Fish Hosts and Culture of Mussel Species of Special Concern: Annual Report for 1999

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SUMMARY

This report describes results of the second year of a 3-year investigation of reproductive biology of freshwater mussels (unionoids). At least 21 North American unionoids are already extinct and 69 species are federally classified as endangered (Williams et al. 1993, Neves et al. 1997). The purpose of this project is to provide information that will facilitate conservation and management of these unique organisms. Parasitism of larval unionoids on fish is a central feature of their biology. Knowledge of the host fish and the requirements of the juvenile life stages are prerequisite for propagation and restoration of endangered species. Therefore, we are attempting to identify fish hosts and key reproductive behaviors and to investigate the biology of cultured juveniles.

During the past year we investigated hosts of four mussel species. Laboratory host tests with spectaclecase (*Cumberlandia monodonta*) on 26 potential host species were all negative. Examination of natural infestations of glochidia on fish revealed a few *Cumberlandia* glochidia on bigeye chub (*Notropis amblops*) and a single glochidium on shorthead redhorse (*Moxostoma macrolepidotum*). These host associations must be considered tentative until transformation is observed. A natural infestation of threehorn wartyback (*Obliquaria reflexa*) was found on goldeye (*Hiodonta alosoides*). This host association is highly probable because the glochidia were numerous and had grown while encysted. To our knowledge, this is the first host that has been identified for *Obliquaria*. Laboratory tests showed that black sandshell (*Ligumia recta*) transforms well on walleye and less well on largemouth bass. Laboratory tests also indicated a new host association for rabbitsfoot mussel (*Quadrula c. cylindrica*), which transformed successfully on blacktail shiner (*Cyprinella venusta*). These are apparently the first host tests for the nominate subspecies of this mussel.

A pilot propagation project was carried out with Neosho muckets (*Lampsilis rafinesqueana*). The Neosho mucket is not yet federally classified as endangered, but is being considered for listing. Glochidia were collected in the Fall River, KS, and transformed on largemouth bass at the Chesapeake Fish Hatchery. Approximately 400 fish were inoculated in two experiments. Methods for the production, collection, transportation and release of juveniles were tested. Over 19,550 juveniles from this study were released in the Fall River Wildlife Refuge in Kansas. This release was a reintroduction of this species to historic habitat, from stock collected downstream in the same river. The release site currently (but not historically) lacks this species, and is separated from the source population by Fall River Reservoir, reducing concern regarding genetic "swamping". Cooperators in this project included personnel of the Missouri Department of Conservation, Kansas Wildlife and Parks Department, and the U.S. Fish and Wildlife Service.

Other mussel projects in progress or completed in 1999 include 1) genetic and life history study of *Venustaconcha pleasii* and *V. ellipsiformis*, 2) experimental hypoxia tolerance of juvenile mussels, and 3) demography of *Cumberlandia monodonta* in the Meramec and Gasconade river systems.

1: HOST STUDIES

Introduction

In 1999 we investigated the host relationships of 4 mussel species. These are the spectaclecase (*Cumberlandia monodonta*), the black sandshell (*Ligumia recta*), the rabbitsfoot mussel (*Quadrula cylindrica*), and the three-horned wartyback (*Obliquaria reflexa*). These investigations included both laboratory host tests and a field survey of natural glochidia infections in the Meramec and Gasconade rivers. In addition to work with native host species, we investigated the ability of several unionids to transform on an introduced fish, the round goby (*Neogobius melanostomus*. This fish was introduced into the Great Lakes in the early 1990's and is expanding its range in streams and lakes of eastern North America. It appears to be a serious threat to native fishes, and therefore, potentially, threatens native mussels by displacing their hosts.

Methods

Laboratory host tests: Gravid female mussels were obtained from stream reaches in Missouri, by permission from Missouri Department of Conservation and the U.S. Fish and Wildlife Service. Mussels were checked in the field for gravidity by inspecting the gills, except in the case of *Cumberlandia,* which were difficult to assess visually (see below). Individuals that bore mature glochidia were returned to the lab and held in aquaria until host tests were performed. Mussels in the lab were kept at low temperature (10 °C) to reduce metabolic rate and starvation stress. Following host tests, individuals were either released at the capture site or were preserved for genetic studies.

Glochidia were recovered from mussels and handled as described previously (Barnhart 1997, 1998). Viability of a sub-sample of 50-100 individuals was tested by observing the closing response to saline solution, immediately before infection of fish. The proportion of viable glochidia was recorded and usually exceeded 95%.

Most fish species used for laboratory host tests were wild-caught individuals. Efforts were made to obtain fish from the same river system as the mussel being tested, because recent studies suggest that host specificity may vary among populations (Riusech 1999). The locality of origin of the fish was recorded. Fishes were identified according to Pflieger (1975) and Page and Burr (1991). Hatchery fishes, particularly largemouth bass (*Micropterus salmoides),* walleye (*Stizostedion vitreum*), and channel catfish (*Ictalurus punctulatus*), were used when convenient and appropriate. Specimens of the round goby, *Neogobius melanostomus,* were collected from the St. Clair River in Michigan by Dr. David Jude. Mudpuppies (*Necturus maculosus*), were obtained from the Carolina Biological Supply Company. According to the supplier, the locality of origin of these individuals was either Wisconsin or Minnesota. Their coloration was consistent with the northern subspecies (*N. m. maculosus*) that is also present in the Meramec drainage (Petranka 1998).

Wild-caught fishes were acclimated for at least one week after capture before use and were usually treated with antibiotics (Maracyn®) during this time. The fishes were maintained in 10 or 20-gallon aquaria, in most cases segregated by species. Fishes were fed with commercial flake or pellet food, frozen brine shrimp, frozen bloodworms, live blackworms, earthworms, or other fishes, depending upon species.

Methods for infection of the hosts varied with species. In most cases, test fishes were anaesthetized briefly with tricaine methanesulfonate (Finquel®, Argent Chemical) and infected by pipetting a suspension of glochidia directly onto the gills. In some cases fish were placed directly in a bath containing glochidia. Immediately after infection the fish were rinsed and examined to be sure that glochidia had attached.

One to five days after infection the fishes were again anaesthetized and examined under a dissecting microscope to determine whether glochidia were attached. Fishes infected with *Cumberlandia* glochidia, which are very small, were sacrificed and the gills were examined with a compound microscope to be sure that glochidia were not present. Fishes that retained glochidia during the initial period were segregated by species. Thereafter, the bottom of the aquarium was vacuumed daily to recover any detached juveniles. The water was filtered through 20-micron Nitex mesh, or through a 60-micron brass soil sieve, depending on the size of the juveniles. Glochidia and juveniles were recovered from the filtrate under a dissecting microscope, using polarizing filters to increase visibility of the shells. Movements of the foot were used to distinguish juveniles from glochidia.

Seasonal reproduction in *Cumberlandia*: Spectaclecase were collected ~monthly in the Meramec and Gasconade rivers, Missouri, from September 1998 to June 1999 (Table 1, Figure 1). Collections were not made in January or March 1998 due to high spring flows. Following collection, mussels were immediately placed individually in water in labeled plastic bags, which were placed in an insulated cooler. Mussels were transported to Southwest Missouri State University (SMSU), and transferred to aerated water in plastic shoeboxes in a 10^0 C incubator. Mussels were monitored weekly for conglutinate release.

Conglutinates were quantitatively collected from on eight individuals that were collected April 1-2, 1999 from the Meramec and Gasconade rivers (Table 1). Individuals were measured to determine total shell length, width, height, and total wet weight (Table 3). Thereafter, these mussels were held individually in shallow water in plastic shoeboxes, with just enough water to cover them. Water changes were performed as needed, and containers were checked daily for expelled conglutinates. Upon discovery, conglutinates were extracted from the water with a plastic pipette and placed in appropriately labeled jars containing 70 percent ethanol. Individuals were checked daily, until no more conglutinates were found. The conglutinates were counted, and measurements were made on a subset of 10 conglutinates from each set (Table 4).

Natural glochidia infestations: We examined fishes for natural infestations of glochidia in the Meramec River (site #8, Table 1) and the Gasconade River (site #5, Table 1). The primary goal was to identify potential hosts of *Cumberlandia*. Therefore, fishes were collected at and near known *Cumberlandia* beds, using boat and backpack electro fishing units and a seine. The collections were made on June 9-10, 1999. These dates are approximately 2 weeks after the last date on which gravid *Cumberlandia* were observed at these sites. From 1- 20 individuals of each fish species were captured per drainage. Fish were immediately placed on ice in insulated coolers, transported to SMSU, identified to species, and measured to total length (mm). Gills were dissected, given a unique identification number, and preserved in 90% ethanol until they could be examined for glochidia.

Preserved gills were later examined for attached glochidia. The gill arches from each fish were placed in Petri dishes in 1N NaOH solution. Sodium hydroxide hydrolyzes the gill tissue, freeing the glochidia shells. Gills were left in NaOH for 30 minutes to several days, depending on size. Glochidia shells were recovered from the digest and transferred to 70% ethanol. The shells were measured using an ocular micrometer, and were identified by comparison with a reference collection of glochidia of known identity.

Results

Seasonal reproduction in *Cumberlandia*: Gravid spectaclecase were collected during the first week of April through May 24 in 1999 (Table 1). A single small fragment of conglutinate, with mature glochidia, was recovered from the container in which Cumberlandia had been transported from the Meramec River on November 17 of 1998. The 20 mussels in this collection did not produce more conglutinates. Thus it appears that most individuals release glochidia in April and May, but that at least a few may also do so in November. Biannual reproduction has been suggested previously for this species (see Discussion).

Conglutinates were collected quantitatively from eight individuals. These individuals each released between 53 and 88 conglutinates (mean 64.5 ± std 13.9). The conglutinates were white and had a complex branched shape (Figure 1). The glochidia of *Cumberlandia* are extremely small (Figure 2).

Laboratory host tests: *Cumberlandia monodonta*: Glochidia obtained in April and May of 1999 were used for laboratory host tests. Thirty-five separate host tests were performed on 25 fish species, and one amphibian (Table 3). The glochidia encysted on all species tested, but the infections generally did not persist more than 3 days. Four species held encysted glochidia more than 4 days: these were flathead catfish (*Pylodictus olivaris*), channel catfish (*Ictalurus punctatus*), longear sunfish (*Lepomis megalotis*), and redear sunfish (*Lepomis microlophus*). These host tests did not identify a probable host. However, natural infestations of *Cumberlandia* glochidia were observed on two fish species (see below).

Ligumia recta: Glochidia from a single female from the Big River was tested on 4 fish species. Over 65% of attached glochidia transformed on hatchery walleye (Stizostedium vitreum). Low transformation success was observed on largemouth bass and round goby (Table 2).

Quadrula cylindrica: Four gravid females were obtained from the Black River in Old Davidsonville State Park near Pocahontas, Arkansas, on May 21, 1999. These individuals had mature glochidia at the time of collection and later released conglutinates. The glochidia were tested on 5 fish species, which were collected at the same site as the mussels. Transformation was observed on blacktail shiners (Cyprinella venusta), but not on 3 other minnow species or on bluegill (Table 3).

Obliquaria reflexa: Glochidia from a Sac River female were tested on 3 fish species, none of which produced juveniles (Table 4). These glochidia had very low viability, so the host tests are inconclusive. However, a probable host, the goldeye, was identified from the observation of a naturally infested fish collected in the Gasconade River (see below).

Round goby: Glochidia of five unionid species were tested on round gobies obtained from St. Clair River in Michigan. Low rates of transformation were observed in three of these species: *Lampsilis siliquoidea, L. reeveiana,* and *Ligumia recta* (Table 5).

Natural infestations: Gills from 690 individual fish (Gasconade n = 321, and Meramec n = 369) were examined for glochidia. These fish represented 32 species in ten families (Table 7). A total of 49 of 690 individuals (7.1%) yielded 1 or more glochidia (Table 8). Most of these glochidia were identified only to subfamily. However, two of the species recovered are sufficiently morphologically distinctive to allow species identification. *Cumberlandia* glochidia were found encysted on two fish species from the Meramec River: bigeye chub, and short-head redhorse. A single big-eye chub (*Notropis amblops*) carried at least 17 *Cumberlandia* glochidia on its gills, and one short-head redhorse (*Moxostoma macrolepidotum*) yielded a single *Cumberlandia* glochidium (Table 8). None of the 18 recovered *Cumberlandia* glochidia showed any sign of growth. No *Cumberlandia* glochidia were recovered from the fishes collected in the Gasconade River.

The other species positively identified in the natural infestations was the three-horn wartyback (*Obliquaria reflexa*). The glochidia of this species have a very distinctive shape that permits unambiguous identification. Nearly 70 glochidia were recovered from the gills of a single specimen of goldeye, (*Hiodonta alosoides*) in the Meramec River collection. These individuals had grown substantially on the host, which greatly strengthens the evidence that goldeye is a suitable host of *Obliquaria*. *Obliquaria* glochidia failed to attach to goldeye or other potential hosts in one lab test (Table 5), but those glochidia were in poor condition, with less than 20% closing in response to salt. We plan further tests with this species.

Discussion

Cumberlandia: Identification of the hosts of *Cumberlandia* has proven to be a difficult task. Host tests have also been performed on this species from the St. Croix River in Minnesota. Hove et al. (1998, and personal communication) tested 24 species without success, including 17 species not tested in our study. Thus at least 43 possible hosts have been tested, and to our knowledge, transformation of *Cumberlandia* has not yet been observed. The conglutinates produced by *Cumberlandia* are conspicuous enough to attract the attention of a wide variety of fish, and therefore provide little evidence as to which species may be the host. However, the habitat preference of these mussels is unusual and may provide some clues. *Cumberlandia* are strongly associated with large boulders and cobble at the base of bluffs on outside river bends. In these areas the adult mussels congregate in very dense clusters (up to $120/m^2$) often concentrated under rocks and in crevices. These are generally areas that combine high substrate stability and shelter with fairly strong current. An effective host species would presumably frequent these same areas.

We were able to recover *Cumberlandia* glochidia from natural infestations of two potential hosts. These were the bigeye chub, *Notropis* (*Hybopsis*) *amblops*, and the shorthead redhorse (*Moxostoma macrolepidotum*). Whether either or both of these two species are actually hosts of *Cumberlandia* remains to be established. *Cumberlandia* glochidia are extremely small (Figure 2). We assume that *Cumberlandia* glochidia will remain encysted for a relatively long period (weeks to months) and will grow to several times their initial length while encysted on the host, because these are characteristics of other Margaritiferids. The case for identifying these fish as hosts would be strengthened, if the encysted glochidia had grown, but this was not the case. On the other hand, the lack of growth could indicate that the glochidia had only recently attached, or that our assumption that this species grows while encysted may be in error. In any event, it is clear that these two species should be tested with laboratory infections, and we hope to accomplish that task in the coming year.

<u>*Ligumia recta*</u>: We found high transformation success of this species on walleye. Recent studies at Tennessee Tech also demonstrated high transformation success on sauger, which produced means of 881 and 3157 juveniles per fish in two different trials (Dr. James Layzer, personal communication). In those studies, largemouth bass, bluegill, and white & black crappie also produced a few juveniles but had relatively poor transformation success. Black sandshell glochidia from the St. Croix River in Minnesota were also able to transform on walleye, largemouth bass, and bluegill sunfish (Mark Hove, U. Minnesota, personal communication). Although older literature (pre-1930) suggests several that other centrarchid species may also be potential hosts (reviewed by Watters 1994), the recent work indicates that *Stizostedion* may be the most important hosts. The hybrid “saugeye” have not yet been tested, but should also prove to be suitable.

Like many other lampsilines, female black sandshells display a lure to attract fish hosts for their glochidia larvae. During the summer of 1995, we observed the lure behavior of a gravid female black sandshell in an aquarium at SMSU for several weeks. The display occurred primarily at night. The mussel lay on her side on the surface of the substrate. The posterior third of the ventral mantle edge was fringed with papillae that inflated to nearly an inch in length. Waves of movement ran quickly along the mantle, spreading and closing these fringes, and exposing the gravid marsupial gills within. The edges of the marsupial gills were beaded and snow white in color, appearing like two rows of pearls just inside the waving fringes. This large and conspicuous lure is consistent with the use of a relatively large, piscivorous host.

The conservation status of *Ligumia recta* in Missouri is not well understood but should be viewed with caution. This species is relatively uncommon in Missouri, comprising only 0.5% of unionids recovered in a major survey of the Meramec River basin in 1997 (A. Roberts, USFWS and S. Bruenderman, MDC, in preparation), 0.5% in the lower Osage system (Grace and Buchanan 1981), and 0.1% in the Little Black River system (Buchanan 1979).

The black sandshell is one of at least five species of unionids that have apparently been extirpated in Kansas in historic times. This species formerly occurred commonly in the rivers of SE Kansas (Scammon 1906). However, it apparently has not been collected alive in KS since 1912. Extensive recent surveys of rivers in eastern Kansas recovered only weathered shells of this species (Obermeyer et al. 1997). The loss of black sandshell from Kansas might be directly related to the loss of host fish populations. According to Cross & Collins (1995): "Walleye were recorded from Kansas as early as 1865 and may have occurred naturally in rivers of eastern Kansas at that time. If so, they soon disappeared. Efforts were made to establish the species by reintroduction at least as early as the 1880s, but all attempts were unsuccessful until the 1960's."

Although walleye and saugeye have been stocked in several Kansas reservoirs and lakes in the last decade, the Kansas Stream Survey of the Neosho basin in 1997 failed to locate walleye or saugeye at any site (Edwin Miller, KDWP, personal communication). Restoration of black sandshell in Kansas or elsewhere might be linked with stocking programs to establish walleye or saugeye populations in selected streams that historically supported *Ligumia recta*.

Quadrula cylindrica: The rabbitsfoot mussel is classified as endangered in Missouri and Kansas, but not in Arkansas. It is probably now restricted to the Black and St. Francis river systems in Missouri and Arkansas, and portions of the Spring and Neosho rivers in western Missouri and eastern Kansas (Oesch 1984, Obermeyer et al. 1997). It has apparently been extirpated from other Neosho River tributaries in Missouri including Shoal Creek and Center Creek, and in Kansas from most of its former range (Obermeyer et al. 1997). The western populations of this species appear to in serious trouble, and the genetic identity of these mussels with the more abundant population in the Black River should be determined as soon as possible.

Our results are apparently the first host tests for populations of this species west of the Mississippi. The blacktail shiner has not previously been reported as a host. Previous studies of Tennessee populations showed that glochidia were able to transform on bigeye chub (*Notropis amblops*), spotfin shiner (*Cyprinella spiloptera*), and whitetail shiner (*Cyprinella galactura*) (Yeager and Neves 1986).

Obliquaria reflexa: The threehorn wartyback is a very distinctive species that produces a small number of very large, compact, and unusually solid conglutinates. Although *Obliquaria* is a relatively common mussel in many rivers, no hosts have previously been reported for this species. We have had difficulty obtaining viable glochidia. On at least two occasions, gravid individuals were collected and produced conglutinates that appeared healthy, with no signs of fungus or protist infections, but with most glochidia unresponsive and unable to attach effectively to host fish. Further laboratory investigations of potential hosts of this species are needed. However, the observation of a heavy natural infestation of glochidia on goldeye, coupled with the fact that these glochidia had grown within the cysts, makes a compelling argument that this fish is a host. The relatively large mouth and well-developed teeth of goldeye could easily rupture the solid conglutinates of *Obliquaria*. The two species are both distributed in Mississippi River and Missouri River tributaries in Missouri.

Round gobies: The host tests with round goby suggest the possibility that this invading species might be able to serve as a host for at least some native unionids. These results were somewhat less discouraging than our previous host work with carp, which we have not found to be a suitable host for any of 4 unionids that we tested (Barnhart 1997, 1998). Carp may be a suitable host of some anodontine unionids that encyst on fins (Watters 1994). Displacement of native fishes by intentionally or accidentally introduced alien species could be a major factor impacting unionid reproduction.

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Figures and tables

Figure 1. Conglutinates of *Cumberlandia monodonta* are shown alongside the female mussel that released them. Each conglutinate is approximately 10-15 mm long.

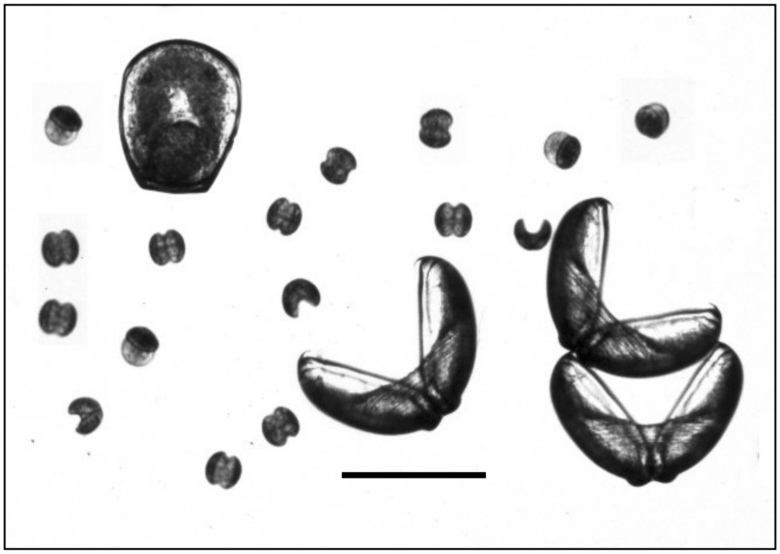

Figure 2. The glochidia of *Cumberlandia monodonta* are shown alongside the much larger larvae of *Lampsilis siliquoidea*. Scale line = 250 microns. Other Unionoid species with very small glochidia typically grow to > 200 microns before leaving the host.

Table 1. Reproductive periodicity in *Cumberlandia*. Collections are listed chronologically. Numbers of individuals examined = n. Condition: G= gravid (i.e. bearing glochidia in the marsupial gills), N/G = not gravid; * = see results.

Site #	Drainage	Legal description	Date	n	Condition
1	Meramec	Franklin T40NR01WS07	09-08-98	18	NG
2	Meramec	Jefferson T43NR4ES19	09-09-98	19	NG
3	Gasconade	Pulaski T36NR10WS13	09-29-98	11	NG
4	Gasconade	Maries T40NR8WS8	10-27-98	20	NG
5	Gasconade	Osage T42NR8WS15/16	10-27-98	20	NG
6	Gasconade	Pulaski T36NR13WS22/23	10-29-98	20	NG
7	Meramec	Franklin T43NR2ES20SE	11-17-98	20	NG*
8	Meramec	Crawford T39NR2WS15	11-17-98	20	NG
6	Gasconade	Pulaski T36NR13WS22/23	12-30-98	10	NG
8	Meramec	Crawford T39NR2WS15	02-25-99	7	NG
6	Gasconade	Pulaski T36NR13WS22/23	02-25-99	8	NG
6	Gasconade	Pulaski T36NR13WS22/23	04-01-99	10	G
7	Meramec	Franklin T43NR2ES20SE	04-02-99	12	G
6	Gasconade	Pulaski T36NR13WS22/23	05-03-99	10	G
8	Meramec	Crawford T39NR2WS15	05-03-99	10	G
8	Meramec	Crawford T39NR2WS15	05-19-99	17	G
6	Gasconade	Pulaski T36NR13WS22/23	05-19-99	12	G
2	Meramec	Jefferson T43NR4ES19	05-24-99	10	G
7	Meramec	Franklin T43NR2ES29SE	05-24-99	10	G
5	Gasconade	Osage T42NR8WS15/16	06-09-99	6	NG

Table 2. Numbers and dimensions of conglutinates expelled by 8 individual *Cumberlandia*. Conglutinate measurements are means ± 1 standard deviation.

Drainage	Mussels		Conglutinates			
	Length (mm)	Mass (g)	N	Length (mm)	Diameter (mm)	Mass (g)
Gasconade	138.10	149.02	60	15.78± 4.19	3.70± 1.23	0.15± 0.06
	124.45	98.36	64	13.69± 2.56	2.51±1.11	0.07± 0.03
	148.35	145.00	60	10.02± 5.62	2.45± 0.87	0.09± 0.05
	N/A	N/A	53	15.10± 3.43	2.98± 1.55	0.12± 0.09
Meramec	166.50	262.48	88	14.14± 3.88	2.36± 0.52	0.11± 0.04
	190.90	381.34	84	15.10± 3.68	2.72± 0.66	0.12± 0.06
	139.00	118.5	54	12.32± 2.20	3.09± 1.24	0.06± 0.02
	N/A	N/A	53	12.42± 2.91	2.97± 0.94	0.13±0.10

Table 3. Host tests for spectaclecase mussel, *Cumberlandia monodonta.* Hosts were kept at 20°C. Glochidia did not transform on any of the 26 host species tested. N = number of individuals infected.

Locality of mussel collection	**Test date(s)**	**Number or sex ratio (M/F)**	**Viability of glochidia**	**Method of infection & remarks**
Gasconade River (T36NR13WS22/23)	4-01-99 4-09-99 4-15-99	N=10; 30/70	100% 100% 100%	Female mussels were newly collected. Glochidia were pipetted onto gills.
Meramec River (T43NR2ES29SE)	4-5-99	N=10; 50/50	98.2%	Mussels were kept at 10°C for three days. Glochidia were pipetted onto gills.
Big River	5-24-99 12-2-99	N=10; 70/30	97.6% 100%	Female mussels were newly collected. Hosts were kept at 20°C. Fishes were fed viable conglutinates.

Host species	**Host locality and collection date**	**Date Infected**	**N**	**Success**	**Remarks**
Catostomidae					
Hypentilium nigricans Northern hogsucker	James River 04-21-99	05-24-99	2	No	Sac. On 05-31-99. No encysted glochidia found.
Moxostoma duquesnei Black redhorse	James River 04-21-99	05-24-99	1	No	Sac. On 05-31-99. No encysted glochidia found.
Centrarchidae					
Micropterus salmoides largemouth bass	Chesapeake Hatchery 06-19-98	04-01-99	3	No	Cysts off within two days.
Micropterus punctulatus Spotted bass	Meramec River 04-02-99	04-05-99	2	No	Cysts off within two days.
Micropterus dolomieu Smallmouth bass	Gasconade River 10-15-99	12-02-99	2	No	Sac. On . No glochidia found.
Lepomis megalotis Longear sunfish	Meramec River 04-02-99	04-05-99	2	No	Cysts lasting for four days.
	James River 04-21-99	05-24-99	1	No	Sac. On 05-31-99. No encysted glochidia found.
	Gasconade River 10-15-99	12-02-99	2	No	Sac. On 05-31-99. No encysted glochidia found.
Lepomis macrochirus Bluegill	Midwest Science Center – Columbia 04-12-99	05-24-99	6	No	No remarks.
Cottidae					
Cottus carolinae Mottled sculpin	N/A	04-05-99	1	No	Cysts off within two days.
Cottus bairdi Banded sculpin	Pearson Creek 04-21-98	04-05-99	3	No	Cysts off within two days.

Host tests for *Cumberlandia monodonta*, continued.

Cyprinidae

Notropis chrysocephalus striped shiner	Meramec River 04-25-98	04-01-99	2	No	Cysts off within two days.
Pimephales promelas fathead minnow	Midwest Science Center – Columbia 04-12-99	05-24-99	6	No	Cysts off within four days.
Cyprinella venustus blacktailed shiner	Black River, Arkansas, 05-21-99	05-24-99	4	No	Cysts off within two days.
Campostoma oligolepis largescale stoneroller	Starks Creek 04-12-99	05-24-99	2	No	Sacrificed 05-31-99. No encysted glochidia found.
	James River 04-21-99	05-24-99	1	No	Sacrificed 05-31-99. No encysted glochidia found.
Nocomis biguttatus Hornyhead chub	James River 04-21-99	05-24-99	3	No	Sacrificed 05-31-99. No encysted glochidia found.
Luxilus zonatus Bleeding shiner	Meramec River 04-25-98	04-01-99	3	No	Cysts off within two days.
Notropis nubilus Ozark shiner	James River 04-21-99	05-24-99	7	No	Sacrificed 05-31-99. No encysted glochidia found.

Gobiidae

Neogobius melanostomus Round goby	Lake St. Clair River	04-1-99	3	No	Cysts off within four days.

Ictaluridae

Pylodictus olivaris Flathead catfish	Missouri River 10-02-98	04-01-99 04-05-99	4 3	No	Cysts off within four days.
Ictalurus punctatus Channel catfish	Missouri River 10-02-98	04-01-99	4	No	Cysts off within four days.
Noturus exilis Slender madtom	James River 02-21-99	05-24-99	1	No	Sacrificed 05-31-99. No encysted glochidia found.
	Starks Creek 04-12-99	05-24-99	1	No	Sacrificed 05-31-99. No encysted glochidia found.

Percidae

Etheostoma spectabile Orangethroat darter	Pearson Creek 04-21-98	04-01-99 04-09-99	4 3	No No	Cysts off within two days. Cysts off within two days.
Percina caprodes logperch	Meramec River 04-25-98	04-05-99	2	No	Cysts off within two days.
Etheostoma tetrazonum Missouri saddled darter	Meramec River 04-25-98	04-05-99 04-09-99	1 1	No No	Cysts off within two days. Cysts off within two days.
Etheostoma blennioides Greenside darter	Meramec River 04-25-99	04-05-99	4	No	Cysts off within two days.
Etheostoma caeruleum Rainbow darter	Meramec River 04-25-98	04-09-99	9	No	Cysts off within two days.

Sciaenidae

Aplodinotus grunniens Freshwater drum	Missouri River 10-02-98	04-01-99	2	No	Cysts off within two days.

Table 4. Host tests for black sandshell, *Ligumia recta*. Hosts that were tested on 03-29-99 were infected simultaneously with *L. recta* (on right gills) and *L. siliquoidea* (on left gills). N = number of individual fish that were tested.

Locality of mussel collection	**Test date(s)**	**Number of females**	**Viability of glochidia**	**Method of infection & remarks**
Big River 04-11-98	03-04-99 03-29-99 09-24-99	1	90% 93.3% 68.2%	Glochidia were pipetted onto gills.

Host species	**Host locality and collection date**	**Date Infected**	**N**	**Success**	**Remarks**
Centrarchidae					
Micropterus salmoides Largemouth bass	Chesapeake Hatchery 06-19-98	03-04-99	4	Yes	23.4% transformation of those that attached.
Gobiidae					
Neogobius melanostomus Round goby	Lake St. Clair River 1998	03-29-99	3	Yes	A few juveniles were found.
Percidae					
Stizostedion vitreum walleye	Chesapeake Hatchery 09-13-99	09-24-99	5	Yes	65.5% transformation of those that attached.

Table 5. Host test for rabbit's foot mussel, *Quadrula cylindrica cylindrica.* Glochidia transformed on one of five host species tested, the blacktail shiner. N = number of individual fish that were tested.

Locality of mussel collection	**Test date(s)**	**Number of females**	**Viability of glochidia**	**Method of infection & remarks**
Black River near Pocahontas, AR 05-21-99	05-31-99	4	~100%	Glochidia were pipetted onto gills.

Host species	**Host locality and collection date**	**Date Infected**	**N**	**Success**	**Remarks**
Centrarchidae					
Lepomis macrochirus Bluegill	Black River (AR) 05-21-99	05-31-99	2	No	Cysts off within two days.
Cyprinidae					
Cyprinella venusta Blacktail shiner	Black River (AR) 05-21-99	05-31-99	6	**Yes**	A few juveniles were found.
Pimephales promelas Fathead minnow	Black River (AR)	05-31-99	4	No	Cysts off within two days.
Notropis volucellus Mimic shiner	Black River (AR)	05-31-99	1	No	Fish died within a day. Glochidia were still present.
Notropis atherinoides Emerald shiner	Black River (AR)	05-31-99	1	No	Fish died within a day. Glochidia were still present.

Table 5. Host tests for three-horn warty-back, *Obliquaria reflexa*. Glochidia did not transform in any of the laboratory host tests, but the condition of the glochidia was poor. Goldeye was identified as a host from natural infestations (see results). N = number of individual hosts infected.

Locality of mussel collection	Test date(s)	Number of females	Viability of glochidia	Method of infection & remarks
Sac River 08-29-99	08-30-99	1	Roughly 20%	Mussels released conglutinates during transport. Kept conglutinates at room temperature for one day before use. Pipetted glochidia on gills.

Host species	Host locality and collection date	Date Infected	N	Success	Remarks
Cyprinidae					
Luxilus chrysocephalus Striped shiner	Bull Creek 06-01-99	08-30-99	1	No	Glochidia were off by day 3.
Cyprinella venusta Blacktail shiner	Black River (AR) 05-21-99	08-30-99	5	No	Glochidia were off by day 3.
Hiodontidae					
Hiodon alosoides Goldeye	Missouri River 07-26-99	08-30-99	7	No	Glochidia were off by day 3.

Table 6. Host suitability tests using the round goby. N = number of fish infected.

Mussel species	Host locality and collection date	Date Infected	N	Success	Remarks
Strophitus undulatus Creeper				No	
Lampsilis siliquoidea Fat mucket	Stockton reservoir Fall 98	01-21-99	1	No	Cysts gone within four days.
Lampsilis siliquoidea **Fat mucket**	Stockton reservoir Fall 98	03-29-99	3	Yes	A few juveniles were found.
Ligumia recta **Black sand shell**	Spring River 04-99	03-29-99	3	Yes	A few juveniles were found.
Cumberlandia monodonta Spectaclecase mussel	Gasconade River 04-99	04-01-99	4	No	Cysts off within four days.
Potamilus alatus Pink heelsplitter	Elk River Fall 99	03-04-99	4	No	Cysts off within four days.
Lampsilis reeveiana **Broken-rays mussel**	Little Black River 10-98	03-04-99	4	Yes	31% transformation of those that attached.

Table 7. Fishes collected from the Gasconade River to assess natural glochidia infections.

Family	Species	n	Size range (mm)	Mean length (mm)
Atherinidae	*Labidesthes sicculus*	1	83	83.00± 0.00
Catostomidae	*Moxostoma duquesnei*	15	124-351	207.00± 55.08
	Moxostoma erythrurum	28	102-453	224.39± 94.16
	Moxostoma carinatum	3	227-593	410.00± 258.80
	Moxostoma macrolepidotum	12	143-390	289.33± 91.00
	Ictiobus bubalus	12	137-485	351.33± 90.23
	Ictiobus cyprinellus	1	372	372.00± 0.00
	Carpiodes carpio	1	375	375.00± 0.00
	Carpiodes cyprinus	3	307-390	357.33± 44.23
	Carpiodes velifer	4	304-371	328.25± 29.41
	Hypentelium nigricans	4	111-265	159.5± 71.00
Centrarchidae	*Lepomis macrochirus*	18	64-184	119.00± 33.11
	Lepomis megalotis	25	84-186	130.48± 21.59
	Ambloplites rupestris	6	141-221	181.67± 33.64
	Micropterus salmoides	7	225-298	259.43± 24.07
	Micropterus punctulatus	11	196-367	276.18± 53.33
Clupeidae	*Dorosoma cepedianum*	14	230-364	303.29± 39.13
Cyprinidae	*Luxilus zonatus*	25	64-102	81.68± 9.20
	Cyprinella whipplei	11	62-87	73.64± 8.90
	Notropis rubellus	26	55-70	60.85± 3.93
	Campostoma oligolepis	23	70-106	88.70± 9.03
	Pimephales notatus	9	58-80	66.22± 8.41
Hiodontidae	*Hiodon alosoides*	1	294	294.00± 0.00
Ictaluridae	*Ictalurus punctatus*	14	246-545	456.14± 70.15
	Pylodictus olivaris	2	346-440	393.00± 66.47
Lepisosteidae	*Lepisosteus platostomus*	3	524-640	588.33± 59.03
	Lepisosteus osseus	1	405	405.00± 0.00
Percidae	*Percina caprodes*	1	125	125.00± 0.00
	Etheostoma blennioides	1	74	74.00± 0.00
	Etheostoma tetrazonum	26	52-65	58.96± 0.00
Sciaenidae	*Aplodinotus grunniens*	13	265-563	377.15± 102.11

Table 7. Fishes collected from the Meramec River to assess natural glochidia infections. These fishes were collected at on 10 June 1999 Fish Trap Rapids…?

Family	Species	n	Size range (mm)	Mean length (mm)
Catostomidae	*Moxostoma duquesnei*	30	122-382	291.67± 62.21
	Moxostoma erythrurum	12	284-385	349.83± 28.52
	Moxostoma carinatum	7	410-630	499.14± 72.06
	Moxostoma macrolepidotum	12	285-362	317.17± 26.51
	Ictiobus bubalus	10	247-402	288.00± 59.30
	Ictiobus cyprinellus	1	420	420.00±0.00
	Hypentelium nigricans	8	260-390	345.00± 45.61
Centrarchidae	*Lepomis macrochirus*	8	67-151	113.34± 35.05
	Lepomis megalotis	28	72-156	110.00± 24.94
	Ambloplites rupestris	1	145	145.00± 0.00
	Micropterus salmoides	2	255-319	287.00± 45.25
	Micropterus dolomieu	8	209-343	278.86± 48.26
Clupeidae	*Dorosoma cepedianum*	22	175-280	224.14± 31.04
Cottidae	*Cottus carolinae*	25	32-101	50.76± 16.85
Cyprinidae	*Luxilus zonatus*	25	65-106	82.68± 12.85
	Cyprinella whipplei	3	96-125	108.67± 14.84
	Notropis rubellus	16	57-70	62.25± 3.30
	Campostoma oligolepis	25	68-92	79.20± 5.57
	Pimephales notatus	11	53-84	67.00±10.56
	Notropis amblops	25	60-76	65.28± 3.66
	Notropis nubilus	11	60-68	64.00± 2.61
	Luxilus chrysocephalus	1	145	145.00± 0.00
	Notropis ludibundus	1	153	153.00± 0.00
	Notropis volucellus	21	58-70	64.76± 4.01
Ictaluridae	*Pylodictus olivaris*	1	310	310.00± 0.00
	Noturus exilis	11	57-91	71.36± 10.50
Lepisosteidae	*Lepisosteus osseus*	1	790	790.00± 0.00
Percidae	*Etheostoma blennioides*	2	63-66	64.50± 2.12
	Etheostoma tetrazonum	17	50-77	63.88± 8.98
	Etheostoma caruleum	11	47-63	52.00± 5.23
	Percina evides	2	66	66.00± 0.00
Scianidae	*Aplodinotus grunniens*	11	245-500	367.64± 89.40

Table 8. Glochidia recovered from fishes collected in the Meramec and Gasconade Rivers. N = number of glochidia recovered per fish. Glochidia shell length, height, and hinge length are mm (mean ± standard deviation).

Fish species	Mussel family	n	Length	Height	Hinge
I. punctatus (n=11)	Lampsilinae	1	0.24	0.27	0.12
	Lampsilinae	4	0.237±0.006	0.297± 0.006	0.095±0.007
	Lampsilinae	1	0.28	0.31	0.09
	Lampsilinae	1	0.23	0.31	0.10
	Lampsilinae	1	0.28	0.32	0.12
	Lampsilinae	1	0.22	0.26	0.12
	Lampsilinae	6	0.28	0.34	0.13
	?	1	0.21	0.17	N/A
	Lampsilinae	1	0.23	0.29	0.09
	Lampsilinae	2	0.21	0.29	0.09
	Lampsilinae	104	0.284±0.008	0.341±0.006	0.120±0.005
	Lampsilinae	9	0.24	0.29	0.095
M.macrolepidotum (n=3)	***Cumberlandia***	**1**	**0.0625**	**0.0625**	**0.04**
	Unknown	1	N/A	N/A	N/A
	Lampsilinae	1	0.22	0.26	0.11
M. erythrurum (n=4)	Unknown	1	N/A	N/A	N/A
	Amblemidae	1	0.23	0.23	0.14
	Unknown	1	0.225	0.295	0.10
	Amblemidae	1	0.15	0.14	0.12
	Unknown	1	N/A	N/A	N/A
M. duquesnei (n=2)	Lampsilinae	1	0.23	0.27	0.12
	Amblemidae	1	0.20	0.21	0.15
D. cepedianum (n=7)	Amblemidae	1	0.208	0.23	0.15
	Lampsilinae	1	0.23	0.28	0.12
	Unknown	1	N/A	N/A	N/A
	Amblemidae	1	0.215	0.225	0.15
	Amblemidae	1	0.16	0.16	0.13
	Amblemidae	3	0.20	0.21	0.15
	Amblemidae	1	0.205	0.22	0.14
L. megalotis (n=4)	Amblemidae	16	0.221±0.003	0.238±0.005	0.139±0.003
	Amblemidae	1	0.21	0.22	0.14
	Amblemidae	1	0.22	0.23	0.12
	Lampsilinae	8	0.175	0.20	0.10
	Lampsilinae	1	0.215	0.28	0.095
L. machrochirus (n=2)	Lampsilinae	5	0.163±0.003	0.185	0.098±0.003
	Amblemidae	6	0.18	0.18	0.10
L. zonatus (n=4)	Unknown	1	N/A	N/A	N/A
	Unknown	1	N/A	N/A	N/A
	Amblemidae	1	0.16	0.12	0.14
	Amblemidae	1	0.165	0.17	0.14

Table 8, continued.

A. grunniens (n=4)	Amblemidae	3	0.22	0.24	0.13
	Amblemidae	77	0.306±0.151	0.265±0.120	0.15
	Anodontinae	3	0.37	0.37	0.28
	Unknown	1	N/A	N/A	N/A
C.carpio (n=1)	Unknown	1	N/A	N/A	N/A
C. cyprinus (n=1)	Amblemidae	1	0.21	0.23	0.13
	Lampsilinae	1	0.21	0.23	0.10
H. alosoides (n=1)	***O. reflexa***	**69**	**0.134±0.011**	**0.091±0.035**	**0.07**
I. bubalus (n=1)	Amblemidae	1	0.22	0.225	0.13
M. salmoides (n=1)	Lampsilinae	1	0.22	0.26	0.11
C. oligolepis (n=1)	Unknown	1	N/A	N/A	N/A
L. chrysocephalus (n=1)	Lampsilinae	1	0.28	0.35	0.15
N. amblops (n=1)	***Cumberlandia***	**17**	**0.06**	**0.065**	**0.05**

2. NEOSHO MUCKET RESTORATION

Introduction

This chapter describes a pilot project in which larval Neosho muckets (*Lampsilis rafinesqueana*) were transformed on fish at the Chesapeake State Fish Hatchery in Missouri and then released into historic habitat in Kansas. Freshwater mussels (Unionoida) are among the most endangered freshwater organisms in North America. The rapid decline of many species in this family has prompted calls for propagation and stocking to prevent further extinctions (NNMCC 1998). Unionids seem to be well suited to these interventions because of their parasitic life cycle and long lifespan. Mussels produce very large numbers of glochidia larvae (10^4 - 10^7 per female: Bauer 1994). Typically over 99.99% of these glochidia fail to reach a suitable host fish (Young and Williams 1984, Jansen and Hanson 1991). Of the few individuals that reach the host and transform, many juveniles presumably fall into unsuitable habitat (silty or unstable substrate) after leaving the fish. Therefore, it should be possible to drastically increase recruitment by putting glochidia on the proper host, recovering the transformed juveniles, and then releasing them in suitable habitat. These actions could generate very large numbers of individuals in a single generation. Most mussels have a long lifespan, so that a stocked cohort could persist for decades, buying time for investigating and correcting factors that limit natural reproduction.

The Neosho mucket is endemic to the Neosho, Spring and Elk river systems in southeastern Kansas, northeastern Oklahoma, and southwestern Missouri. It is state-listed as endangered in Kansas and Oklahoma, and S2 (imperiled) in Missouri. The Neosho mucket is not listed federally, although it was formerly classified as C2. Recent discussions with USFWS officials suggest that this species will be considered for federal listing (Paul McKenzie, Paul Hartfield, USFWS, personal communication). We chose to work with this species for several reasons. The draft recovery plan for endangered mussels in Kansas identifies reintroduction and augmentation as critical needs for the Neosho mucket, and Kansas state officials have been supportive of work with unionids. The distribution and conservation status of this species are well understood in Kansas because of recent survey work (Obermeyer et al. 1997). Neosho muckets are also attractive for a restoration project because of their large body size and large numbers of glochidia. Finally, this species lends itself to propagation in fish hatcheries because largemouth bass, which are commonly propagated in hatcheries, are suitable host fish (Barnhart and Roberts 1997).

Methods

Collection of mussels: Neosho muckets were sought in the Fall River in Kansas on July 27, 1999. The first site searched was below an old iron bridge northwest of Neodesha (0.8 mi N and 2.3 mi W; SW 1/4 Sec. 13 T30S R15E Wilson Co, KS). At this site the most abundant species were *A. plicata*, *L. rafinesqueana*, *T. verrucosa*, *Q. pustulosa*, *F. flava*, and *O. reflexa*. Five *Cyprogenia aberti* were located and tissue samples were taken from these and also from 6 Neosho muckets. One gravid Neosho mucket was collected. Ten other female Neosho muckets were examined and were not gravid on this date. A second site was visited on the Fall River below the RR bridge SW of Fredonia, just downstream from the Fredonia City dam (1.25 mi S, 0.8 mi W Fredonia; NW1/4 Sec. 23 T29S R14E). At this site we found approximately 5 Neosho muckets, none of which were gravid, along with several old *M. nervosa* and one more *Cyprogenia*.

Host fish and transformation: Transformation on host fish was carried out at the Chesapeake Fish Hatchery of the Missouri Department of Conservation, with help from personnel of both MDC and the Neosho National Hatchery. Two experiments were conducted. The first began on 8/20/99 and the second on 9/13/99. The host fish were fingerling largemouth bass (~10 cm total length). A single Fall River Neosho mucket (see above) was the source of glochidia for both experiments. In both experiments, glochidia were removed from the mussel just prior to inoculating the fish. The shell was opened gently using a nasal speculum and wedged open approximately 1.5 cm. Two short cuts were made in one marsupial gill, parallel to the gill filaments, using a scalpel. The glochidia were then flushed from the gill into a glass dish by directing a stream of water at the openings from a pipette. Clumps of glochidia were separated by drawing them in and out of the pipette. The glochidia were then distributed into several glass dishes. Approximately 200 fish were inoculated in each of the two experiments (~400 fish total). The fishes were anaesthetized with MS-222 in groups of 6-10. As the fish became anaesthetized they were removed from the water by hand and the glochidia were pipetted directly on to the gills on both sides.

After inoculation the fishes were placed in a cylindrical fiberglass tank in approximately 300 gallons of water. Water was delivered to the tank continuously and volume was regulated with a standpipe. Temperature was measured every 1-2 days using a YSI digital thermometer. During the second experiment, inlet water temperature had declined, and a heater unit (3000 Watts) with thermostat was used to regulate the tank temperature. In the first trial, the fish were fed at least daily. In the second trial, the fish were not fed after the first week following inoculation. Several test fishes were sacrificed approximately 1 week following each inoculation and examined to determine the number of attached glochidia.

Juveniles were recovered by siphoning water from the bottom of the tank through a filter. The siphon head was a 3x3-inch block of ½-inch Plexiglas with milled out on one side to 1/8-inch depth, leaving a 1/16-inch thick wall. Small screws were set in each corner so as to lift the edge approximately 1 mm from the surface being vacuumed. The head was drilled and tapped at center to accept a barbed fitting, and was attached to a 4-foot length of ½-inch PVC pipe, which was in turn attached to a length of ½-inch ID rubber hose for siphoning. The other end of the siphon hose was directed into a Newark #120 brass soil sieve (125 micron mesh) to recover juveniles.

Juveniles were collected at approximately 2-day intervals. The number of individuals in each collection was quantified by concentrating the juveniles in a known volume of water (0.5-1.0 L), suspending them by vigorous stirring, and then removing 10 ml samples of the suspension to a small dish for counting under a dissecting microscope. Live juveniles and dead individuals (empty shells) were counted separately. Three to six 10-ml samples of each collection were counted. These counts were averaged to determine the number per ml, which was then multiplied by the total volume of the suspension to estimate the total number of glochidia in the collection. The juveniles were stored in water in 1-gallon Ziploc bags kept at 15 °C in an incubator. Several collections were transported to the Fall River in Kansas where they were released by using turkey basters to distribute them into the substrate in suitable habitat (see below).

Results

Two transformation experiments were carried out (Tables 1 & 2). The mean numbers of glochidia attached were 407 and 153 glochidia per fish in the first and second trials, respectively (Table 1). Despite the rather heavy infestations, the fish did not appear to be stressed by the glochidia and appeared to behave normally. Only one fish died during the first experiment, possibly due to effects of being dropped during handling. No fish died during the second trial.

In the first experiment, drop-off of juveniles from the fish began within 15 days after inoculation. A large number of juveniles were collected from this experiment, but it proved to be very difficult to remove the juveniles from fish wastes that accumulated in the tank. The frass and debris made it difficult to observe and count the juveniles. Moreover, the juveniles died within 8 hours when left in a static volume of water containing this material. Therefore, the second batch of fish was inoculated and feeding of this group was suspended after 1 week in order to minimize waste production during the drop-off period. Temperature averaged 22.2 °C (71.9 °F). The first juveniles from this group were recovered 13 days after inoculation (Table 2). Thereafter the tank was siphoned at 2-day intervals. The rate of drop-off of juveniles increased and peaked on or before 19 days post-inoculation (Figure 1). Juveniles were last recovered 30 days after inoculation, when the experiment was terminated.

Releases: Approximately 19,550 juveniles were transported to Kansas and released in the Fall River Wildlife Refuge above the Fall River Reservoir (Table 3, Figure 1). This area presents several advantages as a site for reintroduction. The area supports several unionid species, indicating that the habitat is suitable. Neosho muckets formerly occurred throughout the Fall River, based on the presence of dead shells, but have apparently been extirpated above the reservoir in recent times (Obermeyer et al. 1997). The Fall River Reservoir constitutes a dispersal barrier, so that recolonization from the surviving population in the lower river is unlikely. Likewise, the introduced population should have no genetic effect on the population below the reservoir.

The juveniles were transported in 3 batches. Siphonate from the first experiment, which was heavily contaminated with fish wastes, was diluted in approximately 5 gallons of water in a picnic cooler, aerated with a battery-powered pump, and transported by car to Kansas for release. Juveniles from the second experiment were largely free of debris, and two batches were shipped overnight in small volumes of water in Ziploc bags, which were packed in insulated containers with “blue ice” to maintain low temperature. Juveniles kept at low temperature in the lab survived for several weeks. The condition of the latter two batches of transported juveniles was briefly checked with a microscope after shipment, but viability was not quantified before release.

Table 1. Neosho mucket transformation experiments. Data are mean ± standard error (N).

Fish Dimensions (mm)	First experiment	Second experiment
Total length	98.8 ± 1.72 (10)	105.3 ± 3.6 (6)
Standard length	81.9 ± 1.54 (10)	89.3 ± 2.81 (6)
Mass	12.2 ± 0.66 (10)	14.2 ± 1.57 (6)
Inoculation		
Glochidia per fish	407 ± 55.9 (10) range = 206-761	155 ± 29.6 (6) range = 59-284
Estimated total number of glochidia attached to 200 fish	81,440 ± 11,185	31,067 ± 5930
Temperature and timing		
Temperature °C	23.1 ± 0.25 (16)	22.2 ± 0.45 (18)
Temperature °F	73.6 ± 0.45 (16)	71.9 ± 0.80 (18)
Time to first drop-off	15 days	13 days

Table 2. Juvenile Neosho muckets recovered from hatchery largemouth bass in the second experiment. Data for each collection are mean ± SE (n samples). The standard deviation of the totals was estimated as the square root of the total of the variances of the individual collections. These data are graphed in Figure 1.

Collection Date	Days post-inoculation	Number of live juveniles	Number of dead juveniles	Percent live
1-Oct	13	183 ± 31 (6)	558 ± 79 (6)	24.7
3-Oct	15	838 ± 118 (6)	575 ± 54 (6)	59.3
5-Oct	17	3520 ± 378 (3)	1440 ± 46 (3)	71.0
7-Oct	19	6800 ± 1097 (3)	2500 ± 351 (3)	73.1
9-Oct	21	4333 ± 145 (3)	1000 ± 153 (3)	81.2
11-Oct	23	5967 ± 731 (3)	1600 ± 451 (3)	78.9
13-Oct	25	3867 ± 318 (3)	1633 ± 384 (3)	70.3
18-Oct	30	3000 ± 503 (3)	2267 ± 371 (3)	57.0
TOTALS		28,508 ±2,619 SD	11,573 ±1,403 SD	71.1%

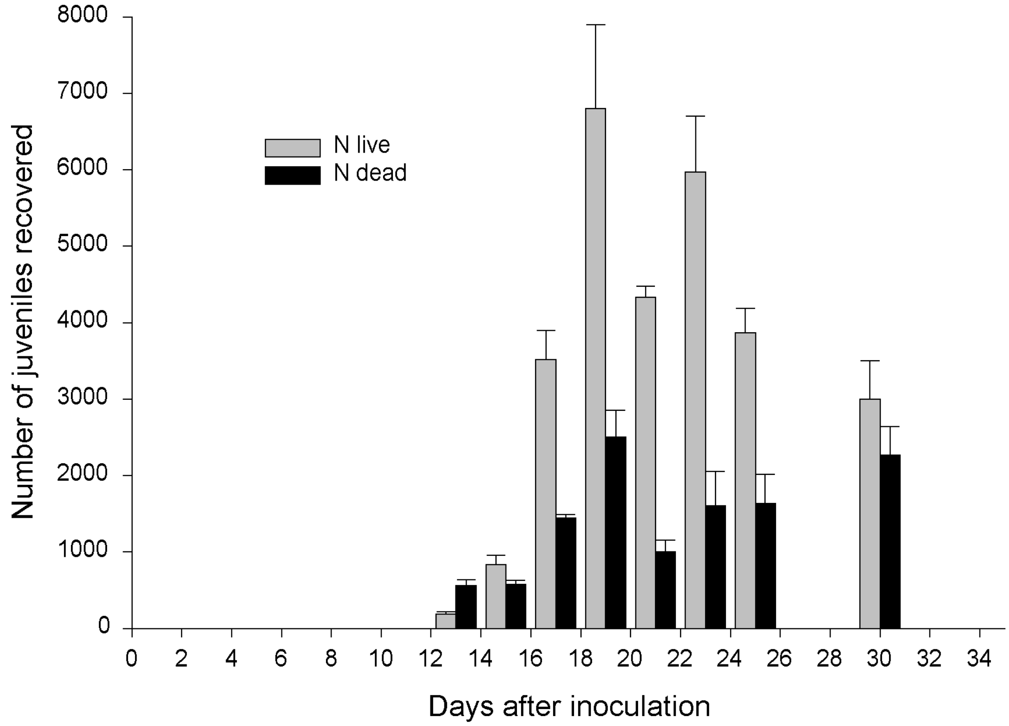

Figure 1. Timing of drop-off of juvenile Neosho muckets from largemouth bass. These data are from the second experiment. Each bar represents the estimated number of juveniles (± SE) that dropped off during the preceding two days, except the bar at 30 days, which represents the preceding 5 days. Temperature during the transformation period was approximately 22 C.

<u>Discussion</u>

Transformation and recovery: Transformation success of Neosho muckets on hatchery largemouth bass was high (71%, Table 2). It appeared that recovery of detached juveniles by siphoning from the fish tank was also efficient. The estimated total number of live and dead juveniles recovered in the second experiment (~40,000, Table 2) exceeded the estimated number of glochidia that attached to the host fish (~31,000, table 1). Logically, these two figures should be equal, but both estimates have large variance. Juvenile mussels settle to the bottom rapidly because of the density of the calcareous shell. The cylindrical tank that we used at Chesapeake was equipped with a central drain and a standpipe that can be pulled to drain the tank. We experimented with draining the tank through a Nitex filter bag attached to the outlet, but this recovery method proved to be more difficult than siphoning. The standpipe was equipped with an outer sleeve perforated at the lower end, so that water leaving the tank enters the sleeve at the bottom of the tank, and then rises to the top of the standpipe. We reversed the sleeve so that the perforations were at the top of the tank, in order to ensure that no juveniles would be lost in the outlet water.

Table 3. Releases of Neosho mucket juveniles in the Fall River Wildlife refuge. A total of approximately 19,550 juveniles were released. See Figure 2 for a map of sites.

Date	Workers	Number	Locality
9/14/1999	Ed Miller and Rick Tush	~3,500	Site A: Fall River Wildlife Area N37 45.220' W96 11.261', T26 R11 Sec27 Greenwood Co. KS
9/14/1999	Ed Miller and Rick Tush	~3,500	Site D: Fall River Wildlife Area N37 46.486 W96 13.187, T26 R11 Sec20 Greenwood Co. KS
10/8/1999	Brian Obermeyer and John Bills	~3,200	Site D: Fall River Wildlife Area N37 46.486 W96 13.187, T26 R11 Sec20 Greenwood Co. KS
10/8/1999	Brian Obermeyer and John Bills	~850	Site C: side channel on west side of stream
10/15/1999	Brian and Bernice Obermeyer	~5,000	Site D: Fall River Wildlife Area N37 46.486 W96 13.187, T26 R11 Sec20 Greenwood Co. KS
10/15/1999	Brian and Bernice Obermeyer	~3,500	Site B: a side channel, north side of stream, immediately below an abandoned ford.

Several potential problems and solutions were identified during this pilot project. The first experiment showed clearly that it is necessary to starve the host fish for several days prior to and during the period when juveniles drop off the host fish. This was important in order to minimize the amount of waste settling to the bottom of the tank. The juvenile mussels are extremely small (~250 microns). They tend to become entangled in debris and fungal hyphae, forming clumps that made it very difficult to separate and count the juveniles. For this reason, we counted only a fraction of the juveniles from the first experiment.

Fish wastes also increased mortality of juveniles, particularly if the water was not renewed continuously. In one test, a volume of ~1L of filtrate from the first experiment was left overnight (8 hours) in a dish without aeration, and all of the juveniles died. This effect may be caused by ammonia produced by the decomposing waste, rather than to hypoxia, because the juvenile mussels are very resistant to hypoxia (Barnhart, unpublished). In the second experiment the fish were starved and the amount of waste was greatly reduced. As a result, it was much easier to observe and count the juveniles, and they were able to survive several days in the filtrate.

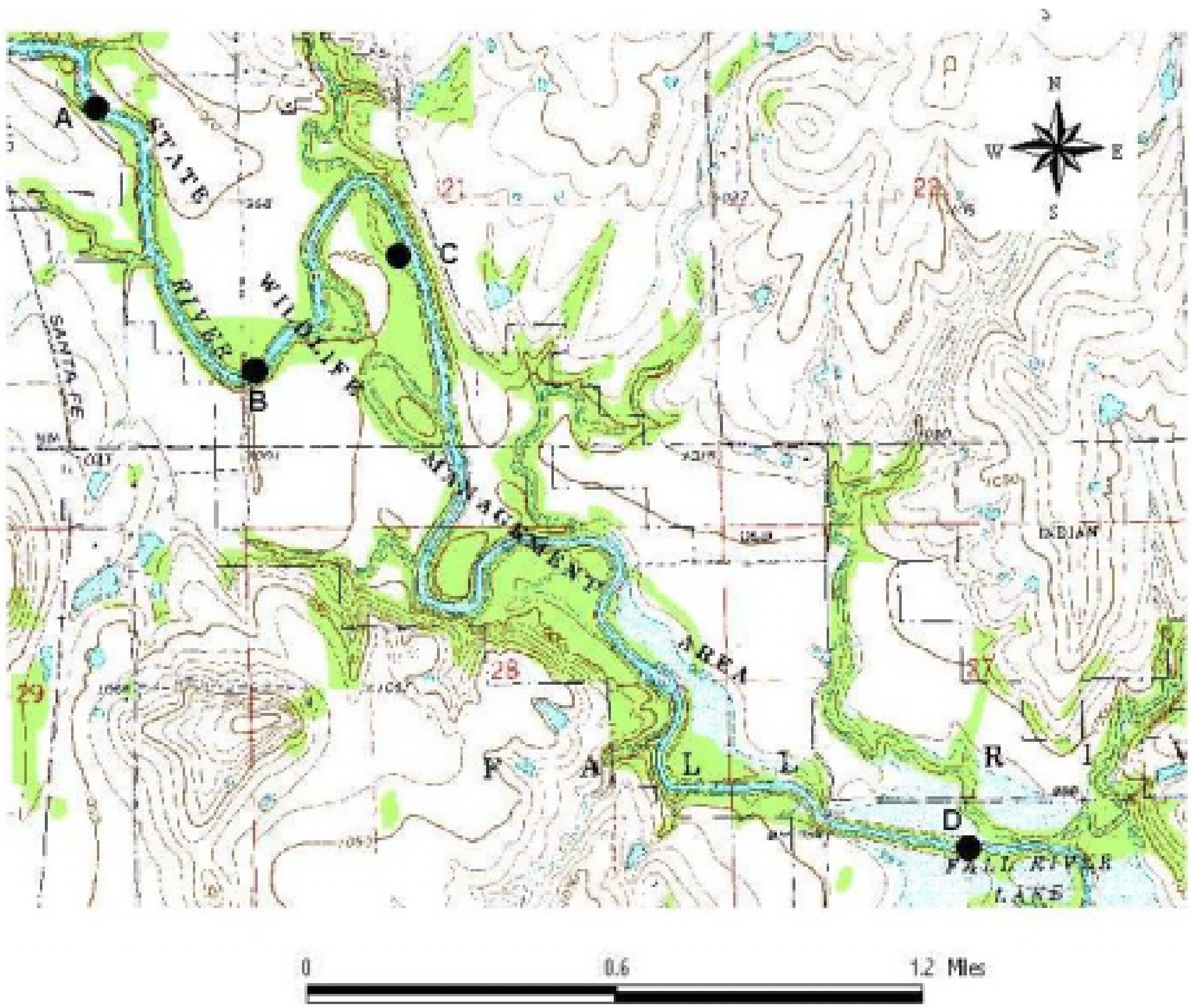

Figure 2. Sites where Neosho muckets were released. See Table 3 for site descriptions.

<u>Predatory flatworms:</u> Another problem identified in this study was predation on the juveniles by turbellarian flatworms. At least three species of Turbellaria (tentatively identified as *Macrostomum sp.*, *Microstomum sp.*, and *Dugesia sp.*) and one Nemertean (*Prostoma sp.*) were collected from the filtrate and were identified using the keys in Kolasa (1991). *Macrostomum* and *Microstomum* were both abundant in the fish tank, particularly if wastes were allowed to accumulate, and both of these species

were observed to feed upon the juvenile mussels. Photographs were obtained of both species showing juveniles in their gastrovascular cavities (Figure 3). Sickel (1998) previously reported predation by *Macrostomum* on juvenile *Corbicula*. Losses to these predators were not quantified, but may have been substantial. The worms tended to migrate to the bottom of glass containers and would attach to the glass fairly tightly by means of their adhesive glands. Therefore, it was possible to remove many of the worms by allowing the filtrate to stand for several minutes, then swirling and pouring it into another container. This treatment tended to leave the worms behind in the first container, and several repetitions could remove a large fraction of the worms from the filtrate.

Releases: This pilot project was intended mainly to explore methods for propagation, as a prelude to later production and release projects. However, we were able to obtain permission from Kansas and USFWS officials to release many of the propagated juveniles in the Fall River Wildlife refuge. Work by Obermeyer et al. (1997) indicates that Neosho muckets were extirpated in this portion of the river (above Fall River reservoir) in historic times. Other unionid species are present. Release of the juveniles in this area obviated several potential problems that must be considered in the future. For example, we used only a single individual mussel as a source for the glochidia. It would not have been appropriate to release such a large number of genetically similar individuals into an existing population, because of concerns about altering the gene pool. However, the release site is isolated from existing Neosho mucket populations by the Fall River Reservoir. Also, the fact that this release is a reintroduction, rather than an augmentation of an existing population, will make it easier to document our success or failure. We anticipate that these individuals will reach a size large enough for recovery within about 3 years. We hope to make further releases at these sites during that time.

We chose to release juveniles to the river as soon as possible following transformation. Allowing juveniles to grow in culture before release might be useful, if larger juveniles are less susceptible to size-limited predators such as the flatworms. However, there are substantial drawbacks to culturing the juveniles. Culture requires extra time, space and expense. Mortality rates in lab culture are high. Both survival time and growth rates vary greatly among individuals (O'Beirn et al. 1998, Barnhart 1999). Therefore, mortality in culture may leave a particular subset of individuals that have been selected for characteristics that are related to survival in artificial conditions, and not necessarily suited for survival in natural conditions.

Factors affecting juvenile mussels after transformation and settlement may also limit recruitment, and presently are poorly understood. However, indirect evidence suggests that conditions for juveniles may still be good even in areas where unionid populations are declining. The introduced Asian clam, *Corbicula fluminea*, has spread rapidly and reproduces successfully in many unionid habitats. The early juveniles of *Corbicula* are identical in size to most juvenile unionids (~250 microns in length), occupy similar habitat, and are likewise pedal feeders in sediments. The ability of *Corbicula* juveniles to thrive in an area may indicate that habitat conditions are also suitable for juvenile unionids. It seems likely that juvenile *Corbicula* are no less susceptible than juvenile native mussels to predation or environmental stresses. In fact, adult *Corbicula* are generally more susceptible to extremes of temperature, pH, and hypoxia than are native sphaeriids and unionids (McMahon 1991 and references therein). *Corbicula* has no parasitic stage, and its success has been attributed mainly to its direct reproductive habits (McMahon 1991).

<u>Demands on hatchery operations:</u> This pilot project demonstrated the feasibility of producing large numbers of *Lampsilis* juveniles in a hatchery setting without making major demands on space or personnel. The numbers of fish involved are modest. New groups of fish may be needed for each round of transformation, because the fish must be starved during the drop-off period, and because fishes to acquire immunity to glochidia after exposure. After serving as hosts, these fish could be placed back into production. The most significant demand on the hatchery appears to be space for holding the host fish during transformation. Fish that are being used as mussel hosts must be isolated, because of the need to interrupt feeding during the drop-off period and provide a clean surface and low-flow environment for recovering juveniles. Therefore, a suitable tank with several hundred-gallon capacity is needed for approximately one month per batch. The time necessary might be reduced to 3 weeks at higher temperature, or even less with species that transform more quickly. These tanks must be either aerated static tanks or, if water delivery is continuous, equipped with standpipes so that outlet water leaves only from the surface. Isolation of host fish from regular production should minimize the possibility of introducing disease or parasites as well the possibility of inadvertent escape of mussels. The risk of inadvertent escape of mussels is minimal even if infected host fish are subsequently released, because largemouth bass are stocked in ponds and lakes, which do not provide suitable habitat for Neosho muckets.

The timing of propagation work is important and must consider both the reproductive periodicity of the mussels and the schedule of fish production at the hatchery. Reproductive timing varies among mussels, but many species, including *Lampsilis*, are gravid with glochidia throughout the winter, spring and summer months. We delayed collection of Neosho muckets until late in the summer this year because of persistent high flows in the lower Fall River. Most females were spent by July 27, and next year we plan to collect earlier in the summer if possible. Gravid females can be stored for weeks or even months at low temperature (10 C), allowing some flexibility in timing of work at the hatchery. According to Jim Maenner, manager of the Chesapeake Hatchery, tank space is more likely to be available in late summer and fall than at other times.

Summary

This pilot project demonstrates the feasibility of propagating a threatened mussel species, the Neosho mucket, using facilities at the Chesapeake State Fish Hatchery and using fingerling largemouth bass as hosts. Minimal requirements for production of approximately 30,000 juveniles include 200 bass fingerlings and the use of a suitable tank for about 1 month. Approximately 19,550 juveniles from this study were released in the Fall River Wildlife Refuge in Kansas. This release is a reintroduction of this species to historic habitat, from stock collected downstream in the same river. Assuming that permission and cooperation can be obtained from MDC Fisheries Division and Chesapeake Hatchery, we hope to carry out a similar project next summer. We also hope to initiate discussion on possible source and release sites for this species in the Spring and Elk River systems in Missouri.

Acknowledgements

This project was a cooperative effort involving the Missouri Department of Conservation, the Kansas Department of Wildlife and Parks, the US Fish and Wildlife Service, Neosho National Fish Hatchery, and the SMSU Biology Department. Brian Obermeyer provided the essential information and advice regarding collection and release sites, and also conducted most of the actual releases of juveniles. Ed Miller led the collecting trip and released the first batch of juveniles. Jerry Horack, Dan Mulhern, Brigit Mulhern, Ben Mulhern, Bryan Simmons, and Diana Sheridan helped with the collecting trip. Jim Maenner and his crew at Chesapeake Hatchery provided fish, tank space, and other essentials, as well as inoculating and caring for both finfish and shellfish. Dave Hendrix, Roderick May and colleagues at Neosho Hatchery helped with fish inoculation, transport of juveniles, and provided heaters. Michael Baird helped with infecting fish and with handling and counting juveniles at SMSU. I am grateful to Steve Eder, Norm Stuckey, and Gary Novinger of Fisheries Division for giving their permission to carry out this work on short notice. Thanks to Amy Salveter for coordinating funding. Special thanks are due to Sue Bruenderman and to Paul McKenzie, for sharing our goals and for promoting this project and others.

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From the left: Rod May, Neosho National Hatchery, Jim Maenner, Chesapeake Hatchery, and Chris Barnhart, SMSU.

3. WORK IN PROGRESS AND RELATED STUDIES

Summaries

In addition to the host and restoration studies described above, work progressed on several other studies and activities during 1999. These studies are all related to the goals of the project and were supported directly or indirectly by our Section 6 funding from USFWS and MDC.

Effects of hypoxia: We performed two 1-month long experiments investigating the effects of multiple levels of chronic hypoxia on the survival and growth of juvenile mussels (*Lampsilis reeveiana* and *Lampsilis siliquoidea*). Results thus far show that survival rates are impaired below 26% of air saturation. However, over half of juveniles are able to survive continuous low DO (3% of air saturation) for over 3 weeks. Further hypoxia experiments will be performed this spring.

Demography of *Cumberlandia*: Coauthor Michael Baird is currently writing his thesis, which is a study of the life history and population demography of *Cumberlandia* in the Meramec and Gasconade rivers. Much of the information included in this report derives from that work. Another major part of Mike's thesis work involves analysis of age and growth and quantitative sampling of populations at eight sites. This work was reported at the Missouri Natural Resources conference and is summarized in an abstract (attached).

Venustaconcha: Graduate student Frank Riusech completed his thesis work on life history and genetics of *Venustaconcha* (Riusech 1999). Frank's work was reported at the Missouri Natural Resources Conference and received the Best Thesis Award from the Missouri Chapter of the American Fisheries Society. Part of the thesis, investigating host specificity of two closely related mussel species, was accepted for publication (Riusech and Barnhart, in press). Other work reported in the thesis includes a genetic study comparing *V. pleasii* and *V. ellipsiformis*, which confirms the identity of *V. pleasii* as a distinct species endemic to the White River system, and clarifies the identity of *Venustachoncha* in the Spring River (Neosho system) as *V. ellipsiformis*. Frank also investigated age and growth in *Venustaconcha* using shell growth lines. This study provides evidence of environmental influences on growth as well as a validation of the annual nature of growth lines (see abstract attached).

Outreach activities: Conservation of endangered species requires public interest and support. Unionids may lack the charisma of large carnivores, but the interaction of mussels with fish is very compelling, and can capture public attention. The relationships between mussels and their hosts are excellent examples of the interrelationships in nature, where no species lives (or dies) without effects on others. Mussels have amazing strategies for attracting host fish, including a variety of lures, baits, nets and traps. These remarkable adaptations can impress almost anyone, and they are effective in focusing attention on endangered species, the biology of streams and water quality issues. We maintain the Unio Gallery, an Internet site presenting photographs of unionids and their life history stages. The purpose of the site is to give conservation professionals, educators, and other interested parties access to pictures for illustrating presentations about endangered species. This year we have expanded the site, and also added video clips that illustrate lure display of *Lampsilis* and *Villosa*. The Unio Gallery URL is http://courses.smsu.edu/mcb095f/gallery/. Our work has received some media attention in 1999, including several radio and television interviews, and two visits from a professional film crew from England, who filmed the lure display, glochidia, and hosts *Lampsilis reeviana* as part of a documentary for public television (PBS). We are also currently developing a permanent display of unionids for the visitor center of Meramec State Park.

Grant-related reports, publications, and presentations for 1999-2000:

Baird, Michael S., and M. Christopher Barnhart. 2000. Population age structure of the spectaclecase mussel, *Cumberlandia monodonta.* Missouri Natural Resources Conference, Lake of the Ozarks.

Baird, M. S., and M. C. Barnhart. 1999. Population age structure of the spectaclecase mussel, *Cumberlandia monodonta.* Great Plains Limnology Meeting, Columbia, MO.

Barnhart, M. C. 1999. Black sandshell- missing, but not forgotten. Kansas Pearly Mussel Newsline 1999:8.

Barnhart, M.C. Unio Gallery. A pictorial resource for conservation professionals and educators working with endangered species. URL: http://www.smsu.edu/mcb095f/gallery/

Barnhart, M. C. 1999. Overview of freshwater mussels. Invited presentation at public hearing for proposed listing of the scaleshell mussel as endangered by the U. S. Fish and Wildlife Service. Runge Nature Center, Jefferson City, MO. 12/8/99.

Barnhart, M. C. 1999. Pearls and Perils in Ozark Streams. Showcase on Faculty Research. SMSU Office of Academic Affairs.

Barnhart, M. Christopher, and Frank A. Riusech. 2000. Age and growth of freshwater mussels inferred from shell annuli: effects of the 1993 flood. Missouri Natural Resources Conference, Lake of the Ozarks.

Barnhart, M. C. and A. R. Roberts. 1999. Life history of the flat floater mussel *Anodonta suborbiculata.* Great Plains Limnology Meeting, Columbia, MO.

Barnhart, M.C. 1999. Ecology and conservation of freshwater molluscs. Invited lecture. Tri-Beta National Biological Honor Society, Northcentral District II Convention. Lay Field Station, St. Louis University. April 10.

Barnhart, M.C. 1999. Captive rearing of native mussels: last chance for endangered species in Missouri? Platform presentation, Missouri Natural Resources Conference, Lake of the Ozarks.

Baird, M.S. and M.C. Barnhart. 1999. Life history of the freshwater bivalve, *Cumberlandia monodonta*. Platform presentation, Missouri Natural Resources Conference, Lake of the Ozarks. (Winner, best student presentation, Missouri Chapter of the American Fisheries Society)

Baird, M. S. and M. C. Barnhart. 1999. Survival and growth of *Lampsilis* species in a recirculating rearing system. Platform presentation, Symposium of the Freshwater Mollusk Conservation Society, March 17-19, Chattanooga, TN.

Barnhart, M.C. 1999. Potential hosts and reproductive characteristics of some unusual unionoids. Platform presentation, Symposium of the Freshwater Mollusk Conservation Society, March 17-19, Chattanooga, TN.

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Barnhart, M. C. and F. A. Riusech. 1999. *Venustaconcha* in the Spring River in Kansas. Kansas Mussel Meetings, Emporia, KS.

Riusech, F. A. 1999. Genetic and life history characteristics of the freshwater bivalves, *Venustaconcha ellipsiformis* and *Venustaconcha pleasii*, in the Ozark Plateaus region. MS thesis, SMSU.

Riusech, F.A. and M.C. Barnhart. In press. Host suitability differences among populations of *Venustaconcha* from the Ozark region. Proceedings of the Captive Care, Propagation, and Conservation of Freshwater Mussels Symposium.

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Population age structure of the spectaclecase, *Cumberlandia monodonta.*

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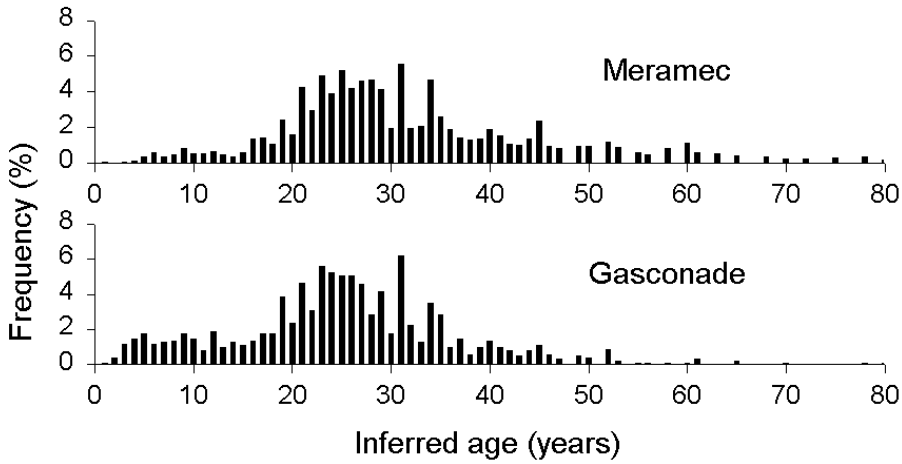
The spectaclecase is the only Margaritiferid mussel in the Mississippi drainage. Although formerly widespread, this species has declined dramatically throughout its range (Williams et al. 1992). Some of the largest remaining populations occur in Missouri in the Gasconade and Meramec rivers, where *Cumberlandia* is the most abundant mussel at many sites (Buchanan 1980). We have studied these populations over the past 2 years in order to determine distribution, abundance and age structure. Eight sites were examined quantitatively. Sites were delimited by the presence of mussels. Mean site area was ~1000 m^2. Quadrats were placed using an adaptive design, excavated to 15 cm depth, and searched visually for mussels. Over 6,000 live specimens were examined and measured (total shell length, height, width, hinge length, and wet weight). A sub-sample of ~35 individuals per site was sacrificed and aged by counting growth lines in the hinge ligament. Apparent ages ranged from 1-56 years and were correlated with shell length. The length/age relationship was similar among sites (ANCOVA), so a single hyperbolic growth model was derived: length =201.4*[age/(15.4+age)], (n = 278, R^2 = 0.83). This relationship was then used to estimate age (years) from shell length (mm) of the other individuals and infer population age distributions by site and drainage basin. Results for the two river basins are presented below:



Inferred age distributions were similar in the Meramec and Gasconade. The most abundant age classes were approximately 20-35 years. Among sites, population densities ranged from 1.2 to 12.8 (mean = 6.7) individuals per m^2, while local (i.e. single quadrat) densities ranged up to 120 m^2. Although a few young individuals were found at all sites, individuals less than 20 years old were relatively rare. Thus, it appears that these populations might be in decline, despite remarkable densities of adults. Pictures of *Cumberlandia* beds, as well as conglutinates and glochidia, can be viewed on the Internet at: http://courses.smsu.edu/mcb095f/gallery/.

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Effects of the 1993 flood on growth of *Venustaconcha*.

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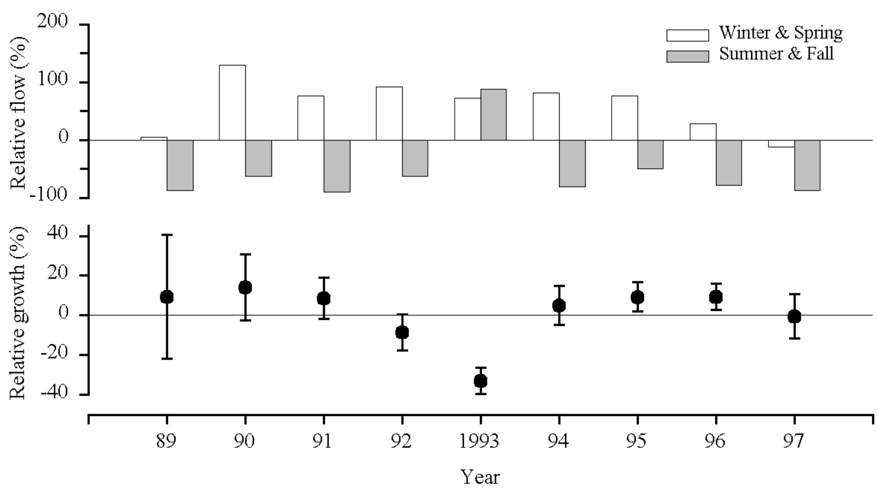
The shells of freshwater mussels preserve a record of growth throughout the lifespan of the individual. However, annual formation of shell growth lines has been questioned (Kesler and Downing 1997). We investigated age and growth *Venustaconcha ellispisformis* and *Venustaconcha pleasii* from 7 sites in the Ozarks region (Riusech 1999). Length at each year of age was determined from measurements of external shell growth lines of approximately 60 individuals per site. Inferred age and growth were then compared among sexes, species, sites, and calendar years. Growth rates increased with age from 0-4 years, then declined precipitously, coincident with sexual maturity. Ellipse mussels were consistently larger than Pleas mussels at similar age. Males were consistently larger than females at similar age. Average peak growth rates were 9.4 and 9.7 mm/y for female and male ellipse, and 7.9 and 9.5 mm/y for female and male Pleas' mussel, and, respectively.

Comparison of growth rates by calendar year showed that growth rate was significantly depressed in 4 of the 7 populations during 1993, relative to growth in other years between 1985 and 1997. This depression of growth coincided with unusually high flows during the summer and fall in these streams, as documented by gage records. The figure below shows results for *V. pleasii* at one representative site, in the James River. Relative flow is the mean difference of monthly flow from mean flow during 1985-1997. Relative growth is the difference from predicted growth based on sex and age of individuals (means±95% C.I., n=7-85).



The appearance of 1993 as a "signature year" in these data supports the annual nature of external shell growth lines in *Venustaconcha*. Analysis of annual growth from shell annuli in longer-lived species might be used to document the effects of both natural and man-made disturbances on growth.

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