Identification of suitable host fishes for the round hickorynut mussel (*Obovaria subrotunda*) from Kentucky

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DEDICATION

This thesis is dedicated to my parents

William G. Shepard & Donna J. Mitchell

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ABSTRACT

The round hickorynut mussel, *Obovaria subrotunda,* is a common species found throughout the Tennessee, Cumberland, and Ohio River systems, and in the Lake St. Clair and Lake Erie drainages in North America. However, it is critically imperiled thoughout much of its northern range. Ortmann (1919) reported this species to be a long-term brooder with eggs present in September and glochidia in June, but no host has been identified. We collected four males and one female from Buck Creek (Cumberland River) in Pulaski County, Kentucky in the fall 2002. Individuals were returned to the Center for Mollusk Conservation, Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky, where they were held in semi-natural conditions in flow-through river tanks. In early winter, the female was examined and contained mature glochidia. Potential hosts were collected from several sites within the mussel's known range (primarily tributaries to the Cumberland and Kentucky Rivers), returned to the Center, acclimated to ambient room temperature, and held in (1 to 9 L tanks, depending on the size of the fish). We extracted glochidia from the female and pipetted 10-150 (depending on the size of the fish) onto the gills of each fish. Infested fish were held in tanks at room temperature (23-24ºC) and siphoned daily to check for metamorphosed juveniles or rejected glochidia. We tested 48 fish species, including 22 genera and 9 families (Percidae, Cyprinidae, Ictaluridae, Fundulidae, Catostomidae, Cottidae, Sciaenidae, Centrarchidae, and Atherinidae). *Obovaria subrotunda* had a high degree of host specificity for the family Percidae. Suitable hosts include: the variegate darter, *Etheostoma variatum*, frecklebelly darter, *Percina* s*tictogaster*, emerald darter, *E. baileyi*, greenside darter, *Etheostoma blennoides*, and speckled darter (longhunt), *Etheostoma cf stigmaeum.* All five species were identified as possible natural host for *Obovaria subrotunda* by promoting transformation of glochidia to active juvenile mussels.

TABLE OF CONTENTS

SECTION **PAGE**

LIST OF TABLES

TABLE **PAGE**

LIST OF FIGURES

FIGURE **PAGE**

INTRODUCTION

Freshwater mussels (Bivalvia: Unionidae) are one of the most endangered group of organisms in North America. In the United States, about 70 % of the freshwater mussel fauna are listed either as extinct, endangered, threatened, or special concern (Williams et al. 1993). Most of the decline has occurred among endemic species of southeastern United States drainages, such as the Tennessee and Cumberland rivers, and the Mobile Bay basin (Bogan 1993).

The decline of unionid populations can be attributed to both biological and anthropogenic factors. In the early 1900's, mussel populations were over exploited by the commercial harvest from the pearl button industry. Other factors contributing to population declines are agricultural and industrial pollution, such as wastewater effluents, acid mine seepage, and sedimentation (Zale and Neves 1982b; Havlik and Marking 1987). The impoundment of major rivers has been detrimental to unionids through alteration of water temperature regimes, flow velocity, water depth, increased siltation, depleted dissolved oxygen, and limited access to host fish (Miller et al. 1984; Havlik and Marking 1987; McMurray 1997). Dams also restrict natural migration and mobility of fish-hosts, and may isolate unionid populations (Kat 1984).

Kentucky has a high diversity of unionid species, including representatives of the Cumberlandian and Mississippian faunal regions. Of the freshwater unionid taxa recognized from the United States and Canada, 35% (103 taxa) are known to occur or have occurred in Kentucky. Kentucky ranks third in unionid richness behind Tennessee and Alabama (Cicerello et al. 1991). However, habitat loss, degradation and exploitation have caused severe declines in unionid populations during the last 200 years, making this group of organisms the most endangered in the Commonwealth (Cicerello et al. 1991). In Kentucky fourteen species are listed by the United States Fish and Wildlife Service as endangered, 12 are extinct, and 8 are extirpated (USFWS 1999).

The complicated life cycle of mussels has contributed to the demise of many species (Fig. 1). Unionids are parasitic for a portion of their life cycle and require a vertebrate host, usually a fish. The parasitic larva or glochidia, attach to gills or fins during a transformation process to juveniles. Unionids may parasatize a specific fish species, a family of fish (Zale and Neves 1982a, b, Yeager and Saylor 1995),

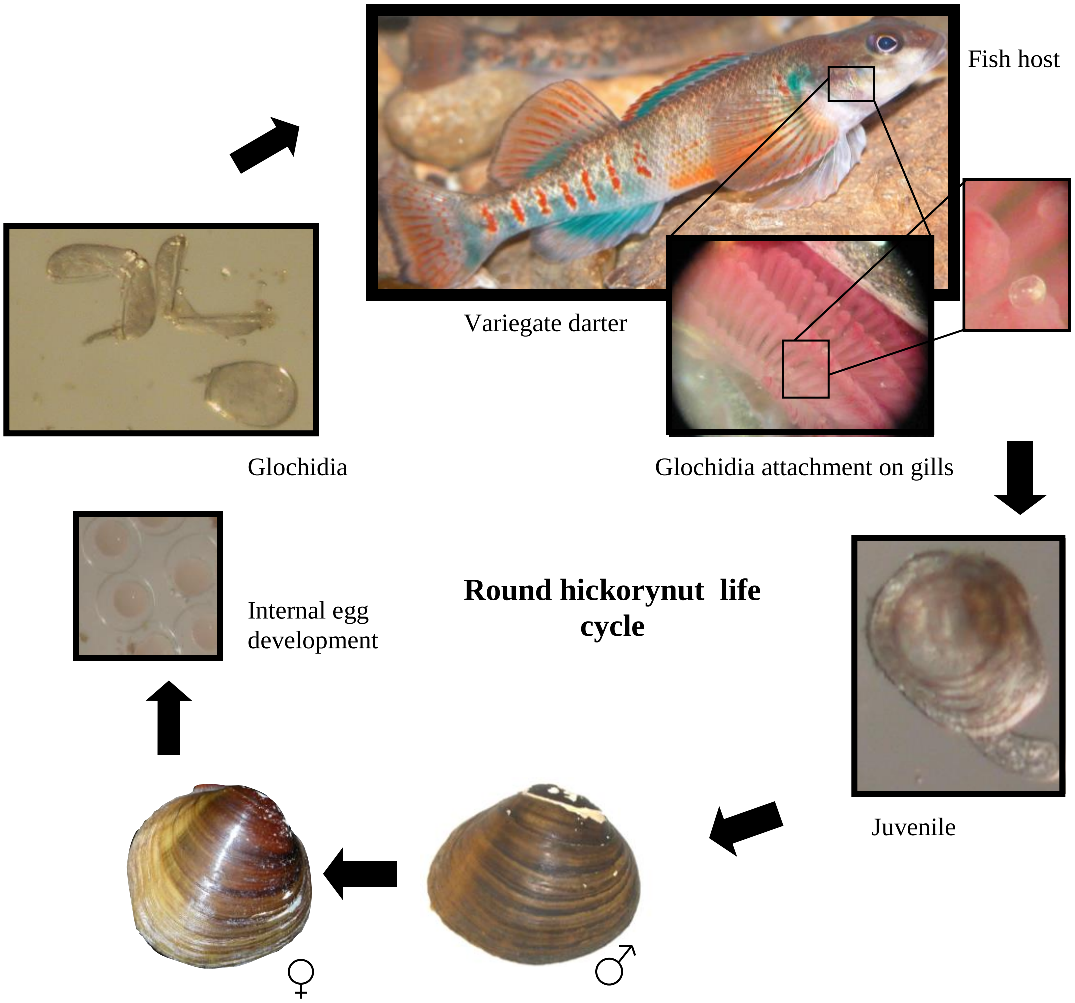

Figure 1. Life history of the round hickorynut, *Obovaria subrotunda*.

or taxonomically wide varieties of fish (Trdan and Hoeh 1982). About 300 glochidia-host relationships have been suggested, mostly based upon glochidia identification from naturally infested fish hosts. However, glochidia of closely related species within the same genus are hard to distinguish from one another and proper identification can be difficult (Hoggarth 1994). In addition, the identified fish host may not overlap in distribution with current mussel populations. Thus, additional natural hosts must be identified to assess the appropriate conservation and management strategy.

Another method of identifying host fish is to inoculate them in a laboratory. This technique involves exposing potential fish hosts to glochidia and monitoring the infested fish in aquaria during the transformation period. The host and non-host fishes are determined by monitoring the production of juvenile mussels or rejection of glochidia (Zale and Neves 1981). About one-third of host fish relationships have been confirmed by laboratory glochidia-host experiments (Watters 1994). This method has proven successful and provides useful information for mussel conservation efforts (Neves et al. 1985; Yeagor and Saylor 1995; Hove et al. 1998; Khym and Layzer 1998; Haag and Warren 2001; Rogers et al. 2001; Jones and Neves 2002).

Several mussel species, such as the round hickorynut have not had a host identified. The round hickorynut, *Obovaria subrotunda*, was described by Rafinesque in 1820. The round hickorynut is a common species found throughout the Tennessee, Cumberland, and Ohio River systems, and in the Lake St. Clair and Lake Erie drainages in North America. However, it is critically imperiled throughout much of its northern range. In Kentucky, the round hickorynut is rare, but can be found in the Green, Licking, upper Kentucky, and upper Cumberland Rivers. *Obovaria subrotunda* has a G4 ranking and is considered to be endangered in Canada (NatureServe 2005).

In addition to *O. subrotunda*, within the genus *Obovaria*, there are four species: *O. olivaria* Rafinesque (hickorynut), *O. retusa* Lamarck (ring pink), *O. unicolor* Lea (Alabama hickorynut), and *O. jacksoniana* Frierson (southern hickorynut). Three of these, *O. subrotunda*, *O. olivaria*, and *O. retusa* can be found in Kentucky. The hickorynut is considered to have stable populations in Kentucky (Ohio R.). The rink pink was listed as an endangered species by the USFWS in 1989 (USFWS 1999) and the only recent collections of live specimens are from the upper Green River in Kentucky. The other representatives, *O. unicolor* and *O. jacksoniana* have G3 and G1 rankings, respectively (Nature Serve 2005), and are limited to the southern

United States. Only one of the five species within the genus *Obovaria*, (*O. olivaria*), is considered to have stable populations. All *Obovaria* species are in a rapid decline, and there is limited life history information available. Fish hosts are known for *O. olivaria* (Clark 1981; Brady et al, 2004) and *O. unicolor* (Haag and Warren 2003). *Obovaria subrotunda*, occurs sympatric with *O. retusa* throughout much of its range (Tennessee R., Cumberland R., Ohio R., and Green R.) and both have similar habitat requirements, and each may have similar fish hosts. In this study, we examined the life history characteristics of *O.subrotunda* to determine fish host relationships. The findings may assist with recovery of the species and others within the genus *Obovaria*.

METHODS

Mussel and Fish Collection

Mussels were collected by snorkeling, in late March to mid November 2003. Five males and one female round hickorynut mussels were collected from Buck Creek (upper Cumberland River, below Cumberland Falls) in Pulaski County, Kentucky. Ortmann (1919) noted *O. subrotunda* gravid and discharging in May. Individuals were transported in temperature controlled insulated coolers containing aerated stream water. All mussels were measured and placed into a plastic "tool tray" container (40.2 cm L × 20.6 cm W × 12.4 cm D) filled with sand and gravel. The trays were stream-fed, submersed in a flow-through raceway (3.7 m L × 1.2 m W × 0.8 m D) located at the Center for Mollusk Conservation (CMC), a facility operated by the Kentucky Department of Fish and Wildlife Resources in Frankfort, KY.

Depending on stream conditions, fish were collected either with Smith Root® backpack electro-fishing gear or with a seine (Murphy and Willis 1996). Potential host fishes were collected from several sites within the mussel's distribution in Kentucky: Red River (Upper Kentucky River), Menifee Co., Elkhorn Creek (lower Kentucky River) Franklin Co., upper Green River (Main-stem) Casey Co., Horse Lick Creek (upper Cumberland River) Rockcastle Co., and Taylorsville Lake State Park, (Salt River) Spencer, County. All fish were held in temperature controlled coolers containing aerated stream water and transported to the

CMC, temperature acclimated, and held in Aquatic habitats® tanks (1 to 9 L, depending on the size of the fish). Only healthy fish were retained for host relationships.

Infestation Methods

The infestation procedures generally follow those used by Zales and Neves (1982a), but were modified to conserve glochidia from the single female of *O. subrotunda*. A maximum of 1,500 glochidia (average of ~ 800 per infestation) were extracted as potential host fish became available for induced laboratory exposures. To reduce any loss of glochidia from premature abortion (i.e., handling), the female was held in a submersible container (153.3L mm × 153.3W mm × 49.6D mm) with a 200-μm mesh lid, which allowed continual freshwater exchange. The container was checked daily for viable glochidia. Any released larvae were counted and used in exposure trials for potential host fishes. Additional glochidia were extracted from the gravid female by flushing the gills, using a water-filled pippet. After extraction, glochidia were checked for maturity (closing response from exposure to salt crystals) and counted. To conserve glochidia, most fish were exposed individually and follow procedures described by Rogers (2001). Potential host fishes were placed into a 2.5 L plastic container and anesthetized with a dilute MS-222 solution to reduce stress before exposure. An appropriate number of glochidia (~10-150 per fish, depending on the size of fish) were introduced to each individual fish by pippeting a known quantity onto the gill filaments of one branchial cavity. After exposure each fish was placed into another 2.5 L container with freshwater for recovery. When adequate numbers of glochidia were available fish were exposed to glochidia in an aerated 2.5 L plastic container filled with just enough water to cover the dorsal fins of the fish. Depending on the number of glochidia present, all exposures ranged from 5 to 60 minutes. All fish were placed into an Aquatic Habitats® multi-tank (1-9L), stand alone re-circulating system containing temperature controlled (23-25º C) aged well water. Each tank held ~1 to 10 fish of a single species, depending on the size of the fish. The contents of the aquaria were siphoned into a 200-μm mesh sieve until juvenile mussels were collected. Glochidia transformation to the juvenile stage was determined when juveniles were observed in the tanks. Daily siphoning continued until no juveniles were recovered.

For each test, I recorded, the number of individual fish exposed that remained alive for the duration of the infestation period, the number of glochidia exposed to each fish, and the number of rejected or sloughed glochidia. Suitable host-fish species were identified as those that produced live and active juvenile mussels. Fish that rejected or sloughed the glochidia (without any transformations) were considered as unsuitable hosts. Potential host-fish species that facilitated partial transformations (i.e., majority of glochidia rejected before transformation) were labeled as marginal hosts. I recorded the total infestation period, total number of juveniles recovered from each host fish species, and observations of glochidial encystment.

Age and Growth Determination

Round hickorynut valves from Buck Creek, Kentucky (collected November 1983, July and August 1984, Branson Museum, Eastern Kentucky University), were measured for length. *Obovaria. subrotunda* have relatively distinct external growth rings (annuli), which were counted visually by two experienced biologists. Based on studies of Neves and Moyer (1988), we assumed that one annuls/year is formed. Length-at-age regressions were examined along with computing a von Bertalanffy growth equation to compare growth rates of males and females. Valves of *O. subrotunda* exhibit sexual dimorphism, in which the females have a less pointed, rounded posterior margin. We estimated age at maturity by noting which annulus (growth year) started showing this dimorphic characteristic.

RESULTS

Reproductive Behavior, Glochidia size, and Fecundity

Adult *O. subrotunda*, individuals ranged in size from 34 to 48 mm total length. The males measured (42.27L mm × 36..84H mm × 24.10W mm; 42.32 L mm × 36.65H mm × 25.96W mm; 44.63L mm × 37.39H mm × 24.64W mm; 48.79L mm × 40.83H mm × 27.46W mm) and the single female (34.80L mm × 29.90H mm × 18.38W mm). The female was examined in September 2003 upon arrival at the CMC and was not gravid. After being held in close proximity to the four males, for several months, the female reproduced and was gravid upon examination in January 2004.

There were no obvious mantle displays or any host-attracting behavior, but I did observe orange-brown coloration on the outer edge of the mantle, which agrees with Ortmann's (1912) observations in Ohio River drainage specimens. He also noted the mantle as being "slightly lamellar and crenulated". The soft parts are considered essentially the same as *O. retusa*, with the ovisacs being narrower than the regular water tubes in non-gravid females, and numbering thirty or more (Ortmann 1912). The glochidia were held individually within the water tubes of the gills and once released by the female they fell to the substrate (bottom of container).

Glochidia (*n* = 25) were (mean ± SD), 182 ± 3.5µm (length), 223 ± 5.3 µm (height), and 85 ± 7.8 µm (hinge length) (Fig. 2). These measurements were similar to Ortmann's (1911), measurements 200 µm length and 230 µm height (individuals from the Ohio, Monongahela, Beaver Rivers in Pennsylvania). The female released glochidia (< 200 per daily examination of container) from May to mid-June 2004, after extracting ~895 glochidia May 1, 2004 for the first host infestation trial. Based on these observations, *O. subrotunda* is considered to be a long-term or winter brooder. Spawning in the fall and releasing glochidia in the spring. Approximately 5,725 glochidia were extracted from the female *O. subrotunda* used during all infestation trials.

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Age, Growth, and Maturity

Male *O. subrotunda* ranged from 4 y to 16 y in age and females ranged from 5 y to 13 y. Total lengths of male *O. subrotunda* ranged from 24.62 mm to 53.19 mm and 26.43 mm to 38.33 mm for females. Length-at-age regressions for *Obovaria subrotunda* males in Buck Creek revealed higher growth rates and larger size compared to females (Fig. 3), which is similar to the observations made by Ortmann (1911) from specimens collected in Pennsylvania. Valves of females exhibited evidence of maturity or change in shell growth, from ages 3 to 5 years. Mean lengths (L) by age were fitted to a von Bertalanffy growth equation: male lengths [$L\ (\text{mm}) = 50.8\ (1 – e^{-0.253\ (t-0.125)})$] ($r^2 = 0.99$) and for females [$L\ (\text{mm}) = 37.8\ (1 – e^{-}$

$^{0.247(t+0.51)}$)] ($r^2 = 0.98$). Male annual growth averaged 6.0mm/y through age 6, and decreased to 2.1mm/y thereafter (Table 1), while females averaged 4.7mm/y through age 6, and decreased to 1.2mm/y (Table2).

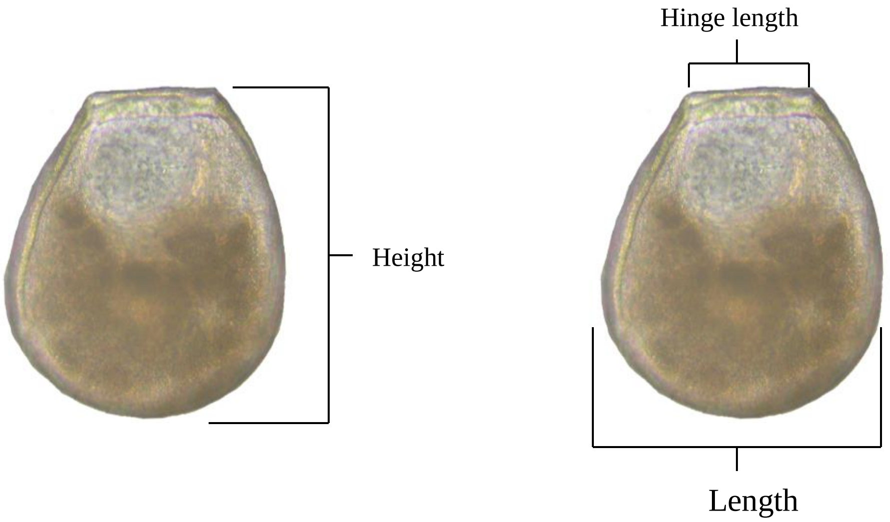

Figure 2. Glochidia of *Obovaria subrotunda* from Buck Creek (upper Cumberland River drainage) showing length, height, and hinge length.

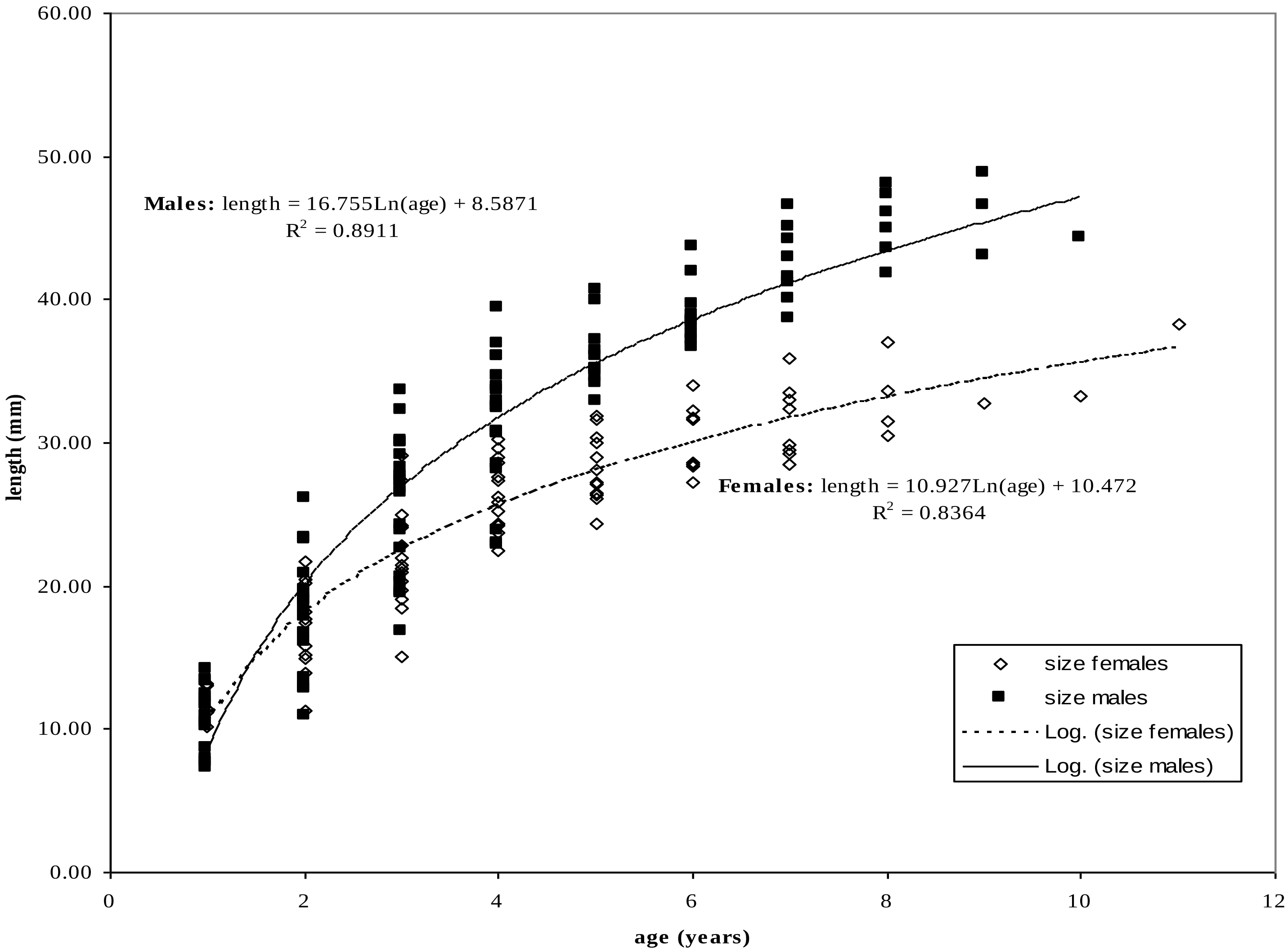

Figure 3. Length (mm)-at age regressions for male (upper line) and female (lower line) *Obovaria subrotunda* determined from external annuli. Shells collected from Buck Creek, Pulaski County, Kentucky, from 1983 to 1984, Eastern Kentucky University Branson Museum.

Table 1. Observed and predicted lengths-at-annuli (mm) of male *Obovaria subrotunda* shells collected from Buck Creek, Pulaski County, Kentucky, 1983 to 1984.

Annulus	Number of individuals	Observed length		Predicted length	Growth Increment
		Mean	Range		
0					
1	15	10.8	7.3 – 14.2	8.6	8.6
2	17	18.2	10.9 – 26.1	16.6	8
3	21	25.9	16.8 – 33.7	23.1	6.5
4	19	31.4	22.9 – 39.4	28.4	5.3
5	11	36.1	32.9 – 40.7	32.6	4.3
6	10	39	36.6 – 43.7	36.1	3.4
7	8	42.5	38.6 – 46.6	38.9	2.8
8	6	45.3	41.8 – 48.1	41.1	2.3
9	3	46.1	43.0 – 48.8	43	1.8
10	1	44.3	44.3 – 44.3	44.5	1.5

Table 2. Observed and predicted lengths-at-annuli (mm) of female *Obovaria subrotunda* shells collected from Buck Creek, Pulaski County, Kentucky, 1983 to 1984.

Annulus	Number of individuals	Observed length		Predicted length	Growth Increment
		Mean	Range		
0					
1	5	11.8	10.1 – 13.2	10.3	10.3
2	11	17	11.3 – 21.7	15.5	5.2
3	16	22	15 – 29.1	19.8	4.2
4	15	26.4	22.4 – 30.2	23.2	3.4
5	12	28.2	24.3 – 31.9	26	2.8
6	9	30.4	27.2 –34	28.2	2.2
7	8	31.5	29.2 – 36	30.1	1.8
8	4	33.2	30.5 – 37	31.5	1.5
9	1	32.8	32.8 –32.8	32.7	1.2
10	1	33.2	33.2 – 33.2	33.7	1
11	1	38.3	38.3 – 38.3	34.5	0.8

Fish Hosts

We tested 48 species, including 22 genera and 9 families (Percidae, Cyprinidae, Ictaluridae, Fundulidae, Catostomidae, Cottidae, Sciaenidae, Centrarchidae, and Atherinidae) as potential hosts for *Obovaria subrotunda* (Table 3). All scientific and common names of potential host fishes were derived from (Nelson et al. 2004) and current distributions from (Etnier and Starnes 1993).

Five species of darters (Percidae) were identified as hosts from induced exposures of glochidia (*Etheostoma baileyi*, *E. blenniodes*, *E. cf stigmaeum*, *E. variatum*, and *Percina stictogaster*). All five species are sympatric with *O. subrotunda* in parts of its range.

Three of the five species of host fishes have restricted distributions. The longhunt darter, *Etheostoma cf stigmaeum* is endemic to the upper Cumberland River (Warren et al. 2000). The emerald darter, *E. baileyi* is endemic to the upper Kentucky and upper Cumberland Rivers. The frecklebelly darter, *Percina stictogaster* is found only in the Barren and upper Green Rivers and the upper Kentucky River (Burr and Page 1993).

One freshwater drum, *Aplodinotus grunniens* died 6 days post-infestation. Gill s were excised and examined with a microscope for encysted glochidia. Some encysted glochidia were observed. The fantail darter, *E. flabellare* and the striped darter, *E. virgatum* may need to be retested, because all individuals died 11 and 10 days respectively, post-infestation, with no sloughed glochidia detected.

Table 3. Results of fish-host tests using glochidia of *Obovaria subrotunda*

Fish species	No. of fish	No. of glochidia exposed	No. of fish alive	No. of days post infestation	No. of rejected glochidia	Glochidial encystment	No. of juveniles recovered	Days to transform
Atherinidae								
Labidesthes sicculus (Cope)	1	20	1	30	0	no	0	0
Catostomidae								
Hypentilium nigricans (Lesueur)	10	20	3	8	0	no	0	0
Moxostoma eurythyrum (Rafinesque)	10	20	4	16	0	no	0	0
M. macrolepidotum (Lesueur)	1	150	1	30	0	no	0	0
Minytrema menalops (Rafinesque)	1	120	0	1	0	no	0	0
Centrarchidae								
Amblopites rupestris (Rafinesque)	2	50	2	30	0	no	0	0
Lepomis cyanellus (Rafinesque)	2	30	2	30	2	no	0	0
L. gulosus (Cuvier)	2	50	2	30	0	no	0	0
L. macrochirus (Rafinesque)	2	50	2	30	0	no	0	0
L. megalotis (Rafinesque)	2	50	2	30	0	no	0	0
L. microlophus (Guenther)	1	50	1	30	4	no	0	0

Table 3, continued.

Fish species	No. of fish	No. of glochidia exposed	No. of fish alive	No. of days post infestation	No. of rejected glochidia	Glochidial encystment	No. of juveniles recovered	Days to transform
Micropterus dolomieu Lacepede	1	30	1	30	4	no	0	0
M. salmiodes (Lacepede)	2	50	2	30	0	no	0	0
Pomoxis annularis Rafinesque	1	200	0	4	0	no	0	0
Cottidae								
Cottus carolinae (Gill)	4	30	4	30	0	no	0	0
Cyprinidae								
Campostoma anamalum (Rafinesque)	1	20	0	5	0	no	0	0
Carrassius auratus (Linnaeus)	1	50	1	30	0	no	0	0
Cyprinella spiloptera (Cope)	4	20	0	10	0	no	0	0
C. whipplei Girard	3	20	3	30	0	no	0	0
Luxilus chrysocephalus Rafinesque	5	30	5	30	0	no	0	0
Lythurus fasciolaris Gilbert	10	15	1	30	0	no	0	0
Nocomis micropogon (Cope)	4	30	4	30	0	no	0	0
Notropis rubellus (Agassiz)	11	15	5	30	0	no	0	0

Table 3, continued.

Fish species	No. of fish	No. of glochidia exposed	No. of fish alive	No. of days post infestation	No. of rejected glochidia	Glochidial encystment	No. of juveniles recovered	Days to transform
Pimephales notatus (Rafinesque)	1	20	1	30	0	no	0	0
P. promelas Rafinesque	2	20	2	30	0	no	0	0
Semotilus atromaculatus (Mitchill)	3	30	3	30	0	no	0	0
Fundulidae								
Fundulus catenatus (Storer)	1	40	1	30	1	no	0	0
Ictaluridae								
Ameirurus melas (Rafinesque)	2	95	1	30	0	no	0	0
Ameiurus natalis (Lesueur)	2	50	2	30	0	no	0	0
Noturus elegans Taylor	2	50	2	30	0	no	0	0
Percidae								
Etheostoma baileyi Page and Burr	17	15	6	30	2	yes	8	10
E. bellum Zorach	2	30	2	30	0	no	0	0
E. blennioides Rafinesque	10	25	7	30	1	yes	3	13
E. caeruleum Storer	10	15	5	30	0	no	0	0

Table 3, continued.

Fish species	No. of fish	No. of glochidia exposed	No. of fish alive	No. of days post infestation	No. of rejected glochidia	Glochidial encystment	No. of juveniles recovered	Days to transform
E. cinereum Storer	1	50	1	30	0	no	0	0
E. flabellare Rafinesque	3	15	0	3	0	no	0	0
E. nigrum Rafinesque	11	15	3	11	17	no	0	0
E. spectabile (Agassiz)	4	20	4	30	0	no	0	0
E. stigmaeum (Buck Cr.) (Jordan)	2	15	1	30	0	yes	1	18
E. stigmaeum (Green R.)	4	25	4	30	4	no	0	0
E. variatum Kirtland	10	20	3	22	0	yes	12	13
E. virgatum (Jordan)	10	15	3	10	0	n/a	0	0
E. zonale (Cope)	12	15	2	21	0	no	0	0
Percina caprodes (Rafinesque)	4	30	2	30	0	no	0	0
P. maculata (Girard)	3	25	3	30	0	no	0	0
P. sp. (*P. stictogaster* undescribed)	7	20	6	30	0	yes	19	10
Sciaenidae								
Aplodinotus grunniens Rafinesque	1	60	0	6	0	yes	0	0

DISCUSSION

The round hickorynut from Buck Creek (Cumberland River) is very host specific. Five darters (Percidae) were the only hosts that produced juveniles out of nine families and 48 species fishes that were infested with glochidia. Of the five darter species that were identified as suitable hosts for *O. subrotunda*, *Etheostoma variatum*, *Percina stictogaster*, and *E. baileyi*, facilitated a higher efficiency of glochidial transformation. These species were considered good hosts. *Etheostoma blenniodes* and *E. cf stigmaeum* had few glochidia transform, and both were considered marginal hosts.

It is likely that *O. subrotunda* females from the upper Kentucky River used some of same host species as *O. subrotunda* in the upper Cumberland River, since many of the potential host fishes are found in both rivers. It's also probable that other metapopulations of *O. subrotunda* use similar riffle dwelling darters. For other species in the genus *Obovaria*, few host fishes are known. Haag and Warren (2003) found that a related Mobile Basin species, *O. unicolor*, used three darters as hosts, *Ammocrypta beani* (Jordan), *A. meridiana* (Williams), and *Etheostoma artesiae* (Hay). The eastern sand darter, *Ammocrypta pellucida* (Girard) may serve as a natural host for *O. subrotunda* in parts of the Ohio River drainage.

From our results, certain subgenera of darters are more likely to serve as natural hosts for the round hickorynut, (i.e., *Etheostoma* and *Ulocentra*). Sixteen darter species were tested for host specificity and of the five host species that produced juveniles, are in the subgenera *Etheostoma* (*E. blenniodes* and *E. variatum*) and *Ulocentra* (*E. baileyi*). Other darters belonging to these subgenera which overlap in distribution with *O. subrotunda* that may serve as potential natural hosts are: (*Ulocentra*) *E. barrenense* (Burr and Page) (Barren R. in Ky., and Tenn.), *E. flavum* (Etnier and Bailey) (Tenn. R. and lower Cumberland R), *E. simoterum* (Cope) (Tenn. R. and Cumberland R.), *E. rafinesquei* (Burr and Page) (Green R. tributaries in Ky.), and (*Blenniodes*) *E. blennius* (Gilbert and Swain) (Tenn. R.). Due to *P. stictogaster* producing the most juveniles from any of the fish species tested, it is likely other species of *Percina* may serve as natural hosts. These include, *P. copelandi* (Jordan), *P. evides* (Jordan and Copeland), *P. oxyrhyncha* (Hubbs and Raney), *P. sciera* (Swain), *P. shumardi* (Girard), in the Ohio River drainage and *P. squamata* (Gilbert and Swain), which occurs in both the upper Cumberland and Kentucky Rivers. In our tests, two darter species of the subgenus /*Catenotus*/ *E. flabellare* and *E. virgatum*, died before glochidial transformation could occur. Since many species of the subgenus *Catenotus* overlap in distribution with *O. subrotunda*, further tests are needed among this group of darters.

In our tests, *E. cf stigmaeum*, the longhunt darter from Buck Creek (Cumberland R.), one fish yielded a single juvenile mussel, while four individuals *E. cf stigmaeum*, the bluegrass darter, from the upper Green River, did not produce any juveniles. It is not evident whether the longhunt darter is a good host for *O. subrotunda* populations in the Cumberland River system. Even though the bluegrass darter was unsuccessful in glochidial transformation, this darter still may serve as a host for *O. subrotunda* in the Green and Barren Rivers. More testing is needed for both forms of this species, using preferably more individuals and glochidia for more conclusive results.

Since *O. subrotunda* occurs in the main-stem of large rivers (i.e., Ohio River) some large river dwelling fish species may be worth investigating as natural hosts. For example, the freshwater drum, *Aplodinotus grunniens*, is a known host for twelve species of unionids (Watters 1994). We tested one freshwater drum which facilitated glochidial encystment, but died six days post-infestation. Since only members of the family Percidae proved to serve as hosts for *O. subrotunda*, the walleye *Sander vitreus* (Mitchill), sauger *Sander canadensis* (Griffith and Smith) and yellow perch *Perca flavescens* (Mitchill) which are also percids, could potentially serve as hosts for *O. subrotunda* in larger rivers throughout its range (NatureServe 2005).

It is important to identify the natural host species to aid conservation efforts for declining mussel species, and to identify the most suitable host fish species for propagation and recovery. Factors that determine the most suitable host fish for propagation of freshwater mussels are availability, sensitivity to handling and captive care, and most importantly, the efficiency of glochidial transformation to juveniles. Our tests showed *E. baileyi*, *E. virgatum* and *P. stictogaster* yielded the best results. *Etheostoma baileyi* is smaller, and may yield fewer transformed juveniles per individual fish compared to the larger *E. variatum* and *P. stictogaster*. Since the variegate darter is more common than the frecklebelly darter and relatively easy to keep in aquaria, the variegate darter proves to be the best host fish species for propagation. This species may not be the most suitable host in other parts of its range (e.g., outside the variegate darter's range). If another larger percid species (i.e., sauger) can serve as a host (facilitating high efficiency of metamorphosis), it may serve as the best host for propagation. efforts because of its larger size, availability, and handling sensitivity. The best host fish species may be found outside the distribution of the target mussel species. Jones (2002) found the roanoke darter, *Percina roanoka* (Jordan and Jenkins), an Atlantic Slope species, to be the most suitable host fish for the endangered fanshell *Cyprogenia stegaria* (Rafinesque), which is found in the Ohio and Tennessee Rivers. However, the natural host should be the first choice.

In some streams, low mussel densities may be attributed to absence or decline of host fishes (Khym and Layzer 1998). In Buck Creek, where the round hickorynut has rapidly declined (G.A. Schuster, Eastern Kentucky University, personal comm.), its demise may partially be a result of few host present. Three darter species *E. baileyi*, *E. blenniodes*, and *E. stigmaeum*, which proved as hosts for *O. subrotunda* are uncommon in Buck Creek in recent surveys. However, other studies with rare mussels appear to have healthy natural host fish population (Haag and Warren 2003).

Habitat degradation resulting from many types of environmental disturbances (e.g., pollution, habitat loss, altered flow regimes), have contributed to the declines of many mussel and host fish populations in nearly all streams. Future freshwater mussel recovery plans must address the host fish populations to ensure successful recruitment among mussel populations. Recovery efforts will likely be unsuccessful unless dedicated efforts toward habitat restoration and conservation are implemented.

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