

# Mate-guarding behavior in clam shrimp: a field approach

Chiara Benvenuto,<sup>a,b</sup> Brenton Knott,<sup>c</sup> and Stephen C. Weeks<sup>b</sup>

<sup>a</sup>Department of Biological Sciences, Kent State University, 256 Cunningham Hall, Kent, OH 44242-0001, USA, <sup>b</sup>Integrated Bioscience Program, Department of Biology, The University of Akron, Akron, OH 44325-3908, USA, and <sup>c</sup>School of Animal Biology, The University of Western Australia, 35 Stirling Highway, Crawley, 6009 WA, Australia

Precopulatory mate guarding is a complex behavior, influenced by many social and physiological factors, representing a case of intersexual conflict. Mate guarding has often been analyzed with the aid of theoretical models. In these models, it is commonly predicted that mate-guarding time is influenced by encounter rates between males and females, the contenders' relative sizes, and the possible interaction among males (i.e., "takeovers": one guarding male displacing another). The factors influencing male and female guarding decisions have been measured in laboratory experiments but never under natural field conditions. In this field-based study, we observed mate-guarding couples of the clam shrimp *Limnadia badia* in ephemeral pools on granite rock outcrops in Western Australia. We recorded guarding duration, focusing on the factors considered important in mate-guarding models: male and female size, population density, sex ratio, operational sex ratio, and the status of female receptivity. We also estimated time budgets for males, the possibility of male takeovers, and the potential role of female resistance. We found that female receptivity stage (how close the female was to molting), small male size, and low absolute female presence are key factors in decreasing mate-guarding duration. This study adds a field dimension to manipulative laboratory projects and theoretical models of mate guarding. We were able to observe the simultaneous interactions of multiple factors in the field and to make a robust examination of the ideas of intersexual conflict during mate guarding in these crustaceans. *Key words*: branchiopoda, density, female resistance, field observations, intersexual conflict, *Limnadia badia*, male size, sex ratio, Western Australia. [*Behav Ecol* 20:1125–1132 (2009)]

Females across a wide range of taxa are receptive to copulation for only a specific and brief period of time, and thus males maximize their chances of reproduction by guarding a potentially receptive female ("precopulatory mate guarding") and defending their primacy of access to her against other males (Parker 1974). In anurans, female receptivity is restricted to a short breeding season and "amplexus" (the "lengthy association of the sexes before actual mating," Ridley 1983) is an adaptive behavior. Similarly, copulation in many arthropods is possible for only a brief period after a female molts (Ridley 1983; Jormalainen 1998) and males closely follow premolt females (mate-attenders) or maintain physical contact with them (mate-carriers), waiting for females to become receptive (Parker 1974; Conlan 1991; Jormalainen 1998).

Precopulatory mate guarding is not just a mating strategy for males; the role of females is also important (e.g., Jormalainen, Tuomi, and Merilaita 1994; Jormalainen 1998; Sparkes et al. 2000). Females would benefit from being guarded only just before molting but males are willing to invest more time in a guarding event so that they will not lose a mating opportunity. Long guarding times can be detrimental to females who can struggle by kicking and contorting their bodies to dislodge early guarding males (Jormalainen and Merilaita 1993; Sparkes et al. 2000). Therefore, there is possibility of intersexual conflict when male and female optimal guarding times do not coincide (Jormalainen, Tuomi, and Yamamura 1994;

Jormalainen et al. 2000; Yamamura and Jormalainen 1996; Jormalainen 1998; Watson et al. 1998).

Mate guarding has been analyzed through a variety of theoretical models (Parker 1974; Grafen and Ridley 1983; Yamamura 1987; Jormalainen, Tuomi, and Yamamura 1994; Yamamura and Jormalainen 1996; Jormalainen 1998; Härdling et al. 1999, 2004). Guarding time is generally predicted to be negatively correlated with female encounter rates (Parker 1974; Jormalainen 1998). As the probability of encounters increases in the population, males should guard for shorter durations because of the higher likelihood of encountering receptive mates. This prediction is supported by studies on density (Alonso-Pimentel and Papaj 1996), sex ratio (Dick and Elwood 1996), and operational sex ratio or OSR (Iribarne et al. 1995; Alonso-Pimentel and Papaj 1996; Vepsäläinen and Savolainen 1995), defined as the "average ratio of fertilizable females to sexually active males at any given time" (Emlen 1976; Emlen and Oring 1977).

Size is another important parameter. Larger males can sustain longer guarding times (Ridley and Thompson 1979; Ward 1983; Hatcher and Dunn 1997). Alternately, larger females can vigorously resist male attempts to guard (Ward 1984; Jormalainen and Merilaita 1993). Male size can have a strong effect on both male–female interactions and on male–male competition (Elwood and Dick 1990; Jormalainen, Tuomi, and Merilaita 1994; Jormalainen 2007). In the latter case, male size may affect guarding time in differing ways depending on the possibility of takeovers. Takeovers are explicit examples of direct male–male competition, with larger males experiencing a higher OSR than smaller males because larger males can dislodge paired small males and thus can gain access to a higher proportion of receptive females than small males. Because larger males can expect to displace smaller males, they should mate guard for shorter times than smaller males (Grafen and Ridley 1983). Without takeovers,

Address correspondence to C. Benvenuto, Department of Biology, The University of Akron, Akron, OH 44325, USA. E-mail: cbenvenu@kent.edu.

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Table 1

Summary of the samples taken for behavioral observations, size ranges (males and females in precopula pairs), and physical parameters per pool

Outcrop	Pool	Couples	Volume (dm <sup>3</sup> )	Number of adults	Number of juveniles	Sex ratio <sup>a</sup>	OSR <sup>b</sup>	Mean male size (mm)	Male size range (mm)	Mean female size (mm)	Female size range (mm)
Dingo	2D	2 (4)	460.18	104	58	0.27 <sup>c</sup>	0.27 <sup>c</sup>	7.55	7.5–7.6	7.20	6.5–7.9
Dingo	6D	2 (1)	252.72	371	129	0.08 <sup>c</sup>	0.09 <sup>c</sup>	6.70	6.5–6.9	6.00	5.6–6.4
Dingo	8D	11 (8)	105.83	98	348	1.23	2.27	7.00	6.5–7.5	6.63	6.0–7.6
Holland	4H	25 (7)	446.15	435	40	1.06	1.37	6.50	5.8–7.3	6.01	5.1–6.8
Holland	10H	22 (3)	123.21	568	4	1.02	1.30	6.39	6.1–7.0	5.74	4.5–6.5
Holland	16H	15 (7)	62.34	48	368	0.92	4.55	7.75	7.0–8.6	6.88	6.3–7.4
Puntapin	9P	5 (21)	150.39	234	305	0.75	1.39	8.02	7.8–8.3	7.56	7.3–7.8
Puntapin	13P	13 (19)	398.5	17	697	1.04	1.92	7.14	6.7–7.5	6.55	6.3–6.8
Wave	8W	8 (19)	438.76	283	47	0.95	1.54	7.31	7.1–7.5	6.96	6.5–7.2
Wave	9W	11 (4)	1008.62	117	30	1.33	4.00	8.27	7.8–8.6	8.09	7.1–8.6
Wave	11W	8 (15)	18.23	82	0	1.10	8.33	8.72	8.2–9.2	7.57	7.2–8.1

The number of couples refers to those observed through the complete guarding phase. In brackets, number of couples which did not complete mate guarding during our window of observation.

<sup>a</sup> Males/females.

<sup>b</sup> Males/receptive females.

<sup>c</sup> Denotes outliers in the sex ratio estimates (observations excluded from the analysis).

mate-guarding duration again should be related strictly to relative size, with larger males guarding for longer times than smaller males (Elwood and Dick 1990).

Mate-guarding models assume the ability of males to detect different stages of female receptivity, otherwise mate guarding cannot be analyzed in an optimality context. If this is the case, males can decide whether to guard one female or search for another closer to receptivity. Unguarded females closer to molting should be more valuable because the overall guarding time will be shorter (Parker 1974; Jormalainen 1998). If all receptive females are guarded and takeovers are not successful, males with a longer guarding criterion (sensu Grafen and Ridley 1983) can obtain a fitness advantage. So the guarding time can be adjusted differently among species (because of variation in reproductive cycles) and populations (because of variation in sex ratio and density).

Many predictions of mate-guarding models have been tested through manipulative laboratory experiments, predominantly on amphipods and isopods (e.g., Dunham et al. 1986; Jormalainen 1998, 2007; Cothran 2004). We use clam shrimp (branchiopod crustaceans) which have the same advantages of other crustaceans, but also are easy to breed under laboratory conditions, have a fast life cycle (with concomitantly short mate-guarding durations), and have a female receptivity state that can be easily assessed by researchers (see Material and Methods). All of these qualities enhance their usefulness in laboratory-based studies of mating behavior (Knoll 1995; Knoll and Zucker 1995; Weeks et al. 2004; Weeks and Benvenuto 2008). Their short guarding duration also makes them ideal for field-based research. Because many other crustaceans guard for long times (from days to weeks), only a few studies have analyzed natural populations (Ward 1986; Dick and Elwood 1996; Bollache and Cezilly 2004; Sutherland et al. 2007). In these studies, mate-guarding couples were sampled in the field and then preserved or moved to the laboratory for mate-guarding analyses. Clam shrimp mate guard from minutes to a few hours, enabling direct behavioral observations to be made in the field.

To test the validity of some assumptions (possibility of takeovers; role of female resistance) of mate-guarding models and verify their predictions (influence of male and female size, population density, sex ratio, OSR, and status of female receptivity on guarding duration), we performed a field-based study

on granite rock outcrops in Western Australia, recording precopulatory mate-guarding times in *Limnadia badia* Wolf, 1911 (Spinicaudata: Limnadiidae).

## MATERIALS AND METHODS

*Limnadia badia* is a dioecious species characterized by a pyriform dorsal organ on the head, no spines on the telson, and male “claspers” bearing a sucker-like projection (Wolf 1911; Richter and Timms 2005). Claspers are dimorphic, secondary sexual characters, missing in females and not developed in juveniles, used by males to obtain and maintain physical contact with their mates. This contact is necessary for copulation to occur (Weeks et al. 2004). Once fertilized, the eggs are moved to the dorsal brood chamber of females where they are visible through the transparent carapace. Throughout this study, females were divided into 2 categories: with and without eggs in the brood chamber. This distinction is an easy way for the observer to estimate roughly female receptivity stage: Females drop their eggs on the bottom of the pool during or immediately before molting, which is a sign of receptivity to mating (Weeks and Benvenuto 2008).

Our field study was conducted on 4 granite rock outcrops, after localized rain events in April and May 2007, in the Wheatbelt area east of Perth, southwestern Western Australia: Holland Rock (Shire of Kent, 33°21.259'S; 118°44.639'E), Dingo Rock (Shire of Lake Grace, 33°0.558'S; 118°36.321'E), Wave Rock (Shire of Kondinin, 32°26.712'S; 118°53.836'E), and Puntapin Rock (Shire of Wagin, 33°19.495'S; 117°23.941'E). Rock basins (termed “gnammas” by the local Aborigines) varied in diameter, depth, population size, and sex ratio, providing a range of natural replicates (Table 1). The minimal presence of substrate at the bottom of the pools resulted in crystal clear water, ideal for focal behavioral observations. The pools were visited at subsequent phases of an inundation cycle, from 6–25 days after rainfall (Holland Rock and Dingo Rock) to 27–36 days (Wave Rock and Puntapin Rock).

Observations to assess mate-guarding duration were performed on 1–11 (mean ± standard error [SE] = 5.6 ± 0.35) couples per pool per day, which we repeated for 4–6 days (mean ± SE = 21.9 ± 2.76 total couples measured per pool). As soon as a male clasped a female, the couple was gently

moved, using a plastic pipette, to a transparent, color-coded container filled with water from the pool. Once the guarding phase was started, males held strongly onto their mates and did not release them, even when slightly disturbed via pipetting. The presence/absence of eggs in the brood chamber of the female was recorded. Each couple was checked every 15 min until the male released the female. Confining pairs in individual containers allowed for repeated observations through time and quick detection of the end of the guarding phase. Clam shrimp were then measured with calipers (maximum carapace length, in mm), marked with a permanent marker (to avoid repeated observations of the same individuals), and returned to the pool (not to alter sex ratio or density). A total of 241 couples were followed, but in 108 couples the guarding phase was not concluded by the end of the daily observations (Table 1) and these data were therefore excluded from the analysis. This excluded group contained both couples that were established later in the day as well as those that were paired for the longest duration.

On the last day of observations, we measured the area of the pool and the average water depth. All clam shrimp were then removed from the pool using a fine mesh net, counted, sexed, and returned to the pool. In order not to stress the animals and to avoid altering their behaviors, this sampling of the population was performed just once per pool after mating observations were concluded. Two pools had estimated sex ratios that were highly female-biased. Both pools had high numbers of juveniles, which can sometimes be mistaken for females with no eggs. This can happen when juveniles are large enough to be comparable in size with adults: hatching is usually simultaneous (i.e., within a 24-h period) in a pool but some shrimp may reach maturity slightly before the rest of the population so that mature and immature individuals of the same size are present concurrently; otherwise, when 2 cohorts are present at the same time in a pool, juveniles are much smaller and it is unlikely that they can be mistaken for adults. We performed Grubb's test (extreme Studentized deviate test) for sex ratio values. Both pools with highly female-biased sex ratios were detected as outliers ( $P < 0.05$ ) and thus were excluded from analyses concerning sex ratios and OSR (Table 1). Sex ratio was calculated as the number of males relative to females; OSR was calculated as the number of males relative to receptive females, considering females without eggs as "receptive" (Weeks and Benvenuto 2008). We also considered the absolute presence of males and females in the population (in percentage) that could be an indicator for males to assess the absolute availability of mates (number of females divided by the total number of individuals) or the occurrence of rivals (number of males divided by the total number of individuals). In *L. badia*, there is evidence that males require direct contact with another individual to assess if this individual is a male or a female (Weeks and Benvenuto 2008). Thus, males are assessing the entire population, and the absolute presence of mates could be an important parameter to assess the encounter rate with mates. On the last day of observations, we also measured water temperature, at 11 AM, as a reference temperature for the pool. Pool age, estimated by the date of the rain events in the area, was used as an estimate of clam shrimp age.

Focal observations (5 min long) were performed on unpaired males to assess male time budgets. We observed 116 focal males in 11 pools, for a total of 580 min. We focused on social interactions with conspecifics: The number of encounters with other individuals was recorded, as well as the sex of the interacting individuals. We also estimated the likelihood of male takeovers by isolating groups of 3 clasping animals (1 female with 2 males clasping her, always with each male

on opposite sides of the female's carapace). We gently placed the trios into isolation containers and followed the same protocol as for the regular couples noted above. When 1 of the 2 males "released" the female (or was displaced by the competing male), we then measured its size (male 1, "loser"). We waited for the couple to separate and then measured the sizes of male 2 ("winner") as well as the female. Because the trio was already formed before observations began, we did not measure when the second male joined the couple, and therefore we do not have precise data on their total mate-guarding time.

Statistical analyses were performed using JMP 7.0 (SAS Institute, Inc., Cary, NC). To meet criteria of normality, mate-guarding time was natural logarithm-transformed. We used a nested, 1-way analysis of variance (ANOVA; mixed effect model) on mate-guarding time for clam shrimp in outcrops and pools nested within outcrops. Additionally, we used a 2-way ANOVA to assess the influence of female receptivity stage and result of mate guarding (positive: successful egg fertilization and egg movement to the brood chamber; negative: the couple broke with no fertilization and no egg movement) on mate-guarding duration.

As an initial exploratory analysis of all the measured variables across all pools, we performed a stepwise multiple regression on the raw data (both the mixed- and forward-models returned the same results). We considered the dependent variable of guarding time and the following independent variables: 1) size (guarding male size, guarded female size, and relative size [i.e., size differences between sexes]), 2) number of individuals present (sex ratio, OSR, and absolute number of males and females), 3) pool age, 4) water temperature, and 5) population density (juveniles included). Of all these variables, we determined those that were significantly related to guarding time and included these in the final model. Because the input variables were grouped by population, we repeated the multiple regression analysis with mean values for populations for these variables. In order to graph each term of the regression model individually, we used 2 partial leverage plots (Sall 1990). In this way we can visualize the effect of just one factor on mate-guarding time while correcting for the effect of the other factor. To check for guarding differences among males of different size within-pools, we performed simple linear regressions by pool. We then considered the 108 couples which did not complete mate guarding during our window of observation (with the exclusion of animals from pool 2D and 6D, which were outliers for sex ratio). We compared the 2 groups (complete and not complete mate guarding during observational period) in order to assess differences in 1) size of the guarding male and 2) absolute female number in the population. Because the residuals were not normally distributed, we ran a nonparametric Wilcoxon/Kruskal-Wallis test.

We performed linear regressions to address the possible interactions between 1) body size and density; 2) male versus female size; and 3) size of guarding versus nonguarding males. A nominal logistic regression was used to interpret variation in time budgets among the different social behaviors.

Finally, we investigated the influence of relative male size on clasping duration when 2 males were clasping the same female. Because we were not able to normalize residuals, we performed a nonparametric Wilcoxon/Kruskal-Wallis test on "winner" size, "loser" size, and female size to detect any differences among the 3 groups. To identify which of the 3 groups differed from the others, we performed a pairwise Mann-Whitney test, adjusting the  $\alpha$  level using a sequential Bonferroni correction for multiple comparisons (Holm 1979). Comparing the size of all groups, we were able to test for the possibility that size affects intrasexual competition (takeovers: male winner vs. male loser size) and/or intersexual conflict (male vs. female size).

Table 2

Results of the statistical analyses performed (see Materials and Methods for details): (a) 1-way ANOVA (mixed effect model) on mate-guarding time in outcrops and pools nested in outcrops; (b) multiple regression on the predictors selected by stepwise regression for mate-guarding time (ln transformed); (c) multiple regression on mean values of significant predictors per pool for mate-guarding time (ln transformed); (d) 2-way ANOVA on mate-guarding time considering the female receptivity stage and the result of the guarding phase

	Source	SS	df	F ratio	Prob > F
(a)	Outcrop	9.0569	3	0.8665	0.4938
	Pool (outcrop)	27.3946	7	2.1144	0.0477
	Error	205.4479	111		
(b)	Guarding male size (mm)	16.881025	1	11.0459	0.0012
	Absolute female number	9.596656	1	6.2794	0.0137
	Error	160.46779	105		
(c)	Mean guarding male size (mm)	1.3638207	1	12.6522	0.0120
	Absolute female number	1.4182338	1	13.1570	0.0110
	Error	0.6467583	6		
(d)	Result of MG	10.488675	1	5.8934	0.0167
	Female receptivity stage	10.334136	1	5.8066	0.0175
	Result of MG × Fem receptivity stage	0.682772	1	0.3836	0.5369
	Error	208.22784	117		

SS = sum of squares; MG = mate guarding.

## RESULTS

### Mate-guarding time

Observed guarding durations varied from a few minutes up to 10 h (overall mean [all pools combined]  $\pm$  SE:  $163.60 \pm 15.10$  min]. We did not find any statistically significant influence of the 4 outcrops on mate-guarding duration, but there were significant differences among pools nested within outcrops (Table 2a).

Of the 10 parameters assessed in the field, only the size of guarding males and absolute number of females were significantly related to guarding time, either when considering all the individual data (Table 2b) or when considering means for populations in different pools (Table 2c). Increased male size correlated with longer mate-guarding durations (Figure 1). No significant guarding differences among males of different sizes were found within-pools (in the linear regressions for each pool,  $b$  ranged from  $-2.2$  to  $1.7$ ;  $r^2$  from  $0.003$  to  $0.15$ ; and  $P$  from  $0.10$  to  $0.93$ ), with the exception of Holland pool 4 ( $b = 2.43$ ;  $r^2 = 0.3$ ;  $P = 0.006$ ,  $n = 24$ ). The size of guarding males in this pool varied considerably (1.5 mm difference). Only one other pool, Holland pool 16, had such high variation, but all males were larger than 7 mm. Holland pool 4 was the only pool with a mixture of small and large males, and in this pool large males again guarded longer than smaller males (in accordance with the general trend among pools). Contrary to expectations, the absolute number of females

correlated positively with guarding duration (Figure 2). The significant predictors in the model were sex-specific: male size played a more important role than female size, whereas the absolute female number was more important than absolute number of males. These results were confirmed also considering the couples which did not conclude mate guarding during our observations. Couples from this group included those having the longest guarding times, even though we could not record exact guarding durations. Overall, this group had significantly larger males (mean  $\pm$  SE; male size:  $7.60 \pm 0.06$  mm vs.  $7.17 \pm 0.07$  mm;  $\chi^2 = 18.9725$ ;  $df = 1$ ;  $P < 0.001$ ) and higher absolute number of females in the population (female absolute number: 50.7% vs. 49.2%;  $\chi^2 = 8.4999$ ;  $df = 1$ ;  $P = 0.0036$ ) than the other group (which concluded mate guarding during our observational period).

As predicted, size and density values were correlated: both average male size and average female size decreased significantly as density increased (Figure 3). We performed the same analysis without Holland pool 10 and Wave pool 11, which were characterized by extremely high densities, to note whether these pools alone were driving the correlation, but we obtained similar results without these 2 pools included (male:  $b = -0.51$ ;  $r^2 = 0.1$ ;  $P = 0.0034$ ,  $n = 87$ ; female:  $b = -0.59$ ;  $r^2 = 0.11$ ;  $P = 0.0020$ ,  $n = 86$ ). Male and female sizes were positively correlated: larger males usually guarded larger females (Figure 4a). This is not a sign of size-assortative

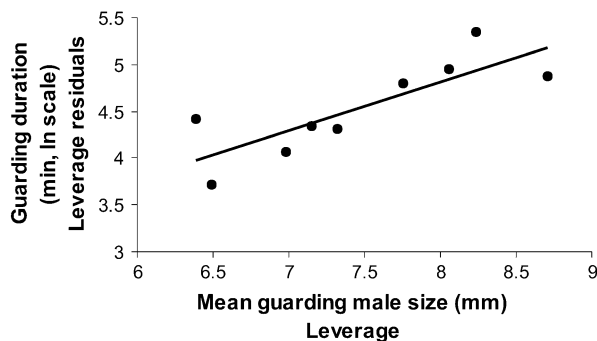


Figure 1

Leverage plot of mean guarding male size (mm) and length of mate guarding (ln transformed), controlling for the effect of absolute female number.

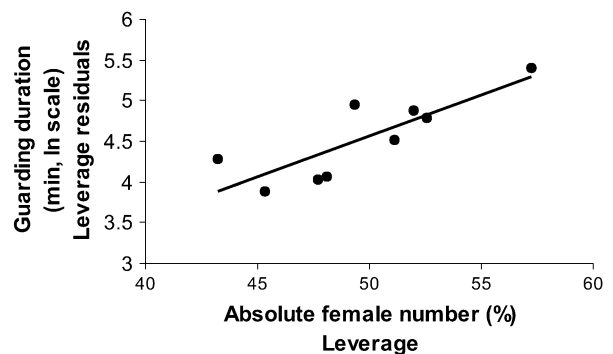
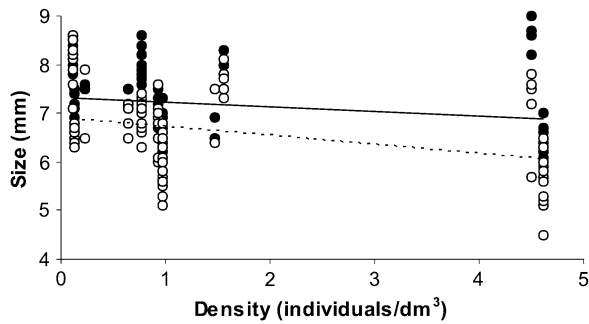


Figure 2

Leverage plot of absolute female number (%) and length of mate guarding (ln transformed), controlling for the effect of male size.



**Figure 3**

Correlation between population density (total adults/dm<sup>3</sup>) and size (mm) for males (black circles) and females (white circles). The linear regressions indicate a negative relationship between the variables for both sexes (male:  $y = -0.0982x + 7.3231$ ,  $P = 0.0277$ ,  $n = 112$ ,  $r^2 = 0.0433$ , best fit: continuous line; female:  $y = -0.1669x + 6.8708$ ,  $P = 0.0003$ ,  $n = 111$ ,  $r^2 = 0.1132$ , best fit: dotted line).

mating, however, because there was significant pool-to-pool variation in size (Figure 4b), and after analyzing size-data within each pool, we did not find any significant correlation in size between the pairs, with the exception of 1 pool out of the 11 investigated: Dingo pool 8 ( $b = 1.58$ ;  $r^2 = 0.68$ ;  $P = 0.019$ ,  $n = 11$ ).

Finally, we examined the length of mate-guarding time relative to the female receptivity stage and the result of the guarding phase (successful vs. unsuccessful mating). Receptive females, closer to molting, were subject to shorter guarding duration (Table 2d, Figure 5). Also the mating result correlated with guarding duration: successful mating events took longer than unsuccessful mating events (Table 2d, Figure 6). There was no significant interaction between female receptivity state and the result of mate guarding in these pools (Table 2d).

### Male time budgets

We recognized 7 types of social interactions: clasping a male, clasping a female with eggs, clasping a female without eggs, clasping an empty carapace (from a molt), clasping a carcass, clasping a juvenile, interacting with a paired couple, and being clasped by a male. Male time budgets varied from pool to pool (denoted with an asterisk in Figure 7): A nominal logistic regression found differences among pools for clasping males ( $P = 0.0041$ ,  $n = 116$ ), clasping females with eggs ( $P = 0.0126$ ,  $n = 116$ ), interacting with couples ( $P < 0.001$ ,  $n = 116$ ) and clasping carcasses ( $P = 0.0169$ ,  $n = 116$ ). We did not find a significant difference between the size of nonguarding males (mean  $\pm$  SE:  $7.53 \pm 0.09$  mm) and guarding males (mean  $\pm$  SE:  $7.39 \pm 0.05$  mm;  $P = 0.4440$ ,  $df = 1$ , all pools combined; also no significant differences were found when analyzing each pool individually).

### Male takeovers

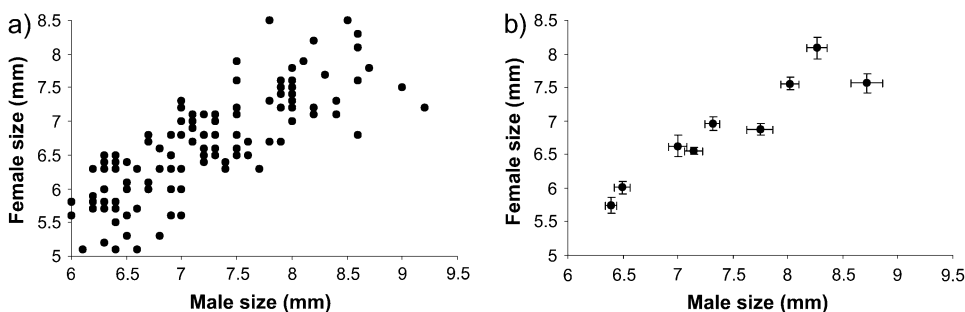
We also observed and isolated a total of 19 trios. We detected a size difference between the 3 groups (mean  $\pm$  SE; female:  $6.57 \pm 0.17$  mm; male winner:  $7.09 \pm 0.17$  mm; male loser:  $6.99 \pm 0.17$  mm;  $\chi^2 = 7.8879$ ,  $df = 2$ ;  $P = 0.0194$ ). Female size was significantly different from male winner size ( $P = 0.0129$ ,  $df = 1$ , significant after sequential Bonferroni correction), but the size of the losing male was not significantly different from the size of the winning male ( $P = 0.3553$ ,  $df = 1$ ) or the size of the female ( $P = 0.0280$ ,  $df = 1$ , non-significant after sequential Bonferroni correction).

### DISCUSSION

Precopulatory mate guarding exemplifies an intersexual interaction where males and females attempt to optimize their investment in reproduction through minimizing time and energy spent prior to copulation. The “optimal” guarding time does not coincide between the sexes: females benefit from short guarding time while males are willing to invest longer times in guarding to minimize the probability of losing a mating opportunity. Relative time investment strategies are the main focus of this study. We investigated the effect of various parameters that could influence mate-guarding time in natural populations of the clam shrimp *L. badia*. Pool age was not recognized as a significant parameter in determining the guarding strategy recorded in the pools. The influence of size of guarding male, absolute number of females, OSR, female receptivity stage, and whether the mating event was successful are discussed below, together with an analysis of male time budgets and male–male competition.

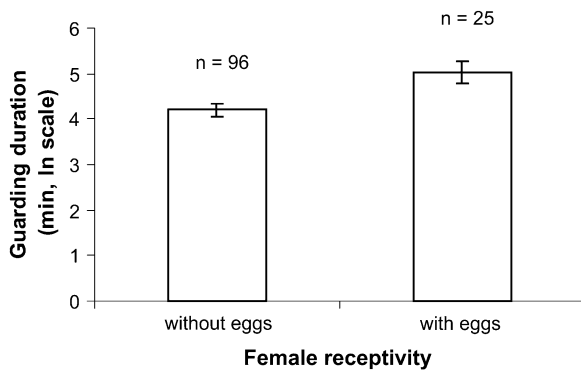
### Male size

Size (specifically relative size between the sexes, and variation in male size) is predicted to influence the guarding duration in mate-guarding models (e.g., Grafen and Ridley 1983; Jormalainen, Tuomi, and Merilaita 1994; Jormalainen 1998; Härdling et al. 2004). Relative size differences between the sexes influence the mate-guarding period in a range of Crustacea (Elwood and Dick 1990; Jormalainen, Tuomi, and Merilaita 1994; Jormalainen 2007) but we did not find evidence for a similar influence in the *L. badia* populations that we studied. Indeed, we detected neither strong size dimorphism between sexes in pools nor evidence of size-assortative mating as reported for amphipods (Dick and Elwood 1996; Bollache and Cezilly 2004), even though, if female fecundity is size related, there would be a benefit for larger males to guard larger females. Size, however, was correlated with density, consistent with competition for limited resources in such pools, as noted by Weeks and Bernhardt (2004). Mate guarding was generally longer in pools with large males and, when there was significant variation in male size within a pool, larger



**Figure 4**

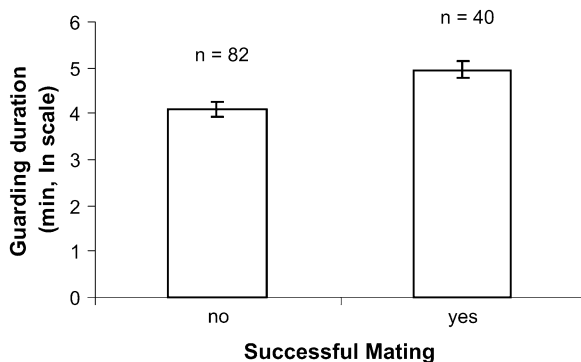
Relationship between male and female size (mm) for couples observed in mate-guarding behavior. (a) All data together; (b) pool by pool variation (mean  $\pm$  1 SE).



**Figure 5**  
Effect of female receptivity on mate-guarding duration (mean  $\pm$  1 SE).

males guarded longer than smaller males. The relationship between male size and mate-guarding duration is thus supported among pools and within pools. The significantly longer guarding time by larger males reported herein is consistent with both their ability to overcome female resistance and/or dislodge smaller guarding males (Grafen and Ridley 1983).

Male size could correlate with temperature: lower temperatures could induce larger size in individuals (Hartnoll 2001). Unfortunately, we could not assess the effect of temperature on size because water temperature was recorded during the behavioral observations when clam shrimp were already mature and not during initial growth. There could also be an interaction between water temperature and population density, but again we could not assess such a potential effect because temperature measurements were taken at adulthood. Nevertheless, the important point is that larger males guarded longer than smaller individuals; the factors mediating the differences in growth rates among males (e.g., temperature, density) were not the focus of this study. Moreover, these factors were consistent within each pool. Because we noted the influence of male size on mate-guarding duration both within and among pools, we are confident that there is a real size effect regardless of the influence of these additional factors. However, pool-to-pool temperature variation could affect female intermolt interval. If this was the case, guarding duration at lower temperatures might be partly ascribable to increased intermolt interval (the longer time the female takes to molt, the longer the guarding). We do not have information on the effect of temperature on intermolt interval in this species, but



**Figure 6**  
Effect of mating result on mate-guarding duration. Successful mating: eggs fertilized and moved to the brood chamber; nonsuccessful mating: eggs not fertilized (mean  $\pm$  1 SE).

temperature was not detected as an important factor in the stepwise multiple regression model of guarding duration, and thus the temperature variation measured in this project did not explain a significant proportion of the observed variation in guarding duration among these pools. To assess the effects of developmental temperature variation completely, a finer-scale experimental design that measured pool-to-pool variation in daily temperature throughout the course of development in these shrimp would have to be undertaken.

#### Absolute number of females

Sex ratio affects mate guarding because it is highly correlated with encounter rates (Parker 1974). Consequently, shorter guarding durations should occur in populations with a female-biased sex ratio. However, our results on *L. badia* contradict this prediction: Mate guarding was significantly longer in populations with higher absolute numbers of females. This unexpected outcome could be explained if females decreased the strength of resistance to being guarded in conditions where fewer males were available for mate guarding (to increase the possibility of being fertilized). A decrease in female resistance has been postulated in an opposite situation (high male density) in the water strider genera *Aquarius* and *Gerris* (Arnqvist 1992a, 1992b; Lauer et al. 1996), where the attention of males towards females can be very persistent. In this situation, Amano and Hayashi (1998) have proposed that it is less detrimental for a female to be guarded longer by one male than resist the continuous harassment of multiple males. Thus, in this insect species and in clam shrimp, sex ratio variation not only affects encounter rates but also likely modifies female strategies in complex ways not initially considered in early mate-guarding models (Parker 1974).

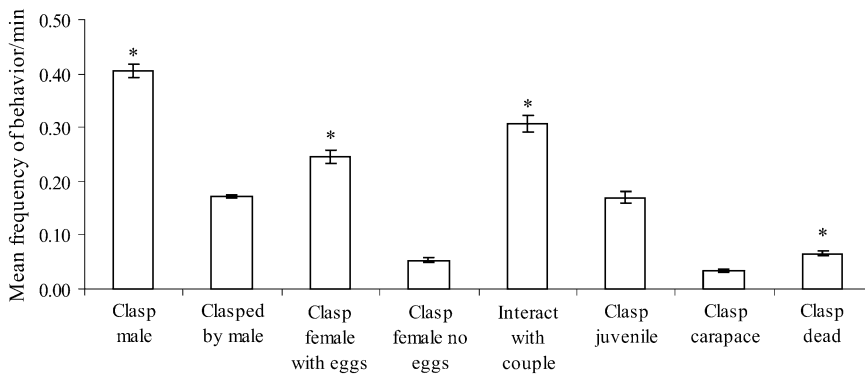
Some authors use OSR to underline the importance of the abundance of receptive females (Iribarne et al. 1995; Vepsäläinen and Savolainen 1995; Alonso-Pimentel and Papaj 1996). Although we did not find evidence of a correlation of mate-guarding time with OSR, we calculated OSR just once during the observation, assuming overall OSR does not change significantly through time. This assumption might have been wrong, or there might be no effect of OSR due to asynchrony of female receptivity in the population. Female clam shrimp start a new reproductive cycle as soon as the previous one is completed and thus reproduction is continuous during their short life cycle (Weeks et al. 1997). If OSR is continuously changing in an unpredictable way, it could be difficult for males to gauge the likelihood of the number of receptive females in the population, and thus adjust their guarding time appropriately.

#### Female receptivity

In *L. badia*, females closer to receptivity were guarded for shorter periods of time than those further from receptivity (i.e., relative to the time of egg extrusion). This is the same pattern as described for the related clam shrimp, *Eulimnadia texana* (Weeks and Benvenuto 2008). When females are closer to egg extrusion, both sexes gain advantage from a short mate-guarding phase: females because of a reduction in costs of being guarded (e.g., less interruption to their feeding) and males because of a faster turnover of mates. Thus, the conflict between the 2 sexes is reduced when mate-guarding duration is low (Jormalainen 1998).

#### Consequences of guarding

Failure to fertilize eggs resulted in shorter guarding times than when mating was successful. Whether cessation of mate



**Figure 7**  
Male time budget (mean frequency of behavior/min  $\pm$  1 SE). Asterisks denote significant difference from pool to pool.

guarding is male- or female-driven needs to be elucidated. The longer the period that a male spends guarding a female, the more valuable she should be. However, males might have a specific amount of time they will allot per mating, beyond which they may assume failure and move on to seek another mate. This guarding threshold may vary from male to male, indicating different male tactics. From the female perspective, being guarded by a “low-quality” male would reduce her genetic fitness, so there might be some sort of female choice, communicated by a specific cue or the lack of a cue (maybe behavioral), that might convince the male to start looking for another female.

#### Male time budgets

In clam shrimp, males swim constantly searching for mates (Medland et al. 2000). Their searching behavior does not seem mediated by diffusible cues in the water. They appear to need physical contact to assess the identity and receptive state of potential mates (Weeks and Benvenuto 2008). During our observations, males established a variety of contacts, ranging from clasping other males, carapaces, dead clam shrimp, and juveniles. In some pools, where overlapping cohorts were present, males clasped immature conspecifics at length (Benvenuto et al. 2009). What the cues might be for establishing a guarding phase detected through physical contact by males is still unclear. Some aquatic crustaceans, like the stream-dwelling isopod *Lirceus fontinalis*, key on chemicals released during molting to assess the receptivity state of their mates (Sparkes et al. 2000) and we are currently assessing the effect of the molting hormone as a possible cue in clam shrimp. This hormone could explain the clasping of other males, juveniles (which molt frequently), and empty carapaces (where possibly traces of the molting hormone could still be present).

#### Male–male competition

We attempted to quantify “direct” competition by observing male–male interactions in clasping trios and thereby assessing the importance of male takeovers. Two males clasping a female kick each other vigorously, attempting to dislodge the rival. Contrary to expectations, there were no significant size differences between “loser” and “winner” males. Thus, it appears that size is not the sole determining factor in deciding which male wins the takeover contest. However, we observed few trios in this field study and we cannot exclude the possibility of a sampling bias: We may have only found trios that had persisted for a comparatively prolonged time. If equally size-matched males are more likely to remain in trios for longer periods, then we may have missed the cases where larger males quickly dislodged smaller males in trios (even though we did

not detect a significant difference between mate-guarding and non-mate-guarding males). Future quantifications of male takeovers should emphasize observing trios from the initial pairings to remove this potential bias.

#### Summary

Precopulatory mate guarding is a complex behavior, involving interactions between males and females with objectives that both overlap and conflict. Males need to ensure paternity and also maximize fitness; females, when receptive, need to ensure a male for fertilization and maximize fitness. Mate guarding has been the focus of several theoretical models (Parker 1974; Grafen and Ridley 1983; Yamamura 1987; Jormalainen, Tuomi, and Yamamura 1994; Yamamura and Jormalainen 1996; Jormalainen 1998; Hårdling et al. 1999, 2004) and this field study was undertaken to assess the applicability of the modeling predictions to natural populations of clam shrimp. Our results are generally consistent with these predictions, with mate-guarding duration in *L. badia* decreasing when females were closer to receptivity and when males were small. We found shorter mate-guarding duration when female absolute abundance was low. This last result possibly provides evidence of a modulation of female resistance with variation in sex ratio. We also recorded male–male competition for females, although the underlying reasons for which male “wins” such competitions have yet to be determined. There might be greater female active involvement than resistance during the mating interaction (i.e., mate choice) but that has not been assessed in the present study. The results presented here point toward planning manipulative, laboratory-based studies designed to unravel the contribution to successful reproduction for each of these parameters in this crustacean.

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