11-15-2003

Culture and Restoration of Mussel Species of Concern: Final Report

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Final Report

Culture and Restoration of Special Concern Mussel Species
Section 6 Grant E-1-35, Amendment No. 1
Year Three of Three

CULTURE AND RESTORATION OF MUSSEL SPECIES OF CONCERN

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Department of Interior
United States Fish and Wildlife Service

November 15, 2003

SUMMARY

This report describes the third and final year of a 3-year project (E-1-35) to propagate and augment populations of mussel species of concern, including the Neosho mucket (*Lampsilis rafinesqueana*, the pink mucket (*Lampsilis abrupta*), and the scaleshell (*Leptodea leptodon*). Work with these species is continuing under a new Section 6 grant (E-1-43, Propagation and Restoration of Special Concern Mussel Species).

A new large-scale recirculating system for mussel propagation was developed and tested. This system improves the efficiency of propagation, reduces the labor involved, and will aid mussel propagation efforts at hatcheries. Renewed Section 6 funding will permit the installation of similar equipment at Lost Valley Hatchery in 2004.

Releases of propagated juveniles in 2003 included 374,250 Neosho muckets at 5 sites in the Spring and Verdigris rivers, 122,000 pink muckets at 4 sites in the Meramec River, and 13,864 scaleshell at 3 sites in the Gasconade River. These numbers exceeded those of each species released in any previous year. Quantitative tests were performed on each species to determine transformation success and timing. As in previous years, the propagation of pink mucket and scaleshell in 2003 was limited mainly by the low number of brooding females that were available. An expanded field effort in the late summer and fall of 2003 resulted in locating 15 adult female pink muckets and 5 adult female scaleshell in the Meramec River. These individuals were sequestered at selected sites and marked to facilitate relocation for propagation next year.

Kansas Wildlife and Parks carried out quadrat sampling of a site in the Verdigris River where 11,600 juvenile Neosho muckets were released in year 2000. The results indicated a surviving population of approximately 75 individuals per 1000 m^2. These individuals are now approximately 80 mm long and will probably be sexually mature next summer.

Evidence was found that otters preyed heavily on adult Neosho muckets at a site in the Spring River. The form of the predation was unusual and has not been reported previously. The otters pull up the adult mussels and bite the extended foot before the mussel can retract into the shell. Neosho muckets appear to be particularly vulnerable to this form of predation.

Information on mussel propagation was disseminated through publications, Internet websites, public programs, and professional presentations at local and national meetings.

ACKNOWLEDGEMENTS

I am indebted to Andy Roberts, Sue Bruenderman, and Scott Faiman, who played essential roles in planning and carrying out this project. Scott, Andy, and Sue all spent many hours in the field locating rare mussels. Andy Cornforth and Dennis Whelan at Chesapeake Hatchery provided fish, tank space, and helped with the care of brood stock. Dave Waller and his staff at Lost Valley Hatchery developed methods and provided facilities, fish and logistic support. SMSU students and staff including Christian Hutson, Bob Brown, Nathan Eckert, Ben Dodd, and Todd Fobian made numerous vital contributions in the field and in the lab. Bob Holmes and Bill Goodman provided expert technical assistance. Dr. Conrad Kleinholtz at Langston University and Doug Aloisi at Genoa National Hatchery provided freshwater drum for propagation of scaleshell. Dave Hendrix and his colleagues at Neosho Hatchery helped with transport of fish. Ed Miller and Brian Obermeyer carried out Neosho mucket fieldwork in Kansas, provided information and advice regarding collection and release sites, and conducted releases of juveniles. 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 Southwest Missouri State University.

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INTRODUCTION AND OBJECTIVES

Native freshwater mussels (Superfamily Unionoida, families Unionidae and Margaritiferidae) are the most imperiled freshwater fauna in North America. According to current USFWS listings, 70 of 297 North American species are federally categorized as threatened or endangered. Over 30 species have apparently become extinct during this century, and this trend is continuing. For example, the Ozark-endemic Curtis Pearly mussel has apparently become extinct within the last decade, despite efforts by the Missouri Department of Conservation to protect its last known habitat in the Little Black River. Some workers believe that over half of the nearly 300 North American species are in danger of extinction (Williams et al. 1993, Stein et al. 2000).

The Missouri Department of Conservation has been involved in efforts to document and conserve mussel populations since the early 1980's (Buchanan 1980, 1982). Several rivers in Missouri are strongholds of mussel diversity, including the Meramec, Gasconade, Black, St. Francis, Osage, and Spring Rivers (Roberts and Bruenderman 2000, Bruenderman et al. 2001, Barnhart and Hutson 2002, Barnhart and Hutson 2003, ESI 2003, Obermeyer et al. 1997). These rivers are believed to hold the best remaining populations of several federally listed and candidate mussel species, including the scaleshell, pink mucket, Neosho mucket, spectaclecase, and sheepnose (Szymanski 1998, Roberts 2003, Obermeyer et al. 1997, Butler 2002a,b).

Population augmentation and reintroduction of mussels to native habitats are recovery objectives for nearly all endangered mussel species (NNMCC 1998). The present project is a program of propagation, population augmentation, and related research objectives for three species of concern: the federally endangered scaleshell and pink mucket, and federal candidate Neosho mucket (Barnhart 2001, 2002). The work is continuing under a new Section-6 funded project (E-1-43, *Propagation and Restoration of Special Concern Mussel Species*, September 2003-August 2006).

RECIRCULATING PROPAGATION SYSTEM (RPS)

One of the major accomplishments of this project is the development of a recirculating propagation system (RPS) for recovering juvenile mussels from large numbers of host fish. This system was designed and constructed at SMSU in 2002-2003 and was first used for propagation in summer 2003. The need for a large-scale recirculating system arose from three factors; 1) need to use large numbers of host fish, 2) problems stemming from zooplankton in hatchery water supplies, and 3) inefficiency of recovering juveniles by vacuuming tanks.

Each of the three target species can produce 1 million or more larvae per female. At least several hundred host fish (first-year drum or bass, 3-5 inches length) can be used to propagate the glochidia from a single female mussel. The fish can be held in flow-through raceways during the period of encystment but must be kept in low-flow or recirculating tanks during the drop-off period to avoid losing the juveniles, which are very small and prone to

drift. In the past we used aerated tanks with limited flow-through and vacuumed the bottom of the tanks through filters to recover juveniles. Problems arose with zooplankton in the water and with the vacuuming process.

Hatchery water supplies typically carry a wide variety of zooplankton in the same size range as mussel glochidia and juveniles. Some zooplankton, including rhabdocoel flatworms and hydra, are predators on the juvenile mussels (Delp 2002, Barnhart 2002). Other species in the same size range as glochidia and juveniles are recovered by filtration in large numbers along with the mussels and are very difficult to separate. These include various cladocerans, ostracods, the shelled amoeba *Arcella*, and bryozoan statoblasts. These zooplankton collected along with the juvenile mussels interfere with counting, deplete dissolved oxygen, degrade the water quality, and generally reduce survival of the juveniles. Efforts to remove the zooplankton by pre-filtering hatchery water supplies were unsatisfactory. Vacuuming the tanks to recover juveniles was likewise labor-intensive and apparently missed a large proportion of the juveniles (Barnhart 2002).

The RPS was designed to hold fish in groups of several hundred and to recover glochidia and juveniles continuously from a recirculating flow of water. Fish can be held in the RPS during the entire encystment period, or they can be moved to the RPS shortly before drop-off occurs. This system eliminates most problems with zooplankton because these organisms do not enter the system. Vacuuming is also eliminated, because recirculation of water is used to recover the juveniles by moving them efficiently to a filtration system. The juveniles can be removed from the filters easily to facilitate counts and expedite their return to the river.

The basic RPS unit (*Figure 1*) consists of 1) two conical-bottom 250-gallon tanks to contain the fish 2) a sump containing 3) a biological filter to maintain water quality 4) recovery filters to recover juveniles from each tank and 5) a pump and associated plumbing to recirculate the water. Two 2-tank units and one 1-tank unit are currently in use at SMSU.

Tanks & stands

The conical bottom tanks and stands (*Figure 2*) are made of high density polyethylene (Polytank Corporation, Litchfield, MN, #CB-6028 and #CBS-6028). The tank bottom has a 20^{o} slope to a central opening. It is designed to be self-cleaning when equipped with a double standpipe that draws water from the bottom. A venturi (Polytank #VD-240) is attached beneath the central opening has sockets for the outer standpipe, inner standpipe, and drain plumbing. The outer standpipe is 3-inch PVC and has slots cut in the lower end to allow water to enter. The inner standpipe is 2-inch PVC that fits into a bushing within the venturi and is continuous with the drain plumbing. Water enters the outer standpipe at its base, rises in the space between the outer and inner standpipes, and then falls through the inner standpipe to the drain (*Figure 2*).

The venturi and drain kit currently manufactured by Polytank cannot be used with the CBS-6028 stand as designed, because the drain attachment extends too far below the bottom of the tank to clear the ground. The manufacturer was notified of this problem but did not indicate whether it would be corrected in the near future. It was necessary to mill out the venturi and

cut down a pair of 3x2-inch PVC bushings to fit inside of it and accept the inner standpipe and the drain plumbing (*Figure 3a*). The bushings were sealed into the venturi with silicon adhesive and the lower one was secured with set screws. These modifications reduced the length of the venturi assembly by approximately 4 inches so that a "street el" fitting cleared the ground by approximately 1 inch. The completed venturi assembly was welded to the outside of the tank, rather than bolted as suggested by the manufacturer, in order to avoid possible leaks (*Figure 3b*).

Sump

The sump is a 22" by 36" polyethylene drum with cover (similar to Chemtainer Industries #TC2236). The sump contains the biological filter and recovery filters. A bulkhead fitting at the base connects to the pump inlet on the outside and the pump intake manifold on the inside. Openings are cut near the top for the return risers and the tank bypass (*Figures 4, 5*).

Recirculating pump and plumbing

The recirculating pump is a magnetic drive unit rated at 20 GPM at 4 feet of head (Iwaki WMD-40RLXT). Water leaves the pump vertically through a 1-inch delivery. A variable portion of the total flow is recirculated to the sump through the tank bypass. The tank bypass is used to balance water level in the sump and provide flow through the upper part of the biological filter. Above the bypass the main delivery includes a check valve to prevent possible backflow and siphoning of the tanks. Above the check valve the main delivery is divided into two tank deliveries. Ball valves on the bypass and the tank deliveries allow adjustment of flow. Typical rate of water flow through each tank of a two-tank unit is approximately 8 GPM, with 4 GPM through the tank bypass.

Water entering the tank is distributed through a manifold that is angled to produce a slow rotation of the water in the tank. Water leaves the tank in a 2-inch return at floor level and a riser delivers the water to the recovery filter inside the sump. The return includes a drain valve at floor level for emptying the tank, and the riser has a return valve for interrupting flow to the sump when the recirculating pump is turned off.

Biological filter

The biological filter is a hollow cylinder (*Figure 6*). The lower portion is a rolled rectangular sheet of open-cell foam, which measures 48 inches by 24 inches by 2.5 inches when unrolled (Aquatic Ecosystems PF7 filter foam). This portion of the filter has an effective surface area of 300 square feet. The lower end of the foam roll is capped with the cut-off bottom of a 10-inch diameter plastic bucket, which is anchored to the foam roll with stainless steel wire hooks. The upper end of the foam roll is inserted into the remainder of the plastic bucket, which forms a collar that extends approximately 10 inches above the roll. A bag of filter carbon is placed within the collar, between discs of filter foam.

The biological filter stands on end in the sump with the water level at or above the lower edge of the plastic collar. The water pump inlet is plumbed through the side of the foam cylinder near its base, in order to draw water from within the cylinder and cause water from

the sump to flow through the foam. The bypass flow from the pump is sprayed onto the top of the cylinder to pass over the filter carbon.

When conditioned, this filter system is adequate to keep NH_3 and nitrite at negligible levels with tank occupancy of 300-500 4-inch bass or drum. The fish are fed a minimal diet while in the RPS to avoid accumulation of waste in the recovery filters.

Recovery filters

The filters for recovering juveniles are similar to plankton nets (*Figure 7*). Each filter consists of a cylindrical Nitex bag and a "cod end" made of two 1-L plastic bottles nested together. The upper end of the net bag is attached to a PVC ring with a wire "bucket handle" that hangs from the riser. The lower end of the bag is sewn to the cod end bottle. The cod end of the filter creates a dead space into which the glochidia and juveniles collect, and in which they are protected from turbulence in the net. Juveniles are removed at daily intervals by lifting the filter out of the sump, hanging the cod end over a container, and opening the cap of the outer bottle to drain it.

Efficiency of the system

The RPS was completed in May and used for each of the rounds of propagation described below, as well as for production of fat pocketbook mussels in a separate project. The results from groups of fish in the RPS were compared with subsets of host fish monitored in the AHAB system (see below). The results from the RPS were generally closely similar to those in the AHAB, indicating that the system recovers essentially all of the glochidia and juveniles.

PROPAGATION SUMMARY

Propagation refers to the collecting of wild glochidia, placing them on suitable host fish, recovering them after transformation, and releasing them back into the field. A "round" of propagation refers to a group of glochidia obtained from one or more female mussels of a species, placed on a group of host fish on the start date, and then recovered after transformation and excystment. Seven rounds of propagation for release and population augmentation of threatened species were carried out in 2003 (*Table 1*). In total we released 612,204 juveniles of federally listed or candidate species in 2003 (*Table 2*). These included Neosho muckets, pink muckets, scaleshell and fat pocketbook. The fat pocketbook juveniles (*Potamilus capax)* were produced under separate contract with the Memphis District USACE. Details of that round of propagation are not included in this report.

The glochidia propagated this year were derived from 20 individual mussels of 4 species. The small number of females available for propagation continues to be a source of concern. Scaleshell, in particular, has proven extremely difficult to locate for propagation, even though the populations sampled are considered to be the best that remain in existence. Although the process of "head starting" larval mussels by propagation is not captive

breeding, propagation has the potential to alter the genetic makeup of wild populations. Therefore, it is important that a large proportion of the local gene pool be represented.

A subset of fish from each inoculated group was monitored in a modified AHAB system (Aquatic Habitats, Inc) to determine the timing of transformation and transformation success of glochidia on individual fish. The modified AHAB system was developed at SMSU in 2001 and was described previously (Barnhart 2002). It uses small unit tanks (1-3 L) equipped with individual filters in a recirculating system to capture the glochidia and juveniles shed by each fish and allow their quantification. The AHAB results allow examination of individual variability in suitability of host fish. The AHAB results were also compared with the RPS results to check the performance of the RPS system.

Juvenile mussels were released at the site of their origin and at other sites deemed suitable by MDC and USFWS. These sites were chosen based on the presence of suitable habitat and strong populations of other mussel species. In each case, the release sites were within the known, current range of the species involved, and no mussels were moved outside of the river system in which they were collected. Therefore, these releases were augmentations, not reintroductions. Juveniles were also supplied to the USGS Columbia Environmental Research Center (CERC) for toxicology testing. Female mussels were returned to the site of origin or in some cases sequestered at other, more accessible sites to facilitate future propagation. The females were photographed and genetic samples (mantle tissue clips) were retained in ethanol for future identification using microsatellite markers or other genetic studies.

SCALESHELL PROPAGATION - 6/2/03

Fish Hosts

Drum were obtained from Dr. Conrad Kleinholtz at Langston University. The fish were pond-reared from Missouri River stock and were approximately 1 year old. Drum were delivered to SMSU and Lost Valley by Neosho Hatchery personnel on May 21, 2003. Approximately 72 pounds (32.7 kg) were brought to SMSU. Mean size of a subsample of 50 fish was 17.5 ± SD 9.7 g and 88.6 ± SD 16.7 mm standard length. Based on these measures, the estimated number delivered was 1,869. The remainder (approximately 1,500 fish) were taken to Lost Valley. On arrival the fish were in poor condition, apparently due to trauma and a period of hypoxia during loading. Mortality at SMSU was 5-12%/day for 6 days, during which time 863 fish died (~46%). Fish were treated continuously with 0.5-1% salt and beginning on day 5 with Kanamycin antibiotic. The surviving fish were healthy and feeding well on live blackworms by day 8. Lost Valley also experienced substantial losses of these fish after delivery but had less trouble with bacterial infection (Dave Waller, personal communication). According to Dr. Kleinholtz, these fish are delicate but can be transported without unusual losses if they are kept normoxic and treated with salt during transport. Based on our experience, prophylactic treatment with antibiotic might also be advisable.

A second small group of drum was obtained from Genoa National Hatchery in early May. These fish originated at Gavins Point National Hatchery where they had "volunteered" in ponds used for paddlefish propagation. The fish were held at Genoa for several months before we obtained them (Herb Bollig and Doug Aloisi, USFWS, pers. comm.). These fish were held separately from the Langston fish. After delivery they showed Ich infection and were treated with RidIch®.

Female and glochidia

A single brooding female was obtained by Scott Faiman, Andy Roberts, and Christian Hutson at Fishtrap Rapids on the Meramec River, on September 12, 2002. The mussel was transported to Chesapeake Hatchery and placed in a container of substrate in an unheated indoor raceway, where it was kept over the winter and spring. The animal was checked periodically through the winter. It was siphoning each time observed and remained in apparently good condition. This scaleshell was nearly 70 mm in length which is unusually large for females of this species (*Figure 8*). Estimated age based on growth lines was 5 years.

Glochidia were removed for propagation by flushing the gills with water from a syringe. A small number were removed on June 2 and used for a trial inoculation of 9 drum from Gavins Point at SMSU. Most of the brood was removed on June 3 and used for inoculating 132 Langston drum at Lost Valley Hatchery. In total, over 1.5 million eggs and glochidia were removed (*Table 3*). Inspection showed that about 1/4 of the total brood remained in the gills, so that estimated total fecundity was approximately 2 million. The female was then transported to CERC where the remaining glochidia were removed and used for toxicity testing (Chris Ingersoll and Ning Wang, pers. comm.) The female was apparently in good health and active for several weeks afterward, but died after transfer to an outdoor raceway (Andy Roberts, personal communication).

Only about 29% of the brood of this female had developed into glochidia. The other 71% were undeveloped eggs (*Table 3*). Such low fertilization is atypical but not unprecedented in rare species of mussels. It appears likely that fertilization is sometimes limited by lack of males upstream.

The glochidia appeared to be in good condition- only 3% were dead (*Table 3*). However, a relatively high proportion of glochidia were closed after being removed from the marsupium. This amounted to about 20% of the glochidia collected on June 2 and 40% of the glochidia used at Lost Valley (*Table 3*). The differing number that closed might reflect difference in the water to which the glochidia were exposed. Synthetic fresh water used at SMSU and raceway water at Lost Valley. Closed glochidia are presumed to be unable to attach to the host fish. Only the number of glochidia that were initially alive and open was used in calculating attachment success.

Inoculation and attachment success

Nine Gavins Point fish were inoculated with scaleshell at SMSU on 6/2/03 and were monitored in the AHAB system. This group of fish was intended as a trial run and it was used for comparison of transformation success with the Langston fish. A group of 132 Langston fish was inoculated on June 3. Because of concern about the effects of salt and antibiotic treatment of the fish at SMSU, we decided to use the fish that had been kept at Lost Valley. Ten of these fish were brought to SMSU and monitored continuously in the AHAB system to determine transformation success. The other 122 fish were brought to SMSU on day 6 and placed in the RPS. Juveniles were recovered from the RPS beginning on day 16 and ending on day 25.

In both inoculations, the fish were swum for 30 minutes in an aerated suspension of glochidia. The infective (open) glochidia concentration used was about 2 times higher with the Gavins Point fish; the bath volume per fish was 40% less, and the number of glochidia per fish 50% higher. It appears that more than double the proportion of the glochidia attached to the Gavins Point fish at SMSU (25-30%: *Table 4A-9 and 4B-3*) than to the Langston fish inoculated at Lost Valley (11%, *Table 5B-3 and 5C-3*).

The low rate of attachment of scaleshell is a problem that should be investigated. Attachment success with the *Lampsilis* species is typically higher (25-70% see below). Glochidia tend to close over time in inoculation baths, perhaps because they respond to chemical cues from the fish in the water, or perhaps because of turbulence due to aeration or fish movement in the inoculation bath. This closing response reduces attachment success. It is possible that scaleshell glochidia are particularly sensitive in this regard. Experiments are needed to determine what factors limit attachment success and to determine optimal concentrations of glochidia, bath volume per fish, and other variables relating to inoculation.

Transformation success:

The transformation success of attached glochidia was similar between the two groups of fish. Estimates of transformation success ranged from 69% for the Gavins Point fish and 61-70% for the Langston fish (*Table 4B-5 and Table 5B-5, 5C-6,*). The similarity between the two groups is interesting because the number of glochidia attached per fish was nearly 4 times higher on the Gavins Point fish (692) than on the Langston fish (179). It appears that the higher rate of infection did not lower transformation success.

Comparison of RPS and AHAB recovery:

The recovery success in the RPS was high and similar to that in the AHAB system. The estimated number of sluffs and juveniles recovered per fish was somewhat higher in the RPS (206: *Table 5C-2*) than in the AHAB (179 ± 69: *Table 5B-2*), but not significantly different. This result indicates that the RPS is efficient in recovering glochidia and juveniles as they leave the fish.

Transformation timing:

The peak of juvenile drop-off in the AHAB at 20.1 °C was at 15 days post-inoculation (*Figure 9*). The peak of juvenile recovery from the RPS was a day later at 16 days (*Figure 10*). The RPS fish were kept at Lost Valley for the preceding 13 days, and the slower transformation was probably due to lower temperature in the raceway at Lost Valley. Temperature records from that period are not available.

Sluffs:

An interesting pattern is evident in the drop-off of incompletely transformed glochidia (sluffs) from scaleshell. The number of sluffs increased markedly in the last few days preceding the appearance of juveniles (*Figure 9, 10*). Many of these late sluffs had a well-developed foot, but were apparently unable to osmoregulate, because they became edematous either before or shortly after death ("bloaters") (*Figure 11*). Twelve bloaters and 12 normal individuals that excysted on day 17 were isolated individually in a cell well plate. In 24 hours all of the bloaters were dead, while only 1 of the normal individuals had died. A similar pattern of numerous late sluffs that become edematous occurs in fat pocketbooks transforming on drum, but not in *Lampsilis* (personal observations). In *Lampsilis*, the largest number of sluffs occurs in the first 1-3 days following inoculation and late sluffs are rare (see below).

Releases of scaleshell:

All releases were made within 1-4 days of juvenile drop-off. In total, 13,864 live juvenile scaleshell were released at 3 sites, one in the Bourbeuse River and two in the Meramec River (Table 2). Another 1,500 juveniles were given to CERC for toxicity testing. The other recovered juveniles were casualties. Mortality was relatively high during the first few days after drop-off.

Recapture of propagated scaleshell:

During the search for brood stock this fall, a single immature scaleshell was found by Nathan Eckert in the Gasconade above Wrinkle Springs Access of USFS. This mussel was about 25 mm long. We released several thousand juvenile scaleshell at this site on June 4 last summer. No other juvenile scaleshell have been found at this site (or any other site) in previous years. The size and apparent age of this individual is consistent with a recapture from last year's release.

NEOSHO MUCKET PROPAGATION 7-3-03

Fish Hosts

The fish hosts were largemouth bass from Chesapeake Hatchery. Mass was approximately 5 grams and standard length 64 mm (*Table 6B-1,2*). A total of 976 fish were inoculated at Chesapeake on 7/3/03. Twelve fish were brought to SMSU immediately for monitoring in

the AHAB system. The rest of the fish were left at Chesapeake for one week. On 7/10/03 the main group of fish was delivered to SMSU and placed in the RPS. After propagation was complete, these bass were returned to Chesapeake on 7/23/03.

Female mussels and glochidia

Six females were collected from the Spring River at Highway 96 in Cherokee Co. KS on 6-27-03. The mussels were brought to SMSU and held at 22 C. Glochidia were collected on 7-3-03. Four of the mussels were brooding mature glochidia. One mussel (K-3) held only embryonated eggs and another (K-2) was not brooding. The observation of 4 females with mature glochidia and 1 with embryos shows that reproduction is not always synchronous in this species. Most female Neosho muckets spawn in the spring and release in the summer (Shiver 2002) but a small fraction of Spring River females can be found with brood in the winter months (see below).

After removing glochidia for propagation, the females were held separately until they had expelled all remaining glochidia from the gills. These were collected and counted to complete the measurement of fecundity. The mussels were marked by engraving ID numbers on the left valve. They were returned to the site of collection on July 17.

A total of 4,250,000 glochidia were collected on 7-3-03 from the 4 females by flushing the gills. These glochidia were in excellent condition (*Table 6*). Over the next week the mussels voided more glochidia which were also quantified in order to measure total fecundity. The average fecundity of these 4 females was 1.27 million glochidia per female (*Table 7*).

Inoculation and attachment success:

The inoculation baths were prepared within fiberglass raceways at Chesapeake. Dennis Whelan constructed a pair of partitions that were used to isolate a raceway segment for inoculating the fish. The partitions were wooden frames covered with plastic sheet. The fish were first herded into a segment of the raceway with screens. The flow was then turned off, and the partitions placed inside the screens. After inoculation the partitions and screens were removed to free the fish.

Inoculating fish directly in the raceway, rather than moving them to a separate tank is advantageous in several ways. Volume can be adjusted to match the number of fish and glochidia available simply by moving the partitions. Handling stress on the fish is reduced. The fish can be kept in the raceway and fed normally during most of the encystment period (usually 1-2 weeks depending on temperature) and later moved to the RPS for recovery of juveniles. Raceway temperature can be controlled by switching the raceway between spring water or solar pond water to control temperature and the speed of transformation. The ability to delay excystment can be advantageous if, for example, high water in the river delays the release of juveniles.

Only half of the available glochidia were used to inoculate fish, because there were not enough host fish available at the time to support all of them. A total of 976 fish were

inoculated in two groups. The volume of the raceway segments was estimated geometrically at 294 L each. Approximately 1 million glochidia and 490 fish were added to each compartment (*Table 8*). The inoculation baths were aerated vigorously to keep the glochidia in suspension. Exposure time was 17 minutes.

This inoculation used a moderate concentration of glochidia (3,414/L). Attachment success was estimated for both the AHAB and RPS groups and these estimates were consistent at 24% (*Table 8B-4, 8C-3*). Estimates of the number attached were also consistent at about 500 per fish (*Table 8B-3, 8C-2*).

Transformation success

Transformation success of attached glochidia was high, as is typical for this mussel and fish species combination. Transformation success in the AHAB (91% ± 2.1, *Table 8B-6*) was somewhat higher than estimated in the RPS (83%, *Table 8C-6*). A total of 402,900 juveniles was recovered from the RPS, or 418 per fish (*Table 8C-4, 5*).

Comparison of RPS and AHAB recovery:

The recovery success in the RPS was similar to that in the AHAB system. The total number of sluffs and juveniles recovered per fish 507 ± 95% CI 129 from the fish in the AHAB (*Table 8B-3*), compared to 503 per fish in the RPS (*Table 8C-2*). This result indicates that the RPS was efficient in recovering glochidia and juveniles as they were shed by the fish.

Transformation timing

The peak of drop-off of juveniles occurred at 16 days post-inoculation in the AHAB, where the mean temperature was 20.5 ± SD 0.7 °C (*Figure 13*). Peak of juvenile recovery from the RPS was 2 days earlier at day 14 (*Figure 14*). The difference reflects higher water temperatures at Chesapeake during the first 8 days of encystment and subsequently in the RPS (23.5 °C).

Releases

A total of 302,250 juveniles from this round of propagation were released at 4 sites in the Spring River between 7/17/03 and 7/20/03 (Table 2).

PINK MUCKET PROPAGATION 7-3-03

Fish Hosts

The fish hosts were largemouth bass from Chesapeake Hatchery. Mass was approximately 4.2 grams and standard length 60 mm (*Table 10B-1, 2*). A total of 349 fish were inoculated at Chesapeake on 7/3/03. Twelve fish were brought to SMSU immediately for monitoring in

the AHAB system. The rest of the fish were left at Chesapeake for one week. On 7/10/03 the main group of fish was delivered to SMSU and placed in the RPS. After propagation was complete, these bass were returned to Chesapeake on 7/23/03.

Female mussel and glochidia

The glochidia for propagation were obtained from a single female collected by Andy Roberts on 6/24/03 in the Meramec River at Opechee Beach. This female was marked "LA". The gills were not fully charged, perhaps because of glochidia released before collection. No unfertilized eggs were noted in this individual (*Table 9*). Fertilization success was also high in a second female collected at this site later in the summer. Therefore, it appears that the Opechee Beach site has a sufficient population of male pink muckets upstream to fertilize females relocated to the site.

A total of 385,000 glochidia were collected. Glochidia condition was fair, with about 5% dead and 5% prematurely closed (*Table 9*). The gills were not fully charged, and not all glochidia were removed. Therefore the number of glochidia collected is not an estimate of fecundity.

Inoculation and attachment success:

The inoculation was carried out in two 35-L picnic coolers with ~175 fish in each. These were aerated vigorously to suspend the glochidia. The bath concentration was 5,185 glochidia per liter (*Table 10A*). Exposure time was 17 minutes. Attachment success was estimated using data from both the AHAB and RPS groups and these estimates were fairly similar at 39% and 33% respectively (*Table 10B-4, 10C-3*). Estimates of the number attached were also consistent at 404 ± 105 (95% CI) from the AHAB fish and a total of 317 per fish from the RPS (*Table 10B-3, 10C-2*).

Transformation success:

Estimates of transformation success of attached glochidia from the AHAB fish and the RPS group were similar at 86% and 86.9% respectively (*Table 10B-6 and 10C-6*).

Comparison of RPS and AHAB recovery:

The recovery success in the RPS was similar to that in the AHAB system. The total number of sluffs and juveniles recovered per fish 404 ± 105 95% CI from the fish in the AHAB (*Table 10B-3*), compared to 317 per fish in the RPS (*Table 10C-2*).

Transformation timing

The peak of drop-off of juveniles occurred at 14 days post-inoculation in the AHAB, where the mean temperature was 20.5 ± SD 0.7 °C (*Figure 15*). Peak of juvenile recovery from the RPS was 3 days earlier at day 11 (*Figure 16*). The faster transformation in the RPS is

consistent with higher water temperatures at Chesapeake during the first 8 days of encystment and subsequently in the RPS (23.5 °C).

Releases of pink muckets:

84,000 juveniles from this female were released at 3 sites in the Meramec River on 7-17-03 (*Table 2*). The gage reading at Eureka was 2.45 ft, 1,300 cfs. Several thousand juveniles were also sent to CERC for toxicity testing.

NEOSHO MUCKET PROPAGATION 7-23-03

Fish Hosts

The fish hosts were largemouth bass from Chesapeake Hatchery. Mass of the fish was approximately 4.9 grams and standard length 64 mm (*Table 12B-1,2*). A total of 401 fish were inoculated at SMSU on 7/23/03. Six fish were monitored in the AHAB system. After propagation was complete, the bass were returned to Chesapeake.

Female mussel and glochidia

The glochidia were obtained from a single Verdigris river female, ID# D-3. This female was collected from the Verdigris River at the KDWP refuge site on February 11, 2003 and was found to be brooding glochidia at that time. Most Neosho muckets spawn in the late spring and release the brood in the summer (Shiver 2002) but occasionally a winter brooding female can be found. Adult Verdigris Neosho muckets are very rare so we elected to propagate this female. The mussel was stored at 7 °C for over 6 months before propagation. A large proportion of the brood was unfertilized eggs but the glochidia were in good condition. The female mussel was returned to the Verdigris in early August.

Only about 40% of the brood was fertile. The low fertilization success is consistent with the fact that adult Neosho muckets are very rare at the site where this individual was collected in the Verdigris River. Over 93% of the glochidia were live and over 83% of these were open (Table 11). Thus, the brood seemed to be in good condition despite the fact that this animal had been stored at low temperature for over 6 months. This result shows that Neosho muckets are capable of long-term brooding, although most individuals are tachytictic summer brooders (Shiver 2002).

Inoculation and attachment success

This inoculation and that of pink muckets on the same date (below) were carried out in the RPS tanks. The fish were already in the RPS tank. The water volume was reduced to 132 liters (to the top of the tapered portion of the tank), the glochidia added, and the water was aerated and stirred manually for 15 minutes. Thereafter, the tank was refilled to its total

volume of 917 L and the circulation restored to remove leftover glochidia. Six of the 401 fish were moved to the AHAB for monitoring.

Although it seemed like a good idea at the time, inoculating the fish in the RPS tanks proved to be inefficient. Percent attachment success was low (*Table 12B-4*). It was difficult to keep the inoculation bath stirred, and many of the glochidia might have settled into the central depression around the standpipes. Another disadvantage was that the unattached glochidia were in the RPS, so that it was impossible to distinguish the unattached glochidia from sluffs. Therefore, it was not possible to calculate attachment success or transformation success. The number of glochidia attached per fish was also low (98, *Table 12B-3*), probably because of low attachment success as discussed above but also because the number of glochidia used per fish was low (581 per fish, *Table 12A-6*).

Transformation success:

Transformation success of glochidia on the monitored fish was good at about 80% (*Table 12B-6*). This result supports the conclusion that the 6-month cold storage of the female had not compromised the glochidia.

Comparison of RPS and AHAB recovery:

Somewhat more juveniles per fish were recovered from the RPS (105, *Table 12C-5*) than from the AHAB fish (77 ± 20; *Table 12B-5*). The difference is probably not significant given the small number of fish (6) that were monitored in the AHAB.

Transformation timing

The peak of drop-off of juveniles from the AHAB fish was at 16 days at 20.4 °C (*Figures 17*). Peak of drop-off in the RPS was also at 16 days post-inoculation although the temperature was significantly warmer at 24.2 ± 0.6 °C (*Figure 18*).

Releases

A total of 27,000 juveniles from this round of propagation was released on August 13 at the same site the female was collected, on the Verdigris River in Montgomery Co KS (*Table 2*).

PINK MUCKET PROPAGATION 7-23-03

Fish Hosts:

The fish hosts were largemouth bass from Chesapeake Hatchery. A total of 444 fish were inoculated at SMSU on 7/23/03 (*Table 14A-2*). Standard length of the fish was approximately 61 mm and mass was 4.3 grams (*Table 14B-1, 2*). Six fish monitored in the AHAB system. After propagation was complete, the bass were returned to Chesapeake.

Female and glochidia

This pink mucket (#P1) was collected near Opechee Beach on the Meramec River on 7-17-03 by Chris Barnhart. Glochidia were removed twice, on 7/23 and again on 7/25. The brood was over 95% fertile. Over 95% of glochidia were living and about 96% of live glochidia were open.

Inoculation and attachment success:

The first inoculation was carried out on 7-23-03 in the RPS tank. As described above for the 7-23-03 Neosho mucket propagation, inoculating the fish in the RPS tanks was inefficient and the percent attachment success was low (18.5%; *Table 14B-4*). Because the unattached glochidia were in the RPS, it was not possible to calculate attachment success or transformation success from the RPS catch.

Transformation success:

Transformation success for these glochidia was 66% ± 11 (95% CI) on the AHAB fish (*Table 14B-6*). This result was significantly lower than that observed for glochidia from pink mucket LA (86% ± 4, *Table 10B-6*).

Comparison of RPS and AHAB recovery:

Recovery of juveniles from the AHAB was 59 ± 28.4 (95% CI) juveniles per fish (*Table 14A-5*). The number of juveniles recovered from the RPS (77 per fish) was similar but not really comparable, because two other small groups of inoculated fish were added to the RPS on 7-25 (see below; and *Table 14C*).

Transformation timing

The peak of drop-off of juveniles from the AHAB fish was at 14 days at 20.4 °C (*Figure 19*). The pattern of drop-off in the RPS was complicated by the addition of a second group of inoculated fish on 7-25. However, the first peak of drop-off, which presumably corresponded to the fish inoculated 7-23-03, was also at 14 days even though the temperature was significantly warmer at 24.2 ± 0.6 °C (*Figure 20*). This result of similar timing despite different temperature in the AHAB and RPS was also observed in the Neosho muckets inoculated 7-23-03 (Figures 17, 18).

Releases of pink muckets

A total of 37,500 juveniles from this round of propagation were released at two site s in the Meramec River on 8/11/03 (*Table 2*). Other juveniles were sent to CERC for toxicity testing.

Effect of glochidia concentration and bath volume on pink mucket attachment

Host fish are routinely inoculated with glochidia by placing them in an inoculation bath. The bath is a suspension of glochidia that is aerated or otherwise stirred to keep the glochidia suspended. As the fish ventilate, suspended glochidia encounter the gill filaments and attach. The concentration of glochidia in the bath declines as the glochidia attach to the fish. However, some glochidia will not attach because they close prematurely, presumably in response to chemical cues from the fish or the agitation of the bath. This response increases over time and may be exacerbated if the fish are over crowded.

When the number of glochidia available is limited, the goal is to optimize attachment success (the proportion of glochidia that attach to the host fish). When the number of host fish available is limited, relative to the glochidia, the goal is to optimize number attached per host fish. The number of attached glochidia that can be tolerated by the host fish is surprising large, but there are limits. For the species that we work with, attachment of up to 500-1000 glochidia per fish on bass and drum weighing 5-15 g seems to be well tolerated.

There is a need to establish the most efficient combinations of bath volume and concentration for inoculating host fish. Increasing glochidia concentration by reducing bath volume should result in a higher attachment success, because the fish will ventilate a larger proportion of bath and the glochidia per unit time. However, this must be balanced against any increased tendency of the glochidia to close when the fish are crowded into a small bath volume. Experiments are needed to test these effects.

Two inoculations of bass with pink mucket glochidia were carried out simultaneously on 7/25/03. This inoculation used glochidia from mussel P-1, collected from the same female that provided glochidia used on 7-23-03. The inoculated fish were placed in the RPS along with the group inoculated on 7-23-03.

The inoculations on 7-25-03 were used to test the effect of changing glochidia concentration and also the time course of attachment (below). Glochidia concentration was 3-fold higher and bath volume per fish 1/3 lower in the second inoculation than in the first (*Table 15*). The attachment success in the more concentrated bath was more than twice as high as the first. This result is consistent with the prediction that attachment success of glochidia can be increased by reducing bath volume.

Time course of attachment

The time course of attachment was also recorded in the inoculation described above. Four 20-ml samples were taken from the inoculation bath at each of several timed intervals in order to record the rate of disappearance of glochidia from the bath. The results showed an exponential decline and were closely fitted by a second-order exponential equation (*Figure 21*). The predicted intercept of the equation (Y_o) should reflect the proportion of glochidia that close prematurely in the inoculation bath and are therefore unable to attach. Similar experiments could be used to test variables and determine the optimum conditions for inoculation with various species.

Observations on heavily infested fish

The number of glochidia attached to the fish used for the second test inoculation on 7-25-03 was quite high at about 1,300 per fish (*Table 15-8*). Several of these heavily infested fish appeared to be stressed 2 weeks later, during the period of juvenile excystment. Some of these individuals were sacrificed and their gills were examined. In many cases the cysts were crowded to the point that several glochidia shared a common cyst (*Figure 22*). Interestingly, each of these fish not only had large numbers of attached glochidia, but Monogenea (Dactylogyridae) were also present on the gills in high numbers (*Figure 23*). Such large numbers of Monogenea were not observed on other fish examined.

RECAPTURES OF PROPAGATED NEOSHO MUCKETS

Neosho muckets have been recovered from year 2000 release sites in the Fall and Verdigris Rivers over the past 3 years. These animals are identified as recaptures because they are the only juvenile Neosho muckets observed at these sites in over 10 years, belong to a single cohort based on size and growth lines, and correlate with the expected age of the propagated juveniles (Barnhart 2002).

During the summer of 2003, Kansas Wildlife and Parks carried out quantitative (quadrat) sampling in one of these reaches on the Verdigris River. The reach was seeded with 11,600 juveniles on August 2, 2000. More than 30 of these individuals have been recovered during incidental sampling over the past 2 years. This summer, KDWP excavated 40 1-m^2 quadrats within a 10 by 100 meter plot. Three of the young Neosho muckets were found within quadrats, indicating a population of approximately 75 individuals in this 1000 m^2 area (Edwin Miller, KDWP, pers. comm.) The area over which the mussels dispersed downstream is presumably much larger, so it is not possible to estimate the total surviving population.

These mussels have grown rapidly and are now over 8 cm in length (*Figures 24, 25*). It appears that they are reaching sexual maturity, so it may be possible to detect reproduction in 2004 and determine whether the population density at the release sites is sufficient for successful fertilization to occur.

OTTER PREDATION ON NEOSHO MUCKETS

On 12/17/02 my students and I visited the K-96 bridge site on the Spring River (R25E T33S S11 Cherokee County KS) on invitation from Kansas Wildlife and Parks, to examine the site prior to bridge replacement. One of the richest mussel beds on the Spring River is found upstream of this bridge site. We found many large Neosho muckets that were lying out of the substrate in shallow water. When I examined these mussels I noticed that nearly all of them had suffered damage to the soft tissues (*Figure 26*). Some of the wounds were very severe, as though the extended part of the foot had been bitten off. Others were just notched.

Some appeared to have healed, while others were very fresh wounds. In most cases the mussels were still living, although a few fresh-dead individuals were also found.

We also noted abundant deposits of otter scat associated with fresh shell and live mussels on shore in sheltered areas, suggesting that otters were feeding on mussels. The scat was recognizable by form and contained *Corbicula* shell fragments, which are not normally found in raccoon scat.

The damaged Neosho muckets were probably too large to be opened directly. Rather, it appears that the otters uproot the mussel and bite the foot before the mussel can retract it into the shell. Neosho muckets may be particularly susceptible to this sort of predation because they tend to have the foot extended in the substrate (personal observations). They are probably especially susceptible in winter because their movements are slowed by low temperature. We found a few mussels of other species that were similarly damaged, but the overwhelming majority were adult Neosho muckets.

On February 10 Edwin Miller (KDWP) and I returned to the site and collected 58 adult Neosho muckets mussels that were lying on the surface. We also found 5 more animals that were buried in the substrate, in their normal posture. We examined each of these and categorized them with respect to the severity of foot damage. Sex was also recorded. Females and males were distinguished by pigmentation along edge of marsupial gill and by shell shape.

We found that 54/58 (93%) of Neosho muckets found on the surface were amputees (*Figure 27*). None of the 5 animals found buried were damaged. Of the damaged mussels, 60% were male and 40% female. All males found on the surface were damaged (32/32). Of the females, 22/26 on the surface were damaged. Of the 26 females we examined, 2 were gravid with mature glochidia.

We investigated the possibility that damage to the foot may leave the mussel unable to rebury. All of the animals were marked and laid on the substrate in shallow water and left overnight to see if they would re-bury themselves. When I returned to the site the next day, none of the damaged mussels had reburied, while 5 of the 9 undamaged mussels had reburied. Three days later, on Feb. 14, none of the damaged animals had reburied and one had died. All but 2 of the undamaged animals had reburied.

Whether or not these animals are able to recover from such injury is unknown, although the appearance of some of the wounds suggests that healing may occur. Clearly, the impact of otters on mussel beds should be monitored and control measures should be considered in situations where endangered mussel species are involved.

BROOD STOCK SEQUESTERED IN 2003

Difficulty in locating gravid scaleshell and pink mucket has limited propagation efforts to date. Therefore, a more extensive effort was made this year to locate females for propagation

in 2004. Fieldwork in the Meramec River this summer and fall resulted in locating 17 adult female pink muckets and 9 adult female scaleshell. These animals were sequestered at selected spots in the river in order to facilitate relocation for propagation next year. At least 2 of the female scaleshell are brooding. Further searches for scaleshell are anticipated as conditions permit in November.

DISSEMINATION OF PROJECT INFORMATION IN 2003

Media and popular press

- Barnhart, M. C. 2003. Making mussels. Missouri Conservationist 64(8):4-9. Article can be viewed on-line at: http://www.conservation.state.mo.us/conmag/2003/08/.
- Shipley, Sarah. 2003. Scientists wage war to save rare mussel. St. Louis Post-Dispatch, Sunday edition, Oct. 5. 830 words, 3 photos. This newspaper article was paired with another article describing the Nature Conservancy's decision to name the Meramec as a top priority for conservation of biological diversity. Text of the articles is available on line at http://biology.smsu.edu/announcements/bionews.
- KOLR 10 Morning Show (76 mile radius). Television interview, tour of facilities, and discussion of endangered species and mussel propagation. Aired September 10.
- Barnhart, M.C. Unio Gallery. A pictorial resource for conservation professionals and educators working with endangered species. http://www.smsu.edu/mcb095f/gallery/
- Barnhart, M. C. Propagation and Restoration of Freshwater Mussels. Resources for conservation professionals working with endangered species. http://unionid.smsu.edu

Public presentations

- Barnhart, M. C. and J. S. Faiman. 2003. Public demonstration on mussels of the Meramec at Castlewood State Park on August 2.
- Barnhart, M. C. and J. S. Faiman. 2003. Public demonstration on mussels of the Meramec at Castlewood State Park for annual Meramec Expedition group. September 19.

Professional presentations

- Barnhart, M. C. and M. A. Shiver. 2003. Progress in the reproductive biology, propagation, and stocking of the Neosho mucket, *Lampsilis rafinesqueana.* Freshwater Mollusc Conservation Society Symposium, Durham NC.
- Eckert, N. E. and M. C. Barnhart 2003. Comparison of host compatibility in two populations of Western fanshell, *Cyprogenia aberti* . Freshwater Mollusc Conservation Society Symposium, Durham NC.
- Barnhart, M. C. 2003. Reproductive biology of native freshwater mussels. Invited lecture at USGS Columbia Environmental Research Center.
- Hutson, C. A. and M. C. Barnhart. 2003. Survey of unionoids in regulated rivers in Southwestern Missouri. Freshwater Mollusc Conservation Society Symposium, Durham NC.

- Barnhart, M. C., Sue Brunderman and Christian Hutson. 2003. Mussel Conservation update. Fisheries Division Training Conference, Missouri Department of Conservation, Lake Ozark, MO.
- Eckert, N. E. and M. C. Barnhart. 2003. Reproductive biology of Western fanshell mussels in Kansas and Missouri. Missouri Natural Resources Conference, Lake Ozark, MO.
- Barnhart, M. C. 2003. Progress in the propagation of endangered native mussels. Kansas Pearly Mussel Meeting, Pittsburg State, KS.
- Eckert, N. and M. C. Barnhart. 2003. Reproductive biology and host requirement differences among isolated populations of *Cyprogenia aberti* (Conrad 1850). Kansas Pearly Mussel Meeting, Pittsburg State, KS.
- Dodd, B. and M. C. Barnhart. 2003. Susceptibility of channel catfish to Ich and glochidia: Implications for artificial propagation of freshwater mussels. Kansas Pearly Mussel Meeting, Pittsburg State, KS.

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Barnhart, M. C. and C. Hutson. 2002. Survey for endangered and special concern mussel species in the Sac and Pomme de Terre rivers in Southwestern Missouri. Report to Missouri Department of Conservation, Endangered Species Grant No. E-1-36. Year 1 of 3.

Barnhart, M. C. and C. Hutson. 2003. A survey of endangered and special concern mussel species in the St. Francis and Black rivers in Southeastern Missouri. Report to Missouri Department of Conservation, Endangered Species Grant No. E-1-36. Year 2 of 3. 17 pp.

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Table 1. Propagation of federally listed and candidate mussel species at SMSU in 2003. Note: fat pocketbook juveniles were produced under a separate project funded by USACE.

Species (n females)	Start date	River	Juveniles released
Neosho mucket (1)	3-25-03	Spring River, MO	45,000
Neosho mucket (4)	7-3-03	Spring River, MO	302,250
Neosho mucket (1)	7-23-03	Verdigris River, KS	27,000
		Subtotal	374,250
Pink mucket (1)	7-3-03	Meramec River, MO	84,500
Pink mucket (1)	7-23-03	Meramec River, MO	37,500
		Subtotal	122,000
Scaleshell (1)	6-2-03	Meramec River, MO	13,864
		Subtotal	13,864
Fat pocketbook (11)	6-17-03	St. Francis River, AR	101,990
		Subtotal	101,990
All species		Total	612,204

Table 2. Releases of federally listed and candidate mussel species propagated at SMSU in 2003. Species codes are LR = *Lampsilis rafinesqueana* (Neosho mucket), PC = *Potamilus capax* (fat pocketbook), LA = *Lampsilis abrupta* (pink mucket) and LL = *Leptodea leptodon* (scaleshell). Note: fat pocketbook juveniles were produced under a separate project funded by USACE.

Date	Species	Site	GIS coordinates (datum)	N released
4/16/03	LR	Spring River, MO	15 424740 4103002	22,500
4/16/03	LR	Spring River, MO	15 415778 4110290	22,500
6/23/03	LL	Bourbeuse River, MO	15 656171 4248570	1,150
6/24/03	LL	Meramec River, MO	15 691885 4257100	6,754
7/1/03	LL	Meramec River, MO	15 697359 4259363	5,960
7/8/03	PC	St. Francis River AR	15 708267 3877150	35,282
7/8/03	PC	St. Francis River AR	15 722163 3917529	33,354
7/8/03	PC	St. Francis River AR	15 723302 3923640	33,354
7/17/03	LA	Meramec River, MO	15 709800 4267944	33,600
7/17/03	LA	Meramec River, MO	15 712763 4268762	25,450
7/17/03	LA	Meramec River, MO	15 697359 4259363	25,450
8/11/03	LA	Meramec River, MO	15 697359 4259363	15,000
8/11/03	LA	Meramec River, MO	15 712763 4268762	22,500
7/17/03	LR	Spring River, KS	15 354095 4116024	90,500
7/20/03	LR	Spring River, MO	15 424740 4103002	78,650
7/20/03	LR	Spring River, MO	15 415778 4110290	54,450
7/20/03	LR	Spring River, MO	15 384680 4116011	78,650
8/13/03	LR	Verdigris River, KS	15 263603 4139591	27,000

Table 3. Fecundity and brood condition of scaleshell propagated 6/02 and 6/03/03. A small portion of the brood was sampled on 6/02/03 and used in a trial infection of 9 Gavins Point drum. Most of the remaining brood was sampled on 6/03/03 and used to inoculate 132 Langston fish. In total, over 1.5 million eggs and glochidia were sampled. Approximately ¼ of the brood was left in the marsupia for use at CERC in toxicity testing. Therefore, fecundity (eggs and larvae) is estimated at 2 million.

	Collection Date		
A.Numbers	**6/2/03**	**6/3/03**	**Total**
1. Total brood	55,050 ± 511	1,464,000 ± 10,169	1,519,050 ± 10,680
2. Undeveloped eggs	24,050 ± 494	1,051,000 ± 8,481	1,075,050 ± 8,975
3. Glochidia	31,000 ± 247	413,000 ± 2,514	444,000 ± 2,761
4. Live, open glochidia	24,600 ± 264	235,000 ± 3,733	259,600 ± 3,997
5. Live, closed glochidia	6,300 ± 181	165,000 ± 3,040	171,300 ± 3,220
6. Dead glochidia	100 ± 20	13,000 ± 588	13,100 ± 608
B. Proportions			
1. Percent of brood fertile	56.3%	28.2%	29.2%
2. Percent of brood infertile	43.7%	71.8%	70.8%
3. Percent of glochidia live	99.7%	96.9%	97.0%
4. Percent of glochidia dead	0.3%	3.1%	3.0%
5. Percent of live glochidia open	79.6%	58.8%	60.2%
6. Percent of live glochidia closed	20.4%	41.3%	39.8%

Table 4. Propagation of Meramec scaleshell on Gavins Point drum. Nine fish were inoculated as a group and monitored individually in the AHAB system. AHAB results are means ± 95% confidence interval.

A. INOCULATION		
1.	Bath volume	3 L
2.	N fish inoculated	9
3.	Bath volume per fish	0.333 L
4.	N infective glochidia	24,600
5.	Glochidia per L	8,200
6.	Glochidia per fish	2,733
7.	Initial – final number of glochidia in bath	7,300
8.	Estimated number attached per fish	811
9.	Estimated attachment success (A7/A4*100)	29.7%
B. AHAB RESULTS (9 fish monitored individually)		
1.	Standard length of fish (mm)	84.3 ± 3.3
2.	Total sluffs and juveniles recovered per fish	692 ± 185.4
3.	Estimated attachment success [(B2*A2)/A4]*100	25.3%
4.	Juveniles recovered per fish	500 ± 193
5.	Transformation success	69% ± 16.0

Table 5. Propagation of Meramec scaleshell on Langston drum. A) 132 fish were inoculated as a group. B) Results from 10 fish monitored individually in the AHAB system: values are means ± 95% confidence interval. C) Results from 122 fish that were held together in the RPS.

A. INOCULATION		
1.	Bath volume	62.4 L
2.	N fish inoculated	132
3.	Bath volume per fish	0.473 L
4.	N infective glochidia	235,000
5.	Glochidia per L	3,766
6.	Glochidia per fish	1,780
B. AHAB RESULTS (10 fish monitored individually)		
1.	Standard length of fish (mm)	86.4 ± 15.8
2.	Total sluffs and juveniles recovered per fish	179 ± 69.2
3.	Estimated attachment success	11.1% ± 4.7
4.	Juveniles recovered per fish	120 ± 49.2
5.	Transformation success	61% ± 27.1
C. RPS RESULTS (group of 122 fish)		
1.	Total sluffs and juveniles recovered	25,114
2.	Total sluffs and juveniles recovered per fish	206
3.	Estimated attachment success	11.6%
4.	Juveniles recovered	17,640
5.	Juveniles recovered per fish	145
6.	Transformation success	70%

Table 6. Brood condition of 4 Spring River Neosho muckets that were propagated 7/03/03. Numbers are in thousands. Each figure is derived from a mean ± 95% CI of counts of 10 volumetric subsamples from the total suspension.

	Female ID#			
A.Numbers (thousands)	K-1	K-4	K-5	K-6
1. Total brood	1,255 ± 13.1	1,175 ±14.2	1,030 ±13.5	975 ±16.0
2. Undeveloped eggs	15 ± 1.5	60 ±5.6	95 ±6.1	15 ± 1.5
3. Glochidia	1240 ± 1.3	1,115 ±13.0	935 ±9.5	960 ± 16.0
4. Live, open glochidia	1150 ± 9.2	1,065 ±12.	890 ±8.7	910 ± 16.8
5. Live, closed glochidia	90 ± 6.2	45 ±3.1	45 ±2.7	15 ± 2.1
6. Dead glochidia	0	5 ±1.0	0	35 ± 2.9
B. Proportions				
1. Percent of brood fertile	98.8%	94.9%	90.8%	98.5%
2. Percent of brood infertile	1.2%	5.1%	9.2%	1.5%
3. Percent of glochidia live	100%	99.6%	100%	96.4%
4. Percent of glochidia dead	0%	0.4%	0%	3.6%
5. Percent of live glochidia open	92.7%	95.9%	95.2%	98.4%
6. Percent of live glochidia closed	7.2%	4.1%	4.8%	1.6%

Table 7. Size and fecundity of Spring River Neosho muckets collected near the K-96 bridge in Cherokee County Kansas on 6-27-03. Four of the females provided glochidia for propagation carried out on 7-3-03. The counts include all brood that were voided within 1 week after propagation as well as those that were removed for propagation.

Female	Length (mm)	Width (mm)	Height (mm)	Whole mass (g)	Fecundity
K-1	109.4	46.5	70.9	264.4	1,466,000
K-2	121.9	47.9	73.7	309.3	-
K-3	124.2	51.2	76.9	379.1	-
K-4	112	45.3	71.6	257	1,189,000
K-5	116	48.6	75.4	302.6	1,173,000
K-6	114.7	44.5	71.4	272.5	1,268,000

Table 8. Propagation of Neosho muckets on largemouth bass 7-3-03. Glochidia were pooled from 4 females. The fish and glochidia were divided equally between two inoculation baths of 294 L each. The fish were left in the inoculation bath for 20 minutes.

A. INOCULATION		
1.	Bath volume	294 L x 2
2.	N fish inoculated	976
3.	Bath volume per fish	0.602 L
4.	N infective glochidia	2,007,500
5.	Glochidia per L	3,414
6.	Glochidia per fish	2,057
B. AHAB RESULTS (12 fish monitored individually, means ± 95% CI)		
1.	Standard length of fish (mm)	63.7 ± 5.0
2.	Mass of fish (g)	5.1 ± 0.9
3.	Total sluffs and juveniles recovered per fish	506.8 ± 128.5
4.	Attachment success (percent) [(B3*A2)/A4 *100]	24.6%
5.	Juveniles recovered per fish	463.1 ± 118.4
6.	Transformation success (percent)	91.3% ± 2.1
C. RPS RESULTS (group of 964 fish)		
1.	Total sluffs and juveniles recovered	484,993
2.	Total sluffs and juveniles recovered per fish	503
3.	Estimated attachment success (C1/C4)*100	24.1%
4.	Juveniles recovered	402,900
5.	Juveniles recovered per fish	418
6.	Transformation success (C4/C1)*100	83.1%

Table 9. Brood condition of pink mucket (#LA) propagated 7/03/03. The gills were not fully charged, and not all glochidia were removed. Therefore these numbers are not an estimate of fecundity.

A. Numbers	
1. Total brood	385,000 ± 5,175
2. Undeveloped eggs	0
3. Glochidia	385,000 ± 5,175
4. Live, open glochidia	345,000± 3,564
5. Live, closed glochidia	20,000± 1,222
6. Dead glochidia	15,500 ± 1,756

B. Proportions	
1. Percent of brood fertile	100%
2. Percent of brood infertile	0%
3. Percent of glochidia live	95.5%
4. Percent of glochidia dead	4.5%
5. Percent of live glochidia open	94.6%
6. Percent of live glochidia closed	5.4%

Table 10. Propagation of pink muckets on largemouth bass 7-3-03. The fish and glochidia were divided between two inoculation baths of 35.2 L each. The fish were left in the inoculation baths for 20 minutes.

A. INOCULATION		
1.	Bath volume	35.2 L x 2
2.	N fish inoculated	349
3.	Bath volume per fish	0.202 L
4.	N infective glochidia	365,000
5.	Glochidia per L	5,185
6.	Glochidia per fish	1,046
B. AHAB RESULTS (12 fish monitored individually, means ± 95% CI)		
1.	Standard length of fish (mm)	59.9 ± 4.1
2.	Mass of fish (g)	4.2 ± 0.8
3.	Total sluffs and juveniles recovered per fish	404.3 ± 105.3
4.	Estimated attachment success [(B3*A2)/A4 *100]	38.7%
5.	Juveniles recovered per fish	351 ± 96.8
6.	Transformation success	86.0% ± 4.3
C. RPS RESULTS (group of 337 fish)		
1.	Total sluffs and juveniles recovered	106,793
2.	Total sluffs and juveniles recovered per fish	317
3.	Estimated attachment success (C1/A4)*100	30.3%
4.	Juveniles recovered	92,800
5.	Juveniles recovered per fish	275
6.	Transformation success (C4/C1)*100	86.9%

Table 11. Brood condition of Verdigris Neosho mucket (#D3) propagated 7/23/03. Numbers are based on the means ± 95% CI of 10 volumetric subsamples from the total suspension.

A. Numbers	
1. Total brood sampled	746,000 ± 6,489
2. Undeveloped eggs	446,000 ± 7,731
3. Glochidia	300,000 ± 3,469
4. Live, open glochidia	233,000 ± 3,079
5. Live, closed glochidia	47,000 ± 776
6. Dead glochidia	20,000 ± 1,012
B. Proportions	
1. Percent of brood fertile	40.2%
2. Percent of brood infertile	59.8%
3. Percent of glochidia live	93.3%
4. Percent of glochidia dead	6.7%
5. Percent of live glochidia open	83.2%
6. Percent of live glochidia closed	16.8%

Table 12. Propagation of Verdigris Neosho mucket on largemouth bass 7-23-03. The fish were inoculated in the RPS. Water volume was reduced to 132 liters and the water was aerated and stirred for 15 minutes. Thereafter, the tank was refilled (total volume 917 L) and the circulation restored to remove leftover glochidia. Six of the 401 fish were moved to the AHAB for monitoring.

A. INOCULATION		
1.	Bath volume	132 L
2.	N fish inoculated	401
3.	Bath volume per fish	0.329 L
4.	N infective glochidia	233,000
5.	Glochidia per L	1765
6.	Glochidia per fish	581
B. AHAB RESULTS (6 fish monitored individually, means ± 95% CI)		
1.	Standard length of fish (mm)	64.3 ± 6.0
2.	Mass of fish (g)	4.9 ± 1.2
3.	Total sluffs and juveniles recovered per fish	97.8 ± 23.5
4.	Estimated attachment success [(B3*A2)/A4 *100]	16.8%
5.	Juveniles recovered per fish	77.0 ± 19.9
6.	Transformation success	78.8 ± 5.9
C. RPS RESULTS (group of 395 fish)		
1.	Total sluffs and juveniles recovered	237,488
2.	Total sluffs and juveniles recovered per fish	*
3.	Estimated attachment success (C1/A4)*100	*
4.	Juveniles recovered	41,538
5.	Juveniles recovered per fish	105.1
6.	Transformation success (C4/C1)*100	*

* Because the fish were inoculated in the RPS, unattached as well as attached glochidia were recovered. Therefore, calculation of attachment success and transformation success from RPS catches was not possible.

Table 13. Brood condition of pink mucket #P1 propagated 7/23/03. Glochidia were removed twice, first on 7-23-03 and again on 7-25-03. Not all glochidia were removed so these numbers are not an estimate of fecundity. Numbers are the means ± 95% CI of 10 volumetric subsamples from the total suspension.

	Date sampled		
A.Numbers	7-23-03	7-25-03	Total
Total brood sampled	232,000 ± 3,380	139,050 ± 952	371,050 ± 4,332
Undeveloped eggs	11,000 ± 849	6,750 ± 421	17,750 ± 1,270
Glochidia	221,000 ± 3,438	132,300 ± 1,094	353,300 ± 4.532
Live, open glochidia	203,000 ± 3,319	122,400 ± 1,130	325,400 ± 4,449
Live, closed glochidia	9,000 ± 989	3,600 ± 176	12,600 ± 1,165
Dead glochidia	9,000 ± 543	6,300 ± 377	15,300 ± 919
B. Proportions			
Percent of brood fertile	95.3%	95.1%	95.2%
Percent of brood infertile	4.7%	4.9%	4.8%
Percent of glochidia live	95.9%	95.2%	95.7%
Percent of glochidia dead	4.1%	4.8%	4.3%
Percent of live glochidia open	95.8%	97.1%	96.3%
Percent of live glochidia closed	4.2%	2.9%	3.7%

Table 14. Propagation of pink muckets on largemouth bass 7-23-03. The fish were inoculated in the RPS. Water volume was reduced to 132 liters and the water was aerated and stirred for 15 minutes. Thereafter, the tank was refilled (total volume 917 L) and the circulation restored to remove leftover glochidia. Six of the 444 fish were moved to the AHAB for monitoring. Two other smaller groups of bass were inoculated separately and added to the RPS on 7-25-03 (Table 15).

A. INOCULATION		
1.	Bath volume	132 L
2.	N fish inoculated	444
3.	Bath volume per fish	0.297 L
4.	N infective glochidia	203,000
5.	Glochidia per L	1,538
6.	Glochidia per fish	457
B. AHAB RESULTS (6 fish monitored individually, means ± 95% CI)		
1.	Standard length of fish (mm)	60.8 ± 4.0
2.	Mass of fish (g)	4.3 ± 0.7
3.	Total sluffs and juveniles recovered per fish	84.7 ± 29.7
4.	Estimated attachment success [(B3*A2)/A4 *100]	18.5%
5.	Juveniles recovered per fish	59 ± 28.4
6.	Transformation success (%)	66.2 ± 10.7
C. RPS RESULTS for 582 fish*		
1.	Total sluffs and juveniles recovered	352,225
2.	Total sluffs and juveniles recovered per fish	**
3.	Estimated attachment success (C1/A4)*100	**
4.	Juveniles recovered	44,913
5.	Juveniles recovered per fish	77
6.	Transformation success (C4/C1)*100	**

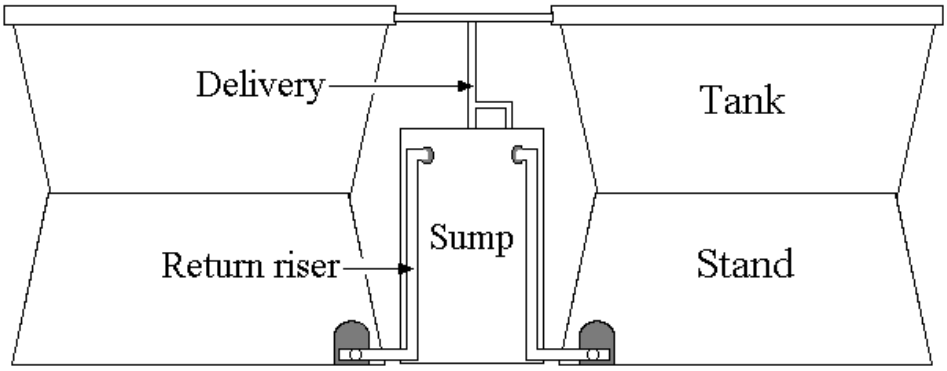
* These RPS results include fish added 7-25-03 which were inoculated separately (see below). Therefore, they are not comparable with the AHAB results, which included only fish inoculated on 7-23-03.

** Because the fish were inoculated in the RPS on 7-23-03, unattached as well as attached glochidia were recovered. Therefore, calculation of attachment and transformation success from RPS catches was not possible.

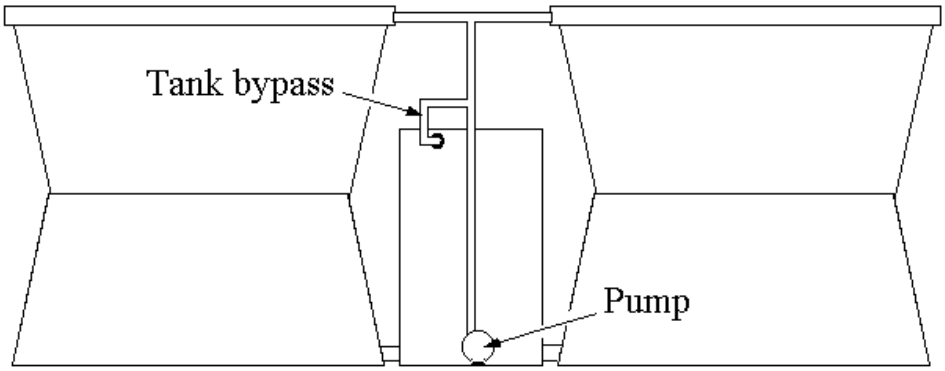
Table 15. Comparison of attachment success of pink mucket glochidia in two inoculations on bass on 7-25-03. Attachment success was calculated by comparing the initial and final numbers glochidia in the inoculation baths. The inoculations were carried out simultaneously using portions of the same group of glochidia and host fish. Inoculation #2 used a 3-fold higher concentration of glochidia and twice as many glochidia per fish as Inoculation #1. The number of glochidia attached per fish was more than 5 times higher and the percent attached was more than twice as high in #2 compared to #1.

Measurement	Inoculation #1	Inoculation #2
1. Number of fish	90	48
2. Bath volume (L)	46	15
3. Exposure time (minutes)	17	17
4. Bath volume per fish (L)	0.511	0.313
5. Glochidia concentration per liter	1,957	6,241
6. Number of glochidia per fish	1,000	1,950
7. Total glochidia attached	20,813	62,687
8. Glochidia attached per fish	231	1,306
9. Attachment success (%)	21.4%	56.2%

A. Front View



B. Rear view



C. Top view

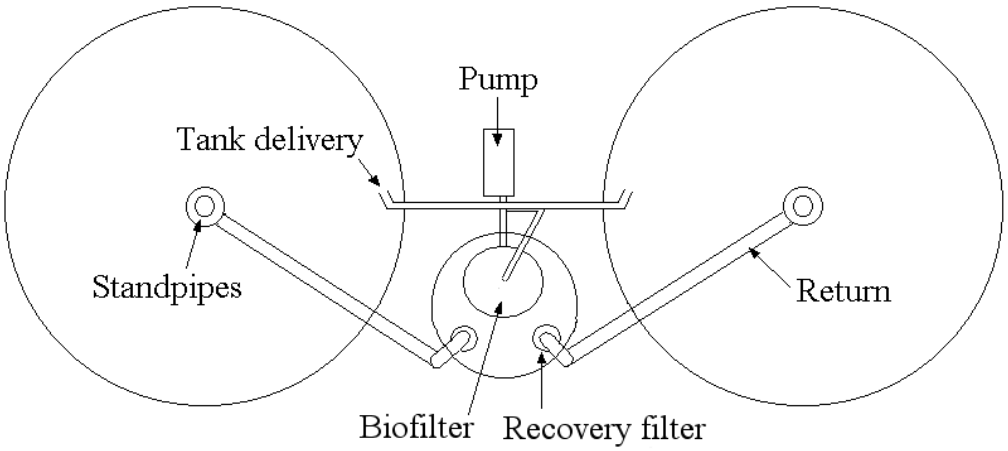


Figure 1. Diagrammatic views of recirculating propagation system (RPS). A) front view, B) back view, and C) overhead view. The tanks are 60 inches in diameter, and 55 inches tall including stands. The footprint of a 2-tank unit is 60 by 150 inches.

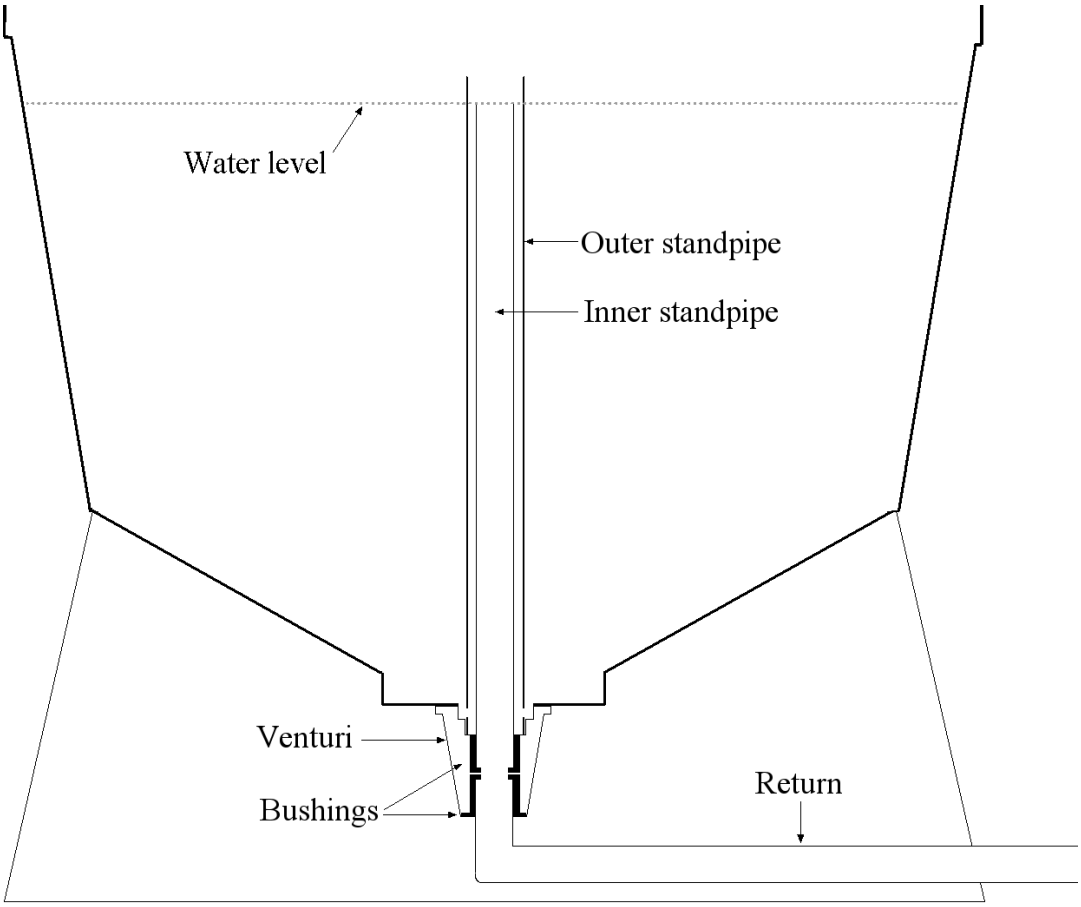


Figure 2. Tank cutaway showing details of standpipes, venturi and return. Tank diameter is 60 inches (5 feet).

A.

B.

Figure 3. A) Venturi and venturi bushing inserts. B) Venturi assembly welded to the base of the tank.

A

B.

C.

Figure 4. Sump and associated plumbing. A) View from front showing delivery and return lines. Pump is behind the sump. B) Sump from above with lid removed to show tank bypass and biological filter. C) Inside view showing biological filter with bypass spray and recovery filters hanging from returns. Refer to Figures 5, 6, and 7 for details.

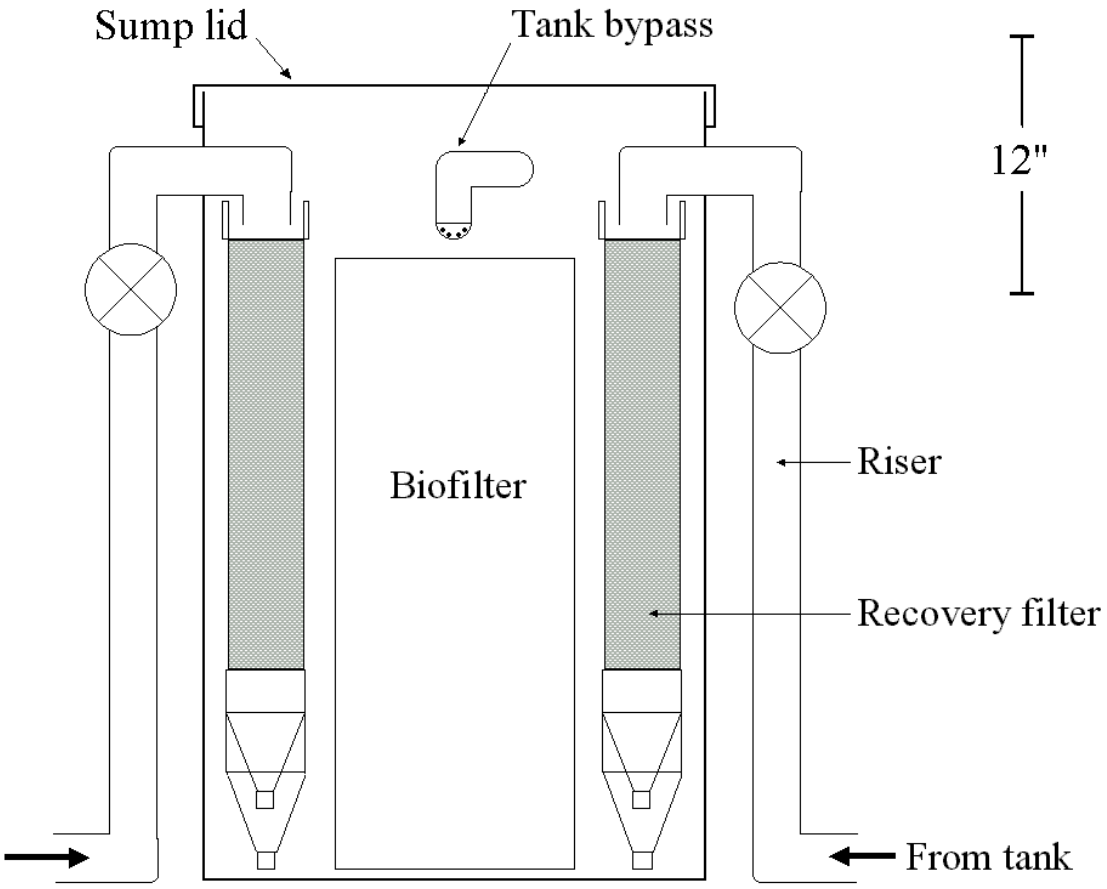


Figure 5. Sump in cut-away front view. The return lines deliver water from the tanks to the recovery filters, which hang in the sump on either side of the biological filter. The tank bypass incorporates a spray head that delivers water to the top of the biological filter. The sump has a removable top to allow access.

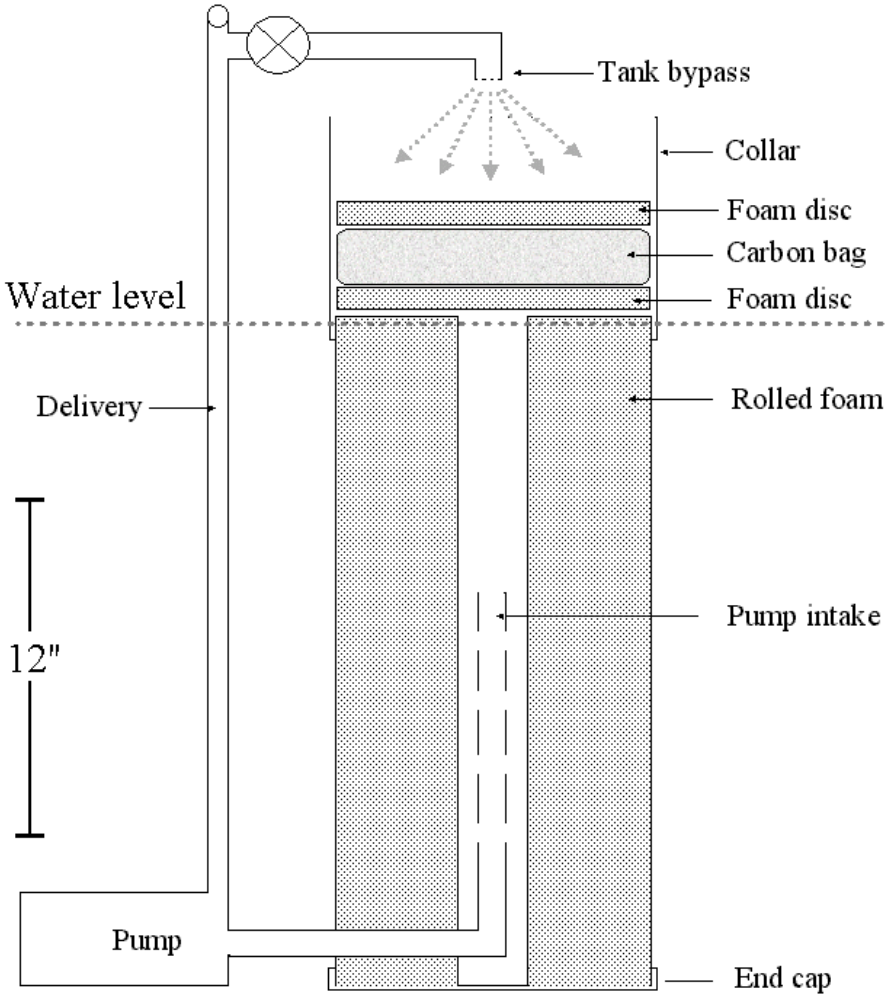


Figure 6. Biological filter in cut-away view. The main filter is a 24 x 48 x 1 inch piece of open-cell foam that is rolled to form a hollow cylinder 24 inches tall. The base of the cylinder is capped and the top is extended above the water level in the sump by a plastic collar. The pump draws water from inside the cylinder creating flow through the sides. Flow from the tank bypass is sprayed over foam discs and a net bag of activated carbon granules supported above the water line in the collar.

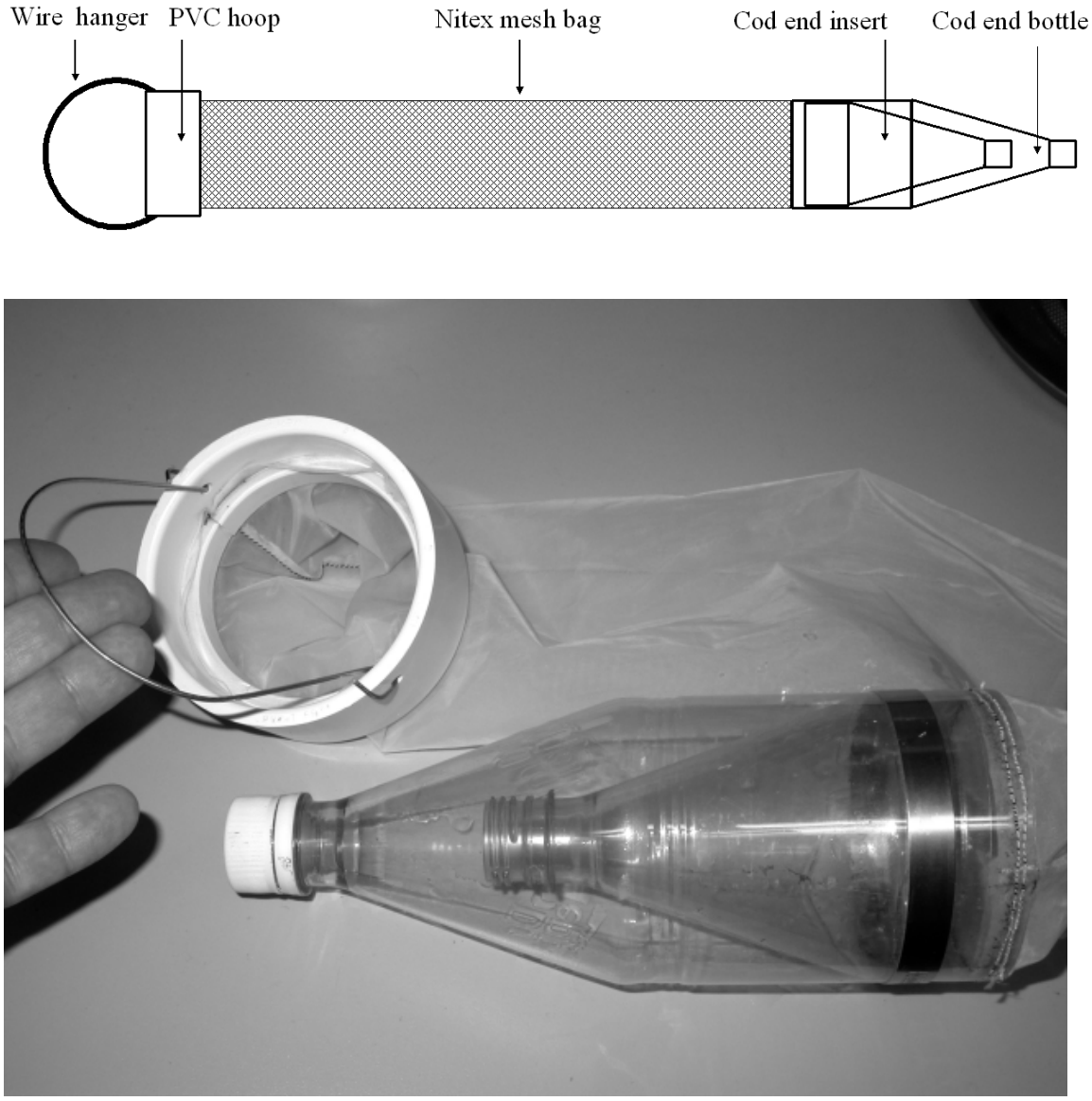


Figure 7. Recovery filter diagram and photograph. The net bag is 125 micron mesh Nitex cloth*. The hoop is a 3-inch PVC coupling cut in half, with a split ring of 3-inch PVC nested into the coupling to hold the end of the bag. The cod end is made of the cut-off ends of two 1-L plastic soda bottles. One cut-off end (the cod end insert) is nested inside the other (the cod end bottle) and secured with a hose clamp. The net bag is sewn to the cod end bottle.

*Mesh size must be matched to the size of the species being propagated.

Figure 8. Adult female scaleshell, the source of glochidia propagated in June 2003. This mussel was collected from the Meramec River at Fishtrap Rapids on September 12, 2002. Total mass was 18.5 g, and shell measurements were 69.6 mm length, 28.5 mm height, 18.3 mm width.

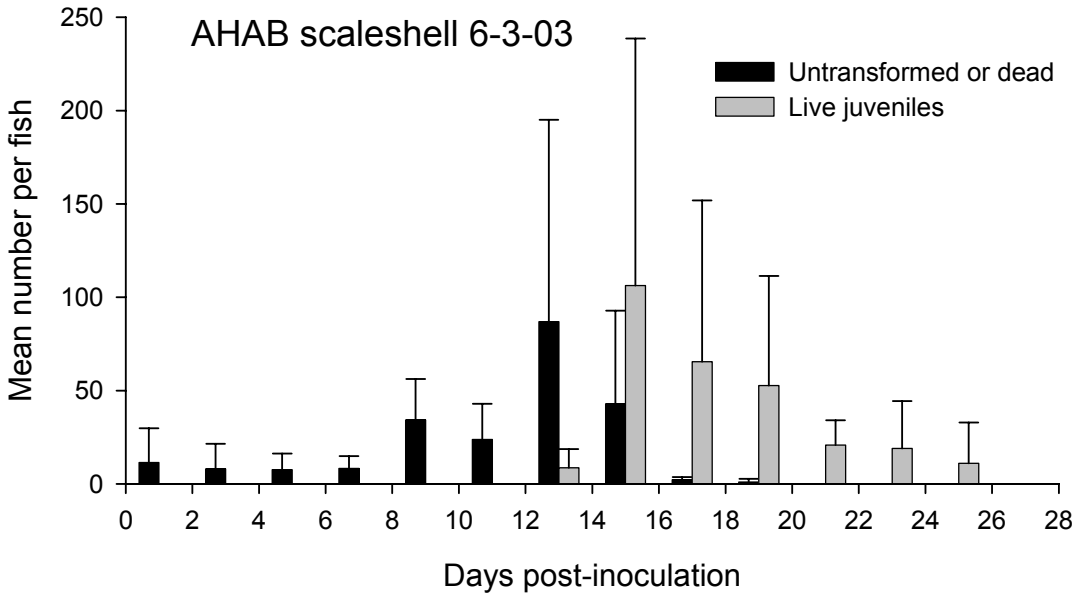


Figure 9. AHAB recovery of scaleshell from drum inoculated on 6-3-03. Mean temperature was 20.1 ± SD 0.75 °C. Data are means ± 95% CI (n=6).

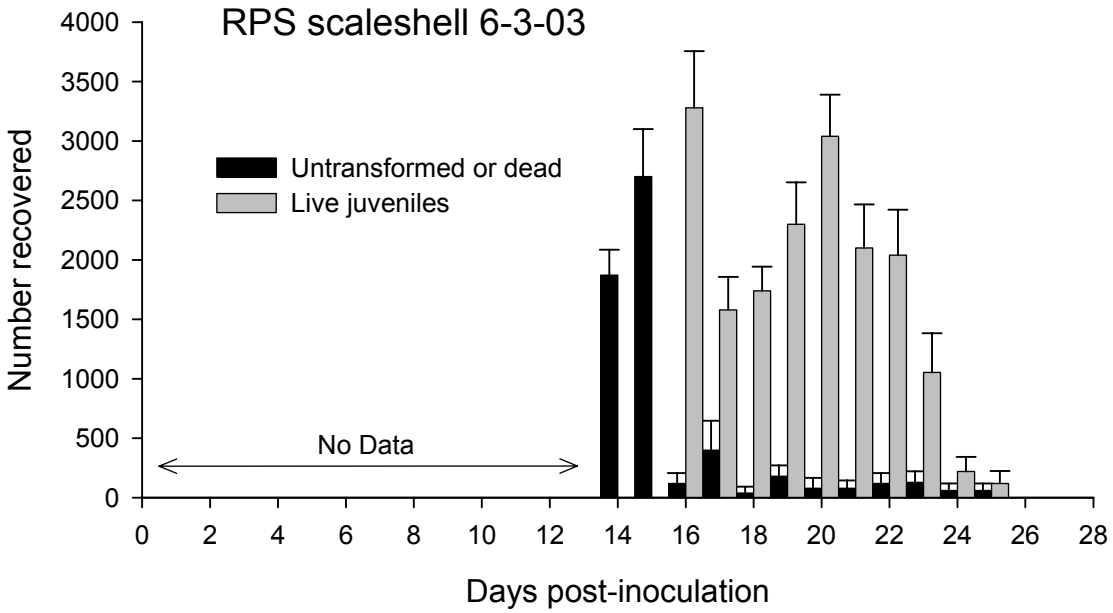


Figure 10. Recovery of scaleshell from 122 drum in RPS. Fish were inoculated and kept at Lost Valley from 6/3- 6/17. Drop-off and temperature during that time were not recorded. Temperature in the RPS was 23.0 ± SD 0.40. Data are total ± 95% CI.

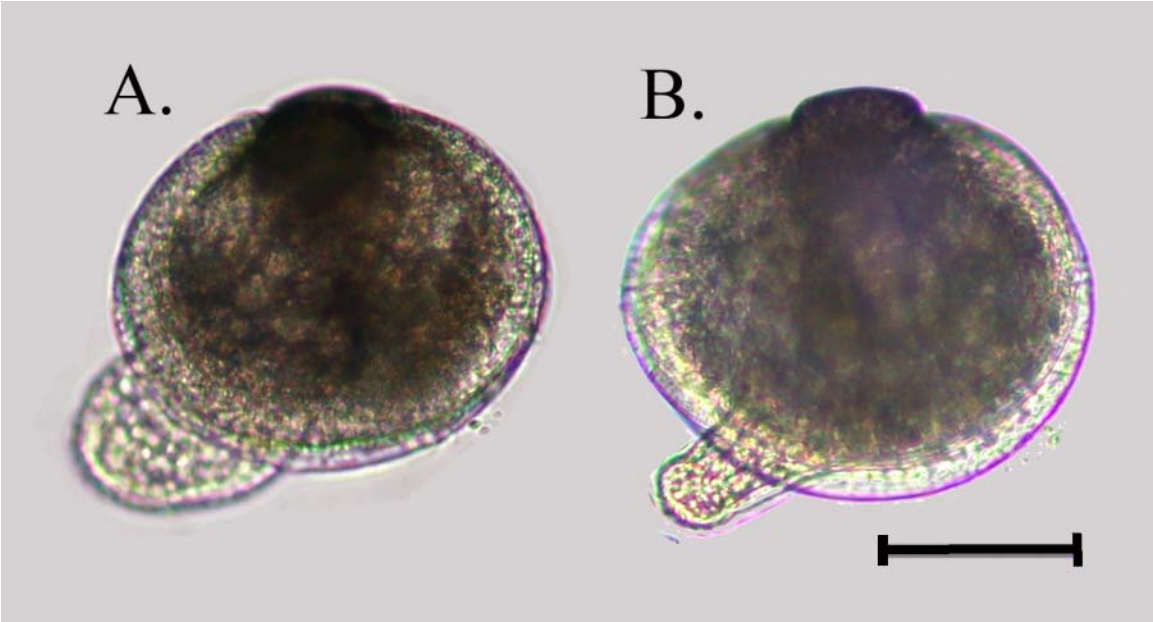


Figure 11. Newly excysted scaleshell juveniles. A) Edematous individual ("bloater") that excysted on day 15. B) Normal juvenile that excysted on day 16. Bloating appears to be characteristic of prematurely excysted scaleshell and fat pocketbooks. Scale line is 100 microns.

Figure 12. Immature scaleshell from the Gasconade River, 0.5 miles upstream of Wrinkle Spring Access, XX County, MO. This individual is assumed to be a recapture from a group of juveniles released at this site in June 2002.

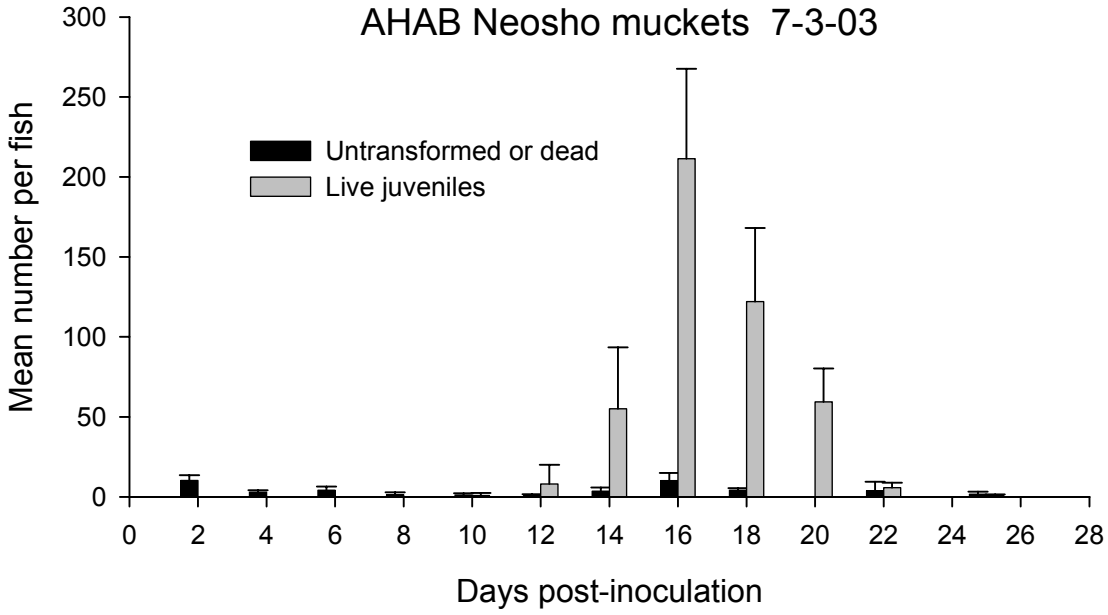


Figure 13. AHAB recovery of Neosho muckets from bass inoculated 7-3-03. Mean temperature was 20.5 ± 0.7 °C. Data are means ± 95% CI (n=12).

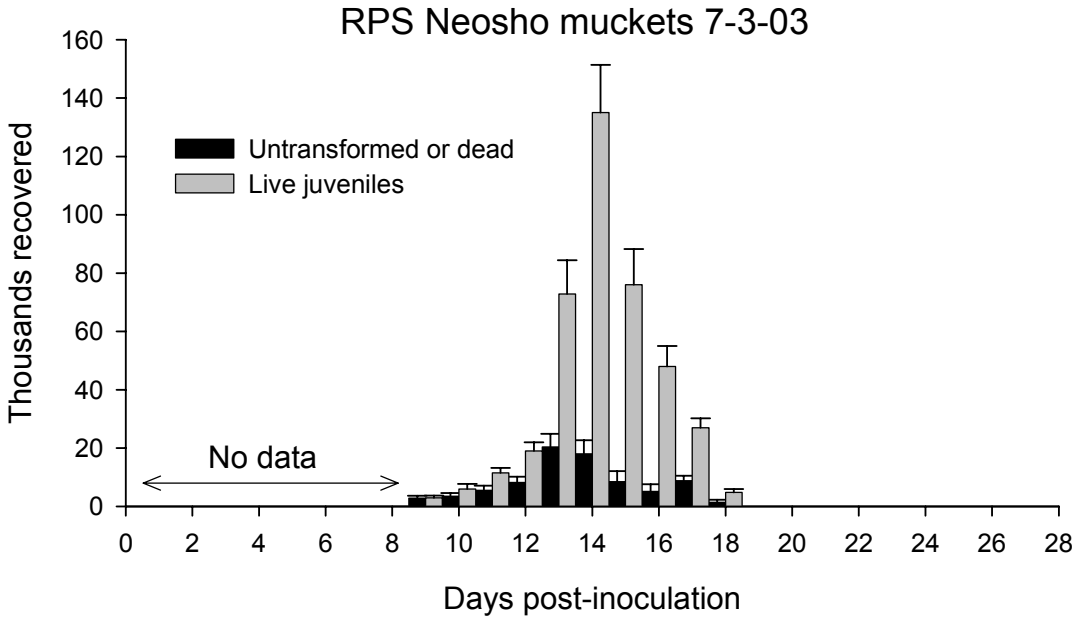


Figure 14. Recovery of Neosho muckets from 964 bass in the RPS. Fish were inoculated and kept at Chesapeake from 7/3- 7/10. Drop-off and temperature during that time were not recorded. Temperature in the RPS tanks was 23.5 ± SD 0.15. Data are daily totals ± 95% CI.

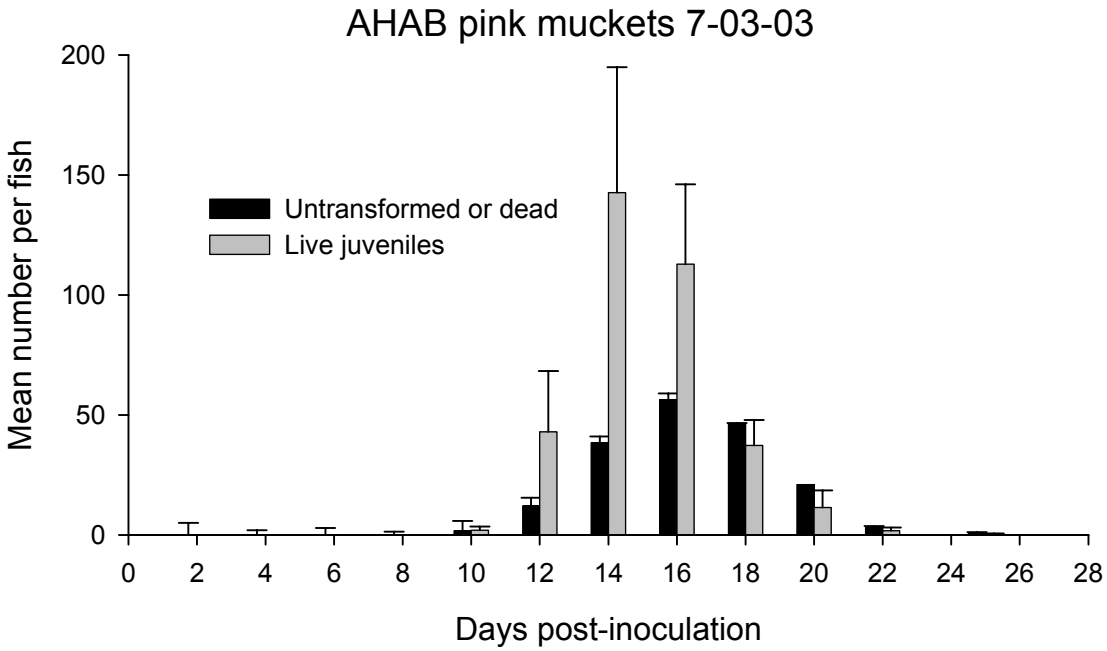


Figure 15. AHAB recovery of pink muckets from bass inoculated 7-3-03. Mean temperature was 20.5 ± 0.7 °C. Data are means ± 95% CI (n = 12 fish).

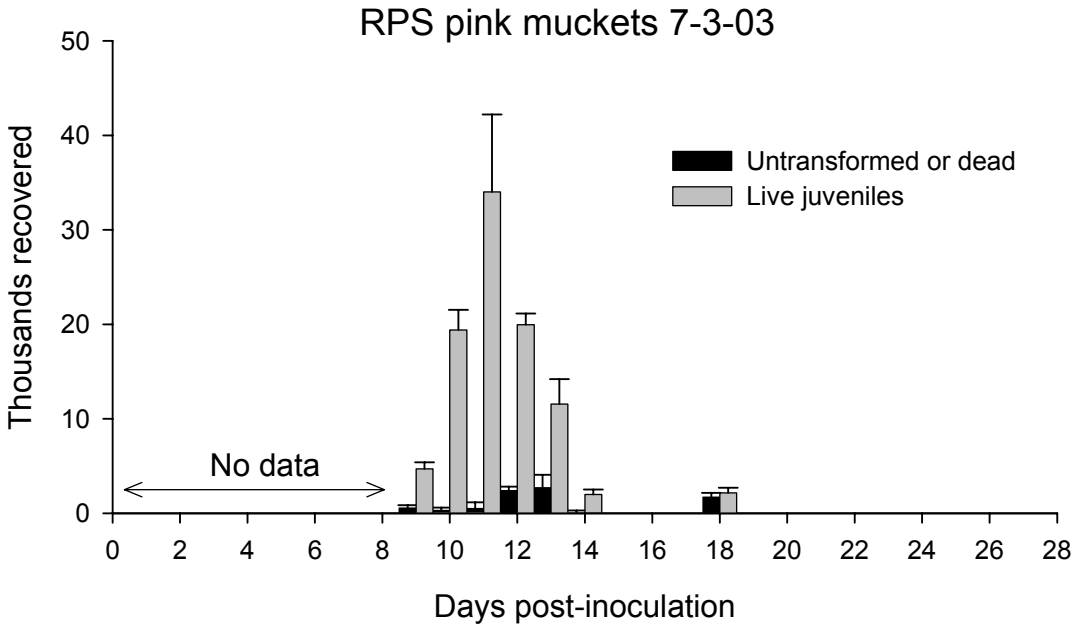


Figure 16. Recovery of pink muckets from 349 bass in the RPS. Fish were inoculated and kept at Chesapeake from 7/3- 7/10. Drop-off and temperature during that time were not recorded. Temperature in the RPS tanks was 23.5 ± SD 0.15. Data are daily totals ± 95% CI.

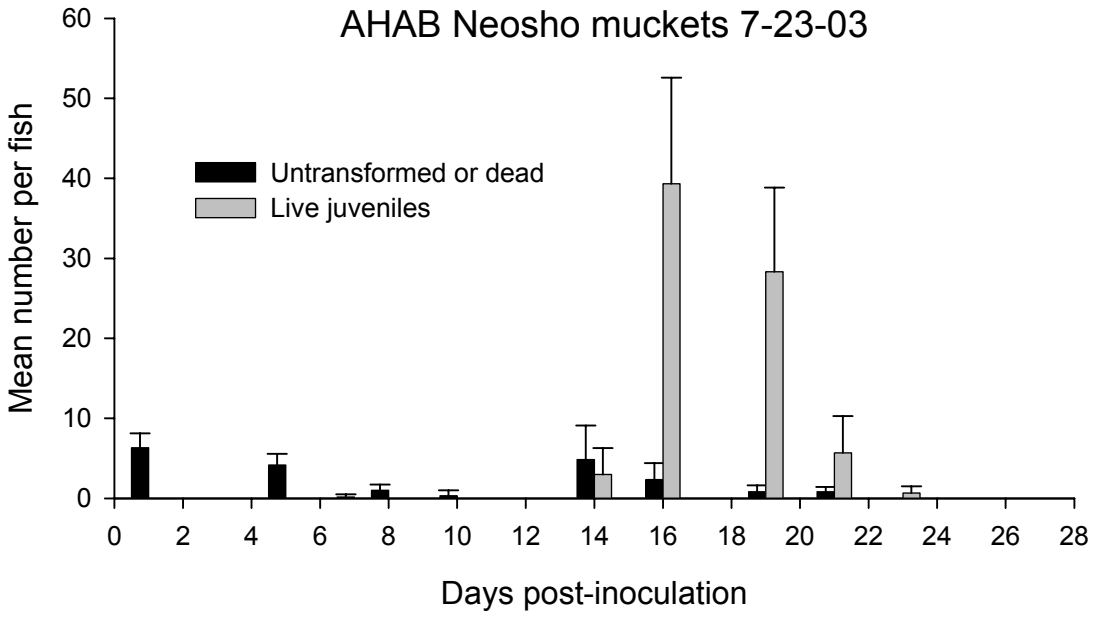


Figure 17. AHAB recovery of pink muckets from bass inoculated 7-23-03. Mean temperature was 20.4 ± SD 0.8 °C. Data are means ± 95% CI (n=6). * Indicates day when count was made but no sluffs or juveniles were found

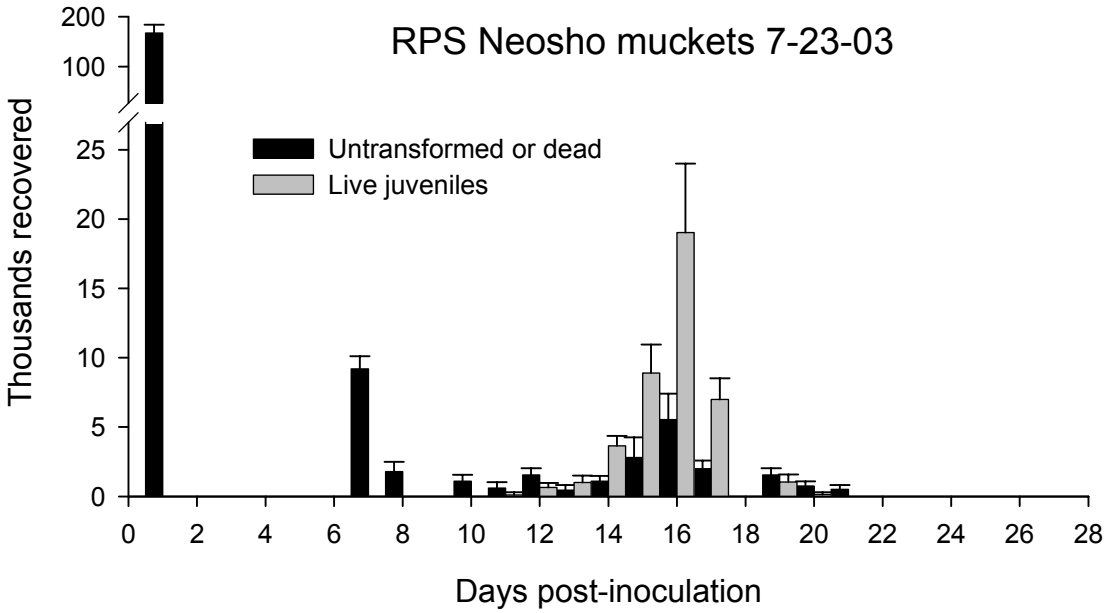


Figure 18. Recovery of Neosho muckets from bass in the RPS. The fish were inoculated in the RPS on 7/23/03. The large peak of sluffs recovered on day 1 are the glochidia that did not attach. No subsequent counts were made until day 7. Temperature was 24.2 ± 0.6 °C.

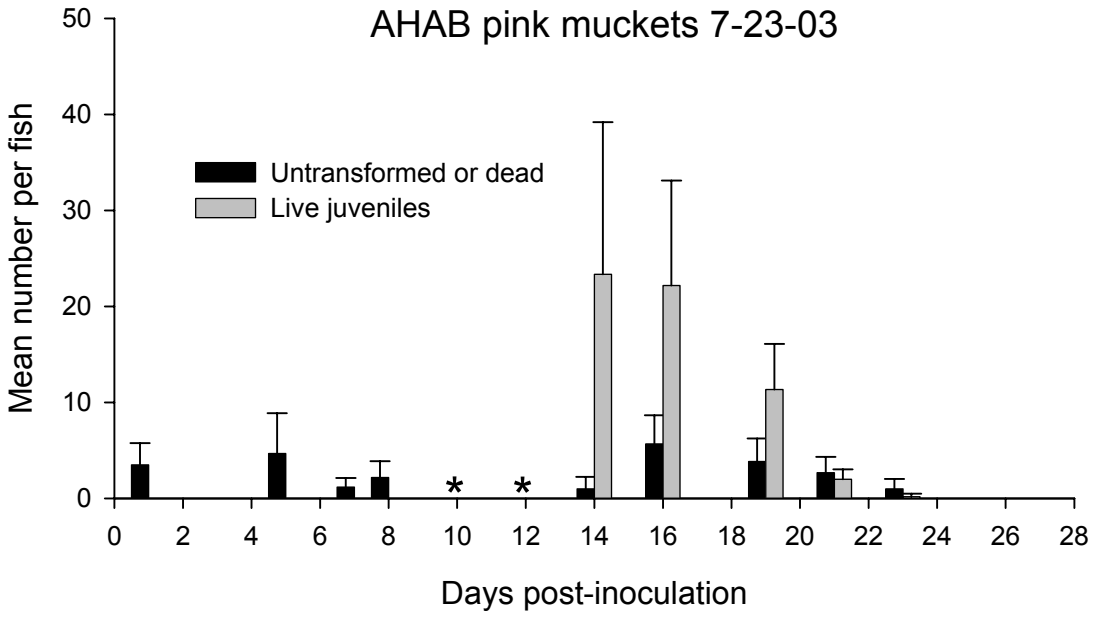


Figure 19. AHAB recovery of pink muckets from bass inoculated 7-23-03. Mean temperature was 20.5 ± 0.7 °C. Data are mean ± 95% CI (n=6). * Indicates day when count was made but no sluffs or juveniles were found

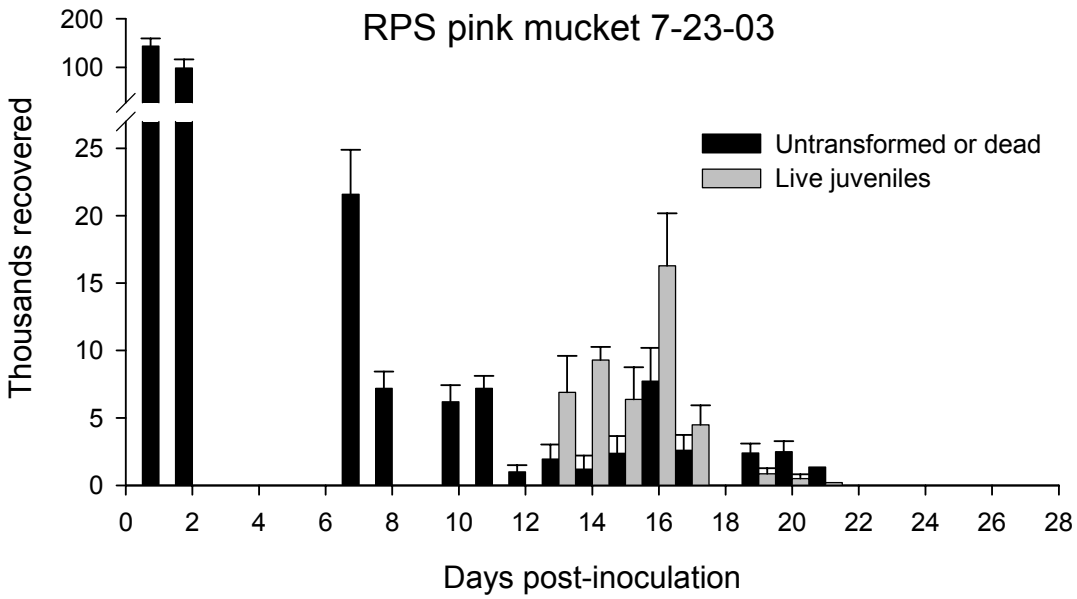


Figure 20. Recovery of pink muckets from the RPS. The drop-off pattern is complicated because 1) the main group of fish were inoculated in the RPS on 7-23, so that unattached glochidia from the inoculation were recovered, and 2) another group of inoculated fish was added on 7-25. The first group of 349 fish was inoculated in the RPS on day zero. The large number of sluffs on day 1 and 2 are the glochidia that did not attach. A second group of 138 fish was inoculated separately and added to the RPS on day 2. No subsequent counts were made until day 7, when a large number of sluffs was removed, probably mainly from the second group of fish. Two peaks of juvenile dropoff are evident on day 14 and day 16, presumably corresponding to the two inoculation groups.

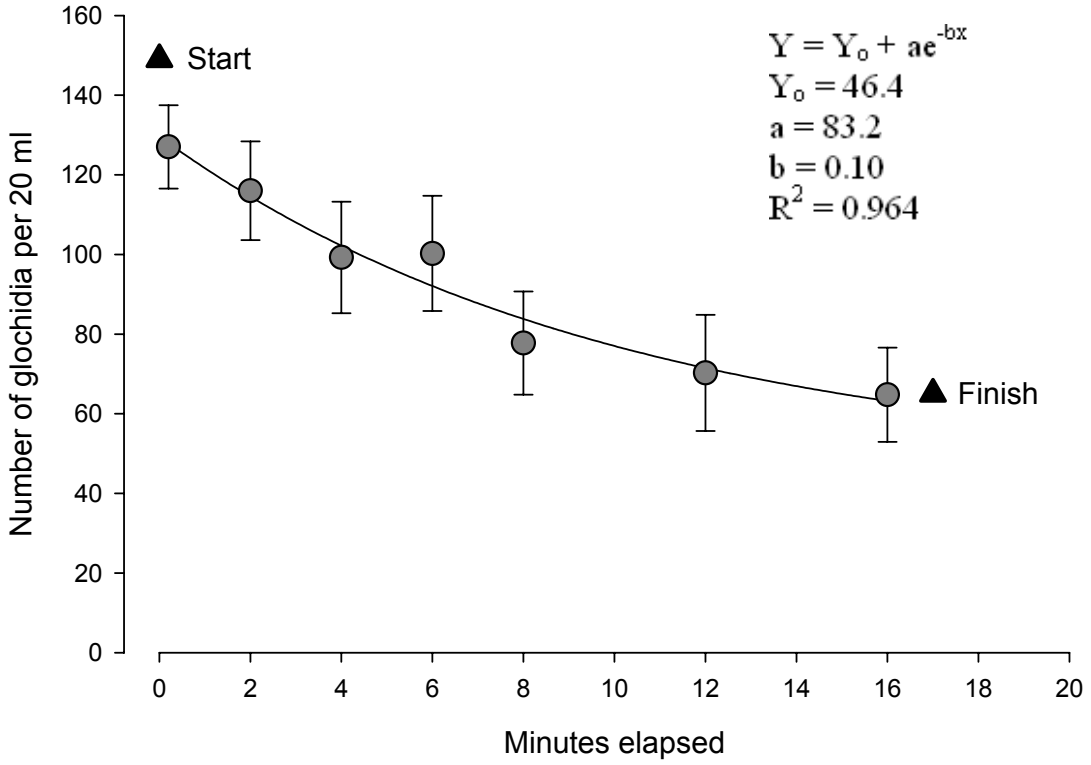


Figure 21. Time course of pink mucket attachment to bass during inoculation #2 (*Table 16*). Glochidia concentration in the bath declined over time as glochidia attached to the fish. Each symbol with error bars is the mean ± 95% CI of four 20-ml samples. The line was fitted by regression on these means. "Start" and "finish" are the concentrations calculated from bath volume and the total number of glochidia before and after the exposure.

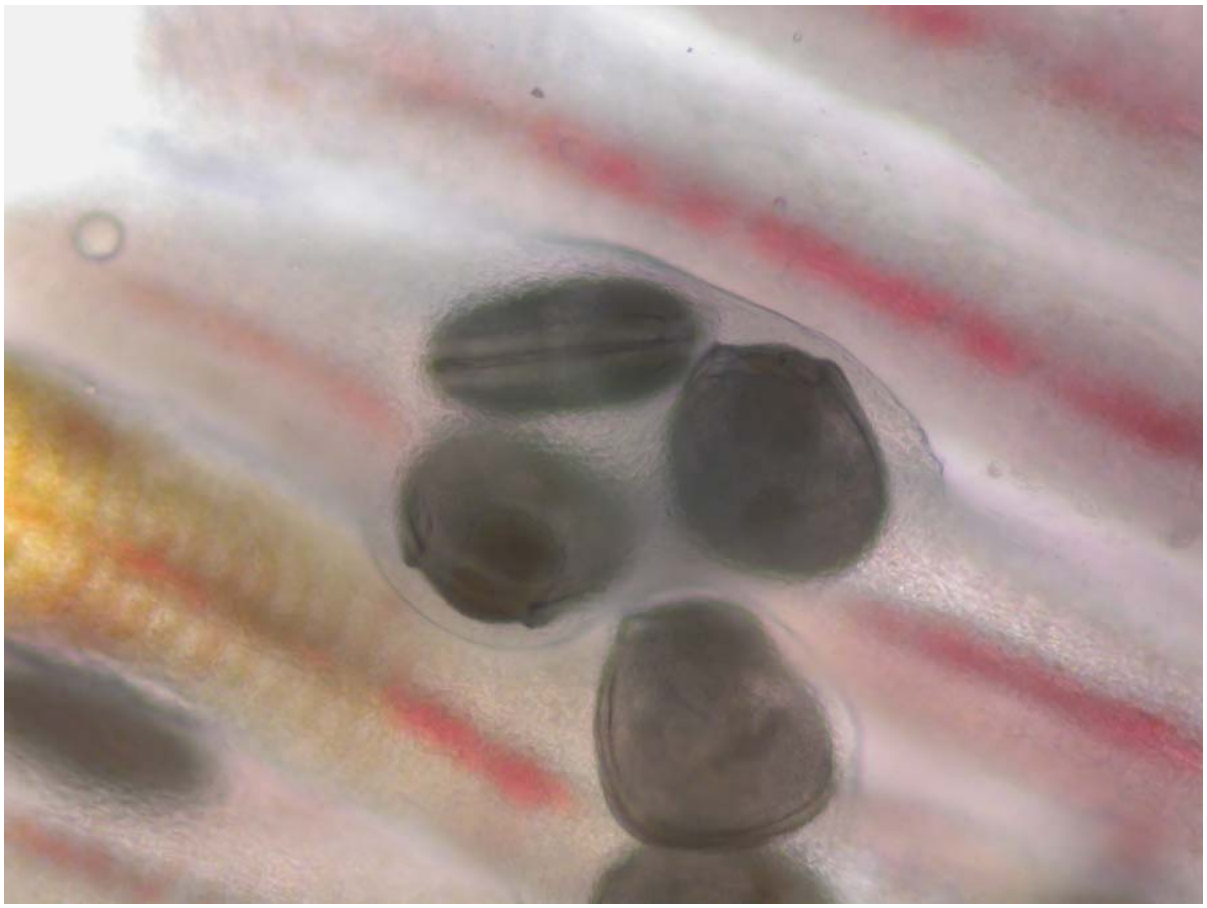


Figure 22. Example of a multiple cyst from a bass heavily infested with pink mucket glochidia. The upper group of 3 glochidia are all enclosed within a shared cyst.

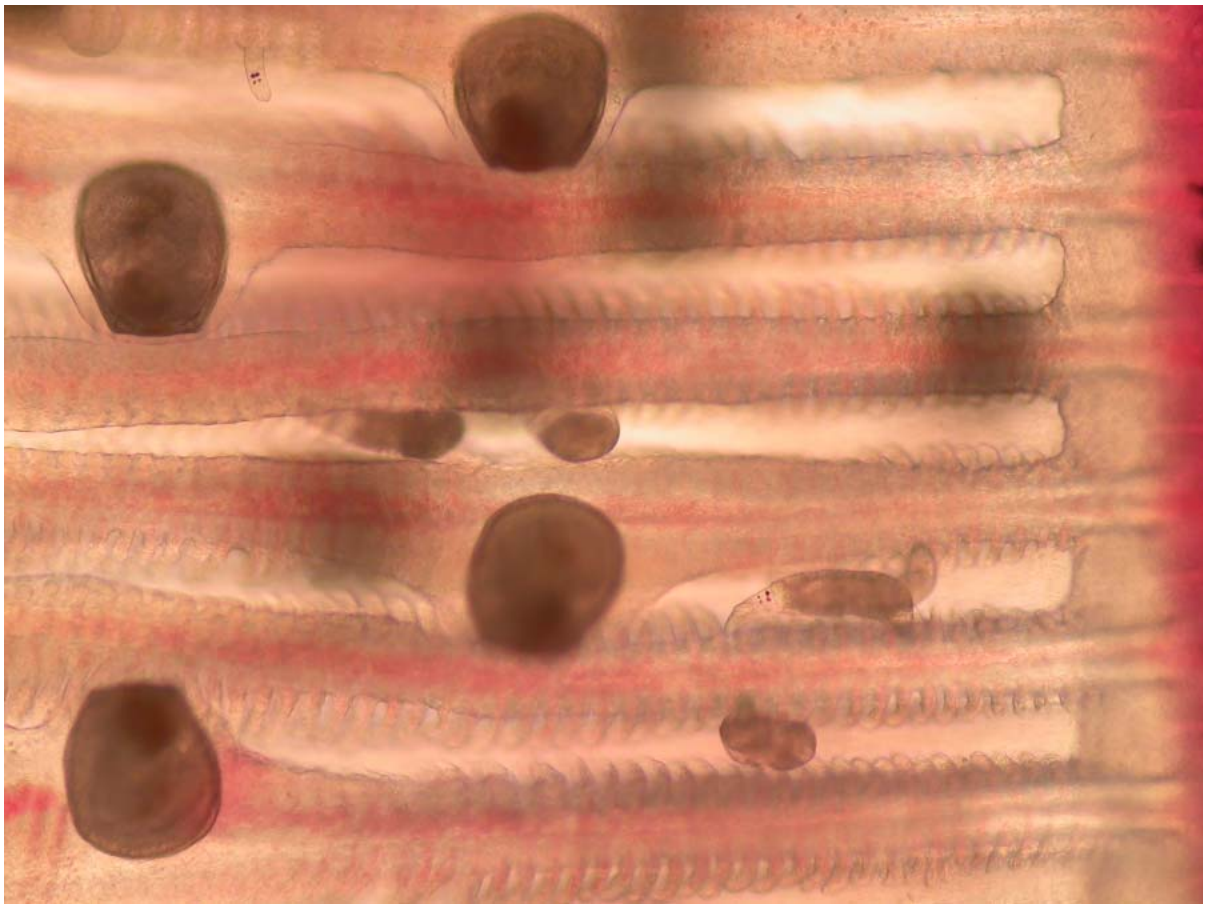


Figure 23. Heavy infestation of Monogenea (Dactylogyridae) coincident with a heavy infestation of pink mucket glochidia on bass.

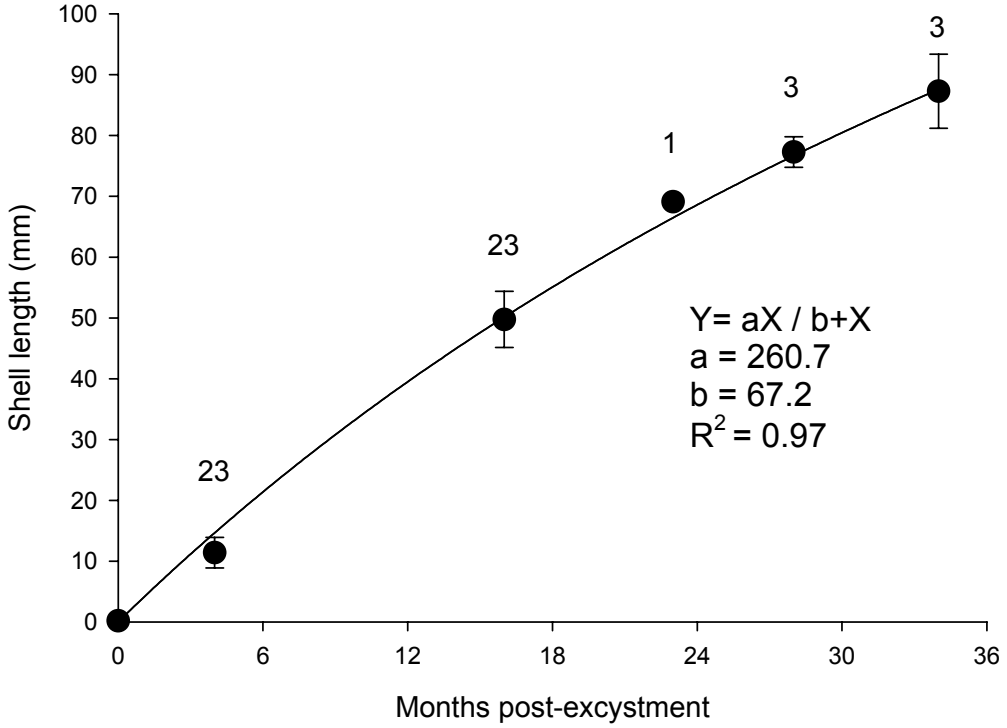


Figure 24. Growth of propagated Neosho muckets at a release site in the Verdigris River, Montgomery Co. KS. These mussels were released in August 2000 and have grown to 86 mm shell length at 34 months of age. Measurements are means ± SD, n is indicated. The line was fitted by regression.

Figure 25. A pair of 3-year old propagated Neosho muckets from the Verdigris River in Montgomery Co. KS. These individuals were released in summer 2000 and are approaching sexual maturity.

Figure 26. Neosho muckets damaged by otters. Upper: view through the shell gape of a live Neosho mucket with foot damaged, probably by otter. The edge of the foot has been torn away. Remarkably, this animal was still living. Lower: a recently dead animal from the same site, opened to show extensive laceration of the foot.

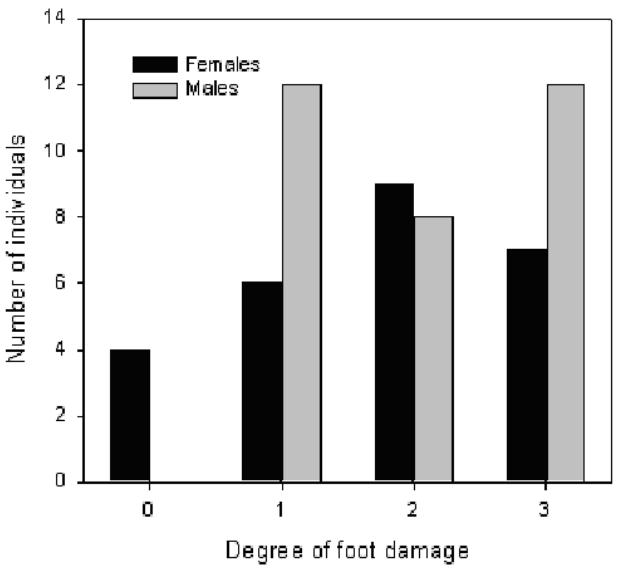


Figure 27. Frequency and degree of foot damage observed in Neosho muckets found out of the substrate at a site in the Spring River, Cherokee Co. KS. Nearly all live, exposed individuals exhibited foot damage, presumably the result of otters pulling animals up and biting the exposed foot. Damaged animals did not rebury themselves during a 3-day observation period.

0 = no damage.
1 = ¼ or less of foot edge damaged.
2= ¼-1/2 of foot edge damaged.
3= more than half foot edge damaged.