Oecologia (1992) 91:214–219

Oecologia

Causes of a non-random pairing by size in the brine shrimp, *Artemia salina*: (Crustacea: Anostraca)

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Received November 13, 1991 / Accepted in revised form March 27, 1992

Summary. Brine shrimp (*Artemia salina*) males and females entered precopula assortatively by size in the laboratory; large males also had a pairing advantage over smaller males. We investigated the causes of such non-random pairing to test hypotheses on size-assortative mating.

We found precopulatory biases with respect to male size in the absence of direct competition among males (which produces pairing biases in other species). Large males encountered females significantly more often than did small males. Similarly, large females encountered males more often than did small females, but showed less 'willingness' than small females to enter precopula when housed with small males. Consequently, large females took longer than small females to enter precopula with small males. Although large males entered precopula readily with small females, such size-mismatched pairs appeared short-lived.

We conclude that non-random pairing by size in *A. salina* is determined by several factors including: encounter rates between males and females of different sizes, female behavior, and time following initial pair formation. Our results are likely applicable to other species and can help explain variation for selection on size or other traits.

Key words: *Artemia salina* – Size advantage – Size-assortative pairing – Encounter rates – Reproductive success

Evolutionary biologists are interested in patterns of non-random mating within animal and plant populations because such patterns can result in evolutionary change (Fisher 1958). One of the most common patterns of non-random mating in animal populations is a large male mating advantage (Partridge and Halliday 1984). Large male mating advantages often occur because male competitiveness for access to mates increases with male size and age (Cox and LeBoeuf 1977; Thornhill and Alcock 1983) or because females show mating preferences for large males (Howard 1981; Downhower and Brown 1981).

Large males from populations that show size-assortative mating often have a mating advantage over their smaller rivals (Crespi 1989). In natural populations, large male mating advantages should act to remove genetic variation for male size (Simmons 1987), male growth rates, timing of onset of male reproduction, or all of the above. In contrast, size-assortative mating may act to maintain such genetic variation (Fisher 1958). Thus, the occurrence and relative strength of a large male mating advantage versus size-assortative mating can determine the extent to which there is strong selection on male size (or other traits) in natural populations.

Hypotheses on size-assortative mating often invoke behavioral determinants such as active male choice of large mates coupled with a large male competitive advantage (Ridley 1983), a large male mating advantage coupled with greater availability of large females (Crespi 1989), or spatial and temporal overlap between males and females of similar sizes mediated by size-constrained choice of habitat (Christy 1983). Other hypotheses suggest that size-mismatched pairs are unlikely to form because small males cannot (effectively) court or mate large females, or because small males cannot (afford the energy required to) guard or carry large mates (Alcock and Gwynne 1987; Adams and Greenwood 1983, 1987; but see Ward 1988).

By knowing the causes of non-random mating patterns, researchers can predict the impact of environmental change on the direction and strength of selection for size (or other traits) in natural populations. An example will help to illustrate this point. If small males *choose* small females because of energetic or mating constraints imposed on them, then increased availability of large females should have little effect on the occurrence or strength of size-assortative mating (Crespi 1989). In contrast, if small males can guard or carry large females

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and benefit by doing so (by gaining greater paternity), then increased availability of large females should dampen patterns of size-assortative pairing (assuming female mate choice is weak).

In this study, we determined whether a large male pairing advantage and size-assortative pairing occur in laboratory-reared brine shrimp, *Artemia salina* (Crustacea: Anostraca). We then determined whether time to enter precopula depended on the sizes of males and females housed together, encounter rates of small versus large individuals, and female responses to encounters with males. We also examined whether egg number covaried with female size to determine whether males might benefit from entering precopula with large females. Finally, we examined whether size-mismatched individuals formed stable pairs. We discuss our results in relation to hypotheses on assortative mating and with respect to variation for selection on size or other traits.

Study species

Brine shrimp (*A. salina*) are branchiopod crustaceans which commonly live in highly saline, temporary environments unsuitable for fish predators. *A. salina* is distributed worldwide; both parthenogenetic and sexual races have been described (Browne 1982). For sexual races, sexes are dimorphic. Females are longer than males (see below) whereas males have enlarged secondary antennae. These antennae are used to clasp ovigerous females at the junction of the last thoracic segment and the abdominal trunk where the base of the ovisac is located (Lochhead 1950). Males will remain in precopula with females for 1 to several days before the female molts; copulation with the attendant male occurs after molt of the female (Lochhead 1950). Both sexes can live up to three months in laboratory cultures under conditions of high food (Browne 1982); over this time period, females have, on average, approximately nine broods (Browne 1982).

Methods

Culture of A. salina

A. salina can be reared easily in the laboratory from the San Francisco strain of desiccation-resistant eggs (available from most aquaria suppliers). In early January 1990, 50 nauplii (free-swimming larvae) of *A. salina* hatched from eggs were added to two 19-L tanks filled with artificial sea water (Aquarium Systems Inc. Canada). Water temperature was maintained at 26±2° C and photoperiod (florescent lighting) was 10:14 h; L:D. The tanks were inoculated once with a mixed culture of green algae (*Ankistrodesmus* spp., principally *A. convolutus*) to provide food. In addition, we added a small amount of dried Brewers' yeast to tanks every other day to provide other food. Water was aerated and circulated throughout tanks by air supplied through polyethylene tubing and air stones. A second and third culture of *A. salina* was founded (as described above) in single tanks in October 1990 and July 1991.

Animals from the first culture were used to examine whether a large male pairing advantage existed for *A. salina* and whether time to enter precopula was contingent on sizes of males and females housed together as pairs. Animals from the second culture were sampled to determine whether assortative pairing by size occurred for *A. salina*. Males and females from the last culture were used in experiments to determine the causes of non-random pairing by size in this species.

Experiments and observations on precopula in A. salina

Precopulatory pairs of *A. salina* collected from the first culture were separated into single males and females by gently forcing each pair through a large eyedropper into a petri dish (height = 15 mm, diameter = 100 mm) filled with artificial sea water. Individuals collected in this way were known to be sexually active because they were paired upon collection. Upon separation, animals were transferred to another dish within which ca. 0.4–0.8 g of Alka-Seltzer (Miles Canada Inc.) had been dissolved in seawater to provide anaesthetic. When individuals stopped moving, they were measured from the tip of their head to the tip of their abdominal furca using a binocular microscope (power 25×) provided with an ocular micrometer. Animals were measured to within one ocular unit (i.e. 0.04 mm).

We first determined whether the size of a male influenced his probability of entering precopula with either a small or large female (i.e., whether there was a large male pairing advantage regardless of female size). We used 10 500-mL jars (height = 13.5 cm, diameter = 8 cm) for each 'run' of this experiment. Each jar was filled with 100 mL of artificial sea water and aerated. One small (7–9 mm) and one large (10–11 mm) male was placed in each jar; 30 minutes later, a small (7–9 mm) or large (11–14 mm) female was added to the jar. We made observations twice daily (at ~12:00 and 18:00 h) and recorded which male, if either, had successfully entered precopula.

After pairs formed in all jars, another experimental run was made. We made 5 runs giving a maximum of 25 replicates for each size class of female. However, only 22 and 23 replicates were run for jars housing 'small' and 'large' females, respectively. A total of 5 replicates was excluded from the analysis because at least one shrimp died during those trials. Each live animal was used only once in the above experiment.

Next, we examined whether time to enter precopula depended on the sizes of males and females housed together as pairs. We wanted to determine whether pairing biases existed in the absence of intrasexual competition (behavioral interactions) between males. For this experiment, only one female and one male was added to each jar. Both size classes of males and females were used resulting in four possible treatment categories (Table 1). Ten replicates per treatment were made, and we checked jars 4 times daily (at ~08:00, 12:00, 16:00, and 20:00 h) to determine when pairs formed. [If pairs were found at 08:00 h on the first day, then time to pair formation was 0.25 days; if pairs were found at 12:00 h on the second day, then time to pair formation was 1.5 days, etc.]. A trial ended after a pair formed; trials were also concluded if no pairs formed after 5 days. As before, animals were only used once.

Finally, we examined whether assortative pairing by size occurred in laboratory-reared *A. salina*. If >10 pairs (which we regarded as a minimum sample size) were present in the second culture tank on a given day, then they were removed and separated as described above. On 21 Nov. and 5 Dec. 1990, 12 and 16 pairs of *A. salina* were collected, respectively; individuals were then measured and transferred to a holding tank.

Experiments on causes of non-random pairing in A. salina

Having found large male pairing advantages and assortative pairing by size in *A. salina* (see Results), we investigated the causes of such non-random pairing through a series of experiments. First, we examined whether male encounter rates with females depended on male size. Both males and females swim upside down nearly constantly using paired appendages (known as phyllopodia) located on the venter of the animal's thorax (Russell-Hunter 1979). However, we suspected that large individuals would swim faster than small individuals. For each trial of this experiment, we removed 4–6 pairs of *A. salina* from the July culture. Males and females were separated by gently prying the male off the female with an 0 insect pin. [We decided against anaesthetizing animals (as described above) because some animals died in preliminary experiments]. The largest male (7.6–8.8 mm) and the smallest male (5.5–7.5 mm) were taken from the 4–6 pairs and housed together in a petri dish filled with artificial seawater. 2–4 females were added to the dish 5–10 min later. We then recorded the number of female encounters made by males over 10 min.

Encounters were defined as the focal male touching a female, and either swimming away or trying to clasp the female with his antennae. Encounters with other males were excluded from totals. Only one male was observed at a time. Thus, each trial lasted 20 min. The order of observation for large and small males was reversed from trial to trial, so that large males were observed first in half of the trials. Following a trial, the males and females were placed in a holding tank and another 4–6 pairs were selected.

We next investigated whether female encounter rates with males depended on the female's relative size. Relatively small males were used exclusively in this and the preceding experiment because large males were not available at the time these experiments were conducted. During previous experiments, we noted that females often curled their abdomen rapidly towards their head during encounters with males. This rapid abdomen-to-head movement made capture of females by males difficult (pers. obs.). We also investigated whether large or small females were more likely to make rapid abdomen-to-head movements in response to encounter with males.

As before, 4–6 pairs of *A. salina* were removed from the July culture for each trial. The largest female (7.9–10.5 mm) and smallest female (7–8.5 mm) from the 4–6 pairs were placed together in a petri dish filled with artificial seawater for 5–10 min before introducing 2–4 males. The number of male encounters made by large and small females was recorded in the same way as the number of female encounters made by males in the previous experiment. After the trial ended, males were returned to a holding tank. Females were killed in 90% ethanol; their ovisacs were dissected and their eggs counted.

Finally, we investigated whether the strength of size-assortative pairing depended on time of sampling which would occur if size-mismatched pairs formed, but were not long-lived. To address this question, we ran two similar experiments. Pairs were removed from the July culture, separated and placed as single animals into either of two experimental tanks (open plastic tubs: $30 \times 18 \times 12$ cm). For one experiment (the daily removal experiment), males and females from 48 pairs were used, whereas males and females from 34 pairs were used for the second experiment (weekly-removal experiment).

For the daily-removal experiment, pairs were removed from one experimental tub at 16:00–17:00 h each day; males and females were separated and measured. This experiment was terminated at the end of 7 days by which time 36 new pairs had formed. For the weekly-removal experiment, pairs were not removed (from the second experimental tub) until after 7 days had elapsed; only 14 pairs were available at that time. We decided not to measure animals before the onset of each experiment in order to minimize handling.

Statistical analyses

For categorical data, binomial tests were used (Zar 1984). Where possible, parametric tests (paired and unpaired *t*-tests, analysis of variance followed by pairwise Tukey tests, and Pearson correlations) were used based on restrictions and assumptions outlined in Zar (1984) and Wilkinson (1989). Tests are two-tailed and standard errors (or S.E.) are reported.

Results

Non-random pairing in A. salina

Overall, female *A. salina* found in precopula were longer than males (Fig. 1). Although small males were found in precopula with females, these same males were unlikely to enter precopula with either small (4 of 22 trials: $p<0.02$, Binomial test) or large females (3 of 23 trials: $p<0.005$) when housed with a large male rival. Thus, there was a large male pairing advantage in *A. salina* irrespective of female size.

Males and females housed together as pairs showed considerable variation in time to enter precopula. Time to formation of precopula was shortest for large males and large females, and almost a day longer ($p<0.05$) for large males and small females. Formation of precopula between a small male and large female also took significantly longer than that between a small male and small female (Table 1).

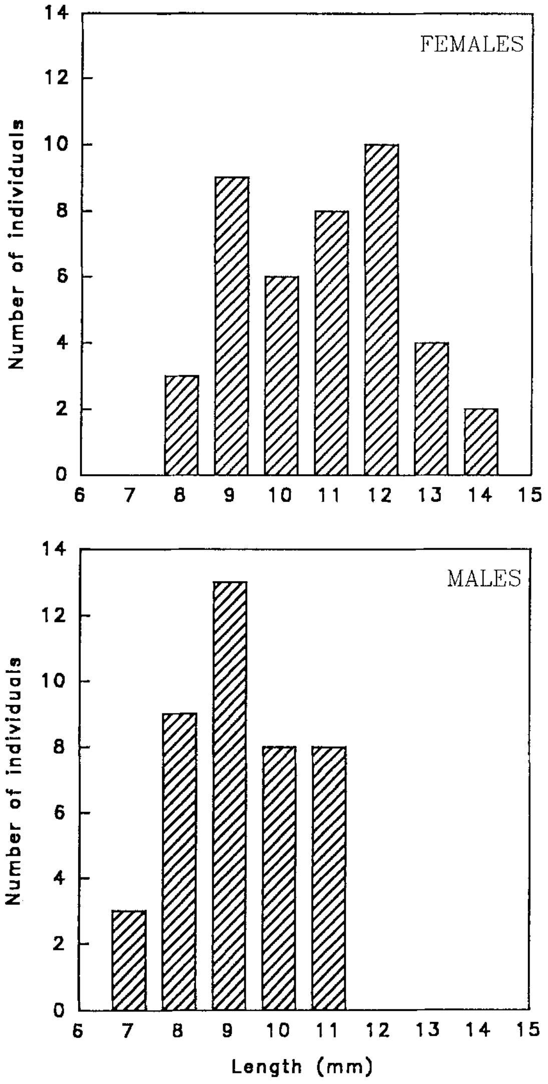

Fig. 1. Size distributions of females (upper panel) and males (lower panel) found in precopula in the first culture. Many of these animals were used in experiments described in the text

Table 1. Average time in days (±standard error) for males and females to enter precopula depending on sizes of individuals. Small males and females were 7–9 mm in this experiment, whereas large males were 10–11 mm and large females were 11–14 mm. *N* refers to the number of trials

Treatment	N	Time to precopula
Small male: small female	10	3.25±0.03
Small male: large female	7	3.97±0.05
Large male: small female	10	1.43±0.07
Large male: large female	10	0.45±0.02

All means were significantly different at $p<0.05$ [Tukey's test, Wilkinson (1989)]

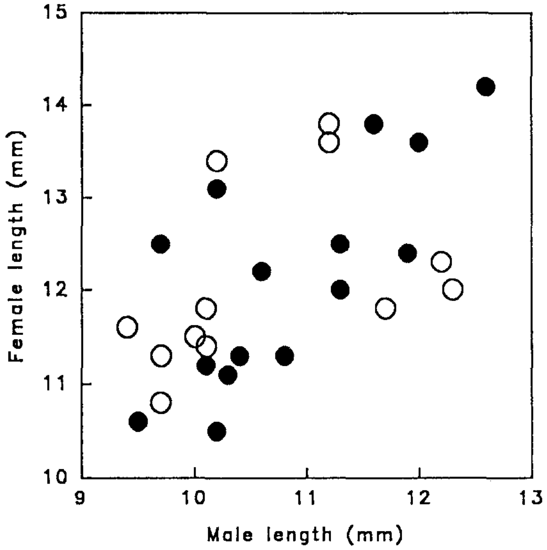

Fig. 2. Size of female *A. salina* plotted in relation to size of males with which they were paired on 21 Nov 1990 (*open circles*) and 5 Dec 1990 (*closed circles*)

Pairs in precopula from the second culture showed size assortment (Fig. 2). On 5 Dec., the correlation between sizes of males and their female partners was highly significant ($r=0.74$, $p<0.005$, $n=16$); there was also a strong trend for assortative pairing by size on 21 Nov. ($r=0.42$, $0.05<p<0.1$, $n=12$).

Causes of non-random pairing in *A. salina*.

Encounter rates, female behavior and female fecundity

Large males encountered females more times (mean = 13.3 times, S.E. = 1.01) than did small males (mean = 10.3, S.E. = 0.82) when housed together with 2–4 females (paired $t=2.69$, $p<0.01$). Similarly, large females encountered males more times (mean = 21.4, S.E. = 0.97) than did small females (mean = 17.9, S.E. = 1.46). This latter result was nearly significant (paired $t=2.09$, $p=0.052$). Large females also made significantly more rapid abdomen-to-head movements (mean = 5.4, S.E. = 0.59) than did small females (mean = 2.9, S.E. = 0.42, paired $t=2.44$, $p<0.001$) in response to encounters with relatively small males.

We also found a strong correlation ($r=0.57$, $p<0.001$, $n=38$) between the number of eggs a female carried (mean = 22.6, S.E. = 1.75) and female body length (mean = 8.50, S.E. = 0.16). Although these eggs were probably already fertilized, they are an indication of the size of the subsequent clutch that could have been fertilized by the attendant male provided he remained in precopula with the female until she molted (Lochhead 1950).

Time and assortative pairing by size

We found no assortative pairing by size for animals collected during the daily-removal experiment ($r=-0.11$, $p>0.25$, Fig. 3a). Both males and females used in that experiment were small relative to animals sampled from the second culture [compare size range of males (and

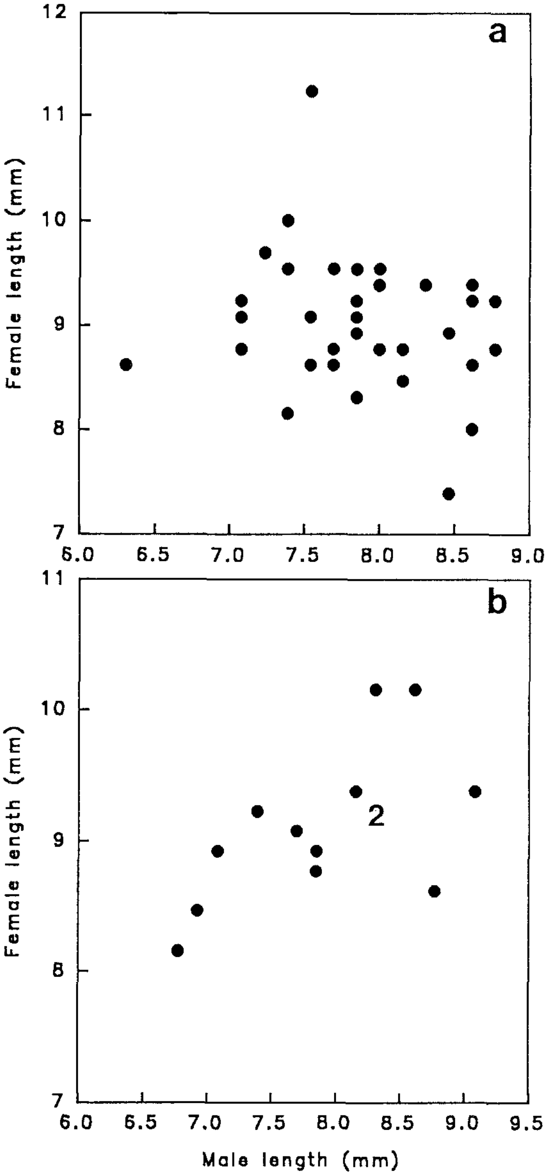

Fig. 3 a, b. Size of female *A. salina* plotted in relation to size of males with which they were paired. In *a*, pairs were removed from the experimental tub each day, or soon after they formed. In *b*, pairs were not removed from the tank until after 7 days had elapsed females) in Fig. 2 and Fig. 3a]. Thus, our inability to find assortative pairing could have been due to a lack of large competitive animals. However, males found in precopula during the daily-removal experiment were larger (mean = 7.9 mm, S.E. = 0.10) than single males (mean = 7.4, S.E. = 0.14) remaining at the end of that experiment (unpaired $t = 2.6$, $p < 0.05$), indicating that size differences among relatively small animals can still affect their pairing success. Similarly, females found in precopula were larger (mean = 9.0 mm, S.E. = 0.11) than single females (mean = 8.3 mm, S.E. = 0.22) sampled at the end of the daily-removal experiment (unpaired $t = 3.3$, $p < 0.01$).

We found strong size-assortative pairing ($r = 0.56$, $p < 0.05$, Fig. 3b) for precopulatory pairs collected during the weekly-removal experiment. Animals collected during the weekly-removal experiment were of similar size to animals collected during the daily-removal experiment [compare size range of males (and females) shown in Fig. 3a and b]. Thus, the size ranges used in the daily-removal experiment should have been sufficient to allow assortative pairing by size. Yet size-mismatched pairs were not present in the weekly-removal experiment.

Discussion

Patterns of non-random mating in animal populations are important inasmuch as they can identify direction of selection for such characters as male and female size, growth rates, or timing of onset of reproduction. By knowing the causes underlying patterns of non-random mating, researchers can predict whether selection should be fairly uniform or variable for such traits. Recently, Olson et al. (1989) demonstrated variation in the occurrence and strength of size-assortative mating in the western toad (*Bufo boreas*) and other amphibians [see also Crespi (1989) for similar findings for arthropod populations]. Such spatial and temporal variation indicates that mating pattern is neither species-specific nor population-specific (Olson et al. 1989; Crespi 1989).

The causes of non-random mating patterns, therefore, may be many, even for single populations. Experiments on mate choice or male-male competition with respect to male size can indicate whether or not size-assortative mating should occur, but can fall short of predicting variation in non-random mating patterns if other important variables are not assessed. Studies have not examined whether encounter rates between males and females of different sizes can account for pairing biases with respect to size in the absence of direct male-male competition, mate choice, or size-related variation in habitat choice, i.e., factors often hypothesized to produce size-assortative mating (see citations in the Introduction).

In this study, we found both a large male pairing advantage and assortative pairing by size for laboratory-reared *A. salina* despite extreme temporal and spatial overlap between males and females of various sizes. In our first experiments, we demonstrated that small males were unlikely to enter precopula with either small or large females when housed in the presence of a large male rival. This result occurred despite the fact that small males could enter precopula with females of both sizes (and were, in fact, initially collected in precopula).

Studies have shown that large males are often favored in mate competition either by their ability to win aggressive encounters with rivals, or by their superior ability in attracting, courting, and mating with females (Thornhill and Alcock 1983; Partridge and Halliday 1984). Some researchers have shown that large males displace smaller males from pairs in other species of aquatic crustaceans (e.g., Elwood et al. 1987). During our experiments, we did not observe any overt forms of male-male competition. However on occasion, two males were clasped to a single female in culture tanks, where densities reached 30–40 animals/litre. We do not know whether more subtle forms of male-male competition occur in *A. salina*, but were not observed during our experiments. However, we do know that direct intrasexual competition between males was not required to produce non-random pairing by size.

During this study, large males had higher encounter rates with females than did small males. Moreover, large females tended to have higher encounter rates with males than did small females. These results suggest that large males and large females should enter precopula sooner than other pair combinations, in the absence of direct male-male competition or female choice. We found that this was the case. Those same results suggest that large females and small males should enter precopula sooner than small females and small males. However, large females showed less 'willingness' than small females to enter precopula, at least when housed with small males. In summary, our results suggest that encounter rates between males and females of different sizes can account for most of the variation in times to enter precopula that we observed, and that behavior of large females (when housed with small males) can account for the unexpected finding that small males and large females took longer to enter precopula than any other pair combination.

In some species, large males provide more material resources than small males to mates (Thornhill and Alcock 1983). Material resources include access to feeding or oviposition sites (Thornhill and Alcock 1983), nuptial gifts (Simmons and Parker 1989) or even paternal care (Downhower and Brown 1981). Male *A. salina*, however, do not defend resources essential for female reproduction nor do they provide paternal care. No information is currently available as to whether male *A. salina* provide nutritious offerings in ejaculates, or protect females from harassment by mate-searching rivals. Thus, it is too early to determine whether females can benefit directly from mating with large males.

Reproductive benefits are predicted by mate choice hypotheses. Male mate choice is advantageous if female fecundity is size-related as it is in *A. salina* (this study) and other arthropods. Further, males should show mate discrimination whenever male investment is substantial (Gwynne 1981). Precopulatory guarding for several days is probably costly for males in several species of aquatic crustaceans. For at least some species such as *A. salina*, time to female molt should dictate the amount of male investment prior to fertilization. In at least one gammarid, time to female molt is believed to be important in determining a male's 'decision' to enter precopula (Poulton and Thompson 1987).

In the final part of our study, we demonstrated that strength of assortative pairing depended on when pairs were sampled. We showed that assortative pairing by size was not evident if pairs were sampled soon after their formation because size-mismatched pairs were present in the collection. Mismatched pairs between large males and small females formed much faster than large female: small male pairs. Although large males paired readily with large or small females, time to pair formation was longer if the female was small. By mating with large females, male *A. salina* should gain greater paternity. Yet matings with small females would be advantageous if those females were close to molt, or if few large females were available.

When pairs were sampled at the end of 7 days, we found strong size-assortative pairing. This latter protocol was quite similar to sampling of pairs from the second culture; animals from the second culture also entered precopula assortatively by size. Thus, our results suggest that time following formation of precopula can affect the likelihood of finding size-mismatched pairs. These results are important inasmuch as patterns of precopula may not reflect eventual patterns of mating. By entering precopula readily with any female, large male *A. salina* can ensure at least some reproductive success when large females are not abundant. Because pairs swim constantly, *A. salina* males in precopula should be afforded opportunity to assess the availability of other females and may simply let go of females that provide lower reproductive returns (for time spent guarding) than expected from other prospective mates.

In conclusion, our results show that several factors interact not only to determine whether size-assortative pairing occurs, but also the strength of such non-random patterns. Such variation in mating pattern is likely to be present in other crustaceans and has clear implications for selection on traits such as body size.

Acknowledgments. Research was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) operating grant to R.L. Baker and by NSERC postgraduate scholarships to the senior author. W.D. Brown, I. Robertson, the Arthropod Behavior Group at Erindale College, and two anonymous reviewers gave many helpful comments and criticisms. S. Goel provided technical assistance.

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