



# Mating behaviour of male rock shrimp, *Rhynchocinetes typus* (Decapoda: Caridea): effect of recent mating history and predation risk

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When males mate so often that they risk sperm depletion, strategic allocation of sperm across multiple matings should be favoured. Recent studies have demonstrated that past mating history and predation risk can affect both male and female mating behaviour, but the interaction between these two factors has not been addressed explicitly. We allowed some socially dominant male rock shrimp, *Rhynchocinetes typus* (the robustus morph), to mate and prevented others from doing so. We then added them to an assemblage of individuals of mixed sex and social rank and gave them the opportunity to mate again in both the absence and the presence of predation risk. Recently mated males guarded females for significantly less time and they transferred significantly fewer spermatophores to these females than did unmated males. However, neither predation risk nor recent mating history affected the rate of spermatophore transfer, and there was no interaction between these variables. The results suggest that recently mated robustus males change their mating behaviour according to their perceived mating opportunities. The behaviour that we observed in robustus male *R. typus* is consistent with that of males of other species. Females and males are affected differently by mating history: with multiple matings, females become choosier and search for higher-quality mates, but males appear to conserve their reproductive resources to be able to increase mate quantity.

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Theoretically, male lifetime reproductive success should be limited only by mate-encounter rates (Simmons 2001). However, sperm is often delivered in batches (e.g. spermatophores) to the female, each with high sperm counts. These batches are limited, so males are restrained in the number of females they can inseminate (Dewsbury 1982). A male's available sperm reserves may have important consequences for its abilities in mate competition, sperm competition and fertilization success. How often and how recently a male has mated affects male reproductive behaviour in several ways, including latency before copulation (Ortigosa & Rowe 2003), sperm release (Pitnick & Markow 1994; Savalli & Fox 1999;

Kendall et al. 2002), successful pairing (Kendall & Wolcott 1999), copulation duration (Hughes et al. 2000; Ortigosa & Rowe 2003) and fertilization success (Arnqvist & Danielsson 1999; Kendall & Wolcott 1999; Savalli & Fox 1999; Kendall et al. 2002). In the water strider *Gerris buenoi*, recently mated males have shorter copulation durations than do recently unmated males (Ortigosa & Rowe 2003). Moreover, sperm count decreases in recently mated males and increases with increasing recovery period (Dewsbury 1982; Arnqvist & Danielsson 1999; Kendall & Wolcott 1999; Kendall et al. 2002). To avoid complete sperm depletion, recently mated males or males with high mating rates should invest their sperm reserves more carefully than recently unmated males or males with low mating rates (Marconato et al. 1995; Wedell & Cook 1999).

Not only mating opportunities, but also predation risk can affect male mating behaviour. Males may diminish their activities in the presence of predators (Fuller & Berglund 1996; Koga et al. 1998; Martín et al. 2003), even

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when this might have negative consequences for their mating success. Although the effects of both recent mating history and predation risk on mating behaviour are becoming evident, the interaction between them has not yet been explicitly addressed. Risk-taking behaviour with respect to access to food is negatively correlated with how recently an animal has fed (reviewed in Lima & Dill 1990; Lima 1998). If animals behave similarly with respect to access to mates, then males that have not mated recently may accept a higher predation risk to obtain a mate than do recently mated males.

We investigated the effects of both recent mating history and predation risk on the mating behaviour of male rock shrimp, *Rhynchocinetes typus*. Male *R. typus* go through several ontogenetic stages and become sexually mature in the female-like 'typus' stage. Subsequently, each male passes through several morphologically intermediate stages, collectively termed 'intermedius', before it reaches the final distinctive moult stage, the 'robustus', in which they possess highly developed chelae (first pereopods) and third maxillipeds (Correa et al. 2000, 2003). Female moulting is asynchronous, so the operational sex ratio is heavily male biased, resulting in intense mate competition (Correa & Thiel 2003). Females moult year-round, but the rate peaks during the austral spring/summer, probably because of increased water temperature, and declines during the austral winter (personal observations). Mating occurs continuously as well, but the mating frequency is greater during the summer months when females moult more frequently (personal observations). In contests for mates, dominant robustus males easily displace males of inferior rank, and then retain the female between their walking legs (the 'cage state') during the entire mating process, which may last over 100 min. Typus and intermedius males use alternative mating tactics, opportunistically and rapidly transferring spermatophores to the female, thereby creating ample opportunity for sperm competition (Correa et al. 2003). Hinojosa & Thiel (2003) found that robustus males have a much larger vas deferens (including sperm and seminal fluids) than do subordinate typus males. Moreover, one successful mating reduces the weight of the vas deferens in typus but not robustus males. In multiple mating trials, few typus males were able to complete more than two successive matings, but all robustus males completed five successive matings (Hinojosa & Thiel 2003). Experiments with *R. typus* have been conducted under controlled laboratory conditions without predators. In their natural environment, *R. typus* are eaten by a wide range of fish (Caillaux & Stotz 2003) and predation risk is high. We therefore predicted that rock shrimp would change their mating behaviour in the presence of predators to reduce predation risk.

In this study, we manipulated both predation risk and recent mating history. We predicted that recently mated males, having a perception of both high mate-encounter rates and high possibility of mating, should (1) take fewer risks when exposed to a predator and (2) have shorter mating durations and transfer fewer spermatophores than males that had not mated recently.

## METHODS

### Collection and Maintenance of Rock Shrimp

Shrimp were collected from the shallow subtidal zone of Bahía La Herradura, Coquimbo, Chile (29°59'S, 71°22'W). Females and typus males were collected using an airlift sampling device while scuba diving (Correa & Thiel 2003) or baited traps hanging from the jetty of Universidad Católica del Norte. Male robustus were collected individually by hand and kept in a handheld net during diving. Shrimp were transferred to the laboratory and sexed. Sexes were kept in separate tanks with flowing aerated water and ad libitum food supply (dead fish, ascidian colonies and crushed molluscs). Tanks containing females were checked daily for shed exoskeletons revealing newly moulted individuals. Newly moulted females were isolated and 12–36 h later were paired with a robustus male to check for receptivity. The female was considered receptive if she allowed the robustus male to seize her to form the 'cage state', in which the male confines the female between his pereopods (Correa et al. 2000). Male subjects were classified into the typus and robustus morphs using criteria established by Correa et al. (2000). To use males with the most similar mating experience, robustus males of similar age (time in robustus moult stage) were selected by the presence of epibionts and coloration of hair on chelipeds. We used the fish *Auchenionchus microcirrhis* (Labrisomidae) as the predator in our experiments. This is a relatively small, benthic fish (20–25 cm) that eats medium-sized crustaceans. *Auchenionchus microcirrhis* has been observed preying on *R. typus* in the laboratory and shrimp give strong escape responses when they see the fish. The fish was kept together with some *R. typus* in a tank with constant water flow and fed daily with dead and live shrimp.

### Experimental Set-up and Manipulation of Male Mating History

The experiment was done in two experimental tanks (L × W × H = 140 × 65 × 26 cm) that had aquaria (50 × 40 × 20 cm) in their centres. The water level was set so that there was some flow over the top rim of the aquaria. The shrimp were in the aquarium while the predator was placed in the experimental tank. Thus, the shrimp and fish were physically separated but could see each other. Four small rocks, one in each corner of the aquarium, provided refuges for the shrimp. The experiment was conducted so that predation risk (predator absence versus presence) and mating history (unmated versus mated) alternated at random in a factorial 2 × 2 design between the two tanks and aquaria. During experiments, the fish moved freely around the experimental tank. The shrimp followed the movements of the fish, directing their heads towards the fish, similar to what can be observed in the field when fish are near a group of shrimp. Occasionally, when the fish made sudden movements towards the aquarium, the shrimp reacted with escape movements. These reactions were observed throughout the experiments, but the possibility that the shrimp got

accustomed to the presence of the fish cannot be excluded.

Each experimental replicate consisted of a standard assemblage of *R. typus*: one robustus, five typus and five nonreceptive females. The 1:5:5 ratio was selected because it is close to the natural ratio found in the field during spring/summer (Correa & Thiel 2003). All typus and nonreceptive females that moulted during the 24-h acclimatization phase were removed and replaced by new individuals to maintain the original proportions (1:5:5) in the assemblage. Each typus male was marked with a differently coloured plastic tag so that we could distinguish individuals. Nonreceptive females were all marked with small white plastic pieces to distinguish them from the typus males and the receptive female. All marks were glued to the cephalothorax using fast-setting cyanoacrylate glue. These marks had no detectable effects on shrimp behaviour.

Recent mating history was manipulated by letting one group of robustus mate twice each day over four consecutive days (eight accumulated copulations), while the other group had no chance to mate (they were given nonreceptive females). Both mated and unmated robustus males were kept individually in trays ( $L \times W \times H = 35 \times 35 \times 20$  cm) with flowing sea water, and received a receptive and a nonreceptive female, respectively, once in the morning and again in the afternoon. The males given the receptive females were observed until they finished transferring spermatophores. The pair was not disturbed until the male released the female from the cage state. All females retained their eggs for at least 2 days, confirming that the eggs were fertilized. After allowing males to mate, or not, for four consecutive days, they received 2 days of rest before we used them in the experiments. Following these 2 resting days, we placed each robustus male in the central aquarium with a standard assemblage of *R. typus*, and after one additional day (i.e. 3 days after the mating manipulation ended), the group was submitted to its corresponding treatment.

### Observations of Shrimp Behaviour

Before starting a replicate, the receptive female was put into the aquarium for a 10-min acclimation period. During this time, she was held under a transparent plastic

bell cut from the top of a bottle. In replicates with predation risk, the predator was introduced into the experimental tank immediately after the receptive female was placed under the bell. The replicate started when the bell was lifted clear of the water, letting the receptive female roam freely in the glass aquarium among the other shrimp (Fig. 1, 'Release'). We took the following observations (Fig. 1).

First contact: established when the antennae of the receptive female and the robustus male touched each other.

Seizure: the moment when the robustus male seized the female and confined her between his pereopods in the so-called cage state (Correa et al. 2000).

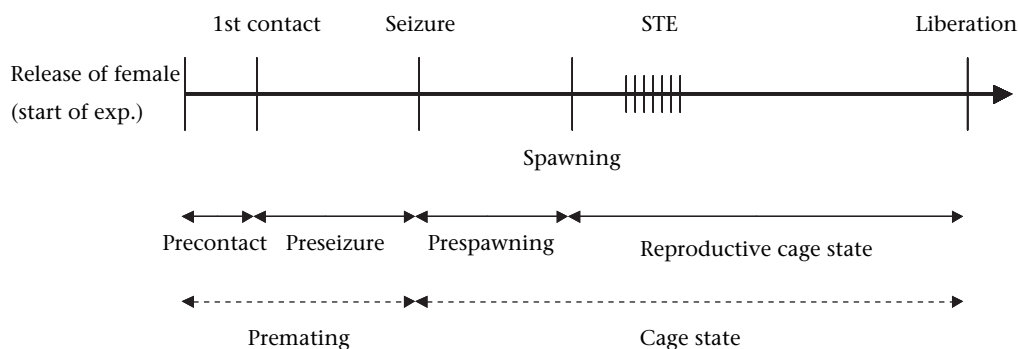
Spawning: a characteristic inward arching of all the segments of the body of the female, followed by a couple of steps backwards. Arching movements performed by the female during spawning differ from those performed in response to manipulation of the abdominal region as described in Thiel & Hinojosa (2003).

Spermatophore Transfer Events (STE): transfer of spermatophores to the female's ventral region of her abdomen.

Liberation: the moment at which the robustus male liberates the female from the cage; this normally occurs when the female has spawned the majority of her eggs.

Based on this ethogram, we calculated the relative timing of each event during a typical mating interaction (Fig. 1). Replicates were terminated either when (1) the female had initiated spawning and subsequently was liberated from the cage state by the robustus male or (2) the robustus male did not form the cage state within 60 min after making contact with the female. If a cage state was interrupted before the robustus transferred spermatophores and then formed again, we continued the observation.

During the last 5 min before the start of the experiment (i.e. after placing the female with the plastic bell in the shrimp aquarium and after adding the fish to the experimental tank), the observer sat near the tank, allowing the shrimp and the fish to become accustomed to his presence. Video recording is inadequate for imaging spermatophore transfers, because this behaviour is rapid and can be seen clearly only from an appropriate and changing angle. Therefore, we observed shrimp directly and at very close range. Although our presence did not appear to affect the shrimp, we cannot exclude this possibility.



**Figure 1.** Ethogram for the behaviours of robustus males of rock shrimp. Vertical lines on the thick, solid line indicate the relative timing of observed events. Solid lines with arrows indicate the observed time variables; the dashed lines with arrows show the corresponding definitions used by Correa et al. (2000). STE: spermatophore transfer event. Ethogram is not to scale.

Nevertheless, we observed shrimp in the same way during all treatments, so any unknown effects due to the observer would be present in all treatments.

## Data Analysis

All statistical analyses were performed using SPSS 10.0 (Chicago, Illinois, U.S.A.). If necessary, data were transformed to meet requirements for parametric tests. Only a subset of the dependent variables observed describe the behaviour of each involved shrimp stage (i.e. robustus, typus, female), so we subdivided the dependent variables into 'families' of related dependent variables (Chandler 1995). For example, the robustus behaviour was described by five dependent variables (precontact time, preseizure time, prespawning time, reproductive cage and number of spermatophore transfers), which were grouped into a family of variables. Following this concept, three families of variables were identified; the robustus family included the five dependent variables related to robustus behaviour, the typus family included the five dependent variables related to typus behaviour, and the two independent variables related to the female spawning behaviour formed the female family. For each family of variables, a two-way MANOVA was conducted, examining the general hypotheses that mating history or predation risk affected the mating behaviour of robustus males, typus males and females, respectively. Subsequently, post hoc Tukey tests were used to determine which group means differed significantly from others.

Robustus males are the most physically powerful male morph, and male competition for receptive females is intense. It is therefore likely that the duration of the cage state (from initiation of female spawning until liberation of the female; Fig. 1) is under the control of the robustus male (see also Correa et al. 2003). The duration of the cage state usually is very consistent and highly predictable (Correa et al. 2000, 2003; I. Hinojosa, T. van Son & M. Thiel, unpublished data). Accordingly, anomalous behaviour of robustus males during the cage state (e.g. very short or very long duration) was taken as an indication that the male was in poor health or condition. Hence, observations that were identified statistically as outliers (according to either SPSS 10.0, the Dixon's test or both) were eliminated from the analyses. By these criteria, observations of the behaviour of two males (one unmated robustus and one recently mated robustus, both in the presence of a predator) were eliminated from all subsequent analyses. Additionally, one replicate in which a robustus failed to seize the receptive female within 60 min after first contact had to be excluded from all analyses. This male was not only an outlier, but it also had an abnormally long preseizure time compared to other studies of rock shrimp (Correa et al. 2000; Thiel & Hinojosa 2003). A two-way ANOVA was applied to test for equal female carapace length between treatments ( $\bar{X} \pm \text{SE}$  carapace length: predation risk: absence:  $13.2 \pm 0.5$  mm; presence:  $13.2 \pm 0.4$  mm;  $F_{1,18} = 0.001$ ,  $P = 0.981$ ; mating history: unmated:  $13.8 \pm 0.5$  mm; mated:  $12.6 \pm 0.4$  mm;  $F_{1,18} = 3.455$ ,  $P = 0.079$ ). Female size can have a significant effect

on the number of STEs transferred by a robustus male (Hinojosa & Thiel 2003). However, we did not use female size as a covariate because some of the correlations in the different treatment groups were not significant, either for STEs or for one of the other dependent variables.

## RESULTS

### Behaviour of Robustus Males

In contrast to what was predicted, only recent mating history of robustus males had a significant effect on the mating behaviour of robustus males (Table 1). First contact between the robustus male and the female often occurred after typus males had transferred spermatophores to the female (17 of 21 cases). In eight of 21 cases (38%), first contact between the robustus male and the female occurred within a minute of female release (range 0.6–18 min; Fig. 2a). Several robustus males seized the female immediately after first contact had been established while others delayed seizure. This delay was most pronounced in the presence of the predator (Fig. 2b).

The robustus males guarded the female in the reproductive cage state from 74 to 176 min (Fig. 2d). Unmated robustus males held the receptive female significantly longer in the reproductive cage than did recently mated males (Table 2). The duration of the reproductive cage was shorter, but not significantly so, and tended to decrease when robustus males were exposed to predation risk (Table 2). Recently mated robustus males transferred significantly fewer spermatophores than did recently unmated robustus males (Fig. 3a, Table 2). However, there were no differences in spermatophore transfer rates (Table 2). In general, between attachment of the first spermatophore and the last, robustus males transferred about one spermatophore/min (range 0.2–3.0/min; Fig. 3b, Table 2).

### Behaviour of Typus Males

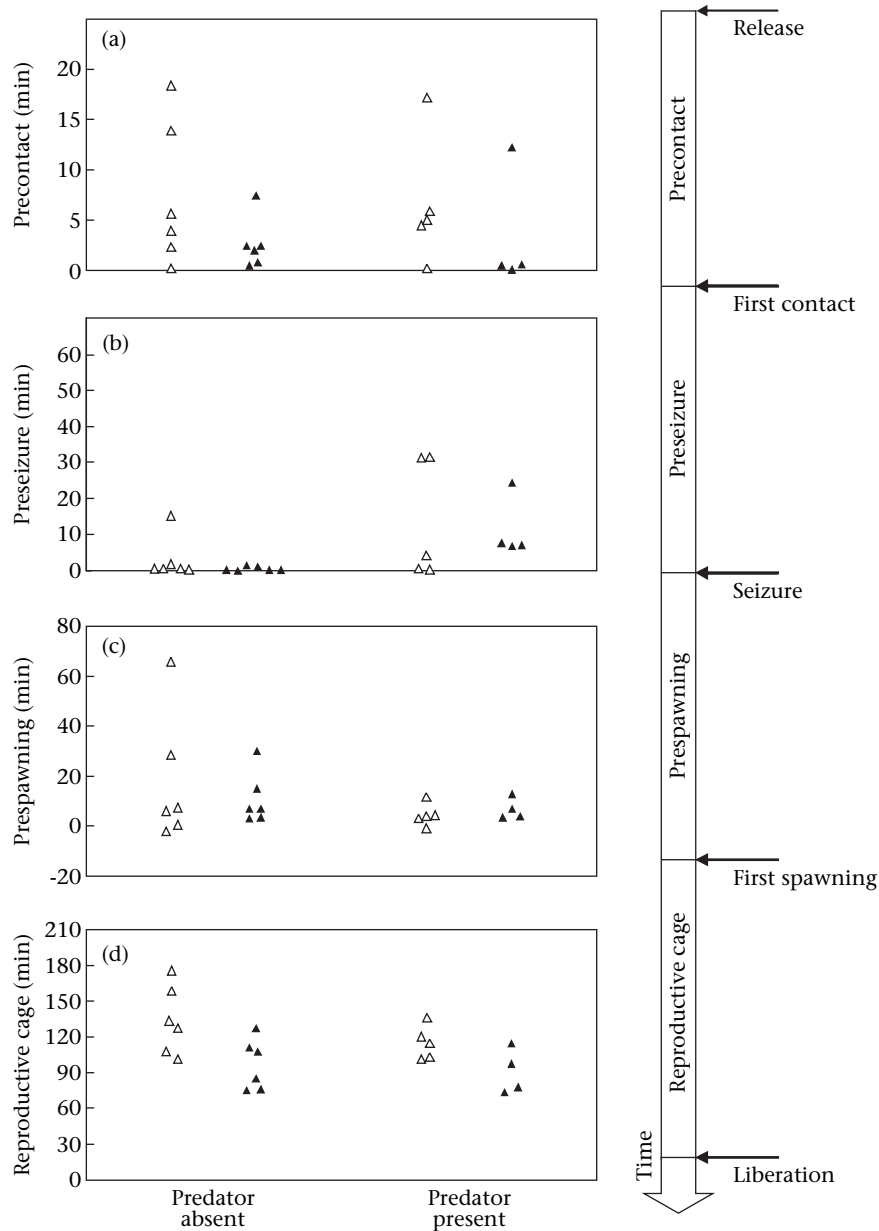
Neither predation risk nor recent mating history of robustus males significantly affected the mating

**Table 1.** Effect of predation risk and mating history on mating behaviour of robustus males, typus males and females tested by MANOVA

'Family'	Factors	$F^*$	df	$P$
Robustus	Predation risk	1.211	5, 13	0.358
	Mating history	3.036	5, 13	0.049†
	Interaction	0.150	5, 13	0.977
Typus	Predation risk	2.172	5, 13	0.121
	Mating history	0.409	5, 13	0.834
	Interaction	2.119	5, 13	0.128
Female	Predation risk	4.058	2, 16	0.038†
	Mating history	1.474	2, 16	0.259
	Interaction	0.066	2, 16	0.936

\*Pillai-Bartlett trace.

† $P < 0.05$ .



**Figure 2.** Effect of predation risk and male mating history on (a) precontact time, (b) preseizure time, (c) female prespawning time and (d) time in reproductive cage. Triangles represent individual responses (open: unmated robustus, filled: mated robustus). Ethogram is not to scale.

behaviour of the typus males (Table 1). Typus males usually were first to contact (touch with their antennae) the receptive females, typically within 30 s of the release of the female (range 5 s–3 min). At the moment of first contact, most typus males detected that the female was receptive. They seized the female and quickly transferred several spermatophores to her abdomen. The total number of spermatophores transferred by all typus males to the receptive females was affected neither by predation risk nor by recent mating history of robustus (range 0–45 spermatophores accumulated among typus; Table 2). After termination of spermatophore transfer, and if not disturbed by other males, typus males often maintained the female in the cage state and behaved as do robustus males

when in the cage state. The percentage of typus achieving the cage state ranged from 0 to 80%.

### Female Spawning Behaviour

Predation risk significantly affected the spawning behaviour of females (Table 1). However, no effect was detected for recent mating history of robustus males, nor was there an interaction. The females initiated spawning independent of the treatments to which they were exposed. Most females (16 of 21 females; 76%) initiated spawning within 30 min of their release (range 2–72 min). In contrast to the MANOVA, a post hoc Tukey test showed no significant effect of predation risk on

**Table 2.** Mean  $\pm$  SE effect of predation risk and recent mating history of robustus males on observed variables of robustus males, typus males and females

Dependent variable	Predation risk			Recent mating history		
	Absence	Presence	<i>P</i> *	Unmated	Mated	<i>P</i>
<b>Robustus</b>						
Precontact (min)	5.0 $\pm$ 1.6 (12)	5.2 $\pm$ 2.0 (9)		7.0 $\pm$ 1.9 (11)	3.0 $\pm$ 1.2 (10)	0.135
Preseizure time (min)	1.8 $\pm$ 1.2 (12)	12.6 $\pm$ 4.2 (9)		7.8 $\pm$ 3.8 (11)	5.0 $\pm$ 2.4 (10)	0.592
Reproductive cage (min)	116 $\pm$ 9 (12)	104 $\pm$ 7 (9)		125 $\pm$ 7 (11)	95 $\pm$ 6 (10)	0.006†
STE	5.9 $\pm$ 0.7 (12)	6.0 $\pm$ 0.6 (9)		6.9 $\pm$ 0.7 (11)	4.9 $\pm$ 0.5 (10)	0.046†
STE per minute	1.1 $\pm$ 0.2 (12)	0.9 $\pm$ 0.1 (9)		0.9 $\pm$ 0.2 (11)	1.2 $\pm$ 0.2 (10)	0.402
<b>Typus</b>						
Precontact (min)	0.5 $\pm$ 0.2 (12)	1.0 $\pm$ 0.3 (9)		0.6 $\pm$ 0.2 (11)	0.8 $\pm$ 0.3 (10)	
STE	13.2 $\pm$ 3.0 (12)	12.4 $\pm$ 3.6 (9)		12.8 $\pm$ 3.7 (11)	12.9 $\pm$ 2.5 (10)	
Percentage involved in STE	32 $\pm$ 8 (12)	33 $\pm$ 8 (9)		29 $\pm$ 7 (11)	36 $\pm$ 9 (10)	
Time in cage (min)	3.8 $\pm$ 2.1 (12)	6.1 $\pm$ 2.1 (9)		6.1 $\pm$ 2.6 (11)	3.4 $\pm$ 1.5 (10)	
Percentage involved in cage	12 $\pm$ 4 (12)	30 $\pm$ 8 (9)		20 $\pm$ 6 (11)	20 $\pm$ 7 (10)	
<b>Female</b>						
Initiation of spawning (min)	21.3 $\pm$ 5.8 (12)	23.3 $\pm$ 3.0 (9)	0.280	26.5 $\pm$ 5.9 (11)	17.3 $\pm$ 3.0 (10)	
Prespawning time (min)	14.4 $\pm$ 5.5 (12)	5.5 $\pm$ 1.4 (9)	0.403	11.7 $\pm$ 6.0 (11)	9.4 $\pm$ 2.6 (10)	

Numbers in parentheses refer to the number of subjects included in the analysis. STE: spermatophore transfer event.

\**P* values are shown only for independent variables that were significant in MANOVA.

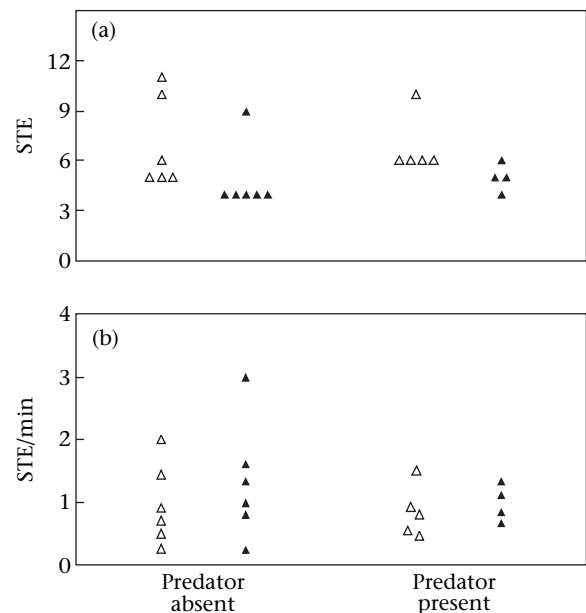
†*P* < 0.05.

initiation of female spawning (Table 2). On two of 21 occasions (10%; one in the absence and one in the presence of a predator, both times in the treatment with recently unmated robustus), the receptive female started to spawn before the robustus male seized her to form a cage state, as indicated by negative values for prespawning time (Fig. 2c). Prespawning duration (from seizure of female by the robustus until initiation of spawning) was, in general, shorter than 10 min (15 of 21 cases; 71%; range 2–66 min). Also for prespawning duration, the Tukey test failed to show an effect of predation risk (Table 2).

## DISCUSSION

Recently mated robustus males guarded the female in the cage state for less time and transferred fewer spermatophores than did unmated robustus. This difference could mean that mated males' sperm reserves had been depleted, as has been shown for recently mated males of other organisms (Pitnick & Markow 1994; Savalli & Fox 1999; Kendall et al. 2002). However, mating history did not affect the number of spermatophore transfers per minute, as expected if repeated mating depletes reserves of stored sperm. As a consequence, an alternative explanation may be that recently mated males perceive a relatively high abundance of receptive females and thus apportion fewer spermatophores to each so that they have enough sperm to fertilize the eggs of relatively many females. Such judicious apportionment has been observed in males of snow crabs, *Chionoecetes opilio* (Rondeau & Sainte-Marie 2001), the butterfly *Pieris rapae* (Wedell & Cook 1999) and the bucktooth parrotfish, *Xyrichthys novacula* (Marconato et al. 1995). After initiation of mating, predation risk

had no further effect on the number of spermatophore transfers, or the duration of prespawning time or the cage state. The results of our study thus suggest that, in dominant rock shrimp, male investment during a mating (time and spermatophores) is not only influenced by female requirements (Hinojosa & Thiel 2003) but also by their chances for future matings.



**Figure 3.** Effects of predation risk and mating history of robustus on (a) spermatophore transfer events (STE) by robustus and (b) STE per minute by robustus. Triangles represent individual responses (open: unmated robustus, filled: mated robustus).

### Effect of Recent Mating History on Mating Behaviour of *Robustus* Males

The shorter time in the cage state by mated robustus males of *R. typus* is consistent with a recent study on the effect of mating history on copulation duration in the water strider *Gerris buenoi*. Ortigosa & Rowe (2003) found that mated males of *G. buenoi* had shorter copulation durations than did unmated males. Copulation time is affected by mate-search time (Parker & Stuart 1976), and hence different operational sex ratios also affect copulation or guarding times (Vepsäläinen & Savolainen 1995; Jirotkul 1999; Wada et al. 1999). Recently mated males may reduce mating time because they perceive that females are relatively abundant. Hence, they may reduce the time and resources allocated to each female in favour of interacting with potentially many. This hypothesis may explain why recently mated robustus males in our study tended to be more 'eager' to mate than were recently unmated robustus males. Furthermore, the decrease in duration of the cage state and number of spermatophore transfers by mated robustus may be a result of their altered perception of mate-search time and mating opportunities (Ortigosa & Rowe 2003). Recently mated robustus males should have a perception of much lower mate-search time than recently unmated robustus males and should alter their behaviour accordingly. In our study, robustus males' perception of mating opportunities, mate competition and mate-search time may have been fixed during the preconditioning phase, when they were offered multiple receptive females, and probably was still the same during the experimental phase. Alterations of the sociosexual context (sensu Rondeau & Sainte-Marie 2001) and their consequences have also been observed and discussed for the hermit crab, *Pagurus middendorffii* (Wada et al. 1999) and the snow crab (Rondeau & Sainte-Marie 2001). Both the hermit crab and the snow crab decrease their guarding time with increasing perception of mating opportunities (i.e. increasing mate-encounter rates).

Studies of several species report a decrease in sperm reserves in males that had recently mated (Pitnick & Markow 1994; Savalli & Fox 1999; Kendall et al. 2002). However, the consequence of sperm depletion and the strategies to avoid it should differ between species. Behavioural adaptations to avoid sperm depletion may be more developed in species where both multiple matings and female promiscuity are normal and frequent. Pitnick & Markow (1994) reported different sperm depletion rates in related *Drosophila* species, and found that male ejaculate size was negatively related to female remating. The avoidance of sperm depletion is likely to be an adaptive strategy for dominant individuals (Marconato & Shapiro 1996; Wedell & Cook 1999). For example, dominant robustus males of *R. typus* are able to mate successfully once every day over five consecutive days, but the mating success of the subordinate typus males drops dramatically with successive matings (Hinojosa & Thiel 2003). Moreover, females of *R. typus* experience less fertilization success when inseminated by recently mated typus males, but not when mated with recently mated robustus males (Hinojosa 2001). In species where

some males have a higher chance for multiple matings, strategic sperm expenditure and avoidance of complete sperm depletion appear to be common (Shapiro et al. 1994; Marconato et al. 1995; Marconato & Shapiro 1996; Alonzo & Warner 2000; Hinojosa & Thiel 2003). Although we cannot exclude the possibility that mated robustus males had diminished sperm supplies, these males have both a morphophysiological adaptation (large testes) and a behavioural adaptation (strategic spermatophore allocation) to minimize the risk of sperm depletion (Hinojosa & Thiel 2003). Such sperm economy may maximize male fitness at the cost of female interests and fitness (Marconato et al. 1995).

### Effect of Predation Risk on Mating Behaviour of *Robustus* and *Typus* Males

Numerous studies have shown that predation risk may affect reproductive behaviours in several ways (reviews in Lima & Dill 1990; Magnhagen 1991; Sih 1994; Lima 1998; Taborsky & Foerster 2004). Mate searching may be especially risky because it usually requires higher activity levels (Magnhagen 1991). In the present study, recently mated robustus males showed a tendency to delay seizure of the female, possibly because they perceived a high possibility of finding another when no predator was around. Robustus males might adjust their risk-taking behaviour in the presence of a predator. Other studies have also shown that organisms are capable of adjusting their risk-taking behaviour according to perceived predation risk in both mating decisions (e.g. Magnhagen 1990; Kålås et al. 1995; Fuller & Berglund 1996; Koga et al. 1998; Martín et al. 2003) and foraging decisions (e.g. Sih 1980; Rochette & Himmelman 1996; Dill & Fraser 1997).

The time that robustus males spent in the cage state was not affected by the presence of a predator. This result suggests that males, after having decided to take the risk to engage in a mating, invested enough time to ensure that they fertilized the female's clutch without exposing her to other males and the consequent risk of sperm competition. However, these results are not consistent with those of other studies. For example, the water strider *Gerris remigis* decreases its copulation duration when exposed to increased predation risk (Sih et al. 1990). Similarly, Maier (1996) reported variable mating durations in cyclopoid copepods. Species living close to the water surface had shorter mating durations than did species living deeper in the water column. Maier (1996) proposed that fast mating close to the water surface, where there is more light and the animals are more visible to their predators, might decrease their risk of predation. These observations suggest that the predation risk of a mating couple differs between species and habitat. A robustus male holding a female in the reproductive cage in its benthic habitat might be exposed to less predation risk than would a species mating in the water column or at the water surface.

The mating behaviour of the robustus males could also be affected by the behaviour of typus males, who are

usually the first to contact both females and resources such as food. As a consequence, robustus males often are visually attracted to females and food by the tumults that the typus males form around these resources (sensu Díaz & Thiel 2004). Thus, the presence and behaviour of typus males may have induced robustus males to accept higher risk so as not to lose the reproductive opportunity presented to them, as had been suggested for dominant lizards (Martín et al. 2003).

## Conclusions

Males in this study appeared to be more reluctant to initiate the mating process in the presence of a predator, but once a commitment had been made to a given female,

predation risk had no effect on their subsequent investment. A male's recent mating history had little influence on the initiation of mating, but affected the investment in a mating. Recent studies have shown that mating history may change reproductive behaviour in both females and males in several ways (Table 3). Recently mated *R. typus* males showed mating behaviours similar to those reported in recently mated males of other organisms. In many species, males appear to reduce mating time and sperm release during multiple matings. Females and males appear to be affected differently by mating history (Table 3). Females often become choosier and seek high-quality mates when they mate multiply. Males appear to reduce the resource allocation per mating and instead increase their number of mates. In females, this shift in mating

**Table 3.** Recent studies examining changes in mating behaviour in response to recent mating history

Species	Sex*	Matings†	Effect of multiple matings	Source
Isopod ( <i>Lirceus fontinalis</i> )	M	1–2	Decreased fertilization success	Sparkes et al. 2002
Beetle ( <i>Callosobruchus maculatus</i> )	M	1–4	Decreased fertilization success, sperm release	Savalli & Fox 1999
Beetle ( <i>Leptinotarsa decemlineata</i> )	M	1–6	Decreased copulation duration	Radtke & Rutowski 2002
Moth ( <i>Helicoverpa armigera</i> )	M	0–3	Decreased fertilization success	Hou & Sheng 1999
Moth ( <i>Zeiraphera canadensis</i> )	M	0–5	Decreased sperm release, fertilization success	Carroll 1994
			Increased preoviposition period (F)‡	
Moth ( <i>Plodia interpunctella</i> )	M	1–3	Decreased sperm release	Cook & Gage 1995
Fruit flies ( <i>Drosophila</i> spp.)	M	1–9	Decreased sperm release	Pitnick & Markow 1994
Blue crab ( <i>Callinectes sapidus</i> )	M	1	Decreased sperm release, vas deferens	Kendall et al. 2001, 2002
Shrimp ( <i>Rhynchocinetes typus</i> )	M	1–5,8	Decreased sperm release, vas deferens, copulation duration	Hinojosa & Thiel 2003; this study
Butterfly ( <i>Pieris</i> sp.)	M	1–2	Decreased sperm release	Bissoondath & Wiklund 1996
Butterfly ( <i>Jalmenus evagoras</i> )	M	1–4	Decreased sperm release, increased copulation duration	Hughes et al. 2000
Water strider ( <i>Gerris buenoi</i> )	M	≥ 1	Decreased premating time, copulation duration	Ortigosa & Rowe 2003
Spider ( <i>Latrodectus hasselti</i> )	M	1–3	Decreased courtship duration	Andrade & Banta 2002
Beetle ( <i>Photinus ignitus</i> )	F	1,3	Increased fecundity	Rooney & Lewis 2002
Beetle ( <i>Ellychnia corrusca</i> )	F	1,3	Increased fecundity	Rooney & Lewis 2002
Beetle ( <i>Tenebrio molitor</i> )	F	1,2,5	Increased fecundity	Worden & Parker 2001
Beetle ( <i>Callosobruchus maculatus</i> )	F	1–4	Increased fecundity and longevity	Savalli & Fox 1999
Beetle ( <i>Adalia bipunctata</i> )	F	2	Increased rejection behaviour	de Jong et al. 1998
Water strider ( <i>Gerris buenoi</i> )	F	≥ 1	Increased rejection behaviour	Ortigosa & Rowe 2003
Spider ( <i>Micrathena gracilis</i> )	F	1–2	Decreased copulation frequency (M)§	Bukowski & Christenson 2000
Spider ( <i>Gasteracantha cancriformis</i> )	F	1–2	Decreased copulation frequency, sperm investment (M)§	Bukowski et al. 2001
Cricket ( <i>Gryllus integer</i> )	F	1	Decreased phonotactic response (movement and direction)	Lickman et al. 1998
Moth ( <i>Helicoverpa armigera</i> )	F	1–3	Increased fecundity, decreased longevity	Hou & Sheng 1999
Moth ( <i>Heliothis virescens</i> )	F	1–2	No effect on sperm storage in spermatheca	LaMunyon 2000

\*The sex having mating history manipulated.

†The number of matings to which an individual was exposed.

‡F = female was affected by male mating history.

§M = male was affected by female mating history.

behaviour is expressed through increased rejection behaviour and decreased receptivity towards male courting. Males, on the other hand, decrease both sperm release and copulation duration. This observed shift in recently mated males might be a result of a change in perception of mating opportunities (e.g. Rondeau & Sainte-Marie 2001; Ortigosa & Rowe 2003; this study), the depletion of sperm reserves (e.g. Arnqvist & Danielsson 1999; Kendall et al. 2002) or a combination of both.

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### References

- Alonzo, S. H. & Warner, R. 2000. Allocation to mate guarding or increased sperm production in a Mediterranean wrasse. *American Naturalist*, **156**, 266–275.
- Andrade, M. C. & Banta, E. M. 2002. Value of male remating and functional sterility in redback spiders. *Animal Behaviour*, **63**, 857–870.
- Arnqvist, G. & Danielsson, I. 1999. Postmating sexual selection: the effects of male body size and recovery period on paternity and egg production rate in a water strider. *Behavioral Ecology*, **10**, 358–365.
- Bissoondath, C. J. & Wiklund, C. 1996. Effect of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). *Functional Ecology*, **10**, 457–464.
- Bukowski, T. C. & Christenson, T. E. 2000. Determinants of mating frequency in the spiny orbweaving spider, *Micrathena gracilis* (Araneae: Araneidae). *Journal of Insect Behavior*, **13**, 331–352.
- Bukowski, T. C., Linn, C. D. & Christenson, T. E. 2001. Copulation and sperm release in *Gasteracantha cancriformis* (Araneae: Araneidae): differential male behaviour based on female mating history. *Animal Behaviour*, **62**, 887–895.
- Caillaux, L. M. & Stotz, W. B. 2003. Distribution and abundance of *Rhynchocinetes typus* (Crustacea: Decapoda), in different benthic community structures in northern Chile. *Journal of the Marine Biological Association*, **83**, 143–150.
- Carroll, A. L. 1994. Interactions between body size and mating history influence the reproductive success of males of a tortricid moth, *Zeiraphera canadensis*. *Canadian Journal of Zoology*, **72**, 2124–2132.
- Chandler, C. R. 1995. Practical considerations in the use of simultaneous inference for multiple tests. *Animal Behaviour*, **49**, 524–527.
- Cook, P. A. & Gage, M. J. 1995. Effects of risks of sperm competition on the numbers of eupyrene and apyrene sperm ejaculated by the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). *Behavioral Ecology and Sociobiology*, **36**, 261–268.
- Correa, C. & Thiel, M. 2003. Population structure and operational sex ratio in the rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea). *Journal of Crustacean Biology*, **23**, 849–861.
- Correa, C., Baeza, J., Dupré, E., Hinojosa, I. & Thiel, M. 2000. Mating behavior and fertilization success of three ontogenetic stages of male rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea). *Journal of Crustacean Biology*, **20**, 628–640.
- Correa, C., Baeza, J. A., Hinojosa, I. A. & Thiel, M. 2003. Male dominance hierarchy and mating tactics in the rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea). *Journal of Crustacean Biology*, **23**, 33–45.
- Dewsbury, D. A. 1982. Ejaculate cost and male choice. *American Naturalist*, **119**, 601–610.
- Díaz, E. R. & Thiel, M. 2004. Sexual communication and mating system in the rock shrimp *Rhynchocinetes typus* (Crustacea, Decapoda). *Biological Bulletin*, **206**, 134–143.
- Dill, L. & Fraser, A. H. 1997. The worm returns: hiding behavior of a tube-dwelling marine polychaete, *Serpula vermicularis*. *Behavioral Ecology*, **8**, 186–193.
- Fuller, R. & Berglund, A. 1996. Behavioral responses of a sex-role reversed pipefish to a gradient of perceived predation risk. *Behavioral Ecology*, **7**, 69–75.
- Hinojosa, I. 2001. Habilidad de apareamientos múltiples de los machos y elección criptica de la hembra en el camarón de roca, *Rhynchocinetes typus* Milne Edwards 1837 (Crustacea: Decapoda: Rhynchocinetidae). Tesis – Biólogo Marino, Universidad Católica del Norte.
- Hinojosa, I. & Thiel, M. 2003. Somatic and gametic resources in male rock shrimp, *Rhynchocinetes typus*: effect of mating potential and ontogenetic male stage. *Animal Behaviour*, **66**, 449–458.
- Hou, M. & Sheng, C. 1999. Fecundity and longevity of *Helicoverpa armigera* (Lepidoptera: Noctuidae): effects of multiple mating. *Journal of Economic Entomology*, **92**, 569–573.
- Hughes, L., Siew-Woon Chang, B., Wagner, D. & Pierce, N. E. 2000. Effects of mating history on ejaculate size, fecundity, longevity, and copulation duration in the ant-tended lycaenid butterfly, *Jalmenus evagoras*. *Behavioral Ecology and Sociobiology*, **47**, 119–128.
- Jirotkul, M. 1999. Operational sex ratio influences female preferences and male–male competition in guppies. *Animal Behaviour*, **58**, 287–294.
- de Jong, P., Brakefield, P. M. & Geerinck, B. P. 1998. The effect of female mating history on sperm precedence in the two-spot ladybird, *Adalia bipunctata* (Coleoptera, Coccinellidae). *Behavioral Ecology*, **9**, 559–565.
- Kålås, J., Fiske, P. & Sæther, S. A. 1995. The effect of mating probability on risk taking: an experimental study in lekking great snipe. *American Naturalist*, **146**, 59–71.
- Kendall, M. S. & Wolcott, T. G. 1999. The influence of male mating history on male–male competition and female choice in mating associations in the blue crab, *Callinectes sapidus* (Rathbun). *Journal of Experimental Marine Biology and Ecology*, **239**, 23–32.
- Kendall, M. S., Wolcott, D. L., Wolcott, T. G. & Hines, A. H. 2001. Reproductive potential of individual blue crabs, *Callinectes sapidus*, in a fished population: depletion and recovery of sperm number and seminal fluid. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 1168–1177.
- Kendall, M. S., Wolcott, D. L., Wolcott, T. G. & Hines, A. H. 2002. Influence of male size and mating history on sperm content of ejaculates of the blue crab *Callinectes sapidus*. *Marine Ecology Progress Series*, **230**, 235–240.
- Koga, T., Backwell, P., Jennions, M. & Christy, J. 1998. Elevated predation risk changes mating behavior and courtship in a fiddler crab. *Proceedings of the Royal Society of London, Series B*, **265**, 1385–1390.
- LaMunyon, C. W. 2000. Sperm storage by females of the polyandrous noctuid moth *Heliothis virescens*. *Animal Behaviour*, **59**, 395–402.
- Lickman, K., Murray, A. M. & Cade, W. H. 1998. Effect of mating on female phonotactic response in *Gryllus integer* (Orthoptera: Gryllidae). *Canadian Journal of Zoology*, **76**, 1263–1268.

- Lima, S. 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Advances in the Study of Behavior*, **27**, 215–290.
- Lima, S. & Dill, L. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.
- Magnhagen, C. 1990. Reproduction under predation risk in the sand goby, *Pomatoschistus minutus*, and the black goby, *Gobius niger*: the effect of age and longevity. *Behavioral Ecology and Sociobiology*, **26**, 331–335.
- Magnhagen, C. 1991. Predation risk as a cost of reproduction. *Trends in Ecology and Evolution*, **6**, 183–186.
- Maier, G. 1996. Variable mating durations in cyclopoid copepods: an adaptation to changing predation risk? *Archiv für Hydrobiologie*, **137**, 349–361.
- Marconato, A. & Shapiro, D. Y. 1996. Sperm allocation, sperm production and fertilization rates in the bucktooth parrotfish. *Animal Behaviour*, **52**, 971–980.
- Marconato, A., Tessari, V. & Marin, G. 1995. The mating system of *Xyrichthys novacula*: sperm economy and fertilization success. *Journal of Fish Biology*, **47**, 292–301.
- Martín, J., López, P. & Cooper, W. E. 2003. Loss of mating opportunities influences refuge use in the Iberian rock lizard, *Lacerta monticola*. *Behavioral Ecology and Sociobiology*, **54**, 505–510.
- Ortigosa, A. & Rowe, L. 2003. The role of mating history and male size in determining mating behaviours and sexual conflict in a water strider. *Animal Behaviour*, **65**, 851–858.
- Parker, G. A. & Stuart, R. A. 1976. Animal behavior as a strategy optimizer: evolution of resource assessment strategies and optimal emigration thresholds. *American Naturalist*, **110**, 1055–1076.
- Pitnick, S. & Markow, T. A. 1994. Male gametic strategies: sperm size, testes size, and the allocation of ejaculate among successive mates by the sperm limited fly *Drosophila pachea* and its relatives. *American Naturalist*, **143**, 785–819.
- Radtke, M. G. & Rutowski, R. L. 2002. Variation in the number of sperm transferred during mating among males of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Insect Physiology*, **48**, 1087–1092.
- Rochette, R. & Himmelman, J. 1996. Does vulnerability influence trade-offs made by whelks between predation risk and feeding opportunities. *Animal Behaviour*, **52**, 783–794.
- Rondeau, A. & Sainte-Marie, B. 2001. Variable mate-guarding time and sperm allocation by male snow crabs (*Chionoecetes opilio*) in response to sexual competition, and their impact on the mating success of females. *Biological Bulletin*, **201**, 204–217.
- Rooney, J. & Lewis, S. M. 2002. Fitness advantage from nuptial gifts in female fireflies. *Ecological Entomology*, **27**, 373–377.
- Savalli, U. M. & Fox, C. W. 1999. The effect of male mating history on paternal investment, fecundity, and female remating in the seed beetle *Callosobruchus maculatus*. *Functional Ecology*, **13**, 169–177.
- Shapiro, D. Y., Marconato, A. & Yoshikawa, T. 1994. Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. *Ecology*, **75**, 1334–1344.
- Sih, A. 1980. Optimal behavior: can foragers balance two conflicting demands? *Science*, **210**, 1041–1043.
- Sih, A. 1994. Predation risk and the evolutionary ecology of reproductive behaviour. *Journal of Fish Biology, Supplement A*, **45**, 111–130.
- Sih, A., Krupa, J. & Travers, S. 1990. An experimental study on the effects of predation risk and feeding regime on the mating behavior of the water strider. *American Naturalist*, **135**, 284–290.
- Simmons, L. W. 2001. *Sperm Competition and its Evolutionary Consequences in the Insects*. Princeton, New Jersey: Princeton University Press.
- Sparkes, T. C., Keogh, D. P. & Orsburn, T. H. 2002. Female resistance and mating outcomes in a stream-dwelling isopod: effects of male energy reserves and mating history. *Behaviour*, **139**, 875–895.
- Taborsky, B. & Foerster, K. 2004. Female mouthbrooders adjust incubation duration to perceived risk of predation. *Animal Behaviour*, **68**, 1275–1281.
- Thiel, M. & Hinojosa, I. 2003. Mating behavior of female rock shrimp *Rhynchocinetes typus* (Decapoda: Caridea): indication of convenience polyandry and cryptic female choice. *Behavioral Ecology and Sociobiology*, **55**, 113–121.
- Vepsäläinen, K. & Savolainen, R. 1995. Operational sex ratios and mating conflict between the sexes in the waterstriders *Gerris lacustris*. *American Naturalist*, **146**, 869–880.
- Wada, S., Tanaka, K. & Goshima, S. 1999. Precopulatory mate guarding in the hermit crab *Pagarus middendorffii* (Brandt) (Decapoda: Paguridae): effects of population parameters on male guarding duration. *Journal of Experimental Marine Biology and Ecology*, **239**, 289–298.
- Wedell, N. & Cook, P. A. 1999. Strategic sperm allocation in the small white butterfly *Pieris rapae* (Lepidoptera: Pieridae). *Functional Ecology*, **13**, 85–93.
- Worden, D. & Parker, P. G. 2001. Polyandry in grain beetles, *Tenebrio molitor*, leads to greater reproductive success: material or genetic benefits? *Behavioral Ecology*, **12**, 761–767.