Male acoustic display in the sand goby – Essential cue in female choice, but unaffected by supplemental feeding

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\textbf{A B S T R A C T}

Many teleost fishes use acoustic and visual signalling during courtship. Such displays may convey information about body condition. Here we experimentally altered body condition of sand goby (\textit{Pomatoschistus minutus}) males to examine effects on acoustic and visual courtship and subsequent spawning decisions. Over two weeks, males fed in excess were fed daily, whereas food-deprived males were fed once a week. Females only spawned with males that produced courtship sound. However, there were no treatment effects on the occurrence of spawning and males fed in excess did not invest more in visual or acoustic courtship than food-deprived males. That said, males fed in excess built more well-covered nests, with more sand piled on top, compared to food-deprived males. Male condition measured as lipid content differed significantly between treatments. However, only males fed in excess differed in lipid content from wild caught males, indicating that in nature, males are of similar condition to males in the low condition treatment group. Apart from the importance of courtship sound, the only male or female behaviour predicting reproductive success was if male displayed in the nest opening. Males often produce courtship sounds together with a visual display in this position. A female dark-eye display did not associate with reproductive success which, together with previous results, suggest a non-ornamental function of this trait. We conclude that male courtship sounds appear to be crucial in female mate choice, but the information content of the courtship sounds and how it relates to male condition remains elusive.

\textbf{1. Introduction}

Animals such as frogs, birds, mammals and insects produce sound during courtship and, like visual courtship, the acoustic courtship may carry information about nutritional condition (Scheuber et al., 2003), body size (Charlton et al., 2007), parasite load (Spencer et al., 2005), developmental stress (Spencer and MacDougall-Shackleton, 2011), ‘good’ genes (Welch et al., 1998) and other male quality traits. Teleost fish also produce sound in reproductive contexts, but the function of these vocalizations are not fully understood (Amorim et al., 2015). Aspects of the sound, such as spectral characteristics and sound pressure level, correlate with body size, at least in some species (Lindström and Lugli, 2005; Malavasi et al., 2008; Pedroso et al., 2013; Verzijden et al., 2010). Accordingly, playback experiments have shown females to prefer males with certain aspects of the courtship sound including those associated with size (McKibben and Bass, 1998; Myrberg et al., 1986). In addition, Mann and Lobel (1995) observed that in the damselfish \textit{Dascyllus aruanus} the maximum male calling rate observed in the day prior to spawning was positively correlated with brood size. Male condition has been shown to correlate with aspects of acoustic courtship (Amorim et al., 2013b; Pedroso et al., 2013). The best example may be female Lusitanian toadfish (\textit{Halobatrachus didactylus}) that prefer males with a
higher vocal activity, which also reflects body condition (Amorim et al., 2010; Vasconcelos et al., 2012). In addition, courtship sounds vary between species and are potentially used in species recognition (Amorim et al., 2004; Blom et al., 2016; Crawford et al., 1997; Pedroso et al., 2013; Verzijden et al., 2010).

Gobies in the genus Pomatoschistus show male parental care in nests built by the male (Amorim et al., 2013b; Kvarnemo et al., 1998; Svensson et al., 2004). These species are well-studied regarding sound production and associated behaviours (Blom et al., 2016; Malavasi et al., 2012). Another research focus has been the condition (body fat reserves) of the male and its relevance in reproductive behaviours. Male condition has been shown to affect or be positively correlated with nest building (Kvarnemo et al., 1998; Lehtonen and Wong, 2009; Olsson et al., 2009), male nest defence (Svensson et al., 2004), mating success (Svensson and Forsgren, 2003) and hatching success (Kvarnemo et al., 1998). The well-covered nests associated with high condition appear to be preferred by females as spawning sites (Jones and Reynolds, 1999; Svensson and Kvarnemo, 2005). On the other hand, male visual display appears to be unaffected by manipulated condition (Olsson et al., 2009) though correlative studies have found it to correlate negatively (Amorim et al., 2013b; Svensson et al., 2004) with condition. Finally, natural variation in condition appears to affect acoustic display rate positively (Amorim et al., 2013b; Pedroso et al., 2013). Accordingly, previous studies have shown that females prefer (Amorim et al., 2013a) and only spawn with males that produce courtship sounds (Amorim et al., 2013b), and seem to prefer males with a higher drumming rate (Amorim et al., 2013b; Pedroso et al., 2013).

In this study, we manipulated body condition of sand goby, Pomatoschistus minutus (Pallas), males by feeding regime, to examine whether body condition affects nest building, visual courtship and acoustic courtship and if it influences female mating decision in a single male ‘no choice’ setup. Based on previously published results reviewed above we predicted that males fed in excess would invest more than food-deprived males in 1) nest building and 2) acoustic courtship and 3) achieve higher mating success. We also predicted that 4) visual courtship would be unaffected by treatment or that food-deprived males would display more than males fed in excess. In addition, we analysed how nest characteristics and courtship behaviours predicted the occurrence of dark eyes in females, a trait displayed by ready to mate females (Blom et al., 2016; Olsson et al., 2017; Olsson et al., 2022), and spawning success.

2. Materials and methods

2.1. Study species

The sand goby is a small (max length 9.5 cm TL), marine fish distributed in lagoons, coastal areas and estuaries of the NE Atlantic, Mediterranean and Baltic region (Kullander et al., 2012; Miller, 1986). These short-lived fish have a single breeding season between March and August (Kullander et al., 2012), during which both sexes can reproduce sequentially with different mates. Males develop a nuptial colouration with black pigmentation on pelvic, anal and tail fins. The anal fin has a clear blue colouration with a black trim, and there is a eye-spot on the first dorsal fin. This colouration is lacking in the cryptic female (Forsgren, 1999; Kullander et al., 2012). Males build a nest by covering bivalve shells with sand and compete for mating opportunities with females, which in turn are the choosier sex. Male courtship behaviour normally starts with a visual display in which the male approaches the female with erect ornamented fins. Typically, the male swims back to the nest in a conspicuous manner, considered a lead display (‘lead swim’), and the female may choose to follow the courting male to his nest (reviewed in Blom et al., 2016; Forsgren, 1999). The male might produce a series of sounds both when the female is outside the nest and when she has entered it (Blom et al., 2016; Lindström and Lugli, 2000; Pedroso et al., 2013). In the sand goby, the sound consists of trains of pulses (drums) which typically are grouped in bursts with <0.3 s interval between drums (Lindström and Lugli, 2000; Pedrosso et al., 2013). During courtship and before entering the nest, the females sometimes get conspicuous dark eyes (Blom et al., 2016; Olsson et al., 2017; Olsson et al., 2022). After entering the nest, females may still choose not to spawn and leave (Lehtonen and Kvarnemo, 2015). The video clip in the supplementary material includes male visual and acoustic courtship as well as female dark eyes.

2.2. Experimental design

The experiment was conducted at Kristineberg Marine Research Station on the west coast of Sweden (58°15′ N, 11°27′ E) between May and July 2013–2014. All fish were caught by hand trawling in bays nearby the station. Males were randomly assigned to one of two treatments. During treatment, males were kept in same sex groups in four 50-l aquaria. In a food excess treatment males were fed every day for two weeks and in a food-deprived treatment males were fed twice during the same period (on day 1 and day 7). In the food excess treatment, the uneaten food was removed once a day and replaced with new food. The food-deprived males had not been fed for a week when entering the experiment. These males were thereafter either used in the behavioural experiment or analysed for lipid content (see below). Females were housed in same sex 50 l storage aquaria and fed daily for 7 days before the experiment started. All fish were fed commercial fish food granules (Nutra HP, Skretting). Any uneaten food was removed the day after feeding. All aquaria had a continuous flow of natural seawater (salinity 22–31 PSU, 11–12 °C) and natural daylight was provided through windows.

In the behavioural experiment, we used 20 experimental glass aquaria (dimensions (LxWxH): 30 cm × 25 cm × 30 cm) filled to 20 l. Each aquarium contained a 5-cm layer of sifted sand and was equipped with an artificial nest site, made of a half grey polypropylene tube (Ø 56 mm) with a pipe attached to a hole, like a chimney (Ø 20 mm). A hydrophone was later placed in the chimney. The video clip in the supplementary material shows the experimental set-up. Each nest site was fitted with an acetate sheet lining the ceiling for females to lay eggs on. The experimental aquaria were placed on top of two marble layers interspaced with silicon balls for sound insulation from ground borne vibrations. The aquaria were separated by opaque screens to avoid visual contact between fish in adjoining replicates.

Males were placed individually in an experimental aquarium in the evening. To encourage nest building, a ripe female in a perforated transparent vial was placed in front of the nest site and the nest was photographed 12 h later. Thereafter, the female was removed and replaced with two new ripe females. These new females were left in a vial inside the aquarium for 1 h to acclimatise before they were released in the aquarium.

Behavioural interactions and courtship sounds were recorded in the first 60 min after the females were released, using a camcorder (Canon Legria HF M56, Ota, Tokyo, Japan) placed in front of the aquarium at a 90 cm distance. Courtship sounds were registered using a calibrated hydrophone (HTI-96-MIN with pre-amplifier, High Tech Inc., Gulfport MS; sensitivity: −165 dB re. 1 V/μPa, frequency range: 0.02–30 kHz) connected to a digital audio recorder (Song Meter SM2+, Wildlife Acoustics, Inc., Massachusetts, USA, sampling frequency 16 kHz 2013 and 32 kHz 2014).

The replicates were terminated 12 h after the females were released. At this point, the fish were removed from the aquaria and the nests were examined for eggs. All fish used in the experiment were fed and returned to their natural environment. We successfully filmed 15 males from the food excess treatment and 16 males from the food-deprived treatment.

2.3. Lipid analysis

To measure the effect of the feeding treatment on lipid reserves, we extracted the lipid contents from 11 males fed in excess, 12 food-
were euthanized using MS222 and frozen individually to previous studies (e.g. Lehtonen and Wong, 2009; Olsson et al., 2009; E.-L. Blom et al., 2005). Nest opening width was measured from the same photographs using the program ImageJ 1.51 (https://imagej.net) with the diameter of the chimney used as a scale. Visual courtship of males (the total time period displaying outside and inside the nest) and the occurrence of dark eyes in females were measured from the videos for the whole 60 min using an event recorder (JWatcher video 1.0; https://www.jwatcher.ucla.edu) (see Blom et al. (2016) for a description of the behaviours). The observer was blind to treatment. All males were monitored for acoustic displays. Out of the 31 filmed males, 17 males produced sound. However, four sound files did not have good enough quality for sound analysis due to high background noise levels and we also lost two sound files due to an accidentally reformatted hard drive. Therefore, 6 well fed and 5 food-deprived males were analysed regarding number of and duration of sounds (drums and bursts) and sound pressure level (SPL), from which 5 well fed and 5 food-deprived males were analysed for number of pulses per drum (train) and pulse repetition rate (number of pulses per second in the drum). Calling effort, i.e. the proportion of the time spent vocalising was tallied for the total time period. Active calling rate (number of drums per minute) was measured during the periods when the males produced sounds. No burst of drums continued for longer than one minute and we counted the number of drums during one minute starting with first drum of the drum burst. We used the average for all drum bursts in the 1 h recording. Duration of sound bursts (a sequence of drums) was the average of the first three bursts (3 being the minimum of bursts per male). For the analysis of pulse rate (number of pulses per second), we used the average of the first three successful analyses in the sequence of drums. Relative SPL was based on the average of three drums produced when the males were in the same position relative to the hydrophone i.e., head in the entrance of the nest and the body inside, while the female was outside the nest. We used the Aquatic acoustic metrics interface (AAMI) software to calculate the maximum (SPLmax) and the average (root mean square, SPL rms) sound pressure levels (dB re 1 μPa). For the other sound analyses, we used Matlab R2009b (The Mathworks Inc., Natick, Massachusetts, USA).

3. Results

3.1. Effect of food regime on nest-building effort, courtship and mating success

Nest cover differed significantly between treatments, with males fed in excess building more well-covered nests. Likewise, nest openings differed in width, with males fed in excess building smaller openings, although this difference was not significant. There were no significant treatment effects on the time the male spent on visual courtship, the occurrence of female dark eyes, or on male mating success (Table 1). Furthermore, there were no significant treatment effects on the occurrence of acoustic courtship or on any of its properties including active calling rate (Fig. 1, Table 2).

3.2. Effect of acoustic and visual displays on female dark eyes

Females were not more likely to display dark eyes in response to visual courtship or in replicates where the male produced courtship sound. However, nest-building effort, measured as nest cover and nest opening width, was higher in replicates where females showed dark eyes than in those where females did not. For nest cover this was significant, whereas for nest opening size it was a non-significant trend (Table 3).
3.2. Effect of food regime on male lipid content

There was a significant effect of feeding treatment on lipid content of the males (generalized linear model: treatment: Wald $\chi^2 = 708.32$, df = 2, $p < 0.001$, dry weight: Wald $\chi^2 = 61.62$, df = 1, $p < 0.001$). In the post hoc tests, food excess males had significantly more lipids than food-deprived and wild caught males (both $p < 0.001$) whereas food-deprived males did not differ significantly from wild caught males ($p = 0.095$) (Fig. 2).

3.3. Effect of acoustic and visual displays on male mating success

Nest cover, nest opening width or visual courtship outside the nest by the male, or dark eyes being displayed by the female did not predict the occurrence of courtship sound correlated positively with spawning. In fact, spawning only happened in replicates where the male performed acoustic courtship. We thereby confirm previous findings of males from the same genus that female

*P. minutus* and *P. pictus* do not spawn with silent males (Amorim et al., 2013b; Lindström and Lugli, 2000; Pedroso et al., 2013). Of 15 males fed in excess, 10 males (67%) produced courtship whereas 7 of 16 food deprived males (44%) did so. How- ever, this difference was not significant and male spawning success was independent of feeding treatment. Our food excess treatment affected the energy reserves of the fish as intended, such that the lipid content of food-excess males was significantly higher than that of food-deprived males. However, food-deprived males were of similar condition as wild caught males, which limits the inferences of our results. Food-deprived males performed acoustic courtship as often as males fed in excess (Table 4).

4. Discussion

In the present study, sand goby females only spawned with males performing acoustic courtship. We thereby confirm previous findings from the same genus that female *P. minutus* and *P. pictus* do not spawn with silent males (Amorim et al., 2013b; Lindström and Lugli, 2000; Pedroso et al., 2013). Of 15 males fed in excess, 10 males (67%) produced sound whereas 7 of 16 food deprived males (44%) did so. However, this difference was not significant and male spawning success was independent of feeding treatment. Our food excess treatment affected the energy reserves of the fish as intended, such that the lipid content of males fed in excess was significantly higher than that of food-deprived males. However, food-deprived males were of similar condition as wild caught males, which limits the interpretations of our results. Food-deprived males performed acoustic courtship as often as males fed in excess (Table 4).
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In the present study, it was observed that well-fed males and males fed in excess once a day. Furthermore, no measures of courtship sound or visual courtship suggested any increased investment in courtship among males fed in excess. Still, males fed in excess piled more sand on top of the nest, and tended to have smaller nest openings, but nest-building effort did not affect spawning success.

The finding that males fed in excess did not increase their acoustic signalling effort compared to food-deprived males is in contrast with previous correlational studies. In the same sand goby population as used in the present study, natural variation in body lipid content has been shown to correlate positively with the active calling rate (number of drums per minute) (Pedroso et al., 2013). The same correlation was found in the congener painted goby as well as a correlation between the information content of the courtship sound is not condition per se severe enough to affect the males’ acoustic and visual courtship. Indeed, wild-caught males had similar lipid content as food-deprived males. This shows that our restricted diet resulted in fish with energy reserves that correspond to natural levels, whereas the excess diet resulted in unnaturally fat fish. Furthermore, in the lipid analyses, a few males deviated from the expected positive relationship between lipid content and lean body mass, and were therefore treated as outliers. If they indeed are part of the natural variation a few individuals of the wild population may be of as high condition as the food-excess males of the present study. Alternatively, in natural populations, it is possible that lipid content, display rate and calling rate co-vary with other factors and that the information content of the courtship sound is not condition per se but instead reflects other aspects of male quality including ‘good genes.’

In addition, previous studies have indicated that, at least without competition, males in low condition may increase their signalling effort (Candolin, 2000; Svensson et al., 2004). When other males are present, male signalling become more honest (Candolin, 2000; Gavassa et al., 2012). Indeed, one difference between the present study and Pedroso et al. (2013) is that in the latter, males had both visual and acoustic access to other nesting males. Furthermore, males may strategically focus their signalling effort on certain traits or aspects of the trait (Amcoff et al., 2009; Rebar and Rodriguez, 2016; Svensson and Kvarnemo, 2005). Therefore, future studies are needed to further investigate information content of the courtship sounds and the relation between male condition and acoustic behaviours.

Previous work on the genus Pomatoschistus in both aquarium experiments (Kvarnemo et al., 1998; Olsson et al., 2009) and in the field (Lehtonen and Wong, 2009) have shown that well-fed males and males with high lipid content pile more sand on top of their nests than other males, and that females prefer to spawn in more well-built nests (Jones and Reynolds, 1999; Svensson and Kvarnemo, 2005). It makes sense that...