

REGULAR ARTICLE

HOST FISHES AND LIFE HISTORY OF THE ROUND HICKORYNUT (*OBOVARIA SUBROTUNDA*)

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ABSTRACT

The Round Hickorynut, *Obovaria subrotunda*, is declining throughout its range, but little life history information exists for the species. We examined host use and glochidial size in Buck Creek (Cumberland River drainage) and the Licking River (Ohio River drainage), Kentucky, and we examined age and growth in Buck Creek. Glochidia of *O. subrotunda* from Buck Creek metamorphosed on five darter species (Percidae)—*Etheostoma baileyi*, *Etheostoma blennioides*, *Etheostoma gore*, *Etheostoma variatum*, and *Percina stictogaster*—but not on 43 other fish species from nine families; host use was broadly similar to previous studies in the Duck River, Tennessee, and Lake St. Clair, Ontario. Glochidia from the Licking River metamorphosed only on Eastern Sand Darters (*Ammocrypta pellucida*) and not on 11 other darter species, including two that produced juveniles in the Buck Creek trial. Glochidial metamorphosis was higher on *A. pellucida* than on any other species, suggesting that sand darters are primary hosts for *O. subrotunda*, but sand darters do not occur in several streams occupied by *O. subrotunda*. Such major differences in host use may indicate phylogenetic divergence between these populations. Glochidial size differed significantly between the Buck Creek and Licking River populations, suggesting that it may have taxonomic value also. *Obovaria subrotunda* was relatively short-lived (13 yr) and fast-growing ($K = 0.22$), and age-at-maturity was estimated at 2–3 yr. Males and females had similar growth rates, but males were substantially larger (L_{∞} : males = 53.7 mm; females = 39.2 mm). Life-history data support categorizing *O. subrotunda* as a periodic life-history strategist, and this view provides benchmarks for assessing population health and responses to watershed conditions.

KEY WORDS: Unionidae, conservation, age and growth, age-at-maturity, *Ammocrypta*

INTRODUCTION

The Round Hickorynut, *Obovaria subrotunda*, is distributed throughout the Ohio River basin and in the Lake St. Clair and Lake Erie drainages (Williams et al. 2008). Although the species has a G4 global ranking of “apparently secure” (NatureServe 2019), it is declining throughout much of its range and is currently under review for listing under the U.S. Endangered Species Act (USFWS 2011).

Life-history information for *O. subrotunda* is scant and exists primarily for populations in the Duck River, Tennessee (Tennessee River drainage; Ehlo and Layzer 2014), and Lake

St. Clair, Ontario (McNichols 2007). In the Duck River population, males grew faster than females, female sexual maturity was reported at one year of age, and glochidia transformed on three darter species (*Etheostoma blennioides*, *Etheostoma obama*, and *Etheostoma flabellare*) and the Banded Sculpin (*Cottus caroliniae*). In Lake St. Clair, glochidia transformed on three darter species (*Etheostoma exile*, *E. flabellare*, and *Percina maculata*). Glochidia of a related species, the Alabama Hickorynut, *Obovaria unicolor*, transformed primarily on sand darters (*Ammocrypta* spp.) and the Gulf Darter (*Etheostoma artesiae*, Haag and Warren 2003). Glochidia of *O. subrotunda* were described by Ortmann (1911, 1919), Surber (1915), and Hoggarth (1988, 1999), but no one

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Table 1. Results of host identification trial for *Obovaria subrotunda* from Buck Creek, Pulaski Co., Kentucky. The number of fish inoculated in parentheses is the number that survived to the end of the trial. Metamorphosis success is the proportion of inoculated glochidia that metamorphosed into juvenile mussels.

Fish Species ¹	No. of Fish Inoculated	No. of Glochidia Inoculated/Fish	No. of Juveniles Recovered	Days to Metamorphosis	Metamorphosis Success	No. of Juveniles/Fish
<i>Etheostoma baileyi</i>	17 (6)	15	8	10	0.089	1.3
<i>Etheostoma blennioides</i>	10 (7)	25	3	13	0.017	0.4
<i>Etheostoma gore</i>	2 (1)	15	1	18	0.067	1.0
<i>Etheostoma variatum</i>	10 (3)	20	12	13	0.200	4.0
<i>Percina stictogaster</i>	7 (6)	20	19	10	0.158	3.2

¹Fish families and species that did not produce juvenile mussels (number of fish inoculated): Atherinidae, *Labidesthes sicculus* (1); Catostomidae, *Hypentelium nigricans* (10), *Moxostoma erythrurum* (10), *M. macrolepidotum* (1), *Minytrema melanops* (1); Centrarchidae, *Ambloplites rupestris* (2), *Lepomis cyanellus* (2), *L. gulosus* (2), *L. macrochirus* (2), *L. megalotis* (2), *L. microlophus* (1), *Micropterus dolomieu* (1), *M. salmoides* (2), *Pomoxis annularis* (1); Cottidae, *Cottus caroliniae* (4); Cyprinidae, *Camptostoma anomalum* (1), *Carassius auratus* (1), *Cyprinella spiloptera* (4), *C. whipplei* (3), *Luxilus chrysocephalus* (5), *Lythrurus fasciolaris* (10), *Nocomis micropogon* (4), *Notropis rubellus* (11), *Pimephales notatus* (1), *P. promelas* (2), *Semotilus atromaculatus* (3); Fundulidae, *Fundulus catenatus* (1); Ictaluridae, *Ameiurus melas* (2), *A. natalis* (2), *Noturus elegans* (2); Percidae, *Etheostoma caeruleum* (10), *E. flabellare* (3), *E. jimmycarter* (4), *E. maydeni* (1), *E. nigrum* (11), *E. spectabile* (4), *E. virgatum* (10), *E. zonale* (12), *Nothonotus bellus* (2), *N. camurus* (9), *Percina caprodes* (4), *P. maculata* (3), Sciaenidae, *Aplodinotus grunniens* (1).

has assessed variation in glochidial size among populations. Additional life-history information for *O. subrotunda* is needed to better understand its ecology and to inform conservation strategies for the species.

We examined life-history aspects of *O. subrotunda* in two populations in Kentucky: Buck Creek and the Licking River. We examined host use and glochidial size in both populations, as well as age and growth and age-at-maturity in the Buck Creek population. We discuss our results in the context of existing information for *O. subrotunda* and other *Obovaria*.

METHODS

Mussel and Fish Collection for Host Identification

We collected one female and five male *O. subrotunda* from Buck Creek, Pulaski County (hereafter Co.), Kentucky (Cumberland River drainage), between March and November 2003; the female was not gravid at the time of collection (September). We transported mussels to the Center for Mollusk Conservation (Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky) and placed them together in sand and gravel substrate in a flow-through raceway fed by water from South Elkhorn Creek. The female became gravid in captivity by January 2004.

We collected 3 female and 13 male *O. subrotunda* from the Licking River, Bath Co., Kentucky, in September 2015. We transported mussels to Minor Clark Hatchery (Kentucky Department of Fish and Wildlife Resources, Farmers,

Kentucky) and placed them together in sand and gravel substrate in a flow-through raceway fed by water from the Licking River. Two females were gravid by March 2016.

For the Buck Creek host trials, we collected 48 potential host-fish species (22 genera, 9 families; Table 1) with a backpack electrofisher or seine in September 2003. We collected fishes from the Red River, Menifee Co., Kentucky; Elkhorn Creek, Franklin Co., Kentucky (both Kentucky River drainage); Green River, Casey Co., Kentucky; Horse Lick Creek, Rockcastle Co., Kentucky (Cumberland River drainage); and Taylorsville Lake (Salt River), Spencer Co., Kentucky. The Buck Creek host trials showed that *O. subrotunda* was a specialist on darters (Percidae; see Results). Therefore, for the Licking River host trials, we collected 11 species of darters (Table 2) with a backpack electrofisher or seine from September to October 2015, from the Licking River, Bath Co., Kentucky. For both trials, we maintained fishes over the winter in aquaria in the laboratory until initiation of host trials. We fed fishes blackworms, crayfish, minnows, or pelletized fish food, depending on the dietary habits of each species.

Host Identification

We extracted glochidia from female *O. subrotunda* on May 1, 2004, and May 17, 2016, for the Buck Creek and Licking River host trials, respectively. For the Licking River trial, we combined glochidia from two females. We extracted glochidia by flushing the gills with water through a syringe. We tested

Table 2. Results of host identification trial for *Obovaria subrotunda* from the Licking River, Bath Co., Kentucky. The number of fish inoculated in parentheses is the number that survived to the end of the trial. Metamorphosis success is the proportion of inoculated glochidia that metamorphosed into juvenile mussels.

Fish Species ¹	No. of Fish Inoculated	No. of Glochidia Inoculated/Fish	No. of Juveniles Recovered	Days to Metamorphosis	Metamorphosis Success	No. of Juveniles/Fish
<i>Ammocrypta pellucida</i>	9 (8)	125	366	20–23	0.366	45.8

¹Fish species (all Percidae) that did not produce juvenile mussels (number of fish inoculated, days to rejection): *Etheostoma blennioides* (10, 2), *E. caeruleum* (10, 2), *E. flabellare* (3, 2–4), *E. nigrum* (4, 2–4), *E. variatum* (12, 2–4), *E. zonale* (10, 2), *Nothonotus camurus* (1, 2), *Percina copelandi* (3, 2–4), *P. oxyrhynchus* (2, 2), *P. sciera* (1, 2).

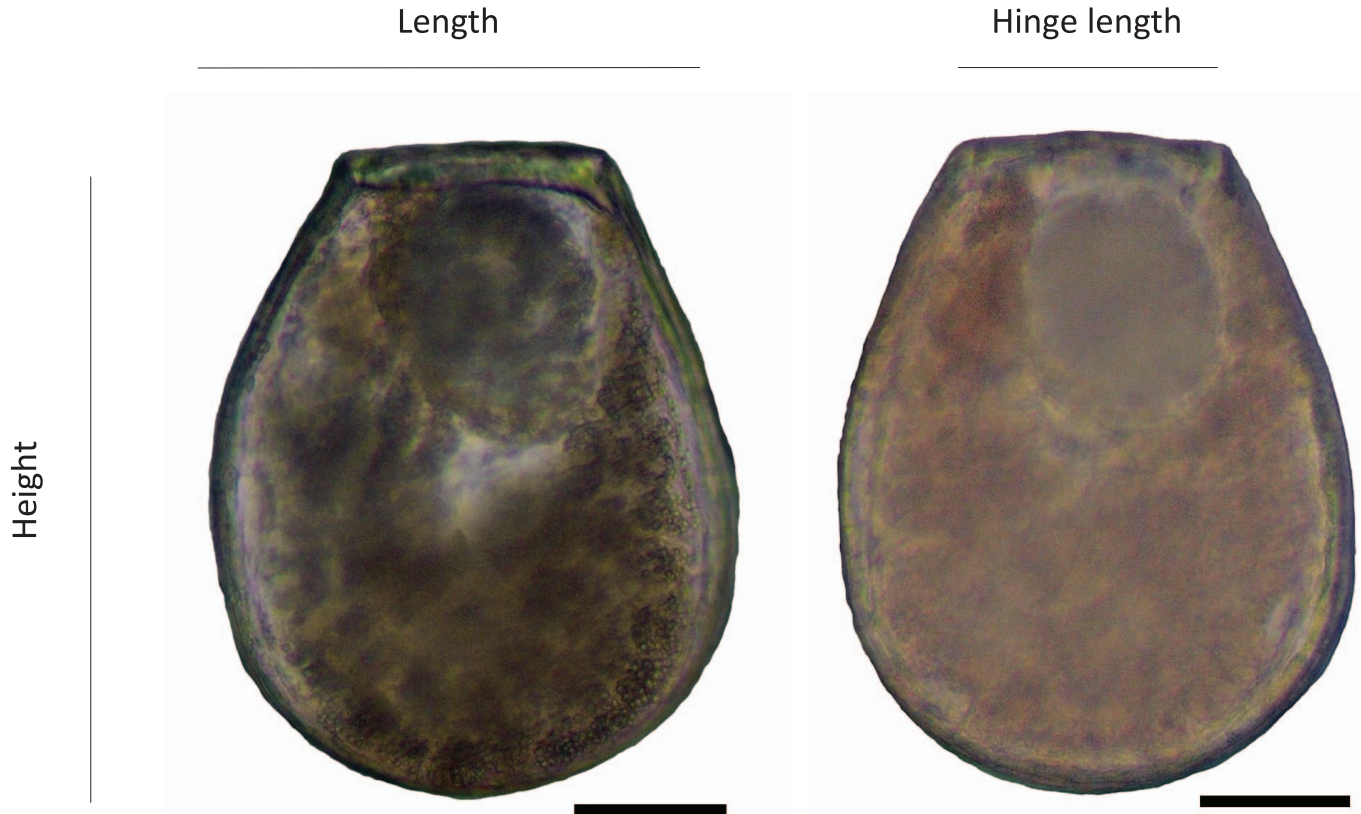


Figure 1. Glochidia of *Obovaria subrotunda* from Buck Creek, Kentucky (left) and the Licking River, Kentucky (right). Scale bar = 50 μm . Darker coloration of the Buck Creek specimen is due to alcohol storage.

viability of glochidia by exposing a subset to a NaCl solution. Glochidia for the Licking River trial exhibited 100% viability. Viability was not quantified for the Buck Creek trial, but most glochidia exhibited rapid valve closure in response to salt.

We conducted host trials for both populations generally following Zale and Neves (1982) and Rogers et al. (2001). We anesthetized fishes in MS-222 (0.15 g/L) and inoculated them by pipetting glochidia directly onto the gill filaments of each fish. For the Buck Creek trial, we inoculated each fish with 15–200 glochidia, depending on fish size (Table 1), and we counted glochidia in a Petri dish before drawing them into a pipette. For the Licking River trial, we inoculated each fish with about 125 glochidia, determined volumetrically based on prior estimates of the number of glochidia in a 0.125 ml aliquot. After inoculation, we placed fishes in a 5-L recovery chamber for about 10 min and then transferred them to 1- to 9-L chambers in a recirculating AHAB system (Pentair Aquatic Habitats, Apopka, FL). We placed 1 to 12 individual fish in each chamber, depending on the size of fishes, maintained them in the AHAB systems at 23–25°C, and fed them as described previously.

For the Licking River trial, we anesthetized all fishes on days 2 and 4 postinoculation and examined their gills for encysted glochidia. All individuals of all species except *A. pellucida* had rejected all glochidia by day 4 and were removed from the trial; we did not examine *A. pellucida* after

day 4 because all individuals were heavily infested on that day. We did not examine fishes after inoculation in the Buck Creek trial. In both trials, we examined material on the bottom of each chamber daily for transformed juveniles, beginning 10 days after inoculation and continuing until 7 days after the last juvenile mussels were observed. We collected material from the bottom of each AHAB chamber on a 100- μm filter screen and examined the material under a binocular microscope. We considered suitable host-fish species as those that produced active juveniles with a well-developed foot. We evaluated the robustness of juvenile mussel production from each suitable host species by calculating a measure of metamorphosis success as total number of juveniles produced/number of inoculated fish that remained alive at the end of the experiment/number of glochidia inoculated on each fish. We also calculated the number of juveniles produced/fish to allow comparison of our results with those of McNichols (2007) and Ehlo and Layzer (2014).

Glochidial Size

We collected a haphazardly selected sample of 25 glochidia each from Buck Creek and the Licking River; Licking River glochidia were from the composited sample from two females. We measured glochidial shell length, height, and hinge length (Fig. 1) of each individual to the

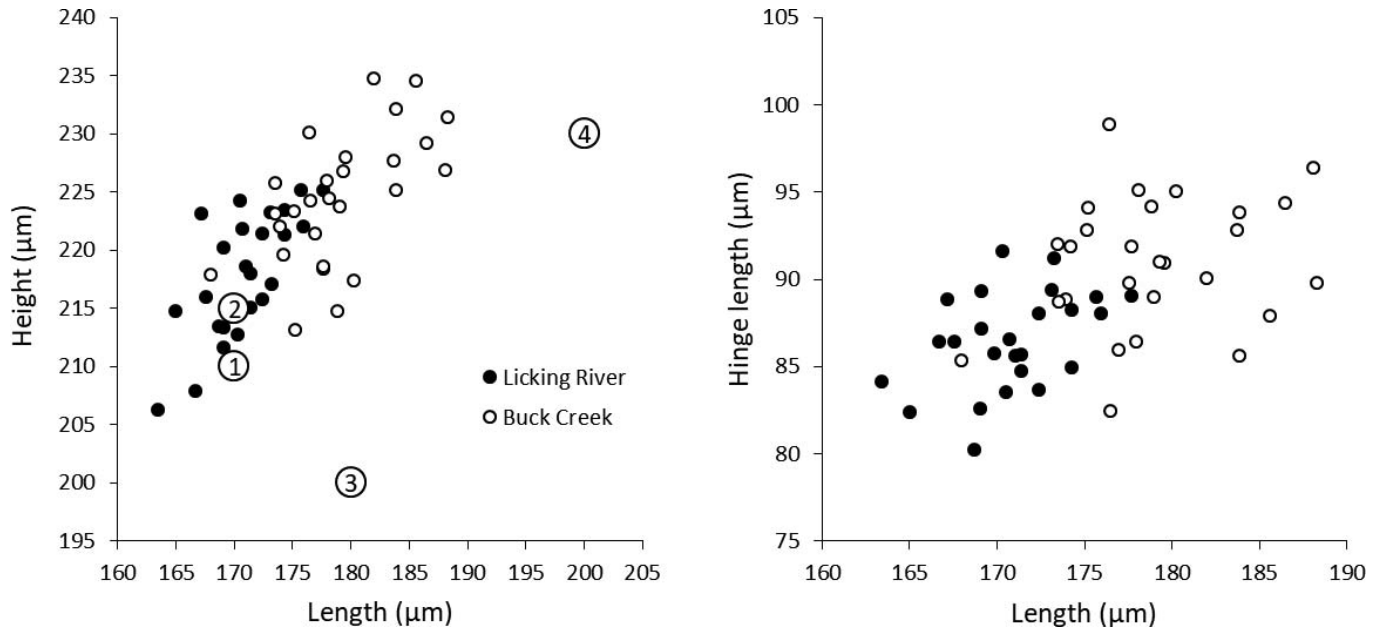


Figure 2. Glochidial size of *Obovaria subrotunda* from Buck Creek and the Licking River, Kentucky. Numbered points refer to single measurements for (1) Fish Creek, Ohio; (2) Wabash River, Indiana; (3) Big Darby Creek, Ohio; and (4) Crooked Creek, Indiana. Hinge length was not available for all of the last four populations. See text for data sources.

nearest 0.1 μm at 20× magnification using a compound microscope and imaging software. We examined bivariate plots between size measurements and tested for differences in size between populations using MANOVA and ANCOVA. We also compared glochidial size in our study populations with published glochidial measurements for *O. subrotunda* from Fish Creek and Big Darby Creek, Ohio (Hoggarth 1988), the Wabash River, Indiana (Surber 1915), and Crooked Creek, Indiana (Ortmann 1911, 1919).

Age and Growth

We obtained shells of 21 male and 17 female *O. subrotunda* collected from Buck Creek in 1983 and 1984 from the Branley A. Branson Museum of Zoology, Eastern Kentucky University (EKU collection numbers 118, 127, and 130). We measured shell length (greatest anterior-posterior dimension, nearest 0.1 mm) of each individual and determined its sex based on shell sexual dimorphism (Ehlo and Layzer 2014). We estimated the age of each individual by counting external growth rings (annuli), assuming one annulus/year (Haag and Commens-Carson 2008). To obtain additional information about length-at-age, particularly for younger ages, we back-measured shell length at all interpretable annuli for all individuals (Michaelson and Neves 1995; Haag and Rypel 2011). We constructed von Bertalanffy growth models separately for each sex. We tested for differences in length-at-age between sexes using ANCOVA with length as the dependent variable, sex as the independent variable, and $\ln(\text{age})$ as the covariate. We estimated age-at-maturity by noting the earliest external annulus at which shell sexual dimorphism became evident.

RESULTS

Host Identification

Glochidia of *O. subrotunda* from Buck Creek metamorphosed only on five darter species (Percidae): *E. baileyi*, *E. blennioides*, *E. gore*, *E. variatum*, and *Percina stictogaster* (Table 1). Metamorphosis success was highest on *E. variatum* (0.200) and *P. stictogaster* (0.158) and was about 50% less or lower on the other three species. No juvenile mussels were produced on any other fish species, including 12 other darter species and *Cottus caroliniae*. Glochidia from the Licking River metamorphosed only on *A. pellucida* (Percidae) and not on 11 other darter species, including two species that produced juveniles in the Buck Creek trial (*E. blennioides* and *E. variatum*; Table 2). Metamorphosis success was substantially higher on *A. pellucida* (0.366) than on any species in the Buck Creek trial.

Glochidial Size

Glochidial size differed significantly between populations (MANOVA: Wilks' $\lambda = 0.480$, $F_{3,46} = 16.64$, $P < 0.0001$; Fig. 2). All three measurements of glochidial size differed significantly between populations, and mean values were 3–5% smaller in the Licking River than in Buck Creek (univariate ANOVA, $F_{1,48} = 18.09\text{--}45.37$, $P < 0.0001$ for all tests; Table 3). Despite overall size differences, glochidia were proportionally similar in both populations (Fig. 2). Glochidial height was strongly related to length (two-factor ANOVA: $F_{1,46} = 31.01$, $P < 0.0001$), but stream and the length $\times$ stream interaction were not significant (stream, $F_{1,46} = 1.82$, $P = 0.182$; length $\times$ stream, $F_{1,46} = 1.83$, $P = 0.183$).

Table 3. Glochidial size of *Obovaria subrotunda* from six populations. Values for Buck Creek and the Licking River are means $\pm$ SD. Means with different superscripted letters within a column are significantly different (all $P < 0.0001$). Values for the other four streams are single reported values; a dash (—) indicates that the value was not reported. See text for data sources.

Stream	Height (μm)	Length (μm)	Hinge Length (μm)
Buck Creek, Kentucky	224.5 $\pm$ 5.7 ¹	179.0 $\pm$ 4.9 ¹	90.9 $\pm$ 3.8 ¹
Licking River, Kentucky	217.9 $\pm$ 5.3 ²	170.8 $\pm$ 3.4 ²	86.5 $\pm$ 2.8 ²
Fish Creek, Ohio	210.0	170.0	80
Wabash River, Indiana	215.0	170.0	—
Big Darby Creek, Ohio	180.0	200.0	90
Crooked Creek, Indiana	230.0	200.0	—

An ANCOVA model omitting the interaction term similarly showed a strong effect of length on height ($F_{1,47} = 28.85$, $P < 0.0001$), but no effect of stream when the effect of length was accounted for ($F_{1,46} = 0.01$, $P = 0.929$; least square mean heights: Licking River = 221.2 μm , Buck Creek = 221.1 μm). Glochidia from Fish Creek, Ohio, and the Wabash River, Indiana, were similar in size to glochidia from the Licking River (Table 2). Glochidia from Big Darby Creek, Ohio, and Crooked Creek, Indiana, differed substantially in size from each other and from other populations.

Age and Growth

Males ranged from 4 to 13 yr old (median age = 5.0 yr), and females ranged from 4 to 12 yr old (median = 6.5). The von Bertalanffy growth constant K was lower for males (0.239 ± 0.026 SE) than females (0.272 ± 0.055), but asymptotic length (L_∞) was substantially higher for males (52.8 mm $\pm$ 1.7 SE) than females (36.9 $\pm$ 1.8; Fig. 3). The slopes of the regressions of length on $\ln(\text{age})$ differed between males and females (ANCOVA, age $\times$ sex interaction: $F_{1,207} = 62.14$, $P <$

0.0001), indicating that male and female size did not differ by a constant factor across ages; rather, length-at-age relationships showed that males became increasingly larger than females with increasing age (Fig. 3 and Table 4). Female shells first showed discernible evidence of sexual dimorphism between the ages of 3 and 5.

DISCUSSION

Our host trials, and those from the Duck River and Lake St. Clair (McNichols 2007; Ehlo and Layzer 2014), support *O. subrotunda* as a host specialist largely on darters (Percidae). The only nondarter host that facilitated glochidial metamorphosis was *Cottus caroliniae* (Cottidae), but that species produced juvenile mussels only in the Ehlo and Layzer (2014) study and not in our Buck Creek trial. We did not test sculpins in the Licking River trial, but *C. caroliniae* does not occur in the Licking River system, and *Cottus bairdi* occurs only sporadically in small streams in the system and is not reported from the mainstem Licking River in association with *O. subrotunda* (Burr and Warren 1986). *Cottus* spp. were not tested by McNichols (2007).

In addition to the lack of metamorphosis on *C. caroliniae*, results of our host trial from Buck Creek differed in several ways from those from the Duck River and Lake St. Clair (McNichols 2007; Ehlo and Layzer 2014). Together, all three studies show that *O. subrotunda* is able to successfully parasitize relatively few darter species, and host use only loosely follows phylogenetic relatedness in darters. No species within the genus *Nothonotus* (*Nothonotus aquali*, *Nothonotus bellus*, *Nothonotus camurus*, *Nothonotus rufilineatus*) or the subgenera *Allohistium* (*Etheostoma maydeni*) or *Boleosoma* (*Etheostoma nigrum*) served as hosts in any study. No species in the subgenus *Oligocephalus* (*Etheostoma caeruleum*, *Etheostoma spectabile*) served as hosts, with the exception of *Etheostoma exile*, which produced the highest number of juveniles in Lake St. Clair (4.8/fish). The species that produced the highest numbers of juveniles in Buck Creek and the Duck River were in the subgenus *Etheostoma* (*Etheostoma blennioides*, Duck River, 1.3 juveniles/fish; *Etheostoma variatum*, Buck Creek, 4.0 juveniles/fish). However, *E. blennioides* produced the lowest number of juveniles of any suitable host species in Buck Creek (0.4 /fish) and did not serve as a host in

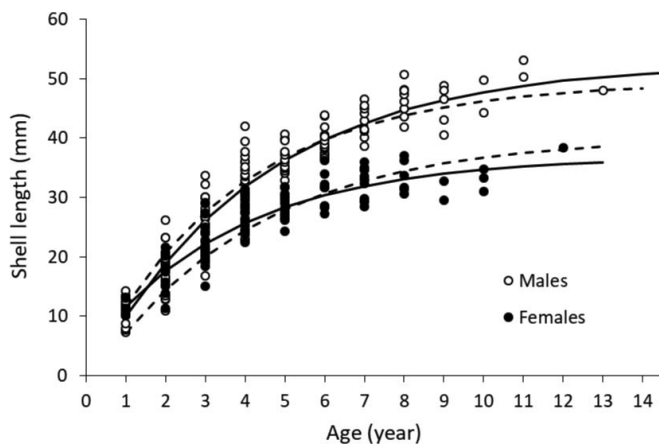


Figure 3. Length-at-age for *Obovaria subrotunda* from Buck Creek, Pulaski Co., Kentucky. Data points are direct estimates of length-at-age inferred from external shell rings. Solid lines are length-at-age predicted by von Bertalanffy growth models for each sex [$L_t = L_\infty(1 - e^{-K(t-t_0)})$, where L_t = length at time t , and t = age (year); males: $L_\infty = 52.8$, $K = 0.239$, $t_0 = 0.118$; females: $L_\infty = 36.9$, $K = 0.272$, $t_0 = -0.391$]. Dashed lines are von Bertalanffy growth curves for *O. subrotunda* from the Duck River, Tennessee (Ehlo and Layzer 2014).

Table 4. Length-at-age data for *Obovaria subrotunda* from Buck Creek, Pulaski Co., Kentucky. Ages without direct observations are indicated by a dash (—). Sample size (*N*) refers to the number of observations for each age. Predicted mean length is from a von Bertalanffy growth model (see Fig. 3). Growth increment refers to the predicted increase in shell length relative to the previous year.

Age (Year)	<i>N</i>	Mean Observed Length (Range, mm)	Predicted Mean Length (mm)	Growth Increment (mm)
Males				
1	15	10.8 (7.3–14.2)	10.0	10.0
2	17	18.2 (10.9–26.2)	19.1	9.1
3	21	25.8 (16.8–33.7)	26.2	7.1
4	19	31.7 (22.9–42.0)	31.9	5.6
5	11	36.5 (32.9–40.7)	36.3	4.4
6	10	39.4 (36.7–43.9)	39.8	3.5
7	8	42.8 (38.6–46.6)	42.5	2.8
8	6	46.3 (41.8–50.7)	44.7	2.2
9	3	47.4 (40.5–48.8)	46.4	1.7
10	2	47.1 (44.4–49.8)	47.8	1.3
11	2	51.8 (50.3–53.2)	48.8	1.1
12	0	—	49.7	0.8
13	1	48.0	50.3	0.7
Females				
1	5	11.8 (10.1–13.2)	11.6	11.6
2	11	17.0 (11.3–21.7)	17.6	6.0
3	16	22.0 (15.0–29.1)	22.2	4.6
4	17	26.9 (22.4–31.4)	25.7	3.5
5	16	28.3 (24.3–31.9)	28.4	2.7
6	10	31.0 (27.2–36.2)	30.4	2.0
7	9	32.4 (28.5–35.9)	31.9	1.5
8	6	33.4 (30.6–37.0)	33.1	1.2
9	2	31.2 (29.6–32.8)	34.0	0.9
10	3	33.0 (31.0–34.7)	34.7	0.7
11	0	—	35.2	0.5
12	1	38.3	35.6	0.4

Lake St. Clair; two other species in the subgenus were not suitable hosts (*Etheostoma blennioides*, *Etheostoma zonale*). Similarly, *Etheostoma flabellare* (subgenus *Catonotus*) produced low numbers of juveniles in the Duck River (0.3/fish) and Lake St. Clair (0.1/fish), but it did not produce juveniles in Buck Creek, and two other members of the subgenus were unsuitable (*Etheostoma crossopterygion*, *E. virgatum*). Two members of the *E. stigmaeum* complex (subgenus *Doration*) produced moderate numbers of juveniles (*Etheostoma obama*, Duck River, 0.6/fish; *Etheostoma gore*, Buck Creek, 1.0/fish), but a third species in the complex was not a suitable host (*Etheostoma jimmycarteri*). *Percina stictogaster* produced the second-highest number of juveniles in the Buck Creek trial (3.2 juveniles/fish), but no other *Percina* were suitable hosts, including *Percina maculata*, which produced the highest number of juveniles in Lake St. Clair (4.8/fish); *Percina* spp. were not tested by Ehlo and Layzer (2014). It is difficult to directly compare juvenile production in our study with that

reported by McNichols (2007) or Ehlo and Layzer (2014) because those studies did not quantify the number of glochidia inoculated on each fish. Nevertheless, inconsistencies between these studies suggest subtle differences in host use between the Buck Creek, Duck River, and Lake St. Clair populations.

Results of our host trial from the Licking River were radically different from all other studies. *Obovaria subrotunda* from the Licking River metamorphosed only on Eastern Sand Darters, *A. pellucida*, and the number of juveniles produced (45.8/fish) was substantially higher than on any fish species from other populations. Higher production is due in part to the greater number of glochidia inoculated on each fish in the Licking River trial, but the higher metamorphosis success (0.366) suggests that *A. pellucida* is a more robust host for *O. subrotunda*. Furthermore, no metamorphosis occurred on fish species that were suitable hosts for the other populations (*E. blennioides*, *E. flabellare*, and *E. variatum*). Host use in the Licking River was similar to *Obovaria unicolor*, which metamorphosed consistently only on two *Ammocrypta* species and *E. artesia*, and 10 other darter species were either marginal hosts or unsuitable (Haag and Warren 2003). *Ammocrypta* spp. were not tested in the Buck Creek, Duck River, or Lake St. Clair trials.

The higher metamorphosis success on *Ammocrypta* in the Licking River trial and the variable results from other darter species in other trials could indicate that *Ammocrypta* is a primary host for *O. subrotunda*, and other darter species are only marginally suitable hosts. This explanation is plausible for the Lake St. Clair population, where *O. subrotunda* co-occurs with *A. pellucida* (Derosier 2004). However, no species of *Ammocrypta* are reported from Buck Creek or the Duck River, and there are few (mostly historical) records of that genus anywhere in the Cumberland or Tennessee river systems (Burr and Warren 1986; Etnier and Starnes 1993). The current or former presence of large populations of *O. subrotunda* throughout those river systems (Parmalee and Bogan 1998; Ehlo and Layzer 2014; Haag and Cicerello 2016) suggests that other darter species are capable of supporting those populations and host use may differ substantially from populations elsewhere in the Ohio River basin, such as the Licking River, where sand darters may be a primary host. The distributions of *O. subrotunda* and *A. pellucida* in the middle and upper Ohio River basin are remarkably concordant (compare distributional maps in Burr and Warren 1986 and Haag and Cicerello 2016, and Trautman 1981 and Watters et al. 2009), and Clark (1977) proposed *A. pellucida* as a host for *O. subrotunda* based on the frequent co-occurrence of the two species in the Maumee River system (Lake Erie drainage). Declines in sand darter populations may partially explain declines in *O. subrotunda* populations in this region (see Trautman 1981). Additional studies of *O. subrotunda* host use in the middle and upper Ohio River basins and Great Lakes basin are needed to better evaluate this potential relationship.

Glochidial size differed significantly between the Buck Creek and Licking River populations. Glochidial size and proportions reported from two other populations (Fish Creek,

Ohio; Wabash River, Indiana) were similar to the smaller glochidia from the Licking River. Glochidial size and proportions from two other populations differed substantially from either of our populations: glochidia from Big Darby Creek had much lower height but were similar in length to Buck Creek glochidia; glochidia from Crooked Creek, Indiana, were similar in height to Buck Creek but had much greater length. Data from these four other populations appear to be based on observations of single glochidia and may suffer from several sources of error. Although our data represent measurements of multiple glochidia, they are from a small number of females and may reflect differences among individual females rather than differences among populations. Nevertheless, these findings are the first to our knowledge to suggest differences in glochidial size among populations.

The Cumberland River system supports high endemism of aquatic species, and recently, several cryptic species have been described from the region (e.g., Powers et al. 2004; Lane et al. 2016). The differences in host use and glochidial size between Buck Creek and Licking River *O. subrotunda* raise the possibility of some degree of phylogenetic divergence between those populations. Host use can vary among populations of the same mussel species from different drainage basins in some cases but not in others (Riusech and Barnhart 2000; Caldwell et al. 2016; St. John White et al. 2017). However, differences are usually manifested as differences in glochidial metamorphosis success on shared host species, rather than radically different patterns of host use, such as those we observed. More data from other populations are needed to examine patterns of variation in glochidial size in more detail, but glochidial size should be considered as a potentially informative taxonomic character (see O'Brien et al. 2019).

Our age and growth data depict *O. subrotunda* as a relatively short-lived, fast-growing species. Our maximum observed age (13 yr) was similar to the median life span for the tribe Lampsilini (15 yr) but is substantially lower than median life span for any other North American unionid tribe except the Anodontini (Haag and Rypel 2011). Similarly, the value of the von Bertalanffy growth constant, K (0.25, mean of males and females), is near the median for the Lampsilini (0.27), and higher than other tribes except the Anodontini. It is possible that our age and growth data are biased due to our reliance on external shell annuli. However, *O. subrotunda* have distinctive external annuli because of their light shell color and relatively rapid growth, and external annuli can provide accurate length-at-age measurements in those situations (Haag and Commens-Carson 2008). Furthermore, our estimates of life span, K , maximum size (L_{∞}), and length-at-age were very similar to those of Ehlo and Layzer (2014) from the Duck River based on internal annuli (maximum age = 14 yr; K : males = 0.272; females = 0.247; L_{∞} : males = 49.5 mm; females = 40.3; see Fig. 3).

We did not see shell sexual dimorphism until three to five years of age, but it is possible that maturity occurs before shell characters become evident. Ehlo and Layzer (2014) reported

female maturity at age one based on the minimum observed size of mature individuals (20 mm) and von Bertalanffy predictions of length-at-age. However, their von Bertalanffy model predicts that a 20-mm female would be slightly less than 3 yr old (predicted mean size of a 3-yr-old individual = 20.1 mm). The smallest mature male they observed (22 mm) is predicted by their von Bertalanffy model to be 2+ yr old. Our estimates of K for males and females predict sexual maturity at age 3.0 and 2.6, respectively, based on a relationship generated for several unionid species [age-at-maturity = $0.69(K^{-1.031}) - 1$; Haag 2012). Direct observations are necessary to determine age-at-maturity more precisely, but our data and those of Ehlo and Layzer (2014) support a relatively early age-at-maturity of about 2–3 yr.

Our data and those of Ehlo and Layzer (2014) support categorizing *O. subrotunda* as a periodic life-history strategist, similar to *O. unicolor* (Haag 2012). In this view, *O. subrotunda* is expected to have periodic, but large, bouts of recruitment and has the potential for rapid population growth under favorable conditions. For example, up to 40% of individuals in populations in the Duck River were recruits <1 yr old, and recent population increases in that river are attributed to watershed and landscape improvement (Ehlo and Layzer 2014). The population of *O. subrotunda* in Buck Creek has declined rapidly in the last 30 yr (Haag and Cicerello 2016; M. McGregor, unpublished data), but the causes of this decline are unknown.

Our host-use findings provide the impetus for assessing host availability as a potential causal factor in widespread declines of *O. subrotunda*. This information also improves our ability to propagate *O. subrotunda* for conservation, but a better understanding of population-specific host use across the range of the species is needed. Phylogenetic divergence between populations of *O. subrotunda* would have important conservation implications, but no firm conclusions about this can be made at this time; assessing this possibility requires a molecular genetics approach. The life-history information we provide for *O. subrotunda* can help derive more explicit benchmarks for assessing population health and responses to watershed conditions.

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