

Chemical and Visual Communication During Mate Searching in Rock Shrimp

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Abstract. Mate searching in crustaceans depends on different communicational cues, of which chemical and visual cues are most important. Herein we examined the role of chemical and visual communication during mate searching and assessment in the rock shrimp *Rhynchocinetes typus*. Adult male rock shrimp experience major ontogenetic changes. The terminal molt stages (named “robustus”) are dominant and capable of monopolizing females during the mating process. Previous studies had shown that most females preferably mate with robustus males, but how these dominant males and receptive females find each other is uncertain, and is the question we examined herein. In a Y-maze designed to test for the importance of waterborne chemical cues, we observed that females approached the robustus male significantly more often than the typus male. Robustus males, however, were unable to locate receptive females *via* chemical signals. Using an experimental set-up that allowed testing for the importance of visual cues, we demonstrated that receptive females do not use visual cues to select robustus males, but robustus males use visual cues to find receptive females. Visual cues used by the robustus males were the tumults created by agitated aggregations of subordinate typus males around the receptive females. These results indicate a strong link between sexual communication and the mating system of rock shrimp in which dominant males monopolize receptive females. We found that females and males use different (sex-specific) communicational cues during mate searching and assessment, and that the sexual communication of rock shrimp is similar to that of the American lobster, where females are first attracted to the dominant males by chemical cues emitted by

these males. A brief comparison between these two species shows that female behaviors during sexual communication contribute strongly to the outcome of mate searching and assessment.

Introduction

Mating systems are expressions of the strategies that both sexes use to find each other and mate (Shuster and Wade, 2003). These strategies, mediated by population demography and ecological variables, depend on specific communication systems between the sexes, which facilitate mate finding and subsequently aid in regulating the mating process (Salmon, 1983). In crustaceans, sexual communication is based on visual, chemical, and acoustic cues (Salmon, 1983; Hughes, 1996; Bushmann, 1999), but chemical signals are of major importance in most aquatic species (Atema, 1995). In many species, individuals are dispersed over variable distances, and mating partners need to locate each other during the searching phase. To better understand the mating system of a species, it is particularly important to know which sex is searching for the other and to identify the communicational cues used during mate searching and assessment.

Chemical communication in aquatic crustaceans may act (1) over distance *via* waterborne odors or (2) by direct contact *via* chemo-tactile signals (Salmon, 1983). Odors may be emitted by either sex to attract potential mates over variable distances (*e.g.*, Dunham, 1978; Atema and Cobb, 1980; Bamber and Naylor, 1996; Bushmann and Atema, 1997). For example, in *Homarus americanus* (Cowan, 1991) and *Callinectes sapidus* (Bushman, 1999), the female is guided towards the male by a pheromone in the male’s urine. In contrast, in *Chionoecetes opilio*, the male is guided by an ecdysteroid from pubescent and multiparous

Received 15 December 2003; accepted 15 March 2004.

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females (Bouchard *et al.*, 1996). Similarly, in *Carcinus maenas*, waterborne signals from the premolt female evoke searching in males (Bamber and Naylor, 1996). Contact chemoreception, which usually occurs when males and females touch each other during assessment and mating (see Salmon, 1983), has been reported for many crustaceans (Borowsky, 1991; Kelly and Snell, 1998; Correa and Thiel, 2003a).

Visual sexual communication involves cues such as color, shape, and size of morphological structures or resources (*e.g.*, shelters), often in connection with elaborate courtship behaviors (*e.g.*, Latruffe *et al.*, 1999; Christy *et al.*, 2003). Visual signals are reported mostly for crustaceans from terrestrial environments (Salmon, 1983; Burggren and McMahon, 1988; Backwell *et al.*, 1998; Pope, 2000), but also occur in some species from shallow aquatic environments (Hatzios and Caldwell, 1983; Hughes, 1996; Acquistapace *et al.*, 2002). When visual signals are used in the aquatic environment, they are often accompanied by chemical cues (see review by Salmon, 1983). Combined visual and chemical signals have been reported in the snapping shrimp *Alpheus heterochaelis* (Hughes, 1996), in smasher stomatopods (Christy and Salmon, 1991; Marshall *et al.*, 1999), and in freshwater crayfish (Acquistapace *et al.*, 2002).

Most studies examining sexual communication in crustaceans focus on signal perception in only one sex, either the males or females (Salmon, 1983; Christy and Salmon, 1991; Bamber and Naylor, 1996; Bouchard *et al.*, 1996; Kamio *et al.*, 2002). Similarly, many studies focus exclusively on either visual cues (*e.g.*, Marshall *et al.*, 1999) or chemical cues (*e.g.*, Cowan, 1991), these being the two most important ones in aquatic crustaceans. Several studies, though, have demonstrated that both sexes are involved in signal exchange (Atema and Voigt, 1995; Bushmann, 1999) and that often more than one sense is employed during crustacean communication (Hughes, 1996). In particular, when individuals assess each other, they may base their decision on multiple signals (Sneddon *et al.*, 2003). This should be expected primarily during sexual communication in those species where members of one or both sexes show strong preferences for specific individuals of the opposite sex.

The rock shrimp *Rhynchocinetes typus* Milne Edwards 1837, which is abundant on shallow subtidal hard bottoms along the coasts of the southeastern Pacific (*e.g.*, Caillaux and Stotz, 2003), presents a mating system described as “neighborhood of dominance” (Correa and Thiel, 2003a). Male rock shrimp reach sexual maturity in the female-like typus stage, and during growth they pass through several intermedium stages before reaching the terminal molt stage, named robustus. The robustus males feature highly developed 1st pereopods and 3rd maxillipeds, and they are dominant over the ontogenetically younger stages. Robustus

males have high resource-holding potential and can defend females during the entire mating process (Correa *et al.*, 2003), and they also have larger sperm supplies than subordinate typus males (Hinojosa and Thiel, 2003). Receptive females prefer to mate with robustus males (Díaz and Thiel, 2003; Thiel and Hinojosa, 2003) even though these are comparatively rare in natural populations (Correa and Thiel, 2003b). Consequently, it can be expected that both robustus males and receptive females have developed efficient sexual communication to find each other. Receptive females might utilize visual signals such as the distinct morphological characteristics of robustus males to identify them. The robustus males might in turn use visual cues such as tumults developing around receptive females to identify and locate them. Tumults are agitated aggregations of several typus males that attempt to gain access to the receptive female. These tumults are visible over distances of several shrimp body lengths, and it is possible that robustus males perceive these tumults and approach the receptive female. However, since visual cues might be of minor importance in coastal habitats with limited visibility, chemical cues might also be important during sexual communication of rock shrimp.

In the present study, we examined whether receptive females and robustus males of the rock shrimp utilize chemical cues, visual cues, or both to locate and assess a potential mating partner.

Materials and Methods

Experiments were conducted during austral summer (February to April, water temperatures: 14.8–18.9 °C) and spring (September to October, 13.0–15.2 °C) of 2002 in a flowing seawater laboratory located near Bahía La Herradura, Coquimbo, Chile (29°59'S, 79°21'W). Shrimp were collected from the field by using a diver-operated suction sampler and were maintained in the laboratory in tanks with flowing and aerated seawater. They were fed fish and molluscs *ad libitum*. Females and males were held in separate tanks each containing up to 30 shrimp. Every morning the tanks with the females were examined to identify recently molted females, which are receptive 24 h after molting. The molted females were individually held in containers (surface area 20 cm × 20 cm and height 15 cm) with flowing seawater until the following day, when they were used in the experiments (for further details, see Correa *et al.*, 2003; Hinojosa and Thiel, 2003). After each replicate, the receptivity of the female was confirmed by allowing it to mate with a robustus male—if the female did not mate during this opportunity, the replicate was eliminated. The males used for the experiments were either in the terminal molt stage (robustus males) or in the intermolt phase (typus males). All individuals were used only once in these experiments, except where noted otherwise.

Chemical communication experiments

We used a Y-maze (Fig. 1A) to examine whether water-borne chemical cues play a role during mate searching in receptive females and robustus males. Seawater entered the Y-maze through two small branches, which converged in the choice chamber. The flow speed in the two branches was $\sim 1 \text{ cm s}^{-1}$. In the upstream part of each branch was a shelter for the shrimp designated as a potential sender of chemical cues; shrimp were randomly assigned to one of the two branches. The shelter was separated from the branches by a barrier of multiple layers of black mesh that prevented mechanical and visual contact, but allowed water to pass. The shrimp to be tested was placed downstream in the choice chamber under a plastic bell with holes allowing contact with the surrounding water. The acclimation period was 30 min for females and 60 min for males: preliminary experiments had shown that robustus males require more time to calm down after handling than females. Following release, the tested shrimp was observed for 30 min, and after

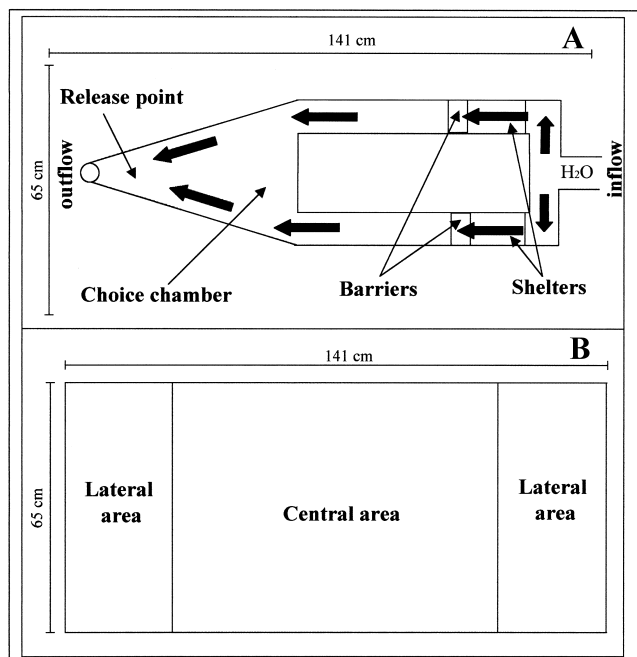


Figure 1. The experimental set-up. (A) The Y-maze used to study the importance of chemical cues that receptive females and dominant robustus males employ to locate potential mating partners. The tested individual was released downstream in the choice chamber; from there it could select one of the upstream branches leading to the shelter of a target individual from the opposite sex. (B) The seawater tank used to study the importance of visual cues employed by receptive females and dominant robustus males to locate their respective potential mating partners. The tested individual was released in the central compartment, while the target individuals from the opposite sex were placed in the lateral compartments. The central compartment was hermetically separated from the lateral compartments by a sealed glass window.

each experiment the tank was washed three times with fresh water.

Receptive females: Are receptive females guided by chemical cues in locating robustus males? One typus male was placed in the upstream shelter in one branch of the Y-maze, and one robustus male was placed in the upstream shelter of the other branch. After releasing a receptive female, we observed it for a maximum of 30 min. If the female spent 10 continuous minutes in one branch of the Y-maze, the male in the corresponding shelter was considered to be the chosen male. If the receptive female did not show a preference for either branch during the 30 min of observation, the replicate was considered as a no-choice. We conducted 12 replicates and used a χ^2 goodness-of-fit test to determine whether females chose robustus males more frequently than they chose typus males.

Robustus males: Are robustus males guided by chemical cues in locating receptive females? This experiment was divided into two parts, which differed by the absence or presence of typus males with the females. In part 1, a receptive female and a nonreceptive female were separately placed in the shelters at the upstream part of each branch of the Y-maze. After releasing the robustus male, we observed it for a maximum of 30 min. If the male stayed in one branch of the Y-maze for a continuous 10-min period, the female in the corresponding shelter was considered to be the chosen female. At the conclusion of part 1, the robustus male was again placed at the end of the choice chamber, where it was re-acclimated for 15 min. During this time, two typus males were added to the shelter of each female to induce mating interactions with the receptive female. Following release of the robustus male, observations were conducted as in part 1. In both parts ($n = 18$ replicates for part 1, $n = 17$ for part 2), we used a χ^2 goodness-of-fit test to determine whether robustus males oriented to the receptive female more often than to the nonreceptive female. In addition, we used a two-tailed Student's t -test for independent samples to compare the reaction times (start of experiment until the robustus male chooses a female).

Visual communication experiments

The visual cues used during mate searching were examined by providing visual signals to the receptive female and the dominant robustus male. The experiments were conducted in a large indoor tank (surface area $141 \text{ cm} \times 65 \text{ cm}$ and height 30 cm) filled to a water level of 20 cm. This tank was divided into three compartments separated from each other by hermetically sealed glass windows (Fig. 1B). The observations were conducted during daylight hours (1100 to 1600). The tested shrimp (receptive female or robustus

male) was placed in the central compartment where it was acclimated behind a glass fence (allowing it to see in all directions). The lateral compartments contained the shrimp that were used to generate visual signals (see below). The duration of the observation differed between the sexes (60 min for robustus males, 90 min for females) since previous studies had shown that robustus males usually seize receptive females within 60 min (Correa *et al.*, 2000), but females may delay mate choice for more than 60 min (Díaz and Thiel, 2003).

Receptive females: Can receptive females distinguish between typus and robustus males via visual cues? Each experiment used two males—a robustus and a typus—and randomly assigned one to each lateral compartment, where they were tethered to the bottom of the experimental tank. Tethering prohibited the males from actively courting the female behind the glass window and limited the visual signal to the morphological characteristics of the male. During the experiment, some males attempted to move once the female approached their glass window, but they were kept in place by the tether. Males were tethered 24 h before the start of the experiment, which was sufficient for them to acclimatize.

Following an acclimation period of 30 min, the receptive female was released in the central compartment of the tank, and her behavior was registered for 90 min. We quantified the following variables: (a) time of first visit to each male, (b) duration and frequency of individual visits to each male, and (c) male attended by the female at the end of the experiment. We use the term visit to refer to the female touching the glass window of the respective male. Finally, we tested the null hypothesis that the frequencies of the first visit and the last visit to either male were similar by using a χ^2 goodness-of-fit test. We also tested whether the total duration of visits by the female to each male differed between typus and robustus males by using the two-tailed Student's *t*-test for dependent samples.

Robustus males: How important are visual cues for the robustus male? We examined this question using tumults generated by typus males around a receptive female. These tumults are visible over distances of several shrimp body lengths, and we hypothesized that they could indicate the presence of a receptive female to robustus males. We used different numbers of typus males together with a receptive female to test whether (1) tumults were produced, (2) the frequency of visual cues increased with increasing numbers of typus males, and (3) robustus males reacted to these visual cues.

Different numbers (2, 3, 4, 8, and 12) of typus males were placed in each lateral compartment 24 h before the start of the experiment. For a given treatment, the same number of

typus males were always put in each lateral compartment. At the end of the acclimation period of the males, a receptive female was introduced into one lateral compartment, while a nonreceptive female was introduced into the other lateral compartment. Females were assigned randomly to each lateral compartment, where they were acclimated under a transparent plastic bell (made from the upper part of a plastic bottle) for 15 min before the start of the experiment. Because of a shortage of receptive females, replication of the different treatments (number of typus males) was unequal, ranging from $n = 8$ (two typus males) to $n = 5$ (two treatments, with four and eight typus males, respectively). The experiment lasted for 60 min after release of the females.

To assess whether the number and intensity of visual signals depended on the number of typus males, we counted the number of tumults per treatment and determined the total mating time of the receptive female with different numbers of typus males. Two Kruskal-Wallis tests (both two-tailed) were conducted to test for significant differences in these variables. While the robustus male was being acclimated for 15 min behind a glass fence in the central compartment, the females were introduced to the lateral compartments. After release of the male, we observed its behavior for 60 min and counted the number and duration of its visits to each of the two lateral compartments. Using a χ^2 goodness-of-fit test, we compared the number of males visiting the receptive and the nonreceptive female during the first and last visits. To examine whether the robustus male used visual signals to locate the receptive female, we calculated the total duration of visits to each female. Three independent parametric tests were run: (a) a one-way ANOVA to compare the total visit duration by the robustus male to the receptive female between the different typus treatments, (b) a parallel one-way ANOVA to compare the total visit duration by the robustus male to the nonreceptive female between the different typus treatments, and (c) a one-tailed Student's *t*-test for dependent samples to determine whether the total visit duration by the robustus male (pooling all typus treatments) was longer for the receptive than for the nonreceptive females (Zar, 1999).

Statistical analysis

To assess frequencies of choices made by either robustus males or receptive females in the different experiments, we conducted χ^2 goodness-of-fit tests. All other data were tested for homogeneity of variances using the Cochran *C*-test. If the original data failed the normality test, they were $\ln(x + 1)$ transformed. A Student's *t*-test was used when variances were homogeneous. One-way ANOVAs were used to test for significant difference between treatments, followed by a *post hoc* Tukey test. If variances were

not homogeneous after transformation, we conducted non-parametric Kruskal-Wallis tests, followed by a *post hoc* Dunn test. All tests were carried out with a significance level of $\alpha = 0.05$.

Results

Chemical communication

Receptive females: Are receptive females guided by chemical cues in locating robustus males? Following release, most females (10 of 12) chose the branch with the robustus male. When the plastic acclimation bell was removed, these ten females went directly to the robustus male (mean \pm SD: 1.22 \pm 2.14 min). Only one female remained for 30 min in the choice chamber. The female that chose the typus male entered and left the typus branch twice before finally staying with the typus. The difference between the females choosing the robustus male and those choosing other options was significant ($\chi^2 = 13.5$, $\chi^2_{0.05,2} = 5.991$, $P = 0.001$).

Robustus males: Are robustus males guided by chemical cues in locating receptive females? Following release, most robustus males in part 1 (female without typus males) failed to make a choice for the receptive female ($\chi^2 = 4$, $\chi^2_{0.05,2} = 5.991$, $P = 0.135$). Of the 18 animals tested, 4 chose the receptive female, 4 chose the nonreceptive female, and 10 made no choice. The robustus males that selected the branch with the receptive female were not faster to react (mean \pm SD: 4.33 \pm 2.75 min) than the robustus males that selected the nonreceptive female (mean \pm SD: 6.85 \pm 5.63 min) (t -test: $t = -0.805$, $t_{0.05(2),6} = 2.447$, $P = 0.452$); the other males reacted late or never left the choice chamber during the 30-min observation period. After the introduction of two typus males to the female shelters (part 2), more robustus males chose the branch with the receptive female, but differences were not significant ($\chi^2 = 2.24$, $\chi^2_{0.05,2} = 5.991$, $P = 0.329$). Of the 17 animals tested, 8 chose the receptive female, 3 chose the nonreceptive female, and 6 made no choice. Also during the second part, robustus males that selected receptive females were not faster to react (mean \pm SD: 6.69 \pm 5.37 min) than those that selected nonreceptive females (mean \pm SD: 3.06 \pm 3.43 min) (t -test: $t = 1.062$, $t_{0.05(2),8} = 2.306$, $P = 0.319$).

Visual communication

Receptive female: Can receptive females distinguish between typus and robustus males via visual cues? Following their release, receptive females started to move continuously without staying for long near the lateral compartments of the aquarium. There was no clear pattern of choice in favor of either of the two male forms (typus or robustus). When comparing the number of visits to the two males,

there was no significant preference for either male, neither during the first visit ($\chi^2 = 0.0769$, $\chi^2_{0.05,1} = 3.841$, $P = 0.78$ after Yates correction) nor during the last visit ($\chi^2 = 2.48$, $\chi^2_{0.05,2} = 5.991$, $P = 0.28$) (Table 1). Similarly, there were no significant differences in the total duration of visits to either male (t -test: $t = -0.432$, $t_{0.05(2),12} = 2.179$, $P = 0.674$). Females spent on average less than 10 min near the compartment of either male (mean \pm SD: robustus 8.3 \pm 24.4 min; typus 6.3 \pm 11.6 min).

Robustus males: How important are visual cues for the robustus male? As predicted, tumults were generated by the typus males around the receptive female, but no tumults were observed near the nonreceptive female. Each tumult lasted only a few seconds, and generally the larger typus male succeeded in mating with the female. The frequency of tumults increased positively in relation to the number of typus males in the different treatments. Significant differences in the number of tumults were found between treatments with 2 and 8 typus males (Kruskal-Wallis $H = 12.602$, $df = 4$, $n = 30$, $P = 0.013$) (Fig. 2A). Another possible visual signal for the robustus male might be generated by mating of the receptive female with one of the typus males. Matings were observed in all treatments, and there were no significant differences in the total mating time between treatments (Kruskal-Wallis $H = 6.301$, $df = 4$, $n = 30$, $P = 0.178$) (Fig. 2B). In summary, many visual signals potentially attractive for the robustus males were generated in the lateral compartment with the receptive female.

When comparing the number of visits between the two females, there was no significant preference for either female, neither during the first visit (see Table 2) nor the last visit (see Table 3). The total duration of visits by the robustus males to the receptive female was not significantly affected by the numbers of typus males in each treatment (one-way ANOVA $F_{4,25} = 1.962$, $P = 0.131$) (Fig. 3A). The visit duration of robustus males to the receptive female reached lowest values in the treatment with the highest densities of typus males, but there were no significant differences to the other treatments (the absence of significant

Table 1

Visual communication experiment: choices made by receptive females when presented with a robustus male and a typus male

Choice	First visit	Last visit
Robustus male	7	7
Typus male	6	3
No choice	—	3

Presented are the first visit and the last visit to one of the lateral compartments with a male behind the glass window.

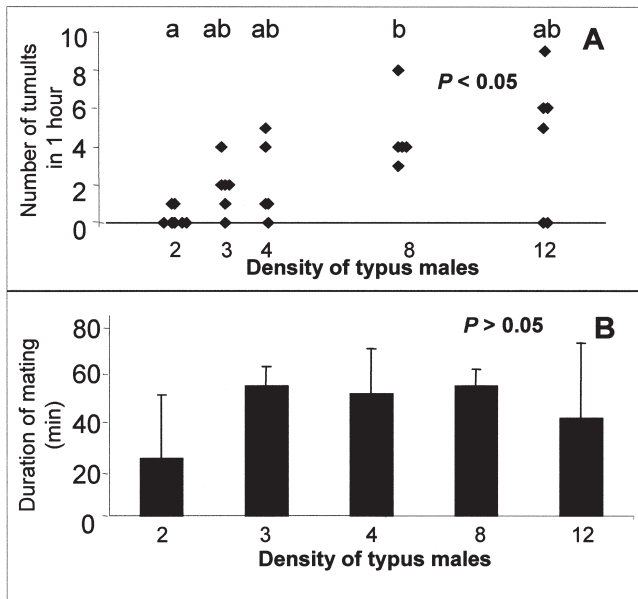


Figure 2. (A) Number of tumults produced in 1 h; different letters indicate treatments with significant differences (*post hoc* test, Dunn $q = 3.073$, $P < 0.05$); (B) Total mating time (mean + SD) of the receptive female with typus males in the respective treatments; note that occasionally a female mated with more than one male.

differences should be interpreted cautiously since the power of the statistical test is very low; $P = 0.253$). The total duration of visits by robustus male to the nonreceptive female also was not affected by the typus treatments (one-

Table 2

Visual communication experiment: number of robustus males that chose, in the first visit, either a receptive or a nonreceptive female when the females were presented in the presence of various numbers of typus males

Treatment (# of typus males)	Female		χ^2	df	P
	Receptive	Nonreceptive			
2	3 (3.5)	4 (3.5)	0.1	1	0.705
3	2 (3)	4 (3)	0.6	1	0.414
4	3 (2.5)	2 (2.5)	0.2	1	0.654
8	4 (2.5)	1 (2.5)	1.8	1	0.179
12	5 (3)	1 (3)	2.6	1	0.102
TOTAL			$\chi^2 = 5.4$	5	
χ^2 grouped	17 (14.5)	12 (14.5)	0.8	1	0.353
Heterogeneity χ^2		4.6	4.6	4	>0.05

In parentheses are the expected frequencies of first visit. The P value corresponding to heterogeneity >0.05 means that treatments are homogeneous and can be analyzed as a whole. In the treatment with 2 typus males, one robustus male did not make any choice for either female during the observation time, and consequently was not included in this analysis.

Table 3

Visual communication experiment: number of robustus males that chose, in the last visit, either a receptive or a nonreceptive female when the females were presented in the presence of various numbers of typus males

Treatment (# of typus males)	Female		χ^2	df	P
	Receptive	Nonreceptive			
2	3 (3.5)	4 (3.5)	0.14	1	0.705
3	4 (3)	2 (3)	0.67	1	0.414
4	4 (2.5)	1 (2.5)	1.8	1	0.179
8	4 (2.5)	1 (2.5)	1.8	1	0.179
12	4 (3)	2 (3)	0.67	1	0.414
TOTAL			$\chi^2 = 5.08$	5	
χ^2 grouped	19 (14.5)	10 (14.5)	2.79	1	0.094
Heterogeneity χ^2			2.29	4	>0.05

In parentheses are the expected frequencies of last visit. The P value corresponding to heterogeneity >0.05 means that treatments are homogeneous and can be analyzed as a whole. In the treatment with 2 typus males, one robustus male did not make any choice for either female during the observation time, and consequently was not included in this analysis.

way ANOVA $F_{4,25} = 0.089$, $P = 0.984$) (Fig. 3B). Since no differences between typus treatments were found, we pooled all replicates and calculated the total visit duration of the robustus males to each lateral compartment (with receptive female and nonreceptive female, respectively) (Fig. 3C). The total visit duration to the receptive female glass window was on average twice as long as to the nonreceptive female (t -test: $t = -2.039$, $t_{0.05(1),29} = 1.699$, $P = 0.025$).

Discussion

Our results show that receptive females and robustus males use different cues during sexual communication. Receptive females locate their potential mating partners by using chemical cues, whereas robustus males find females mostly by using visual cues. The results suggest that females and males adopt sex-specific roles during the searching and assessment phase. Thus *Rhynchocinetes typus*, like the lobster *Homarus americanus* (Bushman and Atema, 2000), is a crustacean species in which females locate dominant males *via* chemical signals. In both species, females preferentially mate with dominant males, which defend females during the mating process. This suggests that sexual communication in *R. typus* (and *H. americanus*) is closely linked to their mating system, as will be discussed in the following section.

Chemical communication

Chemical cues are used by a variety of crustaceans during sexual communication. In many species such as the shore

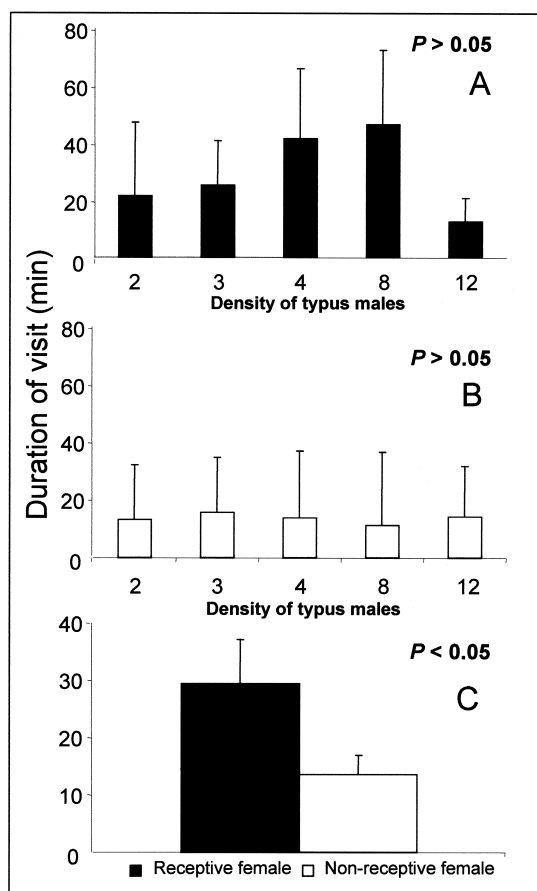


Figure 3. Total visit duration (mean + SD) of robustus males to the (A) receptive female and (B) nonreceptive female in respective treatments; $P > 0.05$ indicates no significant differences between treatments. (C) Total visit duration (mean + SD) of robustus males to the respective females after pooling among all treatments; $P < 0.05$ indicates significant differences between visits to respective females.

crab *Carcinus maenas*, snow crab *Chionoecetes opilio*, and helmet crab *Telmessus cheiragonus*, females advertise their reproductive status and attract males *via* waterborne chemical signals (Bamber and Naylor, 1996; Bouchard *et al.*, 1996; Kamio *et al.*, 2002). This does not appear to be the case in *Rhynchocinetes typus*, where males do not locate receptive females *via* chemical cues. Most crustacean species possess highly efficient chemoreceptive capabilities that allow them to evaluate their environment (Derby and Steullet, 2001). We believe that this is also true for *R. typus*, and that males would be able to perceive the presence of a receptive female if appropriate chemical signals were available. Since males of this species apparently cannot identify a receptive female *via* waterborne chemical cues, it appears that receptive females do not release waterborne chemicals advertising their reproductive status. When typus males were together with females, there was a slight tendency for robustus males to approach the receptive females, but the

experimental set-up did not allow distinguishing whether this was in response to chemicals released by the females or by typus males attending them. Rock shrimp typically live at high densities (Caillaux and Stotz, 2003), and there are usually many males for each receptive female (Correa and Thiel, 2003b). Female rock shrimp might have no problem in obtaining a mating partner, and thus the adaptive advantage in attracting males *via* waterborne chemical signals would be slight. It is also possible that *R. typus*, which inhabits complex and wave-exposed rocky shore environments, lives in a habitat where communication *via* chemical cues is complicated by turbulent flow. However, the fact that female rock shrimp locate males *via* waterborne chemical cues suggests that chemical communication in the habitat of *R. typus* is not negatively influenced by the hydrodynamic regime.

In some crustacean species, males advertise their presence to females. For example, in the lobster *Homarus americanus*, females are attracted to dominant males *via* chemical signals (Bushman and Atema, 1997, 2000). Similarly, in the blue crab *Callinectes sapidus*, females appear to key in on chemical signals from males (Gleeson, 1991; Bushmann, 1999). Males of these crustacean species typically advertise their status to other individuals *via* chemical signals released in the urine (*e.g.*, Breithaupt and Atema, 2000; Zulantz Schneider *et al.*, 2001; Breithaupt and Eger, 2002). These signals often are used during agonistic encounters to establish dominance status, and females might exploit these signals to locate dominant males. This could explain why and how females of *R. typus* find dominant robustus males *via* chemical signals. Robustus males occasionally engage in agonistic interactions (Correa *et al.*, 2003), and during these encounters they may employ chemical signals similar to those of male lobsters and crayfish. During the searching phase, female rock shrimp may utilize these chemical signals to locate robustus males, which they prefer as mates (Díaz and Thiel, 2003; Thiel and Hinojosa, 2003).

Visual communication

Since robustus males feature distinct morphological characteristics, it could have been expected that receptive females would use visual cues to distinguish between males. Females of some other decapod crustaceans choose males on the basis of visual cues such as size of body structures or shelter, which are reliable indicators of the fitness of an individual (Atema and Cobb, 1980; Christy, 1987). The results of the current study suggest that females do not approach the robustus males on the basis of visual signals. In general, visual signals are uncommon during sexual communication of aquatic crustaceans (see Salmon, 1983). Only in particular groups of stomatopods (Hatzioles and

Caldwell, 1983; Christy and Salmon, 1991; Marshall *et al.*, 1999) and in the snapping shrimp *Alpheus heterochaelis* (Hughes, 1996) are visual signals known to play an important role during intersexual communication, probably because both species live in tropical waters where visibility usually is high. Similarly, females of terrestrial crustaceans use visual signals to find males (Christy and Salmon, 1991; Pope, 2000). The importance of visual signals for sexual communication in terrestrial and clear-water environments appears to be related to the better light conditions and visibility. The apparent inability of female rock shrimp to select robustus males *via* visual cues in our study does not prove that females do not use visual cues to recognize robustus males. Female rock shrimp that received both chemical and visual cues avoided robustus males initially (see Díaz and Thiel, 2003). The experiments with the robustus males demonstrated that visual cues also play a role during intersexual communication in *R. typus*. Robustus males are guided visually to the tumults or matings developing around receptive females. A similar phenomenon has been reported for the horseshoe crab *Limulus polyphemus*, where additional males are attracted to satellite males around the receptive female apparently by visual cues (Barlow *et al.*, 1982), but this occurs in shallow waters and is aided by chemical signals (Hassler and Brockmann, 2001). These observations suggest that in the marine environment, once crustaceans are close to potential mating partners, that is, during the assessment phase, visual cues (often accompanied by chemical cues) may gain in importance.

Visual cues are important for judging the size of an opponent during agonistic encounters (*e.g.*, Caldwell and Dingle, 1979; Atema and Cobb, 1980). This occurs when individuals are within a few body lengths of each other. In addition to chemical signals (Breithaupt and Atema, 2000) and moderate agonistic interactions (Karavanich and Atema, 1998), visual signals may also serve to establish and maintain dominance status. In rock shrimp, visual communication occurs during agonistic encounters when robustus males appear to gauge the size of their opponents (*e.g.*, Correa *et al.*, 2003). This might explain why male lobsters and shrimps have evolved visual skills to distinguish conspecifics (opponents and receptive females).

Sexual communication and mating system of rock shrimp

The mating system of rock shrimp has been characterized as “neighborhoods of dominance” (Correa and Thiel, 2003a) in which dominant males monopolize a receptive female and defend it against subordinate males (Correa *et al.*, 2003). In *Rhynchocinetes typus*, females have a strong preference for these dominant males (Díaz and Thiel, 2003; Thiel and Hinojosa, 2003). The mating system of *R. typus* thus shows strong similarities to that of *Homarus ameri-*

canus. In this species, dominant males that inhabit shelters also form neighborhoods of dominance (*e.g.*, Karnofsky *et al.*, 1989), and reproductive females exhibit preferences for dominant males (Atema *et al.*, 1979; Atema, 1986; Bushmann and Atema, 2000), even leading to female molt-staggering (Cowan and Atema, 1990).

In contrast to many other decapod species, female rock shrimp and lobsters do not attract males *via* waterborne chemical signals. In both species, harassment from subordinate males may represent a high cost for females (*e.g.*, Cowan, 1991; Thiel and Hinojosa, 2003). To avoid approaches and rambunctious harassment from subordinate males, female rock shrimp and lobsters may thus hide their reproductive status (they remain “chemically quiet” *sensu* Atema, 1995) until they reach the vicinity of a dominant male. Male lobsters are residents in shelters and accept only mature females into their shelter (Cowan, 1991). The same might be true for rock shrimp, where one or several robustus males often occupy large spaces in open shelters (*pers. obs.*). These dominant males usually are dispersed and difficult to locate visually in their shelters. Consequently, receptive females are attracted to these areas by chemical cues, as shown herein for rock shrimp and by Bushmann and Atema (1997) for *H. americanus*. Once in the vicinity of a dominant male, females may no longer hide their reproductive status but rather permit assessment by that male. In lobsters, this appears to occur *via* chemical cues (Atema and Cowan, 1986), which reduce male aggression and later facilitate successful mating (Bushman and Atema, 1997). Waterborne chemical signals most likely play only a minor role during mate assessment in *R. typus*, since they would attract large numbers of subordinate males. Visual cues arising from activities developing around the receptive female are apparently sufficient to attract a dominant male within a short period of time and induce it to monopolize the female. Thus the assessment phase seems to be brief and simple in rock shrimp, even though in other crustaceans it involves sophisticated signals and requires substantial time (Christy, 1987; Cowan, 1991; Bushmann, 1999). For rock shrimp, we propose a hypothetical mating scenario in which females molt at night and subsequently (or before molting) approach areas with dominant males. Females then become receptive during the day, when visual cues can be used by the robustus males to locate and monopolize receptive females in their vicinity.

In summary, this comparison of sexual communication in rock shrimp and lobster further underlines the important role of female behavior during mate searching and assessment. Females may significantly influence the outcome of the mating process by hiding their reproductive status before reaching the neighborhood of dominant males.

Acknowledgments

We are grateful to I. Hinojosa and T. Chr. van Son for help in collecting shrimp. Our special thanks go to W. Stotz and D. Lancellotti for their unconditional support during this study. Helpful comments from A. Baeza and from two anonymous reviewers helped to improve the original manuscript.

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