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Life history and population biology of the endangered tan riffleshell (*Epioblasma florentina walkeri*) (Bivalvia: Unionidae)

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Abstract. The tan riffleshell (*Epioblasma florentina walkeri*) is listed by the US Fish and Wildlife Service as endangered, and is restricted to only 1 reproducing population in Indian Creek of the upper Clinch River, Virginia. We investigated its fish hosts, efficacy of infestation methods, suitability of host populations, and population demographics. Fish were collected and infested with tan riffleshell glochidia to examine potential hosts. Juveniles transformed on at least 5 of the tested species: fantail darter (*Etheostoma flabellare*), greenside darter (*E. blennioides*), redline darter (*E. rufilineatum*), snubnose darter (*E. simoterum*), and 1 or 2 cottids: banded sculpin (*Cottus bairdi*) or mottled sculpin (*C. carolinae*). Fantail darters from 4 drainages were collected and infested with glochidia to compare the suitability of various populations of the most successful host. The number of juvenile mussels obtained from fantail darters collected from Indian Creek ($\bar{x} \pm 1$ SD = 59.22 ± 10.01) was significantly higher than those transformed on fantail darters from the Roanoke River ($\bar{x}$ = 9.45 ± 10.64), where the tan riffleshell does not occur. Variation in transformation success supports the hypothesis that host fish suitability is mediated by varying immune responses, and that coadaptation of sympatric host fish and mussel populations seemingly enhances compatibility. No significant difference (ANOVA, p = 0.39) existed between the numbers of juvenile tan riffleshells produced by fish using 2 different infestation techniques. The population of tan riffleshells in Indian Creek was ~2000 adults using Schumacher's modification of Schnabel's maximum likelihood estimator. Sex ratio was near 1:1, and size-frequency distributions of males and females were not significantly different. Maximum age of the population, determined by thin-sectioning of valves, was 11 y; ~70% of the population was <6 y.

Key words: tan riffleshell, freshwater mussels, host fish, population demographics, endangered species.

Riffleshells of the genus *Epioblasma* (Bivalvia: Unionidae) are considered to be the most highly specialized and most endangered genus of unionids in the United States (US Fish and Wildlife Service 1984). Sixteen of the 25 recognized taxa in the genus are presumed to be extinct, and all but the snuffbox (*E. triquetra*) are listed as endangered by the US Fish and Wildlife Service (Turgeon et al. 1998). Subspecies of the *E. florentina* complex survive as the Curtis pearlymussel (*E. f. curtisii*) in Missouri and tan riffleshell (*E. f. walkeri*) in eastern Tennessee and southwest Virginia. However, the last collection record of the Curtis pearlymussel was in 1993, so this subspecies seems to be on the brink of extinction (US Fish and Wildlife Service 1986, Buchanan 1993).

Populations of the tan riffleshell have been recorded recently from the Duck and Hiwassee rivers in eastern Tennessee, and Middle Fork Holston River, Clinch River, and Indian Creek in southwest Virginia (US Fish and Wildlife Service 1984, Winston and Neves 1997, Parmalee and Bogan 1998). Another population, yet to be confirmed, is reported from the Big South Fork, Cumberland River, Tennessee (S. Ahlstedt, US Geological Survey, Knoxville, Tennessee, personal communication). However, the status of most of these populations has changed drastically in the last 10 y. Neither the Duck nor Hiwassee

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river populations is thought to be selfsustaining (Parmalee and Hughes 1994) because only a few, large specimens have been collected recently. The population in the Middle Fork Holston River is nearly extirpated, with only 1 specimen documented during a recent survey of the river (Henley et al. 1999). The Clinch River population, restricted to a 1-km reach at Cedar Bluff, Tazewell County, Virginia, was eliminated by a toxic spill in 1998 (Jones et al. 2000). Thus, the only known reproducing population of the tan riffleshell occurs in a 2-km reach of Indian Creek, tributary to the Clinch River at Cedar Bluff, Virginia.

Because of the precarious status of this species throughout its range, we conducted a biological investigation of the tan riffleshell population in Indian Creek between 1996 and 1999. The objectives of the study were to identify fish hosts, evaluate methods of host infestation, compare suitability of host populations, and describe population demographics and life-history observations.

Methods

Host fish identifications

Gravid females of *E. f. walkeri* were collected in spring 1996 from Indian Creek, Tazewell County, Virginia. Once a female was found, it was checked for gravidity by slightly opening the valves by hand or with modified O-ring expanders. The mussel then was measured to the nearest 0.1 mm, marked with an individually numbered tag (Hallprint Tags, Holden Hills, Australia), and its location was plotted using landmarks at the site. We placed gravid females in small-mesh cloth bags or zip-lock bags and transported them to the laboratory in insulated coolers containing cooled stream water. Each zip-lock bag had holes punched in the top to facilitate water flow but did not permit the release of the more dense glochidia. Females were placed in 500- or 1000-mL beakers in the laboratory and then submerged in Living Streams (Frigid Units Inc., Toledo, Ohio). The beakers captured any glochidia released naturally from the females. Keeping the temperature in the holding tanks at ~10°C inhibited glochidial release. Cultured algae (*Neochloris oleoabundans*) were introduced approximately weekly to feed the mussels. Mortality of 1 gravid female (44.6 mm long, 6 y) provided an opportunity to determine fecundity. We removed both of its gravid gills, ruptured them in a water-filled dish, and counted all glochidia teased from the marsupia.

The choice of fishes to be infested with glochidia was based on previous research with riffleshells. Neves et al. (1985) and Yeager and Saylor (1995) showed that hosts, specifically *Etheostoma*, were shared by species of the genus *Epioblasma*. Hosts for the tan riffleshell had not been identified, so we focused on darter species. However, other fish species also were tested to provide a suite of natural hosts. A fish survey conducted in Indian Creek from 1996 to 1997 provided a complete record of resident species (Pinder and Jones 2000). Fish species outside of the tan riffleshell's historic range (e.g., *Nocomis leptocephalus*, *Cottus bairdi*) also were tested to determine if allopatric congeneric species would serve as hosts.

We collected fishes either with backpack electrofishing gear or with a seine, as stream conditions permitted. We collected from streams in the Clinch River and Holston River drainages that did not support mussels or that contained populations only at low densities to avoid the possibility that the fishes were immune as a result of previous infestations (Arey 1932, O'Connell and Neves 1999). We inspected fishes on site or in the laboratory for glochidial attachment or disease, and we retained only parasite-free and disease-free fish. We collected: banded and/or mottled sculpin (*Cottus bairdi/carolinae*), greenside darter (*Etheostoma blennioides*), fantail darter (*E. flabellare*), redline darter (*E. rufilineatum*), snubnose darter (*E. simoterum*), northern hogsucker (*Hypentelium nigricans*), channel catfish (*Ictalurus punctatus*), redbreast sunfish (*Lepomis auritus*), bluegill (*L. macrochirus*), largemouth bass (*Micropterus salmoides*), rock bass (*Ambloplites rupestris*), bluehead chub (*Nocomis leptocephalus*), black jumprock (*Scartomyzon cervinus*), bigeye jumprock (*S. ariommus*), and margined madtom (*Noturus insignis*). The healthy fishes were placed in insulated coolers containing river water and supplied with air for transport to the laboratory. Once at the laboratory, fishes were placed in Living Streams or in 38-L aquaria for an acclimation period of ~1 wk before infestations were induced. Fishes were fed live larval *Tilapia* spp., frozen blood worms

(Chironomidae), pieces of earthworm (*Lumbricus cambrus*), or fish pellets.

The infestation procedure was adapted from Zale and Neves (1982). Mature glochidia from gravid females were obtained by water temperature manipulation or, primarily, by expulsion using a hypodermic needle. We flushed the gills with water after inserting the needle, which caused the gills to rupture. The resulting glochidia were captured in a petri dish. Activity of the larvae was tested by introducing a minute amount of salt to a sample of glochidia within water in the dish. Mature, active glochidia rapidly snap shut in a saline solution, as observed with a dissecting microscope.

Two methods of infestation were used, depending on abundance of mature glochidia. If ≥1 gravid females were collected, the fishes were placed in a 19-L bucket of aerated water. The bucket contained only enough water to submerge the fishes. The fish then were exposed to the glochidia, which were emptied into the bucket over a period of 30 to 60 min, depending on the number of glochidia present. Every few minutes, we induced the fish to swim around in the bucket to facilitate a greater exposure to the glochidia. If only a partially gravid female was collected, then several hundred glochidia were pipetted directly into the right branchial cavity of each fish, out of water. Small fishes (e.g., darters and minnows) were exposed to reduced numbers of glochidia to avoid lamellar inflammation and possible death. Fishes to be infested were anesthetized first with MS-222 (~100–200 mg/L, depending on the size and species) and placed in clean water to rinse away the chemical. After exposure, the fishes were placed in another 19-L bucket of water for recovery. Once recovered, we placed the fishes in 38-L aquaria with aeration provided by air stones and pieces of PVC pipe cut in half lengthwise to provide cover. Fish species that required flowing water were placed in Living Streams at water temperatures of 12 to 19°C for ~2 wk. After 2 wk, we moved the fishes into 38-L aquaria with aeration and flow (provided by air stones and electric power heads, respectively). Each aquarium contained individuals of a single fish species. All aquaria were devoid of substrata.

A few individuals in each aquarium were checked immediately after exposure and every few days to determine whether encystment and development had occurred. These fishes were anesthetized (MS-222) and inspected visually under strong light. If the glochidia were being sloughed off, glochidial incidence on test fishes decreased rapidly during the first few days after exposure. After ~1 wk, the bottom of each tank was siphoned every 1 to 2 d with a flexible hose. The siphoned water was filtered through a 300-μm- and then 150-μm-mesh sieve to capture any juvenile mussels (the 300-μm sieve was used to remove large debris siphoned from the aquaria). About ½ of the water in each aquarium was removed and replaced with conditioned water after each siphoning. The siphoned debris in the 150-μm-mesh sieve then was flushed into a gridded petri dish and examined with a dissecting microscope to search for juvenile mussels. We continued this process until no more transformed juveniles were collected or until a fish species was eliminated as a host. A fish species was considered a host if the glochidia attached, encysted, and metamorphosed into juveniles. We released all metamorphosed juveniles in the field at the site of collection of the gravid females. If an infested fish died before transformation had occurred, its gills and fins were removed and examined with a dissecting microscope. Encysted glochidia ~1 wk post-infestation indicated that the fish species was a possible host.

Comparison of infestation methods

Gravid female tan riffleshells collected from Indian Creek were placed in the coarse gravel of a Living Stream set at ~15°C. Fantail darters (*E. flabellare*) were collected with a backpack electrofisher from various locations in the Roanoke and Tennessee drainages. We transported fish to the Aquaculture Center in aerated coolers and placed them in 38-L aquaria with airstones and no substratum. Pieces of 5-cm PVC pipe, cut in half lengthwise and placed in the tanks, provided cover for the fish.

After several days, we infested the darters with glochidia using 1 of 2 methods to determine which yielded more juveniles. In both cases, we flushed glochidia from the female's gills using a water-filled syringe, as described above. In the 1st infestation method, the fish were placed together in a 19-L bucket with water ~5 cm deep and with a large airstone. The glochidia then were flushed into the water, and the air-

stone kept them in suspension. We left the fish in the bucket for ~1 h and then returned them to the aquaria. In the 2nd method, the fish were placed into a dilute MS-222 solution to anesthetize them before infestation. We pipetted the glochidia onto each gill and then placed the fish in another 19-L bucket with water for recovery. We returned all the fish to the aquaria, once they had been infested and recovered.

For the first 7 d post-infestation, the fish were fed frozen chironomids, and the water was changed every other day. After 7 d, the aquaria bottoms were siphoned every other day and examined for transformed juveniles. We siphoned the water through a 300-μm sieve to remove large particles and then through a 120-μm sieve to collect the juveniles. About ¼ of the water in each aquarium was removed and replaced with conditioned water after each siphoning. We flushed the contents of the 120-μm sieve into a petri dish to be examined under a dissecting microscope at ~10× magnification.

Suitability of fantail darter populations as hosts

As a follow-up experiment to host fish testing, we initiated a study in 1998 to compare the suitability of various populations of fantail darters to host glochidia of the tan riffleshell. We collected fantail darters at 4 sites in Virginia: Indian Creek (Tennessee River drainage), Elk Garden (Tennessee River drainage), South Fork Holston River (Tennessee River drainage), and North Fork Roanoke River (Atlantic slope drainage). These streams were chosen to represent an array of distributional overlaps with the mussel: Indian Creek has a resident population, South Fork Holston River was historic habitat for the species, Elk Garden is a tributary of the Clinch River with no historic record of the species, and North Fork Roanoke River is outside the historically inhabited drainage of the species. All streams had a cobble and gravel substratum except Elk Garden, which had predominantly fine sediment.

Fantail darters were collected from these streams with a backpack electrofisher. The fish from each stream were randomly distributed into 38-L aquaria according to their stream of origin, to produce 4 aquaria per stream and approximately equal numbers of fish per aquarium. Before infestation, each fish was given a fin clip unique to its stream. Fish could thus be placed together in the same tank to ensure equal infestation and then separated by stream after infestation. Once clipped, we placed the fish in a 19-L bucket with ~5 cm of water and an airstone to keep the glochidia in suspension. The glochidia, extracted from the gills of 4 gravid tan rifflesheells, were added to the bucket. Fish and glochidia were left in the bucket for 90 min and then separated by stream of origin and returned to the aquaria. We infested 17 fish from Indian Creek, 22 from Elk Garden, 31 from South Fork Holston River, and 34 from North Fork Roanoke River.

Beginning on day 8 and approximately every 2 d thereafter, we siphoned the bottoms of aquaria and filtered the contents through 300-μm and 105-μm sieves; the coarse sieve size allowed removal of coarse particles, and the fine sieve retained the juveniles, which were carefully removed and counted. Juveniles were collected in this manner through day 23, when it was apparent that all glochidia had completed transformation. Sloughed, untransformed glochidia also were counted from each tank, and the numbers were compared among the 4 populations.

For each day that juveniles were collected, we divided the number of juveniles that transformed per tank by the number of fish that survived up to that day. We summed the resulting numbers for the entire juvenile collection period for each tank. Least squares means of the juveniles per fish from each stream were compared using analysis of variance with repeated measures (RM-ANOVA), and Tukey's multiple comparison test (Tukey's MCT) was used to compare numbers among the streams (Ott 1993), with a significance level of $p < 0.05$.

Population demographics

A composite collection of *E. f. walkeri* shells, collected following this population's discovery in 1995 (Winston and Neves 1997), was used to estimate size-class distribution. The shell collection (~400 specimens) was divided into 10-mm size categories, and we randomly selected 25% of the shells of each size category for age analysis. Shells were aged first by counting external growth rings. The 1st year's growth ring was eroded in most specimens, so we added 1 y to the age of each specimen based on the location of the age 1 growth line visible on young spec-

imens. Two experienced biologists aged the shells independently.

Shells then were thin-sectioned with a Buehler Isomet low-speed saw (~200 rpm), using a diamond-impregnated blade that allows precise cuts to be made at reduced speeds (Clark 1980, Neves and Moyer 1988). We set the width of the thin-section at ~250 μm, although occasionally the epoxy used to attach the shell to a slide for cutting or a thicker shell deflected the blade to produce a slightly thicker cut. Once the cuts were completed, all thin-sections were polished on 1600-grit sandpaper to remove scratches formed during cutting. In addition, we ground sections that had slightly thicker cuts to ~250-μm width with coarser sandpaper.

Three biologists read each thin-section independently, using a bright field dissecting microscope with ~10× magnification, by counting the rings that originated in the umbo and extended along the shell to the periostracum. Rings that clearly did not originate in the umbo or extend to the periostracum were considered to be false annuli. We excluded specimens for which at least 2 biologists disagreed on the ages or when both valves did not yield the same age. Age-class distributions and length-at-age regressions were examined separately for males and females to compare growth rates and cohort structure by gender.

Population estimate

A complete survey of Indian Creek was conducted for the tan riffleshell from June to September in 1996 and 1997 to determine range and reaches with greatest abundances (Watson 1999). Survey results indicated that 99% of the population occurred in the lowermost 1185 m of the stream. During the 1996 survey, 112 tan rifflesheIls were tagged with individually numbered plastic tags (Hallprint Tags) within a 930-m section of stream near its confluence with the Clinch River. These tags were attached to the left valve of the mussels using cyanoacrylate glue. We pooled these tagged specimens with specimens tagged after 1997 to provide a population estimate for the entire system.

We surveyed Indian Creek about once per month from 1997 to 1999. Surveyors snorkeled along the streambed, searching for mussels visible in the substratum. Snorkeling was useful only for finding adult mussels because juveniles were generally too small to be observed at the surface. Tan rifflesheIls found were collected, aged, sexed when possible, and measured lengthwise with calipers to the nearest 0.1 mm. We attached tags to the left valve of the shells using cyanoacrylate glue and then returned the mussels to their original locations. Recaptured tagged mussels were measured and returned to the stream. The observations also provided informative data on movements of the species vertically in the substratum during various seasons.

We estimated the population size of adult tan rifflesheIls in a 500-m section of stream extensively surveyed between 1996 and 1999 using Schumacher's modification of Schnabel's maximum likelihood estimate (Schumacher and Eschmeyer 1943, Caughley 1977). This method is appropriate when only a few individuals can be collected in 1 sampling event. The population size is estimated from the rate at which the proportion of marked individuals rises as progressively more are marked (Caughley 1977). This estimate accounts for variation in the number of captured individuals on any 1 sampling occasion (Schumacher and Eschmeyer 1943). We calculated total population size using this population estimate for the stream reach that contained 54% of the population (Watson 1999) and the relative abundance of tan rifflesheIls in the remaining 685 m of stream.

Results

Life-history observations

Several insights into the behavior of tan rifflesheIls can be gleaned from the monthly sampling of the same 100-m reach of Indian Creek under constant survey effort (8 person-h) (Table 1). Adults of both sexes burrow into the substratum for 3 mo (November–January) of the year. No tan rifflesheIls were visible at the surface during this period. Many males and some females were present at the surface in August and September, the putative spawning period of long-term brooders such as rifflesheIls. Females were most evident at the surface in May and June, when glochidial release occurs. It seems that most female tan rifflesheIls became gravid by late fall or early winter, overwintered below the substratum surface, began to emerge in February, and released glochidia principally in May

TABLE 1. Number of tan riffleshells (*Epioblasma florentina walkeri*) observed monthly from September 1997 to August 1999 in a 100-m reach of Indian Creek, Virginia, using 8 person-h of snorkeling effort. – = not applicable.

Month	No. observed	Males	Females	% females gravid
January	0	0	0	–
February	10	2	8	75
March	4	0	4	100
April	5	0	5	100
May	15	2	13	92
June	16	1	15	93
July	3	1	2	50
August	14	10	4	50
September	16	12	4	0
October	5	4	1	100
November	0	0	0	–
December	0	0	0	–

and June. Gravid females with mature glochidia protruded well out of the substratum with valves gaped and the mottled gray mantle tissue covering the visceral mass. This interpretation of collection data corroborates our qualitative observations in other reaches of Indian Creek (Watson 1999).

Of the 88 tan riffleshells collected, 56 were females and 45 of these were gravid (Table 1). Only the outer demibranchs served as marsupia for this species, which were swollen and milky-white when gravid. Examination of the glochidia from 7 of the gravid females revealed that all were mature and held individually within the water tubes of the gills. These glochidia were rounded to horseshoe-shaped, but were longer (anterior to posterior) than wide (dorsal to ventral). Glochidia (n = 25) were 260 ± 22.3 ($\bar{x}$ ± SD) μm long, 230 ± 16.9 μm wide, and had a hinge length of 180 ± 19.7 μm. None of the gravid tan riffleshells aborted their glochidia when transported and held in the laboratory. A fecundity estimate of nearly 20,000 mature glochidia was obtained from the 2 marsupia of a gravid female that died in the laboratory.

Fish hosts

At least 5 (likely 6, because of the confusion of *C. bairdi* and *C. carolinae*) of the 16 fish species tested served as successful hosts (Table 2). Fish hosts were benthic, riffle-dwelling fish (darters and sculpins) that occupy the same habitat as the mussel species. Periods of transformation ranged from 9 to 21 d (Table 2), with peak transformation occurring around day 13 (Fig. 1). Water temperatures ranged from 22.0 to 23.2°C. The number of transformed juveniles appeared to be species-dependent, with the fantail darter serving as the most suitable host fish for the tan riffleshell (Table 2). Comparison of *E. f. walkeri* juveniles to glochidia revealed that the same shape was retained after transformation, and that no visible growth occurred (length ≅ 280 μm, width ≅ 255 μm, and hinge length ≅ 200 μm). All juveniles were released into Indian Creek in July 1996. We found no significant difference (ANOVA, p = 0.39) between the numbers of juvenile tan riffleshells produced by fish using the 2 different infestation techniques.

TABLE 2. Fish species confirmed as hosts for the tan riffleshell (*Epioblasma florentina walkeri*).

Fish species	No. infested	No. of juveniles	Days to transform
Cottus bairdi/carolinae[a]	24	95	11–19
Etheostoma blennioides	11	248	9–19
E. flabellare	13	1181	9–21
E. rufilineatum	10	106	9–13
E. simoterum	8	43	9–13[b]

[a] Individuals of both species were inadvertently infested together

[b] All fish died before glochidia transformed

Suitability of fantail darter populations

There were no differences among streams in the number of glochidia that sloughed from the fish (range: 38–73/fish) before transformation (ANOVA, p = 0.669). In addition, there were no differences between the lengths of darters from each stream. Only darters from Indian Creek produced significantly more juveniles than those from the Roanoke River (RM-ANOVA, p = 0.024; Fig. 2).

Age-class structure

Seventy-seven of 102 shells were aged successfully from thin-sections (Fig. 3). Lengths of individuals not included in the analysis were not distributed differently from the rest of the sample. Age 3 individuals were most common

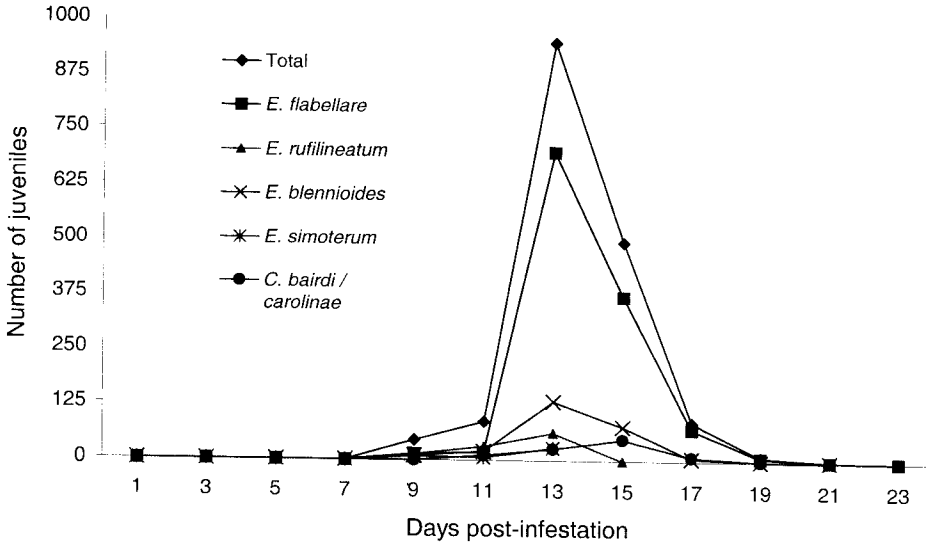

FIG. 1. Periods of transformation on various fish species infested with *Epioblasma florentina walkeri* glochidia at 22 to 23°C.

in the sample, constituting 28.6% of sectioned shells, followed by age 5 individuals (19.5%), and ages 4 and 6 (14.3% each). More males (47) than females (30) were included in the analysis, but their age and length distributions were similar. Males and females were analyzed separately because of the obvious sexual dimorphism in shell shape. Shell length was strongly correlated with age for males, but the relationship was weaker for females (Fig. 4). However, both sexes had similar growth rates. Only 5 individuals (all males) were observed with false annuli in thin-sections. False annuli also were visible on the exterior of both valves and could have been mistaken for external annuli.

Ages determined from thin-sections compared to those from external ring counts showed that, in general, ring counts tended to underestimate true ages, assuming thin-section counts reflected true ages. However, variability

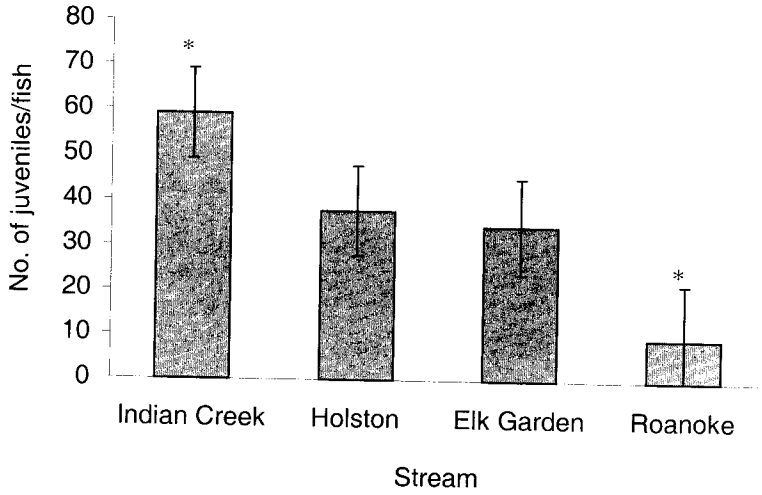

FIG. 2. Mean number (±1 SD) of juvenile *Epioblasma florentina walkeri* that transformed on fantail darters from various streams. The 2 streams with asterisks were significantly different from one another ($p = 0.024$).

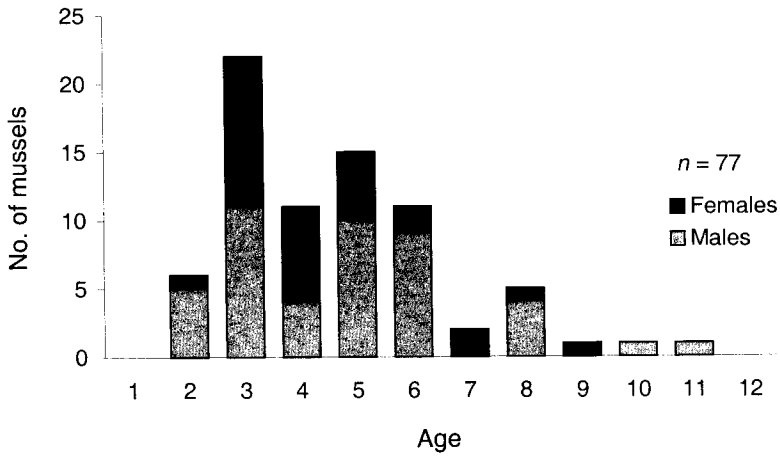

FIG. 3. Age (y) distribution of male and female *Epioblasma florentina walkeri* determined from thin-sections of shells.

between the 2 methods was small until after age 8, when external growth rings consistently underestimated true ages of adult mussels (Fig. 5).

Population estimate

We tagged 156 tan riffleshells between 1996 and 1999: 112 in 1996 and 44 from 1997 to 1999. Only 12 individuals were recaptured throughout the resampling effort from 1997 to 1999. Using Schumacher's modification of Schnabel's maximum likelihood population estimate, the tan riffleshell population size in Indian Creek was estimated to be 1078 adults (95% CI = 760–1853) for the 500-m section of creek. We calculated that the entire population in Indian Creek was ~2000 tan riffleshells by extrapolating the estimate for 54% of the population determined

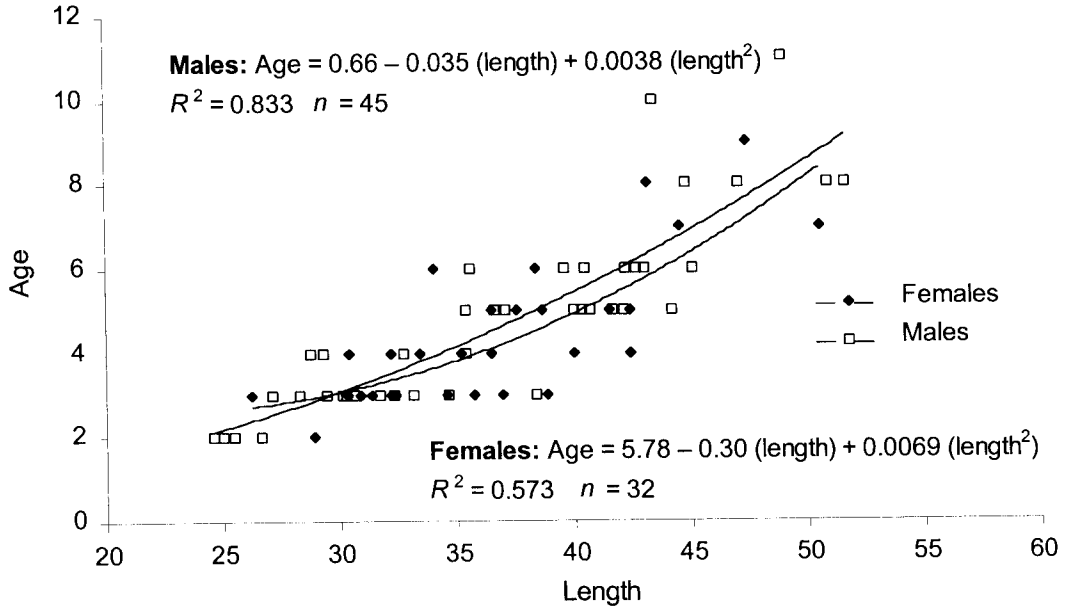

FIG. 4. Length (mm)-at-age (y) regressions for male (top line) and female (bottom line) *Epioblasma florentina walkeri* determined from thin-sections of shells.

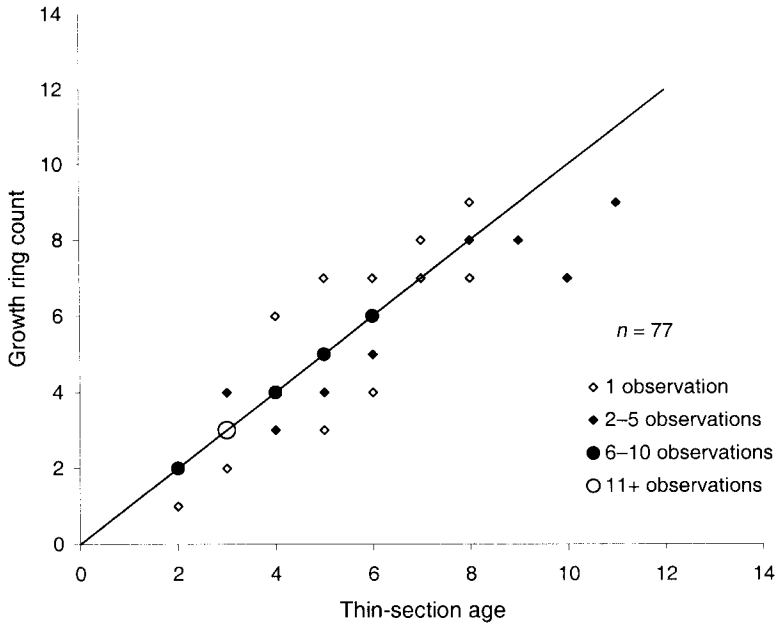

FIG. 5. Thin-section ages versus external growth ring counts for *Epioblasma florentina walkeri* collected in Indian Creek, Tazewell County, Virginia. Line indicates 1:1 ratio (same ages, y).

in the full survey to the remaining 46% in other reaches. We emphasize that only individuals at the surface could be collected during sampling, so this population estimate is a minimum estimate of actual population size.

Tagged tan riffleshells were 20 to 54 mm long, with most individuals measuring 38 to 44 mm (Fig. 6). Approximately equal numbers of males and females were tagged (70 and 86, respectively), but the 2 sexes had different size distributions. Significantly more females than males were 40 to 44 mm long, and almost twice as

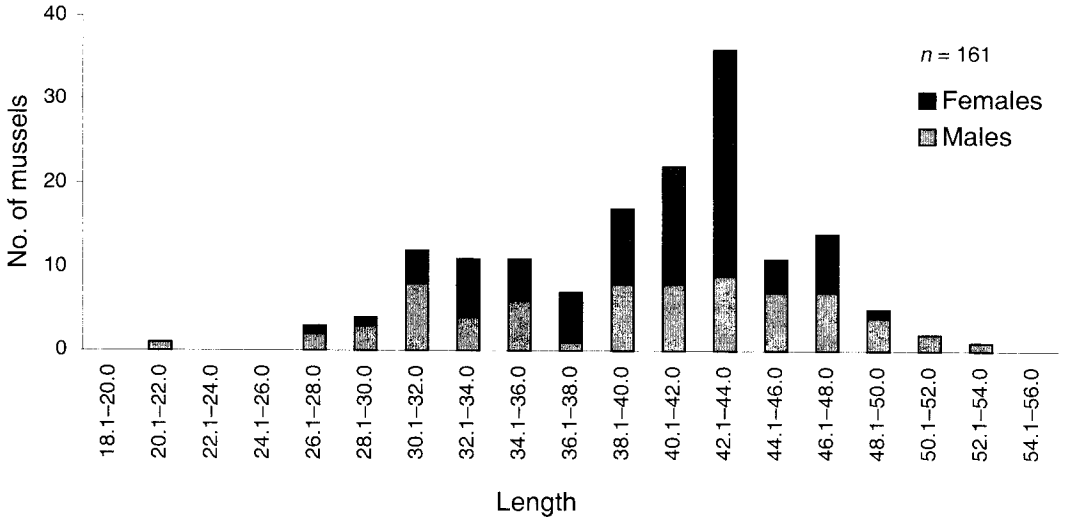

FIG. 6. Lengths (mm) of male and female *Epioblasma florentina walkeri* tagged in Indian Creek, Tazewell County, Virginia, 1996 to 1999.

many males as females were >44 mm long. The low number of small individuals does not indicate a lack of recruitment; rather, few small (<30 mm) individuals were captured because of the snorkeling technique used. Watson (1999) estimated reproductive size to be 36 mm; therefore, 26% of the tagged sample (excluding juveniles) was at or below reproductive size, indicating a fairly young and healthy population.

Discussion

Fish hosts

The tan riffleshell is a host-fish specialist, using only benthic, riffle-dwelling species. Although fish species from 6 different families were infested with glochidia, only 4 darter species and 2 sculpin species were suitable host fish. These results are similar to what Yeager and Saylor (1995) reported for the oystermussel (*E. capsaeformis*), Cumberland combshell (*E. brevidens*), and snuffbox (*E. triquetra*). Only 1 species of sculpin (Cottidae), 4 darters, and 2 logperches (Percidae) served as hosts for these species of mussels (Yeager and Saylor 1995). Therefore, it is likely that *E. f. walkeri* uses only specific darters and sculpins as fish hosts, which may explain the overlap in types of substratum and flow regimes inhabited by both the tan riffleshell and its host species. Sympatry in host fish and mussel microhabitat use and the richness and abundance of fish assemblages are perhaps more important than microhabitat availability in explaining the relative abundance of mussels in stream communities (Haag and Warren 1998).

Suitability of fantail darter populations

Freshwater mussel glochidia have a wide range of host specificity, with some mussel species being highly host-specific and others being more eurytopic (Zale and Neves 1982, Isom and Hudson 1984, Neves et al. 1985). However, it is not known whether mussels are so specific in their host requirements that they could be restricted to particular populations of host fish rather than simply to the fish species. Although only the rudimentary aspects of incompatibility between mussel glochidia and nonhost fish are known (Neves et al. 1985), the results of our experiments suggest that the host immune system influences compatibility, and that this relationship may be the result of coadaptation among the mussels and fish populations in the stream.

Various hypotheses may explain the phenomenon of host specificity. Most researchers agree that it is likely regulated by the fish's immune response, and several observations support that theory. First, fish previously exposed to glochidia are generally less suitable as hosts, which can be explained best by adaptive (acquired) immune responses (Reuling 1919, Bauer and Vogel 1987). Adaptive immunity is specific to an antigen and is marked by memory cells, which elicit an enhanced response on repeated encounters. Further, the blood components necessary for glochidial transformation are present in most fish because glochidia are able to transform in the absence of an immune response from the fish (Isom and Hudson 1984). However, there are species-level differences in fish immune responses to glochidia (Meyers and Millemann 1977), which implies that genetic differences among fish immune systems play a major role in fish host specificity (O'Connell and Neves 1999). Fish immune systems exhibit a high degree of differentiation among species (Rijkers 1981), which lends support to this hypothesis.

The Roanoke River and Indian Creek are in separate river drainages, so there are likely differences in the pathogens experienced by the respective populations of fishes. Fantail darters and tan riffleshells likely have resided together in Indian Creek for millennia. The darters have put selective pressure on the mussels to avoid rejection by the fish's immune system, and in turn, the glochidia likely have imposed a relatively small degree of selective pressure on the fantail darters to develop immunity to avoid overinfestation, which can result in death. However, because it appears that natural levels of glochidial infestation have little detrimental effect on fish, the fantail darter's immune system likely is influenced more by a variety of other parasite and disease organisms in the drainage. The genetic basis of the immune system would vary from drainage to drainage because disease prevalence varies from drainage to drainage.

Fantail darters in the South Fork Holston River and Elk Garden are not contiguous with the Indian Creek population, so genetic exchange can occur rarely, if ever, among these populations, resulting in distinct genetic differences. In

addition, the Roanoke River population of fantail darters is isolated physically from the other 3 populations, and genetic exchange is prevented. Differential selection and genetic drift would cause fish in the Roanoke River to exhibit different immunological characteristics than those in the other systems. Therefore, we presume that if Indian Creek fantail darters are the most suitable host fish, then populations of fantail darters in proximity would serve as less suitable hosts, and those completely isolated from the tan riffleshell would be poor hosts.

In summary, we hypothesize that variations in mussel antigens and host immunogenetic variation may mediate success of transformation of juvenile tan riffleshells among populations of fantail darters. It is likely that variation occurs in both parasite and host, in the antigens (proteins and carbohydrates) present on the surface of glochidia, and in the fish's immune recognition of these antigens. The inferences drawn in this discussion require further research, to include examining host specificity among populations of a mussel species, as well as immunogenetic studies of host fish and mussels. In addition, information on the minimum number of attached glochidia needed to sustain a population of mussels is requisite to understanding whether our results are biologically significant or simply an artifact of maximum infestation. If our hypothesis is correct, then mussels and their host fish are more highly coadapted than previously demonstrated.

Population demographics

Internal growth rings are much more reliable indicators of age than those on the exterior of the shell (MacDonald and Thomas 1980, Neves and Moyer 1988). Older tan riffleshells (7+ y) lay down growth rings close together, so thin-sectioning allowed for easy differentiation of those rings at the shell margin. In addition, the early growth rings that typically eroded from the periostracum were readily identifiable in thin-sections. Relatively few false annuli were detected in this study.

A comparison of thin-sections with externally aged individuals reinforces the conclusion that counts of external rings are an imperfect aging technique when used alone. Erosion of the periostracum, dark shell coloration, inability to differentiate false annuli, and closely laid rings in older individuals result in dubious age estimates. However, at streamside one frequently needs a quick method of aging, and it is often not feasible or legal to sacrifice individuals for thin-sectioning. An alternative is to collect shells, determine ages from thin-sections, and then create an age-length key for the species.

The greater variation in length-at-age of females than males may have arisen from the higher energetic cost of reproduction for females, especially since tan riffleshells brood their offspring over winter. This variation seems to be especially evident when one examines the size-frequency diagram; the mode of females was in the 42 to 44 mm size class (~7 y old). Females may grow to a certain size (i.e., 40–44 mm) and greatly decrease somatic growth for reproductive output. Conversely, males may continue to grow because less energy is required for sperm production. There were many more males than females >44 mm in length. However, males and females appear to have similar life spans, with the oldest female aged as 10 y and the oldest male as 11 y.

The maximum age for tan riffleshells is not known, although this species may live to ~15 y. If so, then tan riffleshells are among the shortest-lived riverine mussel species. Similarly, fecundity of this species is within the lower range of fecundity estimates determined for a suite of species (McMahon 1991). The combination of short lifespan and low fecundity has implications for the sustainability of the population in the face of predation and environmental stochasticity. This species has few cohorts, which would cause the loss of ≥1 cohorts to have a highly adverse effect on the population. Muskrats tend to prey on adults because they are elevated within the substratum during reproduction. The loss of many individuals of reproductive size therefore can have a large negative effect on the population over time.

Population estimate

Several assumptions are inherent in Schumacher's method of population estimation. First, there could be no loss of marks during the sampling period, which was seemingly met. Second, there must be random mixing of marked and unmarked individuals and all individuals must have an equal probability of capture. A violation of this assumption would cause the population

size to be underestimated. This assumption may have been violated because tan riffleshells were tagged and returned to their exact point of collection. However, mussels migrate vertically and horizontally in the substratum and surveyors spent equal time searching the entire stream segment during each visit, such that the increased likelihood of finding a previously marked individual was relatively small. Last, the population could not experience mortality or recruitment throughout the sampling time. This assumption was violated because recruitment and mortality were continuous in our 3-y sampling effort. However, it is reasonable to assume that this population was near equilibrium for the 3 y, so that mortality and recruitment were similar. The length distribution of the tagged mussels revealed that few mussels were at the upper limit of the size range, suggesting that mortality rate from old age was low. In addition, only 5 mussels <30 mm were captured after the initial sample, suggesting that the recruitment of juveniles into the adult population for this 2-y period was low as well. Therefore, it does not seem that this 3rd assumption was violated egregiously, and the population estimate is probably close to the actual population size. The Schumacher estimator remains preferable to other estimates that allow for dynamic population sizes because of the very low recapture rate. Other methods, such as Jolly–Seber, require a minimum recapture rate of 10% (Caughley 1977), which was not achieved in our study. The mortality of tagged individuals would cause the Schumacher estimate to be an overestimate of the true population size, whereas the recruitment of juveniles into the adult, sampled population would tend to underestimate the true population size. Therefore, the abundance estimate produced by the Schumacher method can be considered a valid approximation of adult population size.

The tan riffleshell population in Indian Creek of ~2000 adults is small but reproducing. However, it is the only known reproducing population of the species, so the potential threats loom large. Predators such as muskrats and raccoons are prevalent in the area, and although an intensive trapping effort has reduced their impact on the population, even low predation rates can be detrimental. In addition, deep coal mines in the upper portion of the drainage present the threat of eventual water-quality degradation. Protection of this population and restoration of other populations is of utmost priority, so that the species can recover to the point of being considered a candidate for downlisting from endangered to threatened status.

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