RESEARCH PAPER

Chorusing Behaviour in the Lusitanian Toadfish: Should I Match My Neighbours' Calling Rate?

Joana M. Jordão*, Paulo J. Fonseca* & Maria Clara P. Amorim†

* Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Bloco C2, Lisboa, Portugal

† Unidade de Investigação em Eco-Etologia, Instituto Superior de Psicologia Aplicada, Instituto Universitário, Lisboa, Portugal

Correspondence

Joana M. Jordão, Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Bloco C2, Campo Grande, 1749-016 Lisboa, Portugal.
E-mail: jjordao@fc.ul.pt

Abstract

Choruses have been described mostly in birds, anurans and insects but have been poorly studied in fish. Research in batrachoidid (toadfishes) species suggest vocal facilitation among neighbouring males, but whether chorusing fish present more complex interactions is unknown. In this study, we test the hypothesis that chorusing fish males compete actively to increase attractiveness to females. We first describe vocal interactions in natural choruses of Lusitanian toadfish males. Our analysis found positive correlations between the calling rates of neighbouring males in several occasions. However, we also found that males that showed an overall low vocal activity throughout the observation period exhibited peaks of increased calling activity when neighbours decreased their calling rate, suggesting an opportunistic maximisation of attractiveness. We further test with playback experiments how toadfish males adjust calling activity relative to their neighbours'. We observed that males silent at the time of the playbacks but who had an overall high vocal performance tended to start calling when exposed to playbacks in contrast to low-activity males. Playback experiments further showed that males initially calling at a high rate adjust their calling rate according to the neighbour's vocal activity level, that is, they increased calling rate when exposed to a high calling rate and decreased it when confronted with a low calling rate. However, males calling at a low rate did not significantly alter their calling rate when presented with a low (similar) or higher calling rate, probably due to temporary physiological and/or ecological constraints. We argue that Lusitanian toadfish males tend to optimise calling effort in relation to their neighbours when they are actively advertising. Further studies are necessary to better understand vocal behaviour with increased chorus size.

Introduction

Advertisement to attract mates is often performed in choruses consisting of two or more individuals signalling at the same time. Vocal choruses have been described for a number of species, but they are especially common in insects, anurans and birds. There are several advantages for sexually active males to aggregate: improved detection of areas with the necessary physical resources for breeding (e.g. Muller 1998), improved mate location by females (e.g. Wells 1977), lower mate assessment costs and reduced individual predation risk (e.g. Ryan et al. 1981). A drawback of these aggregations is the increased competition experienced by neighbouring males. The strategies to cope with competition can be diverse, but males that produce signals that are heard against the background chorus increase their chances of attracting a mate. Finer-scale calling interactions, often matching or anticipating neighbours' sound sequences, are also a common behavioural response found in several taxa (Whitney & Krebs 1975; Greenfield 1983; Gerhardt et al. 2000; Schwartz et al. 2002; Foote et al. 2008). As a generalisation, a number of studies show that

females prefer males that initiate chorus signalling, perform longer calls and exhibit higher calling rates (reviewed by Gerhardt & Huber 2002).

Vocal communication is widely used by fishes, and choruses have been described for several fish families. The family Sciaenidae form large active calling choruses during the spawning season to the point that their mating calls are globally used by fishermen to locate fish aggregations (Takemura et al. 1978; Lagardère & Mariani 2006). Several passive acoustic studies have focussed on daily and seasonal timing of such choruses (e.g. Connaughton & Taylor 1995), but no studies have described the adjustments and alterations of individual temporal patterns of calling activity.

During the reproductive season, batrachoidids establish a nest (usually in a cleft or under a rock) and use advertisement calls to attract mates (Bass & McKibben 2003). Females attracted by the calls (Vasconcelos et al. 2012) spawn in the nest, and the resident male provides parental care to the young until they are free-swimming (Brantley & Bass 1994). The sedentary nature of these males makes it possible to extract detailed information about their individual calls and interactions (Fine & Thorson 2008). Studies with the Gulf toadfish, *Opsanus beta*, have shown that neighbouring calling males compete by increasing their calling rates while avoiding overlaps by making shorter and simpler calls (Thorson & Fine 2002; Fine & Thorson 2008). Overlapping is also rare in the Oyster toadfish (*Opsanus tau*), but Winn (1967) suggests that call alternation in the Oyster toadfish does not differ from what is expected by chance.

A recent study with the Lusitanian toadfish (*Halobatrachus didactylus*) has shown that call rate is affected by social environment (Amorim et al. 2011). Males calling alone mostly call at low rates, while males calling in a chorus call at all rates, but show higher rates on average. The temporal patterns of male calls are also affected by both social environment and internal motivation status (i.e. call rate). For example, males calling alone do not change boatwhistle duration, but males calling in a chorus significantly lengthen call duration when they increase their calling rate (Amorim et al. 2011). In addition, calling rate is condition dependent in this species, and only males with a good body condition can sustain prolonged bouts of vocal activity (Amorim et al. 2010). Sustained high calling activity is also predictive of high reproductive success in this species (Vasconcelos et al. 2012).

In this study, we first asked whether chorusing toadfish males compete actively to improve their attractiveness to females by standing out from the chorus background. Moreover, we investigated whether vocal strategies within a chorus are dependent on calling rate. We first approached these questions by examining vocal interactions in natural occurring choruses. Second, we performed playbacks simulating conspecifics calling at different rates to test how advertising males adjust their vocal activity. Given that males from other batrachoidid species are known to start calling when faced with a calling environment (Remage-Healey & Bass 2005), we predicted that playbacks would elicit calling behaviour in the present species. We also expected that vocally active males would alter their calling activity to maximise competition. However, as calling rate is condition dependent (Amorim et al. 2010) and changes with social environment (Amorim et al. 2011), we predicted that adjustments to calling competition would be dependent on the male's quality and advertisement motivation (i.e. calling rate).

Methods

Recording Natural Choruses

Six (2008) to eight (2010) artificial hemicylinder-shaped concrete shelters (internal dimensions: 50 cm long, 30 cm wide and 20 cm high) were placed about 2.5 m apart in a row on an intertidal area of the Tagus estuary (Air Force Base 6, Montijo, Portugal; 38°42′N, 8°58′W) that was only exposed during low spring tides. Breeding males spontaneously occupy such shelters and use them as nests during the breeding season (Amorim et al. 2006). The group of shelters with subject males were at least 15 m away from other shelters that could be occupied by conspecific males. They were wrapped in a medium-sized grid plastic mesh with a small opening at the front. This opening was large enough to allow gravid females to access the breeding male (i.e. some males had fertilised eggs in the end of the confinement period, see Vasconcelos et al. 2012), but small enough to prevent the subject male to leave the nest, thus ensuring the subject's identity throughout the recording period. The mesh also allowed for crabs and other small prey to enter. Nine groups of six to eight males (N = 57) were recorded over an average period of 11 d (range: 7–17 d) between May and July 2008 and 2010, which is the peak of the reproductive season in Portugal (Amorim et al. 2006). A typical recording session would start 3 h prior to high tide and finish 3 h after (in a total of 6 h/d), because this is the period when males vocalise most actively (Amorim et al. 2011).

Each shelter was fitted with a hydrophone (High Tech 94 SSQ; High Tech Inc., Gulfport, MS, USA; sensitivity −165 dB re. 1 V/μPa, frequency response within ± 1dB from 30 Hz to 6 kHz) to localise vocalising males. Hydrophones were attached to partially buried iron rods and positioned about 10 cm from the shelter entrance and from the substrate. Simultaneous multichannel recordings were made to a laptop connected to USB A/D converter devices (in 2008: Edirol UA25; Roland, Osaka, Japan; 16 bit, 44.1 kHz acquisition rate per channel, and in 2010: M-Audio Fast Track Ultra 8R; 16 bit, 44.1 kHz acquisition rate per channel but subsequently down-sampled to 8 kHz for analysis) controlled by Adobe Audition 3.0 (Adobe Systems Inc., Mountain View, CA, USA). Recorded sounds could be attributed to each male owing to the high acoustic attenuation observed in shallow water (Fine & Lenhardt 1983).

In 2008, water temperature was measured with a hand-held thermometer every 3 h. In 2010, a temperature data logger (Iotech, USB-501-LT) was sealed in a jar placed by the nests, and water temperature was logged every 15 min. Water temperature averaged 22.2°C (range: 17.0–28.0°C) in 2008 and 23.0°C (range: 19.5–28.0°C) in 2010. All subjects experienced similar water temperature variability during recordings. Water depth varied between 0.0 m (peak of low spring tides when the shelters were exposed) and 2.8 m (peak of high spring tides).

At the end of the recording period, in the following spring tide, males were removed from their nests and their weight and total length (TL) were measured. The size of the recorded males ranged from 34.0 to 49.5 cm (TL) (average ± SE: 44.1 ± 0.44), and the body mass varied between 627 and 2097 g (Average ± SE: 1419 ± 43). All subjects were sexually mature. Fish were then sacrificed with an overdose of MS-222 (Tricaine methanesulfonate). The procedures used in this study comply with the current laws of Portugal.

Vocal Interactions in Natural Choruses

We have explored vocal interactions in chorus recordings of 2010. We have counted the number of boatwhistles per minute produced by every monitored fish. We selected all periods when two or more fish were vocally active with a call rate of above nine boatwhistles per minute (BW per min) for at least 20 min at a time and analysed how their calling rate was related. We have recorded four groups of males for a total recording time of 196 h. From this time, only 7% (831 min) referred to recordings of a chorus of two (or more) vocally active fish, and only in 5.8% of the time was at least one of the males in the chorus calling at a high rate (≥ 9 BW per min). This was not evenly distributed across the four groups of males. In groups 3 and 4, chorus time was 2% and 0%, respectively. For this reason, vocal interactions focussed on males from either group 1 or group 2. Also, typically each group had 1–3 vocally active males. So usually, vocal interactions in the same group repeatedly involved the same individuals. The studied vocal interactions concerned a total of 280 min of chorus time. Although this was about 35% of the total registered chorusing time, bouts of chorusing were scattered across the recordings and some bouts were as short as 2 min. Our analysis considered only the bouts of long uninterrupted interactions (larger than 20 min) where chance associations are progressively less probable.

Playback Experiments

An underwater speaker (Electrovoice UW-30; Lubell Labs Inc. Columbus, OH, USA; Frequency Response: 0.1–10 kHz) was firmly attached to iron rods deeply inserted into the sand and kept slightly above the substrate at 80 cm from the opening of each of six subject's shelters. The speakers, which played back the stimuli, were connected to an amplifier (Blaupunkt GTA 260) and fed through the D/A subsystem of an USB Edirol UA25 controlled by Adobe Audition 3.0 (Adobe Systems Inc).

Playback (PBK) stimuli consisted of sounds mimicking a conspecific neighbour calling at different rates. Six boatwhistles (BW) from different fish, all with the second harmonic as the dominant frequency (see Amorim & Vasconcelos 2008), were selected from our recordings archive (2008: average duration = 737 ms, range: 701–771 ms; 2010: average duration = 680 ms, range: 664–705 ms). Each one was looped to create two 5-min sound files: one with 5 BW per min (CR5) and another with 20 BW per min (CR20), using Adobe Audition 3.0. Each playback session used stimuli constructed from boatwhistles of only one individual, but different males were exposed to boatwhistles of different individuals. A 5-min file with white noise (WN) sound stimuli (2008: 737 ms; 2010: 680 ms) delivered at a rate of 20 sounds per minute was also used as a control. In this way, we could control for the subjects' ability to discriminate between conspecific calls vs. WN of similar duration at a rate that was likely to induce significant changes in the subjects' calling rate (Winn 1967; Fish 1972). The presentation of the stimulus playback was opportunistic, that is,

dependent on the fish vocal activity. The three treatments were presented to the subjects in a randomised order only when all the following conditions were met: at least 48 h after confinement of the subject to the shelter; the subject was either silent or calling at a steady rate during 5 min (the PRE period); the immediate left and right neighbours of the subject were quiet. Calling males were classified as silent, calling at a low rate (3–8 BW per min) or calling at a high rate (≥ 9 BW per min) immediately before the PBK experiment (PRE). The criteria for low- and high-calling-rate states followed Amorim et al. (2011) and mimicked a calling rate lower and higher than the population average.

All vocally active males (calling at a low or a high rate at the time of PBKs) had similar vocal performance across the 2 wk of recording: 80% of the males showed periods with high vocal activity (≥ 9 per BW per min), but two-thirds of the remaining fish also obtained eggs in their nests, indicating that they possibly had high vocal activity during no-recording periods (Vasconcelos et al. 2012). Males used in 'silent males PBK trials' were more heterogeneous in their vocal activity: only 40% of males showed high vocal activity periods and only 18% of the remaining fish had eggs in the nest, suggesting that they had overall low calling activity (Vasconcelos et al. 2012).

Ambient noise levels were low and constant between trials across the study. As the output of the speakers changes with water level, the amplitude of the playback was manually adjusted so that the amplitude recorded by the hydrophone, and visually monitored in the laptop screen, was approximately half the amplitude of the boatwhistles recorded from the subject male. Afterwards, we discarded all tests where the average RMS amplitude of the PBK was less than one-third of the RMS amplitude of the subjects' call; thus, we only used PBKs that mimicked the amplitude of a conspecific neighbour calling from no further than about 1.5 m away.

The speakers used for stimuli playbacks may alter the amplitude modulation of the stimuli boatwhistles and do not respond well to frequencies below about 100 Hz, cutting off the fundamental frequency, but maintained the second harmonic as the dominant frequency (Fig. 1), which is the typical main frequency of mating boatwhistles (Amorim & Vasconcelos 2008; Vasconcelos et al. 2010).

Sound Analysis

Calling rate (BW per min) was tallied for all subjects and recording sessions. We also analysed acoustic parameters of boatwhistles produced by the subjects in the 5 min prior to (PRE), during (SOUND) and
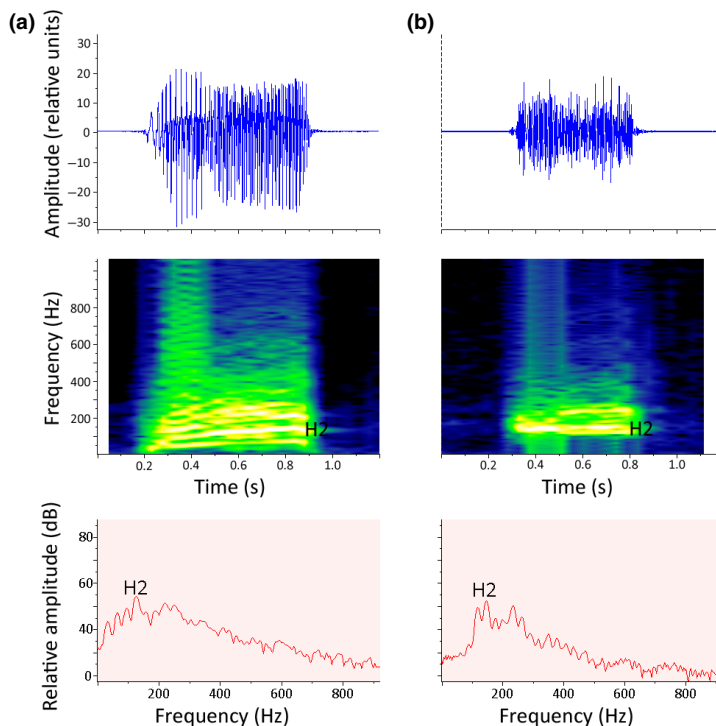
Fig. 1: Typical waveform, sonogram and power spectrum (of the middle tonal phase) of a recorded boatwhistle emitted by the Lusitanian toadfish (a) and by the underwater loudspeaker (b). The dominant frequency of both the original boatwhistle and the speaker sound corresponded to the second harmonic (H2), as indicated. Sampling frequency 8 kHz, filter bandwidth 10 Hz (sonogram and power spectrum), 50% overlap, Hamming window. (A colour figure is available in the online version of the journal).

after (POST) stimuli presentation (totalling 15 min for each PBK trial) to test whether PBK treatments had any effect on call characteristics. When the males were calling at a high rate (≥ 9 BW per min during PRE), 5 BW per period with a good signal-to-noise ratio were randomly selected. In cases where the calling rate dropped to <1 BW per min or when the number of boatwhistles eligible for analyses was <5, all available boatwhistles were analysed. The following acoustic variables were measured using Adobe Audition 3.0 (Adobe Systems Inc): sound duration (ms), measured from the start of the first pulse to the end of the last pulse; pulse period (ms) of the tonal phase (see classification by Amorim & Vasconcelos 2008), calculated as the average peak-to-peak interval between six consecutive pulse units; dominant frequency (Hz) of the entire sound; dominant frequency (Hz) of the tonal phase; and peak amplitude (dB) and average amplitude (RMS) of the entire sound, both parameters measured in an arbitrary but consistent scale across recordings. Temporal variables were measured from oscillograms and the dominant frequencies from power spectra [fast Fourier transform (FFT) size 2048 points; Hamming window].

Statistical Analysis

Vocal interactions in natural choruses

Data were checked for the assumptions of normality (normal probability plots for within-cell residuals) and homogeneity of variances (Levene's test for homogeneity of variances) but, because our data set did not follow the assumptions of parametric statistics, we have used the nonparametric gamma statistic (G), which is appropriate for measuring the degree of association between two ordinally scaled variables (Siegel & Castellan 1988), to analyse whether the changes in calling rate of two chorus attending males were positively or negatively correlated during a given period of time.

We have also generated random calling patterns to test the validity of the significant associations that we expected to find. We have measured the degree of association between an observed and a random call pattern and between two random call patterns. In cases where we compared a natural calling pattern with a randomly generated one, we opted to keep the natural calling pattern of the fish calling at a higher rate and generate a low call rate pattern. Random call patterns were created by generating randomised numbers on a minute basis, falling between the minimum and maximum call rate for the 'real' fish in that call bout. For example, we have observed calling rates

between 5 and 22 BW per min for Fish 2.7 (Table 1), so the calling rates simulating this fish were generated randomly between 5 and 22 BW per min.

Playback effect on calling rate

For 'silent males PBK trials', we used a one-tailed Wilcoxon's matched-pairs test, W (Siegel & Castellan 1988), to compare the average calling rate of the combined period SOUND + POST with PRE, that is, 0 BW per min, to establish whether treatments evoked a calling response in previously silent males.

For 'vocally active PBK trials', we first compared the PRE calling rate with a one-way ANOVA, F, to ensure there were no major differences in the pre-stimuli calling rate among individuals within each calling rate state. We then used a repeated-measures ANOVA, F, to test for changes in calling rate among PRE, SOUND and POST periods of each treatment. Data were checked for the assumptions of normality (normal probability plots for within-cell residuals)

Table 1: Results of the gamma statistics for bouts of interactions between males in a natural chorus, showing both positive and negative associations. All males were recorded in 2010. Individuals are identified by group and nest (i.e. fish 1.1 belongs to group no. 1 and nest no. 1)

Individual	Average calling rate (BW per min)	N (min)	G	p
Fish 1.1	15	66[a]	−0.29	0.002
Fish 1.3	12			
Fish 1.1	15	66[a]	−0.26	0.007
Fish 1.6	8			
Fish 1.3	12	66[a]	0.46[c]	0.000
Fish 1.6	8			
Fish 1.2	3	45[b]	0.40	0.002
Fish 1.3	11			
Fish 1.2	3	45[b]	−0.46	0.000
Fish 1.6	9			
Fish 1.3	11	45[b]	−0.26[c]	0.020
Fish 1.6	9			
Fish 1.3	5	41	−0.61[c]	0.000
Fish 1.6	11			
Fish 2.3	13	24[d]	0.65	0.000
Fish 2.7	12			
Fish 2.3	9	64	0.36	0.000
Fish 2.7	6			
Fish 2.3	11	40[e]	0.43	0.000
Fish 2.7	15			

[a,b]Pairwise correlation values for three vocally active males in the same period of time.
[c]Positive and negative interactions involving the same two males, at different occasions.
[d]Refers to period of time depicted in Fig. 2a (0–24 min).
[e]Refers to period of time depicted in Fig. 2b.

and homogeneity of variances (Levene's test for homogeneity of variances).

Playback effect on sound parameters

Statistical analysis of acoustic features of calls when males were calling at a low rate was not possible because of reduced boatwhistle numbers. For males calling at a high rate, we used a repeated-measures ANOVA, F, to test for changes in the measured boatwhistles' temporal and spectral parameters among the PRE, SOUND and POST playback periods of each treatment. The amplitude measurements were only used for *post hoc* validation of the playback trial (see the Playback experiments section) and not for statistical analysis. In all statistical analyses, we considered temperature and tide level as covariates. Temperature may potentially affect sonic muscle contraction and

consequently acoustic parameters (Amorim 2006). Tide level significantly affects the temporal features of boatwhistles (Amorim et al. 2011). However, because their effect was not significant, we repeated the analysis without these covariates.

All statistical tests were performed using Statistica 7.0 for Windows (StatSoft, Inc., 2004, STATISTICA data analysis software system, Tulsa, USA).

Results

Vocal Interactions in Natural Choruses

Vocal interactions between males in a natural chorus were complex, and the same individuals interacted in different ways at different times (Table 1, Fig. 2a). We identified periods of time when the calling rates of two males were positively correlated (Table 1,
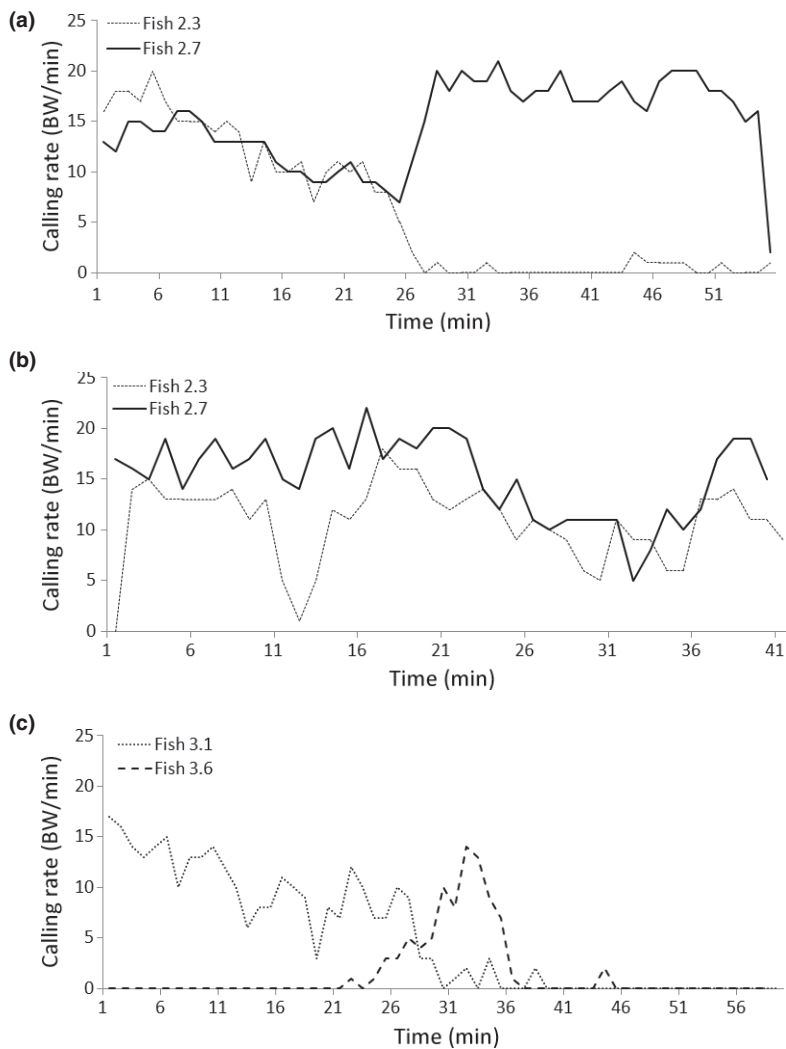
Fig. 2: Examples of vocal interactions in natural choruses. Individuals are identified by group and nest (i.e. fish 2.2 belongs to group no. 2 and nest no. 2). (a and b) Typical example of how two neighbours vocalising at a high rate adjust their calling rates to a point when the interaction pattern abruptly changes (a only), with one significantly dropping and the other significantly increasing the calling rates. (c) Typical example of how a male calling at a low rate and with a low calling effort (Fish 3.6) does not adjust its own vocalisation to the calling rate of neighbours calling at higher rate, but instead increases calling rate during a period of time when neighbours are calling less actively.

0–24 min to Fig. 2a,b) and both individuals matched their calling rate, and other periods when one individual's calling rate increased just after its neighbours' calling rate dropped (see example depicted in 25–60 min of Fig. 2a). All fish in this case had high maximum observed calling rates higher than 9 BW per min. Calling effort (percentage of time spent calling out of total recording time) was 23% and 35% for males 2.3 and 2.7, respectively. This is a typical example of how two neighbours calling at a high rate adjust their calling rates up to a point when the interaction pattern abruptly changes, with one male decreasing acoustic activity and the other significantly increasing it.

Another frequently observed pattern was a brief calling rate increase by a less vocally active male when the vocal activity of more active neighbours was temporarily lowered (26–36 min of Fig. 2c). The fish depicted in this example had high maximum observed calling rates higher than 9 BW per min. However, calling effort for male 3.6 was 2.5%, much lower than the calling effort of male 3.1 (21%). This is a typical example of how a male calling at a low rate and with a low calling effort (Fish 3.6) does not adjust its own calling rate to the calling rate of neighbours calling at higher rate, but instead increases calling rate during a period of time when neighbours are calling less actively.

None of the associations between randomly generated calling patterns were significant. We have tested the significance of the degree of association between 20 pairs of calling rates, representing the pairs presented in Table 1. In 10 of these cases, we have tested one naturally occurring calling pattern against a randomly generated one (G varied between −0.28 and 0.17, p > 0.05). In the other 10 cases, we have measured the significance of the degree of association between two randomly generated calling patterns (G varied between −0.16 and 0.20, p > 0.05). For that reason, we strongly believe that our data support true association between calling rates of chorusing fish.

Silent Males' Playback Trials

Statistically, silent males tended to vocalise with playback of all acoustic stimuli (Wilcoxon's matched-pairs test; CR5: N = 17, Z = 2.02, p = 0.02; CR20: N = 17, Z = 1.83, p = 0.03; WN: N = 16, Z = 1.83, p = 0.03). Nonetheless, there was a high variability in the magnitude of the response, and most males remained silent during and after the PBK stimuli (percentage of tested males that responded to playback treatments: CR5: 30%; CR20: 24%; WN: 25%; Fig. 3). There was,

however, a tendency for a higher average response to the CR20 treatment than to the other treatments. In fact, only CR20 elicited responses higher than 3 BW per min.

Calling Males' Playback Trials

Calling rates (BW per min) observed in the PRE period did not differ significantly between males that were subject to the different treatments (low rate: $\bar{X} \pm$ SD BW per min: CR5 = 4.89 ± 1.57, N = 7; CR20 = 4.76 ± 1.75, N = 8; WN = 4.70 ± 2.16, N = 6; one-way ANOVA, $F_{(2,18)}$=0.10, p = ns; high rate: $\bar{X} \pm$ SD BW per min: CR5 = 15.23 ± 2.65, N = 7; CR20 = 15.56 ± 1.94, N = 5; WN = 17.40 ± 3.42, N = 5; one-way ANOVA, $F_{(2,18)}$ = 1.00, p = ns), within each calling rate class.

Males initially calling at a low rate did not significantly alter their calling rate from PRE to SOUND, or POST periods (repeated-measures ANOVA: CR5, N = 7, $F_{(2,12)}$ = 0.49, p = ns; CR20, N = 8, $F_{(2,14)}$ = 0.28, p = ns; WN, N = 6, $F_{(2,10)}$ = 0.78, p = ns). However, when calling at a high rate, males significantly lowered their calling rate in the POST period, compared to PRE, when presented with CR5 treatment (repeated-measures ANOVA, $F_{(2,12)}$ = 5.17, p = 0.024; *post hoc* comparison Tukey's HSD: CR5, N = 7, p = 0.024, Fig. 4). In contrast, males significantly increased their calling rate during SOUND of CR20 presentation (repeated-measures ANOVA, $F_{(2,8)}$ = 5.41, p = 0.033; *post hoc* comparison Tukey's HSD, CR20, N = 5, p = 0.028, Fig. 4). In the POST period of CR20, males continued to show an elevated calling rate, but it did not statistically differ from the PRE period (Tukey's HSD, p = ns, Fig. 4). In contrast to boatwhistles (CR5 and CR20), the WN treatment did not significantly affect the male's calling rate when they were calling at a high rate (repeated-measures ANOVA: N = 5, $F_{(2,8)}$ = 1.24, p = ns, Fig. 4).
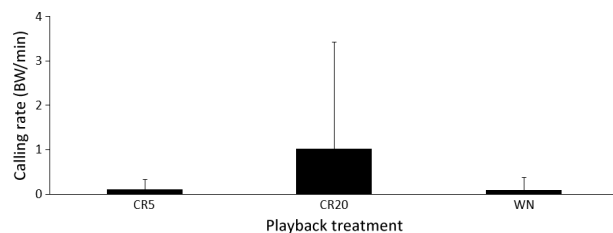
Fig. 3: Average calling rate observed in the 10 min (5 min during + 5 min after) stimuli presentation to silent males (including non-responses). Error bars indicate standard deviations. Maximum elicited calling rates for CR5, CR20 and WN were 0.7, 8.4 and 1.1 BW per min, respectively.
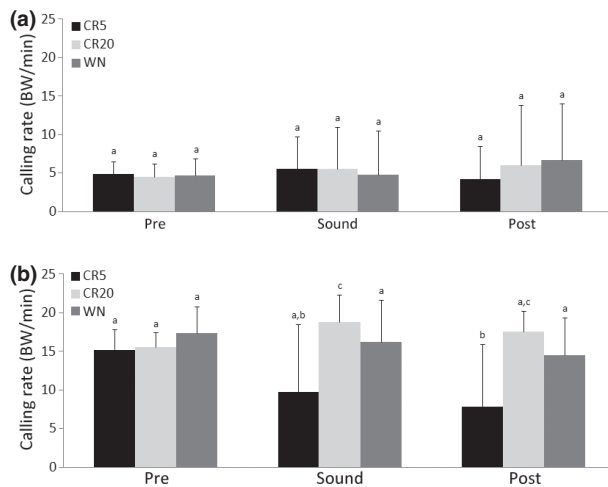
Fig. 4: Average calling rate observed in the period prior to (PRE), during (SOUND) and post (POST) playback periods when (a) males are calling at a low rate and (b) males are calling at a high rate. Males calling at a low rate did not alter their calling rate when presented with any playback treatment. Males calling at a high rate significantly lowered their calling rate, during the POST period in the CR5 treatment (playbacks of a simulated neighbour calling at a rate of 5 BW per min), but significantly increased calling activity during the SOUND period in the CR20 treatment (playbacks of a simulated neighbour calling at a rate of 20 BW per min). The white noise (WN) control (playbacks of white noise at a rate of 20 bouts per min) had no effect on the subjects' calling rate. Error bars indicate standard deviations. Different letters represent pairwise significant differences between each treatment given by Tukey's HSD tests, i.e. factor levels with the same letter show no significant differences.

The temporal features of the boatwhistles did not significantly change when males calling at a high rate were exposed to any PBK treatment (note that data on males calling at a low rate were too sparse to allow analysis). Also, the dominant frequency of both the entire sound and of the tonal phase did not change for any treatment (Table 2).

Discussion

In this study, we present examples of different interaction patterns among neighbouring Lusitanian toadfish nest holder males. The same two individuals may engage either in positive (when the calling rate of the two males are very closely matched) or in negative (when calling rates vary inversely) vocal interactions at different occasions. Positive correlations of calling activity suggest that males may stimulate neighbours to call at a similar rate, suggesting calling facilitation or competition. The examples given in Fig. 2 illustrate that males can very closely match the calling activity of another male for long periods of time. On the other hand, the negative correlations often observed between males capable of calling at a high rate that have just interacted positively suggest males may increase vocalisation investment upon a reduction in a neighbour calling rate, possibly taking advantage of a temporary decrease in competition. This is consistent with the observation that males with a low calling effort throughout the recording period show an opportunistic brief peak of activity when neighbours calling at a high rate lower their calling rate (i.e. Fig. 2c, Fish 3.6). It is likely that these males in general have a low body condition and low sonic muscle mass (Amorim et al. 2010) and are only able to produce opportunistic brief bouts of calling activity. Mitchell et al. (2008) show that sonic muscle contraction in the oyster toadfish (*O. tau*) is fatigue-limited owing to the depletion of glycogen reserves. Moreover, males double glycogen content on sonic muscle mass from winter to the reproductive season (Mitchell et al. 2008) undoubtedly to match the energetic challenge of maintaining a constant high and attractive (Vasconcelos et al. 2012) calling rate. Low-quality males that do not possess such energetic reserves are constrained in their ability for maintaining a high calling rate for a long period of time and may opt to produce these costly bursts of signalling only when competition is low, thus optimising their effort to outstand from the background chorus.

All PBK treatments in 'silent male PBK trials' evoked responses within 10 min (i.e. during and/or immediately after the trial) on about 25% of the subject males. Remage-Healey & Bass (2005) also observed that Gulf toadfish males placed in a nesting site exposed to other vocally active conspecifics resumed vocal activity within 48 h, whereas males placed in a control 'silent' site did not vocalise at all during the same period of time. A closer inspection of the overall vocal performance of the males that did respond revealed that these males were generally vocally active throughout the recording period and called on average at a rate above the chorus average. This suggests that, although at the moment of the playback these males were not vocally active, their condition and advertisement motivation were probably higher than those of other males, prompting them to react to changes in the acoustic environment. Condition-dependent signalling is widely documented across several taxa. Food-supplemented field cricket males (*Gryllus campestris*) showed a significant increase in body condition and called more frequently when compared to control males (Holzer et al. 2003). Male túngara frogs provided with extra food were also more likely to call than males without supplemental food (Marler & Ryan 1996). In this line of thought,

Table 2: Descriptive statistics and comparison between PRE, SOUND and POST of the acoustic parameters measured for the boatwhistles emitted by the playback subjects. P2 refers to the middle tonal phase of the boatwhistle

Calling rate	Treatment	Acoustic feature	Before ($\bar{x} \pm$ SE)	During ($\bar{x} \pm$ SE)	After ($\bar{x} \pm$ SE)	Repeated-measures ANOVA (F, p)
HIGH	CR5	Sound duration (ms)	722.55 ± 83.20	687.20 ± 105.10	677.70 ± 86.11	($F_{(2,6)} = 1.60$, $p = 0.28$)
		Pulse period P2 (ms)	16.67 ± 0.47	17.17 ± 0.47	16.83 ± 0.47	($F_{(2,6)} = 3.36$, $p = 0.11$)
		Dominant frequency (Hz)	122.61 ± 5.14	109.90 ± 8.57	124.57 ± 6.43	($F_{(2,6)} = 1.17$, $p = 0.17$)
		Dominant frequency P2 (Hz)	121.44 ± 4.32	108.93 ± 9.48	128.48 ± 9.92	($F_{(2,6)} = 1.15$, $p = 0.38$)
	CR20	Sound duration (ms)	657.08 ± 24.70	716.64 ± 38.20	706.12 ± 46.82	($F_{(2,8)} = 1.10$, $p = 0.38$)
		Pulse period P2 (ms)	16.62 ± 0.36	16.43 ± 0.51	16.56 ± 0.40	($F_{(2,8)} = 0.46$, $p = 0.65$)
		Dominant frequency (Hz)	155.25 ± 24.89	141.96 ± 12.78	146.85 ± 19.32	($F_{(2,6)} = 1.17$, $p = 0.37$)
		Dominant frequency P2 (Hz)	153.69 ± 25.32	141.76 ± 13.82	147.04 ± 19.76	($F_{(2,6)} = 1.04$, $p = 0.41$)
	WN	Sound duration (ms)	729.36 ± 69.70	749.80 ± 95.39	691.00 ± 69.52	($F_{(2,8)} = 1.36$, $p = 0.31$)
		Pulse period P2 (ms)	16.96 ± 0.54	17.35 ± 0.88	16.75 ± 0.56	($F_{(2,8)} = 1.67$, $p = 0.25$)
		Dominant frequency (Hz)	143.87 ± 18.93	132.77 ± 7.39	129.50 ± 4.72	($F_{(2,8)} = 0.88$, $p = 0.45$)
		Dominant frequency P2 (Hz)	141.83 ± 18.90	128.41 ± 5.48	126.84 ± 4.04	($F_{(2,8)} = 0.97$, $p = 0.42$)

CR5, Playback treatment simulating a neighbouring male calling at a rate of 5 BW per min; CR20, Playback treatment simulating a neighbouring male calling at a rate of 20 BW per min; WN, Playback treatment simulating of white noise bouts with the duration of a typical boatwhistle at rate of 20 BOUTS per min.

one would expect males to exhibit a high calling rate if in a good-enough body condition or in socially relevant occasions. This is also consistent with our observations of natural vocal interactions in natural Lusitanian toadfish choruses where only some males sustained high calling rates for long periods and both low- and high-calling-rate males showed opportunistic peaks of activity when competition dropped. A high calling effort (percentage of time spent calling) and a high calling rate pay off as these males show a significantly higher reproductive success (Vasconcelos et al. 2012).

'Vocal male playback experiments' showed that males calling at a high rate assessed the calling rate of other males and matched their own calling rate to the perceived acoustic environment, increasing it when exposed to a high 'neighbour's' calling rate and decreasing it when confronted with 'neighbours' calling at a low rate. Consistently, Winn (1967) and Fish (1972) showed that in the Oyster toadfish, playbacks simulating vocal neighbours increased the calling rates of nesting males, as long as the playback rates were equal or above a 18-BW per min threshold. Gulf toadfish males also increased their calling rates when exposed to playbacks of vocal 'challenges' simulating territorial intrusions (Remage-Healey & Bass 2005), suggesting that a high calling rate can be elicited in both sexual or agonistic contexts. However, neither Winn (1967) nor Fish (1972) observed a decrease in *O. tau* males' advertising rate when exposed to playbacks of males calling at low rates. This may be partially due to the naturally low calling rates of *O. tau* (i.e. from three to 10 boatwhistles per minute (Fine

et al. (1977)), when compared to the higher calling rates of the Lusitanian toadfish. None of the playbacks in these authors' experiments consisted of a calling rate actually lower than that exhibited by the tested subjects.

Lusitanian toadfish males calling at a low rate at the time of the PBK trials did not significantly alter their own calling rate when exposed to PBKs of males calling at either high or low rates. Based on the results obtained with males calling at a high rate, it was not surprising that the subjects calling at a low rate did not change their calling rate when presented with the CR5 treatment, because their calling rate already matched the playback. However, in contrast to males calling at a high rate, they did not attempt to compete with a more active male simulated by the CR20 treatment. Calling rate could be momentarily limited by physiological constraints such as low androgen levels (Remage-Healey & Bass 2004, 2005) and/or the relative higher costs to match a competitor's high calling rate. Elevated androgen levels associated with high vocal activity have been shown to be linked with increased energy consumption, immunosuppression and interference with parental care (Oliveira 2005). Apart from physiological costs and the already mentioned energetic demands, there are also other probable ecological drawbacks, such as the attraction of predators (Ryan 1988; Gannon et al. 2005) and the reduction in time for other activities (e.g. feeding). Alternatively, males with steady but low calling levels could be just investing at signalling their presence in the nest to females and other males and not motivated to engage in competitive sexual attraction. As we did

not have access to the male at the moment of the PBK trials, we could not assess all these potential variables that might be at play in these cases.

Vocally active males only reacted to conspecific sounds but not to non-biological white noise sequences. Therefore, the frequency content or other fine parameters of the calls may be perceived by the subject males because white noise bouts with an identical duration to boatwhistles, and played at a rate that should elicit an increase in calling rate, did not affect the subjects' vocal activity. This is consistent with the study of Vasconcelos et al. (2011) that showed from auditory evoked potentials (AEP) recordings that the Lusitanian toadfish can resolve details of the calls in both the time and frequency domains.

Unlike observed by Amorim et al. (2011), changes in the calling rate by males vocalising at a high rate did not change other acoustic parameters of their calls, including boatwhistle duration. Boatwhistle duration is probably modulated by levels of circulating 11KT (Remage-Healey & Bass 2004). Although vocal challenges simulating an intruder elicited rapid (within 5–20 min) increases in the circulating levels of steroid hormones in the Gulf toadfish (Remage-Healey & Bass 2005), the physiological response to a decrease in calling rate (i.e. the observed change elicited by our playbacks) may be slower. Our short time frame analysis (only 5 min after the playback) may have not been long enough to observe differences in boatwhistle duration and spectral content of the calls that usually accompany changes in calling rate (Remage-Healey & Bass 2005; Amorim et al. 2011).

In summary, we show that male *H. didactylus* respond selectively to different temporal calling patterns of other vocal males. We present strong evidence that this species calling behaviour seems dependent on the males' immediate calling rate and probably on its overall ability to sustain a high vocal performance. As chorus size increases, adjustment of calling behaviour is bound to be more complex than we report here, and further studies are necessary to better understand the complex relations of all contributing variables. Future playbacks and passive acoustics studies are planned to monitor acoustic interactions in subjects with manipulated circulating steroid levels to further ascertain how males modulate calling behaviour in response to social interactions.

Acknowledgements

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