Population Biology of the Tan Riffleshell (*Epioblasma florentina walkeri*) and the Effects of Substratum and Light on Juvenile Mussel Propagation

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Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Fisheries and Wildlife Sciences

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December, 1999

Keywords: freshwater mussels, tan riffleshell, juvenile propagation, host fish, light regime, sediment preferences

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(ABSTRACT)

The federally endangered tan riffleshell (*Epioblasma florentina walkeri*) is restricted to only one known reproducing population, in Indian Creek, Tazewell County, Virginia. Attempts to recover this species by augmenting relic populations throughout its historic range are aided through knowledge of its population biology and requirements in culture environments. Infestations of host fish (fantail darters, *Etheostoma flabellare*), obtained from four river drainages, with tan riffleshell glochidia showed that significantly more juveniles transformed per fish from infestations on fantail darters from Indian Creek (mean = 59.22 ± 10.01) than on fantail darters from the Roanoke River (mean = 9.45 ± 10.64) ($p = 0.024$). Number of juveniles from fantail darters collected from Elk Garden and the South Fork Holston River were not significantly different from those of either Indian Creek fish or Roanoke River fish. These results support the hypothesis that mussel – host fish relationships are likely mediated by fish immune responses. Furthermore, this study suggests that this compatibility has resulted from coadaptation between the tan riffleshell and fantail darter populations in Indian Creek.

The tan riffleshell population in Indian Creek was estimated to be 1078 adults (95% CI= 760 - 1853), using Schumacher's modification of Schnabel's maximum likelihood estimator. The sex ratio and size distribution of males and females were approximately equal. Specimen ages, determined from thin-sections of shells, showed that mussels aged by external annuli on shells likely underestimates the true ages of individuals.

Appropriate culture conditions for this species were examined using juveniles of the wavyrayed lampmussel (*Lampsilis fasciola*) as a surrogate. In the first experiment, juvenile growth and survival was compared between four substratum types (fine

sediment, < 120μm; fine sand, 500 μm – 800 μm; coarse sand, 1000 μm – 1400 μm; and mixed sediment, < 1400 μm) and two light treatments in open versus covered recirculating troughs (2.8 m). Juveniles in fine sediment substratum and covered troughs fared poorest, with 7% survival and growth to only 0.86 mm in length after 16 wk. Juveniles in mixed sediment and open troughs fared best, with 26% survival and growth to 1.09 mm after 16 wk. Additionally, juveniles in fine sand in covered troughs had significantly higher survival (23.1%) than juveniles in fine sediment ($p = 0.04$), and juveniles in fine sand survived consistently better between light treatments than in the other substrata. There were no significant differences among the other treatments.

A second experiment was performed to determine whether juveniles were responding directly to the presence of light or whether only the increased autochthonous production improved growth and survival. One-half of each of three 2.8 m troughs were covered with 50% shade cloth, while the other sides were left open to ambient light. Additionally, the best and worst sediments from the first experiment (fine sand and fine sediment) were used again to verify the results from the previous experiment. In this case, juveniles in both sides of the troughs grew equally well, but juveniles in the open sides had significantly poorer survival (open mean: 1.78%, sd = 5.01; covered mean: 7.4%, sd = 5.01) ($p = 0.046$). Fine sediment yielded significantly higher growth of juveniles than fine sand ($p = 0.009$), with shell lengths of 2.63 mm (sd = 0.075) in fine sediment and 1.94 mm (sd = 0.102) in fine sand. The differences in survival and growth between the two experiments were attributed to differential numbers of chironomids and platyhelminths, which are predators of young juveniles. Additionally, the fine sediment was more tightly packed in the first experiment than in the second, which may have restricted movement and subsequently reduced survival. Light alone likely did not affect juvenile survival and growth; rather, it was seemingly the greater abundance of aufwuchs available as food. This hypothesis was corroborated by a juvenile behavior experiment, which showed that juveniles did not act differently when in tanks not exposed to light versus those open to ambient light.

Acknowledgements

The U. S. Fish and Wildlife Service and the Tennessee Wildlife Resources Agency provided funding for this project. I would like to thank my committee members, Dr. Dennis Lemly and Dr. George Libey, for their technical help, guidance, and support. Dr. Eric Hallerman supplied invaluable assistance in the immunogenetics section of this thesis. Most importantly, I am grateful to Dr. Richard Neves, my advisor, for providing me the opportunity and support to pursue research on such fascinating animals.

This project would not have been possible without the help of several people. The guidance of Francis O'Beirn, Brian Watson, and Michelle Steg was invaluable as I began learning about the techniques integral to mussel biology. Shane Hanlon and Lora Zimmerman accompanied me on numerous field excursions and have been great friends as well as coworkers. Additionally, the technicians on this project, Jen Rohrer, Rachel Mair, and Becca Winterringer, worked long hours on the dirtiest of tasks, always with a smile. Their dedication increased the quality of this project several times over.

I owe a great deal of thanks to the graduate students in this Department. They have become good friends as well as mentors. Their friendliness and intelligence are unmatched by any other group of people I have worked with. In particular, Kevin Goodwin, Brett Albanese, Ryan Smith, Gyasi Quince, Craig Roghair, Tim Copeland, Amanda Rosenberger, and Kirk Krueger have provided companionship that has made my time here very enjoyable. Many thanks to George Bumann and Scott Haulton, my officemates, for supplying comic relief and enlightening conversation. Also, Mark Rogers offered a significant amount of patience and moral support throughout my work on this project.

Lastly, but certainly not least, I thank my parents and family for supporting me in all my endeavors, academic and otherwise. It is their love and encouragement that has given me the courage to pursue my goals.

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Introduction

Freshwater mussels are among the most imperiled taxa in North America, as well as one of the most diverse (Neves 1991). In North America, there are 297 recognized freshwater mussel taxa (Turgeon *et al.* 1998), and 55% of these species are considered "extinct or imperiled" by The Nature Conservancy. This relatively recent and severe decline can be attributed primarily to habitat loss (Williams *et al.* 1993). Because mussels rely on host fish in the early stages of their life cycle, suitable habitat must exist for them, as well as for their specific host fish. Narrow host specificity of some mussel species results in even greater susceptibility to environmental changes.

The mussel life cycle is atypical for mollusks. Males release sperm into the water column to be picked up by females while filtering. The eggs are fertilized in the female's marsupium, where they develop into parasitic larvae (glochidia). Species may be long-term brooders (bradytictic), in which the eggs are fertilized in late summer, brooded over winter, and released in the spring; or they may be short-term brooders (tachytictic), in which the progeny are released in the summer immediately following fertilization in late spring (Neves 1991). Glochidia are released into the water column and must come into contact with a suitable fish host in order to attach and metamorphose. Depending on the species of mussel, the glochidia attach to the fins or gills of the fish and become encysted. Many factors affect the duration of metamorphosis, which may take as little as a week or as long as a month (Neves 1991), after which the newly metamorphosed juveniles drop from the fish and begin the free-living stage of the life cycle. Freshwater mussels generally reach reproductive maturity after three to five years (Neves 1991).

Transformation to the juvenile stage of the life cycle is often considered a "weak link" in the mussel life cycle (Lefevre and Curtis 1912), due to the low probability of glochidia coming into contact with and encysting on fish hosts. However, other periods of vulnerability likely exist. Juvenile mussels are fairly sensitive to poor water quality; thus, recruitment is typically considered a sign of a healthy population and environment. This is underscored by the bottleneck in survival that is observed before juveniles reach four weeks of age in the laboratory (Steg 1998). Additionally, because it takes several years for the animals to reach sexual maturity, there is a significant amount of time

during which the individuals must survive and grow before they can contribute their offspring to the population. The above factors necessitate a comprehensive effort towards recovery that includes habitat improvement as well as attention to the more sensitive stages of the life cycle.

Many attempts have been made to counteract the decline of mussels. The Endangered Species Act of 1973 led official conservation efforts for mussel restoration. The United States Fish and Wildlife Service (USFWS) has drafted recovery plans for all listed species, which identify improved water quality, habitat enhancement, and relocation of adults into historically inhabited reaches as goals for recovery. Relocation efforts have had varied success (e.g., Ahlstedt 1979, Sheehan *et al.* 1989), often with rather high mortality of transplanted mussels (Cope and Waller 1995). Lately, mussel conservationists have looked to artificial propagation for population augmentation and establishment. Artificial propagation can increase the number of juveniles that encyst on fish hosts through manually induced infestation. In this way, large numbers of juveniles can be collected and then used for species recovery through augmenting or reintroducing populations.

The tan riffleshell (*Epioblasma florentina walkeri* Wilson and Clark) was listed as an endangered species in 1977. It is a highly restricted species with relic populations reported in the Duck River, Red River, Middle Fork Holston River, and Hiwassee River (USFWS 1984, Parmalee and Hughes 1994). The only known reproducing population is in Indian Creek, a tributary of the Clinch River in Tazewell County, Virginia. Of the recovery objectives listed in its recovery plan (USFWS 1984), the establishment of new populations is prominent. This primarily involves the propagation of juveniles and their subsequent reintroduction into previously inhabited reaches. It is necessary to establish other populations of the species in order to help ensure the species' persistence; one catastrophe can eliminate the population and, possibly, the species.

Statement of Objectives

The primary goal of this project was to conduct research useful to recovery of the endangered tan riffleshell. Information on species requirements, both for reproduction and survival, is essential for successful recovery. Additionally, knowledge of appropriate

culture techniques is necessary to successfully propagate the species to augment and restore populations.

The objectives of this project were as follows:

1. To describe the population biology of tan riffleshells in Indian Creek, including compatibility with host fish, age class distribution, length distribution, sex ratio, and population size.
2. To test the feasibility of propagating juvenile tan riffleshells for release in the Hiwassee River in Polk County, Tennessee, and to recommend appropriate culture systems and infestation techniques.
3. To determine whether sediment composition and light regime play significant roles in juvenile mussel survival or growth in culture systems.

Chapter 1

Tan Riffleshell (*Epioblasma florentina walkeri*) Population Biology

Introduction

The genus *Epioblasma* has been considered to be the most highly developed and recently evolved genus of freshwater mussels (USFWS 1984). All of its members have highly specific habitat requirements that cause them to be notably susceptible to habitat alterations (Sickel 1980). Of the 25 taxa within the genus *Epioblasma,* 16 are presumed to be extinct, and all but *E. triquetra* of the remaining 9 are federally listed (Turgeon *et al.* 1998). The tan riffleshell may be the last extant subspecies in the *Epioblasma florentina* complex, as *E. f. curtisi* has not been found alive in many years (S. Bruenderman, Missouri DOC, personal communication).

The tan riffleshell was once found throughout Tennessee and southwestern Virginia in the Tennessee River system (USFWS 1984). Currently, the only known reproducing population is in Indian Creek, a tributary of the Clinch River, in Tazewell County, Virginia (Figure 1-1). Live specimens have been found in the Clinch River, Middle Fork Holston River, and the Hiwassee River, but these are not believed to be members of viable populations. The Indian Creek population is an extremely vulnerable one; the stream drains the town of Cedar Bluff, VA, and inadequate buffer strips occur between the stream banks and the lawns of nearby houses.

Adult tan riffleshells are sexually dimorphic, with females displaying a pronounced marsupial swelling posteriorly. The species is bradytictic; female eggs are fertilized in autumn and glochidia are released in early spring through early summer. Fantail darters (*Etheostoma flabellare*) are the tan riffleshell's primary fish host. Other hosts include sculpin species (*Cottus bairdi, C. carolinae*), greenside darters (*Etheostoma blennioides*), redline darters (*Etheostoma rufilineatum*), and snubnose darters (*Etheostoma simoterum*) (Watson 1999).

Artificially propagating juveniles and releasing them at sites where they were found historically can prove to be an important facet of this species' recovery. To this end, information on the tan riffleshell's host requirements can be used to increase the number of juveniles that transform. Knowledge of the population size, age structure, and

host fish suitability are integral to monitoring the existing population and recovering the species.

Fantail Darter Population Suitability

The mechanism that governs host specificity remains poorly understood. However, the rudimentary aspects are fairly well known. When a glochidium attaches to a gill lamella of a fish, the tissues surrounding the parasite swell and it becomes encysted (d'Eliscu 1972, Waller and Mitchell 1989). In host species, the glochidium remains encysted and assimilates components of the host's blood while transforming into a juvenile mussel (O'Connell and Neves 1999). In non-hosts, the glochidium is subsequently sloughed or destroyed via antibodies and/or leukocytes (Arey 1932, O'Connell and Neves 1999). Fish immune systems are sufficiently developed to have a high degree of differentiation among species (Cushing 1970, O'Connell 1991). Whether a species of fish may serve as a host depends on the level and type of the immune response (Reuling 1919, Arey 1932) and the level of glochidial resistance (Neves *et al.* 1985).

The range of fish species that may serve as glochidial hosts varies among mussel species. Some mussel species are highly specific, with few hosts available (Zale and Neves 1982), while others are more eurytopic in their use of fish hosts (Trdan and Hoeh 1982, Gordon and Layzer 1989). It has been shown that closely related species of *Venustaconcha* (*V. ellipsiformis* and *V. pleasii*) from different river drainages exhibit differential host specificity, and this specificity might be extended to populations of a species from different drainages (Riusech and Barnhart 1998). This specificity is presumed to be immunological in origin (Reuling 1919, Arey 1932, Kirk and Layzer 1997), as the components of fish blood necessary for glochidial transformation are present in all species of fish (Isom and Hudson 1984). However, the exact immunological mechanism that defines specificity is unknown.

Acquired immunity to glochidial infestation has been demonstrated in fish previously exposed to glochidia (Reuling 1919, Arey 1932, Watters and O'Dee 1996), and this has been a primary factor in selecting host fish for mussel propagation. Because it is impossible to know which fish have been infested previously, collectors have often

collected fish in streams lacking the target mussel. This is the only way of ensuring that collected fish have not been infested previously with glochidia of that species (Neves *et al.* 1985). However, some mussel species may be so highly host-specific as to be restricted to populations of fish within their own drainage. Neves *et al.* (1985) postulated that cohabitation of mussel and suitable hosts is not necessary for successful transformation. However, it is typically the more common mussels, which are generally eurytopic in their host specificity, that can transform on allopatric fish species. This low degree of host specificity may be a factor in the ubiquity of those species. Conversely, less widespread mussel species tend to have a more narrow range of available hosts (Neves *et al.* 1985, O'Connell 1991).

Presently, the tan riffleshell is extremely limited in its range. However, its predominant host, the fantail darter, is widespread in eastern drainages that are not known to have contained populations of this mussel species. Recent results of an experiment have suggested that the tan riffleshell transforms more effectively on fantail darters from populations within the Tennessee River drainage system. Fantail darters from the Tennessee River system, used in host fish identification experiments, yielded six times more juveniles than those from the Atlantic slope during the same time of year (Brian Watson, NCWRC, unpublished data). These results led to the hypothesis that the tan riffleshell may transform best on hosts within the Tennessee River drainage system or even within streams in which it occurs.

Data on host suitability of endangered mussels are integral to the success of population augmentation and reintroduction. It is desirable to obtain the maximum number of juveniles possible when culturing them for release. Therefore, determining which species and populations of host fish are most suitable for glochidia transformation will have a considerable positive effect on the availability of juveniles for culture.

Aging

Various methods have been used to determine the ages of freshwater mussels, to include using mark-recapture data, growth-interruption line counts, and size-frequency to determine age-classes (Haskin 1954). However, most of these methods are fraught with problems. The complete absence of a year class in a size-frequency diagram may lead to

error in the estimated ages, especially when younger individuals are not adequately represented due to decreased probability of capture, as is often the case with freshwater mussels. Additionally, mark-recapture studies require several years to examine the number of rings added during the release period. Therefore, the use of growth checks has become the easiest and most reliable method of aging freshwater mussels, especially when combined with size-frequency determination or mark-recapture (Haskin 1954, Neves and Moyer 1988).

Many attempts have been made to attribute the external growth rings on freshwater mussel shells to annual cycles. Growth rates in mussels can vary according to environmental factors such as oxygen level, turbidity, substratum type, food availability, and temperature, among others (McCuaig and Green 1983). As growth rates slow, the concentric rings on the valves are thought to be formed by proportional changes in levels of calcium and organic matter caused by temperature variations and other changes (Crowley 1957) or by anaerobiosis (Lutz and Rhoads 1977). The primary difficulty with the counting of external growth rings is the inability to distinguish annual growth lines from those that arise from spawning, warming or cooling of the water, or other disturbances that cause pseudoannuli or false annuli to form (Lutz and Rhoads 1980). In addition, use of these external growth checks is hindered by shell erosion, dark periostracum, and the inability to distinguish closely deposited checks in old specimens (Neves and Moyer 1988). Because few aging studies have validated these checks as true annuli, published age and growth data are questionable (Downing *et al.* 1992). Negus (1966) and Haukioja and Hakala (1978) found that significant proportions of recaptured mussels (50% and 36%, respectively) formed an inappropriate number of “annuli.” Neves and Moyer (1988) found that only 12% of their tagged and recovered specimens formed one growth band annually, but attributed much of the lack of annual formation to the low recovery rate (36%) and slow growth of older individuals (<1 mm/yr).

Thin-sectioning of mussel shells has provided an avenue to circumvent several of the above problems. Neves and Moyer (1988) found that this technique was the most accurate of the three tested: external annuli, acetate peels, and thin-sections. Older individuals could be more reliably aged, and true annuli could be distinguished from false annuli. Thin-sectioning exposes internal growth rings, which extend from the umbo to

the shell margin. In this manner, false annuli are easily distinguishable, as they are typically incomplete (Neves and Moyer 1988), and they are lighter in color than true growth rings (Haukioja and Hakala 1978). Additionally, the first few annuli, which generally have eroded externally in individuals over 4 years of age, are observable in thin sections. Downing *et al.* (1992) found that thin-sections increased the accuracy of ring counts, although these rings might not correspond to annual cycles in some species. Thin-sections should not be considered an absolute appraisal of an individual's age unless annulus formation has been validated for that species in that locality (Neves and Moyer 1988). However, they do provide a more accurate count of the number of true growth rings within the shell.

Watson (1999) conducted a survey of the tan riffleshell population in Indian Creek, and measured and aged the specimens by the external ring method. He found a strong correlation between shell length and age, but questioned the accuracy of external aging. No thin-sections were made to more correctly determine the ages of individuals and to provide a correction factor for aging live tan riffleshells at streamside. Additionally, he used mark-recapture information to calculate a Petersen estimate of abundance; an estimated 683 adults occurred within the approximately 100 meters of Indian Creek where the majority of the tan riffleshell population is found.

Statement of Objectives

The goal of this chapter is to provide information on the biology of the tan riffleshell population in Indian Creek so that the species can be effectively managed and recovered. Specific objectives are as follows:

1. To determine whether fantail darters (*Etheostoma flabellare*) from a population sympatric with the tan riffleshell are more suitable hosts than darters from allopatric populations.

   H_o: Fantail darters in Indian Creek are as compatible as glochidial hosts as allopatric populations of fantail darters.
2. To describe the Indian Creek population of the tan riffleshell using ages obtained from thin-sections, shell lengths, sex ratios, and mark-recapture information.

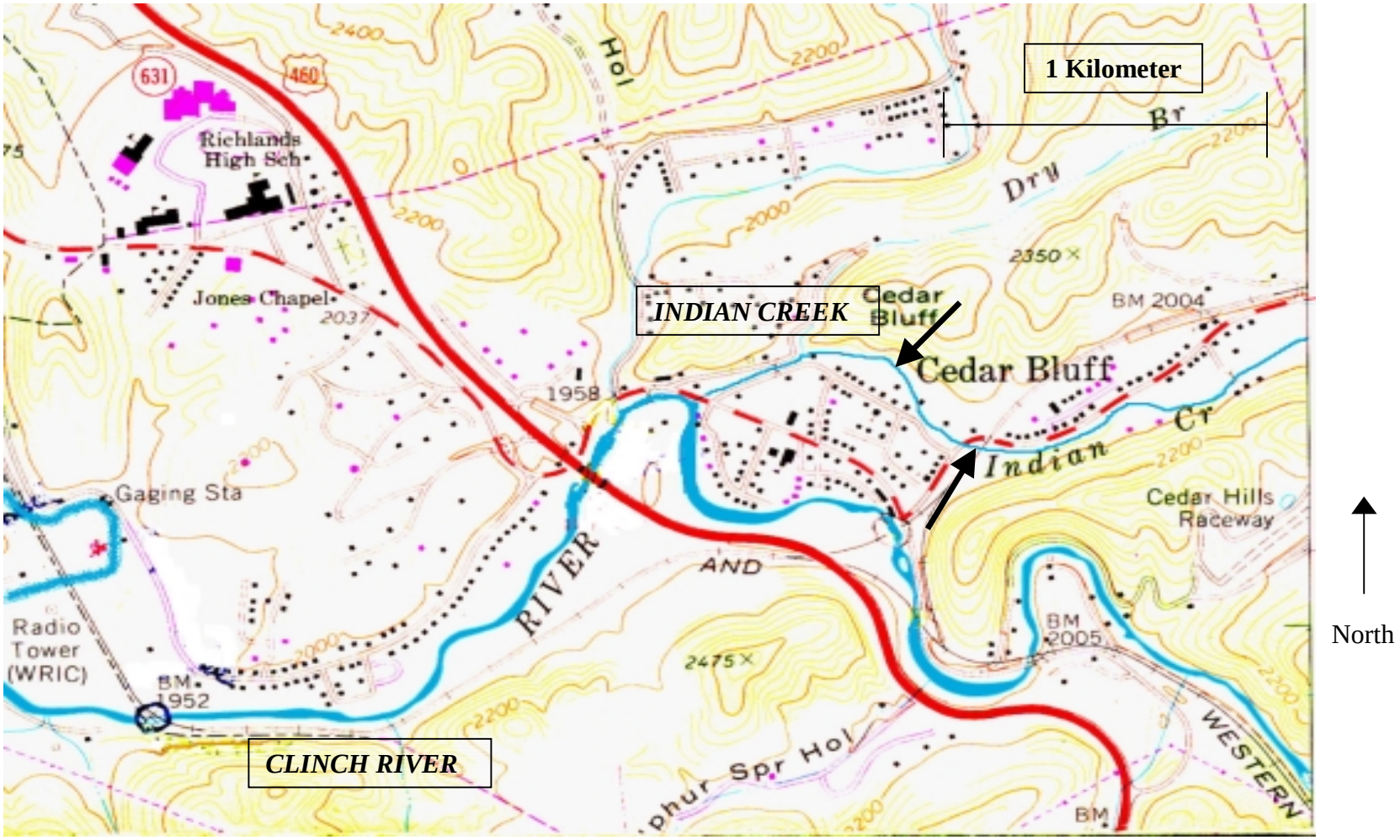

Figure 1-1. Location of Indian Creek, Tazewell County, VA (Richlands Quadrangle). Area between arrows denotes primary location of tan riffleshell (*Epioblasma florentina walkeri*) population.

Methods

Fantail Darter Population Suitability

Collection Sites

Fantail darters to be tested as host fish for the tan riffleshell were collected at four sites: Indian Creek (Clinch River drainage), Elk Garden (Clinch River drainage), South Fork Holston River, and North Fork Roanoke River (Figure 1-2). The first three streams are within the Tennessee River drainage, whereas the last stream is within the Roanoke River drainage. These streams were chosen according to the following criteria: Indian Creek has historically contained tan riffleshells (high potential for contact); Elk Garden is a tributary of the Clinch River that has not been known to contain tan riffleshells (low potential for contact); South Fork Holston River is within a parallel drainage system as the tan riffleshell population in Indian Creek (unlikely potential for contact); and North Fork Roanoke River is within an entirely different river drainage that has never contained tan riffleshells (no possible contact).

Indian Creek is a clear, shallow stream located in Tazewell County, Virginia, and contains the only known reproducing population of the tan riffleshell, discovered in 1995 (Winston and Neves 1997). It is a third-order stream that joins the Clinch River in the town of Cedar Bluff. This stream has viable populations of the tan riffleshell, the endangered purple bean (*Villosa perpurpurea*), and other common species (Watson 1999). Darters were collected in the 1 km section upstream of the Route 627 bridge crossing, upstream of the area known to be inhabited by tan riffleshells. The stream in this area was approximately 5 m wide, and average depth was about 0.5 m. The substratum consisted primarily of cobble.

Elk Garden is a fourth-order stream in Russell County, Virginia, that joins Big Cedar Creek east of the town of Lebanon. Its watershed consists of primarily agricultural land, with very little vegetation in the riparian zones along most of its length. Siltation is a major impact on the stream, and erosion has caused it to become fairly entrenched. Fantail darters were collected within the section of stream that runs parallel to Route 19 and Route 80 for about 3.5 km. The average width of the stream in this section was

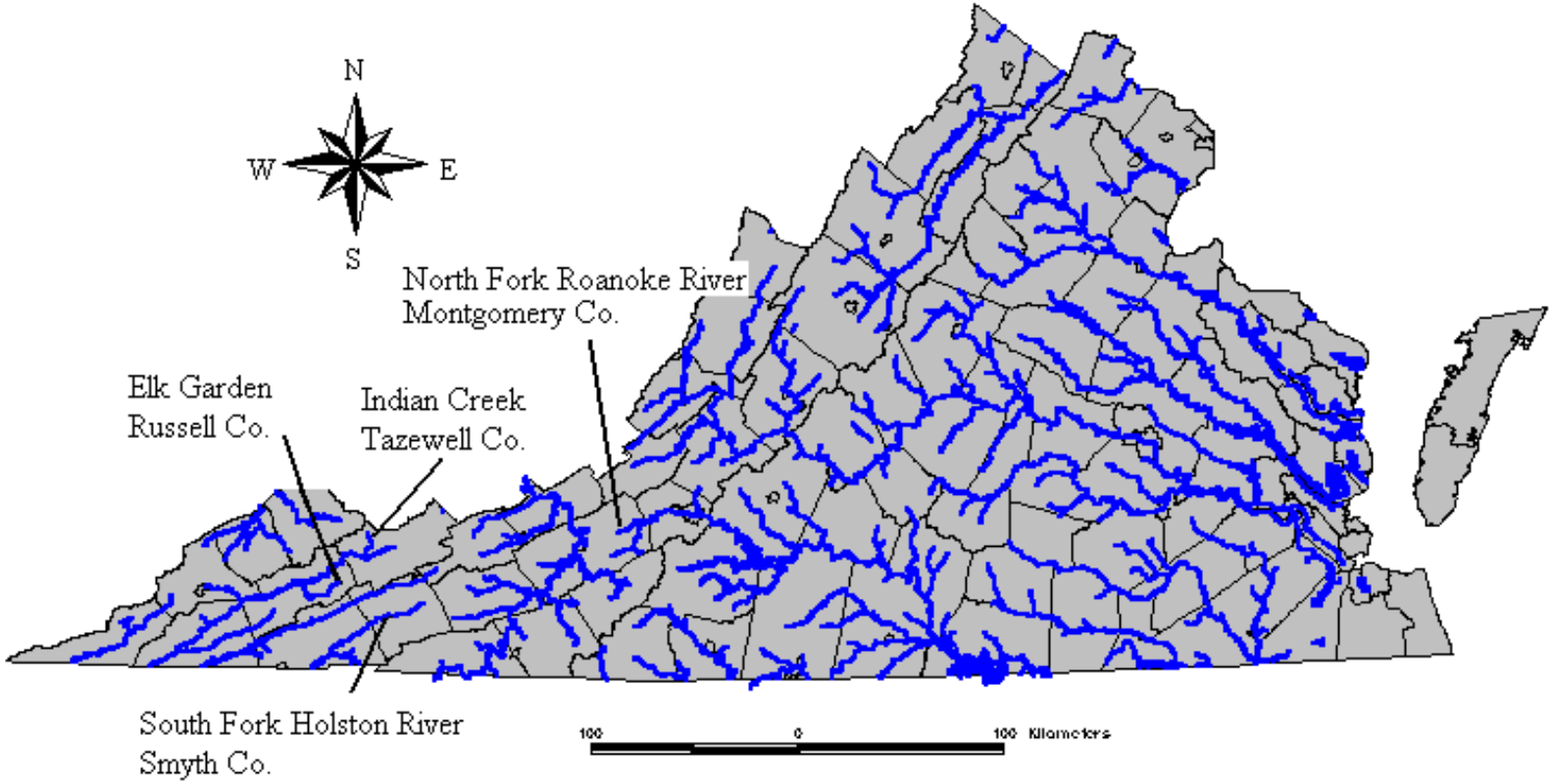

Figure 1-2. Collection sites for fantail darters (*Etheostoma flabellare*) used to determine the suitability of these populations as hosts for tan riffleshells (*Epioblasma florentina walkeri*) from Indian Creek.

approximately 2 m, and the average depth was approximately 1 m. The substratum was gravel mixed with silt.

The South Fork Holston River flows through Smyth and Washington counties in Virginia. It is a fourth-order stream with typically cool water that joins the Middle Fork Holston River in Washington County, Virginia. Fantail darters were collected in the section of the South Fork just south of Marion in Smyth County, near Thomas Bridge at the Route 650 bridge crossing. The habitat in this section is comprised mostly of riffles and runs. The stream's average width was about 10 m, and the average depth was approximately 0.25 m. The substratum was principally cobble.

The North Fork Roanoke River is also a third-order stream located in Roanoke and Montgomery counties, east of Blacksburg, Virginia, and joins the South Fork to form the Roanoke River near the town of Ironto. Agriculture and development have impacted this stream heavily, and siltation is a common problem. Fantail darters were collected from the North Fork at the Route 603 bridge crossing in Ellett, near the stream's confluence with Wilson Creek. This section is primarily riffle and run habitat, with mostly cobble and gravel substratum. The average width of the stream at this site was roughly 5 m, and the average depth was about 0.5 m.

Experimental Design

Fantail darters were collected from Indian Creek, Elk Garden, South Fork Holston River, and North Fork Roanoke River with a backpack electrofisher. The fish were kept in aerated coolers and transported to Virginia Tech's Aquaculture Center. The fish from each stream were randomly distributed into 38 L aquaria according to their stream of origin, such that there were four aquaria per stream and approximately equal numbers of fish within each. The darters were held in aquaria for an acclimation period of approximately 7 days. I collected 33 fantail darters from Indian Creek, 51 from Elk Garden, 45 from South Fork Holston River, and 53 from North Fork Roanoke River. After the 7 day holding period and subsequent mortality, I infested 17 fish from Indian Creek, 22 from Elk Garden, 31 from South Fork Holston River, and 34 from North Fork Roanoke River.

Before infestation, each fish was given a fin clip unique to its drainage. Pectoral fin clips consisted of two small cuts to the fin that removed a triangle-shaped area from either the right or left pectoral fin. Fish that received caudal fin clips had 2 mm removed from either the top or bottom corner of the caudal fin. The clips were given accordingly: Indian Creek, right pectoral fin; Elk Garden, top of caudal fin; South Fork Holston River, left pectoral fin; and North Fork Roanoke River, bottom of the caudal fin. With these clips, fish could be placed together into a tank during infestation to ensure equal infestation among fish from all streams and then easily separated after infestation. Once clipped, the fish were placed in a 19 L bucket with approximately 5 cm of water and an airstone to keep the glochidia in suspension. The glochidia, extracted from the gills of four gravid tan rifflesheIls, were added to the water with the fish. Fish and glochidia were left in the bucket for 90 min and then separated by stream of origin and returned to the aquaria.

Aquaria were not inspected for juveniles during the first 7 days after infestation. Partial water changes were performed every other day, and water temperatures were taken during this period. Beginning on day 8 and approximately every 2 days thereafter, the bottoms of the aquaria were siphoned and filtered through 300 and 105 μm sieves; the large sieve size removed wastes but allowed the juveniles to pass through, and the finer sieve captured the juveniles. The contents of the sieve were examined with a dissecting microscope, and the number of juveniles from each tank was counted. The juveniles were then put in a petri dish with fine sand and placed into a trough with recirculating water. Juveniles were collected in this manner through day 23, when it was apparent that all juveniles had transformed and dropped from the fish. Sloughed, untransformed glochidia also were counted for each tank, and the numbers were examined for differences among the four populations.

For each day that juveniles were collected, the number of juveniles that transformed per tank was divided by the number of fish that survived up to that day. For each tank, the resulting numbers were summed for the entire juvenile collection period. Least squares means of the juveniles per fish estimates from each stream were compared using analysis of variance with repeated measures, and Tukey's multiple comparison method was used to compare numbers among the streams (Ott 1993). The experimental

units were the aquaria; thus, there were four replicates per stream. The significance level was set at $p < 0.05$.

Tan Riffleshell Population

Age Class Structure

Shells of tan riffleshells have been collected for several years from Indian Creek and along the banks in muskrat middens, following this population's discovery in 1995. Shells of 102 individuals were randomly selected as a stratified 25% sample of the collection of over 400 shells to assess age structure of the population. In order to represent adequately the size class distribution of shells in the collection, the shells were divided into 10 mm size categories, and 25% were randomly selected from each category for analysis. Shells were aged first by counting external annuli. Because the first year's growth ring was eroded in most specimens, one year was added to each age of those specimens. Two experienced biologists independently aged the shells by external growth rings.

Shells were thin-sectioned with a Buehler Isomet low-speed saw, using a diamond-impregnated blade that allows precise cuts to be made at low speeds (Clark 1980). Blade speed was set at approximately 200 rpm. The initial cut was made by placing a single valve on the plastic guide plate and manually feeding the shell into the rotating blade. One side then was selected for aging based on the cleanliness of the cut. The selected side was attached to a petrographic slide with five-minute epoxy and left to dry overnight. This was done for both valves per specimen. The second cuts were made after the epoxy had fully cured. The slides with the shells attached were vacuum-sealed to a petrographic chuck on the specimen arm of the saw. The arm then was lowered slowly toward the blade to produce uniform pressure on the blade. The width of the thin-section was set at approximately 250 μm, although occasionally the epoxy or a thicker shell deflected the blade to produce a slightly thicker cut. Once the second cut was completed, all thin-sections were polished on 1600-grit sandpaper to remove scratches formed during cutting. In addition, those sections that had slightly thicker cuts were ground with coarser paper to approximately the same width as the others.

Three biologists read each thin-section independently with a microscope by counting the rings that originated in the umbo and extended along the shell to the periostracum. False annuli were considered to be those checks that clearly did not originate in the umbo or extend to the periostracum. Those specimens for which at least two biologists disagreed on the ages, or when both valves were determined to be of different ages, were excluded. Age class distributions and length-at-age regressions were examined for males and females to compare growth rates and population structure.

Population Estimate

Indian Creek has been surveyed periodically for tan riffleshells since the population's discovery in 1995. Nearly 110 tan riffleshells had been tagged using individually numbered plastic tags (Hallprint Tags, Holden Hill, Australia) prior to 1997 (Watson 1999), within a 200 m section of stream near its confluence with the Clinch River. These tags were super-glued to the left valve of the mussels and were used to obtain an estimate of population size using the Petersen mark-recapture method of population estimation. In order to provide a more accurate estimate, those data were pooled with my data from individuals collected and tagged after 1997.

From 1997-1999, Indian Creek was surveyed about once per month. Surveyors snorkeled along the streambed, searching for mussels visible in the substratum. For the 100 m of stream, the entire width was surveyed by spreading the surveyors equidistant across the stream and then traversing the area between observers. Thus, only mussels visible to snorkelers were included in my abundance estimate. Additionally, this technique only was useful for finding adult mussels, as juveniles were generally too small to be observed by snorkeling. When tan riffleshells were found, they were collected and brought to the bank. Their location was marked with flagging tape so they could be returned to the exact location of collection. Individuals were aged, sexed when possible, and measured lengthwise (anterior-posterior) to the nearest 0.1 mm with vernier calipers. The tags were attached to the left valve of the shells using superglue. When the glue had dried completely, the mussels were returned to their original locations. When tagged mussels were recaptured, they were measured and returned to the stream. Shells from muskrat middens along the streambanks were collected as well during these surveys.

The population size of adult tan riffleshells in the 100 m of stream extensively surveyed between 1996 – 1999 was estimated using Schumacher's modification of Schnabel's maximum likelihood estimate (Schumacher and Eschmeyer 1943, Caughley 1977). This method is appropriate when only a few individuals can be caught on one sampling occasion. The population's size is estimated from the rate at which the proportion of marked individuals rises as progressively more are marked (Caughley 1977). This estimate accounts for variation in the number of captured individuals on any one sampling occasion (Schumacher and Eschmeyer 1943). Schumacher's modification increases accuracy, as it depends less on random mixing of marked and unmarked individuals than Schnabel's original estimate. Population size was estimated from

$$N = \frac{\sum M_i^2 n_i}{\sum M_i m_i}$$

where N was the constant size of the population, M_i was the number of individuals marked prior to the ith sampling occasion, and n_i was the number of individuals captured on the ith occasion of which m_i had been marked previously.

The 95 percent confidence limits were computed from the standard error of N indirectly. The standard error of 1/N was computed as $s/\sqrt{\sum M_i^2 n_i}$ where

$$s^2 = \frac{\sum (m_i^2 / n_i) - (\sum M_i m_i)^2 / (\sum M_i^2 n_i)}{j - 1},$$

j being the number of recapture samples. Confidence limits were obtained by multiplying the standard error and the t value corresponding to j-1 degrees of freedom. The reciprocals of the upper and lower limits then were calculated.

The information from the tagged mussels was used to determine the sex ratio and size distribution of the *E. f. walkeri* population in Indian Creek. Shell lengths and thin-sections were used to calculate ages of tagged mussels using the regression equations obtained from size-at-age data. Mussels outside of the range of sizes used to compute the equation (30 – 51 mm for females and 24 – 52 mm for males) were not included.

Results

Suitability of Fantail Darter Populations

A total of 808 juvenile tan rifflesheIls were collected from the infested fantail darters from all streams. The mean numbers of juveniles per fish were as follows: Indian Creek, 59.22 ± 10.01; South Fork Holston, 37.81 ± 10.01; Elk Garden, 34.51 ± 12.25: North Fork Roanoke, 9.45 ± 10.64 (Figure 1-3). An analysis of variance with repeated measures indicated that fish from Indian Creek produced significantly more juveniles than those from the Roanoke River (p=0.024). The juveniles per fish from the South Fork Holston River and those from Elk Garden were not significantly different from those of either Indian Creek or the Roanoke River, nor were they significantly different from each other. The majority of the juveniles dropped from the fish at 17 days post-infestation, and the drop-off period occurred between 8 and 23 days post-infestation. There were no differences among the streams in the number of glochidia that were sloughed from the fish before transformation (p = 0.669), which ranged from 38 to 73 glochidia sloughed per fish.

Although the average temperature in the tanks was about 21°C, in some cases the temperature reached 25°C, which resulted in mortality of fish in some tanks. One tank of Elk Garden fish experienced mortality of all fish, and one tank of Holston fish and one tank of Indian Creek fish experienced no mortality. The tank in which all fish died was not included in the analysis. The other 13 tanks had an average of two fish die over the course of the experiment (Table 1-1). Mortality was accounted for by examining the data in terms of juveniles recovered per live fish at each time of juvenile collection.

Tan Riffleshell Population

Age Class Structure

Shells of 102 individual tan rifflesheIls were successfully aged from thin-sections. Of these, both valves were sectioned for 72 individuals. For the remaining 30, only one valve was sectioned because either only a single valve was available or one of the two valves cracked during the sectioning process. Of the 102 individuals sectioned, 77 were included in the analysis. Both valves from two of the specimens cracked while on the

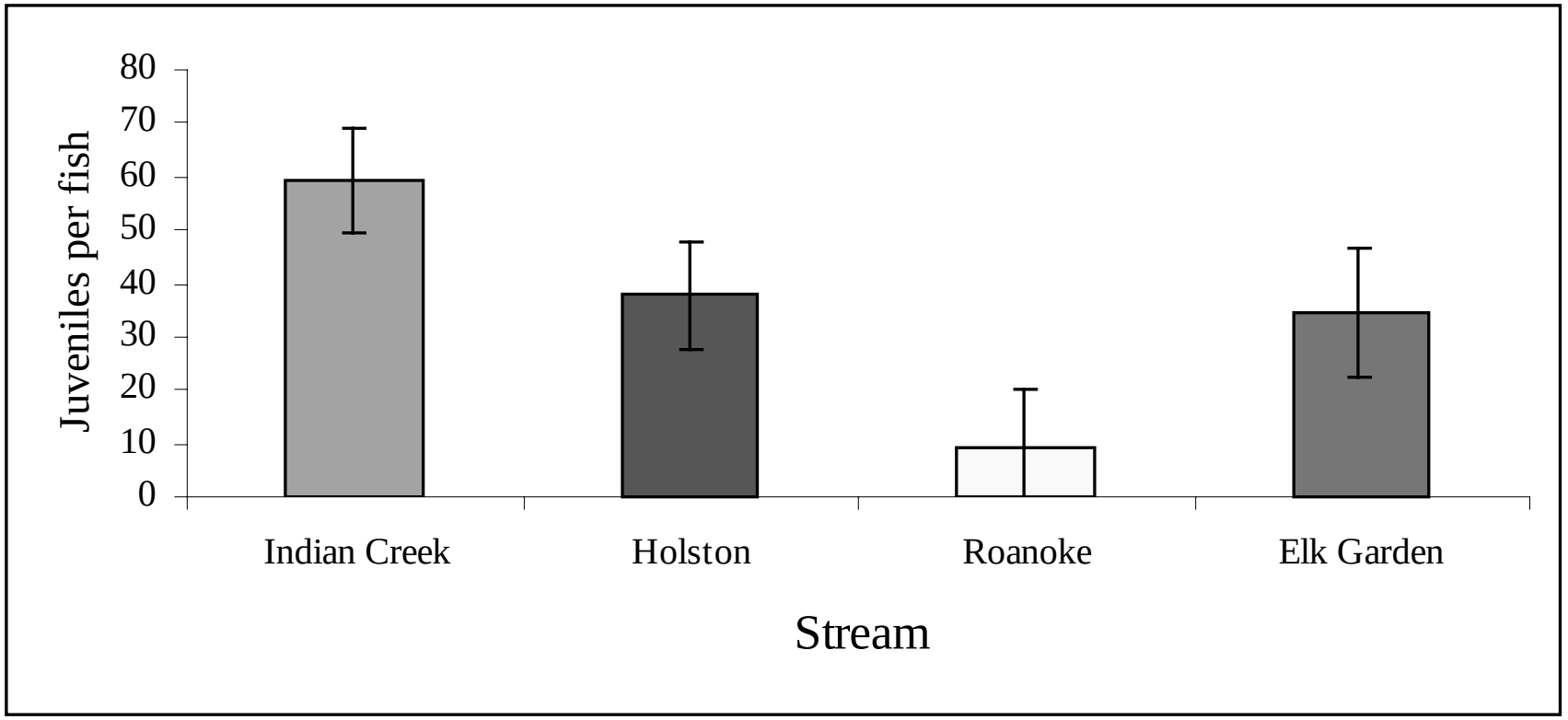

Figure 1-3. Least-squares means of the number of juvenile *Epioblasma florentina walkeri* that transformed on fantail darters from each stream. The two streams with asterisks were significantly different (p=0.024).

Table 1-1. Summary of transformation of tan riffleshell (*Epioblasma florentina walkeri*) glochidia on fantail darters (*Etheostoma flabellare*) from different watersheds.

Stream	Tank Level	Average Temperature (°C)	# Mortalities	Juveniles/ fish
Indian Creek	upper	21.7	0	52.0
Indian Creek	upper	21.5	1	24.0
Indian Creek	lower	20.8	1	56.0
Indian Creek	lower	21.3	1	105.0
Elk Garden	upper	20.8	5 (complete mortality)	0
Elk Garden	upper	21.2	1	13.5
Elk Garden	lower	20.5	1	33.5
Elk Garden	lower	21.0	1	27.0
South Fork Holston	upper	21.7	3	50.9
South Fork Holston	upper	21.7	2	19.0
South Fork Holston	lower	20.5	2	33.0
South Fork Holston	lower	21.0	0	48.4
North Fork Roanoke	upper	20.9	3	7.6
North Fork Roanoke	upper	21.8	2	17.6
North Fork Roanoke	lower	20.4	1	2.2
North Fork Roanoke	lower	20.7	2	7.6

saw, and the remaining 23 were not included because a single age was not agreed upon among biologists. Those individuals not included in the analysis were not distributed differently from the rest of the sample (Figure 1-4). Age 3 individuals were most common in the sample, comprising 28.6% of sectioned shells (Figure 1-5). Second most common were age 5 individuals (19.5%), followed by ages 4 and 6 (14.3% each). More males than females were included in the analysis (30 females, 47 males), but their age and length distributions were similar (Figure 1-6). Because of the obvious sexual dimorphism, it was prudent to analyze males and females separately. Analysis of the relationship between shell length and age shows that shell length was strongly correlated with age for males (Figure 1-7), but the relationship was weaker for females (Figure 1-8). Both sexes appeared to have similar growth rates. Only five individuals (approximately 5%) were recognized as having produced false annuli. All five were males, and the false annulus was visible on the exterior of both valves and could have been mistaken for an external annulus.

A comparison of the ages determined from thin-sections versus those from external ring counts showed that the two methods yielded nearly equal results until the mussels grew older (Figure 1-9). The majority of the data points lie below the 1:1 ratio line, indicating that counting growth rings typically yields younger ages than thin-sectioning. Young mussels could be aged about as accurately from the external growth rings as from thin-sections. However, after age 8, external growth rings more consistently underestimated the true ages of the individuals. In some cases, an age 11 mussel was determined to be only 8 or 9 years by external growth rings.

Population Estimate

Using Schumacher's modification of Schnabel's maximum likelihood population estimate, the tan riffleshell population size in Indian Creek was estimated to be 1078 adult individuals (95% CI= 760 - 1853). Throughout the 3 year sampling time, only 12 individuals were recaptured (Table 1-2). Mussels less than two years old (juveniles) were excluded from the estimate because they could not be sampled effectively by snorkeling. This estimate exceeds Watson's (1999) Petersen estimate of 683 adults (95% CI = 316 –

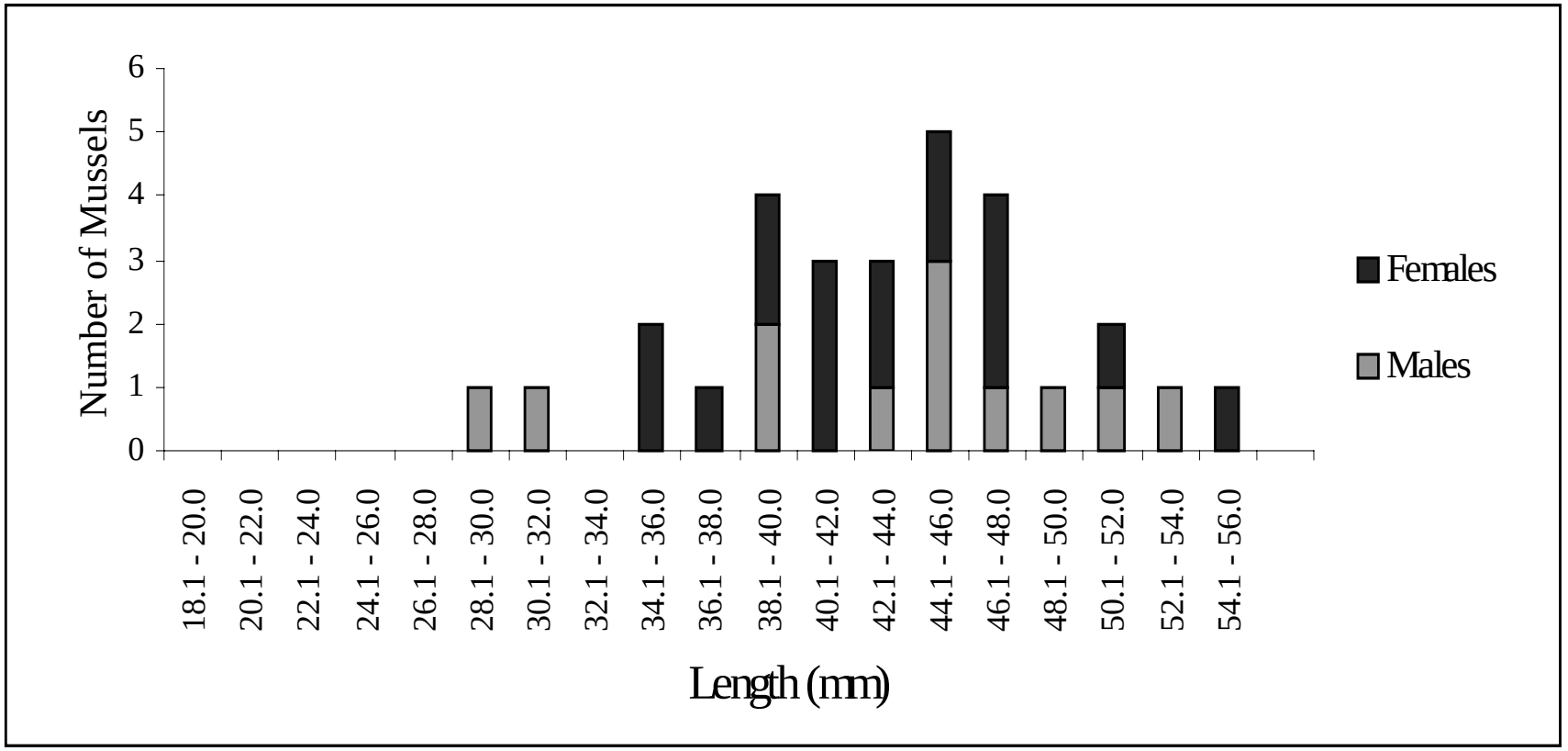

Figure 1-4. Size distribution of tan riffleshells not included in thin-section analysis. There was no significant difference between the size distribution of individuals included in the analysis and those excluded at $p < 0.05$.

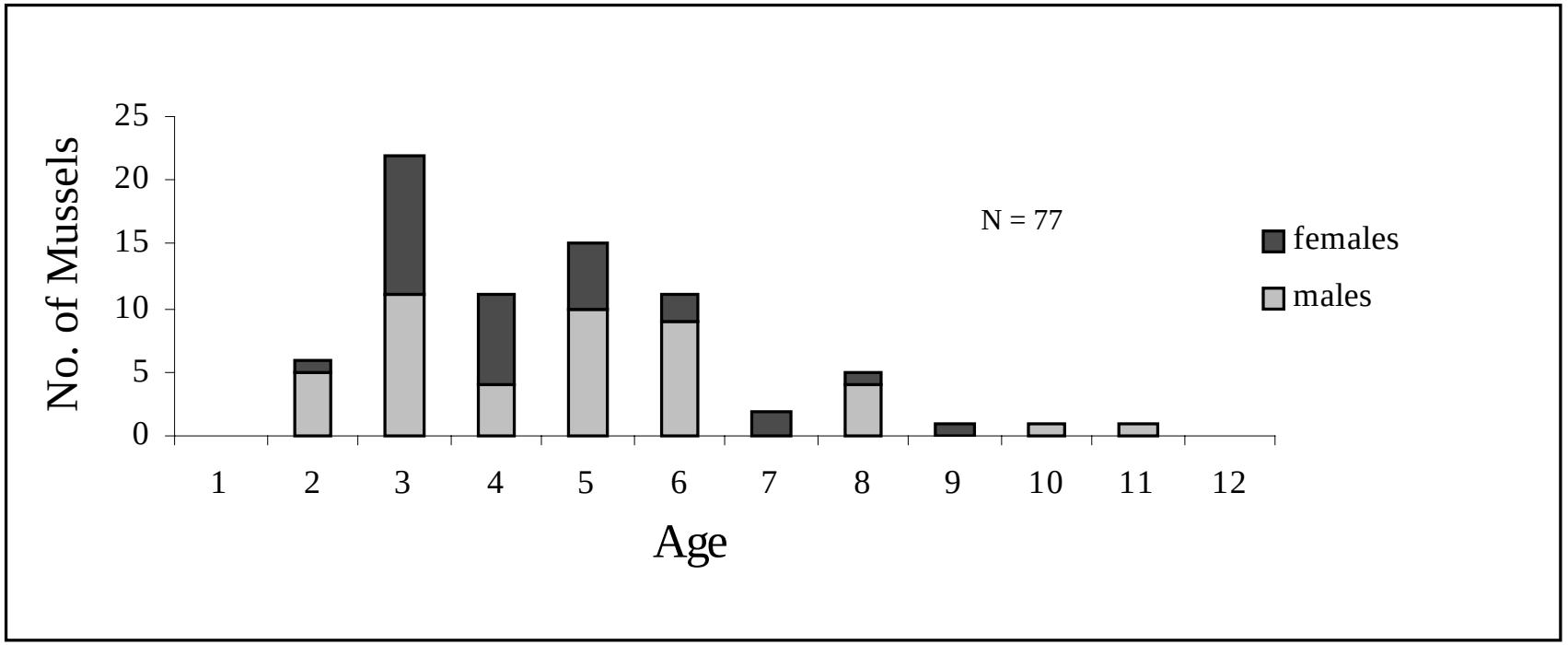

Figure 1-5. Age distribution of male and female *Epioblasma florentina walkeri* determined from shell thin-sections.

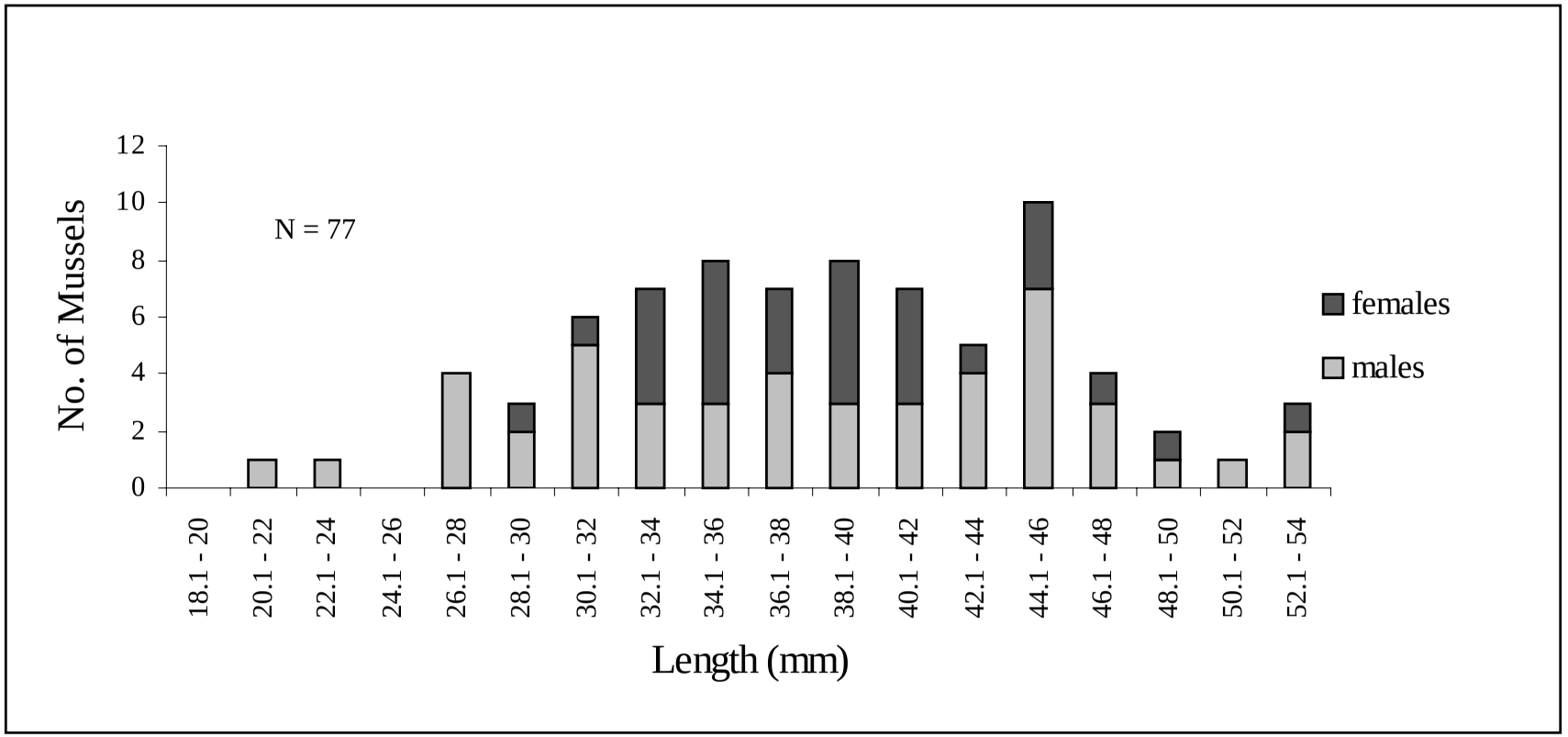

Figure 1-6. Lengths of *Epioblasma florentina walkeri* aged by thin-sections.

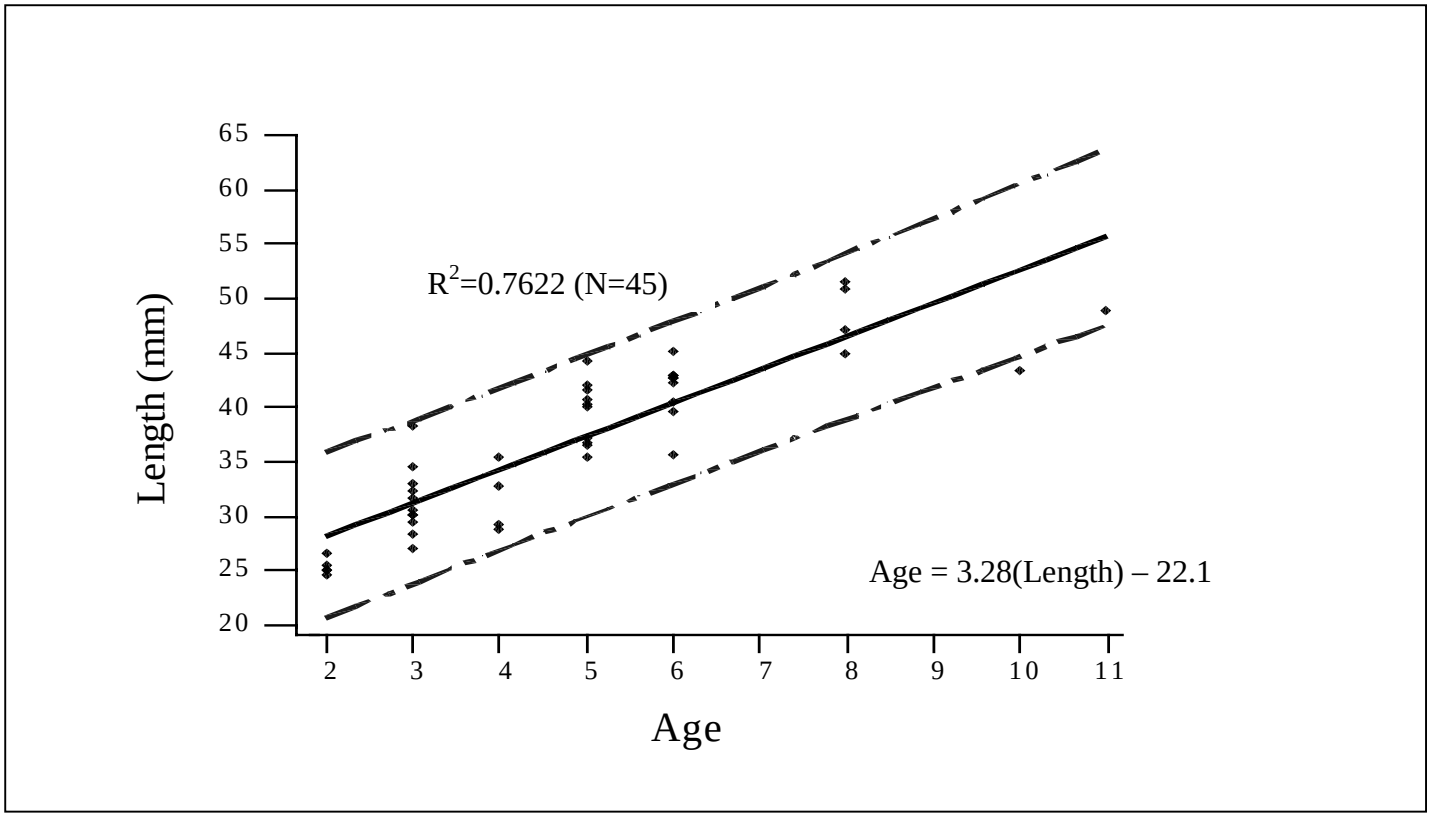

Figure 1-7. Length-at-age regressions for male *Epioblasma florentina walkeri* determined from shell thin-sections. Dashed lines indicate 95% prediction bands.

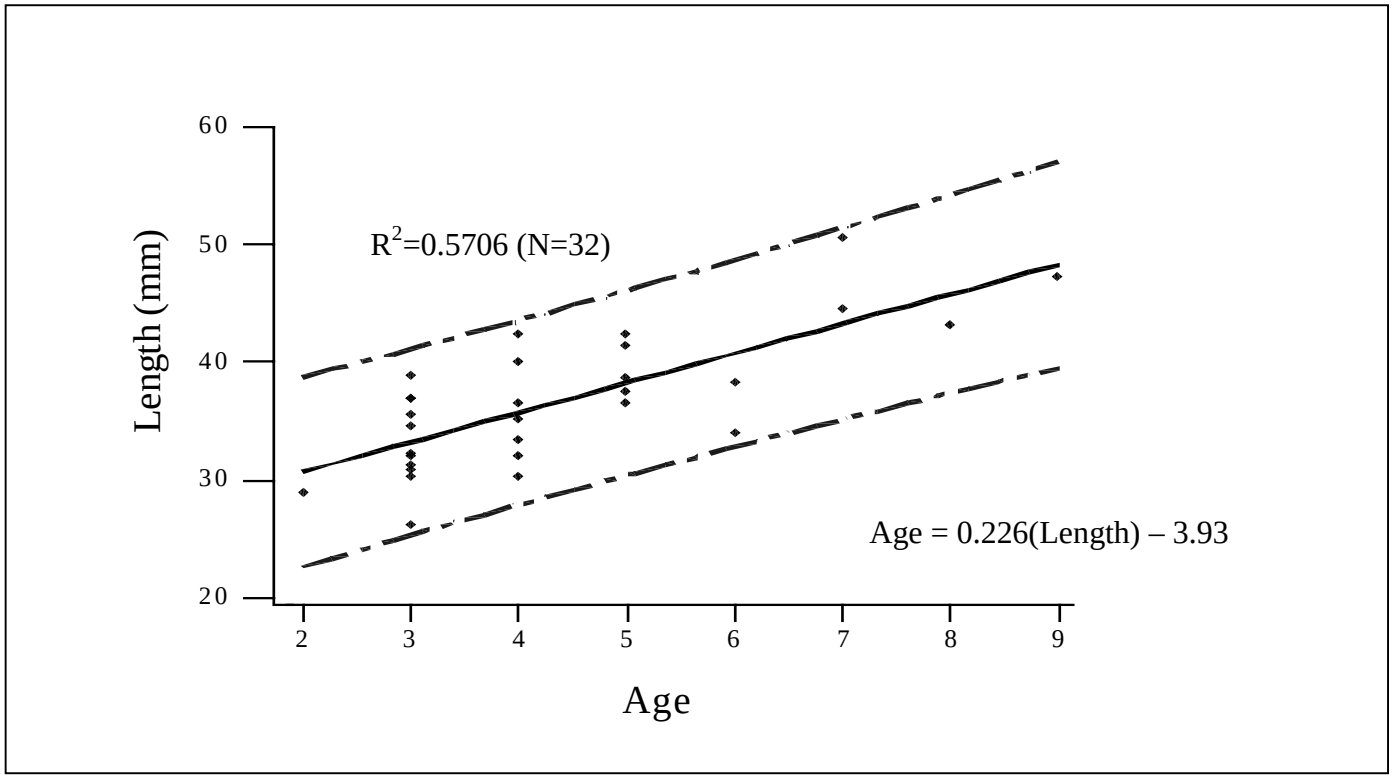

Figure 1-8. Length-at-age regressions for female *Epioblasma florentina walkeri* determined from shell thin-sections. Dashed lines indicate 95% prediction bands.

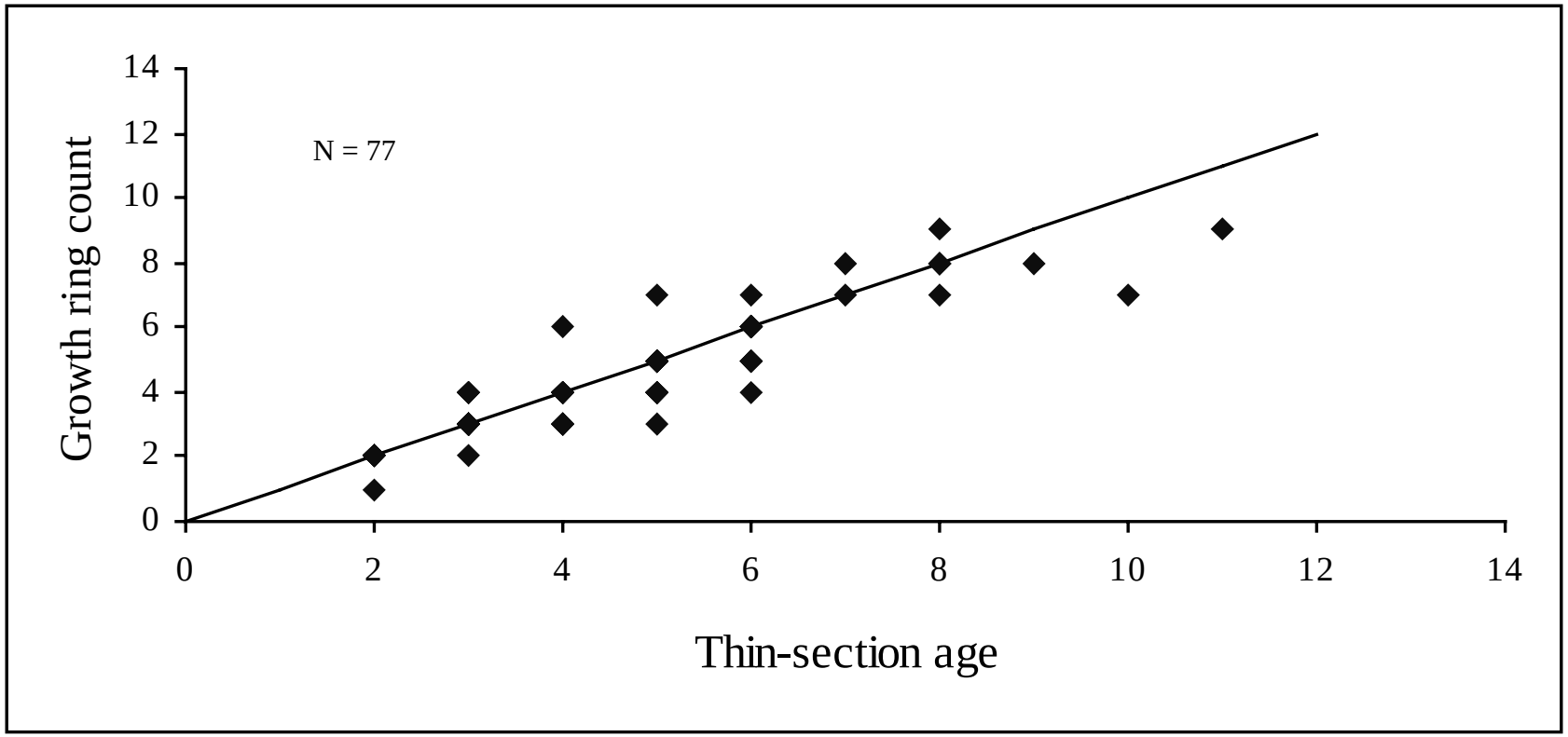

Figure 1-9. Thin-section ages versus external growth ring counts for *Epioblasma florentina walkeri* collected in Indian Creek, Tazewell County, Virginia. Line indicates 1:1 ratio (same ages).

Table 1-2. Summary of recaptures used to compute Schumacher's modification of Schnabel's maximum likelihood estimate. M_i refers to the total number of marked individuals in the population, n_i denotes the number of individuals captured on each sampling occasion, and m_I refers to the number of marked individuals captured in each sample.

Sample #	Date	M_i	n_i	m_I
0	6/96	0	56	0
1	8/96	56	66	5
2	6/97	117	13	2
3	10/98	128	8	1
4	5/99	135	23	2
5	6/99	156	17	2

1762) by nearly 60%, perhaps because the multiple mark-recapture estimate accounted for variation in the numbers of mussels collected on any one sampling occasion. Additionally, it is important to note that only individuals at the surface could be collected during sampling, so this population estimate is a minimal one for actual population size.

Tagged tan riffleshells ranged from 20 mm to 54 mm in length, with the bulk of the individuals measuring between 38 mm and 44 mm (Figure 1-10). The low number of small individuals does not indicate a lack of recruitment; rather, few small (<30 mm) individuals were captured because of the sampling technique employed. Watson (1999) estimated reproductive size to be 36.0 mm; therefore, 26% of the tagged sample (which does not include juveniles) were at or below reproductive size, which corroborates his statement that this is a fairly young and healthy population.

There were 156 individuals tagged in the population, with approximately equal numbers of males and females (70 and 86, respectively), but distributed unequally in size. There were significantly more females than males in size ranges between 40 and 44 mm (Figure 1-10). Additionally, there were almost twice as many males as females in the size classes above 44 mm. Calculated ages of males and females in the tagged sample of tan riffleshells in Indian Creek were obtained by inserting the lengths of the mussels into the regression equations for males and females. Most of the individuals were ages 6-8, with the mode of females at age 7 (Figure 1-11). There was not a similar spike in cohort abundance for males.

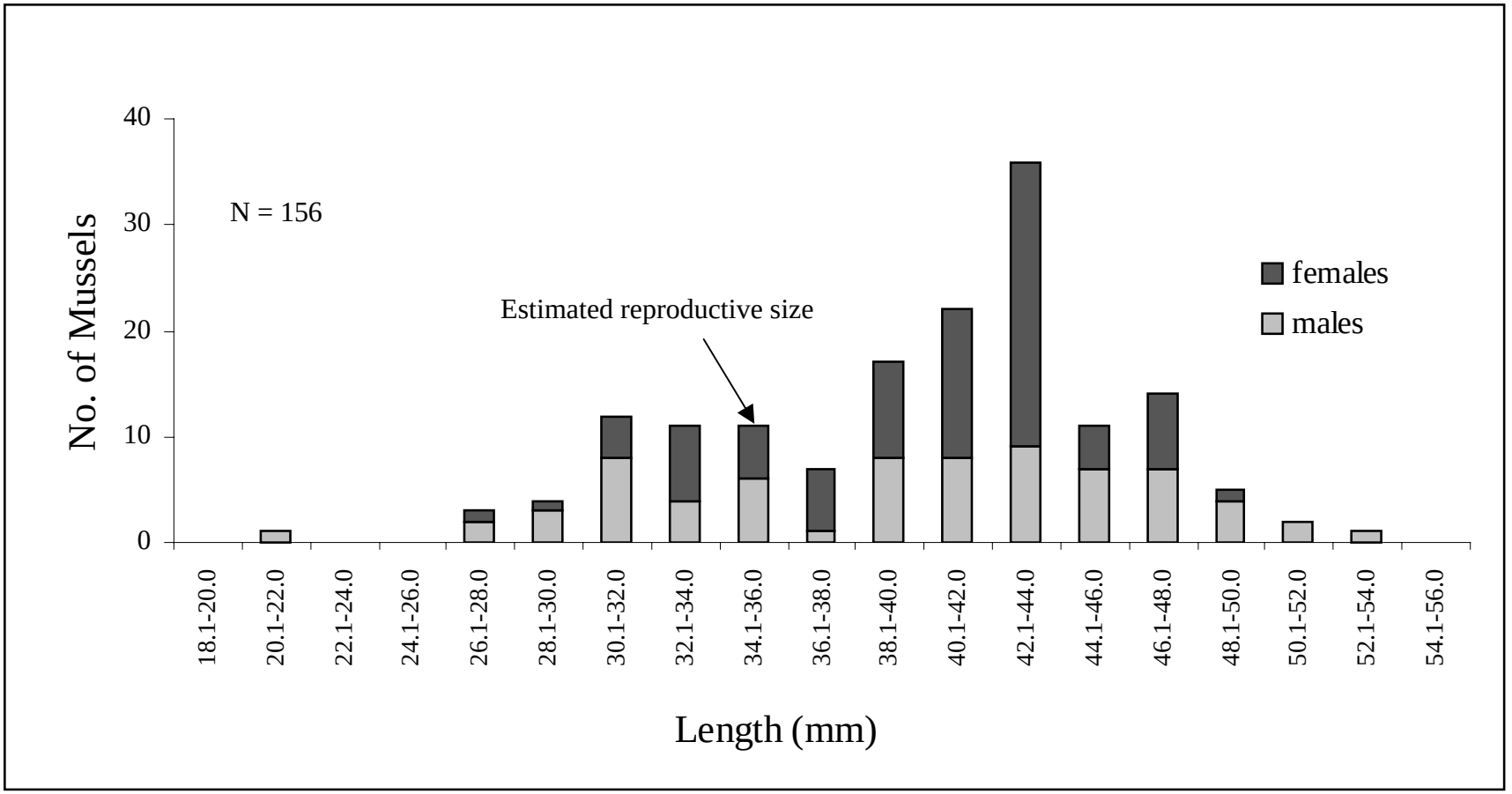

Figure 1-10. Sizes of male and female *Epioblasma florentina walkeri* tagged in Indian Creek, Tazewell County, Virginia.

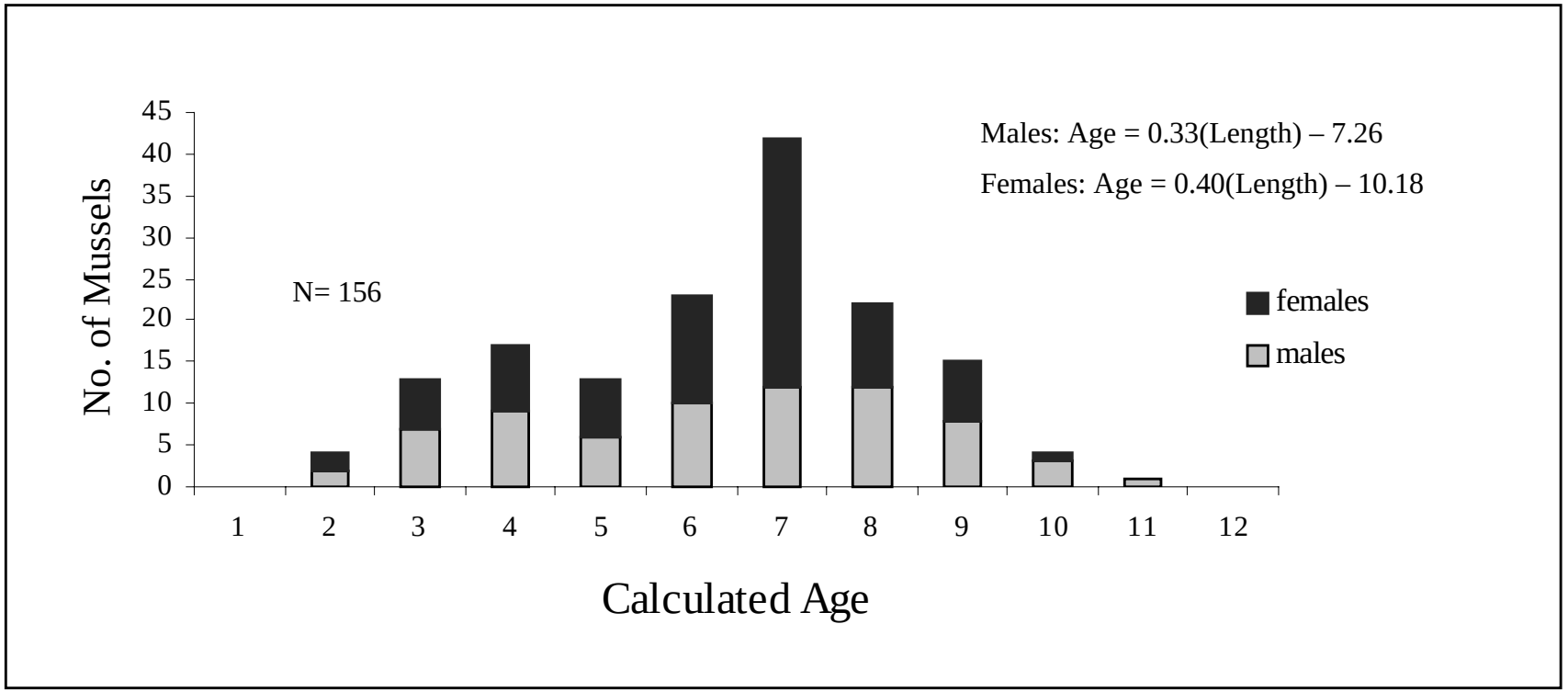

Figure 1-11. Ages calculated for male and female *Epioblasma florentina walkeri* tagged in Indian Creek, Tazewell County, Virginia.

Discussion

Suitability of Fantail Darter Populations

It is commonly known that freshwater mussel glochidia have specific host requirements to transform into juvenile mussels. There is wide variation in the degree of specificity, with some mussel species being highly host-specific and others more eurytopic in their use of hosts (Zale and Neves 1982, Isom and Hudson 1984, Neves *et al.* 1985). However, it is not known whether mussels are so specific in their host requirements that they could be restricted to particular populations of fish rather than simply to the fish species. Although the rudimentary aspects of incompatibility between mussel glochidia and non-host fish are known (Neves *et al.* 1985), the results of my experiment suggest that the host's immune system influences compatibility, and that this relationship may be the result of coadaptation among the mussel and fish populations in the stream.

This study demonstrated differences in the ability of tan riffleshell glochidia to transform on fantail darters from different populations. Various hypotheses have been suggested to explain the phenomenon of host specificity of mussels. Most researchers agree that it is likely regulated by the fish's immune response. Although this hypothesis has not been proven, several observations support it. First, fish previously exposed to glochidia are generally less suitable as hosts, which can be explained best by an acquired immunological response (Reuling 1919, Bauer and Vogel 1987). Acquired immune responses occur when the recombination of certain genes in B-cells results in the production of antibodies against a particular antigen (Lewin 1987). Once the challenged organism has synthesized the antibody, it is protected when re-exposed to that antigen in the future. Further, the blood components necessary for glochidial transformation are present in most fish; glochidia are able to transform in the absence of an immune response from the fish (Isom and Hudson 1984). However, there are species-level differences in fish immune responses to glochidia (Meyers and Milleman 1977), which implies that genetic differences among fish immune systems play a major role in fish host specificity (O'Connell and Neves 1999). Fish immune systems exhibit a high degree of differentiation among species (Rijkers 1981), which lends support to this hypothesis. The

mechanism of fish immunology and immunogenetics presumably are similar to those of higher vertebrates (Rijkers 1981, Danska and McDevitt 1987, Kaastrup *et al.* 1989, Hashimoto *et al.* 1990). The piscine immune system exhibits non-specific and specific defense mechanisms. Non-specific reactions include inflammation at the affected site, which has been observed at the site of glochidial attachment (Arey 1932). Specific reactions may be either humoral, in which antibodies circulate throughout the body; cell-mediated, in which specialized cells are responsible for the defense activity; complement-based, in which a system of molecules works together to lyse invader cells; and non-specific phagocytosis, in which the invading material is destroyed by specialized white blood cells (Cushing 1970, O'Connell 1991). Serological studies performed on fish-glochidia relationships have suggested that the likely defense mechanisms are humoral and/or cell mediated (Meyers *et al.* 1980, Bauer and Vogel 1987, Danska and McDevitt 1987, O'Connell and Neves 1999).

When a glochidium attaches to a fish, its antigens are present on the glochidial surface. In a non-host fish, these are recognized as "non-self" antigens by receptor molecules encoded by the major histocompatibility complex (MHC), which are expressed on the surface of B-cells. These receptors bind the foreign antigen, and the B-cells present these antigens to T-cells, which produce antibodies against the antigen. The antibodies attach to the antigens and mark them for destruction by phagocytes (Corbel 1975, Rijkers 1981, van Muiswinkel 1992). Immune systems of fishes, regardless of their suitability as hosts, exhibit some level of humoral response to glochidial attachment. Meyers *et al.* (1980) showed that after infestation, anti-glochidial antibodies were present even in moderately and highly suitable host fishes.

There are several possible mechanisms by which a fish's immune system may govern host specificity. Antigen mimicry, in which the glochidium has antigens in common with its host and, therefore, is not detected as foreign, seems a likely mechanism. Anemonefish (*Amphiprion clarkii*) have been shown to exhibit antigens that mimic the external mucus of their hosts, the sea anemone, to avoid being stung (Elliott *et al.* 1994). Antigen mimicry also would explain the phenomenon of closely related mussel taxa transforming on closely related fish taxa, although O'Connell and Neves (1999) did not find any evidence to support this hypothesis. Additional hypotheses

include antigenic variation and the synthesis of immunosuppressive chemicals to avoid detection by the host (O'Connell and Neves 1999), but these hypotheses have not yet been investigated. Relating the results of my study to possible explanations, it seems possible that immunity might be related to fantail darter population variability at the MHC and its recognition of glochidial antigens as foreign (Eric Hallerman, VPI & SU, personal communication).

The MHC has been shown to play a key role in the recognition aspect of the immune response (van Muiswinkel 1992). This group of genes is highly polymorphic in vertebrate species and, therefore, high levels of individual variation exist (Wiegertjes *et al.* 1996). It is a tightly linked complex that is inherited as a unit (Fudenberg *et al.* 1984). Because the probability of two individuals sharing a combination of all alleles at these loci is extremely low (Danska and McDevitt 1987), it is possible for nearly every individual of a species to have a unique MHC genotype. For example, goldfish (*Carassius auratus*) exhibited extremely high genetic diversity at the MHC, even between sublings (Maxey *et al.* 1997). The MHC, with its two major groups of genes, is responsible for recognizing antigens as foreign material (Class I) and presenting these antigens to the T-cells (Class II). It is the MHC that is responsible for initiating rejection or non-rejection of transplanted tissue (Austyn 1989). In this capacity, the possibility exists that the MHC, in the immune systems of susceptible populations of host fish, does not recognize juveniles as "non-self," which allows glochidia to encyst and transform into juveniles. If the MHC does not recognize the glochidial antigens to be non-self antigens and, therefore, does not present them to T-cells, the immune response does not occur, and the glochidium remains.

The MHC primarily is responsible for parasite and pathogen resistance, and this is of particular adaptive significance (Hedrick 1994, Hedrick and Parker 1998). The high allelic diversity observed at the MHC is presumed to be maintained through a diversifying selective force, such as the selective pressure incurred by parasites and pathogens (Hedrick 1994, Paterson *et al.* 1998). Presence or absence of various parasites and pathogens in different streams would cause MHC haplotype frequencies to vary between the populations in those streams.

Population-level immunogenetic variability, analogous to that discussed here, has been observed previously in fish. Coho salmon (*Oncorhynchus kisutch*) from different drainages in British Columbia have exhibited differences in natural resistance to *Renibacterium salmoninarum*, the primary cause of bacterial kidney disease, and these differences were attributed to genetic variation (Wiegertjes *et al.* 1996). Similarly, Sovenyi *et al.* (1988) noted the significance of genetics when carp (*Cyprinus carpio*) were infected with the parasite *Aeromonas salmonicida*, which causes erythrodermatitis. In their experiment, homozygous individuals were significantly more affected by the bacterium than were hybrids. Finally, heritability of tolerance to infectious hematopoietic necrosis was determined to be around 30% in sockeye salmon (*Oncorhynchus nerka*) under natural selection pressures (McIntyre and Amend 1978), suggesting a complex genetic basis for resistance.

Because the Roanoke River and Indian Creek are in two entirely different river drainages with no connectivity, there presumably are differences in the pathogenic challenges experienced by the respective populations of fishes. The selective pressures posed by these pathogens in the watersheds are probably stronger than those posed by glochidial parasitism, and the fish populations likely have adapted to those stronger pressures. Fantail darters and tan riffleshells have been together in Indian Creek for millennia. The fantail darters have put a great deal of selective pressure on the mussels to avoid rejection by the fish's immune system, and in turn, the glochidia likely have imposed a relatively small degree of selective pressure on the fantail darters to develop immunity to avoid over-infestation (which can result in death). Parasites have been shown to have a regulatory effect on their host populations. In yellow perch (*Perca flavescens*), the nematode parasite *Raphidascaris acus* caused disproportionate mortality in maturing males because they had higher weight-specific metabolic demands than females. This resulted in females dominating the adult population, and growth rates were reduced in both sexes. This type of regulation has genetic implications, such as selection for individuals with these slower growth rates (Szalai and Dick 1991). However, because natural levels of glochidial infestation have little detrimental effect on fish, the fantail darter's immune system likely is not regulated by glochidia and instead is influenced by various disease organisms in the watershed. Because disease prevalence varies from

watershed to watershed, the genetic makeup of the fantail darter immune system would vary from watershed to watershed.

The South Fork Holston River has historically contained tan riffleshells (Ortmann 1918), but the species has not been collected from this stream since then. Elk Garden has never contained a tan riffleshell population. Thus, the intermediacy of fantail darters from these streams as suitable hosts may be explained by the "stepping stone" model of population genetics (Kimura and Weiss 1964), in which populations of a species are distributed discontinuously over the landscape. This model is especially appropriate in streams, where suitable habitat can be considered as having a one-dimensional distribution. Individuals may move between populations, but the likelihood of mating with an individual in an adjacent population is much greater than mating with an individual in a non-adjacent population. Genetic variation according to the stepping-stone model has been encountered in various fish species. The least killifish (*Heterandria formosa*) in Florida demonstrated significant genetic variation at three highly polymorphic loci between two forks of the St. Johns River (Baer 1998). Additionally, the Mediterranean killifish (*Aphanius fasciatus*) demonstrated a significant negative relationship between gene flow and geographic distance and was genetically structured according to the stepping-stone model (Maltagliati 1998).

Because fantail darters in the South Fork Holston River and Elk Garden are not contiguous with the Indian Creek population, genetic exchange occurs rarely among these populations, resulting in distinct genetic differences. Additionally, the Roanoke River population of fantails is physically isolated from the other three populations, and genetic exchange is prevented. Differential selection and genetic drift would cause fish in the Roanoke River to exhibit different immunological characteristics than those in the other systems. Therefore, if Indian Creek fantail darters are the most suitable host fish, then populations of fantail darters in proximity would serve as less suitable hosts, and those completely isolated from the tan riffleshell would be poor hosts.

Neves *et al*. (1985) stated that there are three requirements that must be met for a successful fish-glochidia relationship: contact must occur between glochidia and host fish; fish must be suitable for encystment and metamorphosis of glochidia; and glochidia must be resistant to host fish responses. The first of these requirements is underscored by

the situation in Indian Creek; contact between fantail darters and tan riffleshells has been continuous. Because of this sympatry, coadaptation likely has occurred and resulted in glochidia not exhibiting antigens that elicit immune responses.

That differential disease pressures among populations of a species can result in different immunogenetic makeup is demonstrated by the Gila topminnow (*Poeciliopis occidentalis occidentalis*) (Hedrick and Parker 1998). This federally endangered fish is found in four isolated Arizona watersheds. Variation at the MHC has been found to be fairly extensive among the four populations, with only two instances of shared MHC alleles. This variation was attributed to differential disease pressures within each watershed and is indicative of adaptive differences among fish populations. A similar example exists in Soay sheep (*Ovis aries*) (Paterson *et al.* 1998). The unmanaged population on the Scottish island of Hilda is subject to high levels of intestinal parasitism by strongyle nematodes, which cause high over-winter mortality of infected individuals, especially juveniles. Because parasite resistance is governed primarily by the MHC, this relationship suggests that the parasites influence the sheep to maintain high levels of MHC diversity. The sheep with genes associated with parasite resistance (and, therefore, high juvenile survivorship) will survive to pass on those genes. In this case, selection is occurring at the MHC and causing that population to differ genetically from other sheep populations not experiencing this selection.

In freshwater mussels, there are several examples of mussel-host compatibility in which the ranges of the mussel and host do not overlap. Obviously, these relationships are not natural but occur only when infestation is induced. For example, Neves *et al.* (1985) found that the slimy sculpin (*Cottus cognatus*) served as a suitable host for the mountain creekshell, *Villosa vanuxemensis*. Additionally, the Florida largemouth bass (*Micropterus salmoides floridanus*) and Suwannee bass (*M. notius*) were parasitized successfully by the Alabama rainbow (*V. nebulosa*). In both cases, the species of fish that served as hosts for the mussels were in the same genera as their natural hosts. For *V. vanuxemensis*, the number of juveniles that transformed per fish were approximately the same for all *Cottus* species tested. It is worthy to note that *C. cognatus* is most closely related to the mottled sculpin, *C. bairdi* (Jenkins and Burkhead 1994), which is a natural host for *V. vanuxemensis*. For *V. nebulosa* (=*V. iris*), its natural host, the largemouth bass

(*M. salmoides*), yielded more juveniles per fish than the other *Micropterus* species tested. *V. nebulosa* did not transform on any exotic fish species tested. While these mussels were able to transform on allopatric fish species, they were only able to do so on fish closely related to their natural hosts, again suggesting a genetic basis for the host relationship.

The fanshell (*Cyprogenia stegaria*) is found in the upper Tennessee River drainage (Parmalee and Bogan 1998), and suitable hosts include the blotchside logperch (*Percina burtoni*) and tangerine darter (*P. aurantiaca*) from the Tennessee drainage (Jess Jones, VPI & SU, personal communication). These fish are considered highly suitable hosts, but they are not useful for propagation because of their rarity and difficulty to maintain in captivity. Therefore, the fish used most successfully for fanshell propagation is the Roanoke darter (*P. roanoka*), which does not occur in the Tennessee River drainage. There are no quantitative data on the suitability of the Roanoke darter as a host species relative to the tangerine darter and blotchside logperch, but it is believed that the Roanoke darter produces fewer juveniles (J. Jones, VPI&SU, personal communication). However, because of their abundance and their success in the laboratory, the Roanoke darter is used for fanshell propagation. Even though these species are not sympatric, the Roanoke darter produces a large number of juvenile fanshells. This does not necessarily contradict the hypotheses presented here. If glochidial antigens escape detection by a species of fish, and this elusion is genetically based, then it makes sense that related species would serve as hosts to some degree. The degree of host specificity varies among mussel species (Zale and Neves 1982, Isom and Hudson 1984), and, therefore, the degree to which allopatric species serve as hosts likely varies as well.

The Tennessee River drainage contains the most diverse mussel assemblage in North America (Parmalee and Bogan 1998), while the Roanoke River contains relatively few mussel species, which sets the stage to explain differences in host fish suitability. The fish within the Tennessee River system have likely been resisting glochidial parasitism by both host and non-host species for millenia, which may have resulted in an "arms race" between the fish and the mussels within the system. The biological arms race theory is a widely accepted model for the evolution of host-parasite interactions (Stahl *et al.* 1999). As the host species develops resistance to the parasite, the parasite

evolves new adaptations to avoid resistance. This relationship has been observedfrom wasps and flies (Weis *et al.* 1989) to birds (Redondo *et al.* 1995), and likely exists between fish and parasitic glochidia. Fantail darters in the Tennessee River system, because of this potential arms race, may have continuously had to adapt to glochidial attachment. In turn, tan riffleshells would have to adapt to the changing immunological characteristics. Fantail darters in the Roanoke River would not be continuously adapting to the presence of glochidia, and therefore, mussel glochidia would be more highly adapted to the fantail darters from the Tennessee River system.

Exotic species have been shown to be suitable hosts for some mussel species. The paper pondshell (*Utterbackia imbecillis*) is considered a host generalist (Trdan and Hoeh 1982). This mussel successfully transformed on 30 of the exotic species tested (Watters 1997). *U. imbecillis* glochidia, like all anodontines, attach to the fins of their hosts. Fins are less vascular than gills (Moyle and Cech 1996) and therefore may not elicit as strong an immunological response as glochidia that attach to gills. Additionally, most of the exotic species tested were not allopatric with any members of the unionid family, and thus were naïve to glochidial infestation. This may have facilitated transformation: the fish, having had no experience (individually or evolutionarily) with rejecting glochidial infestation, were unable to slough the glohidia. Furthermore, the pocketbook (*Lampsilis cardium*) transformed successfully on only 6 exotic species tested, although the most suitable host was the largemouth bass, which is a natural host. These six fish species were either related to natural hosts of other North American unionids, or they were found within the range of Asian unionoids, where they perhaps serve as hosts (Watters 1997)

In summary, I hypothesize that variations in mussel antigens and host immunogenetic variation mediate success of transformation of juvenile tan riffleshells among populations of fantail darters. It is likely that variation occurs in both parasite and host, in the proteins and carbohydrates present on the surface of glochidia and in the fish's immune recognition of these proteins and carbohydrates. The inferences drawn in this discussion require further research, to include examining host specificity between populations of a mussel species, as well as immunogenetic studies of host fish and mussels. Additionally, information on the minimum transmission level necessary to

sustain a population is necessary to understand whether these results are biologicaly significant, or simply an artifact of maximum infestation. If my inferences are correct, then mussels and their host fish are more highly coadapted than previously demonstrated.

Management Implications

Glochidia often are removed from a gravid female in a healthy population, infested onto host fish, and the juveniles are released at another site. However, if differences in host suitability among populations are widespread, the transplanted species may not produce as many offspring at the new site. If the new site is within the same drainage as the source population, my results do not suggest a conflict, as all fantail darters from upper Tennessee River sites were not significantly different. Additionally, when hosts are being infested with glochidia in the laboratory, fish from drainages where the mussel does not occur often are used in order to avoid the possibility of acquired immunity. However, results of this study suggest that using fish from external watersheds also may reduce the production of juveniles of highly host-specific species, versus using fish from watersheds within the species range.

Tan Riffleshell Population

Aging

The use of thin-sections to ascertain ages in mollusks has been well demonstrated in marine bivalves. Internal growth rings are considered to be much more reliable indicators of age than those on the exterior of the shell. MacDonald and Thomas (1980) found that the numbers of growth lines in thin-sections directly corresponded to known ages of the softshell clam (*Mya arenaria*), and that these internal lines were more reliable than external rings. While not as well documented, thin-sectioning of freshwater mussels seems to be the most accurate technique for age determination (Neves and Moyer 1988). The results of my study seem to substantiate this. Although there were no known-age specimens to validate the technique, it was apparent that thin-sectioning was the more appropriate technique for older individuals. Because older tan riffleshells (7+ years) lay down growth rings close together, thin-sectioning allowed for easy differentiation of

those rings at the shell margin. Additionally, the early growth rings that typically eroded from the periostracum were readily identifiable in thin-sections.

A comparison of thin-sections with externally aged individuals reinforces the conclusion that counts of external rings are not a dependable aging technique. Erosion of the periostracum, dark shell coloration, the inability to differentiate false annuli, and closely laid rings in older individuals result in dubious age estimates. However, at streamside one often needs a quick method of aging, and it is often not feasible or legal to sacrifice individuals for thin-sectioning. An alternative is to collect shells and compare external versus internal ages. Thin-sections improve accuracy of aging and may validate external ring counts (McCuaig and Green 1983, Neves and Moyer 1988).

Comparing thin-section ages with the size-frequency histogram illustrates the necessity of using growth rings instead of dominant size classes to determine ages. The size-frequency histogram showed no prominent year classes, probably due to slow growth and individual variation. The oldest individual that was thin-sectioned was 11 years and 49 mm in length. This individual was smaller than four other mussels, each of which was determined to be at least three years younger. Therefore, lengths of mussels are not reliable indicators of age, particularly in old specimens.

Relatively few false annuli were detected in this study. The five that were detected appeared to be due to the incorporation of particles of the substratum into the shells, which resulted in a line that extended out to the shell margin (Neves and Moyer 1988).

The tan riffleshell exhibits rapid growth within the first two years of life. Unfortunately, individuals of that age are difficult to capture by snorkeling. Because of this, the length-at-age relationship is linear and does not show the growth rate exhibited during these first two years. Tan riffleshells propagated in the laboratory were generally 0.25 – 0.5 mm in length post-metamorphosis. Those captured at 2 years old were approximately 25 mm. This slowing of growth as mussels age has been well documented (Grier 1922, McCuaig and Green 1983, Day 1984, Neves and Moyer 1988). As the adults begin expending energy on reproduction through gametogenesis, less energy is allocated for body growth and shell construction (Haukioja and Hakala 1978). This is evidenced in older individuals, where the last few growth rings are laid down very close

together and are nearly impossible to distinguish visually, leading to underestimates of the individuals' ages. A thin-section, however, allows the observer to distinguish the growth lines of these last few rings easily and prevents underestimations.

Neither males nor females had very strong correlations between length and age. This variability likely is due to environmental and genetic factors. The greater variability in length-at-age of females than males probably was due to the higher energetic cost of reproduction for females, especially since tan riffleshells brood their offspring over winter. This is especially evident when one examines the size-frequency diagram. The mode of females was in the 42 – 44 mm size class. When the ages of the tagged sample were calculated, most of these females were determined to be approximately seven years of age. However, the sample of mussels used to calculate the regression equation for females included very few individuals between 40 and 44mm. Hence, I am hesitant to state that the tan riffleshell population in Indian Creek is comprised primarily of seven year old females. More likely, females grow to a certain size (in this case, between 40 and 44 mm) and greatly decrease somatic growth but instead put more energy into reproduction. Conversely, males continue to grow because less energy is required for sperm production. There were many more males than females above 44mm in length. However, males and females appear to have similar life spans, with the oldest female aged as 10 years and the oldest male aged as 11 years. Watson (1999) found individuals up to 9 years old. The maximum age for tan riffleshells is not known, although this species likely can live to about 15 years. If this is the case, then tan riffleshells are among the shortest-lived mussel species. This has implications for the sustainability of the population in the face of predation. This species has fewer cohorts, which would cause the loss of a single cohort to have a stronger effect on the population, as one cohort is a larger proportion of the population in a short-lived species. Muskrats tend to prey on reproducing individuals, because they are elevated within the substratum in order to attract host fish (for females) and to release sperm (for males). The loss of many individuals of reproductive size can have a large negative effect on the population.

Population Estimate

Several assumptions are inherent in Schumacher's method of population estimation. First, there must be random mixing of marked and unmarked individuals,

which was likely the case. Mussels migrate vertically and horizontally in the substratum, such that there was not an increased likelihood of finding a previously marked individual. Second, there could be no loss of marks during the sampling period, which was likely met as well. Finally, the population could not experience mortality or recruitment throughout the sampling time. This assumption was violated, as recruitment and mortality are continuous in this stream. However, it is reasonable to assume that this population is stable. The Schumacher estimator remains preferable to other estimates that allow for dynamic population sizes, however, because of the very low recapture rate. Other methods, such as Jolly-Seber, require a minimum recapture rate of 10% (Caughley 1977), which was not achieved in this study. The mortality of tagged individuals would cause the Schumacher estimate to be an overestimate of the true population size, while the recruitment of juveniles into the adult, sampled population would tend to underestimate the true population size. Therefore, the abundance estimate produced by the Schumacher method must be considered an approximation of adult population size. Examining the length distribution of the tagged mussels reveals that few mussels were at the upper limit of the size range, suggesting that mortality due to old age was likely low. Additionally, only five mussels below 30 mm were captured after the initial sample, suggesting that the recruitment of juveniles into the adult population is low, as well. Therefore, it does not seem that this third assumption was strongly violated, and this estimate may be considered a close approximation.

Watson (1999) estimated that muskrats were responsible for a large amount of tan riffleshell mortality in Indian Creek. Between 1996 and 1997, he collected nearly 500 valves of the species, and he attributed most of this mortality to muskrat predation. Muskrat trapping has occurred since 1997, and this seems to have reduced their impact on the population. Since late 1997, approximately 20 fresh valves have been collected which have been attributed to muskrat predation, suggesting that this trapping has been highly successful. If that mortality is taken into account, then our estimates of population size are very similar. The estimate of 683 adults, plus the estimated mortality of 500 adults from predation, approximates my estimate of 1078 adults. Additionally, the length distribution of individuals in Watson's sample is very similar to mine, except that there are approximately 50 more individuals in the size classes between 38 and 44 mm. More

individuals have entered these size classes since predation on adults has been reduced. It is obvious that trapping muskrats in Indian Creek is beneficial to the population.

Management Implications

The tan riffleshell population in Indian Creek of 1078 adults is small but reproducing. However, because this is the only known reproducing population of the species, the potential threats loom large. Predators such as muskrats and raccoons are prevalent in the area, and while an intensive trapping effort has reduced their impact on the population, even low predation rates can be detrimental. Additionally, deep mines in the upper watershed present the threat of water quality degradation upstream. Protection of this population and restoration of other populations is of utmost priority, such that the species can persist despite potential threats.

Chapter 2

Tan Riffleshell Propagation

Introduction

The recovery plan for the tan riffleshell (USFWS 1984) specifies that viable populations of the species must be established in at least three additional rivers from those known at the time the recovery plan was written. Because adult translocations have been generally unsuccessful (*e.g.*, Ahlstedt 1979, Sheehan *et al.* 1989), juvenile propagation and release has been considered a more feasible means of population reestablishment (Gatenby *et al.* 1996). This method customarily produces more individuals for release than adult translocations and has greater potential for restoration of populations.

Tan riffleshells are sexually dimorphic, with adult females displaying a marsupial swelling of the posterior shell margin. They are long-term brooders (bradytictic) and become gravid in the fall. Glochidia are brooded over winter and are released in early spring through mid-summer, with a peak in April. Host fish have been identified as the greenside darter (*Etheostoma blennioides*), fantail darter (*E. flabellare*), redline darter (*E. rufilineatum*), snubnose darter (*E. simoterum*), and various sculpin species (*Cottus bairdi* and *C. carolinae*). Fantail darters have yielded the most juveniles in host fish identification trials and thus have been the primary species used for juvenile production. Glochidia remain attached to the gills of host fish for approximately 2 – 3 weeks until transformation is complete, and they fall from the fish as juveniles. The year of first reproduction is believed to be age 4 (Watson 1999). This species is relatively short-lived; individuals can probably live for about 15 years.

Statement of Objectives

The goal of this study was to demonstrate the feasibility of propagating juvenile tan riffleshells in the laboratory for release into the Hiwassee River in Tennessee. Specific objectives were to test two methods of infesting host fishes and compare two recirculating culture systems for sustaining juvenile tan riffleshells. The null hypotheses were: (1) there were no significant differences in the numbers of juveniles produced by

the two infestation methods, and (2) there were no significant differences in the number of juveniles surviving in either recirculating system. Information on the growth and survival of tan riffleshells in the laboratory can then be applied to improve propagation techniques in subsequent recovery efforts.

Methods

Gravid female tan riffleshells were collected by snorkeling in Indian Creek, Tazewell County, Virginia. They were transported to the Aquaculture Center at Virginia Tech in aerated coolers and placed in the large gravel of a Living Stream set at approximately 15°C. Fantail darters were collected with a backpack electrofisher from various locations in the Roanoke and Tennessee drainages. Fish were transported to the Aquaculture Center in aerated coolers and placed in 38 L aquaria with airstones and no substratum. Pieces of 5 cm PVC pipe were cut in half lengthwise and placed in the tanks to provide cover for the fish.

After several days, the darters were infested with glochidia using one of two methods to determine which yielded more juveniles. In both cases, the glochidia were flushed from the female's gills using a water-filled syringe. The hypodermic needle was inserted into the anterior portion of the gill and the water ruptured the gill chambers, causing the glochidia to flush out into a petri dish. In the first infestation method, the fish were placed together in a 19 L bucket with water approximately 5 cm deep and with a large airstone. The glochidia were then flushed into the water, and the airstone kept them in suspension. The fish were left in the bucket for about 1 h and then returned to the aquaria.

In the second method, the fish were placed into a dilute MS-222 solution to anesthetize them before infestation. The glochidia were pipetted onto each gill, and the fish were placed into another 19 L bucket with water for recovery. Once all the fish had been infested and recovered, they were returned to the aquaria.

For the first 7 days, the fish were fed frozen chironomids, and the water was changed every other day. After 7 days, the aquaria bottoms were siphoned every other day for transformed juveniles. The water was siphoned through a 300 μm sieve to

remove large particles, and then through a 120 μm sieve to collect the juveniles. About one-quarter of the water in each aquarium was removed and replaced by conditioned water after each siphoning. The contents of the 120 μm sieve were flushed into a petri dish and examined with a dissecting microscope at approximately 10x magnification. Juveniles were collected and measured using an ocular micrometer and placed into 9 cm square petri dishes with 1 cm of substratum comprised of particles <500 μm. They were held in recirculating culture troughs until ready for release.

Two different culture systems were used to rear the juveniles. The primary type was a large 175 L trough connected to a 225 L reservoir tank (Figure 2-1). Water was pumped from the reservoir tank to the trough, flowed over the culture dishes, and was gravity-fed back into the reservoir tank. Depth of water in the trough was approximately 18 cm. The second type of culture system held 145 L of water (Figure 2-2). The water was air-lifted from the reservoir into the trough and then gravity-fed back into the reservoir. This culture trough was smaller, with a water depth of approximately 12 cm. Water flow was more turbulent in this system than in the other culture system. The flow rates were approximately 9.5 L/min in the first raceway and 12.6 L/min in the second. Mussels in both troughs were fed an algal mix of *Scenedesmus* spp. and *Neochloris oleoabundans* daily. Algae was introduced to the systems daily at a level of approximately 20,000 cells/mL.

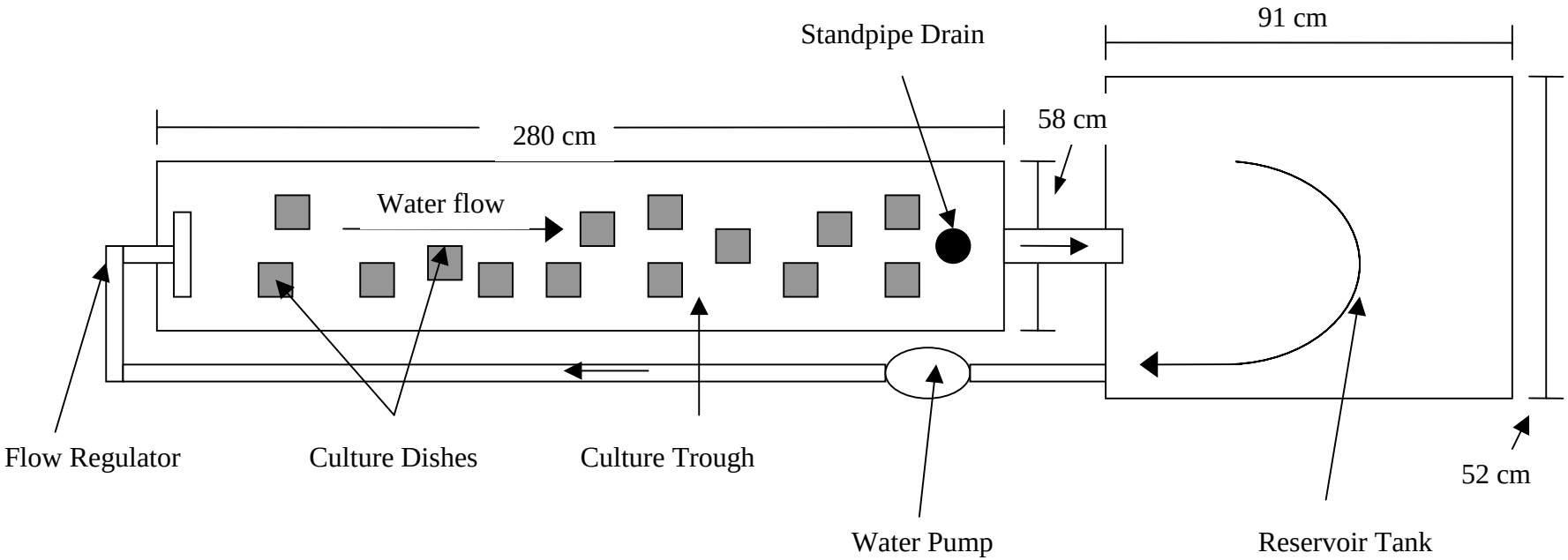

Figure 2-1. Top view of the first, larger recirculating culture system for juvenile mussel propagation. Water was pumped from the reservoir tank through the flow regulator and over the dishes in the trough. It then flowed down the standpipe drain and back into the reservoir tank.

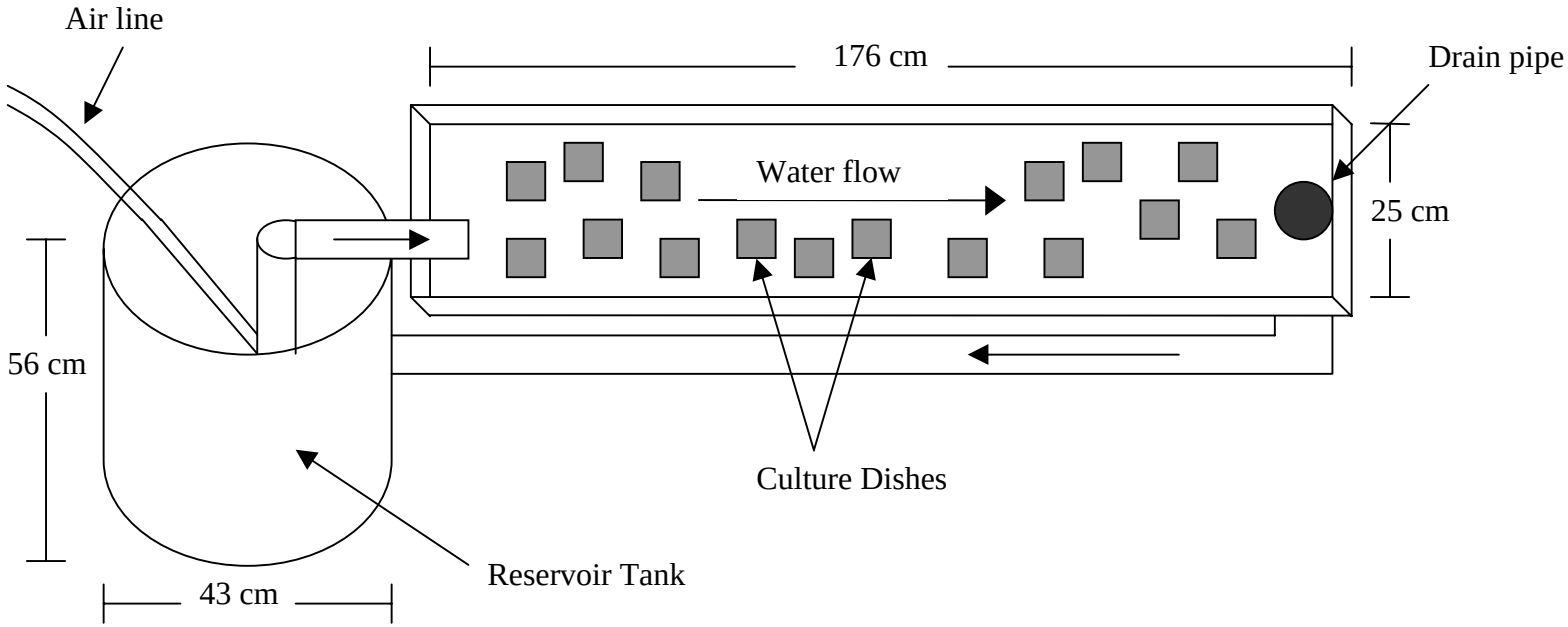

Figure 2-2. Top/side view of second, smaller recirculating culture system for juvenile mussel propagation. Water was air-lifted from the reservoir tank into the culture trough and then was gravity-fed back through the drainpipe to the reservoir tank.

Results

Infestation Technique

Fantail darters were infested with tan riffleshell glochidia four times with each infestation method. The pipetting method (mean = 10.23, sd = 8.56) was slightly more successful than the bucket method (mean = 5.74, sd = 2.82) for infesting fish (Table 2-1). Because different numbers of fish were used during each infestation, the number of juveniles per infestation method was standardized by computing the number of juveniles that transformed per fish infested. The pipetting method yielded more juveniles per fish than the bucket method nearly every time, although the two methods were not significantly different ($p = 0.39$).

Culture System

The large culture troughs were used for tan riffleshell propagation five times, and the small culture troughs were used three times. In comparing the survival in each culture system, it is obvious that the small troughs are inadequate for tan riffleshell propagation (Table 2-2). The small troughs resulted in 0% survival for each of the three trials. Conversely, the larger systems averaged 9.9% survival (sd = 7.56), although the duration of time the juveniles were held varied. The previous calculation of mean survival for the large troughs excluded the infestation conducted on March 30 because those juveniles were held for only 9 days and had not reached the survival bottleneck that typically occurs between 2 and 4 wk of age. The mean survival of each system could not be compared statistically because the small troughs yielded 0% survival each time, and no variation could be calculated.

The Hiwassee River, in Polk County, Tennessee, was selected as the first site for augmentation of a tan riffleshell population. Tan riffleshells have inhabited this stream historically (Parmalee and Hughes 1994), but no evidence of recruitment has been observed in recent years. Appropriate host fish, including *Cottus* spp., *Etheostoma blennioides*, *E. rufilineatum*, and *E. simoterum*, occur within the Hiwassee system (Hitch and Etnier 1974). The selected release site is within the approximately 13 km of stream from which the Tennessee Valley Authority diverts water at the Apalachia Dam. The

TABLE 2-1. Number of juvenile tan riffleshells collected from the two infestation techniques. Numbers of juveniles per fish infested between the two techniques were not significantly different (p = 0.39).

Date	Infestation method	Number of fish	Number of females	Juveniles collected	Juveniles/ fish
6/18/97	bucket	58	2	261	4.50
7/18/97	bucket	44	1	226	5.14
3/30/99	bucket	120	4	418	3.48
5/18/99	bucket	82	4	808	9.85
				Mean:	5.74 (sd = 2.82)
4/7/98	pipette	23	3	520	22.61
5/14/98	pipette	55	3	202	3.67
5/25/98	pipette	26	2	238	9.15
10/27/98	pipette	41	1	226	5.51
				Mean:	10.23 (sd = 8.56)

TABLE 2-2. Summary of transformation of *Epioblasma florentina walkeri* on fantail darters, and fate of juveniles. Valve height was measured dorso-ventrally, and length was measured anterio-posteriorly.

Date infested	Number of fish infested	Number of females used	Total number of juveniles collected	Culture System	Percent Survival (days)	Average size (mm) Height	Length	Fate
6/18/97	58	2	261	large	11.0 (81)	1.09	0.83	released
7/18/97	44	1	226	large	18.4 (53)	0.72	0.56	released
4/7/98	23	3	520	small	0 (65)	-	-	died in lab
5/14/98	55	3	202	small	0 (32)	-	-	died in lab
5/25/98	26	2	238	small	0 (21)	-	-	died in lab
10/27/98	41	1	226	large	0 (63)	-	-	died in lab
3/30/99	120	4	418	large	~100 (9)	0.31	0.26	released
5/18/99	82	4	808	large	10.3 (114)	1.31	1.78	released
		Total Collected:	2899					

water is sent to the Apalachia Powerhouse and returned to the Hiwassee River about 13 km downstream of the dam. The cut-off reach has been deemed highly suitable as habitat for mussels because the dam prevents extremely high or low flows. Tan riffleshells were released at this site on three occasions (Table 2-3). Although there were four infestations in 1998, none of the propagated juveniles survived for release, likely due in part to the culture conditions. Juveniles collected from the first three infestations in 1998 were held in the small recirculating systems, which were poorly suited for tan riffleshell culture. Mussels were released in a backwater above the riffle along the left ascending bank of the Hiwassee River just above the Route 68 bridge crossing in Polk County, Tennessee.

TABLE 2-3. Summary of releases of juvenile *Epioblasma florentina walkeri* in the Hiwassee River, Polk County, Tennessee. Valve height was measured dorso-ventrally, and length was measured anterio-posteriorly.

			Average size (mm)	
Date	Number of juveniles	Age	Height	Length
9/24/97	52 (2 infestations)	2-3 months	1.09	0.83
4/28/99	418	~ 1 week	0.31	0.26
9/16/99	83	3.5 months	1.31	1.78
Total Released	553			

Discussion

Infestation Technique

Although the difference in the mean numbers of tan riffleshell juveniles obtained by infesting fantail darters using the bucket method versus the pipetting method was not statistically significant, some qualitative comparisons can be made. The pipetting method yielded more juveniles per infested fish than the bucket method; however, more fish died when the pipetting method was used. This higher number occurred likely because pipetting the glochidia directly onto the gills ensured contact between the glochidia and the gills of the fish, and thus increased the number that attached and transformed. However, this technique was probably more stressful to both fish and glochidia than the bucket method. The fish were anesthetized and handled more with this method, and the glochidia were likely exposed to the anesthetic when pipetted on the fish gills. Additionally, the pipetting method was time-consuming, taking approximately one hour for the infestation of 50 fish. When very large numbers of fish and glochidia are used, this amount of time can be detrimental to the organisms, as the stress is incurred over a long period of time. Therefore, although the pipetting method often yielded more juveniles per fish, I recommend the bucket technique for infestation.

Culture System

The small culture systems were unsuitable for tan riffleshell propagation. Tan riffleshells seemingly are sensitive to environmental conditions and are difficult to culture successfully. The high amount of turbulence within the small systems was likely a cause of mortality for the majority of the juveniles in those troughs, and also possibly washed them out of the culture dishes and into the reservoir trough. Although the bottoms of the troughs were siphoned for juveniles occasionally, very few juveniles were recovered. This low recovery rate indicates that either the juveniles had died previously and their valves decomposed, or they had been washed into the reservoir tank and were not recovered. Regardless, all recovered juveniles were found dead. Therefore, whether light levels or the turbulence directly caused mortality or did so indirectly by washing the juveniles from the dishes, these conditions are unacceptable for tan riffleshell culture.

Because the small troughs were kept indoors at the Aquaculture Center at Virginia Tech, low light levels reached the culture troughs. The low light levels may have depressed juvenile survival, as direct sunlight aids the production of algae and other biological material on which juveniles potentially feed (see Chapter 3).

Few culture studies have examined the performance of rare mussel species in culture environments. Information on the performance of these more sensitive species in culture is important to ensure that maximum numbers of juveniles survive to be released to natal rivers. The threatened snuffbox, *Epioblasma triquetra*, was cultured in lake water and fine sediment in individual containers without a recirculating component for 42 days, with fairly good growth and survival (Hudson and Isom 1984). At 18 days, survival was approximately 80%. Unfortunately, percent survival was not quantified at 42 days, and was only referred to as "good." Additionally, the federally endangered oyster mussel, *Epioblasma capsaeformis*, has been successfully transformed on host fish in the laboratory, but survival in culture has been very low (Jess Jones, VPI &SU, personal communication). The bottleneck in survival that typically occurs between 2 and 4 wk has tended to reduce survival of the oyster mussel in culture situations to less than 10%.

Management Implications

The tan riffleshell is not a species maintained easily in the laboratory. Attempts to hold this species for longer than approximately 3 mo have had very low success, and this likely is due to inappropriate culture techniques. It seems that using the bucket infestation method and holding the juveniles in the large culture troughs with a source of sunlight should increase juvenile survival, but much more work needs to be done. Many other factors need to be examined, including optimal temperature and food suitability. Culturing endangered mussels in order to release them into their natural habitat is a promising means of population recovery, but much more testing of appropriate culture conditions is necessary to reduce mortality during the early juvenile stage.

Chapter 3

Effects of Substratum and Light on Juvenile Propagation

Introduction

A procedure for culturing endangered species of mussels has been developed to reduce sources of high mortality and provide sufficient progeny to implement recovery plans (Beaty and Neves 1996, Gatenby *et al.* 1997, O'Beirn *et al.* 1998, Steg 1998). To increase glochidia-host contact, glochidia of endangered mussels have been infested manually onto gills of appropriate host species. The transformed juveniles then have been held in culture systems to increase post-transformation survival, as the first several weeks after metamorphosis have proven to be periods of high mortality (O'Beirn *et al.* 1998, Steg 1998). After reaching sufficient size, the juveniles can be released into suitable habitats to augment existing populations or to reintroduce the species into historic habitat of a river reach.

The recirculating aquaculture systems for rearing juvenile mussels have had varying degrees of success. Survival tends to range from approximately 2% to as high as 35-40%, and was typically 20% (O'Beirn *et al.* 1998, Steg 1998). Conditions in culture systems may be manipulated to increase survival and growth. Ideally, laboratory conditions should mimic environmental conditions in natural habitats. Factors such as flow rate, substrate size and composition, light availability, temperature, and food availability all can be manipulated in attempts to increase juvenile survival.

The culture of mussels to supplement existing populations began after the turn of the century. Lefevre and Curtis (1910, 1912) investigated artificial propagation to counteract declining mussel populations in the Mississippi River, although they had little success. Howard (1915) had some success in rearing juveniles in floating crates in the Mississippi River. Most of these historical culture efforts were performed in semi-natural environments; *i.e.*, concrete ponds, metal troughs, or crates floating in streams or ponds (Coker *et al.* 1921). These systems did not allow for control of many variables, such as temperature or input of material (organic or inorganic), and substratum was rarely included. The juveniles were not provided with any supplemental food, which, in conjunction with the above factors, may explain why these attempts had limited success.

Much work has been done on various aspects of mussel culture since that time. Concerning growth, larger individuals of juvenile freshwater pearl mussels (*Margaritifera margaritifera*), cultured for transplanting in formerly inhabited streams, survived their first winter, whereas all individuals under 700 μm (at 3 mo) did not (Buddensiek 1995). Additionally, a suitable water hardness for juvenile *Lampsilis fasciola* in culture is approximately 250 mg/L (Steg 1998). Other factors, such as flow rate, food suitability, substratum suitability, and dish size and type, are being evaluated (J. Jones and S. Hanlon, VPI&SU, personal communication).

Importance of Sediment in Culture

Several studies have focused on the role of sediment in culture systems for juvenile mussels. Sediment in culture systems facilitated feeding in juvenile rainbow mussels (*Villosa iris*), and these juveniles yielded higher growth and survival than those observed in the wild (Gatenby *et al.* 1996). Juvenile freshwater mussels feed pedally until their gills are formed fully and they begin filter-feeding. Hence, for juveniles, sediment facilitates collection of food materials, as interstitial spaces promote feeding by the juveniles by providing areas for the deposition of food particles (Gatenby *et al.* 1996), especially since the juveniles burrow into the substratum and are not exposed to the overlying water (Yeager *et al.* 1994). Survival of juvenile *Anodonta imbecilis* increased when fine sediment was added to the culture dishes (Hudson and Isom 1984). As these studies show, the presence of sediment is important in juvenile culture. However, the composition of sediment that is best for culture is unknown.

Different species of mussels, both marine and freshwater, have been observed to exhibit different substratum preferences. Substratum composition has been demonstrated to influence freshwater mussel species assemblages (Negus 1966, Tevesz and McCall 1979, Lewis and Riebel 1984), and mussels likely are adapted to particular environments (Coker 1921). Some species of mussels have fairly restricted substratum preferences (*i.e.*, sand or gravel), while others are more ubiquitous (Sickel 1980, Huehner 1987). Differential sediment size selection between groups of adult *Lampsilis radiata siliquoidea* has been observed (Bailey 1989). Juveniles of *Villosa iris* held in an artificial stream system with various sediment sizes (<120 μm and 120 μm – 600 μm) did not

show differences in growth or survival, but this may have been because a fine layer of fine sediment was deposited on all of the dishes used (Beaty and Neves 1996). Sediment preferences likely differ between juveniles and adults of the same species, as juveniles tend to be found in finer substrata than adults (Neves and Widlak 1987).

Effects of Light

Convenience or presumption often evokes the use of various culture techniques rather than the knowledge that those methods yield healthy juveniles. One of these techniques includes the covering of culture tanks to reduce algal growth. Open tanks quickly become covered with algae, especially when the tanks are exposed to direct sunlight. However, covering the tanks generally results in a decrease of direct light and a change in the natural light regime. These factors may be important in juvenile growth and survival, especially if photoperiod acts as a cue for circadian rhythms. Additionally, algal growth is reduced when culture troughs are covered, which may affect food availability. Conversely, the abundance of algal production in uncovered troughs might be detrimental to the juveniles for several reasons. First, when unicellular and filamentous algae proliferate in the systems, they tend to settle on the bottom of the troughs. The settled algae can become thick enough to effectively smother young juveniles. Additionally, the production of large amounts of algae results in high amounts of decaying matter, which may reduce the amount of dissolved oxygen in the bottom of the troughs. Finally, predatory organisms may thrive in the lighted environment.

Freshwater mussels have been documented to exhibit daily rhythms, which may be cued by daylight. *Carunculina texasensis* was observed to show diurnal fluctuations in sodium and chloride concentrations in the blood, and these fluctuations were attributed to photoperiod (Graves and Dietz 1980). Additionally, *Elliptio complanata* displayed increased opening behavior within an hour after light onset and light termination (Imlay 1969). *Anodonta cygnea* increased activity during night hours, although they were not exclusively active during this time (Salanki 1977). These periodic rhythms may not be tied to photoperiod and instead may be driven by oxygen saturation and temperature fluctuations, which also fluctuate between day and night. Finally, zebra mussels (*Dreissena polymorpha*) raised in artificial rearing tanks migrated to the underside of

rocks on the opposite ends of the tanks from the light sources (Zhang *et al.* 1998).
The above examples demonstrate that freshwater mussels exhibit responses to light in the form of circadian rhythms and phototactic behaviors. The interference with these rhythms through covering culture troughs could influence survival and growth of juveniles.

Statement of Objectives

This study aims to determine whether sediment composition and light regime play a role in juvenile survival or affect their health, as assessed through shell growth. These factors may help to further refine mussel propagation as a feasible avenue for species recovery.

Specific objectives are as follows:

1. To determine whether substratum composition and light regime play a significant role in survival or growth of juvenile *Lampsilis fasciola*.
   H_o: Survival and growth of juveniles do not differ when juveniles are reared with different light regimes and substrata of different sizes.
2. To determine whether high versus low light conditions yield different levels of survival and growth for juvenile *Lampsilis fasciola*.
   H_o: There is no difference in survival and growth of juveniles under high versus low light levels.
3. To determine whether juvenile *Lampsilis fasciola* behave differently in observation tanks exposed to the natural light regime versus observation tanks that are covered.
   H_o: Juveniles behave similarly in open versus covered observation tanks.

Methods

Part 1: Sediment Preferences and Light Regime in Culture

To examine the effects of sediment particle size and light regime on freshwater mussels in culture environments, the wavyrayed lampmussel (*Lampsilis fasciola*) was used as a test species. Five gravid females were collected from Indian Creek, Tazewell County, Virginia and held at approximately 18°C in a Living Stream at Virginia Tech's Aquaculture Center. Sixty host fish (10 cm largemouth bass, *Micropterus salmoides*) from a hatchery were infested with glochidia. The infestation procedure consisted of holding the fish together in a tank with an airstone and approximately 15 cm of water, just enough to cover the fish. The glochidia were extracted from the females by inserting a water-filled syringe into the first of the gill chambers in which the glochidia were brooded. By pressing on the plunger, water ruptured the chambers and the glochidia were flushed out. The glochidia were collected in a petri dish and introduced into the water with the bass. The bass were left in the water with the glochidia for approximately 20 min, which was long enough to ensure infestation but not so long as to over-infest the fish (a source of mortality). At the end of 20 min, the bass were removed from the tank and returned to the recirculating system in which they were held. This system provided a way to hold large numbers of fish with minimal maintenance. It consisted of three 800 L tanks connected to an ultraviolet filter, a biofilter, and a chiller unit. A standpipe drained the water from each tank and connected each to the main system. This system, as well as the weekly partial water changes, kept ammonia levels low in the system. The water was kept at approximately 24°C. After 14 days, the bottom of the tank was siphoned for juveniles. The water was passed through a 300 μm sieve to remove debris and then through a 120 μm sieve to collect the juveniles. Juveniles were collected between days 12 and 25, and over 8,000 juveniles were collected by day 17.

The sediment to be used for this experiment was collected from the Clinch River near the mouth of Indian Creek, in the town of Cedar Bluff in Tazewell County, Virginia. The occurrence of two federally endangered species at this location was presumed to indicate that substratum was suitable for mussels. It was sieved into the following four categories: <120 μm (fine sediment), 500 μm – 800 μm (fine sand), 1000 μm – 1400 μm

(coarse sand), and <1400 μm (mixed sediment). Approximately 0.5 cm of sediment was placed into 9 cm square plastic dishes. Fifty juveniles were placed into each of 40 dishes of each sediment type, for a total of 160 dishes. Ten dishes of each sediment type were then placed into one of four recirculating raceways, which were composed of 175 L culture troughs connected to 225 L reservoir tanks (Figure 3-1). Two raceways were covered with black plastic to block all light, while the other two remained open to ambient light. The mussels were fed at a level of 20,000 cells/mL of algae (*Scenedesmus* spp.) daily. The water was changed in the systems, and debris was cleaned from the sides and bottom of the tanks and troughs every 2 wk.

Two dishes of each sediment type from each raceway were sampled at weeks 2, 4, 8, 12, and 16 by elutriation. Elutriation separates particles by density; as water flows up a 5 cm diameter clear PVC pipe, the less dense juveniles are lifted up and out of the pipe and are collected in a sieve. The number of survivors was noted, and the first 5 juveniles encountered were measured from each dish. Differences in growth and survival of the juveniles in each treatment were compared using an analysis of variance according to the split-plot design with subsampling (Ott 1993). The troughs were considered to be the whole plot, and the sediment types were the split plots. The dishes containing juveniles were the experimental units, and significance was determined to be $p < 0.05$.

Part 2: High versus Low Light Conditions

This trial was conducted to determine whether survival and growth of juvenile *L. fasciola* increased because of direct light or because of the increased production that results from direct sunlight. Therefore, high-light and low-light conditions were examined. Additionally, the sediment sizes that yielded the highest and lowest survivals in Part 1 (fine sand and fine sediment) were used in this experiment in order to replicate the previous results. Three of the raceways described above were used for this experiment. Each was divided lengthwise into two sections: one half was covered with 50% shade cloth to create low-light conditions, and the other half was left open to full sunlight. The half that received the shade cloth was randomly determined for each trough.

Approximately 100 largemouth bass, purchased from a hatchery, were infested with glochidia from 8 gravid *L. fasciola*. The infestation technique and holding facility for the bass were identical to those described in Part 1. The tank bottoms were siphoned for juveniles beginning on day 14, and the water was passed through a 300 μm sieve to remove debris and then through a 120 μm sieve to collect the juveniles.

The sediment used for this experiment was from the same source as for Part 1. Dishes (9 cm square) were filled with 0.5 cm of either fine sediment (<120 μm) or fine sand (500 μm – 800 μm). Fifty juveniles were placed into each of 180 dishes. Thirty dishes of each sediment type were placed into each raceway, with 15 of each under the shade cloth and 15 of each on the open side. The juveniles were fed *Scenedesmus* spp. daily at a level of 20,000 cells/mL. The troughs were cleaned approximately every 2 wk, which consisted of changing the water and cleaning the bottom and sides of the troughs and reservoirs.

Survival and growth were monitored at weeks 2, 4, 8, 12, and 16. Six different dishes of each sediment type from each raceway (three from the shaded side and three from the open side) were sampled at each interval. Differences in survival and growth of the juveniles in each treatment were examined with an analysis of variance according to the split-split-plot design with subsampling (Ott 1993). The troughs were considered to be the whole plots, light treatment was the split plot, and sediment type was the split-split plot. Experimental units were the dishes containing juveniles, and the significance level was set at $p < 0.05$.

Part 3: Response of Juveniles to Light

One-year-old juveniles were used in this experiment because they were large enough to be seen in observation tanks. Twenty one-year-old *L. fasciola* were divided between two observation chambers constructed to resemble visual ant farms. Each chamber was built from a 38 L aquarium with a piece of acrylic cut the same size as the long side of the aquarium. The acrylic was glued to the inside of the aquarium approximately 1 cm from the glass. The space between the acrylic and the glass was filled 75% full with fine sand to a depth of 35 cm. The entire aquarium was filled with water, and the mussels were fed daily with algae (*Scenedesmus* spp.) at a concentration of

about 20,000 cells/mL. These chambers allowed an observer to note the location of the juveniles in the sand, and their depths could be recorded.

Ten juveniles were placed into the sand of each observation chamber. One chamber was covered with black plastic, and the other was left open. An airstone was placed into each chamber. The juveniles were allowed to acclimate for 24 hr before any observations were taken. After the acclimation period, observations such as juvenile depth and water temperature were recorded daily at 8:00 am, 4:00 pm, and 12:00 am for 5 days. Juvenile depths were placed into one of three categories: at the surface, between 0 and 2 cm, and >2 cm. Juveniles were determined to be at the surface if any part of their shell or siphon was visible above the sand level. The second category, 0 – 2 cm, consisted of juveniles in which the midlines of the shells were between these depths but were not visible above the sand. The third category, >2 cm, contained those juveniles in which the midlines of the shells were deeper than 2 cm. At the end of 5 days, the cover was switched to the tank that had previously been open. Again, the juveniles were allowed to acclimate for 24 hr, and observations at the same intervals were recorded for another 5 days. Differences between the depths of the juveniles in each treatment were analyzed using logistic regression over time, with temperature as a covariate (Ott 1993). Significance was determined to be $p < 0.05$.

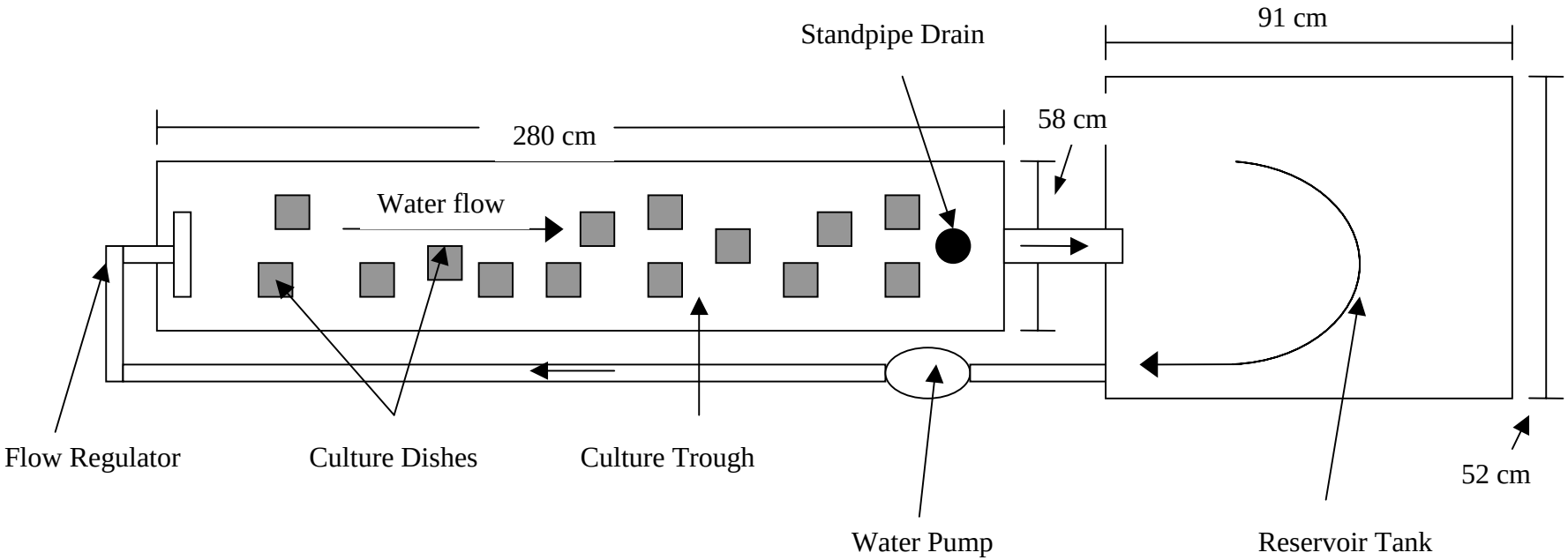

Figure 3-1. Top view of the recirculating culture system for juvenile mussel propagation. Water was pumped from the reservoir tank through the flow regulator and over the dishes in the trough. It then flowed down the standpipe drain and back into the reservoir tank.

Results

Part 1: Sediment Preferences and Light Regime in Culture

Effects of Sediment

Fine sediment yielded significantly poorer survival of juveniles ($p = 0.023$) than coarse sand and mixed sediment, especially in light treatments (Figure 3-2). Survival over the 16 wk period ranged from 7% in fine sediment in the covered raceways to 26% in mixed sediment in the open raceways, and were significantly different ($p = 0.04$). After 16 wk, juvenile shell lengths were 0.86 mm (sd = 0.23) in fine sediment, 1.10 mm (sd = 0.13) in fine sand, 1.58 mm (sd = 0.11) in coarse sand, and 1.09 mm (sd = 0.11) in mixed sediment. Shell heights were 0.73 mm (sd = 0.16) in fine sediment, 0.87 mm (sd = 0.09) in fine sand, 1.15 mm (sd = 0.07) in coarse sand, and 0.78 mm (sd = 0.08) in mixed sediment. However, because the juveniles in all replicates of the fine sediment in covered raceways died by week 16, growth data could only be compared between all treatments up to 12 wk (Figures 3-3 and 3-4). There were no significant differences in growth among the sediment types in either height ($p = 0.59$) or length ($p = 0.32$) up to 12 weeks. It appeared that growth was more affected by sediment type in the absence of light, although the differences were not statistically significant ($p = 0.32$) (Figure 3-5). Temperatures ranged from a minimum of 14°C to a maximum of 28°C throughout the course of the experiment, with a mean of 21.1°C. There were no differencesin survival based on the placement of dishes within the troughs.

Effects of Light

Light had more of an effect on growth of the juveniles than it did on their survival. Survival remained moderate in the light treatments through week 8 (21%), and appeared lower in all weeks in the dark treatments, although there was not a significant difference ($p = 0.45$) (Figure 3-6).

Availability of light affected juvenile growth considerably. Mean shell lengths in light treatments in each sediment type were significantly greater than those in covered treatments ($p = 0.03$) (Figure 3-5). At the end of the 16 wk period, mean shell heights across all sediment treatments reached 0.98 mm (sd = 0.075) in open treatments and 0.78

mm (sd = 0.068) in covered raceways (Figure 3-7). Mean shell length was 1.27 mm (sd = 0.106) in light and 1.04 mm (sd = 0.097) in dark (Figure 3-8). Juveniles grew faster in both dimensions (length and height) in the raceways that were open to ambient light. While the mean measurements at any given sampling period did not differ significantly, their overall growth rates were significantly different at $p = 0.03$. Juvenile mussels in the dark troughs grew in a linear fashion ($R^2 = 0.703$), whereas juveniles exposed to light grew in a non-linear manner ($R^2 = 0.574$). They grew quickly in the first 8 wk and then slowed thereafter.

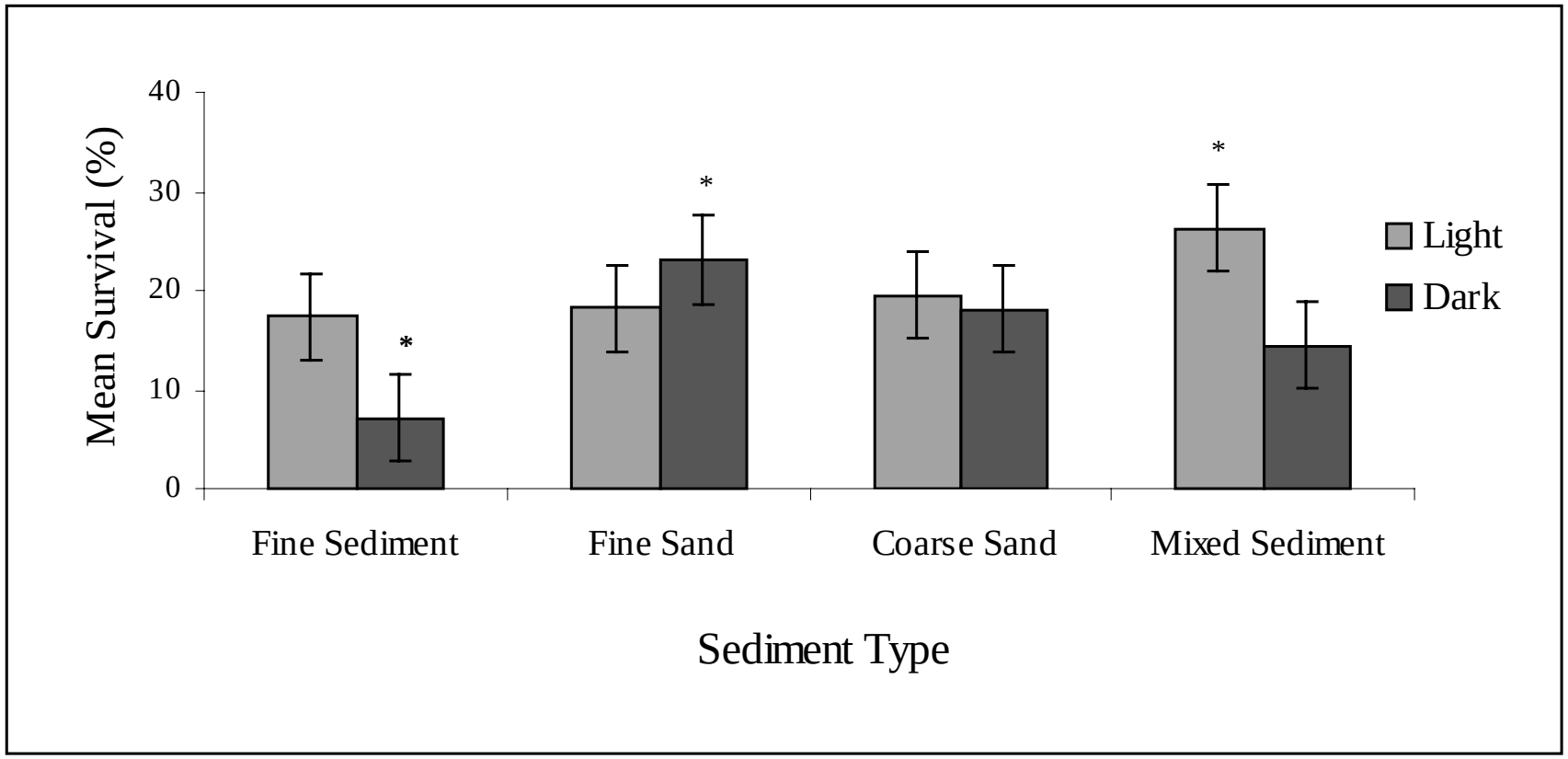

Figure 3-2. Survival of juvenile *Lampsilis fasciola* in different sediment types and light regimes over 16 weeks. * Fine sand in dark raceways and mixed sediment in light raceways were significantly different from fine sediment in dark raceways ($p = 0.04$).

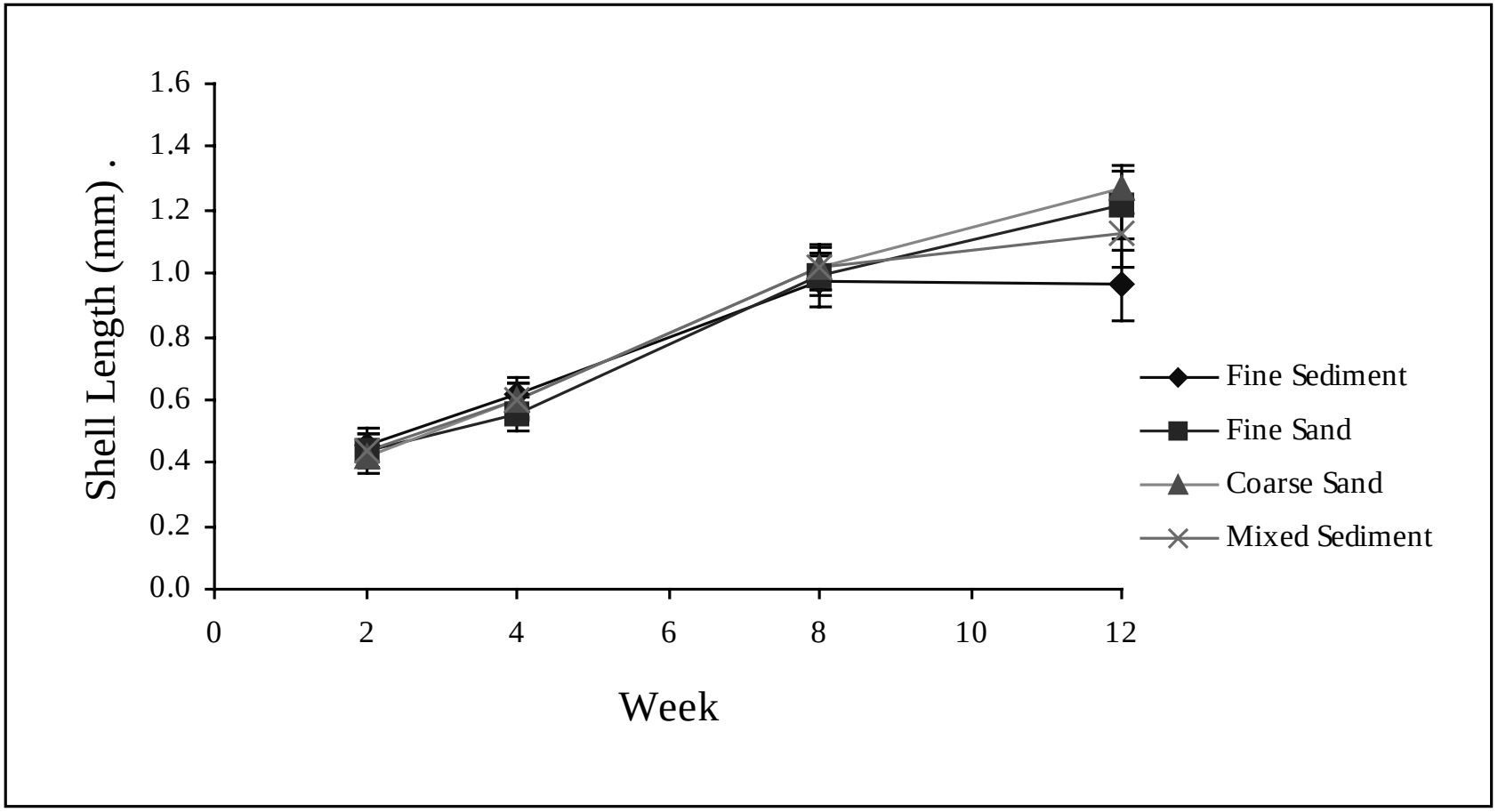

Figure 3-3. Mean shell lengths of juvenile *Lampsilis fasciola* in different sediment treatments after 12 wk. There were no significant differences in lengths among the sediment types ($p = 0.32$).

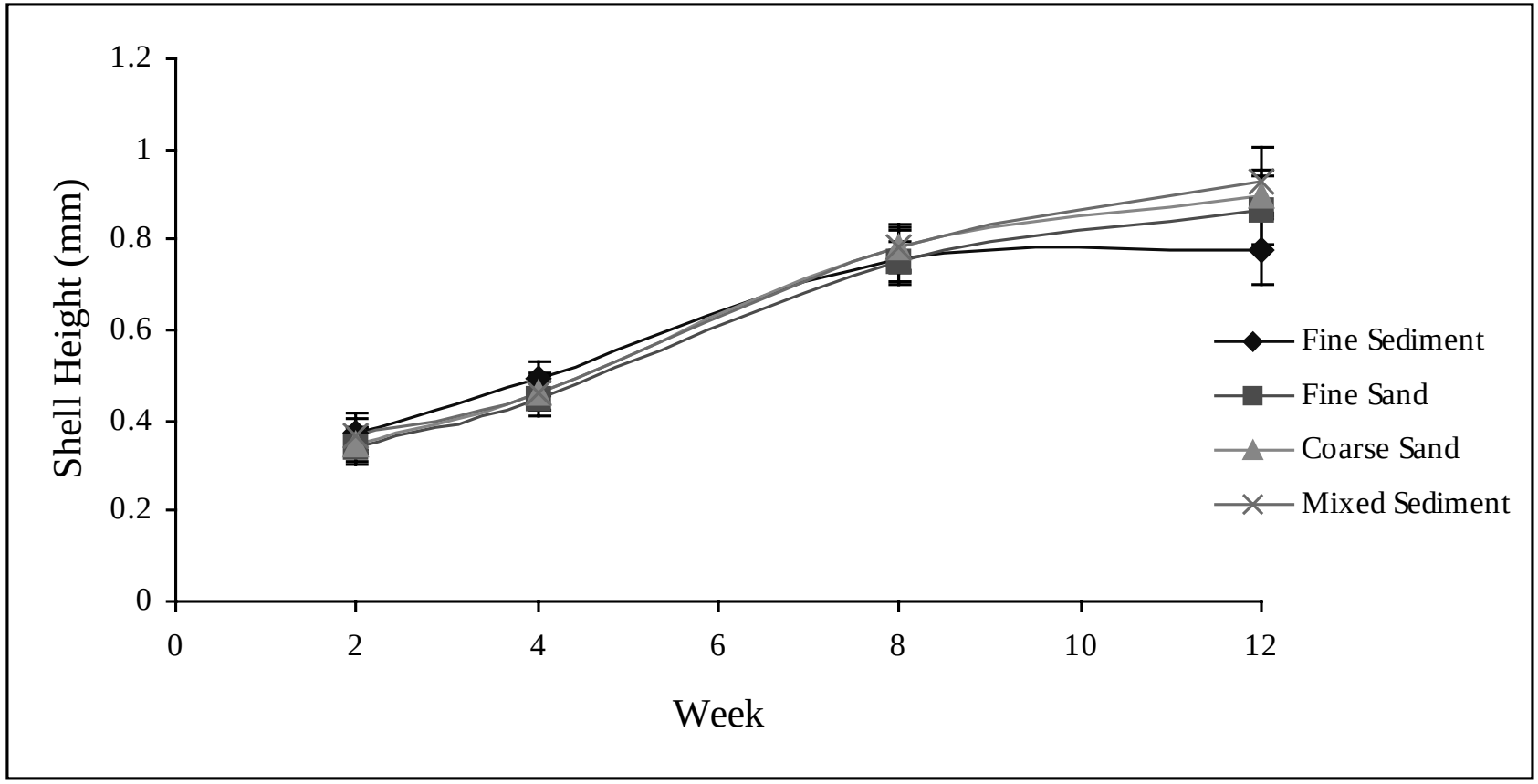

Figure 3-4. Mean shell heights of juvenile *Lampsilis fasciola* in different sediment treatments up to 12 wk. There were no significant differences in heights among the sediment types ($p = 0.59$).

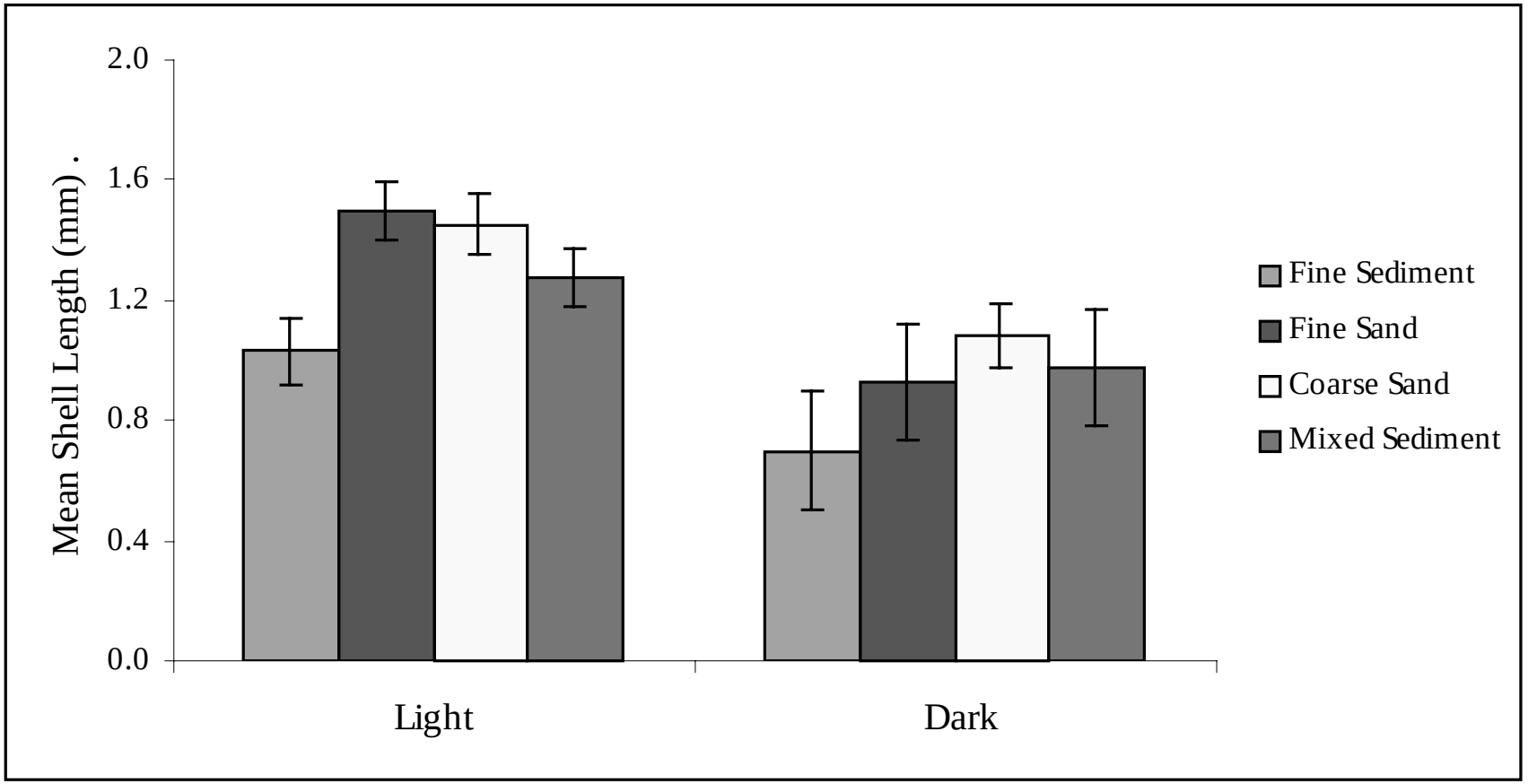

Figure 3-5. Mean lengths of juvenile *Lampsilis fasciola* after 12 wk in different sediment and light treatments. There was a significant difference between the light and dark treatments ($p = 0.03$). There were no significant differences in growth among the sediment types ($p = 0.32$).

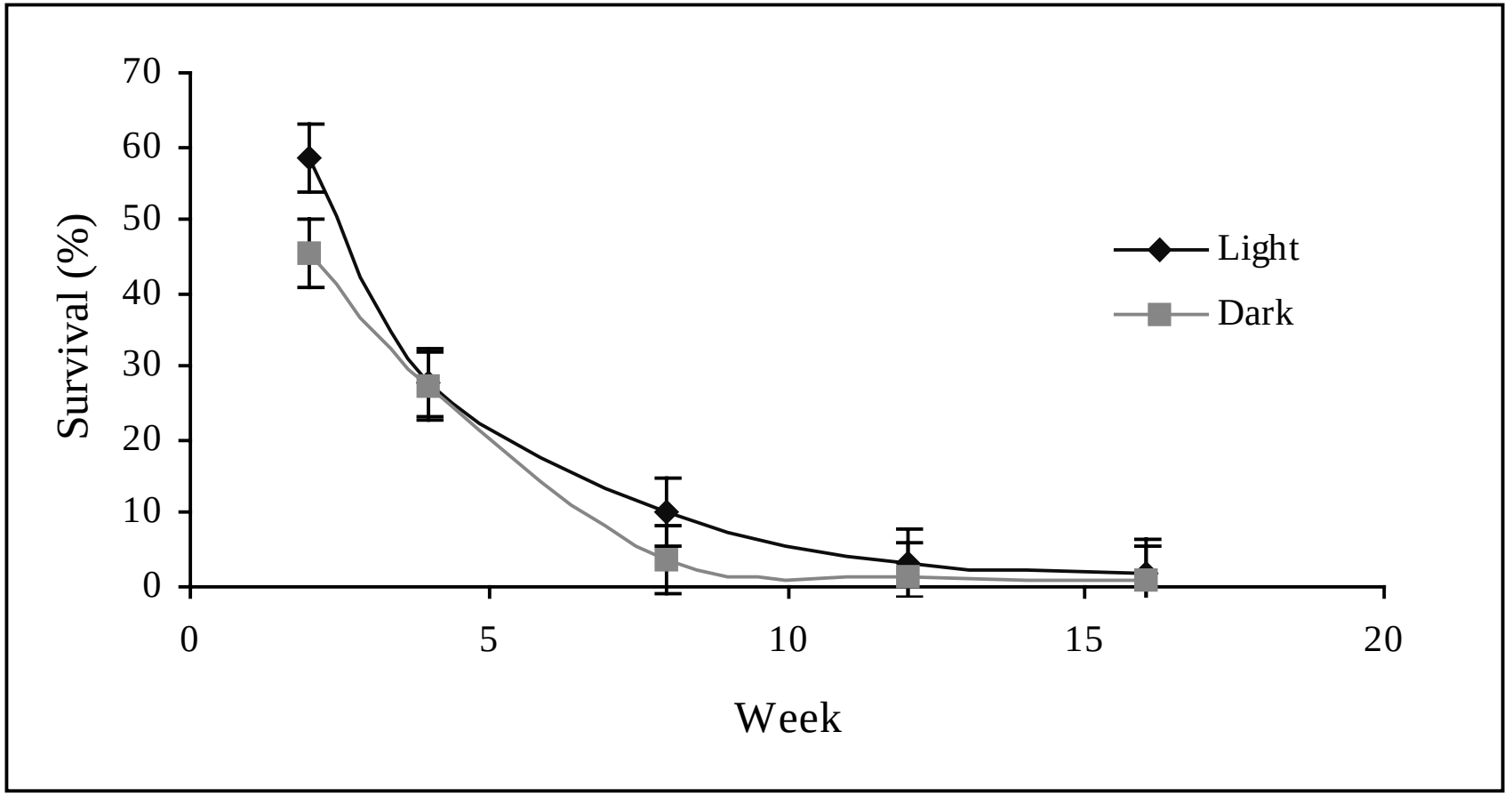

Figure 3-6. Survival of juvenile *Lampsilis fasciola* in light and dark treatments over 16 wk. There was no difference in survival between the two light treatments ($p = 0.45$).

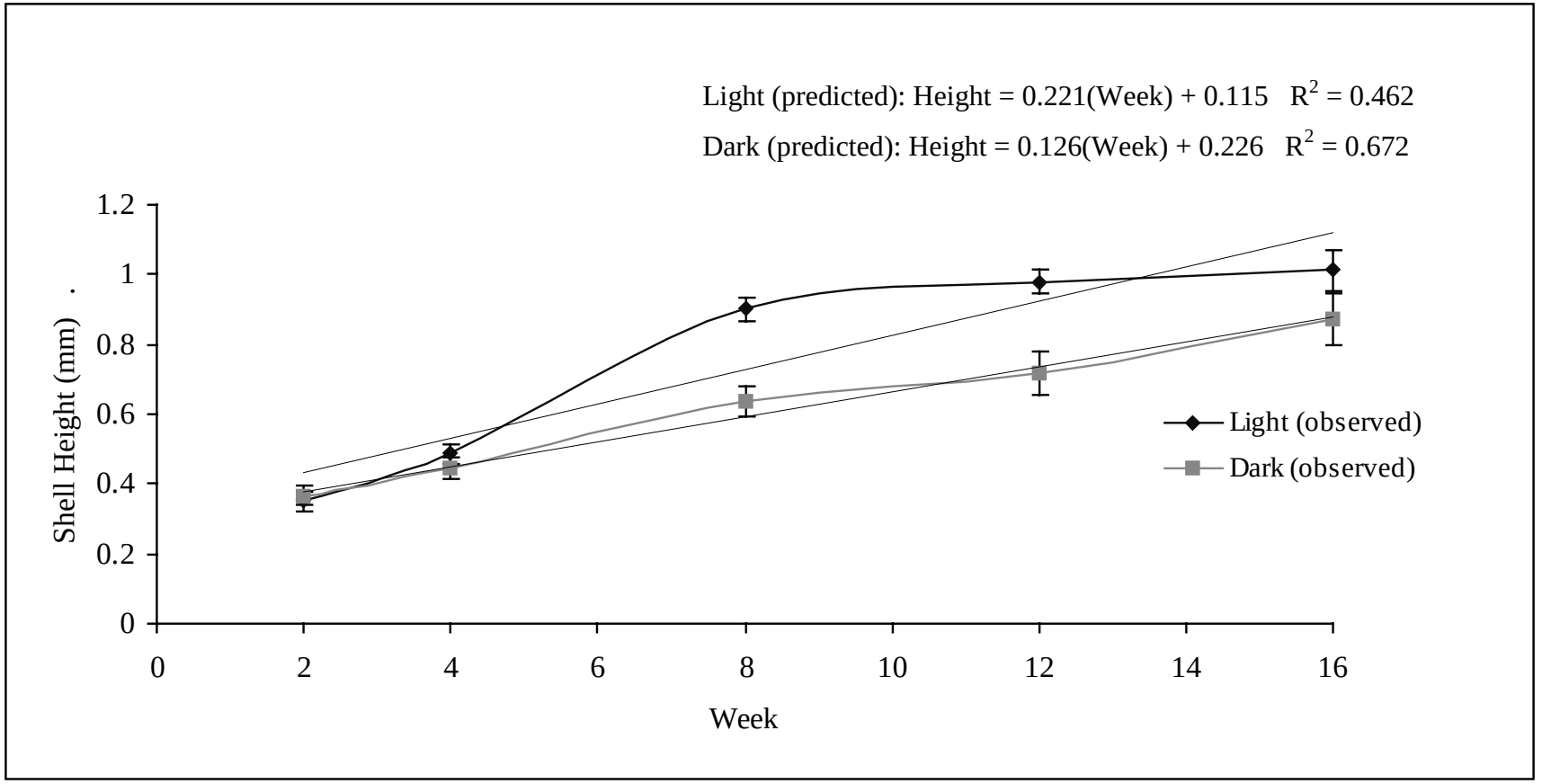

Figure 3-7. Mean growth in shell heights of juvenile *Lampsilis fasciola* in light and dark treatments over 16 wk. Juveniles in light treatments demonstrated significantly faster growth rates ($p = 0.05$). Straight lines indicate predicted linear regression lines.

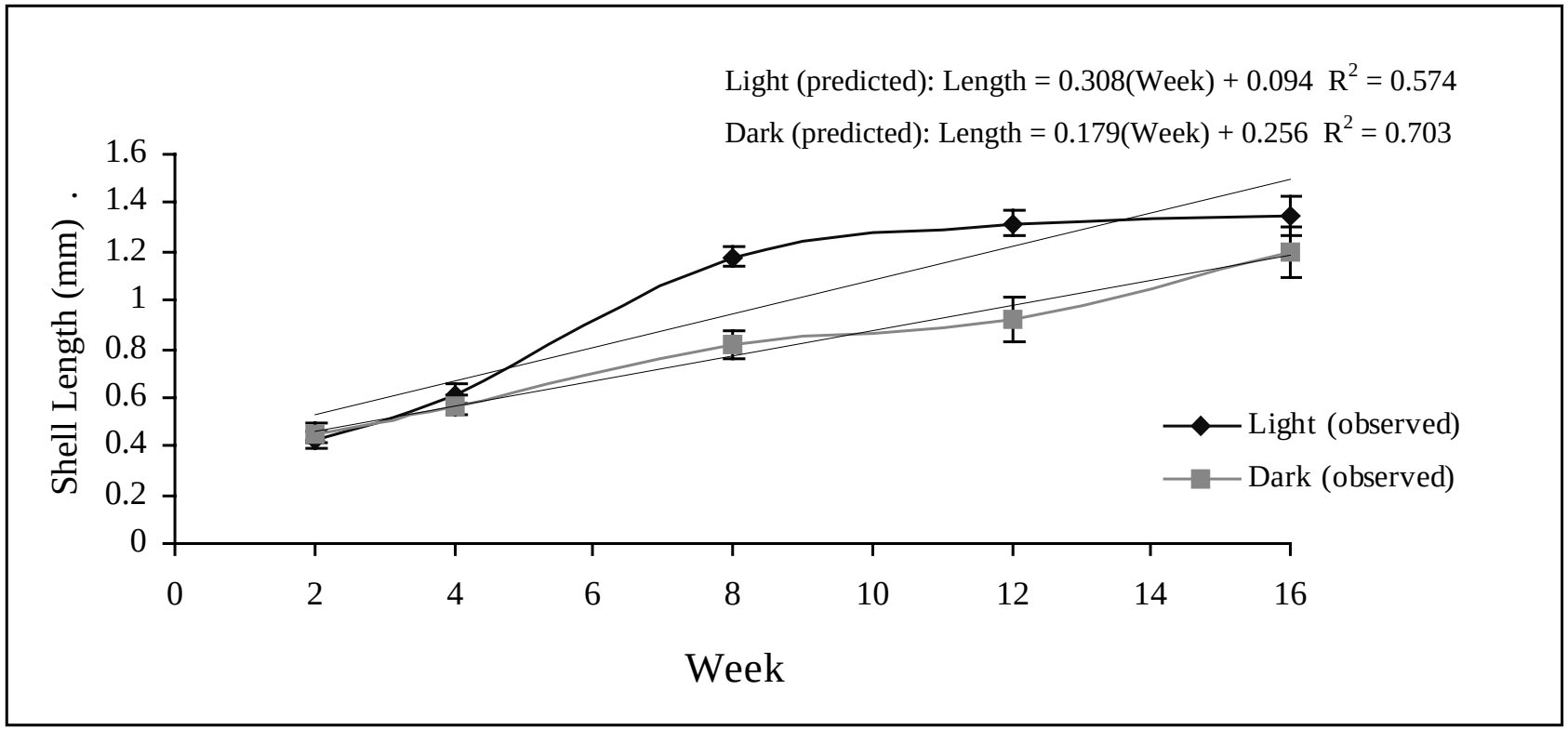

Figure 3-8. Mean shell lengths of juvenile *Lampsilis fasciola* in light and dark treatments over 16 wk. Growth rates between the light and dark troughs were significantly different ($p = 0.03$). Straight lines indicate predicted linear regression lines.

Part 2: High-light versus Low-light Conditions

Effects of Sediment

Overall survival of juvenile *Lampsilis fasciola* averaged 31% at 2 wk and dropped to approximately 5% after 16 wk. Substratum type did not yield significantly different survival at the end of 16 wk (fine sediment mean = 5.8%, sd = 4.78; fine sand mean = 3.4%, sd = 4.78) ($p = 0.32$) (Figure 3-9). There were no differences in mean survival over all of the weeks in fine sediment (16.27%, sd = 2.21) versus in sand (14.06%, sd = 2.21) ($p = 0.262$).

Shell length was significantly affected by sediment type over 16 wk ($p = 0.009$) (Figure 3-10). Shell height was affected similarly ($p = 0.005$), with juveniles in both substratum types demonstrating linear growth rates in both dimensions. Mean shell length of juveniles was 2.63 mm (sd = 0.075) in fine sediment substratum and 1.94 mm (sd = 0.102) in fine sand at the end of 16 wk, and mean shell height was 1.81 mm (sd = 0.042) in fine sediment and 1.50 mm (sd = 0.018) in sand. Temperatures ranged from 3.4°C to 35.1°C, with a mean of 23.1°C. There were no differences in survival based on placement of dishes within the troughs.

Effects of Light

Light intensity affected juvenile survival more than growth, with juveniles in both sediment treatments in the shaded troughs having significantly higher survival than juveniles in fine sand in the open troughs ($p < 0.01$) (Figure 3-11). Survival differed over the 16 wk period between the two light treatments, with juveniles in shaded tanks (mean = 7.4%, sd = 5.01) consistently surviving better than those in open tanks (mean = 1.78%, sd = 5.01) ($p = 0.046$) (Figure 3-12).

Light intensity did not appear to affect juvenile growth. After 16 wk, mean shell height was 1.72 mm (sd = 0.068) in open raceways and 1.58 mm (sd = 0.035) in shaded raceways ($p = 0.693$) (Figure 3-13). Shell length after 16 weeks was 2.37 mm (sd = 0.11) in open raceways and 2.20 mm (sd = 0.068) in shaded raceways ($p = 0.747$) (Figure 3-14). Shell lengths in shaded treatments in this experiment were about 1.0 mm larger at the end of 16 wk than those in the covered troughs of the first experiment (Figure 3-15).

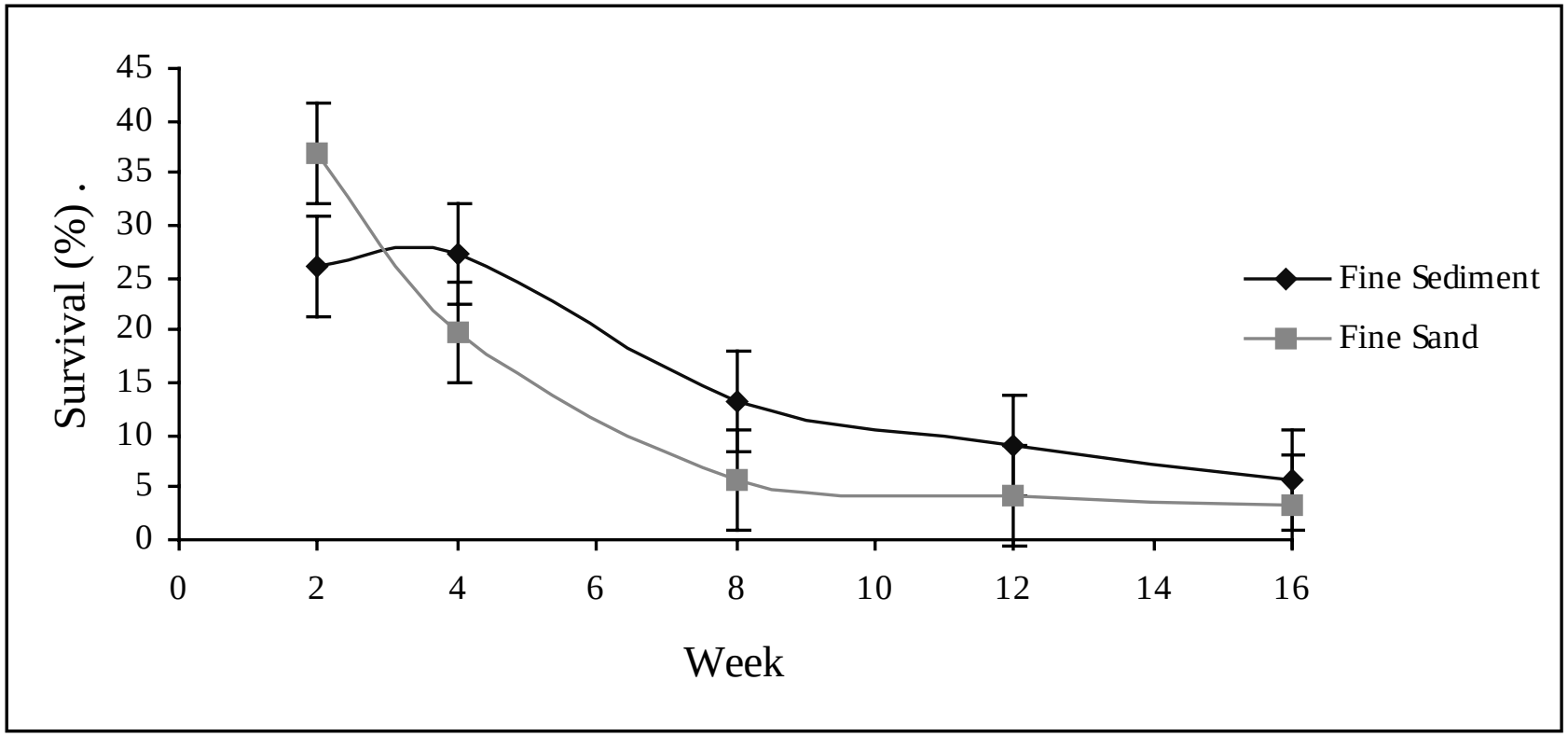

Figure 3-9. Survival of juvenile *Lampsilis fasciola* reared in two sediment treatments. There were no differences in survival between juveniles in fine sediment and those in fine sand when averaged over the 16 wk ($p = 0.262$).

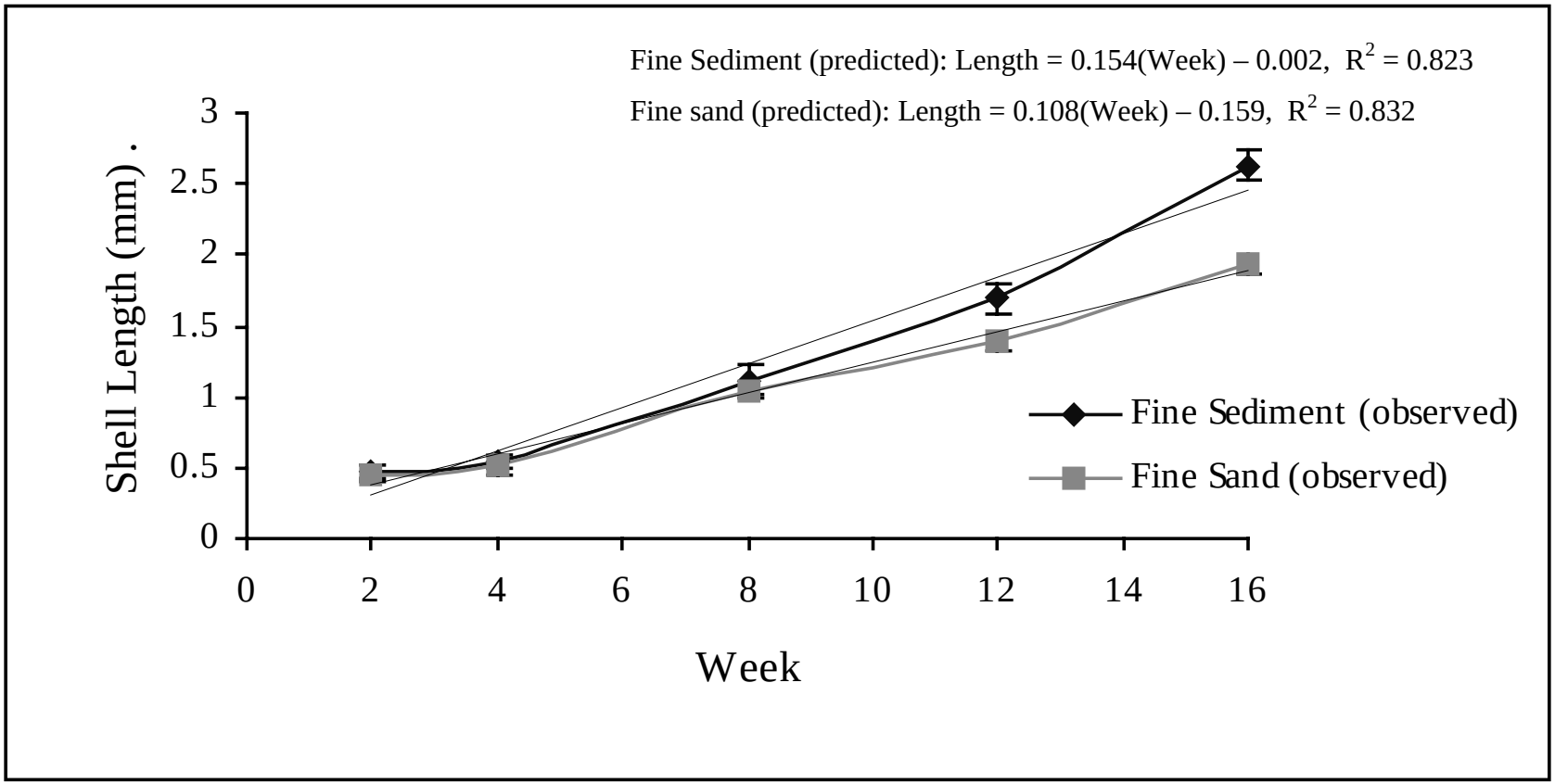

Figure 3-10. Shell growth of juvenile *Lampsilis fasciola* in two sediment treatments. Growth rates of juveniles in fine sediment were significantly higher than growth rates in fine sand over the 16 wk period (p = 0.009). Straight lines indicate predicted linear regression lines.

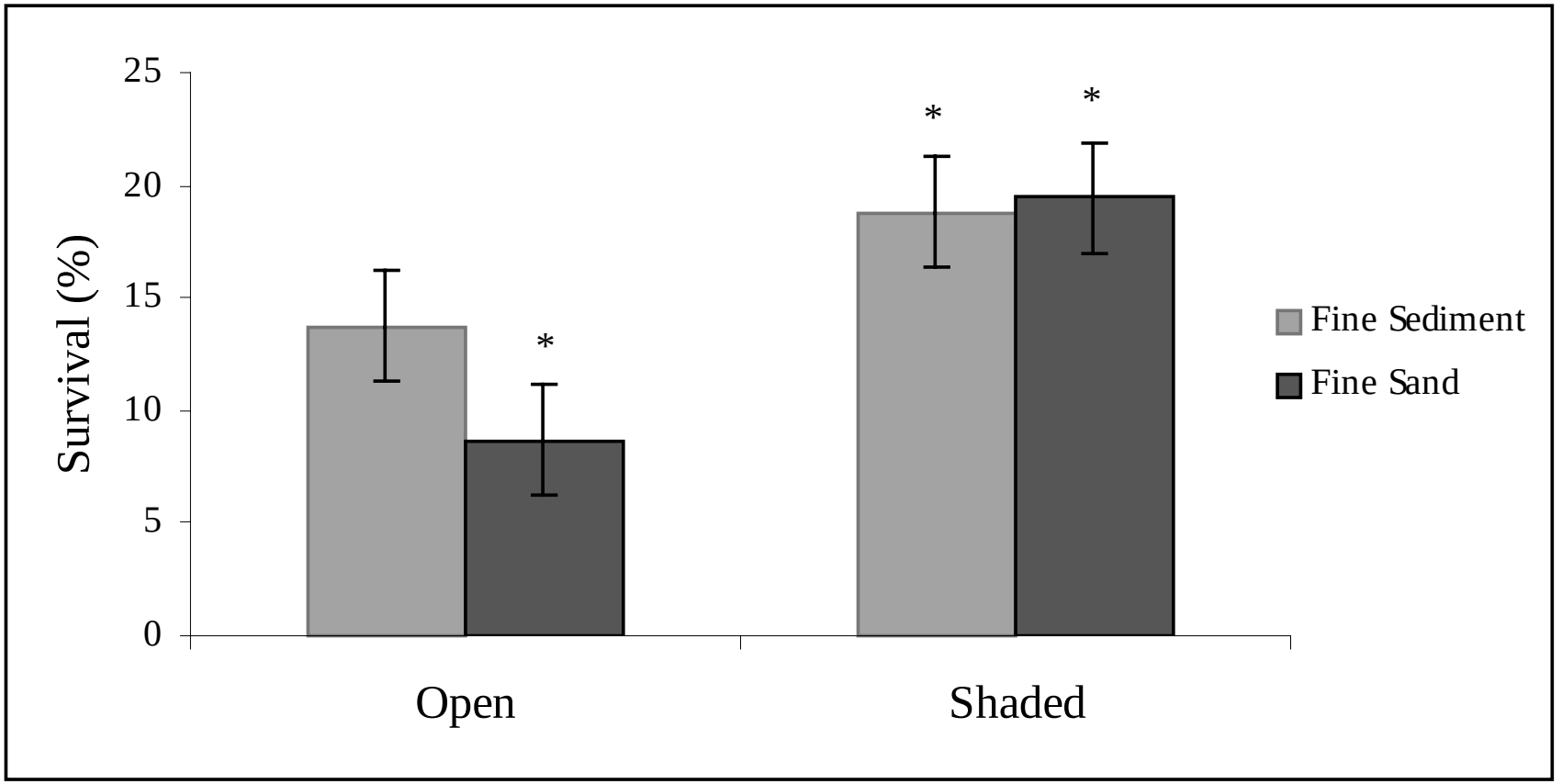

Figure 3-11. Mean survival of juvenile *Lampsilis fasciola* in different sediment and light treatments over 16 wk. *Survival of juvenile mussels in both sediment treatments in shaded troughs was significantly higher than those fine sand in open troughs ($p < 0.01$).

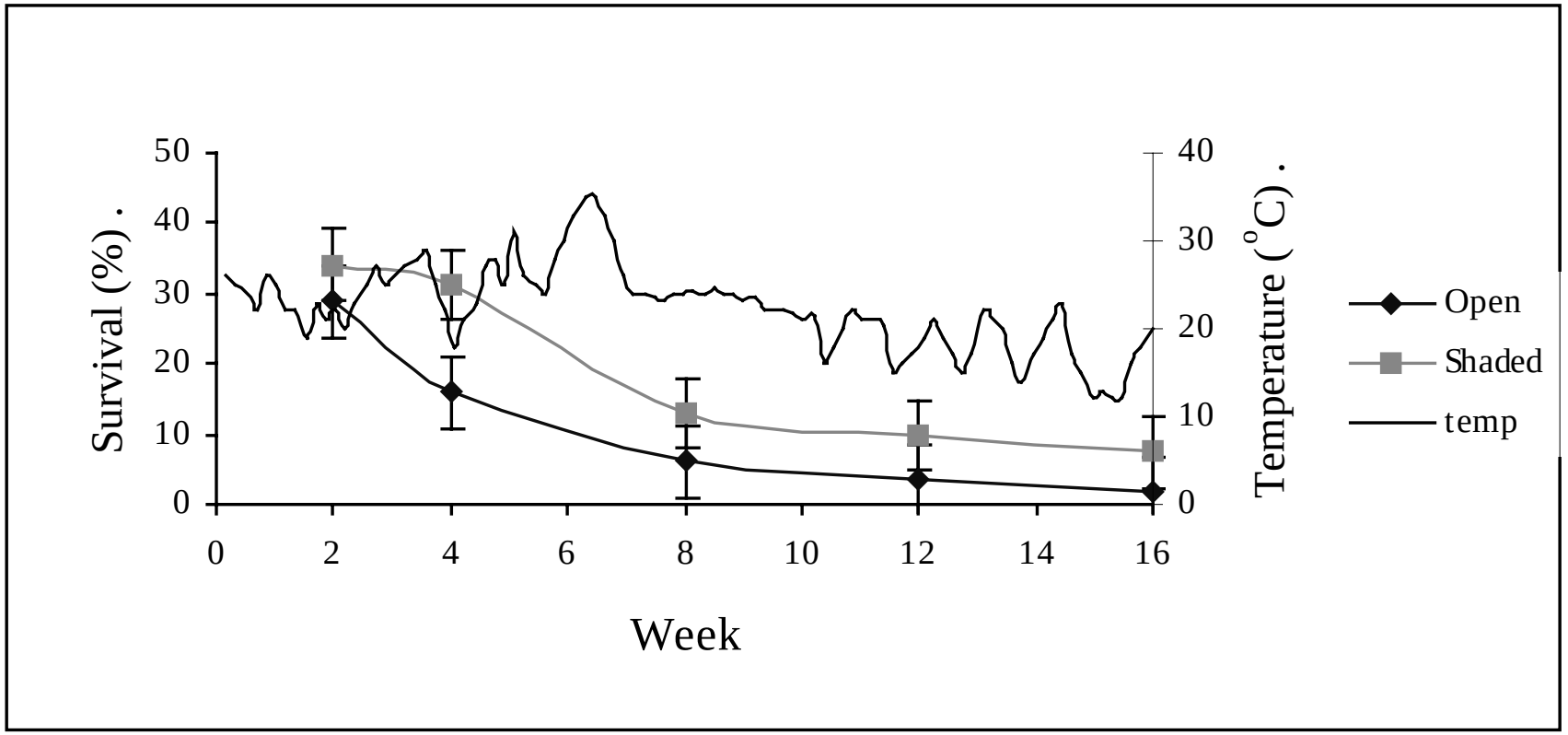

Figure 3-12. Survival of juvenile *Lampsilis fasciola* exposed to two light treatments, and the range of temperatures. Juveniles in shaded troughs had significantly higher survival than those in open troughs ($p = 0.046$).

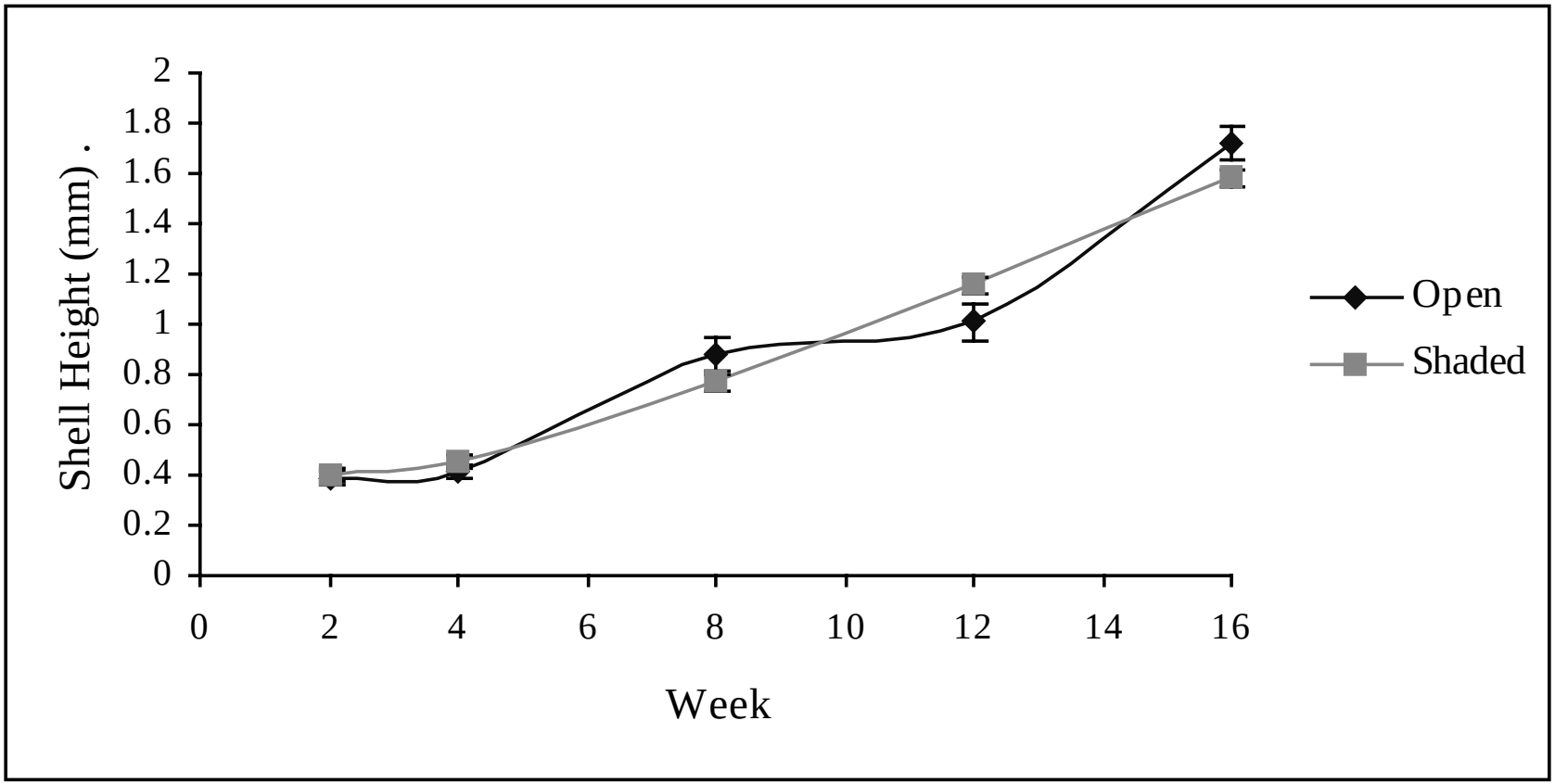

Figure 3-13. Shell heights of juvenile *Lampsilis fasciola* exposed to two light treatments. There were no significant differences between juveniles in open and shaded troughs ($p = 0.693$).

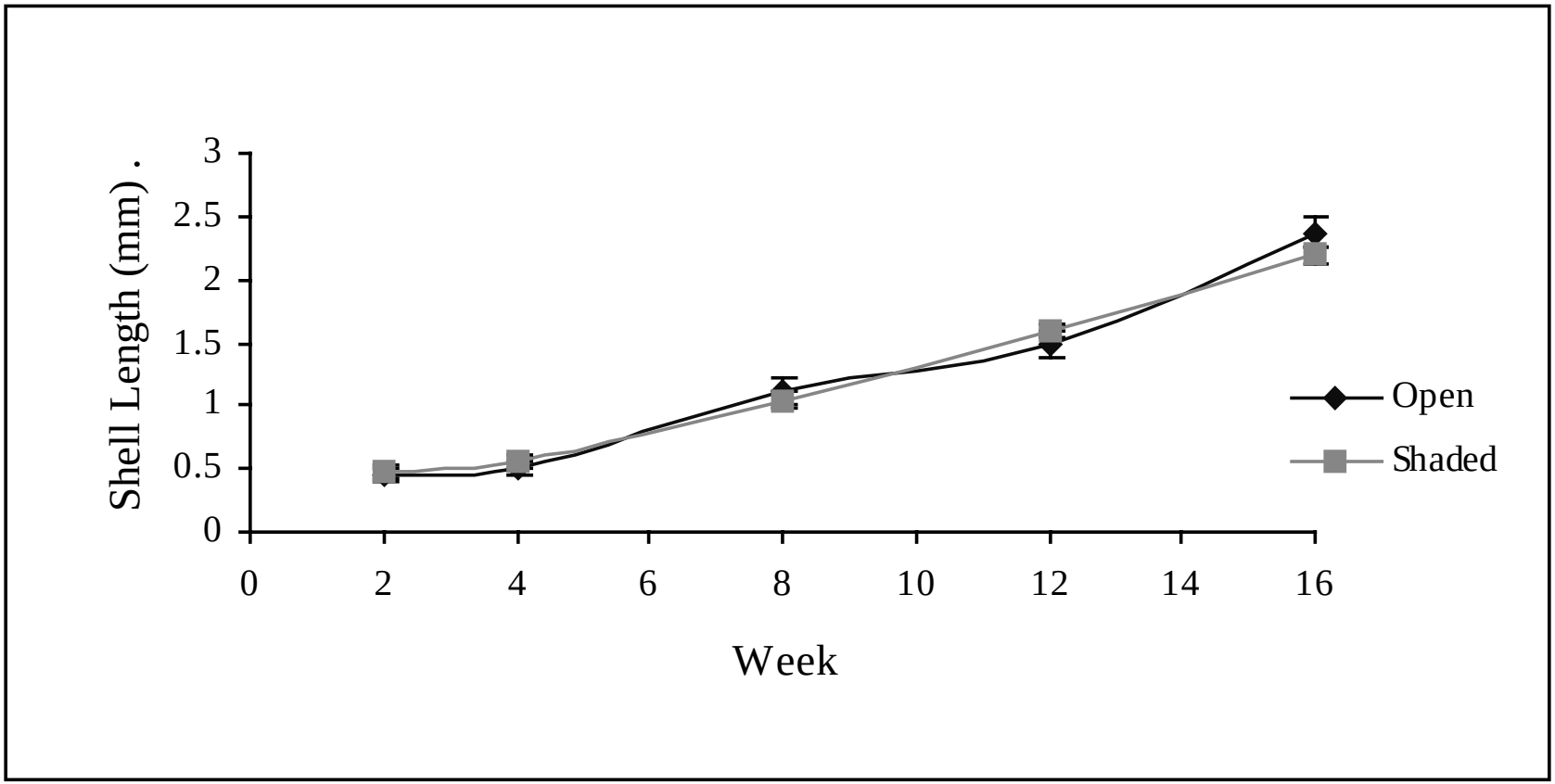

Figure 3-14. Shell lengths of juvenile *Lampsilis fasciola* exposed to two light treatments. There were no significant differences in shell length between the two treatments (p = 0.747).

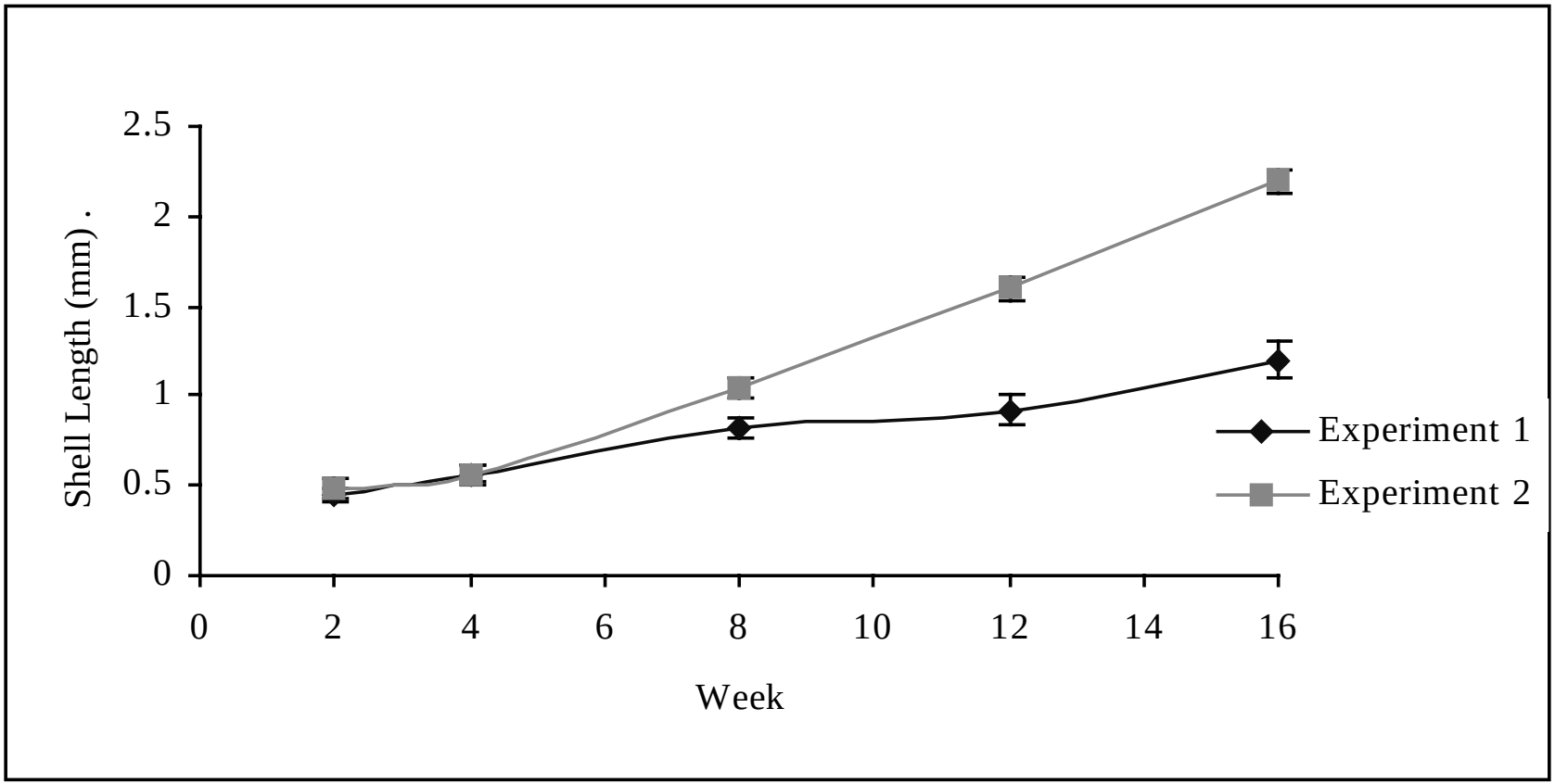

Figure 3-15. Shell lengths of juvenile *Lampsilis fasciola* in shaded versus dark treatments of experiment 1 and 2. Juveniles in dark treatments in experiment 1 were not exposed to any light, whereas juveniles in dark treatments of experiment 2 were exposed to 50% of ambient light.

The combination of sediment composition and light intensity seemed to have the most effect on juvenile growth rates. In open troughs, juveniles in fine sediment and fine sand substrata had similar growth rates ($p = 0.476$) (Figure 3-16). However, in shaded troughs, juveniles in fine sediment had significantly higher growth rates ($p = 0.018$) (Figure 3-17). Predicted regression lines for growth were linear, with high R^2 values (fine sediment, $R^2 = 0.854$; fine sand, $R^2 = 0.825$).

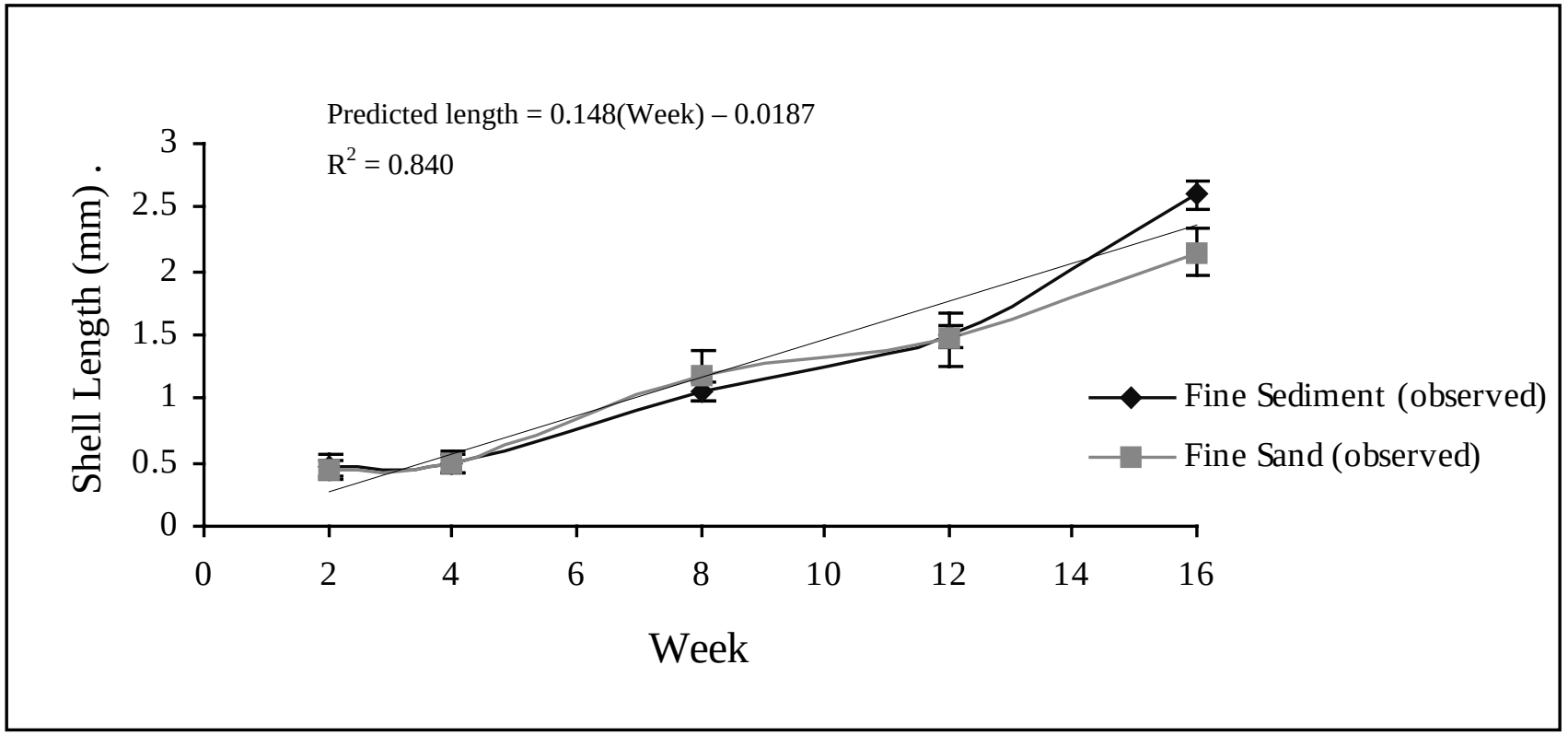

Figure 3-16. Shell lengths of juvenile *Lampsilis fasciola* in two sediment treatments in open troughs. There were no differences between growth rates of juveniles in either substratum type (p = 0.476). The straight line indicates the predicted linear regression line.

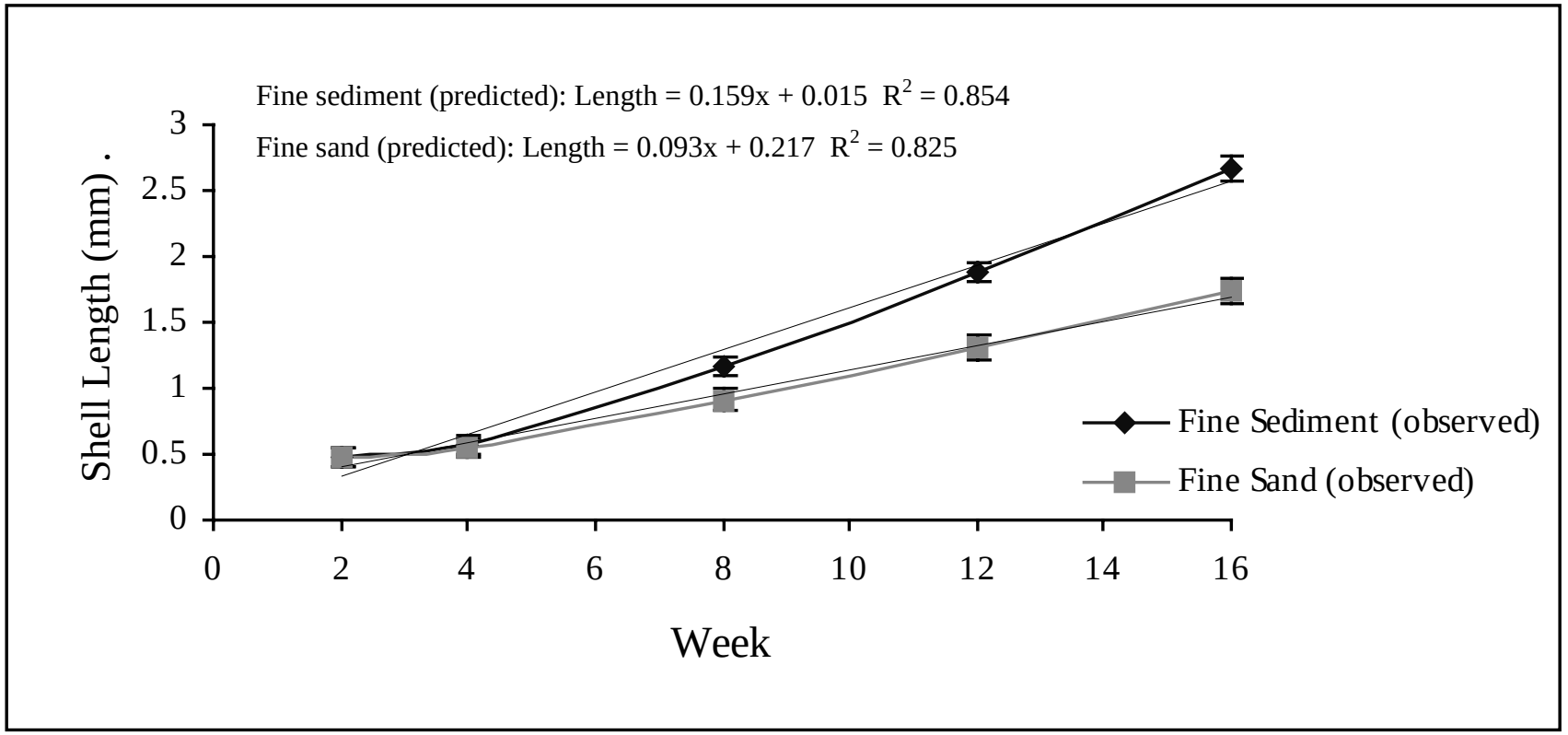

Figure 3-17. Shell lengths of juvenile *Lampsilis fasciola* in different sediment treatments in shaded troughs. Growth rates between the two substratum types were significantly different (p = 0.001). Straight lines indicate predicted linear regression lines.

Part 3: Response of Juveniles to Light

According to the logistic regression model, there were no differences in the depths of the juveniles in the tanks, regardless of light level ($p = 0.17$), time of day ($p = 0.07$), or temperature ($p = 0.34$). The juveniles were distributed between 0 and 13 cm deep, with the majority between 0 and 2 cm. Time of day appeared to have the most notable effect, although the differences were not statistically significant at $p = 0.05$. At 12:00 am, the juveniles in both tanks were more evenly distributed among the depths than at any other time period, although the majority of juveniles were burrowed 0 – 2 cm deep. Most juveniles were within 0 – 2 cm at each observation period in open and covered tanks (Figure 3-18). Additionally, there were no differences in the depths of the juveniles over the course of the 5 days for each trial ($p = 0.55$). On average, however, juveniles tended to be more evenly distributed throughout the depth categories in the covered tanks, with fewer individuals in the 0 – 2 cm category at any one time. The temperature in the two tanks ranged from 21°C to 36°C, with a mean of 26.9°C, and the tanks never differed by more than 3°C at any time.

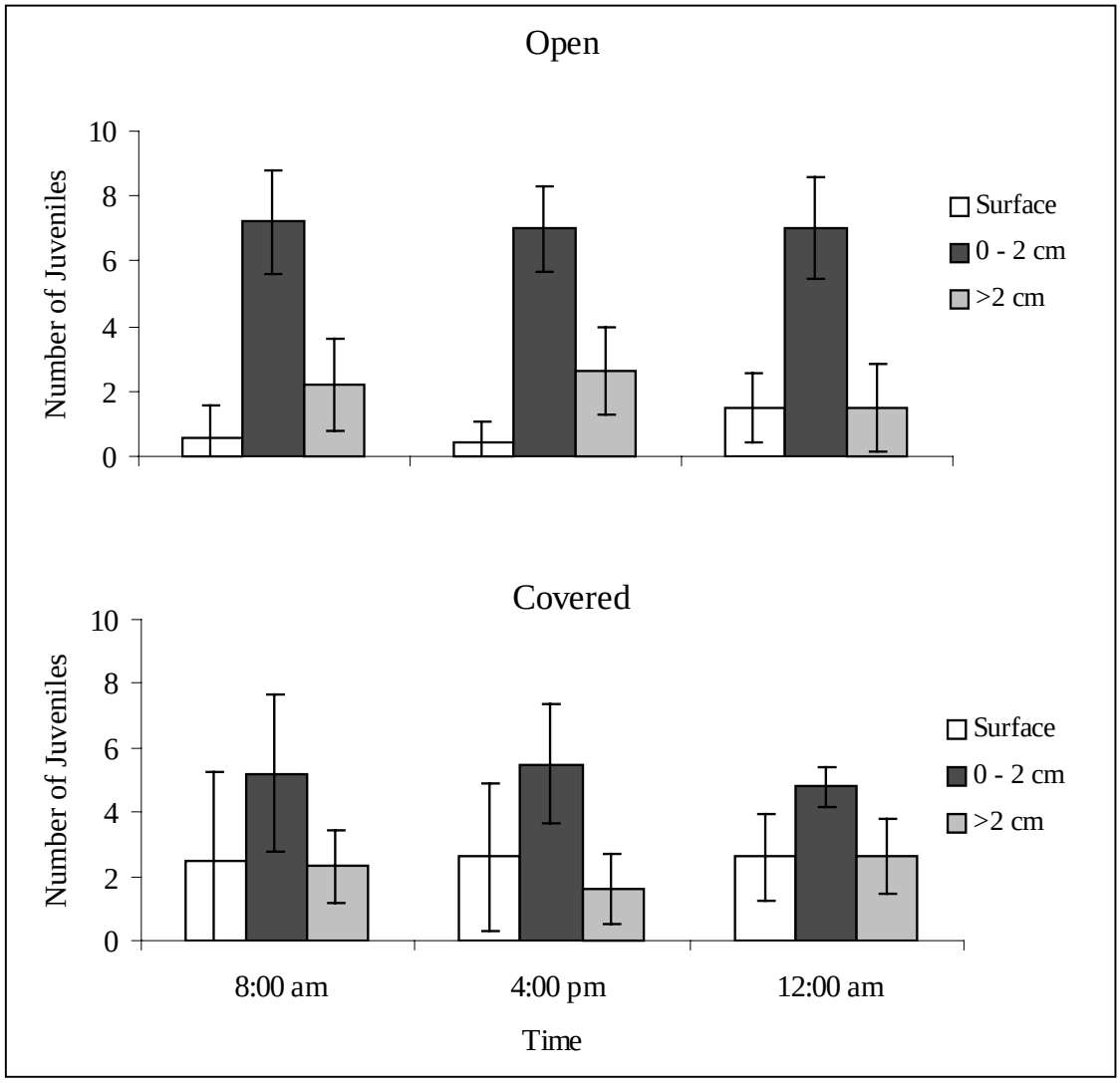

Figure 3-18. Number of juvenile *Lampsilis fasciola* in the observation chambers in each depth category per observation period.

Discussion

Effects of Sediment

Although the results from the first culture trial indicated that fine sediment was a poor substratum for rearing juvenile *Lampsilis fasciola*, this was not the case in the second experiment, in which survival in fine sediment was not significantly different from survival in fine sand. Additionally, growth of juveniles in fine sediment was significantly higher in the second experiment, while in the first experiment substratum type did not significantly affect growth. There are several explanations for this discrepancy in sediment suitability between the two experiments. In the first experiment, chironomids were more abundant in fine sediment than in the other sediment treatments. However, in the second culture trial, the numbers of chironomids were comparable among treatments and were much lower in abundance than in the first experiment. Although I do not have an explanation for the lower numbers of these chironomids in the second experiment, their low abundance may have been beneficial. Chironomids build cases out of surrounding material (Buddensiek 1995), and can use juvenile mussels in these cases. This may not be a direct cause of mortality but it likely interferes with juvenile feeding efficiency.

In addition to the difference in chironomid density, the fine sediment was more tightly packed in the first experiment than in the second. During the first trial, the fine sediment was very tightly packed, which likely impeded both feeding and mobility of the juveniles. Juveniles pedal-feed early in life until their gills can support filter-feeding (Yeager *et al.* 1994, Gatenby 1996). Therefore, packed sediment can hinder feeding as it lacks interstitial spaces in which algae, bacteria, and detritus can collect and be obtained easily by the juveniles. Additionally, packed fine sediment can reduce flow through the hyporheic zone of a streambed, thus resulting in lowered dissolved oxygen levels available to mussels buried in the substrate (Gordon *et al.* 1992, Box and Mossa 1999). This likely applies to fine sediment within a culture system as well. In the second experiment, the fine sediment was much looser than it was in the first trial, and this may have been a factor in the inter-experimental variance of fine sediment as a substratum for juvenile mussel culture.

The above factors only explain why survival in fine sediment substratum improved, and not why growth in fine sediment was higher than growth in sand in the second trial and not in the first. The sediment used in both trials was collected at the same time from the same source, and therefore, the differences cannot be attributed to sediment composition. However, fine sediment may be preferable to fine sand when it is loosely packed because juveniles can move through it more readily. However, when the fine sediment is tightly packed, and feeding and movement are hindered, fine sand may be favored. This may explain why growth rates were significantly higher in fine sediment than they were in fine sand in the second trial, and there were no differences in growth rates in the different sediment types in the first trial. The fine sediment likely yielded better growth in the second trial for the same reasons survival was higher; food collection was easier in loose fine sediment because the juveniles could move about easily.

These results contradict typical mussel-sediment relationships. Detrimental effects of fine sediment from run-off and erosion on freshwater mussels have been documented. Heavy sediment loads in the water column can interfere with feeding activity (Kat 1982, Box and Mossa 1999), as mussels in turbid waters remained closed about 50% longer than mussels in silt-free water, reducing the time available to feed (Ellis 1936). *Amblema plicata* demonstrated a slower growth rate in turbid waters (Stansbery 1970), which may be related to reduced feeding under high sedimentation levels. Fine sediment plumes may also reduce feeding in mussels by diluting the density of food particles in the water column (Widdows *et al.* 1979).

Very few studies have examined the relationship between juvenile mussels and sediment. Neves and Widlak (1987) found the majority of juveniles located in substratum that consisted primarily of pebble-sized particles (6 - 63 mm) and very little fine sediment. However, fine sediment has yielded fairly high survival in various juvenile culture studies, and has yielded higher survival than juveniles reared with no substratum. Juveniles reared in fine sediment survived better than those in an artificial substratum (kaolin) or those with no substratum (Gatenby *et al.* 1997). Additionally, *Anodonta imbecilis* juveniles had markedly higher survival when fine sediment was included (89%) than when there was no fine sediment (10%) (Hudson and Isom 1984).

The interstitial spaces in loose fine sediment have been observed to provide areas in which juveniles may feed (Yeager *et al.* 1994), which would explain the higher survival I observed in the second trial when the fine sediment remained loosely packed.

Lampsilis fasciola adults, as well as associated species, are rarely found in areas of streams with heavy sediment loads in the substratum or the water column, likely because of the detrimental effects of fine sediment described above. Larger particle sizes in the substratum provide more stability in fast water, where many adult riverine mussels are found (Harman 1972, Lewis and Riebel 1984). Small particle sizes, including fine sediment, would not provide this stability and would be unsuitable for adults that prefer fast-water habitats. Fine sediment would not likely be found in these fast-water habitats because the water velocity is usually above the critical velocity necessary to move fine sediment particles. Juveniles have been documented to have different habitat requirements than adults, with juveniles being found in areas of streams with slower water (Neves and Widlak 1987), which would be more likely to have fine sediment deposited on the substratum.

Effects of Light

In the first experiment, survival of juveniles was not significantly affected by light availability, although shell growth was influenced considerably. Those juveniles in raceways open to ambient light were significantly larger than those in raceways that were not open to any light, probably in response to greater food availability. There was an obvious difference in the amount of algae produced in the light treatments versus the dark treatments. The raceways left open to ambient light had algae, microbes, and detritus in the water column, raceway bottom, treatment dishes, and reservoir tank. Those raceways that were covered did not have any significant algae or microbes growing within the systems. It is likely that the mussels benefited from this presence of greater food resources rather than from the light itself. The second culture trial and the behavior trials appeared to confirm this. In the second culture experiment, survival was lower in the open parts of the troughs, which received direct sunlight, than in the shaded parts of the troughs, which received 50% of direct sunlight. Both light treatments were present in each trough, so mussels in each treatment within the same trough received the same

water. Therefore, any biota resulting from direct sunlight would be circulated throughout the system and would be available to juveniles in both treatments. The higher survival in the covered sides of the troughs indicates that light is not directly beneficial to juvenile mussels. Instead, the increased level of biological production in the tanks probably increased survival. In fact, direct light may hinder survival by producing too much algae, because survival was actually lower in the open sides of the troughs.

Different groups of algae are sensitive to different light levels (B. Parker, VPI&SU, personal communication). Filamentous algae, because they require calcium for attachment to the substratum, have a high energy requirement relative to unicellular algae, which do not require as much calcium (Turner *et al.* 1989, Stevenson 1997). Therefore, the filamentous algae were unable to thrive in the shaded sides of the troughs, while the unicellular algae were able to conserve energy and succeed in the shaded sides. Filamentous algae attached to the sediment in the dishes in the open sides of the troughs and likely negatively influenced survival on those sides. Unicellular algae also were present in large quantities in the open sides, and also likely had an effect on the survival of juveniles in the open sides. A large amount of algae settled out of the water and formed a 0.5 cm layer on the substratum of all of the dishes on the open sides. These algae were present in quantities not seen in nature and may have smothered the juveniles. More biota, such as chironomids and platyhelminths, were present in these dishes as well, probably because of the high levels of settled algae. These chironomids and platyhelminths probably had a detrimental effect on juvenile survival. Chironomids build cases that can include juvenile mussels, as described above, and platyhelminths have been observed to ingest juveniles (Shane Hanlon, VPI & SU, personal communication). These flatworms may have had a negative impact on juveniles in direct sunlight. Conversely, juveniles in low light were not inundated by algae, nor were there as many chironomids or platyhelminths in these treatments.

Equal growth rates in high and low light conditions in experiment 2 was intuitive, considering all treatments were within one trough and therefore were exposed to the same levels of suspended material. Juveniles in dark treatments in the second experiment were nearly twice as large as those in dark treatments in the first experiment, suggesting that growth is related more to food availability than to light availability.

The juvenile behavior study yielded no differences in depths of juveniles between open and covered treatments. Because the juveniles used in the observation study were one year old, they were beyond the pedal-feeding stage; therefore, filter-feeding or deposit-feeding most likely occurred at the substrate surface. Hence, juvenile depths were presumed to be indicators of feeding behavior; juveniles at the surface were assumed to be actively feeding, while buried juveniles were likely not. Light did not affect juvenile behavior; however, time of day seemed to make a difference. Juveniles in both open and covered treatments were at the surface more often during night hours. This suggests that while light, or absence thereof, may not directly affect juvenile feeding behavior, the juveniles have an internal clock that regulates behavior. This could possibly be an adaptation to avoid predation. Muskrats are crepuscular feeders (Burt and Grossenheider 1980), and are primary predators of mussels. Mussels that are buried during dawn and dusk and at the surface at night would be less likely to be detected by muskrats than mussels at the surface during dawn and dusk. This may also be the case with other mussel predators, including turtles, some species of waterfowl, and freshwater drum, which possibly feed on juveniles as well as larger adults (Parmalee and Bogan 1998). Trials of five days in length may not have been sufficient to determine whether light is the clock-setting mechanism. After a longer period of darkness, the juveniles may lose the regularity of their activity and exhibit atypical behavior. The activity period was more pronounced in the open tanks, although the differences between the tanks were not significant.

Increased activity at night may be triggered by mechanisms other than light. Temperatures drop at night in the wild as well as in this experiment; temperatures at night averaged 5°C less than during the day in the observation tanks. Cooling temperatures could cue mussels to increase activity, which was the case for *Anodonta cygnea* (Salanki 1977). However, temperature would not necessarily be a reliable cue. In lakes, oxygen levels drop at night due to decreased photosynthetic activity, and mussels may respond to this cue, although Salanki (1977) found that decreased oxygen resulted in decreased mussel activity. Regardless, mussels in riffles and runs would not likely encounter this oxygen decrease because stream oxygen levels are generally at or near saturation from turbulence (Simmons *et al.* 1997), and thus oxygen levels would not act as a cue.

Additionally, various mussel species spend much of the time burrowed in the substrate or under rocks (Lewis and Riebel 1984), which would often preclude their ability to detect daylight. Juveniles, in particular, remain buried much of the time (Neves and Widlak 1987). These studies, along with my data, show that factors other than light set the internal clocks of mussels.

Management Implications

Because juvenile *Lampsilis fasciola* appear to be unaffected by the presence or absence of light in their feeding behavior, and juveniles in low-light conditions survive better than those in high light conditions, it seems that low-light conditions are favorable for mussel propagation. It is probably necessary to include a reservoir tank or other water source that is open to direct light, however, to facilitate algal and bacterial growth in the water column. The combination of low-light and fine sediment as a substratum yielded the largest juveniles. Conversely, high light and fine sand yielded the smallest juveniles, as well as the poorest survival. However, more research needs to be done on substrate suitability in order to determine which substrate conditions are optimal for survival and growth. Although fine sediment appears suitable for juvenile *Lampsilis fasciola*, it may not be suitable for all species, especially those species that are more sensitive to environmental conditions. This includes several species of *Epioblasma*, which have been observed to fare poorly in fine sediment (J. Jones, VPI&SU, personal communication).

Summary

1. Differences in host fish suitability for thetan riffleshell (*Epioblasma florentina walkeri*) were shown between allopatric and sympatric populations of fantail darters (*Etheostoma flabellare*), with fish from an allopatric population being most suitable as hosts. These differences are hypothesized to stem from differences in the immunogenetic makeup of the host fish.
2. Counts of external growth rings on shells tended to underestimate ages of older mussels when compared with ages obtained from thin-sections. The mode of tan riffleshells in Indian Creek was between 38 and 44 mm, and there were many individuals below the estimated reproductive age of 4 years old, which signifies recruitment. The maximum age for tan riffleshells is likely near 15 yr.
3. There were an estimated 1078 adult tan riffleshells in Indian Creek, Tazewell County, Virginia, with approximately equal numbers of males and females. Watson (1999) estimated this population to be 683 adults in 1996, although an estimated 500 individuals were preyed upon by muskrats during the study. Since muskrat trapping has reduced the number of tan riffleshells lost to predation, it seems that the population estimates are similar and muskrat trapping has been successful.
4. Tan riffleshells were propagated for release into the Hiwassee River in Polk County, Tennessee. The bucket method was the most appropriate infestation technique, and culture troughs with little turbulence exposed to direct sunlight yielded relatively good juvenile survival. Three releases took place, for a total of 553 juveniles released into the Hiwassee River in 1997 - 1999.
4. Culture conditions were examined to increase juvenile growth and survival. The effects of substratum composition were variable between experiments, whereas sunlight availability had a direct positive effect, seemingly due to increased production of algae and microbes in the culture systems. However, an overabundance of biotic colonization was detrimental to juvenile survival likely because it settled and smothered the juveniles.
5. A juvenile behavior observation experiment showed that juveniles do not behave differently when exposed to sunlight versus being in covered tanks. Most juveniles in

both covered and open treatments were buried between 0 and 2 cm in the sand. This substantiates the above statement that juveniles are not highly phototropic.

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Vita

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