Naturwissenschaften (2009) 96:103–110
DOI 10.1007/s00114-008-0458-9

ORIGINAL PAPER

Sex identification in female crayfish is bimodal

Laura Aquiloni · Alessandro Massolo · Francesca Gherardi

Received: 16 August 2007 / Revised: 13 August 2008 / Accepted: 20 September 2008 / Published online: 11 October 2008

Abstract Sex identification has been studied in several species of crustacean decapods but only seldom was the role of multimodality investigated in a systematic fashion. Here, we analyse the effect of single/combined chemical and visual stimuli on the ability of the crayfish *Procambarus clarkii* to identify the sex of a conspecific during mating interactions. Our results show that crayfish respond to the offered stimuli depending on their sex. While males rely on olfaction alone for sex identification, females require the combination of olfaction and vision to do so. In the latter, chemical and visual stimuli act as non-redundant signal components that possibly enhance the female ability to discriminate potential mates in the crowded social context experienced during mating period. This is one of the few clear examples in invertebrates of non-redundancy in a bimodal communication system.

Keywords Bimodal communication · Sex identification · Crayfish · *Procambarus clarkii*

Introduction

Many animals communicate via composite signals emitted through more than one sensory channel (i.e. multimodal signals; Partan and Marler 2005). "Signal" is here defined in a broad sense to include all the modalities used to transmit information (sensu Dawkins 1995), independently of their being specifically elaborated by the sender for communication purposes (but see Wisenden and Stacey 2005). "Stimulus" denotes a detectable change in the internal and external environment that might stimulate behavioural response in an organism (Immelmann and Beer 1989).

When compared to unimodality, multimodality affords new opportunities for communication (Partan and Marler 1999) particularly by enhancing the detection, discrimination, and memorability of signals (Rowe 1999). For instance, the distance at which females of the wolf spider, *Schizocosa ocreata*, detects mates is higher when vibratory components of male displays are associated with their vision (Scheffer et al. 1996). The production of two or more types of stimulus often refines the ability to perceive a signal against background noise: the crayfish *Orconectes propinquus* can recognise a predator at a distance when the vision of the latter is combined with the detection of alarm substances emitted by conspecifics (Bouwma and Hazlett 2001). Finally, multimodality may improve the ability of a receiver (e.g. a predator) to learn and remember the association between another animal (e.g. a prey) and some properties of the latter (e.g. unpalatability; Guilford and Dawkins 1991).

The literature is crowded of studies that analyse the diverse sensory channels used by crustacean decapods. The role of olfaction in sex identification and mating has been investigated in several species, including clawed lobsters (e.g. Bushmann and Atema 1997, 2000), crabs (e.g. Bamber and Naylor 1997), and crayfish (e.g. Bechler 1995; Stebbing et al. 2003). The shrimp *Palaemonetes pugio* uses contact pheromones: individuals have to touch the cuticle of a conspecific with their antennae in order to recognise its sex and reproductive status (e.g. Caskey and Bauer 2005). In

L. Aquiloni (✉) · F. Gherardi
Department of Evolutionary Biology, University of Florence,
Via Romana 17,
50125 Florence, Italy
e-mail: laura.aquiloni@unifi.it

A. Massolo
Department of Ecosystem and Public Health,
University of Calgary,
G362, 3330 Hospital Drive,
NW Calgary, AB T2N 4N1, Canada

several decapod species, antennule ablation (Cowan 1991; Christofferson 1970; Bamber and Naylor 1996), but not blindfolding (Snyder et al. 1993; Bushmann 1999), affects the animal's ability to identify the other's sex.

However, notwithstanding the surge of interest in the communication systems of crustaceans (Atema and Steinbach 2007), multimodality has been only rarely analysed in a systematic fashion. An exception is the study by Hughes (1996a) on the shrimp *Alpheus heterochaelis*. Males of this species respond aggressively to visual stimuli alone, such as an open claw, and do not respond to chemical stimuli alone; but when the two are combined and the odour is released by a female, aggressive responses are suppressed.

Here, we analyse the effects of chemical and/or visual stimuli emitted by conspecifics of either sex on the behaviour of the red swamp crayfish *Procambarus clarkii* during mating interactions. Males (Ameyaw-Akumfi and Hazlett 1975; Dunham and Oh 1992) and females (Dunham and Oh 1996) of this species use chemical stimuli to identify the sex of a potential mate and even to assess its quality (i.e. body size, Aquiloni and Gherardi 2008a). On the contrary, little is known about the role of visual stimuli during sex identification of this taxon (but see Itagaki and Thorp 1981; Pavey and Fielder 1996; Corotto et al. 1999; Acquistapace et al. 2002; Cronin and Hariyama 2002). Finally, signal redundancy still remains unexplored in crayfish, notwithstanding its recognised central role in assuring the accurate reception and recognition of messages by the numerous species studied so far (Partan and Marler 2005).

We designed an experiment in which crayfish of the two sexes were offered with either chemical or visual stimuli from a potential mate or with the two stimuli combined. We expected that, if sex identification relies on "redundant" signal components, each type of stimulus should elicit equivalent (or enhanced) responses from the receiver regardless of whether the stimuli are presented separately or in combination (Partan and Marler 2005). Conversely, as a result of the combination of "non-redundant" signal components, distinct responses may be still elicited or one component may dominate the other or modulate its effect; alternatively, a new response may emerge.

Materials and methods

Experimental protocol

Form-I males (i.e. reproductive males, cephalothorax length, mean±SE: 48.5±2.7 mm) and sexually mature females (cephalothorax length: 46.0±2.3 mm) were collected from Massaciuccoli Lake (Tuscany, Italy) and isolated in individual plastic aquaria (25×20×20 cm; water level: 10 cm). We only used sexually responsive individuals, i.e. crayfish that, once offered with a conspecific of the other sex, displayed pre-copulatory behaviours (Kasuya et al. 1996). Before copulation took place, crayfish (120 males and 120 females) were separated and isolated in individual aquaria as above for 48 h before being used in the experiment.

The experimental apparatus consisted of glass aquaria (40×25×25 cm) supplied with an aerator and filled with 12.5 L of well water at the temperature of 20 °C (Fig. 1). Aquaria contained the test crayfish in Area A, a circular glass container chemically isolated from the rest of the apparatus (10×4 cm bottom and 22 cm high) placed at the opposite side of the experimenter in Area B, and a black plastic wall. Aquaria were visually isolated from each other and from possible sources of disturbance. All tests were conducted between 0800 and 1400 h during July 2007. Crayfish were kept under an artificial light with a 12L:12D-hour cycle.

Responsive crayfish were randomly assigned to one of three treatments: single chemical stimuli (C), single visual stimuli (V), and chemical plus visual stimuli (CV). These stimuli were produced by source crayfish of either the same (Hom) or the opposite (Het) sex. In each treatment, we tested 20 males and 20 females for both Hom and Het stimuli so that each individual was subject to two tests in two consecutive days in a random sequence (HomC and HetC, HomV and HetV, or HomCV and HetCV).

Twenty trials were run each day, the order of treatments being determined using a random number table. After 24 h acclimation, we observed the behaviour of test crayfish in two consecutive phases of 3-min each (time sufficient for a crayfish to show a clear change in its behaviour as a response to a stimulus; see Acquistapace et al. 2002): (1) a control phase following the injection of 20 ml of well water (control) into the right-hand corner of the aquarium

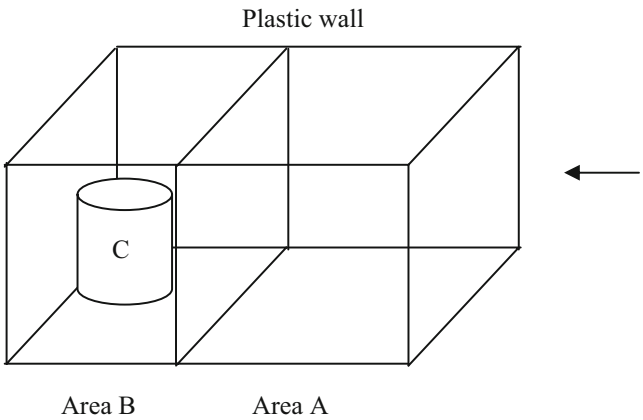

Fig. 1 Experimental apparatus composed of an aquarium (40×25×25 cm) with a glass container, C (10×4 cm bottom and 22 cm high, area B), on the side opposite the experimenter, and a plastic wall. Single test crayfish was inserted in area A. The *arrow* indicates the position from which the experimenter observed crayfish

near the experimenter in Area A; during this time, the plastic wall was left in place; and (2) a test phase corresponding to one of the six treatments; this phase started with the removal of the plastic wall. To ascertain that the injection of 20 ml of well water and the removal of the plastic wall did not disturb the test crayfish, we first conducted a preliminary test (20 replicates per sex) that followed the same procedure as above but in the absence of chemical and visual stimuli from any source crayfish.

Following the results of previous studies (e.g. Bushmann and Atema 1997; Simon and Moore 2007), we assumed that sex pheromones were released in the urine. Therefore, we kept single sexually receptive males and females as source crayfish in 2 L of well water for at least 24 h to obtain "whole body water", i.e. water conditioned by the odour of the whole crayfish body that should also contain the putative sex pheromone. For HomC, HetC, HomCV, and HetCV treatments, 20 mL of whole body water were injected with a syringe into the right-hand corner of the aquarium near the experimenter. In HomC and HetC, the glass container in Area B remained empty. For HomV, HetV, HomCV, and HetCV, the source crayfish was either a male or a female of the same size as the test crayfish that was placed in the glass container (C in Fig. 1). This container was small enough to impede any movement to the source crayfish, thus, reducing the influence of its behaviour on the test crayfish. At the end of each session, crayfish were fed, aquaria were carefully cleaned, and the water was changed.

During the control and the test phases, we measured the time spent by test crayfish in locomotion and in aggressive posture. Locomotion was recorded because its changes in intensity denote the detection by the test crayfish of the stimuli emitted by the source conspecific (Acquistapace et al. 2002). Aggressive posture (consisting in crayfish having their body raised, their chelipeds held off the substratum and parallel to it, or higher, and the abdomen and tail fan extended; Ameyaw-Akumfi and Hazlett 1975) is used here as an indicator of sex identification: *P. clarkii* males and females (Ameyaw-Akumfi and Hazlett 1975; Dunham and Oh 1992) and *Orconectes virilis* males (Hazlett 1985) display this posture when they perceive individuals of the same—but not of the other—sex.

Statistical analyses

The data collected in the preliminary test were analysed for significance using a Wilcoxon signed ranks test (statistic: Z; Siegel and Castellan 1988). To compare the control and the test phase, we first computed the difference (in seconds) between the time that test crayfish spent in locomotion or in the aggressive posture during the test phase and the time spent in the same behaviour and posture during the control. Crayfish were then classified into two groups, i.e. (1) individuals that spent, in the test phase, the same time (±15 s) as in the control phase in locomotion or in the aggressive posture and (2) individuals that either increased (>15 s) or decreased (<−15 s) that time. Since time most often increased (see Table 2 for the few exceptions), the analyses were done on pooled data. G tests after Williams' correction (statistic: G; Siegel and Castellan 1988) were then used to test the significance of the differences between crayfish showing and not showing temporal changes.

General Linear Models for repeated measures (GLMs, statistic: F), followed by Tukey post hoc tests, were used for each sex to analyse differences among treatments (C, V, and CV) and between sexes of the source crayfish (Hom, Het), where treatments were taken as between-subject factors and the sex of the source crayfish as a within-subject factor (Quinn and Keough 2002). Wilcoxon signed ranks tests (statistic: Z) were then used to compare the effects of each treatment between sexes of the source crayfish. Finally, the data from Hom and Het, averaged per test crayfish, were pooled to analyse differences among treatments between sexes of the test crayfish using a Mann–Whitney test (statistic: U; Siegel and Castellan 1988). The level of significance is $\alpha=0.05$.

Results

Preliminary test

In both sexes, the time spent in locomotion (males, $N=20$, $Z=-0.663$, $p=0.541$; females, $N=20$, $Z=-1.268$, $p=0.216$) or in the aggressive posture (males: $N=20$, $Z=-1.0$, $p=1.0$; females: $N=20$, $Z=0$, $p=1$) did not change after the injection of well water and the removal of the plastic wall. This denotes that manipulation does not have a detectable effect.

Comparison between control and test phases

In the presence of each type of stimulus (except in the case of visual stimuli when test crayfish were males), the number of crayfish recorded in locomotion significantly increased from the control to the test phase. While visual stimuli alone had no clear effects on male aggressiveness, chemical stimuli (alone or combined with visual stimuli) either increased the number of aggressive males (when released by another male) or kept it at a relatively low value (when released by a female). This suggests that males require chemical stimuli to identify the sex of a conspecific. On the contrary, visual (but not chemical) stimuli from crayfish of either sex or chemical plus visual stimuli from a female increased the number of aggressive females, which

pinpoints the importance of vision for females. Interestingly, the combination of the two types of stimulus from a male was associated with a very low number of aggressive females. This might denote that females, in the presence of chemical and visual stimuli, have been able to identify the other's sex (Tables 1 and 2).

Comparisons among treatments and between sexes of the source crayfish

The time spent in locomotion was always independent of the sex of the source crayfish (Fig. 2a, b). Conversely, males and females differed in that responses were stronger (i.e. they showed a more intense locomotion) in the presence of chemical stimuli (alone or combined with visual stimuli) when the test crayfish were males ($F_{2,57}$= 10.152, p=0.0002; Tukey post hoc test: p<0.05; Fig. 2a), and in the presence of visual stimuli (alone or combined with visual stimuli) when they were females ($F_{2,57}$=3.751, p=0.029; Tukey post hoc test: p<0.05; Fig. 2b; Table 3).

Aggressiveness in males (as denoted by the time spent in the aggressive posture; Fig. 2c) did not vary with the type of stimulus ($F_{2,57}$=0.821, p=0.445), but, at least in the presence of chemical stimuli (alone or combined with visual stimuli), was significantly more intense when the source crayfish was a male. Females were more aggressive in the presence of visual stimuli (alone or combined with chemical stimuli; $F_{2,57}$=7.448, p=0.001; Tukey post hoc test, p<0.05). However, the intensity of aggressiveness was significantly lower when the two types of stimulus were emitted by a male rather than by a female, as a confirmation that sex identification in females requires the co-occurrence of olfaction and vision.

Comparison between sexes of the test crayfish

Significant inter-sexual differences were found when test crayfish were offered with chemical and visual stimuli but not with the two stimuli combined. The time spent by females in locomotion and in the aggressive posture was shorter in the chemical treatment but longer in the visual treatment (Fig. 3, Table 3).

Discussion

This study shows that, to identify the sex of a conspecific, *P. clarkii* females rely on chemical and visual stimuli as non-redundant signal components, olfaction and vision acting in concert to evoke an adaptive response. We found, in fact, that visual (and not chemical) stimuli alone of either a male or a female conspecific elicit the aggressive behaviour of females. It is the combination of chemical and visual stimuli that suppresses female aggressiveness, but only when the source conspecific is a male.

Our results also confirm previous studies (Ameyaw-Akumfi and Hazlett 1975; Dunham and Oh 1992, 1996) showing that crayfish males identify a female through the detection of chemical stimuli alone: *P. clarkii* males exhibit a lower aggressiveness when smelling a female rather than a male. In other decapod species, odours were also found to be used by males to assess the receptivity (in crayfish: Villanelli and Gherardi 1998) or the ovigerous status of females (in fiddler crabs: Goshima et al. 1996). Taken together, these results might suggest that in *P. clarkii*, similarly to the crabs *Carcinus maenas* and *Telmessus cheiragonus* (Bamber and Naylor 1996; Kamio et al. 2000), the male is the sex involved in mate search. The exclusive use of olfaction by *P. clarkii* searching males is not surprising given that (1) chemical stimuli may be detected at a longer distance than visual stimuli and (2) this species lives in turbid water conditions (Gherardi 2006). Conversely, in crayfish species that inhabit clearer waters such as *Austropotamobius pallipes*, searching males use both olfaction and vision (Acquistapace et al. 2002); visual stimuli might provide male crayfish with additional information about, for instance, the size of the encountered female as an index of her fecundity (Nobblitt

Table 1 Control vs. test phase

		Stimulus					
		C Male	C Female	V Male	V Female	CV Male	CV Female
Locomotion	Male	+++	+++	0	0	+++	+
	Female	++	+++	++	+++	++	++
Aggressive posture	Male	+++	+++	0	0	++	+++
	Female	0	0	+	++	+	++

Differences between the control and the test phase in the time spent in locomotion and in the aggressive posture per sex of the test crayfish (in column) and per treatment (in row). One, two, and three + denote significant differences at p<0.05, p<0.01, and p<0.001, respectively, after G tests. 0 means no difference between the control and the test phase

Table 2 Control vs. test phase

		TC+ Males	TC=	*G*	*p*	TC+ Females	TC=	*G*	*p*
Locomotion	Hom C	18	2	14.363	0.0002	18	2	14.363	0.0002
	Hom V	12	8	0.786	0.251	19	1	19.303	0.00002
	Hom CV	18	2	14.363	0.0002	15 (1)	4	7.522	0.005
	Het C	18	2	14.363	0.0002	16 (1)	3	10.554	0.001
	Het V	14	6	3.211	0.057	17	3	10.544	0.001
	Het CV	15	5	5.105	0.020	15 (1)	4	7.522	0.005
Aggressive posture	Hom C	18	2	14.363	0.0002	9	11	0.195	0.411
	Hom V	11	9	0.195	0.411	16	4	7.522	0.005
	Hom CV	17	3	10.544	0.001	16	4	7.522	0.005
	Het C	1	19	19.303	0.00002	8	12	0.786	0.251
	Het V	10	10	0.000	0.588	14	6	3.211	0.057
	Het CV	2	18	14.363	0.0002	5 (1)	14	3.211	0.057

Statistical results after *G* tests with Williams' corrections between the numbers of test crayfish that showed (TC+) or not showed (TC=) a change, from the control to the test phase, in the time spent in locomotion and in the aggressive posture per sex of the test crayfish and per treatment. Treatments are: single chemical stimuli, C, single visual stimuli, V, and chemical and visual stimuli combined, CV. Hom and Het denotes the sex of the source crayfish, which was either the same as the test crayfish (Hom) or the opposite (Het). The numbers in parentheses denote the number of crayfish showing a decrease in TC, if any

et al. 1995). The importance of odours in mate search is confirmed by the relatively few cases of decapods, in which sexual roles are inverted, such as *Homarus americanus* (Bushmann and Atema 1997). In this species, it is the female that, at about 5 m, identifies the other sex by odour; in the American lobster, odours are also used by the sheltering males to identify the sex of the approaching individual and to decide of whether to accept it (if a female) or to drive it out of the shelter (if a male intruder; Bushmann and Atema 2000).

Sexes differed in their responses also in the presence of visual stimuli alone. In fact, the vision of a conspecific had a slight effect on male behaviour, whereas females were found to respond to visual stimuli from either sex. The social life of females, thus, mostly relies on vision. This confirms previous evidence showing that, during mate selection (exceptions are the American lobster and the rock shrimp; Bushmann and Atema 1997, 2000; Díaz and Thiel 2004), females use visual stimuli (e.g. body or chela size) as indices of male

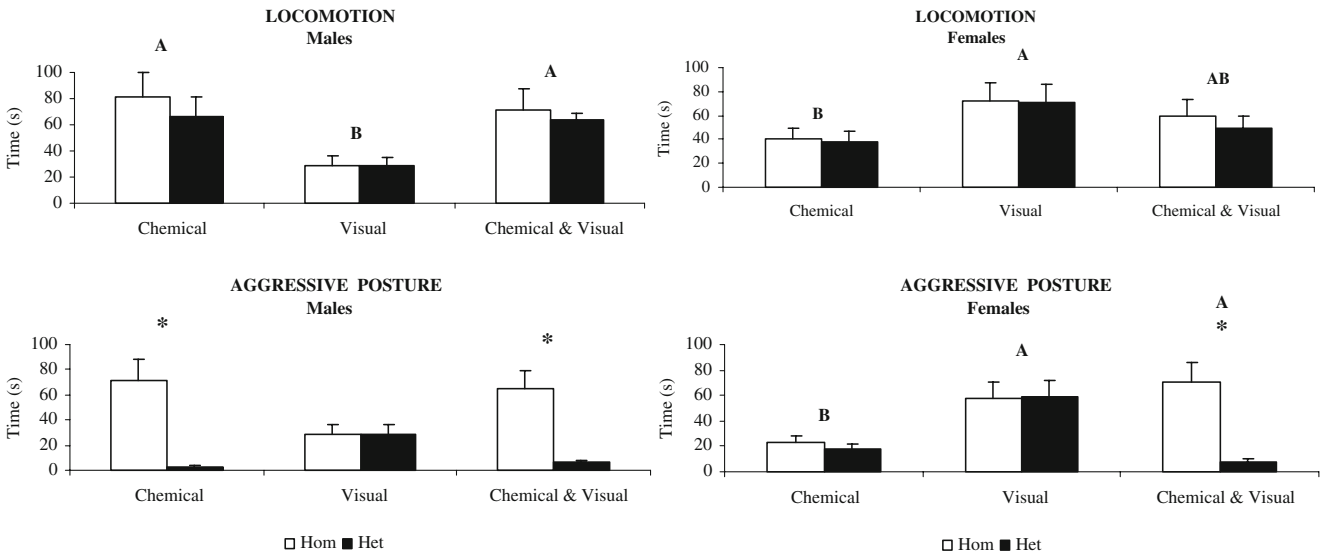

Fig. 2 Differences between the control and the test phases (mean± SE) in the time spent (in seconds) by *P. clarkii* males and females in locomotion (*A*, *B*) and in the aggressive posture (*C*, *D*): comparisons among treatments and sex of the source crayfish (Hom the same sex as the test crayfish; Het the other sex) per sex of the test crayfish. *Letters over bars* denote the hierarchy among treatments (C, V, and CV); *asterisk* denotes significant differences at $p<0.001$ after a Wilcoxon signed ranks test between Hom and Het. $N=20$ per treatment

Table 3 Comparisons between sexes of the source crayfish and between sexes of the test crayfish (b) in each treatment

		Between sexes of the source crayfish				Between sexes of the test crayfish	
		Z Male source	*p*	*Z* Female source	*p*	*U* Males vs. Females	*p*
Locomotion	C	−1.373	0.178	−0.604	0.758	−2.828	**0.005**
	V	−0.355	0.738	−0.443	0.672	−3.45	**0.001**
	CV	0.567	0.587	−1.176	0.250	−0.907	0.365
Aggressive posture	C	−3.724	**0.001**	−0.64	0.540	2.817	**0.005**
	V	−0.491	0.641	−0.118	0.927	−2.574	**0.01**
	CV	−3.636	**0.001**	−3.309	**0.001**	1.083	0.279

Time spent in locomotion and in the aggressive posture by males and females of test crayfish during the test phase: comparisons between sexes of the source crayfish after Wilcoxon signed ranks test (*Z*) and between sexes of the test crayfish after Mann–Whitney test (*U*) in the presence of chemical (C), visual (V), and chemical plus visual (CV) stimuli. Significant values in bold

quality (see, e.g. Forbes et al. 1992; Villanelli and Gherardi 1998; Sneddon et al. 2003; Galeotti et al. 2006; Gherardi et al. 2006; Aquiloni and Gherardi 2008a) and even eavesdrop on fighting males before choosing dominant mates (Aquiloni et al. 2008).

However, visual stimuli are not sufficient for *P. clarkii* females to identify the sex of a conspecific and/or to elicit their disposability to mate: only when they are combined with the odour of a male is female aggressiveness suppressed. A likely advantage offered by the non-redundant combination of odour and vision in this sex is to enhance the female ability to discriminate potential mates in the crowded social context experienced during mating period (Gherardi et al. 1999). Thanks to the enhanced discrimination ability, females might also avoid mating with low-quality mates, i.e. small-sized males (Aquiloni and Gherardi 2008b). Indeed, the reproductive investment of *P. clarkii* females is relatively high (Aquiloni and Gherardi 2008c) in terms of time (parental care may last 3 months; Huner and Barr 1991) and energy (each clutch is composed of 200 eggs on average).

To the best of our knowledge, sex identification in female *P. clarkii* is one of the few examples of non-redundancy in a bimodal communication system of invertebrates in which one signal component (odour) modulates the "message" of another (vision; Hughes 1996a, b).

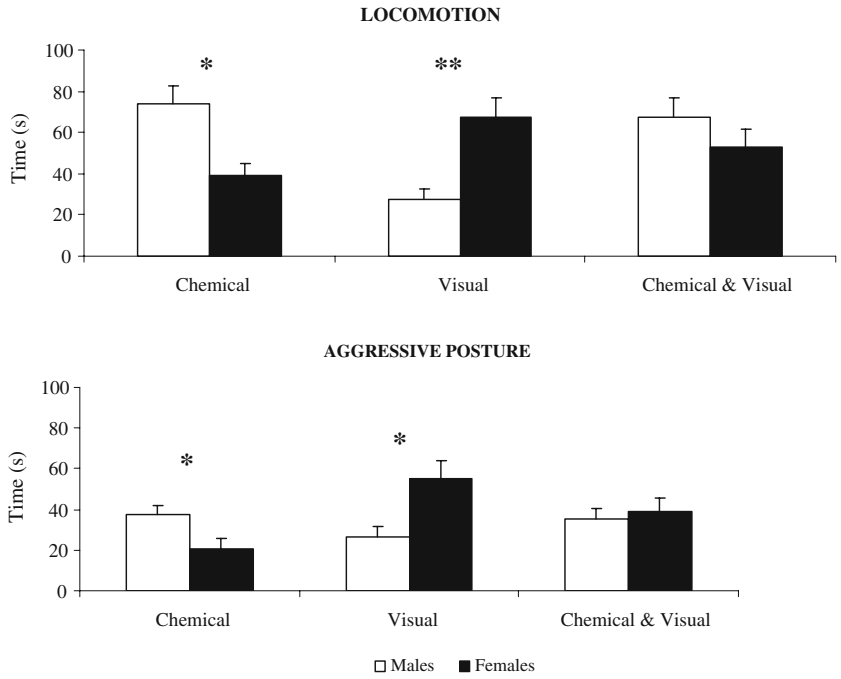

Fig. 3 Differences between the control and the test phases (mean± SE) in the time spent (in seconds) by *P. clarkii* males and females in locomotion and in the aggressive posture: comparisons between sexes per treatment. *One* and *two asterisks* denote significant differences at $p<0.01$ and $p<0.001$, respectively, after Mann–Whitney tests. $N=20$ per treatment

Acknowledgement We are grateful to three anonymous referees for their helpful suggestions. The study complies with the Italian laws of animal treatment in research.

References

Acquistapace P, Aquiloni L, Hazlett BA, Gherardi F (2002) Multimodal communication in crayfish: sex recognition during mate search by male *Austropotamobius pallipes*. Can J Zool 80:2041–2045

Ameyaw-Akumfi C, Hazlett BA (1975) Sex recognition in the crayfish *Procambarus clarkii*. Science 190:1225–1226

Aquiloni L, Gherardi F (2008a) Assessing mate size in the red swamp crayfish *Procambarus clarkii*: effects of visual versus chemical stimuli. Freshw Biol 53:461–469

Aquiloni L, Gherardi F (2008b) Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. J Zool 274:171–179

Aquiloni L, Gherardi F (2008c) Evidence of cryptic mate choice in crayfish. Biol Lett 4:163–165

Aquiloni L, Buřič M, Gherardi F (2008) Crayfish females eavesdrop on fighting males before choosing the dominant mate. Curr Biol 18:462–463

Atema J, Steinbach MA (2007) Chemical communication and social behaviour of the lobster *Homarus americanus* and other decapods. In: Duffy JE, Thiel M (eds) Evolutionary ecology of social and sexual systems. Oxford University Press, Oxford, UK, pp 116–190

Bamber SD, Naylor E (1996) Chemical communication and behavioural interaction between sexually mature male and female shore crabs (*Carcinus maenas*). J Mar Biol Assoc UK 76:691–699

Bamber SD, Naylor E (1997) Sites of release of putative sex pheromone and sexual behaviour in female *Carcinus maenas* (Crustacea: Decapoda). Est Coastal Shelf Sci 44:195–202

Bechler DL (1995) A review and prospectus of sexual and interspecific pheromonal communication in crayfish. Freshwater Crayfish 8:657–667

Bouwma P, Hazlett BA (2001) Integration of multiple predator cues by the crayfish *Orconectes propinquus*. Anim Behav 61:771–776

Bushmann PJ (1999) Concurrent signals and behavioral plasticity in blue crab (*Callinectes sapidus* Rathbun) courtship. Biol Bull 197:63–71

Bushmann PJ, Atema J (1997) Shelter sharing and chemical courtship signals in the lobster *Homarus americanus*. Can J Fish Aquat Sci 54:647–654

Bushmann PJ, Atema J (2000) Chemically mediated mate location and evaluation in the lobster, *Homarus americanus*. J Chem Ecol 26:883–899

Caskey JL, Bauer RT (2005) Behavioral tests for a possible contact sex pheromone in the caridean shrimp *Palaemonetes pugio*. J Crust Biol 25:35–40

Christofferson JP (1970) Evidence for the controlled release of a crustacean sex pheromone. J Chem Ecol 4:633–639

Corotto FS, Bonenberger DM, Bounkeo JM, Dukas CC (1999) Antennule ablation, sex discrimination, and mating behavior in the crayfish *Procambarus clarkii*. J Crustacean Biol 19:708–712

Cowan DF (1991) The role of olfaction in courtship behavior of the American lobster *Homarus americanus*. Biol Bull 181:402–407

Cronin TW, Hariyama T (2002) Spectral sensitivity in crustacean eyes. In: Wiese K (ed) The crustacean nervous system. Springer, Berlin, pp 499–511

Dawkins MS (1995) Unravelling animal behaviour. Longman, Harlow

Díaz ER, Thiel M (2004) Chemical and visual communication during mate searching in rock shrimp. Biol Bull 206:134–143

Dunham DW, Oh JW (1992) Chemical sex discrimination in the crayfish *Procambarus clarkii*: role of antennules. J Chem Ecol 18:2363–2372

Dunham DW, Oh JW (1996) Sex discrimination by female *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae): use of chemical and visual stimuli. Crustaceana 69:534–542

Forbes MRL, Pagola H, Baker RL (1992) Causes of a non-random pairing by size in the brine shrimp, *Artemia salina* (Crustacea: Anostroca). Oecologia 91:214–219

Galeotti P, Ruolini D, Fea G, Ghia D, Nardi PA, Gherardi F, Fasola M (2006) Cryptic female choice in a freshwater crayfish: females produce larger eggs for small, large-clawed males. Proc R Soc Lond B 273:1105–1110

Gherardi F (2006) Crayfish invading Europe: the case study of *Procambarus clarkii*. Mar Freshw Behav Physiol 39:175–191

Gherardi F, Raddi A, Barbaresi S, Salvi G (1999) Life history patterns of the red swamp crayfish, *Procambarus clarkii*, in an irrigation ditch in Tuscany. In: von Vaupel Klein JC, Schram FR (eds) The biodiversity crisis and Crustacea. Balkema, Rotterdam, pp 99–108

Gherardi F, Renai B, Galeotti P, Rubolini D (2006) Nonrandom mating, mate choice, and male–male competition in the crayfish *Austropotamobius italicus*, a threatened species. Arch Hydrobiol 165:557–576

Goshima S, Koga T, Murai M (1996) Mate acceptance and guarding by male fiddler crabs *Uca tetragonon* (Herbst). J Exp Mar Biol Ecol 196:131–143

Guilford T, Dawkins MS (1991) Receiver psychology and the evolution of animal signals. Anim Behav 42:1–14

Hazlett BA (1985) Chemical detection of sex and condition in the crayfish *Orconectes virilis*. J Chem Ecol 11:181–189

Hughes M (1996a) The function of concurrent signals: visual and chemical communication in snapping shrimp. Anim Behav 52:247–257

Hughes M (1996b) Size assessment via a visual signal in snapping shrimp. Behav Ecol Sociobiol 38:51–57

Huner JV, Barr JE (1991) Red swamp crawfish: biology and exploitation, 3rd edn. Sea Grant College Program, Baton Rouge, Louisiana

Immelmann K, Beer C (1989) A dictionary of ethology. Harvard University Press, Cambridge, p 106

Itagaki H, Thorp JH (1981) Laboratory experiments to determine if crayfish can communicate chemically in a flow-through system. J Chem Ecol 7:115–126

Kamio M, Matsunaga S, Fusetani N (2000) Studies on sex pheromones of the helmet crab, *Telmessus cheiragonus* 1. An assay based on precopulatory mate-guarding. Zool Sci 17:731–733

Kasuya E, Tsurumaki S, Kanie M (1996) Reversal of sex roles in the copulatory behavior of the imported crayfish *Procambarus clarkii*. J Crustacean Biol 16:469–471

Nobblitt SB, Payne JF, Delong M (1995) A comparative study of selected physical aspects of the eggs of the crayfish *Procambarus clarkii* (Girard, 1852) and *P. zonangulus* (Hobbs and Hobbs, 1990) (Decapoda, Cambaridae). Crustaceana 68:575–582

Partan S, Marler P (1999) Communication goes multimodal. Science 283:1272–1273

Partan S, Marler P (2005) Issues in the classification of multimodal communication signals. Amer Natural 166:231–245

Pavey CR, Fielder DR (1996) The influence of size differential on agonistic behaviour in the freshwater crayfish, *Cherax cuspidatus* (Decapoda, Parastacidae). J Zool 238:445–457

Quinn DGP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, p 556

Rowe C (1999) Receiver psychology and the evolution of multicomponent signals. Anim Behav 58:921–931

Scheffer SJ, Uetz GW, Stratton GE (1996) Sexual selection, male morphology, and the efficacy of courtship signalling in two wolf spiders (Aranae: Lycosidae). Behav Ecol Sociobiol 38:17–23

Siegel S, Castellan NJ (1988) Nonparametric statistics for the behavioural sciences. McGraw-Hill, New York

Simon JL, Moore PA (2007) Male–female communication in the crayfish *Orconectes rusticus*: the use of urinary signals in reproductive and non-reproductive pairings. Ethology 113:740–754

Sneddon LU, Huntingford FA, Taylor AC, Clare AS (2003) Female sex pheromone-mediated effects on behavior and consequences of male competition in the shore crab (*Carcinus maenas*). J Chem Ecol 29:55–70

Snyder MJ, Ameyaw-Akumfi C, Chang ES (1993) Sex recognition and the role of urinary cues in the lobster, *Homarus americanus*. Mar Behav Physiol 24:101–116

Stebbing PD, Bentley MG, Watson GJ (2003) Mating behaviour and evidence for a female released courtship pheromone in the signal crayfish *Pacifastacus leniusculus*. J Chem Ecol 29:465–475

Villanelli F, Gherardi F (1998) Breeding in the crayfish, *Austropotamobius pallipes*: mating patterns, mate choice and intermale competition. Freshwater Biol 40:305–315

Wisenden BD, Stacey NE (2005) Fish semiochemicals and the evolution of communication networks. In: McGregor PK (ed) Animal communication networks. Cambridge University Press, Cambridge, pp 540–567