

Article

Agonistic Behaviour and Sound Production during Male–Male Varunid Crabs (*Cyrtograpsus angulatus*, Dana 1851) Encounters

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Abstract: Crustacean sounds are the main contributor to shallow water soundscape biophonic components. Here, we investigate the sound production of *Cyrtograpsus angulatus*, one of the most important intertidal decapods of the Southwestern Atlantic Ocean. Underwater audio and video were recorded during encounters among male pairs. Two kinds of experimental settings were conducted involving same size (SS) and different-size (DS) male crabs. Behavioural states, behavioural events and sound emission were investigated. SS spent more time in contact and still behaviour and showing competitive interactions than did DS. Crabs presented body displays involving chelae, legs, and maxillae movements with the same frequency in both settings. Our results demonstrate the production of impulsive sounds in male *C. angulatus* with high rates during contact and still behaviour (specifically during agonistic interactions and body displays without contact between individuals). Furthermore, we were able to describe behaviour-related variability within impulsive sounds. These outcomes provide a better understanding of the role and behavioural context of these sounds in the natural soundscape.

Keywords: decapods; crabs; bioacoustics; agonistic behaviour



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1. Introduction

Acoustic communication in the water environment is one of the most efficient mechanisms to transmit information [1,2]. Sound production and perception adaptations to the environmental propagation properties are crucial during key phases of the lifecycle of marine and freshwater animals [3,4]. Traditionally, research has focused on marine mammals and fish, but studies referring to invertebrate acoustic activity and their role in soundscapes composition have been growing in the last years [5].

The marine coastal water soundscape is dominated by impulsive sounds generated by several invertebrate species. The spatial and temporal patterns of sounds can be proxies of animals presence and activities. Crustaceans produce sounds in different behavioural contexts, such as prey-predatory behaviour [6–8], aggressive territorial behaviour [9–11], defence [12], and reproductive behaviour [13,14]. Sound is generally produced by striking a body part on a substratum, vibrating appendages, during respiration, and via percussion and rasping involving the cephalothorax or the appendages [15–18].

Sounds generated by crustaceans are generally broadband impulsive signals, characterized by short duration and changing peaks of frequency according to the species. They can be emitted alone or in trains of several impulses (called rasps), depending on the behavioural context [19,20]. For example, males of *Neohelice granulata* (Dana, 1851) produce underwater impulsive signals by the rasping of merus and the pterygostomial area, increasing their acoustic activity in the presence of receptive females [14,18]. Coquereau

et al. [19,20] reported the most comprehensive acoustic description of large crustaceans in temperate coastal habitats, highlighting that crustacean sounds are differentiable by acoustic features according to species and behaviour. The description of sounds emitted under different behavioural contexts may allow the differentiation of crab species and help to identify the source of biological components in the soundscape.

Members of the infraorder Brachyura produce drumming, thumping, and rasping sounds during courtship or agonistic activity in a mating context [14,18,21–23], but few acoustic studies were focused on other behavioural contexts [23–26].

Cyrtograpsus angulatus (Varunidae) lives in intertidal areas from Rio de Janeiro (22°55' 46.953" S, 43°8'48.306" W; Brazil [27]) to Puerto Deseado (47°45'20.275" S, 65°53'9.093" W; Argentina). Juveniles inhabit rocky shores and brackish waters, sheltering under rocks or other structures [28,29]; adults live superficially buried in muddy habitats [30]. The reproductive period starts from early summer until late summer with subsequent egg clutches according to its seasonal reproductive activity pattern [31]. During this period, large aggregations of solitary males, ovigerous females and mating pairs are found [28]. Males involved in mating pairs hold females displaying pre-copulatory guarding behaviour [32]. Solitary males are commonly involved in fights among themselves and with males already engaged in mating pairs, trying to “capture” females [32]. Males in the mating pairs are always larger than the solitary males [32]. These mechanisms suggest a female-centred competitive mating system (sensu Christy, 1987) [33]. Field experiments showed that larger males outcompeted smaller ones in contests for females when they had intact appendages [34].

In Mar Chiquita coastal lagoon (37°44'28.138" S, 57°24'58.549" W, Buenos Aires province, Argentina), *C. angulatus*, jointly with another Varunidae crab species, *N. granulata*, are the most important macrofaunal species present in the lagoon and is recognized as a keystone species [31]. Recent studies have shown the importance of crab impulsive sounds contributing as unique biological components of the lagoon soundscape over 2 kHz and being used by larvae and juveniles *C. angulatus* in their orientation to the recruitment areas [35,36]. The sounds increased during spring along the entire lagoon and especially in the mouth where a strong circadian rhythm also occurred [35]. The origin of these sounds is still unknown. Laboratory experiments demonstrated that *Neohelice granulata* emits impulsive sounds in a competing mating context [14]. However, the spatial and temporal patterns of impulsive sound occurrence suggest a different emitting species [35]. Indeed, the impulsive signals that compose the soundscape of the lagoon were recorded in areas with high abundances of *C. angulatus*: the mouth of the lagoon and reefs built by the invader polychaete *Ficopomatus enigmaticus* (Fauvel 1923) [35].

To date, no studies have been conducted to investigate the sound production by *C. angulatus*. Considering the extension of the temporal and spatial distribution of impulsive signals out of the mating period, we aimed to test the hypothesis that *C. angulatus* males emit impulsive sounds during their interaction behaviours. In particular, considering the relevance of body size for the agonistic behaviour in this species [34], we investigated: (1) the behaviour of *C. angulatus* males interacting with a similar and different sized male; (2) the acoustic emission of *C. angulatus* males interacting with a similar and different sized male; and (3) the temporal and spectral acoustic characteristics of sounds emitted by *C. angulatus* males during different behaviours.

2. Materials and Methods

2.1. Animal Collection and Maintenance

Male crabs were hand collected in the shallow subtidal of the Mar Chiquita coastal lagoon. Individuals were maintained in holding aquaria (30 cm × 60 cm × 25 cm, seawater depth: 15 cm) with aerated seawater; rocks were placed at the bottom to provide crabs with refuge from stress. A maximum of two similar-sized crabs were placed per aquarium during maintenance. Individuals were fed three times per week with pieces of squid and the water was changed shortly after feeding. The crabs were maintained under natural

daylight conditions at 22 ± 2 °C and were used in the trials (see below) within the week of capture. At the end of the experiment, crabs were released in the field and if necessary, new animals were collected and maintained as described above.

2.2. Experimental Setup

Two kinds of experimental settings were conducted by using two Same-Sized (SS) or two Different-Sized (DS) male crabs. Individuals belonging to different holding aquaria were randomly selected and released in the centre of the experimental tank (65 cm wide × 100 cm long × 65 cm deep) using a net. The experimental tank was filled with filtered seawater. To avoid substratum-borne vibrations produced by crab locomotion, and to reduce visual reverberation during video recordings, the tank was internally covered with ethylene-vinyl acetate foam 10 mm thick. Twelve trials for each setting were carried out and each crab was used during only one trial to meet the assumption of experimental independence (Table 1).

Table 1. Average (\pm SD) of chelae length and carapaces width of male crabs (M1 and M2) involved for 12 trials for Same Sized (SS) and Different Sized (DS) experimental setting and their differences.

Replicates	Trials	Experimental Setting	Carapace M1 (mm)	Chelae M1 (mm)	Carapace M2 (mm)	Chelae M2 (mm)	Carapax Difference (mm)	Chelae Difference (mm)
24	12	SS	30.9 (\pm 3.05)	20.8 (\pm 3.94)	30.9 (\pm 3.94)	21.5 (\pm 4.03)	1.6 (\pm 1.35)	1.4 (\pm 1.27)
	12	DS	35.2 (\pm 4.12)	25.9 (\pm 3.6)	25.7 (\pm 3.08)	15.9 (\pm 1.5)	9.46 (\pm 4.5)	10.1 (\pm 4.12)

The trial lasted 1 h and started after an acclimation period of 10 min. Following studies on the size frequency distribution and relative growth of this species [32], males forming an experimental pair were considered of different sizes (i.e., DS) when their carapace width differed by at least 4 mm (\pm 0.1 mm) and the length of the chelae differed by at least 2 mm (\pm 0.1 mm). In SS trials, crabs’ carapace width was on average 30.9 mm (ranging between 25–37 mm) and chelae length was 21.1 mm (ranging between 15–28 mm); in DS trials, carapace width was on average 30.45 mm (ranging between 20–44 mm) and chelae length was 20.9 mm (ranging between 13–31.5 mm; Table 1). Experiments were carried out during daylight, alternating the experimental settings to equally distribute the trial conditions. Synchronized audio and video recordings were collected during each trial. The synchronization was achieved by triggering a visual and acoustic signal, visible from the cameras and audible from the hydrophone emitted before each trial. For audio recordings, a calibrated hydrophone (Reason TC4013, Teledyne Marine, Houston, TX, USA, with a sensitivity response of 211 ± 3 dB re 1 V/ μ Pa from 1 Hz to 150 kHz) was located in the centre of the tank at 20 cm under the water surface. It was coupled with a preamplifier (1-MHz bandwidth single-ended voltage and a high-pass filter set at 10 Hz, 20 dB gain, Avisoft Bioacoustics (Glienicke, Germany)) connected to a digital acquisition card (Avisoft UltraSoundGate 116h) managed by the Avisoft Recorder USGH software (Avisoft Bioacoustics). The sampling frequency of the hydrophone was set at 300,000 samples per second. For video recordings, two systems were used: an external security camera (AVTech (Taipei, Taiwan), KPD136ZALTP model, connected to a generic digital recorder H.264 V2.08, set 5 frames per second) located out of the water at 30 cm above the tank water surface, and a GoPro camera (GoPro, San Mateo, CA, USA) (set at 30 frames per second) positioned inside the tank (Figure 1). The first camera recorded the entire tank from the top, allowing to discriminate individuals and their general behaviour; the GoPro camera recorded part of the experimental tank, allowing it to monitor the details of movements (Figure 1).

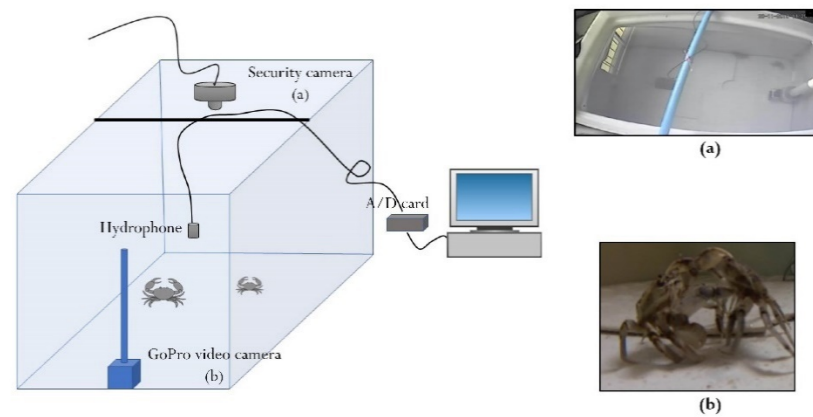


Figure 1. Schematic draft of experimental set-up including video frame collected by (a) external security camera and (b) GoPro video camera.

2.3. Data Analysis

Acoustic and video data were analysed respectively by two different operators. They were synchronised, and to make the analysis independent, they were only subsequently matched to obtain the number of sounds recorded during each behaviour.

2.3.1. Behavioural Analysis

The video data were manually screened by two expert operators blinded to treatment using VLC media player software with the same setting and considering the same variables previously clearly defined.

From the analysis of the external camera, considering the distance of the camera to animals and its resolution, three behavioural states were identified and characterised:

- Moving (M): one or both crabs were walking, without contact between each other;
- Contact (C): both crabs touched each other;
- Still (S): both crabs were not moving or walking and had no contact between them.

The duration of each behaviour was recorded.

When one or both crabs touched or were close (≤ 2 cm) to the GoPro camera, this period was excluded from the acoustic analysis since it caused noise unrelated to animal behaviour.

From the analysis of the GoPro camera, when both crabs were clearly within the visual field, detailed behavioural events were identified as follows:

- Body Movement (BodyMov): defined each time the crabs were still and moving chelae/legs without contact between each other;
- Agonistic Interaction between the crabs (AgoInt): defined as each time the pair of males contacted each other with their chelae, or only one of the males using its chelae to contact the other male;
- Non-agonistic Interaction between crabs (Int): defined as every time both males contacted each other without using their chelae, for example, contact through their pereopods, or one crab walking over the other crab.

The frequency of each behavioural event and the duration of each behaviour, from the first movement/contact until both males stopped/separated, were recorded.

2.3.2. Acoustical Analysis

All acoustic recordings were visually screened using Raven PRO 1.5.0 (Raven Sound Analysis, Ithaca, NY, USA), with a spectrogram window size of 512 points and keeping brightness and contrast fixed during the visualisation. All impulsive sounds were marked with Raven selection mode. The sum of sounds detected during each behaviour (state and event) was calculated and reported. Based on the behaviour duration, the Emission Rate of Sounds was estimated by dividing the number of sounds counted by the behaviour duration.

In order to analyse the temporal and spectral acoustical characteristics of recorded sounds, the sounds with a high signal-to-noise ratio and absence of superimposition to other sounds were selected. The characterisation was carried out using a modified Matlab code (version 2019b, The MathWorks, Inc., Natick, MA, USA) already applied to describe temporal and spectral properties of impulsive crustacean, fish, and dolphin sounds [37–41].

Following the procedure described below, five parameters were measured for each sound:

1. Maximum Sound Pressure Level (Lp–dB re 1 μ Pa peak);
2. Sound duration (s);
3. 1st peak frequency (kHz);
4. 2nd peak frequency (kHz);
5. 3 dB bandwidth (kHz).

The code first applies a high-pass filter (cut-off frequency 500 Hz, order 200) and then the Teager Kaiser operator (Kaiser 1990). This operator amplifies the impulsive signals characterised by a high frequency and an increase in instantaneous energy. On this modified signal, the time position of the maximum amplitude is identified. Then, a window around the maximum position is extracted from the 500 Hz high-pass filtered waveform. Within this window, we calculated: (1) the maximum Sound Pressure Level (Lp–dB re 1 μ Pa peak), and (2) the sound duration in seconds (s), measured by applying the ‘envelope’ function and defining the points at which 20% of the peak value was reached. Moreover, the MATLAB function ‘pwelch’ was applied to the window extracted from the waveform filtered signal ranging between the onset and end of the pulse. Here, the Power Spectral Density (PSD) was calculated, and the MATLAB code allowed the operator to check and note if the signal window was correctly evaluated and decide if the sound should be counted, the duration should be corrected, and the PSD should be calculated. For each checked sound, (3–4) the 1st and 2nd peak frequencies (defined as the frequencies corresponding to the highest and second-highest amplitude values, respectively) determined from the PSD, and (5) the 3 dB bandwidth (the frequency band between the lower and upper half-powers or 3 dB down from the maximum PSD) were also calculated.

2.4. Statistical Analysis

Behavioural and acoustic parameters were tested for goodness-of-fit to the normal distribution using the Shapiro–Wilk normality test.

The duration of each behavioural state in the SS and DS experimental settings was compared using the Mann–Whitney U test. Moreover, the differences between the duration of each behaviour were evaluated by using the Kruskal–Wallis rank sum test and Dunn multiple comparison post-hoc test. The duration of each behavioural event was compared using the Kruskal–Wallis rank sum test and Dunn multiple comparison post-hoc test, and the frequencies of each behaviour in the SS and DS experimental settings were compared using the chi-squared tests.

To investigate the relationship between behaviour and sound emission, Generalized Linear Mixed Models (GLMMs) for zero-inflated data were run in R (package glmmTMB) due to the high number of frames with no-signals emission. Two different data sets were considered: the first was based on the emission rate of sounds produced during the behavioural states, and the second was based on the emission rate of sounds produced during the behavioural events. The emission rate was considered as a dependent variable, and behavioural state and event, the experimental setting, and the day of the experiment were included as possible explicatory fixed effects. Replicates ID was included as a random factor to take into account repeated measures from the same individuals. The best fitting model was chosen based on the Akaike information criterion (AIC), both for behavioural and acoustic data. Best models were validated, graphically assessing the residuals (e.g., residuals versus fitted values, Q–Q plots, and residuals versus the original explanatory variables). For selected models, pairwise multiple comparisons using Tukey’s HSD were performed in R (package multcomp, glht function).

The five acoustic characterisation parameters (the maximum Sound Pressure Level (Lp), the sound duration (s), the 1st peak of frequency, the 2nd peak of frequency, and the 3 dB bandwidth) of sounds recorded during the different behavioural events were compared using the Kruskal–Wallis rank sum test and Dunn multiple comparison post-hoc test.

3. Results

In total, 24 h of acoustic and GoPro video data were analysed, together with 16 h of analysable data from the security camera, equally distributed between SS and DS trials.

Crabs involved in SS trials spent more time in contact (Mann–Whitney U, $W = 24$, $p < 0.05$) and still (Mann–Whitney U, $W = 10.5$, $p < 0.05$) than those involved in DS trials (Figure 2). The experimental setting did not affect the time spent moving (Mann–Whitney U, $W = 38$, $p = 0.57$). In general, crabs spent more time moving than in contact or still behaviours (Kruskal–Wallis test, $\chi^2 = 33.30$, $df = 2$, p -value < 0.0001 ; Dunn multiple comparison, M vs. C: $Z = -4.05$, $p < 0.001$; M vs. S: $Z = 5.58$, $p < 0.0001$; C vs. S: $Z = 1.53$, $p = 0.377$).

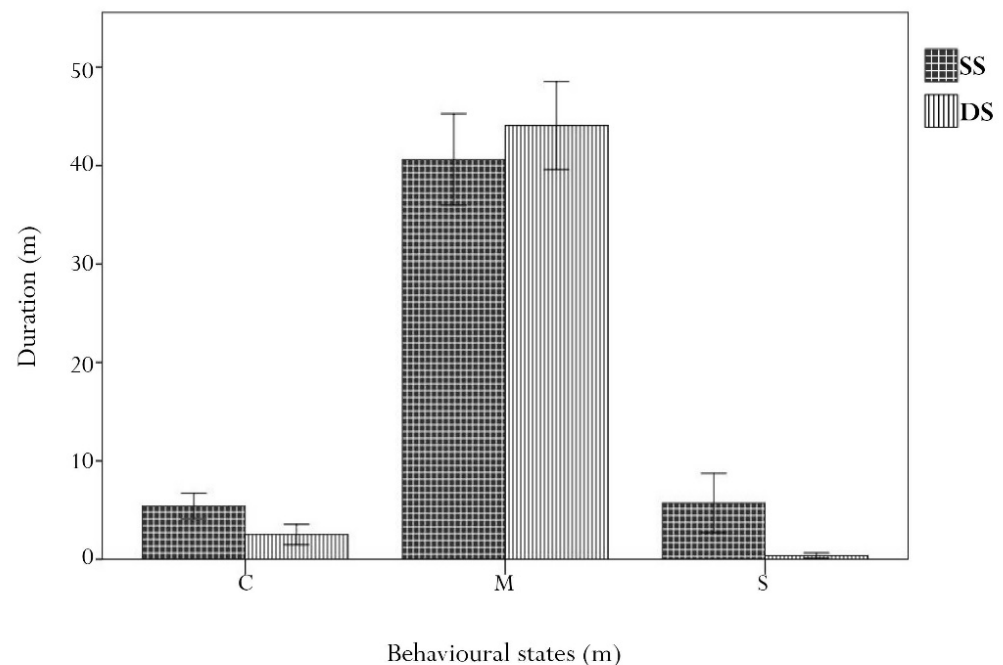


Figure 2. Comparison of average duration (\pm SE) of behavioural states: Contact (C), Moving (M), and Still (S) during SS and DS experimental settings.

Considering behavioural events, the frequency of agonistic interactions (AgoInt) was higher in SS than in DS trials (Chi-square test, $\chi^2 = 64.47$, $p < 0.001$, Figure 3). The frequency of non-agonistic interactions (Int) was instead higher in DS than in SS trials (Chi-square test, $\chi^2 = 6.54$, $p = 0.001$, Figure 3). The frequency of body movements (BodyMov) in the two settings was comparable (Chi-square test, $\chi^2 = 2.25$, $p = 0.134$, Figure 3). Moreover, the duration of events resulted comparable (Kruskal–Wallis $\chi^2 = 0.13$, $df = 2$, p -value = 0.9359).

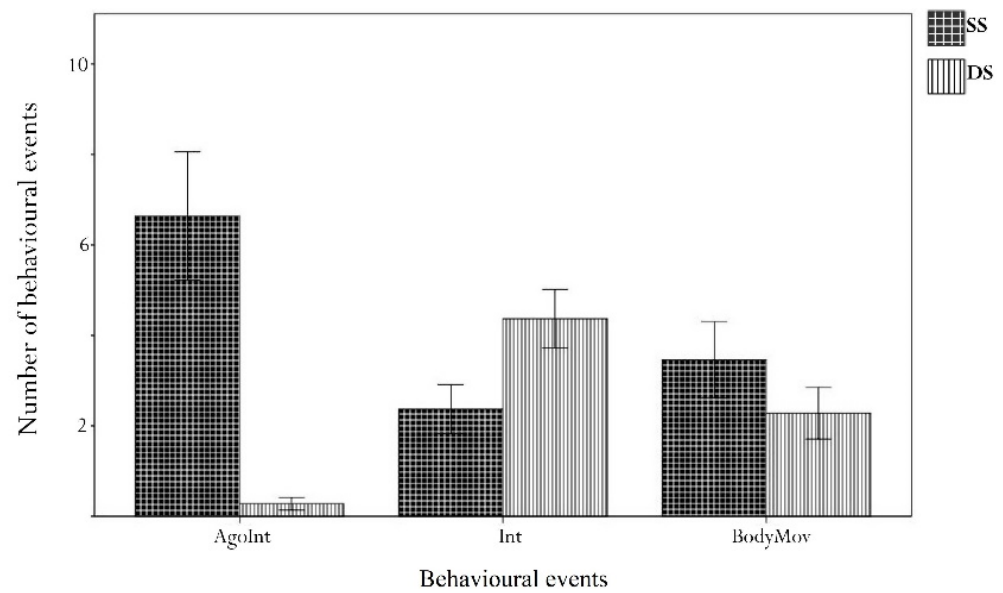


Figure 3. Comparison of average number (\pm SE) of behavioural events: Agonistic Interaction (AgoInt), non-agonistic Interaction (Int), and Body Movements (BodyMov) during SS and DS experimental settings.

From acoustic data, 2184 impulsive signals were recorded (Figure 4). For both sounds recorded during behavioural states and behavioural events, the best models included the behaviour as fixed effect (for states—C, M and S; for events—AgoInt, Int and BodyMov).

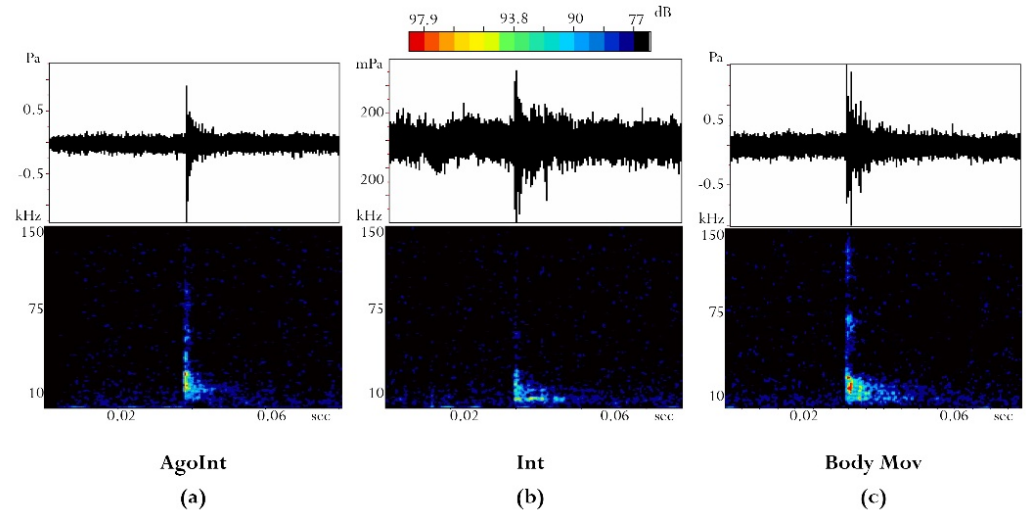


Figure 4. Example of waveforms and spectrograms of impulsive signals emitted during: (a) Agonistic Interaction (AgoInt), (b) non-agonistic Interaction (Int), and (c) Body Movements (BodyMov). FFT length 512 points, Hann window, frequency resolution 586 Hz. Graphs were realized with Avisoft SAS-Lab Pro software 5.3.01 (Glienicke, Germany).

For behavioural states, the emission rate was significantly affected by different behaviours (Table 2, $\chi^2 = 96.57$, $df = 2$, $p < 0.001$). In particular, crabs emitted more signals when involved in contact interactions or still behaviour than during movement. There were no significant differences between the emission rate recorded during contact behaviour and when the male crabs were still. The increase in emission rate during contact behaviour was higher, although non-significantly, both in same-sized trials than different-sized ones (Table 2, Figure 5).

Table 2. Result of Generalized Linear Mixed Model on emission rate among Contact (C), Moving (M), and Still (S) behavioural states including the trials as the random effect.

Analysis of Deviance Table (Type II Wald Chi-Square Tests)			
	χ^2	Degree Freedom	<i>p</i> -Value
Behavioural states	96.57	2.00	<0.001

Generalized Linear Mixed Model (GLMM), Multiple Comparisons of Means: Tukey Test Dependent Variable: Emission Rate				
	Estimate	SE	z-Value	<i>p</i> -Value
Intercept	2.64	0.45	5.84	<0.001
Moving (M) vs. Contact (C)	11.74	1.20	9.78	<0.001
Still (S) vs. Contact (C)	2.06	1.45	1.42	0.319
Still (S) vs. Moving (M)	−9.68	1.84	−5.26	<0.001

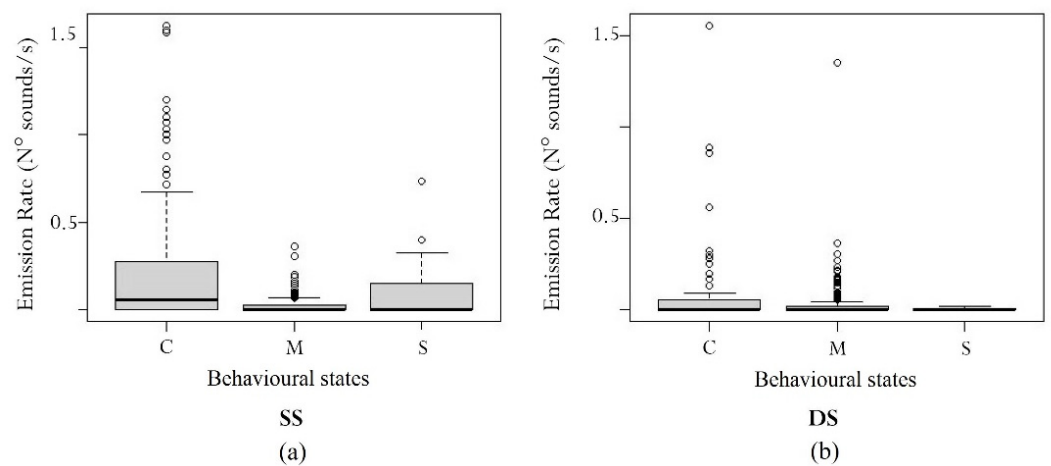


Figure 5. Box plots of the signal emission rates during Contact (C), Moving (M), and Still (S) behaviours considering (a) SS and (b) DS experimental settings.

For behavioural events, the best model showed significant effects of different behaviours on emission rate (Table 3, $\chi^2 = 20.56$ df = 2, $p < 0.001$), with the highest emission rates recorded during agonistic interactions and body movements and the lowest emission rate during non-agonistic interactions (Table 3, Figure 6).

Table 3. Result of Generalized Linear Mixed Model on emission rate among non-agonistic Interaction (Int), Body Movements (BodyMov), and Agonistic Interaction (AgoInt) behavioural events including the replicates as random effect.

Analysis of Deviance Table (Type II Wald Chi-Square Tests)			
	χ^2	Degree Freedom	<i>p</i> -Value
Behavioural events	20.56	2.00	<0.001

Generalized Linear Mixed Model (GLMM), Multiple Comparisons of Means: Tukey Test Dependent Variable: Emission Rate				
	Estimate	SE	z-Value	<i>p</i> -Value
Intercept	−0.83	0.28	−2.97	<0.001
BodyMov vs. AgoInt	0.30	0.30	1.00	0.578
Int vs. AgoInt	−1.14	0.34	−3.31	<0.001
Int vs. BodyMov	−1.42	0.32	−4.46	<0.001

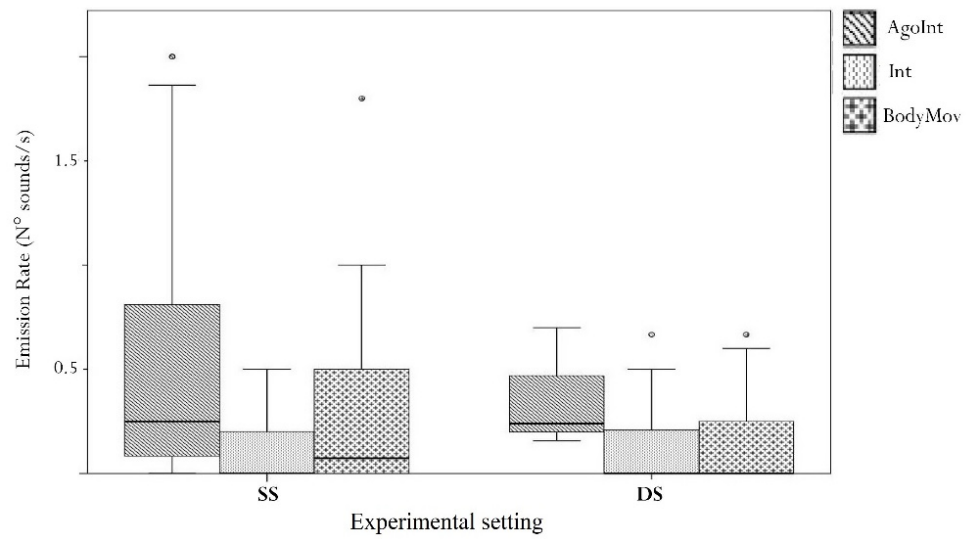


Figure 6. Box plots of the emission rate during agonistic interaction (AgoInt), non-agonistic interactions (Int), and body movements (BodyMov) events considering SS and DS experimental settings.

For characterisation, 297 impulsive sounds were selected. The impulsive sounds lasted on average 0.004 s (SD = 0.005 s), 3 dB-bandwidth of 1.5 kHz (SD = 3.1 kHz), Sound Pressure Level (Lp) of 147 dB rel 1 μ Pa (SD = 10 dB), and two peaks of frequency, respectively, of 45 kHz (SD = 37.5 kHz) and 80 kHz (SD = 32.8 kHz).

The sounds recorded during behavioural events resulted in being significantly different for all the acoustic parameters (Kruskal–Wallis test, Duration: $\chi^2 = 32.95$ df = 2, $p < 0.001$; 3 dB-bandwidth $\chi^2 = 17.27$, df = 2, $p < 0.001$; Sound Pressure Level $\chi^2 = 21.10$, df = 2, $p < 0.001$; I Peak of frequency $\chi^2 = 24.23$, df = 2, $p < 0.001$; II Peak of frequency $\chi^2 = 6.04$, df = 2, $p = 0.05$). Sounds emitted during non-agonistic interactions (Int) were significantly different from those emitted during agonistic interactions (AgoInt) and body movements (BodyMov) that, conversely, were comparable for all parameters (Figure 7). In particular, the sounds emitted during non-agonistic interactions showed lower first peaks of frequency, bandwidth and energy, but higher duration and second peak of frequency.

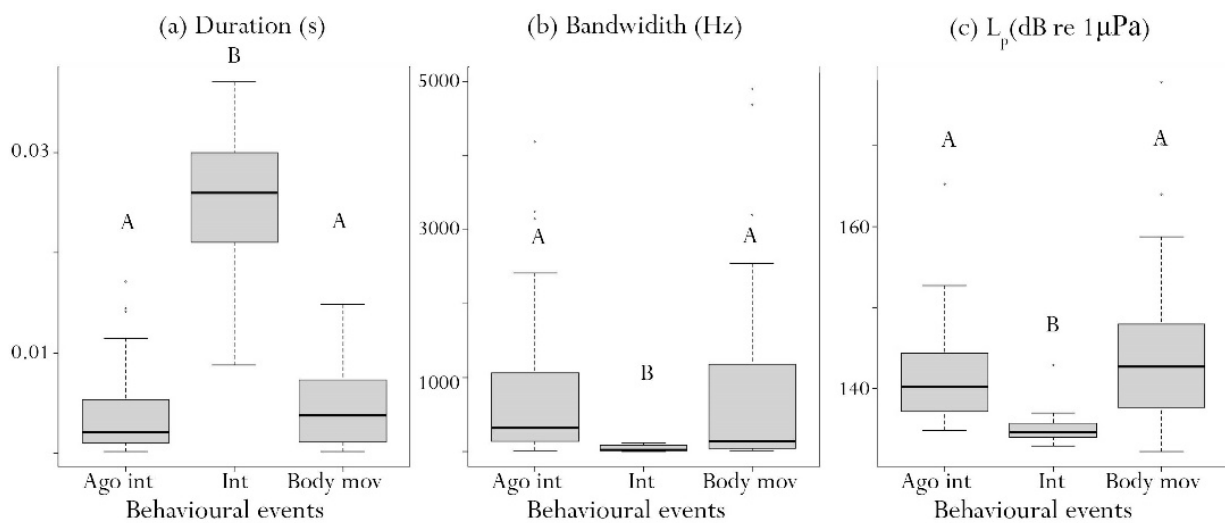


Figure 7. Cont.

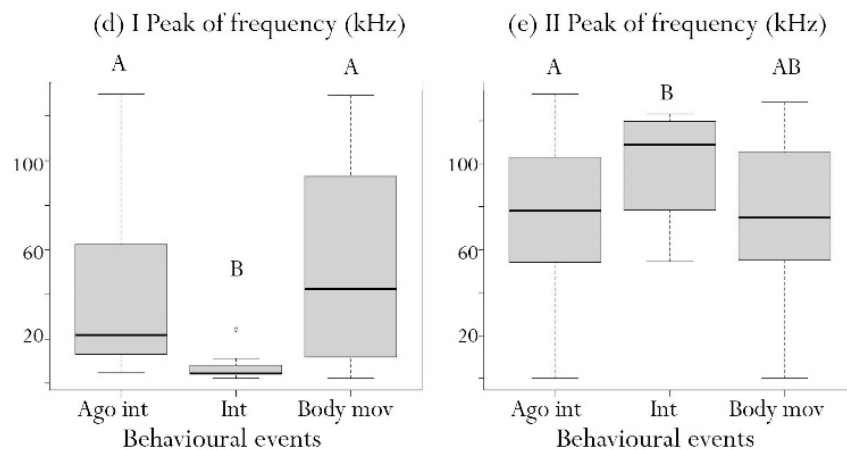


Figure 7. Comparisons of sounds acoustic parameters emitted during agonistic interaction (AgoInt), non-agonistic interactions (Int), and body movements (BodyMov) (a) duration, (b) bandwidth, (c) Sound Pressure Level, (d) I peak of frequency and (e) II peak of frequency. Different capital letters indicate significant differences between groups ($p < 0.05$).

4. Discussion

The benthic crustaceans' sounds are the main contributor to shallow water soundscape biophonic components [42]. This study investigated for the first time the relation between male–male interactions and sound production of *C. angulatus*, one of the most important macrofaunal species of intertidal South Atlantic regions.

During this study, three kinds of behavioural states have been described, and behavioural events have been detailed showing that *C. angulatus* males involved in males encounters spent most (86%) of the time moving, whereas they interacted among them or were still only a limited time (8% and 6% of the time, respectively). When same-size males interacted, the time spent in contact and still behaviour (10% and 11%, respectively) was proportionally higher than for different-size males interactions (5% and 1%, respectively). The size difference also influenced behavioural events in our experiment: similar-sized males interacted agonistically more than different-sized males. Agonistic interactions in crustaceans are one of the main social behaviours [43], determining injuries that can affect the competitiveness and survival rate of individuals [44–46]. Based on the observations from the natural environment where larger males outcompete smaller ones during mating competitions [34], we may hypothesise that in a small experimental tank, the agonistic encounters between different size males are avoided to reduce the risks linked to fighting. In addition, our results showed how *C. angulatus* carries out body movements involving claws and legs in comparable numbers during same-size and different-size encounters and that movements are associated with a high rate of emission of sounds, suggesting a preference for interactions that avoid direct conflict. The study of behavioural temporal sequence during the agonistic interactions of a portunid crab (*Portunus tuberculatus*, Miers, 1876) highlighted the presence of a stale mate stage during which the crabs evaluate each other, showing alert and demonstration behaviours involving body displays with chelipeds and legs moving without contact with antagonist [47]. Moreover, Goh et al. [48] showed the role of sounds in the agonistic context of *Episesarma versicolor* (Tweedie, 1940), where signals and vibrations were used to transmit information about the sender, minimising the costs associated with direct conflicts. More focalised studies on *C. angulatus* behaviour could help to highlight a possible relation between the role of body movements, such displays associated with acoustic signals, and the probability of a consequent agonistic interactions.

Our results on acoustic behaviour showed that *C. angulatus* emits isolated impulsive sounds and that the rate of emission is driven by the behavioural context more than the reciprocal size of competitors. Signals emitted during agonistic interactions and body displays were characterised by a short duration (0.004 s on average), an intensity of 143 dB re 1 μ Pa, and two high peaks of frequency over 20 kHz (39.9 kHz and 50.8 kHz for the

first peak of frequency and 77 kHz on average for the second, respectively). During non-agonistic interactions, signals were longer (0.2 s on average), and the first peak of frequency was lower (7 kHz on average). Coquereau et al. [19] described the acoustic behaviour of large crustaceans common in Northeast Atlantic coastal areas, revealing that every species emitted acoustic signals characterised by species-specific acoustic parameters. In these species, the first peak of frequency resulted in being the most important for species and behavioural discrimination. Sounds characterized by frequency peaks < 20 kHz were typically associated with feeding activities and movements on the rock. On the contrary, sounds characterized by a high peak of frequency (25–45 kHz) were associated with species-specific behaviours, such as antennae, chelipeds, and mandibles rubbing [19]. These findings support the idea that sounds recorded during non-agonistic interactions by *C. angulatus* are likely unintentional, whereas sounds emitted during agonistic encounters and body movements could have different purposes and be used as acoustic cues by competitors.

From an ecological point of view, a previous soundscape study conducted in Mar Chiquita Lagoon showed the presence of impulsive signals broadly spread from the mouth to the inner side of the lagoon during spring, autumn, and winter [35]. Moreover, a particular circadian pattern of the same signals was detected in the mouth of the lagoon during spring, suggesting the presence of a particular ecological phenomenon [35]. The lagoon is inhabited by four crab species, but *N. granulata* and *C. angulatus* are dominant [28,49]. When in a mating context, *N. granulata* emits impulsive signals [14] by rubbing its chelipeds against the pterygostomial area of the carapace [18]. However, since impulses in *N. granulata* are emitted in trains (defined as rasps), and they are characterised by an average frequency peak of 4917 Hz (± 1508.95) [14], an important proportion of the impulsive signals recorded in the lagoon is probably provided by *C. angulatus*. The hypothesis is also supported by the different distribution of the two species in the lagoon. *C. angulatus* inhabits the subtidal area, whereas *N. granulata* occupies the upper intertidal area, being less conspicuous in the shallow subtidal areas [28,31] where the hydrophones were deployed [35]. Furthermore, although the mating activity of both *C. angulatus* and *N. granulata* occurs during the austral spring in November, only *C. angulatus* adults aggregate for mating near the mouth of the lagoon [28,31,32], suggesting a possible correlation with the circadian pattern recorded. Moreover, larvae and juveniles *C. angulatus* are attracted by impulsive sounds recorded in the lagoon [36], suggesting a key role of sounds in the ecology of *C. angulatus*.

Our results in laboratory conditions provide information on the sounds production of *C. angulatus* and its emission-behavioural context, contributing to the understanding of the acoustic activity of this species and laying the groundwork for future research hypotheses on its role in the soundscape of a natural environment. This is the first study that describes the acoustic emission in *C. angulatus*, providing base data that can be used in future research evaluating the acoustic behaviour of this species and the effects of biotic and abiotic (including anthropogenic) sound sources on its behaviour.

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