

Visceral sex, hermaphroditism, and protandry in a population of the freshwater bivalve *Elliptio complanata*JOHN A. DOWNING, J.-P. AMYOT, M. PÉRUSSE¹, AND Y. ROCHON²Département de Sciences Biologiques, Université de Montréal,
C.P. 6128, Succursale 'A', Montréal, Québec, Canada H3C 3J7

Abstract. Sexual allocation was examined in a dense (28/m²) population of *Elliptio complanata* (Lightfoot) in Lac de l'Achigan, Québec. The gonads of individuals in this population contained mixtures of male and female tissue with 6% containing >10% and <90% female tissue. The sex ratio was determined by microscopic examination of gonads and was found to be skewed toward males. The sexual composition of the population varied significantly with body size. Protandry was indicated because the gonads of 80% of animals <50 mm in shell-length were composed primarily of male tissue, while larger animals had a significantly higher proportion of females and hermaphrodites. The fraction of the population producing eggs or glochidia increased with the fraction of the gonad composed of female tissue, such that animals whose gonads contained <40% female tissue had a low probability of egg production. There was no significant tendency for dioecious organisms to be found in denser aggregations than hermaphrodites.

Keywords: hermaphroditism, reproduction, protandry, sex ratio, aggregation, freshwater, Unionidae, Pelecypoda, Mollusca.

Hermaphroditism is very common in the animal kingdom, occurring in more than 80% of the phyla examined by Ghiselin (1969). Hermaphroditism is most frequently found in animals that occur at low densities, brood their young, have small, genetically isolated populations, and are sedentary as adults (Ghiselin 1969, Charnov 1982). It is not surprising, therefore, that bivalves in general (Coe 1943) and freshwater bivalves in particular (van der Schalie 1970, Heard 1979, Mackie 1984) are commonly hermaphroditic. Van der Schalie (1970) examined microscopic sections of the gonads of 97 species of freshwater mussels and found that in 4% of the species nearly all of the gonads contained both male and female tissue, in 23% of the species a few of the organisms contained both male and female tissue, while 73% of the species were dioecious. Heard (1979) examined the gonads of individuals from 13 populations of nine species of *Elliptio*, a common genus, and found that hermaphroditism was often present but was never the dominant state. Four of the *Elliptio* species produced no hermaphrodites,

while the frequency of hermaphroditism in the remaining five species averaged about 2%. The occurrence of hermaphroditism in *Elliptio complanata* (Lightfoot 1786) is controversial. Heard (1979) found occasional hermaphrodites in two populations while Kat (1983) stated that "none of the 560 individuals from 28 populations . . . examined exhibited any evidence of hermaphroditism".

Isolated, nearly sedentary populations such as freshwater bivalves may inbreed. Sex change or sequential hermaphroditism is a means of benefitting from hermaphroditism while decreasing the probability of inbreeding (Policansky 1982). Sex change is predicted for animals in which the age- or size-specific reproductive success rates are different for males and females (Policansky 1982) and is fairly common in non-motile filter feeders (Ghiselin 1974). Species of molluscs are more frequently sequential hermaphrodites than any other group except the fishes (Policansky 1982). Protandrous sex change has been discovered in many species of marine bivalves (Policansky 1982) but evidence of sex change in freshwater bivalves has been presented only for *Anodonta cygnea* (van der Schalie 1970) although Kat (1983) suggested that most young unionids are phenotypically male. Because sperm could be produced in great abundance by small male bivalves and egg production in females might increase rapidly with size,

¹ Present address: Department of Biology, McGill University, 1205 Ave. Docteur Penfield, Montréal, Québec, Canada H3A 1B1.

² Present address: INRS-eau, Université de Québec, 2700 rue Einstein, C.P. 7500, St-Foy, Québec, Canada G1V 4C7.

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investigation of the possibility of protandrous sequential hermaphroditism in freshwater bivalves seems warranted.

If simultaneous hermaphroditism is to be a successful strategy then hermaphrodites must be capable of fulfilling both female and male sexual functions. Some evidence exists, however, that this is not true in freshwater bivalves. For example, hermaphroditic *Carunculina parva* produced eggs that were smaller than those of pure females (van der Schalie 1970). Kat (1983) suggested that some hermaphroditic animals may not develop phenotypically female gills and are therefore not functionally hermaphroditic. Whether the ability of bivalves to produce eggs is affected by hermaphroditism is unknown, because quantitative estimates of sexual allocation in freshwater bivalves have never been coupled with measures of reproductive success.

The most long-standing explanation for the development of hermaphroditism in animal populations is that it would increase the reproductive success of individuals found at low population densities. This is because, where encounter frequency among individuals is low, being both male and female would double the encounter probability with an individual of the "opposite sex". If sperm survival time is short, one might expect, therefore, that in a spatially heterogeneous population, dioecious organisms might increase their reproductive success by being more frequently found in dense aggregations. Such a suggestion presupposes that spatial behaviour and sexual allocation are determined to some degree by genotype. A purely female individual could insure successful reproduction only by being within the sperm dispersal and survival distance of a male or hermaphrodite during spawning. An organism that is hermaphroditic could insure successful outcrossing by being sufficiently close to any other population member. On the other hand, Kat (1983) suggested that hermaphroditism in unionids might arise as a result of alteration of hormone levels caused by developmental errors or parasite infestation of the gonad.

The purpose of this research was to test four hypotheses about sexual allocation in a population of *Elliptio complanata*, a common freshwater bivalve. First, we postulate that hermaphroditism occurs in this population with a frequency of about 2%, the level seen in other

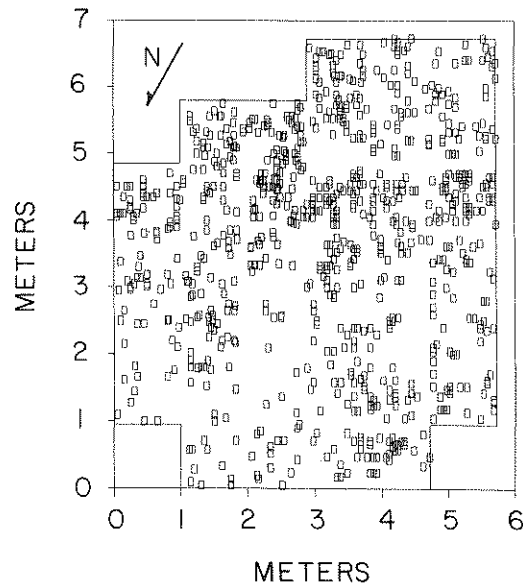


FIG. 1. Spatial distribution of individuals of *Elliptio complanata* in the study population in Lac de l'Achigan, Québec. Each square represents the position of one mussel. Positions were determined using geometric triangulation from reference stakes. Shore is toward the bottom of the figure and prevailing winds sweep diagonally from upper right to lower left.

Elliptio populations (Heard 1979). Second, we test the hypothesis that the relative frequency of the sexes of *Elliptio complanata* varies significantly with body size (a correlate of age). Third, we test the hypothesis that the probability of ovum production is proportional to the fraction of female tissue in the gonad. Fourth, we examine the idea that purely male or female organisms are found more closely spaced than hermaphrodites.

Methods

Elliptio complanata was examined during early July in a dense ($28/m^2$) and highly aggregated (Fig. 1) population in Lac de l'Achigan, Québec, Canada ($45^{\circ}57'N$, $73^{\circ}58.4'W$). Sediments were clean uniform sand, water depth was 1-2 m, and the study site was 100 m from a tributary stream and 5 m inshore from a narrow macrophyte bed. Inspection of the shore indicated no predation by mammals.

The frequency of hermaphroditism was tested by collecting all 810 individuals from within

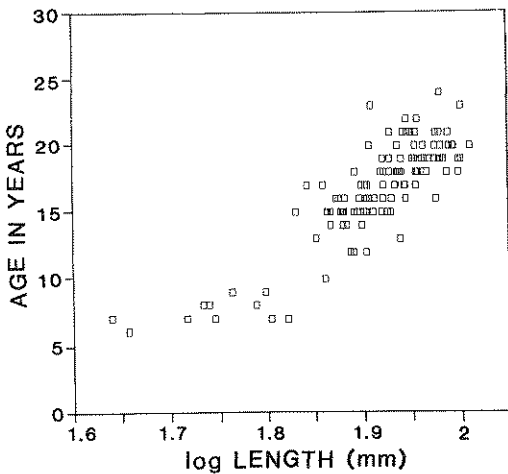


FIG. 2. Relationship between the logarithm (base 10) of maximum linear dimension of shells of *Elliptio complanata* and age determined from growth annuli.

the approximately 6-m \times 7-m study area. Underwater, each mussel was sealed immediately in an individual plastic bag (Whirlpak[®]) to avoid the loss of eggs and glochidia due to spontaneous abortion (Matteson 1948). Gonads were removed and preserved in 95% ethanol. Gonads were serially sectioned and stained and mounted following the technique of Heard (1975). Several different sections of each gonad were examined microscopically and the approximate proportion of the gonad area occupied by male and female tissue was noted by sorting the animals into five categories: <10% female, 10 to 40% female, 40 to 60% female, 60 to 90% female, and >90% female. Comparisons of proportions and frequency distributions of sex categories were made using χ^2 tests (Conover 1971).

The test of the hypothesis that the sex of *Elliptio complanata* changes with age or size was made using a randomly chosen subset ($n = 371$) of the same data. Shell length was used as a surrogate of age and was measured using a digital micrometer (± 0.01 mm) as the maximum linear dimension of the shell. Age could be determined for many of the animals by counts of growth annuli, but the shells of most were too eroded to permit accurate aging. The animals were thus sorted into three size/age categories (small: 15–50 mm, medium: 50–90, mm, large: 90+ mm) and the frequency of visceral males, females and hermaphrodites was calculated

within each size category. These size categories correspond approximately to ages of 2–7 years, 7–22 years and >22 years (Fig. 2). The hypothesis of equivalent frequency distributions of sexes was tested using a χ^2 test (Conover 1971).

The hypothesis that ovum production probability is related to sexual allocation was tested on the same population of 810 animals as above. Samples were taken on 5 July, which is 1–2 months after spawning and a few weeks before the release of glochidia (Matteson 1948). The contents of the plastic bag in which the animal was collected and the suprbranchial chamber of each mussel were examined microscopically for the presence or absence of eggs and glochidia. The relative frequency of presence of ova or glochidia in the suprbranchial chamber was tabulated for each of the five sex classes and the relationship examined graphically.

The hypothesis that males and females are found in denser aggregations than hermaphrodites was tested using the 543 mussels that were at least 0.5 m interior from the periphery of our study site. The degree of aggregation was measured as the number of other mussels found within a 0.5 m radius of each individual. Tests for differences in the spatial density frequency distribution among sex categories were made using a χ^2 test (Conover 1971).

Results and Discussion

Elliptio complanata was more frequently hermaphroditic in Lac de l'Achigan than has been previously observed in this genus. We found that *E. complanata* was commonly hermaphroditic which contrasts directly with the research of Kat (1983). Figure 3 shows that gonadal tissue sections contained from 100% male tissue to 100% female tissue, and covered the range of quantitative combinations of hermaphroditism. Only about 20% of these organisms appeared purely male or female. The population of *Elliptio complanata* in Lac de l'Achigan was composed of about 6% visceral hermaphrodites (Table 1), using our quantitative sex categories, which is greater than the proportion of hermaphrodites in any other population of *Elliptio* yet investigated. Heard (1979) found a population of *E. icterina* with 5.6% hermaphrodites and a population of *E. buckleyi* with 5% hermaphrodites. The two populations (266 animals) of *E. complanata* examined by Heard (1979) revealed only

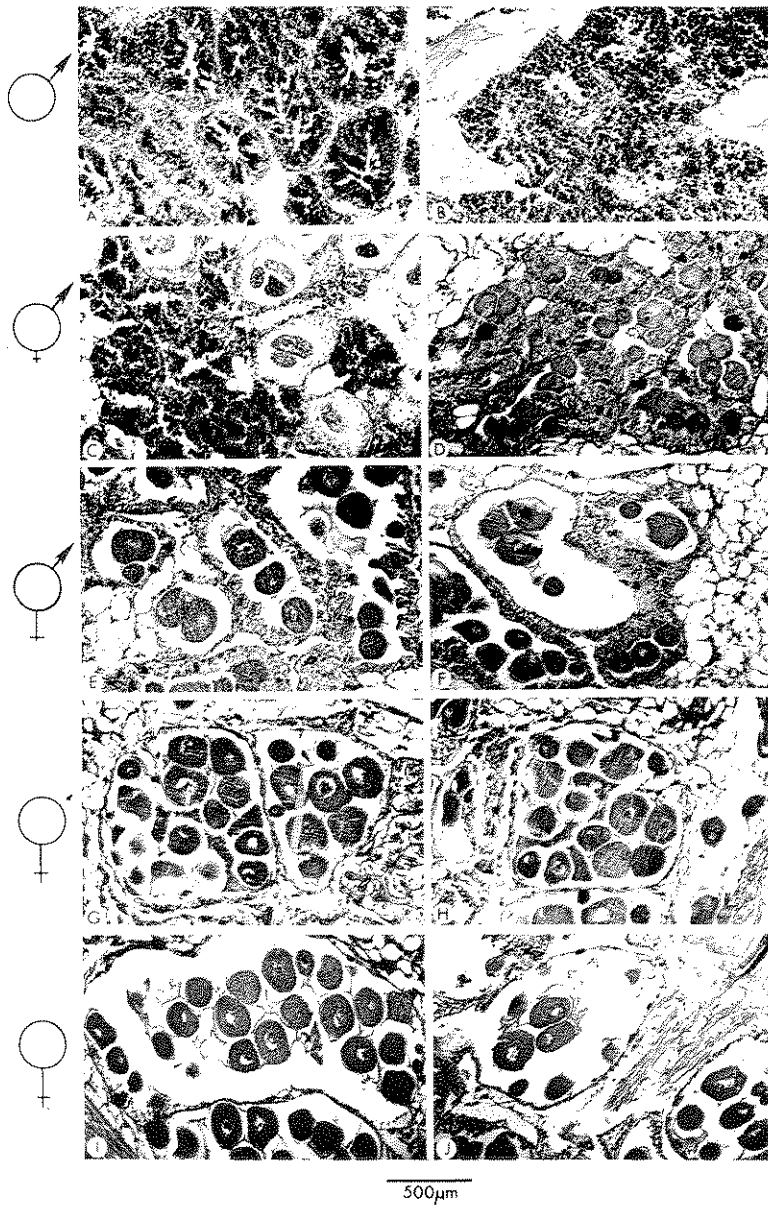


FIG. 3. Examples of tissue allocation in the gonads of *Elliptio complanata* found in Lac de l'Achigan, Québec. Organisms were considered to be "male" or "female" if their gonad contained >90% of that tissue. Other sexual categories are defined in text.

1.4 and 1.6% hermaphrodites. A χ^2 test of Heard's data shows no significant difference between the sexual allocation among the species or populations of *Elliptio* that he examined ($p = 0.86$). The overall average occurrence of hermaphroditism in the 1178 animals of the genus *Elliptio*

examined by Heard was 1.9%. Taken together, Heard's 13 populations of nine species of *Elliptio* yielded 577 males, 579 females and 22 hermaphrodites. Comparison of these data with Table 1 demonstrates that *E. complanata* in Lac de l'Achigan has a significantly higher percentage

TABLE 1. The frequency of individuals in the population of *Elliptio complanata* of Lac de l'Achigan possessing various combinations of male and female tissue in their gonad. $n = 810$.

Sex	% Female Tissue	% of Population
Female	90-100	36
Female hermaphrodite	60-90	3
Hermaphrodite	40-60	2
Male hermaphrodite	10-40	1
Male	0-10	58

of hermaphrodites than the average for *Elliptio* species ($\chi^2 = 25.49$, $df = 1$, $p < 0.0001$). In addition, other populations of *Elliptio* tend to have equal numbers of males and females, whereas our population showed significantly more males than females ($\chi^2 = 27.51$, $df = 1$, $p < 0.0001$). *Elliptio complanata* in Lac de l'Achigan has significantly more hermaphrodites and males than other populations of *Elliptio* and has a visceral sex allocation that is heavily skewed towards maleness (Table 1). This finding is similar to the data of Cvancara (1972) and Cvancara and Freeman (1978) who showed that populations of *Lampsilis* had up to 11 times more males than females.

Our data agree with the conclusion of Bloomer (1939) that sexuality is not species specific but may be highly variable among populations. Kat (1983) associated hermaphroditism with rates of parasitism of the gonads of *Lampsilis radiata* by trematodes. We found no evidence of such parasitism in *Elliptio complanata*. Hermaphroditism in this population thus seems a natural component of its life history. It would be interesting to know whether rates of hermaphroditism in populations vary systematically with density, spatial aggregation, or environmental characteristics. This research is currently being performed in our laboratory.

Our data suggest that *Elliptio complanata* is a protandrous, sequential hermaphrodite. Table 2 shows that samples of smaller animals contain as much as 4.3 times as many males as females. The male:female sex ratio decreases somewhat in medium sized animals but never reaches the equality seen in other populations of mussels (e.g., Heard 1979) and most animal populations (Hamilton 1967). A comparison of male:female frequency distributions of different size cate-

TABLE 2. Variation in the frequency of sexes of *Elliptio complanata* in different size classes.

Size class	n	% Female	% Hermaphrod.	% Male
15-50 mm	83	18	3	79
50-90 mm	255	40	5	55
90+ mm	83	29	11	60

gories shows that this trend is statistically significant ($\chi^2 = 7.58$, $df = 2$, $p = 0.02$). A similar empirical trend could arise if there were massive differential mortality of small males or if small females occupy a separate habitat. Although we have no reason to believe either of these possibilities, we cannot specifically rule them out. If *E. complanata* were not protandrous, however, we would expect to find either isolated populations of small females or empty shells of mostly small organisms, due to high mortality of small males. Despite exhaustive sampling by SCUBA, the former has not been found and the empty shells are usually from larger organisms (Fig. 4). Given that protandry is common in pelecypods (Policansky 1982), it is therefore plausible that *Elliptio complanata* is protandrous. Such sex reversal may be advantageous, allowing greater reproductive success of small organisms than they could attain by producing eggs and brooding developing glochidia. Other possible adaptive advantages and disadvantages of protandry are reviewed elsewhere (Charnov 1982, Policansky 1982).

Elliptio complanata seems to combine sequential and simultaneous hermaphroditism. If sequential hermaphroditism were the only strategy employed, then organisms with male and female tissue in their gonads would represent the transition between male and female stages and would therefore be concentrated in small or small and medium sized organisms. Instead, the degree of simultaneous hermaphroditism seems to increase from 3% of the population in small organisms to 11% in large ones (Table 2). Van der Schalie (1970) felt that hermaphroditism in *Actinonaias ellipsiformis* was associated with senescence but further investigations failed to demonstrate it. Both the relative frequency of females and hermaphrodites increased with increasing size and age in *Elliptio complanata* in Lac de l'Achigan.

Hermaphrodites function as females but with

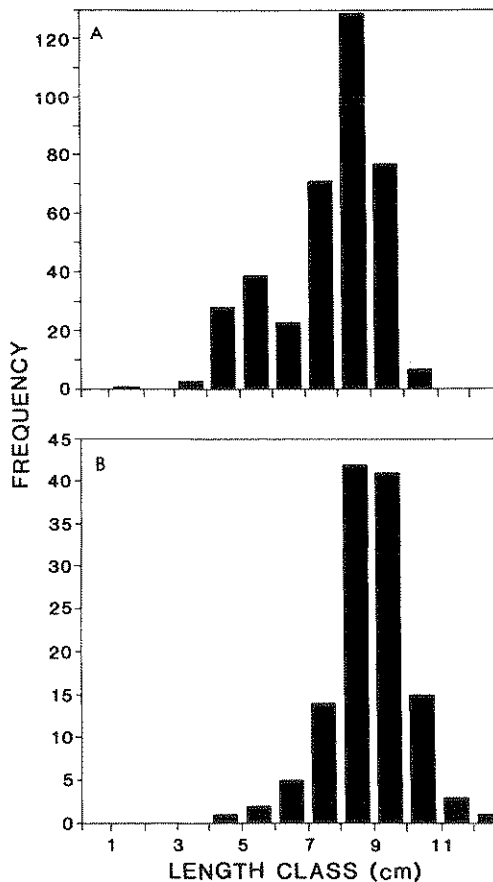


FIG. 4. Frequency distribution of total shell length measurements of (A) living members ($n = 378$) of this population of *Elliptio complanata* and (B) empty shells ($n = 124$) found in the same sampling area.

an efficiency that is correlated with the relative abundance of female tissue in their gonads. Figure 5 shows that most of the mussels classed as females were found with either fertilized or unfertilized ova in their suprabranchial chamber. The fraction of organisms that succeeded in producing or retaining female gametes decreased with the decreasing relative abundance of female tissue in the gonad. Mussels with about 50% female tissue, however, had an ovum production probability nearly 70% of that of "pure" females. Animals with <50% female tissue had little probability of producing female gametes. Hermaphroditism appears to reduce the probability of female reproductive success.

Dioecious organisms and hermaphrodites seem to distribute themselves similarly in space.

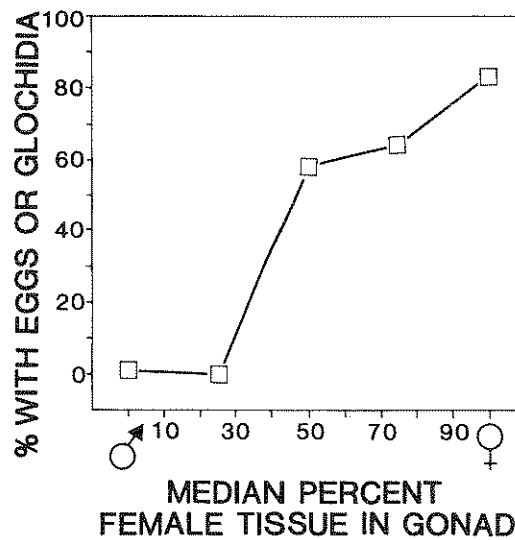


FIG. 5. The relationship between the fraction of *Elliptio complanata* found to possess either ova or developing glochidia in their supra-branchial chamber and the allocation of male and female tissue in the gonad. Animals were collected between the time of spawning and release of glochidia. Sample size given in text.

Frequency distributions of the number of other mussels within 0.5 m of mussels of different sex categories suggest that hermaphrodites might even be found in slightly denser aggregations than dioecious organisms (Fig. 6). χ^2 analysis of these data shows, however, that males, females and hermaphrodites have spatial density frequency distributions that are not significantly different from each other ($\chi^2 = 4.074$, $p = 0.25$). Within this population there is no tendency for relatively isolated organisms to be hermaphroditic or for clumps to contain proportionately more dioecious organisms.

Such a tendency might have existed during spawning but may have been erased by subsequent displacement of mussels. In a preliminary study of mid-summer locomotion in 171 members of this population, we found that after 17 days of observation, only 37% of the population had moved at all, and the average rate of horizontal displacement in those that moved was only 1.2 cm/d. Although rates of locomotion in this population are low, movement appears sufficient to offset any tendency that might exist for dioecious organisms to decrease their distance from potential mates. It seems reason-

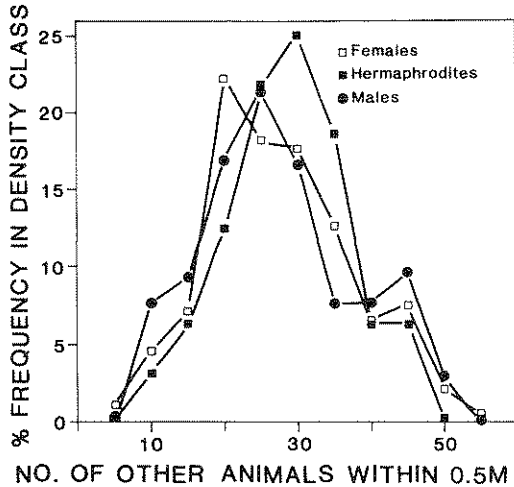


FIG. 6. Local density frequency distributions of 198 female (>90% female tissue in gonad), 313 male (<10% female tissue), and 32 hermaphroditic (10–90% female tissue) *Elliptio complanata* in Lac de l'Achigan, Québec. Densities indicated are the upper boundary of each density class.

able though, that differences among sexes in spatial aggregation would be small, as aggregative behaviour might also be advantageous to hermaphrodites if self fertilization does not occur (Charnov 1982). The classical Low Density Model (Ghiselin 1969) would suggest that, among isolated populations, the occurrence of hermaphroditism would be inversely proportional to spatial density. This would be an interesting topic for future research.

Visceral sex in *Elliptio complanata* varies through every possible quantitative combination of male and female tissue (Fig. 3). Most members of the population appear to start life as males but the relative frequency of female and hermaphroditic organisms increases with increased size. Despite this shift in sex, males, females, and hermaphrodites are present in every size category. Males form the majority throughout the age structure of the population. Fisher (1930) suggested that such a situation would be impossible for panmictic populations of sexually reproducing organisms if the cost of rearing males and females is equal. In addition, Hamilton (1967) suggested that organisms that inbreed may have sex ratios that are biased toward one sex or the other. The degree of panmixia, the relative costs of rearing male

and female young, the relative mortality of male and female glochidia, and the degree of inbreeding are unknown for this species. Hamilton (1967) also suggested that competition for mates might also bias sex ratios. The preponderance of males, for example, suggests that the fertilization of females is a highly critical step in the reproduction of this population. If fertilization of eggs is rare and egg production is not very costly, then selection may favour those individuals that produce large proportions of males. This might be especially probable because some males apparently change to females or reproducing hermaphrodites later in life.

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