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14 June 1993

Dr. Richard Neves
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Dear Dr. Neves,

Would you please review the enclosed manuscript, entitled "Periods of gravidity and fish hosts for four species of freshwater mussels (Pelyccypoda: Unionidae) in the upper Tennessee River drainage", for the *American Midland Naturalist*. I realize that you are busy but we wish to get top reviewers to maintain quality in the journal.

Please submit your primary comments for the authors on a separate sheet in addition to any marginal comments on the manuscript that you may wish to add. In addition, I have enclosed a "Reviewer's Evaluation Sheet" to guide your comments to me regarding the suitability of publication. Your review will be kept confidential.

If possible, please complete the review in about three weeks. If you have any questions, please contact me. Thanks for your help on this manuscript.

Sincerely yours,

Anthony Joern, Associate Editor
Invertebrate Zoology
American Midland Naturalist

Enclosures

General Comments

This is an important piece of research with new data on several rare species. In addition to numerous comments and revisions of writing style on the manuscript, I provide the following recommendations to the authors.

1. The Introduction is lengthy and not well structured. State the objectives of the study rather than a woozy "report information useful to future conservation efforts."

2. If you do any statistical comparisons in Results, mention that in Methods. Would a table for glochidia data be better than a narrative on dimensions?

3. Use citations when you make statements that are not common knowledge or subjective.

4. Give common names for mussels the first time the scientific name is used in text.

5. The Discussion rambles and could be more definitive and less speculative (see comments). There is much speculation that is unrelated to objectives of this study.

6. Check format on Literature Cited for journal.

7. Tables

    a. The small sample sizes for tested fishes leaves me uneasy in eliminating some species as hosts. A sample size of 1 isn't very definitive. For example, the other 2 species of Hybopsis had only 2 and 3 specimens infested versus 21 fish for the confirmed hosts. How confident are the authors of their non-host conclusions? I think some statement on sample size and the possibility of other hosts needs to be made.

    b. Delete many of the footnotes from tables.

    c. Table 4 needs to be redone; delete cryptic abbreviations and reduce the length of table. These raw data can be combined per month within years (give range for dates sampled and water temperature). Does the site of sample really make any difference? Can that be deleted? Is any analysis possible on this table?

8. The authors are to be commended for finally getting these data into publishable format.

PERIODS OF GRAVIDITY AND FISH HOSTS FOR FOUR SPECIES OF FRESHWATER MUSSELS (PELECYPODA:UNIONIDAE) IN THE UPPER TENNESSEE RIVER DRAINAGE[1]

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ABSTRACT

Fish hosts were identified for glochidia of four species of freshwater unionid mussels, Epioblasma brevidens, E. capsaeformis, E. triquetra and Quadrula intermedia from the upper Tennessee River drainage. Epioblasma spp. were gravid from early May to early June, and Q. intermedia was gravid from late April to late June. All four species exhibited high degrees of host specificity in laboratory tests. Host fish for Epioblasma spp. were restricted to several percids, i.e. the greenside darter (Etheostoma blennioides), spotted darter (E. maculatum), redline darter (E. rufilineatum), Tennessee snubnose darter (E. simoterum), logperch (Percina caprodes), dusky darter (P. sciera), and the sculpin (Cottus carolinae). Fish hosts for Q. intermedia were restricted to two congeneric cyprinids, the streamline chub (Hybopsis dissimilis) and blotched chub (Hybopsis insignis).

Host fishes for all four mussel species were restricted to riffle and run-dwelling, fast-water species occupying the same habitats as the mussels. Stenotopic use of host fishes could contribute to the vulnerability of these declining mussel species to environmental perturbations.

Key Words: Epioblasma brevidens, Epioblasma capsaeformis, Epioblasma triquetra, Quadrula intermedia, freshwater mussels, mussel life history endangered species

INTRODUCTION

The decline or recent extinction of several species of Epioblasma (=Dysnomia=Plagiola) and some Quadrula spp. indicates a synecological problem exists between these groups of freshwater mussels and changes in North American rivers and streams. Most species of Epioblasma are either extinct (Stansbery 1970, 1971, 1976) or candidates for federal listing as endangered (Clark 1981; Ahlstedt 1983a, 1985; Neves 1983). At present two rare and endangered members of the genus Quadrula also continue to survive as remnant populations in a few larger tributaries of the Tennessee River drainage. Both endangered species of this widespread group are riverine, also found only in shallow riffle and shoal areas in moderate to fast flowing current.

The pattern of distribution for the 17 species of the genus Epioblasma infers a Tennessee-Cumberland River origin, with all members either belonging to, or descending from, the ancient Cumberlandian fauna (Ortmann 1924, 1925; Johnson 1978). Epioblasma brevidens (Lea 1834) and E. capsaeformis (Lea 1834) are endemic species, restricted in distribution to the Tennessee and Cumberland River systems. E. triquetra (Rafinesque 1820), occurring in the upper White, Missouri, Mississippi, Illinois, Tennessee, Cumberland, Ohio, Green and St. Lawrence river systems and Lakes Michigan and Erie, is the most ubiquitous and abundant member of the genus.

The two rarest Quadrula, Q. intermedia and Q. sparsa, are closely related and were formerly considered as one species (Ortmann 1918). However, Stansbery (1973) considered both valid species and reported them from the upper Tennessee River drainage. Ahlstedt (1983b) recorded Q. sparsa in the upper Clinch and Powell rivers, with the largest population occurring in the Powell River. Q. intermedia is presently known only from the upper Clinch, Powell, Duck and Elk rivers (Ahlstedt 1982), with the largest extant population also occurring in the Powell River.

Knowledge of mussel-fish host relationships, a critical period in the life cycle of freshwater mussel species, is imperative in effective conservation efforts for either declining mussel populations or attempts to establish transplanted populations. The reproductive biology of the sexually dimorphic Epioblasma species has not been previously reported, and most ~~assumptions about~~ influences on reproduction are based on the taxonomic position of the genus in the subfamily Lampsilinae (Heard and Guckert 1970). As part of the Cumberlandian Mollusk Conservation Program of the Tennessee Valley Authority (TVA 1980), the life history and reproductive biology of Quadrula species were briefly reviewed by Yeager and Neves (1986) in an intensive study using Q. cylindrica strigillata as a surrogate species for development of field and laboratory techniques. These methods were then used to conduct the investigations described herein for the endangered Q. intermedia. Our purpose is to report life history information useful to future conservation efforts for these declining taxa and to identify fish hosts for glochidia of Epioblasma brevidens, E. capsaeformis, E. triquetra and Quadrula intermedia.

METHODS AND MATERIALS

All specimens of the three Epioblasma species were collected by snorkeling near the Virginia-Tennessee border in the Powell River between river miles 106.8 and 117.4. Searches and collections of Q. intermedia were conducted between Powell River Miles (PRM) 99.2 and 117.4. Mussels were opened slightly by hand or with modified O-ring expanders to check for gravidity. Because of the propensity of Quadrula spp. to readily abort glochidia (Yeager and Neves 1986), gravid mussels were placed in small-mesh cloth bags or plastic zip-lock bags and transported in insulated coolers of river water to a TVA laboratory in Norris, TN. The Epioblasma species were held in Living Streams (Frigid Units Inc., Toledo OH)[2] with flow-through spring water at a mean temperature of 17.8 C. Quadrula intermedia was held in 3.8 liter aquaria at a mean temperature of 21.0 C. After aborting glochidia, all Q. intermedia ~~taken to the laboratory~~ were returned to their collection sites unharmed. ~~Following each day's searches~~ All non-gravid ~~mussels~~ and ~~following their laboratory use,~~ all gravid ~~mussels~~ were ~~replaced in the substratum~~ in an area of each shoal ~~from which~~ the highest densities of Q. intermedia ~~had been found over the season~~. Males were placed a few yards upstream of females. Ages of mussels were estimated by the external growth ring method (Chamberlain 1931, Crowley 1957).

When possible, fish to be tested as hosts were collected from mussel-free sites, or from ~~areas~~ known to have ~~only~~ low mussel densities, to avoid incidences of prior exposure to infestations with glochidia. Before testing, fish were maintained in either Living Streams[2] with flow-through spring water or, if tolerant of slack water conditions, in 36.8 or 66.6 liter aquaria equipped with filtration and aeration ~~systems~~. Rheophilic fish species ~~more dependent on current (e.g. members of the cyprinid genera~~ Hybopsis and Phenacobius were successfully held in specially constructed circular wooden tubs (approximately 400 L capacity) equipped with electrical water pumps (1140 L/h) to create adequate current velocities. A reservoir of potential test fish also was maintained in large flow-through concrete raceways of approximately 3900 L and 7700 L capacities.

Experimental Procedures

For the tests with Epioblasma spp., mature glochidia were obtained by excising the marsupial gills from gravid female mussels and rupturing the ovisacs with a probe. Mature glochidia were obtained from Q. intermedia by daily siphoning the bottom material of holding aquaria through a 100 micron mesh sieve. All material from tanks of Q. intermedia was examined through a binocular microscope to determine if glochidia were present. Glochidia were tested for maturity by exposing a small subsample to salt crystals, and were deemed suitable if they showed a strong, immediate closing response (Zale and Neves 1982, Yeager and Neves 1986).

Prior to infestation, experimental fish were anesthesized with tricaine methanesulfonate (MS-222), and the gills and fins were carefully inspected for any attached glochidia or other parasite infestations. Fishes so infested were excluded from experimental trials.

Criteria used to select fish species ~~for~~ to as hosts of testing ~~with~~ Q. intermedia were: 1) the species was implicated as a potential host previously (Koch et al, 1986); 2) the species was observed in the immediate vicinity of Q. intermedia populations; or 3) the fish species was identified as a host for a congener of Q. intermedia. Fish species tested as hosts for Epioblasma spp. were essentially those species likely to be sympatric with the particular mussel species ~~being~~ tested, and were available or collectable by investigators when mature glochidia were obtained. Fish were fed frozen brine shrimp (Artemis) and freeze-dried tubifex worms daily during the tests. Short sections of PVC pipe of appropriate diameter were provided as cover for fish in the test chambers.

Individual fish were infested ~~by~~ anesthesized ~~ing~~ them with MS-222 and by pipetting about 100 to 300 glochidia into the right branchial chamber. Each fish was exposed to glochidia only once. Fish were immediately checked for successful attachment of glochidia, held in a recovery chamber with fresh-filtered spring water for a few minutes, and then returned to their holding chambers.

Mixed species assemblages of fish infested with glochidia of a single species of Epioblasma were held in Living Streams for ten days after infestation. Five and ten days post-infestation, all fish were anesthesized and inspected for retention of glochidia. Fish not found to have retained glochidia were preserved in ten percent formalin and re-examined. Fish retaining glochidia after ten days were sequestered by individual fish species in 38 L aquaria containing spring water filtered through a 5 micron aperture bag filter.

Beginning ~~eleven~~ (11) days after infestation and every second day thereafter, material from the bottoms of aquaria was siphoned through a 35 micron nylon-mesh sieve. Sloughed glochidia and juvenile mussels from the test chambers were examined under a stereomicroscope. Representatives of transformed juvenile specimens were preserved in a five percent formalin solution buffered to pH 7.0 with ammonium hydroxide. All test fish were eventually preserved in identical solutions. Measurements of glochidia and juvenile mussels were made under a stereomicroscope equipped with an ocular micrometer.

In host experiments with Q. intermedia, all test fish were sequestered by individual species immediately after infestation. Test fish were anesthesized at five day intervals and inspected for retention of glochidia. If infestation was detected, that chamber was siphoned daily to check for sloughing of glochidia or juvenile mussels. Except for preserving only a very limited number of specimens, transformed juveniles and sloughed material were examined and handled as described previously.

The methods of Castanaga and Kraeuter (1981), as modified by Hudson and Isom (1984), were used to culture metamorphosed juvenile mussels. Juvenile mussels were placed in 2 L Nalgene trays with about 1.5 L of filtered (5 micron) cultured pond water containing a mixed culture of ~~several species of~~ unicellular algae and diatoms. Silt from the Powell River, strained through a 50 micron sieve, was added to each tray until it formed a fine silt coat on the bottom of the tray. ~~Each day~~ Juvenile mussels were washed on a 100 micron sieve daily followed by a change of food-laden water and silt.

RESULTS

Genus Epioblasma

During the spawning period, adults of the three Epioblasma spp. often lay exposed on the substratum surface or were only partially buried in the firm cobble-gravel in the swifter currents of large riffles. Observations spanning several successive years in the Powell and Clinch Rivers ~~by the authors~~ indicate that these species are typically ~~more completely and deeply buried~~ well burrowed in the substratum during other seasons of the year. Females of E. capsaeformis lay partially open, displaying a brilliant bluish-cream colored mantle that was often observable from the stream bank. This brilliantly colored mantle displayed by E. capsaeformis may serve to attract potential host fish

for infestation with glochidia. While snorkeling at Powell River collection sites, the senior author observed ~~individuals of the~~ gilt darters, Percina evides, ~~the~~ greenside darters, E. blenniodes, and ~~the~~ spotted darters, P. maculatum repeatedly strike or touch ~~this~~ mantles.

All three species of Epioblasma were gravid with mature glochidia in May or June, and were spent by July (Table 1). Because As gravid females were found on the first collection date in early May, the initial occurrence of gravidity was undoubtedly earlier. Gravid specimens of E. brevidens and E. triquetra were found from May 1 to June 5 at water temperatures ranging from 15.0 to 17.8 C. Gravid E. capsaeformis were collected only until May 18. During these periods, 45 percent of E. brevidens, 58 percent of E. capsaeformis and 31 percent of E. triquetra females were gravid. Estimated ages of gravid females were as follows: E. brevidens, 8 to 13 years; E. capsaeformis 7 to 10 years; and E. triquetra, 5 to 10 years.

Only the outer demibranchs served as marsupia for these all three species. Water tubes containing mature glochidia were swollen, whitish and granular in appearance. Unfertilized eggs accounted for less than one percent of conglutinates. Fully mature glochidia were nearly transparent and subcircular, with a definitely truncated dorsal hinge line. Glochidia are apparently released as conglutinates disintegrate; intact conglutinates could not be teased from water tubes. Mean dimensions of 100 mature glochidia from two females of each species were as follows: E. brevidens, length 0.24 mm (SE=0.01),

depth (dorsal to ventral) 0.23 mm (SE=0.05)and hinge length 0.17 mm (SE=0.04); E. capsaeformis, length 0.25 mm (SE=0.06), depth 0.23 mm (SE=0.05), hinge length 0.17mm (SE=0.05); E. triquetra length 0.22 mm (SE=0.03), depth 0.22 (SE=0.02), and hinge length 0.16 (SE=0.03). There were no statistically significant differences (ANOVA P> .05) in size or shape among species.

During tests, glochidia typically attached to the distal portion of gill lamellae on experimental fish, although on the banded sculpin, Cottus carolinae, most glochidia attached and encysted in the epithelial tissue lining the branchial cavity. Degrees of infestation were light to moderate depending upon the species and size of fish. Immediately after infestation, as few as six to ten glochidia were observed on smaller percids and cyprinids and as many as several hundred on centrarchids and larger percids.

Numbers of fish species exposed to glochidia of E. brevidens, E. capsaeformis and E. triquetra were 26, 20 and 25 species, respectively (Table 2). Newly metamorphosed juveniles of E. brevidens, E. capsaeformis and E. triquetra were obtained from six, five and two species of fish, respectively (Table 3). All identified hosts were percids or a cottid. Periods of transformation were temperature dependent and ranged between 16 and 45 days (Table 3).

Newly metamorphosed juveniles of Epioblasma spp. were nearly identical to glochidia in size and shape. Juveniles however had two adductor muscles, gill lamellae, functioning cilia, and other internal organs apparent. Juveniles moved by extending the extremely adhesive foot, then drawing the body to the foot. Within one to seven days after dropping from the host fish, juveniles showed evidence of shell growth (Figure 1a). ~~Twenty-seven-day-old~~ Juveniles of E. brevidens after 27 days had the characteristic hatchet-shaped foot of adult mussels.

Genus Quadrula

Except during spawning, Q. intermedia is typically found buried in the substratum with only its siphons exposed. However, while spawning in May and June, individuals were most often found partially or totally exposed on the substratum surface. Although work with Q. intermedia was directed at identifying fish hosts, ~~much valuable~~ ancillary observations ~~information was gained~~ were recorded during ~~the course of~~ field work completing. Within the study area the male to female ratio of the sexually dimorphic species (Ahlstedt 1984) was 0.8:1.0 in 1983 and 1984. Within the defined deficient period of gravidity for each year, the percentage of gravid females was 5 percent in 1983; 35 percent in 1984; and applying the above male to female ratio to 1985 data, was estimated to be 46 percent in 1985 (Table 4). In 1983 the first gravid individual contained only unfertilized eggs, whereas the other two contained a few mature

glochidia and many unfertilized eggs. However, most mussels in 1985 contained predominantly mature glochidia at roughly the same stage of development. Ages of gravid females were estimated to be 14 to 22 years.

In all years, the spawning period occurred between the last week of April and the third week of June at water temperatures between 16 and 24 C. The major period of gravidity occurred in May and early June at temperatures between 18 and 24 C (Table 4).

Gravid Q. intermedia used both the inner and outer (all 4) demibranchs as marsupia for developing glochidia. However, most females held glochidia only in the outer demibranchs. The water tubes of mussels containing mature glochidia were whitish, swollen, and granular in appearance. Glochidia were contained in lanceolate-shaped conglutinates packed in the water tubes ~~of the gills~~. An average of 309 (range 233 to 352) eggs per conglutinate was calculated for five conglutinates. Mature glochidia were expelled already released from conglutinates. Five conglutinates averaged 5.9 mm in length, 1.0 mm in width, and 0.4 mm in thickness. The hookless, mature glochidia were similar to those of its congener Q. cylindrica strigillata (Yeager and Neves 1986); namely whitish with a light tinge of red or peach color. Glochidia were subcircular in shape, and 100 specimens averaged 0.22 mm (S.E.=.04) anterior to posterior, 0.24 mm (S.E.=.04) dorsal to ventral and 0.10 (S.E.=.03) in hinge length. A single adductor muscle was apparent, and mantle cells were spread over the interior of the valves.

~~Glochidia of Q. intermedia were gill parasites~~. A total of 211 fish representing 34 species were tested as potential hosts for Q. intermedia (Table 2). Only two closely-related cyprinid species, the streamline chub, Hybopsis dissimilis, and ~~the~~ blotched chub, Hybopsis insignis, served as hosts for glochidia of Q. intermedia. Periods for complete transformation were as follows: 24-29 days at 19 C and 42-47 days at 15 C for H. dissimilis; and 26-41 days at 17 C for H. insignis. After 19-22 days, 101 partially transformed unviable glochidia were sloughed from ten of the H. dissimilis. Five to ten days after excystment, thirty-nine of the fully transformed juveniles (Figure 1b) produced were subsequently released into adult mussel habitat at McDowell Ford (PRM 106.4). They ranged in size from 0.35 to 0.40 mm in diameter (1.7 to 2.0 times larger than their excystment diameter).

DISCUSSION

The low fertilization success of Q. intermedia ~~seen in the field~~ in field samples prior to 1984 was as expected for low density populations of rare mussels. ~~This low fertilization rate initially~~ and limited the availability of glochidia to test fish hosts during our study. Unfertilized eggs are commonly found in summer breeders, and are especially common in Quadrula spp. (Lefevre and Curtis 1910). We observed a dramatic increase in fertilization success for Q. intermedia in subsequent sampling latter stages ~~of this work~~. The greater numbers of gravid females ~~we encountered~~ examined in 1984 and 1985 were likely due to three influences. Our field efforts began earlier in these ~~latter~~ years to sample the peak period of gravidity.

We placed males upstream of females during previous year's investigations to enhance fertilization success. Finally, the rapid warming and then extended spring temperature conditions in 1985 also could have contributed to greater occurrence of gravidity or higher rates of fertilization. Although no documentation was provided, Coker et al. (1921) suggested that release of sperm by males, their dispersal by water currents, and subsequent entry into females during normal siphoning may stimulate ovulation. Greater synchrony of male and female spawning, ~~to within a short time period~~ and higher densities of sperm entering females could conceivably have increased both fertilization success ~~of individuals~~ and the percentage of gravid females ~~gravid~~.

Handling and movement of mussels, thermal changes (slowly warming), and increased turbidity all induced abortion of glochidia and conglutinates by gravid Q. intermedia. After the propensity of Quadrula spp. to abort eggs and glochidia in response to stimuli was reported (Yeager and Neves 1986) and somewhat controlled by handling and laboratory procedures, this behavioral response was used to our advantage in obtaining mature glochidia for screening of fish hosts.

Glochidia of Q. intermedia exhibited a high degree of host specificity, successfully metamorphosing only on two closely related cyprinids among thirty-four species of fish in ten families tested. The two identified hosts, the streamline and blotched chubs, are sympatric with the three ~~presently known~~ populations of Q. intermedia. Both hosts have greater geographical distributions than

the mussel (Lee et al. 1980), indicating that factors other than declining ranges for fish hosts have effected a range decline for the Cumberland monkeyface mussel.

Both chubs occur ~~in the same habitat as~~ Q. intermedia~~; i.e.,~~ the moderate to swifter currents of large cobble and gravel shoals, but H. dissimilis was most commonly encountered by snorklers in immediate proximity to Q. intermedia during mussel searches in the Powell River. Host specificity ~~to the few fish species typically occurring in the same habitat as the mussel~~ has also been reported for the rabbits foot mussel, Q. cylindrica (Yeager and Neves 1986). For this mussel the obligate fish hosts, Notropis galacturus and Hybopsis amblops, also occupied the same habitat as the mussel, i.e. eddies of mid-stream currents or areas adjacent to emergent or submerged vegetation near current.

Fish hosts for Epioblasma spp. were restricted to a few species of benthic, riffle-dwelling darters and a sculpin living in the same habitat as the mussels. Obligate fish hosts for the more endangered ~~members of this mussel genus~~ are also likely to be found within these two groups of fishes. The fish hosts for Q. intermedia have a wider geographical range than the mussel (Lee et al. 1980), indicating that ~~additional factors are contributing to the decline of this taxon.~~

Conservation efforts for maintaining freshwater mussel diversity require critical knowledge of specific mussel-fish host relationships. Fish provide not only the obligate hosts for particular mussels, but also serve as a dispersal mechanism into or within suitable habitats. Use of specific host fishes occupying the same particular habitats as the mussel species improves the chances of attachment and the excystment of juveniles into suitable habitat. This situation appears true for both Epioblasma spp. and Q. intermedia. Conversely, mussel species such as Anodonta grandis and A. imbecilis, tolerant to a broad range of ecological habitats (Trdan and Hoeh 1982), exhibit eurytopic host use, successfully transforming on a greater number of fish species in diverse habitats (e.g., over 30 known fish hosts have been reported for A. grandis). These eurytopic species such as Anodonta spp. seem to partition overlapping fish host resources by differences in seasonality of glochidial releases (Trdan and Hoeh 1982). Coevolution of mussels with stenotopic host requirements and reproductive periods synchronized with the occurrence of host fishes, would contribute to greater reproductive success in pristine environments, but make them more vulnerable to anthropogenic disturbances of shoal and riffle habitats.

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reproductive conditions

Table 1. ~~Collection records~~ of females of Epioblasma spp. collected in the Powell River in 1984.

Date	River mile Site[1]	Water Temperature (°C)	Number ~~Females~~ Collected (Number Gravid) E. brevidens	E. capsaeformis	E. triquetra
1984					
May 1-2	BI 111.7	15	6(4)	4(3)	8(3)
May 17-18	FF 117.1	16	2(0)	7(4)	3(1)
May 19	MF 106.4	16	0(0)	1(0)	3(0)
June 5	BI	19	3(1)	0(0)	2(1)
July 19	BI	23	4(0)	1(0)	4(0)
Total ~~Number Inspected~~ collected (total gravid)			15(5)	13(7)	20(5)

1. ~~Bales Island (BI) = PRM 111.7; Fletcher Ford (FF) = PRM 117.4; McDowell Ford (MF) = PRM 106.4~~

~~0069y~~

Table 2. Fish species ~~and numbers laboratory~~ and number tested as potential hosts for glochidia of ~~Epioblasma~~ ~~brevidens~~, ~~E. capsaeformis~~, ~~E. triquetra and O. intermedia.~~ the four mussel species.

Fish species	Mussel Species			
	E. brevidens	E. capsaeformis	E. triquetra	O. intermedia
	Number of ~~test~~ fish infested			
Dorosoma cepedianum	-	-	-	1
Campostoma anomalum	1	2	4	1
Hybopsis aestivalis	-	-	-	2
H. amblops	1	4	1	3
H. dissimilis	2	2	2	21
H. insignis	-	-	-	21
Nocomis micropogon	1	-	1	3
Notropis ardens	-	-	-	8
N. ariomus	3	-	1	-
N. chrysocephalus	1	-	1	6
N. coccogenis	3	1	2	1
N. galacturus	2	3	1	8
N. leuciodus	2	-	2	6
N. lirus	-	-	-	2
N. rubellus	2	1	2	2
N. spectrunculus	-	-	-	4

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Table 2. (continued)

	Mussel Species Number of test fish infested			
Fish species	*E. brevidens*	*E. capsaeformis*	*E. triquetra*	*Q. intermedia*
N. spilopterus	2	1	2	22
N. telescopus	1	-	1	-
Phenacobius uranops	1	-	-	-
Pimephales notatus	4	2	2	6
P. vigilax	-	-	-	1
Rhinichthys atratulus	-	-	-	3
Carpiodes sp. (juvenile)	-	-	-	1
Hypentelium nigricans	2	2	3	-
Moxostoma duquesnei	-	-	-	3
M. erythrurum	1	-	-	-
Ictalurus punctatus	-	-	-	3
Fundulus catenatus	-	2	-	4
Morone mississippiensis	-	-	-	1
Lepomis auritus	2	-	2	-
L. machrochirus	2	-	3	-
L. megalotis	-	2	-	-
Pomoxis annularis	-	-	-	1
Etheostoma blennioides	2	-	2	6

Table 2. (continued)

Fish species	Mussel Species: Number of test fish infested			
	E. brevidens	*E. capsaeformis*	*E. triquetra*	*Q. intermedia*
E. caeruleum	-	-	2	-
E. camurum	-	-	-	5
E. jessiae	3	3	1	-
E. maculatum	2	1	1	-
E. rufilineatum	12	5	9	-
E. simoterum	10	1	15	10
E. zonale	-	-	5	
Percina caprodes	1	2	3	6
P. copeland	-	1	3	-
P. evides	4	-	2	
P. sciera	1	-	-	
Cottus carolinae	13	1	4	5
Aplodinotus gruniens	-	-	-	1

- species not tested

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species confirmed as hosts for the four mussel species.

Table 3. Fish hosts identified for glochidia of Epioblasma brevidens, E. capsaeformis, E. triquetra or Quadrula intermedia.

Host Fish	Mussel Species											
	E. brevidens			E. capsaeformis			E. triquetra			Q. intermedia		
	No. Juveniles[1]	Period[2] Days to transform	Mean Temp.[3] (°C)	No. Juveniles[1]	Period[2]	Temp.[3] °C	No. Juveniles[1]	Period[2]	Temp.[3] °C	No. Juveniles[1]	Period[2]	Temp.[3] °C
Etheostoma blennioides	9	34-37	17.0	-			-			-		
E. maculatum	2	17	15.4	10	19-31	16.2	-			-		
E. rufilineatum	28	16-33	16.3	1	25	15.8	-			-		
E. simoterum	3	25-34	16.9	-			-			-		
Percina caprodes	104	28-45	16.7	-			804	24-33	17.1	-		
P. sciera	-			4	25	15.8	-			-		
Cottus carolinae	123	20-48	17.2	14	20-34	16.9	26	25-44	17.2	-		
Hybopsis dissimilis	-			-			-					
Test 1	-			-			-			26[4]	24-29	19.2
Test 2	-			-			-			19	42-47	15
H. insignis	-			-			-			41	26-41	17.0

1 - A dash indicates not a host for that mussel species.
2 - Period in days for transformation of glochidia to excysted juveniles.
3. Mean temperature.
4 - An additional 101 transformed juveniles excysted earlier on days 19-22, but all these earlier excysted juveniles died within four days.

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Table 4. Specimens of Quadrula intermedia examined in the Powell River, 1981-1985.

Date	~~Powell~~ River Site	Water Temperature (°C)	Number Total-females (gravid)	Period of gravidity by year (Temp. °C)
1981				
6-23	MF	20	2-1(0)	
6-24	MF	20	4-0(0)	
7-1/2	MF	22	0-0(0)	
		Total	6-1(0)	
1982	No collections made			
1983				
6-9	MF	21	19-9(0)	
6-15	FC	21	19-9(1)	June 15-16
6-16	FC	22	3-2(0)	(21-22 C)
6-16	FF	22	12-8(2)	
6-17	BF	24	14-11(0)	
6-18/19	BD	24	12-8(0)	
6-20	BI	23	10-7(0)	
7-1	FF	24	4-2(0)	
		Total	93-56(3)	

I didn't see how temp was taken in Methods

Table 4. (continued)

Date	Powell River[1] Site	Water Temperature °C	Number Total-females (gravid)	Period of gravidity by year (Temp. °C)
1984				
4-16	BI	16	7-0(0)	April 30-June 20
4-30	BD	16	4-3(0)	(16 - 21 C)
4-30	BI	16	13-6(1)	
5-1	FF	16	13-5(1)	
5-17	FF	16	16-9(8)	
5-18	BD	18	16-10(1)	
5-19	MF	16	1-1(1)	
5-24	FF	18	9-3(0)	
6-5	BI	19	13-8(6)	
6-20	BI	21	11-8(1)	
6-26	BF	23	2-2(0)	
		Total	105-55(19)	

Table 4. (continued)

Date	Powell River[1] Site	Water Temperature °C	Number Total-females (gravid)	Period of gravidity by year (Temp. °C)
1985				
4-26	BI	19	11-?(2)	April 26 - June 11
5-6	BI	18	11-?(2)	(19 - 24 C)
5-7	BI	18	3-3 (3)	
5-14	FF	21	11-?(4)	
5-29	BI	22	9-?(1)	
5-31	MF	22	0-0(0)	
5-31	BD	22	1-1(1)	
6-5	FF	25	6-?(2)	
6-11	BF	24	4-?(1)	
6-21	BI	20	3-?(0)	
6-26	FF	26	4-?(0)	
10-29	BF	20	0-0(0)	
		Total	63-35?(16)[2]	

1. McDowell Ford (MF) = PRM 106.4; Fletcher Cliff (FC) = PRM 117.9; Fletcher Ford (FF) = PRM 117.4; Buchanan Ford (BF) = PRM 99.2; Bales Ford (BD) = PRM 112.3; Bales Island (BI) = PRM 111.7

2. Field data was recorded as total number found, number gravid in 1985. Total number females calculated from previous years sex ratios.

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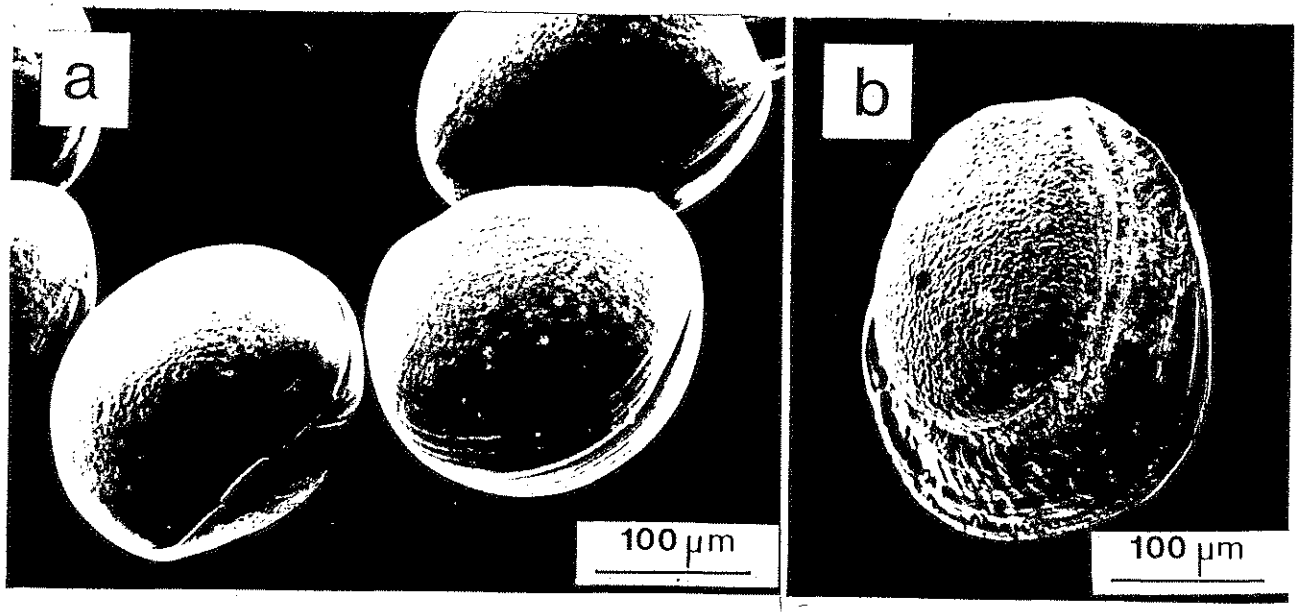
LIST OF FIGURES

Figure 1

a. Photograph (scanning electron micrograph) of transformed juveniles of Epioblasma triquetra.

b. Photograph (scanning electron micrograph) of transformed juvenile of Quadrula intermedia showing growth beyond the shell valve.

It would be better to show a + b for Epioblasma sp and Q intermedia i.e. 4 photos.


a
100 µm
b
100 µm