Lusitanian toadfish song reflects male quality

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SUMMARY

Lusitanian toadfish males that provide parental care rely on acoustic signals (the boatwhistle) to attract females to their nest. We test the hypothesis that male quality, namely male size and condition that are relevant for parental success, is reflected in vocal activity and boatwhistle characteristics and thus advertised to females. We recorded 22 males over a week during the peak of the breeding season. Calling rate and calling effort (percentage of time spent calling) strongly reflected male condition (lipid content of somatic muscles) and to a smaller extent sonic muscle hypertrophy and larger gonads. Males in better condition (increased body lipid and relative higher liver mass) also contracted the sonic muscles at faster rate as shown by the shorter boatwhistle pulse periods. Amplitude modulation reflected the degree of sonic muscle hypertrophy. None of the measured male quality parameters were good predictors of boatwhistle duration and dominant frequency. Altogether this study strongly suggests that Lusitanian toadfish males advertise their quality to females primarily with boatwhistle calling rate and calling effort, which mainly reflect male condition. Because pulse period had low variability, consistent with the existence of a vocal central pattern generator, we suggest that males that sustain sonic muscles contraction at a very fast rate close to their physiological limit may be honestly advertising their quality (condition). Similarly, males that produce boatwhistles with higher amplitude modulation, a feature that seems dependent on sonic muscle hypertrophy, could be more attractive to females.

Key words: fish, Batrachoididae, *Halobatrachus didactylus*, acoustic communication, mate choice, male condition, muscle lipid content.

INTRODUCTION

Females are often the choosy sex and select mates based on direct benefits such as food, parental care or a good territory, or on indirect benefits such as genetic quality (Andersson, 1994). Males advertise their attributes through courtship displays, which inform their mates about their quality. Signals can evolve to be reliable or honest if they bear a cost to the sender either because of production costs or through increased vulnerability of attack by heterospecific or conspecific receivers (Zahavi, 1975). Alternatively, index signals are reliable because they cannot be faked due to physical or physiological constraints that force the signal to reveal honest information (Vehrencamp, 2000; Maynard Smith and Harper, 2003). Honest signals can also be relatively cost-free if signallers and receivers share a common interest or when there is a threat of retaliation by the receiver (Vehrencamp, 2000).

Acoustic signals are good examples of sexually selected traits predominantly used by females of several taxa to identify, locate and choose between potential mates (Andersson, 1994; Bradbury and Vehrencamp, 1998). Different components of vocalisations seem to convey honest messages that are relevant for mate choice but may also be maladaptive for females if female preference has evolved, for example, by male sensory exploitation (Ryan et al., 1990). Examples of honest acoustic signal components are high calling rates that are energetically costly to maintain or may attract predators or parasites, and the fundamental frequency of advertisement calls that may be dependent on the vocal apparatus size and thus on the sender size (Maynard Smith and Harper, 2003).

Fish probably represent the largest group of sound-producing vertebrates and often emit acoustic signals during courtship (Ladich, 2004). However, there are few studies that relate calling rate and call characteristics with male quality and even fewer that show the role of acoustic signals in mate choice. This is probably due to technical limitations related to underwater recordings (in natural contexts) and playbacks, rather than to the lack of a role of fish calls on mate choice (Ladich, 2004). For example, female of the bicolour damselfish *Stegastus partitus* (Pomacentridae) prefer courtship chirps of lower frequency that indicate a larger male body size (Myrberg et al., 1986). Females of the same species also favour males with high courtship rates who have better body condition and are less prone to cannibalise their eggs (Knapp and Kovach, 1991). Although it has not been tested, the studies by Myrberg et al. (Myrberg et al., 1986) and Knapp and Kovach (Knapp and Kovach, 1991) both suggest that *S. partitus* females could be also selecting males with a high courtship chirping rate, which could be indicative of a better male condition and parental ability. McKibben and Bass have also shown that females of the midshipman *Porichthys notatus* (Batrachoididae) prefer the more intense of two hums, the male mating signals (McKibben and Bass, 1998). In addition, the percentage of females responding to sound playback increased as duration of calls increased and the pauses between calls decreased, indicating that longer continuous calls resembling natural hums are more attractive to females (McKibben and Bass, 1998).

Breeding males from the family Batrachoididae (toadfishes and midshipmen) nest under rocks and produce advertisement calls

(boatwhistles or hums) from their nests to attract females by contracting a pair of sonic muscles embedded in the sides of the swimbladder (Fish, 1972; Brantley and Bass, 1994; dos Santos et al., 2000). Batrachoidid males provide uniparental care until the young are free-swimming and call to attract females until the nest's ceiling is fully covered with eggs and embryos (Brantley and Bass, 1994). Batrachoidids have become a model group in the study of acoustic communication in teleosts (Bass and McKibben, 2003; Sisneros, 2009) but little is known on the role of acoustic signals in mate choice and reproductive success. The Lusitanian toadfish *Halobatrachus didactylus* (Bloch and Schneider 1801) produces a long (about 1s) tonal low-frequency advertisement sound, the boatwhistle, which is highly stereotyped and shows considerable inter-individual differences during short periods of time (<10 min) (Amorim and Vasconcelos, 2008). A recent study has shown that the male's sound-producing muscles are highly variable (c.v.=40%) and that its mass depends mostly on male length and condition, suggesting that sounds could advertise male quality in this species (Amorim et al., 2009). Because the boatwhistle seems to be the only courtship signal in this species (M.C.P.A., P.J.F. and J.M.S., personal observation) [see also Brantley and Bass (Brantley and Bass, 1994) for a detailed description of the mating behaviour of *P. notatus*] we have hypothesised that it should contain information relevant to the female for mate choice. In the present study, we test the hypothesis that calling activity (calling rate and calling effort – percentage of time calling) and boatwhistle characteristics (duration, pulse period, dominant frequency and amplitude modulation) reflect male quality. For that purpose we recorded 22 males, each over a week during the peak of the breeding season, and measured their body condition, body size, sonic muscle hypertrophy and reproductive condition (gonad and accessory gland mass). Because Lusitanian toadfish females seem to produce only one clutch of a few large eggs per reproductive season (Modesto and Canário, 2003a; Costa, 2004) and have to probably rely on the parental ability of one single male for their reproductive success, we predict that boatwhistles should inform females of male size and condition, which are relevant for nest defence and parental care.

MATERIALS AND METHODS
Study species
Breeding males of the Lusitanian toadfish, *H. didactylus*, form conspicuous choruses from May to July (in Portugal) and defend nests in estuarine shallow waters that can contain clutches from different females (Amorim et al., 2006). Parental care is provided until the young are free-swimming. Besides the nest-guarding male ('type I') morphotype, there are sneaker males ('type II') that have larger testis (sevenfold), smaller accessory glands (threefold; the accessory glands are part of the male reproductive apparatus, secrete mucosubstances and are connected to the spermatic duct) and lower (sixfold) 11-ketotestosterone levels than nesting males (Modesto and Canário, 2003a; Modesto and Canário, 2003b). Only type I males produce boatwhistles during the breeding season. Sonic muscles of type I males but not of type II males or of females suffer hypertrophy during the breeding season (Modesto and Canário, 2003a), concurrent with an increase in vocal activity (Amorim et al., 2006).

Sound recording
Sixty artificial hemicylinder-shaped concrete shelters capped at one end were deployed 1.5 m apart in an intertidal area of the Tagus estuary (Portugal, Montijo, Air-Force Base 6; 38°42′N, 8°58′W) that was only exposed to air during spring low tides. Water level varied between 0 m and 2.8 m in the study area. The shelters were

large enough (internal dimensions: 50 cm long×30 cm wide×20 cm high) to house a large male and several females and were readily used as nests during the breeding season (Amorim et al., 2010). Three groups of 6–8 males (*N*=22) that spontaneously occupied these artificial concrete shelters were recorded over a period of eight days in June and July in 2006 and 2007, during the peak of the reproductive season (May to July in Portugal) (Modesto and Canário, 2003a). Shelters (6–8) containing subject males were placed 1.5 m apart in two rows and were at least 15 m apart from the remaining shelters. The entrance of the subject males' shelters were closed with a plastic mesh preventing fish from abandoning the nest during recordings but allowing prey items to enter and possible visual interactions with conspecifics.

One hydrophone (High Tech 94 SSQ hydrophone, High Tech Inc., Gulfport, MS, USA; sensitivity –165 dB re. 1 V/μPa, frequency response within ±1 dB from 30 Hz to 6 KHz) was firmly attached to an iron rod partially buried in the sand substrate, placed ~10 cm from each shelter entrance and from the substrate. Simultaneous multi-channel recordings were made to a laptop connected to USB audio capture devices (Edirol UA25, Roland, Osaka, Japan; 16 bit, 6 kHz acquisition rate per channel) controlled by Adobe Audition 2.0 (Adobe Systems Inc., Mountain View, CA, USA). Recorded sounds could be attributed to each male due to the high acoustic attenuation observed in shallow water (Fine and Lenhardt, 1983). Sounds from a neighbouring male were ~27 dB lower than of a subject male. Water temperature was measured every 3 h during recording periods and averaged 23°C (range: 19.5–28°C). All subject fish experienced similar water temperature variability during recordings; hence, the effect of temperature on call parameters should be similar for all fish. Each male was recorded for an average of 35 h (range: 11–56 h).

Sound analysis
Boatwhistles have been described in detail in Amorim and Vasconcelos (Amorim and Vasconcelos, 2008) and, as in other batrachoidids (e.g. Thorson and Fine, 2002), are characterised by an initial shorter pulsed part followed by a longer tonal segment (dos Santos et al., 2000; Amorim and Vasconcelos, 2008). We analysed boatwhistles for total sound duration (ms, measured from the start of the first pulse to the end of the last pulse), pulse period of the tonal segment (ms, average peak-to-peak interval of six consecutive pulses in the middle of this segment), dominant frequency of the tonal segment (Hz, the frequency with maximum energy in this part of the sound), and amplitude modulation [the ratio between the mean amplitude (root mean square, r.m.s.) of the initial and of the tonal segments; r.m.s. amplitude is a measurement native to Raven software, Cornell Laboratory of Ornithology, Ithaca, NY, USA]. Temporal variables and amplitude modulation were measured from oscillograms, and dominant frequency from power spectra computed with a 2048 points FFT conditioned by a Hamming window, with a time overlap of 50.0% and a 10 Hz filter bandwidth. These acoustic parameters are depicted in Fig. 1. Calling rate (number of boatwhistles emitted per hour) was tallied for each fish. Calling effort (number of hours calling / number of hours recorded × 100) was also calculated per fish. Sound analysis was carried out with Adobe Audition 2.0 and Raven 1.2.1 for Windows.

Morphometric analysis
At the end of recordings subject males were killed with an excessive dosage of MS 222 (tricaine methane sulphonate; Pharmaq, Skøyen, Oslo, Norway). In the laboratory, each subject male was measured to the nearest mm for total length (*TL*), and to the nearest g for
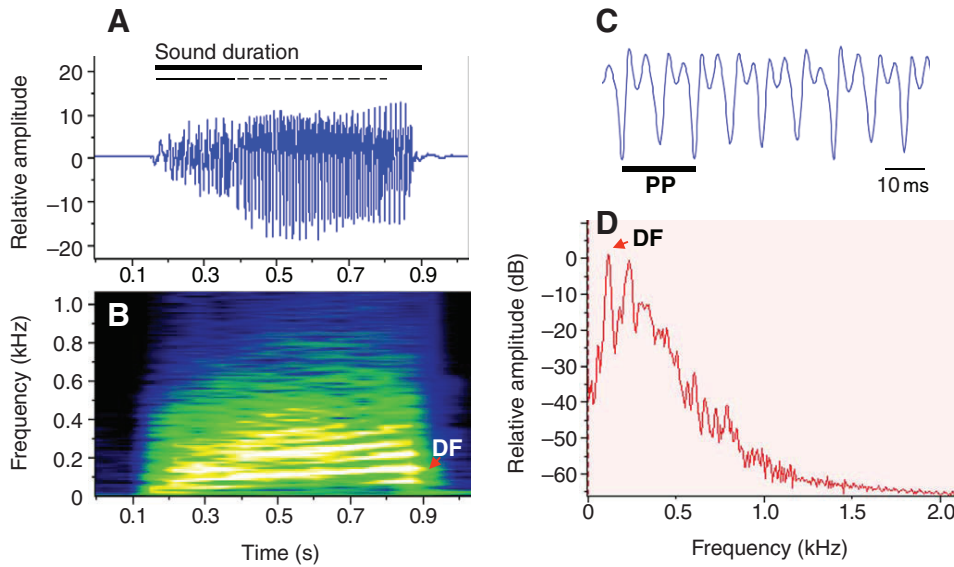
Fig. 1. Oscillogram (A), sonogram (B) and power spectrum (D) of a boatwhistle. Sound duration, the initial (fine continuous line) and the tonal (fine dashed line) phases of the boatwhistle are depicted in the oscillogram. In the sonogram and in the power spectrum the dominant frequency (DF) of the sound is shown. (C) Detail of the boatwhistle tonal phase waveform depicting the pulse period (PP). Note that the pulse period is inversely related to the fundamental frequency and not to the dominant frequency of the boatwhistle.

eviscerated body mass (M_E). Males (N=22) used in this study averaged 42.9 cm (range: 37.9–47.7 cm) in total length and 1207 g (range: 857–1612 g) in eviscerated mass. The gonads (M_G), the accessory glands (M_{AG}) and the liver (M_L) mass were tallied to the nearest mg. Sonic muscles, which are embedded in the sides of the swimbladder, were gently cut from the swimbladder wall with a pair of fine dissection scissors and were also weighed to the nearest mg (M_{SM}). The mass of the accessory glands was included in the measurements as they are part of the male reproductive apparatus and increase in mass during the breeding season in nesting males (Modesto and Canário, 2003a).

A sample of body muscles was taken (hypaxial muscle fibres) and the lipid fraction of both somatic and sonic muscles was quantified as an additional measurement of body and sonic muscle condition. Lipids are an important source of energy in fish and are often used as a direct measure of body condition (Chellapa et al., 1995). Lipids are also one of the major metabolic substrates of sonic muscles during prolonged aerobic activity (Fine et al., 1986).

All experimental procedures comply with Portuguese animal welfare laws, guidelines and policies.

Lipid analysis

The fish somatic and sonic muscle homogenates (10 g) were extracted using 30 ml of chloroform: methanol (1:2 vol./vol.) with a polytron system. In addition, a saturated sodium chloride solution (4 ml), chloroform containing 50 p.p.m. (parts per million) of butylhydroxytoluene (10 ml) and water (10 ml) were added to split the system into an aqueous and an organic phase (Bligh and Dyer, 1959). The mixture was sonicated during 15 min. Complete separation of the two phases was obtained by adding isopropanol; the total mixture was centrifuged and the chloroform phase transferred to a weighed tube. The chloroform was evaporated under nitrogen prior to gravimetrical determination of total lipids.

Statistical analysis

We examined eight potential predictors of call parameters. We included total length ($\log_{10}TL$) as a metric of body size. We used residuals of the simple linear regression of sonic muscle mass on eviscerated body mass (RM_{SM}) as a metric of sonic muscle hypertrophy. This metric gives a measure of an observed sonic muscle mass relative to a mean expected value (given by the regression model) for a given body size. In other words, a male with a high positive residual of RM_{SM} will have heavier than average sonic muscles for his size. Likewise we used the residuals of the simple linear regressions of gonads, accessory glands and liver mass on eviscerated body mass (RM_G, RM_{AG}, RM_L, respectively) as metrics of these parameters controlled for the influence of body size. In addition, we used the residuals of M_E on TL (COND) as a metric of body condition. We $\log_{10}$-transformed TL and mass data to meet the assumptions of normality and to linearise allometric relationships. We further considered the lipid content of somatic and sonic muscles (LipidM and LipidSM, respectively) as possible predictors of call parameters.

We first generated a correlation matrix of the six measured call parameters (calling rate and effort, and boatwhistle characteristics) and morphological traits to examine general relationships among the variables across all individuals. We then used multiple regression analysis to assess the statistical significance of each physical parameter as a predictor of male mating call parameters with a stepwise selection procedure ($P \leq 0.05$ to add and $P \geq 0.10$ to remove).

Because three of our dependent variables, calling rate, calling effort and pulse period, were highly correlated (Table 1), we included these variables in a factor analysis to generate a single factor that would combine and explain most of the variance in this group of variables. Pulse rate (the inverse of pulse period and equivalent to sonic muscle contraction rate) was used instead of pulse period because the former had a positive loading in the first principle component, similar to the other two variables. The scores of the first factor can be viewed as an index of 'vocal performance', i.e. of length and rate of calling and of muscle contraction rate during sound production. The first factor explained 75% of the total data variance with call rate, effort and pulse rate presenting a factor-loading score of 0.90, 0.89 and 0.81, respectively. We used this first factor (hereafter called vocal performance) in a multiple regression analysis with the same eight aforementioned predictors to further analyse data.

Our final regression models complied with all assumptions of multiple linear regression. All model residuals were normally distributed. Further residual analysis was performed using

Table 1. Correlations between the six acoustic variables showing the strong relations between calling rate, calling effort and pulse period

	Calling rate	Calling effort	Duration	Pulse period	Dominant frequency	Amplitude modulation
Calling rate	–	0.76***	0.00	−0.67***	0.27	0.41
Calling effort		–	−0.06	−0.55*	0.20	0.33
Duration			–	−0.31	0.00	0.00
Pulse period				–	−0.12	−0.52*
Dominant frequency					–	−0.07
Amplitude modulation						–

Values shown are Spearman rank correlation coefficients. Significant differences are indicated by asterisks, i.e. *$P<0.05$; ***$P<0.001$; P-values are uncorrected for multiple tests. $N=21$ except for the correlation between calling rate and calling endurance where $N=22$.

Durbin–Watson statistics, residual plots as well as multicollinearity tests (variance inflation factors, VIF).

All statistical analyses were performed using SPSS for Windows (16.0, SPSS Inc., Chicago, IL, USA).

RESULTS
Acoustic activity
Acoustic activity varied greatly among subject males. All males produced boatwhistles (BW) during the study period but calling rate varied markedly among males and within males. The average calling rate varied from 0.1 to 361.7 BW h⁻¹ per male (overall mean calling rate=39.9 BW h⁻¹; Table 2). Only seven out of the 22 recorded males exhibited average calling rates higher than 10 BW h⁻¹ during the study period, and the maximum calling rate observed for each male varied between 2 and 1071 BW h⁻¹ (mean=244.5 BW h⁻¹). Males vocalised for a different number of days [mean (range)=5 (2–8)] and for a different number of hours (calling effort, Table 2) but remained in silence most of the time.

Boatwhistle characteristics were consistent with previous descriptions (e.g. Vasconcelos et al., 2010) and showed a large between-individual variation (Table 2).

Predictors of male acoustic characteristics and activity
Correlation analysis showed that both calling rate and calling effort were significantly positively related with lipid content of the somatic muscles, relative gonad mass and relative sonic muscle mass (Table 3). Boatwhistle duration was positively correlated with body condition (Table 3). Pulse period was negatively correlated with lipid content of the somatic muscles and relative sonic muscle mass (Table 3), indicating that males that exhibited an average faster sonic muscle contraction rate (i.e. shorter pulse period) had larger sonic muscles and higher lipid levels in the body.

The best regression models for each dependent variable showed that calling rate, calling effort and pulse period strongly reflect male condition measured by the lipid content in the somatic muscles (Table 4). Body lipid showed high partial correlations with calling rate, calling effort and pulse period ($r=0.89$, 0.94 and −0.61, respectively, Table 4) and accounted for most of the variation explained by each regression model (Table 4). Lipid content of the somatic muscles explained 77% (out of 82% explained by the full model, Table 4), 88% (out of 94%) and 31% (out of 46%) of calling rate, calling effort and pulse period variability, respectively. Males with higher body lipid content showed a significantly higher calling rate, called for more hours and contracted the sonic muscles faster during sound production, exhibiting shorter pulse periods in a boatwhistle (Fig. 2). Calling rate was further predicted by relative sonic muscle mass, which explained a further 5% of its variability. Males with heavier-than-average sonic muscles called at a higher rate (Fig. 2). Gonad mass also explained 3% of calling effort variability, and males with relatively larger gonads spent longer periods calling (Fig. 2). Liver mass accounted for an additional 15% of pulse period variation, and males with heavier livers for a given size tended to contract the sonic muscles faster during sound production as observed by their shorter pulse periods (Fig. 2). Consistently, vocal performance, a new variable that combined the former dependent variables was only predicted by the lipid content of the somatic muscles, the strongest predictor for these three acoustic parameters (Table 4).

Additionally, relative sonic muscle mass showed a weaker but significant positive effect on amplitude modulation explaining 22% of its variation (Table 4; Fig. 2). Both boatwhistle duration and dominant frequency were not predicted by any of the independent variables as regression models were not significant ($P>0.05$).

DISCUSSION
The boatwhistle produced by Lusitanian toadfish nesting males is the major mate attraction signal and therefore essential for male reproductive success (dos Santos et al., 2000) [see Bass and McKibben (Bass and McKibben, 2003) for other batrachoidids]. This call shows a large inter-individual variation (Amorim and Vasconcelos, 2008) (present work) and could thus be used to discriminate among males. But can females use acoustic cues to choose a mate? Our work shows that male vocal activity and mating call characteristics reflect several aspects of male quality.

Calling rate and calling effort
High calling rate and increased calling effort strongly reflected good male body condition measured by the lipid content of the somatic muscles. The lipid fraction of the body muscles explained 77% and 88% of the variability of calling rate and effort observed over a week in our focal males. In animals where males provide parental care, indicators of male parental ability such as body condition are expected to play a substantial role in intersexual communication and be under strong mate selection by females (Andersson, 1994). Fish unguarded eggs are quickly eaten by

Table 2. Descriptive statistics for the dependent acoustic variables

	N	Mean	s.d.	Range	c.v.
Calling rate	22	39.9	87.7	0.1–361.7	2.19
Calling effort	22	30.7	20.1	2.4–67.7	0.66
Boatwhistle duration (ms)	21	681.9	154.4	383.2–1049.7	0.23
Pulse period (ms)	21	19.2	1.1	17.4–22.4	0.06
Dominant frequency (Hz)	21	117.9	35.0	52.7–181.1	0.30
Amplitude modulation	21	0.7	0.2	0.5–1.3	0.26

Coefficient of variation (c.v.)=s.d./mean.

Table 3. Relationship between male physical and acoustic characteristics (Spearman rank correlation)

		Calling rate	Calling effort	Duration	Pulse period	Dominant frequency	Amplitude modulation
LipidM	r	0.51*	0.61**	0.03	−0.60**	−0.05	0.50*
	N	20	20	20	20	20	20
LipidSM	r	−0.06	−0.09	−0.25	0.39	0.33	−0.37
	N	22	22	21	21	21	21
$\log_{10} TL$	r	−0.27	−0.18	−0.02	0.30	−0.15	−0.21
	N	22	22	21	21	21	21
RM_G	r	0.49*	0.63**	0.05	−0.31	0.07	0.14
	N	22	22	21	21	21	21
RM_{AG}	r	0.36	0.42	0.28	−0.29	−0.08	0.41
	N	22	22	21	21	21	21
RM_L	r	0.12	−0.09	0.15	−0.30	−0.19	0.09
	N	22	22	21	21	21	21
RM_{SM}	r	0.58**	0.47*	0.21	−0.53*	0.13	0.41
	N	22	22	21	21	21	21
COND	r	0.04	0.08	0.51*	−0.35	−0.35	0.07
	N	22	22	21	21	21	21

Significant differences are indicated by asterisks, i.e. $*P<0.05$; $**P<0.01$; P-values are uncorrected for multiple tests. LipidM – total lipid content of somatic muscles; LipidSM – total lipid content of sonic muscles. TL – total length; RM_G – residuals of gonad mass; RM_{AG} – residuals of accessory gland mass; RM_L – residuals of liver mass; RM_{SM} – residuals of sonic muscle mass; COND – body condition.

Table 4. Table for predictors of male call parameters (calling rate, calling effort and boatwhistle characteristics)

Dependent variable	Included predictor	B	s.e.m.	t	P	r	F	Model significance	R^2	DW	VIF
Calling performance	Intercept	−1.50	0.36	−4.19	0.001						
	LipidM	3.65	0.78	4.68	<0.001	0.74	$F_{1,18}=21.90$	$P<0.001$	0.55	1.7	1.00
Calling rate	LipidM	2.11	0.26	8.18	<0.001	0.89					1.04
	RM_{SM}	0.27	0.12	2.38	0.03	0.49	$F_{2,18}=41.92$	$P<0.001$	0.82	1.8	1.04
Calling effort	LipidM	73.69	6.11	12.06	<0.001	0.94					1.05
	RM_G	6.46	2.68	2.41	0.03	0.49	$F_{2,18}=85.86$	$P<0.001$	0.91	1.3	1.05
Pulse period	Intercept	20.49	0.44	46.22	<0.001						
	LipidM	−3.03	0.96	−3.14	0.006	−0.61					1.00
	RM_L	−0.44	0.21	−2.15	0.046	−0.46	$F_{2,17}=7.13$	$P=0.006$	0.46	2.0	1.00
Amplitude modulation	Intercept	0.736	0.039	18.69	<0.001						
	RM_{SM}	0.086	0.038	2.27	0.036	0.47	$F_{1,18}=5.16$	$P=0.04$	0.22	1.9	1.00

Calling rate was $\log 10\,(x+1)$-transformed to meet the linear regression model assumptions. r – partial correlation between the dependent variable and the predictor, controlling for the effects of the other predictors in the model. LipidM – total lipid content in the somatic muscles. RM_{SM} – residuals of sonic muscle mass. RM_G – residuals of gonad mass. RM_L – residuals of liver mass. Results are from multiple regression analysis (stepwise procedure). Regression models for boatwhistle duration and dominant frequency were not significant.

predators and females must rely on male brood protection for the survival of their offspring (Sargent and Gross, 1993). Consequently, females benefit from choosing good fathers, and more so if they are single spawners such as batrachoidids (Brantley and Bass, 1994; Modesto and Canário, 2003a). Parental care in the Lusitanian toadfish is costly because type I males experience reduced feeding, fan the eggs and defend their nest vigorously for at least 30 days (till the fry becomes free swimming) (Modesto and Canário, 2003a; Vasconcelos et al., 2010), consistent with the marked decrease in the male's condition (hepatossomatic index and the Fulton's condition factor, K) during the spawning season (Modesto and Canário, 2003a). Our results suggest that Lusitanian toadfish females should favour males that call at a higher rate and for prolonged periods, as they would be in better condition and could provide better parental care. Consistently, larger nesting males of the batrachoidid *P. notatus* sampled at the end of the breeding season presented higher body condition (K) and a larger number of viable late-stage embryos

in the nest (Sisneros et al., 2009), suggesting that body condition is an honest indicator of parental ability in batrachoidids. Similarly, in the sand goby (*Pomatoschistus minutus*), another teleost with prolonged male parental care, only males with adequate body condition initiate nest building and breeding (Lindström, 1998a), and food supplemented males stay longer at the nest, mate sooner and manage to get more eggs than non-fed males with lower condition (Lindström, 1998b). Parental common goby (*Pomatoschistus microps*) males with higher energy reserves are also probably less likely of filial cannibalism than males with lower condition, as observed in other fish (Kvarnemo et al., 1998). Future work will need to address whether calling rate and effort reflect male parental ability in the Lusitanian toadfish. In birds, call rate and other song features may be reliable indicators of parental quality (Dolby et al., 2005).

In our study species, the ability to call at higher rates and to sustain calling for longer periods also reveal, although to a much smaller
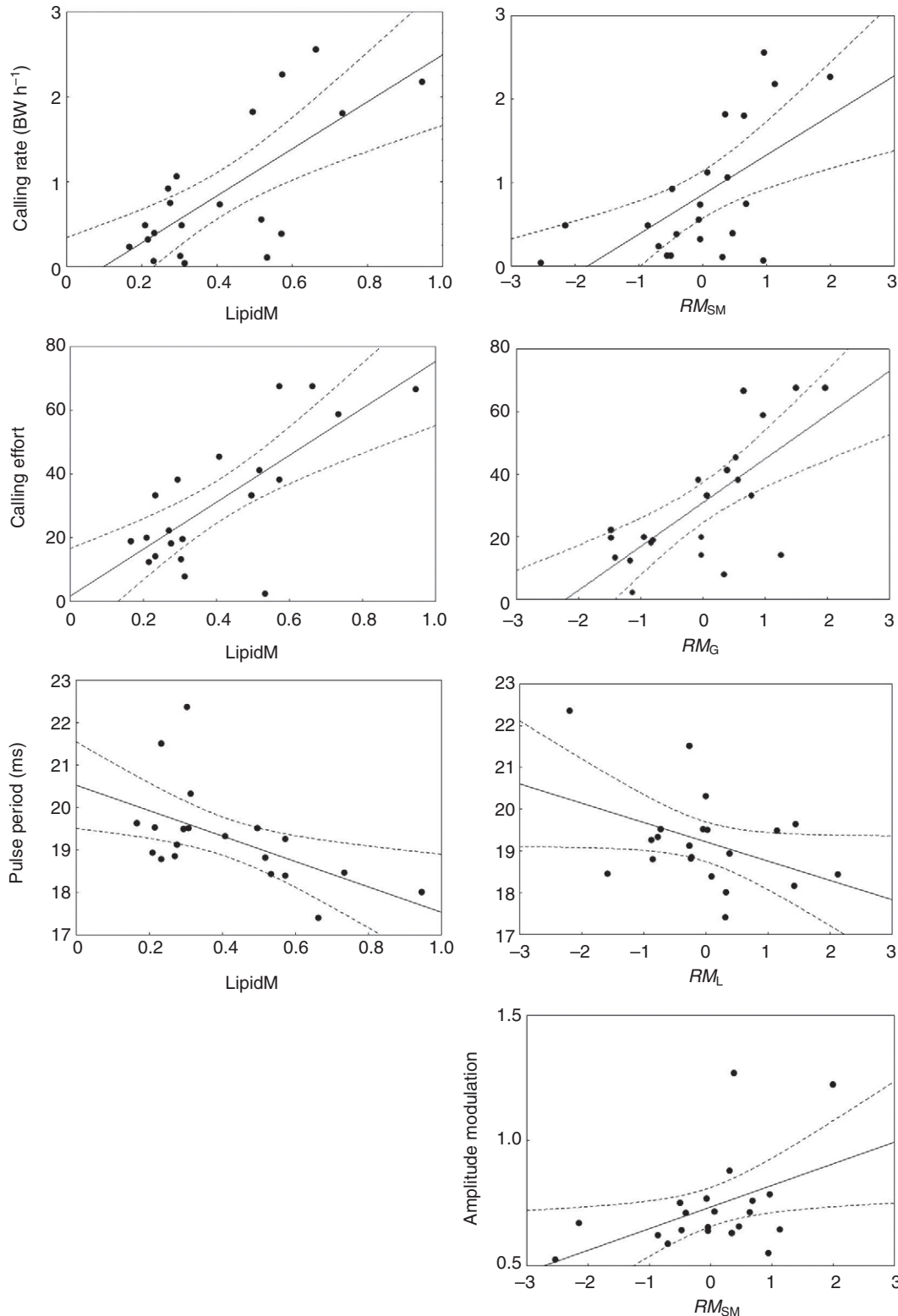
Fig. 2. Relationship between predictor variables of male quality and call parameters. Lines of univariate regressions and 95% confidence interval bands are shown. LipidM – lipid content of body muscles; RM_{SM} – residuals of sonic muscle mass; RM_G – residuals of gonad mass; RM_L – residuals of liver mass.

extent, a higher degree of sonic muscle hypertrophy and relative larger gonad mass. Muscle hypertrophy has been generally associated with a higher capacity for calling (e.g. Connaughton et al., 1997). The sonic muscle hypertrophy in *H. didactylus* males observed in association to the reproductive season is accompanied by an increase of total myofibril and sarcoplasm area and breeding males show a larger sarcoplasm/myofibril area ratio than females (Modesto and Canário, 2003b). A higher sarcoplasm/myofibril area ratio has been interpreted as an adaptation to the increased speed and fatigue resistance needed for boatwhistle production in the oyster toadfish, *Opsanus tau* (Fine et al., 1990) and an increased myofibrillar area should allow for more forceful contractions resulting in higher amplitude calls (Connaughton et al., 1997). Hence, Lusitanian toadfish males with larger sonic muscles should

sustain high calling rates and call at higher amplitudes, resulting in a more conspicuous vocal output. The significant effect of relative gonad mass suggests that calling rate and effort signal male mating motivation as shown for fish and other vertebrates [e.g. fish (Fish, 1972); anurans (Burmeister and Wilczynski, 2001); mammals (Vannoni and McElligott, 2009)]. Also, a higher than average calling rate may signal other male traits such as a better immune system [e.g. insects (Jacot et al., 2004)] or a higher fertilisation success [e.g. anurans (Pfennig, 2000)].

Interestingly sonic muscle lipid content did not seem to predict differences in the ability to sustain high calling rates over a week but perhaps differences in lipid concentration would be visible in the vocal performance of males in a longer time scale. For example, Connaughton et al. (Connaughton et al., 1997) observed a pronounced decrease in sonic muscle lipid in the weakfish (*Cynoscion regalis*) during the peak of acoustic activity, which is a month past the start of a high calling activity in this species.

Boatwhistle characteristics

Males in better condition (body lipid and higher liver mass) had shorter pulse periods and hence contracted the sonic muscles faster during sound production. However, we found a low between-male variability for this parameter (c.v.=6%), consistent with the existence of a vocal central pattern generator in the hindbrain of batrachoidids that establishes the patterned activity of the sonic muscles and hence the pulse period of their calls (Bass and Baker, 1990). Consistently, *O. tau* males also present a similar variability of fundamental frequency of boatwhistles (c.v.=6%) (Barimo and Fine, 1998), which is the inverse of the pulse period. Lusitanian toadfish males that contract the sonic muscles at a very fast rate could reliably be indicating to females their better quality (condition) with the ability to sustain sonic muscle contraction close to their physiological limit. Consistent with this suggestion, males of the non-passerine bird brown skuas that produce long difficult calls close to their performance limit are honestly advertising their quality because they have a higher breeding success and fledge more chicks (Janicke et al., 2008).

Relative sonic muscle mass showed a significant positive effect on boatwhistles amplitude modulation. Amplitude modulation is an important characteristic to distinguish boatwhistle emitted by nesting males in different motivational contexts. Vasconcelos et al. (Vasconcelos et al., 2010) have shown that the Lusitanian toadfish also emits boatwhistles during territorial intrusions by other males but these lack amplitude modulation, which seems characteristic of a mating context. Consequently, it is possible that males can also advertise their quality and motivation by increasing the amplitude modulation of the mating boatwhistle, although this suggestion needs to be tested.

Both boatwhistle duration and dominant frequency showed high variability and were not predicted by any of the considered independent variables. Boatwhistle duration was very variable among males (see also Barimo and Fine, 1998; Amorim and Vasconcelos, 2008). This parameter seems motivation dependent in batrachoidids (M.C.P.A., P.J.F. and J.M.S., unpublished data) (Thorson and Fine, 2002; Remage-Healey and Bass, 2005) and probably translates the male's physiological state (Remage-Healey and Bass, 2005). Although pulse period (and hence the fundamental frequency) showed little variability, the same was not true for the dominant frequency because it may be represented by the fundamental or by the first or the second harmonic (Amorim and Vasconcelos, 2008).

Here, we have shown that calling rate, calling effort, pulse period and amplitude modulation may honestly signal male quality, namely male condition, spawning readiness and sonic muscle hypertrophy in a batrachoidid. A recent study with *P. notatus* has shown that females reveal best hearing sensitivity matching the higher harmonic components of male advertisement calls during the breeding season (Sisneros, 2009). This suggests the action of selective pressures for these females to better detect and probably choose among different males in dense breeding aggregations that are typical of batrachoidids and other sound-producing teleosts. Although not tested in fish, calling rate and time spent calling may influence female choice in other species such as in insects and anurans (reviewed in Gerhardt and Huber, 2002), in birds (e.g. Gentner and Hulse, 2000) and in mammals (e.g. McComb, 1991). Calling activity is also likely to be an important parameter for mate choice in fish because sound duty cycle (the proportion of sound in a stimulus) influences female preference (McKibben and Bass, 1998). Whether females benefit from better brood care, better territories, good genes or just ease of male location, remain still to be addressed in fish.

LIST OF SYMBOLS AND ABBREVIATIONS

BW	boatwhistle
COND	residuals of the simple linear regressions of eviscerated body mass on total length
LipidM	lipid content of somatic muscles
LipidSM	lipid content of sonic muscles
M_{AG}	accessory glands' mass
M_E	eviscerated body mass
M_G	gonads' mass
M_L	liver mass
M_{SM}	sonic muscle mass
RM_{AG}	residuals of the simple linear regressions of accessory glands on eviscerated body mass
RM_G	residuals of the simple linear regressions of gonad on eviscerated body mass
RM_L	residuals of the simple linear regressions of liver on eviscerated body mass
RM_{SM}	residuals of the simple linear regressions of sonic muscles on eviscerated body mass
TL	total length

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