RESEARCH ARTICLE

Mate preference in the painted goby: the influence of visual and acoustic courtship signals

M. Clara P. Amorim[1,*], Ana Nunes da Ponte[2], Manuel Caiano[2], Silvia S. Pedroso[1], Ricardo Pereira[2] and Paulo J. Fonseca[2]

[1]Unidade de Investigação em Eco-Etologia, Instituto Superior de Psicologia Aplicada – Instituto Universitário, Rua Jardim do Tabaco 34, 1149-041 Lisboa, Portugal and [2]Departamento de Biologia Animal and Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Bloco C2, Campo Grande, 1749-016 Lisboa, Portugal

*Author for correspondence (amorim@ispa.pt)

SUMMARY

We tested the hypothesis that females of a small vocal marine fish with exclusive paternal care, the painted goby, prefer high parental-quality mates such as large or high-condition males. We tested the effect of male body size and male visual and acoustic courtship behaviour (playback experiments) on female mating preferences by measuring time spent near one of a two-choice stimuli. Females did not show preference for male size but preferred males that showed higher levels of courtship, a trait known to advertise condition (fat reserves). Also, time spent near the preferred male depended on male courtship effort. Playback experiments showed that when sound was combined with visual stimuli (a male confined in a small aquarium placed near each speaker), females spent more time near the male associated with courtship sound than with the control male (associated with white noise or silence). Although male visual courtship effort also affected female preference in the pre-playback period, this effect decreased during playback and disappeared in the post-playback period. Courtship sound stimuli alone did not elicit female preference in relation to a control. Taken together, the results suggest that visual and mainly acoustic courtship displays are subject to mate preference and may advertise parental quality in this species. Our results indicate that visual and acoustic signals interplay in a complex fashion and highlight the need to examine how different sensory modalities affect mating preferences in fish and other vertebrates.

INTRODUCTION

Mate choice plays a major role in sexual selection and can influence the genetic structure of a population (Andersson, 1994; Parker and Partridge, 1998). When parental care is exclusive to males, females should prefer high parental-quality mates as this will maximize their offspring survival (Trivers, 1972). An important trait used by females in mate choice, including in species with paternal care, is body size (Andersson, 1994). It may confer direct benefits including better territories and enhanced offspring protection from predators (Magnhagen and Kvarnemo, 1989; Lindström and Pampoulie, 2005), or indirect benefits such as offspring with large body size and improved reproductive performance (Ruiz et al., 1991; Reynolds and Gross, 1992). In addition, preference for large males should dominate if they provide better parental care (e.g. Côte and Hunte, 1989; Lindström and Hellström, 1993) or are more fertile (e.g. Howard et al., 1998).

In teleost fish, male parental care can be energetically costly as they face feeding restrictions during the often-prolonged periods of paternal care (e.g. Lindström, 2001; Sisneros et al., 2009). Empirical studies with several fish species have shown that female choice is based on courtship intensity, which is an honest indicator of male quality (energetic reserves) and male reproductive success (Knapp and Kovach, 1991; Vasconcelos et al., 2012). Females may be choosing better fathers as low-condition males may not only provide lower parental care quality but also engage in filial cannibalism

(Manica, 2004). Rogers (Rogers, 1995) also demonstrated that females of the cichlid *Amphilophus citrinellus* choose males based on traits that result in higher juvenile survival rates.

Fish often engage in elaborate courtship dances that culminate in spawning events. During courtship, different signal types, including bright colours, conspicuous motor patterns, pheromones and acoustic signals, lure the females into the male territory and stimulate them to release eggs (Knight and Turner, 1999; Plenderleith et al., 2005; Amorim, 2006). Accounting for the interplay of these multimodal signals in female choice is essential to understanding the evolution of traits by sexual selection (Candolin, 2003). Although the roles of body size and other visual traits have received considerable attention in the study of mate choice in fishes, the influence of acoustic signals on mating preferences or its combination with other signals has been poorly studied (e.g. Verzijden et al., 2010; Maruska et al., 2012).

The study organism, the painted goby *Pomatoschistus pictus* (Malm 1865), is a small marine fish with exclusive paternal care that uses at least visual and acoustic signalling to attract mates (Amorim and Neves, 2007). Males compete aggressively over nesting sites during the breeding season (Amorim and Neves, 2008) and have few breeding opportunities, as they only live up to 2 years (Miller, 1986). Because paternal care is essential for the reproductive success of this species, traits that advertise paternal ability should be subject to female choice. The aim of the present study was to test the hypothesis that painted
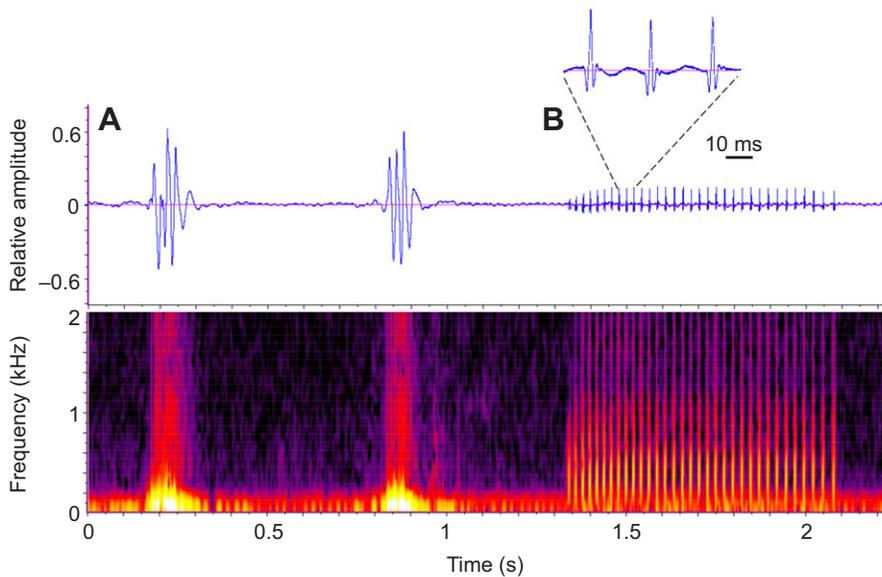
Fig. 1. Oscillogram (above) and sonogram (below) of a sequence of two thumps (A) followed by a drum (B). Detail of three drum pulses is depicted.

goby females prefer high-parental-quality mates such as large or high-condition males. We first tested female preference for larger males because they are likely to be more successful in defending territories including nests with offspring. We further tested the contribution of acoustic and visual signals (courtship displays) to mate choice with playback experiments. We predicted the existence of female preference for males with higher visual and acoustic courtship efforts as visual courtship is likely to be condition dependent (Knapp and Kovach, 1991) and a recent study has shown that male painted goby vocal activity reflects body fat reserves and seems subject to female preference (Amorim et al., 2013).

MATERIALS AND METHODS
Model system and test subjects
The painted goby, *P. pictus*, is an exclusively coastal benthic species inhabiting shallow gravel and sand substrate areas in the Eastern Atlantic Ocean and in some areas of the Mediterranean Sea (Miller, 1986). They are polygamous small gobies with a short life span. Starting in January, males establish territories and build nests under bivalve shells (Bouchereau et al., 2003). Nest owners actively defend their nests from other male intruders (Amorim and Neves, 2008) and attract females for spawning with vibrant displays that include visual and acoustic signals (Amorim and Neves, 2007). Females lay their eggs in a single layer on the nest ceiling and leave the male to provide parental care that takes up to 3 weeks (Bouchereau et al., 2003; Miller, 1986). Male visual courtship includes conspicuous displays outside the nest such as approaching the female, quivering, leading the female to the nest, nudging the female flank, and rapid figure-of-eight swimming manoeuvres, and nest displays that include jerky movements or body quivering while the male is raised on the pelvic fins with the head at the nest entrance (Amorim and Neves, 2007). Acoustic courtship, which is only observed after the male detects a female, consists of the emission of two types of acoustic signals to attract passing females and also when the female is already inside the nest: thumps (Fig. 1A), short, low-frequency non-pulsed sounds associated with nest displays, and drums, low-frequency pulsed sounds (trains of pulses; Fig. 1B) associated with quivering displays both outside and inside the nest (Amorim and Neves, 2007; Amorim et al., 2013).

We captured fish using hand nets in spring low tides at Parede (38°41′N, 9°21′W) and by SCUBA diving in shallow water at Arrábida (38°28′N, 8°58′W), Portugal, during the breeding season (January–June 2011 and 2012). Genetic analysis (A. M. Pereira and V. C. Almada, unpublished data) has shown that this is a single population. We separated fish by gender and housed them under a natural light regime (12 h:12 h light:dark) in glass stock aquaria of *ca.* 18 l (24×24×32 cm) provided with sand substrate, shelters and a closed-circuit flow of artificial filtered seawater kept at *ca.* 16°C. We fed fish daily *ad libitum* with chopped mussels, clams and cockles.

Size preference experiments
To test whether painted goby females show preference for males of different sizes, we presented test females with a choice of two males differing by at least 20% in standard length (SL) (i.e. in SL ratio). Experimental aquaria (35 l, 26×51×31 cm; Fig. 2), provided with a 2-cm-deep sand substrate and filtered artificial seawater (16°C, as stock aquaria), were divided in three distinct compartments by two perforated transparent acrylic partitions. In the central compartment next to the partitions we marked two 5 cm response zones (corresponding approximately to one fish length) with a permanent pen (Fig. 2A). We provided the outer compartments with a shelter. Males were assigned randomly to one lateral compartment of an experimental tank 2 days prior to the start of a trial to allow them to become territorial. To stimulate nest building and territorial behaviour we placed a pregnant stimulus female in the central compartment during those 2 days. Trials were only carried out when both males showed territorial behaviour (nest building and occupation). In order to standardize nest attributes across trials, we assured that nest coverage was similar between each pair of males' nests (and in different trials) by adding or removing sand as this is an attribute that can influence female preference in other *Pomatoschistus* (Svensson and Kvarnemo, 2005). We replaced the stimulus female with a focal pregnant female 60 min before the trial to allow acclimatization to the aquaria and added an extra opaque partition next to each transparent partition to prevent focal females from having visual access to males before being tested. Ten minutes before the start of trials, aeration and filters were switched off to allow sound recording (see below). Trials started by removing the opaque partitions and lasted 20 min. During the 60 min acclimatization pre-trial period and during the trial it was likely that females had access to chemical cues from the males. As test males and females were changed between trials, we do not believe that
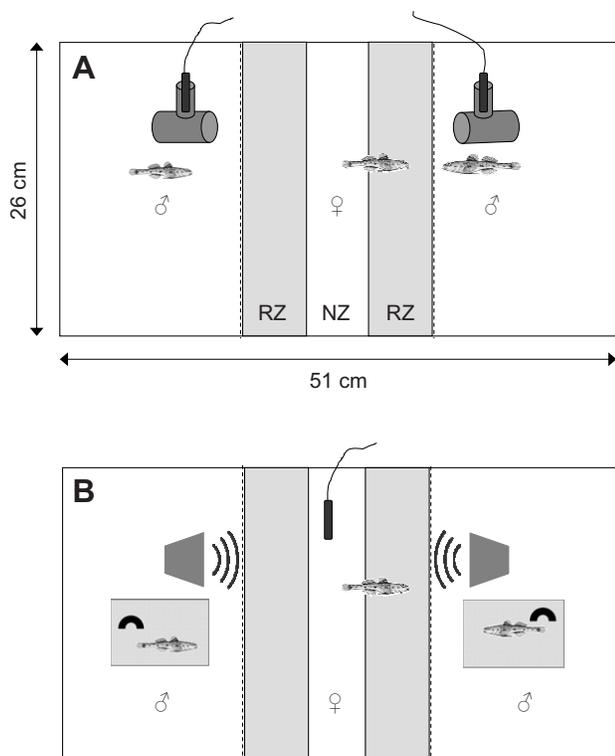
Fig. 2. Experimental setup for the assessment of female preference for male size (A) and sound playback (B). The two setups differ in the stimuli given to subject females. (A) Males of different sizes are present in each lateral compartment. Male assessment is allowed through a transparent partition (dashed lines). (B) The female receives sound playback from one side *versus* a control (silence or white noise). Additional visual stimuli (a male in a small confinement aquarium) are present in some experiments. For detailed procedures, see Materials and methods. Grey areas in the central compartment of the tank depict the response zones (RZ) and the white area the neutral zone (NZ).

there was any systematic effect that may have affected the outcome of experiments.

All behaviour was videotaped with a video camera (Sony DCR-HC39, Sony, Tokyo, Japan) positioned 50 cm in front of the experimental aquarium. The video and synchronized audio signals (derived from the audio recording chain) were digitized with Pinnacle Dazzle DVD Recorder Plus (Pinnacle Systems, Mountain View, CA, USA) to a laptop. We analyzed videos using EthoLog (v. 2.2, University of São Paulo, São Paulo, Brazil). The activity of all individuals, including males' courtship behaviour and sound production, were noted with continuous focal observations of the three individuals. To estimate female choice, we recorded how long females spent in each response zone during trials. For a trial to be considered valid for analysis, the focal female had to spend more than 25% of the time in the response zones (we discarded 1 in 22 trials) and had to associate with both males, i.e. a female that would remain stationary near one male would be discarded. We registered the following behavioural parameters for males: total male courtship, i.e. the total number of courtship displays including approach, quiver outside the nest, lead (leading the female to the nest), eight displays (where the male swims rapidly in front of the female in a figure-of-eight pattern), nest exhibitions (including rubbing the belly in the ceiling of the nest and quivering with only the head outside the nest), rubbing the belly in the sand and sound production (see Amorim and Neves, 2007).

We registered sounds produced by the stimulus males with an audio recording chain consisting of two hydrophones connected to an A/D converter device (Edirol UA-25, Roland, Tokyo, Japan; 16 bit, 8 kHz) controlled by a laptop through Adobe Audition 3.0 (Adobe Systems, San José, CA, USA), allowing simultaneous two-channel recordings. Both hydrophones [Brüel & Kjær 8104, Brüel & Kjær, Nærum, Denmark; sensitivity −205 dB re. 1 V/μPa; frequency response from 0.1 Hz to 180 kHz; connected to a Brüel & Kjær Mediator type 2238, and a custom-made hydrophone (Fonseca and Maia Alves, 2011)] were housed inside chimneys in the male's shelter (a PVC tube 5.5 cm long with 3 cm inner diameter and with a chimney; Fig. 2A). To significantly minimize the conduction of room-floor-borne noise to the tank and improve sound recordings, experimental aquaria were placed on top of two marble layers interspersed with two levels of rubber foam shock absorbers.

Females were measured for SL and body mass (M) after each trial. Twenty-two females were tested, ranging in SL between 2.9 and 3.7 cm (mean ± s.d.=3.3±0.23 cm) and in M between 0.33 and 1.02 g (0.60±1.02 g). Female roundness was estimated as M/SL^3 (i.e. the condition factor). All tested females ranged in roundness between 5.7 and 16.7 (8.2±2.2). SL ranged in small-stimulus males between 2.5 and 3.4 cm (mean ± s.d.=2.8±0.25 cm) and in large males between 3.1 and 4.2 cm (3.7±0.31 cm). Male condition was estimated by calculating the condition factor.

Playback experiments

A female was placed 24 h before trials in the central area of an experimental aquarium similar to the ones used in the size preference experiment, fitted with double partitions (one opaque and one transparent partition) that divided the tank into three equal compartments. Lateral compartments contained a nest and an underwater speaker in close proximity of the nest and the partition.

We stopped aeration and filters 15 min before trials. We started trials by removing only the opaque partitions to allow the female access to visual and acoustic stimuli presented from the lateral compartments. The playback protocol lasted 15 min and was divided into three periods: 5 min prior to playback (PRE), 5 min of stimuli presentation (PBK) and the subsequent 5 min with no stimuli presentation (POST).

Each female was tested only once with paired-choice tests, in which it was presented with a sound and a control stimulus, which was either white noise (WN) or silence. When females were faced with sound playback from both sides, we ensured that the emission of drums and WN did not overlap to avoid masking and interference. The test stimuli (sound *versus* control) were assigned randomly to the left and right sides of the experimental tank.

We ran two independent experiments (always with different test females), one with only courtship sound or control stimuli (playback only; above) and another with additional visual stimuli (playback + visual). The additional visual stimuli were present throughout the 15 min and consisted of a male inside a small confinement aquarium (8×8×22 cm) provided with a nest, placed beside the speaker in each lateral compartment (Fig. 2B). Confinement aquaria were high enough to prevent chemical communication during experiments. Males from the two lateral compartments were matched in size (difference in SL ratio <10%) and had enough space to perform part of the courtship repertoire including quivering inside or outside the nest, nest building and attempts to approach the female (approaching and touching the walls of their confinement aquarium).

We only used drums in the playback treatment as previous work has shown that high-quality and motivated males invest mostly in drum production, while thumps are more common in lower-quality
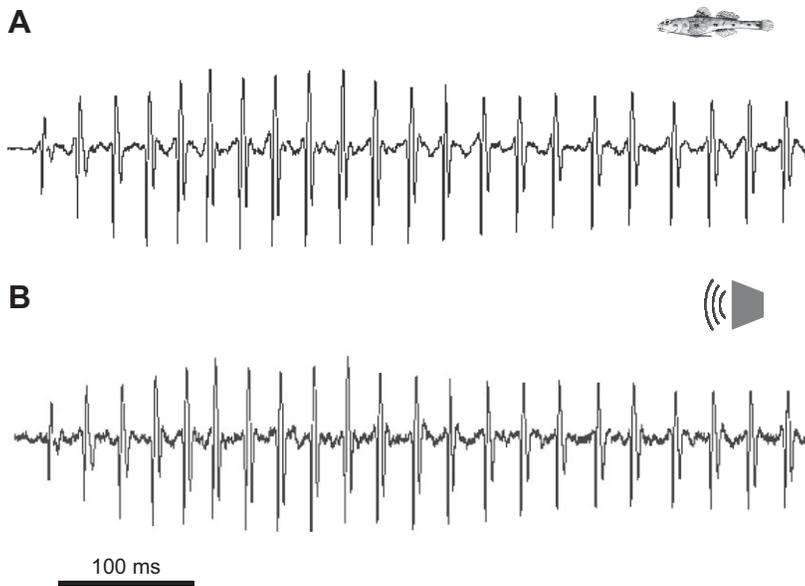
A

B

Fig. 3. Oscillogram of a drum sound produced by the painted goby (A) or played back by the speaker (B).

100 ms

males (Amorim et al., 2013). Drum PBK consisted of a 5 min sequence of drums mimicking a conspecific neighbour calling at a high rate (*ca.* 8 drums min^{-1}). To define a high drumming rate we selected the 5 min period with the highest drum rate from nine males in our sound archive (2010) and calculated the 75% quartile. Spacing between sounds in a playback sequence followed the pattern observed in courting males. Drums from four different males (mean SL 3.4 cm, range 3.0–3.6 cm) were used to produce four 5 min playback sequences. Each sequence was assembled with three different sounds from a single male. All four files had the same time of drum sound playback, i.e. approximately the same acoustic energy. Four playback sequences of WN segments similar in terms of sound duration and interval to the drum sequences were also built. We equalized sounds in both drum and WN sequences to the same sound amplitude. Playback was carried out with custom-made speakers (Fonseca and Maia Alves, 2012) that are able to accurately reproduce low-frequency pulsed fish sounds such as the painted goby drums (Fig. 3). The speakers, 3 cm in diameter, were connected to a custom-made amplifier (Fonseca and Maia Alves, 2012) fed through a D/A device (Edirol UA-25) controlled by Adobe Audition 3.0. The amplitude of the sound playback (drum or WN) was previously adjusted to mimic that of a painted goby male at 1–2 cm distance (*ca.* 130 dB) with the above-mentioned Brüel & Kjær 8104 hydrophone and sound level measuring device (Brüel & Kjær Mediator type 2238).

Fish behaviour was videotaped and analyzed with EthoLog (v. 2.2) as above. We assessed female preference by measuring how long females spent in each response zone during the PRE, PBK and POST periods. As above, we only considered trials in which the focal female interacted with both males (i.e. did not remain stationary in a single position) and spent more than 25% of the time in the response zones (we discarded none of a total of 28 trials). We also quantified male courtship, as the number of quivers, nest building movements and approach attempts to the female. Twenty-eight females were tested in total and ranged in SL between 3.0 and 3.9 cm (mean ± s.d.=3.5±0.24 cm).

Statistical analyses

For size preference experiments, female choice was investigated with Wilcoxon paired tests that compared the time spent within the response zones next to each male (small or large). Because males were allowed

to display courtship behaviour, we compared total courtship between the preferred (the male with whom the female spent more than 50% of the time with) and the non-preferred male with paired *t*-tests. We carried out Spearman correlations to check whether the time spent near the preferred male was related to the preferred male quality (condition and total courtship), relative quality between both males (ratio of SL, condition and total courtship between the preferred and the other male), female features (SL and roundness) and the ratio between female SL and the preferred male SL or the non-preferred male SL. We finally tested the effect of the factor courtship intensity (high and low, i.e. above or below the median observed for all males) on the time spent near the preferred male with a one-way ANOVA.

Regarding playback experiments, we calculated the proportion of time the female spent in the response zone where drums were played back (sound response zone) in relation to the total time spent in both response zones for each PRE, PBK and POST period. For the playback-only experiments, we compared the relative time spent in the sound response zone in the three periods with a repeated-measures ANOVA. Experiment type (sound *versus* WN or sound *versus* silence) was used as a factor to test for the effect of control type. For playback + visual experiments we carried out a similar repeated-measures ANOVA that also included a covariate to control for the possible influence of male behaviour on female preference: the relative courtship of the 'sound male', i.e. the ratio between the mean courtship of the 'sound male' and the 'control male' observed in the three trial periods. Male courtship ratio did not vary among trial periods (repeated-measures ANOVA, $F_{2,26}$=0.05, $P>0.05$). Pearson correlations between the covariate (relative male courtship) and female preference (relative time spent in the sound response zone) were carried out for each experimental period as the interaction between the dependent variable and the covariate was significant.

We conducted statistical analyses with Statistica (version 10, Statsoft, Tulsa, OK, USA). We transformed data when necessary to meet assumptions of the used parametric tests. Data are presented as means ± s.d. unless otherwise indicated.

RESULTS
Size preference

We found no significant differences between the time spent near larger males in relation to smaller males (Wilcoxon test, *N*=21,
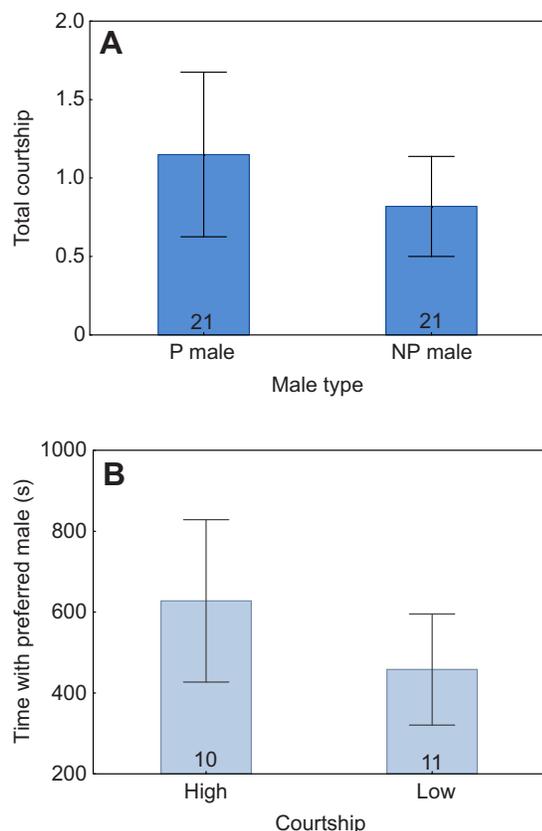
Fig. 4. (A) Total courtship [data were log(x+1) transformed] exhibited by preferred (P male) and non-preferred (NP male) males and (B) the time females spent with the preferred males according to their courtship activity. Data are means ± s.d. Sample sizes are shown in each bar.

T=111.0, P>0.05). Time spent near the larger males averaged 336.7±257.8 s (range 40.7–1087.3 s) and time spent with smaller males averaged 351.7±217.3 s (9.6–801.4 s).

Preferred males showed higher courtship effort (total courtship) than non-preferred males (t=2.63, d.f.=17, P<0.05; Fig. 4A). Also, the time females spent with the preferred male was significantly related to the courtship performed by the preferred male and by both males (N=21, r=0.48–0.50, P<0.05) and was not related to the remaining considered parameters (see Materials and methods; N=21, r=–0.14–0.39, P>0.05). In addition, females spent more time with the preferred male when it showed high levels of courtship (one-way ANOVA, $F_{1,19}$=5.21, P<0.05; Fig. 4B).

Only four males produced sounds (thumps) while courting the females. Although data on the number of sound-producing males were too small to statistically test female preference for vocal males, a qualitative analysis of the data showed that females stayed longer near the vocal male than near the alternative silent male in three out of four occasions (Table 1). In the one experiment where the vocal male was non-preferred, its vocal activity was very low, producing only eight sounds in comparison with 17–99 sounds produced by the other three vocal males that enjoyed female preference.

Overall, these results suggest that in contrast with fish size, courtship effort and perhaps vocal activity may be important factors in mate choice in this species.

Playback experiments

In these experiments we tested whether conspecific courtship sounds presented as the only stimulus were attractive to gravid

Table 1. Time spent near large and small males in the four trials where acoustic activity (thumps) was observed

No. of thumps	Time near large male (s)	Time near small male (s)
8	336.99*	466.9
26	1059.98*	84.7
99	1087.26*	9.57
17	212.87	801.42*

In three out of the four cases the female preferred the vocal male.
*Indicates vocal activity.

females or whether an additional visual stimulus was required for evoking female attraction. Repeated-measures ANOVA revealed no significant change in the percentage of time spent by the female in the courtship sound response zone during the playback-only treatment ($F_{2,24}$=0.26, P>0.05). Experiment type (whether the control is WN or silence) also did not have a significant effect ($F_{1,12}$=0.004, P>0.05), but the interaction between variables was significant ($F_{2,24}$=3.56, P<0.05) because there was a non-significant tendency for females to increase the percentage of time spent in the sound response zone from PRE to POST periods when the control used was silence, but the reverse was observed when the control was WN (Fig. 5).

When visual stimuli were added (playback + visual), we found an increase of the percentage of time spent in the sound response zone from PRE to POST periods ($F_{2,22}$=5.11, P=0.02, LSD test for differences between PRE and POST, P=0.07; Fig. 6) and no effect of the control (WN or silence) ($F_{1,11}$=0.02, P>0.05). The relative courtship activity between the 'sound' and the 'control' males had a significant effect on female spatial distribution ($F_{1,11}$=7.78, P=0.02) and there was a significant interaction between this covariate (relative male courtship) and the playback effect ($F_{2,22}$=4.81, P=0.02). In the PRE period, when there were no acoustic stimuli, female preference (relative time in the sound response zone) increased with relative male courtship (ratio between the mean courtship of the 'sound' and the 'control' males) (Fig. 7). In the PBK period, this positive relationship was still present, but the slope of the tendency was weaker. In the POST period, this relationship was reversed and the data showed a large dispersion in relation to the tendency line (Fig. 7), indicating that female presence in a response zone was unrelated to male courtship. Also, the spread of observations in the x-axis (Fig. 7), which depicts female preference for the sound response zone, became smaller and shifted to the right side of the axis (i.e. with larger values) from PRE to POST periods. This indicates that the increase in preference for the sound side was accompanied by a decrease in preference variability among females. These results suggest that females show a preference for more actively courting males when faced with visual stimuli alone (PRE), but when they are simultaneously or have recently been exposed to courtship sound (PBK and POST), their preference goes to the side from where conspecific sounds were presented. In the POST period, the effect of male courtship effort seems to have disappeared.

DISCUSSION
Male size
We found that females did not show a preference for male size as there were no differences between the time spent near larger males and that spent near smaller males. Instead, preferred males showed higher courtship efforts than non-preferred males, and the time females spent near the preferred male was dependent on its courtship effort, as they spent significantly longer periods near higher-courting than lower-courting preferred males. These results point
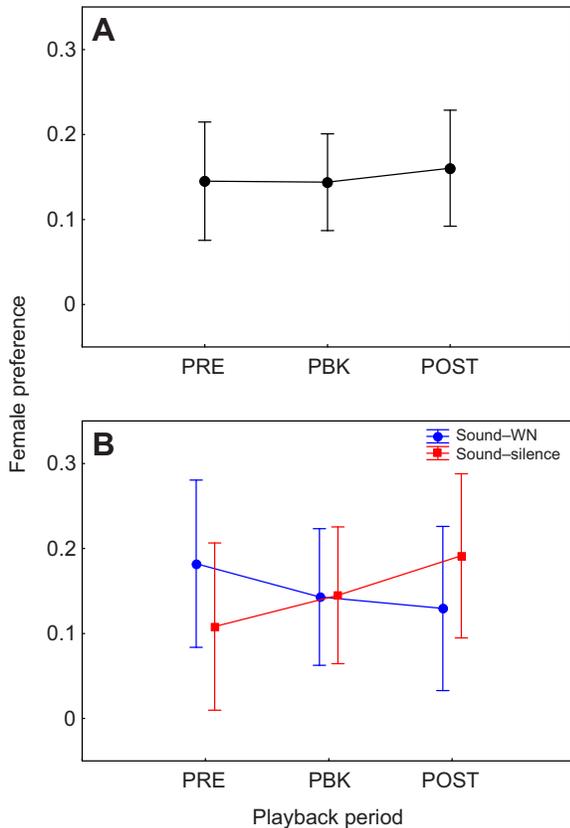
Fig. 5. Effect of sound playback (only) on female preference. Two experiments were performed: sound *versus* white noise (WN) and sound *versus* silence. Preference was assessed as the percentage of time a female spent in the sound response area in relation to the total time spent in both response areas [data were $\log_{10}(x+1)$ transformed]. Means and 95% confidence intervals are shown for female preference during the PRE (5 min before playback), PBK (5 min of stimuli presentation) and POST (5 min following PBK) periods of playback. In A, the overall effect of the playback treatment on female preference is shown (*P*>0.05), hence combining both experiments. In B, the interaction between the variables experimental period (PRE, PBK and POST) and experiment type is illustrated.
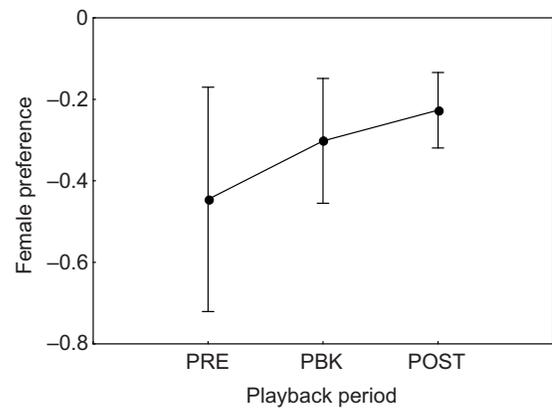

Fig. 6. Effect of sound playback (playback + visual) on female preference. Preference was assessed as the percentage of time a female spent in the sound response zone in relation to the total time spent in both response zones [data were $\log_{10}(x)$ transformed]. Means computed for the covariate relative male courtship mean (see Materials and methods for details) and 95% confidence intervals are shown for female preference during the PRE (5 min before playback), PBK (5 min of stimuli presentation) and POST (5 min following PBK) periods of playback.

than inter-sexual selective pressures, may have driven larger male size selection. In *P. minutus* and other goby species, a consistent preference for paternal quality (fanning intensity) and male courtship effort has been found (Forsgren, 1997; Suk and Choe, 2002; Pampoulie et al., 2004; Lindström et al., 2006), suggesting that paternal ability and traits that advertise this feature are among the primary traits under mate selection.

In this context, the lack of preference for male size may be explained if body size is independent of parental investment in our study species. Alternatively, our experimental design may not have been adequate to test the role of size in mate choice as females were only presented with a dichotomous choice and did not have direct access to males as in natural situations. Similar dichotomous choice experimental designs are common practice (e.g. Gabor and Page, 2003; Borg et al., 2006; Maruska et al., 2012). However, they may not always translate true mating choices or mating outcomes, as females may gain access to other relevant cues while approaching the males or as a result of mutual assessment (Gonçalves and Oliveira, 2003). Another possibility is that uncontrolled traits associated with male size interfered with the results (Baldauf et al., 2009).

Other parameters of male or female quality did not seem to influence mate choice in the studied species. Painted goby female size and roundness (used as a proxy for reproductive status) were not related with time spent with the preferred male, although the perception of own quality may influence mating preferences in other species (Parker, 1983). Also, the time females spent with the preferred male was not related to absolute or relative male condition, although male energetic condition has proved important in determining male mating success in the sand goby (Lindström, 1998). However, male energetic condition (lipid content) was not assessed in the painted goby and the condition factor does not seem to be related with body lipid content in this species (Amorim et al., 2013).

Only four males produced courtship sounds in this study and one of these showed a weak vocal activity (eight sounds). Interestingly, the remaining three more vocal males, which produced 17–99 sounds in the same 20 min period, were preferred by females over the

to male courtship effort as an important trait for mate choice by painted goby females.

Although mate choice based on body size is reported across taxa (Andersson, 1994), in several other goby species with paternal care the absence of preference for male size has also been found (e.g. Forsgren, 1997; Suk and Choe, 2002; Takahashi and Kohda, 2004). Females are expected to choose mates based on indirect benefits, such as genetic quality, or direct benefits, such as good territories or better parental care. In *Pomatoschistus* spp., genetic contribution to adult body size is probably small compared with the environmental influence associated with the time fish hatched during the previous breeding season (Kvarnemo and Forsgren, 2000). Hence body size may not be a reliable indicator of genetic quality in these short-lived species. In the closely related sand goby (*Pomatoschistus minutus*), however, a large body size proved beneficial in resource competition (Magnhagen and Kvarnemo, 1989; Lindström and Pampoulie, 2005), as commonly found in other animals (e.g. Davies and Halliday, 1979). Larger *P. minutus* males can hold larger nests that can receive more eggs (Lindström, 1988). Thus in sand gobies, male–male competition for large nests, rather
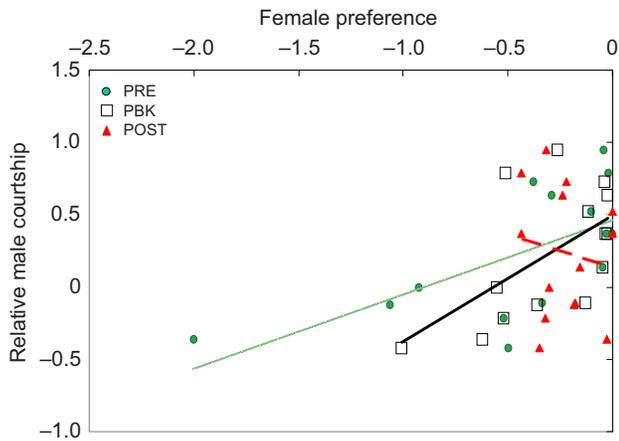
Fig. 7. Relationship between female preference and relative male courtship. Female preference was assessed as the percentage of time a female spent in the sound response zone in relation to the total time spent in both response zones [data were $\log_{10}(x)$ transformed]. Relative male courtship was calculated as the ratio between the mean courtship of the 'sound' and the 'control' males [data were $\log_{10}(x)$ transformed]. Filled circles, open squares and filled triangles refer to PRE, PBK and POST trial periods, respectively. Green fuzzy line, solid black line and dashed red line are tendency lines for the PRE, PBK and POST trial periods.

alternative silent male. This suggests that, besides visual courtship, acoustic signalling may also be an important male trait for female choice in this species, consistent with the findings of Amorim et al. (Amorim et al., 2013). This hypothesis was tested with the playback experiments.

Acoustic and visual courtship signals

Our playback experiments have shown no significant change in the percentage of time females spent in the response zone associated with playbacks of conspecific courtship sounds (playback only). However, additional visual stimuli caused an increase in preference for the courtship sound side from PRE to POST periods in relation to the control (WN or silence). In addition, the relative courtship activity between the 'sound' and the 'control' males had a significant effect on female spatial distribution; in fact, a significant interaction between male courtship and playback effect was found. These results suggest that females showed a preference for more actively courting males when faced with visual stimuli alone (during the PRE period), which is consistent with the results of the size preference experiment. However, when females were, or had recently been, simultaneously presented with courtship acoustic signals, they preferred the side associated with the acoustic stimuli. Indeed, in the POST period the effect of male courtship effort seems to have disappeared as there was prevalence towards the courtship sound side (Fig. 7).

Sound production in reproductive contexts is widespread among teleost fish (Myrberg and Lugli, 2006), pointing to an important role in mate recognition and choice (Amorim et al., 2008). While the influence of acoustic signalling in mating decisions has been established in other taxa (Catchpole and Slater, 2008; Gerhardt and Huber, 2002), there is still little empirical evidence for the effect of sound on mate choice in teleost fish (Myrberg et al., 1986; McKibben and Bass, 1998; Verzijden et al., 2010; Maruska et al., 2012). This is likely because the majority of fish do not produce advertisement signals similar to those of crickets, frogs and songbirds and, as a result, typically do not approach sound sources without

additional stimuli. Indeed, only few studies have shown phonotaxis of female fish towards male mating sounds (e.g. Tavolga, 1958; Lugli et al., 1996; McKibben and Bass, 1998; Rollo et al., 2007; Zeddies et al., 2010), and a recent work has revealed vocal activity to be determinant of reproductive success in a highly vocal fish species, the Lusitanian toadfish (Vasconcelos et al., 2012). This species does not make conspicuous visual displays during courtship, but seems to rely mostly on advertisement acoustic signals to attract females.

Acoustic signals probably have an important role in both multimodal and cross-modal communication in fish. Playback experiments have shown that acoustic signals are relevant for behavioural modification in most fish species only when presented with another stimulus, such as a visual or chemical cue (Lugli, 1997; Lugli et al., 2004; Bertucci et al., 2010; Verzijden et al., 2010). Two recent experiments have demonstrated that females prefer to affiliate with males that have been previously associated with courtship sounds (Verzijden et al., 2010; Maruska et al., 2012), providing the first experimental evidence of sounds affecting female preferences in fish (see also Myrberg et al., 1986; McKibben and Bass, 1998). Both studies have presented acoustic and visual signals uncoupled in time. This prevented testing the role of sounds as a component of a more complex multimodal signal, but put into evidence the cross-modal function of acoustic signals. In fact, other studies have shown that in order for fish to respond behaviourally to conspecific sounds, they must be pre-exposed to other sensory modalities involved in courtship (Lugli, 1997; Lugli et al., 2004). In contrast, a set of elegant playback experiments that used robotic frogs to test cross-modal and multimodal communication in anurans has shown that temporally asynchronous visual and acoustic signals failed to elicit the appropriate behavioural responses in both the agonistic and mating contexts, although the degree of failure depended on the level of temporal displacement (Narins et al., 2005; Taylor et al., 2011). In the Túngara frog, *Physalaemus pustulosus*, for example, the visual cue of the vocal sac inflation that accompanies vocalizations is neither necessary nor sufficient for mate attraction as it does not elicit female attraction when presented alone nor does it enhance female preference when it accompanies a mating call (Taylor et al., 2011). However, matching the visual (inflation of the vocal sac) and the acoustic signals may still play a role in modulating mate choice because Túngara frog females strongly discriminate against asynchronous multimodal signals in favour of the male call alone (Taylor et al., 2011).

To the best of our knowledge, the role of the acoustic component in multimodal courtship communication has so far remained unravelled in fish. We have shown that while courtship sound alone does not seem to influence mate attraction in this goby, when combined with visual cues it becomes a salient feature overriding visual information. This suggests that visual courtship may function as an 'attention grabber' (alerting signal hypothesis) or it may enhance the perception of acoustic cues (receiver psychology hypothesis) (Candolin, 2003; Bro-Jørgensen, 2010). However, the interplay of acoustic signals with visual and/or chemical traits still needs to be addressed in fish.

As vocal activity in fish might be energetically expensive (Amorim et al., 2010; but see Amorim et al., 2002) and is condition-dependent in the painted goby (Amorim et al., 2013), it may function as an honest signal of male quality, including paternal ability, as found in other taxa (e.g. Dolby et al., 2005). However, how fish mate decisions are influenced by call characteristics is still poorly understood (Myrberg et al., 1986; McKibben and Bass, 1998).

In conclusion, we demonstrate that while male size does not appear to be under selection by painted goby females, both visual and acoustic courtship signals are relevant in mate choice. Moreover, when presented together, acoustic signalling seems to override visual courtship. Addressing the role of multimodal communication in a fish courtship context may contribute to a better understanding of the evolutionary forces underlying sexual selection and to clarify the mechanisms by which non-random mating is achieved in fish. Future studies should investigate whether visual and mainly acoustic courtship reflect parental ability in this and other fish species.

LIST OF SYMBOLS AND ABBREVIATIONS

M	mass
PBK	playback, playback period
POST	post-playback period
PRE	pre-playback period
SL	standard length
WN	white noise

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AUTHOR CONTRIBUTIONS

M.C.P.A. and P.J.F. were involved in conception of the study and experimental design. P.J.F. prepared and calibrated the setup. A.N.P., M.C., S.S.P. and R.P. conducted the study. M.C.P.A. carried out statistical analyses and drafted the article. M.C.P.A. and P.J.F. revised the article.

COMPETING INTERESTS

No competing interests declared.

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