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# Fish Hosts and Culture of Mussel Species of Special Concern: Annual Report for 2000

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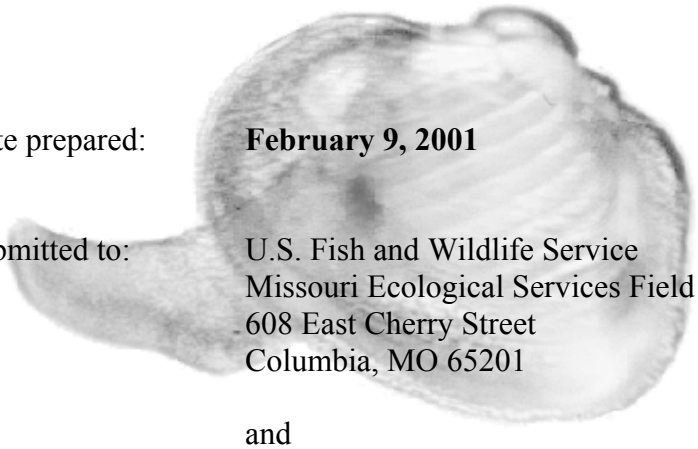
**Fish Hosts and Culture of Mussel Species of Special Concern:**
**Annual Report for 2000**



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## SUMMARY

This report describes results of the third year of a 3-year investigation of reproductive biology of freshwater mussels (unionoids). The first two years of this project focused on identification of hosts and on development of methods related to propagation of threatened species (Barnhart 1998, Barnhart and Baird 1999). In the third year of the grant we continued propagation efforts with Neosho muckets (*Lampsilis rafinesqueana*), and began propagation efforts with scaleshell (*Leptodea leptodon*). We also investigated the effects of chronic exposure to low oxygen on survival of juvenile *Lampsilis*. We plan to continue propagation of threatened species and investigations of the biology of juvenile mussels over the next three years, with continued support from U. S Fish and Wildlife Service and the Missouri Department of Conservation (MDC).

Production of Neosho muckets was again successful in 2000. Glochidia were obtained from females collected in the Fall River, Kansas and were transformed on largemouth bass at the MDC Chesapeake Fish Hatchery. Hatchery largemouth bass were inoculated with glochidia from two female mussels. Approximately 33,000 juvenile mussels resulted and were released at sites in the Fall River Wildlife Refuge and the Verdigris River in Kansas. The total number of juvenile Neosho muckets that we have released over the past 2 years is approximately 52,000. Cooperators in this project included personnel of the Missouri Department of Conservation, Kansas Wildlife and Parks Department, and the U.S. Fish and Wildlife Service.

An unsuccessful attempt was made to propagate scaleshell for release. Only a single gravid female scaleshell and 9 live males were located during 45 man-hours of search time in the Gasconade and Bourbeuse rivers. Difficulties were also experienced in obtaining host fish (drum). Glochidia from the female were eventually placed on a single host fish, but only a few juveniles were produced. A source of captive-propagated drum was later located and 150 fish are presently being held at Neosho Hatchery. Therefore, host availability is less likely to limit efforts to propagate scaleshell during the coming year.

Fieldwork yielded a large collection of shells of scaleshell, permitting analysis of morphometrics and sex ratio. Differences were found in the shape and size of scaleshell between Gasconade and Meramec populations. The sex ratio of scaleshells at the Gasconade study site appears to be skewed heavily toward males, with females comprising only 15% of the population. A previous study (Roberts and Bruenderman 2000) also indicates a skewed sex ratio in the Meramec population. Rarity of females and small body size will complicate efforts to locate this species for propagation. However, in spite of small body size of females, fecundity appears to be high (~400,000 glochidia per female).

The tolerance of juvenile mussels to chronic hypoxia (low oxygen) was tested in *Lampsilis siliquoidea* and *Lampsilis reeveiana*. Young juveniles of both species (1-6 weeks post-transformation)

were surprisingly tolerant of hypoxia. Significant depression of survival time ($LT_{50}$) occurred only at dissolved oxygen (DO) levels below 1 mg/L (less than 10% of air saturation). Individuals survived for weeks, even at the lowest DO tested (<0.4 mg/L). *Lampsilis siliquoidea* also grew at similar rates at DO ranging from 4-82% of air saturation. These results suggest that juveniles could occupy interstitial habitats that are relatively hypoxic.

**ACKNOWLEDGEMENTS**

I wish to thank several persons without whom this work would not have been possible. Michael Baird made numerous important contributions in the field and in the lab. Kuniko Yamada spent long hours carefully gathering most of the data on hypoxia effects on juveniles. Brian Obermeyer and Ed Miller helped with all aspects of Neosho mucket fieldwork, provided essential information and advice regarding collection and release sites, and conducted the releases of juveniles. Andy Roberts helped with collection of scaleshell and provided numerous insights on the biology of this species, as well as making strenuous efforts to supply it with hosts. Jim Maenner, Andy Cornforth and others at Chesapeake Hatchery provided fish, tank space, and other essentials, and inoculated and cared for both finfish and shellfish. Mitch Henson and his staff at Bass Pro generously donated their time helping us to inoculate fish. Dave Hendrix, Roderick May and colleagues at Neosho Hatchery also helped with loans of equipment and time, and are working to maintain host fish. I am grateful to Norm Stucky, Steve Eder, Gary Novinger, and Al Buchanan of Fisheries Division for supporting this work. Al Buchanan made useful editorial comments on the manuscript. Special thanks are due to Sue Bruenderman and to Paul McKenzie, for sharing our goals and for promoting this project and others. This project was a cooperative effort involving the Missouri Department of Conservation, the Kansas Department of Wildlife and Parks, the US Fish and Wildlife Service, Neosho National Fish Hatchery, and the SMSU Biology Department.

## 1. NEOSHO MUCKET PROPAGATION

### Introduction

This was the second year in which we propagated and released Neosho muckets (*Lampsilis rqfinesqueana*). The Neosho mucket is endemic to the Neosho, Spring and Elk river systems in southeastern Kansas, northeastern Oklahoma, and southwestern Missouri. It is state-listed as endangered in Kansas and Oklahoma, and S2 (imperiled) in Missouri. The distribution and conservation status of Neosho muckets in Kansas and Missouri are well understood because of recent surveys (Obermeyer et al. 1997). We chose to begin our propagation efforts with this species not only because of its conservation status, but also because of characteristics that make it relatively easy to work with as we develop our methodology. These features include relatively easy access to females, large numbers of glochidia, and a host fish (largemouth bass) that is readily available in hatcheries (Barnhart and Baird 1999).

### Methods

Two gravid female Neosho muckets were collected on June 23, 2000 from the Fall River at Huser Bridge in Wilson County, Kansas (Figure 1). This site was the source of Neosho muckets previously used for propagation (Barnhart and Baird 1999). Inoculation of host fish took place on July 18 at Chesapeake Fish Hatchery. Glochidia were removed from one marsupial gill from each of the two gravid females. Each gill was emptied as completely as possible by flushing with water, so that a count of the contents could be made. The contents of each gill were suspended in 500 ml water, stirred vigorously, and five 200-ul samples were withdrawn with a volumetric micropipette while stirring the suspension. These samples were stored in vials in ethanol. Later, each sample was dispersed in a small Petri dish and digitally photographed using a Nikon 950 digital camera attached to a dissecting microscope. The glochidia and undeveloped eggs were then counted using a graphics program to mark each glochidium and egg in the image. The sample counts from each gill were averaged and multiplied by 2500 to estimate the number of glochidia and eggs in the gill. These figures were multiplied by 2 to estimate the total number of glochidia and undeveloped eggs in each female (Table 1).

We inoculated a total of 637 bass with Neosho mucket glochidia. Approximately equal numbers of fish were inoculated from each of the two batches of glochidia. The fish were anaesthetized briefly with MS-222 (Finquel®) and a plastic Pasteur pipette was used to apply ~5ml of glochidia suspension directly onto both gills via the opercula and the mouth. After inoculation the fish were counted and then returned to a 4 by 20 foot rectangular fiberglass tank. Three workers required approximately 2.5 hours to complete this process. We sacrificed 14 of the fish by placing them in 95% ethanol immediately after

inoculation. Another 7 fish were sacrificed 1 week later and examined without preservation in ethanol. These fish were used for counts of attached glochidia. Each gill arch was dissected free and placed in a ~1% solution of KOH for a few minutes to make the gill tissues transparent. The attached glochidia were then counted using a dissecting microscope.

Following inoculation, the fish were held in a 4' by 20' rectangular fiberglass tank. Water was delivered to the tank continuously at one end, and exited via a standpipe behind a screen at the opposite end. Feeding was stopped 2 days after inoculation and thereafter the fish were not fed. The tank walls were cleaned by brushing and vacuuming at 4-days post-inoculation. Beginning at 6 days post-inoculation, the bottom of the tank was vacuumed at 2-3 day intervals and the water was run through two brass soil sieves. The first sieve had 250 micrometer mesh to remove larger frass, and the second sieve had 125 micron mesh to recover the juveniles.

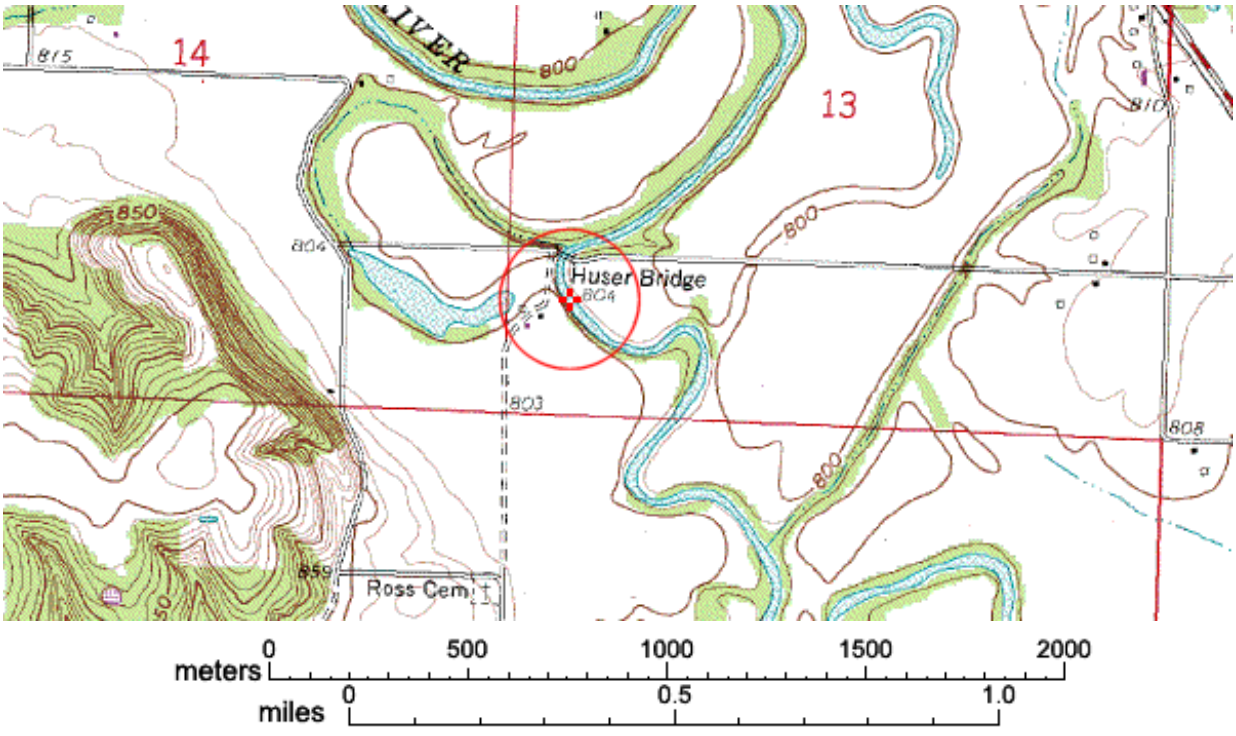


Figure 1. Neosho mucket collection site below Huser bridge, 2 miles east of Neodesha, Wilson County, KS (NEODESHA KS quadrangle 1:24000). Map coordinates are UTM 15 259095E 4145991N (NAD27 datum). This site was also the source of glochidia that were collected last year.

## Results

Collection of mussels & glochidia: Flow at the Huser Bridge site was ~80 cfs on June 23, which was just low enough to permit searching by groping without snorkeling. Three workers searched for approximately 9 man-hours total and recovered 5 Neosho muckets, two of which were gravid females. Other rare and endangered species observed included 2 live *Cyprogenia aberti* and 3 live *Ptychobranchus occidentalis*. The two Neosho mucket females were transported to SMSU for study.

One of the two gravid Neosho muckets had an unusually large number of undeveloped ova (Table 1). This female released several intact conglutinates, which were photographed to document the distribution of these ova within the conglutinates. The undeveloped ova tended to be concentrated in particular areas of the conglutinate (Figure 2).

Table 1. Estimates of the number of glochidia and sterile eggs in the two Neosho muckets used for propagation. Values for one gill are the mean ± standard error of subsamples (n=5) of a suspension of the gill contents. The means were doubled to provide estimates for totals in both marsupial gills of each female.

| Female # | Gills | Glochidia | Ova | Total | % Ova |
|---|---|---|---|---|---|
| 6-23-00-5 | One | 375,440 ± 25,733 | 83,720 ± 19,342 | 459,160 ± 37,277 | 18.2% |
| | Both | 750,880 | 167,440 | 918,320 | |
| 6-23-00-6 | One | 333,900 ± 15,274 | 18,060 ± 7,171 | 351,960 ± 10,638 | 5.1% |
| | Both | 667,800 | 36,120 | 703,920 | |

Attachment to host fish: Although the range of fish body size was small (85-110 mm) the number of glochidia that attached to individual fish was significantly dependent on body size (Figure 3). Those fish placed into ethanol immediately after inoculation with glochidia had only 64% as many glochidia attached as those that were examined 8 days after inoculation (Table 2). The difference was not statistically significant after removing the effect of length (ANCOVA), but the sample size was small. We feel that it is probable that placing the fish into ethanol immediately after inoculation dislodged some of the attached glochidia, which had not had time to encyst.

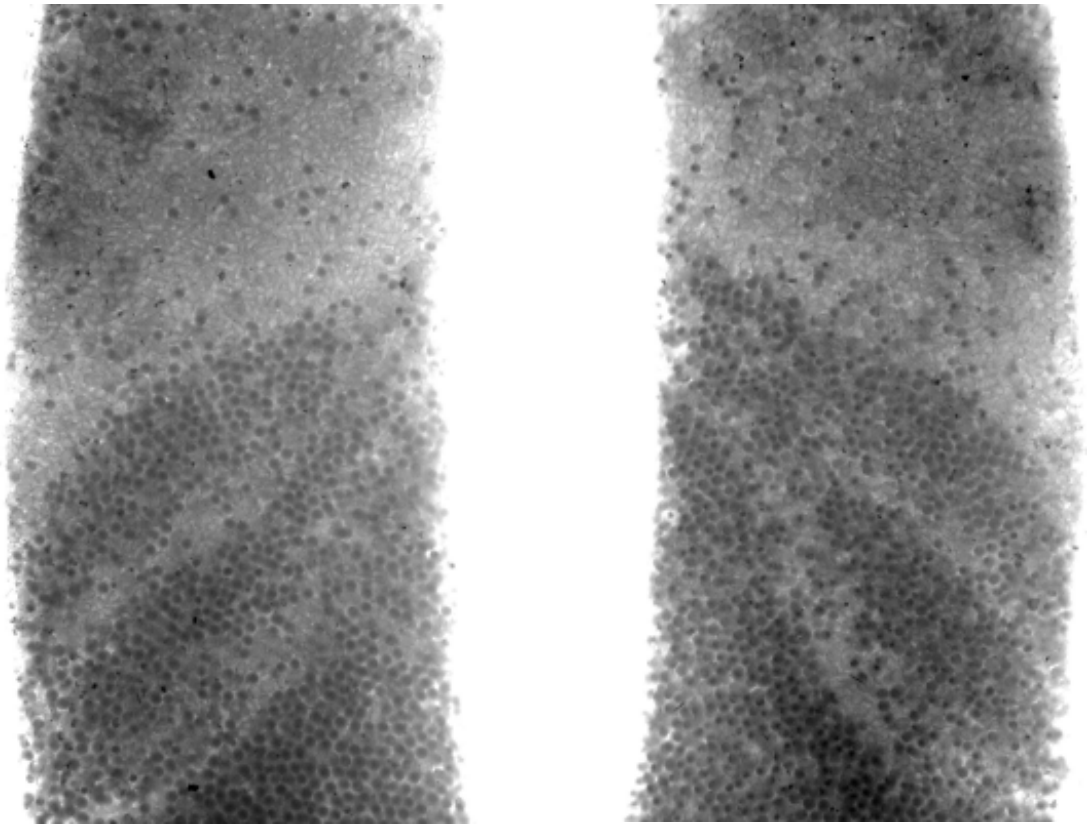

Figure 2. Portion of a Neosho mucket conglutinate viewed from two sides. The whole conglutinate was measured about 25 x 7 x 2 mm. Eggs containing glochidia are bright. Undeveloped eggs are dark. Note that the dark areas with high proportions of undeveloped ova are continuous through the conglutinate and show on both sides.

The mean number of glochidia attached to each host (244) was in the range of last year's results with Neosho muckets. Inoculations of two batches of similar size fish last year gave averages of 407 and 155 attached glochidia per fish (Barnhart and Baird, 1999). These results could be affected by a number of factors, particularly the concentration of glochidia in the suspension and the technique used in pipetting the glochidia onto the gills. The number of glochidia attached per fish was extrapolated to estimate the total number and proportion of glochidia that were successfully placed on the host. Assuming 244 glochidia per fish and 637 fish inoculated, a total of 155,428 glochidia attached to the hosts. Approximately 709,340 glochidia were in the suspension used to inoculate the fish (Table 1). Therefore, approximately 22% of glochidia attached.

Table 2. Fish length and number of attached glochidia. Fish were sampled on two dates. The first group (7-18-00) was preserved in ethanol immediately after inoculation. The second group was examined 8 days later and was not preserved in ethanol. Values are mean ± standard error.

| Sample date | N fish | Total length | N glochidia |
|---|---|---|---|
| 7-18-00 | 14 | 96.1 ± 1.8 | 205 ± 34.2 |
| 7-26-00 | 7 | 99.7 ± 2.6 | 321 ± 41.9 |
| All | 21 | 97.3 ± 1.7 | 244 ± 28.9 |

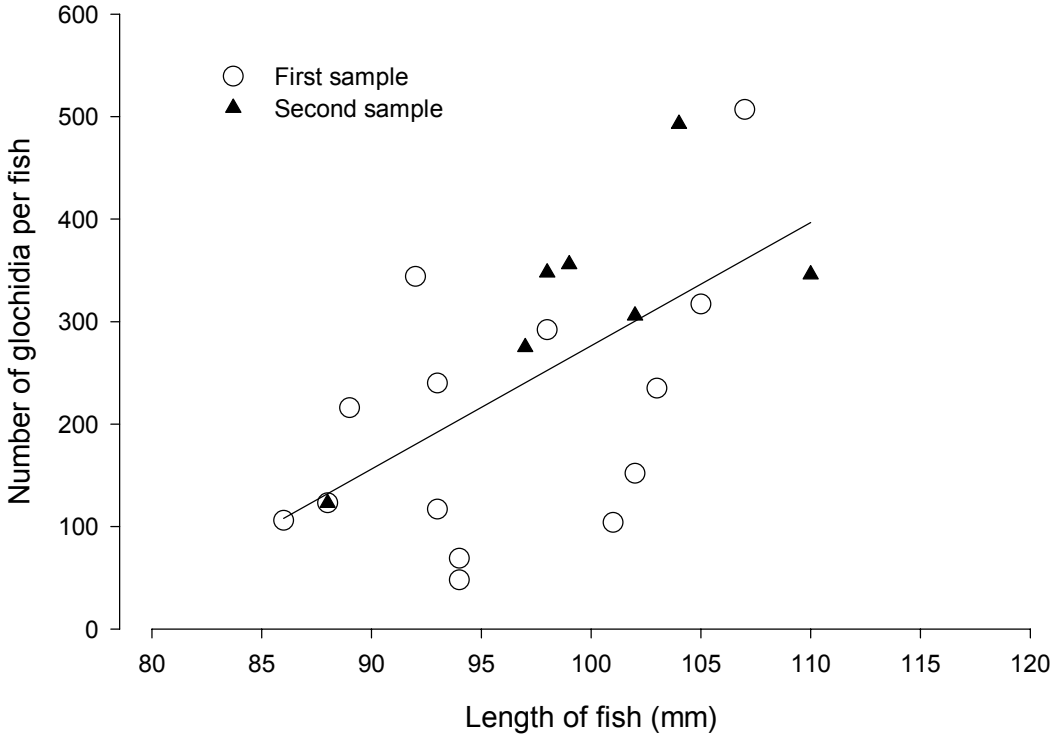


Figure 3. Number of glochidia attached to individual bass versus size of fish. Fish in the first sample were preserved in ethanol immediately after inoculation. Fish in second sample were sacrificed at 8 days post-inoculation and were not preserved in ethanol. The line is the linear regression of number of glochidia on length, pooling the two samples (N= 12[length]-927, $R^2$ = 0.34, P = .003).

Recovery of glochidia: Juveniles were first recovered at 11 days after inoculation. It is probable that drop-off began a day or two earlier, because some individuals showed growth and because no collection was attempted on July 27-28. The number of juveniles recovered peaked on day 13, and had dropped to a low level by 17 days post-inoculation, at which time collections were terminated (Figure 4). The 614 inoculated fish that were held for recovery of juveniles carried an estimated 149,816 encysted glochidia. Of these, an estimated 41,250 (~27% of those attached) were eventually recovered either live or dead. Of these, 32,830 (over 79%) were recovered alive (Table 3).

Table 3. Juvenile Neosho muckets recovered from largemouth bass. Data for each collection are mean ± standard error (n samples). These data are graphed in Figure 1.

| Collection Date | Days post-inoculation | Number of live juveniles | Number of dead juveniles | Percent live |
|---|---|---|---|---|
| 7-24-00 | 6 | 0 | 0 | - |
| 7-26-00 | 8 | 0 | 0 | - |
| 7-29-00 | 11 | 10750 ± 1510 (5) | 2250 ± 250 (5) | 82.7 |
| 7-31-00 | 13 | 13520 ± 1516 (5) | 1040 ± 637 (5) | 92.9 |
| 8-2-00 | 15 | 8000 ± 1159 (5) | 3750 ± 884 (5) | 68.1 |
| 8-4-00 | 17 | 560 ± 160 (5) | 1360 ± 349 (5) | 29.2 |
| TOTALS | | 32830 | 8400 | 79.6% |

Flatworms: Similar to last year, rhabdocoel turbellarian flatworms increased in abundance in the holding tank during the drop-off period. They were observed and photographed feeding on the juvenile mussels, as well as on cladocerans, ostracods, and annelids. These flatworms are a source of concern because they appear to be responsible for at least some of the mortality of juveniles after leaving the fish. We attempted to remove the flatworms from collections before they were shipped for release. Although the worms locomote nearly constantly in quiet conditions, they tend to adhere quickly and tenaciously to surfaces when exposed to turbulence. Passing the juveniles through a 250-micron screen removed many of the flatworms. If a collection was left sitting in a glass dish for a few minutes, then swirled and poured into another dish, many of the flatworms would remain behind, adhering to the glass.

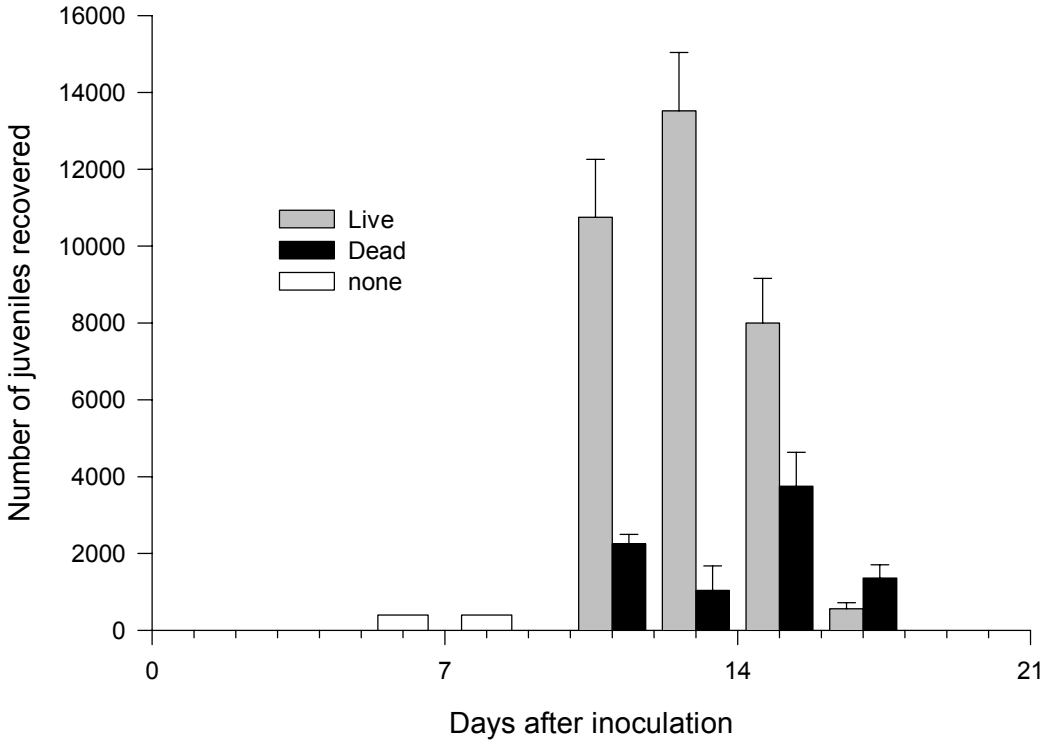


Figure 4. Timing of drop-off of juvenile Neosho muckets from largemouth bass. Each pair of bars represents the estimated number of juveniles that dropped off during the preceding two days, except the pair at 11 days, which represents the preceding 3 days. Error bars indicate standard error. Temperature during the transformation period was approximately 25 C.

Shipping and release: Juveniles were placed in groups of several thousand in 1-gallon ziplocks, with ~250 ml of water and ~1 liter of air. These bags were stored at 15 C for 0-2 days before shipping. For shipping, the bags were "double-bagged", and placed in an insulated shipping box along with 2 or more "blue-ice" packs. The ice packs were wrapped in cloth to prevent direct contact with the bags. The collections were shipped overnight (Federal Express) to Brian Obermeyer and Ed Miller (KWP), who carried out their release within 1-2 days of receipt (Table 4, Figures 5 and 6). In each case, the juveniles were released within 2-5 days after recovery at the hatchery. Cursory microscopic examination indicated good survivorship after shipping, but this was not quantified.

Table 4. Sites and releases of juvenile Neosho muckets 1999-2000. See Figures 4 and 5 for site maps. Note: the previous report on these sites (Barnhart 1999) switched the description and coordinates of Sites A and D. The positions marked on the map for these sites were correct.

| Locality | Date | Workers | Number |
|---|---|---|---|
| Site A: Fall River. E½ S20 T26 R11 Greenwood Co. KS. Map coordinates: UTM 14 744743E 4184452N (NAD27 datum). | Sept-14-99 | Ed Miller and Rick Tush | 3500 |
| Site B: Fall River. SW¼ S21 T26 R11 Greenwood Co KS. Map coordinates: UTM 14 745266E 4183543N (NAD27 datum). Side channel north of stream, just below abandoned ford. | Oct-15-99 | Brian and Bernice Obermeyer | 3500 |
| Site C: Fall River. SW¼ S 21 T26 R11 Greenwood Co KS. Map coordinates: UTM 14 745790E 4183924N (NAD27 datum). Side channel on west side of stream | Oct-8-99 | Brian Obermeyer and John Bills | 850 |
| Site D: Fall River. S½ S27 T26 R11 Greenwood Co. KS. Map coordinates: UTM 14 747725E 4181958N (NAD27 datum). | Sept-14-99<br>Oct-8-99<br>Oct-15-99<br>Aug-2-00<br>Aug-8-00 | Ed Miller and Rick Tush<br>Brian Obermeyer and John Bills<br>Brian and Bernice Obermeyer<br>Brian Obermeyer<br>Brian Obermeyer | 3500<br>3200<br>5000<br>8800<br>3800 |
| Site E: Verdigris River. SW¼ S4 T36 R16 Montgomery County KS. Map coordinates: UTM 15 263603E 4139591N (NAD27 datum). (property of Dan Small). | Aug-2-00<br>Aug-8-00 | Ed Miller<br>Ed Miller | 11600<br>8600 |

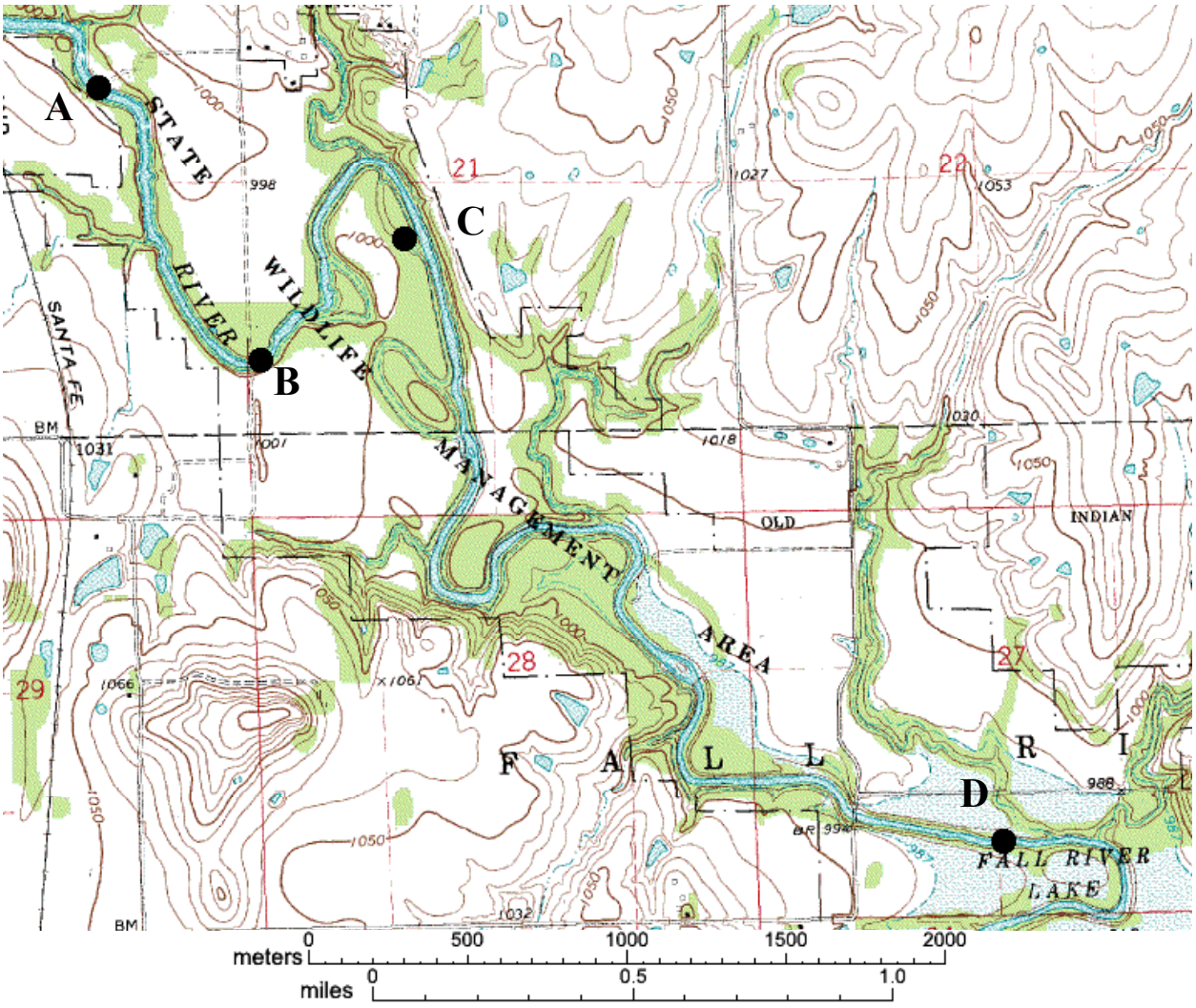


Figure 5. Neosho mucket release sites in the Fall River, Greenwood County, Kansas. TONOVAY MO quadrangle 1:24000.

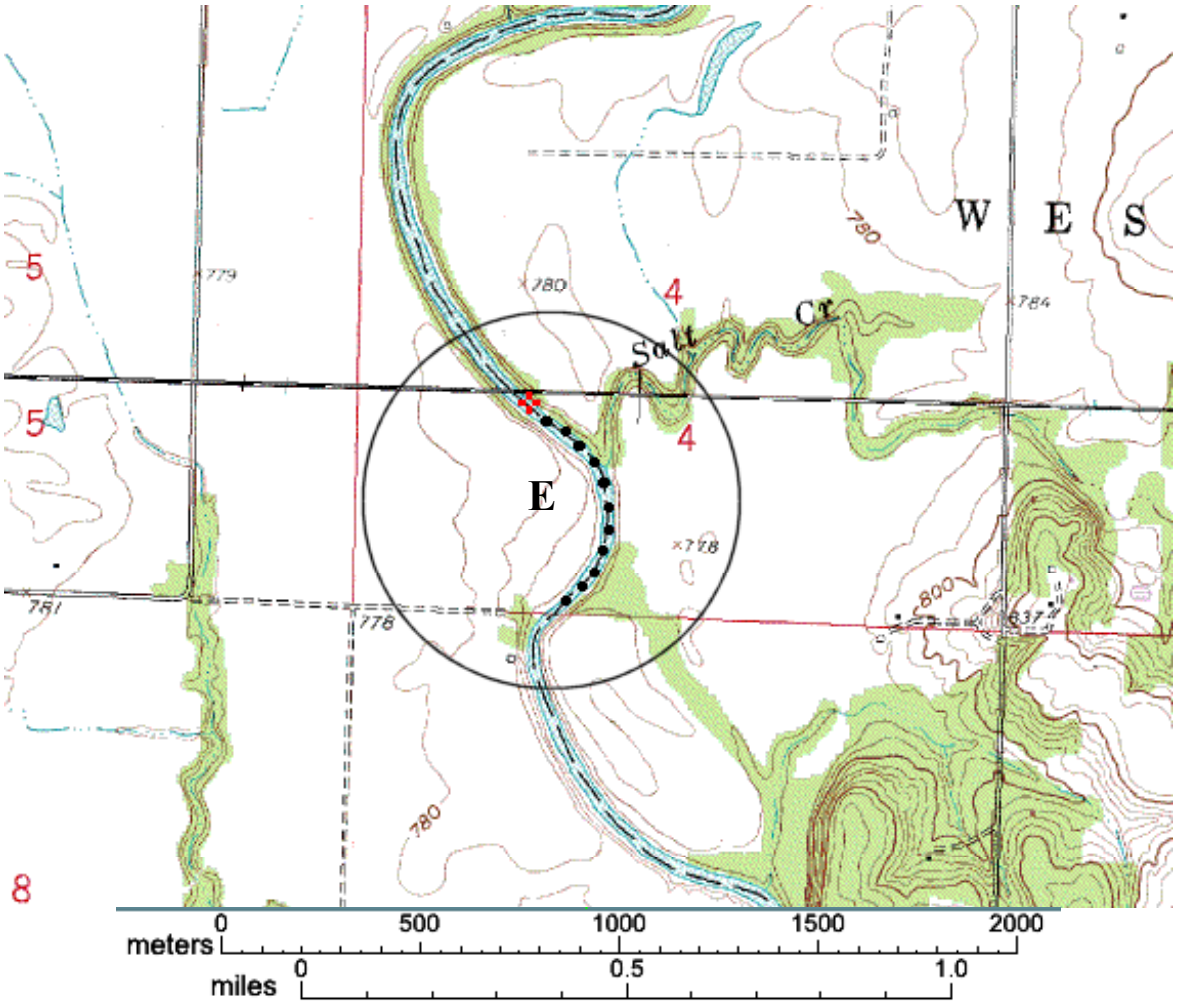


Figure 6. Neosho mucket release site E on the Verdigris River, Montgomery County, KS. Dotted line indicates the reach in which the juveniles were released. SYCAMORE MO quadrangle 1:24000.

**Discussion**

We have now released approximately 52,000 juvenile Neosho muckets at 4 sites in the Fall River and 1 site in the Verdigris river. These juveniles are the offspring of 3 Fall River females and an unknown number of males. Presumably, multiple males may fertilize each female, increasing the proportion of the gene pool represented in the glochidia. It should be possible to relocate the oldest of these juveniles in one or two years when they reach a size that can be easily spotted. Over the next 3 years we hope to continue stocking these sites as well as begin propagating the glochidia of Spring River females for stocking in the Spring and North Fork Spring rivers in Missouri. These glochidia will probably be taken from a site in Shoal Creek near Joplin, where we recently marked 35 individuals in conjunction with an MDOT relocation project.

The high proportion of unfertilized ova observed in one of the Huser Bridge females is unusual for *Lampsilis* (personal observations). This condition could be caused by an insufficient number of sperm available to fertilize the eggs during the period when they were entering the marsupial gills. The banding pattern of the distribution of fertilized and unfertilized eggs might reflect the time course of egg production and sperm availability. The observation is interesting, because apparently nothing is known about the time course of oviposition into the marsupial gills or the timing of fertilization. It appears that eggs that have become incorporated into the conglutinate without being fertilized cannot subsequently be fertilized when sperm become available.

Some refinements of technique are notable from this year's work with Neosho muckets. We learned that it is not advisable to preserve fish for counts of attached glochidia immediately after inoculation, because many of the unencysted glochidia apparently fall off when the fish is placed in ethanol. In the future, we will allow at least 1 day for encystment to take place before counting attached glochidia. About 22% of glochidia pipetted onto the gills were able to attach. Possibly this number could be improved. The number attached was significantly correlated with length of fish, even over a small size range, indicating that using larger fish may improve the proportion that attach.

The proportion of juveniles that were recovered after drop-off was much lower this year (~27%) compared to last year (~100%). The loss is probably attributable to use of the rectangular holding tank rather than the circular tank that was used last year. It was difficult to do a thorough job of vacuuming the lower end of the rectangular tank, around the screen and standpipe, and a substantial number of shells were noted in an accumulation of frass in these areas when the tank was later cleaned. In the future we hope to use several 4-foot diameter cylindrical tanks, which will allow separation of different batches of juveniles and facilitate their recovery.

Drought conditions in eastern Kansas during the summer impacted at least one of the release sites, as was described by Brian Obermeyer:

"I first noticed a stranding problem for mussels [at Fall River 'D' release site] on 26 August 2000. Low flows combined with extremely high air temperatures, especially between 14-31 August, were severely stressing mussels that had become stranded. Most were killed unless at least half of the shell was immersed in water. There were even some fully immersed mussels killed at the site, especially where they were covered by algal mats or in stagnant water. The only sanctuary for mussels was in a narrow chute on the north side (left bank) of stream, deeper glide habitat (above the riffle), and run habitat immediately downstream from the riffle. The highest concentration of live mussels was found in the chute. Even though temps weren't quite as severe, low flow conditions remained until we finally got some rains in October. Our first rain, after 67 days, was on 5 October (0.85"). However, the river didn't resume "normal" flows until mid-October after 3 more rain events: 1.6", 1.7" and 1.7" (16th, 22nd and 24th, respectively).
The chute where the majority of remaining mussels was found receded to about a 1 - 2 m wide channel, with maximum depth of about 8" (average 3-4"). Obviously, raccoons were taking advantage of the situation. We probably rescued 200+ live mussels (most were placed in the chute), but many of these were later harvested by coons. Just guessing, I'd estimate that about 200 - 300 mussels were killed from the event. Unfortunately, I have no way of knowing how many, if any, juveniles were affected. The vast majority of the habitat where I had released juvenile Neosho muckets was exposed. The only release areas not severely impacted by the adverse conditions were the upstream glide and downstream run habitats. I had avoided the chute when we made the releases because the current was too swift."

The other Fall River release sites were not visited during this period, so we don't know if they were similarly affected by the drought. It is likely that they were, because the other 3 sites are upstream of site D. It is impossible to know whether small juveniles were affected as severely as adult mussels. Although this drought event was unfortunate, it was also unlikely, and it should probably not discourage us from continuing to stock Neosho muckets in these reaches in the future. The upper Fall River has several advantages as a site for reintroduction of Neosho muckets (Barnhart 1999). Obviously, it will be wise to distribute some of the juveniles in the deepest parts of the available habitat. Here, and at other sites, we feel that the presence of populations of adult mussels of long-lived species is probably the best indicator of long-term suitability of habitat.

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## 2. SCALESHELL PROPAGATION

### Introduction

We intend to propagate this species over the next 3 years. We previously identified the fish host, which is freshwater drum (Barnhart 1998). There are several reasons for working with scaleshell. This species has undergone one of the most dramatic range reductions of any unionid. It formerly occurred in at least 53 rivers in 13 states throughout most of the eastern US (Williams et al. 1993). Currently, scaleshell is known to occur in only 13 rivers in 3 states, and is rare at most of these sites. These trends led to the proposal of scaleshell for federal endangered species (ES) status. The listing process is presently near completion but has been delayed by controversy regarding designation of critical habitat and the current freeze on ES listings.

Presently, we have a unique opportunity to work with this species because of our location, and because recent surveys by MDC in the Gasconade and Meramec basins have given us knowledge of scaleshell distribution. The best populations of scaleshell that remain are in these rivers (Symanski 1998, Roberts and Bruenderman 2000). However, this opportunity might not last for long. Scaleshell are apparently unusually short-lived mussels, meaning that failure of recruitment for even a few years could lead to local extirpation. This may be one reason that the species has declined so rapidly range-wide, and it is reason to assume that the remaining populations are not secure. Therefore, we feel that it is important to work with this species as soon as possible.

### Methods and results

Scaleshell were sought in June and early July at two sites, the Gasconade River near Schlicht Springs Access in Pulaski County, and the Bourbeuse River at Schmitt Ford in Franklin County. Three workers spent 45 man hours of search time snorkeling in shallow water in good conditions of water level and light. Dead shells were collected and measured for analysis of size distribution, sex ratio, and for comparison of size and shape between the Gasconade and Bourbeuse River sites. We found only 1 live female and 9 live males. However, a large number of dead shells were recovered, suggesting that the populations at these sites are reasonably numerous. These collections are detailed below.

Gasconade site: Site MB12 is on the Gasconade River about 1 mile south of Schlicht Springs Access, Pulaski County (see map, Figure 1). The river makes a large sweeping bend from the southeast. The site is a riffle, below the head of which is a mussel bed containing approximately 25 species (see table 1). This site was visited twice (June 7 and July 7, 2000). Water level was unusually low on both

dates. The Richland gage, a few miles upstream, read 475 cfs on June 7 (vs. 2000 cfs median for that date) and 650 cfs on July 7 (vs. 1100 cfs median for that date). Three workers spent 37 man-hours total searching for live and dead scaleshell. Most time was spent snorkeling. In all, we found intact shells of 43 dead individuals and fragments of several others. We found 8 live individuals, 1 male and 1 gravid female on June 7, and 6 males on July 7. Most search time was spent in the riffle and the live individuals were found there. The dead shells were found in the riffle and also upstream and downstream. They were one of the more common dead shells, and were easily spotted because of the bright purple nacre. We found 25 unionoid species at this site, 23 live and 2 from dead shells only.

Bourbeuse site: The second site where we sought scaleshell was Schmitt Ford on the Bourbeuse River (Figure 2). Two workers searched for 8 man-hours on June 8, mainly at the head (upstream) end of the ford, which is a long riffle. We collected 10 fresh shells of scaleshell and found 2 live males, but no females. Andy Roberts recalled finding 4 scaleshell in a short time here on a previous visit. We found 25 species at this site, including 2 live males and several dead shells of snuffbox (*Epioblasma triquetra*).

Sexual dimorphism: The relatively large number of dead shells recovered permitted analysis of morphometrics, including allometry of shape and comparisons between sites (Gasconade males and Bourbeuse males), and between sexes (Gasconade males and females). Sexual dimorphism was marked. Female shells were distinguished by the presence of a broad, thin, and ruffled posterior margin of the shell (Figures 3, 4). Gasconade females were 33% smaller than males (Table 1) and were also less tall relative to length than Gasconade males (Figure 5). The umbones of females were significantly further anterior (Figure 6). Both of these measurements reflect the elongation of the posterior margin of the shell in females. Plots of shell height versus length were linear (Figure 7) showing that scaleshell have approximately isometric growth.

Size distribution: The size distribution of male and female scaleshell differed. Large male shells were more common than large females and small male shells were generally lacking. A similar pattern is evident among live scaleshell from the Meramec river system (Figure 8). At least two explanations for this difference are possible. First, female scaleshell may have a shorter lifespan than males, so that the female shells that we collected tended to be younger and therefore smaller. Second, female scaleshell may grow more slowly than the males and therefore be smaller at similar age. Both factors may be at work. Female mussels of some other species grow more slowly than males after sexual maturity (Riusech 1999). The lack of small male shells cannot be attributed to taphony (differential preservation), because small female shells were present.

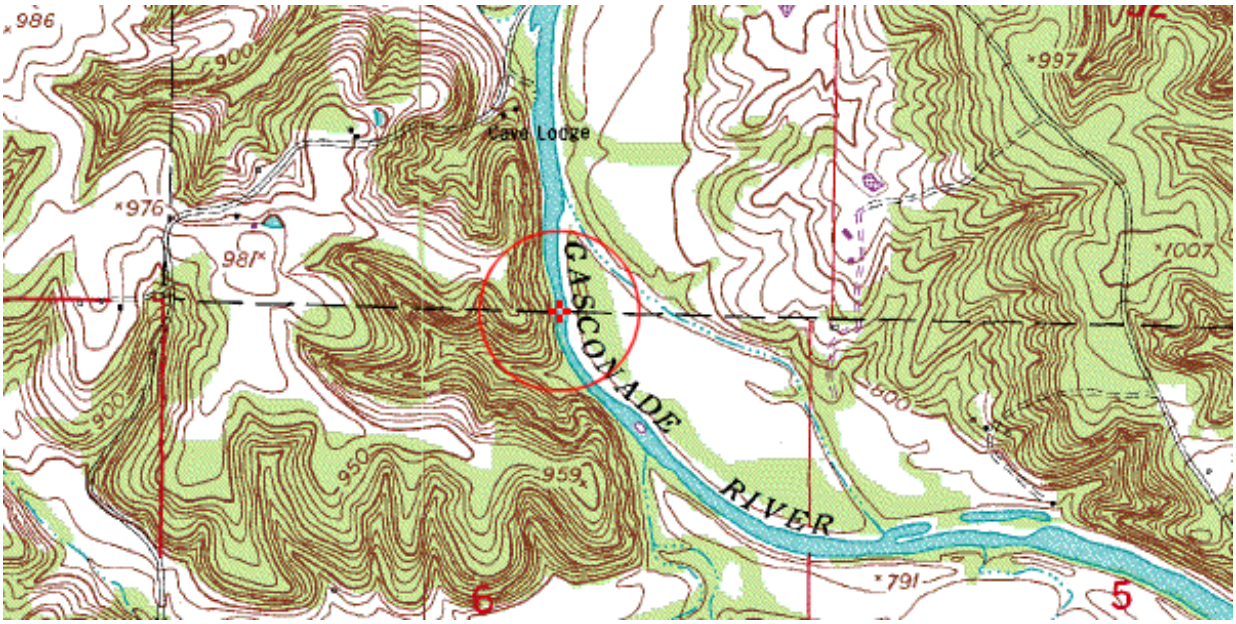


Figure 1. Collection site MB12 (CROCKER MO quad 1:24000). Coordinates are UTM 15 562635E 4193311N (NAD27 datum). SE¼ S31, T37N R12W, Pulaski Co MO.

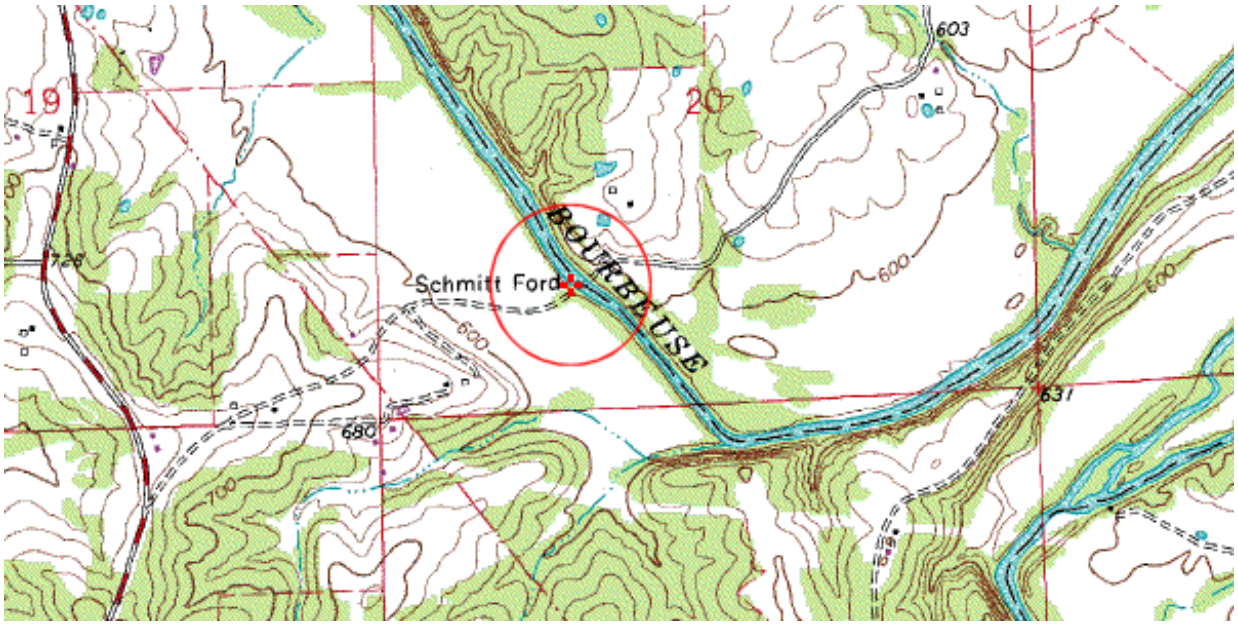


Figure 2. Collection site at Schmitt Ford (SPRING BLUFF MO quad 1:24000). Map coordinates: UTM 15 659287E 4247540N (NAD27 datum). SW¼ S20 T42N R02W Franklin Co MO.

Figure 3. Shells of female (left) and male (right) scaleshell from the Gasconade River. Note the broad, thin, uncalcified, posterior extension of the female. Female shells also tended to be less tall, relative to length, and the umbones tended to be positioned slightly more anterior.

Figure 4. Ventral view of gravid female scaleshell. Foot is partly extended at left. Note the gravid marsupium, right of center. The posterior extension of the shell is damaged on one side (top right of photograph).

Table 1. Morphometrics of scaleshell from the Gasconade and Bourbeuse Rivers. Values are mean ± standard error of mean.

| Measurement | Gasconade Males (N=47) | Gasconade Females (N=9) | Bourbeuse males (N=9) |
|---|---|---|---|
| Height (mm) | 28.1 ± 0.552 | 20.0 ± .074 | 40.6 ± 2.86 |
| Length (mm) | 55.8 ± 1.11 | 43.1 ± 1.86 | 77.1 ± 5.24 |
| Height/length | 0.505 ± 0.002 | 0.465 ± 0.007 | 0.526 ± 0.008 |
| Umbone/length | 0.219 ± 0.0213 | 0.185 ± 0.0260 | 0.225 ± 0.0244 |

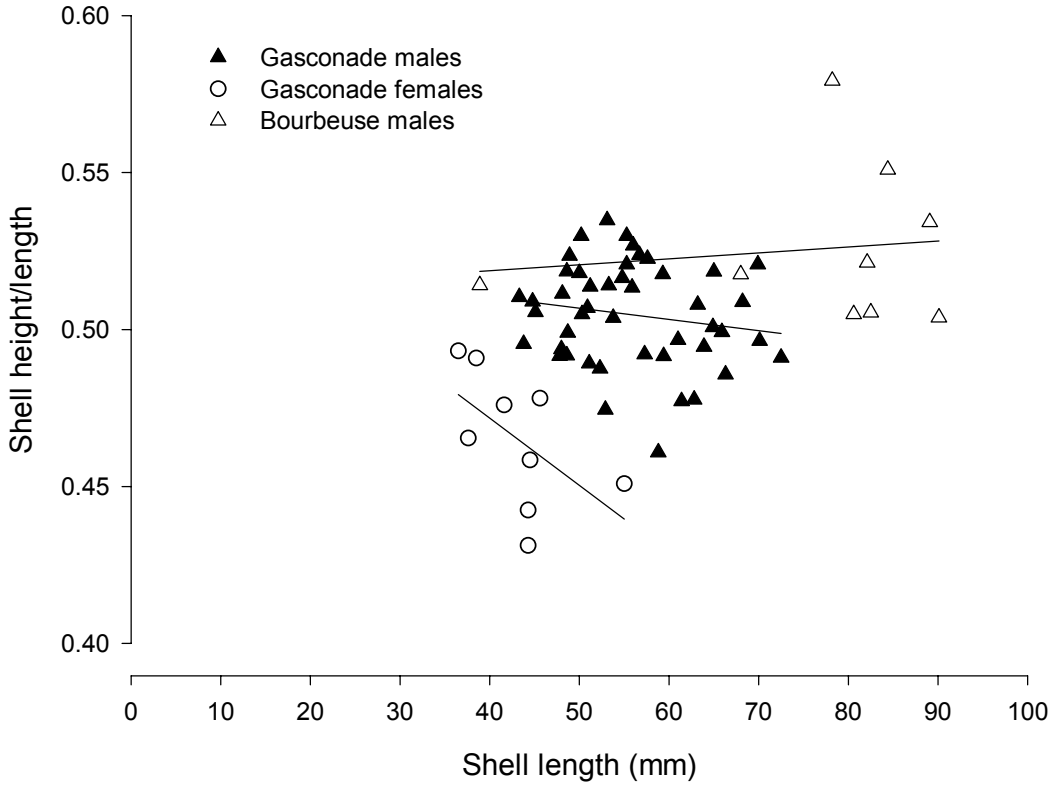


Figure 5. *L. leptodon* shell shape (H/L) versus shell length. Although H/L appeared to increase with length among groups, the slopes of the regression lines within groups did not differ significantly from zero. Therefore, differences in shape among groups were not attributed to length. All pairwise comparisons of means among the 3 groups were significant (ANOVA , $P<0.05$).

Male scaleshell from the Bourbeuse River site were larger than those from the Gasconade (Table 1) and had slightly different shape (Figure 6). A collection of live scaleshell from the Meramec and its tributaries also shows larger body size than the Gasconade specimens (Figure 8).

Skewed sex ratio: Very few females were found, either live (1) or dead (9). At the Gasconade site, we recovered 47 male and only 10 female specimens. At the Bourbeuse site, 8 males and no females were found. For both collections combined, the tally was 15% female. This proportion differs significantly from 50:50 (binomial distribution; $P<0.0001$).

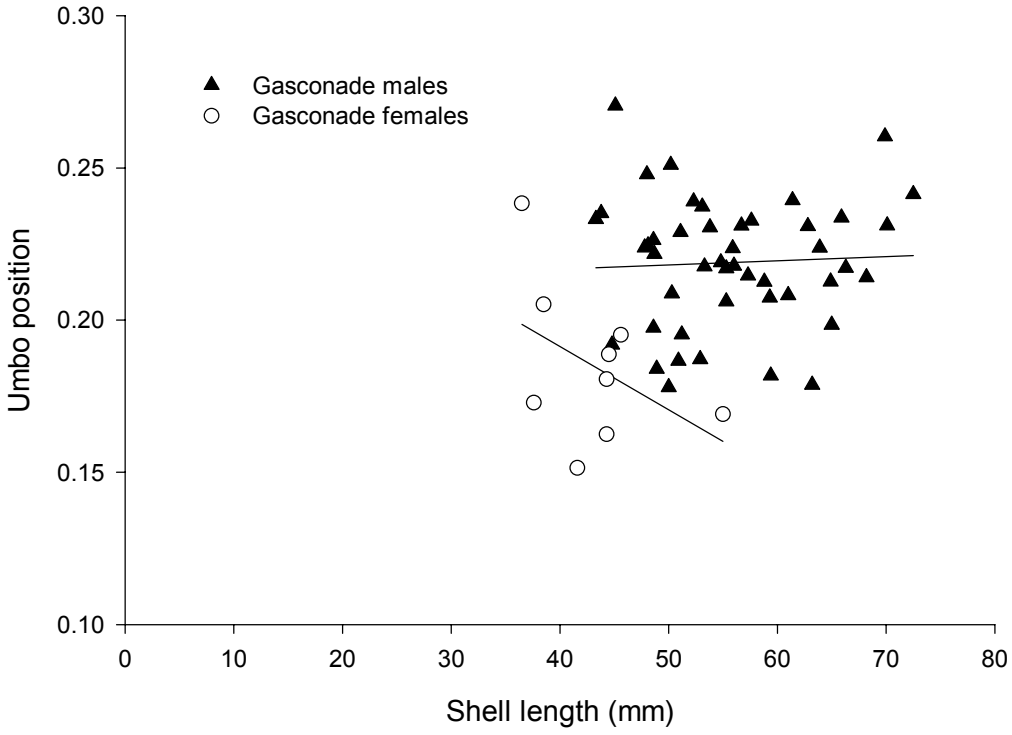


Figure 6. *Leptodea leptodon* umbo position ([distance from anterior margin]/length), versus shell length. Umbo position did not change significantly with length within groups. Therefore, differences in shape among groups were not attributed to length. The umbones were significantly more anterior in females than in males (ANOVA, $P<0.05$).

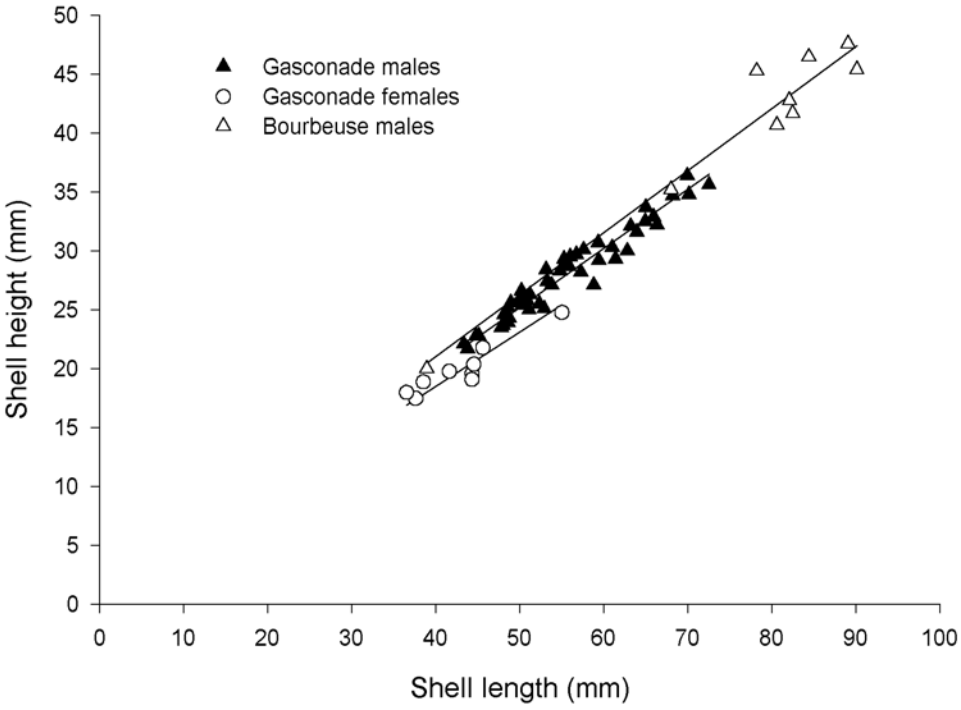


Figure 7. *Leptodea leptodon* shell height vs. length. Lines are linear regressions through the origin. Within each group, shell growth was isometric. However, Gasconade males were significantly larger and proportionately taller than the females. Bourbeuse males were significantly larger and proportionately taller than Gasconade males. (Table 1, Figure 5).

Propagation: The single gravid female recovered on June 7 was returned to the laboratory to provide glochidia for propagation on host fish (freshwater drum). The female was held at 15 C while attempts were made to capture drum. However, after about 2 weeks the scaleshell appeared unhealthy and it became necessary to act quickly. A captive drum was located at Bass Pro Shops and permission was granted to use this fish as a host. On June 28 glochidia were sampled from both marsupial gills of the female. Glochidia from one side appeared healthy, while those on the other side were heavily infested with small ciliate protists and had low viability. Glochidia from the healthy side were harvested and used to inoculate the Bass Pro fish, which weighed approximately 4 pounds. The fish was anaesthetized and the gills were inoculated with several thousand glochidia on both sides.

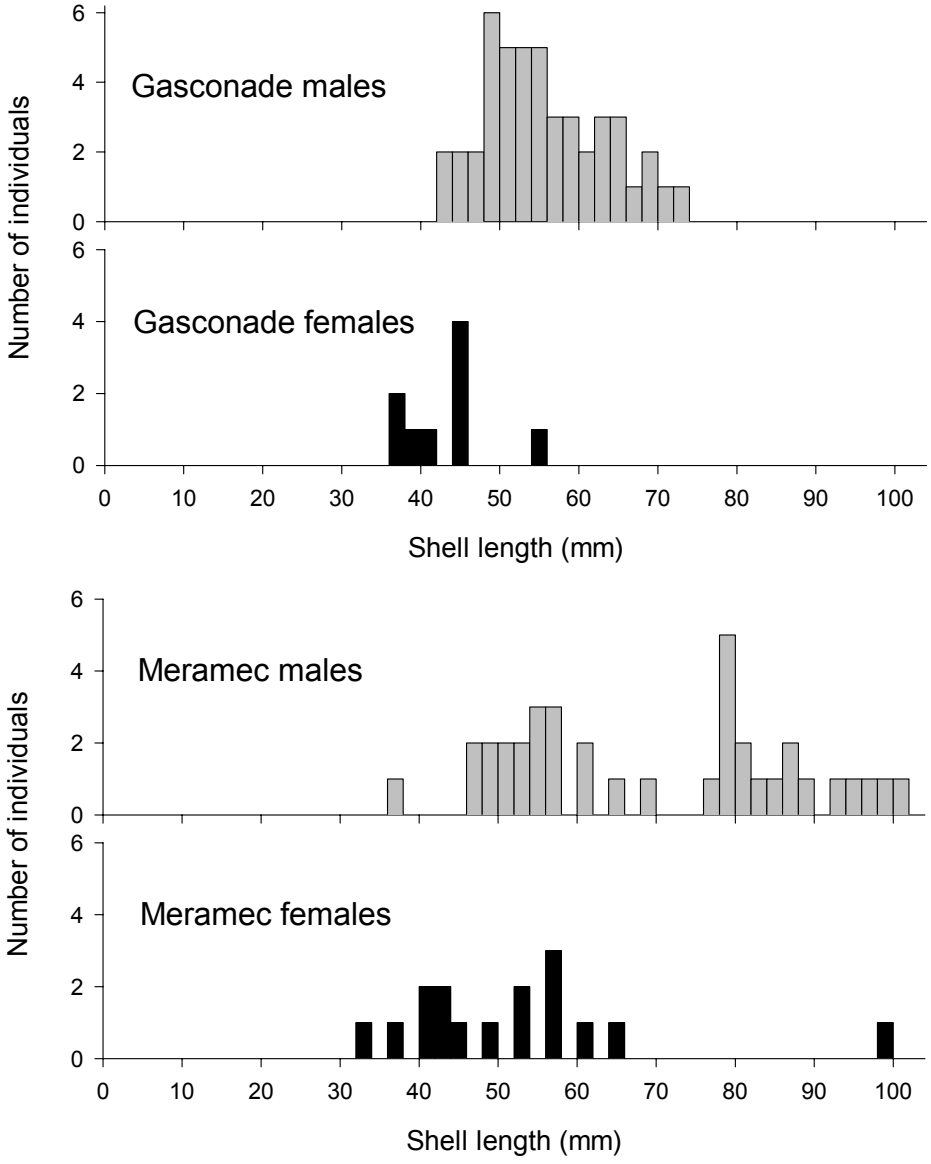


Figure 8. Shell length frequency distributions for scaleshell from Gasconade site MB12 (51 dead and 5 live- present study) and for specimens from multiple sites in the Meramec river system (8 dead [present study] and 44 live specimens [data from Roberts and Bruenderman 2000]). Bin width = 2 mm.

The next day (July 29) several 6-8" long drum were obtained by Andy Roberts (USFWS) from hoop nets in the Missouri River and immediately driven to Springfield. Preparations were made to inoculate these fish, and glochidia were again harvested from the healthy gill of the female. Unfortunately, the fish were dead on arrival in Springfield, apparently as a result of trauma during capture. The female scaleshell also died on July 30, so that no further inoculations of hosts were possible. The death of the female may be attributable to an injury suffered before capture. The posterior margin of one valve of the shell was broken away (Figure 4, top panel). The presence of the ciliates in the marsupium on the broken side may also have contributed to its poor condition.

The Bass Pro fish was monitored at 2-3 day intervals by vacuuming the bottom of its tank through a 40-micron filter and examining the filtrate for excysted juvenile mussels. An empty shell was found on July 12, two more on July 15, and one more on July 18. These shells showed considerable growth, which was expected because *Leptodea* grow during the period of encystment on the host fish Table 2, Figure 9). Based on previous experience, most of the juveniles were expected to excyst within 3 weeks of inoculation. Unfortunately, these empty shells were the only evidence of excystment seen over 4 weeks. On August 3 the drum was anesthetized and examined, and no attached juveniles were seen.

Numbers of glochidia: Although scaleshell are small, the glochidia are also very small and the number produced can therefore be large. The number of glochidia was estimated as follows: The female had one marsupial gill apparently fully charged, while the other gill, which was infested with ciliate protists, had several empty water tubes. Therefore, glochidia were harvested and counted only from the healthy, fully charged, gill. The glochidia were flushed from the water tubes by inserting a hypodermic needle and injecting sterile water. The removal of the glochidia was carried out in two stages for the two rounds of host inoculation. Each of the two batches was suspended in 100 ml of water, and agitated with a pipette to suspend the glochidia evenly in this volume. While the glochidia were suspended, five 200-ul samples were removed and stored in vials of ethanol. Later each of these samples was counted and multiplied to provide an estimate of the total number in the 100 mL suspension. These 100-ml estimates were combined to estimate the total in one marsupial gill and this number was then doubled, on the assumption that the other gill had originally contained a similar number of glochidia. The total estimated fecundity was 419,000 ± SD 6,500 glochidia. This fecundity exceeds that of some other unionids with larger glochidia, even species having much larger adult body size (Table 3).

Table 2. Dimensions of the 4 scaleshell juvenile shells recovered from the Bass Pro drum.

| Days post-inoculation | Initial length | Final length | Final height |
|---|---|---|---|
| 12 | 0.0644 | 0.119 | 0.111 |
| 15 | 0.0691 | 0.177 | 0.148 |
| 15 | 0.0692 | 0.182 | 0.148 |
| 18 | 0.065 | 0.194 | 0.175 |

Figure 9. Scaleshell glochidia and juvenile shells recovered from drum, showing growth. Numbers indicate days post-inoculation (zero = glochidia before encystment).

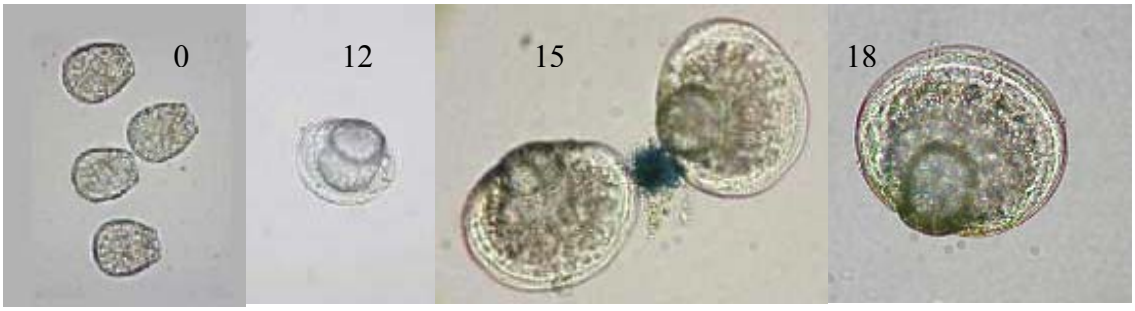


Table 3. Estimated fecundity (number of glochidia) for some individual unionids. Scaleshell have relatively high fecundity despite their small size because of their very small glochidia. Numbers are means ± standard deviation of n=5 estimates from subsample counts.

| Species | Body dimensions | Total number of glochidia |
|---|---|---|
| *Leptodea leptodon*<br>Gasconade River, Pulaski Co MO | L = 44.1, W=11.2, H= 21.0 | 419,000 ± 6,500 |
| *Venustachoncha pleasii*<br>James River, Green Co MO | L = 37.3, W=14.3, H=22.3<br>Tissue mass = 3.04 grams | 46,947 ± 3,268 |
| *Pyganodon grandis*<br>Fellows Lake, Green Co MO | L=125.5, H=81.1, W=56.5<br>Tissue mass = 211.6 grams | 235,210 ± 33,117 |

**Discussion**

Scaleshell from the Gasconade site and the Meramec and Bourbeuse sites differed significantly in size and shape. These differences could reflect either genetic differences among populations or differing conditions for growth. Population genetic differences appear unlikely, given the mobility of the host fish and the proximity of the two drainage systems. Nonetheless, it is probably not desirable to mix stocks between the two drainages. Further morphological and genetic comparison among the remaining scaleshell populations is certainly desirable, given the broad geographic range and the range-wide decline of the species.

The smaller number of female scaleshells recovered is puzzling. Most specimens collected were dead shells. Therefore, a possible explanation for the skewed sex ratio is differential taphony (preservation after death). Female shells are smaller and thinner, and might therefore disintegrate faster after death than male shells. If so, the dead shells may not accurately represent the sex ratio of living animals. However, a collection of 44 live scaleshell from the Meramec River system was only 36% female, which also differs significantly from 50:50 (binomial P=0.048) (data from Roberts and Bruenderman 2000). Thus, it appears that female scaleshell are relatively rare, or at least more difficult to find than males. Small mussels are easy to miss in qualitative sampling, and this may account for the apparent rarity of females. Another possible explanation is that the host fish (drum) prey differentially upon females, and destroy the shells. It is interesting to speculate that the small size, thin shell, and peculiarly elaborated posterior mantle and shell margin in female scaleshell all might be related to a habit of "female sacrifice" in which the host is infected by feeding on the female. The elaborated posterior mantle might be adapted to produce chemical attractants for the host fish. Luckily, females produce large numbers of glochidia (Table 3). This high fecundity is possible in spite of small body size, because the glochidia are also very small. Scaleshell glochidia grow during encystment and increased more than 4-fold in length before excysting (Barnhart 1998).

Growth lines of the Gasconade scaleshells were relatively indistinct, so that it was difficult to infer age. Roberts and Bruenderman (2000) used growth lines to estimate age of 44 live specimens from the Meramec and Bourbeuse Rivers. Their estimates range from 2-6 years, with a mean of 3.07 years. It is probable that the first year growth line was overlooked in these counts, so that the estimate might be adjusted upward by one year (A. Roberts, personal communication). There is strong suspicion that these small, thin-shelled mussels are very short-lived, but estimates of their age and lifespan are speculative because the relationship between growth lines and age has not been validated in this species.

The difficulty of finding female scaleshell presents problems for propagation efforts. Low water conditions in summer 2000 made conditions nearly ideal for fieldwork, but only a single female was

located. Greater effort and an earlier start will be necessary in 2001. Gravid females could potentially be collected throughout the winter and spring months. Scaleshell apparently follow the winter-brooding habit that is typical of lampsilines. Three females observed in the Meramec River were gravid with ova by mid-August and mature glochidia the following spring (Barnhart 1998). Glochidia release probably normally occurs by early July, so that late-summer collecting may not be productive.

The failure of scaleshell glochidia to transform on the single drum that we inoculated is problematic. In previous laboratory host tests, scaleshell transformed only on drum (*Aplodinotus grunniens*) of 24 fish and one amphibian species that were tested. It is not clear why the glochidia failed to transform on the Bass Pro fish. Similar inoculation of a smaller, wild-caught, drum produced over 3300 juveniles (Barnhart 1998). Although a few glochidia apparently attached and developed in this trial, it appears likely that most of the glochidia were lost during the first few hours after inoculation. Loss of the glochidia occurs if the host fish is either genetically unsuitable or if it has developed immunity through previous exposure to glochidia. There is also some evidence in the literature that fishes exposed to gill-parasitic copepods can develop immunity to glochidia as well as copepods (Wilson 1916). The Bass Pro fish was a long term captive and had been exposed to copepod infections, which were formerly common in the display tanks (Mitch Henson, Live Exhibits Director, personal communication). The possibility of acquired immunity is an argument for use of young fish for mussel propagation.

Although drum are abundant in many rivers, it is difficult to capture young fish in large numbers without damaging them. Wild-caught drum are delicate and nervous and difficult to acclimate to aquaria and artificial food. Luckily, Conrad Kleinholtz of Langston University has recently been successful in spawning and pond-rearing drum. He has generously supplied us with 150 fish, which are being held at Neosho National Hatchery. The staff of the Neosho Hatchery acclimated these fish to artificial food and they appear to be thriving. We anticipate having a reliable supply of fish for propagation of scaleshell and other species that require drum (e.g. the federally endangered, *Potamilus capax*), in the future.

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## 3. EFFECTS OF CHRONIC HYPOXIA ON SURVIVAL AND GROWTH OF JUVENILE *LAMPSILIS*.

### Introduction

Juvenile unionids are very small and occupy the interstitial spaces among sediment particles in streambeds (Yeager et al.1994). This microhabitat is one of the most poorly understood of stream environments (Hynes 1983, Hendricks and White 1991). One of the significant factors in the interstitial environment may be the availability of oxygen. In stream substrates, macroscopic gradients of dissolved oxygen (DO) occur with depth in the streambed and also longitudinally along riffle-pool systems, and are strongly influenced by hyporheic flow (Whitman and Clark 1982, Hendricks and White 1991). Less well understood are microscopic gradients that may occur within the interstitial spaces in the streambed. It is reasonable to expect that local flow patterns and biological oxygen demand may result in gradients of DO at scales of millimeters. Microenvironments on this scale are certainly significant for post-metamorphic unionids, which have body length ranging from 0.06- 0.3 millimeters.

Oxygen availability may limit metabolism directly and may also directly or indirectly influence other chemical parameters such as ammonium and nitrate concentrations and pH. Benthic organisms respond to oxygen gradients by altering their position and their behavior (Rees 1972, Hoback and Barnhart 1996). Little is known regarding the tolerance of young juvenile unionids for hypoxia. The heart rates of 14-day old *Utterbackia imbecillis* and *Pyganodon cataracta*, 400-600 microns long, were constant over a wide range of DO but fell by up to 75% at DO below 5% of air saturation. The lack of compensatory change in heart rate above this limiting level suggested that these juveniles were unable to regulate oxygen uptake during hypoxia (Polhill and Dimock 1996). If so, aerobic metabolic rate might be affected by DO even at higher levels.

Organic pollution reduces oxygen availability, because of increased biological oxygen demand from the higher biomass that results from increased nutrient availability. Eutrophication of streams resulting in hypoxic habitat is a potential contributing factor in the decline of unionids, but the possibility is difficult to assess without information on tolerance of unionids for hypoxia. Therefore, we investigated the effects of chronic hypoxia on survival and growth in recently metamorphosed juveniles of *Lampsilis siliquoidea* and *Lampsilis reeveiana*.

### Methods

Control of dissolved oxygen. Water was deoxygenated using a gas-stripping column (Barnhart 1995) and then re-oxygenated by passing over a series of partitions and pools (aeration ladder, Nebeker 1972). Water was continuously recycled through the system from a ~100 liter reservoir at a flow rate of

approximately 0.5-L•min$^{-1}$. A thermostat controlling a small refrigeration compressor regulated temperature. Water in the reservoir was circulated over a titanium heat exchanger attached to the compressor, and a 100W aquarium heater in the reservoir provided a heat load to buck the cooling system. Water temperature was similar among pools ± 0.5 $^{o}$C and ranged from 19.5 to 21.0 °C during the experiments.

The aeration ladder consisted of an inclined rectangular acrylic box, 4 feet in length and 5 inches square. Eleven 4-inch high partitions separated the water flowing through the box into 12 pools. Water entering the ladder at the upper end flowed over the partitions and through the pools before exiting at the lower end of the ladder and returning to the reservoir. Containers holding juvenile mussels (see below) were placed in six of the 12 pools having the desired levels of oxygenation. Supplemental aeration was provided in some pools using an air pump and air stones to help oxygenate the water as it flowed down the aeration ladder, but containers were not placed in those pools.

Homogeneity of $PO_2$ within each pool was measured prior to experiments using a Cameron oxygen meter (Model OM-201) with a semi-micro oxygen electrode (Microelectrodes, Inc., Model MI-730). Oxygen varied less than 1 Torr (0.7%) with position within each pool. During experiments, oxygen in each pool was checked every 1-3 days using an Orion Model 820 oxygen meter. Calibration of the oxygen meters was checked with nitrogen gas and air. All oxygen measurements were recorded as percent of air saturation and later converted to mg/L based upon barometric pressure and temperature.

Containers. Juvenile mussels were kept in custom-made acrylic containers (Figure 1). Each container consisted of two rectangular Plexiglas® plates joined face-to-face by a pair of alignment pins and a connecting bolt. The dimensions of the back plate were 3½" x 2½" x ½". The dimensions of the front plate were 3½" x 1¾" x 5/16". A series of eight holes was drilled through the joined plates. These holes were tapered slightly from the front to the back (5/8" diameter on the facing side and ½" diameter on the back side). The front and back of each hole was covered with Nitex® nylon screening (202 $\mu$m mesh) attached with acrylic cement, to form 8 tapered cylindrical compartments in each container. When the plates were separated, juvenile mussels could be placed in the compartments on the back plate using a pipette. When the plates were joined with the connecting bolts, the flat surfaces of the plates abutted closely to form a seal adequate to retain the juveniles. The 8 compartments in each container allowed the juveniles to be divided into small groups, which greatly facilitated observations.

The closed containers were placed vertically in the pools of the oxygen ladder. Water flowing through the oxygen ladder also flowed through the compartments via the screened openings. The flow rate of water through the oxygen ladder was measured volumetrically and was approximately 0.5 liters per minute. The containers spanned the width and depth of the raceway, so that most of this flow passed

through the chambers. Flow was checked before the experiments by injecting dye (food color) in front of each compartment and observing the passage of the dye through the compartments.

Figure 1. Container for hypoxia experiments. Similar units were placed at each of 6 different levels of dissolved oxygen in a flowing system. The facing plate is removable for placing juveniles in the compartments and for observations. The compartments are bounded on both ends by nylon screen to allow water to pass through.

Juvenile mussels were obtained by artificially infecting largemouth bass with glochidia. Gravid female *Lampsilis siliquoidea* were collected from Stockton Lake, Cedar County, MO, and *Lampsilis reeveiana* were collected from the James River, Greene County Missouri. Glochidia from a single female were used for each experiment. Glochidia were removed from the female mussels by injecting sterile water into the marsupia. The glochidia were transformed on largemouth bass and then placed in a rearing system for 1-5 days before the experiments began. During this time, and during the experiments, the alga *Neochloris oleoabundans* was provided as food. Algal cell concentration was checked every 1-2 days using a hemocytometer, and algal culture was added as necessary to maintain a concentration of

approximately 40, 000 cells/ml. Half of the water in the system was replaced every 10 days to maintain water quality.

Groups of six juveniles were placed into each of 4 wells in each of six containers (24 juveniles per container, 144 juveniles total). Juveniles were observed every 1-2 days by removing the containers from the oxygen ladder and placing them face up in a tray in shallow water a few mm deep. A squirt bottle was used to rinse each compartment and wash the juveniles into the back half of the compartments. The facing plate was then removed and the backing plate and tray were placed under a dissecting microscope. At ~1-week intervals the juveniles were pipetted out of the compartments temporarily and the Nitex screens were cleaned of any debris or attached protists to prevent the screens from becoming clogged.

Juvenile growth and mortality were recorded. The number of surviving individuals was recorded every other day for the first two weeks, then every three days for the following three weeks (five weeks total). Dead individuals soon opened and bacteria and protists rapidly emptied the shells. In some cases, recently dead individuals remained closed but were recognized by the presence of protists within the shell. Live individuals were typically closed, and cilia movements could be seen. Stalked ciliate protists (*Vorticella)* appeared on the juvenile shells during and following the third week of the experiment. If abundant, these protists were gently removed with a fine paintbrush while the shell measurements or survival checks were conducted. In the experiment with *Lampsilis siliquoidea,* shell length was measured prior to the beginning of the experiment, and three more measurements were conducted approximately every ten days. Shell length was measured with an ocular micrometer (precision of measurement was ± 0.025 mm).

Statistics: The time to 50% mortality ($LT_{50}$) and 95% confidence intervals were calculated at each level of DO by linear regression of log (mortality) versus time, and interpolation of $LT_{50}$ from these regressions. Values for mortality were calculated pooling all 4 groups at each DO. The effect of DO on growth rate was tested as the interaction term in an analysis of variance (GLM) model of the effects of DO and time on shell length. Statistical analyses were carried out using Minitab® version 13.

## Results

Survival: *Lampsilis* juveniles did not survive indefinitely in the test system regardless of DO. Survival time was affected by DO, but the juveniles were surprisingly resistant to even the most severe hypoxia tested, surviving for weeks at DO as low as 4% air saturation (0.36 mg/L) (Tables 1, 2; Figures 2, 3). The effect of hypoxia on survival can be quantified by the reduction of $LT_{50}$ (time to 50% mortality).

Table 1. Effect of dissolved oxygen on estimated time to 50% mortality ($LT_{50}$) of juvenile *Lampsilis reeveiana* at 20 °C. Units of DO are % air saturation and mg/L. Units of $LT_{50}$ are days.

| DO | 7.1 % A.S. 0.63 mg/L | 9.1% A.S. 0.81 mg/L | 18.5% A.S. 1.65 mg/L | 29.4% A.S. 2.62 mg/L | 42.5% A.S. 3.79 mg/L | 75.0% A.S. 6.69 mg/L |
|---|---|---|---|---|---|---|
| $LT_{50}$ (95% CI) | 20.3 (18.4-22.1) | 25.9 (24.1-27.8) | 26.2 (23.8-28.6) | 27.3 (25.4-29.2) | 27.9 (26.5-29.4) | 26.3 (24.7-27.9) |

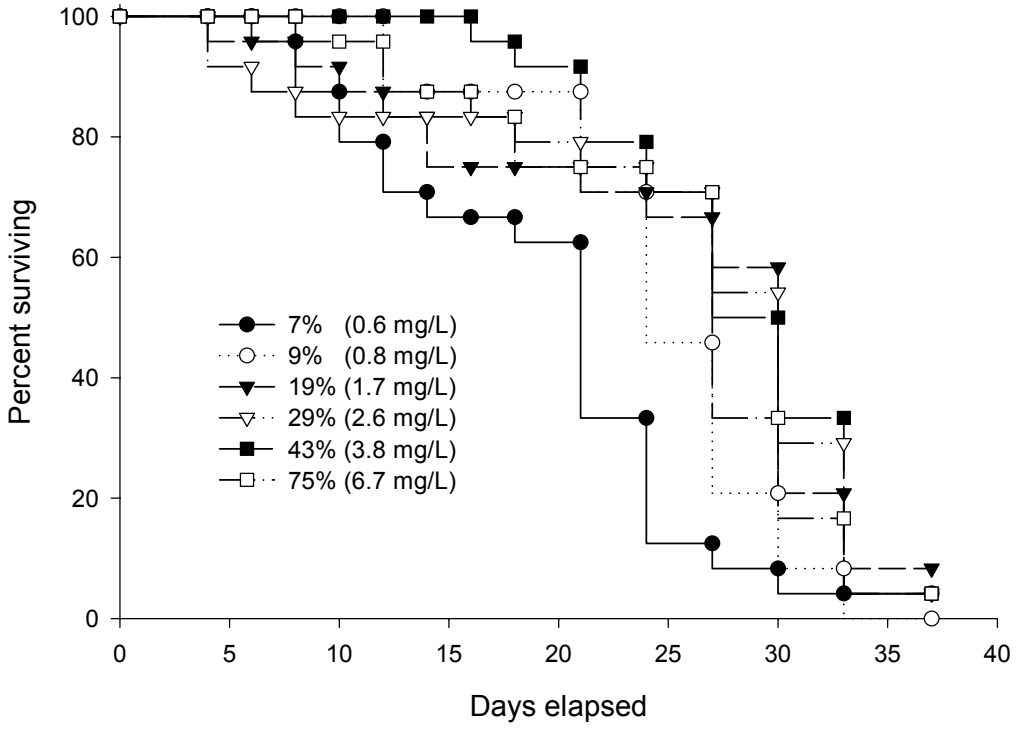


Figure 2. Survival of *Lampsilis reeveiana* juveniles versus time during 37 days at 20 °C. Legend indicates DO treatment group in % air saturation and concentration.

Table 2. Effect of dissolved oxygen on estimated time to 50% mortality ($LT_{50}$) of juvenile *Lampsilis siliquoidea* at 20 °C. Units of DO are % air saturation and mg/L. Units of $LT_{50}$ are days.

| DO | 4.0% A.S. 0.36 mg/L | 6.8% A.S. 0.60 mg/L | 15.3% A.S. 1.36 mg/L | 26.5% A.S. 2.37 mg/L | 37.5% A.S. 3.35 mg/L | 82% A.S. 7.32 mg/L |
|---|---|---|---|---|---|---|
| $LT_{50}$ (95% CI) | 25.6 (24.2-27.0) | 27.6 (25.9-29.2) | 29.0 (27.6-30.4) | 30.9 (26.9-34.9) | 33.3 (29.2-37.5) | 31.5 (28.9-34.0) |

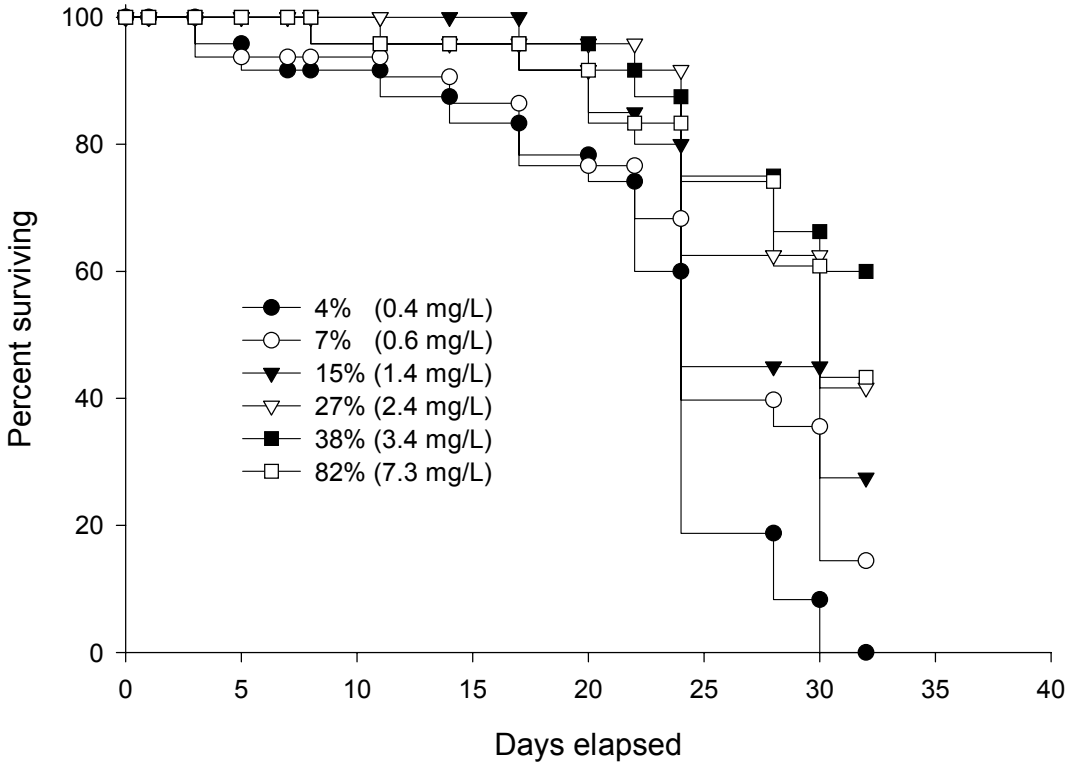


Figure 3. Survival of *Lampsilis siliquoidea* juveniles versus time during 34 days at 20 °C. Legend indicates DO treatment group in % air saturation and concentration.

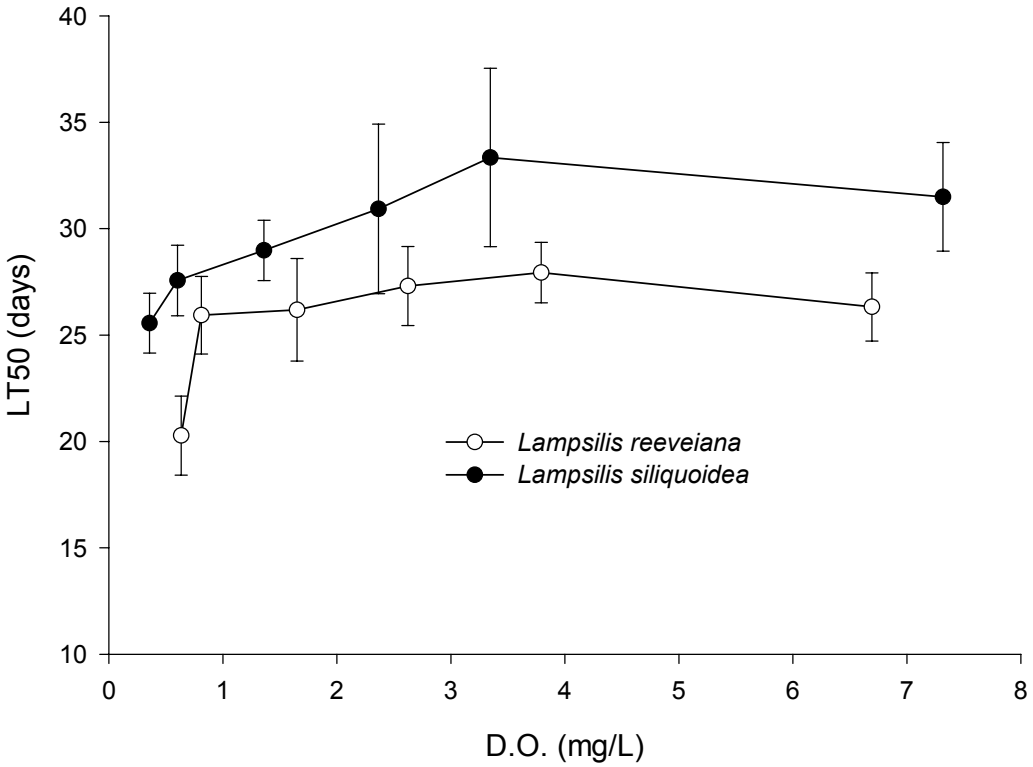


Figure 4. Time to 50% mortality of *Lampsilis* juveniles ($LT_{50}$, days) versus dissolved oxygen (DO, mg/L) at 20°C. Bars indicate 95% confidence intervals of $LT_{50}$ estimated from regressions of mortality on time.

Table 3. Effects of dissolved oxygen on growth of juvenile *Lampsilis siliquoidea* at 20 °C. Days = days of exposure. Units of D.O. are % air saturation and mg/L. Values are mean length in micrometers ± standard error of mean (N surviving).

| Days | 3% A.S. 0.27 mg/L | 6% A.S. 0.53 mg/L | 15% A.S. 1.34 mg/L | 26% A.S. 2.32 mg/L | 39% A.S. 3.48 mg/L | 84% A.S. 7.50 mg/L |
|---|---|---|---|---|---|---|
| 0 | 391 ± 7.3 (24) | 391 ± 5.7 (24) | 400 ± 7.5 (24) | 393 ± 8.9 (24) | 384 ± 5.0 (24) | 371 ± 8.4 (24) |
| 9 | 423 ± 8.4 (22) | 416 ± 8.9 (24) | 412 ± 7.2 (24) | 410 ± 8.3 (24) | 420 ± 5.9 (24) | 391 ± 7.9 (24) |
| 23 | 438 ± 8.6 (17) | 441 ± 7.9 (20) | 436 ± 10.5 (16) | 419 ± 10.0 (23) | 435 ± 6.4 (22) | 427 ± 6.6 (20) |
| 34 | 468 ± 12.7 (2) | 460 ± 7.4 (10) | 459 ± 16.3 (6) | 450 ± 10.5 (15) | 446 ± 7.8 (11) | 428 ± 9.3 (13) |

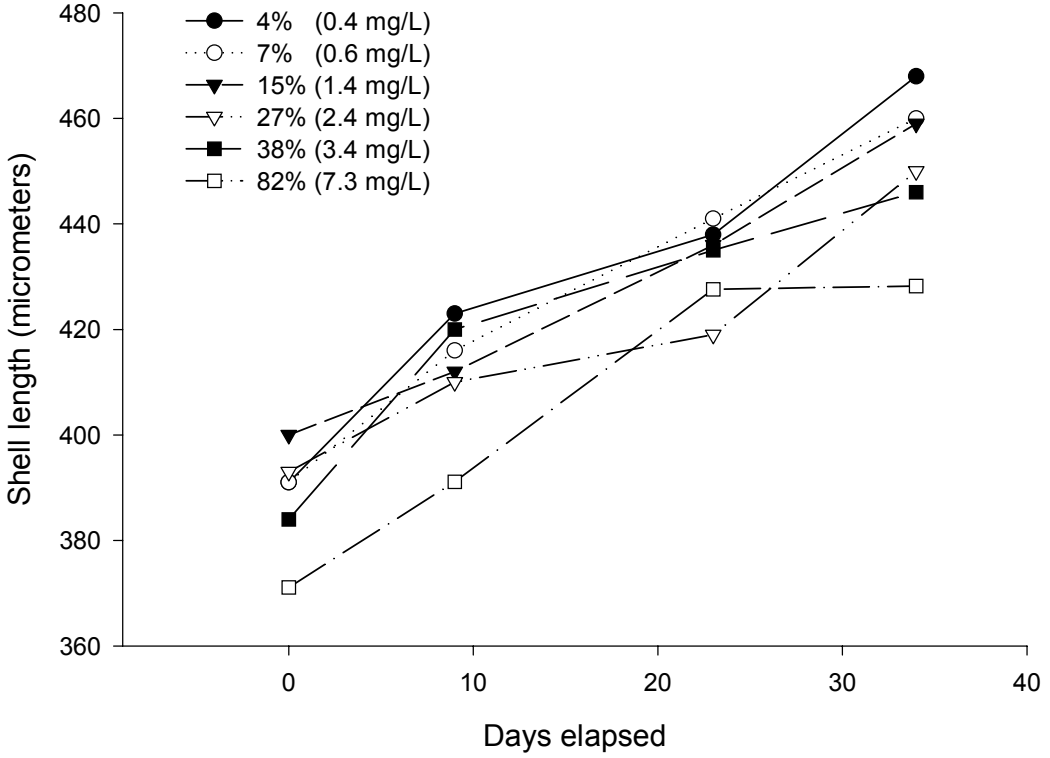


Figure 5. Growth of *Lampsilis siliquoidea* juveniles during 34 days at 6 levels of hypoxia. There were no significant differences in slope (growth rate) among treatment groups (GLM, P=0.49). Data are shown in Table 3.

DO had little effect on $LT_{50}$ at levels above 1 mg/L (Figure 4). $LT_{50}$ was consistently higher for the *L. siliquoidea* juveniles than for the *L. reeveiana* juveniles. *Lampsilis siliquoidea* juveniles grew approximately 15% in length during the 34 days of the hypoxia experiment (Table 1). Surprisingly, the rate of growth was not significantly affected by DO (Figure 5).

## Discussion

These experiments suggest that juvenile unionids are remarkably resistant to hypoxia. Small body size and consequently large surface/volume ratio probably facilitate oxygen uptake in these tiny bivalves (Herreid 1980). The constant renewal of the water boundary layer by cilia action must also facilitate oxygen uptake. Tolerance of low oxygen by juvenile mussels is more likely to be explained by efficient oxygen transport than anaerobic metabolism. Juvenile *Pyganondon cataracta* survive less than 24 hours in anoxia, suggesting that anaerobic metabolic scope is limited (Dimock and Wright 1993).

These experiments are compromised somewhat by the fact that the juveniles did not survive indefinitely even at high levels of DO. Presumably the *Neochloris* that was provided as food is not a sufficient diet. Some individuals of *Lampsilis siliquoidea* may survive up a year or more eating *Neochloris,* but not all individuals thrive, and other species that we have tested do not do as well on this diet (Barnhart 1998). Difference in nutritional condition may explain the difference in $LT_{50}$ between *L. reeveiana* and *L. siliquoidea.* If adequately nourished, these juveniles might be even more resistant to hypoxia.

The ability of young juvenile mussels to tolerate very low DO raises the possibility that they may be adapted to these conditions. It is possible that hypoxic microhabitats might even be favored as a mechanism of predator avoidance. Investigation of DO gradients on a scale of millimeters in benthic microhabitats could reveal some interesting and unexpected relationships.

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