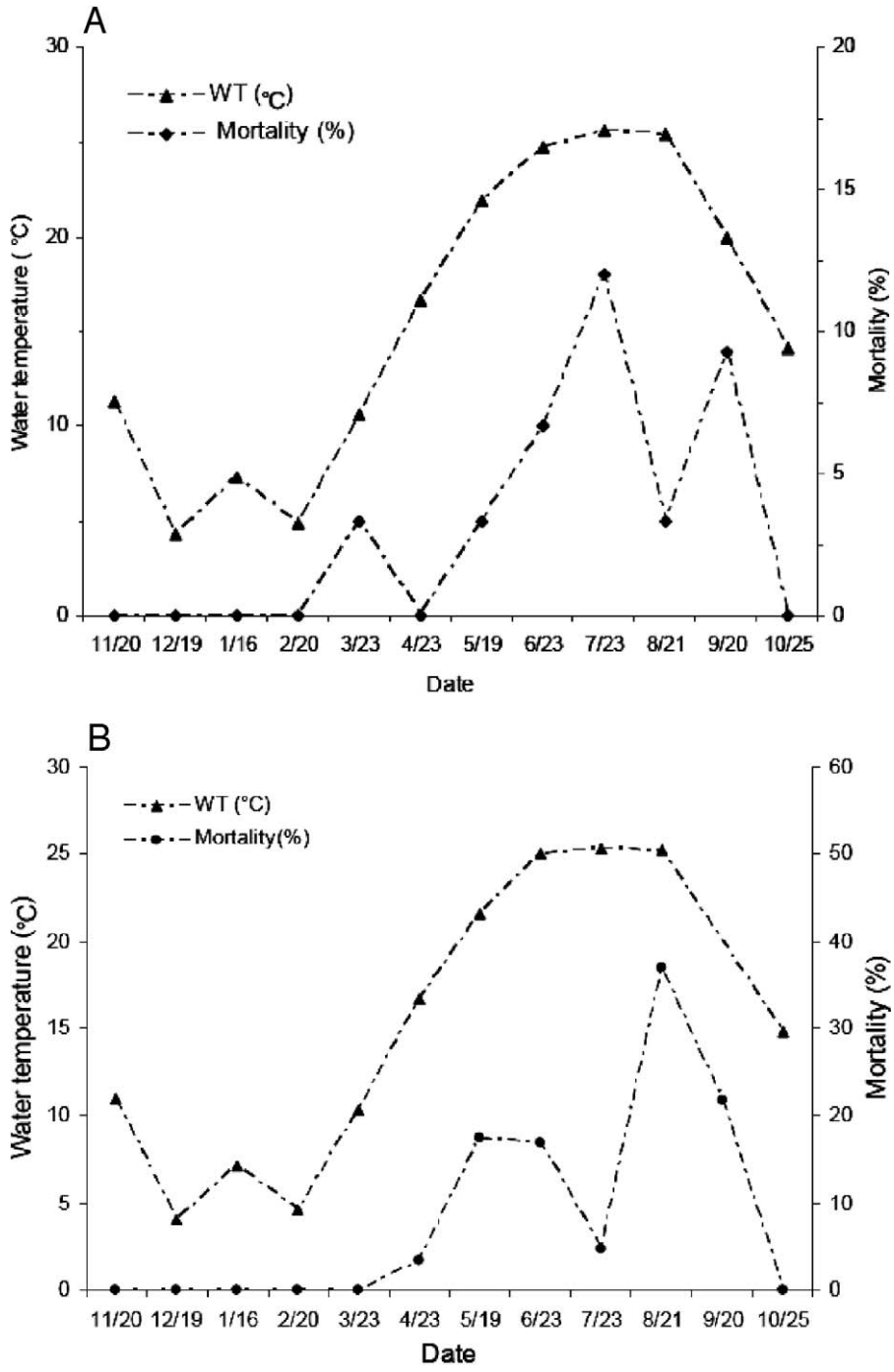

FIGURE 1.—Monthly mortality of pink heelsplitter mussels and water temperature (WT) at the shallow end (upper panel) and the deep end (lower panel) of the Freshwater Mollusk Conservation Center pond, Virginia, November 2003–October 2004. Water temperature data for 20 September 2004 were lost because of a malfunction of the temperature logger.

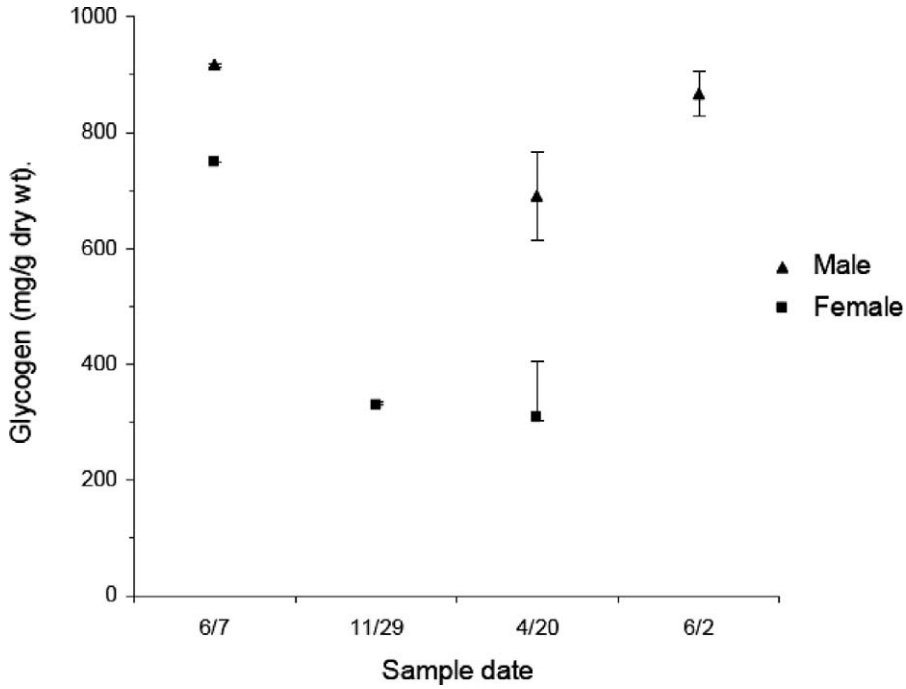

FIGURE 2.—Comparison of mean glycogen reserves (mean ± SE) in female and male pink heelsplitter mussels collected June 2004–June 2005 in the Kentucky Lake portion of the Tennessee River.

the Duck Pond (688.7 ± 60.6 mg/g dry weight; Table 2). Comparison of mussels subjected to surgery and the no-surgery control mussels showed no significant difference in both ponds (Duck Pond: $P = 0.418$; FMCC pond: $P = 0.791$).

Algae were collected monthly to assess species richness and to identify dominant algae in the ponds since they are the primary food source for mussels. There were seven dominant algal genera in the Duck Pond, including *Bracteacoccus*, *Chlorella*, *Cryptomonas*, *Euglena*, *Melosira*, *Navicula*, and *Scenedesmus*, while three genera, *Cylindrotheca*, *Navicula*, and *Dinobryon*, dominated the FMCC pond. Most of these algae were of suitable size for consumption by pink heelsplitter mussels.

TABLE 2.—Mean ± SE monthly survival rates and glycogen content (dry weight basis) of control pink heelsplitter mussels and mussels surgically implanted with image pearl (IP) implants, and nonnucleated implants (NNI) during 10-month period in the Duck Pond and Freshwater Mollusk Conservation Center (FMCC) pond. For the mussels at Duck Pond and the FMCC pond, $n = 9$. For the wild mussels, $n = 3$. Means followed by the same letter are not significantly different at $\alpha = 0.05$.

	Monthly survival rate (%)			
Sample site	NNI	IP	Control	Glycogen (mg/g)
Duck Pond	95.5 ± 1.7	93.1 ± 3.2	95.5 ± 2.0	688.7 ± 60.6 z
FMCC pond	97.6 ± 1.0	97.6 ± 1.3	97.6 ± 1.3	262.6 ±43.4 y
Wild[a]				868.2 ± 38.9 z

[a] From Kentucky Lake, Tennessee.

Discussion

Captive Survival in Pond

Pink heelsplitter mussels had low survival at the bottom of the deep end (30.0%) in the FMCC pond after 1 year, which is comparable to the survival (23%) reported for this species in a pond in West Virginia (Gatenby 2000). Higher survival rates at the shallow end (83.3%) and in pocket nets (63.3%) also concur with results of Gatenby (2000); namely, that six species of mussels held suspended in pocket nets had higher survival rates than those held in racks on the pond bottom.

In the FMCC pond, DO was only 1.9 mg/L at the sediment surface of the deep end in summer, while it was 5.0 mg/L at the shallow end. Low DO conditions in summer may have stressed benthic pink heelsplitter mussels and caused the mortalities recorded at this bottom location. Johnson et al. (2001) reported that four mussel species experienced high mortality when DO concentrations fell below 5 mg/L. Similarly, Chen et al. (2001) reported that mussels were unable to

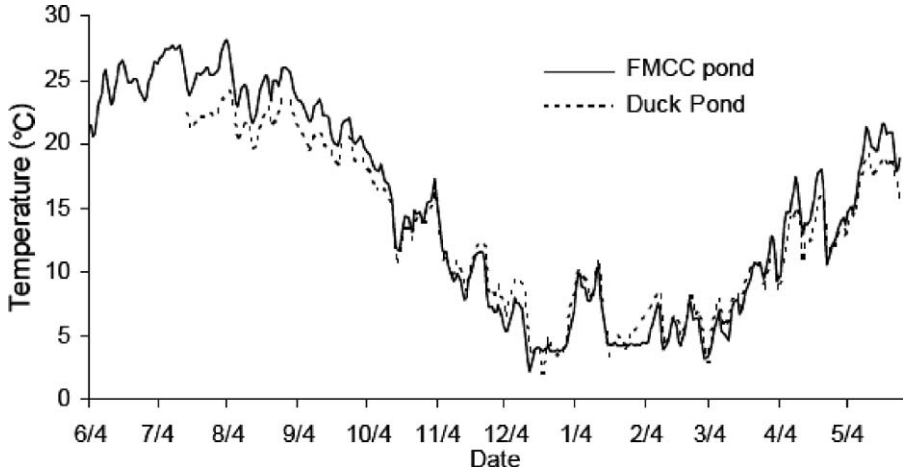

FIGURE 3.—Water temperature at the Freshwater Mollusk Conservation Center (FMCC) pond and Duck Pond during the experimental period June 2004 to May 2005.

maintain normal oxygen consumption under low DO levels and suggested that a critical minimum DO concentration should be above 2–3 mg/L. Otherwise, mussels may become stressed if exposed to low DO for hours or days (Davis 1975). By comparison, mussels placed at the shallow end and in suspended pocket nets had high survival, seemingly unaffected by DO level. Although some freshwater mussels can temporarily lower metabolic activity to accommodate reduced DO (Burky 1983; Sheldon and Walker 1989; McMahon 1991), pink heelsplitter mussels may not be capable of doing this, as judged by high mortality at the deep end.

The paddle wheel used for circulation and aeration facilitated water flow throughout the pond and increased DO and available phytoplankton. It moved water more effectively at the shallow end, which may have positively affected survival. However, the paddle wheel was incapable of providing water flow to the bottom of the deep end, as evidenced by low DO.

TABLE 3.—Mean ± SE values of water quality parameters in the Duck Pond and Freshwater Mollusk Conservation Center (FMCC) pond during the experimental period (July 2004–May 2005). The *P*-values were determined by ANOVA (except pH, which was determined with a Wilcoxon test) at $\alpha = 0.05$. Significant differences between sites are represented by asterisks.

Parameters	Duck Pond	FMCC pond	*P*-value
Dissolved oxygen (mg/L)	8.0 ± 0.9	9.3 ± 0.9	0.2855
Temperature (°C)	14.3 ± 1.9	15.4 ± 2.4	0.7417
pH	7.7 ± 0.1	7.9 ± 0.1	0.0886
Alkalinity (mg/L $CaCO_3$)	182.8 ± 8.1	139.7 ± 6.0	0.0005*
Total ammonia nitrogen (mg/L)	0.25 ± 0.04	0.04 ± 0.01	<0.0001*
Organic matter (mg/L)	4.2 ± 0.6	1.3 ± 0.2	0.0002*

Gatenby (2000) also reported that flow rate was negligible at the bottom of her ponds when a paddle wheel was in operation. Johnson et al. (2001) reported that mortality of unionid mussels increased when velocity at the substrate surface dropped below 0.01 m/s. Therefore, pink heelsplitter mussels at the deep end of the pond may have experienced higher mortality because of limited flow and resultant DO sag when compared with those at the shallow end and in suspended pocket nets.

In addition to low DO conditions at the bottom, the presence of H_2S was noted at the deep end of the pond, which had blackened sediment and an H_2S smell during summer. Hydrogen sulfide is highly toxic to cellular respiration and disrupts oxygen transport by aerobic organisms (Berzofsky et al. 1971). Kraus et al. (1996) reported that gutless awningclams *Solemya reidi* showed a reduction in rate of deoxygenation of the cytoplasmic hemoglobin because of sulfides. Consequently, lower survival of pink heelsplitter mussels at the deep end of the FMCC pond also may be related to H_2S production from decomposition of organic matter under low DO conditions.

Based on monthly samples, survival remained high during winter 2003, declined as water temperature increased in summer, and was again high in winter 2004. The direct and indirect effects of temperature are well documented for a suite of bivalves (Bayne 1976; Newell 1979; Newell and Branch 1980). Comparing the correlation of water temperature and survival of pink heelsplitter mussels at both bottom locations, mortality increase was associated with increasing temperature ($R = 0.72$). Gatenby (2000) reported that the high mortality of mussels in summer in her

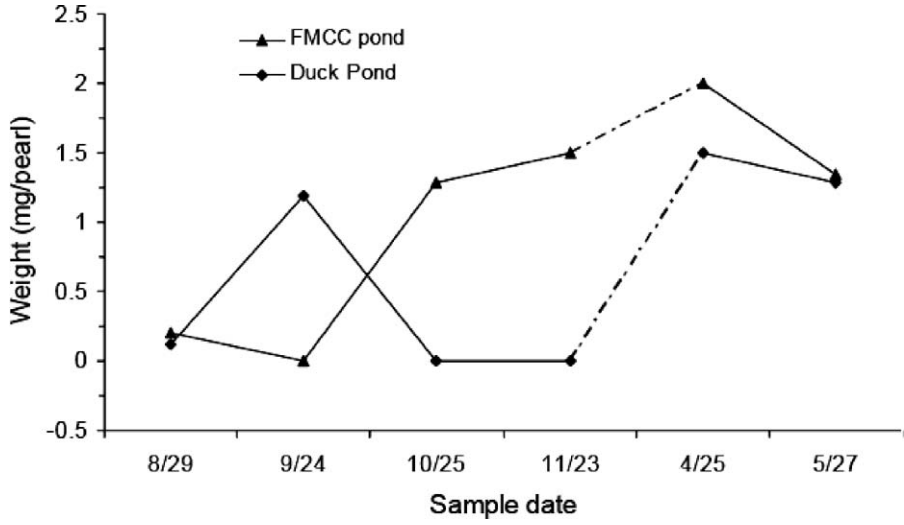

FIGURE 4.—Mean pearl weight (mg/pearl) in pink heelsplitter mussels surgically implanted with mantle tissue (nonnucleated) in the Duck Pond and Freshwater Mollusk Conservation Center (FMCC) pond over the period August 2004 to May 2005.

experiment could be attributed to the lethal effect of high temperature (which exceeded 28°C for 4–5 d) and enhanced by poor condition in mussels with a low glycogen level (mean glycogen = 10.2 ± 4.2 mg/g dry weight). Although not well documented for mussels, lethal temperature was reported at 29°C for cylindrical papershell mussels *Anodontoides ferussacianus* (Salbenblatt and Edgar 1964). In this study, water temperatures exceeded 29°C for eight consecutive days in July and four consecutive days in August, probably contributing to stressful conditions or mortality in summer.

To assess the variation in survival among containment locations, it is essential to consider the body condition of mussels. In previous research, glycogen levels declined when wild bivalves were brought into captivity (Lomte and Jadhav 1982; Gatenby 2000; Monroe and Newton 2001), indicating that mussels can be stressed after relocation. Patterson et al. (1999) reported that glycogen levels of adult threeridge mussels and pimpleback mussels *Quadrula pustulosa* were extremely low in starved mussels compared with fed mussels. Glycogen reserves were lower in pink heelsplitter mussels contained at the deep end of the FMCC pond when compared with wild pink heelsplitter mussels from Kentucky Lake that were collected and measured concurrently. Likewise, Gatenby (2000) also reported that all mussels held in dish racks on the bottom and in pocket nets appeared to terminate feeding and gradually starved to death. Pink heelsplitter mussels at the shallow end and in suspended nets of our pond may have had more food available because of the

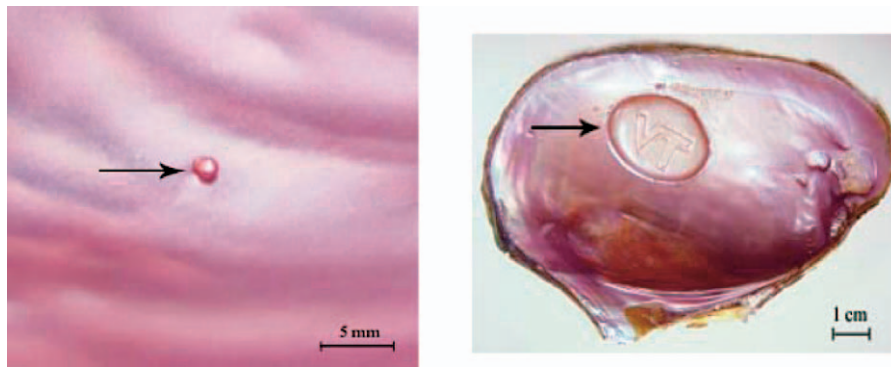

FIGURE 5.—A purple nonnucleated pearl (left) and purple image pearl with a VT (Virginia Tech) symbol (right) collected 27 May 2005 from pink heelsplitter mussels.

paddle wheel, resulting in relatively higher glycogen reserves compared with those at the deep end of the pond. The low glycogen of pink heelsplitter mussels at the deep end is indicative of poor condition and survival, perhaps due to reduced feeding. Because there was no significant difference in suitability of locations between shallow and suspended pocket nets, based on mean survival rate and glycogen content, suspended nets were used to hold pink heelsplitter mussels in the subsequent pearl culture experiment.

Haag et al. (1993) reported that eastern lampmussel females had lower glycogen content (0.08 mg/g tissue wet weight) than males (0.13 mg/g tissue wet weight). Likewise, in this study, glycogen content differed ($P = 0.001$) by sex in wild pink heelsplitter mussels collected from Kentucky Lake; glycogen content was higher in males. This may be explained by the reproductive traits of pink heelsplitter mussels, since this is a long-term brooder in the subfamily Lampsilinae (Ortmann 1919). Female mussels often brood glochidia in gills for 9–12 months (Clarke 1981), so they demand more energy (e.g., glycogen) to sustain brooding glochidia (Mackie 1984).

Pearl Formation in Ponds

Mean survival rate of the implanted pink heelsplitter mussels was comparable to that of the no-surgery control pink heelsplitter mussels in both ponds during the 10-month period, indicating that neither the NNI nor the IP operations influenced survival. Survival of mussels from the NNI was as high as for the no-surgery control mussels, perhaps because the implanted pieces of mantle tissue were soft, thin, and small (2×2 mm). Pieces of mantle tissue from the same species usually are accepted by grafted mussels because antigens from the mantle piece of the donor are likely to be compatible with antibodies of the grafted mussels. For example, mantle tissues of the Chinese triangle sail, Biwa pearly *H. schlegeli*, swan *Anodonta woodiana,* and *A. calipygos* mussels can create pearl sacs and produce pearls in grafted mussels when they are transplanted within the species (Zhang 1975; Xiong et al. 1980; Hua et al. 2001).

The mantle tissue of mussels is critical for pearl production (Hua 2001), shell growth (McMahon 1991), and physiological function and energy storage (Gabbott and Bayne 1973; Fraser 1989); therefore, it was used for glycogen determination. The surgically implanted pink heelsplitter mussels would presumably consume more energy to support pearl growth, as reported for gravid female mussels or for mussels fouled by zebra mussels (Haag et al. 1993; Hallac and Marsden 2001). However, there was no significant difference in glycogen levels in implanted pink heelsplitter mussels and no-surgery control pink heelsplitter mussels in both ponds. Mussels probably undergo nacre deposition within embedded mantle tissues as a normal physiological function. Furthermore, the glycogen levels of mussels from the Duck Pond showed no significant difference from those of wild mussels, indicating that environmental conditions in the Duck Pond generally met the needs of pink heelsplitter mussels. Water quality in both ponds was mostly suitable for mussel growth by having sufficient DO (8.0 mg/L), stable pH (<8), and suitable water temperatures. However, algal richness and abundance, organic matter content, and alkalinity differed in the ponds. Mussels extract $CaCO_3$ from water to meet their great demands for shell development and growth as well as for pearl growth. Consequently, the higher alkalinity in the Duck Pond (183 mg/L $CaCO_3$) may have facilitated better growth and condition of pink heelsplitter mussels than those mussels in the FMCC pond (140 mg/L $CaCO_3$).

Algae and detritus are the main dietary components for mussels (Way 1989; Gatenby 2000), and particle concentration and size affect filtration rate (Navarro and Winter 1982; Roper and Hickey 1995; Vanderploeg et al. 1995). Therefore, pink heelsplitter mussel nutrition probably was affected by differences in the abundance of food resources in the ponds. Based on relative abundance and diversity of species, algae in the Duck Pond provided more food than that in the FMCC pond. Furthermore, dominant genera varied between the two ponds throughout the experiment; there were seven genera in the Duck Pond and only three genera in the FMCC pond. Most of these algae were suitable as food, especially because of their small cell sizes. Miura and Yamashiro (1990) indicated that *A. calipygos* ingested food ranging from 0.5 μm to 100 μm. Similarly, Parker et al. (1998) concluded that unionids selected algae particles between 4 and 80 μm in length, and the dominant genera were *Chlorella*, *Cyclotella*, *Navicula*, *Melosira*, and *Scenedesmus* based on gut analyses. In addition, the relative abundance of algal genera in unionid guts was very similar to the water sample collected from the river. Consequently, algae of the seven dominant genera could be ingested by pink heelsplitter mussels in the Duck Pond, while only the cell sizes of *Navicula* and *Dinobryon* were most suitable for ingestion in the FMCC pond.

The significantly greater quantity of organic matter in the Duck Pond would tend to support better growth there, resulting in improved physiological condition and high glycogen content in pink heelsplitter mussels. Previous studies on the selection of particulate organic matter by freshwater mussels reported that organic matter is an important food resource, capable of being ingested and then digested or expelled (Parker et al.

1998; Dettman et al. 1999; Christian et al. 2004). Water in the FMCC pond was clear and low in suspended organic matter, resulting in oligotrophic conditions and lower body condition of mussels. Therefore, ponds with an adequate supply of suitable algae, organic matter, and water quality parameters would provide excellent conditions for the production of pearls in implanted specimens of the pink heelsplitter mussel.

Variations in the pearls formed by pink heelsplitter mussels were very high, as some pearls were still tiny and unmeasurable in November 2004 owing to the complicated processes of pearl formation. Growth rate of pearls is based on the rate of nacre secretion and deposition by the pearl sac, which varies by mussel species, health condition, and environmental quality. Hu and Shi (1995) found that, even within a pearl sac of grafted swan mussels, epithelial cells showed inconsistent development; a few irregular cells developed after 35–42 d, while others exhibited orderly columnar shapes. Irregular cells can negatively affect pearl quality. In our experiment, pearls sampled from the same mussels were highly variable in size and weight, which supports previously reported results. However, results from monthly pearl samples in both ponds showed an increase in size with culture time. The largest pearl reached 1×3 mm from a mussel in the FMCC pond after 35 weeks. Pearls were colored purple and pink, matching the nacre color of sacrificed pink heelsplitter mussels used for mantle tissue preparation.

The NNI pearls were first obtained from pink heelsplitter mussels with transplanted pieces of mantle tissue after 44 d, when water temperature was about 21°C. However, some pearls were not detected in their pearl sacs, and the pearl sacs were still soft in mussels in which some pearls had already been formed. Therefore, pearl formation in pink heelsplitter mussels was estimated to require approximately 40 d post-implantation. Pearl sac formation should occur in less than 40 d, since pearls were generated from the nacre deposited by the pearl sac. Formation of the pearl sac varies among mussel species and environmental conditions, such as temperature and season; it requires 14 d in swan mussels and 20 d in Chinese triangle sail mussels as water temperature approaches 20°C in spring (Hu and Shi 1995). The rate of pearl sac formation increases as water temperature rises. Likewise, it takes some time for pearly nacre to be deposited after pearl sac formation; for example, it requires about 10–12 d for Chinese triangle sail mussels at 18–20°C (Hua et al. 2001). By comparison, the pink heelsplitter mussel may form a pearl sac in 25 d at 21°C and 15–20 d for pearl deposition after the pearl sac is formed. Therefore, pink heelsplitter mussels have suitable traits for pearl sac formation and pearl deposition and are comparable with the pearl-producing Chinese triangle sail mussel, although rate of pearl formation is somewhat slower. In addition to species physiology, water temperature and food availability directly affect pearl growth. Pearl sac formation and nacre deposition become slow when water temperature declines, and mussels discontinue nacre deposition when water temperature is below 10°C (Hua et al. 2001). Therefore, the pearl growth season is only about 7 months, from April to November, in Virginia. By comparison, pearls will grow much faster in southern and southwestern states (Neves 1999). Another important strategy is to maximize food availability in the pond to meet the needs of implanted mussels. A fertilization regimen is highly recommended to increase algal growth and abundance. Management of environmental conditions is critical to both the well-being and production potential of implanted mussels, so that pearls of marketable quality (size, shape, luster, color, and surface complexion) can be obtained.

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