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# Determination or Verification of Host Fish for Nine Species of Unionid Mussels

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ABSTRACT.—Identification of host fish for native freshwater mussels (Family: Unionidae) is increasingly important because of the rapid decline of these mollusks. To date, hosts have been identified for fewer than a third of all unionids inhabiting the United States and Canada. We identified previously unknown hosts for *Elliptio buckleyi, E. icterina, Lampsilis straminea claibornensis, Villosa lienosa* and *V. villosa*. Successful transformations also were achieved for *Lampsilis siliquoidea, L. teres, Megalonaias nervosa* and *Utterbackia imbecillis*. Fish hosts for these species have been listed in previous studies but many were deduced from circumstantial evidence, or if based on laboratory experiments, have not been verified.

## INTRODUCTION

The life cycle of unionid mussels was described as early as the 1860s (Leydig, 1866; Fo 1866). Larval mussels, called glochidia, must attach to a vertebrate host, usually a fish undergo organogenesis (transformation) and complete their development to the juve stage (Stein, 1971). Once this transformation is complete, mussels become filter-feed members of river and lake benthos (Fuller, 1974). While many unionid hosts have b identified (Young, 1911; Surber, 1913; Wiles, 1975; Yeager, 1986; Bruenderman and Ne 1993; Watters, 1994), hosts for over two-thirds of the unionids distributed in North Ame N of Mexico are still unknown (Watters, 1994). As part of a mussel culture and toxi testing program, our laboratory has performed many infections of fish with glochidia so doing, host designations cited in earlier literature were verified and new hosts w recorded for mussel species that have not been previously cultured. Such research is n essary before successful culture of this highly endangered fauna is possible and is a n identified in many endangered species recovery plans (U.S. Fish and Wildlife Service, 19 1984, 1987, 1994). Based on both distributional information and habitat preference number of fish species from which juvenile mussels were collected probably are not natural hosts but could serve as hosts in propagation studies.

## MATERIALS AND METHODS

Fish used for mussel infections were obtained from several sources. Largemouth b (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), channel catfish (*Ictalurus pun tus*), brown bullhead (*Ameiuris nebulosus*), long-nosed gar (*Lepisosteus osseus*) and gol shiners (*Notemigonus crysoleucas*) were purchased from fish hatcheries. Gulf sturgeon ( *penser oxyrincus desoti*) were obtained from the University of Florida. The eastern mos tofish (*Gambusia holbrooki*), weed shiner (*Notropis texanus*), Florida gar (*Lepisosteus p rhincus*), chain pickerel (*Esox niger*), warmouth (*Lepomis gulosus*), redear sunfish (*Lepo microlophus*) and *Lepomis* sp. were collected from the wild.

Mussels were collected from the Suwannee River (*Elliptio icterina, Utterbackia imbec Lampsilis straminea claibornensis, L. teres, Villosa villosa, V. lienosa, V. vibex*) and Appal icola River, Florida (*Megalonaias nervosa*), and several lakes near Gainesville, Fla. (*U*

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*backia imbecillis, Elliptio buckleyi*); Kinchafoonee Cr., Georgia (*V. lienosa*); St. Croix I Minnesota (*Lasmigona costata*); Spain Creek, Ohio (*Lampsilis siliquoidea*); and Ken Lake, Tennessee (*Megalonaias nervosa*). Voucher specimens of shells for mussels us infections are available at the Florida Natural History Museum, Gainesville, Fla.

Juveniles harvested from our cultures were to be used for growth studies and to tests. Therefore, two or three mussels were generally used for each infection effort t crease the genetic diversity of the juveniles. Subsamples of 50–100 of each mussel's gloc were tested for viability by adding NaCl crystals to the water in which they were dispe If viable, glochidia respond by snapping or completely closing their valves (Jones, 19

If subsamples of glochidia from one mussel were at least 90% viable, the rema glochidia were mixed with one or two others of the same species and rinsed in a beak well water (except *Lampsilis teres* described below). Next, several milliliters of water taining glochidia were either pipetted onto the gills of a fish or placed in a container an air stone, water and host fish for 15–90 min. This permitted a more natural exp of fish to the larvae, provided an opportunity for glochidia to attach to external sur in case that was their preferred mode of fixation and it was the only way to infect sm species because their gills were difficult to access with a pipette. Infected fish were he 18–22 C in flow-through tanks that were siphoned daily until juvenile mussels were dete Once juveniles were present, they were counted daily until no more were found.

For infections with *Lampsilis teres*, a slightly different method was used. Cultures initiated by obtaining glochidia directly from mussel marsupia with a pipette and pl them on the fishes' gills without rinsing. Glochidia from two or three mussels were us produce juveniles when possible to increase the potential for genetic diversity.

## RESULTS AND DISCUSSION

The goal of the infections was to produce juvenile mussels for multiple uses. There the potential overlap of habitat or range between the fish and mussels used in infec was not considered. However, according to Lee *et al.* (1980), Cummings and Mayer (1 and Burch (1975), all but three species of fish used as hosts were present in the re where the mussels were collected. The exceptions are *Notropis texanus* and *Lampsi claibornensis, L. siliquoidea* and *Lepisosteus platyrhincus* and *L. siliquoidea* and *Esox* throughout most of its range.

Infections of fish with glochidia were not performed quantitatively. That is, no att was made to quantify the number of glochidia pipetted onto hosts vs. the number transformed to juveniles because such an effort is time-consuming and was not the i of the research. The goal was to produce juvenile mussels. Therefore, statistical an were not performed on juvenile counts. However, based on counts of juvenile mussels each individual or group of fish from a species, it appears that some fish species prod more juvenile mussels than others. In general, more juvenile mussels were siphoned tanks containing fish onto which glochidia were directly pipetted than from tanks cor ing fish exposed to glochidia via an air stone (Table 1).

Hosts for five species of mussels were identified for the first time. These included *E. icterina*, which is distributed along the Florida and Atlantic coastal drainages, *Villosa lie* which ranges from Texas to Florida N into the Ohio River and *E. buckleyi, V. villosa Lampsilis straminea claibornensis* whose distributions are limited to parts of Georgia Florida (Burch, 1975). Largemouth bass and bluegill were included among the hos all of these mussel species.

New hosts were identified for *Lampsilis siliquoidea, L. teres, Megalonaias nervosa Utterbackia imbecillis*. The Florida gar was added to the list of gar species previously k

TABLE 1.—Summary of laboratory infections with unionid mussels performed during 1994–1996

| Mussel species<br>Fish host | Infection* method | Total no. juveniles | No. fish used | Days to transform | Months of infection |
|---|---|---|---|---|---|
| *Elliptio icterina* | | | | | |
| *Lepomis macrochirus* | p | 709 | 11 | 14–20 | Jun–Aug |
| *L. macrochirus* | a | 620 | 5 | 14–17 | Jul |
| *Micropterus salmoides* | p | 296 | 6 | 16–19 | Jun, Aug |
| *Elliptio buckleyi* | | | | | |
| *Lepomis macrochirus* | p | 226 | 5 | 14–17 | May, Jun |
| *Micropterus salmoides* | p | 92 | 5 | 17 | May, Jun |
| *Lepisosteus platyrhincus* | p | 75 | 3 | 17 | May, Jun |
| *Lampsilis s. claibornensis* | | | | | |
| *Lepomis macrochirus* | p | 105 | 10 | 18–28 | Apr, May, Dec |
| *L. microlophus* | p | 0 | 1 | — | Apr |
| *Micropterus salmoides* | p | 5584 | 441 | 9–30 | Apr, May, Dec, Jan |
| *Lepisosteus platyrhincus* | p | 0 | 1 | — | Mar |
| *Ictalurus punctatus* | p | 4 | 6 | 25 | Mar |
| *Ameiuris nebulosus* | p | 0 | 6 | — | Mar |
| *Gambusia affinis* | a | 9 | 2 | 25 | Mar |
| *Notropis texanus* | a | 10 | 2 | 25 | Mar |
| *Lampsilis siliquoidea* | | | | | |
| *Lepomis macrochirus* | p | 2 | 16 | 15 | Jul, Sep, Oct |
| *L. macrochirus* | a | 5 | 10 | 15–19 | Jul, Dec |
| *L. microlophus* | p | 0 | 1 | — | Jul |
| *L. gulosus* | p | 0 | 1 | — | Jul |
| *Micropterus salmoides* | p | 1617 | 15 | 17–25 | Jul, Sep, Oct, Dec |
| *M. salmoides* | a | 42 | 2 | 15–23 | Dec |
| *Lepisosteus platyrhincus* | p | 25 | 1 | — | Jul |
| *Esox niger* | p | 0 | 1 | — | Jul |
| *Lampsilis teres* | | | | | |
| *Lepomis macrochirus* | p | 0 | 63 | — | May, Jul, Aug, Dec |
| *L. macrochirus* | a | 0 | 48 | — | Jun, Jul, Dec |
| *Micropterus salmoides* | p | 97 | 18 | 23 | Dec, Apr–Aug |
| *M. salmoides* | a | 0 | 2 | 17–23 | Dec |
| *Lepisosteus platyrhincus* | p | 832 | 9 | 19–22 | Apr, May, Jul |
| *L. osseus* | p | 961 | 20 | 20–25 | Jun, Jul |
| *Ictalurus punctatus* | p | 0 | 7 | — | May, Jun |
| *Notemigonus crysoleucas* | a | 0 | 1 | — | Jul |
| *Lasmigona costata* | | | | | |
| *Lepomis macrochirus* | p | 0 | 10 | — | Sep |
| *Micropterus salmoides* | p | 0 | 7 | — | Sep |
| *Acipenser oxyrincus desoti* | p | 0 | 3 | — | Sep |
| *Megalonaias nervosa* | | | | | |
| *Lepomis macrochirus* | p | 0 | 18 | — | Oct, Nov |
| *L. macrochirus* | a | 0 | 19 | 25 | Oct, Nov |
| *Micropterus salmoides* | p | 13 | 8 | — | Oct |
| *M. salmoides* | a | 0 | 9 | — | Oct |
| *Ictalurus punctatus* | p | 0 | 2 | — | Oct |

TABLE 1.—Continued

| Mussel species<br>Fish host | Infection* method | Total no. juveniles | No. fish used | Days to transform | Months of infection |
|---|---|---|---|---|---|
| *Ameiuris nebulosus* | p | 0 | 3 | — | Oct |
| *A. nebulosus* | a | 0 | 4 | — | Oct |
| Utterbackia imbecillis | | | | | |
| *Lepomis macrochirus* | p | 171 | 13 | 9–14 | Jul, Aug |
| *L. macrochirus* | a | 490 | 12 | 9–14 | Jun, Jul, Aug |
| *Lepomis* sp. | p | 574 | 9 | 7 | Jul, Aug |
| *Lepomis* sp. | a | 301 | 3 | 7 | Aug |
| *Micropterus salmoides* | p | 1251 | 18 | 9–27 | Jul, Aug |
| *Ictalurus punctatus* | a | 9 | 4 | 25 | Jun |
| *Notemigonus crysoleucas* | p | 145 | 4 | 8–11 | Jun |
| *Villosa lienosa* | | | | | |
| *Lepomis macrochirus* | p | 996 | 132 | 5–28 | Jul, Sep, Dec, Jan |
| *L. macrochirus* | a | 37 | 5 | 27 | Sep, Dec |
| *Lepomis* sp. | p | 918 | 19 | 18–21 | Aug, Sep |
| *Lepomis* sp. | a | 19 | 3 | 20 | Jul, Aug, Sep |
| *Micropterus salmoides* | p | 831 | 312 | 5–27 | Jul–Sep, Dec, Jan |
| *M. salmoides* | a | 285 | 8 | 27 | Aug, Dec |
| *Ictalurus punctatus* | a | 1 | 17 | 26 | Sep |
| *Ameiuris nebulosus* | a | 16 | 0 | — | Sep |
| *N. crysoleucas* | p | 0 | 6 | — | Jul |
| *Villosa villosa* | | | | | |
| *Lepomis macrochirus* | p | 584 | 29 | 22–28 | Apr, May, Jul, Sep |
| *Micropterus salmoides* | p | 600 | 91 | 8–24 | May, Jul |
| *Lepisosteus platyrhincus* | p | 0 | 2 | — | May |
| *Villosa vibex* | | | | | |
| *Lepomis macrochirus* | p | 0 | 5 | — | Jul |
| *L. macrochirus* | a | 0 | 4 | — | Jul |
| *Micropterus salmoides* | p | 0 | 3 | — | Jul |

* a = fish were placed in a container with water, glochidia and an air stone for 15 to 90 min glochidia were pipetted directly onto fish gills

to be hosts for *Lampsilis teres* and *L. siliquoidea* (Howard, 1914; Coker *et al.*, 1921; Ellis Ellis, 1926; Watters, 1994), and was also a host for the narrowly distributed, *L. strai claibornensis*. A few juveniles were collected from largemouth bass infected with *Megalo nervosa* glochidia. However, in general, attempts to transform glochidia of this species with little success. The golden shiner and channel catfish are new hosts for *Utterb imbecillis*. Several hosts determined in earlier studies either by infection of fish in the oratory (Trdan and Hoeh, 1982) or identification of glochidia on fish gills (Stern Felder, 1978; Trdan and Hoeh, 1982), were verified in this study (Table 1).

Production of juvenile mussels of several species was very difficult or unsuccessfu juvenile *Villosa vibex* or *Lasmigona costata* were produced; however, infections were formed on only a single day with one group of mussels and a few fish species. Consid the number of attempts and the variety of fish we used, the production of juvenile *Lam*

*teres* was the most difficult. Transformations were attempted during 5 separate mo in 3 different yr on six species of fish before juveniles were produced (Table 1). Methods tha were productive with other mussels, *i.e.*, direct application of rinsed glochidia onto fish gill or the use of air stones, never produced juvenile *L. teres*. This species' glochidia only sur vived a few hours outside of the marsupia, and closed their valves when exposed to light vibrations or temperature changes. Therefore, glochidia were not prepared in any way be fore being put on hosts. Instead, glochidia were withdrawn from the marsupia and imme diately placed on the gar's gills. This method was so successful that during April, May and June of 1996, nearly 1800 juveniles were produced from 29 Florida gar and long-nosed gar (Table 1).

In several cases, species within the same unionid genus had similar hosts, as has been noted in *Anodonta* (Trdan and Hoeh, 1982), *Ligumia* (Lefevre and Curtis, 1912; Young, 1911; Stern and Felder, 1978) and *Potamilis* (Howard, 1913; Howard and Anson, 1923; Cummings and Mayer, 1993). Often, the number of juveniles produced from infections of a particular species of fish differed markedly among congener mussel species. For example, virtually no *Lampsilis siliquoidea* or *L. teres* juveniles were produced on bluegill, while an average of 10 *L. straminea claibornensis* juveniles were transformed per infected bluegill. More juvenile *L. s. claibornensis* were produced from infections of largemouth bass than any other fish; the bluegill was also an effective host. The largemouth bass was also the most productive host for *L. siliquoidea*, followed by *Lepisosteus platyrhincus*. The most productive hosts for both *Elliptio icterina* and *E. buckleyi* were largemouth bass, followed by bluegill. No comparison can be made with their transformation success on other fish because the Florida gar was the only other fish used, and only for *E. buckleyi*. In contrast, 95% of the *Lampsilis teres* were produced from infections of Florida gar and long-nosed gar. The bluegill was the best host for *Villosa lienosa*, while largemouth bass produced more *V. villosa*.

Whether or not these infection results indicate what occurs in the natural environment is impossible to determine without field validation. For example, fish hosts must both come in contact with glochidia and provide the proper environment for transformation. Since habitat and prey preferences are similar for largemouth bass and bluegill during part of their lives, lures observed on the mantle flaps of many mussels (*e.g.*, *Lampsilis* sp., *Villosa* sp.) (Haag *et al.*, 1995) probably attract both fish species. Their suitability as hosts has been verified in these laboratory infections. The relative number of juvenile mussels produced by bluegill and largemouth bass in natural infections, however, may differ from that seen in this study because the success of artificial infections depends in part on the accessibility of the gills to a pipette. Therefore, the size of the operculum and gills affects their exposure to glochidia. However, the fact that Florida and longnose gar produced virtually all of the juvenile *Lampsilis teres* in the laboratory strongly suggests that these fish are its primary or only hosts. Finally, both gar and largemouth bass developed immunity to glochidial infections after several exposures. No attempt was made to count the number of exposures it took or how much time was required to lose immunity, but both fish species were used successfully for juvenile production after not being exposed for several months.

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