The Role of Agonistic Sounds in Male Nest Defence in the Painted Goby Pomatoschistus pictus

6 AUTHORS, INCLUDING:

P.J. Fonseca

University of Lisbon

87 PUBLICATIONS   **698** CITATIONS

SEE PROFILE

M. Clara P Amorim

ISPA Instituto Universitário

79 PUBLICATIONS   **1,172** CITATIONS

SEE PROFILE

RESEARCH PAPER

The Role of Agonistic Sounds in Male Nest Defence in the Painted Goby *Pomatoschistus pictus*

Ricardo Pereira*, Stefania Rismondo*, Manuel Caiano*, Silvia S. Pedroso†, Paulo J. Fonseca* & Maria Clara P. Amorim†

* Departamento de Biologia Animal e Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

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

Abstract

Animals often vocalize during territorial challenges as acoustic signals may indicate motivation and fighting ability and contribute to reduce aggressive escalation. Here, we tested the function of agonistic sounds in territorial defence in the painted goby. *Pomatoschistus pictus,* a small vocal marine fish that defends nests during the breeding season. We first measured the number of times a male approached, avoided, explored, entered and exited two unattended nests associated with either conspecific agonistic sounds or a control: silence or white noise. Acoustic stimuli were played back when the male approached a nest. In a second experimental set, we added visual stimuli, consisting of a conspecific male in a small confinement aquarium near each nest. Even though we found no effect of the visual stimuli, the sound playbacks induced similar effects in both experimental conditions. In the sound vs. silence treatment, we found that when males approached a nest, the playback of conspecific sounds usually triggered avoidance. However, this behaviour did not last as in longer periods males visited nests associated with agonistic sounds more often than silent ones. When the control was white noise, we found no significant effect of the playback treatment in male behaviour. Although we cannot exclude the possibility that other sounds may dissuade nest occupation, our results suggest that agonistic sounds act as territorial intrusion deterrents but are insufficient to prevent nest intrusion on their own. Further studies are needed to test the significance of sound production rate, spectral content and temporal patterns to deter territorial intrusion in fish.

Introduction

In nature, resources such as those needed for breeding (e.g. food, water accessibility, vegetation, shelter availability and nesting sites) are often limited and of variable quality. Hence, in order to have a chance to reproduce, individuals (typically males) may compete intensely over these limited resources (Huntingford & Turner 1987; Andersson 1994). This competition is often solved through confrontations that frequently start with a mutual assessment phase, characterized by low-level agonistic displays and are only expected to escalate to overt aggression if asymmetries between individuals' fighting ability and resource ownership are small (Huntingford & Turner 1987; Briffa & Sneddon 2007). Low-level aggressive behaviour involved in such contests is often accompanied by sound production in a variety of different taxa (insects - Gerhardt & Huber 2002; fish - Ladich & Myrberg 2006; anurans - Davies & Halliday 1978; birds – de Kort et al. 2009; and mammals - Clutton-Brock & Albon 1979). In most vertebrates, such as frogs, birds and mammals, the sounds are the result of the passage of air through specialized vocal organs (larynx, syrinx) driving the vibration of membranes. These sounds typically exhibit strong frequency and amplitude modulation (Ladich 2004). By contrast, teleost fishes have evolved a diversity of sound-producing

mechanisms that often generate low-frequency pulsed sounds lacking complex patterns of frequency and amplitude modulation (Amorim 2006; Ladich & Fine 2006; Rice & Bass 2009). This makes fish particular suitable models to study the function of acoustic signals with conceptually simple playback experiments. Nevertheless, carrying out sound playback experiments with fish can be technically challenging due to the lack of available commercial underwater loudspeakers that can reproduce fish sounds accurately (Fonseca & Maia Alves 2012).

Sounds produced by fishes during territorial defence usually function as a complement to visual behaviour such as colour alterations or aggressive visual displays, including fin erection or quivering (Ladich & Myrberg 2006). Though sound seems to play an important role in fish communication there is little experimental evidence demonstrating the function of acoustic signals in fish territorial defence (Ladich & Myrberg 2006; Vasconcelos et al. 2010). Experiments with muted specimens and with sound playback suggest that in an agonistic context acoustic signals can reveal valuable information about the sender and may help avoid overt confrontation and thus energy depletion or even injury and death (Valinsky & Rigley 1981; Riggio 1981; Ladich et al. 1992; Ladich 1998; Raffinger & Ladich 2009; Bertucci et al. 2010; for a review see Ladich & Myrberg 2006). For example, body size can be advertised by the production of longer sounds with lower dominant frequencies and higher amplitudes that result from larger body structures (Myrberg et al. 1993; Lobel & Mann 1995; Connaughton et al. 2000; Amorim & Neves 2008; Colleye et al. 2009; Amorim et al. 2013). Also, fish sounds may potentially inform the receiver of the sender's motivation during a confrontation (Ladich 2004). Indeed, steroid circulating levels that affect territorial behaviour and aggressiveness may also influence the patterns of sonic muscles contraction or of fin stridulation (Fine & Pennyacker 1986; Connaughton et al. 1997; Remage-Healey & Bass 2006).

In this study, we aimed at examining the role of fish agonistic sounds in territorial defence using playback experiments in a small marine benthic species, the painted goby (*Pomatoschistus pictus*). Painted goby males produce drums and thumps to attract and court females and only drums during agonistic interactions (Amorim & Neves 2007, 2008). During the breeding season (January–May), males compete aggressively over nesting sites and have few breeding opportunities, as they only live up to 2 years (Miller 1986). Males provide exclusive paternal care and use visual and acoustic behaviour to defend nests that are used

both in mate attraction and to hold the eggs laid by females (Bouchereau et al. 2003; Amorim & Neves 2007, 2008). As agonistic drums duration and drum bout duration increase with male size, it is likely that acoustic signals are involved in male-male assessment (Amorim & Neves 2008).

We experimentally examined whether males approached and intruded vacant nests (silent or associated with white noise) more readily than 'occupied' nests. We mimicked nest occupation by playing back a sequence of conspecific agonistic sounds whenever a male approached a nest within a body length. We checked for several reactions of the subject male to vacant and 'occupied' nests, including approaching, avoiding, exploring, entering and exiting the nests. We further checked for nest intrusion behaviour in a similar-design experiment that used additional visual stimuli, consisting of a male in a confinement aquarium placed next to the nest. We predicted that males would be more reluctant to intrude nests associated with agonistic sound, especially in the presence of a conspecific male. Specifically, we expected that males avoided more often and intruded less frequently nests associated with agonistic sounds.

Materials and Methods

Fish Collection and Maintenance

We captured approx. 120 adult fish from January to April 2012 at Parede (38°41′N, 9°21′W), during low spring tides at night with the help of hand nets and flashlights. We separated fish by gender which we assessed by examining the external papilla (rounder in females and longer and pointed in males) and by the presence of nuptial colours in males and swollen bellies in ripe females (Bouchereau et al. 2003). We kept a maximum of eight fish per stock 18 l aquarium (24 × 24 × 32 cm), provided with sand, shelters and a closed-circuit flow of artificial filtered sea water kept at 16°C. Fish were maintained under a 12L/12D photoperiod and fed daily *ad libitum* with chopped shrimps. At the end of experiments (May/June 2012), the fishes were returned to their collection location in a healthy condition.

Experimental Procedure

Thirty-five-litre glass experimental aquaria (26 × 51 × 31 cm) also provided with a sand substrate were divided into three compartments by transparent or opaque partitions. Water, conditioned by dedicated filter, pumps and cooler, was similarly kept at 16°C.

The aquaria were placed above two 3-cm-thick stone slabs separated by two levels of rubber foam shock absorbers. This helped to insulate the aquaria from floor born vibrations and increased acoustic signal-to-noise ratio, thus reducing possible interference with our sound playbacks. Twenty-four hours before an experiment, one male was placed in the central compartment of the aquarium to allow it to become resident. Only males that showed nuptial colours and territorial behaviour in the stock tanks were used. Each lateral compartment was equipped with one artificial PVC shelter (5.5 cm length and 3 cm inner diameter) partially covered with sand and one custom made speaker (see below). The speaker was protected by a plastic net to exclude fish from the speaker area. We also covered both lateral walls of the experimental aquarium with a fine net to reduce areas where a fish might interact with its own reflection.

Playback Treatment

Prior to all trials, we presented a 2 min sound pre-stimulus during which subject fish were exposed to conspecific agonistic sounds and a control, white noise (WN) or silence. The 2 min sound pre-stimulus intended to inform the subject fish of territorial ownership in the lateral compartments before the test period. Both the playback treatment (silence vs. sound or WN vs. sound) and the stimuli assignment to the lateral sides of the aquarium were randomly selected. When sound was emitted at both sides of the aquarium, that is, during WN vs. sound treatment, we insured that the stimuli did not overlap to avoid acoustic interference and masking. Different males were used for each playback treatment.

Agonistic sounds used in the playback treatments consisted of drums from three different males (standard length: 3.3, 3.3 and 3.8 cm) from our sound archive (2010). We made three pre-stimulus drum playback sequences. Each drum pre-stimulus sequence comprised three different sounds from the same male (Fig. 1a). Only sounds with a good signal-to-noise ratio were used. We also selected sounds that would represent the natural variability in number of pulses, sound duration and sound frequency. All files had the same duration and acoustic energy, and the spacing between sounds followed a typical pattern observed in sound production during territorial defence in this species (Fig. 1b). Each pre-stimulus sequence presented a total of 34 sounds, that is, a sound rate of 17 per min, a typical rate for a motivated male (Amorim & Neves 2008). An
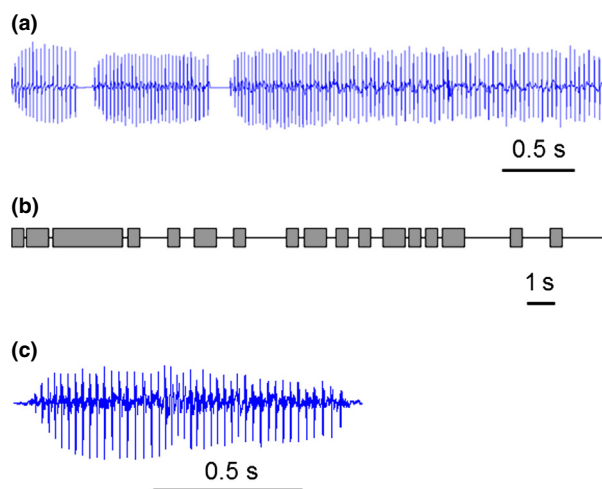

Fig. 1: Oscillogram of three different drums used in a playback sequence (a). A similar sequence of only three drums was played back during trials whenever a subject male approached a nest within a body length. A longer sequence of 2 min containing the same three drums was used in the sound pre-stimulus in which the interval between sounds followed a natural pattern. In (b), approximately 1 min of the 2 min sound pre-stimulus is schematized depicting the rate of 17 sounds per min used in the playback sequence. Note that an agonistic sound sequence comprised three different sounds from the same male. (c) Illustrates a played back drum recorded in the middle of the central compartment. Note that this drum is from a different playback sequence than the one depicted in (a).

identical number of WN files were created and presented similar sound intervals as the drum sequences and were equalized to the same sound amplitude. Also, WN did not contain the pulse pattern of the agonistic drums but had similar acoustic energy and hence a shorter duration.

During the test period, every time a male approached a nest and reached within one body length from the entrance of the nest we immediately played three sounds of the correspondent playback treatment (sound or WN). This stimulus consisted of the first three sounds of the sound pre-stimulus sequence used in a particular trial. Playback experiments were performed using custom made devices composed by an underwater speaker and a driver (Fonseca & Maia Alves 2012), which are able to reproduce low-frequency pulsed fish sounds with great accuracy, such as the ones emitted by the painted goby (cf. Fig. 1 in Fonseca & Maia Alves 2012). These were fed through a D/A converter (Edirol UA-25, Roland, Japan; 16 bit, 8 kHz) controlled by Adobe Audition 3.0 (Adobe Systems Inc., Mountain View, CA, USA), which allowed independent playbacks on two channels. The amplitude of the sound playback (drum or WN) was measured with a

hydrophone (Brüel & Kjær 8104, Brüel & Kjær, Naerum, Denmark, sensitivity −205 dB re1V/µPa; frequency response within ±1 dB from 0.1 Hz to 180 kHz), connected to a Brüel & Kjær 2238 Mediator Sound Level Meter, Naerum, Denmark) and adjusted to mimic that of a painted goby male at 1–2 cm distance measured in previous experiments (approx. 130 dB SPL, re. 1 µPa; Amorim et al. 2013). An additional hydrophone (High Tech 94 SSQ, High Tech Inc., Gulfport, MS, USA; sensitivity −165 dB re1V/µPa; frequency response within ±1 dB from 30 Hz to 6 kHz) was kept on the central compartment of the experimental aquarium, to register any sound produced during trials and to monitor sound playback. Played back sounds reached the central compartment with a good signal-to-noise ratio and quality (Fig. 1c).

Playback Experiments

We carried out two experiments that differed in the presence of visual stimuli during the test period: 'playback only' and 'playback+visual'.

The 'playback only' experiment started 15 min after we turned off all the equipment in the room to reduce noise background in the experimental aquarium. We started video/sound recordings, removed the opaque partitions and exposed the subject fish to a 5 min visual pre-stimulus. This consisted of a male inside a small confinement aquarium (8 × 7 × 22 cm), provided with sand substrate and high enough to prevent chemical communication, placed in each lateral compartment next to the transparent partition (Fig. 2a). Stimulus males were matched in size, that is, difference in standard length ratio was <10%. This visual pre-stimulus intended to arouse territorial behaviour in the subject fish and thus to increase responsiveness during the trial (see Lugli 1997). We finished the visual pre-stimulus period by replacing the opaque partitions. We then removed the lateral stimulus males and allowed a silence period of 8 min which was followed by the 2-min sound pre-stimulus. The PVC and acrylic plastic partitions allowed good transmission of the sound between compartments. Afterwards, all partitions were removed and the fish behaviour was observed and recorded for another 5 min (test period).

In the 'playback+visual' experiment, there was no visual pre-stimulus and trials started with the 2-min sound pre-stimulus, prior to the partitions' removal, followed by the 5-min-test period. The stimulus males stayed between the nest and the side wall of the experimental aquarium throughout the test period (Fig. 2b).
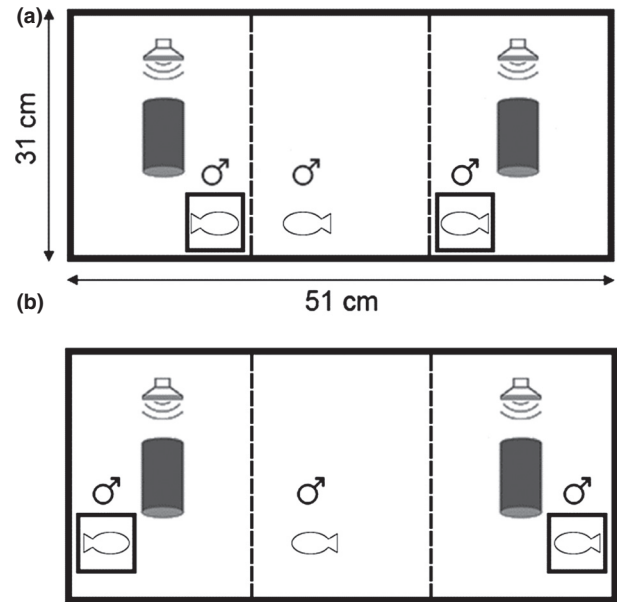

Fig. 2: Top view of experimental setup during the visual pre-stimulus on the 'playback only' (a) and during the whole 'playback+visual' (b) experiments. Males in glass confinement aquaria (8 × 7 × 22 cm) were placed near the transparent partitions (dashed lines) in 'playback only' experiments to provide a visual pre-stimulus before playback procedures. In 'playback+visual' experiments visual stimulus males were placed instead between the nest and the side wall of the experimental tank (26 × 51 × 31 cm) throughout the experiment.

We used different males in the 'playback only' and 'playback+visual' experiments. Fish were measured for standard length (SL) and weighted (W) after each test. A total of 12 subject males with SL ranging from 3.4 to 3.9 cm (mean ± standard deviation [SD] = 3.7 ± 0.18 cm) and W from 0.4 to 0.88 g (0.67 ± 0.12 g) were tested in the 'playback only' experiment. From these, eight and four males were tested in the silence vs. sound and in the WN vs. sound treatments, respectively. A total of 14 subject males were tested in the 'playback+visual' experiment with SL ranging from 3.4 to 3.9 cm (3.7 ± 0.16 cm) and W from 0.48 to 0.88 g (0.7 ± 0.13 g), half of which were tested in the silence vs. sound and the other half in the WN vs. sound treatments. All males interacted with the nests except from two males tested in the 'playback+visual' experiment. These were 3.6 cm SL, 0.58 g W (silence vs. sound treatment) and 3.9 cm SL, 0.62 g W (WN vs. sound treatment).

Behavioural Recording and Analysis

Fish behaviour was recorded with a video camera (Sony DCR-HC39, Sony, Tokyo, Japan) positioned

50 cm in front of the experimental tank and subsequently analysed with Etholog (v.2.2, Ottoni 2000). During the pre-stimuli period video recording captured the entire aquarium, while during the test period, we focused on the behaviour of the test male.

We tallied the frequency of occurrence of the following nest-related behavioural parameters: approach (move within a body length of the nest), avoidance (abruptly swims away from the nest), explore (swim around and on top of the nest), enter and exit the nest. Avoidance, explore and 'initial enter' were considered when they occurred within 5 s of the male's approach to the nest and hence approximately within 5 s of the playback stimulus. Enter and exit refer to the number of times a male entered, following an approach and exited a nest with no such time restriction. Notice that in the case a male left the vicinity of the nest and later approached it again and entered, we considered that the first approach did not result in nest intrusion (enter). The total time spent on each side of the aquarium and the first side explored by the fish were also registered. Trials were considered valid when the tested male approached at least one of the nests.

Statistical Analyses

Playback effects were analysed based on the different behaviours exhibited by the subject males. We carried out repeated measures ANOVAs to compare each male behaviour (approach, avoidance, explore, enter and exit) in relation to control nests and 'sound-associated' nests, that is, using playback treatment (control vs. sound) as the dependent variable (i.e. the repeated measures variable), as males could interact with both nest types. We also used experiment type: 'playback only' and 'playback+visual' as a factor (independent variable). We analysed separately the data for the playback treatments silence vs. sound and WN vs. sound. The two males that did not interact with the nests were excluded from this analysis, and hence, 24 males were considered in total.

We tested male preference for the sound or control sides of the experimental aquarium with Wilcoxon non-parametric tests, where we considered the total time spent on each side during the test period. We carried out Binomial tests to examine whether males first swam to the sound (or the control, i.e. silence or WN) side of the aquarium more often than expected by chance alone. The intention was to determine whether there was a first preference based on the pre-stimuli playback treatment (silence vs. sound or WN

vs. sound). We performed further Binomial tests to examine whether the first nest occupation was made randomly relative to nest type (test or control). In these analyses, we pooled the 'playback only' and 'playback+visual' data because the presence of the visual stimuli had no effect on the male's behaviour towards the nest (see results). We also used the full data set, that is, the 26 tested males in these analyses (except for the first nest occupation), as the two males that did not interact with nests still showed active exploration of the full aquarium.

Statistical analyses were conducted with Statistica (10, Statsoft Inc., Tulsa, USA), and all data were transformed when necessary to meet assumptions of the used parametric tests. When there was no normality of the transformed data, non-parametric tests were used.

Results

During the acoustic pre-stimulus, when the partitions were in place, subject males did not exhibit any measurable reaction to the sound playback. In contrast, during the visual pre-stimulus (in the 'playback only' experiment), tested males repeatedly swam up and down the partition in front of the visual stimulus males. Upon the removal of the partitions, the subject male readily moved towards one side of the experimental aquarium, this behaviour being more obvious when an additional visual stimulus was present. In the absence of conspecifics, males showed a general explorative behaviour swimming around the aquarium. However, when confined conspecific males were present ('playback+visual' experiment), subject males often interacted with the opponent by performing quivering lateral and frontal displays while darkening the chin.

In the treatment, silence vs. sound fish showed a tendency ($p = 0.07$) to approach more often nests associated with sound than silent nests (Table 1, Fig. 3a). Figure 4 depicts the number of times males approached nests associated with different playback treatments during both experiment types ('playback only' and 'playback+visual') and hence the number of received playback stimuli. Interestingly, males avoided nests associated with agonistic drums more frequently than silent nests (Table 1, Fig. 3b). In spite of this, the frequency with which males entered both nest categories within 5 s of an approach showed no significant differences (Table 1), but in longer periods, males entered and exited nests associated with sound more frequently than silent ones (Table 1, Fig. 3c, d). Hence, the first reaction of an approaching male

towards an 'occupied' nest (i.e. associated with sounds) was often avoidance, but this behaviour would not last and could be followed by a nest visit.

Table 1: Effects of playback treatment (PBK: silence vs. sound) and experiment type (EXP: 'playback' only or 'playback+visual') on nest-related behaviours: approach, avoidance, explore, initial enter (upon 5 s of approach), enter (no time restraints) and exit

Dependent variable	Factor	F	p
Approach	PBK	$F_{1,12} = 4.64$	0.07
	Exp	$F_{1,12} = 0.00$	0.23
	PBK × Exp	$F_{1,12} = 1.16$	0.35
√Avoidance	PBK	$F_{1,12} = 5.37$	0.04
	Exp	$F_{1,12} = 2.15$	0.17
	PBK × Exp	$F_{1,12} = 2.15$	0.17
Explore	PBK	$F_{1,12} = 0.02$	0.89
	Exp	$F_{1,12} = 2.44$	0.14
	PBK × Exp	$F_{1,12} = 0.94$	0.35
Log-initial enter	PBK	$F_{1,12} = 0.24$	0.64
	Exp	$F_{1,12} = 0.07$	0.80
	PBK × Exp	$F_{1,12} = 1.64$	0.22
Log-enter	PBK	$F_{1,12} = 4.84$	0.048
	Exp	$F_{1,12} = 0.30$	0.59
	PBK × Exp	$F_{1,12} = 1.91$	0.19
Log-exit	PBK	$F_{1,16} = 4.47$	0.05
	Exp	$F_{1,16} = 0.11$	0.74
	PBK × Exp	$F_{1,16} = 2.01$	0.18

Data were transformed when necessary to meet the ANOVA assumptions.

We found no significant differences between the exploring behaviour towards silent or 'occupied' nests (Table 1). In addition, we found no effect of experiment type for any variable (Table 1).

Regarding the treatment WN vs. sound, there were no significant effects of the variables playback treatment and experiment type for the nest-related behaviours approach, explore, enter (any time frame) and exit performed by males towards the nest (Table 2). Nevertheless, we found a significant effect of experiment type on avoidance behaviour as males avoided nests more often when there was no visual stimulus nearby (Table 2, Fig. 5).

After the pre-stimuli, males swam to one side of the aquaria (sound vs. control) at random, that is, swimming towards the sound side was not different than expected by chance (Binomial test, p > 0.05). Fish swam to the sound side 10 times out of 15 in the silence vs. sound treatment and 6 of 11 in the WN vs. sound treatment. However, when considering only the males that entered a nest during trials (N = 19), it was significantly more likely that the first nest that males entered was a nest associated with agonistic sound rather than a control nest (Binomial test, p = 0.002). The first nest to be intruded was a nest associated with agonistic sound 16 of 19 times: 6 of 8 times when the control was WN and 10 of 11 times
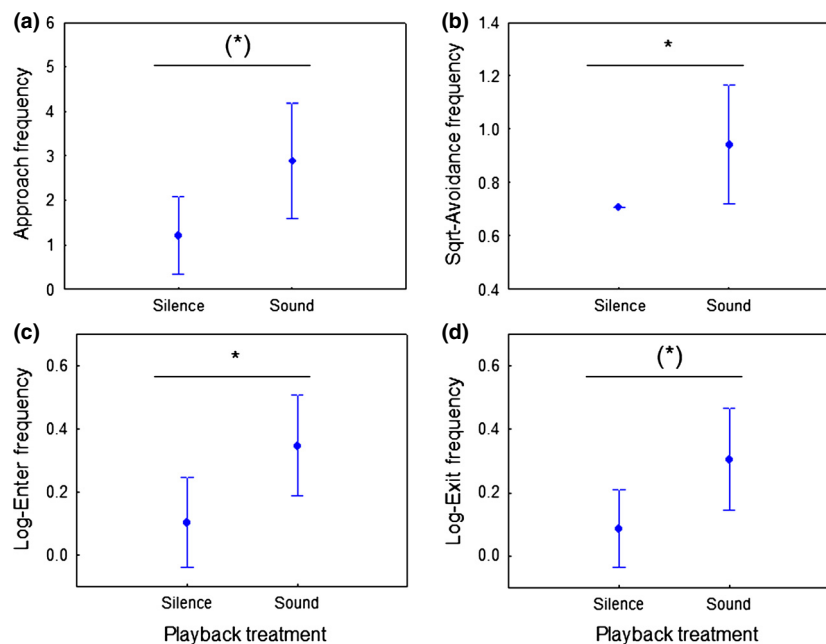

Fig. 3: Comparison of the frequency of the subject male behaviour towards the nest between playback treatments (silence and sound): (a) approach, (b) avoidance, (c) enter and (d) exit. Avoidance was measured in a time window of 5 s after approach, whereas enter and exit were measured with no time restraints. Dots and error bars are means and 95% confidence intervals, respectively. Data were transformed when necessary to meet the ANOVA assumptions. (*) p < 0.10; * p < 0.05.
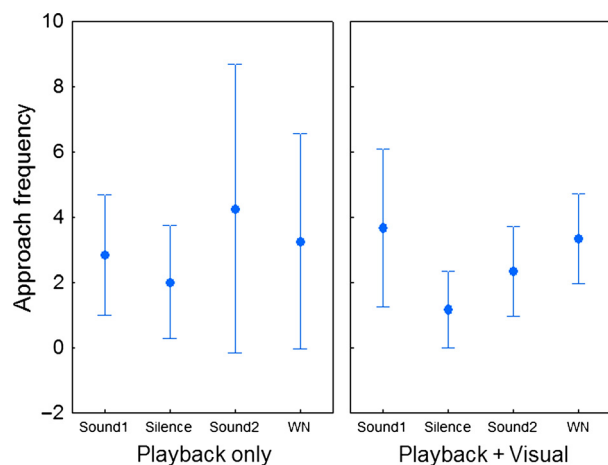
Fig. 4: Mean ± standard deviation of the number of times a male approached a nest associated with different stimuli during the 'playback only' and the 'playback+visual' experiments. Note that the number of approaches to a nest also corresponds to the number of received stimuli. Sound1 and sound2 correspond to the sound playbacks performed during the silence vs. sound and WN vs. sound treatments, respectively.

Table 2: Effects of playback treatment (PBK: WN vs. sound) and experiment type (Exp: 'playback' only or 'playback+visual') on nest-related behaviours: approach, avoidance, explore, initial enter (upon 5 s of approach), enter (no time restraints) and exit

Dependent variable	Factor	F	p
Log-approach	PBK	$F_{1,8} = 0.04$	0.84
	Exp	$F_{1,8} = 0.10$	0.76
	PBK × Exp	$F_{1,8} = 0.24$	0.64
Log-avoidance	PBK	$F_{1,8} = 0.01$	0.92
	Exp	$F_{1,8} = 33.41$	0.00
	PBK × Exp	$F_{1,8} = 0.01$	0.92
Explore	PBK	$F_{1,8} = 0.02$	0.88
	Exp	$F_{1,8} = 1.82$	0.21
	PBK × Exp	$F_{1,8} = 0.60$	0.46
Initial enter	PBK	$F_{1,8} = 0.08$	0.78
	Exp	$F_{1,8} = 0.08$	0.78
	PBK × Exp	$F_{1,8} = 2.11$	0.18
Enter	PBK	$F_{1,8} = 0.73$	0.42
	Exp	$F_{1,8} = 0.49$	0.42
	PBK × Exp	$F_{1,8} = 0.00$	0.97
Exit	PBK	$F_{1,8} = 1.07$	0.33
	Exp	$F_{1,8} = 0.36$	0.56
	PBK × Exp	$F_{1,8} = 0.02$	0.89

Data were log-transformed when necessary to meet the ANOVA assumptions.

when the control was silence. The time until first nest occupation averaged 91.2 s for nests associated with agonistic sound (N = 16) but only 50.3 s for control nests (N = 3). Males also spent more time in the sound than in the control side of the aquarium
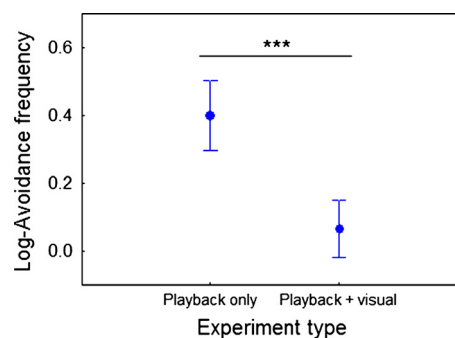

Fig. 5: Avoidance behaviour exhibited by subject males towards the nest according to different experiment types: 'playback only' and 'playback+visual'. Dots and error bars are means and 95% confidence intervals, respectively. *** p < 0.001.
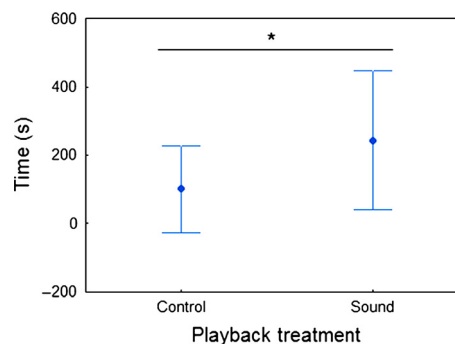

Fig. 6: Mean ± standard deviation of the amount of time spent by tested males on each side of the aquarium according to playback treatment, that is, control vs. sound. * p < 0.05.

(Wilcoxon matched-pairs signed-ranks test: $T = 83.0$, N = 26, p = 0.02; Fig. 6).

Discussion

In fish, sounds play an important role in establishing and defending territories allowing to assess the condition of competitors and to decide whether they should possibly risk injury by engaging in a fight (Ladich 2004). In this study, we used a small marine nest-reproducing fish as a model and observed the effect of playing back conspecific aggressive sounds, which mimicked the presence of a vocalizing nest-holder, on nest occupation.

In the silence vs. sound playback treatment, we found that although subject males first headed randomly to a side of the aquarium (sound or control side), they tended to approach nests associated with conspecific sounds more frequently than silent ones. As expected, a frequent first reaction of males approaching nests associated with agonistic sounds

was avoidance, a behaviour that was significantly less likely to occur on approaching silent nests. However, contrary to our predictions, after the first avoidance reaction, males entered 'occupied' nests more frequently than silent ones, probably because they could not associate the sound to a male nest-holder. Altogether, the results suggest that agonistic sounds have a significant role in territorial defence in the painted goby and may act as a deterrent to nest intrusion until the absence of a nest-holder physically defending the nest is confirmed. Agonistic sounds have been shown to be important in territorial defence in a few other fish species. Schuster (1986) observed that in the dwarf gourami *Colisa lalia* territorial defence was approx. sixfold more effective when attacks chasing intruders were accompanied by sounds than when they were silent. Valinsky & Rigley (1981) muted juveniles of skunk loaches *Yasuhikotakia horae* and showed that muted individuals had fewer chances to chase intruders from their shelters. Consistent with our results, Ladich & Myrberg (2006) and Vasconcelos et al. (2010) suggest that fish agonistic sounds can act as a 'keep-out' signal towards intruders. For example, nest-holding Lusitanian toadfish (*Halobatrachus didactylus*) males defend their territories with loud sounds (boatwhistles), which often elicits fleeing behaviour from the intruder, thus decreasing the chances of escalated fights and nest takeovers (Vasconcelos et al. 2010). Myrberg (1997) also showed with a series of elegant field experiments that playbacks of the chirp sound of bicolour damselfish males (*Stegastes partitus)* made in unguarded territories inhibit territorial intrusion by neighbouring conspecific males for a significantly longer time than when there is no sound playback. Consistent with our study, Myrberg also found that the deterrent effect of the sound was short-term when transmitted in the absence of the resident (Myrberg 1997). Similarly, in birds acoustic signals can slow down, but not prevent, territory intrusion (reviewed in Nowicki et al. 1998). For example, great tit males (*Parus major*) take longer to intrude recently unoccupied territories if they are associated with conspecific song playback than silent territories (Krebs 1976).

The fact that males visited more often and were more likely to first intrude nests associated with conspecific sounds, and spent more time in the sound side of the aquarium, deserves further attention. For example, males could be selecting for good-quality nests already chosen by other males thus saving the time and energy costs of searching for a suitable nesting site (Lindström & Pampoulie 2005). Also, as females of the congeneric *P. minutus* prefer males that

already have eggs in the nest (Forsgren et al. 1996), the search for such nests could be an added value if mate selection in the studied species follows the same pattern.

In the trials using white noise as a control, we registered no significant effect of the playback treatment in any male nest behaviour suggesting that males may not distinguish conspecific sound from white noise. However, the lack of significant differences in the reaction towards nests associated with agonistic sound or white noise should be seen with caution as it is likely due to the low sample size. In the bicolour damselfish, Myrberg (1997) found that white noise had no effect as a territorial deterrent. Consistently, Schwarz (1974) found that agonistic sounds reduced aggression in the cichlid, *Archocentrus centrarchus*, in contrast with control noise that rendered no effect in male–male agonistic interactions. These and other studies (reviewed in Ladich & Myrberg 2006) support our suggestion that low sample sizes hampered the ability to find a treatment effect in this set of experiments.

Fish are believed to mainly use the temporal patterns of calls for communication (Ladich 2004; Amorim 2006). Therefore, we removed the specific temporal pattern from the white noise stimuli used in our experiments to avoid relaying to the receiver information likely contained in the pulse pattern. As a consequence, and to keep the same acoustic energy among the sound stimuli, a parameter that might affect the behaviour by changing the overall stimulation of the hearing pathway, the white noise stimuli were shorter than the agonistic drums. However, by doing this, we were modifying another possible relevant parameter, the duration of the stimuli, which thus did not corresponded to the duration of the average drum and might have influenced the fish response. For example, McKibben & Bass (1998) carried out phonotactic experiments with plainfin midshipman females (*Porichthys notatus*) and concluded that duty cycle (sound on time, i.e. acoustic energy) and also sound duration affected female preference. The decrease in duty cycle and sound duration of mating-like signals decreased the likelihood of phonotaxis possibly because playback sounds decreased their resemblance to the long tonal mate attraction hums made by nesting males.

Although test and visual stimuli painted goby males often engaged in mutual visual displays, the presence of confined males had in general no effect on the male's nest-related behaviour. Most available studies have shown that agonistic sounds usually need to be associated with signals of other modali-

ties to elicit appropriate behavioural responses (Ladich 2004). For example, Bertucci et al. (2010) experimentally separated or coupled visual and acoustic signals to test the role of sounds produced during male–male aggressive interactions in a cichlid fish, *Metriaclima zebra,* and observed that acoustic signals alone never triggered aggression. However, when associated with visual signals agonistic sounds significantly lowered the levels of aggressive behaviour observed when visual signals were presented alone. The general lack of effect of additional visual stimuli further points to a prevalent and independent role of acoustic signals in territorial defence in our study species, a role scarcely documented in fish. However, we cannot rule out that the lack of effects induced by the visual stimulus male might be due to test fish perceiving that the male in the confinement aquarium was not a threat and could not defend the available nest. Nevertheless, Myrberg (1997) found in the aforementioned field experiments with the bicolour damselfish that the visual presence of a confined resident near the shelter (a male in a confinement bottle) was a more effective deterrent to intrusion than sound playbacks alone.

The function of sounds emitted during agonistic behaviour is not fully understood in fishes and the relative paucity of studies have shown that agonistic sounds can have different roles depending on species or individual status (reviewed in Raffinger & Ladich 2009). For example, in the satinfin shiner *Cyprinella analostana,* a small cyprinid where males aggressively defend territories while producing single knocks or series of knocks (Stout 1963), playbacks of rapid series of knocks can increase or inhibit males' aggression depending on the receiver's social status. When dominant males interacting with a mirror received playbacks of agonistic sounds, they increased aggression, in contrast to submissive males where the playback induced the opposite effect (Stout 1975).

This study has revealed that acoustic communication is important in territorial defence in the painted goby and likely other fishes as agonistic sounds slowed down territorial intrusions. Additional experiments are required to test the significance of sound rates, spectral and temporal patterns to deter territorial intrusion in fish and to ascertain the specificity of the acoustic features that can prime territorial behaviour. Other studies not involving direct assessment of the function of particular sound features on territorial defence have, however, shed light on signal parameters that might be used by fish to encode-decode relevant information. For example, sound pressure level,

sound frequency or sound emission rate were demonstrated to give information on fish size and condition and therefore resource holding potential (Ladich 1998; McKibben & Bass 1998; Amorim et al. 2010). However, the best approach to assess the function of signals is the use of playback experiments. This has been common practice to infer the territorial function of acoustic signals and has shown in other taxa the importance of particular acoustic traits in male-male assessment (Davies & Halliday 1978; de Kort et al. 2009). Associating nests with acoustic stimuli differing in the above-mentioned features could shed light to whether agonistic sounds only inform the intruder of the presence of a nest-holder or if they are also used in male-male assessment.

Literature Cited

Amorim, M. C. P. 2006: Diversity of sound production in fish. In: Communication in Fishes, Vol. 1 (Ladich, F., Collin, S. P., Moller, P. & Kapoor, B. G., eds). Science Publishers, Enfield, N.H., pp. 71—104.

Amorim, M. C. P. & Neves, A. S. M. 2007: Acoustic signalling during courtship in the painted goby, *Pomatoschistus pictus*. J. Mar. Biol. Assoc. UK **87**, 1017—1023.

Amorim, M. C. P. & Neves, A. S. M. 2008: Male painted gobies (*Pomatoschistus pictus*) vocalise to defend territories. Behaviour **145**, 1065—1083.

Amorim, M. C. P., Simões, J. M., Mendonça, N., Bandarra, N. M., Almada, V. C. & Fonseca, P. J. 2010: Lusitanian toadfish song reflects male quality. J. Exp. Biol. **213**, 2997—3004.

Amorim, M. C. P., Pedroso, S. S., Bolgan, M., Jordão, J. M., Caiano, M. & Fonseca, P. J. 2013: Painted gobies sing their quality out loud: acoustic rather than visual signals advertise male quality and contribute to mating success. Funct. Ecol. **27**, 289—298.

Andersson, M. 1994: Sexual Selection. Princeton Univ. Press, Princeton, New Jersey.

Bertucci, F., Beauchaud, M., Attia, J. & Mathevon, N. 2010: Sounds modulate males' aggressiveness in a cichlid fish. Ethology **116**, 1179—1188.

Bouchereau, J. L., Houder, V., Marques, A. & Rebelo, J. E. 2003: A new distribution record and the reproductive strategy of *Pomatoschistus pictus* adriaticus (Pisces: Gobiidae) in the Mediterranean Sea. J. Mar. Biol. Assoc. UK **83**, 1157—1161.

Briffa, M. & Sneddon, L. U. 2007: Physiological constraints on contest behaviour. Funct. Ecol. **21**, 627—637.

Clutton-Brock, T. H. & Albon, S. D. 1979: The roaring of red deer and the evolution of honest advertisement. Behaviour **69**, 145—169.

Colleye, O., Frédérich, B., Vandewalle, P., Casadevall, M. & Parmentier, E. 2009: Agonistic sounds in the skunk clownfish *Amphiprion akallopisos*: size-related variation in acoustic features. J. Fish Biol. **75**, 908—916.

Connaughton, M. A., Fine, M. L. & Taylor, M. H. 1997: The effects of seasonal hypertrophy and atrophy on fiber morphology, metabolic substrate concentration and sound characteristics of the weakfish sonic muscle. J. Exp. Biol. **200**, 2449—2457.

Connaughton, M. A., Taylor, M. H. & Fine, M. L. 2000: Effects of fish size and temperature on weakfish disturbance calls: implications for the mechanism of sound generation. J. Exp. Biol. **203**, 1503—1512.

Davies, N. B. & Halliday, T. R. 1978: Deep croaks and fight assessment in toads *Bufo bufo*. Nature **274**, 683—685.

Fine, M. L. & Pennyacker, K. R. 1986: Hormonal basis for sexual dimorphism of the sound-producing apparatus of the oyster toadfish. Exp. Neurol. **92**, 289—298.

Fonseca, P. J. & Maia Alves, J. 2012: A new concept in underwater high fidelity low frequency sound generation. Rev. Sci. Instrum. **83**, 055007.

Forsgren, E., Karlsson, A. & Kvarnemo, C. 1996: Female sand gobies gain direct benefits by choosing males with eggs in their nests. Behav. Ecol. Sociobiol. **39**, 91—96.

Gerhardt, H. C. & Huber, F. 2002: Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. The Univ. of Chicago Press, Chicago and London.

Huntingford, F. & Turner, A. K. 1987: Animal Conflict. Chapman & Hall, London.

de Kort, S. R., Eldermire, E. R. B., Cramer, E. R. A. & Vehrencamp, S. L. 2009: The deterrent effect of bird song in territory defense. Behav. Ecol. **20**, 200—206.

Krebs, J. R. 1976: Bird song and territorial defence. New Scientist **70**, 534—536.

Ladich, F. 1998: Sound characteristics and outcome of contests in male croaking gouramis (Teleostei). Ethology **104**, 517—529.

Ladich, F. 2004. Sound production and acoustic communication. In: The Senses of Fishes. Adaptations for the Reception of Natural Stimuli (van der Emde, G., Mogdans, J. & Kapoor, B. G., eds). Narosa, New Delhi, pp. 210—230.

Ladich, F. & Fine, M. L. 2006. Sound-generating mechanisms in fishes: a unique diversity in vertebrates. In: Communication in Fishes, Vol. 1 (Ladich, F., Collin, S. P., Moller, P. & Kapoor, B. G., eds). Science Publishers, Enfield, N.H., pp. 3—43.

Ladich, F. & Myrberg, A. A. 2006. Agonistic behavior and acoustic communication. In Communication in fishes, Vol. 1 (Ladich, F., Collin, S. P., Moller, P. & Kapoor, B. G., eds). Science Publishers, Enfield, N.H., pp. 122—148.

Ladich, F., Bischof, C., Schleinzer, G. & Fuchs, A. 1992: Intra- and interspecific differences in agonistic vocalization in croaking gouramis (Genus: *Trichopsis*, Anabantoidei, Teleostei). Bioacoustics **4**, 131—141.

Lindström, K. & Pampoulie, C. 2005: Effects of resource holding potential and resource value on tenure at nest sites in sand gobies. Behav. Ecol. **16**, 70—74.

Lobel, P. S. & Mann, D. 1995: Spawning sounds of the damselfish, *Dascyllus albisella* (Pomacentridae), and relationship to male size. Bioacoustics **6**, 187—198.

Lugli, M. 1997: Response of male goby, *Padogobius martensii*, to aggressive sound playback following pre-experimental visual stimulation. Behaviour **134**, 1175—1188.

McKibben, J. R. & Bass, A. H. 1998: Behavioral assessment of acoustic parameters relevant to signal recognition and preference in a vocal fish. J. Acoust. Soc. Am. **104**, 3520—3533.

Miller, P. J. 1986: Gobiidae. In: Fish of the North-eastern Atlantic and Mediterranean, Vol. III (Whitehead, P. J. P, Bauchot, M. L., Hureau, J. C., Nielsen, J. & Tortonese, E., eds). UNESCO, Paris, pp. 1019—1985.

Myrberg, A. A. Jr 1997: Sound production by a coral reef fish (*Pomacentrus partitus*): evidence for a vocal, territorial "keep-out" signal. Bull. Mar. Sci. **60**, 1017—1025.

Myrberg, A. A. Jr, Ha, S. J. & Shamblott, H. 1993: The sounds of bicolor damselfish (*Pomacentrus partitus*): predictors of body size and a spectral basis for individual recognition and assessment. J. Acoust. Soc. Am. **94**, 3067—3070.

Nowicki, S., Searcy, W. A. & Hughes, M. 1998: The territory defense function of song in song sparrows: a test with the speaker occupation design. Behaviour **135**, 615—628.

Ottoni, E. B. 2000: EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions. Behav. Res. Methods Instrum. Comput. **32**, 446—449.

Raffinger, E. & Ladich, F. 2009: Acoustic threat displays and agonistic behaviour in the red-finned loach *Yasuhikotakia modesta*. J. Ethol. **27**, 239—247.

Remage-Healey, L. & Bass, A. H. 2006: A rapid neuromodulatory role for steroid hormones in the control of reproductive behavior. Brain Res. **1126**, 27—35.

Rice, A. N. & Bass, A. H. 2009: Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: insights into the diversity of the Batrachoididae. J. Exp. Biol. **212**, 1377—1391.

Riggio, R. 1981: Acoustical correlates of aggression in the bicolor damselfish, *Pomacentrus partitus*. M.Sc. Thesis, University of Miami, Miami, USA.

Schuster, S. 1986: Untersuchungen zur Bioakustik des Zwergfisches *Colisa lalia* (Perciformes, Belontiidae). Zool. Beitr. **29**, 295—306.

Schwarz, A. 1974: The inhibition of aggressive behavior by sound in cichlid fish, *Cichlasoma centrarchus*. Z. Tierpsychol. **35**, 508—517.

Stout, J. F. 1963: The significance of sound production during the reproductive behaviour of *Notropis analostanus* (Family Cyprinidae). Anim. Behav. **11**, 83—92.

Stout, J. F. 1975: Sound communication during the reproductive behavior of *Notropis analostanus* (Pisces: Cyprinidae). Am. Midl. Nat. **94**, 296—325.

Valinsky, W. & Rigley, L. 1981: Function of sound production by the skunk loach *Botia horae* (Pisces, Cobitidae). Z. Tierpsychol. **55**, 161—172.

Vasconcelos, R. O., Simões, J. M., Almada, V. C., Fonseca, P. J. & Amorim, M. C. P. 2010: Vocal behaviour during territorial intrusions in the Lusitanian toadfish: boat-whistles also function as territorial 'keep-out' signals. Ethology **116**, 155—165.