Differential investment in acoustic communication during social interactions in two closely related sand goby species

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Abstract
Communication signals provide key information for conspecific recognition, mate choice and rival assessment. The painted goby *Pomatoschistus pictus* and the common goby *P. microps* are two closely-related sand goby species, often sympatric and with an overlapping breeding season. In this study we staged male–male and male–female interactions and compared visual, tactile and acoustic behaviour in both species. Sound production in the common goby is here accounted for the first time. We observed some differences in visual behaviour and a striking divergence in the use of tactile and acoustic communication during courtship and agonistic interactions. We further describe differences in drumming signals with social context in the painted goby. This study suggests a divergence in communication in two closely-related sand goby species and emphasizes the importance of further research concerning the role of multimodal communication in closely related species.

1. Introduction

Male advertisement signals can be used by females during conspecific recognition and mate choice, or during the earliest steps of mutual assessment in male agonistic interactions (Bradbury & Vehrencamp, 1998). In addition, animals may exploit multiple sensory channels during social interactions such as visual, acoustic or chemical (Moyle & Cech, 2004).

Divergence in communication during courtship and male–male competition can play an important role in sexual isolation of closely related species (Gerhardt, 1988; Seehausen & Schluter, 2004). Different signal types used in mate choice, including visual and acoustic, have been shown to have a key role in speciation (Gerhardt & Huber, 2002; Hugall & Stuart-Fox, 2012). On the other hand male–male competition has also been proposed to promote signal (colour) diversification in closely related fish species and thereby to set the stage for speciation (Seehausen & Schluter, 2004). Examining the patterns of signal variation among related species may, thus, be useful for assessing the evolutionary history of communication (Brooks & McLennan, 1991; Martins, 1996). In particular, the way multimodal or multicomponent signals (sensu Candolin, 2003) diverge between closely related species and drive evolution is far from being understood.

Fish often rely on visual communication to provide information during intra-sexual contests and reproductive interactions (Bradbury & Vehrencamp, 1998). In this taxon acoustic communication also plays an important role during territorial defence (Myrberg, 1997; Ladich & Myrberg, 2006), mate attraction and mate choice (Myrberg et al., 1986; Myrberg & Lugli, 2006). Although the use of visual and acoustic signals have been described in many different fish species, including closely related species (Boughman, 2001; Myrberg & Lugli, 2006; Amorim et al., 2008; Malavasi et al., 2008; Parmentier et al., 2009; Verzijden et al., 2010), few studies have provided empirical tests of the role of visual (e.g., Shashar et al., 2005) and acoustic (Myrberg et al., 1978) signals in species recognition, and few systematic comparisons of social signals in closely-related species are available in the literature (e.g., Lobel, 1998; Amorim et al., 2008; Malavasi et al., 2008; Verzijden et al., 2010).

Among vocal fish, gobies (Gobiidae) are one of the most studied families that produce both visual and acoustic signals during mating and territorial defence (Myrberg & Lugli, 2006; Amorim & Neves, 2008). Four species of sand gobies (genus *Pomatoschistus*) have been documented to be both vocal

and to make conspicuous visual displays during social interactions (Lugli et al., 1995; Lugli & Torricelli, 1999; Lindström & Lugli, 2000; Amorim & Neves, 2007, 2008; Malavasi et al., 2008, 2009). Further, in at least one sand goby species (*P. minutus*) male visual courtship displays and breeding colouration are well documented mating choice criteria (e.g., Svensson & Kvarnemo, 2005). Sand goby species are very similar morphologically (Kovacic, 2008) and frequently live in sympatry (Miller, 1986), thus offering a very good opportunity to test the role of both visual and acoustic signals in pre-zygotic reproductive isolation in closely related sympatric fish species.

In this study we compared courtship and agonistic behaviour in two closely-related sand goby species, the painted goby *Pomatoschistus pictus* and the common goby *Pomatoschistus microps*. These species belong to a monophyletic group, i.e., the sand goby group, as demonstrated by molecular data (Huyse et al., 2004) as well as from behavioural and life history characters (Malavasi et al., 2012; also see Malavasi et al., 2008). Moreover they are often sympatric, sharing the same habitat including along the Portuguese coast (Edlund et al., 1980; Miller, 1986; Cunha & Antunes, 2008) and they present an overlapping breeding season (Miller, 1986). Considering their genetic affinity and their similar environmental use, we predicted that divergence in particular behavioural traits such as in visual or acoustic signalling may have contributed to reproductive isolation and ultimately to speciation. We staged male–male and male–female interactions and compared visual and acoustic behaviours observed in different social contexts between species. While painted gobies have been previously shown to communicate acoustically during courtship and territorial defence (Amorim & Neves, 2007, 2008), sound production in the common goby is here accounted for the first time. A detailed description of the full reproductive behaviour and of courtship drums made by the painted goby together with a comparison of drum acoustic features among social contexts is also provided here for the first time.

2. Material and methods

2.1. Study species

The painted goby *P. pictus* and the common goby *P. microps* are short lived (up to 1–2 years), exclusively coastal benthic species inhabiting shallow gravel and sand substrate areas (Miller, 1986). Like other sand gobies,
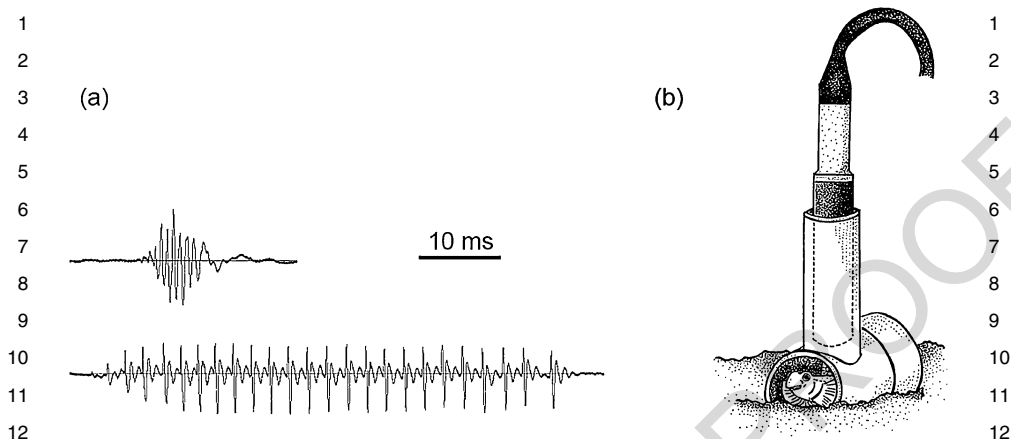
Figure 1. Painted goby courtship sounds and detail of experimental nest. (a) Waveform of a drum sound (low frequency pulsed sound) and a thump sound (low frequency non-pulsed sound). (b) Hydrophone position inside the nest chimney in order to minimize the distance between the recording device and the vocal resident male.

painted goby and common goby males are polygamous species which show high reproductive effort (Rogers, 1988). During the breeding season males build nests under empty bivalve shells, covering them with sediments leaving only one opening (Bouchereau et al., 2003). Nest owners actively defend their nests from other male intruders (Amorim & Neves, 2008). Females enter the nest attracted by male courtship displays and lay their eggs in a single layer on the nest ceiling. After spawning females leave the nest and males provide parental care, i.e., nest defence from intruders and egg fanning until hatching, which usually takes up to 3 weeks (Miller, 1986; Bouchereau et al., 2003). In a courtship context painted gobies make drumming sounds (low frequency pulsed sounds; Figure 1a) during quivering outside the nest and thumps (low frequency non-pulsed sounds; Figure 1a) mostly during displays in the nest (Amorim & Neves, 2007). When defending the breeding territory from other male intruders they emit only drums while quivering outside or inside the nest (Amorim & Neves, 2008).

2.2. Fish collection and maintenance

Fish were caught in shallow water using hand nets at Parede (38°41′N, 9°21′W) and Lagoa de Albufeira (38°30′N, 9°10′W), Portugal, during the breeding season (January to June 2010).

Animals were housed according to species and gender in separate stock tanks (circa 18 l) provided with artificial seawater, internal power filters, sand substrate and shelters, at circa 18°C, with a natural photoperiod. They were daily fed with chopped mussel ad libitum.

2.3. *Mating and territorial intrusion trials*

We tested both the painted goby and the common goby in agonistic and courtship trials. We placed experimental aquaria (circa 35 l) on top of two marble layers interspaced with two levels of rubber foam shock absorbers which significantly minimized the conduction of room floor born noise to the tank. We divided each aquarium in three compartments by means of two opaque removable partitions. All resident males were provided with a nest. In the agonistic trials the resident male was allowed to interact with an intruder male, which was housed in the middle compartment. The intruder was not given a nest and was smaller than the resident to ensure that the resident (subject) male was the winner and, thus, the sound producer (Amorim & Neves, 2008). This size difference also allowed distinguishing the resident from the intruder during trials and video analyses. In the courtship trials the resident male was allowed to interact with two ripe females, which were also housed in the middle compartment. All fish, i.e., resident and intruder males and the females were left to acclimatise in the experimental aquaria for a minimum of 24 h before trials. We stopped aeration approximately 15 min prior of each trial that started by removing one partition. In agonistic trials the intruder was gently pushed towards the resident's compartment where the encounters took place. In courtship trials interactions took place in both compartments. Each trial lasted 20 min.

We used three hydrophones to record any sounds produced by the fish. Two High Tech 94 SSQ hydrophones (High Tech, Gulfport, MS, USA; sensitivity -165 dB re. 1 V/μPa; frequency response within ± 1 dB from 30 Hz to 6 kHz) were placed circa 4 cm above the substrate, one in front of the nest and the other close to the partition in agonistic trials or, in courtship trials, in the middle of each of the two compartments. The DC component of these hydrophones was decoupled by a high-pass filter. We placed the other hydrophone (Brüel & Kjær 8104, Brüel & Kjær, Naerum, Denmark; sensitivity -205 dB re 1 V/μPa; frequency response from 0.1 Hz to 180 kHz) inside a nest chimney (Figure 1b), minimizing the distance to the sound-producing male when he was inside the nest, and conditioned the audio signal with a

sound level meter (Brüel & Kjær 2238 Mediator Sound Level Meter; Brüel & Kjær). All hydrophone signals were digitized with an A/D converter device (M-Audio Fast Track Ultra 8R, M-Audio, Irwindale, CA, USA; 16 bit, 44.1 kHz acquisition rate per channel) controlled by Adobe Audition 3.0 (Adobe Systems, Mountain View, CA, USA), allowing simultaneous multi-channel recordings by a laptop.

We video-recorded all the social interaction experiments with an external camera (Sony handycam DCR-HC39, Sony, Tokyo, Japan) placed 50 cm in front of the experimental aquarium. The entire region of the experimental aquarium in which the interactions took place was framed. The camera output was digitized to a laptop with Pinnacle Dazzle DVD Recorder Plus (Pinnacle Systems, Mountain View, CA, USA).

2.4. Acoustic and visual behaviour analysis

We carried out focal observations of the resident male with a continuous sampling method (Lehner, 1996). We randomly chose six videos with high-quality image per social context of each species (one video per male, 24 videos in total). The videos were synchronized with the corresponding sound recordings to associate sounds with visual behaviour. Based on previous work (Amorim & Neves, 2007, 2008) and ad hoc preliminary observations of the resident male (circa 25 h) made at the beginning of the study the following agonistic behaviours were scored with Etholog 2.2.5 software (Ottoni, 2000): assessment phase — quiver inside or outside the nest, frontal display, frontal display with quiver, lateral display, lateral display with quiver and approach; escalation phase — dart, bite and chase (see Amorim & Neves, 2008 for detailed descriptions). Visual courtship made outside the nest included quiver out of the nest, eight display, approaching the female, lead, and tactile courtship behaviour that consisted in the male nudging the female flank (Amorim & Neves, 2007). We tallied as a nest-related behaviour the periods when the female was not detectable while the resident male lied on the bottom inside the nest with his head outside, often quivering his body ('male and female in the nest'). We further considered other nest-related behaviours including 'rest in the nest' (male lies inside the nest, with its head outside), 'rest close to the nest' (male rests within 5 cm from the nest with spread fins) and 'nest display' (male is in the nest with his head protruding out, quivering the body and the pectoral fins and exhibiting gasping movements). These nest-related behaviours were scored in both agonistic and reproductive interactions. We considered the same behavioural categories for both species as

they share similar behavioural elements. This option also allowed to carry out statistical comparisons.

We aurally and visually inspected all acoustic recordings (circa 32 h) using Adobe Audition 3.0 to assess the number of subject males of each species that emitted sounds. Drums were analyzed using Raven 1.2.1 for Windows (Bioacoustic Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA) for: duration (DUR, ms), measured from the start of the first pulse to end of the last pulse in a sound; number of pulses (NP): total number of pulses in a sound; pulse period (PP, ms): mean interval between the peaks of consecutive pulses in a sound; peak frequency (Hz): frequency where the sound had more acoustic energy. The drum rate (number of sounds per minute) was also calculated.

We tested 6 painted goby males for courtship (mean = 1.5 trials per fish; range: 1–2) and 11 males for agonistic interactions (mean = 3.2 trials per fish; range: 1–11). From these 11 males tested in agonistic context 6 were the same males tested in the courtship context. We registered sounds from 5 painted goby males in the courtship context with mean ± SD (range) = 38.6 ± 0.9 (38–40) mm standard length (SL) and 0.84 ± 0.08 (0.79–0.97) g weight (W); the non-vocal male was 37 mm in SL and 0.65 g in W. We registered agonistic sounds in 6 painted goby males with 35.5 ± 3.21 (31–40) mm SL and 0.64 ± 0.20 (0.49–0.97) g W, from which only two were also vocal during courtship. Painted goby males that did not vocalize during agonistic encounters had 44.6 ± 7.6 (33–52) mm SL and 0.80 ± 0.27 (0.54–1.14) g W and hence overlapped in length and weight with males that vocalized. We analysed a mean of 30.8 ± 16.3 (10–45) courtship drums per male and a mean of 45 ± 30.0 (9–10) agonistic drums per male.

We tested 15 common goby males in the courtship context (mean = 2.3 trials per fish; range: 1–7) with 32.6 ± 0.7 (32–34) mm SL and 0.51 ± 0.03 (0.34–0.54) g W and another set of 8 different males in the agonistic context (mean = 2.0 trials per fish; range: 1–2) with 32.3 ± 1.4 (30–34) mm SL and 0.51 ± 0.03 (0.27–0.54) g W. We only registered (and analysed) 3 sounds from one common goby male with 33 mm SL and 0.48 g W.

2.5. *Statistical analysis*

We compared the duration and the frequency of behaviours between species with Mann–Whitney U-tests. For male–female interactions we compared the males' total duration of nest related behaviour and visual courtship outside

the nest ($N = 6$ males for each species). We further compared the frequency of all courtship behaviours between species (see above). For male–male interactions we compared for each male the total durations of the assessment display phase and the escalated phase ($N = 6$ males for each species).

The association between behaviours and the sound emission during courtship was investigated in the painted goby with a Chi-square test of independence. Painted goby agonistic and courtship drum features were also compared using Mann–Whitney U-tests using mean values of each acoustic parameter per fish.

Statistical analyses were run using STATISTICA Software (version 10, Statsoft, Tulsa, OK, USA).

3. Results
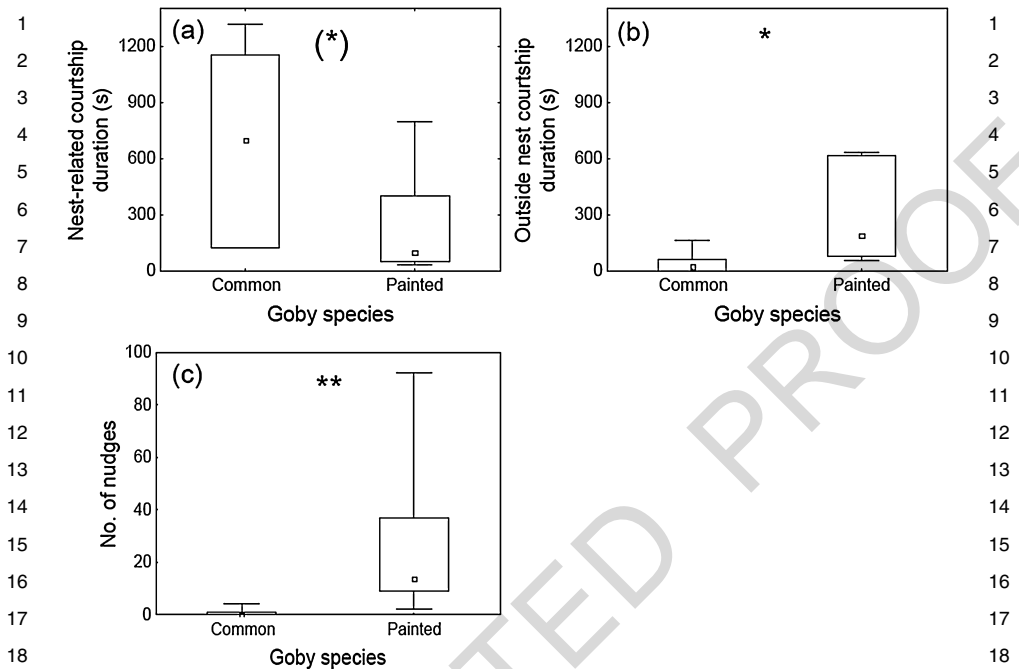
3.1. Courtship interactions

Males of both species exhibited similar visual behaviour when courting the females. Males made a series of jerky jumps towards the females and tried to lure them into the nest by performing straight swimming movements or conspicuous 'dances' (such as the eight display) to lead them to the nest entrance. Males also made quiver displays both outside and inside the nest. We found however some differences between the species. One typical courtship display consisted in staying in the nest with the head outside while quivering (nest display). Although painted gobies performed this display when the female was either outside or inside the nest, common gobies only showed this behaviour with the female outside the nest.

Common goby resident males performed nest related behaviours (rest in the nest, rest close to the nest and nest display) for marginally non-significant longer periods than painted goby males (Mann–Whitney test: $N_{common} = 6$, $N_{painted} = 6$, $U = 6.0$; $p = 0.07$; Figure 2a). In contrast, the painted gobies performed visual courtship outside the nest (quiver out of the nest, eight display, approach and lead) for significantly longer periods than the common goby ($U = 4.0$; $p < 0.05$; Figure 2b). Tactile courtship (nudge) was carried out significantly more often by painted than by common goby males ($U = 1.0$; $p < 0.01$; Figure 2c). Inter-specific differences for the frequency of all other behaviours were not significant ($U = 7.0–17.0$, $p > 0.05$).

The number of specimens that emitted drumming sounds during courtship differed markedly between the studied species. From the 15 tested common

Figure 2. Comparison of behaviours observed during courtship interactions by the common and the painted gobies. (a) Nest-related visual courtship behaviours were longer in common than in painted goby males, whereas (b) the reverse was observed for the duration of visual courtship performed outside the nest and (c) for the frequency of tactile courtship. Asterisks denote significant and marginal non-significant differences (Mann–Whitney U-tests, $^{**}p < 0.01$, $^{*}p < 0.05$; $(^{*})p < 0.1$). Medians, 25% and 75% percentiles and range are depicted.

goby males only one emitted sounds (6.7%). In contrast, five out of the six tested painted goby males produced drumming sounds (83.3%). Common gobies were never heard to make thumping sounds whereas this was a common acoustic signal in the painted goby: four of the five vocal males emitted thumps during courtship. This discrepancy in acoustic signalling is even more striking when considering that more courtship trials were attempted per male in the common than in the painted goby (mean 2.3 vs 1.5 trials per male, respectively).

The vocal common goby male emitted drums only when both the male and the female were inside the nest while painted goby males emitted drums also when they were alone in the nest. A chi-square test revealed that in the latter species sound production was significantly associated with these behaviours ($\chi^2 = 451.2$, df = 7, $p < 0.001$; Figure 3).
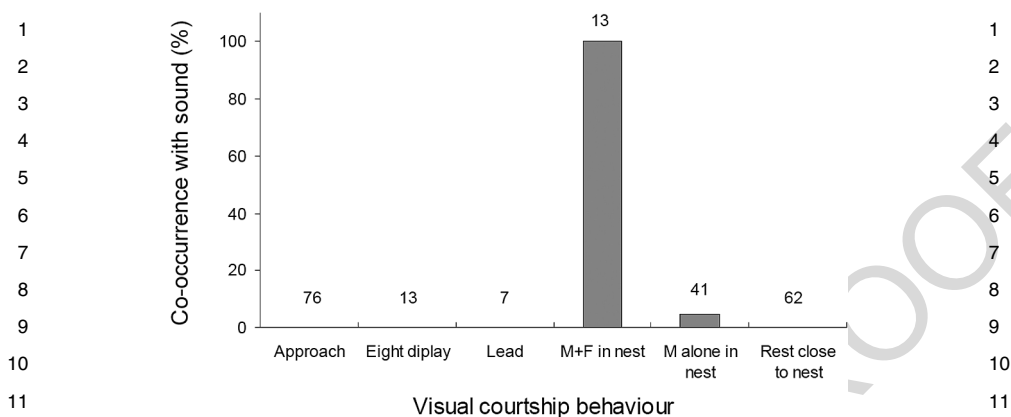
Figure 3. Percentage of visual courtship displays that were accompanied by the emission of drums in painted gobies. Total number of visual behaviour occurrences is depicted on top of the histogram bars. M, male; F, female. Male alone in nest includes both rest in the nest and nest display.

3.2. Agonistic interactions

Agonistic interactions were quite similar in the two species: males from both species started interactions with lateral and frontal displays accompanied with a body quiver; they also displayed in the nest with their head out, extending the fins, darkening the chin and quivering. Fights did not differ in duration between species either in the assessment display phase (Mann–Whitney test: $N_{common} = 6$, $N_{painted} = 6$, $U = 18.0$, $p > 0.05$) or in the escalation phase ($U = 9.0$, $p > 0.18$). In contrast, differences in acoustic behaviour were found: the common goby did not vocalize during the display phase (8 males) whereas painted goby males often did (6 out of 11 males produced sounds). Sounds were made mainly during nest displays as previously described in Amorim & Neves (2008).

3.3. Variability in acoustic signals

Agonistic drums made by the painted goby were longer than courtship drums (Mann–Whitney test: $N_{court.} = 5$, $N_{Agon.} = 6$, $U = 3.0$, $p < 0.05$; Figure 4a, Table 1) and showed a tendency to have more pulses ($U = 5.0$, $p = 0.08$; Figure 4b); the agonistic drums did not differ from courtship drums in pulse period or dominant frequency ($U = 8.0$–12.0, $p > 0.05$; Figure 4c and d). We found a tendency for drums to be produced at a higher rate (Mann–Whitney test: $N_{court.} = 4$, $N_{Agon.} = 6$, $U = 4.0$, $p = 0.06$; Figure 4e) but with
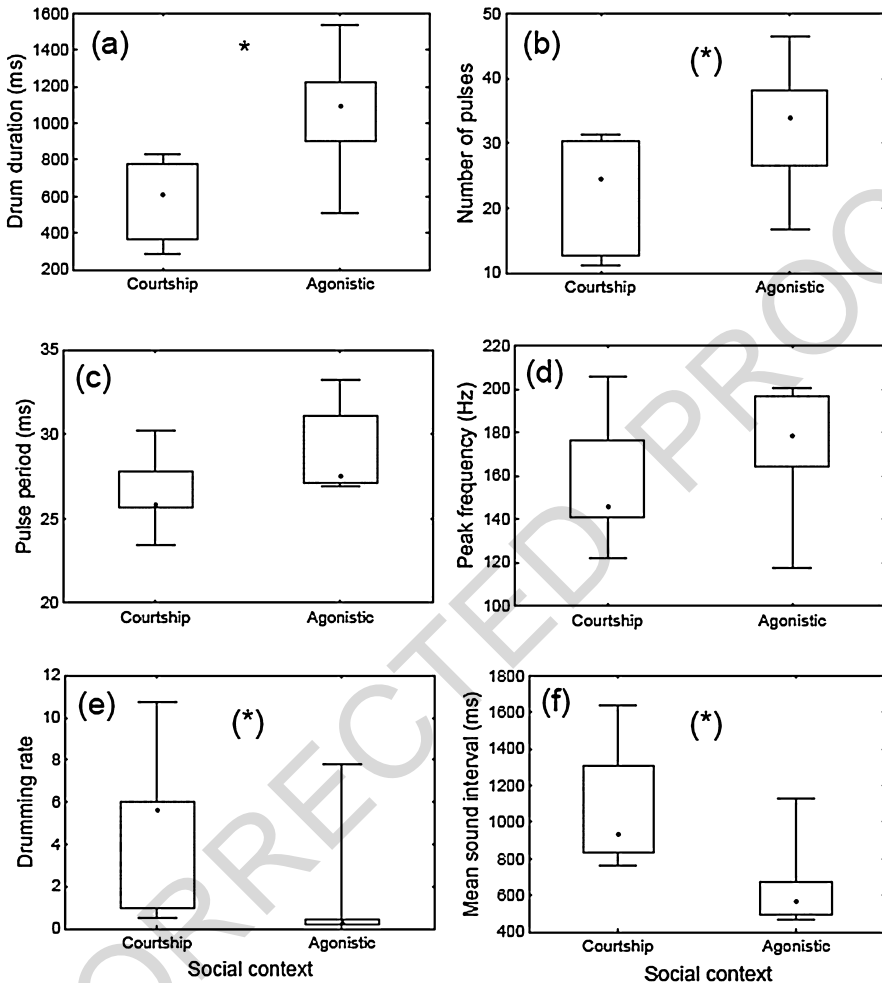
Figure 4. Comparison of drums made during courtship and agonistic contexts. (a) Drum duration, (b) number of pulses, (c) pulse period, (d) peak frequency, (e) drumming rate (drums/min), (f) mean interval between sounds. Asterisks denote significant and marginal non-significant differences (Mann–Whitney U-tests, $^*p < 0.05$; $(^*)p < 0.1$). Medians, 25% and 75% percentiles and range are depicted.

longer intervals between sounds (only intervals up to 3 s were considered; $U = 3.0$, $p = 0.07$; Figure 4f) in the courtship than in the agonistic context. The males that made sounds during courtship and agonistic contexts did not differ in standard length (Mann–Whitney test: $N_{court.} = 5$, $N_{Agon.} = 6$, $U = 6.0$, $p > 0.05$).

Table 1.

Acoustic features and rate of drums produced during courtship and agonistic contexts by *Pomatoschistus pictus* males.

Acoustic parameter	Courtship				Agonistic			
	Mean	SD	Range	Range$_{abs}$	Mean	SD	Range	Range$_{abs}$
Drum duration (ms)	575.2	242.9	286.3–835.1	84.0–1920.0	1062.0	347.0	510.6–1537.0	68.0–7560.0
No. of pulses	22.0	9.6	11.2–31.3	4–86	32.7	10.2	16.7–46.6	4–252
Pulse period (ms)	26.6	2.6	23.4–30.2	17.8–38.0	28.9	2.6	26.9–33.2	22.2–44.1
Peak frequency (Hz)	158.1	33.1	121.7–206.0	88.1–352.3	172.5	31.3	117.2–200.5	93.8–280
Drum rate (drums/min)	4.8	4.2	0.5–10.7	–	1.5	3.1	0.2–7.8	

Descriptive statistics are based on male means, except for absolute range values (Range$_{abs}$) that concern all data (courtship: 154 drums from 5 males; agonistic: 273 drums from 6 males) and for drumming rate that only considers one observation per male.

The one common goby that made drumming sounds ($N = 3$) produced drums that ranged in duration from 320–588 ms, had 11–18 pulses, pulse periods of 31–33 ms and peak frequency of circa 180 Hz.

4. Discussion

4.1. Courtship interactions

The courtship repertoire observed in the two studied goby species is generally consistent with the one found in other species belonging to the sand gobies group, which involves fin displays, jerky swimming towards and around the females and lead swims towards the nest (Kangas & Lindström, 2001). However, while the painted goby is more active in terms of visual and tactile (nudge) courtship performed outside the nest, the courtship in the common goby is restricted to nest related behaviours. Jones & Reynolds (1999) have shown that in the common goby nest coverage with sand increases male attractiveness as females spawned significantly more often in well-covered nests with smaller openings. Only in one of the 35 courtship trials we performed with common goby males a spawning occurred, and in that encounter the nest was completely covered with sand. We suggest that a well built nest may represent an important mate cue in the common goby, while painted goby males appear to invest more in other cues, such as visual and tactile mating signals.

We also found striking differences in terms of acoustic activity with the painted gobies being a lot more vocal than the common gobies. In addition to vocalizing more frequently, the painted gobies also emitted two types of courtship sounds in contrast with only one observed for the common goby. Although we have only observed one spawning event in the common goby we believe that males were sexually motivated as they even showed higher durations of nest related courtship behaviour than the painted gobies (Figure 2a). Nevertheless, we cannot rule out the possibility that acoustic activity of the males depends on the receptive state and motivation of females to mate and might have increased had more spawning events occurred.

We show for the first time that common gobies can vocalise during courtship. The common goby emitted drums when both male and female were inside the nest, consistent with other *Pomatoschistus* spp. (Lugli et al., 1995; Lugli & Torricelli, 1999; Lindström & Lugli, 2000). We observed very few occurrences of sound production in this species suggesting that drums

1   might be mostly produced in pre-spawning context (Lugli et al., 1995). On
2   the contrary, the painted gobies emitted drumming sounds (and thumps)
3   mostly in association with nest display behaviour while females were ei-
4   ther outside or already inside the nest. This is consistent with their use
5   as courtship and pre-spawning sounds and possibly as spawning acoustic
6   signals. Amorim & Neves (2007) suggested that drumming sounds could
7   signal a high motivation for mating. Our results for the painted goby sup-
8   port this idea since, with only one exception, spawning was observed in
9   all trials where drumming production occurred. Similarly, sounds play an
10   important role in the last stage of courtship of other fish species. For ex-
11   ample, in Mozambique tilapia (*Oreochromis mossambicus*; Amorim et al.,
12   2003), in cod (*Gadus morhua*; Rowe & Hutchings, 2006) and in the had-
13   dock (*Melanogrammus aeglefinus*; Hawkins & Amorim, 2000) sounds have
14   been proposed to advertise the spawning readiness of males and probably
15   synchronize gamete release. Also, in some shallow water vocal species, such
16   as the weakfish (*Cynoscion regalis*) and the red drum (*Sciaenops ocellatus*),
17   repetitive courtship sounds have been demonstrated to attract females to a
18   lek formation and facilitate spawning synchronization (Gilmore, 2002). The
19   same is true for other taxa. For example in birds song is known to stimulate
20   females into reproductive condition and to synchronise breeding (Catchpole
21   & Slater, 1995).

4.2. Agonistic interactions

24   The two studied species had similar agonistic behaviour with comparable
25   levels of aggressiveness during nest defence. However, the common and
26   the painted goby resident males appear to invest in different communication
27   channels. Indeed, the painted goby frequently used acoustic communication
28   during agonistic interactions while the common goby did not. Painted goby's
29   agonistic drums were produced at the beginning of agonistic interactions,
30   mostly in association with frontal, lateral and nest displays. Agonistic inter-
31   actions can be costly both for winners and losers of a conflict, and should be
32   of mutual benefit to avoid escalating aggressions (Hurd, 1997; Maghagen,
33   2006). Evaluating the opponent by signalling represents an economic way
34   to solve disputes which otherwise would be decided at much higher costs
35   (injury or death). Numerous species of fishes emit sounds in early phases of
36   agonistic interactions and it has been demonstrated that acoustic signals can
37   be key to the fight outcome in some fish species (Ladich & Myberg, 2006).

For example, in *Halobatrachus didactylus* (boatwhistles) sounds function as active 'keep-out' signals during territorial defence (Vasconcelos et al., 2010) and in the cichlid fish *Metriaclima zebra* the association between visual and acoustic signals lower the level of aggressiveness between opponents (Bertucci et al., 2010). In the painted goby, sound duration (drum and 'drum sequence' duration) has been suggested to give information about male size and perhaps motivation (Amorim & Neves, 2008). If so, painted goby's agonistic drums may convey information about opponent's resource holding potential which can be used in addition to visual behaviour, to solve conflicts without the need to escalate fights. Future studies with playback experiments will need to investigate this hypothesis.

4.3. *Variability in acoustic signals*

Although we could not compare acoustic signals between the two goby species since the common gobies emitted very few drums, we explored differences in drumming features of the painted goby according to social context, which can provide information on the sender's motivation. Agonistic drums were longer and tended to have more pulses than courtship drums. Also, there was a tendency for males to be more vocal when courting than when defending the nest but drums tended to be emitted more spaced apart during reproductive events. These changes in temporal patterning indicate that drumming emission can be modulated to express sexual and agonistic motivation. Variability of acoustic parameters has been associated with different levels of motivation in other vocal fish species (Amorim, 2006). For example, in *Pseudotropheus zebra* male agonistic sounds are also longer and have a slower pulse rate than courtship sounds (Simões et al., 2008). Male motivation and quality may be advertised by differential calling rates, sound duration and pulse repetition rate in various taxa. These features may be evaluated by females during mate choice or by conspecific males during agonistic encounters. For example, in the gray tree frog *Hyla versicolor*, females are more attracted to longer male calls with a higher pulse number than to shorter calls (Gerhardt et al., 2000). Temporal characteristics of calls can also give information on resource holding potential. In the field cricket, *Gryllus bimaculatus* syllable rate within chirps decreases whereas the duration of syllables increase in larger males (Simmons & Zuk, 1992).

4.4. Implications of inter-specific differences

We have shown that these two closely related goby species exhibit small differences in visual behaviour but a striking divergence in the use of the tactile and mainly of the acoustic communication channels during courtship and agonistic interactions. Drumming during pre-spawning and spawning phases of reproductive activities has been reported in seven species of sand gobies belonging to the genera *Pomatoschistus* and *Knipowitschia* (Lugli et al., 1995; Lugli & Torricelli, 1999; Lindström & Lugli, 2000; Amorim & Neves, 2007; Malavasi et al., 2008; present study). Apart from mate choice (Lugli & Torricelli, 1999; Lindström & Lugli, 2000) drums could also be used in species recognition (Lugli & Torricelli, 1999). Sand gobies belong to a monophyletic group (Huyse et al., 2004; Malavasi et al., 2008, 2012) and they often live in sympatry in coastal zones or in freshwater (Miller, 1986; Cunha & Antunes, 2008) suggesting that possible inter-specific differences in breeding signals, including visual but mainly acoustic ones (e.g., Lugli & Torricelli, 1999), could potentially be used in species-specific recognition. Nudging behaviour, which differed between the painted and the common goby, could not only be used to stimulate the female but also to increase chances for chemical communication. Immediately before and during spawning, many teleost fishes respond to the conspecific odour by increasing gonadal development and/or with hormonal changes that induce final gamete maturation (Moore & Waring, 1996; Kobayashi et al., 2002; Stacey & Sorensen, 2002). Reproductively mature males of round gobies (*Neogobius melanostomus*) release a chemical cue that strongly enhances the behavioural response in reproductively mature females, facilitating mating (Gammon et. al., 2005). Further research is required to investigate the importance of the chemical communication channel and its function in intraspecific recognition in the painted and in the common gobies.

In conclusion, this study suggests a divergence in the use of different channels of communication in two closely-related sand goby species that could influence pre-zygotic reproductive isolation, and invites for broader comparative research concerning the role of multimodal communication and of acoustic signals in particular in species recognition and social interactions.

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