Reproduction and Fish Hosts of Unionid Species of Concern

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ABSTRACT

Potential host fish species were determined for four unionids. The federally endangered fat pocketbook, *Potamilus capax*, transformed only on freshwater drum (*Aplodinotus grunniens*) of 28 species fish species tested. Time from encystment to peak of excystment was 19 days at 20 °C and 16 days at 26 °C. Drum that were re-infected with *P. capax* 12 months after the initial infection did not show evidence of acquired immunity. The federally endangered pink mucket, *Lampsilis abrupta*, transformed on four of nineteen fish species tested. These were largemouth bass (*Micropterus salmoides*), spotted bass, (*Micropterus punctulatus*), smallmouth bass, (*Micropteris dolomieu*), and walleye, (*Stizostedion vitreum*). Time from encystment to the peak of excystment from hosts was 12 to 14 days at 25 °C. The western fanshell, *Cyprogenia aberti* transformed successfully on fantail darters (*Etheostoma flabellare*), logperch (*Percina caprodes*) and banded sculpins (*Cottus carolinae*), of twenty-eight fish species and one crayfish species tested as hosts. Time from encystment to peak of excystment was about 27 days at 22 °C. Twelve species of fishes were tested as potential hosts of the Ozark shell, *Fusconaia ozarkensis*. Of these, transformation was observed on bleeding shiner (*Notropis zonatus*), cardinal shiner (*Notropis cardinalis*) and southern red-belly dace (*Phoxinus erythrogaster*). Transformation time was 9-11 days at 26 °C. The conglutinates of both *Cyprogenia aberti* and *Fusconaia ozarkensis* consisted largely of sterile eggs, which appear to serve a functional role in conglutinate structure and attraction of host fish.

INTRODUCTION

Freshwater mussels (family Unionidae) are generally considered to be the most endangered major family of animals in North America. At least 21 species are already extinct and 62 species are federally classified as endangered. Many workers believe that over half of the 297 North American species are in danger of extinction due to the effects of pollution, erosion, siltation, impoundment, and the introduction of non-native species (Williams et al. 1993). A national strategic plan for the conservation of native mussels identifies research on reproductive biology as one of the most critical needs (Biggins et al. 1995, Neves 1997). Most threatened species, even those which are very rare, continue to produce large numbers of viable glochidia

larvae. Therefore, it is logical to suspect that the availability of host fish and the survival of the early juvenile stages of unionids are critical issues (Neves 1997). The identification of fish hosts of threatened and endangered mussels is necessary for the reintroduction of these species to their native habitats, a recovery objective for most endangered species.

The objectives of the present study are to investigate the reproductive biology and identify potential host fishes of selected unionids that are considered to be at risk of extinction. The study of unionid host relationships has accelerated greatly in recent years. Published reports of host relationships were reviewed by Fuller (1974), Hoggarth (1992), and Watters (1994). More recent host studies include Hove and Neves (1994), Michaelson and Neves (1995), Yeager and Saylor 1995, Barnhart and Roberts (1997), Hove et al. (1997), O'Brien et al. (1997), Haag and Warren (1997) and a number of studies reported as abstracts in professional newsletters (Triannual Unionid Report, U. S. Fish and Wildlife Service; Mussel Info Newsletter, Texas Wildlife and Parks).

METHODS

Gravid female mussels were obtained from stream reaches in Missouri, Kansas and Arkansas, under permits from U.S. Fish and Wildlife Service, Missouri Department of Conservation, Kansas Wildlife and Parks, and Arkansas Department of Game and Fish. Mussels were checked in the field for gravidity. Mussels bearing mature glochidia were returned to the lab and held in aquaria until host tests were performed. Mussels were not fed, and those individuals which were held for more than 2 weeks were kept at low temperature (10 C) to reduce metabolic rate and starvation stress. Following host tests, individuals of endangered species were returned to the site of capture and released. Mussels of other species were either released at the site of capture or were preserved for genetic studies.

Glochidia of *Potamilus* and *Lampsilis* were collected as follows: The valves were spread slightly using a nasal speculum and then wedged open with a piece of soft vinyl tubing of appropriate diameter. Sterile water was injected into the marsupia from a syringe to flush glochidia from individual water tubes out the excurrent siphon. Using this method, glochidia could be harvested repeatedly from an individual over a period of weeks or months.

The glochidia of *Cyprogenia* and *Fusconaia* were obtained by different methods. These species normally release unhatched eggs in cohesive aggregates known as "conglutinates", which are formed within the water tubes of the marsupial gills. Mature conglutinates are normally released via the excurrent siphon. *Cyprogenia aberti* is gravid in winter, and the release of conglutinates in the laboratory was induced by increase in water temperature (see results). *Fusconaia ozarkensis* is a summer-breeding species and, like many other amblemines, readily releases conglutinates in response to stress. Gravid individuals were placed in finger-bowls of water. Release of conglutinates generally ensued within 12-48 hours, presumably in response to hypoxia. Glochidia were stripped from the mature eggs by drawing the conglutinates rapidly in and out of a pipette.

Glochidia were generally used immediately for host tests. If not used within one hour, glochidia were kept at 10 °C in shallow water in glass fingerbowls for no more than 3 days before use. In each case, viability of a sample of 50-70 individuals was tested by observing the closing response to saline solution, immediately before infection of fish. The proportion of viable glochidia was recorded. The viability of glochidia that were used for host tests usually exceeded 95%.

Most fish species used for host tests were wild-caught individuals. Hatchery fishes, particularly largemouth bass (*Micropterus salmoides)*, walleye (*Stizostedion vitreum*), and channel catfish (*Ictalurus punctulatus*), were used when convenient and appropriate. Wild-caught fishes were acclimated for at least one week after capture before use and were usually treated with antibiotics (Maracyn) during this time. We used primarily small individuals, typically 2-6 inches in length, for convenience in handling. Care was taken to keep fish healthy and well-fed and to use only healthy individuals for tests. 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 blood worms, live blackworms, or other fishes, depending upon species.

Fish species were chosen for testing based in part upon the habitat and adaptations of the mussel species being tested and the known hosts of related unionids, as well as upon availability. In most cases, several (3-10) individuals of each species were tested. The locality of origin of each fish was recorded. Identifications of fishes were made according to Pflieger (1975) and Page and Burr (1991).

Test fishes were anaesthetized briefly with tricaine methanesulfonate (Finquel, Argent Chemical) and infected by pipetting a suspension of glochidia directly onto the right gill. From 1-5 days after infection the fishes were re-anaesthetized and examined under a dissecting microscope to determine the condition of glochidial infections. Fishes that retained glochidial cysts during this initial period were segregated by species and the bottom of the aquarium was vacuumed daily to recover any excysted juveniles. The water was filtered through 20 micron Nitex mesh. Glochidia and juveniles were recovered from the filtrate under a dissecting microscope, using polarizing filters to increase visibility of the shells. Juveniles were examined microscopically to determine number, stage of development and viability. Both glochidia and juveniles of each species were photographed and samples were preserved in ethanol.

Cages were placed at three sites this year to hold adult mussels and facilitate the collection of glochidia. These cages were placed in the Spring River near Lawton, Kansas (T33S RR25E S1 Cherokee Co.), the Big River near Eureka, Missouri (T43N R4E S18 Jefferson Co.) and the Spring river near Ravenden, Arkansas (T18N R2W S7 Lawrence Co). Caging is especially useful for work with bradytictic species that release glochidia in the early spring. It is necessary to collect these mussels in low water conditions in the fall. Caging makes it possible to leave the animals under nearly natural conditions and recover them later from the cage in the winter or spring during the gravid period. The cages are 1x1x2 foot boxes of 1 inch square steel tubing, to which is welded 1.5 inch mesh diamond-pattern expanded steel panels, with lids of similar construction. These cages are partly buried in the substrate and attached to bedrock or to large boulders with masonry bolts to prevent displacement by floods. Mussels are placed in normal posture in the substrate within the cage. Individuals too small to be retained by the cage are placed in a smaller mesh container within the cage.

RESULTS and DISCUSSION

Potamilus capax

The distribution and status of *P. capax* were reviewed in the Recovery Plan (USFW 1989) by Cummings et al. (1990) and by Roberts et al. (1997). Our specimens were collected in the Belle Fountain Ditch in Dunklin County, Missouri (T16N R10E S20). Two of six individuals examined on June 6-20, 1996 were gravid with mature glochidia. No further observations were

made until August 13-22, 1996, at which time none of 16 individuals examined was gravid. On June 27, 1997, two of six individuals examined at the same site were gravid. Based upon these observations, it appears that the glochidia are probably released in July at this site.

Twenty-nine species of fish were tested as possible hosts (Table 1). Glochidia successfully transformed to juveniles only on freshwater drum (*Aplodinotus grunniens*). The metamorphosis from glochidium to juvenile was completed in 15-26 days at 21 °C, with the peak of excystment at 19-20 days post-infection (Figure 1). Somewhat more rapid development was observed at 26 °C (Figure 1). Unlike most mussel species, the glochidia of *Potamilus* grow substantially during encystment. Shell length (anterior-posterior) of *P. capax* increased from 95 micrometers in glochidia to 334 micrometers in recently excysted juveniles (means of 5 individuals).

The individual drum used in our host tests for *P. capax.* were retained and reinfected one year later, to test whether fish might serve as hosts in successive years or whether aquired immunity might develop. The second infection was relatively heavy, and the gills of the infected fishes were visibly affected for several days with grey areas apparently due to tissue hypertrophy and perhaps excessive mucus production. This response is also observed in incompatible hosts. However, the condition of the gills improved after about one week, and several thousand juveniles were eventually recovered from the six infected fish. Transformation was also successful upon two individuals that, in addition to the previous exposure to *P. capax*, had been infected with and rejected glochidia of *Cyprogenia aberti* eight months earlier. We hope to reinfect these drum with *P. capax* again in 1998.

Further studies of the induction and the persistance of host immunity are clearly needed. Very little is known of the induction and persistence of host immunity. These issues are important for artificial propagation, particularly with hosts such as drum, for which there is no established culture protocol. Reuling (1919) suggested that exposure to glochidia of one species might induce immunity to others. However, recent investigations suggest that this may often not be the case (present study and G.T. Watters, personal communication).

Glochidia attributed to *P. capax* were found encysted on drum in the lower Wabash River by Cummings and Mayer (1993). Our observation of successful transformation of *P. capax* on drum confirms its suitability as a host. The apparent restriction of *P. capax* to a single host, freshwater drum, suggests that the distribution and abundance of drum may be critical in the

survival of this mussel species. Drum are abundant in the agricultural ditches of the Missouri Bootheel and this fact may be important in maintaining the abundance and diversity of the unionid fauna. At least 12 other mussel species are also known to utilize drum as a host, including all members of the genus *Potamilus* investigated to date (Surber 1913, Watters 1994, Weiss and Layzer 1995, Roe et al. 1997).

Cyprogenia aberti

The western fanshell is a former C2 species, and its eastern congener, *Cyprogenia stegaria* (Rafinesque), is federally classified as endangered. *Cyprogenia aberti* is native to the Arkansas (Neosho), Black, St. Francis, Ouachita, Meramec and White river systems (Obermeyer 1996). Over 80 years ago, Utterback (1915) reported that *C. aberti* was "a rather common little shell in the White, Black, and Neosho basins of [Missouri]." and also noted that *C. aberti* was unusually abundant in Indian middens, suggesting that in prehistoric times it was abundant enough to be an important food source. However, recent surveys suggest that *C. aberti* is one of the rarest mussels in its range. It comprised only about 0.2% of individuals in a recent survey of the unionids of southeastern Kansas (Obermeyer et al. 1995). Seitman and Sadler (1994) surveyed sites on the Little Black, Castor, Cane Creek, Current and Strawberry rivers and examined 1,865 live mussels. Only 12 of those individuals were *C. aberti* (0.6%). Ahlstedt and Jenkinson (1986) surveyed 113 sites in the St. Francis River system, examined 13,752 mussels and found 27 *C. aberti* (0.2%). Ahlstedt and Jenkinson (1987) surveyed 252 additional sites in the St. Francis system and examined 14,993 live mussels, and found 2 live *C. aberti* (0.01%). The Spring River near Ravenden and Imboden in Lawrence County, Arkansas may be a present population stronghold (personal observations, 1997).

Cyprogenia are highly distinctive and interesting mussels. Several water tubes of each outer gill are uniquely elongated and produce elongated, wormlike, conglutinates (Chamberlain 1934, Utterback 1915). LeFevre and Curtis (1912) noted that *C. aberti* were gravid with glochidia in November. The locality was not reported but presumably was in Missouri or Arkansas. Chamberlain (1934) described the conglutinates of *C. aberti* from the St. Francis river in Arkansas and reported the release of mature glochidia in February.

We collected six individuals of *Cyprogenia aberti* in the Spring River during three searches on August 9, September 19, and October 8 of 1996. Four of these individuals were found near the

K-96 bridge (T33S R25E S11 Cherokee Co., Kansas) and two at the Pierce site south of Lawton (T33S RR25E S1 Cherokee Co., Kansas). All were caged together at the Pierce site. On December 10 we visited the cage site and found that three of the six animals were gravid with mature glochidia. The gravid individuals were transported to SMSU and stored at 10 °C. On December 17 we warmed one of these gradually over 12 hours from 10 °C to 20 °C, and within 12 hours it began extruding a conglutinate from the excurrent siphon. We subsequently found that we could reliably induce release of conglutinates by warming the animals, and that returning them to 10° C would prevent further conglutinate release.

The conglutinates were white in color, in contrast to the red conglutinates reported from *Cyprogenia* in the St. Francis River by Chamberlain (1934). Lengths of three that were released from a 7.3 cm long mussel were 6.3, 6.4, and 6.6 cm. The "head" end (positioned dorsally in the water tube) was expanded in a flattened oval shape about 2-3 mm wide and 3-4mm long. The structure was solid except for the head end, which was fenestrated with from one to four openings. This portion presumably occupies the dorsal excurrent chamber, and the openings may function to let water pass. The rest of the length is of uniform width or tapers very gradually to the "tail" end. The cross-section is oval or semi-rectangular, so that the conglutinate is somewhat ribbon-like. In some specimens the "head" is hardly expanded in the narrow plane, while in others it is broader in both planes.

Most of the structure of *Cyprogenia* conglutinates is a core of undeveloped eggs which adhere tenaciously to each other. The developed eggs are attached (equally tenaciously) to the outside, primarily in two strips along the narrow sides of the conglutinate. This layer of developed eggs extends nearly the full length of the conglutinate except for the "head". It usually did not extend over the full circumference except for approximately the last quarter of the length. The conglutinates were surprisingly durable. We sucked them vigorously in and out of a pipette to rupture the developed eggs without breaking the conglutinate. The undeveloped eggs did not break, while the vitelline membrane of eggs containing glochidia ruptured easily to release the larvae. The conglutinates did not seem adhesive to glass or to fingers, but sometimes did cling very tightly to the inside of a polyethylene pipette.

Sterile eggs comprised up to 85% of the conglutinate and serve a structural role by providing a tough and elastic support upon which the developed glochidia are arrayed in a superficial layer. This support increases handling time by the host fish and ensures that the

glochidia are easily shed when the conglutinate is bitten. It appears that sterile eggs may also make conglutinates more attractive to the host through taste and scent, because the conglutinates are fed upon avidly when offered to fish. Improved rates of host fish infection by conglutinates bearing sterile eggs presumably offset the consequent reduction in the number of larvae that are produced.

The glochidia of *Cyprogenia* have been described as "morphologically depressed" because of their short lever arm and narrow gape, and it was suggested that these features may make attachment to the fish host difficult (Hoggarth and Gaunt 1988). In fact, it was noticeably difficult to achieve good rates of initial attachment when pipetting *Cyprogenia* glochidia in suspension onto the gills. Much heavier infections were achieved when the conglutinates were fed upon by the fish.

Twenty-eight fish species and one crayfish species were tested as hosts (Table 2). Glochidia transformed successfully only on fantail darters (*Etheostoma flabellare*), logperch (*Percina caprodes*) and banded sculpins (*Cottus carolinae*). Time from encystment to peak of excystment was about 27 days at 22 °C and did not differ between glochidia encysted on logperch and those encysted on fantail darters (Figure 2).

Lampsilis abrupta

Distribution and taxonomic opinions on the pink mucket, *Lampsilis abrupta* (Say, 1831) [formerly known as *Lampsilis orbiculata* (Hildreth, 1828)] were reviewed in the Recovery Plan (USFW 1985). Both the pink mucket and its near relative, *Lampsilis higginsi*, are federally classified as endangered. We located a single gravid female and three males on May 9, 1997 in the Osage River at NW ¼ Sec 2, T41N, R12W Cole County, Missouri. At the time of collection (midday) the female was displaying the mantle flap lure, which was relatively elongate, pale, and not strongly marked. Functional glochidia were present, so this individual was transported to SMSU for host tests. Injection of sterile water into the marsupia dislodged bits of conglutinate that were composed of both fertile and undeveloped eggs. The proportions of undeveloped eggs in two such fragments were 30/80 (37.5%) and 27/60 (45%).

We tested 19 species of fishes as hosts of *Lampsilis abrupta* (Table 3). Of these, four species supported transformation of the glochidia. Suitable hosts were largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropteris dolomieui*), spotted bass, (*Micropterus*

punctulatus), and walleye (*Stizostedion vitreum*). Time from encystment to peak excystment was 12 to 14 days at 25 °C (Figure 3). The fish hosts of the closely related *Lampsilis higginsi* were investigated previously (Waller and Holland-Bartels 1988). In that study, nine species of fishes were tested as hosts. Of these, four were found to support transformation: largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieui*), walleye (*Stizostedion vitreum*), and yellow perch (*Perca flavescens*). The use of piscivorous carnivores such as bass and walleye by these mussels is consistent with the display of a relatively large, fish-like lure to attract the hosts.

Three female pink muckets located during surveys in the Meramec and Big River have been caged for the winter and will hopefully provide glochidia for further host work and rearing studies next year.

Fusconaia ozarkensis

This species is restricted to the Ozark uplifts in southern Missouri and northern Arkansas, where it is found in rivers of the Arkansas, White, Eleven Point and Current river drainages (Oesch 1995). Gravid *F. ozarkensis* were collected in the Spring River near Hoberg in Lawrence County, Missouri (T27N R27W S2). Release of the conglutinates was readily induced by mild hypoxia. The conglutinates were spindle-shaped and subcylindrical in section. The ova were not packed in distinct layers. Both the eggs and developing embryos were usually pink to red in color. However, two of 14 gravid individuals examined had white eggs and embryos. Individuals of *F. flava* and *F. ebena* in Missouri and Arkansas likewise produce either red or white eggs (Barnhart, unpublished observations), as do *F. subrotunda* in the upper Tennessee river basin (S. Bruenderman, personal communication).

Conglutinates of *F. ozarkensis* included a large proportion of sterile eggs, which may serve to attract host fishes. Among 8 individual females, the proportion of undeveloped eggs averaged 40% and varied from 15% to 51% (Barnhart 1997). Eggs bearing glochidia were relatively transparent. Sterile eggs, in contrast, were opaque and colored either pink or white. The color and visibility of the conglutinates was affected strongly by the presence of the sterile eggs. The conglutinates had average length of 6.1 mm and width of 1.1 mm. The average number of conglutinates released per individual was 238 (S.D. 35, n=8).

Eggs of *Fusconaia* containing glochidia ruptured easily, and it was possible to release the mature glochidia by drawing the conglutinate in and out of a Pasteur pipette. This action is similar to that of host fish feeding on prey. The sterile eggs were durable and were not broken by this treatment, and the egg membranes were sufficiently tough that the conglutinate remained intact even when drawn rapidly and repeatedly through a narrow aperture. The mature glochidia each deployed a larval thread upon release from the egg.

Twelve species of fishes were tested as potential hosts of *F. ozarkensis* (Table 4). Of these, transformation was observed on bleeding shiner (*Notropis zonatus*), cardinal shiner (*Notropis cardinalis*) and southern red-belly dace (*Phoxinus erythrogaster*). Recent studies of other *Fusconaia* species have also implicated cyprinids as hosts (Bruenderman and Neves 1993, Kitchel 1984). Curiously, the older literature lists a number of centrarchids and no cyprinids as hosts of *Fusconaia flava* and *F. ebena* (references in Watters 1994).

Temperature effects

The host tests reported herein did not attempt to match temperatures to those of the habitat, and temperature in the aquaria varied somewhat over the course of the year due to lack of consistent air conditioning and heating in the laboratory facility used for host tests. Temperature affects transformation time. Therefore, comparisons of transformation time among species or hosts should be made at similar temperatures. Perhaps more importantly, unionid species which breed in early spring may well be adapted to low temperatures. Recent studies in our laboratory provide evidence that the immune system of host fish may be depressed by low temperature, resulting in improved transformation success. The proportion of glochidia of *Anodonta suborbiculata* that successfully transformed on fish hosts significantly increased at lower temperature. This effect was not seen in *in vitro*, suggesting that the effect was on the interaction with the host rather than the glochidia themselves (Roberts 1997). It is conceivable that temperature could affect both intrinsic and acquired immunity of fishes to glochidia.

Other species

Host relationships were also investigated and hosts were determined for *Strophitus undulatus* and *Venustaconcha ellipsiformis*. This work will be reported elsewhere. We quantified sterile egg production in *Fusconaia ebena*, and *Pleurobema coccineum* (in addition to

C. aberti and *F. ozarkensis* described above) and developed the hypothesis of sterile eggs as a normal feature of reproduction in many unionids (Barnhart 1997). Unsuccessful attempts were made to obtain glochidia of *Cumberlandia monodonta* in the Big Piney, Gasconade, Big River and Meramec on four dates from March-July. This species is a late spring breeder in other localities, and we will give it priority in the coming year. Attempts in April and August to collect *Epioblasma curtisi* from the Little Black River were unsuccessful. We also provided preserved specimens of adult unionids to several labs (University of Alabama, Leetown Science Center, Texas Wildlife and Parks) for genetic studies.

Artificial transformation

Glochidia of *Potamilus capax* and *Cyprogenia aberti* placed in cell culture media (Keller and Zam 1990) both failed to transform. We have not yet attempted *in vitro* transformation with *Lampsilis abrupta* or *Fusconaia ozarkensis.* Although anodontine species often transform readily *in vitro*, lampsiline and amblemine species often do not (Hudson and Shelbourne 1990, personal observations). Transformation in cell culture media may prove to be undesirable, because the highly artificial conditions may select individuals having genetic characteristics unrelated to survival under natural conditions. Such selection might lead to production of populations unsuited for natural reproduction. Of course, similar criticisms can be leveled at any artificial culture process, including the rearing of early juvenile stages in laboratory conditions after transformation on natural hosts.

Culture of juvenile unionids

A cooperative agreement was signed with the Neosho National Fish Hatchery, wherein we will share resources and expertise in attempting to develop rearing methods for juvenile unionids. We began algae culture in preparation for rearing juveniles, and are currently scaling up these cultures to suitable volumes. We have provided algae cultures and juveniles of several species (*Lampsilis abrupta, Lampsilis cardium, Lampsilis rafinesqueana, Cyprogenia aberti* and *Potamilus capax*) to the Neosho National Hatchery for rearing experiments.

Plans for next year

In the upcoming year, I plan to 1) continue host studies, 2) develop a small-scale rearing facility where we can maintain early juveniles for periods of months, and 3) begin investigations of predation on early juveniles by benthic invertebrates. The unionid species for which I anticipate initiating or continuing host work or rearing over the next 2-3 years include the following:

1. *Cumberlandia monodonta*
2. *Leptodea leptodon*
3. *Epioblasma triquetra*
4. *Quadrula cylindrica*
5. *Obliquaria reflexa*
6. *Arcidens confragosus*
7. *Simpsonaias ambigua*
8. *Plethobasus cyphus*
9. *Alasmidonta marginata*
10. *Lampsilis abrupta*
11. *Potamilus capax*
12. *Ligumia recta*

During the summer of 1997, unionid surveys of the Meramec River basin by Missouri Department of Conservation and our field work in Missouri and Arkansas revealed populations of most of these species. We have caged individuals of several priority species in anticipation of host tests. These include pink mucket (*Lampsilis abrupta*), scaleshell (*Leptodea leptodon*), snuffbox (*Epioblasma triquetra*), elktoe (*Alasmidonta marginata*), salamander mussel (*Simpsonaias ambigua*), bullhead (*Plethobasus cyphus*), rabbitsfoot (*Quadrula cylindrica cylindrica*), three horn wartyback (*Obliquaria reflexa*), and western fanshell (*Cyprogenia aberti*).

DISSEMINATION OF RESULTS

The following articles, reports, abstracts, and presentations were derived wholly or in part from work supported by this funding.

Articles, reports, and theses

- Barnhart, M.C. and A.D. Roberts. 1997. Reproduction and fish hosts of unionids from the Ozark Uplifts. *In:* K.S. Cummings, A.C. Buchanan and L.M. Koch, eds. Conservation and management of freshwater mussels II. Proceedings of a UMRCC symposium, 16-18 October 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois.
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SUMMARY

Potential host fish species were determined for four unionids: fat pocketbook (*Potamilus capax*), pink mucket (*Lampsilis abrupta*), western fanshell (*Cyprogenia aberti*), and Ozark shell (*Fusconaia ozarkensis*).

Twenty-nine species of fish were tested as possible hosts of *Potamilus capax*. Glochidia successfully transformed only on freshwater drum (*Aplodinotus grunniens*). Time from encystment to peak of excystment was 19 days at 20 °C and 16 days at 26 °C. Individual fish were re-infected with *P. capax* after 12 months and successfully served as hosts for the second time. This result is important because very little is known of the development or persistence of host immunity to glochidia. The ability to re-use fish for mussel culture is of interest, particularly for host species such as drum for which there is no established culture method. We hope to reinfect these fish a third time in 1998.

Twenty-eight fish species and one crayfish species were tested as hosts of *Cyprogenia aberti*. Glochidia transformed successfully only on fantail darters (*Etheostoma flabellare*), logperch (*Percina caprodes*) and banded sculpins (*Cottus carolinae*). Time from encystment to peak of excystment was about 27 days at 22 °C. The conglutinates of *Cyprogenia aberti* were found to consist largely of sterile eggs, which appear to serve a functional role in conglutinate structure and attraction of host fish.

Nineteen species of fishes were tested as hosts of the federally endangered pink mucket, *Lampsilis abrupta*. Of these, four species supported transformation of the glochidia. Suitable hosts were largemouth bass (*Micropterus salmoides*), spotted bass, (*Micropterus punctulatus*), smallmouth bass, (*Micropteris dolomieu*), and walleye, (*Stizostedion vitreum*). The time from encystment to the peak of excystment from hosts was 12 to 14 days at 25 °C.

Twelve species of fishes were tested as potential hosts of *Fusconaia ozarkensis*. Of these, transformation was observed on bleeding shiner (*Notropis zonatus*), cardinal shiner (*Notropis cardinalis*) and southern red-belly dace (*Phoxinus erythrogaster*). Transformation time was 9-11 days at 26 °C. Conglutinates of *F. ozarkensis* also include a large proportion of sterile eggs, which may serve to attract host fishes.

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TABLE 1. HOST TESTS FOR *POTAMILUS CAPAX*

Locality and date of mussel collection	Date(s) of host test(s)	Number of females	Viability	Method of infection & remarks
Belle Fountain Ditch T16N R10E S20 Dunklin Co. MO. June 6, 1996	7/8/96 7/11/96 8/7/96 8/19/96	1 1 1 1	48/49 56/60 58/60 59/60	Adults kept at 21 C. Glochidia flushed from marsupia by syringe and either used immediately or stored at 10C for up to 3 days. Pipetted unilaterally onto right side gills unless otherwise noted.
Belle Fountain Ditch T16N R10E S20 Dunklin Co. MO. June 27, 1997	8/1/97	1	58/60	

Fish species tested	Locality and date of fish collections	Date Infected	N	Transform Success	Time & Temp.	Remarks
Polyodontidae						
Polyodon spathula paddlefish	Osage Catfisheries 6/10/96	7/8/96	2	No	-	Off within 4 days. One individual died and was not checked.
Lepisosteidae						
Lepisosteus osseus long-nose gar	Pomme de Terre River at Hwy 65, R20W T31N S17 Greene Co., MO 8/5/96	8/7/96	2	No	-	Off within 4 days
Clupeidae						
Dorosoma cepedianum gizzard shad	Stockton Lake at Hwy 123, T32N R24W S3 Polk Co. MO 8/5/96	8/7/96	10	No	-	Infected by bath- some attached to fins. Off within 4 days. MS222 was fatal to these fish.
Cyprinidae						
Notropis venustus blacktail shiner	Belle Fountain Ditch, T16N R10E S9 Dunklin Co., MO 6/20/96	7/8/96	6	No	-	Off within 4 days. One individual carried a glochidium of another species.
Pimephales notatus bluntnose minnow	Belle Fountain Ditch, T16N R10E S9 Dunklin Co., MO 6/20/96	7/8/96	8	No	-	Off within 4 days.
Notropis chrysocephalus striped shiner	Pomme de Terre River at Hwy 65, R20W T31N S17 Greene Co., MO 8/5/96	8/7/96	5	No	-	Off within 4 days.

Table 1. Host tests for *Potamilus capax*, continued

Cyprinidae, continued						
Campostoma anomalum stoneroller	Pomme de Terre River at Hwy 65, R20W T31N S17 Greene Co., MO 8/5/96	8/7/96	3	No	-	Off within 4 days.
Cyprinus carpio common carp	Missouri River S21, Chariton Co., MO 8/18/96	8/19/96	3	No	-	Off within 4 days.
Catostomidae						
Carpiodes cyprinus quillback	Main floodway E of Kennet, Dunklin Co., MO 7/11/96	8/7/96	1	No	-	Off within 4 days.
Carpiodes carpio river carpsucker	Missouri River S21, Chariton Co., MO 8/18/96	8/19/96	3	No	-	Off within 4 days.
Minytrema melanops spotted sucker	Main floodway E of Kennet, Dunklin Co., MO 7/11/96	8/7/96	2	No	-	Off within 4 days.
Ictiobus bubalus smallmouth buffalo	Osage Catfisheries 6/10/96	7/11/96	15	No	-	Off within 5 days. Very small fish.
		8/7/96	10	No	-	Off within 4 days.
Ictaluridae						
Ictalurus furcatus blue catfish	Osage Catfisheries 6/10/96	7/11/96	16	No	-	Off within 5 days.
Ictalurus punctatus channel catfish	Chesapeake Fish Hatchery 6/12/96	7/8/96	20	No	-	Off within 4 days.
Belontiidae						
blue gourami blue gourami	Pet Warehouse 7/8/96	7/8/96	5	No	-	Off within 4 days.
Cyprinodontidae						
Fundulus catenatus northern studfish	Pomme de Terre River at Hwy 65, R20W T31N S17 Greene Co., MO 8/5/96	8/7/96	3	No	-	Off within 4 days.
Poecilliidae						
Gambusia affinis mosquitofish	Osage Catfisheries 6/10/96	8/7/96	1	No	-	Off within 4 days.

Table 1. Host tests for *Potamilus capax*, continued

Percichthyidae						
Morone chrysops white bass	Stockton Lake at Hwy 123, T32N R24W S3 Polk Co. MO 8/5/96	8/7/96	5	No	-	Off within 4 days.
Centrarchidae						
Lepomis macrochirus bluegill	Chesapeake Fish Hatchery 6/12/96	7/8/96	15	No	-	Off within 4 days.
Lepomis humilis orange-spotted sunfish	Belle Fountain Ditch, T16N R10E S9 Dunklin Co., MO 6/20/96	7/8/96	10	No	-	Off within 4 days.
Lepomis megalotis longear sunfish	Stockton Lake at Hwy 123, T32N R24W S3 Polk Co. MO 8/5/96	8/7/96	5	No	-	Off within 4 days.
	Pomme de Terre River at Hwy 65, R20W T31N S17 Greene Co., MO 8/5/96	8/7/96	6	No	-	Off within 4 days.
Pomoxis annularis white crappie	Stockton Lake at Hwy 123, T32N R24W S3 Polk Co. MO 8/5/96	8/7/96	5	No	-	Glochidia encysted well but died within cysts. Off within 4 days.
	Missouri River S21, Chariton Co., MO 8/18/96	8/19/96	5	No	-	Off within 4 days.
Micropterus salmoides largemouth bass	Chesapeake Fish Hatchery	7/8/96	20	No	-	Off within 4 days.
Micropterus dolomieui smallmouth bass	Pomme de Terre River at Hwy 65, R20W T31N S17 Greene Co., MO 8/5/96	8/7/96	1	No	-	Off within 4 days.

Table 1. Host tests for *Potamilus capax, continued*

Fish species tested	Locality and date of fish collections	Date Infected	N	Transform Success	Time & Temp.	Remarks
Percidae						
Stizostedion vitreum walleye	Chesapeake Fish Hatchery 6/12/96	7/8/96	16	No	-	Off within 4 days.
Stizostedion canadense sauger	Missouri River S21, Chariton Co., MO 8/18/96	8/19/96	2	No	-	Off within 4 days.
Percina caprodes logperch	Stockton Lake at Hwy 123, T32N R24W S3 Polk Co. MO 8/5/96	8/7/96	4	No	-	Off within 4 days.
Etheostoma blennioides greenside darter	Stockton Lake at Hwy 123, T32N R24W S3 Polk Co. MO 8/5/96	8/7/96	1	No	-	Off within 4 days.
Sciaenidae						
***Aplodinotus grunniens* freshwater drum**	Belle Fountain Ditch, T16N R10E S9 Dunklin Co., MO 6/20/96	7/8/96	8	-	-	Good encystment and growth within cysts. However, fish died of Ich before transformation.
	Missouri River S21, Chariton Co., MO 8/18/96	8/19/96	6	Yes	-	Excellent transformation success
	Missouri River S21, Chariton Co., MO 8/18/96	8/1/97	6	Yes	-	Same fish as 8/19/96, reinfected heavily. Also infected previously with and rejected *Cyprogenia aberti*. Excellent transformation success-thousands of juveniles recovered.

TABLE 2. HOST TESTS FOR *CYPROGENIA ABERTI*

Locality and date of mussel collection	Date(s) of host test(s)	Number of individuals	Viability	Method of infection & remarks
Spring River at Hwy K-96, Cherokee Co., KS 12/10/96; kept at 10C.	12/17/96 12/20/96 1/3/97 4/9/97	1 1 1 1	60/60 58/60 59/60 65/65	Adults were kept at 10 C and released conglutinates soon after warming to 21 C. Glochidia were stripped from conglutinates and pipetted onto right gills, or conglutinates were fed to hosts (latter gave more effective attachment).

Fish species tested	Locality and date of fish collections	Date Infected	N	Transform Success	Time & Temp.	Remarks
Cyprinidae						
Cyprinus carpio common carp	Missouri River S21, Chariton Co., MO 8/18/96	12/17/96	2	No	-	Off within 3 days.
Notropis lutrensis red shiner	Flat Creek at Highway 65, T45N R21W S20, Pettis Co., MO 5/17/1996	12/17/96	6	No	-	Off within 3 days.
	Spring River at Hwy K-96, Cherokee Co., KS 12/10/96	12/17/96	4	No	-	Off within 3 days.
Notropis zonatus bleeding shiner	Little Sac at Hwy O, T30N R22W S20 Greene Co., MO 9/10/96	12/17/96	6	No	-	Off within 3 days.
Notropis cardinalis cardinal shiner	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	12/17/96	8	No	-	Off within 3 days.
Pimephales notatus bluntnose minnow	Belle Fountain Ditch, T16N R10E S9 Dunklin Co., MO 6/20/96	12/17/96	2	No	-	Off within 3 days.
Campostoma anomalum stoneroller	Little Sac at Hwy O, T30N R22W S20 Greene Co., MO 9/10/96	12/17/96	2	No	-	Off within 3 days.
	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	12/17/96	4	No	-	Off within 3 days.
Phoxinus erythrogaster southern redbelly dace	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	12/17/96	3	No	-	Off within 3 days.

Table 2. Host tests for *Cyprogenia aberti*, continued

Catostomidae						
Ictiobus bubalus smallmouth buffalo	Osage Catfisheries 6/10/96	12/17/96	6	No	-	Off within 3 days.
Ictaluridae						
Noturus flavater checkered madtom	Spring River at Hwy K-96, Cherokee Co., KS 12/10/96	12/17/96	1	No	-	Off within 3 days.
	North Fork White River at Hebron Access, T25N R11W S18 Douglas Co., MO	2/21/97	2	No	-	Off within 4 days.
Ictalurus punctatus channel catfish	Chesapeake Fish Hatchery 6/12/96	12/17/96	6	No	-	Off within 3 days.
Cottidae						
***Cottus carolinae* banded sculpin**	Spring River at Hwy 97, SE4 S14R28W T28N Lawrence Co. MO 12/12/96	12/17/96	10	No	-	Cysts persisted on most fish for more than 3 days.
		1/3/97	2	No	-	Reinfected same fish as above, heavily. Only 1-5 cysts persisted 3 days. Off within 10 days.
	Pearson Creek T29N R21W S35 Greene Co., MO 1/7/97	1/8/97	6	Yes	19-22 d 21C	Only modest success- many did not transform.
		1/17/97	1	Yes	22-25 d 21C	As above.
Centrarchidae						
Pomoxis annularis white crappie	Missouri River S21, Chariton Co., MO 8/18/96	12/17/96	4	No	-	A few cysts persisted on 1 fish for at least 3 days.
Lepomis humilis orange-spotted sunfish	Belle Fountain Ditch, T16N R10E S9 Dunklin Co., MO 6/20/96	12/17/96	5	No	-	Off within 3 days.
Lepomis macrochirus bluegill	Chesapeake Fish Hatchery 6/12/96	12/17/96	6	No	-	Off within 3 days.
Lepomis humilis orange-spotted sunfish	Belle Fountain Ditch, T16N R10E S9 Dunklin Co., MO 6/20/96	12/17/96	1	No	-	Off within 3 days.

Table 2. Host tests for *Cyprogenia aberti*, continued

Centrarchidae, continued

Lepomis megalotis longear sunfish	Pomme de Terre River at Hwy 65, R20W T31N S17 Greene Co., MO 8/5/96	1/8/97	4	No	-	Off within 5 days.
Micropterus salmoides largemouth bass	Stockton Lake at Hwy 160, Polk Co. MO 9/10/96	12/17/96	5	No	-	A few cysts persisted on 1 fish for at least 3 days.

Percidae

***Percina caprodes* logperch**	Little Sac at Hwy O, T30N R22W S20 Greene Co., MO 9/10/96	12/17/96	1	-	-	Many cysts persisted at least 3 days- but the patient died.
	Elk River, T32S R15E S10 Montgomery Co., KS 3/26/97	4/9/97	2	Yes	23-34 days 21 C	Good host. Drop-off period was relatively prolonged- see Figure 2.
Percina poxocephalus slenderhead darter	Spring River at Hwy K-96, Cherokee Co., KS 12/10/96	12/17/96	1	No	-	Off within 3 days.
	Elk River, T32S R15E S10 Montgomery Co., KS 3/26/97	4/9/97	3	No	-	Off within 3 days.
Etheostoma blennioides greenside darter	Little Sac at Hwy O, T30N R22W S20 Greene Co., MO 9/10/96	12/17/97	1	No	-	Off within 3 days
	Spring River at Hwy K-96, Cherokee Co., KS 12/10/96	1/3/97	2	No	-	Infected heavily. Some cysts persisted at least 10 days
		12/17/96	6	No	-	Off within 3 days
		2/21/97	1	No	-	Reinfected fish- off within 4 days
Etheostoma juliae yolk darter	North Fork White River at Hebron Access, T25N R11W S18 Douglas Co., MO	2/21/97	2	No	-	A few cysts persisted on both fish for at least 4 days, off within 7 days.
Etheostoma zonale banded darter	Spring River at Hwy 60,T26N R26W S20 Lawrence Co., MO 12/5/96	2/21/97	2	No	-	Off within 4 days

Table 2. Host tests for *Cyprogenia aberti*, continued

Percidae, continued						
Etheostoma spectabile orangethroat darter	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	12/17/96	4	No	-	Off within 3 days.
	Little Sac at Hwy O, T30N R22W S20 Greene Co., MO 9/10/96	1/3/97	3	No	-	Cysts persisted at least 5 days, less than 10 days
	Pearson Creek T29N R21W S35 Greene Co., MO 1/7/97	1/8/97	2	No	-	A few cysts on 1 fish persisted at least 9 days.
	Elk River, T32S R15E S10 Montgomery Co., KS 3/26/97	4/9/97	7	No	-	A few cysts on 2 fish persisted at least 3 days
Etheostoma caeruleum rainbow darter	Pearson Creek T29N R21W S35 Greene Co., MO 1/7/97	1/8/97	5	No	-	Heavy infection. A few cysts on 1 fish persisted at least 5 days.
	North Fork White River at Hebron Access, T25N R11W S18 Douglas Co., MO	2/21/97	2	No	-	Heavily infected- all cysts off within 4 days
Etheostoma stigmaeum speckled darter	Little Black River at Mudpuppy Conservation Area , Ripley Co., MO 3/24/97	4/9/97	2	No	-	Many cysts persisted on both fish at least 3 days, but all were off within 9 days.
Etheostoma flabellare **fantail darter**	Spring River at Hwy 60,T26N R26W S20 Lawrence Co., MO 12/5/96	12/17/96	5	Yes	20 days 21 C	Only 1-3 cysts each, only 1 live juvenile recovered
		1/3/97	6	Yes	19-22 days 21C	Infected heavily. Good numbers of juveniles recovered
		1/14/97	6	Yes	14-15 days 21C	Same as above, reinfected by feeding conglutinates.
		2/21/97	1	No	-	Same as above, reinfected again (third time). Cysts off within 4 days.
		4/9/97	2	Yes	23-41 d 21 C	Second infection for these fish. Excellent survivorship- good hosts. See Figure 2 for drop-off period.

Table 2. Host tests for *Cyprogenia aberti*, continued

Percidae, continued						
Stizostedion vitreum walleye	Chesapeake Fish Hatchery 6/12/96	12/17/96	6	No	-	Cysts persisted on 2 fish for at least 3 days.
Sciaenidae						
Aplodinotus grunniens freshwater drum	Missouri River S21, Chariton Co., MO 8/18/96	12/17/96	5	No	-	Off within 3 days
Cambaridae						
Orconectes macrurus Neosho midget crayfish	Spring River at Hwy 97, SE4 S14R28W T28N Lawrence Co. MO 12/12/96	12/20/96	7	No	-	No cysts after 11 days.

TABLE 3. HOST TESTS FOR *LAMPSILIS ABRUPTA*

Locality and date of mussel collection	Date(s) of host test(s)	Number of females	Viability	Method of infection & remarks
Osage River, Cole County, Missouri. July 18, 1997.	6/3/97 8/1/97	1 1 (same)	64/64 49/50	Conglutinate fragments flushed from gill, stripped from conglutinates by pipette and pipetted onto right side gills. Roughly 25% undeveloped eggs in conglutinates along with glochidia.

Fish species tested	Locality and date of fish collections	Date Infected	N	Transform Success	Time & Temp.	Remarks
Lepisosteidae						
Lepisosteus osseus long-nose gar	Missouri River near Columbia, MO	8/1/97	4	No	23 C	Off within 3 days.
Cyprinidae						
Notropis zonatus bleeding shiner	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO. 5/29/97	6/3/97	3	No	28 C	Off within 5 days.
Notropis lutrensis red shiner	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/29/97	6/3/97	3	No	28 C	Off within 1 day.
Campostoma anomalum central stoneroller	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/29/97	6/3/97	2	No	28 C	Off within 1 day.
Pimephales notatus bluntnose minnow	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/29/97	6/3/97	3	No	28 C	Off within 1 day.
Pimephales promelas fathead minnow	Chesapeake Fish Hatchery 3/27/97	6/3/97	3	No	28 C	Off within 1 day.
Ictaluridae						
Noturus flavater checkered madtom	James River at Hwy 125, T29N R20W S21 Greene Co., MO 5/22/97	6/3/97	4	No	26 C	Off within 1 day.
Ictalurus melas black bullhead	Elk River, T32S R15E S10 Montgomery Co., KS 3/26/97	6/3/97	3	No	26 C	Off within 1 day.

Table 3. Host tests for *Lampsilis abrupta*, continued

Ictaluridae, continued						
Ictalurus punctatus channel catfish	Osage Catfisheries 6/10/96	6/3/97	4	No	26 C	Off within 1 day.
Cottidae						
Cottus carolinae banded sculpin	Pearson Creek T29N R21W S35 Greene Co., MO 5/12/97	6/3/97	5	No	26 C	Very few attached- all off and no juveniles recovered after 10 days
Centrarchidae						
Lepomis cyanellus green sunfish	Chesapeake Fish Hatchery ponds	8/1/97	1	No	-	Off within 3 days
Lepomis macrochirus bluegill	Chesapeake Fish Hatchery ponds 3/27/97	6/3/97	5	No	-	Off within 5 days.
Lepomis megalotis longear sunfish	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/29/97	6/3/97	5	No	-	Off within 5 days.
Lepomis microlophus redear sunfish	Chesapeake Fish Hatchery 3/27/97	6/3/97	5	No	-	Off within 5 days.
Micropterus punctulatus **spotted bass**	Stockton Lake at Hwy 123, Polk Co. MO 5/12/97	6/3/97	2	Yes	10-17 days 25 C	Good transformation success.
Micropterus dolomieui **smallmouth bass**	James River at Hwy 125, T29N R20W S21 Greene Co., MO 5/22/97	6/3/97	1	Yes	10-16 days 25 C	Good transformation success.
Micropterus salmoides **largemouth bass**	Stockton Lake at Hwy 123, Polk Co. MO 5/12/97	6/3/97	1	Yes	10-17 days 25 C	Good transformation success.
	Chesapeake Fish Hatchery	8/1/97	6	Yes	14-19 days 25 C	Good transformation success.
			2	Yes	14-19 days 25 C	Previously infected 7/22/97 with and rejected *Fusconaia ozarkensis*.
Percidae						
Stizstedion vitreum **walleye**	Chesapeake Fish Hatchery	8/1/97	4	Yes	15 days 23 C	Fair transformation success.
Etheostoma spectabile orangethroat darter	Pearson Creek T29N R21W S35 Greene Co., MO 5/12/97	6/3/97	5	No	25 C	Off within 5 days.

TABLE 4. HOST TESTS FOR *FUSCONAIA OZARKENSIS*

Locality and date of mussel collection	Date(s) of host test(s)	Number of individuals	Viability	Treatment and method of infection
Spring River NE of Highway H bridge, SW4 S2 R27W T27N, Lawrence County, Missouri. July 18, 1997	7/22/97 7/24/97	3 3	45/53 66/72	Glochidia stripped from conglutinates by pipette and pipetted onto right side gills.

Fish species tested	Locality and date of fish collections	Date Infected	N	Transform Success	Time & Temp.	Remarks
Cyprinidae						
Notropis zonatus **bleeding shiner**	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO. 5/27/97	7/22/97	5	Yes	11 days 26 C	Suitable host.
Notropis cardinalis **cardinal shiner**	Elk River, T32S R15E S10 Montgomery Co., KS 3/26/97	7/24/97	4	Yes	9 days 26 C	Previously infected with (& rejected) *Lampsilis abrupta* . Suitable host. Very few cysts were lost.
Notropis lutrensis red shiner	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/27/97	7/22/97	4	No	-	Cysts persisted for 6 days.
Phoxinus erythrogaster **southern redbelly dace**	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	7/24/97	4	Yes	9 days 26 C	Suitable host.
Pimephales notatus bluntnose minnow	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/27/97	7/22/97	7	No	-	Several had no cysts form, others retained cysts for up to 6 days.
Pimephales promelas fathead minnow	Chesapeake Fish Hatchery	7/24/97	2	No	-	Off within 4 days.
Campostoma anomalum central stoneroller	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/27/97	7/22/97	2	(?)	-	Cysts persisted for up to 10 days but no juveniles were found.
	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	7/24/97	2	(?)	-	As above.

Table 4. Host tests for *Fusconaia ozarkensis*, continued

Centrarchidae						
Lepomis megalotis longear sunfish	Bear Creek at Hwy 32, T33N R25W S1 Cedar County, MO 5/27/97	7/22/97	4	No	-	Off within 2 days.
Lepomis macrochirus bluegill	Chesapeake Fish Hatchery	7/22/97	4	No	-	Most were off within 2 days, all off within 4 days
Micropterus salmoides largemouth bass	Chesapeake Fish Hatchery	7/22/97	4	No	-	Most cysts were off within 2 days, all off within 4 days.
Etheostoma caeruleum rainbow darter	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	7/24/97	4	No	-	All were off within 4 days.
Etheostoma flabellare fantail darter	Spring River at Hwy 60,T26N R26W S20 Lawrence County, MO 12/5/96	7/24/97	3	No	-	Some cysts persisted for at least 6 days.

FIGURE LEGENDS

Figure 1. Timing of the excystment of juvenile *Potamilus capax* from drum. Upper and lower graphs describe host tests in at two different temperatures in 1996 and 1997.

Figure 2. Timing of excystment of juvenile *Cyprogenia aberti* from logperch (upper graph) and fantail darters (lower graph).

Figure 3. Timing of excystment of juvenile *Lampsilis abrupta* from spotted bass (upper graph) and largemouth bass (lower graph).

Figure 1.

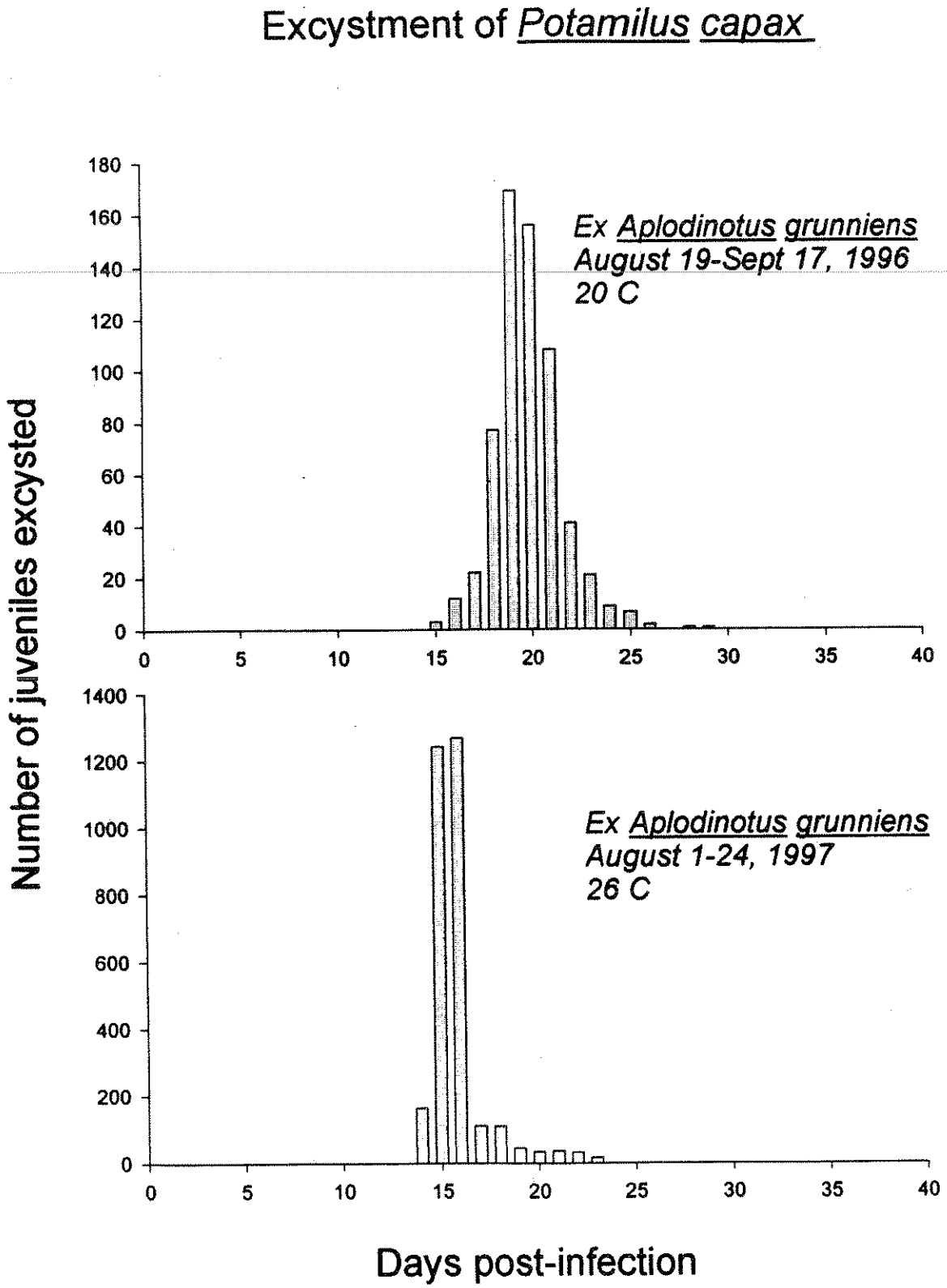
Excystment of Potamilus capax
Ex Aplodinotus grunniens
August 19-Sept 17, 1996
20 C
Ex Aplodinotus grunniens
August 1-24, 1997
26 C
Number of juveniles excysted
Days post-infection
0
20
40
60
80
100
120
140
160
180
200
400
600
800
1000
1200
1400
5
10
15
20
25
30
35
40

Figure 2.

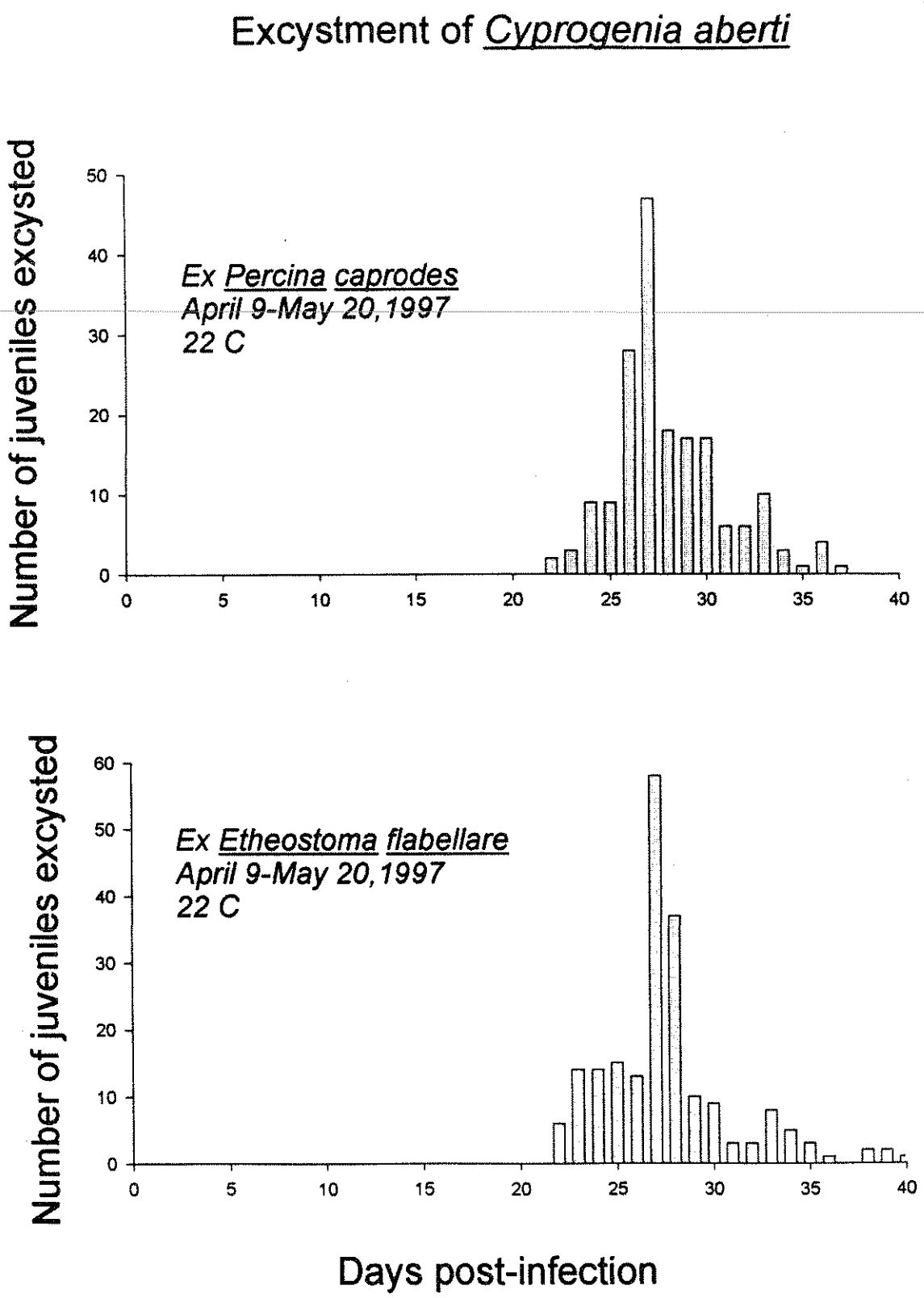
Excystment of Cyprogenia aberti
Number of juveniles excysted
Ex Percina caprodes
April 9-May 20,1997
22 C
0
10
20
30
40
50
0
5
10
15
20
25
30
35
40
Number of juveniles excysted
Ex Etheostoma flabellare
April 9-May 20,1997
22 C
0
10
20
30
40
50
60
0
5
10
15
20
25
30
35
40
Days post-infection

Figure 3.