

The Effect of Male and Female Body Size on the Mating Behavior of Male Fairy Shrimp, *Eubranchipus neglectus*

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ABSTRACT

We studied size-related mating patterns in the freshwater fairy shrimp *Eubranchipus neglectus* in the laboratory. Males were provided with relatively small or large females that they could only sense by vision. We recorded the number of times a male pursued the female ("follows"), the duration of these follows, and the number of times a male attempted to grasp the female. Relatively large males performed more mating behavior than relatively small males. Furthermore, males (regardless of their own size) exhibited more mating behavior towards large than small females. They followed large females more frequently, attempted to grasp large females more often, and had a greater percentage of follows with a grasp attempt with large females. There was no relationship between male to female size ratio and either follows or grasp attempts. These results suggest that male *E. neglectus* have a preference for larger females; the function of this preference may be that large females have more eggs, a benefit which may help compensate for any possible costs of mating to the male.

INTRODUCTION

In crustaceans, as for many other taxa, body size is often a critical determinant of reproductive success in males and females. For example, larger, dominant male rock shrimp (*Rhynchocinetes typus*) have higher reproductive success than smaller, subordinate males because of an increased ability to displace subordinate males from females and because of females behaving such that dominant males are more likely to fertilize their eggs (Thiel and Hinojosa 2003, Thiel and Correa 2004). Similarly, large blue crab (*Callinectes sapidus*) and crayfish (*Orconectes rusticus*) males have an increased ability to displace smaller males paired with females (Berril and Arsenault 1984, Jivoff 2003). Furthermore, some species of freshwater amphipods have an increase in pairing success with increasing size (Wellborn 1995). Female size may also be important. For instance, males may benefit from mating with larger females, perhaps because these females have more eggs (e.g., Diesel, 1988, Koga 1998).

We studied size-related mating patterns in *Eubranchipus neglectus*, a freshwater fairy shrimp (Order Anostraca). Over 40 years ago, Moore and Ogren (1962) stated that there was little information on the breeding behavior in Anostraca; surprisingly, there have been few additions to the literature on this topic since that time, other than descriptions of the behavior involved (but see, e.g., Wiman 1981, Belk 1984 and 1991). Indeed, mating patterns for branchiopods as a whole are still largely understudied. Specifically, in our study we investigated the following two questions: (1) Is there a difference in the mating behaviors displayed by males towards females of different body sizes? (2) Is there a difference in the mating behaviors displayed by different-sized males toward females? Our testing procedure separated males from females and allowed us to study changes in male behavior based on the visual perception of a female; tests therefore controlled for possible chemical cues and possible changes in female behavior that might occur in response to contact with males.

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Belk (1991) described three distinct behavioral patterns displayed in sequence by male fairy shrimp prior to copulation — detection and orientation, station taking, and grasping. We found that these three behaviors were displayed by *E. neglectus* in the laboratory. When a male detects a female, he will orient toward her and line up the longitudinal axis of his body with hers. Station-taking, hereafter referred to as “following”, is when the male positions himself slightly beneath the female and follows any change in her course. Grasping is the final step prior to copulation; this involves a male rapidly extending his second antennae and clasping the female’s body trunk just anterior to her genital segments.

MATERIALS AND METHODS

Fairy shrimp were collected from a fishless, freshwater pond in Coles County, Illinois, in February and March, 2005. Individuals were separated by eye into four groups in the laboratory — large females, small females, large males and small males. Following the use of an individual in an experiment, its precise length (rostrum to anus) was determined with a digital caliper to the nearest 0.01 mm.

Each of the four groups was held in a separate tank that contained filtered water from a local freshwater pond. The containers were stored in an environmental chamber at 10° C and 9.5L:14.5D photoperiod. The shrimp were fed fish food (Hikari Tropical: First Bites) two times per day and held in the environmental chamber for at least one week prior to testing.

Our testing procedure was a modification of that described by Belk (1991). Specifically, a single female was placed in water in the bottom of a plastic Petri dish (10 mm x 95 mm). The dish was then floated in a larger glass dish (45 mm x 110 mm) containing water with a single male. Thus, the male and female were physically and chemically separated, but the clear plastic of the Petri dish allowed visual contact between them. To control for possible differences between males, we tested each male with both a small female and a large female; the order of female presentation was randomized among males.

All trials took place in the environmental chamber under the conditions described above. Twelve to 24 hours prior to testing, a female was placed in the Petri dish. Females thus had acclimated to the dish and appeared to swim normally during trials. A trial began by placing the dish with a large female or small female on the surface of the dish containing either a small male or large male, who was also placed into his dish 12 to 24 hours prior to testing. For the next 7.5 min, we observed the dishes directly and recorded the occurrence and duration of each follow by the male; we also recorded any grasp attempts that occurred during each follow. Following the first test with a female, the female’s dish was removed, and the male remained in the glass dish undisturbed for at least 30 min prior to being tested with the second female.

Data were analyzed in two ways. First, we analyzed males using just the first female with which they were tested; this gave us a range of female sizes relative to the size of the male and controlled for possible experience effects between trials of the same male. Such effects were unlikely to exist, however, because we found no significant correlations between the two trials for the one male (i.e., they appeared to respond independently to the two females; see Results). We also conducted within-male analyses to compare male behavior in response to the large and small female.

For statistical analysis, we used Statview software (v. 4.5; Abacus Concepts, 1994); we conducted nonparametric tests, with tied values taken into account when appropriate. We report all means as \pm SE. Males needed to follow a female in order to make a grasp attempt; therefore, when analyzing grasp attempts, only those males that followed a female at least once were included. Likewise, for comparisons of grasp attempts between a male's two trials, only those males that followed both females were analyzed.

RESULTS

In the first trial, 20 of 21 males followed the female (4.8 ± 0.74 follows/male, range 0–12); 19 of 21 males followed the second female (4.7 ± 0.67 follows/male, range 0–10). The number of follows was not significantly correlated between the first and second trials for a male (Spearman $r_s = 0.23$, $z = 1.05$, $P = 0.29$). Larger males tended to follow the female more frequently (Fig. 1A; $r_s = 0.43$, $z = 1.93$, $P = 0.052$) and for longer total time during the trial ($r_s = 0.40$, $z = 1.79$, $P = 0.073$) than smaller males, but both trends were of borderline statistical significance. Males followed larger females more frequently (Fig. 1B; $r_s = 0.61$, $z = 2.72$, $P = 0.006$) but not for longer total time ($r_s = 0.35$, $z = 1.57$, $P = 0.12$). No pattern was found for the ratio of male to female size and either the number of follows ($r_s = -0.14$, $z = 0.65$, $P = 0.52$) or total time following ($r_s = 0.04$, $z = 0.20$, $P = 0.84$). The same male followed the large female more frequently than the small female (mean difference 1.43 ± 0.80 follows; Wilcoxon signed rank test, $T = 46.5$, $z = 1.96$, $P = 0.05$) but not for more total time (mean difference 2.27 ± 10.4 s; $T = 89$, $Z = 0.60$, $P = 0.55$).

Males attempted to grasp the female in $50.2 \pm 0.09\%$ ($N = 20$) of follows in their first trial. Neither the number of grasp attempts nor the proportion of follows with a grasp attempt, was positively correlated between a male's two trials ($N = 18$ males; number of grasps: $r_s = 0.22$, $z = 0.90$, $P = 0.37$; % of follows with a grasp: $r_s = 0.12$, $z = 0.51$, $P = 0.61$). However, larger males tended to make more grasp attempts ($N = 20$, $r_s = 0.48$, $z = 2.08$, $P = 0.037$) and attempted grasps on a higher proportion of follows (Fig. 2A; $r_s = 0.54$, $z = 2.34$, $P = 0.019$) than smaller males. A positive correlation also existed

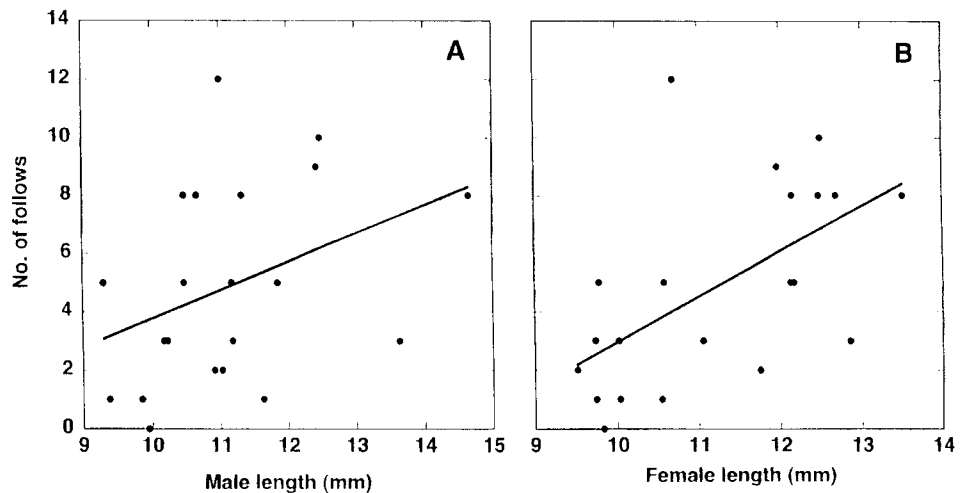


Figure 1. Number of times the female was followed by the male as related to (A) male body length and (B) female body length. Line represents a simple linear fit to the data.

between female size and both grasp attempts ($r_s = 0.57$, $z = 2.50$, $P = 0.013$) and proportion of follows with a grasp (Fig. 2B: $r_s = 0.42$, $z = 1.85$, $P = 0.064$) but only grasp attempts was significant at the 0.05 level. No significant relationship existed between male to female size ratio and either number of grasps ($r_s = -0.013$, $z = 0.057$, $P = 0.95$) or proportion of follows with a grasp ($r_s = 0.14$, $z = 0.61$, $P = 0.54$). The same male made significantly more grasp attempts (mean difference 2.8 ± 0.66 grasps; $N = 17$, $T = 10.5$, $z = 3.15$, $P = 0.0017$) and attempted grasps during a higher proportion of follows (mean difference $27.7 \pm 0.10\%$; $N = 17$, $T = 27.5$, $z = 2.32$, $P = 0.02$) toward the large female than toward the small female.

DISCUSSION

In our study, male *E. neglectus* were physically and chemically separated from females while being tested. This allowed us to determine if, based on vision alone, female size would alter male mating behavior. Our results indicated that all males, whether large or small, tended to follow large females more frequently than small females. Also, males displayed more grasp attempts and had a higher proportion of follows with a grasp attempt toward a large female than toward a small female. No relationship was found between male to female size ratio and any of our measures of male behavior.

These behavioral patterns suggest that male fairy shrimp, regardless of their own size, prefer larger females. The existence of a preference by males for large females has been found in other arthropod mating systems (Alcock and Gwynne 1987, Wen 1993, Bounduriansky and Brooks 1998, Sacki et al. 2005). One common explanation for why males prefer larger females is that female size is often positively correlated with increased egg production (Diesel 1988, Bounduriansky and Brooks 1998, Koga 1998). However, although we do not know the relationship between female size and fecundity in *E. neglectus*, Daborn (1976) found a positive relationship between female size and egg number for *Eubranchipus bundyi*. In addition, we know little about sperm storage or paternity of clutches, but males that are successful in mating may have a high probability of fertilizing the females eggs, for females may become less receptive following mating

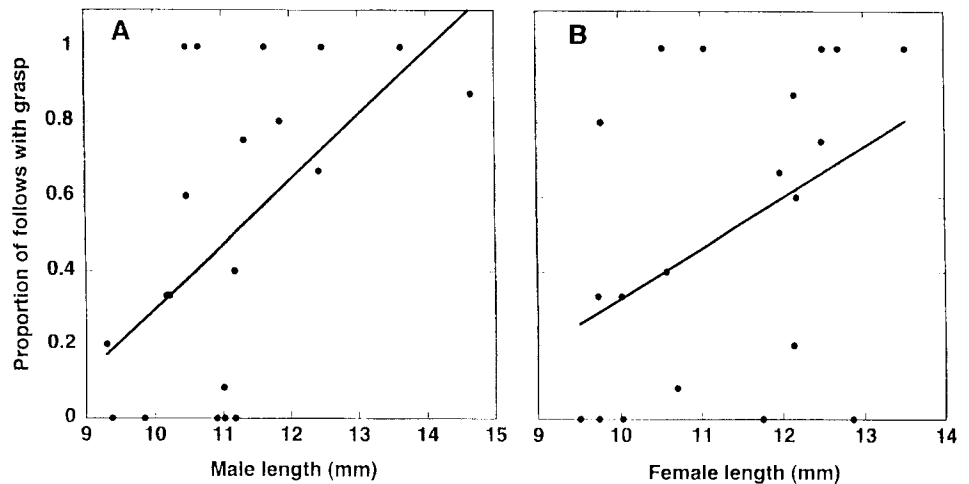


Figure 2. Proportion of times a male attempted to grasp the female when following her as related to (A) male body length and (B) female body length. Line represents a simple linear fit to the data.

in some species (e.g., Pearse 1912) and females may need to copulate prior to producing each clutch (Prophet 1963).

Regardless of the function of male choice, its existence implies that there is a cost to mating for males (Thornhill and Alcock 1983). To date, no studies of the direct costs of mating for male fairy shrimp have been conducted. Potential mating costs to the male fairy shrimp include increased predation risk (e.g., Berril and Arsenault 1984, Ward 1988), missed mating opportunities (e.g., Dickinson 1995), energy expenditure (e.g., Telford and Webb 1998), and sperm depletion (e.g., Jivoff 2003, Mesterton-Gibbons 1999).

Although benefits may exist for preferring larger females, this preference did not depend on male size. Male *E. neglectus* mating behavior (e.g., seemingly struggling with females prior to copulation) and morphology (e.g., large, modified second antennae for grasping) appear consistent with overcoming potential female resistance to mating, and thus sexual conflict may be an important component of the mating system of *E. neglectus* as well as other Anostraca. Such conflict also exists in the mating systems of other crustacean species and may affect male mating success and size-related mating patterns (e.g., Jormalainen and Merilaita 1993, Sparkes et al. 2002). In *E. neglectus*, however, it is apparent that even if such conflict exists, this conflict does not affect male preference for female size (e.g., as opposed to smaller males perhaps preferring relatively small, more easily subdued females), at least for the size range of females included in this study. Because our design intentionally controlled for male-female interactions, future studies, including those in which the success of pair formation and copulation is recorded, are clearly necessary to investigate how a male's mating preference translates to his actual reproductive success.

Finally, large males tended to follow females more frequently and for a longer total time than small males. Large males made significantly more grasps, and grasped on a higher proportion of follows, than small males. All of these trends suggest that larger males are more motivated to mate, at least in our experimental situation, than small males. This pattern cannot be because small males are simply not sexually mature and therefore unable to perform this sexual behavior; even the smallest male included in our study followed and attempted to grasp females. These results bring to mind interesting questions about the development of sexual behavior and the possible existence of size-related, alternative mating strategies in this species.

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