Seasonal variation of sound production in the Lusitanian toadfish *Halobatrachus didactylus*

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Seasonal variation of sound production, which includes boatwhistles, grunts, croaks and double croaks, was studied in the Lusitanian toadfish *Halobatrachus didactylus*. Boatwhistles were emitted during the mating season in contrast with the other sound types, which were emitted all year round.

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Pronounced seasonal patterns of sound production in mature fishes are typically related to reproductive activities. Male members of the toadfish family (Batrachoididae) defend nests in the breeding season, are polygynous, provide parental care and emit a long tonal boatwhistle advertisement call from their nest that is used in male–male competition and mate attraction (Gray & Winn, 1961; Fish, 1972). In the species studied up to date (Bass & McKibben, 2003; Amorim, 2006), both genders produce agonistic broadband pulsed sounds (grunts), but only males emit tonal mating sounds (boatwhistles or hums). These are produced by the extremely fast contraction of the intrinsic sonic muscles upon the swimbladder (Fine *et al.*, 2001). The Lusitanian toadfish *Halobatrachus didactylus* (Bloch & Schneider) (Batrachoididae) is an ubiquitous sound producer that lives along the coast of Portugal in estuarine murky waters (dos Santos *et al.*, 2000). During the reproductive season that lasts from May to July in Portugal (Modesto & Canário, 2003*a*), breeding Lusitanian toadfish males defend nests in shallow water and provide parental care to the eggs in the nest until the young are free-swimming (Brantley & Bass, 1994;

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dos Santos *et al.*, 2000). Recordings made during the breeding season have shown that males of this species have an unusual large acoustic repertoire for fishes, which consists of four distinct sound types: boatwhistles, grunts, croaks and double croaks (dos Santos *et al.*, 2000). Boatwhistles emitted by the Lusitanian toadfish are tonal sounds, *c*. 800 ms long, which start and end with a grunt-like portion. The fundamental frequency is *c*. 60 Hz and dominant frequencies are typically either the second or the fourth harmonic (pers. obs.) (Fig. 1). Grunts are short drumming sounds emitted in trains (typically eight grunts per grunt train) with dominant frequencies around 300 Hz (Fig. 1). Croaks are low frequency pulsed sounds emitted in isolation; they are longer and present higher dominant frequencies (around 650 Hz) than individual grunts and, unlike double croaks, do not show amplitude or frequency modulation (dos Santos *et al.*, 2000). In contrast, double croaks are made up of two croak-like elements that present both amplitude and frequency modulation, with typical durations of *c*. 90 ms (each) and dominant frequencies around 500 Hz (Fig. 1).

Little is known on the function of the different sound types and how sound emissions vary throughout the year. Dos Santos *et al.* (2000) have suggested, in analogy to other batrachoidids, that boatwhistles are mating signals whereas the other sound types are probably agonistic. If this is true, it would be predicted that boatwhistles should only be heard during the breeding season whereas the other sound types should be heard all year round, especially during territory establishment at the start of the breeding season. To test this prediction, seasonal variations of sound production were studied in this species. Several acoustic features of boatwhistles, grunts and double croaks were also measured and correlated with water temperature. These three sound types were the most frequent sound emissions registered in this study.

Recordings of the Lusitanian toadfish sounds were made from piers in two areas within the Tagus estuary, Portugal: Montijo (38°42′ N; 8°58′ W) and Barreiro (38°39′ N; 9°04′ W). An average of five recording sessions (ranging from two to seven) lasting from 5 to 10 min were carried out approximately once a month from July 2001 to September 2002. Recording locations were at least 4 m apart. Water depth varied approximately between 0·8 and 4·0 m, depending on tide. Sounds were registered with a High Tech 94 SSQ hydrophone (sensitivity of −165 dB re 1 V/μPa, flat frequency response up to 6 kHz ± 1 dB) and a Sony TCD-D8 DAT recorder. A thermometer was attached to the hydrophone to register water temperature. Sounds were digitized at 44 kHz (16 bit resolution) and analysed with Raven 1.2 for Windows (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, U.S.A.).

Sound production rate (number of sounds min^{-1}) was determined for each recording session and sound type by listening to the recordings and simultaneously checking the spectrographic representation of the sounds. Only the sounds emitted by fish close to the hydrophone were counted (*i.e.* those that showed a clear representation both in the spectrogram and oscillogram). Because grunts are emitted in trains (dos Santos *et al.*, 2000), the number of grunt trains was counted instead of single grunts. Sound emission rate was compared between recording dates with one-way ANOVAs. The square root transformation was applied to the data because group variances were proportional to the
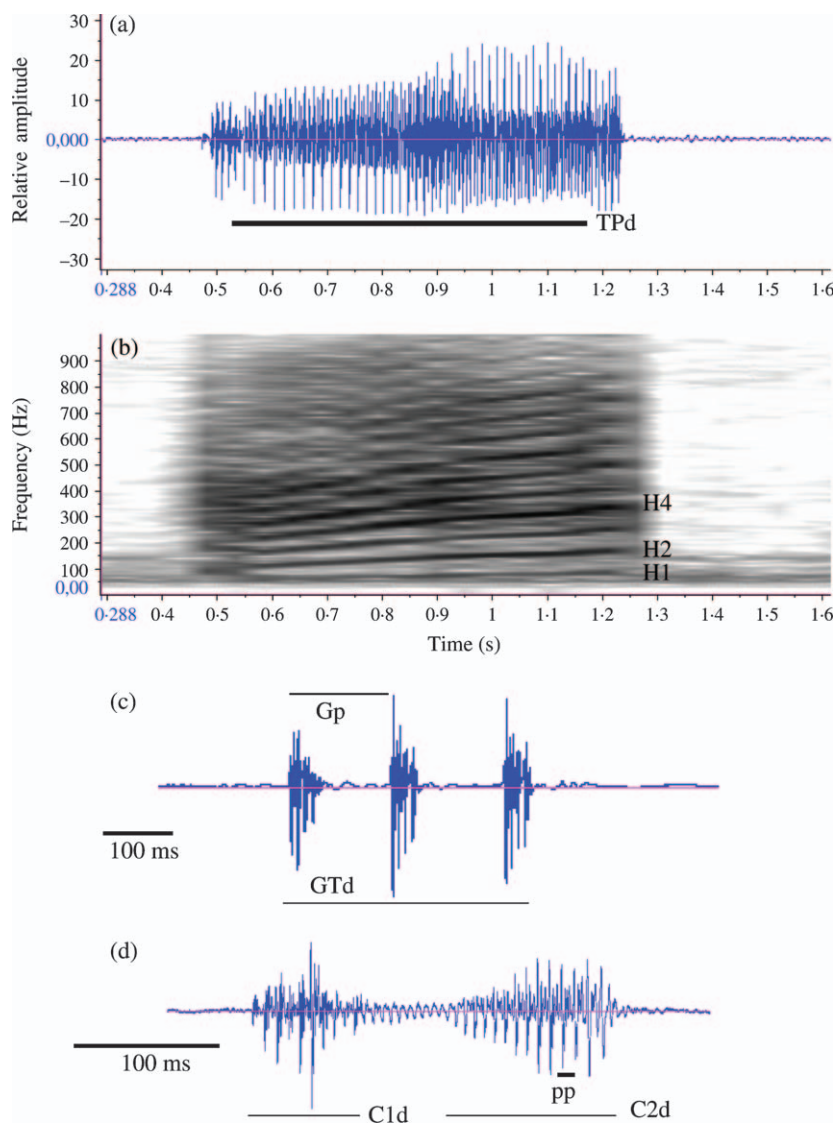
FIG. 1. Acoustic parameters measured in boatwhistles (a) oscillogram and (b) spectrogram, (c) grunt trains and (d) double croaks in *Halobatrachus didactylus*. TPd, tonal phase duration; H1, H2, H4, first, second and fourth harmonic in the boatwhistle tonal phase, respectively (H4 is the harmonic with most energy, *i.e.* the dominant frequency). GTd, grunt train duration; Gp, grunt period; C1d, first croak duration; C2d, second croak duration; pp, pulse period. Note that not all acoustic parameters are depicted. Time bars are given in (c) and (d).

means (Zar, 1984). *Post hoc* multiple group comparisons were conducted with Tukey tests. Boatwhistle, double croaks and grunt trains were analysed for several temporal and frequency features (see Table I and Fig. 1), measured from oscillograms and power spectra (Hamming window, filter bandwidth of 10 Hz), respectively. Sound emission rate and acoustic parameters were correlated with

TABLE I. Acoustic parameters measured in boatwhistles, double croaks and grunt trains emitted by *Halobatrachus didactylus*. The boatwhistle tonal phase is the larger portion of the boatwhistle situated between an initial and an ending grunt-like segment. C1 and C2 are the first and second croak of the double croak, respectively. H2 and H4 are the second and fourth frequency harmonics, which are the typical dominant frequencies in the boatwhistle

Acoustic parameter	Boatwhistle	Double croak	Grunt train
Duration (ms)	Total/tonal phase	Total/C_1 and C_2	Total
Number of pulses	—	C_1 and C_2	Number of grunts per bout
Pulse period (ms)[1]	Tonal phase	C_1 and C_2	Grunt period
Fundamental frequency (Hz)	Tonal phase	C_1 and C_2	Grunt bout
Dominant frequency (Hz)	H2 and H4, tonal phase	C_1 and C_2	Grunt bout

[1]Measured from peak to peak of consecutive pulses or, in the case of grunt bouts, of consecutive grunts.

temperature through Spearman rank correlations (r_s). Bonferroni corrections for multiple tests were applied to P values. All statistical tests were performed with Statistica *v*. 7.1 for Windows (StatSoft, 2005).

The Lusitanian toadfish emitted sounds all year round (except in January 2002) but showed minimum levels of acoustic activity (near 0 sounds min^{-1}) from October to January and February (Fig. 2). All sound types showed significant variation in the emission rate throughout the year (grunt trains: $F_{15,64}$, $P < 0.001$; croaks: $F_{15,64}$, $P < 0.001$; double croaks: $F_{15,64}$, $P < 0.001$; boatwhistles: $F_{15,64}$, $P < 0.001$). Boatwhistle emission showed the most pronounced seasonal activity with no emission registered from October to March and significantly higher calling rates observed during the breeding season, from May to mid-July (Fig. 2; Tukey tests, $P < 0.05$). Boatwhistles were the most conspicuous sound type during the reproductive season (Fig. 2). Grunt trains and double croaks showed similar seasonal patterns, presenting minimum emission rates from October to January (grunt trains were not registered in December and January and double croaks were not registered in January), a slight increase in the pre-reproductive season (February to March), and an emission peak at the start of the mating season, in May (Fig. 2). Grunting levels in May were significantly higher than those observed for October to April and for late June onwards (Tukey tests, $P < 0.01$). Similarly, May double croak emission rates were significantly higher than that observed in 21 July 2001 to March 2002 and in 25 June 2002 to September 2002 (Tukey tests, $P < 0.05$). Croaks were emitted at low rates with slight variations throughout the year. The croak's emission rate was significantly higher in May when compared to the October to February emission levels (Fig. 2; Tukey tests, $P < 0.05$).

Seasonal patterns of acoustic emissions in male Lusitanian toadfish were significantly influenced by changes in water temperature. Sound production rate was positively correlated with this parameter (all sound types: $n = 80$, $r_s = 0.31$ to 0.49, $P < 0.05$). Sound production rate and especially boatwhistles emission, however, dropped at the end of the breeding season (*i.e.* end of July)
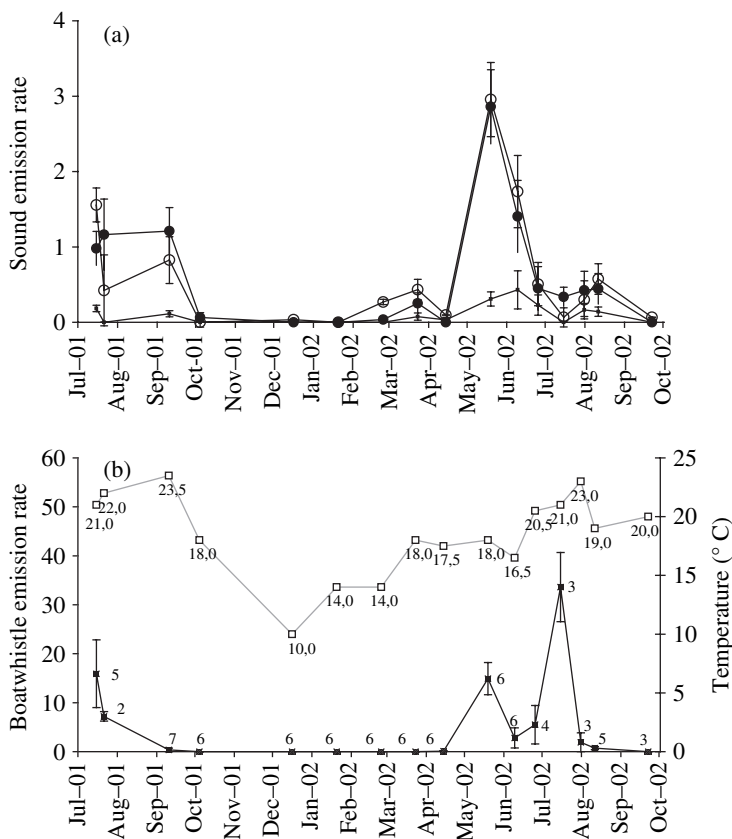
FIG. 2. Mean ± S.E. (a) emission rates (number of sound min^{-1}) of double croaks (●), grunt trains (○), croaks (·) and (b) boatwhistles (■) in *Halobatrachus didactylus*. Water temperature (□) observed in the Tagus estuary (Montijo and Barreiro, Portugal) from July 2001 to September 2002 is given in (b). Numbers given in (b) correspond to the number of recording sessions (sample size).

despite the high water temperatures (Fig. 2). Water temperature also influenced acoustic features of sound emissions. Total boatwhistle duration ($n = 100$, $r_s = 0.46$, $P < 0.001$) and tonal phase (TP) duration ($n = 100$, $r_s = 0.37$, $P < 0.001$) significantly increased with temperature. The TP pulse period decreased ($n = 100$, $r_s = -0.50$, $P < 0.001$) and the TP fundamental frequency increased ($n = 100$, $r_s = 0.48$, $P < 0.001$) with rising temperature values. No significant correlation was found for TP dominant frequency (both H_2 and H_4) and temperature ($n = 100$, $r_s = 0.14$ to 0.16, $P > 0.05$). In grunt trains, grunt period decreased ($n = 47$, $r_s = -0.51$, $P < 0.01$) and the fundamental frequency increased ($n = 47$, $r_s = 0.54$, $P < 0.001$) significantly with temperature. All other grunt features did not vary with temperature ($n = 47$, $r_s = -0.03$ to 0.07, $P > 0.05$). Total double croak and second croak durations ($n = 38$, $r_s = -0.62$ to -0.44, $P < 0.05$), as well as the first and second croak pulse period ($n = 38$, $r_s = -0.76$ to -0.64, $P < 0.001$), decreased with water temperature, but all other analysed features were not affected by temperature ($n = 38$, $r_s = -0.20$ to 0.44, $P > 0.05$).

This study has shown that the acoustic activity and repertoire size of the Lusitanian toadfish has a marked seasonal variation. The emission of boatwhistles was restricted to the breeding season, which is consistent with the suggestion that it is involved in mate attraction (dos Santos *et al.*, 2000) as in other batrachoidids (Winn, 1972; Ibara *et al.*, 1983; Brantley & Bass, 1994). Grunts and double croaks were emitted all year round and showed a pronounced emission peak at the start of the breeding season. It is likely that these sound types are agonistic signals and may play a role in territorial establishment by nesting males. Other members of the Batrachoididae have shown a similar vocal behaviour (Amorim, 2006). *Porichthys notatus* Girard nesting males emit growls only at the start of the breeding season when males are occupying nest sites (Bass *et al.*, 1999). Interestingly, growls have intermediate characteristics to the agonistic grunt and to the mating humming that are heard throughout the breeding season (Ibara *et al.*, 1983; Brantley & Bass, 1994). Agonistic grunts in *Opsanus tau* (L.) are produced by nesting males when conspecific males approach the nest, especially if the nesting males are guarding eggs or young (Gray & Winn, 1961). In this species, grunts are emitted by both males and females outside the breeding season (Winn, 1967).

Sound production rate, regardless of sound type, increased with temperature. Activity in this species is strongly temperature dependent: spawning is only triggered with temperatures >18° C and lethargy is induced by temperatures <14–16 °C (J. L. Costa, pers. comm.). Consistently, some acoustic activity was present all year round, probably because water temperature only dropped below 14° C in January 2002. Likewise, when water temperature rose to 18° C in March 2002, a slight increase in acoustic activity was observed, with few boatwhistles already registered in April 2002 (0·1 ± 0·07 boatwhistles min^{-1}). Moreover, the boatwhistle emission drop observed on 9 June 2002 was most likely caused by the sudden fall of water temperature (to 16·5 °C) in that period. Temperature also influenced acoustic properties of boatwhistles, grunt trains and double croaks emitted by the Lusitanian toadfish. In general, sound duration and fundamental frequency increased, and pulse period decreased with rising water temperatures, which is consistent with other studies (Brantley & Bass, 1994; Connaughton *et al.*, 2000). This is probably related to temperature dependency of muscle contraction properties (Feher *et al.*, 1998) and of pulse pattern generator circuits in the central nervous system (Bass & Baker, 1991). Temperature, however, did not explain all the variability in the data in the present study (the highest r_s was −0·76) suggesting that additional effects are involved. In *O. tau*, boatwhistle duration is not temperature dependent, although it varies seasonally (Fine, 1978), and in *Opsanus beta* (Goode & Bean) the duration and other features of boatwhistles can also change in a daily cycle, unrelated to temperature (Thorson & Fine, 2002). In *H. didactylus,* boatwhistles show clear individual differences (pers. obs.) and boatwhistle individuality can obscure significant temperature and season effects on acoustic parameters. Acoustic features and general acoustic activity observed in the Lusitanian toadfish must have also been influenced by seasonal androgen changes (Fine, 1978; Modesto & Canário, 2003*a*, *b*). In batrachoidids and in other vocal fishes with pronounced seasonal acoustic activity, androgen variations associated with the breeding season cause sonic muscle hypertrophy and

changes in fibre morphology that increase muscle contractile abilities and may affect sound properties (Brantley *et al.*, 1993; Connaughton *et al.*, 2002), although Johnson *et al.* (2000) found no seasonal pattern in the sonic neuro-muscular system (sonic muscle mass and spinal sonic motor nucleus neurone soma size) in *O. tau*.

Vocal fish species that live in dark and turbid waters are difficult to see in their natural environment but can be monitored through passive acoustics (Luczkovich & Sprague, 2001). Seasonal variation in the acoustic activity of the Lusitanian toadfish and other fishes can give information on their general activity, which typically increases around the reproductive season. In particular, detection of distinct seasonal emission patterns for different sound types, as found in the present study, may further elucidate the function of sound production in fish communication.

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