Mate Guarding in the Androdioecious Clam Shrimp *Eulimnadia texana*: Male Assessment of Hermaphrodite Receptivity

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Abstract

Precopulatory mate guarding primarily occurs when males encounter receptive females at a low enough rate that such females become a valuable resource once encountered. Such circumstances are common in aquatic crustaceans wherein females are only receptive for a short period directly after molting. In these species, males commonly mate guard by physically attaching themselves to their prospective mates for hours to days at a time. To be effective in mate guarding, males must be able to assess the time to receptivity in their mates, which is commonly via chemical cues associated with molting. Clam shrimp in the genus *Eulimnadia* exhibit mate guarding, but with an important variation: these species are mixtures of males and hermaphrodites (androdioecy) rather than males and females. Nonetheless, the mate guarding behaviors of these shrimp are much the same as in other aquatic crustaceans. In this study, three projects were undertaken to determine the ability of *Eulimnadia texana* males to assess hermaphroditic receptivity. Males were found to be unable to assess receptivity without physically contacting hermaphrodites. However, after physical contact, males spent a significantly greater amount of time guarding receptive relative to non-receptive hermaphrodites. Additionally, male interest in mate guarding was highest during the period between the dropping of one clutch of eggs and the extrusion of the following clutch. Because this period is also associated with hermaphroditic molting, it is consistent with the notion that males cue into chemicals associated with molting to determine hermaphroditic receptivity. These findings are consistent with previous studies of mating behavior in this species, and we discuss their importance to future tests of optimal mate guarding planned for these shrimp.

Introduction

From Darwin's time, evolutionary biologists have understood that mating behaviors are central to the fitness of most animals. Studies of mating behavior have often centered on the influence of social and ecologic environment on the development of these behaviors. For example, male reproductive behaviors evolve in response to a variety of ecologic factors, such as female distribution in space (influenced by population sex ratio and female behavior) and female receptivity in time (Emlen & Oring 1977; Reynolds 1996; Shuster & Wade 2003). Different searching behaviors, aggressive interactions, defenses of breeding sites or resources, monopolization of mates, and sperm competition are some of the behavioral strategies males can use to increase their reproductive success and thus maximize their fitness (Andersson 1994; Shuster & Wade 2003): once a possible mate has been found, males often compete with each other in order to fertilize eggs and ensure paternity.

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Precopulatory mate guarding is a male strategy to monopolize mates. It is common in aquatic crustaceans because females are often receptive only for a short time after molting (excellent review in Jormalainen 1998). It also ensures paternity for males in the presence of high male–male competition and when females are rare (unbalanced sex-ratio) or difficult to find (Olsson 1993; AlonsoPimentel & Papaj 1996; Engqvist & Sauer 2002; Prenter et al. 2003). If receptive females (i.e., females prepared for mating) are exceptionally rare, monogamy might be expected; when receptive females are very abundant, a pure searching strategy can assure higher mating success (Wickler & Seibt 1981). Mate guarding should only be selected when guarding a female would result in higher mating success than searching for other receptive females (Parker 1974).

Many theoretical models have been developed in the attempt to understand the dynamics of this mating strategy (Parker 1974; Grafen & Ridley 1983; Yamamura 1987; Jormalainen et al. 1994a; Yamamura & Jormalainen 1996; Jormalainen 1998; Härdling et al. 1999, 2004), employing a variety of different approaches including game theory, optimality models, and evolutionarily stable strategies. In fact, mate guarding is a challenging behavioral problem that involves the interaction of multiple parameters. It is a decision-making process with many facets: Is guarding worthwhile? Who should be guarded? When should guarding start? How long should a mate be guarded?

One of the first models of mate guarding was proposed by Parker (1974), who formally analyzed mate guarding purely as a 'male time investment strategy' defined as the 'optimum allocation of time' spent with a female vs. searching for other females in order to maximize the reproductive output of males. However, the maximization of male reproductive success can result in a reduction of female fitness. For example, guarded females can have their fitness reduced by the increased energetic costs associated with guarding (e.g., defensive responses to guarding), increased risk of predation, and suboptimal use of time (e.g., loss of foraging opportunities).

Noting this discordance between the sexes, Jormalainen et al. (1994a) proposed the idea that precopulatory mate guarding can be analyzed as a case of intersexual conflict. A sexual conflict is defined as '*a conflict between the evolutionary interests of individuals of the two sexes*' (Parker 1979). During reproduction, the increase in fitness of one sex does not necessarily mean an increase in fitness in the opposite sex (Daly 1978), so conflicts are then expected between mates.

In many species of crustaceans, fertilization occurs when females move their eggs into 'brood chambers' where they hold on to the fertilized eggs for some period of time (often until hatching; Jormalainen 1998). Males must pair with females during these 'windows of opportunity' to have the highest rates of mating success. When a female is nearing this egg-laying period, they are valuable resources to males, and thus guarding such receptive females should be advantageous (Jormalainen 1998). Costs and benefits of increased mating duration are often asymmetrical, and thus male and female optima may not coincide. In theory, both sexes should gain from a short-guarding time, but males undergo a high risk of losing the mating opportunity and so they are willing to guard longer (Jormalainen et al. 1994a; Yamamura & Jormalainen 1996). In crustaceans, timing of female molting determines receptivity for mating, and as this time approaches, males are maximally willing to incur costs to guard females. Because females also benefit from mating, they do not expend energy to resist males when they are close to molting. As time before molting increases, costs to females of being mate-guarded increase, and thus at some point females are willing to expend energy to avoid being guarded because prolonged guarding is expected to be costly to females (Jormalainen 1998). Essentially there are three different phases to mate guarding: (1) no initial conflict, when females are far from being receptive, (2) initiation of the contest, when males first attempt guarding, but females resist strongly, and (3) a reduction in female resistance leading to eventual uncontested mate guarding and contest conclusion as the female molt approaches and copulation takes place (Jormalainen 1998).

To be effective at mate guarding, males should be capable of assessing the level of female receptivity (Jormalainen 1998). If males can assess approximate time to receptivity, they can decide whether a particular female is valuable enough to mate guard or whether the male should continue searching for other females closer to egg laying (Parker 1974; Grafen & Ridley 1983; Yamamura 1987; Yamamura & Jormalainen 1996; Härdling et al. 2004). Such decisions are likely to be sensitive to the overall availability of receptive mates, with low levels of receptive females leading to longer mate guarding times (Jormalainen & Merilaita 1995). Thus, models of optimal mate guarding time all assume that males have some information on the timing of receptivity.

The freshwater shrimp *Eulimnadia texana* (Branchiopoda: Spinicaudata: Limnadiidae) is an excellent

species with which to test some of the above ideas. These shrimp exhibit mate guarding (Knoll 1995; Knoll & Zucker 1995; Zucker et al. 2002) with an important difference from other systems (Jormalainen 1998): males coexist with and mate guard hermaphrodites in an unusual mating system termed *androdioecy* (Sassaman & Weeks 1993; Weeks et al. 2006). The hermaphrodites are self-compatible, with the posterior portion of their ovotestis-producing functional sperm (Zucker et al. 1997). Hermaphrodites can fertilize their own eggs or outcross with males but cannot outcross with other hermaphrodites (Sassaman & Weeks 1993); only males have the appropriate appendages (termed 'claspers') that can allow pairing for sperm transfer with hermaphrodites (Knoll 1995). Males are constantly swimming, actively searching for mates. Outcrossing is most effective if sperm transfer occurs when the hermaphrodite moves the eggs from the ovotestis to the brood chamber (Weeks et al. 2004).

In *E. texana*, male–male competition is low and hermaphrodites are abundant; sex ratio is variable among populations but is always biased towards hermaphrodites (ranging from populations with no males to populations with ~40% males, average sex ratio is 25–30%; Strenth 1977; Sassaman & Weeks 1993). However, the operational sex ratio (i.e., the 'average ratio of fertilizable females to sexually active males at any given time'; Emlen & Oring 1977) is low. Hermaphrodites seem able to delay their cycle, retaining eggs in the ovotestis while waiting for a male (Zucker et al. 2002), in order to increase the likelihood of outcrossing, but hermaphrodites will successfully self-fertilize all their eggs if not in contact with a male (there is no evidence of sperm limitation in hermaphrodites; Weeks et al. 2001). Thus, even though the overall sex ratio is biased towards hermaphrodites, the operational sex ratio is still conducive to mate guarding by males.

Whether or not *E. texana* males can mate guard optimally completely depend on their ability to detect when hermaphrodites are approaching receptivity to fertilization by males (i.e., when they move eggs into their brood chamber). Thus, in our current investigations of mate guarding behavior in these clam shrimp, we will address the following questions: (1) Can males identify receptive hermaphrodites before physical contact?; (2) Can males identify receptive hermaphrodites after physical contact?; and (3) At what point of the hermaphrodite's reproductive cycle do males mate guard hermaphrodites? In this initial set of experiments, we are not addressing sexual conflict *per se*, but rather are assessing the males' ability to detect hermaphroditic receptivity. Once we confirm (or not) this ability, we can then move on to questions concerning hermaphroditic reactions to male mate guarding attempts and the trade-offs predicted between the predicted optimal guarding time for males and hermaphrodites (Jormalainen 1998).

Methods

Three separate experiments were designed to test three aspects of male mate-searching behavior. All three experiments used clam shrimp raised from resting eggs contained in soil collected from a site in Arizona (previously referred to as the 'WAL' site; Sassaman & Weeks 1993) near Portal in Cochise Co., near the base of the Chiricahua Mountains. These samples were then transported back to the laboratory at the University of Akron (Akron, Ohio). Sub-samples of soil (250 ml) were hydrated using deionized water. Hydrations were done in 37-l aquaria under 'standard' rearing conditions, which consisted of the following. Aquaria were housed in an environmentally controlled room under continuous light (Durotest sunlight-simulating fluorescent bulbs), at 25–27°C, and continuous aeration (Sassaman & Weeks 1993; Weeks et al. 1997). Shrimp were fed 20–40 ml of baker's yeast solution (0.5 g dried yeast and 0.5 g ground Tetramin fish flakes per 100 ml water) per day per aquarium, depending on the density of shrimp per aquarium. Males and hermaphrodites from these hydrations were then used for the three experiments described below.

Expt 1 – Can Males Identify Receptive Hermaphrodites Before Contact?

To determine whether males can identify receptive hermaphrodites, we used three ratios of receptive to non-receptive hermaphrodites. In this context, we refer to receptivity as a purely physiologic state: hermaphrodites with eggs in the brood chamber are not receptive while those without eggs in the brood chamber (but with eggs in the ovotestis, ready to be extruded) are receptive (Weeks et al. 2004). Eggs are readily observed both in the brood chamber and in the ovotestis in these shrimp because of their translucent exoskeleton. The three ratios were 10% receptive (2 hermaphrodites with no eggs plus 18 hermaphrodites with eggs), 20% receptive (4 hermaphrodites with no eggs plus 16 hermaphrodites with eggs), and 30% receptive (6 hermaphrodites

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with no eggs plus 14 hermaphrodites with eggs). Note that the overall sex ratio was not changed, but rather we manipulated the operational sex ratio. The operational sex ratios used were similar to those found in an experiment in semi-natural pools in the desert (Benvenuto unpublished data). The shrimp (1 male and 20 hermaphrodites) were placed into 7-l tanks filled with filtered water from their rearing aquarium. Male mating behavior was recorded from a single male per tank which was followed for 20 min. The numbers of mating encounters between males and hermaphrodites were recorded during this time. Males make physical contact with their mates, using their claspers to hold on to the carapace (clasping). Mating encounters were defined as clasping, in which the male would grasp the hermaphrodite and hold on for 5 s or more. Forty replicates of each treatment were observed for a total of 120 replicates.

The question addressed was whether males could seek out receptive (i.e., no eggs) hermaphrodites at a higher rate than they occurred in the treatments. Thus, for each male, the proportion of encounters was determined to be either greater or lesser than that expected in each of the three treatments (10, 20, and 30% receptive hermaphrodites). There were three 'exact' encounter proportions, which were all coded as less than expected (coding as greater or less than expected did not change any of the results, so less than expected was chosen to be conservative with respect to the alternative hypothesis). Chi-square analyses were then calculated using the expected frequency of >50% and <50% expected frequencies per treatment. These analyses were performed for all observations combined (across treatments) and for each of the three receptivity treatments separately. Bonferroni p-value corrections were made for the latter comparisons.

Expt 2 – Can Males Identify Receptive Hermaphrodites After Contact?

To determine whether males preferentially spent more time with receptive relative to non-receptive hermaphrodites, focal males were followed for 25 min to note their overall time spent with the two categories of hermaphrodites. Treatments were again designed to note whether relative frequency of receptive hermaphrodites had any effect on male behavior; single focal males were placed into treatments of 10, 20, and 30% receptive hermaphrodites in the same 7-l tanks with the same total density of shrimp (20 total). In order to record the duration of encounters, a simple behavioral events recording computer program was used, which allowed an observer to record all pertinent behaviors in the 25 min observation period by simply pressing preassigned computer keys when the males switched from one behavior to another. The time between key-presses was then automatically recorded into a spread sheet. Forty replicates of each treatment were observed for a total of 120 replicates.

Two general categories of mating behavior were recorded: clasping and thrusting. In the former, males used their claspers to hold on to the edge of the hermaphrodite's carapace, while in the latter males performed a prolonged clasping (which represents precopulatory mate guarding), which concluded by a 'thrusting behavior' in which the male attempted to insert his telson between the carapace valves of the hermaphrodite (Knoll 1995; Weeks et al. 2004). If thrusting is successful, males will then release sperm and leave their mate to search for another one. Thus, behaviors were grouped into four categories: (1) clasping, wherein the males would grasp the hermaphrodite's carapace for 5 s or more; (2) Type 1 thrusting, in which the male swings his telson forward unsuccessfully attempting to insert it between the two valves of the hermaphrodites' folded carapace, with quick and irregular motions; (3) Type 2 thrusting wherein the male successfully inserts his telson between the valves of the hermaphrodite's carapace and briefly holds the telson between these valves (Weeks et al. 2004); and (4) other, non-reproductive behaviors.

Total time clasping included the times for Type 1 and Type 2 thrusting because the males must be clasped to perform each of these thrusting maneuvers (Weeks et al. 2004). All four of these behaviors were noted for interactions with receptive and non-receptive hermaphrodites.

To note any differences between time spent with receptive relative to non-receptive hermaphrodites, we used a one-way MANOVA on clasping time. The main effect was receptivity treatment (10, 20, and 30% receptive hermaphrodites) and the two dependent variables compared were the relative amounts of time clasped to receptive and non-receptive hermaphrodites, respectively. To normalize residuals, time spent clasping was square-root transformed.

Type 1 thrusting was quantified as the proportion of the number of clasps per male in which this form of thrusting was observed. This proportion was again compared using the same MANOVA comparison noted above (i.e., comparing time thrusting with receptive and non-receptive hermaphrodites).

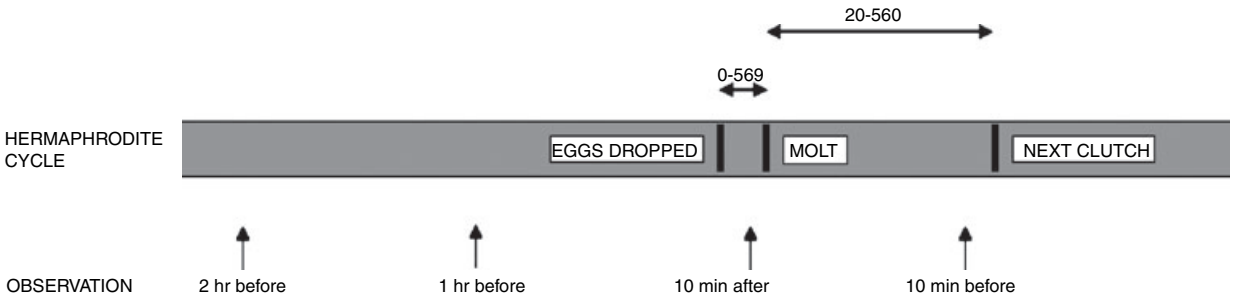

Fig. 1: Hermaphrodite reproductive cycle. Arrows represent starting point of 10 min behavioral observation periods. Black bars represent the moment of eggs dropping, molt, and movement of next clutch in the brood chamber. Drawn in scale with median values. Upper arrows indicate the range (minimum–maximum in minutes) between eggs dropping and molting and molting and moving of next clutch.

Type 2 thrusting was not compared using this analysis because no males exhibited Type 2 thrusting with non-receptive hermaphrodites.

Expt 3 – When Do Males Mate Guard Hermaphrodites?

To note when, during a reproductive cycle, males mate guard hermaphrodites, single-pair mating behavior assays were recorded. One male and one hermaphrodite were placed into 50 ml beakers with black sand backgrounds and videotaped for 24 h using a black and white video camera (Panasonic CCD) connected to a time-lapse VCR (Samsung SSC-1280 Real Time Lapse Recorder) following procedures outlined in Zucker et al. (2002). The black background enhances the contrast with the clam shrimp body, allowing better image quality in the videos. If either of the shrimp died in the 24 h observation period, the tape was not examined for mating behavior.

From Expt 2, we noted that males were most attracted to hermaphrodites without eggs (see below). Thus, the time that hermaphrodites dropped their previous clutch of eggs from the brood chamber determined the focal point of our observations of the video. In ~10% of the taped periods, hermaphrodites did not drop their eggs, and thus data from these tapes were not used in the final analysis. Taping couples for 24 h allowed us to record precisely mate guarding times and focus attention on very specific moments of the hermaphroditic reproductive cycle.

The amount of time the male was clasped to the hermaphrodite and the total number of encounters between males and hermaphrodites were quantified in four, 10 min periods: 2 h before hermaphrodite dropped eggs, 1 h before dropping eggs, the 10 min period directly after dropping eggs, and the final 10 min before the next clutch of eggs were deposited into the brood chamber (Fig. 1). In some cases, the time between dropping one egg clutch and depositing the next into the brood chamber was less than 20 min. Because we required at least 20 min between these periods (or our measurements would overlap), we did not use data gathered from those observations. Thus, we used a total of 60 tapes (i.e., 60 couples) for our analysis.

Because the data could not be normalized under any form of transformation, we used a series of non-parametric Wilcoxon signed rank matched-pairs analyses to compare the time within 10 min observation periods to the final 10 min before the hermaphrodites moved the new clutch of eggs into their brood chambers. Bonferroni corrections were made for multiple comparisons.

Results

Expt 1 – Can Males Identify Receptive Hermaphrodites Before Contact?

There were significantly fewer encounters between males and receptive hermaphrodites than expected by chance: 77 out of 120 replicates ($\chi^2 = 9.63$; $p < 0.01$) had males encountering receptive hermaphrodites in a lower proportion than found in their respective treatments. This lower encounter rate was not evenly distributed across treatments: only in the highest proportion of receptive hermaphrodites (30%) were males significantly less likely to encounter receptive hermaphrodites than expected by chance, interacting with these hermaphrodites in only 25.4% of encounters even though they were 30% of the available hermaphrodites ($\chi^2 = 12.9$;

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$p < 0.001$, which is also significant at the $p < 0.05$ after Bonferroni correction). In the 10 and 20% treatments, males encountered receptive hermaphrodites at rates expected by chance: 11.1% ($\chi^2 = 0.3$; $p > 0.05$) and 20.2% ($\chi^2 = 2.5$; $p > 0.05$), respectively.

Expt 2 – Can Males Identify Receptive Hermaphrodites After Contact?

Males clasped receptive hermaphrodites (i.e., those without eggs) significantly longer than unreceptive hermaphrodites ($F_{1,30} = 13.6$; $p = 0.0009$; Fig. 2). There was no overall effect of the proportion of receptive hermaphrodites on clasping time ($F_{2,30} = 2.2$; $p = 0.123$), nor did the time spent clasping receptive relative to unreceptive hermaphrodites differ across the three treatments ($F_{2,30} = 1.6$; $p = 0.228$).

Not only did males spend less time with unreceptive hermaphrodites (Fig. 2), but in the time they did spend with these hermaphrodites, they showed less reproductive interest: males only displayed Type 1 thrusting $4 \pm 1\%$ of the time with unreceptive hermaphrodites whereas they displayed significantly more Type 1 thrusting ($65 \pm 8\%$; $F_{1,24} = 40.9$; $p < 0.0001$) for receptive hermaphrodites. This difference did not depend on receptivity treatment ($F_{2,24} = 1.2$; $p = 0.317$). Type 2 thrusting was never seen between a male and an unreceptive hermaphrodite, but occurred in $20 \pm 6\%$ encounters with receptive hermaphrodites.

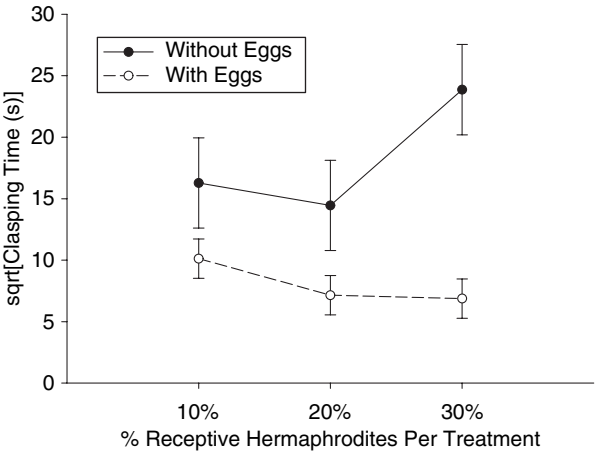

Fig. 2: Clasping times for males clasped to hermaphrodites with eggs (dashed lines and open circles) relative to hermaphrodites without eggs (solid lines and filled circles). Sample size: n = 120 (40 replicates per three treatments). Error bars portray 1 SEM.

Expt 3 – When Do Males Mate Guard Hermaphrodites?

Males guarded hermaphrodites most often during the time between dropping one clutch of eggs and transferal of their next clutch of eggs into the brood chambers (Figs 3 and 4). Both the numbers of encounters and the time spent mate-guarding showed large changes between the 1 h before dropping and the 10 min period directly after dropping

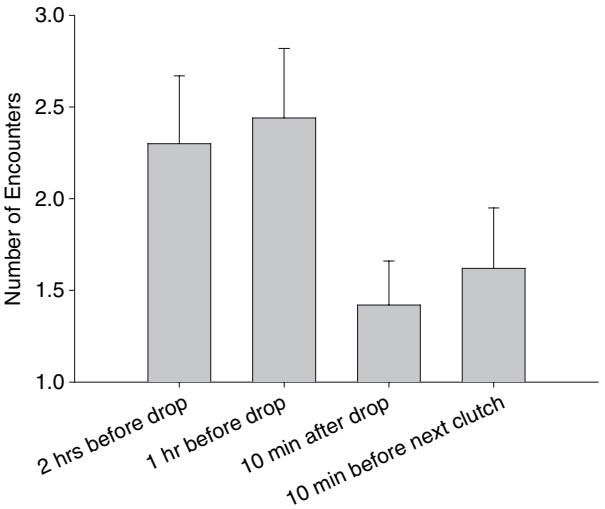

Fig. 3: Number of male encounters with hermaphrodites at various times in the reproductive cycle (see Fig. 1). Sample size: n = 60. Error bars portray 1 SEM.

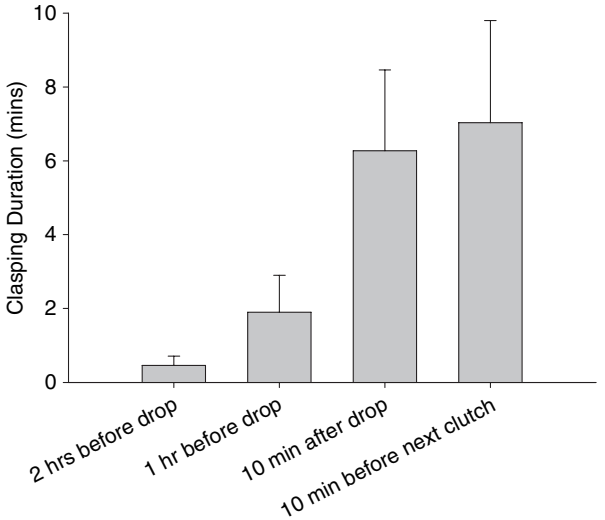

Fig. 4: Time males clasped hermaphrodites at various times in the reproductive cycle (see Fig. 1). Sample size: n = 60. Error bars portray 1 SEM.

Table 1: Non-parametric matched-pairs analyses for Expt 3

Comparison	Wilcoxon signed rank test statistic	p-value
Encounters		
2 h before drop vs. 10 min before next clutch	−106	0.057
1 h before drop vs. 10 min before next clutch	−106	0.057
10 min after drop vs. 10 min before next clutch	0.5	0.990
Claspings		
2 h before drop vs. 10 min before next clutch	292	<0.0001*
1 h before drop vs. 10 min before next clutch	225	<0.0001*
10 min after drop vs. 10 min before next clutch	54	0.302

Analysis of encounters and mate guarding by males at different times of the hermaphrodite reproductive cycle. All matched pairs were compared against the final 10 min of observation (the last 10 min before eggs were moved into the brood chamber). See text for further details. Data were summarized in Figs 3 and 4.
*$p < 0.05$ after Bonferroni corrections.

eggs (Figs 3 and 4). The two observation periods before dropping eggs (2 and 1 h) had significantly shorter mate guarding than in the final observation period before moving the clutch to the brood chamber (Table 1). The same pattern was reflected in the encounter data, but the differences were not significant (Table 1). On the other hand, the number of encounters and the time of clasping were essentially the same in the time sampled between dropping the eggs and moving the next clutch to the brood chamber (Table 1).

Molting was associated with dropping an egg clutch, and occurred in 53 of the 60 (88.3%) observation periods. Molting occurred approx. 14.9 (median) min after the clutch was dropped [range = 0.0 min (i.e., simultaneous with dropping of eggs) to 569 min]. The median time between the dropping of a clutch and the moving of the next clutch into the brood chamber was 71.6 min (range = 20.0 min to 560 min; Fig. 1).

Discussion

Precopulatory mate guarding has been described as a form of 'intersexual conflict,' in that the optimal timing of pairing before fertilization often differs between males and females (Yamamura & Jormalainen 1996; Jormalainen 1998; Härdling et al. 1999; Jormalainen & Shuster 1999; Jormalainen et al. 2000, 2001). It is often assumed that males drive the timing of mate guarding (Parker 1974; Manning 1975; Grafen & Ridley 1983 but see Jormalainen et al. 1994a,b; Yamamura & Jormalainen 1996; Jormalainen 1998; Härdling et al. 1999; Sparkes et al. 2000, 2002), but to do so, males must be able to gauge the timing to receptivity using one or more cues. Several mate guarding crustaceans cue into the chemicals released during molting, which is strongly correlated with female receptivity: in species with internal fertilization, molting allows copulation through enlarged female reproductive openings (e.g., in the isopod *Asellus*; Ridley 1983); in species with external fertilization the exoskeleton after molting is soft enough to allow egg extrusion into the brood chamber (e.g., in the amphipod *Gammarus*; Sutcliffe 1992).

In the current experiments, we have set out to assess the ability of *E. texana* males to distinguish a receptive hermaphrodite from a non-receptive one. We used three separate experiments to test whether males could assess hermaphrodite receptivity (1) without physical contact or (2) after physical contact had been made. For the latter, we also determined when in a reproductive cycle the males were most likely to mate guard a given hermaphrodite. At this stage, we were not concerned with determining the optimal mate guarding time (which is predicted to be a compromise between male and hermaphroditic optima), but were rather concerned with whether a male would initiate mate guarding or not.

Just as in many amphipods (e.g., *Gammarus*; Hartnoll & Smith 1978; Dunham et al. 1986; Sutcliffe 1992) egg fertilization in *E. texana* is external (Weeks et al. 2004). Hermaphrodites will drop their eggs (often by placing them into burrows; Zucker et al. 2002) anywhere from minutes to a few hours before they extrude their next clutch of eggs (Zucker et al. 2002). Thus, noting the presence or absence of eggs in a hermaphrodite's brood chamber is a simple, visual way to determine whether a hermaphrodite is non-receptive or nearing receptivity, respectively. In expts 1 and 2, we recorded male interest in

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hermaphrodites with or without eggs in the brood chamber. In expt 1, males did not interact with hermaphrodites without eggs (receptive hermaphrodites) at rates higher than expected by chance: males encountered receptive hermaphrodites at or below their relative frequencies in the three trials (10, 20, and 30%). This suggested that males were not specifically attracted to receptive hermaphrodites without physical contact. A similar result was found in a related branchiopod, *Daphnia pulicaria*, in which males did not discriminate between males and females without physical contact (Brewer 1998).

This finding is consistent with a previous study that found males which did not spend more time near a partition separating males from receptive hermaphrodites (Medland et al. 2000). In that project, separate trials were performed video-tracking focal animals while individuals of the opposite sex were kept in the same arena separated from the focal animals by a perforated Plexiglas barrier. This setup allowed the focal animal to receive visual and chemical stimuli without any physical contact. Hermaphrodites were found to spend more time next to the partition separating them from males, but not vice versa. Males swam at about twice the speed of hermaphrodites, but showed no specific orientation towards hermaphrodites. Medland et al. (2000) suggested that the male strategy is to increase swimming speed to contact more hermaphrodites, and receptivity is then assessed after contact, similar to that found in *Daphnia* (Brewer 1998). The authors suggested that such a strategy can work because hermaphrodites are abundant enough (60–80% of the population, Sassaman 1989; Weeks & Zucker 1999) so that merely increasing swimming speed is sufficiently effective to allow ample contact with receptive hermaphrodites.

Expt 2 revealed that after contact, males are able to recognize receptive from non-receptive hermaphrodites, spending up to four times as much time with the former relative to the latter. When *E. texana* males clasp hermaphrodites, they often move up and down the carapace using alternate claspers to grasp the carapace (S. C. Weeks, C. Benvenuto, pers. obs.). *Eulimnadia texana* males are also known to clasp briefly and then release other males (Knoll 1995), again suggesting that reproductive assessment does not occur until after physical contact. Male claspers have intricate pads and setae on the grasping portions of the appendages (Olesen et al. 1996). These structures are likely partly sensory in nature (Olesen et al. 1996) and may contain chemoreceptors allowing the males to assess the reproductive status of the hermaphrodites. Antennae could also be used to assess receptivity (S. C. Weeks, C. Benvenuto, pers. obs.). In other crustacea, males also seem to need to physically contact their mates before engaging in mate guarding. For example, contact with antennal flagella or pereopods have been described in *Palaemonetes pugio* (Caskey & Bauer 2005) and calceoli (situated in the antennae) have been found to be involved in the reproductive assessment process in *Gammarus* (Dunn 1998). Thus, although many male crustaceans can locate females using water-borne pheromone cues (Dunham 1978), the results of expts 1 and 2 suggest that male *E. texana* must contact hermaphrodites using their claspers and/or antennae to assess reproductive status. We think that a chemical cue is likely involved in this assessment, but we cannot exclude the possibility of other cues, such as physical changes in the carapace.

Although we used the presence or absence of eggs in the brood chamber as a convenient measure of receptivity in hermaphrodites (Zucker et al. 2002), we have no indication whether males use this dichotomy as a cue to receptivity. Thus, in Expt 3 we filmed interactions between males and hermaphrodites in a confined volume of water and noted when males were most likely to mate guard hermaphrodites. Mate guarding activity was, in fact, most prevalent in hermaphrodites without eggs in the brood chamber. Because of the dichotomy between the time periods before eggs were dropped from the brood chamber (first two observational periods) relative to after dropping (last two observational periods), it does seem that the presence/absence of eggs is a good surrogate of receptivity in these shrimp. Because the hermaphrodites usually molted near to the time when they dropped their clutch of eggs from the brood chamber (within ~15 min of dropping the egg mass), males may use the act of molting as a cue to mate guard. Hormones have been the cue most commonly associated with female receptivity in studies on crustaceans (Dunham 1978) even if they might not be the only stimulus involved in receptivity assessment (Dunham 1986). Molting hormone is a possible cue for amphipods (Hartnoll & Smith 1978, 1980; Borowsky & Borowsky 1985, 1987), isopods (Thompson & Manning 1981), and copepods (Kelly & Snell 1998; Kelly et al. 1998). In the branchiopods, mating is also associated with molting in cladocerans (Dumont & Negrea 2002). Thus, it is likely that *E. texana* males are detecting some chemical associated with molting to determine hermaphrodite receptivity.

In this paper, we have focused on the ability of males to assess the receptive state of their mates. This initial focus on male behavior should not be taken to imply that we assume that mate guarding is a pure male strategy to maximize fitness. Mate guarding has been proposed as an interesting case of intersexual conflict (reviewed in Jormalainen 1998) and we are aware that this conflict is determined by the interaction of both sexes. Our preliminary studies were aimed at verifying that males can assess hermaphroditic receptivity; without this male ability, further studies on optimal mate guarding would be moot. Thus, in this initial stage of exploration, we are concentrating on male interest in hermaphrodites: if hermaphrodites are not receptive, there will be no conflict (both males and hermaphrodites will not want to mate guard). It is possible that the communication between sexes at this level is purely chemical, with the lack of a chemical cue for receptivity resulting in male behavior (non-interest) that suits both males and hermaphrodites. On the other hand, if hermaphrodites are receptive, then males will show some level of interest and conflicts are then possible. For this second phase (receptivity), one needs to measure both male and hermaphroditic responses to the interactions to properly assess optimal mate guarding and the possibility of sexual conflicts (Jormalainen 1998).

In conclusion, clearly *E. texana* males are capable of assessing hermaphroditic receptivity with some precision, but only after physical contact with hermaphrodites. These observations are consistent with previous behavioral studies (Knoll 1995; Medland et al. 2000), and underscore that males should be capable of adjusting mate guarding time to optimize their opportunities for fertilizing hermaphrodites. The next step in our examinations is to observe the mate guarding behavior of these shrimp to note whether they guard optimally, as suggested in a range of other crustaceans (Jormalainen 1998).

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