Behavioral Ecology
doi:10.1093/beheco/arr199
Advance Access publication 12 December 2011

Original Article

Vocal behavior predicts reproductive success in a teleost fish

Raquel O. Vasconcelos,[a] **Rita Carriço,**[a] **Andreia Ramos,**[a] **Teresa Modesto,**[b] **Paul J. Fonseca,**[a] **and M. Clara. P. Amorim**[c]
[a]Departamento de Biologia Animal, Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Bloco C2 Campo Grande, 1749-016 Lisbon, Portugal, [b]Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8000-810 Faro, Portugal, and [C]Unidade de Investigação em Eco-Etologia, I.S.P.A. – Instituto Universitário, Rua Jardim do Tabaco 34, 1149-041 Lisbon, Portugal

The relation between acoustic signaling and reproductive success is important to understand the evolution of vocal communication systems and has been well studied in several taxa but never clearly shown in fish. This study aims to investigate whether vocal behavior affects the reproductive success in the Lusitanian toadfish (*Halobatrachus didactylus*) that relies on acoustic communication to attract mates. We recorded 56 nest-holding (type I) males during the breeding season and analyzed the calling performance and acoustic features of the mate advertising sounds (boatwhistles) exhibited over circa 2 weeks. Hormonal levels of the subjects and the number of eggs (reproductive success) present in the respective nests were quantified. Nesting males attracted both females and other males, namely smaller type I males with significantly lower total length (TL), body condition, sonic muscle mass, gonad mass, and accessory glands mass. Calling rate (CR), calling effort (CE) (% time spent calling), and sound dominant frequency were significantly higher in nesting males with clutches than in those without clutches. Sex steroids (11-ketotestosterone and testosterone) were not correlated with vocal parameters or number of eggs. Maximum CR and CE were the best predictors of the number of eggs. In addition, these vocal variables were best explained by male's TL, condition, and sonic muscle mass. We provide first evidence that vocal behavior significantly determines reproductive success in a vocal fish and show that acoustic signaling at higher and constant rates can operate as an indicator of the male's size and body condition and probably of elevated motivation for reproduction. *Key words:* acoustic communication, Batrachoididae, mate attraction, reproductive success, toadfish. *[Behav Ecol 23:375–383 (2012)]*

INTRODUCTION

Many studies on communication systems have centered on the relationship between signals and reproductive success. Determining the characteristics of signals that lead to enhanced mating success may help in understanding how a communication system may have evolved and how sexual selection may have shaped signaling (Andersson 1994; Bradbury and Vehrencamp 1998).

Acoustic signals are well-known examples of sexually selected traits typically used by females of several taxa to identify, locate, and select between potential mates (Andersson 1994; Bradbury and Vehrencamp 1998). The effect of acoustic signaling on mate attraction has been broadly investigated primarily in insects, anurans, and birds. These studies reported that features of males' calling, such as song complexity, repertoire size, amplitude, singing effort, and conspecific acoustic interactions, can be indicators of individual quality. This can be related with males' resources, health condition, learning ability, developmental resilience to stress or social skills (e.g., Searcy and Andersson 1986; Kroodsma and Byers 1991; Nordby et al. 1999; White et al. 2010).

Teleost fishes most likely represent the largest group of sound-producing vertebrates that have evolved a variety of mechanisms to produce vocalizations crucial to social interactions, including mate attraction (Ladich & Myrberg 2006; Myrberg and Lugli 2006). However, a link between vocal behavior and reproductive success has never been directly shown. Few studies have demonstrated the role of certain acoustic signals in mate choice and related sound features with male size. For example, females of the bicolor damselfish (*Stegastus partitus*, Pomacentridae) prefer not only males that produce courtship chirp sounds of lower dominant frequency, which are positively correlated with body size (Myrberg et al. 1986), but also males with higher rates of courtship visual displays (Knapp and Kovach 1991). A male's courtship rate correlates positively with mating success and subsequent egg survival, suggesting that chirp sound rate may also relate to reproductive success; however, this has never been directly shown (Knapp and Kovach 1991). The existence of redundant multimodal signals has been previously reported (revised in Partan and Marler 2005). Male vocal features, such as dominant frequency, seem to be a common indicator of body size in vocal fish species (e.g., croaking gouramis, Ladich et al. 1992; mormyrids, Crawford 1997).

Representatives of the family Batrachoididae, which include toadfishes and the plainfin midshipman fish (*Porichthys notatus*), have emerged as one of the main study models for both behavioral and neurobiological studies in fish acoustic communication (Bass and McKibben 2003). Winn (1972) reported female phonotaxis toward a male whose mate advertising signals, called boatwhistles, were emitted at higher calling rates (CRs) in

---

Address correspondence to Raquel Vasconcelos. E-mail: rfvasconcelos@fc.ul.pt.

Received 19 May 2011; revised 23 October 2011; accepted 23 October 2011.

Opsanus tau. Female midshipman fish also showed phonotaxis toward hum-like (mate attraction) sounds, namely longer, higher amplitude and higher fundamental frequency tone stimuli (McKibben and Bass 1988). Recently, Amorim et al. (2010) reported that male vocal activity and fine acoustic features of mating call (boatwhistle) reflect several aspects of male morphological characteristics in another batrachoidid, the Lusitanian toadfish (*Halobatrachus didactylus*). Males that contracted the sonic muscles at faster rates, as shown by the shorter boatwhistle pulse periods, were in better condition (increased body lipid and higher liver mass), and boatwhistle amplitude modulation reflected the degree of sonic muscle hypertrophy. Besides, this study also suggested that Lusitanian toadfish males advertise their quality based on boatwhistle CR and calling effort (CE), which was correlated with measurements of physical condition.

The major goal of this work was to verify whether vocal behavior (CR, CE, and signal features) can predict reproductive success, as measured by the number of eggs in a male's nest, in a vocal teleost. For this purpose, we tested the Lusitanian toadfish (*H. didactylus*), which strongly depends on acoustic communication for mating; males are polygynous, defend nests, and provide parental care. We also investigated the influence of sex steroids (11-ketotestosterone [11-KT] and testosterone [T]) that typically peak in the breeding season (Modesto and Canário 2003a), in the vocal performance and reproductive success. Moreover, the morphometric traits of males were also related with vocal parameters and number of eggs.

We used the Lusitanian toadfish as the study species for various reasons. This species relies heavily on acoustic communication to find mates in the breeding season. *Halobatrachus didactylus* exhibits a rich vocal repertoire rare among fishes, which comprises at least 5 different vocalizations (Amorim et al. 2008), including a complex amplitude-modulated advertisement call (Amorim and Vasconcelos 2008). Phylogenetic analysis indicated that Lusitanian toadfish represents a basal lineage in the Batrachoididae, providing an excellent model for understanding integrated mechanisms underlying the evolution of acoustic communication in fishes (Rice and Bass 2009). Moreover, the Lusitanian toadfish is highly tolerant to experimental manipulations, displays the full acoustic repertoire, and mates in seminatural situations (Amorim et al. 2010; Vasconcelos et al. 2010).

METHODS

Study species

The Lusitanian toadfish, *H. didactylus* (Batrachoididae), is a benthic marine fish that inhabits estuaries and coastal zones of the Eastern Atlantic and the Mediterranean (Roux 1986). During the reproductive season, from May to July in Portugal, territorial males ("type I") build nests under rocks in aggregations in shallow waters and attract females to spawn by emitting long advertisement calls (boatwhistles), forming conspicuous choruses (Amorim et al. 2006; Amorim and Vasconcelos 2008). Besides boatwhistles, Lusitanian toadfish also produces other pulsed sounds, such as grunt trains, long grunt trains, croaks, double croaks, and associations between some of these calls (Amorim et al. 2008). Some of these sounds are known to be used during agonistic interactions, such as territorial defense (e.g., grunt train, Vasconcelos and Ladich 2008; Vasconcelos et al. 2010), but the function of such vocal plasticity remains unclear. Females deposit the eggs on the roof of the nest, which are guarded by the male until the offspring are free swimming (dos Santos et al. 2000). Like other batrachoidids, this species is sexually dimorphic: sneaker or "type II" males are typically smaller, with a higher gonadosomatic index and smaller sonic muscles relative to type I male (Modesto and Canário 2003a, 2003b), and attempt opportunistic fertilizations by parasitizing the nests.

Test subjects

Prior to the onset of the breeding season, 60 concrete nests were placed along an intertidal area of the Tagus River estuary (Military Air Force Base, Montijo, Portugal) to create an aggregation of artificial shelters that were easily accessible at low tides during the whole breeding season, from May to July. These hemicylinder-shaped nests (internal dimensions: 50 cm long, 30 cm wide, and 20 cm high) were placed along the shore approximately 1.5 m apart in 2 rows.

We used a group of these nests (6 or 7) to confine type I toadfish males that spontaneously occupied these shelters. In total, we recorded 56 males (34–49.5 cm total length [TL]; 627–2097 g eviscerated body mass [ME]). At the end of the recording protocol, tested type I males did not differ in body mass and body condition from the other territorial type I males found in the nests along the shore in the study area (One-way ANOVA: $F_{1,77} = 0.44$–0.75; $P > 0.05$). The male morphotype was easily identified on the basis of body size and secretion of their large accessory glands when gently pressed near the vent (Modesto and Canário 2003a). All animals used in this study, including conspecifics attracted to the experimental nests, were dissected after being sacrificed with an excessive dosage of MS222 (tricaine methane sulphonate; Pharmaq, Norway) at the end of the study. Each subject was measured to the nearest millimeter for TL and to the nearest gram for ME. The gonads (M_G), the male accessory glands (M_{AG}), and the liver (M_L) mass were tallied to the nearest milligram. Sonic muscles, which are embedded in the swimbladder walls, were gently cut and weighed to the nearest milligram (M_{SM}). All experimental procedures complied with Portuguese animal welfare laws, guidelines, and policies.

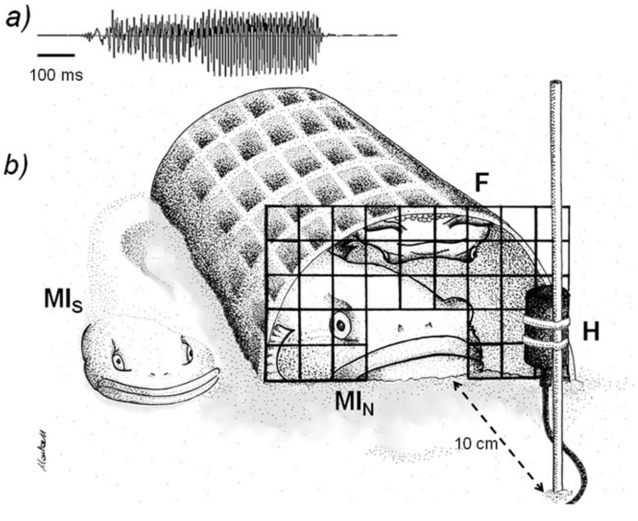

Figure 1
Oscillogram of a typical mate advertisement boatwhistle produced by a type I male Lusitanian toadfish during the reproductive season (a). Experimental setup (b), showing a vocal type I toadfish male (MI_N) confined inside the artificial nest, a female (F) inside the nest laying eggs and a satellite type I toadfish male (MI_S) that was often found outside the nest close to the entrance. A hydrophone (H) was placed in front of the nest entrance in order to record male's vocal activity. Illustration by Marta Bolgan.

Experimental setup

The vocal activity of 9 different groups of type I males was recorded over a period of 6 h per day (centered in the full tide) for about 2 weeks. Each experimental group was composed of 6 or 7 fish. These recordings were performed throughout the peak of the toadfish breeding season (May–July) in 2008 and 2010.

Nests were surrounded with a plastic net to prevent vocal type I males from escaping and to ensure individual identity throughout the recordings (see Vasconcelos et al. 2010; Figure 1). A small opening (10 cm wide, 5 cm high) was created at the entrance of each of these nests to allow females and, eventually, small type I or type II males to enter. These are typically smaller (generally TL <30 cm and body mass <500 g) than the tested type I males (Amorim et al. 2009). Plastic nets did not affect propagation of acoustic signals and allowed possible visual interactions with free-swimming conspecifics, as well as the entrance of prey items in the nest. All unoccupied nests within 15 m from a subject male were also wrapped in plastic nets to prevent further occupations during the study.

One hydrophone (High Tech 94 SSQ, Gulfport, MS; frequency range: 30 Hz–6 kHz, ±1 dB; voltage sensitivity: −165 dB re. 1 V/μPa) was placed at about 10 cm from the entrance of each experimental nest and 10 cm up from the substrate. The recording system also included audio capture devices Edirol UA-25 (Roland, 16 bit, 6 kHz acquisition rate per channel) connected to a laptop to perform simultaneous multichannel recordings, which were controlled with Adobe Audition 2.0 (Adobe Systems Inc., 2005). Sounds captured from each hydrophone were stored in approximately 60-min duration wave files and, therefore, about 6 recording sessions were acquired per day for each fish.

Estuary water temperature during the recording period ranged between 19.5 and 24 °C, and the water level varied from air exposure in the lower spring tides up to 2.8 m at high tide.

Every 2 weeks, when the tide was low enough to access the nesting experimental area (spring tides), any fish found outside close to the subject males' nests (i.e., partially under or on the side near the entrance) were identified (sex and male type), euthanized, and dissected in the laboratory. This procedure was only possible in the first breeding cycle (2008) when there were more specimens spawning in the study area.

Recorded type I males were also removed, anesthetized in an MS222 bath, and blood samples were collected within 5 min of handling the specimen. After finishing the recordings and removing the type I males from the respective nest, a photograph was taken whenever there were eggs attached to the roof of the nest. The number of eggs that seemed intact and viable (e.g., remains of egg membranes without yolk were not considered) was determined using the software Image J (Wayne Rasband, National Institute of Health). This software allowed analyzing the pictures with detail and marking each egg. All eggs were recognized and counted individually.

Sound analysis

All test fish produced several types of vocalizations that included agonistic grunts, croaks, and mate-advertising boatwhistles. We only quantified and analyzed the boatwhistles (Figure 1a) because these are the signals used for nest ownership advertisement and to attract females during the reproductive season in Batrachoididae (McKibben and Bass 1998; Amorim and Vasconcelos 2008). Acoustic analysis was performed using Raven 1.2 for Windows (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY). Recorded sounds could be attributed to particular nest holders due to proximity of the hydrophones to the subject males and because of the high sound attenuation along short distances with low water depth (≥21 dB between nests).

The following parameters were calculated for each individual: mean CR (CR_{mean}), as the averaged number of sounds emitted per hour (i.e., per recording session); maximum CR (CR_{max}), as the maximum CR per hour; active CR (CR_{active}), as averaged number of sounds emitted per hour excluding sessions without subject's vocal activity; and CE, percentage of time spent calling, that is, number of 15 min intervals with calling activity divided by the total number of recorded 15 min intervals multiplied by 100. CRs were tallied on a minute basis.

Moreover, in order to relate sound features with the males' vocal performance and reproductive success, we analyzed 15 boatwhistles per male (with high signal-to-noise ratio) selected randomly from all 56 fish. For the acoustic analysis, we adopted the classification used by Amorim and Vasconcelos (2008) that considers 3 distinct phases in the boatwhistle (beginning [P1], middle [P2 or tonal phase], and end [P3]), based on differences in pulse period and dominant frequency. The acoustic parameters measured were total duration (ms), from the start of the first pulse to the end of the last pulse; amplitude modulation, by dividing the mean (root mean square) amplitude measured in P1 by the one measured in P2; dominant frequency (of P2), as the highest energy component within the sound power spectrum (sampling frequency 8 kHz, Hamming window, filter bandwidth 10 Hz); fundamental frequency (of P2), calculated as the inverse of the average pulse period measured in the tonal phase.

Hormone assays

In order to determine whether sex steroid levels were related to the vocal performance and reproductive success of recorded toadfish, blood samples were collected from the caudal vein in heparinized syringes. This procedure was performed after finishing the recording protocol for each test group recorded in 2010.

Plasma was separated by centrifugation (5000 rpm for 5 min) and stored at −4 °C. Plasma samples (50 μl) were diluted in phosphate buffer (450 μl) containing 0.5 g/L of gelatin (pH 7.6) and denatured at 80 °C for 60 min. After samples cooled, steroids, T (17β-hydroxyandrost-4-ene-3-one), 11-KT (17β-hydroxyandrost-4-ene-3,11-dione), and cortisol (11b,17,21-trihydroxy-pregn-4-ene-3,20-dione) were measured by radioimmunoassay (RIA). Details of the RIAs methodology have been published elsewhere (Scott et al. 1984). RIAs were performed using duplicate amounts (100 μl) of denatured samples. Cross reactions of antisera used in RIAs for T, 11-KT, and cortisol were described previously in Kime and Manning (1982), Scott et al. (1984), and Rotllant et al. (2005), respectively. For each hormone, circulating plasma levels from all animals were measured within the same assay. Average intraassay and interassay coefficient of variations for RIAs were 1.0% and 5.2% for T, 1.3% and 5.5% for 11-KT, 6.4% and 10.3% for cortisol, respectively.

Statistical analysis

Following Amorim et al (2010), we used residuals of the simple linear regression of sonic muscle mass on ME (RM_{SM}) as a metric of sonic muscle hypertrophy. Likewise, we used the residuals of the simple linear regressions of gonads, accessory glands, and liver mass on ME (RM_G, RM_{AG}, and RM_L, respectively) to control for the influence of body size. This metric gives a measure of an observed organ mass relative to a mean expected value (given by the regression model) for a given body size. Moreover, we used the residuals of ME on TL

(COND) as a metric of body condition. We only used the total body mass to calculate COND when comparing recorded males and free-swimming territorial type I males, as the latter were not sacrificed and ME was not determined. We $\log_{10}$-transformed TL and ME to meet the assumptions of normality and to linearize allometric relationships.

We used Mann–Whitney (M-W) *U* tests to compare morphological traits ($\log_{10}$TL, COND, RM_G, and RM_{SM}) between recorded type I males and nest-satellite conspecifics. We also used M-W *U* tests to compare vocal parameters (CR_{mean}, CR_{max}, CR_{active}, and CE) and the morphological traits ($\log_{10}$TL, COND, RM_G, and RM_{SM}) between males with eggs and males without eggs. These tests were also adopted to compare boatwhistle features between males with and without eggs, as well as to compare hormonal levels between tested males and free-swimming males from the same study area.

We examined general relationships among the variables across all individuals, including morphological traits, calling parameters (including boatwhistle features), and steroid levels, by performing Spearman correlations. As a large number of statistical tests were made, the overall level of significance needed to be adjusted for multiple tests (to control the type I error rate). Strict application of the Bonferroni method can strongly reduce the power of statistical tests. Such power loss can be avoided by choosing an experiment wise error rate higher than the usually accepted 5%. We used 10% as suggested by Wright (1992) and Chandler (1995), which has been adopted in similar studies (e.g., Møller and Petrie 2002). So, we adopted a significance criterion of $P < 0.01$. We then considered 9 potential predictors of reproductive success (number of eggs), including morphological traits and calling parameters ($\log_{10}$TL, COND, RM_G, RM_{SM}, RM_L, CR_{mean}, CR_{max}, CR_{active}, and CE). We used multiple regression analysis to assess the statistical significance of each variable as a predictor of number of eggs with a stepwise selection procedure ($P \leq 0.05$ to add and $P \geq 0.10$ to remove). Our final regression modes complied with all assumptions of multiple linear regression. All model residuals were normally distributed. Further residual analysis was performed using Durbin–Watson statistics, residual plots as well as multicollinearity tests (variance inflation factors, VIF).

Parametric tests were only performed when data were normally distributed and variances were homogeneous. Statistical analyses were performed using SPSS for Windows (16.0, SPSS Inc., Chicago, IL).

RESULTS

Vocal behavior of nesting males and attraction of conspecifics

Most of the nesting toadfish males started to vocalize, predominantly with boatwhistles, within 24 to 48 h of confinement and interacted acoustically in a chorus, similarly to free-swimming toadfish. From the total of 56 toadfish males confined in the artificial nests, 51 toadfish (91.1%) showed vocal activity and 16 vocally active fish (28.6%) presented egg clutches in their nests, indicating that they successfully attracted mates. From the 28 males recorded in the first breeding season, 11 vocally active specimens (39.3%) attracted other conspecific males, which were outside the nests, close to the nest's entrance, and partially buried in the substrate (Figure 1b). These fish, which were in a position typically occupied by sneakers (type

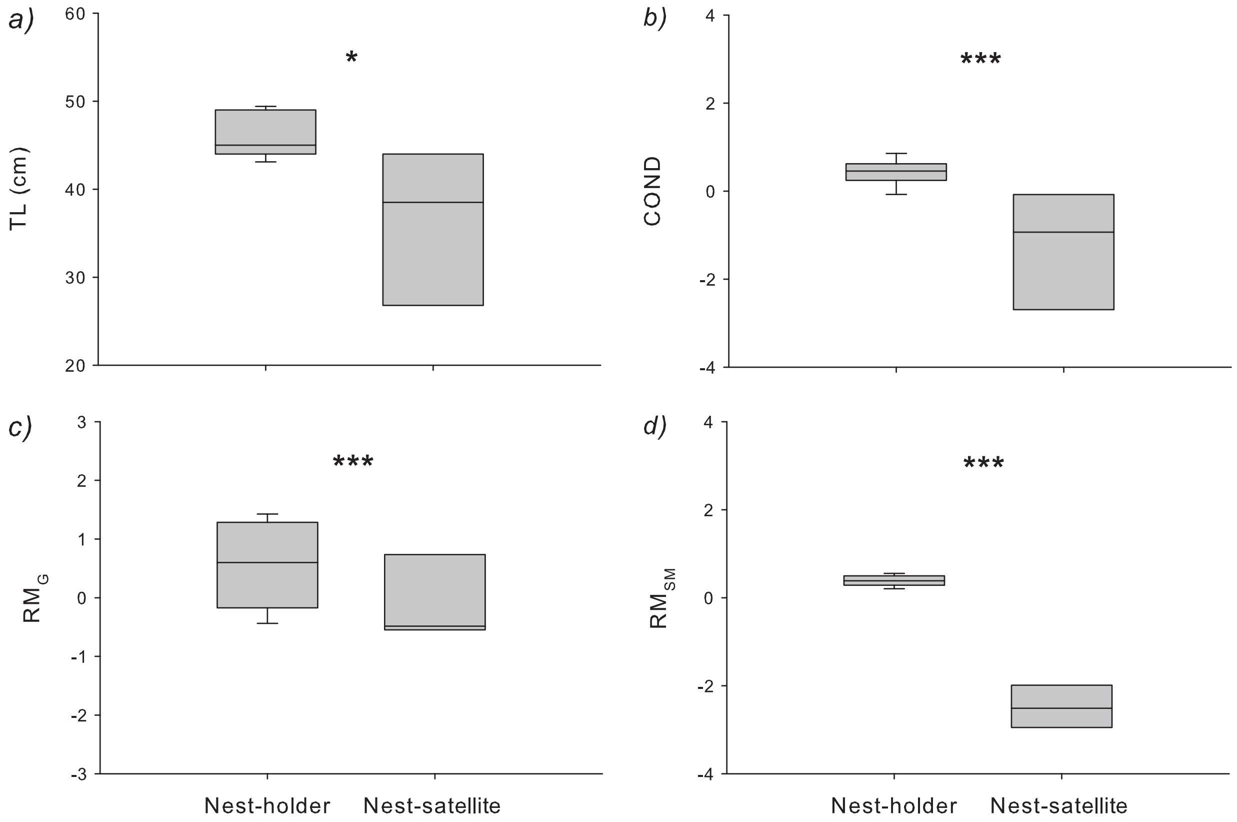

Figure 2
Comparison of total length, TL (a), body condition, COND (b), residuals of gonads mass, RM_G (c), and residuals of sonic muscle mass, RM_{SM} (d) between nest-holder type I toadfish males and nest-satellite type I toadfish males found outside the nests. Plots show medians, 10th, 25th, 75th, and 90th percentiles as boxes and whiskers. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Mann–Whitney *U* tests.

II males), were mostly small type I males ($N = 10$, 91%) with significantly lower TL (M-W U test: $U = 13.5$, $N_{\text{nest-holder fish}} = 11$, $N_{\text{nest-satellite fish}} = 7$, $P = 0.023$) and COND (U test: $U = 2$, $N_{\text{nest-holder fish}} = 11$, $N_{\text{nest-satellite fish}} = 7$, $P < 0.001$, Figure 2a) relative to the vocalizing nesting type I males. Moreover, these type I males found outside the nest also had significantly lower M_{SM} (U test: $U =$ 0–12, $N_{\text{nest-holder fish}} = 11$, $N_{\text{nest-satellite fish}} = 7$, $P <$ 0.001, Figure 2b), M_{AG} (U test: $U =$ 0–12, $N_{\text{nest-holder fish}} = 11$, $N_{\text{nest-satellite fish}} = 7$, $P = 0.016$) and M_G (U test: $U = 16$, $N_{\text{nest-holder fish}} = 11$, $N_{\text{nest-satellite fish}} = 7$, $P = 0.042$). Only one type II male was found outside the nest, among the 11 collected males. Within the same study period, also 3 gravid females were found inside the nests during low tides (see Figure 1b).

The mean and maximum CRs varied considerably among individuals, namely between 0 and 2.81 bw min^{-1} (0.34 ± 0.64 bw min^{-1}, mean ± standard deviation [SD]) and 0–20.46 bw min^{-1} (3.60 ± 5.55 bw min^{-1}), respectively. The CE also differed greatly between males, that is, 0–46% (15% ± 13%, mean ± SD).

Predictors of reproductive success

The mean, active, and maximum CRs were significantly higher in nesting toadfish males with eggs than in males without any clutches (M-W U test, U (CR_{mean}) = 70; U (CR_{active}) = 103; U (CR_{max}) = 66.5; $N_{\text{eggs}} = 14$, $N_{\text{no eggs}} = 40$, $P < 0.001$, Figure 3a). The CE also differed significantly between these 2 fish groups (U test: $U = 102$, $N_{\text{eggs}} = 14$, $N_{\text{no eggs}} = 40$, $P < 0.001$, Figure 3b).

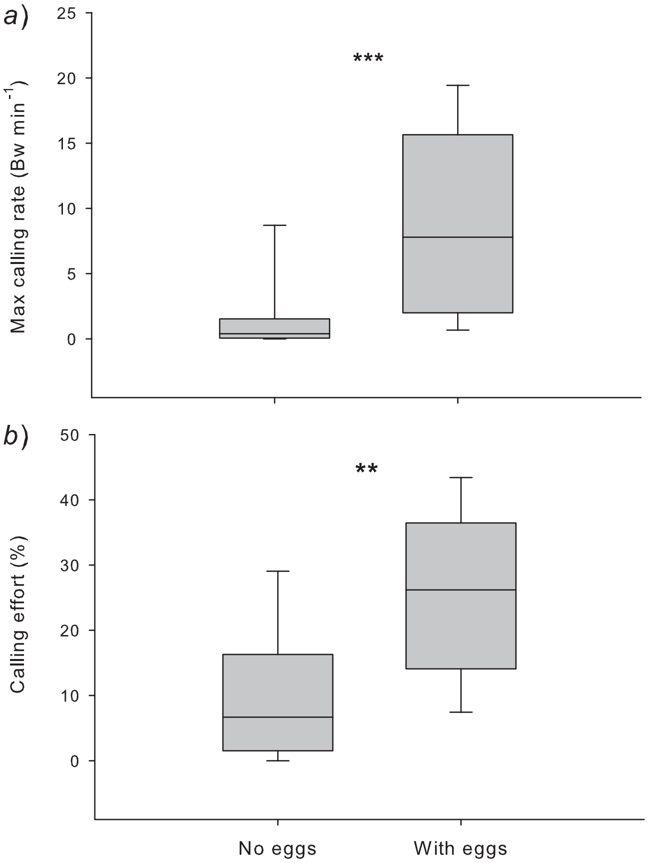

Figure 3
Comparison of maximum calling rate, CR (a) and calling effort, CE (b) between type I toadfish males with and without clutches in the nests. Plots show medians, 10th, 25th, 75th, and 90th percentiles as boxes and whiskers. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Mann–Whitney U tests.

No differences were found in terms of TL, COND, RM_G, and RM_{MS} (U tests: U (TL) = 230.5; U (COND) = 241; U (RM_G) = 277; U (M_{MS}) = 255; $N_{\text{eggs}} = 14$, $N_{\text{no eggs}} = 40$, $P > 0.05$).

Correlation analysis showed that most morphometric features and vocal parameters were not significantly correlated (see Table 1). Calling rates (CR_{mean}, CR_{active}, and CR_{max}) and the CE were highly positively correlated with the number of eggs (Table 1).

Androgen levels (T and 11-KT) were not correlated with any of the several vocal activity parameters nor with the number of eggs (Table 1). Androgen and cortisol levels were compared for tested animals versus free-swimming fish collected in the same study area and showed that tested animals presented significantly higher cortisol levels but T and 11-KT levels were not significantly different (Table 2). In addition, males with clutches obtained similar number of eggs compared with nonrestrained control males (Table 2).

Most boatwhistle acoustic features were neither correlated with the vocal performance nor with the number of eggs (Spearman correlations: $R = -0.49$ to 0.24, $N = 24$, $P > 0.01$). Only pulse period was negatively correlated with CR_{max} ($R = -0.55$, $N = 24$, $P = 0.006$). Nevertheless, toadfish males with eggs produced generally boatwhistles with lower dominant frequencies when compared with males without eggs (M-W U test, $U = 31$, $N_{\text{eggs}} = 13$, $N_{\text{no eggs}} = 11$, $P = 0.018$) but similar duration (U test, $U = 63$, $P > 0.05$), amplitude modulation (U test, $U = 47$, $P > 0.05$) and pulse period (U tests: $U = 65$, $P > 0.05$).

The best predictors for the number of eggs were the maximum CR and the CE—Table 3, Figure 4a,b. CR_{max} was the variable that most explained the variability of the number of eggs, namely 52% (out of 58% explained by the full model). Secondly, CE explained 6% out of 58% by the model.

Although morphometric features did not account for variation of reproductive success, they explained some of the variability found in the vocal parameters. TL, COND and RM_{SM} explained 29%, 6%, and 7% (out of 42% explained by the full model) of the CR_{max} variability, respectively. In addition, TL was the only variable that explained CE variability (56%, Table 3).

DISCUSSION

The relation between characteristics of acoustic signaling that lead to enhanced reproductive success can provide important means to understand how vocal communication systems have evolved. This approach has been focused in several taxa such as insects, anurans, and birds. Although teleost fishes may represent the largest group of sound-producing vertebrates and use acoustic signals during various social interactions including courtship, the relationship between vocal behavior and reproductive success has never been clearly shown. This study is the first to experimentally demonstrate that sound production influences reproductive success in a teleost fish.

Inter- and intrasexual attraction

Almost all Lusitanian toadfish tested in this study showed vocal activity and several presented egg clutches in their nests, indicating that they successfully attracted gravid females. Besides mates, vocal males also attracted other conspecific “satellite” males, which remained outside the nests close to the entrance. In Batrachoididae, nest-parasitizing males have been described as type II males that attempt opportunistic fertilizations, which are characterized by smaller body size, accessory glands, and sonic muscle mass, but larger gonads than type I males (or nest holders) (Brantley and Bass 1994; Modesto and Canário 2003a). Our data, however, showed that the nest-satellite males were mostly type I but with significantly lower TL, condition, sonic muscle, accessory gland mass and gonad

Table 1

Correlations between morphometric features, vocal performance, and number of eggs in the Lusitanian toadfish males

	TL	COND	RM$_{G}$	RM$_{SM}$	CR$_{mean}$	CR$_{max}$	CR$_{active}$	CE	T	11-KT
TL	—	−0.02 (54)	0.27 (54)	−0.05 (54)	−0.22 (55)	−0.25 (55)	−0.34 (55)	−0.06 (55)	–0.42 (24)	−0.17 (24)
COND		—	0.00 (54)	0.19 (54)	0.02 (54)	−0.01 (54)	−0.02 (54)	0.22 (54)	0.00 (23)	0.01 (23)
RM$_{G}$			—	0.36* (54)	0.05 (54)	0.03 (54)	−0.05 (54)	0.11 (54)	0.01 (23)	0.03 (23)
RM$_{SM}$				—	0.23 (54)	0.22 (54)	0.20 (54)	0.21 (54)	0.05 (23)	0.38 (23)
CR$_{mean}$					—	0.97** (55)	0.92** (55)	0.86** (55)	0.11 (23)	0.04 (23)
CR$_{max}$						—	0.94** (55)	0.77** (55)	0.17 (23)	0.12 (22)
CR$_{active}$							—	0.68** (55)	0.08 (23)	0.11 (23)
CE								—	0.26 (23)	0.16 (23)
T									—	0.37 (23)
N° eggs	−0.12 (54)	−0.08 (54)	0.01 (54)	0.07 (54)	0.58** (55)	0.59** (55)	0.49** (55)	0.50** (55)	0.45 (23)	−0.05 (23)

Values shown are Spearman rank correlation coefficients. *N* is indicated below respective correlation coefficients. COND, body condition; CR$_{active}$, active calling rate.

Significant differences are indicated by asterisks: $^{*}P < 0.01$, $^{**}P < 0.001$.

mass than the nest-holder vocal males. Such finding suggests that smaller type I males with inferior quality (body size and condition) may adopt a sneaking behavior due to space competition. The data presented were collected during a summer season with particularly high occupancy rates of the artificial nests placed along the intertidal study area. Alternative mating tactics are not necessarily fixed throughout life and may change depending on the social environment. For instance, in the gobiid fish *Bathygobius fuscus*, larger males are always nest holders but males of smaller size employ both tactics, nest holder or sneaker (Taru et al. 2002). With the decrement of larger males, sneaking males change their mating tactic to nest holding. Also, in cichlid fish, *Oreochromis mossambicus*, small males often adopt alternative female-like mating tactics (Oliveira and Almada 1999). Our results provide evidence of a similar mating behavioral plasticity in type I Lusitanian toadfish males, which probably depends on the social contexts and/or environmental constraints (nest availability). Similar nest-parasitizing behavior has been observed for type I males of the batrachoidid *P. notatus* (Lee and Bass 2006), even though type II males of both batrachoidid species show fixed alternative mating tactics (Brantley and Bass 1994).

Species that breed in aggregations typically exhibit higher levels of competition among males that can ultimately result in higher number of males unable to defend a territory, and also in higher sexually motivated territorial males that lose the ability to discriminate between sexes (Oliveira and Almada 1999). Future studies should investigate whether nest-satellite type I males are truly sneakers and attempt opportunistic fertilizations, whether this tactic is maintained throughout the whole breeding season, and which social and environmental features are responsible for shaping such mating behavior.

Vocal behavior and reproductive success

Our data indicated that the CR (mean, active, and maximum CRs), as well as the CE, were significantly higher in nesting males with eggs than in males without clutches. The physical features of nesting males, such as TL, body condition, and gonads and sonic muscle masses, were not correlated with the number of eggs, which indicates that the reproductive success in *H. didactylus* primarily relies on the vocal performance. Both higher CRs and calling in a regular fashion during long periods of time (increased CE) result in a more conspicuous vocal output or male advertisement, which probably facilitates detection, localization, and selection by females. Higher advertising CRs might be important to indicate spawning readiness or motivation of males and in synchronizing gamete release (Amorim et al. 2003). Previous studies with other batrachoidids revealed that gravid females show phonotaxis when advertising calls are played back at relatively high rates (e.g., *O. tau*, Winn 1972; *P. notatus*, McKibben and Bass 1998).

Ultimately, CR and CE may provide information about the singing males, and it is possible that these vocal features are used as honest signals for mate choice by females. Our data indicated that TL, condition, and sonic muscle mass combined partially explained the variability found in the calling performance among individuals. Moreover, besides the likely higher physiological and metabolic costs (Mitchell et al. 2008; but see Amorim et al. 2002), the production of boatwhistles at high rates and in a regular fashion may also impose ecological costs, such as the attraction of predators and the time spent calling and not in other activities (Ryan 1988; Gannon et al. 2005). Females strongly benefit from choosing good males, especially when they are single spawners as batrachoidids (Brantley and Bass, 1994; Modesto and Canário, 2003a). Fish-unguarded eggs are quickly eaten by predators, and

Table 2

Comparison of steroid levels (T, testosterone and 11-KT, 11-ketotestosterone) between tested confined fish and free-swimming fish with and without eggs present in the nest

		T (ng)		11-KT (ng)		Cortisol (ng)		Number of eggs	
With eggs	Test fish	0.53 ± 0.36 (7)	*U* = 19,	0.43 ± 0.40 (7)	*U* = 13,	16.66 ± 13.32 (5)	*U* = 3,	994 ± 518 (14)	*U* = 78,
	Free-swimming fish	1.00 ± 1.05 (8)	$P > 0.05$	2.75 ± 3.53 (8)	$P > 0.05$	3.99 ± 2.47 (8)	$P = 0.013$	810 ± 692 (14)	$P > 0.05$
No eggs	Test fish	0.47 ± 0.24 (17)		0.63 ± 0.59 (17)		21.82 ± 10.37 (18)			
	Free-swimming fish	0.29 (2)		1.54 (2)		1.18 (2)			

Mann–Whitney *U* tests performed between groups are indicated. Values are means ±SD; *N* is indicated in parentheses.

Table 3

Predictors of reproductive success (number of eggs), CR_{max}, and CE of the Lusitanian toadfish

	Predictor	B	SEM	t	P	r	F	Model significance	R^2	DW	VIF
N eggs	CR_{max}	1.20	0.33	3.67	0.001	0.762	$F_{1,53} = 35.32$	<0.001	0.581	2.20	2.03
	CE	0.28	0.10	2.70	0.009						2.03
CR_{max}[a]	log TL	2.10	0.42	0.536	<0.001	0.651	$F_{3,53} = 12.28$	<0.001	0.424	1.92	1.92
	COND	−1.91	0.73	−0.284	0.012						1.03
	RM_{SM}	1.78	0.70	0.275	0.015						1.03
CE[a]	log TL	9.18	1.13	8.104	<0.001	0.747	$F_{1,53} = 65.68$	<0.001	0.558	1.33	1.00

COND, body condition.

[a] Only morphometric parameters were considered for regression analysis.

females must rely on males' brood protection to ensure the survival of the offspring (Sargent and Gross 1993). Also, the presence of a nesting male is critical for eggs' survival as they need to be aerated for proper development and to prevent fungal infections (Ramos and Amorim, unpublished data). Therefore, in species where males provide parental care, indicators of male parental quality are expected to be important in intersexual communication and be under strong mate selection by females (Andersson 1994).

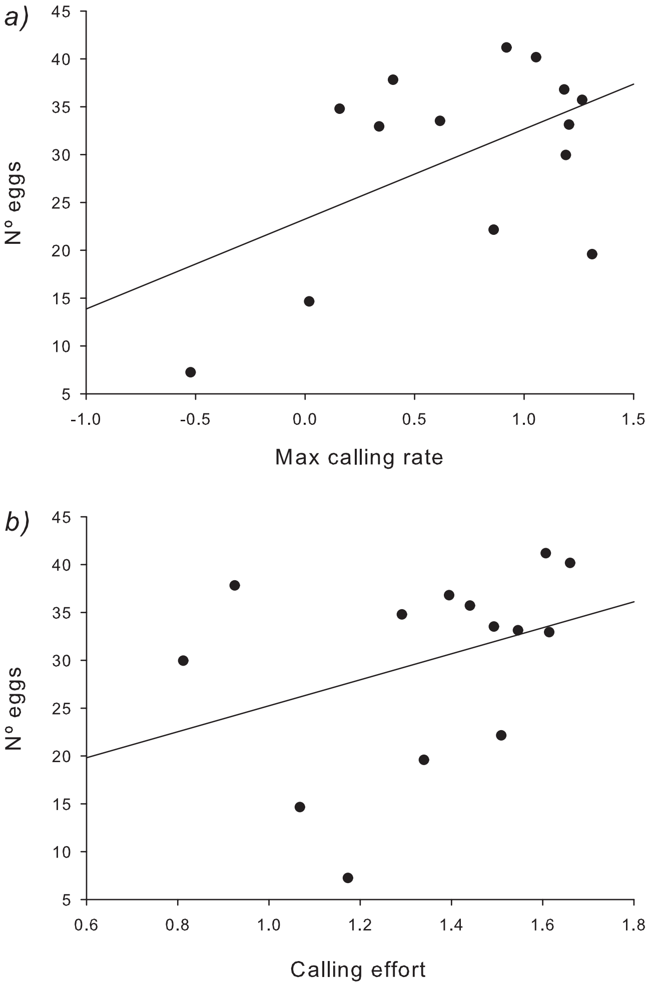

Figure 4

Relationship between the best predictors, maximum calling rate, CR (a) and calling effort, CE (b), and the reproductive success (number of eggs) in the Lusitanian toadfish. The number of eggs was square-root transformed and the maximum CR was log-transformed. Regression equations: (a) SQRT (N° eggs + 0.5) = 9.4 (log CR_{max}) + 23.3; (b) SQRT (N° eggs + 0.5) = 13.6 (CE) + 11.7.

Amorim et al. (2010) also reported that increased boatwhistle CR and CE strongly reflected good male condition given by the lipid content of the somatic muscles in the Lusitanian toadfish. Accordingly, we further demonstrate that these vocal parameters affect the mating success in this species and seem to inform receivers, that is, females and other competing males, about the size and also quality of males. Lusitanian toadfish males that are in good condition advertise their spawning motivation through higher CRs and increased CE, which affects their success in attraction of gravid females for reproduction.

Moreover, our data showed that the toadfish males with eggs that exhibited higher CRs also produced boatwhistles with significantly lower dominant frequencies. This suggests a possible trade-off between CR and muscle contraction rate, as calling more requires slower muscle contraction to avoid muscle fatigue. Changes in muscle contraction rate have been described to lower levels of muscle fatigue (e.g., Brantley and Bass 1994). Moreover, dominant frequency may indicate size of vocal male. Dominant frequency is negatively correlated with body size in several fish species (e.g., Ladich et al. 1992; Myrberg et al. 1993).

Sisneros et al. (2009) reported that, in the batrachoidid *P. notatus* at the end of the breeding season, larger nesting males presented higher body condition (K) and larger number of viable late-stage embryos in the nest, suggesting that body condition is an honest indicator of parental ability in batrachoidids. In our study, we did not find a correlation between condition (comparable to K, but based on residuals—see MATERIALS AND METHODS) and number of eggs. The data obtained in terms of number of eggs was collected after 2 weeks of confinement of reproductive males in the experimental nests. At this point, we were only evaluating the ability to attract mates and to provide early parental care and not the capacity of providing good parental care through later stages of embryo development.

In other taxa, higher CRs may signal male quality such as a better immune system (e.g., insects, Jacot et al. 2004), parental quality (e.g., birds, Dolby et al. 2005) or higher fertilization success (e.g., anurans, Pfennig 2000 and birds, White et al. 2010).

Steroid plasma levels, vocal behavior, and reproductive success

Our data showed that circulating androgen levels were not significantly related with reproductive success or vocal behavior in the studied animals. However, androgen levels in experimental animals were generally lower (but not significantly different) than those obtained from free-swimming toadfish collected in the same study area during low tides (see Table

2). In fact, androgen profiles of these free-swimming animals were similar to those found for the same species in wild animals captured by beam trawler during the reproductive season (Modesto and Canário 2003a).

In contrast, cortisol circulating levels of confined males were significantly higher than those obtained from free-swimming toadfish at the same study site. This suggests that confinement in the experimental nests was probably responsible for the higher cortisol levels, although animals exhibited vocal activity, successfully attracted mates, and had similar body condition compared with the free-swimming fish. In addition, subject males with clutches obtained similar number of eggs as free-swimming males from the same site (Table 2), suggesting that spawning success of the studied males was not altered by any reaction to confinement. Therefore, we consider the hypothesis that in confined animals increased cortisol levels could potentially result in decreased androgen levels concealing any possible relation with the male's reproductive success. In common carp, chronically elevated cortisol levels affected all parts of the brain–pituitary–gonad axis resulting in a strong decrease of testicular production of androgens, including 11-KT (Goos and Consten 2002). Moreover, *in vitro*, physiological levels of cortisol can inhibit the pathways that lead to the production of 11-KT (Consten et al. 2002). In this context, measured androgen levels probably did not reflect accurate hormonal profiles of the specimens throughout the study period and cannot provide precise information about normal relationships between steroid plasma levels, vocal behavior, and reproductive success. Future studies should investigate whether androgens affect calling and spawning success in nonconfined animals.

This study is the first to experimentally demonstrate that sound production influences reproductive success in a teleost fish. We suggest that signaling at higher rates and in a regular fashion can operate as an honest signal of male quality and an indicator of elevated motivation for reproduction in Batrachoididae, and perhaps in other fish. Future studies should address whether vocal behavior also signals parental quality, as in other taxa.

FUNDING

Science and Technology Foundation, Portugal (project PDCT/MAR/58071/2004, pluriannual program UI&D 331/94 and UI&D, grants SFRH/BD/30491/2006 to R.O.V. and SFRH/BPD/41489/2007 to M.C.P.A).

We thank the Air Force Base No. 6 of Montijo (Portugal) for allowing this study in their military establishment. We are grateful to Marta Bolgan for the illustration of the toadfish experimental nest. We also thank Silvia Pedroso and Joana Jordão for their help with the fieldwork.

REFERENCES

Amorim MCP, McCracken Ml, Fine ML. 2002. Metabolic costs of sound production in the oyster toadfish, *Opsanus tau*. Can J Zool. 80:830–838.

Amorim MCP, Fonseca PJ, Almada VC. 2003. Sound production during courtship and spawning of *Oreochromis mossambicus*: male–female and male–male interactions. J Fish Biol. 62:658–672.

Amorim MCP, Vasconcelos RO, Marques JF, Almada F. 2006. Seasonal variation of sound production in the Lusitanian toadfish, *Halobatrachus didactylu*s. J Fish Biol. 69:1892–1899.

Amorim MCP, Vasconcelos RO. 2008. Variability in the mating calls of the Lusitanian toadfish *Halobatrachus didactylus*: cues for potential individual recognition. J Fish Biol. 73:1267–1283.

Amorim MCP, Simões JM, Fonseca PJ. 2008. Acoustic communication in the Lusitanian toadfish, *Halobatrachus didactylus*: evidence for an unusual large vocal repertoire. J Mar Biol Assoc UK. 88: 1069–1073.

Amorim MCP, Vasconcelos RO, Parreira B. 2009. Variability in the sonic muscles of the Lusitanian toadfish (*Halobatrachus didactylus*): acoustic signals may reflect individual quality. Can J Zool. 87:718–725.

Amorim MCP, Simões JM, Mendonça N, Bandarra NM, Almada VC, Fonseca PJ. 2010. Lusitanian toadfish song reflects male quality. J Exp Biol. 213:2997–3004.

Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.

Bass AH, McKibben JR. 2003. Neural mechanisms and behaviors for acoustic communication in teleost fish. Prog Neurobiol. 69:1–26.

Bradbury JW, Vehrencamp SL. 1998. Principles of animal communication. Sunderland (MA): Sinauer Associates.

Brantley RK, Bass AH. 1994. Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish, *Porichthys notatus* (Teleostei, Batrachoididae). Ethology. 96:213–232.

Chandler CR. 1995. Practical considerations in the use of simultaneous inference for multiple tests. Anim Behav. 49:524–527.

Consten D, Keuning ED, Terlou M, Lambert JGD, Goos HJT. 2002. Cortisol effects on the testicular androgen synthesizing capacity in common carp, *Cyprinus carpio* L. Fish Physiol Biochem. 25:91–98.

Crawford JD. 1997. Hearing and acoustic communication in mormyrid electric fishes. Mar Freshw Behav Physiol. 29:65–86.

Dolby AS, Clarkson CE, Haas ET, Miller JK, Havens LE, Cox BK. 2005. Do song-phrase production rate and song versatility honestly communicate male parental quality in the Gray Catbird? J Field Ornithol. 76:287–292.

dos Santos ME, Modesto T, Matos RJ, Grober MS, Oliviera RF, Canário A. 2000. Sound production by the Lusitanian toadfish, *Halobatrachus didactylus*. Bioacoustics. 10:309–321.

Gannon DP, Barros NB, Nowacek DP, Read AJ, Waples DM, Wells RS. 2005. Prey detection by bottlenose dolphins, *Tursiops truncatus*: an experimental test of the passive listening hypothesis. Anim Behav. 69:709–720.

Goos HJT, Consten D. 2002. Stress adaptation, cortisol and pubertal development in the male common carp, *Cyprinus carpio*. Mol Cell Endocrinol. 197:105–116.

Jacot A, Scheuber H, Brinkhof MWG. 2004. Costs of an induced immune response on sexual display and longevity in field crickets. Evolution. 58:2280–2286.

Kime DE, Manning NJ. 1982. Seasonal patterns of free and conjugated androgens in the brown trout *Salmo trutta*. Gen Comp Endocrinol. 48:222–231.

Knapp R, Kovach J. 1991. Courtship as an honest indicator of male parental quality in the bicolor damselfish, *Stegastes partitus*. Behav Ecol. 2:295–300.

Kroodsma DE, Byers BE. 1991. The function(s) of bird song. Am Zool. 31:318–328.

Ladich F, Myrberg AA. 2006. Agonistic behavior and acoustic communication. In: Ladich F, Collin SP, Moller P, Kapoor BG, editors. Communication in fishes. Vol. 1. Enfield (NH): Science Publishers. p. 121–148.

Ladich F, Bischof C, Schleinzer G, Fuchs A. 1992. Intra- and interspecific differences in agonistic vocalization in croaking gouramis (genus: *trichopsis, Anabantoidei, Teleostei*). Bioacoustics. 4:131–141.

Lee JSF, Bass AH. 2006. Dimorphic male midshipman fish: reduced sexual selection or sexual selection for reduced characters? Behav Ecol. 17:670–675.

McKibben JR, Bass AH. 1998. Behavioral assessment of acoustic parameters relevant to signal recognition and preference in a vocal fish. J Acoust Soc Am. 104:3520–3533.

Mitchell S, Poland J, Fine ML. 2008. Does muscle fatigue limit advertisement calling in the oyster toadfish *Opsanus tau*? Anim Behav. 76:1011–1016.

Modesto T, Canário AVM. 2003a. Morphometric changes and sex steroid levels during the annual reproductive cycle of the Lusitanian toadfish, *Halobatrachus didactylus*. Gen Comp Endocrinol. 131:220–231.

Modesto T, Canário AVM. 2003b. Hormonal control of swimbladder sonic muscle dimorphism in the Lusitanian toadfish Halobatrachus didactylus. J Exp Biol. 206:3467–3477.

Møller AP, Petrie M. 2002. Condition dependence, multiple sexual signals, and immunocompetence in peacocks. Behav Ecol. 2:248–253.

Myrberg AA, Mohler M, Catala J. 1986. Sound production by males of a coral reef fish (*Pomacentrus partitus*): its significance to females. Anim Behav. 24:923–933.

Myrberg AA, Ha SJ, Shamblott H. 1993. The sounds of bicolor damselfish (*Pomacentrus partitus*): predictors of body size and a spectral basis for individual recognition and assessment. J Acoust Soc Am. 94:3067–3070.

Myrberg AA, Lugli M. 2006. Reproductive behavior and acoustical interactions. In: Ladich F, Collin SP, Moller P, Kapoor BG, editors. Communication in fishes. Vol. 1. Enfield (NH): Science Publishers. p. 149–176.

Nordby JC, Campbell SE, Beecher MD. 1999. Ecological correlates of song learning in song sparrows. Behav Ecol. 10:287–297.

Oliveira RF, Almada VC. 1999. Male display characters, gonadal maturation and androgens in the Cichlid Fish *Oreochromis mossambicus*. Acta Ethologica. 2:67–70.

Partan SR, Marler P. 2005. Issues in the classification of multimodal communication signals. Am Nat. 166:231–245.

Pfennig KS. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. Behav Ecol. 11:220–227.

Rice AN, Bass AH. 2009. Novel vocal repertoire and paired swimbladders of the three-spined toadfish, *Batrachomoeus trispinosus*: insights into the diversity of the Batrachoididae. J Exp Biol. 212:1377–1391.

Rotllant JPM, Guerreiro AL, Redruello B, Canário AVM, Power DM. 2005. Stimulation of cortisol release by the N terminus of teleost parathyroid hormone-related protein in interrenal cells *in vitro*. Endocrinology. 146:71–76.

Roux C. 1986. Batrachoididae. In: Whitehead PJP, Bauchot ML, Hureau JC, Tortonese E, editors. Fishes of the North-eastern Atlantic and the Mediterranean, Vol. III. Paris: Unesco. p. 1360–1361.

Ryan MJ. 1988. Energy, calling and selection. Am Zool. 28:885–898.

Sargent RC, Gross MR. 1993. William's principle: an explanation of parental care in teleost fishes. In: Pitcher TJ, editor. Behaviour of teleost fishes. London: Chapman and Hall. p. 333–361.

Scott AP, MacKenzie DS, Stacey NE. 1984. Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni.* II. Steroid hormones. Gen Comp Endocrinol. 56:349–359.

Searcy WA, Andersson M. 1986. Sexual selection and the evolution of song. Annu Rev Ecol Syst. 17:507–533.

Sisneros JA, Alderks PW, Leon K, Sniffen B. 2009. Morphometric changes associated with the reproductive cycle and behaviour of the interidal-nesting, male plainfin midshipman fish, *Porichthys notatus.* J Exp Biol. 74:18–36.

Taru M, Kanda T, Sunobe T. 2002. Alternative mating tactics of the gobiid fish *Bathygobius fuscus.* J Ethol. 20:9–12.

Vasconcelos RO, Ladich F. 2008. Development of vocalization, auditory sensitivity and acoustic communication in the Lusitanian toadfish *Halobatrachus didactylus.* J Fish Biol. 211:502–509.

Vasconcelos RO, Simões JM, Almada VC, Fonseca PJ, Amorim MCP. 2010. Vocal behaviour during territorial intrusions in the Lusitanian toadfish: boatwhistles also function as territorial 'keep-out' signals. Ethology. 116:155–165.

White DJ, King AP, West MJ, Gros-Louis J, Tuttled EM. 2010. Effects of singing on copulation success and egg production in brown-headed cowbirds, *Molothrus ater.* Behav Ecol. 21:211–218.

Winn HE. 1972. Acoustic discrimination by the toadfish with comments on signal systems. In: Winn HE, Olla BL, editors. Behavior of marine animals. . Vol. 2. New York: Plenum. p. 361–385.

Wright SP. 1992. Adjusted P-values for simultaneous inference. Biometrics. 48:1005–1013.