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Variation in fecundity and other reproductive traits in freshwater mussels

WENDELL R. HAAG AND J. LEANN STATON
USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, Oxford, MS, U.S.A.

SUMMARY

1. Life histories of the highly diverse and endangered North American freshwater mussel fauna are poorly known. We investigated reproductive traits of eight riverine mussel species in Alabama and Mississippi, U.S.A.: *Amblema plicata, Elliptio arca, Fusconaia cerina, Lampsilis ornata, Obliquaria reflexa, Pleurobema decisum, Quadrula asperata* and *Q. pustulosa*, and compare our results with existing life history information for other species.
2. These eight species had reproductive traits characteristic of large, outcrossing populations: hermaphrodites were rare, we found no evidence of protandry, and sex ratios were even or slightly male-biased.
3. Age at sexual maturity varied among species, ranging from <1 to 2 years for *L. ornata* to 3–9 years for *Q. asperata*. In all species, most mature females participated in reproduction and fertilisation success was high.
5. Fecundity was related positively to both length and age, but length was the best predictor. In six species, fecundity increased exponentially with increasing size; in two species the rate of increase in fecundity declined in larger animals. In four species, fecundity declined in older animals. These latter results indicate weak reproductive senescence; however, in all species, older individuals continued to produce large numbers of offspring. Mean annual fecundity differed widely among species ranging from 9647 to 325 709. Within-species differences in fecundity were found among rivers and among populations within a river.
6. The wide variation in reproductive traits among species indicates the existence of widely divergent life history strategies in freshwater mussels.

Keywords: fecundity, life history, mussels, reproduction, Unionidae

Introduction

Reproductive traits such as fecundity, size at first reproduction and sex ratio are important characteristics of the life history of any organism. In species for which these traits are known, such data form the basis of our understanding of life history evolution and population dynamics (van Groenendael, de Kroon & Caswell, 1988; Stearns, 1992) and inform science-based management and conservation strategies (Crouse, Crowder & Caswell, 1987; Heppell, Crowder & Menzel, 1999). The reproductive biology of many common aquatic organisms is well known, but little quantitative information exists on the reproductive traits of North American freshwater mussels.

Freshwater mussels (family Unionidae) are a diverse and conspicuous element of the benthic fauna in fresh waters of eastern North America. Mussels have a complex life cycle in which modified larvae (glochidia) are obligate parasites on the gills or fins of fishes. Most species are primarily dioecious, although

Correspondence: Wendell R. Haag, USDA Forest Service, Southern Research Station, Center for Bottomland Hardwoods Research, 1000 Front Street, Oxford, MS 38655, U.S.A.
E-mail: whaag@fs.fed.us
Present address: J. Leann Staton, Mississippi Museum of Natural Science, 2148 Riverside Drive, Jackson, MS 39202, U.S.A.

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hermaphrodites are regularly encountered (van der Schalie, 1970; Heard, 1979). Eggs are deposited in the interlamellar spaces (water tubes) of the gills of the female mussel where they are fertilised by sperm filtered from the water column. Glochidia are brooded in the gills until mature then released through the siphons, after which they can survive only a few days before they must find a suitable fish host (Zimmerman & Neves, 2002). The degree of host specificity varies among species from specialists, able to successfully parasitise only one or a few closely related fish species, to generalists which can complete development on a taxonomically wide range of fish species (Haag & Warren, 2003).

Because of a wide variety of anthropogenic modifications to aquatic habitats, freshwater mussels are today the most critically endangered group of organisms in North America. Of 297 recognised taxa, at least 21 (7%) have become extinct in the last 50 years and 194 (65%) are considered imperilled (Williams *et al.*, 1993). In addition, a number of species are exploited commercially for the cultured pearl industry (Anthony & Downing, 2001). Management and conservation efforts for these animals are hampered by a lack of basic life history information for most species, and determination of life history traits is a key research need for their recovery (National Native Mussel Conservation Committee, 1998).

We studied reproductive traits of eight mussel species found in medium-sized to large streams in the southeastern United States: *Amblema plicata* (Say), *Elliptio arca* (Conrad), *Fusconaia cerina* (Conrad), *Lampsilis ornata* (Conrad), *Obliquaria reflexa* (Rafinesque), *Pleurobema decisum* (I. Lea), *Quadrula asperata* (I. Lea) and *Q. pustulosa* (I. Lea). We asked the following questions: (1) What is the sex ratio in these populations? (2) Do mature males and females differ in size? (3) What is the size and age at reproductive maturity for each species? (4) What percentage of females reproduces? (5) What are the patterns of brooding and egg fertilisation within the gills? (6) How does fecundity vary with length and age and (7) How does fecundity differ among species and among populations of the same species?

Methods

We examined reproductive traits of eight freshwater mussel species collected from one site in the Buttahatchee River, Monroe Co., MS, U.S.A. (33°48′N, 88°18′W), one site in the Little Tallahatchie River, Panola Co., MS, U.S.A. (34°23′N, 89° 47′W), and four sites in the Sipsey River, Greene/Pickens Co., AL, U.S.A.(site 1: 33°05′N, 87°57′W; site 2: 33°16′N, 87°45′W; site 3: 33°02′N, 88°06′W; site 4: 33°07′N, 87°54′W), for the 2000 reproductive season (Table 1). In order to maximise the likelihood that we sampled gravid females with their full reproductive complement, we collected all individuals of a particular species during the peak period of brooding for that species, but prior to the period of glochidial release as determined by Haag & Warren (2003). Times of collections for each species were May to June, 2000 (*A. plicata*, *E. arca*, *F. cerina*, *O. reflexa*, *P. decisum*, *Q. asperata* and *Q. pustulosa*) and October 1999 to May 2000 (*L. ornata*) (Haag & Warren, 2003).

We collected animals by snorkeling and using SCUBA. We located animals by looking for siphons and by disturbing the substratum with our hands to dislodge small or buried individuals. For each species, we attempted to collect a series of individuals representing the full range of sizes present at each site. Upon collection, we preserved specimens of most species immediately in 50% ethanol to reduce the release of glochidia associated with handling stress. At the beginning of the study, we placed each specimen in an individual polyethylene bag immediately after removal from the substratum to determine the number of glochidia that were released during collection and preservation. Few (<100) to no glochidia were released in the bags and, for the remainder of the study, we preserved all specimens from a single site in a common container. *Pleurobema decisum* is protected under the U.S. Endangered Species Act and no individuals of this species were killed during the study.

In the laboratory, we dissected each animal from the shell, determined whether it was fully, partially or not gravid, and measured shell length (0.1 mm) with dial calipers. We considered individuals fully gravid if all gill water tubes within the portion of the gill typically used for brooding by that species were fully charged with eggs, embryos or glochidia. We considered individuals partially gravid if portions of the brooding area were empty and not charged with offspring. We considered individuals not gravid if no offspring were found in the gills. We measured shell length as the longest anterior-posterior dimension. We determined reproductive maturity of each individual

Table 1 Summary of lengths and sex ratios in eight mussel species. An asterisk by the site name denotes that sex ratios were significantly different from 1 : 1 (*$P < 0.01$, **$P < 0.0001$). Length range of female *Lampsilis ornata* is based on six animals collected at site 1, plus an additional 23 animals collected at a nearby site to augment fecundity estimates; most males of this species were not measured

Species site	Maximum length (mm) of immature individuals	Length of mature individuals [range and mean ± SE (mm)]		Number examined		
		Female (F)	Male (M)	Immature	F	M
Amblema plicata						
Little Tallahatchie River	44.0	32.2–99.6 (75.6 ± 3.8)	37.8–113.5 (77.5 ± 5.3)	5	22	19
Sipsey River	–	52.5–115.6 (85.4 ± 4.5)	36–111.3 (88.0 ± 5.9)	0	17	13
Elliptio arca						
Sipsey River, sites 1 and 3	23.3	34.6–88.3 (68.1 ± 1.8)	36.8–78.5 (64.9 ± 1.4)	8	42	39
Fusconaia cerina						
Sipsey River, sites 1,3,4	26.8	30.6–58.0 (46.9 ± 1.2)	31.2–57.9 (43.5 ± 1.3)	2	29	32
Lampsilis ornata						
Sipsey River, site 1**	39.8	38.5–95.4 (73.6 ± 2.3)	Minimum = 41.6	8	6	33
Obliquaria reflexa						
Little Tallahatchie River	–	22.4–77.8 (51.3 ± 3.5)	29.2–79.5 (62.5 ± 2.7)	0	21	20
Sipsey River	–	20.0–48.4 (40.2 ± 2.1)	–	0	17	–
Quadrula asperata						
Buttahatchee River	31.8	24.6–52.2 (40.6 ± 1.7)	29.1–53.8 (42.6 ± 1.3)	5	22	24
Sipsey River*†, site 1	27.7	26.9–53.0 (42.1 ± 1.5)	27.5–52.7 (41.4 ± 1.3)	7	25	35
Sipsey River, site 2	29.7	30.0–51.0 (41.3 ± 1.6)	23.7–48.1 (40.1 ± 1.8)	3	15	15
Sipsey River, site 3	32.8	29.5–45.6 (38.3 ± 1.3)	29.9–53.2 (39.9 ± 1.0)	4	17	34
Sipsey River, site 4	18.2	31.7–48.8 (38.6 ± 2.8)	25.1–50.3 (37.1 ± 1.8)	5	5	19
Quadrula pustulosa						
Little Tallahatchie River*	40.2	25.0–74.8 (53.6 ± 2.2)	25.7–79.8 (53.8 ± 1.8)	17	33	58

†For all four Sipsey River populations, pooled. Sex ratios did not differ significantly among these populations.

and the sex of mature individuals by examining two plugs (approximately 2 × 2 mm) of tissue cut from the dorsal region of the visceral mass under a dissecting microscope (Bauer, 1987); these plugs always contained gonadal tissue. We recognised sexually mature individuals by the presence of large gonads organised in distinct lobes; we identified females by the presence of oocytes and males by the presence of creamy-white, fine-textured testes. Immature individuals had clear, granular, undifferentiated gonadal tissue and we did not determine sex for these specimens. We estimated the sex ratio of *P. decisum* by determining, in the field, the ratio of gravid to non-gravid individuals for all those greater than or equal to the size of the smallest gravid individual observed.

We defined fecundity as the full complement of developing eggs produced by a female during a single reproductive event. We assumed that all species in this study produce one clutch of eggs per year (see Discussion); under this assumption, our fecundity values also represent the number of offspring produced per female per year. We estimated the fecundity of gravid females by counting the number of developing eggs in all charged gills in each individual. For all species, except *P. decisum*, we dissected gravid gills whole from each specimen, placed them in a Petri dish, and removed eggs from the gills by tearing gill walls with a fine probe and flushing with water. Individual eggs of *A. plicata*, *E. arca* and *L. ornata* were not bound to each other within the gills, and were flushed singly from the gills in this manner. Individual eggs of *F. cerina*, *O. reflexa* and *Quadrula* spp. were bound tightly to each other within the gills and were flushed from the gills in clumps. We dissolved clumps in 5% NaOH until individual eggs were free, but before eggs themselves were digested (about 1–5 min). We diluted the entire gill contents of a single individual to a total volume of 500 to 900 mL, and total fecundity estimates were extrapolated from the mean number of eggs in two replicate 1 to 2-mL aliquots. Precision of fecundity estimates based on two aliquots (standard error/mean, Andrew & Mapstone, 1987) averaged 0.100 (range = 0.000–0.526). We classified the developmental stage of eggs in each individual as early stage eggs,

embryos, immature glochidia, or mature glochidia (Heard, 1975; Jones, Simpson & Humphrey, 1986). To quantify the percentage of eggs that developed into mature glochidia, we counted separately the number of developed and undeveloped eggs in each aliquot for all individuals that carried immature or mature glochidia. Because it was difficult or impossible to distinguish early-stage developing eggs or embryos from undeveloped eggs, we did not quantify the percentage of developing eggs for individuals that carried earlier developmental stages. In these individuals, we adjusted total fecundity by subtracting the mean proportion of undeveloped eggs for the species, as obtained from individuals carrying later developmental stages. In a small number of individuals, we found glochidia in the suprabranchial chamber, or found eggs only in the dorsal section of the gills and abundant, mature oocytes in the gonads. We assumed that these individuals were collected during glochidial release or deposition of eggs into the gills, respectively, and did not make fecundity estimates for these individuals.

We made fecundity estimates of *P. decisum* by bringing gravid mussels into the laboratory and placing them into individual, aerated beakers at room temperature (21–25 °C). All individuals released the entire gill contents into the beakers within 24–48 h and were returned to their site of origin within 7 days of collection.

We determined the age of *E. arca, F. cerina, L. ornata, Q. asperata* and *Q. pustulosa* by cutting thin-sections from shells and counting internal annuli under a dissecting microscope. We prepared and interpreted thin-sections following Neves & Moyer (1988). We validated annual formation of internal growth rings using a mark–recapture experiment (W.R. Haag, unpublished data). We collected animals of each species in June 2000, filed a shallow notch in the posterior-ventral margin of the shell, and returned animals to the substratum, then collected all animals 1 year later. Shell growth occurred in all specimens, and individuals deposited a single additional annulus beyond the notch at the previous shell margin. In addition, growth rates of these specimens were similar to growth trajectories constructed from length-at-age relationships based on annulus data (W.R. Haag, unpublished data).

We tested for departure from a 1 : 1 sex ratio using goodness of fit tests. We pooled populations of some species to increase sample size (Table 1). We examined the possible occurrence of protandry in *Q. asperata* and *Q. pustulosa* by assigning individuals into one of three length classes, constructed by dividing the total observed range of length into thirds for each species (Table 2) and testing for independence of sex ratio and length class using *G*-tests. The distribution of sexes among size classes was not examined in other species because of low sample sizes of small individuals. We fitted regression models to length–fecundity and age–fecundity data by examining coefficients of determination and residuals, and by testing Type I sums of squares of polynomial model parameters using $P < 0.20$ (Little, Freund & Spector, 1991). In all other statistical tests, including overall significance of polynomial models, we used a significance level of $P < 0.05$. We transformed variables ($\log_{10}$ or square-root) where necessary to satisfy assumptions of normality and homogeneity of variances. We tested for differences in fecundity of a species among sites using analysis of covariance, with length as the covariate. For length– and age–fecundity plots, we pooled populations that did not differ in fecundity. We plotted data for all length– and age–fecundity relationships on linear scales; for relationships best described using transformed variables, regression lines were created using back-transformed y-values predicted for each observed x-value by the regression equation for that species. We used regression equations to calculate mean fecundity for each species by generating a predicted value of fecundity corresponding to the grand mean of length of each species; within species, we made separate fecundity estimates only for populations whose regression equations or size distributions differed significantly.

Table 2 Distribution of sexes among size classes in two species of freshwater mussels. Numbers of *Q. asperata* are for all four study populations in the Sipsey River, combined. There was no association between size class and sex ratio for either species (*Q. asperata* : 2 d.f., $G = 0.400$; *Q. pustulosa* : 2 d.f., $G = 0.551$)

Species	Size class (mm)	*n*	Percentage of female	Percentage of male
Quadrula asperata	23.7–33.7	36	33	67
	33.8–43.8	78	37	63
	>43.9	50	40	60
Q. pustulosa	25.0–43.3	19	37	63
	43.4–61.5	43	39	61
	>61.5	29	31	69

Results

Sex ratios

The sex ratio of four species did not differ from 1 : 1 (Table 1). Sex ratios did not differ significantly among four populations of *Q. asperata* in the Sipsey River ($G = 6.13$, 3 d.f.), and the sex ratio of the pooled populations was male-biased. The sex ratio of *Q. asperata* in the Buttahatchee River did not differ from 1 : 1. Populations of *L. ornata* and *Q. pustulosa* were male-biased. We found only two individuals that appeared to have both male and female gonadal tissue (*L. ornata*, 81.6 mm, and *Q. pustulosa*, 71.0 mm). The ratio of gravid to non-gravid *P. decisum* >26.3 mm (see below) was not significantly different from 1 : 1 (36 gravid : 40 not gravid, $G = 0.211$, 1 d.f.), suggesting an even sex ratio. We found no evidence of protrandry in *Q. asperata* from the Sipsey River or in *Q. pustulosa*; sex ratios did not differ among size classes in these species (Table 2).

In five species, gonads of a low percentage of individuals were completely replaced by parasitic trematode flatworms, and the sex of these mussels could not be determined. Sterilisation by trematodes occurred only in large individuals of *E. arca* (3.3% parasitised; ≥81.7 mm, 95th percentile for length) and *Q. asperata* (1.2%; ≥49.5 mm, 89th percentile). Incidence of trematodes was not restricted to large individuals of *A. plicata* (1.4% parasitised; ≥84 mm, 54th percentile), *F. cerina* (6.6%; ≥45.3 mm, 47th percentile), and *Q. pustulosa* (2.7%; ≥43.7 mm, 34th percentile). *Lampsilis ornata* and *O. reflexa* were not parasitised by trematodes.

The mean size of females and males did not differ in any species or population except one (Table 1). Male *O. reflexa* were significantly larger than females in the Little Tallahatchie River (*t*-test: $t = 2.5$, $P < 0.05$). Male *O. reflexa* were not collected or measured in the Sipsey River.

Size and age at maturity

Size at maturity varied among individuals within most species, but was similar for males and females (Table 1). The maximum length of immature individuals and the minimum length of mature individuals overlapped widely in *A. plicata*, *Q. asperata* and *Q. pustulosa* (Table 1); within ranges of overlap, 60, 83 and 73% of individuals were mature, respectively. Size ranges of immature and mature individuals of *E. arca*, *F. cerina* and *L. ornata* did not overlap widely, although we were unable to examine a wide range of small specimens of these species. We did not find immature specimens of *A. plicata* in the Sipsey River or *O. reflexa* at either site. We examined a wide size range of *P. decisum* during the peak brooding season (15.0–59.2 mm, $n = 85$); the smallest gravid individual found was 26.3 mm. Size at sexual maturity was similar among five populations of *Q. asperata* (Table 1).

Age at sexual maturity differed among four species (Fig. 1). *Elliptio arca* and *L. ornata* matured at an early age; in both species, 100% of individuals 2-years old or greater were mature. In *E. arca*, sexual maturity occurred abruptly between ages 1 and 2. In *L. ornata*, individuals attained sexual maturity as early as age

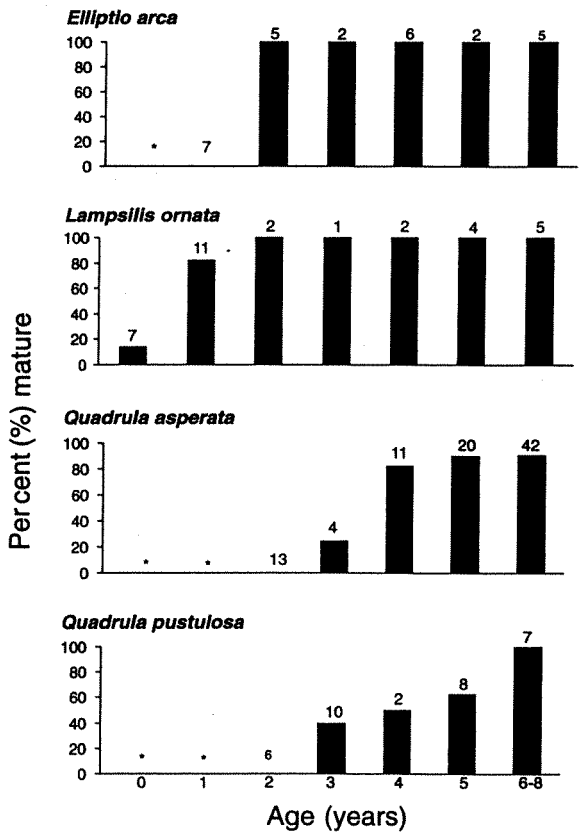

Fig. 1 Age at maturity for four species of freshwater mussels. Numbers above bars indicate sample size for each age class. An asterisk denotes that no specimens of that age class were examined.

0+, followed by a sharp increase in the percentage of maturity in 1-year old individuals. *Quadrula asperata* and *Q. pustulosa* matured later and over a protracted period. In both species, a low percentage of age three individuals were mature, but this percentage increased gradually and 100% maturity was not reached until ages 9 (*Q. asperata*) and 7 (*Q. pustulosa*).

Patterns of gravidity and brooding

A high percentage of mature females were gravid in *E. arca* (93%), *F. cerina* (100%), *L. ornata* (94%), *O. reflexa* (97%), *Q. asperata* (92%) and *Q. pustulosa* (94%). Non-gravid females of these species were all small individuals except for *Q. asperata* and *Q. pustulosa*, in which non-gravid individuals were distributed evenly across size classes. For *P. decisum*, 36 of 76 individuals (47%) ≥26.3 mm were gravid. Assuming a 1 : 1 sex ratio, 94% of females of this species were gravid. For *A. plicata*, 71 and 77% of mature females were gravid in the Sipsey and Little Tallahatchie rivers, respectively. In the Sipsey River, non-gravid *A. plicata* were either small or large individuals. In the Little Tallahatchie River non-gravid *A. plicata* were small to intermediate-size individuals.

The portion of the female gill used for brooding young differed among species but was consistent within species. *Amblema plicata*, *F. cerina*, *Q. asperata* and *Q. pustulosa* brooded young along the entire length of all four gills. In *F. cerina*, *Q. asperata* and *Q. pustulosa*, individuals ranged from having all four gills fully charged to having only scattered charged water tubes and water tubes that were often charged along less than their full length. Gills of *A. plicata* were completely charged in all individuals. *Elliptio arca*, *L. ornata*, *P. decisum* and *O. reflexa* brooded young only in the outer pair of gills. *Elliptio arca* and *P. decisum* brooded young along the entire length of the outer gills, which were fully charged in all individuals. *Lampsilis ornata* brooded young in 17–27 water tubes located at the posterior end of each of the outer gills. *Obliquaria reflexa* brooded young in two to nine modified water tubes located in approximately the middle of each of the outer gills.

In all species, the developmental stage of maturing eggs brooded by a single female varied among, but never within, individuals. We found no individuals of any species brooding more than one developmental stage. However, the percentage of undeveloped eggs varied among species (Table 3). Undeveloped eggs represented a low percentage of the total gill contents in *A. plicata*, *E. arca*, *L. ornata*, *O. reflexa*, *Q. asperata* and *Q. pustulosa*. Exceptions to this pattern were noted only for *E. arca* in which two individuals were found with 47.7 and 85.2% undeveloped eggs. In contrast, the occurrence of undeveloped eggs was consistently high in *F. cerina* and *P. decisum*, with the exception of one individual of *F. cerina* in which 100% of eggs were developing.

Table 3 Percentage of undeveloped eggs in eight species of freshwater mussels. Results are pooled across all sites for each species

Species	Mean percentage (range) of undeveloped eggs per female	*n*
Amblema plicata	1.3 (1.0–10.1)	17
Elliptio arca	1.5 (0.0–10.9)*	14
Fusconaia cerina	48.0 (18.0–68.1)†	13
Lampsilis ornata	1.5 (0.3–6.1)	18
Obliquaria reflexa	0.6 (0.0–4.9)	15
Pleurobema decisum	47.0 (30.3–66.7)	10
Quadrula asperata	2.8 (0.0–10.0)	36
Quadrula pustulosa	3.4 (0.0–8.6)	17

*Mean and range excludes two outliers: 47.7 and 85.2%.
†Mean and range excludes one outlier: 0.0%.

Glochidia of *F. cerina*, *P. decisum* and *O. reflexa* were aggregated within the gills into a number of discrete packages (conglutinates), each containing the entire contents of a single gill water tube. In *F. cerina* and *P. decisum*, conglutinate shape was maintained by strong cohesion between eggs. In *O. reflexa*, conglutinate shape was maintained by a sausage-like membrane that contained the glochidia. The number of conglutinates produced varied among species but varied little within species (*F. cerina* : mean number of conglutinates per individual (±SE) = 134.9 ± 10.0; *P. decisum* : 83.6 ± 5.1; *O. reflexa* : 8.1 ± 0.6). Glochidia of the remaining five species were not aggregated into conglutinates.

Fecundity

Fecundity was related positively to both length and age in all species, but length explained an average of 31% more variation in fecundity than age (Fig. 2). In most species, fecundity was related to length by a power or square-root function in which the rate of increase in fecundity continued to increase along the

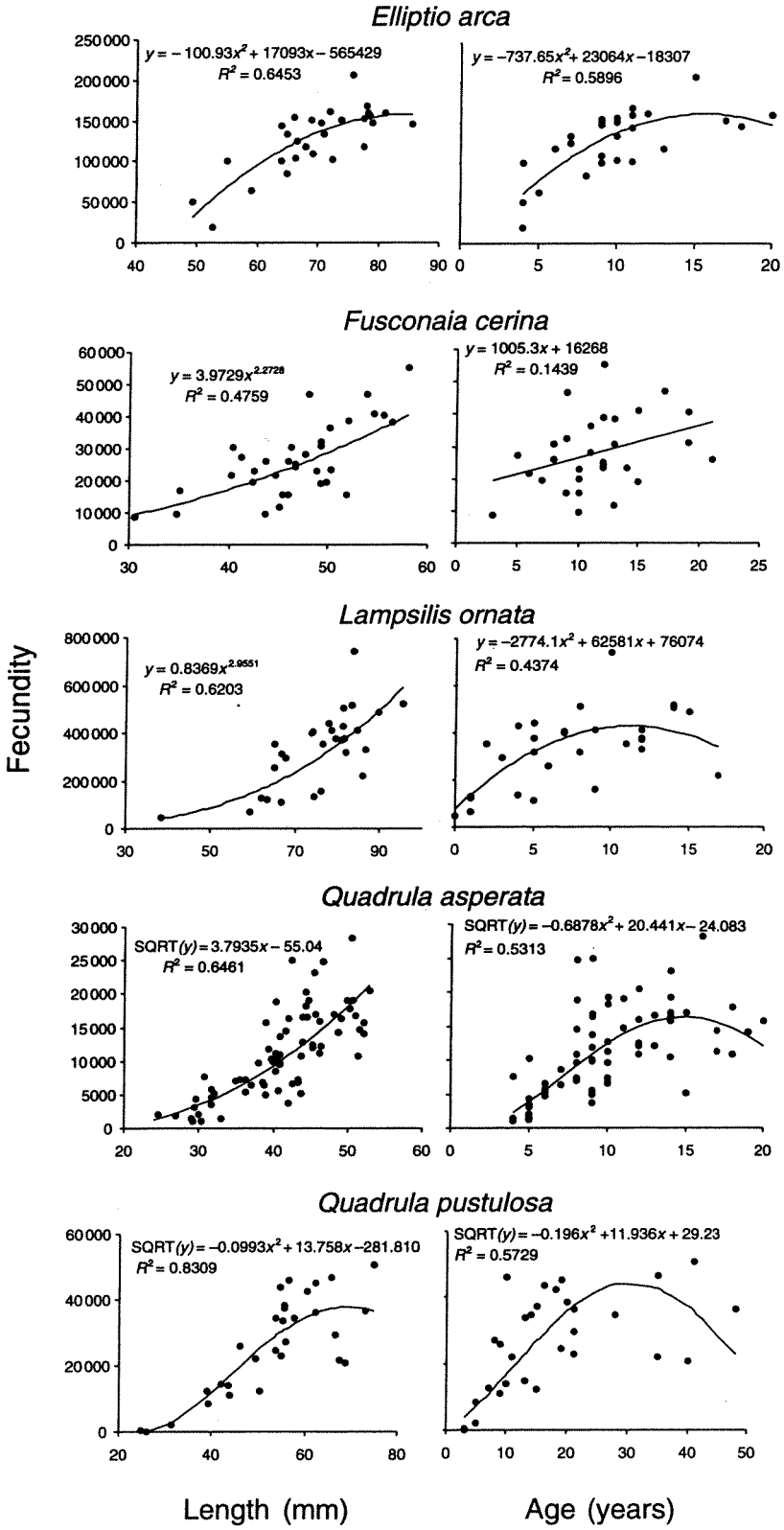

Fig. 2 Length– and age–fecundity relationships for five species of freshwater mussels. For relationships based on transformed data, regression lines were created using back-transformed y-values predicted from each observed x-value using the regression equation for that species. All relationships are significant at $P < 0.0001$, except for *F. cerina*, age, for which $P < 0.05$.

entire observed range of length (Figs 2 and 3). In *E. arca* and *Q. pustulosa*, the length–fecundity relationship was best described by a second-order polynomial that reflected a decline in the rate of increase of fecundity in large animals.

In four out of five species, fecundity was related to age by a second-order polynomial that reflected a decline in fecundity in older animals (Fig. 2). The relationship of fecundity to age in *F. cerina* was best described by a linear relationship. A second-order polynomial model explained more of the variance in fecundity for *F. cerina* ($R^2 = 0.2404$), but was not significant ($P < 0.08$). Older individuals of all species were uncommon and only one gravid female >25-years old was found for each of three species (*E. arca* : 32 years, fecundity = 151 500, *F. cerina* : 34 years, 15 750, *Q. asperata* : 34 years, 5375). Fecundity of these individuals was near or slightly less than the average for each respective species (Table 4). These animals were excluded from analysis because they strongly influenced results because of the paucity of observations for intervening ages. Inclusion of these observations strengthened the polynomial relationship between age and fecundity for *F. cerina* but had little effect on model fit or parameters for *E. arca* and *Q. asperata*. Six *Q. pustulosa* >25-years old were found and were included in the analysis. No *L. ornata* >17-years old were found.

Fecundity varied widely among species (Table 4). Length-specific fecundity and slope of length–fecundity relationships did not differ among populations in four species (*E. arca*, two sites; *F. cerina*, three sites; *O. reflexa*, two sites; *Q. asperata*, five sites). Although length-specific fecundity did not differ between two populations of *O. reflexa*, mean fecundity was higher in the Little Tallahatchie River because of higher mean and maximum size of animals at this site. Length-specific fecundity differed between Little Tallahatchie and Sipsey river populations of *A. plicata* (d.f. = 1, $F = 8.92$, $P < 0.01$), and between two Sipsey River populations of *P. decisum* (d.f. = 1, $F = 7.89$, $P < 0.01$); slopes of length–fecundity relationships did not differ between sites for either species (Fig. 3).

Table 4 Length-standardised fecundity for eight species of freshwater mussels. Mean fecundities are predicted values calculated from length–fecundity regressions (Figs 2 and 3) for each species with mean length of gravid females as the independent variable. For relationships based on transformed variables, means were back-transformed on to a linear scale. Within a species, separate fecundity values are given for populations with significantly different length–fecundity relationships or significantly different lengths

Species	Mean length (mm)	Mean fecundity (observed range)
Amblema plicata		
Little Tallahatchie River	78.5	325 709 (76 050–681 300)
Sipsey River	86.9	229 738 (40 5000–465 300)
Elliptio arca	69.9	136 227 (19 300–206 875)
Fusconaia cerina	46.0	23 890 (8 750–55 422)
Lampsilis ornata	74.2	281 776 (48 625–739 600)
Obliquaria reflexa		
Little Tallahatchie River	48.8	40 975 (447–135 750)
Sipsey River	40.8	25 767 (3250–82 500)
Pleurobema decisum		
Sipsey River, site 1	46.7	29 433 (6721–73 581)
Sipsey River, site 3	47.9	40 887 (27 860–69 553)
Quadrula asperata	40.4	9647 (250–28 250)
Quadrula pustulosa	53.0	28 369 (49–50 625)

Discussion

These eight mussel species exhibited reproductive traits characteristic of large, stable, outcrossing populations. Small, isolated populations of mussels or other organisms, or populations in stressful environments are often characterised by a high incidence of hermaphrodites or by highly skewed sex ratios (Heard, 1975). For the mussel populations we studied, dioecy was the rule, the incidence of hermaphroditism was low, and we found no evidence of protandry. Hermaphrodites occur in many freshwater mussel species but, in most populations, constitute a small percentage (usually $\ll$10%) of total individuals (Downing *et al.*, 1989; Haggerty *et al.*, 1995; Garner, Haggerty & Modlin, 1999). Because we did not quantify gonadal allocation, we may have missed hermaphrodites having a small proportion of gonadal tissue of the opposite sex. However, most hermaphrodites in unionids are predominantly of one sex with only a small amount of tissue of the opposite sex (van der Schalie, 1970), and animals with <50% female tissue have little probability of producing eggs (Downing *et al.*, 1989). Thus, we can conclude that the populations examined in this study were at least functionally dioecious.

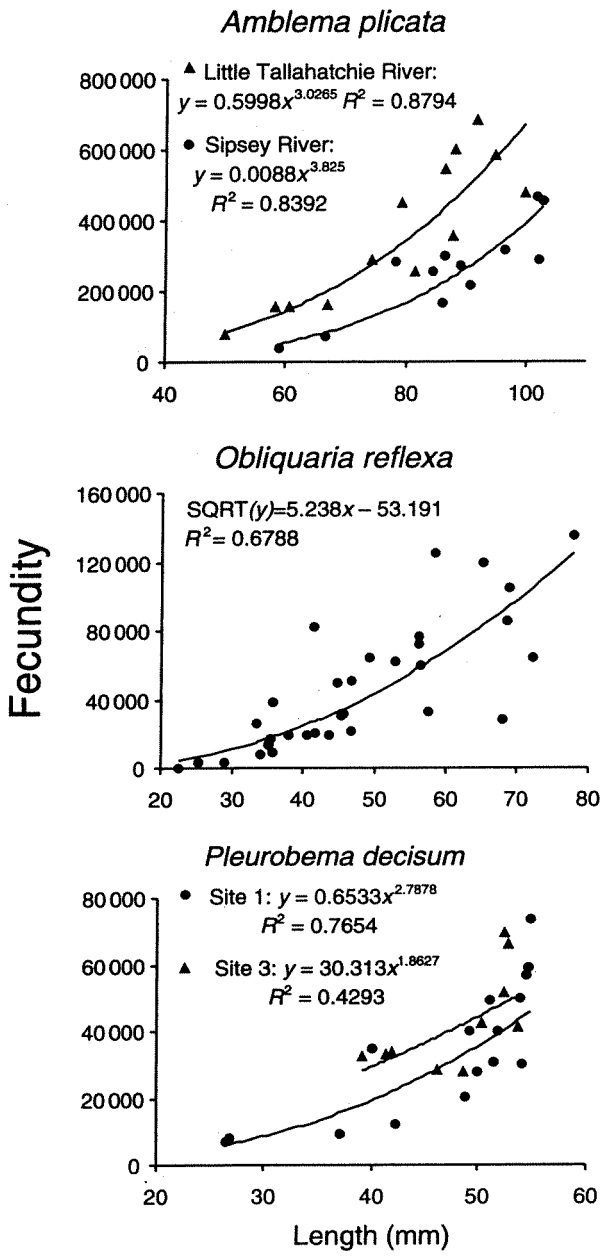

Fig. 3 Length–fecundity relationships for three species of freshwater mussels. Within a species, populations with significantly different length–fecundity relationships are plotted separately. For relationships based on transformed data, regression lines were created using back-transformed *y*-values predicted from each observed *x*-value, using the regression equation for that species. All relationships are significant at $P < 0.0001$, except for *P. decisum*, site 3, for which $P < 0.05$.

Female participation in reproduction was high in all species. In seven species, nearly all mature females in all size classes were gravid during the peak of the brooding season, with the exception of small females that only recently had become sexually mature. The majority of *A. plicata* were gravid, but female participation in reproduction was lower than in the other species. Year to year variation in the percentage of females that reproduce is unknown for these and most other species. In *Margaritifera margaritifera*, an average of 64% of females reproduces in a given year with the remainder of females in a resting stage (Bauer, 1987). For North American unionids, a number of studies reported high female participation in reproduction (>85% of females reproducing in a given year) (Yokely, 1972; Trdan, 1981; Jansen & Hanson, 1991; Bruenderman & Neves, 1993; Woody & Holland-Bartels, 1993; Haggerty & Garner, 2000), whereas in others, the percentage of gravid females during the peak brooding period ranged from 64 to 75% (Yeager & Neves, 1986; Haggerty *et al.*, 1995; Garner *et al.*, 1999). It is unclear whether the low numbers found in these latter studies are indicative of lower female participation in reproduction, or simply a result of a protracted brooding period with low brooding synchrony among individuals. During the peak brooding period, we found few resting or otherwise non-gravid females of any species except *A. plicata*. These data, along with the majority of observations for other North American unionid species, suggest that most females in a mussel population reproduce in most years.

The area of the gills used for brooding was consistent with previous observations for each species or for congeners (Heard & Guckert, 1970). In species that used only a portion of the gills for brooding, this area was always fully charged with eggs and greatly distended. Gills of three species that used the entire gill area for brooding (*F. cerina*, *Q. asperata*, *Q. pustulosa*) tended to be less than fully charged. This phenomenon is known in at least two other species (*Cyclonaias tuberculata* (Rafinesque), Haggerty *et al.*, 1995; *Fusconaia ebena* (I. Lea), personal observation) and was considered to be evidence of a poor reproductive year (Haggerty *et al.*, 1995). We have observed partially charged gills in our study species consistently in several years. Furthermore, *F. cerina*, *Q. asperata* and *Q. pustulosa* were among the most abundant species at the study sites and strong recruitment occurred for these species in 2000 and 2001 (Haag, 2002; W.R. Haag & M.L. Warren, unpublished data). These observations, along with

the prevalence of partially charged gills among years, species and geographic locations, strongly suggests that this is a regular characteristic of brooding in these species and not indicative of poor reproductive success.

Because the fertilisation of eggs in mussels apparently depends on chance encounters of females with water-borne sperm, fertilisation success may be highly sensitive to variations in mussel density and flow conditions (Downing *et al.*, 1993). Fertilisation success was high in all species in our study. Within each species, there was little variation among individuals in the percentage of developing eggs, and the percentage was high in all but two species. A high percentage of eggs of *F. cerina* and *P. decisum* were undeveloped or unfertilised, although this observation does not indicate poor fertilisation. Both of these species release glochidia in discrete packages (conglutinates) that mimic prey items of host fishes, and the structural matrix of these conglutinates is formed by cohesion between unfertilised eggs (Haag & Warren, 2003; C. Barnhart, Southwest Missouri State University, Springfield, MO, U.S.A., personal communication). Thus, the prevalence of undeveloped eggs is characteristic of these and other species that produce this type of conglutinate (e.g. Layzer *et al.*, 2003). In contrast, the percentage of developing eggs is high in species that release glochidia in membrane-bound conglutinates (e.g. *O. reflexa, Ptychobranchus* spp.), the structures of which do not depend on cohesion between undeveloped eggs (Watters, 1999), and in species that do not release glochidia in conglutinates. Although occasional individuals may experience abnormally low fertilisation (e.g. two individuals of *E. arca*), overall fertilisation success is remarkably high in these species. High fertilisation success may be a result of the high density of some of these species in our study populations. *Elliptio arca, F. cerina, P. decisum, Q. asperata,* and *Q. pustulosa* occurred at densities ranging from 2.97 to 8.25 individuals m^{-2} (Haag, 2002; W.R. Haag & M.L Warren, unpublished data). However, *A. plicata, L. ornata* and *O. reflexa* were present at fairly low density (0.15–1.15 individuals m^{-2} ; Haag, 2002; W.R. Haag & M.L Warren, unpublished data). Our extensive experience in handling gravid mussels in host identification trials (Haag & Warren, 1997; Haag, Warren & Shillingsford, 1999; Haag & Warren, 2003), suggests to us that high fertilisation success is ubiquitous in these and many other species, even in sparse headwater populations. The prevalence of high fertilisation in most species, and the predictability of this variable within all species, indicate that highly efficient but as yet unidentified modes of fertilisation exist to increase chances of success in a variable environment.

The annual frequency of spawning in freshwater mussels is an important but poorly known feature of mussel reproductive biology, and no studies have directly evaluated the possibility of multiple clutches. The homogeneity of developmental stages within individuals of the eight species in this study shows that females brood only a single clutch at any one time; this observation, coupled with the short duration of the brooding period for most species (Haag & Warren, 2003), indicates that these species produce a single clutch per year. Production of a single clutch is also indicated in several other species, in which the percentage of females brooding mature glochidia peaks briefly once a year (Lewis, 1985; Yeager & Neves, 1986; Bruenderman & Neves, 1993; Woody & Holland-Bartels, 1993; Hove & Neves, 1994). In two of these species, *Fusconaia cuneolus* (I. Lea) and *Pleurobema collina* (Conrad), numbers of glochidia in stream drift peaked twice during the brooding period, but this was attributed to variation in physical conditions stimulating glochidial release, rather than production of multiple clutches (Bruenderman & Neves, 1993; Hove & Neves, 1994). In long-term brooding species, females brood mature glochidia for much of the year and glochidia are found in stream drift year-round (Zale & Neves, 1982). However, at least some long-term brooders release glochidia primarily in response to physical interactions with fish hosts and release only a small percentage of the reproductive complement during each encounter (Haag & Warren, 2000). Thus, the release of an entire clutch by these species may require an extended time period. Fertilisation of eggs in five, long-term brooding species occurred only once a year during a brief period (Zale & Neves, 1982; Holland-Bartels & Kammer, 1989), providing solid evidence for single clutches in these species. In another long-term brooder, release of glochidia was observed only during a 1-month period, although females were gravid for approximately 8 months prior to release (Haggerty & Garner, 2000). Production of multiple clutches was suggested for *Glebula rotundata* (Lamarck) from south Louisiana (Parker, Hackney & Vidrine, 1984) and in *Cumberlandia*

monodonta (Say) from Missouri and Tennesssee (Gordon & Smith, 1990), but these studies were not conclusive. At this time, evidence from a wide variety of unionid species in temperate eastern North America strongly suggests production of a single clutch per year.

Length was a good predictor of fecundity in all species and explained 43–88% of the variation in this trait. Length-fecundity relationships of organisms with indeterminate growth, including fishes (Bagenal, 1978) and freshwater mussels (Hanson, Mackay & Prepas, 1989; Hochwald, 2001) are commonly in the form of a power function in which the rate of change in fecundity increases throughout the life of the organism. This function, or a similar square-root function best described the length-fecundity relationship for six of the species in this study. Because of their high fecundity, large individuals are probably particularly important for the maintenance of populations of these species.

Length-fecundity relationships for *E. arca* and *Q. pustulosa*, and age-fecundity relationships for all five species examined provided evidence of a decline in fecundity in very large or old individuals, respectively. Reproductive senescence in mussels has not been examined previously but has important conservation implications. Today, many mussel populations are composed exclusively of old individuals, presumably because of human-induced habitat changes that have made conditions unfavourable for juvenile survival (e.g. Layzer, Gordon & Anderson, 1993). In cases where likely causal factors have been identified (particularly stream reaches affected by impoundment or cold, hypolimnetic dam discharges), conservation strategies have focused on restoring habitat conditions that may allow the resumption of mussel reproduction (Heinricher & Layzer, 1999). A critical assumption of this strategy is that the old individuals constituting these populations are reproductively viable and will respond to abatement of stress. We examined few animals >30-years old. Nonetheless, our observations suggest that, although the rate of increase in fecundity declines as predicted by our models, older animals continue to produce large numbers of offspring. Growth of freshwater mussels is almost imperceptible in older animals (Anthony *et al.*, 2001). Consequently, because fecundity is primarily a function of body size, the form of the age-fecundity relationship may actually be asymptotic, in which fecundity is relatively constant among older animals of varying ages. Additional fecundity observations of older females are needed to determine more precisely the degree of reproductive senescence in these individuals.

We found variation in life history traits both within and among the eight mussel species in this study. Intraspecific variation in life history traits is documented in several freshwater mussel species and is often attributed to phenotypic plasticity in response to local conditions (Bauer, 1992; Johnson & Brown, 1998; Hochwald, 2001). Similarly, intraspecific differences in sex ratio and small differences in fecundity among populations in this study may be due to phenotypic plasticity and have little adaptive value. In contrast, interspecific differences in fecundity, age at maturity and brooding pattern are probably adaptive traits indicating divergent life history strategies in these animals. Further elucidation of these traits and the strategies they represent will have important evolutionary, ecological and conservation implications for freshwater mussels.

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