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Propagation and Culture of Mussel Species of Concern Annual Report for 2002

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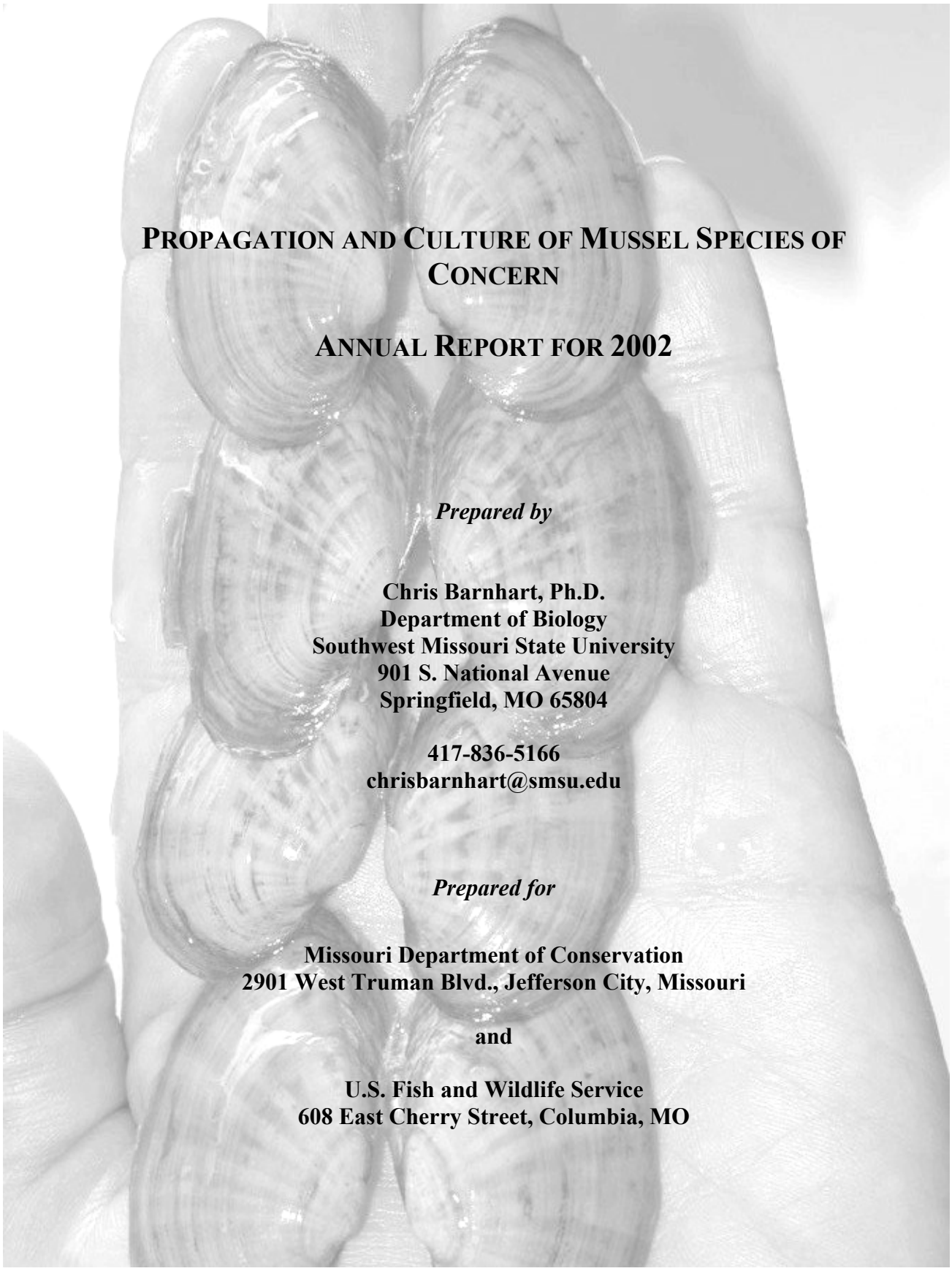

PROPAGATION AND CULTURE OF MUSSEL SPECIES OF CONCERN

ANNUAL REPORT FOR 2002

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SUMMARY

This report describes the second year of a 3-year project. The project objectives are to 1) augment existing populations and to establish new populations of mussel species of concern, 2) use molecular markers to permit genetic identification of the parentage and cohort of recovered juvenile mussels, and 3) test the susceptibility of juvenile mussels to predation by benthic invertebrates and to water quality factors such as low oxygen. The work is a cooperative effort among several agencies, including the Missouri Department of Conservation, U.S. Fish and Wildlife Service, and Southwest Missouri State University. In 2002, this cooperative effort resulted in production and release of 208,550 juveniles of 5 mussel species, including 7,450 scaleshell (*Leptodea leptodon*), 4,600 snuffbox, (*Epioblasma triquetra*), 106,560 Neosho mucket, (*Lampsilis rafinesqueana*), 39,940 black sandshell (*Ligumia recta*) and 50,000 mucket, (*Actinonaias ligamentina*). We recaptured propagated mussels for the first time, demonstrating the survival of propagated juveniles in the field. We developed new equipment and methods for monitoring and quantifying the transformation of glochidia on hosts, and performed quantitative tests on each of the species we propagated, to determine transformation success and timing. Lost Valley Hatchery carried out propagation of two species, and added facilities that will permit an expanded role in mussel propagation next year. We completed studies of reproductive timing in Neosho muckets, flatworm predation on juvenile mussels, and continued ongoing studies of the effects of low oxygen on juveniles. We disseminated information on mussel propagation through publications, Internet websites, and presentations at meetings, including a 2-day workshop that we organized at the National Conservation Training Center, under the auspices of the Freshwater Mollusc Conservation Society.

ACKNOWLEDGEMENTS

Many persons are responsible for the work reported herein. The work would not have been possible without Sue Bruenderman and Andy Roberts, who provided guidance and assistance in all aspects of the project. Sue, Andy, and Scott Faiman all spent long hours in the field locating rare mussels. Dave Waller and his staff at Lost Valley Hatchery helped develop methods, carried out propagation of snuffbox and black sandshell, and provided fish for work at SMSU. Andy Cornforth, Dennis Whelan and others at Chesapeake Hatchery provided fish, tank space, and helped with the care of both finfish and shellfish. SMSU students and staff including Bob Brown, Nathan Eckert, Christian Hutson, Jeremy Myers, and Bryan Simmons made numerous essential contributions in the field and in the lab. Trish Yasger and Mike Smith collected logperch for propagation of snuffbox. Dr. Conrad Kleinholtz and Langston University generously provided drum. Dave Hendrix and his colleagues at Neosho Hatchery helped with transport of these fish. Brian Obermeyer and Ed Miller helped with all aspects of Neosho mucket fieldwork, provided information and advice regarding collection and release sites, and conducted releases of juveniles. Susan Rogers and John Harris did their best to locate *Potamilus capax*. Brian Wilcox released juvenile muckets in the Meramec. I am grateful to Charlie Scott, Paul McKenzie, Norm Stucky, Steve Eder, Ron Dent, and Al Buchanan for supporting this work. This project was a cooperative effort involving the Missouri Department of Conservation, the U.S. Fish and Wildlife Service, the Kansas Department of Wildlife and Parks, Neosho National Fish Hatchery, and the SMSU Biology Department.

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FACILITY IMPROVEMENTS AND INNOVATIONS

A specialized aquarium system was installed at SMSU in 2002. Many of the data in this report were obtained using this system and we feel that it is a significant innovation in mussel culture. The system uses small (1, 3, or 9 liter) molded polycarbonate tanks designed for flow-through (AHAB tanks, Aquatic Habitats, Inc). These tanks have a shape that facilitates flushing of debris from the bottom of the tank. An outlet spout is placed high at one end and allows the recovery of juvenile mussels when a suitable filter is placed under the outlet stream. These tanks are potentially useful both for production and for research on timing and efficiency of transformation. The small volume of the tanks allows individual host fish or groups of small host fish to be monitored. A recirculating AHAB system for mussel culture was purchased with funds from USFWS and installed at SMSU. The system supplies 168 AHAB tanks. We modified the design of the system specifically for mussel propagation by adding extra pumping capacity and manifolds to increase the range of flow rates, expanding the guttering system to accommodate the increased flow, and designing unit filters to permit recovery of juvenile mussels from each tank. AHAB tanks were also obtained by Lost Valley Hatchery through a separate USFWS grant. These innovations were shared with Mammoth Springs National Hatchery, resulting in their decision to purchase AHAB equipment for mussel culture.

PROPAGATION AND RELEASE

Snuffbox, *Epioblasma triquetra*

Four brooding females were obtained from the Bourbeuse River. One brooding and one non-brooding female were found by Roberts, Hutson and Barnhart at sites between Wenkel Ford and Peter's Ford on April 10-12. Sue Bruenderman and Scott Faiman obtained 3 brooding females a week later at Peter's Ford. The mussels were kept in a recirculating aquarium at 12 C before and after harvesting glochidia for propagation. Each mussel was marked by engraving a number (1-4) in the shell and all were released at Peter's Ford by Bruenderman and Faiman on July 25. These females were fully gravid, were emptied of glochidia as completely as possible, providing a measure of fecundity for this species (Table 2).

Snuffbox propagation began at Lost Valley on 5/3/02. Glochidia from 2 of the 4 gravid females were used to inoculate logperch from the Bourbeuse River (n=20) and from Truman Lake (n=60). The fish were exposed to glochidia in suspension and then placed in groups of 10 in 9-L AHAB tanks with filters under the outlets. The initial intent was to compare transformation success on these two host populations, but mortality of the Bourbeuse fish over the next 2 weeks and difficulties with the filters made this comparison impractical. On 5/31/02 I visited Lost Valley and counted 3,200 juveniles that had been collected and were being held in aerated, shallow water. Over the weekend the AHAB tanks were cleaned and more captures were added, but no further counts were made. On 6/3/02, Sue Bruenderman examined the total collection of juveniles and estimated that about 1/3 were alive. The number of live juveniles was conservatively estimated at 1,000, or 1/3 of the total counted on May 31. These juveniles were released on 6/3/02 in the Bourbeuse River at Peter's Ford (Table 1).

A second round of snuffbox propagation began at Lost Valley on 6/19/02. Logperch from the Bourbeuse River (60) and from Truman Lake (40) were inoculated by swimming in a suspension of glochidia harvested from 2 females. After inoculation the logperch were transferred to a raceway in order to facilitate feeding them during the period of encystment. They were later moved to AHAB tanks to recover excysting juveniles.

Three of the fish inoculated on 6/19/02 were brought to SMSU and monitored in the AHAB system to determine the timing and efficiency of transformation (Table 3, Figure 1). Drop-off of transformed juveniles began 15 days after inoculation, and continued until 39 days after inoculation at 21 C (Figure 1). An average of 40 live juveniles per fish were recovered. Therefore, we expected that approximately 40*100=4,000 juveniles would be obtained from the 100 fish at Lost Valley. In fact, 3,600 live juveniles were recovered by 7/7/02 (pers. comm. J.R. Booth). Bruenderman and Faiman released these juveniles on 7/8/02 at Peter's Ford (Table 1).

Scaleshell, *Leptodea leptodon*

Scaleshell propagation has been challenging because of the rarity of the species, the difficulty of locating small mussels, the male-skewed sex ratio (Barnhart 2001), and the difficulty of

obtaining host fish (drum). After considerable effort, a single gravid female mussel was collected by Scott Faiman on September 20, 2001 at the US Forest Service Wrinkle Spring Access (N of Hwy. 32, off Hwy. K, LaClede County). This female was kept over the winter at Chesapeake Hatchery in a basket of fine gravel in a raceway, in flowing spring water. Glochidia were harvested on 5/29/02. This small mussel (L 42.5, H 23.2, W 11.5 mm) yielded a surprisingly large number of glochidia (814,000). Viability was tested by the closing response to saline and was nearly 100% (110/111).

Propagation at Chesapeake: Host fish were pond-reared freshwater drum (*Aplodinotus grunniens*) donated by Dr. Conrad Kleinholtz of Langston University. Average standard length of these fish was 12.3 ± SD 1.2 cm. The fish were inoculated with scaleshell glochidia on 5/29/02. The 500 fish were split into 5 groups of 100 that were each exposed to a concentration of approximately 150,000 glochidia in 50 liters (2,500/L and 1,500/fish) for 15 minutes. Thereafter, the fish were held in two 400-gallon circular tanks at Chesapeake in filtered solar pond water. After 2 weeks the fish were transferred into clean tanks. They were not fed thereafter, in order to avoid accumulation of feces during drop-off. Temperature was monitored with an Onset recorder and averaged 22.5 C over the incubation period. Approximately 450 fish survived until the juveniles were recovered. The fish were transferred into clean tanks and the tanks were vacuumed at 1-d intervals beginning 14 days after inoculation. Transformed juveniles were first recovered 16-d after inoculation (6/14/02) and they continued to excyst for approximately 1 month. The juveniles were held in the AHAB system at SMSU until release.

Monitoring at SMSU: Six of the inoculated fish were brought to SMSU for monitoring drop-off in the AHAB system. They were not monitored during the first 10 days after inoculation, because of difficulties with particulates in the new system clogging the 50-micron mesh filters needed to capture the very small (60 micron) scaleshell glochidia. This problem was solved before the juveniles excysted. The fish produced an average of 142 juveniles each (Table 4a). The number of juveniles per fish ranged from 0 to 435 and the transformation success from 0-82%. The pattern of drop-off differed from that observed in other species, in that the drop-off of incompletely transformed juveniles increased just prior to the appearance of fully transformed juveniles (Figure 2). A small number of fully transformed juveniles were noted at 15 days, and

after 16 days essentially all individuals observed were fully transformed. Drop-off of juveniles was confined mainly to 16-22 days post inoculation. Observations were terminated after 26 days.

Cold-stored glochidia: We tested the effect of cold-storing scale-shell glochidia on transformation success. After inoculation of fish at Chesapeake, the remaining glochidia were held for 4 days at 4 C before inoculating a group of 6 test fish. Drop-off from these fish was monitored from 8 days after inoculation, but very few untransformed glochidia or juveniles were recovered (Table 4b). Although the glochidia closed readily in saline after the 4-day storage and appeared to be functional, they apparently either did not attach or were sloughed within 8 days.

Efficiency of propagation: Only about 1% of glochidia were recovered as live juveniles (Table 5). There was wide variation in transformation success among the 6 individual host fish that were monitored, and half of the fish did not produce any juveniles at all. Another factor reducing recovery was mortality of host fish. Approximately 10% of the 500 fish died before metamorphosis was complete. After transformation, it appears that only 14% of the transformed juveniles were recovered live from the circular tanks. Losses at this step were presumably due to some combination of predation by flatworms, incidental consumption by the host fish, and losses during handling. These losses appeared to be due to starvation and the low body mass of some fish at the beginning of the experiment.

The variation in transformation among individual host fish is unexplained. The differences could be innate (due to genetic variation among individual host fish). Another possibility is that the differences were acquired, if some of the fish had experienced parasitic infections during culture that resulted in immunity to glochidia. The fish were pond-cultured, and could have been exposed to a variety of parasites including mussel glochidia (a single *Utterbackia* juvenile was recovered from one of the monitored fish). Next year we plan to monitor drop-off and quantify yield at each step. We anticipate receiving drum from Genoa National Hatchery and comparing these with the Langston fish. We also hope to improve yield from the circular tanks by using a net to isolate the fish from the bottom of the tank.

Releases: Scaleshell were released at two sites (Table 1). The first group (3,850 juveniles) was released on 6/19/02 by Christian Hutson in the Gasconade River near Hwy. 7 in Pulaski County (Table 1). There is a bluff on the east side of the river and the substrate at the east upstream end of the bluff is coarse (rock and sand). The mussel community at the site is dominated by *Cumberlandia* and contains at least 11 other unionid species. Water depth ranged from 5-7 feet. The site was 3-18 m from the east bank. The reach was marked with orange rebar placed on the bank at the upstream and downstream ends, and a third piece of rebar in the river at the upstream end of the site. The juveniles were released in areas ranging from 3-18 meters from the east bank. The second batch of scaleshell (3,600 juveniles) was released on 6/24/02 by Faiman and Bruenderman in the Gasconade River ~ 1/2 mile upstream of the US Forest Service Wrinkle Spring Access (N of Hwy. 32, off Hwy. K, LaClede County). This is the same site from which the gravid female was collected.

Black sandshell, *Ligumia recta*

Black sandshell were propagated at Lost Valley Hatchery. Glochidia were obtained from 2 females collected from the Big River by Bruenderman and Faiman on 6/4/02 at mile 1.3 along Hwy. W (Jefferson Co., T43N, R04E, S 19). Walleye were inoculated on 6/19/02 at Lost Valley Hatchery by placing them in a suspension of glochidia. The inoculated fish were held in a raceway in well water for ~1 week to facilitate feeding. The low temperature of the well water during this period probably delayed transformation by several days. The fish were then transferred to AHAB tanks to recover the juveniles. On July 15, 2002, Bruenderman and Faiman released 39,940 juveniles into the Big River @ mile 10.3, just downstream from MDC House Springs Access and Hwy. W crossing (T43N, R04E, no section number).

A subset of glochidia and 7 fish was brought from Lost Valley to SMSU and used for quantitative testing of transformation in the AHAB system. These fish were exposed to 1100 glochidia per liter (370/fish) for 5 minutes. Average total attachment was 61/fish, and yield was 43 juveniles/fish for 74% transformation success (Table 6). The juvenile drop-off period was 15-33 days at 21 C (Figure 3).

Neosho mucket, *Lampsilis rafinesqueana*

Two rounds of propagation were carried out at Chesapeake in 2002. Host fish were largemouth bass (8-10 cm). The first inoculation took place on 7/19/02. Glochidia (534,000) were obtained from a single Spring River female, collected near Carthage, MO. Five hundred host fish were exposed for 15 minutes to a glochidia concentration of approximately 2670/liter (1270/fish). These fish were kept in circular fiberglass tanks at Chesapeake. None of these fish were monitored in the AHAB system. A total of 75,000 live juveniles were recovered by 7/31/02. Efficiency of production was about 14% (Table 8). These juveniles were released at two upper Spring River sites (Table 1).

A second round of Neosho mucket propagation began on 8/19/02. Glochidia (344,000) were obtained from 2 Fall River females. Four hundred fifty host fish were exposed for 15 minutes to a glochidia concentration of 2300/L (690/fish). The fish were kept in circular fiberglass tanks at Chesapeake. A total of 41,700 live juveniles were recovered by 9/5/02. These juveniles were shipped to Ed Miller (KDWP) and were released in the Verdigris River in Kansas (Table 1). Several thousand juveniles collected after 9/5/02 were sent to the Columbia Environmental Research Center (CERC) for use in toxicity tests.

A subset of 10 fish from the 8/19/02 inoculation was monitored in the AHAB system at SMSU to determine the timing and efficiency of transformation and drop-off (Table 9, Figure 5). Each fish produced about 270 juveniles and transformation success on individuals averaged over 70% (Table 9). Transformed juveniles dropped off at 15-25 days after inoculation (at 21 C) (Figure 5).

The overall efficiency of propagation and recovery calculated for the Neosho mucket juveniles at Chesapeake was about relatively high at about 14% (Table 8) and 12% (Table 10). The largest losses apparently occurred during inoculation and during recovery from the circular tanks. An estimated 52% of glochidia attached to the host fish, and estimated 40% of transformed glochidia were recovered (Table 10).

Mucket (*Actinonais carinata*)

Mucket glochidia were obtained from a single Bourbeuse River female collected in April 2002 by Sue Bruenderman. The glochidia were placed on 500 largemouth bass at Chesapeake on 7/8/02. Transformed juveniles were recovered beginning on 7/17/02 (9 days post-inoculation). This rapid transformation is attributed to relatively high temperature at Chesapeake during the transformation period. Most of the juveniles were shipped to Brian Wilcox at Meramec State Park, who released 50,000 in the Meramec River in the park on 7/26/02 (Table 1). Another 8,000 juveniles were sent to the CERC for toxicology testing.

A host test was carried out with mucket on largemouth bass at SMSU. Inoculation took place on 7/15/02, using glochidia sampled from the same female used for propagation on 7/8/02. Eight fish were inoculated and monitored in the AHAB system at 21 C. Drop-off began at 12 days after inoculation. Due to an oversight, monitoring ended after 16 days, so that not all juveniles were recovered (Figure 6). Nonetheless, transformation success was high (average 71%) and consistent among the fish tested, with an average of 473 juveniles recovered per fish.

Fat Pocketbook, *Potamilus capax,* and pink mucket *Lampsilis abrupta*

We propagated both *P. capax* and *L. abrupta* in 2001 and had hoped to do so again this year, but we were unsuccessful because of failure to obtain glochidia. A collecting trip was made on 5/13/02 to Big Lake NWR, Mississippi Co AR with Susan Rogers (USFWS), to obtain fat pocketbook glochidia for propagation. We found one small *capax*, which was not gravid. Later we received a partly gravid *P. capax* on 5/17/02 from John Harris (AHDT) via Susan Rogers. The animal aborted all gill contents and died within 24 hours of arriving at SMSU. The glochidia were few and in poor condition (in a subsample of 134 eggs, 60 were undeveloped, 49 were dead glochidia, and 25 were live glochidia). We decided not to propagate these glochidia because of their poor condition and the limited supply of host fish (drum). We hoped to obtain more *P. capax* from anticipated relocation work contracted by the USACE, but the project did not proceed in time for this year. Hopefully we will be able to obtain *P. capax* in 2003.

Several collecting trips by MDC, USFWS and SMSU personnel failed to secure pink mucket glochidia in summer 2002. High water in the lower Meramec throughout the spring and summer prevented access to the best remaining population of these animals. This site is in the lower Meramec near the I-55 bridge. The site is inaccessible not only when flow is high in the Meramec, but also at times when the Mississippi River is high. Reproduction at this site has also been inconsistent. In 2001, nine animals were collected at the site in late summer but none were gravid. This fall (October 2002) Sue Bruenderman, Scott Faiman and Andy Roberts were able to collect two gravid females in the lower Meramec and transplant these to a site on the Big River, where they are more likely to be accessible next spring.

DESCRIPTION AND ASSESSMENT OF PROPAGATION METHODS

This section of the report is provided as a description and critique of our methods. Hopefully this description will be helpful for others attempting to propagate freshwater mussels.

Useful equipment.

1. Filter cups are made by placing Nitex cloth over the end of a short length of 3-inch PVC pipe and pressing it into a slip-slip PVC coupling. Mesh size should be matched to the size of the glochidia, which varies among species.
2. A small, pressurized garden sprayer, modified by shortening and bending the spray arm, is useful for rinsing filters and dishes.
3. Glass culture dishes sold by Carolina Biological come in several sizes and are very useful for handling and storing glochidia and juveniles.
4. A volumetric pipette is needed for counting (see below). Either an adjustable or a fixed-volume, 200-microliter model can be used. The aperture on the disposable tips must be large enough to pass glochidia easily. The tip can be trimmed to widen the aperture to about 1 mm. Use a balance to check the volume dispensed and ensure that the calibration is accurate.

Counting glochidia and juveniles.

Counts are essential so that results can be quantified and methods improved. We use the following procedures:

1. Mature glochidia can be removed from most lampsiline species (e.g. *Lampsilis, Ligumia, Potamilus, Epioblasma*) by injecting clean water into the marsupia from a syringe. Mature glochidia should discharge easily without clumping. Immature or infertile eggs tend to clump. If clumps appear, check under a microscope to be sure that the glochidia are mature before proceeding.
2. Rinse the glochidia through a 400-micron Nitex filter to disperse any clumps.
3. Place the glochidia in a measured volume of water in a beaker. The volume should be such that the concentration of glochidia is ~150/ml (30/0.2 ml). Check the number in a drop and adjust the volume accordingly.
4. Suspend the glochidia by gently agitating the water with a turkey baster. While continuing to agitate, withdraw ten 0.2-ml samples with a volumetric pipette.
5. Place each sample as a separate drop on a clean, dry polystyrene Petri dish (the lid and base of a 3-inch dish will accommodate 5 drops each).
6. Examine each drop under a dissecting microscope and count each individual present. Make separate counts of open and closed glochidia and of undeveloped eggs.
7. Add a drop of saturated salt solution to each sample, and then count the number of open individuals again.
8. Determine the number of infective glochidia by subtracting the number that failed to close in salt from the number initially open.
9. The number of glochidia in the total suspension is calculated as: (n per sample)*(total volume/sample volume). The individual sample estimates are averaged. If 10 subsamples are counted, and if there are ~10-30 glochidia per sample, the precision of the estimate is typically ±10% (95% CI).
10. Recovered juveniles can be counted in the same way. When counting juveniles, distinguish live and dead individuals by noting color, adductor and gill development, and movement. Learning to discriminate juveniles from untransformed glochidia takes practice, and you

should use a compound microscope to check yourself until you are familiar with the appearance of juveniles.

Timing of transformation

Transformation time varies with temperature and also among species. Data provided in this report can be used to predict transformation times of those species tested. Water temperature can be manipulated to change the timing of transformation and to delay or accelerate the drop-off of juveniles. Control of timing might be used to coordinate the drop-off period with the hatchery work schedule or to work around periods of inclement weather or water levels at release sites.

Juveniles may continue to excyst from fish for weeks beyond the peak period and the number of juveniles in the "tail" of the excystment may be substantial (Figures 1, 4). Therefore, it may be worthwhile to hold host fish for a longer period. We should investigate temperature effects to see if the shape of the drop-off curve changes.

Glochidia load on host fish

Either the concentration of glochidia or the duration of exposure can be used to adjust the number of glochidia attached to host fish. For example, walleye exposed for 5 minutes to 1100 glochidia/liter picked up 61 glochidia/fish (Table 5) while those exposed to 2000 g/l picked up 116 per fish (Table 6).

The numbers of glochidia attached to host fish in this study ranged from about 60-400 per fish (Table 10). The host fish were generally 8-10 cm long. We have attached larger numbers of glochidia to smaller fish in other tests without causing any apparent ill effects in the fish. Therefore, it appears that we could increase the numbers of glochidia placed on bass, walleye and drum. A reasonable inoculation treatment would be 4,000 glochidia/liter, 2,000 glochidia/fish, and 10 minutes exposure. A group of 100 fish inoculated in 50 liters would require 200,000 glochidia. The volume in which fish are inoculated should be at least 2 liters

per fish (8-10 cm). If the fish are excessively concentrated, the glochidia may close prematurely in response to fish mucus, blood, or other chemical cues in the water.

Propagation facilities at Chesapeake

Most propagation has been carried out in six 400-gallon, 4-ft diameter circular fiberglass tanks at Chesapeake. Each circular tank has a central standpipe to control water level and conduct water out of the tank. The standpipe is press-fit into a socket molded into the bottom of the tank. An outer socket is also present to hold a perforated sleeve surrounding the standpipe. We removed the sleeve, and sealed the standpipe into the inner socket to prevent leakage out the bottom.

We vacuum the bottom of the tank with a siphon hose and filter the siphonate through two soil sieves, a coarse one to remove larger debris, and a finer sieve to recover the juveniles. The vacuuming process has been improved with a specialized vacuum head and filter frame, but is still tedious. The outer standpipe socket forms a narrow cavity that accumulates sediment and must be vacuumed separately.

The tanks are supplied with solar pond water, which contains a variety of plankton. The plankton is a problem because it includes predators such as *Hydra* and rhabdocoel flatworms, as well as many cladocerans, bryozoan statoblasts, shelled amoebas, etc. Many of these organisms are in the same size range as the juvenile mussels, making it difficult to isolate the mussels by filtration. The plankton makes it difficult to count the juveniles and creates water quality problems during storage and transport.

We attempt to minimize the plankton problem by filtering the water coming into the tanks. We use two spin-down filters (Rusco Corporation) in series for each tank, one 60 mesh (254 micron) and the other 250 mesh (61 micron). The filters clog up and must usually be cleaned at least once a day. Sand filtration of the water delivered to the circular tanks would be a great improvement. Feeding the fish also generates frass and uneaten food, which further contributes to the problems described above. Therefore, we generally do not feed the fish for several days

before drop-off of juveniles or during the drop-off period. We also transfer the fish to a clean tank just before drop-off is expected to begin.

Efficiency of recovery of excysted juveniles from the circular tanks is apparently low (estimated at about 20% for scaleshell on drum [Table 5] and about 40% for Neosho muckets on bass [Table 10]). There are several possible routes of loss. The vacuuming step misses juveniles that either become suspended or adhere to the tank. It is possible for suspended juveniles to be lost from the standpipe, although we minimize flow through the tanks and aerate only in the upper half of the water column to try to avoid circulating juveniles out the top of the standpipe. We also suspect that some host fish (drum) may eat detritus from the bottom of the tank and thus consume the juveniles.

In addition to the losses described above, mortality of excysted juveniles is also a problem in the circular tanks. We estimated that only about 76% of scaleshell juveniles and 86% of Neosho mucket juveniles were recovered live from these tanks. (Table 5 and Table 10). Possible sources of mortality include flatworm predation and mechanical stresses during vacuuming and filtration.

The ideal recovery system would minimize the influx of plankton, maintain water quality, and filter the water continuously to isolate the juveniles as they drop off. AHAB tanks are very effective, but they are small (up to 9-L) so that only a few fish may be kept in each. An ideal recovery tank for large-scale propagation would 1) hold hundreds of fish, 2) recirculate the water through a filter to recover juveniles, and 3) maintain water quality, either by conditioning the recirculated water or allowing some delivery and flow-through of clean water.

RECAPTURES OF PROPAGATED MUSSELS

Initial efforts were made to recapture propagated mussels at release sites. In January, Brian Obermeyer (TNC), Ed Miller (KWP), and SMSU personnel searched shell middens (shells of mussels eaten by raccoons or muskrats) near our Neosho mucket release sites on the Fall River and Verdigris River. Brian Obermeyer found 9 fresh-dead shells at the Fall River Wildlife

Refuge, and we found 21 fresh-dead shells at a release site on the Verdigris River. Several other live individuals of the same cohort were also located at the Verdigris site by Ed Miller, but these were not collected or measured.

We presume that the shells we recovered in January had all died during the winter, when low water conditions exposed many mussels and made them accessible to raccoons. Approximately half of the shells had tissue remaining in the valves. The shells were all of young individuals, from 42-64 mm in length (Figure 7). The Fall River site was stocked in 1999 & 2000, while the Verdigris site was stocked only in 2000 (Barnhart 2001). Therefore, two age cohorts were potentially present at the Fall River site and only one cohort at the Verdigris site. All of the shells showed a fairly clear growth line approximately 7-15 mm long (Figure 8). Subsequent growth lines were absent or ambiguous.

I compared the size distribution of shells from the two sites in order to determine the number of size cohorts represented. All of the shells from both sites appear to be a single size and age cohort. Measurements of both the first growth line and the total shell length are unimodal within sites (Figure 9). Although the Fall River shells are somewhat larger than the Verdigris shells (~25%), the difference is not sufficient to indicate different age classes. Length is expected to more than double between the second and third winters (Riusech 1999). The 1999 cohort may have been killed by a severe drought and dewatering of the release site in the summer of 2000 (Barnhart 2001).

The identity of these mussels as recaptures is indicated by the following arguments. First, no living Neosho muckets had been found on the Fall River Wildlife Refuge, or any sites above Fall River Lake, in previous mussel surveys (Obermeyer et al. 1997, pers. comm.. Brian Obermeyer, TNC and Ed Miller, KDWP). The lake provides a barrier to any possible recruitment from downstream populations. We chose this site intentionally so that recaptures would be unambiguous. Second, adult Neosho muckets are present at the Verdigris release site, but they are rare and no juveniles have been found previously (Figure 10). Only 13 adult Neosho muckets, and no juveniles, were found among 7,416 mussels sampled at this site in 1991-2001 (pers. comm. Ed Miller, KDWP). In fact, these recaptures are the first juvenile Neosho muckets

to be reported from these rivers, or anywhere in Kansas, for at least 10 years (pers. comm. Ed Miller, KDWP and Brian Obermeyer, TNC). Third, the size distribution of the shells is consistent with a single cohort that is of appropriate size and age to be our year 2000 releases.

We also attempted, unsuccessfully, to recover propagated animals at two other sites this year. On 7/18/02 we visited two sites where we released Neosho muckets in 2001 on Shoal Creek (Hwy 86 near Joplin, and Lime Kiln Access at Neosho). We sampled 35 ¼ m^2 quadrats quantitatively by excavating the substrate into hardware-cloth lined boxes, sifting out the finer material in the water, and then examining the remaining gravel closely to locate juvenile mussels. We examined 13 samples at the Hwy 86 site, and 22 samples at the Lime Kiln site. In all, we recovered no juvenile Neosho muckets, and only 11 unionids, in the total sample of 8.75 m^2.

GENETIC IDENTIFICATION OF RECAPTURES

Juvenile mussels are too small (~0.25 mm) to be marked at the time of release. Therefore, the identity of recaptures must be inferred by means other than direct marking. Recaptures may be unambiguous if they are at a site with no previous population or recruitment. However, releases of endangered species, such as pink mucket, must generally be made in areas where the species is already present. One of the objectives of this project is to use microsatellite markers to ascertain the identity of recaptured mussels, particularly pink mucket. Microsatellite markers are rapidly evolving genetic elements that can be used to determine parental and sibling relationships among individuals. We have retained tissue samples of the female parents and hope to employ this method to identify recaptures at sites where the species is already present. Dr Hsiu-Ping Liu, former co-PI on this project, left SMSU in July 2001 and is no longer involved in developing the microsatellite markers. Fortuitously, Dr. Tim King (USGS Leetown Science Center) has developed suitable markers for *Lampsilis* and has agreed to process our recapture samples when they become available.

HOST TESTS WITH BLACK SANDSHELL

This experiment was prompted by interest in propagating black sandshell in Missouri, Arkansas, and Kansas. Black sandshell is a species of concern in our region and is particularly rare in Kansas, which includes the western edge of the species range. Black sandshell was formerly widespread in the rivers of eastern Kansas (Scammon 1906) but has been nearly extirpated. A single male specimen discovered in the Marais des Cygnes National Wildlife Refuge in 2002 is the only recent record of the species in Kansas (Brian Obermeyer, TNC, pers. comm.).

We investigated three groups of fish: walleye from the Lost Valley Hatchery, sauger from Farlington Hatchery in Kansas, and largemouth bass from Chesapeake Hatchery. Glochidia were obtained from a single female black sandshell, collected in the Sac River near Caplinger Mills, Missouri on July 1, 2002. Viability of 356 glochidia tested with salt was 74%. The host fish were pooled and inoculated simultaneously in two groups (14 fish each) by swimming with 12,000 glochidia in 6 liters of water (2000/L, 860/fish) for 5 minutes. The fish were then kept in 3-L AHAB tanks for 2 months, during which time they were fed daily with blackworms. Drop-off was checked at 1-2 day intervals for the first month and at the end of the second month. After 58 days the fish were sacrificed and measured.

Attachment and transformation success varied significantly among the host species (ANOVA, Tukey's test) (Table 7). Largemouth bass were not a suitable host, transforming only ~1% of attached glochidia. In contrast, walleye and sauger transformed about 50% of attached glochidia. There was no significant difference in the number of juveniles or the percent transformation between walleye and sauger. The drop-off of most juveniles (~70%) took place between 16 and 26 days (Figure 4). However, drop-off continued at a lower rate for several more weeks. About 30% of the total number of juveniles from walleye and sauger were recovered during the second month after inoculation. The rate of drop-off from sauger peaked about 2 days earlier than that from walleye, but this difference was not tested statistically. The prolonged drop-off period observed for black sandshell is significant in two ways. First, at 22 C, the number of juveniles recovered can be increased ~30% by holding the hosts for an extra month. Second, the continued presence of cysts should be considered when host fish are released.

If black sandshell are to be augmented in the Marais des Cygnes system in Kansas, then Sac River populations in Missouri are a likely choice as a source of glochidia. The present study shows that either sauger or walleye could be used for propagation and/or stocked on site as hosts. Previous work by Dr. James Layzer (pers. comm.) indicated that sauger was more suitable than largemouth bass or green sunfish as a host for black sandshell from Tennessee. The present experiment shows quantitatively that both sauger and walleye are suitable hosts for Sac River black sandshell. Transformation success on both *Stizostedion* species was about 50%, while bass were unsuitable.

REPRODUCTIVE BIOLOGY OF NEOSHO MUCKETS

Several experiments relevant to our propagation of Neosho muckets were carried out by SMSU graduate student Melissa Shiver. The results are presented in detail in her Master's thesis (Shiver 2002) and are summarized in here. Timing of reproduction in 2001 was compared at two sites in the Spring River system: Shoal Creek at Joplin, MO and Spring River at Carthage, MO. At least 10 individuals were examined monthly at each field site. Females spawned in May and brooded eggs and larvae from May through July at both sites. This timing is atypical of *Lampsilis* species, which generally spawn in the fall and brood through the winter and spring. Gonad fluid was collected in the field and examined microscopically to determine the timing of gamete development. Neosho muckets appeared to undergo two peaks of ovogenesis, first in the early spring and again in the fall. At Shoal Creek, 90% of females reproduced in 2001, but only 40% of the females at Spring River did so. Sterilizing trematodes (*Rhipidocotyle* sp.) affected a few individuals at both sites, but did not explain the lower reproduction rate at the Spring River site. Shell size differed significantly between the two populations. Mean length of Spring River shells was 96.4 mm compared to 71 mm in Shoal Creek. Growth curves were derived from measurements of external shell annuli and compared among ages, sexes, and sites. Size was consistently smaller at the same inferred age in Shoal Creek.

Glochidia were transformed on hatchery largemouth bass. Host acquisition of immunity to glochidia was tested by repeated infestations. Transformation success differed significantly between fish exposed for the first time and second time to Neosho mucket glochidia.

Transformation success of another species, *L. cardium*, was also significantly lower after exposure of the host fish to Neosho mucket glochidia. These results demonstrate that immunity acquired by host fish to one mussel species can affect transformation of other species.

FLATWORM PREDATION ON JUVENILE MUSSELS

Another objective of this project is to investigate predators of juvenile mussels. Free-living flatworms (Phylum Platyhelminthes, Class Turbellaria) are important predators on small aquatic invertebrates. *Macrostomum tuba*, a predominantly benthic species, feeds on juvenile freshwater mussels in fish hatcheries and mussel culture facilities. SMSU graduate student Angela Delp performed laboratory experiments to assess the predation rate of *M. tuba* on newly transformed juveniles of plain pocketbook mussel, *Lampsilis cardium.* Her results are presented in detail in her thesis (Delp 2002) and are summarized here. Predation rate at 20 ^{o}C in dishes without substrate was 0.26 mussels·worm^{-1}·h^{-1}. Predation rate increased to 0.43 mussels·worm^{-1}·h^{-1} when a substrate, polyurethane foam, was present. Substrate may increase predation rate by altering behavior of the predator and bringing the flatworms in contact with the mussels more often. Presence of an alternative prey, the cladoceran *Ceriodaphnia reticulata*, reduced predation rate on mussels. Flatworms preyed at a higher rate on *Ceriodaphnia* than on mussels, both when only one prey was present and when both were present. The effect of flatworm size (0.7- 2.2 mm long) on predation rate on mussels (0.2 mm) was tested. Predation rate increased with predator size. The slope of this relationship decreased with increasing predator size. Predation rate was near zero in 0.7 mm worms. Juvenile mussels grow rapidly and can escape flatworm predation by exceeding the size of these tiny predators. Captive culture for even a few weeks might improve the survival of propagated juveniles if flatworm predation also occurs in nature.

LITERATURE CITED

Barnhart, M. C. 2001. Fish hosts and culture of mussel species of special concern. Report to U.S. Fish and Wildlife Service and Missouri Department of Conservation. 41 p.

Delp, Angela M. 2002. Flatworm predation on juvenile freshwater mussels. Master's thesis, Southwest Missouri State University. 31 p.

Obermeyer, B. K., D. R. Edds, C. W. Prophet, and E. J. Miller 1997. Freshwater mussels (Bivalvia: Unionidae) in the Verdigris, Neosho, and Spring River basins of Kansas and Missouri, with emphasis on species of concern. Am. Malac. Bull, 14:41-55.

Riusech, Frank. 1999. Genetic and life history characteristics of the freshwater bivalves, *Venustaconcha ellipsiformis* and *Venustaconcha pleasii*, in the Ozark Plateaus region. Thesis, Master of Science, SMSU.

Scammon, R.E. 1906. The Unionidae of Kansas, Part I. An illustrated catalogue of the Kansas Unionidae. Kansas University Science Bulletin 3(9):279-373 + 21 plates.

Shiver, Melissa A. 2002. Reproduction and propagation of the Neosho Mucket, *Lampsilis rafinesqueana.* Master's thesis, Southwest Missouri State University.

RELATED PUBLICATIONS AND PRESENTATIONS

Barnhart, M. C. 2002. Successful stocking of Neosho muckets in the Fall and Verdigris rivers. Kansas Pearly Mussel Newsline 7:1-2. (Newsletter article)

Bruenderman, S., J. Sternberg and C. Barnhart. 2002. Missouri's Freshwater Mussels. Missouri Department of Conservation. 16 p. (Booklet)

Barnhart, M. C. 2002. An introduction to reproduction in Unionoid mussels. Freshwater Mollusc Conservation Society Mussel Propagation Workshop. National Conservation Training Center, Shepherdstown, WV.

Barnhart, M. C. 2002. Mussel propagation in Missouri. Freshwater Mollusc Conservation Society Mussel Propagation Workshop. National Conservation Training Center, Shepherdstown, WV.

Myers, Jeremy and M. C. Barnhart. 2002. Effects of low oxygen on juvenile freshwater mussels. Annual Meeting, Missouri Academy of Science, Springfield, MO.

Shiver, Melissa A. and M. Christopher Barnhart. 2002. Reproduction and Propagation of the Neosho mucket, *Lampsilis rafinesqueana.* American Fisheries Society, Southern Division Annual Meeting, Little Rock.

Shiver, Melissa A. and M. Christopher Barnhart. 2002. Reproduction and Propagation of the Neosho mucket, *Lampsilis rafinesqueana.* Missouri Natural Resources Conference, Osage Beach. **BEST STUDENT PAPER AWARD,** Missouri Chapter, American Fisheries Society.

Table 1. Releases of propagated juvenile mussels in 2002.

Snuffbox, *Epioblasma triquetra*

Date	N juveniles	Release site
6/3/02	1,000	Bourbeuse River at Peters Ford, Franklin County MO
7/8/02	3,600	Bourbeuse River ½ mile downstream of Peters Ford, Franklin County MO

Scaleshell, *Leptodea leptodon*

date	N juveniles	Release site
6/19/02	3,850	Gasconade River 250 m downstream Hwy 7, Pulaski Co., MO. UTM: X=4184045, Y=558035
6/24/02	3,600	Gasconade River, ½ mile upstream USFS Wrinkle Spring Access, Laclede Co., MO.

Black sandshell, *Ligumia recta*

date	N juveniles	Release site
7/15/02	39,940	Big River just downstream Hwy. W, Jefferson Co., MO

Neosho mucket, *Lampsilis rafinesqueana*

date	N juveniles	Release site
7/31/02	32,430	Spring River, 200m upstream of road crossing, NE of Hoberg, SE¼ S2 T27NR27W, Lawrence Co. MO
7/31/02	32,430	Spring River just downstream of Hwy 97 bridge, SE¼ S14 T28N R28W, Lawrence Co. MO
9/4/02	41,700	Verdigris River, SW¼ S4 T36 R16 Montgomery County KS.

Mucket, *Actinonaias ligamentina*

date	N juveniles	Release site
7/26/02	50,000	Meramec River SE ¼S13 T40N R2W Franklin CO MO Above and below Benchmark 599.

Table 2. Snuffbox shell measurements and glochidia counts. The mussels appeared to be fully gravid and the glochidia were removed as completely as possible, so that these counts are an estimate of fecundity. The glochidia were used for propagation on 5/3/02 (#1,2) and on 6/19/02 (#3, 4). Viability was tested with salt.

Female #	length (mm)	height (mm)	width (mm)	# glochidia	% viable
1	35.3	19.3	19.9	14,105	92.2
2	35.1	20.1	17.3	13,440	97.8
3	34.7	19.8	17.3	21,000	88.1
4	36.2	19.9	16.9	13,500	88.9
Mean ± Stdev	35.3 ± 0.6	19.8 ± 0.3	17.9 ± 1.4	15,511 ± 3671	91.8 ± 4.4

Table 3. Snuffbox attachment and transformation success on logperch. These fish were a subset of the 100 fish used for propagation at Lost Valley on 6/19/02.

Fish #	Untransformed or dead	Live juveniles	Total attached	Percent transformed
1	17	57	74	77.0
2	10	27	37	73.0
3	81	36	117	30.8
Mean ± Stdev	36 ± 39.1	40 ± 15.4	76 ± 40	60.3 ± 25.6

Table 4a. Scaleshell transformation success on drum. These fish were a subsample of the fish inoculated at Chesapeake on 5/29/02. Note the variation in transformation success among individual fish.

Fish #	Untransformed or dead	Live juveniles	Total*	Percent transformed*
1	79	356	435	81.8
2	76	39	115	33.9
3	3	0	3	0.0
4	8	0	8	0.0
5	131	313	444	70.5
6	0	0	0	0.0
Mean ± Stdev	59 ± 54	142 ± 178	201 ± 222	37 ± 38

*Drop-off was not monitored until day 11, so glochidia lost earlier were not counted. Therefore, the total underestimates the number of glochidia initially attached, and the percent transformation values are overestimated.

Table 4b. Transformation of cold-stored scaleshell glochidia on drum. These fish were inoculated with glochidia that had been stored at 4 C for 4 days. Drop-off was monitored after day 8. Apparently the glochidia either failed to attach or they were sloughed before day 8.

Fish #	Untransformed or dead	Live juveniles	Total	Percent transformed
1	0	0	0	0
2	1	0	1	0
3	4	1	5	20
4	3	0	3	0
5	1	0	1	0
6	0	1	1	0
Mean ± Stdev	1.8 ± 1.6	0.2 ± 0.4	2.0 ± 2.0	4.0 ± 8.9

Table 5. Efficiency of scaleshell propagation on 450 drum in circular fiberglass tanks at Chesapeake Hatchery. The precision of each estimate is indicated as an approximate 95% confidence interval.

Stage of the process	Number of individuals (± 95% CI)	Percent of previous	Percent of initial
1. Initial (glochidia)	814,000 ± 10%	-	100%
2. Transformed juveniles*	63,900 ± 100%	7.9%	7.9%
3. Total juveniles recovered	12,650 ± 10%	19.8%	1.6%
4. Live juveniles recovered	9,660 ± 10%	76.4%	1.2%
5. Survived to be released	6,650 ± 10%	68.8%	0.8%

**The number of transformed juveniles was estimated from the number produced per fish in a monitored subgroup (Table 3a) and the total number of fish. Precision of the estimate is low because of the high variability in transformation among individual fish.

Table 6. Big River black sandshell attachment and transformation on walleye. These fish were inoculated in parallel with those used for propagation at Lost Valley. They were exposed for 5 minutes in 2.5 liters at 1,100 glochidia /liter.

Fish #	Untransformed or dead	Live juveniles	Total attached	Percent transformed
1	64	68	132	51.5
2	5	19	24	79.2
3	13	51	64	79.7
4	14	34	48	70.8
5	12	42	54	77.8
6	12	56	68	82.4
7	9	29	38	76.3
Mean ± Stdev	18.4 ± 20.3	42.7 ± 16.9	61.1 ± 34.7	73.9 ± 10.5

Table 7. Comparison of Sac River black sandshell attachment and transformation success on largemouth bass, sauger, and walleye. SL= standard length of host fish. Numbers are mean ± standard deviation, n=8. Means with different superscripts (within columns) were significantly different (Tukey's test). Significantly more glochidia attached to walleye than to sauger or bass. The number transformed and the percent transformed were similar on sauger and walleye and higher than on bass.

Host	SL (mm)	N attached	N transformed	% Transformed
Walleye	86.4^{a} ± 7.8	116.1^{a} ± 26.3	58.5^{a} ± 25.5	49.0^{a} ± 14.2
Sauger	90.8^{a} ± 4.9	83.1^{b} ± 16.3	47.1^{a} ± 17.2	55.4^{a} ± 11.1
Lm. bass	68.8^{b} ± 3.4	64.1^{b} ± 16.7	0.7^{b} ± 1.0	1.1^{b} ± 1.3

Table 8. Efficiency of Spring River Neosho mucket propagation (7/19/02). Transformation on 450 largemouth bass in circular fiberglass tanks at Chesapeake Hatchery. The approximate precision of each estimate is indicated as a confidence interval.

Stage of the process	Number of individuals (approximate 95% CI)	Percent of previous	Percent of initial
1. Initial (glochidia)	534,000 ± 10%	-	100%
2. Total juveniles recovered	99,000 ± 20%	18.5%	18.5%
3. Live juveniles recovered	75,000 ± 24%	75.8%	14.0%

Table 9. Fall River Neosho mucket attachment and transformation success on largemouth bass. Glochidia were pooled from two females. These fish were a subset of those used for propagation at Chesapeake on 8/15/02.

Fish #	Total attached	Untransformed or dead	Live juveniles	Percent transformed
1	652	408	244	37.4
2	331	115	216	65.3
3	390	93	297	76.2
4	492	148	344	69.9
5	344	91	253	73.5
6	354	54	300	84.7
7	344	91	253	73.5
8	354	54	300	84.7
9	304	82	222	73.0
Mean ± Stdev	396.1 ± 109.8	126.2 ± 109.5	269.9 ± 42.5	70.9 ± 14.1

Table 10. Efficiency of Fall River Neosho mucket propagation (8/15/02). Transformation on 450 largemouth bass in circular fiberglass tanks at Chesapeake Hatchery. The approximate 95% confidence interval of each mean is given as percent of the mean.

Step of the process	Number of individuals (± 95% CI)	Percent of previous step	Percent of initial
1. Initial (glochidia)	344,400 ± 15%	-	100%
2. Total attached glochidia*	178,245 ± 20%	51.7%	51.7%
3. Transformed juveniles*	121,455 ± 10%	68.1%	35.3%
4. Total juveniles recovered	48,500 ± 20%	39.9%	14.1%
5. Live juveniles recovered	41,700 ± 20%	86.0%	12.1%

*The numbers of attached glochidia and transformed juveniles were estimated from the number per fish in a monitored subgroup (Table 7) and the total number of fish.

Table 11. Effects of inoculation concentration and duration. Host fish were all 8-12 cm standard length. The fish were swum in aerated suspensions of glochidia having the stated concentrations per liter and number of glochidia per fish. The numbers attached were determined by quantitative recovery of glochidia and juveniles in the AHAB system (data from Tables 3a, 5, 6, and 8)

Date	Species	Glochidia /liter	Glochidia /fish	Minutes	Glochidia attached/fish
5/29/02	Scaleshell & drum	2500	1500	15	201
6/19/02	Black sandshell & walleye	1100	370	5	61
7/1/02	Black sandshell & walleye	2000	860	5	116
7/1/02	Black sandshell & sauger	2000	860	5	83
7/1/02	Black sandshell & lm bass	2000	860	5	64
8/15/02	Neosho mucket & lm bass	2300	760	15	396

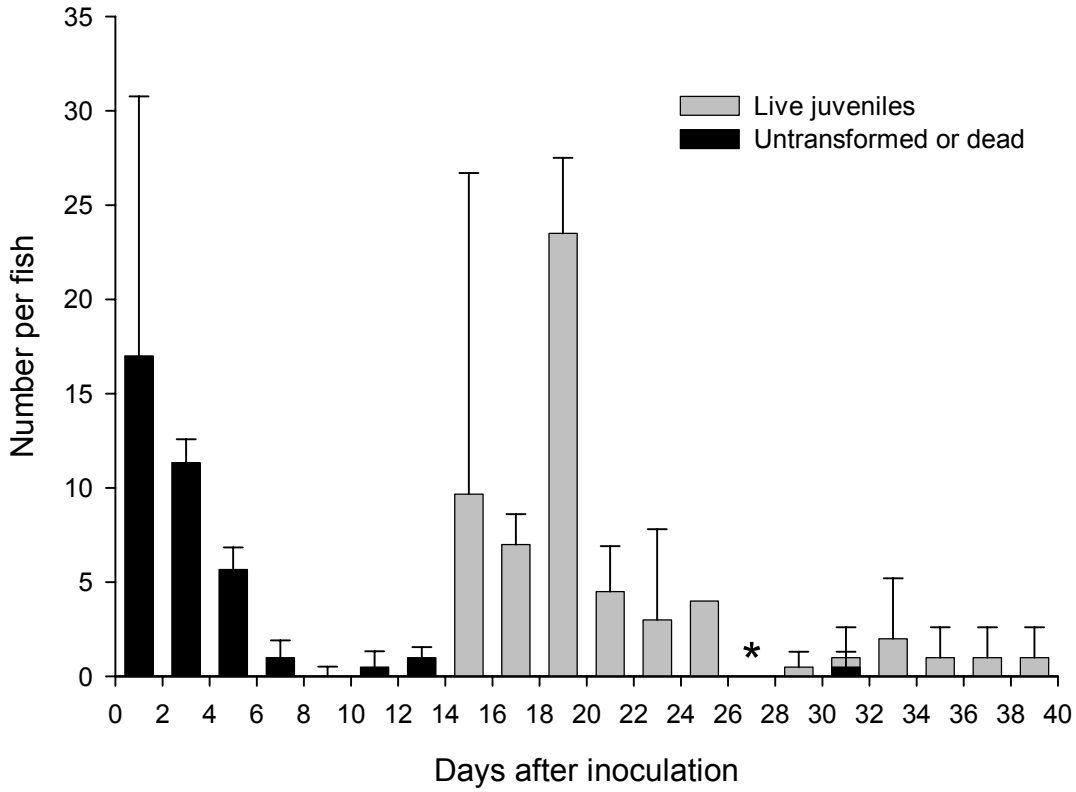

Figure 1. Timing of drop-off of snuffbox from logperch at 21 C. These data were derived from 3 fish that were a subset of those used for propagation at Lost Valley on 6/19/02. Columns represent the mean number of individuals recovered per fish during the preceding 1-2 d interval. Error bars indicate 95% C.I. Stars indicate days when drop-off was checked but no juveniles were recovered. See Table 2 for totals.

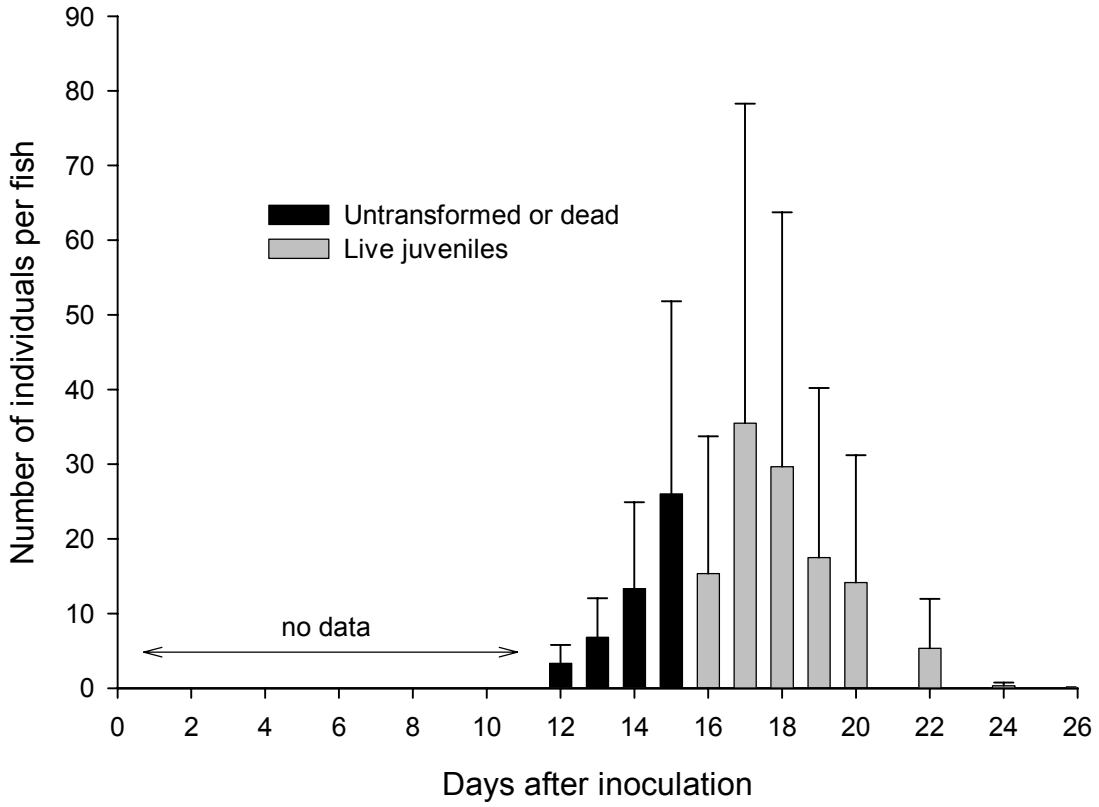

Figure 2. Timing of drop-off of scaleshell from drum. These data are from 6 fish that were a subset of those used for propagation at Chesapeake. Temperature was 21-22 C. These fish were not monitored for the first 11 days after inoculation. Columns represent the mean number of individuals recovered per fish during the preceding 1-2 d interval. Error bars indicate 95% C.I. See Table 4a for totals.

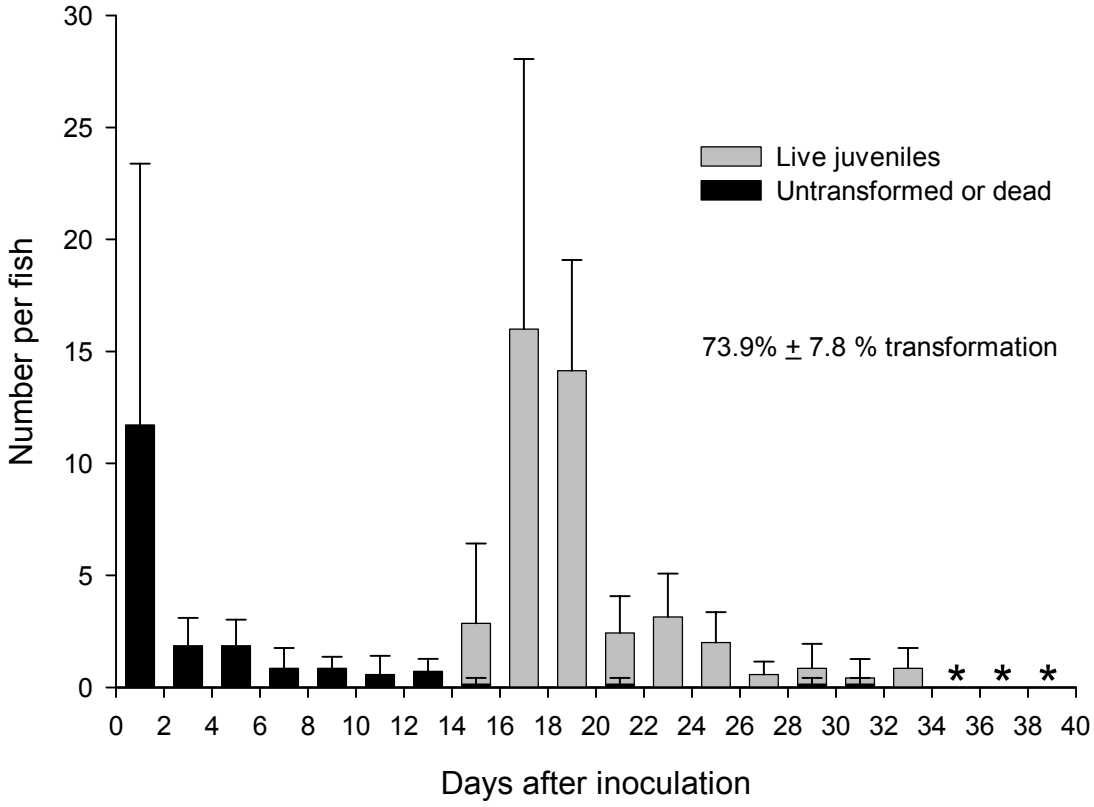

Figure 3. Timing of drop-off of Big River black sandshell from walleye. Temperature was 21 C. These data are derived from 7 fish that were representative of those used for propagation at Lost Valley on 6/19/02. Columns represent the mean number of individuals recovered per fish during the preceding 1-2 d interval. Error bars indicate 95% C.I. Stars indicate days when drop-off was checked but no juveniles were recovered. See Table 6 for totals.

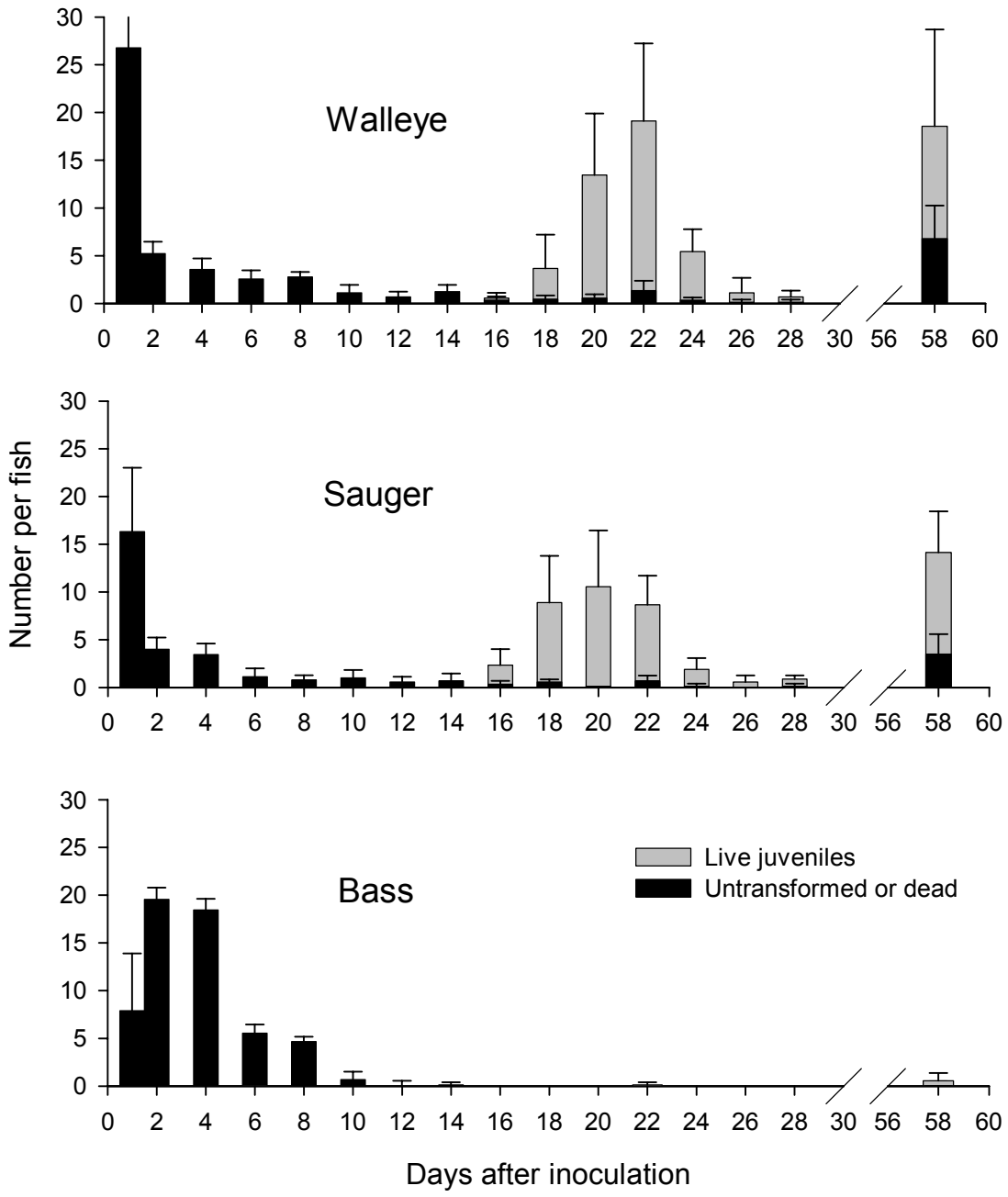

Figure 4. Timing of drop-off of Sac River black sandshell from walleye, sauger and largemouth bass. Temperature was 21-22 C. Recovered glochidia and juveniles were counted at 1-d and 2-d after inoculation (0) and at 2-d intervals thereafter until 28-d. A final count was made at 58-d. Columns represent the mean number recovered per fish during each interval. Error bars indicate 95% C.I. See Table 7 for totals.

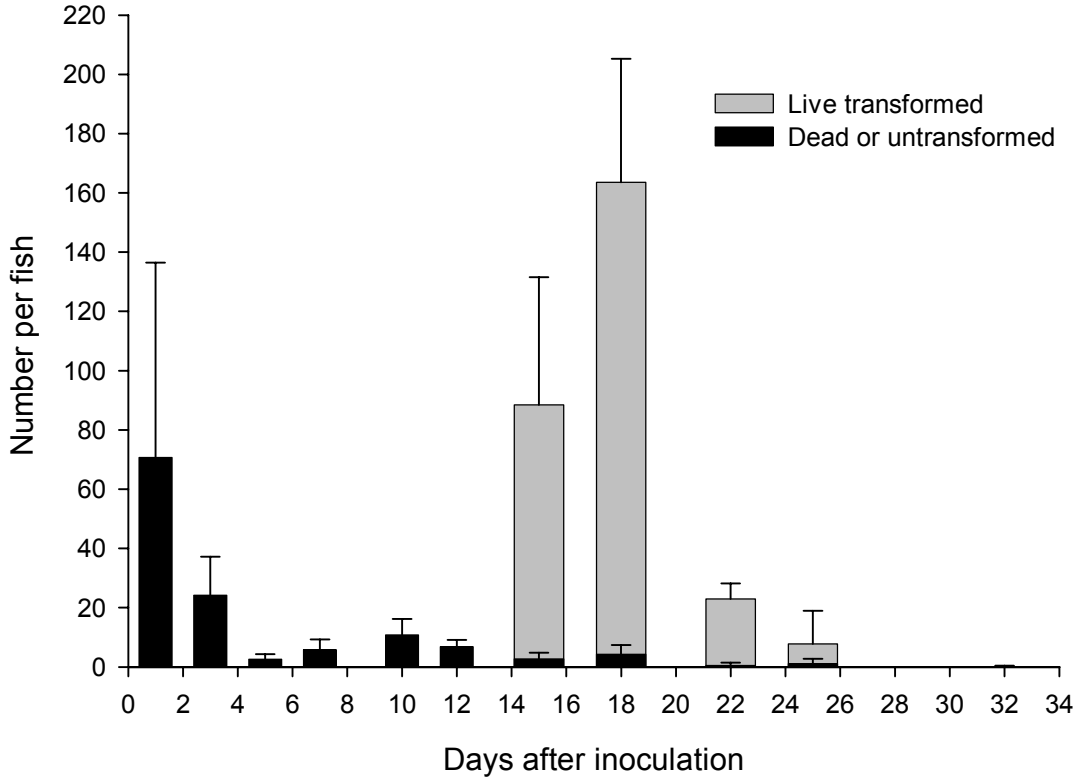

Figure 5. Timing of drop-off of Neosho muckets from bass at 21 C. These data are from 10 fish that were a subset of those used for propagation at Chesapeake on 8/15/02. Columns represent the mean number of individuals recovered per fish during the preceding interval. Error bars indicate 95% C.I. See Table 9 for totals.

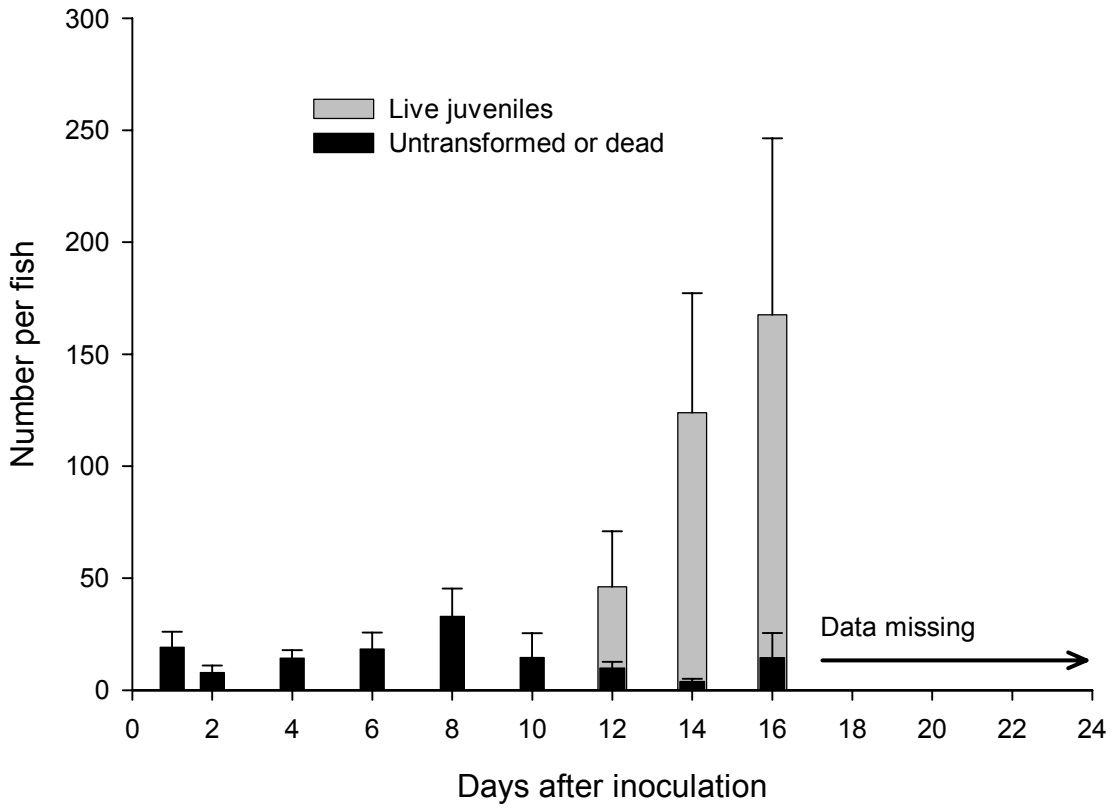

Figure 6. Timing of drop-off of muckets (*Actinonaias ligamentina*) from bass at 21 C. These data are from 10 fish inoculated at SMSU on 7/15/02. Columns represent the mean number of individuals recovered per fish during the preceding interval. Error bars indicate 95% C.I.

Figure 7. Young Neosho muckets recovered from release site on the Verdigris River in January-February 2002. We believe that these are mussels propagated at Chesapeake Hatchery and released on August 2 and August 8, 2000.

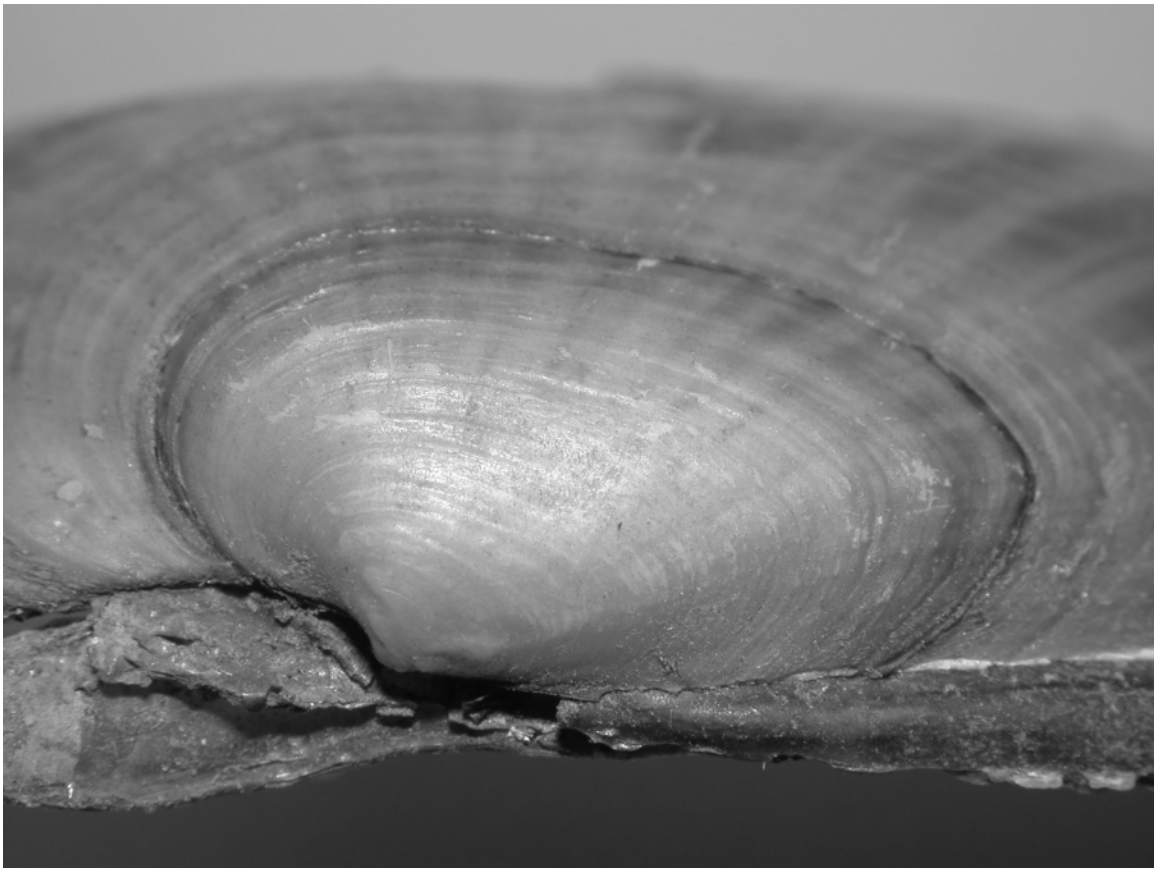

Figure 8. The right umbone of a Neosho mucket recovered from the Verdigris River release site, showing the first growth line. This line presumably marks the size of the mussel when growth ceased during the winter of 2000-2001, following release on August 2-8, 2000. Length of the shell at this line is 13 mm.

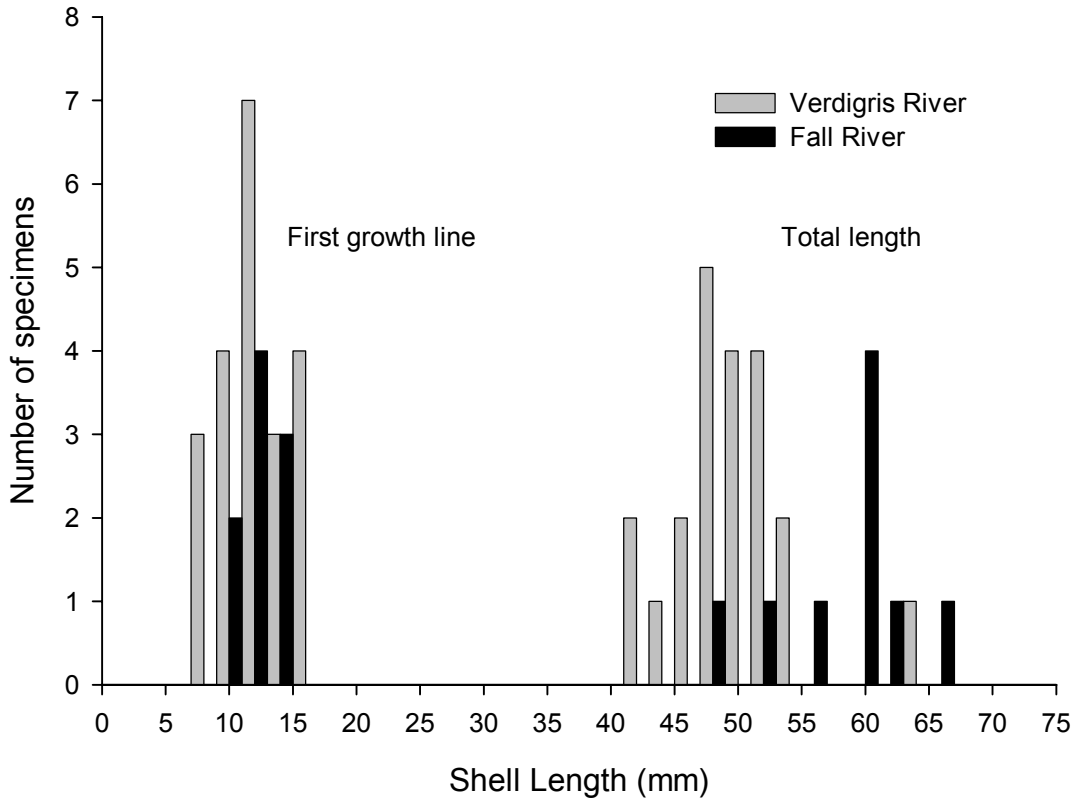

Figure 9. Measurements of Neosho mucket shells recovered from release sites on the Verdigris (grey bars, n=21) and the Fall River (black bars, n=9). Two measurements are presented for each shell. The distribution on the left is the length of the first growth line, indicating size at the end of the first growing season. Distribution on the right is total shell length, at the end of the second growing season. Shells were collected fresh-dead in January 2002. The Fall River shells are significantly larger (T-test, $P<0.001$) but appear to be of the same age cohort.

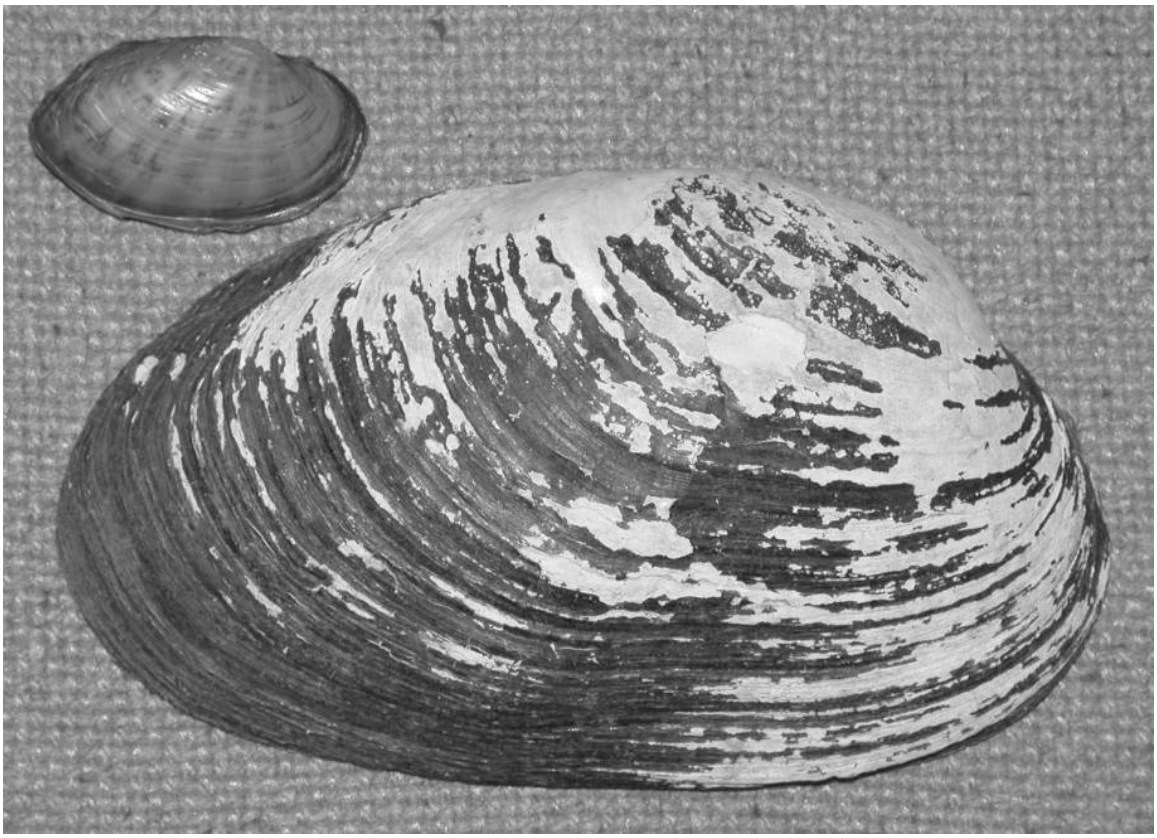

Figure 10. Contrast between ~18-month old Neosho mucket juvenile and aged adult shell from the Verdigris River. The young shell is 63 mm long.