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Acoustic signals and behaviour of *Ovalipes trimaculatus* in the context of reproduction

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ABSTRACT: This study focused on the acoustic behaviour of the paddle crab *Ovalipes trimaculatus* (De Haan, 1833) in relation to its reproductive status and behaviour. Paired males and females (demonstrating pre-copulatory behaviour) as well as solitary animals were collected a few hundred metres from the coast of Puerto Madryn (Patagonia, Argentina) and kept in indoor tanks. Synchronized acoustic and video monitoring systems were used to record the acoustic signals and other behaviours of single and grouped specimens in an experimental tank. Acoustic data were analysed to characterize crab sounds (duration, pulse number and pulse rate, 1st and 2nd peaks in frequency, amplitude of the frequency peaks and bandwidth) and were counted as behavioural events. Video data were analysed to count behavioural events (i.e. agonistic fight, chela spread) and determine status (locomotor indices, inter-crab distance). *O. trimaculatus* produced wide frequency band multi-pulse signals with significant differences between males and females: males showed a lower 1st peak in frequency, with a higher amplitude and a higher bandwidth. The sound emission rate was significantly higher in grouped animals than in single individuals. The sound emissions were not accidental events correlated with locomotor activities. In the trials involving pre-copulatory females, the total number of sounds was significantly higher compared to the trials with non-copulatory (control) females, and the sounds were not correlated with the agonistic events between males. Our data indicate that in *O. trimaculatus* sound emissions play a role in intraspecific communication related to sexual attraction.

KEY WORDS: Acoustic behaviour · Crabs · Reproductive context

INTRODUCTION

Many marine animals use acoustic signals to enact a wide range of biological activities. There is good evidence that sound is used as an orientation cue in fish to guide settlement onto reefs (Simpson et al. 2004), and that dolphins use echolocation to search for prey (reviewed by Au 1993, Miller et al. 2004, Johnson et al. 2008, Buscaino et al. 2015). Several species of marine crustaceans have evolved various sound production mechanisms (reviewed by Greenfield 2002, Patek & Oakley 2003, Buscaino et al. 2011a,b), such as percussion or rubbing (Imafuku & Ikeda 1990), stridulation (Boon et al. 2009), carapace vibrations (Patek & Caldwell 2006), stick and slip friction (Meyer-Rochow & Penrose 1976, Patek 2001, Patek & Baio 2007, Patek et al. 2009), snaps (Knowlton & Moulton 1963), emission of bubbles (Crane 1966), mandible grinding (Meyer-Rochow & Penrose 1976) and contraction of internal muscles (Henninger

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& Watson 2005). However, little is known about the ability of crustaceans to perceive sound and vibration (Meyer-Rochow et al. 1982, de Vincenzi et al. 2015). Many decapods have an extensive array of hair-like receptors that could potentially or actually respond to water displacements (reviewed by Popper et al. 2001) and changes in pressures (Ali 1987), however to date, intraspecific communication in decapods based on acoustic signals is still hypothetical.

Ovalipes trimaculatus (Crustacea, Brachyura, Polybiidae) is a swimming crab adapted to live in high-latitude environments (25 to 45° S; Boschi et al. 1992). It has a wide geographical distribution, dwelling on the sandy bottoms of the east and west coast of South America and South Africa (Arnaud et al. 1972, Nakamura et al. 1986, Boschi et al. 1992, de Melo 1996, Retamal & Arana 2000) from the low intertidal to about 100 m depth (Boschi et al. 1992). A more recent study conducted in the San Jorge Gulf (Argentina) (Vinuesa 2005) reported a more limited distribution to 30 m depth.

Guinot-Dumortier & Dumortier (1960) hypothesized that *Ovalipes* spp. produces sounds via stridulation, and that some species of this genus use the friction of their appendices on the cephalothorax or other body regions to synthesize sounds. Stephenson (1969) stated that these stridulatory structures vary in development with the species group, defining 'A' and 'B' groups. In *O. trimaculatus* and *O. punctatus* (both belonging to group 'A'), it was proposed that the sounding body consists of a large ribbed surface on the lower edge of the cheliped, which can rub on a partial horn surrounding the distal end of the merus of the first pair of walking legs (Guinot-Dumortier & Dumortier 1960). In *O. trimaculatus* males, these structures are bigger than those of females and likely affect the characteristics of the produced sound. However, the sound emission mechanism and the relative structures involved remains only hypothesized. Although sound production is known to occur in this species, the bio-ecological role of these signal emissions is not yet understood.

Some researchers have assumed that marine crustaceans only produce sounds in the context of anti-predator behaviour (Lindberg 1955, Moulton 1957, Hazlett & Winn 1962, Meyer-Rochow & Penrose 1974, 1976, Patek 2001, Patek & Oakley 2003, Bouwma & Herrnkind 2009). Others have proposed alternative functions that include mate attraction, aggregation, locating feeding individuals, and aggressive-territorial behaviour (Moulton 1957, Busnel 1963, Stephenson 1969, Meyer-Rochow et al. 1982, Buscaino et al. 2011a,b, de Vincenzi et al. 2015). For instance, McLay (1988) speculated that the sounds produced by *O. catharus* are involved in courtship display, and Parker et al. (1998) hypothesized that *Ovalipes* spp. use sounds as a sexual signal. Sexual behaviour plays an important role in mate attraction and mating pair formation. Nevertheless, there is neither research on the acoustic signals in the context of mating behaviour of *O. trimaculatus* nor a description of the acoustic parameters that characterize these signals. Information about the context in which the crabs emit acoustic signals is important, because it allows for a greater understanding of the ecological role of these signals. Moreover, the acoustic parameters that characterize the signals, and their energy distribution at different frequencies indicate the potential receivers of these signals, based on their hearing ability (i.e. their audiogram).

In Brachyura, communication during the reproductive period can occur through visual, acoustic, chemical and tactile cues, which may act synergistically (Sastry 1983, Sal Moyano & Gavio 2012, Sal Moyano et al. 2014). During the reproductive period, several changes occur in the biology of crustacean species with consequent changes in behaviour. For example, Pinheiro & Fransozo (1999) showed that in courtship exhibitions, males of the swimming crab *Arenaeus cribrarius* embrace females. They defined this behaviour as a 'precopulatory embrace', during which the male uses its second pereiopod pair to carry the female. Copulation occurs shortly after moulting, when the female has a soft carapace (Asakura 2009). Thus, it is reasonable to assume that some types of signals (chemical, acoustic, visual) occur between males and females to encourage the choice of the pre-moulting female.

In this study, we characterized the acoustic signals emitted by *O. trimaculatus* and examined whether they differed qualitatively and quantitatively between (1) sex, (2) single or grouped individuals, and (3) during the day or the night. We also studied the behavioural context of the acoustic emissions and its relationship to reproductive status.

MATERIALS AND METHODS

Animal housing and experimental design

The study was conducted in the marine laboratory tanks at the CENPAT-CONICET in Puerto Madryn (SE Argentina). A total of 100 specimens of *Ovalipes trimaculatus* were captured in February 2014 by snorkelling at a depth of 5 to 10 m near the beach of Puerto Madryn, including 37 mating pairs, 24 non-copulatory females (control), and 2 non-embracing males. In the mating pairs (with females in the pre-copulatory embrace), the configuration behaviour indicated that the females were almost ready to moult — a necessary condition for copulation (Crothers 1967, Haddon 1994). After capture, the pairs were separated and placed separately in 8 indoor rectangular PVC tanks (70 × 40 × 28 cm) equipped with an aeration system, and supplied with seawater collected in the Nuevo Gulf (where the animals were captured). The specimens were maintained in holding tanks (3 for the 39 males, 3 for the 37 females in the pre-copulatory condition and 2 for the 24 control females) for 1 wk acclimation. The source water supplying the experimental tank had a mean (±SD) salinity of 33 ± 0.1 ppt. The temperature was kept constant at 16 ± 1°C during the entire study period by an air-conditioning system. This temperature is similar to that during early summer in the Nuevo Gulf (Dellatorre et al. 2012), where the bottom temperature in shallow water varies between 9 and 19°C throughout the year.

Crabs were fed frozen molluscs, shrimps and fish ad libitum and were deprived of food for 2 d before the start of the experimental trials. All animals were kept under artificial photoperiods (12 h light, 12 h dark). After acclimation, the crabs (39 males and 61 females) were measured (carapace length [mean ± SD] of 80.1 ± 2.0 cm [females] and 112.9 ± 6.3 cm [males]), and randomly assigned from each group to the 3 experimental trials described below.

The crabs were assigned to the 3 experimental trials described below. Each crab was used in only 1 trial to meet the assumption of experimental independence. For the experimental procedure, we used only crabs that had hard-shelled carapaces. The crabs were released into the centre of a conical PVC experimental tank (Fig. 1) (1 and 0.8 m diameter at the mouth and at the bottom respectively and 0.5 m depth). To avoid the noise produced by the crabs' legs during walking, a rubber mat was placed on the bottom of the tank. No shelter was provided.

We analysed different acoustic, locomotor and agonistic behavioural parameters (Table 1) and performed 3 trial typologies (single, group, caged) to study the behaviour and the characteristics of the acoustic signals produced by *O. trimaculatus*. Trials began immediately following 1 h of acclimation; specimens were monitored for 2 h (trial duration). In total, we recorded 66 h of video and 72 h of acoustic recordings.

Trials

Single trials: the aim of the single trials was to assess whether single males, single control females, and single pre-copulatory females produced sounds. We also compared the sound emission rate with that of the grouped animals, and assessed other behavioural events and states (see Table 1). A total of 9 trials with single animals were carried out: 3 males alone, 3 control females alone, and 3 pre-copulatory females alone. In total, 18 h of video and audio data were analysed.

Group trials: the aim of the grouped trials was to assess the differences in behaviours among the different group types and in the acoustic parameters of the emitted sound. We also compared the sound emission rate with that of the single animals. Additionally, for the mixed sex group, we compared the sound emission rate in the daylight with the night. A total of 12 trials in groups of 5 animals were carried out: 9 mono-sex groups (3 groups of 5 males, 3 groups of 5 control females, 3 groups of 5 pre-copulatory females) and 3 mixed-sex groups (2 pre-copulatory females with 3 males). A total of 24 h of video and audio data were analysed.

Caged trials: the main aim of these trials was to evaluate whether sound emission rate was related to reproductive context. Moreover, we evaluated the agonistic behaviour of the males (see chelae spread, inter-crab distance and agonistic fight in Table 1) and the distance between the caged females and males.

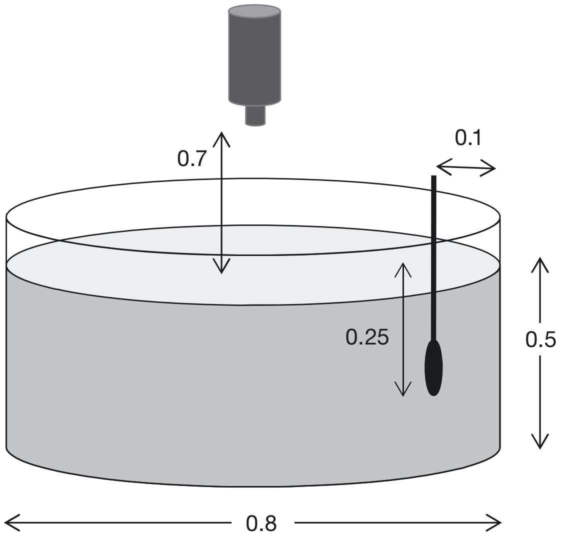

Fig. 1. Schematic representation (in m) of the experimental tank equipped with a hydrophone and video camera

Table 1. Description of *Ovalipes trimaculatus* behavioural events and states measured during different trials, and the number of hours of data analysed

	Description	Trial type	Hours (h) of video and acoustic data analysed
Behavioural event			
Acoustic signal[a]	No. of wide-band frequency multi-pulsed signals that were likely produced by the stridulating mechanism (rasp)	All during daylight Mixed sex group during night	66 (in daylight) 6 (at night)
Chela spread[a]	No. of threat displays using spread chela	Caged trials	24
Agonistic fight[a]	No. of fighting by grasping and pulling the opponent's chela or appendages	Caged trials (with only 2 males)	12
Contact approach[a]	No. of contacts with the other male without an agonistic fight	Caged trials (with only 2 males)	12
Contact cage[a]	No. of contacts with the cage using any part of the body	Caged trials	24
Behavioural status — distances			
Inter-crab distance[a]	Mean distance (cm) between 2 subjects measured from the carapace centre point of each crab	Caged trials (with only 2 males)	12
Cage–crab distance[a]	Mean distance (cm) between the crab and the cage measured from the centre point of the crab and the centre point of the cage	Caged trials	24
Behavioural status — locomotor			
Mobility	Cumulative duration (s) for which animal mean running velocity is between 1.50 and 1.80 cm s^{-1}	All	66
High mobility	Cumulative duration (s) for which animal mean running velocity exceeds 1.80 cm s^{-1}	All	66
Velocity	Mean distance (cm s^{-1}) moved by the centre point of the subject per unit time	All	66
Turn angle	Variation of direction (degree) of the head	All	66
Angular velocity	Variation of direction (degree s^{-1}) of the head per unit time	All	66

[a] Total number or mean distance in 10 min

These analyses allowed us to assess whether the sound emission rate was also linked to the agonistic events in the males that were competing for the females. The cage consisted of a parallelepiped (20 × 15 × 15 cm) of plastic net (mesh size 1 cm) that allowed for circulation of water inside and outside the cage, and also allowed the male to see the female in the tank. A total of 12 trials with 1 caged female (in either pre-copulatory or control condition) with 1 or 2 males outside the cage were carried out: 3 trials with a caged pre-copulatory female and 1 male, 3 trials with a caged control female and 1 male, 3 trials with a caged pre-copulatory female and 2 males, and 3 trials with a caged control female and 2 males. For the caged trials, 24 h of audio and video data were analysed.

Audio and video monitoring system

To avoid disturbing the animals inside the experimental tank, the audio-video monitoring and recording equipment was installed on a table placed 3 m from the tank. The acoustic behaviour of the crabs and the baseline noise of the tank were recorded using a calibrated hydrophone (model 8104; Bruel & Kjer) with a sensitivity of 205.6 dB re 1 V Pa^{-1} ± 4.0 dB in the 0.1 Hz to 80 kHz frequency band. The hydrophone was placed at a depth of 0.2 m, close to the tank wall (0.1 m). The equipment was connected to a digital acquisition card (USGH416HB, Avisoft Bioacoustics, septate with 40 dB gain) managed by a dedicated Avisoft Recorder USGH software (Avisoft Bioacoustics). The signals were acquired at 300 kilosamples s^{-1} at 16 bits and analysed by the Avisoft-SASLab Pro software (Avisoft Bioacoustics).

A video system was used to monitor the behaviours of the crabs, and was synchronised with the acoustic monitoring system. The videos for behavioural monitoring were collected using an analog camera (model PC-IRB23WP, PCBOX, lens: 3.6 mm, sensor: ¼′ CCD, effective picture NTSC: 510 × 492, horizontal resolution 420 TV lines) placed on the top of the experimental tank 0.7 m from the water surface (see Fig. 1). The camera was linked to a computer, and the files were managed by Nero Vision 12.0 (Nero Development & Services).

Acoustic analysis

To individuate the sounds emitted by crabs, an operator visualized the oscillogram and spectrogram of all the acoustic files in wave format from all trials. To avoid counting sounds produced by accidental contact between animals, we considered only sounds with a regular pulse rate and with 3 or more pulses. For the acoustic parameter measurements, we selected the sounds that did not show any overlap with noise generated accidentally by crabs.

For each selected sound, we measured the following parameters: (1) the number of pulses in the pulse train; (2) the pulse train duration (s), determined as the interval time between the first and last pulse of the pulse train; (3) the pulse rate (Hz), determined as the ratio of the number of pulses and the duration of the pulse train; (4) the 1st and 2nd peak frequencies (Hz), which were the frequencies corresponding to the highest and second highest amplitudes respectively, determined from the spectrogram (sampling frequency 300 kHz; FFT length: 512 points; resolution: 586 Hz; window: FlatTop); (5) the 1st and 2nd peak amplitudes (dB re 1 µPa), the amplitude of the 1st and 2nd peak frequencies determined in the spectrogram; (6) the 3 dB bandwidth, determined as the frequency range within which the power spectrum density was above half of its maximum value (that is, above −3 dB relative to the peak); and (7) the 10 dB bandwidth, determined as the frequency range within which the power spectrum density was above 10 dB of its maximum value.

Video analysis

A visual continuous sampling procedure (i.e. each observed event annotated in a sampling table) was only applied for the behaviours ‘chela spread’, ‘agonistic fight’, ‘contact approach’ and ‘contact cage’ (see Table 1), and was performed because the automated software was unable to identify and measure these specific behavioural events. After this phase, the videos were analysed with EthoVision XT 9.0 software (Noldus Information Technology). The experimental arena was calibrated across the bottom wall of the tanks, and the calibration axes were placed to designate the origin (0, 0) at the tank centre. Group trials were analysed by adopting the Social Interaction Module (SIM). The SIM is an add-on to the EthoVision XT program that enables the detection of multiple unmarked animals in a social context. It is capable of assessing the crabs′ shoaling behaviour by simultaneously tracking all specimens and recording dynamic changes in the social behaviour between the subjects. The analysis was performed on each frame to distinguish the object(s) from the background on the basis of their greyscale/brightness values and by extracting the coordinates of the geometric centre and surface area for each object in each frame. The data were acquired at 25 frames s^{-1}. In all experiments, the subject loss due to misdetection by video-tracking software was <2%. Behavioural data were exported to Microsoft Excel to generate the total and per-minute plots for each endpoint. We then pooled the behaviours into 10 min sections. See Table 2 for a description of all behavioural states and events that were evaluated.

Statistical analysis

The data were tested for goodness-of-fit to the normal distribution using a chi-squared test. Different distributions in behavioural data were observed; therefore, nonparametric tests were applied to compare the different values among the trials.

To determine differences in acoustic parameters between male and female sounds, we used a Mann-Whitney *U*-test (sex condition: male or female). In the caged trials, the Mann-Whitney *U*-test was also used to compare all behavioural variables measured in the 1- or 2-male trials (condition: 1 or 2 males), and with different female reproductive status (condition: pre-copulatory or control females).

A Kruskal-Wallis test was used to assess if behavioural parameters among single animals (conditions: male, female control, female pre-copulatory) and among animals in groups (conditions: males, females control, female pre-copulatory, mixed sex) were different. Moreover, a comparison between single and grouped animals was performed to determine any differences in sound emission rate using a Mann-Whitney *U*-test (conditions: single, group).

The statistical tests were performed on data collected every 1 min for the total experimental time procedure. Results were considered statistically significant at $p < 0.05$. All statistical analyses were performed using the STATISTICA 7.0 software package (StatSoft).

RESULTS

Acoustic signals

The mean power spectrum of signals from 10 crabs (from the mixed sex group trial recordings) was distinguishable from the background noise of the experimental tank (Fig. 2a). An oscillogram and spectrogram of a signal from a male are provided as examples (Fig. 2b).

A total of 207 sounds were recorded during all trials. We selected 82 signals from the mono-sexual group or single animals to measure the acoustic parameters, distinguishing males and females (without any distinction between pre-copulatory or control females) (Table 2). Males and females showed similar acoustic characteristics. However, significant differences were found in the 1st peak frequency, its amplitude and the 10 dB bandwidth. Specifically, the median values of the 1st peak frequency was lower in males than females (3.5 and 4.8 kHz respectively; Mann-Whitney *U*-test, $p < 0.05$). Conversely, the median values of the 1st peak frequency amplitude was higher in males than in females (127.0 and 123.5 dB re 1 µPa respectively; Mann-Whitney *U*-test, $p < 0.05$). In addition, males presented higher median values than females for the 10 dB bandwidth (5.55 and 2.90 kHz; Mann-Whitney *U*-test, $p < 0.05$).

Single trials

Individual crabs were monitored for 18 h. Acoustic analysis showed that 2 out of 3 pre-copulatory females emitted signals (3 in 6 h); conversely, males and control females (in a total of 12 h) did not produce any sounds. Video analysis showed that the control females presented significantly higher velocity values than the pre-copulatory females and higher mobility values than the males (Fig. 3) (Kruskal-Wallis multiple comparison test, $p < 0.05$).

Group trials

In the group trials, both the males and females produced acoustic signals. Grouped animals produced pro capite a higher number of sounds than single animals. Grouped males emitted the highest number of sounds (Mann-Whitney *U*-test, $p < 0.05$) compared to females (considering control and pre-copulatory females together). The mean (±SE) number of signals per specimen in 10 min was 0.30 ± 0.05 and 0.13 ± 0.03 for males and females respectively (considering control and pre-copulatory females together). The mixed sex groups presented a higher number of signals during the night than during the day (Mann-Whitney *U*-test, $p < 0.04$; mean no. of signals per 10 min: 0.53 ± 0.11 and 0.30 ± 0.08 respectively).

The grouped males showed lower values of velocity and higher mobility compared to the other 3 group types (Kruskal-Wallis multiple comparison test, $p < 0.05$) (Fig. 4). The pre-copulatory female and mixed-sex groups had higher values of turn angle and angular velocity (Kruskal-Wallis multiple comparison test, $p < 0.05$). The mixed groups had higher values of mobility compared to the female groups (Kruskal-Wallis multiple comparison test, $p < 0.05$).

Caged trials

The signal analysis performed in the caged trials (with 1 male and 2 males) showed a significantly higher number of sound emissions when a pre-copulatory female was present (Fig. 4a).

With 1 male. The mean (±SE) number of acoustic signals per individual in 10 min was higher in the trials with a pre-copulatory female than a control female (0.28 ± 0.09 and 0.03 ± 0.02 respectively; Mann-Whitney *U*-test, $p < 0.01$). In the other behavioural events and states, no significant differences were observed between the trials with the control or pre-copulatory female with the exception of the contact cage event, which was higher in the trial with the pre-copulatory female (Fig. 4d).

With 2 males. The mean (±SE) number of signals per individual in 10 min was significantly higher in the trials with a pre-copulatory female compared with the control female (Mann-Whitney *U*-test, $p < 0.01$), with values of 0.24 ± 0.05 and 0.07 ± 0.02 respectively. In the trials with the caged pre-copulatory female, the number of acoustic signals per animal did not change if we considered the trials with 1 or 2 males (see the previous sub-section) (Fig. 4a).

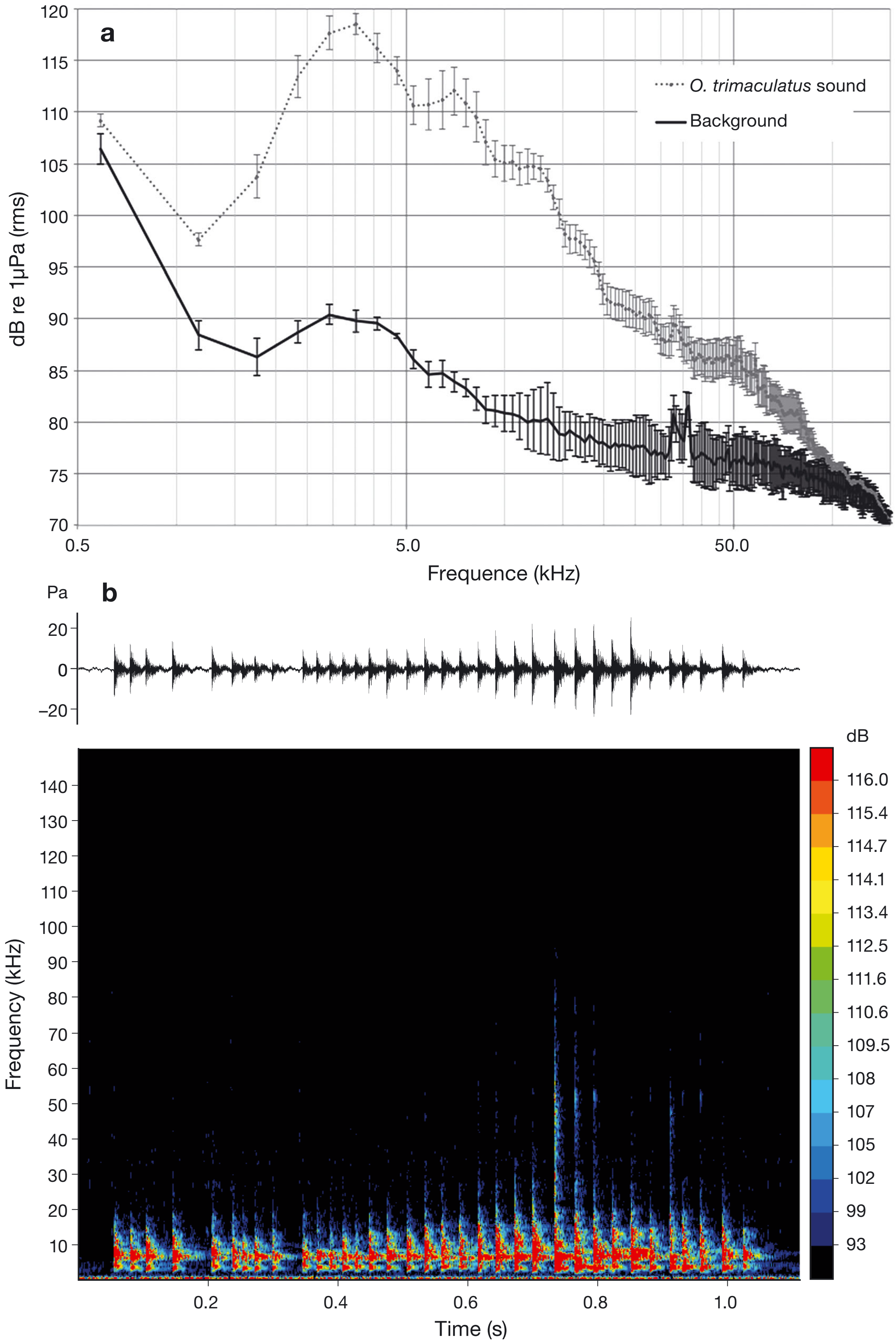

Fig. 2. (a) Mean (±SE) power spectrum of 10 *Ovalipes trimaculatus* signals compared with the background noise of the experimental tank, and (b) the oscillogram (top) and spectrogram (bottom) of a signal from a male. Colour scale: dB re 1µPa (rms)

Table 2. Comparison between acoustic parameters of sound (median ± 10th to 90th percentiles) produced by male (n = 54) and female (n = 28) (considering control and pre-copulatory females together) *Ovalipes trimaculatus*, and associated p-values based on the non-parametric Mann-Whitney *U*-test. Significant ($p < 0.05$) values in **bold**

Parameter	Units	Males		Females		p
		Median	10–90 %	Median	10–90 %	
No. pulses per rasp	no.	11.0	4.0–35.0	8.0	4.0–25.0	0.55
Rasp duration	s	0.150	0.070–0.910	0.145	0.055–0.445	0.60
Pulse rate	Hz	57.1	31.0–105.0	52.9	33.7–97.8	0.82
1st peak frequency	kHz	3.5	1.1–7.6	4.8	3.1–11.4	**0.03**
2nd peak frequency	kHz	9.6	4.1–19.3	9.2	3.5–15.8	0.53
1st peak frequency amplitude	dB re 1µPa	127.0	115.0–139.0	123.5	113.5–132.0	**0.03**
2nd peak frequency amplitude	dB re 1µPa	119.0	108.0–130.0	117.0	108.0–126.0	0.07
Bandwidth 3 dB	kHz	1200	1100–4700	1100	600–1200	0.34
Bandwidth 10 dB	kHz	5550	2900–11650	2900	2300–4100	**0.03**

The comparison of the other behavioural states and events showed significant differences (Mann-Whitney *U*-test, $p < 0.01$) between the trials. In particular, with a caged pre-copulatory female, the males showed higher values of chela spread, velocity, mobility, and high mobility (Mann-Whitney *U*-test, $p < 0.01$) (Fig. 4b,c,e,f).

DISCUSSION

The analysis of our data showed that both sexes of *Ovalipes trimaculatus* produced sounds. Males and females presented a similar sound structure that consisted of a multi-pulsed 'rasp' (Fig. 2, Table 1), which was similar in structure to other rasps produced by stridulating crustaceans (Patek 2001, Buscaino et al. 2011a). For both sexes, the 1st and 2nd peaks in frequencies fell within the human audible range (below 10 kHz); however, ultrasound frequencies were present until 100 kHz (see the power spectrum in Fig. 2a). These higher spectral energies are typical of a stridulation mechanism if we exclude the 'rapping' among them, namely striking the claw against the substrate (Popper et al. 2001). This was avoided in our study by positioning a rubber substrate on the bottom of the tank. Even though male and female sounds revealed similar structures, 3 significant differences in acoustic characteristics between the sexes were found. In particular, in females the median values of the 1st peak frequency and 1st peak frequency amplitude were significantly higher and lower, respectively, than those of the males. Moreover, males produced a sound with a slightly larger bandwidth with respect to females. Although there are exceptions, crustacean males generally grow faster and larger than females (Aiken 1980), and these growth differences are particularly evident in the secondary sexual characters such as the chelae, abdomen and first pleopods (Hartnoll 1974, Vallina et al. 2014). In this genus, stridulation is hypothesized to occur by rubbing the appendages against the carapace (Stephenson 1969). Therefore, it is possible that our results may be correlated with the differences in body size between males and females, because an increase in the size of the sound-producing apparatus should permit the production of louder signals, and consequently less acuteness in frequency (Patek & Oakley 2003).

In the single trial type, in which we had the opportunity to associate sound emission with a specific movement of an animal in the tank, we did not observe any large movement before, during or after sound emission, and the specimen did not walk. However, the quality of the videos did not allow us to assess if there were small movements of the legs against the chela to confirm the stridulation mechanism (Guinot-Dumortier & Dumortier 1960, Stephenson 1969, Parker et al. 1998). In some crustacean decapod species, sound production results from an accidental event due to the movement of the animal with respect to the bottom, and/or is associated with a behavioural event such as a tail flip (e.g. in *Palinurus* or *Procambarus* species) (Buscaino et al. 2011a,b, 2012). However, in *O. trimaculatus* the production of sound did not appear to be correlated with large movements, and thus we assumed that the production of sound was not an accidental event. This hypothesis was confirmed by our results because the males in groups presented lower values of locomotor parameters compared to females, yet had higher sound emission rates.

The mixed sex groups showed a higher rate of sound emission during the night compared to the day. This result is in agreement with other studies of aquatic crustaceans, which have revealed the ten-

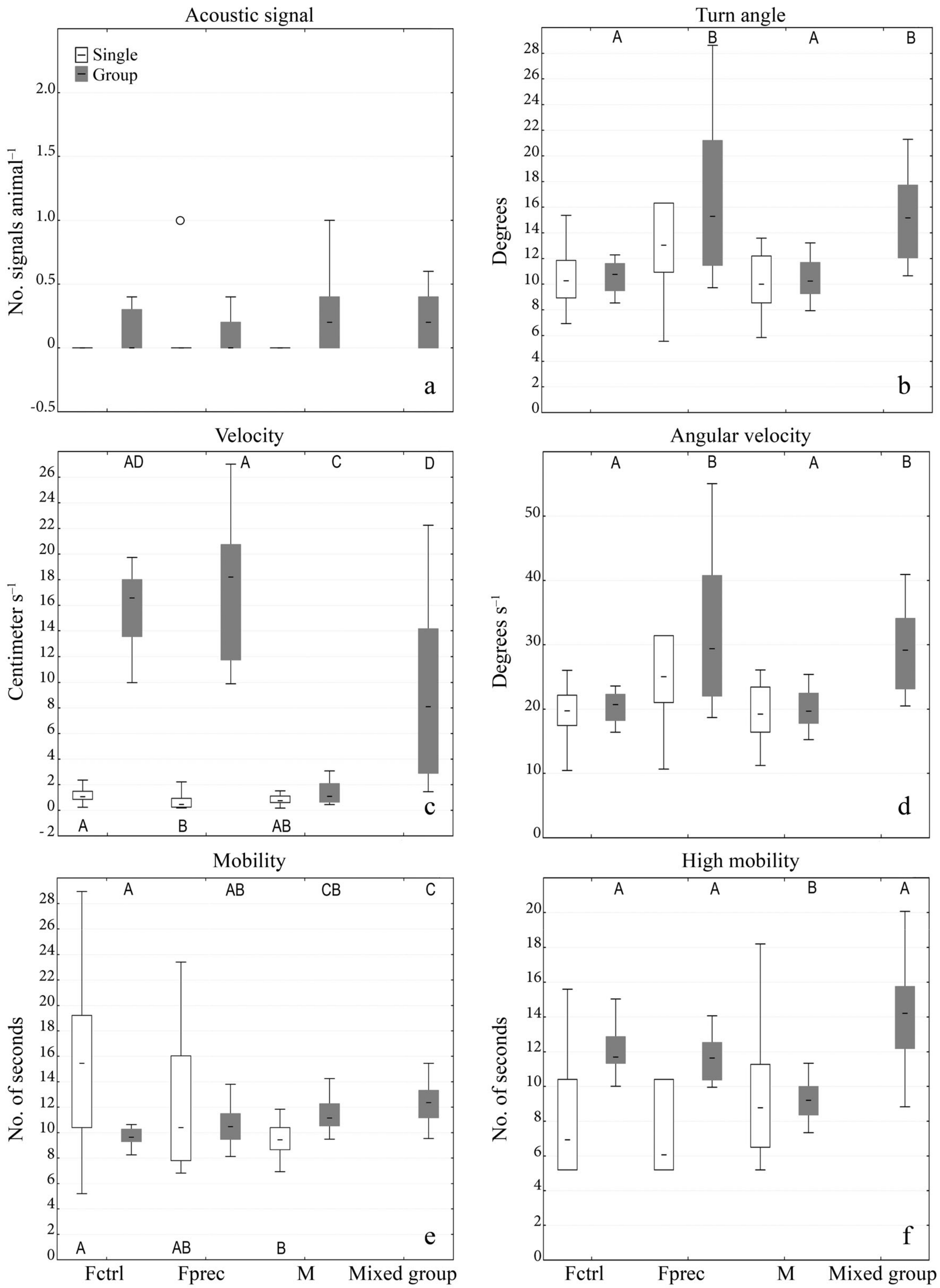

Fig. 3. Behavioural events and states for single and group trials per animal in 10 min, showing median ± 25th to 75th percentiles; error bars: 95 % CI. In (a), the symbol (○) refers to an outlier. Fctrl: control female; Fprec: female in pre-copulatory condition; M: male; Mixed group: group with 3 males and 2 pre-copulatory females. The different letters indicate significant differences among the trials: below for significant differences within grouped animals, above for significant differences between single animals. For example, in (b) the 'A' above indicates significant differences of group Fctrl with regard to the group Fprec and Mixed group but no significant difference with regard to group M

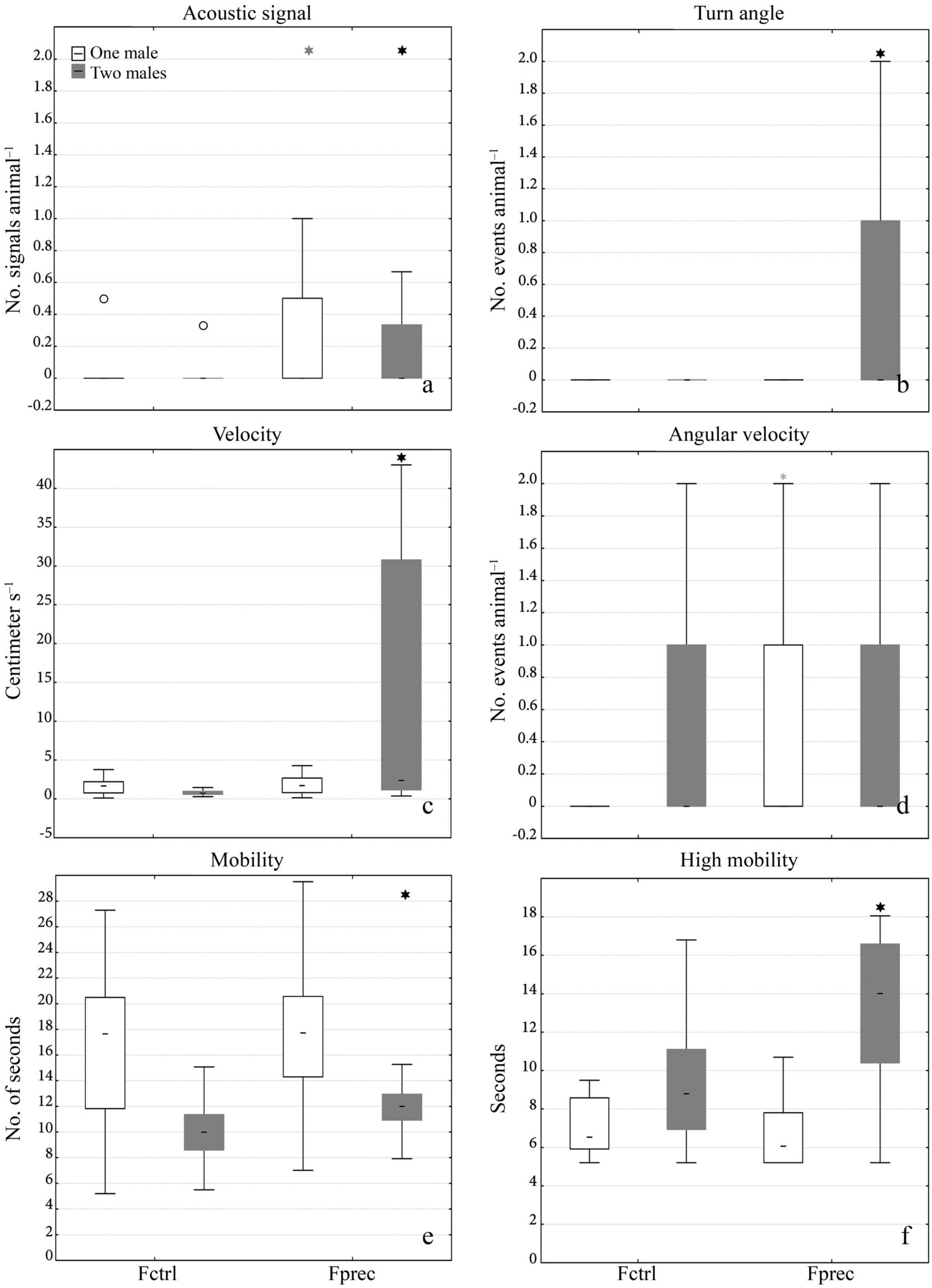

Fig. 4. Male crab behavioural events and states per 10 min in the presence of caged female (Fprec: pre-copulatory female; Fctrl: control female). For the acoustic events, the value refers to 1 animal without the distinction of sex. The plots concern only the behaviours in which differences between control (Fctrl) and test (Fprec) were significant. Median ± 25th to 75th percentiles; error bars: 95% CI. Outliers (°) are presented only for the acoustic signal graph. Asterisks (*) represent significant differences between control and test conditions (grey asterisk for significant differences within the 1-male trials; black for significant differences within 2-male trials)

dency to increase sound emission rate and locomotor activities at night (Buscaino et al. 2012, Giacalone et al. 2015). In animals that are active nocturnally, such as *Ovalipes* spp. (Takahashi & Kawaguchi 2001), visual signals are of limited value, and thus vocal and chemical cues could be used in mate attraction. During the night, sexual partner selection by *O. trimaculatus* could be based on the partner's perceptible signals, such acoustic or chemical.

In brachyurans, most acoustic studies have focused on semi-terrestrial crabs (e.g. Sesarminae, Ocypodidae, Gecarcinidae) (Parker et al. 1998, Boon et al. 2009). In crabs, sounds can play an important role in courtship (Popper et al. 2001), and most of the current literature on mating behaviour has primarily focused on the use of signals by males to attract females. These signals were recorded, especially during phases of the lunar cycle, when females were receptive to sounds (Popper et al. 2001). Salmon (1967) showed that males of Ocypodidae (*Uca rapax* and *U. speciosa*) produce sounds to attract receptive females to their burrows. In the current study, analysis of the single trials indicated that only females in a pre-copulatory state produced sounds. We therefore assume that females in the pre-copulatory phase can use acoustic signals, probably in addition to chemical ones, to attract males. The acoustic, chemical and visual signals could all be cues for intraspecific communication in crustacean species, and the prevalence of one with respect to the others could vary as a function of species distribution and/or ecological condition (i.e. day-nighttime, current, predation risk, sexual stage, presence of sexual partner or conspecifics that compete for the same resources).

In the group trials, control females, males and pre-copulatory females emitted acoustic signals. In particular, a significantly higher number of emissions were found in the male groups compared to the other groups and the single trials. This result was in agreement with another study conducted with Ocypodidae that revealed that undisturbed males produced sounds at relatively slow rates, but when stimulated by sounds of other crabs or by the nearby movements of neighbouring specimens, the rates of sound production could more than double (Popper et al. 2001). Moreover, the male groups presented lower values of locomotor behaviours, such as velocity and high mobility, than the control and pre-copulatory female groups. In mobile marine species, such as paddle crabs, locomotor movement is an important link between the behaviour of individuals and ecological processes (Herrnkind 1983, Spanier et al. 1988, Lawton & Lavalli 1995, Giacalone et al. 2006). In our study, the single pre-copulatory females presented lower velocity values than the control females. These results are in agreement with the results found for *Uca pugilator*, in which individual females in the pre-copulatory condition occupied temporary burrows for approximately 2 wk, presumably to accumulate reserves sufficient to produce a clutch of eggs (Christy & Salmon 1984, Turner et al. 2003) and for muscle growth (Medici et al. 2006). In this way, their locomotor activity was considerably lower than females with the basal condition.

In addition, the results obtained from the group trials with the pre-copulatory females (see the mixed groups and the mono-sexual pre-copulatory female groups) had higher rates of turn angle and angular velocity (Fig. 3b,d). These movements are not finalized to linear displacement in space and could be associated with the search/approach orientation phases. In the presence of pre-copulatory females, higher rates of turn angle of males and/or females could indicate a specific interest in the approach of the sexual partner. Additionally, an increase in angular velocity could favour a better perception of chemical stimuli produced by the female during the pre-copulatory phase.

In the caged trials with both 1 and 2 males, the number of sounds was higher when a female in the pre-copulatory condition was present. However, the emission sound rate did not change between the 1- or 2-male trials in the presence of a pre-copulatory female. Additionally, in the 2-male trials the agonistic parameter 'chelae spread' and some locomotor values increased in the presence of a pre-copulatory female. These 2 results led us to consider that in these crabs, sound emissions are directly connected to sexual activity and not to agonistic behaviours. Considering that in the single trials, the pre-copulatory females produced a higher number of signals than the control females and the males, we hypothesized that in the caged trials, the pre-copulatory females emitted a higher number of acoustic signals to attract males. Otherwise, the male (in the presence of a pre-copulatory female) can be elicited by the female's chemical signals to emit sounds and attract the female to mate. However, in our caged trials, it is unknown whether the males or females produced the sounds.

Crabs are mobile species, and their movements are highly related to ecological and biological aspects. In particular, territoriality, nomadism and migration are locomotor behaviours that have implications for the survival, growth and reproduction of decapod crustaceans (Herrnkind 1983, Spanier et al. 1988, Lawton & Lavalli 1995). The results of our 2-male caged trials showed a significant increase in locomotor behaviours such as mobility, high mobility and velocity when females in the pre-copulatory condition were present. Many genera of crabs, including the portunid *O. trimaculatus*, can only copulate immediately following the moulting period, when the female has a soft carapace (Crothers 1967, Haddon 1994). Male paddle crabs are regularly found carrying and defending pre-moult females (Haddon 1994). Haddon (1994) documented males of the New Zealand paddle crab *Ovalipes catharus* carrying pre-moult females under their body for up to 8 d prior to the female moulting. This pre-moult guarding by the males should increase the chances of a female finding a suitable partner in the 4 d available before her newly secreted exoskeleton becomes too hard. At the same time, immediately post-moult, crabs were extremely vulnerable to cannibalism (Haddon 1994), so that copulating males vigorously defended their partners from attacks by other paddle crabs (Haddon 1994). This evidence suggests that male *O. trimaculatus* may increase their locomotor behaviour in the presence of other males in order to protect his potential partner for copulation. This was defined by Christy (1987) as 'pure search and interception' that is, searching for or intercepting females and attempting to mate with those they encounter.

This study was the first characterization of the acoustic parameters of sounds emitted by *O. trimaculatus*, and revealed differences between males and females. The sound emission rate was higher in grouped animals (relative to individuals housed alone) and was also higher during the night (relative to the day). Sound emission did not occur as a result of accidental events correlated to locomotor activities, and seemed to play a role in intraspecific communication related to sexual attraction.

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