

Seasonal growth and mortality of juveniles of *Lampsilis fasciola* (Bivalvia: Unionidae) released to a fish hatchery raceway

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Abstract: Recent efforts to restore remnant or extirpated populations of freshwater mussels have focused on artificial propagation as an effective and practical conservation strategy. Although artificially cultured juveniles have been produced and released to the wild at various times of the year, no study has investigated the best time of year to release these juveniles. Newly metamorphosed juveniles of the wavyrayed lampmussel (*Lampsilis fasciola*) were released into a stream-fed fish hatchery raceway during March, June, and September. Growth and survival rates were measured 32, 52, 72, and 92 days post-metamorphosis. Juveniles released in June experienced the greatest growth and survival rates. Juveniles released in September and March experienced high mortality within the first month of release and exhibited poor growth in the cool water conditions typical of those seasons. Overwinter survival exhibited a size-dependent relationship.

Key words: *Lampsilis fasciola*, life stage, propagation, overwinter mortality, temperature

Over 70% of North American's freshwater mussel species are considered extinct, endangered, threatened, or of special concern (Williams *et al.* 1993). Overharvesting, pollution, habitat destruction, and the introduction of exotic species have destroyed or altered much of the habitat that once supported a high diversity and abundance of freshwater mussels. As a result of increased environmental protection over the last thirty years, some stream reaches are beginning to recover from the perturbations that occurred over the past century. Some of these recovering stream reaches are currently or anticipated to become suitable habitat for mussel re-colonization. However, the fecundity and density of many mussel populations has been reduced to the point that natural re-colonization is unlikely. Many researchers and resource managers have focused on the use of laboratory-cultured mussels as one method to augment or reestablish these populations. However, the viability of such a technique for successful re-colonization remains severely understudied. One variable that may strongly influence the success or failure of restoration efforts may be the time of year juvenile mussels are released to the natal streams.

In this study, we tested the release of newly metamorphosed juvenile mussels into a fish raceway, simulating natural stream conditions, at three times of the year to identify the optimal time for mussel recruitment.

METHODS

We conducted tests in a fish raceway at the Buller Fish Culture Station near Marion, Virginia, U.S.A. Hatchery water originated from the South Fork Holston River (SFHR) at river kilometer 169 and was piped from an instream impoundment into a 2 ha pond prior to diversion to the raceway. The water from the pond was warmer and richer in algae than that in the SFHR, a coolwater trout stream. The raceway was 18 m long and was partitioned longitudinally with plywood (60 cm wide by 18 mm thick), forming 4 separate 0.6 m wide sub-raceways. Each sub-raceway was divided horizontally into 1.2 m long units using 3 mm mesh galvanized screen to provide designated areas for various experiments. A maximum flow of 720 L/min (velocity = 0.13 cm/sec) and a water depth of 29 cm was maintained throughout the study.

We selected the wavyrayed lampmussel (*Lampsilis fasciola* Rafinesque, 1820) for use in this study because populations of this species occur naturally in the Holston River drainage and gravid females of this species can be collected throughout most of the year. In addition, previous studies have confirmed suitable fish hosts for *L. fasciola* (Zale and Neves 1982) and have successfully reared significant numbers of juveniles under laboratory conditions (O'Beirn *et al.* 1998).

We collected gravid mussels by snorkeling at three locations in the Clinch River basin (Indian Creek, Tazewell County, Virginia, Clinch River at Nash Ford, Russell County, Virginia, and Clinch River at Hancock County,

* The Unit is jointly supported by the Biological Resources Division-USGS, Virginia Department of Game and Inland Fisheries, The Wildlife Management Institute, and Virginia Polytechnic Institute and State University.

Tennessee). Mussels were transported in river water to the laboratory in an aerated cooler and held in a Living Stream (Frigid Units, Inc., 3214 Sylvania Ave., Toledo, Ohio) maintained at 15°C.

We infested hatchery-reared largemouth bass (*Microp-terus salmoides* Lacepède, 1802) (13 cm in length) with glochidia extracted from collected mussels using the procedure described by Zale and Neves (1982). Once infested with glochidia, we returned fish to a tank (100 L) supplied by recirculating water. After 2 weeks, the holding tank was checked daily for metamorphosed juvenile mussels by siphoning the bottom of the tank and capturing juveniles in a 130 µm nylon mesh sieve.

We released propagated juveniles to the hatchery raceway on 26 June and 16 September 1998, and 8 March and 16 September 1999. With the exception of the September 1998 trial, we extracted glochidia from a minimum of 4 gravid females to produce a pooled stock of juveniles for each trial. Because only 1 gravid female was available for the September 1998 release, we repeated this trial in September 1999. For each trial, 500 newly metamorphosed juveniles (<2 days old) were used, and a sub-sample of 10 randomly selected individuals was measured for initial mean shell length using a calibrated ocular micrometer and a stereozoom dissecting microscope. After capture, we immediately transported juvenile mussels in a 1 L container of well water to the hatchery. At the hatchery, we placed 50 juveniles in each of ten 200 mL plastic containers (8 cm² and 3.5 cm deep), each containing 5 mm of limestone sand (1 > 2.5 mm). We partially submerged dishes in hatchery water to allow juveniles to acclimate to the water temperatures. After a 2 h acclimation period, all dishes were submerged within a designated area of the raceway. Each dish was covered with a 120 µm mesh nylon screen over each dish to prevent the loss of juveniles during submersion. Once each dish was placed at the bottom of the raceway and the contents of each dish had settled, the screen was removed. An Onset Optic Stowaway data logger (Onset Computer Corporation, 536 MacArthur Blvd., P.O. Box 3450, Pocasset, Massachusetts) was placed in the raceway to record temperature hourly.

We sampled dishes for juvenile mussels at 32, 52, 72, and 92 days to obtain trends in growth and survival over time. Juveniles introduced to the raceway in June were sampled periodically after 92 days to collect subsequent information on growth and survival. Because sampling may negatively affect juvenile growth (O'Beirn *et al.* 1998), we reduced our sampling efforts by systematically selecting 5 out of the 10 dishes during each sampling period to evaluate growth and survival. Subsets (5 replicates) were alternately sampled for subsequent sampling periods. During sampling events, each selected dish was removed from the raceway and the contents were decanted through two different mesh

sieves (1000 µm, then 120 µm). Samples were rinsed with hatchery water for 1 min to separate juveniles from the substratum. Sediment that naturally accumulated in each dish was discarded during the rinsing process, and the limestone sand (retained in the 1000 µm sieve) was returned to the dish. Juveniles were rinsed from the lower sieve (120 µm) into a petri dish.

We counted live juveniles, with the aid of a stereozoom microscope, to determine survival in each dish. Mean shell length was calculated in each dish with measurements obtained from 10 randomly selected juveniles. Once growth and survival data were obtained for each dish, we placed juvenile mussels into their original dish and returned each dish to the raceway. Because juveniles occasionally escaped from dishes, we vacuumed the bottom of the raceway unit during each sampling period with a shop vacuum to collect emigrant juveniles. The vacuumed matter was poured through a graduated series of sieves to collect juveniles. We assumed that the rate at which juveniles escaped was equal among replicates. Therefore, we reallocated migrant juveniles equally among the 10 dishes and survival was adjusted accordingly. Although we recognized this assumption to be crude, we believe the inclusion of migrant juveniles produced a more accurate analysis of mean survival.

Due to limitations in comparable data and statistical complications caused by escaped juveniles, only data for the 32-day growth and survival were used for statistical comparison among released cohorts. We used a one-way ANOVA followed by Fisher's pairwise comparisons to compare 32-day growth and survival among juvenile mussels released at different times of the year. All statistical tests were performed with the SAS statistical package (SAS Institute, Inc., Cary, North Carolina). Subsequent data were used to illustrate trends in growth and survival over time. We also compared sample variances and used a Chi-square test to compare the growth of June-release mussels measured at 122 days (Fall) and 334 days (Spring) to evaluate size-selective mortality over the winter months (when water temperatures remain below 15°C).

RESULTS

Survival was significantly different at 32 days among juveniles released at different months ($p < 0.0001$) (Fig. 1). Juveniles released in June exhibited the highest survival rate and 13% of these juveniles survived through the subsequent winter months. Presumably as a result of error in systematic sampling, mean survival in June appeared higher at 92 days (48%) than at 72 days. However, June-release survival data among the 52, 72, and 92-day sampling periods were not statistically significant (ANOVA; $p = 0.122$). Juveniles released in September and March exhibited significant mor-

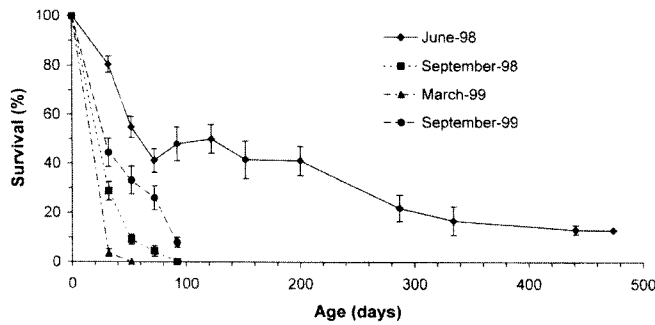


Figure 1. Survival (mean \pm SE) of juveniles of the wavyrayed lampmussel, *Lampsilis fasciola*, released in a fish hatchery raceway during June and September 1998 and March and September 1999.

tality within the first 32 days post-metamorphosis, with only a few survivors reported at 92 days from the September 1999 release.

Juvenile mussels released at different months showed significant differences in mean shell length at 32 days ($p < 0.0001$) (Fig. 2). Fisher's pairwise comparisons revealed that shell length at 32 days was greater for mussels released in

June and least for those released in March. Trends in growth of juveniles released in September 1998 and September 1999 were almost identical. Growth of juveniles released in June was minimal between mid-October to May when water temperatures remained below 15°C, but then increased rapidly from May onward until termination of the study in October 1999. Based on growth values of individual juvenile mussels obtained at 122 and 334 days, pre-winter and post-winter mean shell lengths (mean \pm SE) were 1.73 ± 0.06 mm ($n = 50$, range 1.08-2.92 mm, variance 0.21) and 1.92 ± 0.06 mm ($n = 31$, range 1.33-2.56 mm, variance 0.10), respectively. Based on 10 categories of shell length, the frequency distribution of the sizes of sampled juveniles was significantly different between 122 days and 334 days ($df = 9$, $\chi^2 = 20.41$, $p \leq 0.025$).

Of the juveniles released in June, 9.6%, 15.1%, 8.1% and 1.0% were collected outside of replicate containers at 72, 92, 122, and 152 days, respectively. Little escapement was evident in other trials, with $< 1\%$ observed during any given sampling period. During the 92-day sampling period, mean shell length of June-released juveniles collected outside of containers (1.81 ± 0.28 mm) was significantly greater ($p <$

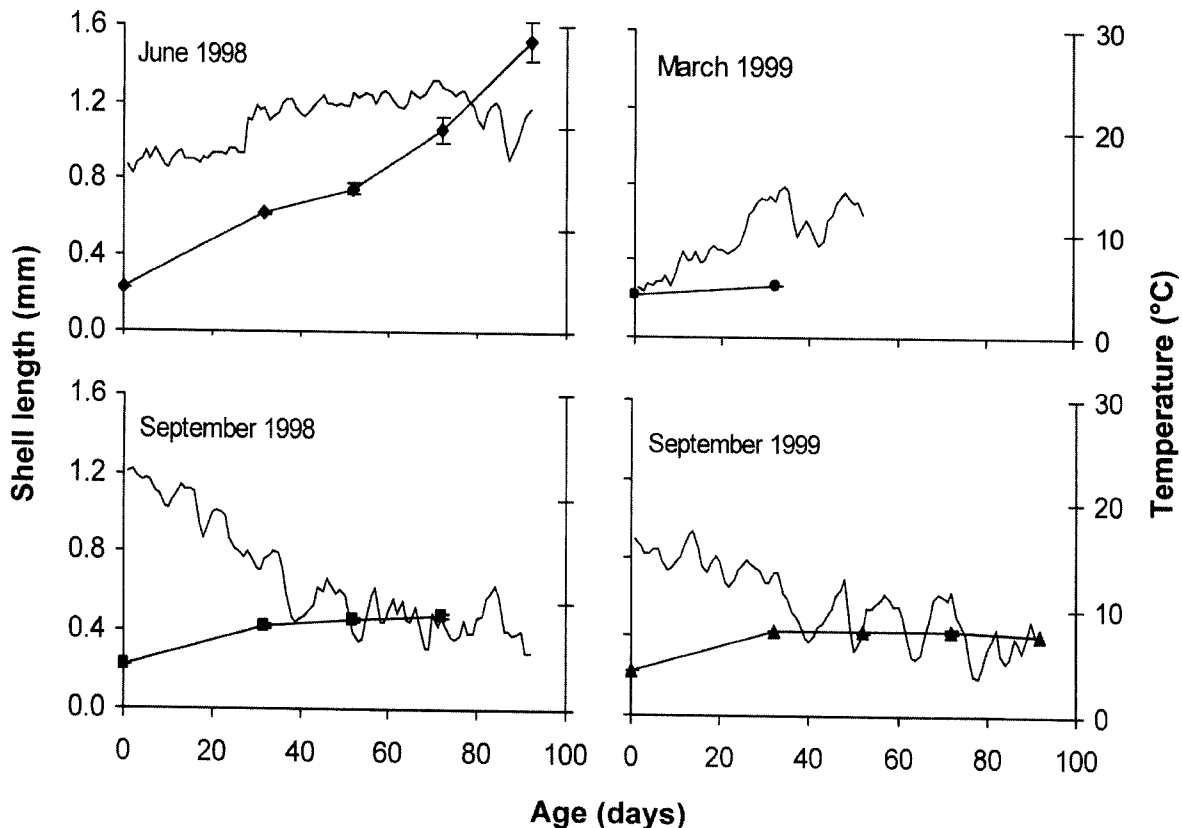


Figure 2. Daily mean water temperatures ($^{\circ}$ C) and mean shell lengths (mm \pm SE) of newly metamorphosed juveniles of the wavyrayed lampmussel, *Lampsilis fasciola*, released in the fish hatchery raceway during June and September 1998 and March and September 1999.

0.0001) than the mean shell length of juveniles that remained in the containers (1.45 ± 0.30 mm).

DISCUSSION

Like most long-term brooders, individuals of *Lampsilis fasciola* spawn in the late summer and brood glochidia over the winter. Gravid females are typically found actively displaying their mantle lure in spring and early summer, and are not commonly found gravid from mid-summer to early fall. Although a small percent of glochidia may be released in mid-summer, most glochidia of *L. fasciola* are released from mid-spring to early summer (Zale and Neves 1982). Glochidia that are released and attach to host fish early in the year (winter and early spring) will probably remain encysted within the gill tissue of the host fish until water temperatures increase in mid-spring (Watters and O'Dee 1999). This brooding cycle ensures that the majority of newly metamorphosed juveniles will excyst from host fish at an opportune time to maximize growth prior to winter.

Results of our study concur with this natural spawning and brooding cycle, and suggest that the success of age-0 recruitment is, in part, dependent on the time of year that juvenile mussels are released. Growth and survival declined precipitously when juveniles were released at cooler water temperatures. An estimated 13% of juveniles released during June were able to survive their first winter, whereas juveniles released in September did not survive the winter, and those released in March did not survive beyond 52 days. Beaty and Neves (2004) reported similar findings with individuals of *Villosa iris* (I. Lea, 1829) cultured in natural river water; juveniles exhibited greater growth and survival when released to the culture system in June compared to subsequent trials initiated later in the summer.

These differences in survival may be influenced by seasonal opportunities for growth and size-selective overwinter mortality. In situations where the possibility of actual growth can be ruled out, an increase in mean size and decrease in variance over winter typically indicate mortality of smaller individuals (Munch *et al.* 2003). Mean shell length of juveniles released in June attained 1.73 mm in mid-October (122 days) and increased over the winter months, reaching a mean of 1.92 mm in late April (334 days). Given the significant decrease in variance in shell length over winter, and assuming that juveniles of *Lampsilis fasciola* do not grow appreciably during winter (water temperature remains below 15°C) (Beaty and Neves 2004), this apparent overwinter increase in mean shell length indicates size-selective overwinter mortality.

Buddenseik (1995) reported that the overwinter survival of age-0 juveniles of *Margaritifera margaritifera* (Linnaeus,

1758) was size-dependent. One hundred percent of juveniles of *M. margaritifera* less than 700 μm in shell length died during winter months, and only juveniles greater than 900 μm had a 50% chance of surviving through the winter. Similarly, our juveniles released in September and March exhibited minimal growth and were unable to survive more than 2 months. Similar size-dependent relationships have been reported in studies of marine bivalves (Beal *et al.* 1995) and many fish species (Toneys and Coble 1979, Gutreuter and Anderson 1985, Post and Evans 1989, McGovern and Olney 1996, Hurst and Conover 1998, Munch *et al.* 2003). In fish, larger members of a cohort possess greater energy reserves and usually exhibit a significant survival advantage over smaller members of the same cohort (Paloheimo and Dickie 1966). Furthermore, weight-specific metabolic costs are usually reduced in larger individuals (Werner and Gilliam 1984). Mortality during the first winter is thought to be a pivotal determination of cohort abundance in many taxa (Munch *et al.* 2003) and is likely to be for young unionids as well.

Based on results of these rearing trials, juvenile mussels of the species *Lampsilis fasciola* will likely have the best opportunity for growth and survival if released to natal streams in spring to early summer when average daily water temperatures exceed 15°C. This window of opportunity may be the same for species with a similar brooding cycle as *L. fasciola*.

Although juveniles that escaped containers were not incorporated into our statistical analysis, we believe the following observation is worth noting. Juveniles collected outside of containers were on average 25% larger than juveniles that remained in the containers at 92 days post-metamorphosis. However, it is not known whether juveniles that escaped from containers were larger, and therefore had a greater capability to escape, or whether their greater size resulted from better food and growth conditions outside of the containers. Greater mobility in the raceway may enhance a juvenile's ability to pedal feed and seek food concentrations not available within the containers. Higher juvenile densities within the containers may also be a contributing factor in retarding growth rates. However, Beaty and Neves (2004) found no significant differences in growth and survival among different stocking densities of juvenile mussels of *Villosa iris* held in their flow-through culture system. Additional investigation of these potential influences will be necessary to further our understanding of the niche requirements for early life stages.

ACKNOWLEDGMENTS

The authors wish to thank Mike Pinder and Monte McGregor for their cooperation with the use of the Buller

Hatchery and Joe Ferraro for renovating and maintaining the hatchery facility. We owe a great deal of gratitude to Lora Zimmerman, Rachel Mair, Rebecca Winterringer, and Mark Kessel for their assistance in data collection. We also thank Susan Rogers, Bill Henley, Jess Jones, and Braven Beaty for sharing their invaluable knowledge of freshwater mussel biology and culture. The Virginia Department of Game and Inland Fisheries, and the United States Fish and Wildlife Service provided funding for this research.

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Accepted: 18 January 2005

