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Effects of temperature on acoustic and visual courtship and reproductive success in the two-spotted goby *Pomatoschistus flavescens*

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ABSTRACT

Fish are ectothermic and small changes in water temperature could greatly affect reproduction. The two-spotted goby is a small semi-pelagic species that uses visual and acoustic displays to mate. Here, we studied the effect of temperature (16 and 20 °C) on acoustic and visual courtship and associated reproductive success in 39 males. Temperature influenced male visual courtship performed outside the nest, but it did not influence calling rate and the number of laid eggs. Interestingly, the number of sounds (drums) was the sole predictor of spawning success. These findings suggest that exposure to different temperatures within the species' natural range affect courtship behaviour but not its reproductive success. We propose that finding the link between acoustic behaviour and reproduction in fishes offers the opportunity to monitor fish sounds both in the lab and in nature to learn how they respond to environmental changes and human impacts, namely global warming.

1. Introduction

Temperature is a critical environmental factor in the ecology of fish. As ectothermic animals, fluctuations in temperature can significantly influence their metabolism, modifying behaviours that can ultimately influence fitness, such as reproductive behaviours (Torricelli et al., 1985; see Conrad et al., 2017; Brandt et al., 2018 for examples in other taxa). Species that reproduce in shallow coastal waters are particularly exposed to temperature fluctuations, as these habitats have lower thermal inertia than open-ocean waters (Vinagre et al., 2018). Thus, temperature changes in coastal ecosystems, which house the most biodiversity in the ocean realm, can have significant repercussion on fish communities and biodiversity. As the initial response to environmental changes is often through changes in behaviour, understanding how fish respond behaviourally to temperature changes is essential for conservation (Tuomainen and Candolin, 2011; Couzin and Heins, 2023).

Communication is fundamental in fish reproduction, and both visual and acoustic signals are known to play an important role in reproductive interactions. Visual courtship plays a key role in mating, namely by influencing female and male mate preferences (Sargent et al., 1998; Romano and Stefanini, 2021). Acoustic courtship also plays a relevant role in mate choice in soniferous fishes (Amorim et al., 2015). In addition, male courtship sounds can modulate female reproductive hormones and spawning behaviours (Crovo et al., 2022), and likely help synchronise milt and egg release (Hawkins and Amorim, 2000). However, there is a paucity of data regarding the effect of temperature on multimodal courtship in fish and on reproduction outcome. Temperature may influence visual courtship behaviour through its effect on general activity (Wilson, 2005; Bartolini et al., 2015) and affect acoustic communication through changes in central and peripheral processes of signal production and reception (Ladich, 2018). For example, the painted goby (*Pomatoschistus pictus*) produces pulsed courtship sounds called drums whose pulse period is linearly related with temperature (Vicente et al., 2015). Also, the Padanian goby (*Padogobius bonelli*) and the Arnogoby (*Padogobius nigricans*) produce tonal courtship sounds that are similarly influenced by temperature, showing a decrease in burst period of 1ms per degree Celsius (Lugli et al., 1997; Torricelli et al., 1990). In a context of climate change and rising sea temperatures, developing a better understanding of the impact of elevated temperatures on fish communication and reproduction is crucial to devising efficient conservation measures.

This study investigates how temperature affects the relationship

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between multimodal courtship behaviour (visual and acoustic) and spawning success in the two-spotted goby, *Pomatoschistus flavescens*. This is a small, semi-pelagic species, with marked sexual dimorphism, and parental care. Males are known to use multimodal communication signals to attract females to their nests that include visual displays and sounds, namely drums and thumps (de Jong et al., 2018a). However, how temperature may change multimodal courtship and reproductive success remains to be clarified. Here, mating sounds and visual courtship were analysed for 39 males recorded under two temperatures, 16 and 20 °C (±1 °C), that are representative of the natural variability experienced by the species during the breeding season.

2. Material & methods

2.1. Ethical note

Experiments were authorized by the Portuguese National Authority for Animal Health - Direção Geral de Alimentação e Veterinária (DGAV reference 0421/000/000/2021) and performed in strict accordance with the EU Directive 2010/63/EU for animal experiments. The study also followed the recommendations of the Animal Care and Use Committee of the University of Lisbon.

2.2. Experimental animals

Two-spotted gobies were collected by scuba diving at depths down to 8 m, in the Arrábida Marine Park, Portugal (38° 28′ N; 8° 59' W), in December 2021. Fish were transported to laboratory facilities where they were housed under a natural light cycle, in 200L tanks filled with artificial filtered seawater, kept at 16 °C by a chiller (Hailea HC300A, Hailea Group Co., Ltd – China). The holding tanks featured sand substrate and shelters and artificial algae as environmental enrichment. The fish were fed daily *ad libitum* with a mix of artemia, chopped mussels and shrimps.

2.3. Experiments

Experiments were carried out in July and August 2022. Experimental setup consisted in six 35L aquaria (51 × 31 × 26 cm) divided into three compartments of equal size by transparent perforated plexiglass partitions, following the design of previous studies (Amorim et al., 2013; Bolgan et al., 2013; Vicente et al., 2015, Fig. 1). Two females were placed in the central compartment to increase the chances of having a ripe female in the experimental tank. A male and an artificial nest made of PVC tubes were placed in each lateral compartment. The inside of each nest was covered with a plastic sheet to facilitate the removal and photography of the eggs in the nest and to prevent males from entering the top chimney used to position one of the hydrophones (see below). The nests were placed on top of 5 cm high bags of pebbles since two-spotted goby courts in the water column (Magnhagen et al., 2014).

Three experimental aquaria were kept at a 16 °C (±1 °C) and 3 others at 20 °C (±1 °C). The water temperature was controlled by six aquaria chillers (Hailea HC300A). A hydrophone HTI-96-Min (High Tech Inc., USA; sensitivity: 165 dB re. 1V/μPa) was inserted in the chimney of the artificial nests during experiments to maximize the signal to noise ratio (SNR) of the fish sound recordings since two-spotted goby males often produce sounds at the entrance of their nests (de Jong et al., 2018a). Experimental aquaria were also placed on top of two 3 cm thick marble slabs interspaced with two levels of rubber foam shock absorbers, which significantly minimized the conduction of floor born vibrations (Amorim et al., 2013; Vicente et al., 2015). Another hydrophone (High Tech 94 SSQ, sensitivity of – 165 dB re. 1V/μPa, flat frequency response up to 6 kHz ±1 dB, High Tech Inc., Gulfport, MS, USA) was positioned near the centre of the aquarium. Experimental animals were transferred from the stock tank to the experimental aquaria at least 24 h before the beginning of an experiment. Animals were slowly acclimatized in aquaria kept at 18 °C for at least 24 h when moved from 16 °C to 20 °C or back to 16 °C to minimize thermal stress.

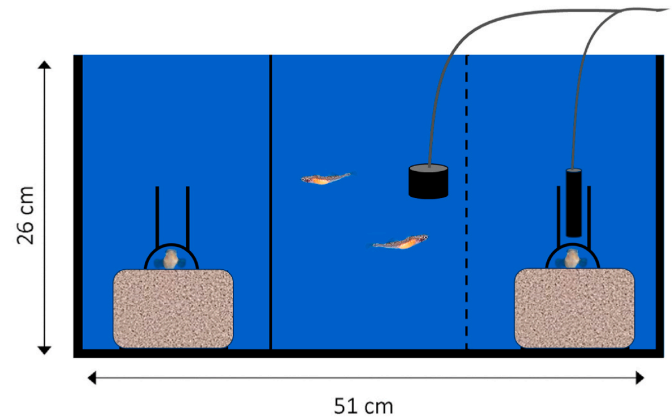

Fig. 1. Schematic diagram of the recording setup used to record reproductive interactions at 16 °C and 20 °C in the two-spotted goby. The tank is divided in three compartments. During trials one opaque partition (continuous line) is added to prevent male-male interactions. Trials begin by removing the transparent perforated partition (dotted line) in the opposite side to allow the territorial male to interact with the females. Two hydrophones, one in the nest chimney and the other in the female compartment, allow the recording of the male's sounds produced inside and outside the nest. A video camera (not shown) is positioned in front of the tank to capture visual behaviour. Synchronous audio and video signals are recorded in a laptop.

The experiment consisted in removing the partition separating the ripe two-spotted goby females from one focal male allowing them to interact for 24 h. During each trial, the fish were recorded on video and through hydrophones for three sessions of 30 min each. The pumps of the filters and chillers were turned off during recording sessions to minimize background noise. One aquarium at 20 °C and one aquarium at 16 °C were tested at each experimental session. The two aquaria were always recorded one right after the other, the order being random. The first recording session started around 10am, the second at 2pm and the third at 6pm. Each male was tested only once while females could be tested several times. Females were chosen from the 200L stock tank based on the colour and volume of their bellies. Females were kept in the same experimental aquaria for a maximum of 1 week if they looked ripe (swollen orange belly) and had not spawned yet. At the end of each experiment, males and females that laid eggs were measured (±1 mm), weighed (±10 mg) and returned to holding tanks, and eggs were photographed. Females which did not look ripe or that had been in the experimental tank for a week were similarly measured, weighed and put in holding tanks as well. The 20 males tested at 16 °C had a mean standard length (SL) of 4.12 cm (sd = 0.33, range = 3.5–4.9 cm), and a mean weight of 1.05 g (0.33, 0.45–1.84 g). The 40 females tested at 16 °C had a mean SL of 3.67 cm (0.16, 3.3–3.9 cm) and a mean weight of 0.58 g (0.13, 0.39–0.87 g). The 19 males tested at 20 °C had a mean SL of 4.11 cm (0.23, 3.8–4.6 cm) and a mean weight of 0.94 g (0.24, 0.63–1.63 g). The 38 females tested at 20 °C had a mean SL of 3.63 cm (0.15, 3.3–3.9 cm) and a mean weight of 0.58 g (0.16, 0.26–0.91 g). There were no significant differences between the two treatments in size or weight for both males and females (Wilcoxon-Mann-Whitney test, W = 145.5–248, $p > 0.05$). Animals were euthanized at the end of the experiment using an excess dose of MS222 buffered with sodium bicarbonate.

2.4. Audio and video recordings and analyses

Sounds captured by the hydrophones were digitized with a Cakewalk UA25EX (Roland, Japan; 16 bit, 6 kHz acquisition rate) to a laptop controlled by Cool Edit Pro (v2.0). Visual behaviour was recorded using two cameras (Sony HDR-CX240E and Sony DCR-SR15), one registering a global view (male and female compartment) and the other zooming in at the nest entrance to capture more detailed behaviour associated with the nest. The video signal from the DCR-SR15 and a synchronized audio signal (derived from the audio recording chain) were digitized with Pinnacle Dazzle DVD Recorder Plus (Pinnacle Systems, Mountain View, USA) to the laptop used for audio recordings.

Sounds (drums and thumps, *sensu* Amorim and Neves, 2007) and visual courtship behaviour were quantified using BORIS (Friard and Gamba, 2016) for each 30 min session. Recorded visual courtship behaviours are presented in Table 1. Two-spotted goby drums are low-frequency (main frequency <300 Hz) pulse trains whereas thumps are low-frequency (<150 Hz) non-pulsed sounds (cf. Fig. 5b in de Jong et al., 2018a), easily distinguishable by the human ear. Note that although small tanks can affect sound features due to reverberation and tank resonance (Akamatsu et al., 2002), we do not consider that it significantly affects the sounds produced by the two-spotted goby males as they are very low in amplitude and attenuate to background levels within a few cm from the source (Amorim et al., 2013, 2015), making it unlikely that they are significantly distorted by the tank. Also, because most sounds are produced when both the male and the female are inside the nest, i.e. in very close proximity to the hydrophone, we are confident that the acoustics of the experimental tanks did not affect the recorded sounds. In addition, this model species is known to present its natural sexual and reproductive behavioural repertoire both in the field and in the laboratory, further suggesting that visual behaviour was likewise unaffected by the experimental setup (Amundsen, 2018).

2.5. Data analysis

Statistical analyses were performed with R version 4.2.2 (R Core Team, 2022), with a significant level $\alpha = 0.05$ for all tests.

The relationship between temperature (water temperatures recorded just before the start of each of the three recording sessions), Julian day, number of eggs, male standard length, male weight, male Fulton's K (used as a proxy for body condition, where K = W/SLˆ3; Chellappa et al., 1995), "thump", "drum", "lead swim", "fast approach", "approach", "fin display", "outside courtship", "near", "push", "male in", "sigmoid display" and "female in" were tested with Pearson correlation tests.

Male courtship behaviours outside the nest ("lead swim", "approach", "fast approach" and "fin display") were significantly positively correlated between themselves (Fig. 2). For this reason, we summed them up in a new variable called "outside courtship".

Preliminary analysis showed that most of the tested behaviours occurred during the first session. However, we chose to present the analysis of the sum of the three sessions in the results because it is a more truthful depiction of the totality of the interactions that occurred during the 24 h of each experiment. The analysis of the data obtained during the first session is available in the Supplementary Material.

The effect of temperature treatment (16 or 20 °C) on courtship behaviours was tested with Wilcoxon-Mann-Whitney tests with continuity correction. The square root of "courtship outside" was used when we tested the effect of temperature treatment on this variable. This transformation rendered the distributions of the two samples closer to normal and made their variances more similar. In turn, it made the Wilcoxon-Mann-Whitney test with continuity correction more powerful.

Differences in courtship behaviours between males that succeeded to mate and males that did not were likewise tested with Wilcoxon-Mann-Whitney tests with continuity correction.

To investigate predictors of spawning success, binomial regressions featuring spawning success as dependent variable were computed and compared using a backward approach from the full model to the simplest model. The explanatory variables featured in the full model were "mean temperature", "male standard length", "drum", "thump", "outside courtship", "near", "push", "male in" and "female in". Binomial regressions were compared two by two using AIC, Bayes factor and p-values.

To investigate predictors of the number of eggs, linear regressions featuring the number of eggs as dependent variable were computed and compared using a backward approach from the full model to the simplest model. The explanatory variables featured in the full model were "mean temperature", "male standard length", "drum", "thump", "outside courtship", "near", "push", "male in" and "female in". Linear regressions were compared two by two using AIC, Bayes factor and p-values.

Table 1
List of courtship behaviours of the two-spotted goby.

Behaviour	Description	Reference	Behaviour type
Lead swim	The male swims away from the female with undulating fin and body movements.	Amundsen and Forsgren (2001); Pélabon et al. (2003)	Event
Fin display	The male swims close to the female with fins erect.	Amundsen and Forsgren (2001); Pélabon et al. (2003)	Event
Fast approach	The male swims rapidly towards the female.	de Jong et al. (2018a)	Event
Approach	The male swims toward the female.	de Jong et al. (2009)	Event
Sigmoid display	The female arches its back.	Amundsen and Forsgren (2001)	State
Male in	Time spent in the nest by the male.	Amorim and Neves (2007)	State
Female in	Time spent in the nest by the female.	This study	State
Near	Time spent near the nest by the male.	This study	State
Push	The male pushes the female out of the nest.	This study	Event
Drum	The male drums.	de Jong et al. (2018a)	Event
Thump	The male thumps.	de Jong et al. (2018a)	Event

3. Results

Two-spotted goby males used only visual courtship (e.g. approaches, fast approaches, fin displays and lead swims) and not acoustic courtship when they were outside the nest. When females were in front of the entrance of the nest the male typically thumped. Once the female was inside the nest, the male sometimes (11 males out of 39 displayed this behaviour) thrusted itself forward from inside the nest towards the entrance (what we called the "pushing" behaviour) and the female usually came out of the nest at this moment (37 out of 48 occurrences). This courtship cycle repeated itself until the female stayed in the nest when the male "pushed". Once both individuals were in the nest, the males drummed on and off. Females typically stayed in the nest for about 45 min when spawning occurred (personal observation).

3.1. Effect of temperature on visual and acoustic courtship

Considering the sum of the three 30 min recording sessions per male, the mean water temperature was significantly and positively correlated with (total) outside courtship (Pearson correlation, $n = 39$, $r = 0.4$, $p = 0.03$), fast approach ($r = 0.4$, $p = 0.03$) and fin display ($r = 0.3$, $p = 0.04$), but showed no relation with behaviours performed inside the nest, including sound production (Fig. 2).

The total number of male courtship behaviour performed outside the nest (outside courtship) was significantly higher (Wilcoxon-Mann-Whitney test, $W = 97.5$, $p < 0.01$, Fig. 3) at 20 °C (mean ± SD, range = 6.23 ± 3.52, 1.00–15.78, $n = 19$) than at 16 °C (3.40 ± 2.22, 0.00–7.62, $n = 20$). There was no effect of treatment in any of the other considered variables (near, push, male in, thump, drum, sigmoid display and female in) (Wilcoxon-Mann-Whitney test, $W = 163.5–248.0$, $p > 0.05$).

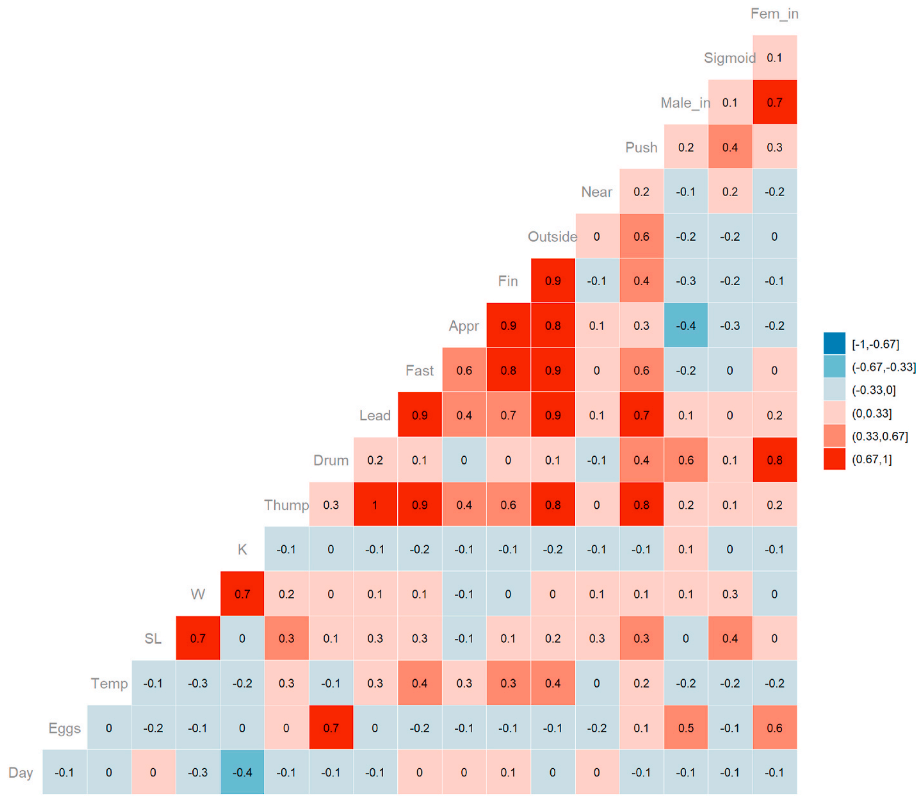

Fig. 2. Heat map of the correlations male size and condition, tested behaviours, Julian day, temperature recorded just before the start of each experimental day and number of obtained eggs. Day = Julian day, Eggs = number of eggs, SL = male standard length, W = male weight, K = male Fulton's K, Lead = Lead swim, Fast = fast approach, Appr = approach, Fin = fin display, Outside = outside courtship, Sigmoid = sigmoid display and Fem_in = female in. Numbers inside squares are Pearson correlation coefficients. N = 39 except for the correlations with number of eggs, where n = 36. The two darker red and blue shades represent significant correlations (p-values are available in the Supplementary Material).

3.2. *Predictors of spawning success*

The number of drums was significantly higher (Wilcoxon-Mann-Whitney test, W = 46.5, $p < 0.01$; Fig. 4A) when spawning occurred (142.37 ± 95.22, 0–304, n = 19) than when spawning did not occur (19.55 ± 46.88, 0–170, n = 20); median number were 142 *vs.* 0 drums. Likewise, the number of thumps was significantly higher (Wilcoxon-Mann-Whitney test, W = 116, p = 0.04; Fig. 4B) when spawning occurred (12.79 ± 22.54, 0–87, n = 19) than when spawning did not occur (19.4 ± 65.25, 0–286, n = 20); median number were 5 *vs.* 0 thumps. Note that the mean number of thumps (but not the median) is higher for males that did not successfully mate because this measure is driven by one male that made 286 thumps.

Females spent more time inside the nest when spawning occurred (1617.48 s ± 897.91 s, 0–3545.59 s, n = 19) than when spawning did not occur (271.50 s ± 612.22 s, 0–2021.61 s, n = 20) (Wilcoxon-Mann-Whitney test, W = 55, $p < 0.01$; Fig. 5A). Similarly, males spent more time inside the nest when spawning occurred (4227.42 s ± 857.16 s, 1625–5400 s, n = 19) than when spawning did not occur (2377.01 s ± 1497.32 s, 225.23–4515.75 s, n = 20) (Wilcoxon-Mann-Whitney test, W = 53, $p < 0.01$; Fig. 5B). The remaining variables (outside courtship, near, push, male SL, weight, and Fulton's K, and female sigmoid display) did not differ between males that succeeded or not in receiving eggs (Wilcoxon-Mann-Whitney test, W = 159.5–246.0, $p > 0.05$).

The binomial regression analysis showed that the number of drums was the only significant explanatory variable for spawning success (Table 2). It showed that an increase of one drum caused an increase of 0.022 in the log odds of spawning success (Fig. 6).

Consistently, the regression analysis investigating significant predictors for the number of eggs showed that the number of drums was the only significant explanatory variable in the model ($F_{1,34} = 41.0$, $p < 0.01$), when considering the sum of the behaviours that occurred during the three sessions. It showed that an increase of one drum caused an increase of 1.46 in the number of eggs (Table 3, Fig. 7).

4. Discussion

In this study we found that in two-spotted gobies visual courtship performed outside the nest was more frequent at 20 °C than at 16 °C. However, there was no temperature mediated difference in the time spent in the nest by males or females or any other courtship variable, including acoustic activity. Also, we found no temperature effect on the other tested variables, including spawning success and the number of eggs. Importantly, we show that from the tested variables, drumming activity was the only that significantly influenced spawning success.

4.1. *Effect of temperature on visual and acoustic courtship*

We only found a temperature treatment effect on visual courtship performed outside the nest, which encompass the most vigorous movements associated with mating in this species. The remaining visual courtship takes place inside the nest and includes pushing the female out of the nest (see Table 1) or staying, mostly still, inside the nest. In fact, males spent most of the time inside the nest (Fig. 5B). The increase of outside courtship with temperature is thus consistent with the known effect of water temperature on fish social and general activity (Bartolini et al., 2015) and on physiological processes underlying reproduction and behaviour (Zahangir et al., 2022). Interestingly, the increase of activity performed outside the nest did not seem to affect mating performance in the studied species as we found no temperature effect on spawning success including in the number of eggs. This finding is in accordance with Lopes et al. (2022) who did not find an effect of

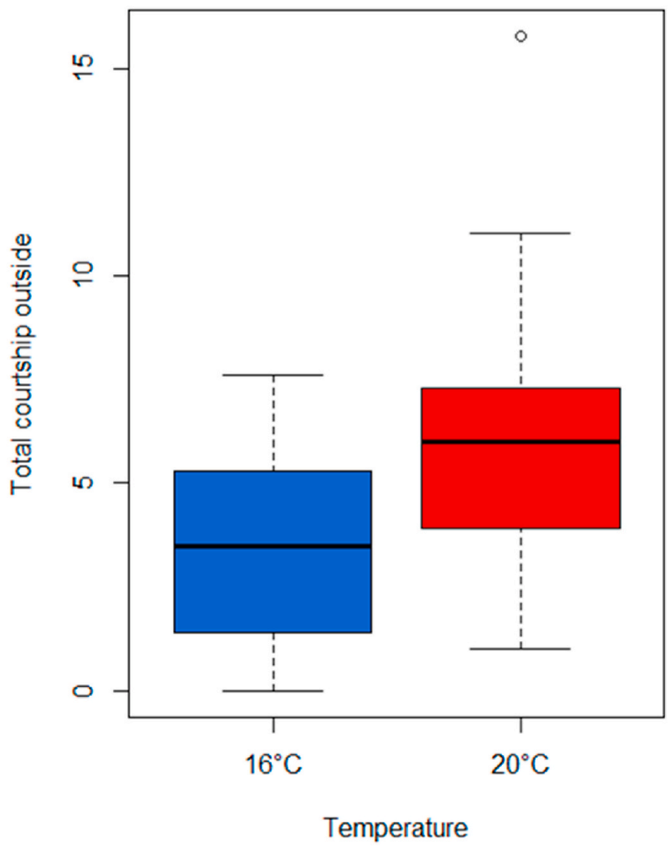

Fig. 3. Comparison between the total number of courtship behaviours performed outside the nest by males at 16 °C and 20 °C, considering the sum of the three 30 min mating sessions. Outside courtship is square root-transformed. The boxplots represent medians (thick middle line), the 25th to 75th percentiles (boxes), range (whiskers) and outliers.

temperature in the two-spotted goby reproductive success either. However, in an earlier paper by Lopes and colleagues (Lopes et al., 2020) breeding pairs of two-spotted gobies kept in water 3 °C warmer than ambient temperature had a lower spawning success and a lower number of eggs. In Lopes et al. (2020), water temperature in the high temperature treatment reached more than 22 °C while in Lopes et al. (2022) and in the present study the high temperature treatment only reached a maximum of 20 °C. This suggests that in the two-spotted goby, spawning success and the number of eggs start to decrease somewhere between 20 °C and 22 °C, i.e. likely when going outside the optimal temperature range (Henriques et al., 2007). This discrepancy could also reflect a population dependent effect. The fish used in Lopes et al. (2020) were sampled from a Swedish population, near the Northern limit of the species' distribution, while the fish used in this study and in Lopes et al. (2022) were from the same Portuguese population near the Southern limit of the species' distribution. Experimenting with more temperature classes, including temperatures outside the species' natural temperature range, and more individuals, could lead to a more precise idea of the effect of temperature on visual behaviour, spawning success and number of obtained eggs.

Two-spotted goby males did not make more sounds at 20 °C than at 16 °C. Temperature has a clear role on the onset and phenology of mating calling activity in fishes (Rice et al., 2016; Monczak et al., 2022) as temperature plays a key role in initiating the spawning seasons by stimulating gonadal maturation and mating behaviour (e.g. McQueen and Marshall, 2017). However, within the mating season, studies report mixed results with either a positive effect of temperature on mating calling activity or no effect (Ladich, 2018). For example, similarly to the present study, Vicente et al. (2015) found that male calling rate (either drums or thumps) was not related with temperature in the closely related painted goby. In contrast, in some sciaenid and batrachoidid species, calling activity increases with temperature within the species-specific temperature range such as in wild and captive spotted sea trout (Montie et al., 2015; 2017), in wild meagre (Vieira et al., 2022), and in wild Lusitanian toadfish (Amorim et al., 2006). Different studies also found contrasting results for the same species. For example, Montie et al. (2015) reported no temperature effect on sound production in the sciaenids red drum, black drum and silver perch recorded in the May River in contrast with the findings of Monczak et al. (2022) who found a significant role of temperature on chorusing activity of the same species, in the same location. Passive acoustic recordings of the oyster toadfish also carried out in the May River have also pointed to

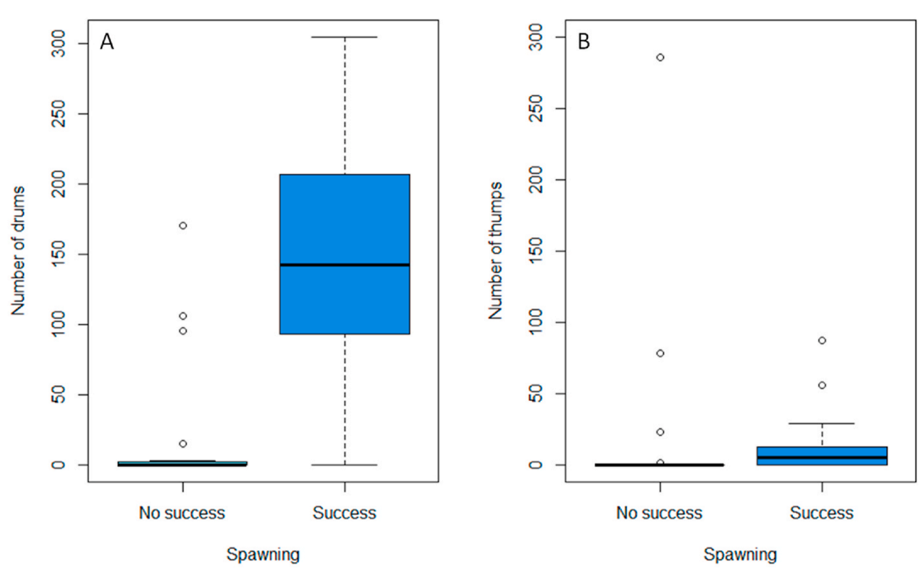

Fig. 4. Comparison of the number of drums (A) and thumps (B) produced by males in the three 30 min mating sessions as a function of spawning success. The boxplots represent medians (thick middle line), the 25th to 75th percentiles (boxes), range (whiskers) and outliers.

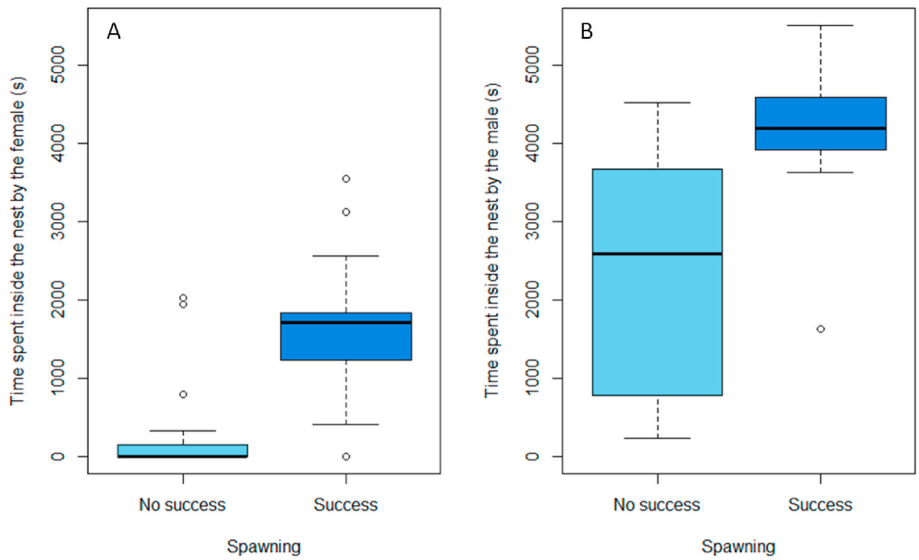

Fig. 5. Comparison of time spent by females (A) and males (B) inside the nest in the three 30 min mating sessions as a function of spawning success. Note that the three sessions duration amount to 5400 s (3 × 30 min). The boxplots represent medians (thick middle line), the 25th to 75th percentiles (boxes), range (whiskers) and outliers.

Table 2
Results from the best fitting generalized linear model (binomial family) predicting spawning success.

Effect	Estimate	Std. Error	Z value	p-value
Intercept	−1.514	0.553	−2.738	0.00619
Number of drums	0.022	0.007	3.278	0.00105

Null deviance: 54.04 on 38 degrees of freedom. Residual deviance: 33.82 on 37 degrees of freedom.

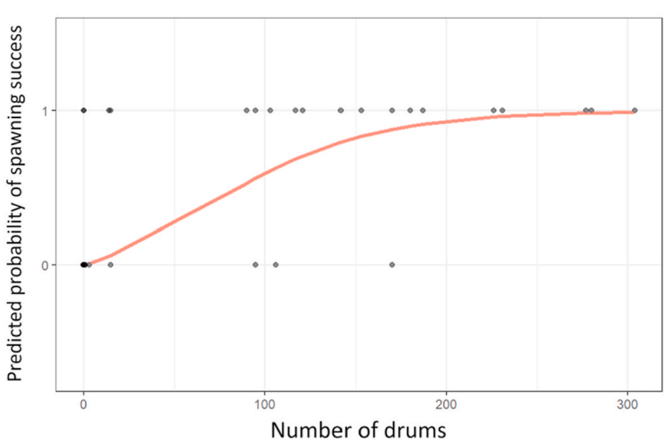

Fig. 6. Plot of the predicted probability of spawning as a function of the number of drums produced in the three recording sessions. The line represents the binomial regression model estimates for the relationship between number of drums and spawning success on data pooled for all temperatures. Note that temperature did not have a significant effect on spawning success.

Table 3
Results from the best fitting linear model predicting spawning the number of laid eggs.

Effect	Estimate	Std. Error	t value	p-value
Intercept	−5.211	28.963	−0.180	0.858
Number of drums	1.465	0.229	6.403	<0.01

Residual standard error: 132.8 on 34 degrees of freedom. Adjusted $R^2 = 0.53$.

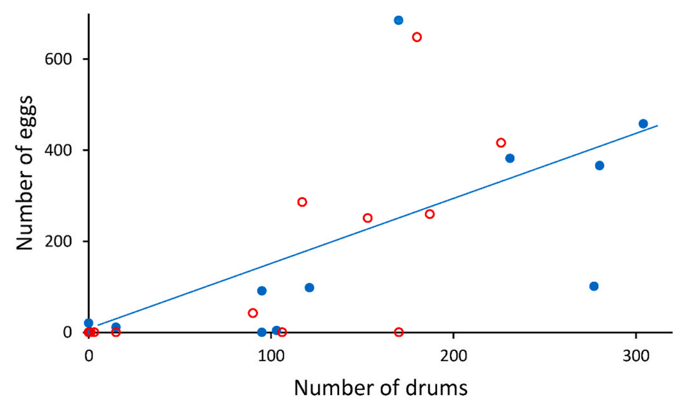

Fig. 7. Relationship between the number of laid eggs and the number of drums on data pooled for all temperatures (16 °C - filled blue circles, 20 °C – open red circles) and recording sessions.

contrasting results of the effect of temperature on mating calling activity (positive effect, Montie et al., 2015; no effect, Monczak et al., 2017). These mixed findings may reflect differences in recordings methodologies (very short (2 min) *vs.* long-term recordings) and the presence of confounding ecological factors that affect sound production, such as seasonal, daily and lunar rhythms, tidal cycles, water depth and salinity (Ladich, 2018). Our study was carried out in a relatively short time span (1.5 months) in the peak of the two-spotted goby breeding activity and in controlled lab environment which should isolate the effect of temperature from other variables, but its interaction with other ecological factors remains to be ascertained. Note that 16–20 °C is within the natural temperature range for mating in this species (Henriques et al., 2007) and that temperature was kept stable. It is likely that an effect of temperature on calling activity would be found if more extreme temperatures or sudden temperature shifts would be tested. Indeed, calling behaviour is typically disrupted outside species-specific optimal temperature ranges (Rice et al., 2016; Monczak et al., 2022) and a rapid rise or fall in temperature can increase or decrease acoustic activity, respectively (Amorim et al., 2006; Ricci et al., 2017; Monczak et al., 2017; Van Wert and Mensinger, 2019; Vieira et al., 2022).

4.2. Predictors of spawning success

Our regression analyses showed that among the tested variables, the number of drums was the sole predictor for spawning success, namely the likelihood of spawning and the number of obtained eggs. Both males and females spent more time inside the nest when spawning occurred, which is when male drumming and likely female mating decisions take place. Our results are in line with the findings of Amorim et al. (2013) for the closely related painted goby in which males that succeed to mate produce more drums than unmated males. These two studies suggest drumming is influencing female mate choice in *Pomatoschistus* spp. Consistently, successful mating depends on the male's calling activity in the Lusitanian toadfish (Vasconcelos et al., 2012; Amorim et al., 2016). In this batrachoidid the number of eggs in the nest is positively associated with the male's maximum calling rate and calling effort, defined as the percentage of time spent calling. As in both the painted goby and the Lusitanian toadfish calling activity presents a positive relationship with male energetic reserves (Amorim et al., 2010; 2013), it is possible that females are using calling activity to evaluate and select males in better condition. Consistent with this hypothesis, male reproductive success (number of egg clutches) in the damselfish *Dascyllus albisella* is correlated with visual and concurrent acoustic courtship rate (Oliver and Lobel, 2013), which in turn is linked with male fat reserves (Knapp and Kovach, 1991). Note that in our study we did not characterise drum acoustic features, but they could also be playing an important role in the female's mating decisions. For example, the plainfin midshipman females are attracted differentially to the playback of male mating hums differing in fundamental frequency, duration, amplitude modulation and fine temporal patterns, suggesting that they could use these acoustic features in mate choice (McKibben and Bass, 1998). Interestingly, this has been the only species thus far where it has been shown that water temperature induces parallel changes in male sound features (e.g. fundamental frequency) and in female preference - known as temperature coupling (Ladich, 2018).

In this study, the remaining tested variables were not correlated with spawning success or the number of eggs, suggesting that visual courtship seems to play a less prominent role than acoustic signalling in mate selection. This is also the case in the painted goby (Amorim et al., 2013). In the present study, spawning only occurred 19 times (12 at 16 °C and 7 at 20 °C) and therefore, the regression analyses featuring the number of eggs as a dependent variable should be considered with caution. Experimenting with more individuals could give a more accurate idea of the effect of the number of drums on the number of laid eggs. de Jong et al. (2018a) showed that both the two-spotted goby and the painted goby males reduced acoustic courtship in the presence of added continuous sound with frequencies overlapping these species' acoustic signals. Interestingly, when the painted goby was exposed to added sound, the females' attention shifted from the males' acoustic displays to the male's visual displays (de Jong et al., 2018b). These authors found that while acoustic courtship was the only predictor of spawning success in the control treatment, male visual courtship was also a significant predictor of spawning success in the added sound treatment (de Jong et al., 2018b). This suggests that the relative importance of visual and acoustic courtship could shift when one of the sensory channels is compromised. It is therefore likely that in our studied species visual displays could have a more prominent role under acoustic masking or under other altered ecological scenarios. But even in unaltered scenarios, visual courtship could have an important role. For example, females might be attracted by the male visual signals but then use acoustic signals for their final mating decision. Also, visual signals could not only be used as back-up message (as suggested above) but could be providing additional information that is only available in the combined signal components (Halfwerk et al., 2019). If so, only more complex experiments using manipulated signals would be able to disentangle the relative roles of the two sensory channels in this species' mating decisions.

4.3. Conclusions and future perspectives

Our results and the ones of de Jong and colleagues (de Jong et al., 2018a, 2018b) suggest that assessing acoustic and visual courtship could give important insights on the effect of global warming and other anthropogenic factors on reproduction outcome in gobies and likely other fishes (e.g. Monczak et al., 2022). To streamline experiments, its design should be optimised. In our study, using the first 30 min session or the sum of all three sessions provided similar results. Therefore, we suggest that using shorter trials (e.g. 30–60 min) and having the filters and chillers placed in another room to avoid having to turn off the pumps and chillers to reduce background noise could greatly improve trial efficiency and allow longer-term experiments in controlled environments.

The effect of temperature on fish acoustic communication and spawning success could be tested using a wider set of temperatures (including non-optimal temperatures) and in a more natural scenario, for example, one in which both males and females would have more available sexual partners to choose from, and/or if experiments were ran outside the peak of the breeding season. This species has exceptionally dynamic sexual selection and displays a complete reversal of sex roles within the reproductive season, being heavily male-biased at the start of the season and heavily female-biased late in the season (Amundsen, 2018). This means that traditional sexual roles are expected at the start of the season when there are few ripe (and choosy) females available but that late in the season male mate choice becomes more prevalent (Forsgren et al., 2004). The relative role of visual and acoustic courtship on mating outcome along the reproductive season in a changing sexual role scenario and the concurrent influence of temperature has never been studied but could elucidate how populations react to temperature variability along the reproductive season. Future studies should thus address more complex scenarios such as by varying the number of available males and females (the operational sex ratio) and by testing a wider range of temperatures as well as temperature variability (e.g. heat waves). Complementary experiments should also be carried out in nature, for example, in Nordic fjords where field work is more amenable than in the Ocean (Forsgren et al., 2004), to investigate how temperature affects the phenology of reproductive behaviour, including acoustic courtship and reproduction outcome.

In our study we tested the effect of two temperature levels that the two-spotted gobies typically experience in the spring-summer period on this species mating behaviour and reproductive outcome. Temperature influenced visual courtship outside the nest, but we found no evidence that it affected either acoustic courtship or mating success. Here, we show that within the natural temperature range, calling activity (drumming) was the main predictor of reproduction success in the two-spotted gobies. Finding the link between acoustic behaviour and reproduction, such as in the present study, offers the opportunity to eavesdrop on fish courtship sounds both in the lab and in nature to learn how fish respond to environmental changes and human impacts such as global warming.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

data is in the supplementary material

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Appendix A. Supplementary data

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