HOST SPECIFICITY OF FRESHWATER MUSSELS:

A CRITICAL FACTOR IN CONSERVATION

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By

Shannon Elizabeth Bigham

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**HOST SPECIFICITY OF FRESHWATER MUSSELS –
A CRITICAL FACTOR IN CONSERVATION**

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Shannon Elizabeth Bigham

ABSTRACT

Freshwater mussels have a unique life cycle in which they parasitize a fish host during their larval stage. This parasitic stage is very precarious and only a small fraction of the larvae survive. Most freshwater mussel species are host-specific and use only one or a few related species of fish. While a considerable amount of research has been done to identify the fish hosts of mussel species, little work has been done to see if this specificity varies among populations of a single mussel species. I investigated variation in host compatibility among populations of *Venustaconcha ellipsiformis*, the ellipse mussel, in the Spring River and Gasconade River in southern Missouri. Field observations suggested that *Etheostoma spectabile*, the orangethroat darter, is a primary host fish for ellipse mussels in the Spring River. I performed a laboratory test and confirmed that orangethroat and rainbow darters, *Etheostoma caeruleum,* from the Gasconade River were suitable hosts for the Gasconade River ellipse mussel (38% and 43% transformation success, respectively). I then compared the suitability of orangethroat darters from the Gasconade and Spring River for both Gasconade and Spring River mussels. I hypothesized that each mussel population would transform most effectively on the fish population with which it co-occurs. Although a few fish in each group transformed a large proportion of attached glochidia, the average transformation success was very low (<5%) in all 4 of the population pairings. Differences in transformation success among these pairings were consistent with the hypothesis, but the differences were not statistically significant. Gasconade glochidia initially attached in significantly larger numbers than Spring River glochidia, and there were also significant differences in attachment success among individual mussels. Spring River glochidia transformed poorly on Spring River orangethroat darters in a subsequent experiment at three different water temperatures. There was a significant positive effect of temperature on transformation success. The available data indicate that the Gasconade ellipse can be compatible with orangethroat darters, but there is no such evidence for the Spring River ellipse. Further study is needed to test the hypothesis of population differences in host compatibility in this species.

This abstract is approved as to form and content

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Dr. M. C. Barnhart
Chairperson, Advisory Committee
Southwest Missouri State University

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Approved:

_____
Dr. M. C. Barnhart

_____
Dr. D. Beckman

_____
Dr. J. Schnake Greene

_____
Dr. F. Einhellig, Graduate College Dean
Graduate College

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INTRODUCTION

Freshwater mussels of the family Unionoida have become increasingly important to conservationists in recent years because of the large number of threatened and endangered mussel species. Mussels are viewed as the "canary in the coal mine" and their plight is seen as a representative of an impending biodiversity crisis in the North American rivers. Unionoids are relatively diverse in North America with 297 species and subspecies (Turgeon et al. 1998). Yet, over half of these species are of special concern, threatened, endangered, or already extinct (Williams et al. 1993). This continuing decline of freshwater mussels has been well documented (Jorgenson and Sharp 1971, Szymanski 1998) and has been attributed to a wide variety of impacts, including increased siltation, toxic chemical waste, channelization of rivers, construction of wing dams, and over-harvesting of commercial species (Neves et at. 1997).

Increasing pressure has been placed on researchers to investigate and document the biology of unionids, so that management plans can be devised. This biology, including the ecology, behavior, and genetics of freshwater mussels, is also potentially crucial in recognizing species diversity. The recognition of species, in turn, is important in conservation because laws that protect biological diversity are based on taxonomy. Species can be nominated for protection under the Endangered Species Act or under state laws that protect endangered species. Under the Endangered Species Act, distinct vertebrate populations, within species, can also be protected to conserve their genetic diversity and ecological/behavioral differences. In contrast, invertebrate populations are not recognized for protection under the ESA, although many argue that this protection should be available to invertebrate populations, as well. Knowledge of invertebrate

biodiversity is generally less detailed than that of vertebrates. Without such information, further losses seem inevitable (Holland-Bartels and Kammer 1989).

Classifications of freshwater mussels have been based largely on morphological characteristics of their shell and a few aspects of soft anatomy. The use of these morphological characteristics alone has led to inconsistent and non-phylogenetic classifications (Hoeh and Gordon 1996, Lydeard et al. 1996). To adequately classify freshwater mussel species, one must use a combination of morphology with genetics, ecology, and behavior.

The life cycle of unionoids is unique, and it is of critical importance to understanding their plight. Most mussels produce a larval stage, the glochidium, which is an obligate parasite on a host, usually a fish. Glochidia development takes place in specialized portions of the female mussel's gills, which are referred to as marsupia. After maturation in the marsupium, these larvae are expelled into the environment where they must parasitize appropriate host fish (Trdan and Hoeh 1982; Figure 1). Most glochidia, after being expelled from the marsupium, perish without successful parasitism, because they fail to reach the proper host (Jansen 1991). The glochidium is not mobile, and it depends on complex behaviors of the female mussel and the fish host to encounter the correct host.

There are several techniques used by the female mussel to deliver her glochidia to the fish host. Some species have sophisticated mantle flap lures to bring the fish into close proximity. Others produce conglutinates, which are packages of glochidia. The lures and conglutinates usually resemble a food organism and may mimic the movement of such an organism. Mussels that tend to be less host-specific usually have either less

sophisticated lures or no lure at all, and some may merely broadcast their glochidia into the water. While fewer of these glochidia are likely to reach an appropriate fish host, the female can compensate for this with the production of more young (Watters 1997).

Those glochidia that do reach a suitable host will remain on the fish from one to several weeks. During this time, the organs of the larva will further develop, and it will grow a second adductor muscle (metamorphosis; Lillie 1985). Following a successful parasitic period, newly metamorphosed individuals (juveniles) begin a free-living existence as members of the benthic community. Mussels usually reach sexual maturity after 3-5 years, and the cycle repeats itself (Neves 1991).

Most species of unionoids are unable to utilize most species of fish. Typically, a few closely related host species, or populations within species, will support the development of the glochidia of many species. Some mussels are highly host specific while others utilize a great variety of hosts (Zales and Neves 1982, Neves et al. 1985, Yeager and Neves 1986). Hosts have been identified for less than half of North American mussel species (Hoggarth 1992). Observations of natural infections may not necessarily show that transformation will occur, and lab studies are needed to confirm this. On the other hand, lab studies may suggest host relationships that seldom or never occur in nature. Approximately half of the suggested mussel host relationships need further study for confirmation (Hoggarth 1992).

Exactly why fish hosts are susceptible to parasitism by certain mussel species and not by others is not well understood. The most likely cause is the immune responses of the host fish to the glochidia (Zales and Neves 1982, O'Connell and Neves 1999). Studies show that fish possess two types of immunity that can terminate parasitism by

glochidia. I will refer to these as innate immunity and acquired immunity. Innate immunity of fish to a parasite is a hereditary tissue response that will result in very early sloughing of the glochidia (Bauer and Vogel 1987). Acquired immunity is a humoral (antibody) response that a fish acquires after one or more past infections. The otherwise suitable fish host recognizes the antigen (the glochidia) and produces antibodies against the glochidia. Tolerance for glochidial parasitism diminishes after each successive infection (Bauer and Vogel 1987, Arey 1932, Watters and O'Dee 1996). The duration and generality for such intolerance seems to vary between studies depending on mussel, host, and conditions. It seems clear however, that in some cases, immunity to one mussel species confers immunity to other related species (Shiver and Barnhart 2002) and that the intolerance can last over five months (Watters and O'Dee 1996, Shiver and Barnhart 2002).

A number of studies have been performed to identify the particular host species of mussels (Hoggarth 1992, Watters and O'Dee 1996, Haag and Warren 1997). This information becomes of great importance for conservation efforts, since introductions and reintroductions of mussels will only be successful if the mussel's host fish is present (Farzaad 1991). In addition to species differences, different populations of the same mussel species may or may not use the same species of host fish (Rogers 1999). This co-adaption between sympatric populations of mussels and fish may cause glochidia from one locality to produce a low yield of transformed juveniles on fish from another locality (Graf 1997).

Another potentially important factor is the influence of temperature on glochidial transformation success. Low temperature is known to suppress immune function in

ectothermic vertebrates (Corbel 1974, Avtalion and Shahrabani 1975, O'Neill 1985) and delay antibody production (O'Connell and Neves 1999). Therefore, the fish in low temperatures might have a decreased immune response that could in turn increase transformation success of glochidia. Because fish are less active at lower temperatures, lowering the temperature of the water would decrease the possibility of the glochidia becoming dislodged from the fish's tissue (Roberts 1997, Avtalion and Shahrabani 1975, O'Neill 1985, and Bly and Clem 1991). Prior investigations also show that at higher temperatures, glochidia transform to juveniles at a quicker rate (Roberts and Barnhart 1999).

Venustaconcha ellipsiformis, the ellipse mussel, is a common mussel in the Ozarks region of Missouri. The ellipse mussel inhabits small to medium-sized streams that have a stable gravel bottom (Oesch 1995). It is most commonly found in tributaries of the upper Mississippi River in southeastern Minnesota, southeastern Wisconsin, eastern Iowa, and northern Illinois. It occurs on the northeast of the Ozark Plateaus in tributaries of the Missouri and Mississippi Rivers, and on the west slope in tributaries of the Arkansas system (Riusech and Barnhart 2000). The ellipse mussel is elliptical in shape and grows to approximately 7 cm as an adult. Its shell is yellow-brown changing to brown with age. Green rays cover most of the shell. This species is bradytictic, meaning that the females carry their glochidia in their marsupium throughout the winter and release them in the following spring, summer, and fall (Oesch 1995). Ellipse mussel females have a simple mantle lure that they flap to lure fish into close proximity to their glochidia. In some cases, the host fish tears the marsupium, releasing the glochidia.

However, it is also possible that female mussels may be able to release glochidia into the mantle cavity and eject them in response to the host fish.

The host fish for *Venustaconcha* include darters, sculpins, and possibly others (Farzaad 1991, Riusech 1999). One possible host for the ellipse mussels is the orangethroat darter (Riusech and Barnhart 2000). *Etheostoma spectabile*, the orangethroat darter, is a moderately stout darter growing to a maximum length of about 8 cm. It is a very common species over much of the Ozarks and in prairie tributaries of the lower Missouri and Mississippi rivers (Pflieger 1997, Robinson 1988). The orangethroat darter is a benthic fish found in clear to moderately turbid streams having mostly a gravely and rocky bottom. It is very similar to the rainbow darter, and the two are often found in the same waterways (Pfleiger 1997). Orangethroat darters of the Spring River and Gasconade River have been described as different subspecies: *E. s. squamosum* and *E. s. spectabile*, respectively (Distler 1968, Wiseman et al. 1978, Ceas and Page 1997).

I examined several aspects of the reproduction of ellipse mussels. First, I compared transformation success of two different populations of ellipse mussels on two different populations of orangethroat darters. Second, I tested the relationship between water temperature and transformation success. Finally, I investigated the fecundity and glochidia dispersal behavior of the ellipse mussel. It is my hope that these experiments and observations will add to the body of knowledge that will enable conservationists to better understand mussels and to ensure their survival.

METHODS

Preliminary host tests

A pilot host study was conducted to make sure that equipment and techniques were adequate and to confirm that rainbow and orangethroat darters were suitable hosts for ellipse mussel glochidia. The laboratory study was run from March 30, 2001 to May 6, 2001. Both rainbow and orangethroat darters were collected from a site on the Woods Fork of the Gasconade River (Figure 2) near the Missouri Department of Conservation (MDC) Odin Access in Wright County, Missouri (UTM 15_534217E_4124579N) on March 23, 2001. A site without mussels was chosen in order to avoid fish having preexisting glochidia infestations. Fish were collected by means of electroshock and seining and then transported to the lab in an insulated, aerated cooler. As an additional precaution to avoid infected hosts, the fish were maintained for several weeks in the lab before the study to allow any possible infection of glochidia to be sloughed off. They were kept in aquariums at room temperature and fed commercial frozen brine shrimp and bloodworms.

Gravid female ellipse mussels were collected from the Gasconade River (Figure 2) at the E-highway crossing in Wright County, Missouri, (UTM15-553273E-4129629N) in late March 2001. The mussels were stored at 4 C in shallow, aerated, containers. Glochidia were removed from one of the parent mussels by injecting sterile water into the marsupial gills from a syringe. The maturity and viability of each batch of glochidia was determined by exposing them to saline solution. The glochidia were assumed to be ready to attach to their host fish if they closed their valves when exposed to saline water (LeFevre and Curtis 1912).

Ten fish (five orangethroat darters and five rainbow darters) were anesthetized with Finquel® and infected by pipetting suspensions of glochidia directly onto their gills through the gill openings and mouth. Infected fish were transferred to a recovery tank for approximately fifteen minutes until fully alert. The infected fish were then transferred into individual aerated 2-liter containers that permitted quantitative recovery of glochidia and juveniles (Roberts 1997).

Water temperature remained fairly constant (20-25 degrees Celsius) throughout the encystment period. Every other day, the containers were half drained through a filter and then refilled with conditioned water. Detached glochidia and juveniles were recovered from the filter and were examined, counted under magnification, and recorded. Untransformed glochidia and transformed juveniles were distinguished by observing foot movement and the number of adductor muscles present. Untransformed glochidia are motionless and have only one adductor muscle, while transformed juveniles show foot movement and have two adductor muscles.

Comparison of transformation success between populations

This laboratory study was run from June 19, 2001 to July 29, 2001. The Gasconade orangethroat darters and ellipse mussels were from the same collections described previously (fish were collected from the Odin site and mussels from E-highway site). Spring River fish and mussels were collected on April 14, 2001, from sites near Hoberg, Missouri. Mussels were collected from the Spring River (Figure 2) branch (UTM15-424022E-4103416N), and the fish were collected from the Honey Creek

branch, which apparently lacks mussels (UTM15-424072E-4105649N). Collection methods and maintenance were similar to those described above.

Four groups of 18 darters each were selected, two groups of Spring River fish and two of Gasconade River fish (72 fish total). Each group included nine males and nine females. Glochidia were obtained from 6 mussels, 3 from Spring River, and 3 from the Gasconade River. Each of the 6 mussels was used to inoculate 12 fish (6 from each river) as described previously (Figure 3).

The fish were held in 2-liter containers as described above. Water temperature remained between 20-25 degrees Celsius throughout the experiment. The bottles were drained every other day though a Nitex filter and glochidia and juveniles that were recovered on the filter were counted under magnification.

Temperature effects on transformation

This laboratory study was run from August 3, 2001 to November 20, 2001. Three gravid ellipse mussels and 36 orangethroat darters from the Spring River were used. Glochidia were extracted and their viability checked in the same manner used earlier. However, the inoculation of the fish differed from the earlier experiment. Twelve darters were placed in each of three two-liter glass beakers. The beakers were filled with conditioned tap water that was highly agitated using an aerator and three air stones to avoid the settling of glochidia to the bottom where they would be less available to the fish. Each mussel provided glochidia to inoculate one beaker of fish. The concentration of glochidia in each beaker was determined by counting the number in volumetric subsamples. The time the fish were allowed to swim in the beakers was

varied according to concentration to achieve similar rates of attachment. In beakers one and three, the fish were allowed to swim in the beakers for a half hour because there was a higher concentration of glochidia and in beaker two for forty-five minutes because the concentration of glochidia was lower.

All thirty-six fish were transferred to 2-liter bottles that allowed for quantitative collection of glochidia and juveniles (the same kind of containers used in the earlier experiment). Four fish from each beaker (twelve total) were placed at three different temperatures: 15, 20, and 25 C. Two groups (15 and 25 C) were hung in incubators, and the 20 C group was hung in the lab (Figure 4). Glochidia and juvenile mussels were collected and quantified as described previously. Temperature was checked and recorded during each collection.

Fecundity Study

I counted the total number of glochidia in the marsupium of six gravid female ellipse mussels. The mussels were collected from the Hoberg site in the Spring River. The females were chosen to be of varying age and size, with full marsupia. Each individual was weighed and measured for height, width, and length. They were then anaesthetized in a solution of water and Finquel for approximately five minutes. They were then sacrificed, and their marsupial gills were dissected. The marsupia were weighed and then carefully dissected in water under magnification to free the glochidia. The glochidia from each female were counted as follows: The glochidia were placed in 250 mL of water. The water was agitated with a large syringe (baster) to keep the glochidia from settling on the bottom. Six 200-μL samples were taken and counted

under magnification. These counts were averaged and multiplied by the total volume to determine the total number of glochidia. The ages of the female mussels were estimated from counts of annual growth lines (Day 1984; Bauer 1991, 1992; Riusech 1999).

Glochidia Release Study

This study was done to characterize the glochidial dispersal technique used by the female mussels. Six Spring River females were placed in river gravel in shallow, aerated water, in order to simulate the natural stream environment. The females were acclimated to their new surroundings for two days. A soft wire loop (~3 cm long) was attached to a plastic pipette so that the loop projected in front of the open end of the pipette. This tool was used to stir the water around the female near the opening of the shell, and to touch the female's mantle and marsupium. Care was taken not to rupture the marsupium. After this stimulus, the pipette was used to suck up the water surrounding the marsupium. Each mussel was treated in this way at four-hour intervals for a twenty-four hour period. Thirty-six total samples, six from each mussel, were collected and their contents examined for glochidia under magnification.

RESULTS

Preliminary Host Tests

In the preliminary test using Gasconade fish and glochidia, both orangethroat darters and rainbow darters were suitable hosts for ellipse mussel glochidia. The mean number of glochidia that attached and transformed did not differ significantly between rainbow and orangethroat darters (t-test, $p > 0.05$). The overall mean number of glochidia

that attached was 41 per fish. The average number of glochidia that transformed on the darters overall was 19 glochidia per fish. The average transformation success was 40% of those that attached (Table 1).

Comparison of transformation success between populations

The attachment, juveniles produced, and transformation success percentage of Gasconade and Spring River glochidia on Gasconade and Spring River fish were compared (Tables 2-5, Figures 5-7). Variation among test groups in number of glochidia attached, number of juveniles produced, and percent of attached glochidia that transformed was analyzed using ANOVA (General Linear Model, Minitab). The independent factors in the analysis were the sources of the glochidia and the fish (Gasconade or Spring River), and the individual mussels from which the glochidia were obtained (3 mussels from each river). Glochidia source and fish source were crossed in the model, and individual mussel was nested within glochidia source. The number of glochidia attached was significantly dependent on glochidia source and on individual mussel, but not on fish source or the interaction of fish and glochidia source (Table 3). Gasconade glochidia attached at a significantly higher rate than Spring River glochidia (Tables 2 and 3, Figure 5). The number of juveniles produced was significantly related only to individual mussel (Table 4). Percent transformation was not significantly affected by any of the independent variables (Table 5 and Figure 6).

Most fish did not transform any of the attached glochidia (Figure 6). The overall means for attachment and transformation were 24.5 and 1.8 per fish, respectively, and the mean transformation percentage was 4.7% (Table 2). Despite this low transformation,

five individual fish (of 72 total) appeared to be fairly good hosts, with more than 20 glochidia attached (Figure 5) and 10-28% transformed (Figure 6).

Most glochidia that did not transform fell off within 2 days after inoculation. Transformed juveniles began appearing 22 days after inoculation and continued to appear for up to 36 days after inoculation. Peak drop-off of juveniles occurred between day 28 and 32 (Figure 7).

Temperature effects on transformation

This experiment used Spring River glochidia on Spring River fish at three temperatures (Table 6). The effects of temperature on glochidia attached, juveniles produced, and percent of attached glochidia that transformed were analyzed using ANOVA (General Linear Model, Minitab). The independent factors in the analysis were temperature and individual mussel. Temperature and individual mussel were crossed in the model. The number of glochidia attached was not significantly affected by temperature or mussel (Table 7). The number of juveniles produced and percent transformation were significantly related to temperature but not mussel (Tables 8 and 9). Tukey's method was used to make pairwise comparisons between temperatures for the number of juveniles produced and percent transformation. The number of juveniles produced differed significantly between 15 C and 25 C. The percent transformation differed significantly between 15 C and 25 C and between 20 C and 25 C (Table 6).

The timing of transformation and drop-off was delayed in lower temperatures. It took 62 days for all the glochidia and juveniles to fall off at 15 C, 34 days at 20 C, and 24 days at 25 C. Peak drop off for juveniles at 15 C was at day 46-58, day 34 at 20 C, and

day 20 at 25 C. As in the prior study, most of the glochidia sloughed off early in the experiment (Figure 8).

Fecundity Study

The mean wet tissue weight of six Spring River females was 4.89 grams and the average total mass (wet tissue and shell) was 15.8 grams. The mean total fecundity was 54,807 glochidia. It should be noted that the mussels were selected for a range of sizes, so that the mean does not represent a random sample of females. There was a positive relationship between fecundity and mass (Table 10, Figures 9, 10).

Glochidia Release Study

I saw four of the six females displaying their lures, but only in the early morning (between 12:00 A.M. and 4 A.M.). Of the other two, I saw no display (Table 11). Of the four that displayed lures, three expelled a few glochidia when stimulated. One female expelled three glochidia at 4:00 A.M. One female expelled two glochidia at both 12 A.M. and 4 A.M. The third female released five glochidia at 12 A. M. Neither of the two females that did not display lures expelled glochidia when stimulated (Table 11).

DISCUSSION

Suitability of host fish

Few other studies of *Venustaconcha* host relationships are available. Riusech and Barnhart (2000) examined the host relationships of *V. pleasii*, which is found in the White River system of the Ozark Plateau. Observations of natural infections and

laboratory host tests indicated that the rainbow darter (*Etheostoma caeruleum*) is a primary host of *Venustaconcha pleasii,* and glochidia were also observed on banded sculpin, yoke darter, and greenside darter. These authors also observed natural infestations of *V. ellipsiformis* on orangethroat darters in the Spring River, where the rainbow darter is absent. Encysted glochidia of *V. ellipsiformis* were also observed on redfin darter, greenside darter, and banded sculpin. Lab tests indicated that James River rainbow darters were suitable hosts for the sympatric *V. pleasii* (31% transformation success) but they transformed far fewer Spring River *V. ellipsiformis* (3%) or Meramec River *V. ellipsiformis* (6%). Hosts for *Venustaconcha ellipsiformis* in Minnesota were reported to include slimy and mottled sculpins, Johnny darter, Iowa darter, fantail darter, blackside darter, logperch, and brook stickleback, but quantitative transformation data are not available (Hove and Anderson 1997, Hove and Kurth 1998).

Results of my pilot study indicated that both rainbow and orangethroat darters were suitable hosts for sympatric ellipse mussel in the Gasconade River. Transformation success on orangethroat darters was 38% (Table 1). However, in the population study, transformation on Gasconade orangethroat darters was only 4% (Table 2). Both experiments placed Gasconade glochidia on Gasconade fish. The numbers of glochidia that attached did not differ (t-test, $p = 0.539$), but the differences between the two experiments in number of juveniles and transformation success were statistically significant (two-sampled t-test, $p = 0.014$, $p = 0.017$, respectively). The cause of this inconsistency in transformation success of Gasconade ellipse on orangethroat darters is not known. One factor that could influence results is condition of the glochidia. Mussels that provided the glochidia used were collected in mid-March and stored at 4 C for the

next several months. Glochidia used in the pilot study had been stored only a week whereas those used in subsequent experiments had been stored longer (up to five months). However, a few of the orangethroat darters were reasonably good hosts (Figure 6). This fact seems to indicate that the glochidia were functional. Individual variation in transformation success was also reported by Riusech and Barnhart (2000).

Another factor that could affect transformation success of glochidia is prior exposure of the host fish to glochidia, resulting in acquired immunity. Both host and non-host species express a humoral response specific to glochidial antigens after induced infestations with glochidia (O'Connell and Neves 1999). Reuling (1918) indicated that, after two successive optimum infections of glochidia on the gills of a fish, the fish no longer would carry glochidia a third time. Other studies have also indicated that tolerance for glochidial parasitism diminishes after each successive infection (Bauer and Vogel 1987, Arey 1932, Watters and O'Dee 1996). The diminished tolerance of a host fish to parasites can last over 5 months and can confer immunity to other related species (Shiver and Barnhart 2002). Although there is no way to be certain that wild-caught fish have never been infected previously, I attempted to obtain fish from stream reaches where mussels did not occur, in order to avoid fish that might have acquired immunity to glochidia. Moreover, in the case of the Gasconade populations, I used fish and mussels from the same collection for the pilot and the population experiment. Therefore, the different results in the two experiments are unlikely to have been caused by acquired immunity.

Other acquired differences among individual fish (as opposed to genetic differences), are possible. For example, some of the fish may have had compromised

immune systems, due to sickness or lack of nutrition, leaving them unable to reject the glochidia. I did not see any evidence of illness during the experiments.

None of the experiments showed good transformation of Spring River glochidia on orangethroat darters. Transformation success ranged from 0.2 to 6.0% with a mean of 4% in 6 different experiments (Tables 2 and 6). Furthermore, a subsequent experiment testing Spring River glochidia on Spring River orangethroats likewise found low transformation success (M. C. Barnhart, personal communication). These observations are at odds with the reported observation of *Venustaconcha* glochidia on a large proportion of orangethroat darters in the Spring River (Riusech and Barnhart 2000). All subsequent observations suggest that Spring River glochidia do not transform well on orangethroats.

Although transformation success was similar among groups in the population experiment, there were differences in the number of glochidia that initially attached to the fish. Significantly more Gasconade glochidia attached than did Spring River glochidia (Table 2, Table 3 and Figure 5). Assuming that attachment reflects compatibility between the glochidium and the host, this result may indicate that the Gasconade River mussels are more compatible with orangethroat darters than are the Spring River mussels. This observation is consistent with the fact that, at least in my preliminary study, Gasconade glochidia transformed well on orangethroats, while Spring River glochidia never did.

The results also showed significant variation in attachment among the individual mussels (Table 3). That is, glochidia from some females attached to the fish in larger numbers than did glochidia from others. The simplest explanation for this variation is

difference in the numbers of glochidia to which the fish were exposed. Although roughly similar numbers of glochidia were used from each mussel, I did not attempt standardize the concentrations of the suspensions that were pipetted onto the fish's gills. Some suspensions may have been more concentrated so that more glochidia attached.

Attachment of Spring River glochidia also differed significantly in one comparison; between the population experiment and the temperature experiment (Tables 2 and 6, two-sampled t-test, $p = 0.000$). These differences were probably caused by a difference in inoculation technique. In the population experiment, the fish were inoculated by pipetting glochidia into their mouth and gills, while in the temperature comparison the fish swam in an aerated beaker with suspension of glochidia. The latter technique was more efficient, inoculating each fish, on average, with seventy-five more glochidia then the former technique (Tables 2 and 6). Swimming the fish with the glochidia, rather than pipeting them onto the fish, was also quicker and less traumatic on the fish.

I hypothesized that sympatric populations of glochidia and hosts would be more compatible than allopatric populations. At least two other studies showed that sympatric host fish populations are better hosts. According to Rogers (1999), tan riffleshell (*Epioblasma florentina walkeri)* from Indian Creek in Virginia transformed more effectively on fantail darters (*Etheostoma flabellare)* from Indian Creek than fantail darters from Roanoke River in Virginia. Secondly, a recent study found differences in host compatibility among populations of fanshell mussels (*Cyprogenia aberti;* Nathan Eckert, SMSU, personal communication). These differences may be caused by a co-adaptation that has taken place between sympatric populations of host and parasite.

It is plausible to expect genetic differences among populations of *Venustaconcha*, because these populations are isolated from one another and probably experience very little if any gene flow between one another. Freshwater mussels, in general, are sedentary animals. Long-distance movements are only likely via the host fish during parasitism. The hosts of *Venustaconcha* are presumably darters and sculpins, which are benthic fish that probably do not travel long distances regularly. Populations of both the mussels and their hosts are found in headwater streams and large river systems would not be habitable for them. For one population to reach the other, the mussel and fish would either have to travel hundreds of miles through the Arkansas, Mississippi, and Missouri River system or have to travel through stream captures, which are probably relatively infrequent on the Ozark Plateau. This isolation would facilitate different evolutionary trends between the two populations. I suggest that the host comparisons study be repeated using more compatible host fish. Recent host studies indicate that suitable hosts for Spring River ellipse include banded sculpin, stippled darter, and fantail darter (personal communication, M.C.Barnhart).

The temperature comparison experiment revealed surprising results as well. As mentioned in the introduction, tests have shown that low temperatures decrease the immunity of vertebrates (Corbel 1974, Avtalion and Shahrabani 1975, O'Neill 1985, O'Connell and Neves 1999). Immunity has a large impact on the ability for a parasite to survive on a host. Roberts and Barnhart (1999), showed that decreasing water temperature slowed transformation but increased the efficiency of transformation of the glochidia on the fish host. However in my study, transformation success decreased from 25 C to 15 C and 20 C to 15 C (Tables 6 and 9, Figure 8).

Fecundity study

The fecundity of *Venustaconcha* is relatively low for a unionoid mussel. Other species with larger body size can produce hundreds of thousands or even millions of glochidia (Bauer 1994). The relatively small number of glochidia produced by *Venustaconcha* appears to be a direct consequence of its small body size. There was a linear relationship between size and fecundity (Table 10, Figures 9 and 10).

Glochidia Release Study

The actual techniques used by *Venustaconcha* species to dispense glochidia are unknown. Close relatives to the ellipse mussel, *Lampsilis* species, dispense their glochidia after a fish ruptures their marsupia (personal observations). This is most likely the case in ellipse mussel, but I wanted to test whether they were capable of releasing a few glochidia at a time with minor tactile stimulation. My results showed that they did expel a few glochidia as a response to tactile stimulation (Table 11, Figure 11). This occurred only in early morning hours (12 A.M. and 4 A.M.). These were the only hours that I personally witnessed some of them displaying their lure (Table 11). This observation suggests that the host fish also feed at this time. Presumably, mussel lure behavior evolved to increase the probability that their glochidia will encounter a fish. The technique of releasing a few glochidia at a time may be advantageous if the female is unsure of a host fish being near. She may release a few of her young, in hopes of its presence but not waste her entire brood if a host fish is not near by. Another advantage of this technique is that one or only a few fish may not be able to carry her entire brood

so she releases only a few at a time with slight stimulation (the stimulation probably caused by only one or a few fish) increasing their ability to successfully attach.

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TABLES

Table 1. Transformation success of Gasconade River ellipse mussels on Gasconade orangethroat and rainbow darters.

Rainbow darters			
fish #	**n glochidia attached**	**n glochidia transformed**	**Percent transformed**
1	**61**	**20**	**32.8**
2	**54**	**29**	**54.7**
3	**32**	**12**	**37.5**
4	**86**	**39**	**45.3**
5	**49**	**23**	**46.9**
Mean ± St. Dev.	**54.6±19.7**	**24.6±10.1**	**43.6±8.21**

Orangethroat darters			
1	**46**	**13**	**28.3**
2	**32**	**9**	**28.1**
3	**29.5**	**22**	**74.6**
4	**24**	**7**	**29.2**
5	**60**	**18**	**30**
Mean ± St. Dev.	**38.3±14.6**	**13.8±6.2**	**38.0±20.5**

Table 2. Comparison of attachment and transformation among populations. G=Gasconade River, S=Spring River. Numbers are mean ± standard deviation (n=18).

Mussel	Fish	n glochidia attached	n glochidia transformed	%transformation
G	G	47.1 ± 52.6	4.4 ± 11.5	4.0 ± 8.4
G	S	29.5 ± 36.2	1.3 ± 2.0	5.7 ± 11.9
S	S	13.6 ± 23.3	1.1 ± 1.9	4.5 ± 8.9
S	G	7.1 ± 7.1	0.4 ± 0.8	4.6 ± 10.0
Grand mean ± St. Dev.		24.3 ± 29.8	1.8 ± 4.1	4.7 ± 9.8

Table 3: ANOVA: Attachment of glochidia to host fish versus glochidia source (gsrc), fish source (fsrc), and individual mussel.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gscr	1	14028.1	14028.1	14028.1	15.78	0.000
mussel (gsrc)	4	22469.7	22469.7	5617.4	6.32	0.000
fsrc	1	550.0	550.0	550.0	0.62	0.434
gsrc*fsrc	1	2616.1	2616.1	2616.1	2.94	0.091
Error	64	56877.3	56877.3	888.7		
Total	71	96541.3				

Table 4: ANOVA: Number of juveniles produced versus glochidia source (gsrc), fish source (fsrc), and individual mussel (mussel).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gscr	1	80.22	80.22	80.22	2.47	0.121
mussel(gsrc)	4	325.56	325.56	81.39	2.50	0.051
fsrc	1	26.89	26.89	26.89	0.83	0.367
gsrc*fsrc	1	68.06	68.06	68.06	2.09	0.153
Error	64	2082.56	2082.56	32.54		
Total	71	2583.28				

Table 5: ANOVA: Transformation success versus glochidia source (gsrc), fish source (fsrc), and individual mussel (mussel).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
gsrc	1	88.9	88.9	88.9	0.42	0.520
mussel(gsrc)	4	1196.2	1196.2	299.1	1.41	0.240
fsrc	1	265.3	265.3	265.3	1.25	0.267
gsrc*fsrc	1	79.4	79.4	79.4	0.37	0.543
Error	64	13562.4	13562.4	211.9		
Total	71	15192.1				

Table 6. Effect of temperature on attachment and transformation. Numbers are mean ± standard deviation (n=12). Tukey's pairwise comparison showed significant difference in number of juveniles produced between 15 C and 25 C (P = 0.0079) and significant difference for % transformation between 15 C and 25 C (P = 0.0021) and 20 C and 25 C (P = 0.0496).

Temperature	n glochidia attached	n glochidia transformed	% transformation
15	67.0 ± 17.1	0.2 ± 0.4	0.2 ± 0.5
20	89.5 ± 42.9	2.0 ± 2.2	2.3 ± 2.6
25	80 ± 37.1	4.4 ± 5.1	6.0 ± 6.1
Mean ± StDev	78.8 ± 32.4	2.2 ± 2.6	2.8 ± 3.1

Table 7: ANOVA: Attachment of glochidia to host fish versus temperature and individual mussel.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
temp	2	3080	3080	1540	1.25	0.303
mussel(temp)	6	5296	5296	883	0.72	0.640
Error	27	33302	33302	1233		
Total	35	41678				

Table 8: ANOVA: Number of juveniles produced versus temperature and individual mussel.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
temp	2	109.264	109.264	54.632	6.39	0.005
mussel(temp)	6	113.875	113.875	18.979	2.22	0.072
Error	27	230.938	230.938	8.553		
Total	35	454.076				

Table 9: ANOVA: Transformation success versus temperature and individual mussel.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Temp	2	196.41	196.41	98.21	6.73	0.004
mussel(temp)	6	57.65	57.65	9.61	0.66	0.683
Error	27	394.04	394.04	14.59		
Total	35	648.10				

Table 10. Fecundity (number of glochidia) versus size and estimated age of female

Estimated Age of Female	Total Mass (g)	Wet Tissue Mass (g)	Fecundity
4	5.5	2.0	10208
6	9.4	2.7	33125
7	11.3	6.8	58333
10	22.5	4.0	71346
12	19.6	4.7	46458
12	25.8	7.2	109370

Table 11: Lure display and glochidia expulsion different times of the day (Y = yes, − = no)

Lure Display						
Mussel	**12 A.M**	**4 A.M.**	**8 A.M.**	**12 P.M.**	**4 P.M.**	**8 P.M.**
1	Y	Y	–	–	–	–
2	Y	Y	–	–	–	–
3	Y	Y	–	–	–	–
4	Y	Y	–	–	–	–
5	–	–	–	–	–	–
6	–	–	–	–	–	–

Glochidia Expulsion						
Mussel	**12 A.M**	**4 A.M.**	**8 A.M.**	**12 P.M.**	**4 P.M.**	**8 P.M.**
1	0	3	0	0	0	0
2	2	2	0	0	0	0
3	5	0	0	0	0	0
4	0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0

FIGURES

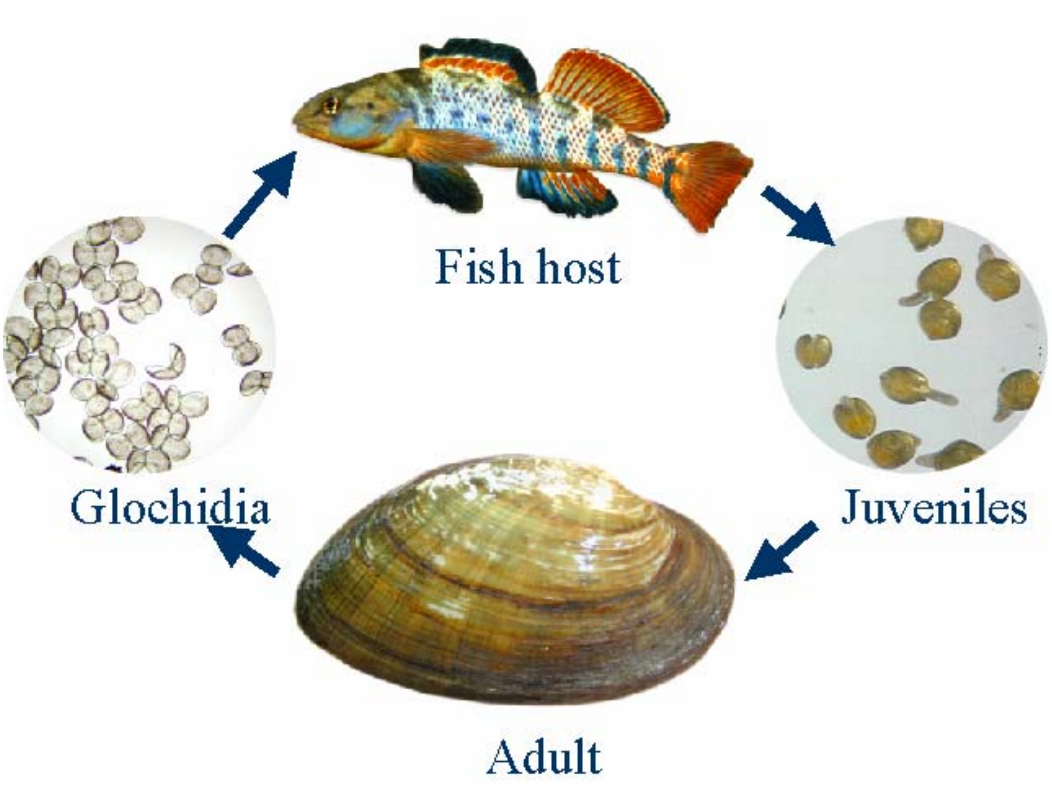

Figure 1. Generalized life cycle of freshwater mussels. The gravid female releases her glochidia, larvae that are unable to survive without parasitizing a compatible fish host. On the fish host, the glochidia will undergo transformation to the juvenile stage. After one to several weeks they will drop off as juveniles and live a benthic existence in the streambed, hopefully reaching maturity and reproducing.

Figure 2. Map showing sites on the Spring and Gasconade River where fish and mussels were collected. The Spring River is part of the Arkansas River system and the Gasconade River is part of the Missouri River system.

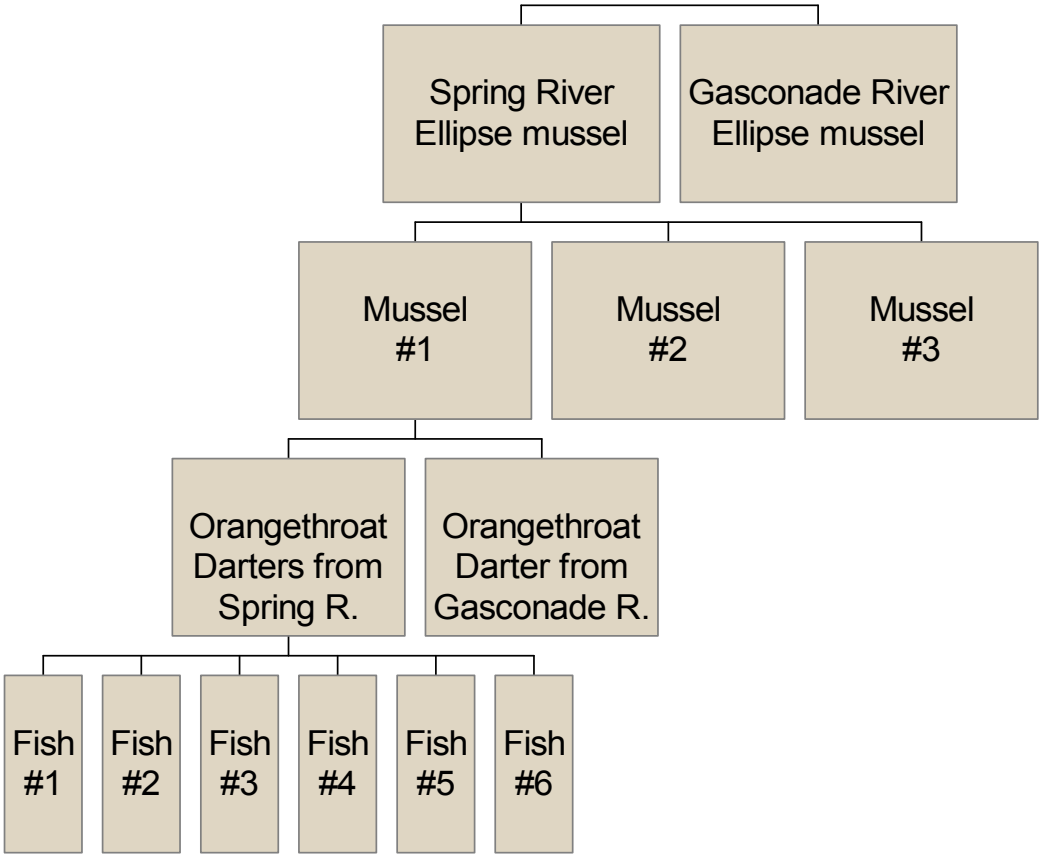

Figure 3. Experimental setup for population comparisons. Detail is shown for only one group at each level but was similar for all groups. Each of the 6 mussels (3 from the Spring River and 3 from the Gasconade River) provided glochidia to inoculate 6 orangethroat darters from the Spring River and 6 from the Gasconade River.

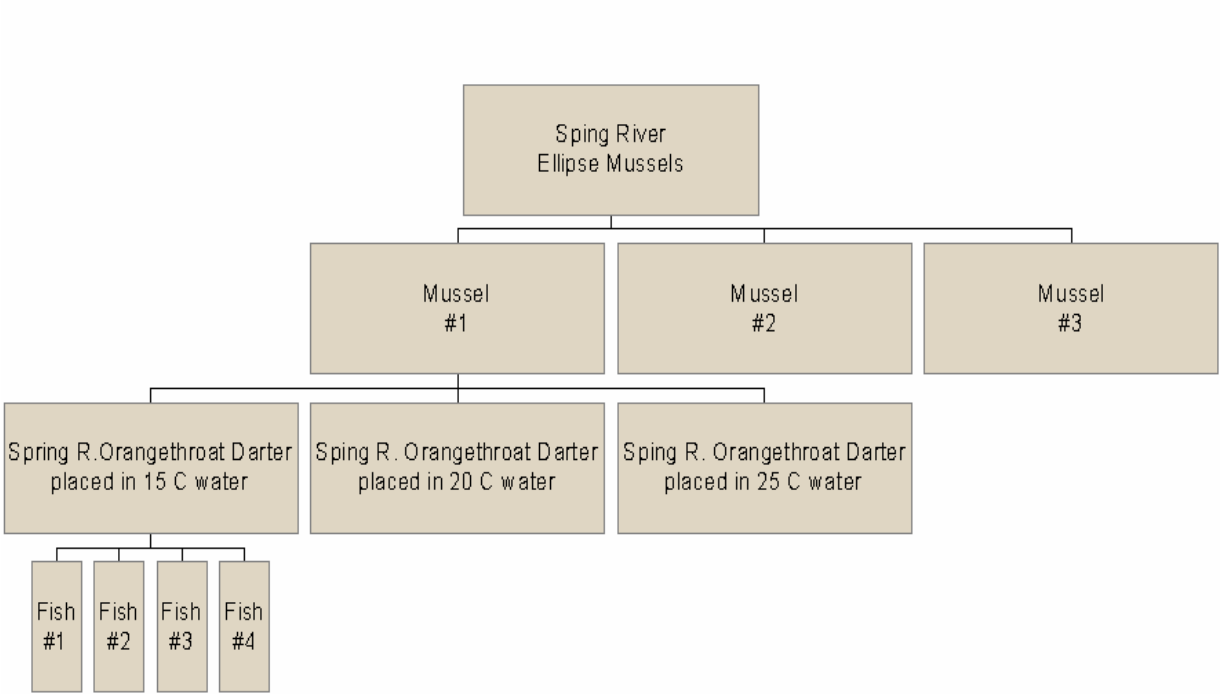

Figure 4. Experimental setup for temperature comparison experiment. Detail is shown for only one group at each level but was similar for all groups. Each of the 3 mussels provided glochidia that were used to inoculate 12 orangethroat darters. Four of the 12 darters from each group were placed in 15 C water, 4 in 20 C water, and 4 in 25 C water.

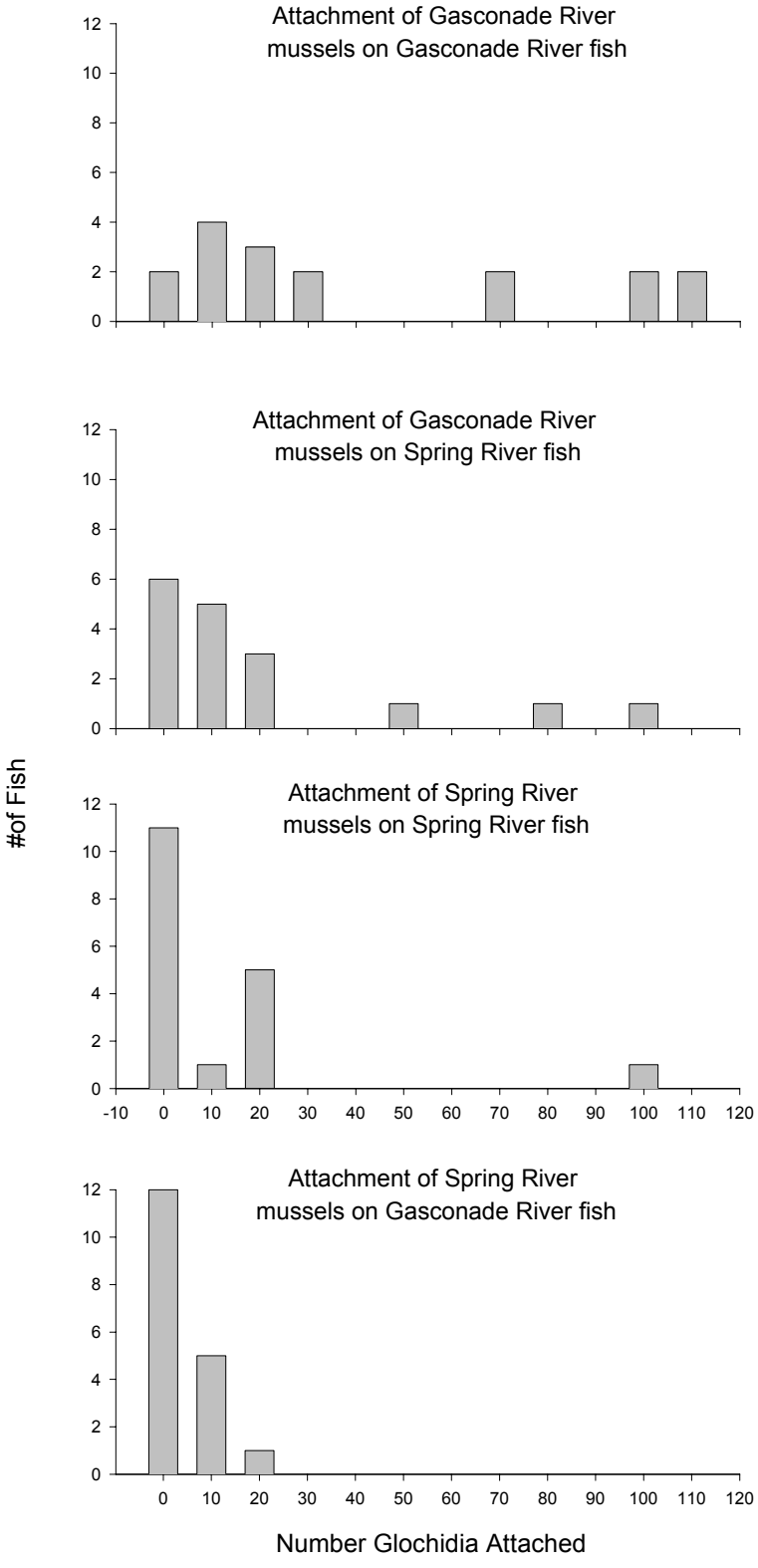

Figure 5. Attachment of Gasconade and Spring River ellipse mussels on allopatric and sympatric populations of orangethroat darters.

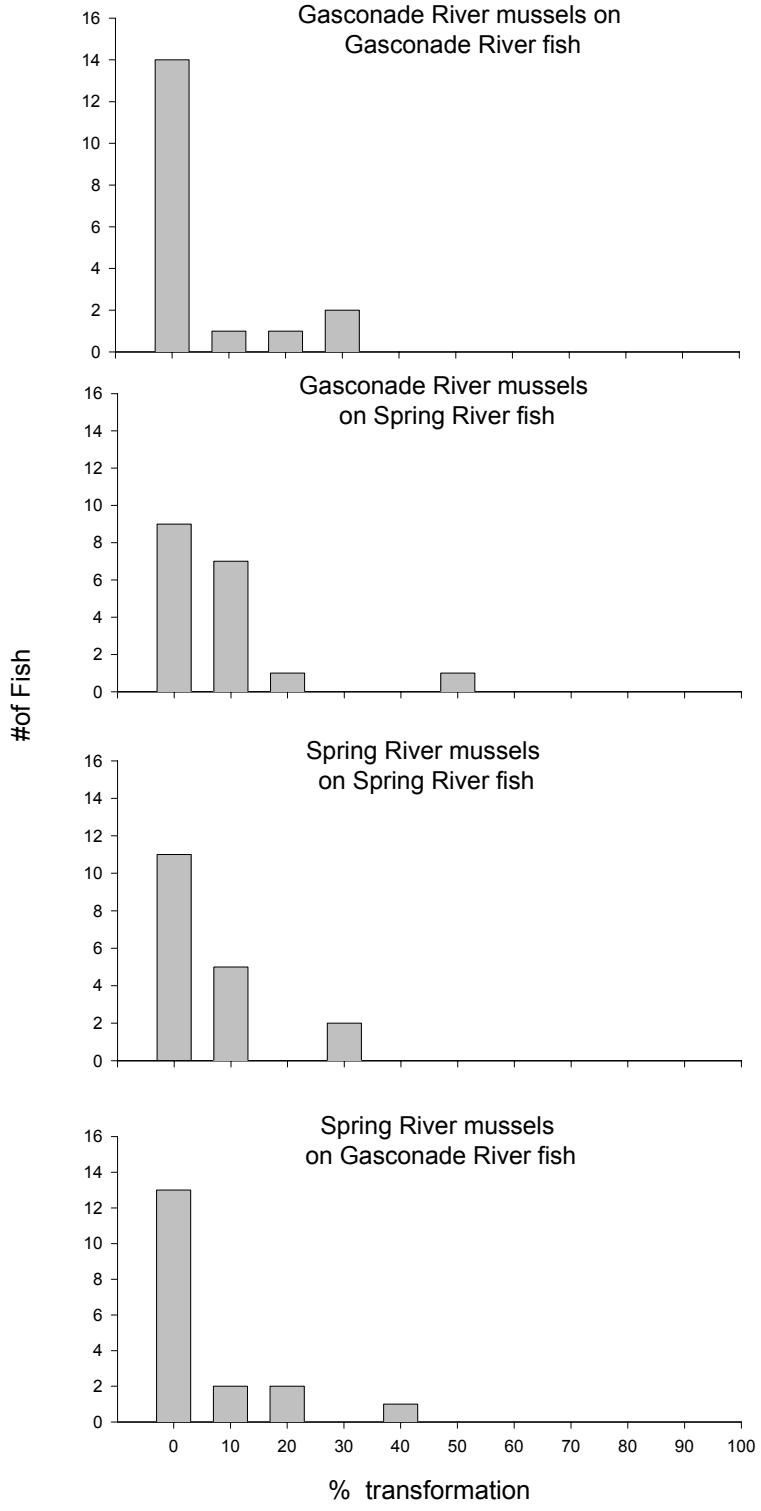

Figure 6. Transformation percentages of Gasconade and Spring River ellipse mussels on allopatric and sympatric populations of orangethroat darters.

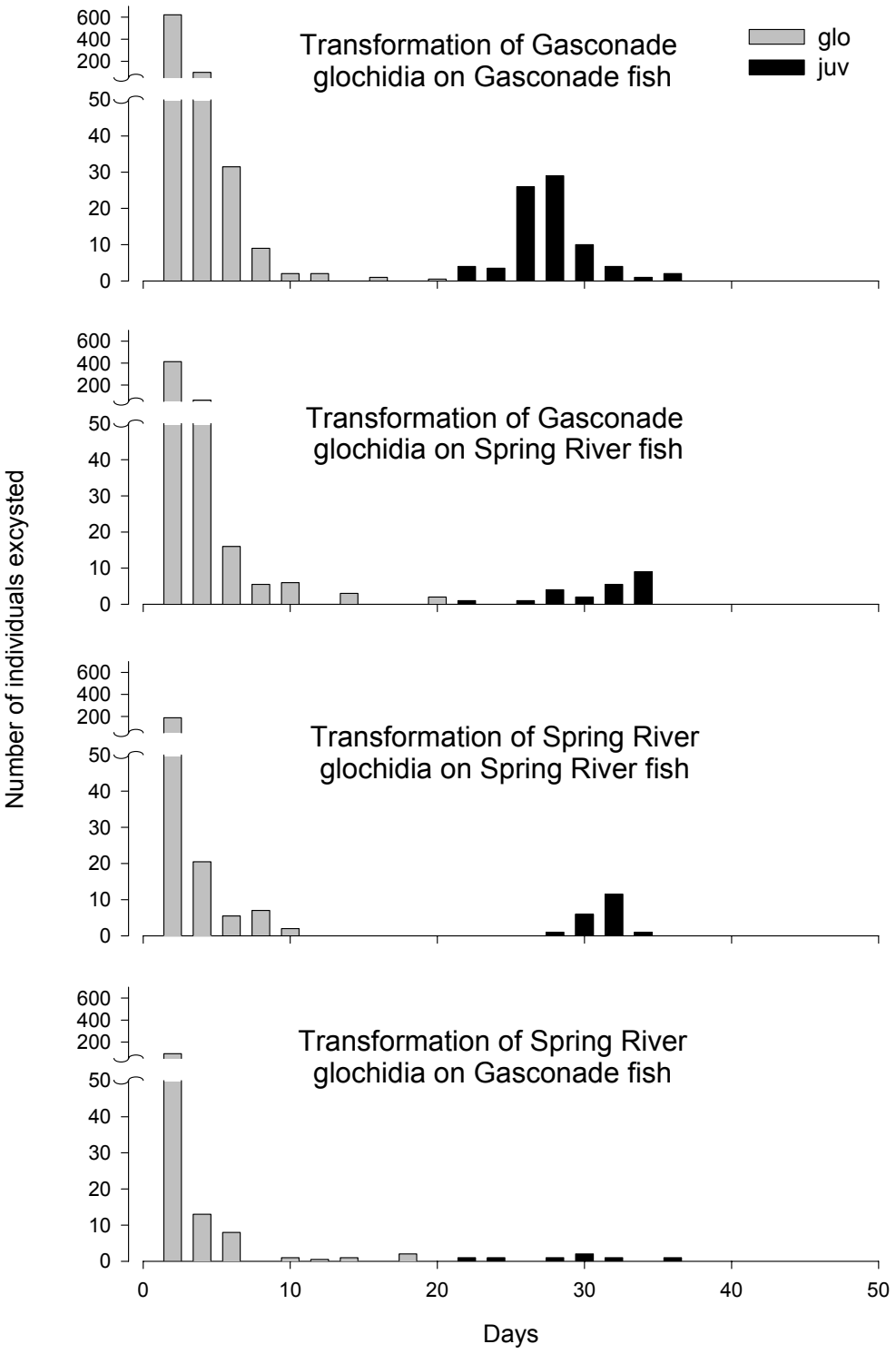

Figure 7: Transformation success of Gasconade and Spring River ellipse mussels on sympatric and allopatric populations of orangethroat darters.

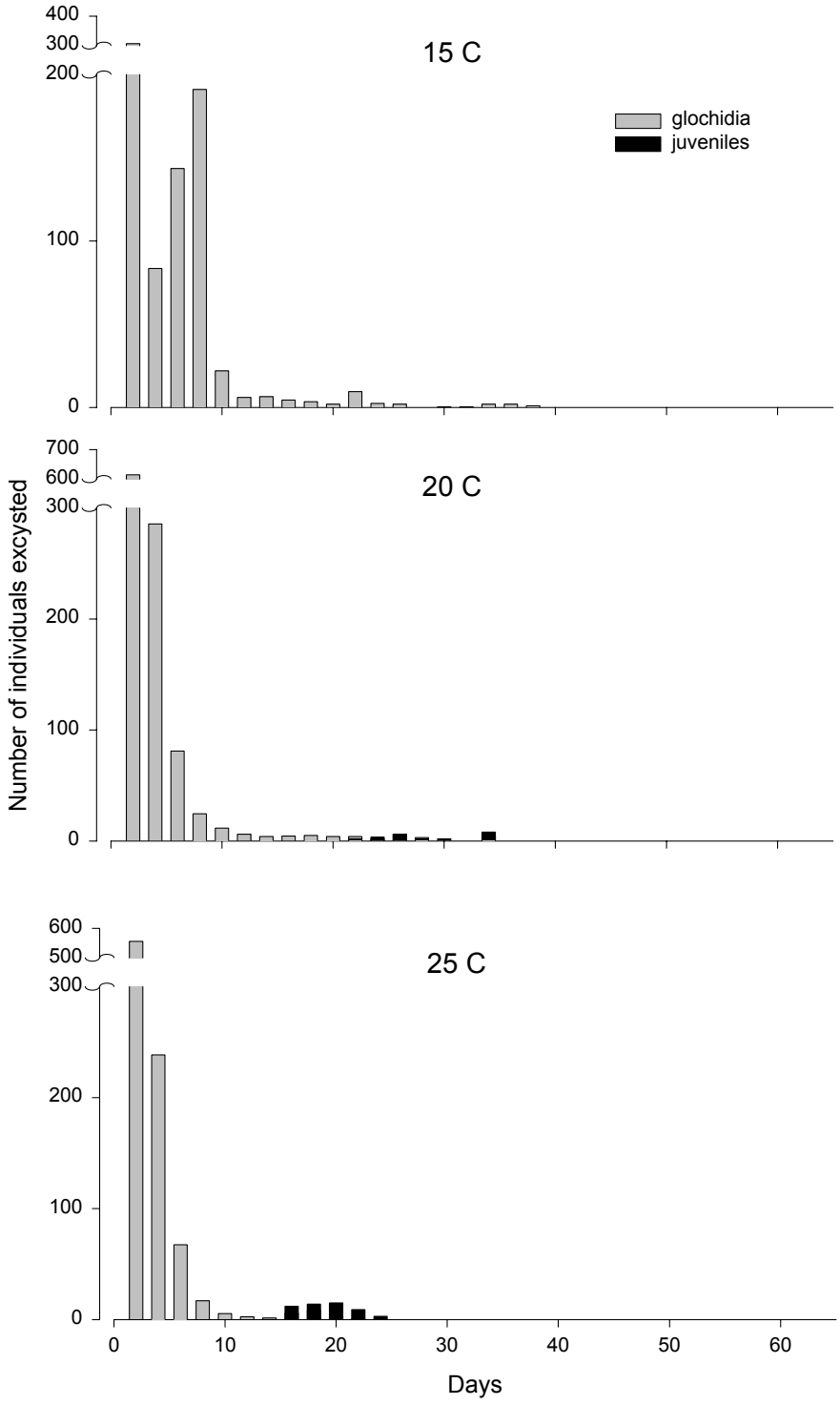

Figure 8: Transformation success of Spring River ellipse mussels on Spring River orangethroat darters at varying temperatures.

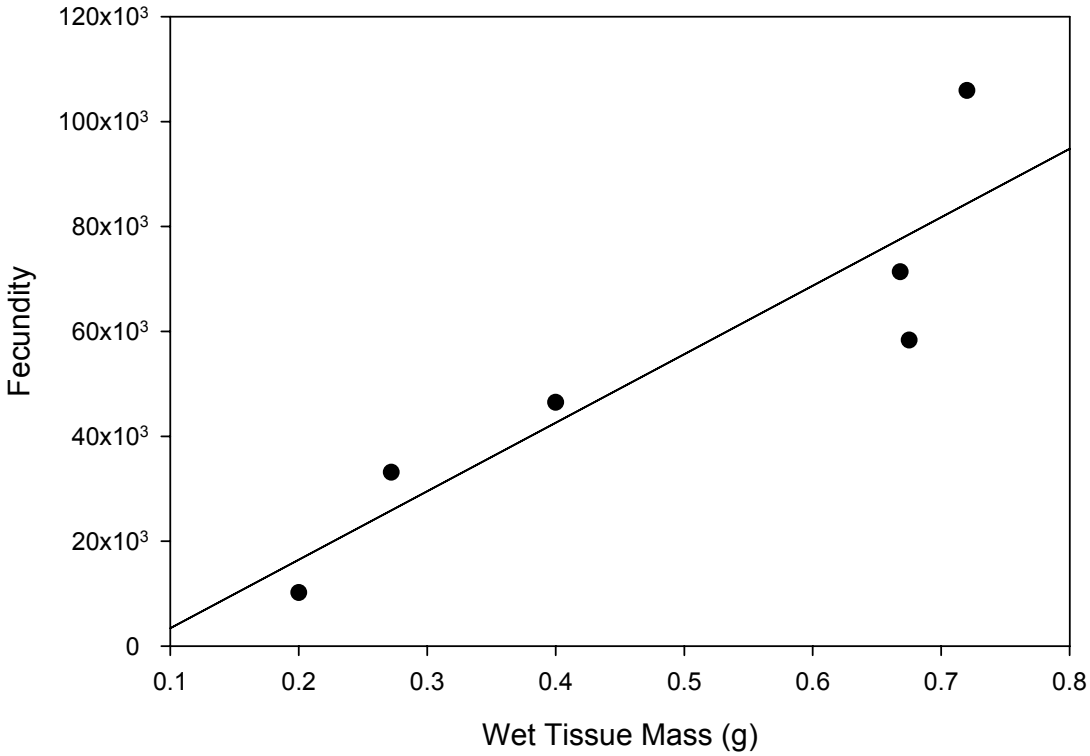

Figure 9. Fecundity versus wet tissue mass of female. Regression equation is: Y=130611X-9654 and the Pearson's correlation coefficient = 0.81.

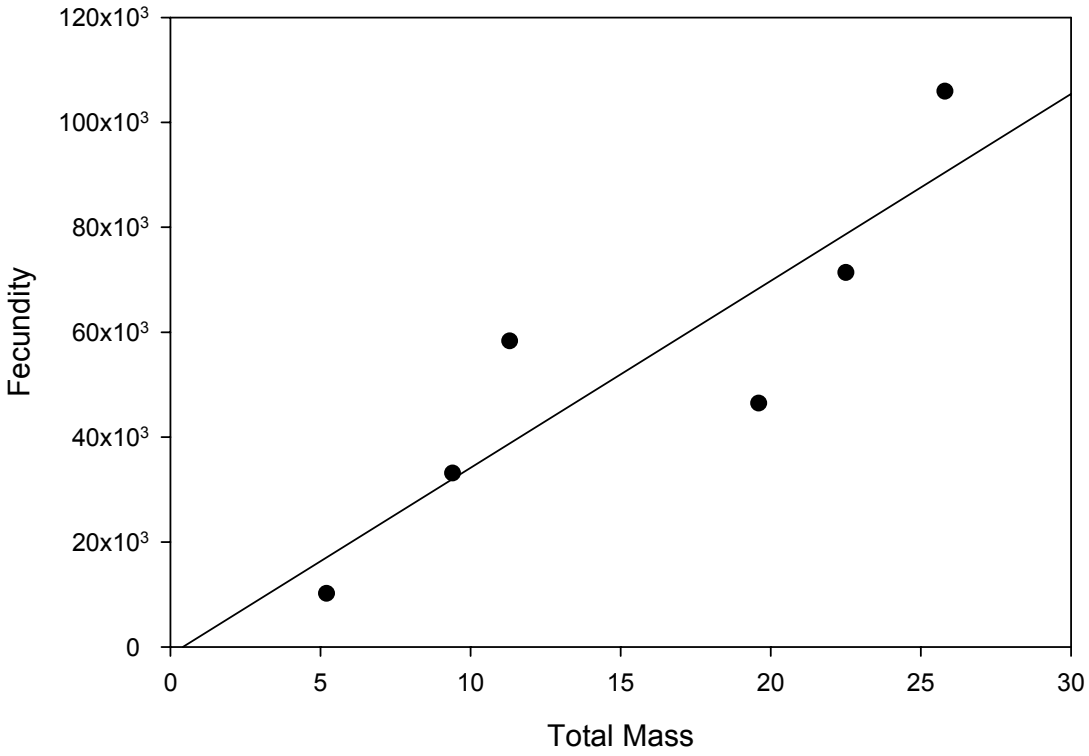

Figure 10: Fecundity versus total mass of female. Mass of the mussel includes shell and wet tissue. They were dried as much as possible but some water may contribute to total mass. Glochidia were counted from both marsupia and combined to get fecundity. Regression equation is: Y=3563X-1465 and the Pearson's correlation coefficient = 0.7783.