Male painted gobies (*Pomatoschistus pictus*) vocalise to defend territories

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Summary

Many fish species emit sounds in agonistic contexts. During direct confrontations sounds are typically produced during the display phase in conjunction with visual exhibitions. Here we studied sound production during territorial defence in captive painted gobies, *Pomatoschistus pictus*, and related acoustic parameters with male traits and the date of recording (Julian day, i.e., with the approach of the peak of the breeding season). Territorial males emitted drumming sounds during displays that involved darkening the chin and fins, spreading fins and quivering the body. Drums were trains of low frequency pulses ($\approx$23 pulses) repeated every 27 ms and usually lasting under a second. Drums were produced in short sequences of sounds (bursts). All acoustic parameters differed significantly among males. Drum and burst duration, and drum number of pulses increased significantly with male size. Calling duration (including drum, burst duration and drum number of pulses) also increased significantly with Julian date and presented a high intra-male variability, suggesting that these parameters may also depend on the individual's motivation. We provide the first report for agonistic sound production in sand gobies and give evidence that sound parameters contain information that can be used during mutual assessment in contests over territories.

Keywords: acoustic communication, sound production, territorial defence, Gobiidae, teleost fish.

Abbreviations

SNR, signal-to-noise ratio; W, fresh weight; SL, standard length; SD, standard deviation; FD, frontal display; CF, condition factor; FDQ, frontal dis-

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play with quiver; LD, lateral display; LDQ, lateral display with quiver; Qnest, Quiver in the nest.

Introduction

In animal contests, asymmetries between the opponents, such as size, previous fighting experience or prior residence, are determinant to the outcome of an interaction (Archer, 1988). Consequently, animals spend a great part of agonistic contests in mutual assessment ultimately avoiding more costly escalated fighting from which physical injuries or even death might occur (Enquist & Leimar, 1983). These early phases of sequential mutual assessment consist of display behaviour that advertises the contestants' fighting abilities, which depend on intrinsic factors such as body size and weaponry (Parker, 1974) or extrinsic components such as motivation, prior residence and experience (e.g., Turner, 1994; Hsu & Wolf, 1999). Therefore, it is not surprising that many species show large agonistic repertoires, combining several sensory modalities by using visual, acoustic or olfactory displays in a complex signalling system, suggesting that multiple fighting ability components can be assessed through multimodal cues (Enquist, 1985; Hack, 1997).

Acoustic signals are emitted in a variety of agonistic contexts by vertebrates (e.g., Clutton-Brock & Albon, 1979), including members of more than 30 families of teleost fishes (reviewed in Ladich & Myrberg, 2006). As other animals, fishes often produce sounds in distress or disturbance situations, as well as in direct confrontations with conspecifics. Agonistic sounds are frequently described as part of displays characteristic of contests for limited resources, such as food, territories or mates, while alarm calls are characteristic of distressful situations such as natural predator attacks, human handling or fishing net confinement (Ladich & Myrberg, 2006).

Unlike amphibians, birds and mammals, that produce sounds by flowing air through a tube (the larynx or the syrinx) associated with membranes (e.g., vocal cords), cavities and sacs (Bradbury & Vehrencamp, 1998), fishes exhibit the largest diversity of sound-producing mechanisms in vertebrates (Ladich & Fine, 2006). These include several swimbladder sonic systems, pectoral-related mechanisms or a variety of other mechanisms such as dorsal fin stridulation, friction of neck vertebrae, or air passage through the anal duct (reviewed in Ladich & Fine, 2006). The most common means of sound generation are associated with the swimbladder that acts as a drum vibrated by either intrinsic sonic muscles attached to its wall or by extrinsic muscles (Ladich & Fine, 2006). Because swimbladder and sonic muscle size increase in larger fish, modifications in acoustic parameters such as lower dominant frequency, increased pulse duration and higher sound pressure level, may result from an increase in fish size and hence give reliable information on the individual's fighting ability (e.g., Connaughton et al., 2002; Amorim et al., 2003; De Jong et al., 2007).

During the exhibition phase of agonistic interactions, fishes typically try to increase their body size appearance by displaying their body parallel or frontally to the opponent, while spreading their fins and extending the opercular cover. Such visual displays are often accompanied by sound production (Ladich & Myrberg, 2006), suggesting that sound may indeed convey information on the fish's fighting ability. A few studies have shown that sound production by territory holders can be effective in territorial defence (reviewed in Ladich & Myrberg, 2006). For example, muting experiments with the skunk loach *Botia horae* (Cobitidae) demonstrated that experimentally muted territorial residents were unsuccessful in repelling intruders from entering their territories, in contrast with intact and sham operated fish (Valinsky & Rigley, 1981). In another study, playback of aggressive pop sounds from within artificial nests significantly deterred territorial intrusion in the damselfish *Stegastes partitus* (Pomacentridae) (Riggio, 1981).

The family Gobiidae includes many vocal species that emit breeding sounds (Lugli et al., 1997; Myrberg & Lugli, 2006). However, only nine species have been documented to emit agonistic sounds during territorial defence (Kinzer, 1961; Protasov et al., 1965; Mok, 1981; Takemura, 1984; Torricelli & Romani, 1986; Casaretto et al., 1988; Guosheng & Takemura, 1989; Ladich & Kratochvil, 1989; Picciulin et al., 2006), although territorial behaviour is widespread in gobies both during and outside the breeding season (Miller, 1986). Agonistic acoustic signals have never been documented in the genus *Pomatoschistus* although four species are known to emit mating sounds (Lugli & Torricelli, 1999; Lindström & Lugli, 2000; Amorim & Neves, 2007). In this work we present a first description of the agonistic sounds of the painted goby *Pomatoschistus pictus* (Miller, 1973). We study the association of sound production with particular displays of territorial defence behaviour and check for the existence of signal variation with associated behaviour. We compare sounds among individuals and correlate their characteristics with male size and condition factor to test if agonistic sounds could give information on male's fighting abilities. We finally correlate acoustic characteristics and frequency of agonistic behaviour with the date of recording (Julian day) to test whether sound parameters could be affected by the approach of the breeding season.

Material and methods

Study species

The painted goby is a coastal benthic species that inhabits shallow gravel and sandy areas (Miller, 1986). It is a small goby with a maximum life span of 2 years reaching up to 57 mm in length, and breeds from approximately February to July depending on location (Miller, 1986). Like other sand gobies, males build nests under empty shells, such as valves from *Pecten* or *Cardium*, and cover them partially or entirely with sediment leaving only one opening (Bouchereau et al., 2003). Females enter the nest attracted by male displays and lay their eggs in a single layer on its ceiling (Bouchereau et al., 2003). Parental care is provided exclusively by the male (Bouchereau et al., 2003). Male nest owners actively defend their nest from intruders both outside and during the reproductive period (personal observation).

Fish collection and care

Fish were collected in December 2005 and February 2006 with hand nets, by scuba diving in shallow waters (2–9 m) at Arrábida, Portugal (38°26′N 9°06′W). After capture, fishes were brought to the laboratory (Universidade Lusofona, Lisbon) and were kept in stock aquaria (11 l; 33 × 22 × 18 cm high), with a maximum density of eight fish of the same sex per tank. Fish gender was recognised by the shape of the external papilla, that is rounded in females and longer and pointed in males, as well as by the existence of nuptial colours in males and swollen bellies in ripe females (Bouchereau et al., 2003). Stock aquaria were provided with seawater, internal power filters, sand substrate and shelters. Photoperiod followed a natural regime and water temperature was kept at 15–16°C. Fish were fed daily with shrimps and bivalves ad libitum.

Recording and analysis of acoustic signals

Recording sessions were carried out in February and March 2006 in two 35 l experimental tanks (29 × 51 × 26 cm high) fitted as described for stock tanks, and placed on top of a 14-cm-thick layer of rock wool. This layer of insulation material improved the signal to noise ratio (SNR) by minimizing the conduction of substrate born noise to the tanks. Experimental aquaria were divided in three compartments of equal sizes by means of opaque removable partitions. Each compartment was provided with one half flower pot of 4.5 cm in diameter that was readily occupied by males as shelter. A single male was introduced in each compartment and left to acclimatise for a minimum of eight days before used in trials. All tested males become territorial within that period. Territorial behaviour was recognised by males spending long periods in the shelter and by covering it with sand. Experimental males were subject to the same water temperature (15–16°C), photoperiod and feeding regime as stock fish.

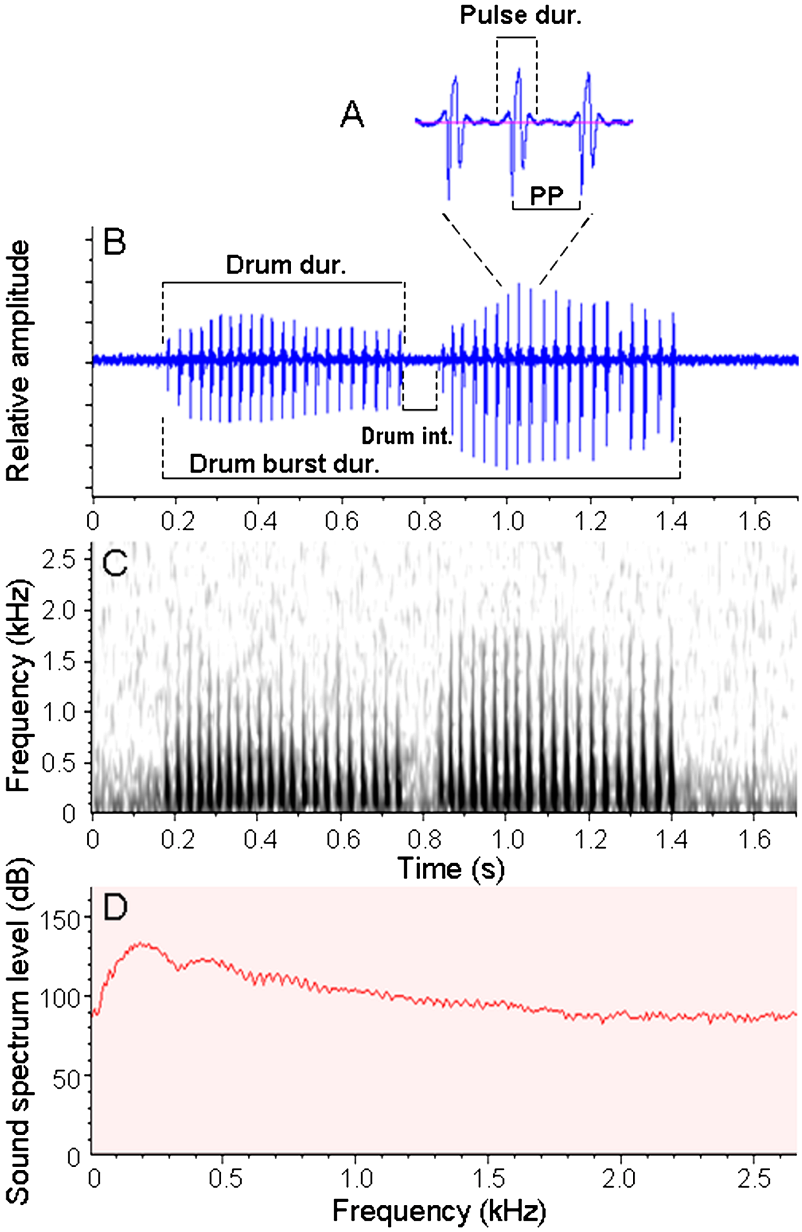
The aeration was stopped approximately 15 min prior to sound recordings. At the start of each recording session one opaque partition was removed allowing interactions between two territorial males. Recordings were carried out for 20 min. In case a fight escalation was detected the fish were immediately separated to prevent physical injury. The identity of the two interacting males was distinguished by the size, the number of spots in the dorsal fin and peculiarities of their behaviour. One to three complete recording sessions were carried out per subject male on the same or consecutive days, with the same opponent. Opponents differed at least by 15% in standard length to insure that it was always the same individual winning the context and emitting sounds (see Results). Once recordings were complete, the subject male was removed, weighed (fresh weight, W) and measured (standard length, SL), returned to a stock tank and replaced with another male. A total of 20 males with mean ± SD (range) $= 29.0 \pm 3.1$ (25–36) mm SL and 0.39 ± 0.09 (0.27–0.57) g W, were recorded.

Sounds were recorded with a High Tech 94 SSQ hydrophone (sensitivity -165 dB re 1 V/μPa, frequency response within ± 1 dB from 30 Hz to 6 kHz) placed just above the rim of the artificial shelter (4 cm above the substrate), connected to a professional digital sound recorder (Marantz PMD670) (sampling frequency of 16 kHz and 16 bit resolution). Sounds were subsequently analysed with Raven 1.2.1 for Windows (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA). Sounds suitable for analysis, i.e., with a good SNR, were typically emitted very close to the hydrophone (≈2 cm). Sounds were unequivocally attributed to a particular male because their intensity attenuated greatly with distance and were consistently associated with particular displays (see Results).

Males emitted drumming sound (drums), consisting of sequences of short low-frequency pulses, during territorial defence (Figure 1). The acoustic pulse is considered the fundamental unit of the drum sound (Lindström & Lugli, 2000). As observed in other *Pomatoschistus* (Lugli & Torricelli, 1999; Lindström & Lugli, 2000), drums were further clustered into drum bursts (i.e., a succession of drum sounds, Figure 1). Because intervals between pulses (i.e., pulse period, see below) within and between sounds were variable it was sometimes difficult to establish the end of sounds or sound bursts. We performed a log-survivor analysis on 72 pulse periods chosen randomly from 12 males, following Lindström & Lugli (2000) and Amorim & Neves (2007). The visual inspection of the log-survivor analysis (Figure 2) showed two abrupt transition points at approximately 30 ms and 90 ms that represent the minimum interval separating successive bouts of events. In other words, pulses with periods larger than ±30 ms and 90 ms were considered to belong to different sounds and different sound bursts, respectively.

We analysed drums for duration (ms), total number of pulses, pulse duration (averaged for 10 pulses, ms), pulse period (average peak to peak interval of 10 consecutive pulses within a drum, ms) and dominant frequency (frequency where the sound has most acoustic energy, Hz) (Figure 1D). Drum bursts were analysed for number of drums and drum interval (measured peak to peak between the last pulse and the first pulse of consecutive drums). Durations were measured from the onset to the end of a pulse, a drum, or a drum burst. Drum temporal features were measured from the oscillograms and dominant frequencies were measured from power spectra (filter bandwidth 10 Hz, FFT size 8192 points, time overlap 50.0% and a Hamming window).

Figure 1. Example of a sequence of two drums (drum burst) produced by a *P. pictus* male. (A) Detail of a sequence of 3 pulses that are part of the second drum. (B) Oscillogram. (C) Sonogram (filter bandwidth 124 Hz, FFT size 512 points, time overlap 50.0% and a Hamming window). (D) Power spectrum of the second drum (filter bandwidth 10 Hz, FFT size 8192 points, time overlap 50.0% and a Hamming window). Some of the sound parameters measured are depicted. Dur., duration; Int., interval; PP, pulse period.


Pulse dur.
A
PP
B
Drum dur.
Drum int.
Drum burst dur.
Relative amplitude
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
C
2.5
2.0
1.5
1.0
0.5
0
Frequency (kHz)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Time (s)
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1.5
2.0
2.5
Frequency (kHz)

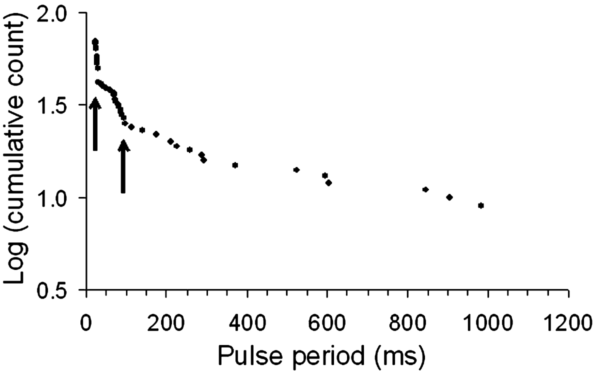

Figure 2. Log survivorship plot of time intervals (pulse period) for drum pulses produced by *P. pictus* males in agonistic contexts. The visual inspection of the plot reveals two abrupt switch points (indicated by arrows) at 30 ms and 90 ms that represent the minimum interval separating successive bouts of events; pulses with periods larger than 30 ms and 90 ms can be considered to belong to different sounds and different sound bursts, respectively.

Recording and analysis of visual behaviour

Agonistic behaviour was observed ad libitum (Martin & Bateson, 1993) in male stock tanks and during preliminary sound recordings for three months. Male behaviour during agonistic interactions was classified into the following categories:

- Frontal display (FD): the male orients his body to the opponent with erected fins. The body is raised on the pelvic and caudal fins. The chin, fins (mainly the pelvics but also the anal and caudal fins) darken. The dark spots along dorsal fins and lateral line become more conspicuous.
- Frontal display with quiver (FDQ): similar to FD, but the male quivers the pectoral fins intensively, while opening the mouth and extending the gill cover, often gasping.
- Lateral display (LD): similar to FD but the male positions his body parallel to the opponent.
- Lateral display with quiver (LDQ): Similar to FDQ, but the male positions his body parallel to the opponent. When the male opens the mouth, it directs the head towards the other male as if to bite him.
- Quiver in the nest (Qnest): the male is in the nest with his head protruding out, and adopts the same posture and coloration as in FDQ, quivering the body and the pectoral fins, exhibiting gasping movements. Often this behaviour precedes attacks.

- Dart: the male dashes rapidly towards the opponent without physical contact.
- Bite: the male bites the opponent.

During sound recording sessions, the aforementioned behavioural categories and the occurrence of sound production were registered in a laptop, using the Observer (version 4.0 for student) software. The number of times each category was performed with or without sound emissions was counted to associate sound emissions with particular behavioural categories. A total of 53 recording sessions were carried that included one to three sessions per male (mean $\pm$ SD $= 1.7 \pm 0.8$).

Statistical analysis

We tested for the independence of behaviour and sound production with χ^2-tests. Sounds that co-occurred with different agonistic displays were compared with Kruskal–Wallis tests. Post-hoc pair-wise comparisons were made with Dunn tests (Zar, 1984). In addition, we studied the existence of significant relations between behaviour frequency (number of the different agonistic behaviours observed per recording session), male traits (SL and CF) and the Julian day (number of days that have passed since the 1^{st} of January) using Pearson correlations.

We compared differences among males in drum parameters with Kruskal–Wallis tests. To assess the individual stereotypy of acoustic parameters we calculated coefficients of variation ($\text{CV} = (\text{SD}/\text{mean}) \times 100$) of drum acoustic features for each male. We used Pearson correlations to test the potential relationship between drum parameters and calling frequency (the average number of drum bursts emitted per min) with male traits: SL and condition factor ($\text{CF} = (\text{W}/\text{SL}^b) \times 1000$; b is the estimated slope from the regression between log(SL) and log(W) from the 20 males group used in the experiments and equals 1.65). Because agonistic sounds were registered throughout February and March, thus approaching the peak of the breeding season (March–July at Arrábida; V. Almada, E. Gonçalves and M. Henriques, personal communication), we also tested the hypothesis that sound parameters could be affected by the recording date, which may reflect differences in the male's physiological state. Possible relations between sound parameters and the Julian day were tested with Pearson correlation tests.

Statistical analysis was performed using Statistica 7.0 (StatSoft, 2005). We used nonparametric statistics whenever the assumptions for parametric tests were not met.

Results

Acoustic and behaviour repertoires

After the removal of the opaque partition one male readily invaded the other male's territory. The invaded male immediately exhibited a series of behaviours that included darkening parts of the body, raising the body on the pelvic fins (see methods for a description of the different displays) and emitting sounds. Acoustic signals produced during agonistic interactions were drumming sounds consisting of a sequence of low-frequency pulses ($\approx$175 Hz). Typical drums lasted 700 ms and were made up of 23 pulses repeated every 27 ms (Table 1). Drums were usually produced singly or in pairs but sequences of up to 5 drums were registered (Table 1).

During the 53 behaviour recording sessions 561 agonistic behavioural acts were registered from which only 27% were accompanied with sound emissions. The emission of drums was significantly associated with the behaviours FDQ, LDQ and Qnest (Chi-square test, $\chi^2 = 498.63$, df $= 6$, $p < 0.001$) and all other behavioural categories never co-occurred with sound production (Figure 3). Drums were longer because they presented more pulses per sound when associated with FDQ than with LDQ and Qnest (Kruskal–Wallis, $N = 147$, $H = 79.38$–81.62, $p < 0.001$; Figure 4). All other acoustic parameters did not differ between associated behaviours (Kruskal–Wallis, $N = 147$, $H = 1.60$–3.16, $p > 0.05$).

Agonistic behaviour frequency (FD, FDQ, LD, LDQ, Qnest, Dart, bite, total frequency) was neither significantly correlated with male size and condition (Pearson correlation, $N = 20$, $r = -0.27$–0.32, $p > 0.05$) nor with the Julian day (Pearson correlation, $N = 20$, $r = -0.33$–0.26, $p > 0.05$).

Acoustic variability

All measured acoustic parameters differed significantly between males, although only the pulse period showed a small intra-male coefficient of variation ($<$10%) (Table 1). Drum duration, the number of pulses in a drum and

Table 1. Drum characteristics for goby *Pomatoschistus pictus* males.

Thump parameters	N	Mean	SD	Range	$\text{Range}_{\text{abs}}$	CV	H	p value
Drum duration (ms)	20	700.3	326.6	278.6–1537.0	29.0–10664.0	93.2	126.3	<0.001
No. of pulses	20	22.9	9.1	9.8–46.6	2–344	94.3	89.0	<0.001
Pulse period (ms)	20	27.3	0.8	25.8–28.9	19.0–34.0	8.6	62.0	<0.001
Pulse duration (ms)	20	7.1	0.9	5.3–8.8	4.0–10.0	15.1	338.1	<0.001
Peak frequency (Hz)	20	175.0	14.1	150.8–198.9	97.0–328.0	19.7	93.2	<0.001
Burst duration (ms)	20	825.0	305.8	339.7–1537.0	34.0–11152.0	89.6	88.1	<0.001
No. of sounds per burst	20	1.2	0.1	1.0–1.6	1–5	35.1	48.8	<0.001
Sound interval (ms)	20	70.6	10.5	51.3–92.9	35.0–122.0	24.3	38.3	<0.01

An average of 40 sounds was considered per male (20 males). Descriptive statistics is based on male means except for absolute range values ($\text{range}_{\text{abs}}$) that concern all data ($N = 802$). H values are the results of Kruskal–Wallis tests comparing drum parameters among males. Water temperature was 15–16°C. SL, standard length; CF, condition factor (= (weight/$\text{SL}^{1.65}$) × 1000); CV, within male coefficient of variation (= (SD/mean) × 100).

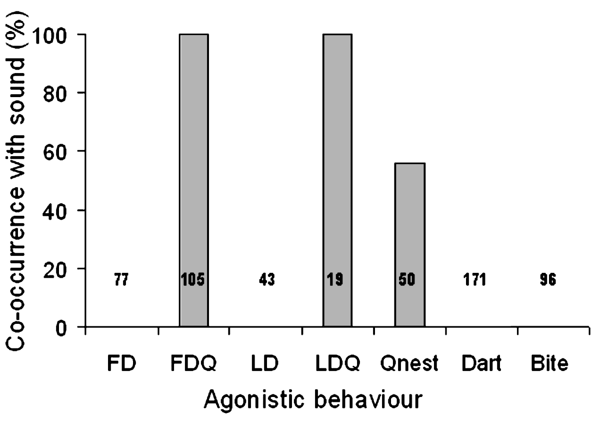

Figure 3. Percentage of agonistic acts that were accompanied by drumming (total $N = 561$). Total number of occurrences is depicted for each behaviour. Note that outside behavioural recording sessions, FDQ and LDQ were sometimes observed to be performed silently.

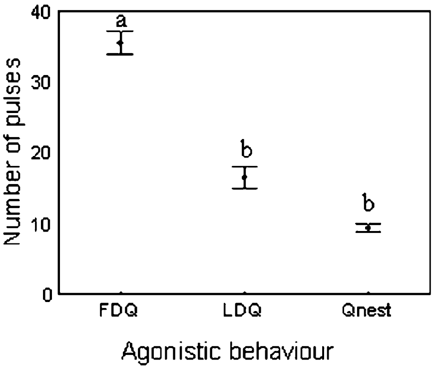

Figure 4. Variation of drum number of pulses (mean ± SE) with associated agonistic behaviour: frontal display with quiver (FDQ), lateral display with quiver (LDQ) and quiver in the nest (Qnest). Different letters indicate pair-wise significant differences (Dunn tests, $p < 0.05$). Variation of sound duration is not depicted because it is highly correlated with the number of pulses ($N = 20$, $r = 0.97$, $p < 0.05$).

burst duration were positively correlated with male length (Pearson correlation, $N = 20$, $r = 0.84$–0.88, $p < 0.001$; for all other parameters including calling frequency: $r = -0.43$–0.29, $p > 0.05$). No significant relation was found between any acoustic parameter and male condition ($N = 20$, $r = -0.22$–0.37, $p > 0.05$). All acoustic parameters, except pulse period ($N = 20$, $r = 0.18$, $p > 0.05$) and calling frequency ($N = 20$, $r = 0.27$, $p > 0.05$) were significantly affected by the Julian day. Drum duration ($N = 20$, $r = 0.61$, $p < 0.01$), the number of pulses ($N = 20$, $r = 0.58$, $p < 0.01$), drum dominant frequency ($N = 20$, $r = 0.46$, $p < 0.05$), burst duration ($N = 20$, $r = 0.54$, $p < 0.05$) and sound interval ($N = 20$, $r = 0.56$, $p < 0.05$) increased significantly whereas pulse duration ($N = 20$, $r = -0.54$, $p < 0.05$) and the number of sounds in a burst ($N = 20$, $r = -0.64$, $p < 0.01$) decreased significantly with the Julian day (Figure 5). Fish length and condition factor were not significantly correlated with the Julian day ($N = 20$, $r = 0.36$–0.39, $p > 0.05$).

Discussion

Dominant territorial males produced drumming sounds accompanying visual displays when conspecific males invaded their territories. Drums were low-frequency (≈175 Hz) pulse trains typically lasting about 700 ms reaching up

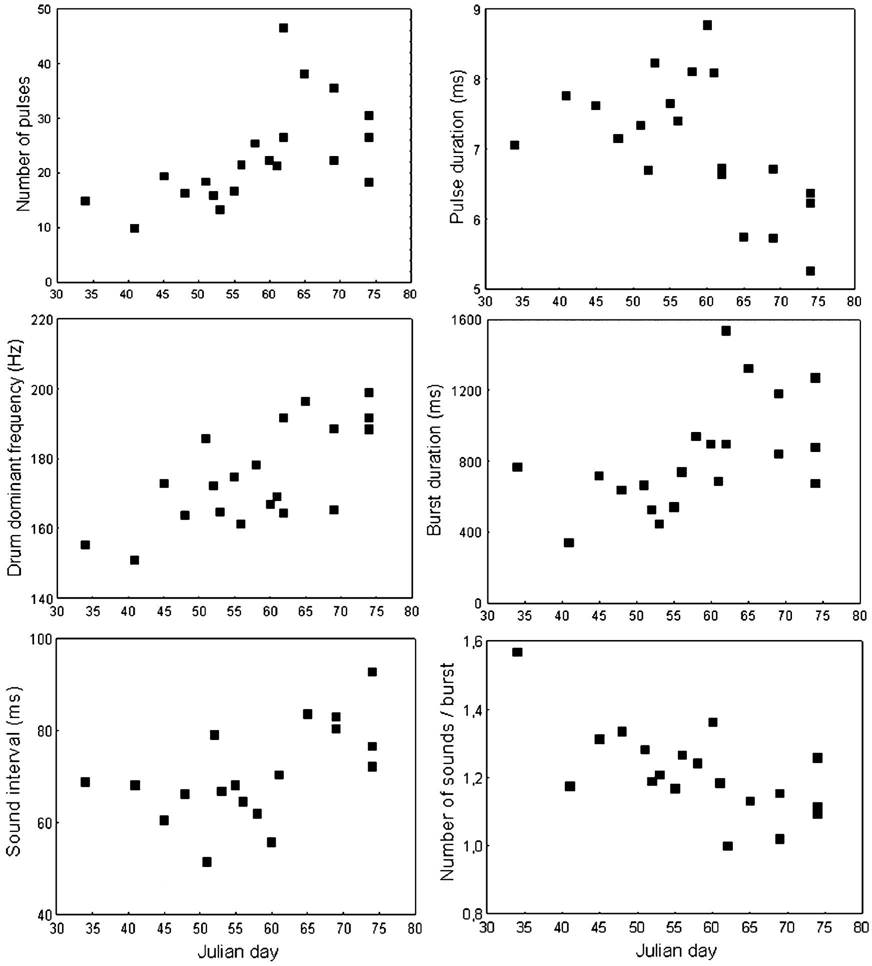

Figure 5. Relation of drum and burst acoustic parameters with Julian day. Sound duration and pulse period are not depicted.

to 10 s. Sound (drum) production has been documented for other sand gobies of the genus *Pomatoschistus* and *Knipowitschia* (Lugli et al., 1995; Lugli & Torricelli, 1999; Lindström & Lugli, 2000), including *P. pictus* (Amorim & Neves, 2007), but only in a reproductive context. Courtship drums of *P. pictus* differ from agonistic drums as they are longer, present a larger number of pulses, shorter pulse periods and have higher dominant frequencies than the latter (Amorim & Neves, 2007). This study represents the first report for the use of sounds during territorial defence in sand gobies, and one of the few reports for gobies in general. Other gobies are also known to use sound production during territorial defence such as the freshwater gobies *Padogobius martensi* (Torricelli et al., 1990) and *Odontobutis obscura* (Takemura, 1984), and the marine red-mouthed goby, *Gobius cruentatus* (Picciulin et al., 2006), suggesting that the use of acoustic signals during agonistic displays are common in gobies.

In the present study drumming was associated with exhibitions that included quivering (FDQ, LDQ and Qnest). Drums were longer and more pulsed when associated with FDQ displays, suggesting that this behaviour may be decisive in providing information on the male's fighting ability. Drumming activity in *P. pictus* was never observed during escalated agonistic behaviour such as attacks and bites. Similarly, agonistic sounds of *P. martensi* are produced during lateral displays but mostly during frontal displays that characterise the earlier exhibition phases of male–male competition for shelters. During frontal displays, *P. martensi* males direct the body towards the opponent, lift the head and enlarge the opercular region, while emitting sounds (Torricelli et al., 1990). Another example is the marmoreal goby (*Proterorhinus marmoratus*) that emit moans during agonistic visual displays that also consist of fin spreading, erecting opercular covers and darkening (Ladich & Kratochvil, 1989). Such visual displays are typically elements of earlier phases of contests when animals try to increase their apparent body size. The present study and the above examples indicate that acoustical signalling in gobies, as in other animals, are produced in the earlier phases of conflict, when animals try to assess each other's fighting ability and motivation without escalating into more costly aggressive behaviour that can incur into injury or death (Krebs & Davies, 1993; Ladich & Myrberg, 2006).

We have shown that all sound features differed significantly among painted goby males, although there was great intra-male variability for all parameters except for the pulse period. In particular, sound and burst duration and the number of pulses in a sound presented the highest CVs ($\geqslant$90%). Similarly, *Pomatoschistus minutus* produce drums during breeding behaviour with high intra-male variability in drum duration and drum pulse number (Lindström & Lugli, 2000), suggesting that these parameters are dependent of male motivation. In *P. pictus*, the pulse period was the most stereotyped acoustic feature since it presented the lowest intra-male CV ($<$10%) and it did not change significantly with either male features (including size) or the Julian day. Consistently, pulse period was also the feature that presented the lowest intra-male variability in *P. minutus* (Lindström & Lugli, 2000). Both Lindström & Lugli (2000) study and the present one suggest that the output of central pattern generators is independent of fish size and that this parameter could be a honest signal of species identity in sand gobies. Pulse period of pulsed sounds has been also proposed as species-identification cue in cichlids (Amorim et al., in press) and in Pomacentridae (Myrberg et al., 1978).

In the painted goby, drum and burst duration, and drum pulse number increased significantly with male size, and male size was unrelated to recording date (Julian day). Information about the opponents' size is extremely important to assess an individual's potential to win a contest and to be able to resolve a conflict without the need for escalated fights, and larger individuals are typically dominant and able to hold better territories (Archer, 1988). Acoustic properties of calls can provide body size information in several taxa. A classical example is given by Davies & Halliday (1978) that showed that male toads, *Bufo bufo*, settle contests for the possession of females by means of their vocalisations that give a reliable signal of body size and hence of fighting ability. In this, like in many other anurans, the dominant frequency of sounds in inversely related with body size. Temporal characteristics of calls can also give information on body size such as in the field cricket, *Gryllus bimaculatus*. In this species syllable rate within chirps decreases whereas the duration of syllables increase in larger males (Simmons & Zuk, 1992). In fish, size information can be encoded in acoustic cues such as sound pressure level and dominant frequency (e.g., Ladich et al., 1992; Lindström & Lugli, 2000; De Jong et al., 2007). In several fish acoustic signals proved effective in keeping out intruders from the signaller's territory (Myrberg, 1997) and in winning fights, especially when size asymmetries are small (Valinsky & Rigley, 1981; Ladich et al., 1992, 1998). In the above studies, both sound pressure level and dominant frequency seemed to be the relevant features in winning contests. In the present study, dominant frequency was not related with male size and we did not measure sound pressure level. However, an increase of drum amplitude with male size was found in the congeneric species, *P. minutus*, suggesting that this relation might also be present in the studied species. In fish, temporal parameters are also known to change with fish size. Consistent with our results, both the grey gurnard (*Eutrigla gurnardus*) and the croaking gourami (*Trichopsis vittata*) show an increase of sound duration and pulse number in larger animals (Henglmüller & Ladich, 1999; Wysocki & Ladich, 2001; Amorim & Hawkins, 2005). Such temporal parameters are not as reliable as sound pressure level and dominant frequency to provide information on body size since they can be affected by the signaller's motivation. However, the outcome of disputes over limited resources depend not only on the contestant's size but also on their motivation (Enquist, 1985), making these important parameters for mutual assessment. All drum acoustic parameters, except for the pulse period, were also affected by the Julian day in *P. pictus*. Sound emissions become longer (i.e., sound duration, number of pulses, burst duration and sound interval) with the approach of the peak of the breeding season, consistently suggesting that calling duration parameters are dependent on male's motivation, which can be affected the male's physiological state. Drum dominant frequency also increased with the recording date, i.e., with the approach of the peak of the mating season. These changes of acoustic characteristics might be related with circulating androgen levels that are known to produce changes in the sound-producing apparatus of fishes and frogs (e.g., Connaughton et al., 2002; Girgenrath & Marsh, 2003). Although the sound generating mechanisms are not known in gobies, in fish with specialised sonic muscles androgens can cause changes in the sonic muscle mass and characteristics such as sonic muscle hypertrophy caused by an increase in fibre cross-sectional area, as well as an increase in the contractile cylinder and in the peripheral sarcoplasm (Connaughton et al., 2002).

In summary, painted goby males drum during the exhibition phase of disputes over territories when males try to gain information about the opponent's resource holding potential. The present results suggest that calling duration (drum and burst duration) can give information about male size and perhaps motivation. Acoustic signals together with the visual information given by lateral, frontal and nest displays may provide critical information for the solving of territorial disputes. Our results add to a growing body of literature on the use of acoustic signals during agonistic interactions by teleosts fish.

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