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Development of vocalization, auditory sensitivity and acoustic communication in the Lusitanian toadfish *Halobatrachus didactylus*

Raquel O. Vasconcelos[1,2,*] and Friedrich Ladich[2]

[1]Departamento de Biologia Animal e Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa. Bloco C2 Campo Grande, 1749-0161 Lisbon, Portugal, [2]Unidade de Investigação em Eco-Etologia, I.S.P.A. Rua Jardim do Tabaco 34, 1149-041 Lisbon, Portugal and [3]Department of Behavioural Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

*Author for correspondence (e-mail: raquel_vasconcelos@ispa.pt)

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SUMMARY

The ontogenetic development of acoustic communication has so far only been investigated in one fish species. In order to determine whether detectability of conspecific sounds changes during growth in a species with limited hearing abilities (generalist), we investigated the development of auditory sensitivity and agonistic vocalizations in the Lusitanian toadfish *Halobatrachus didactylus*. Agonistic grunts were recorded, their sound pressure levels determined, and auditory sensitivities measured in five different size groups ranging from 3 to 32 cm standard length. Hearing thresholds were obtained using the auditory evoked potentials (AEP) recording technique. Dominant frequency, sound duration and number of pulses decreased, whereas pulse period and sound level increased with increasing fish size. The best hearing was below 300 Hz in all groups. Lower hearing sensitivity was found in the smallest juveniles at 100 Hz as well as at higher frequencies (800 and 1000 Hz). Comparisons between audiograms and sound spectra within the same-sized fish revealed that smaller juveniles would be barely able to detect agonistic grunts, while these vocalizations were clearly perceived by larger fish. In the latter, the main energy of sounds was found at the most sensitive frequencies. This study demonstrates that acoustic communication in the Lusitanian toadfish might be absent in early developmental stages and seems to start when juveniles are able to generate grunts of higher sound level and lower dominant frequency.

Key words: ontogeny, sound spectra, hearing, auditory evoked potential, acoustic communication, *Halobatrachus didactylus*.

INTRODUCTION

While numerous studies have examined developmental changes in vocalizations or hearing in mammals and birds (e.g. Dimitrieva and Gottlieb, 1992; Dimitrieva and Gottlieb, 1994; Podos et al., 1995; Ruben, 1995; Reimer, 1996; Moss et al., 1997; Branchi et al., 2001), few have focused on similar changes in other vertebrates such as fishes. Ontogenetic development of vocalization has been investigated in detail in the croaking gourami *Trichopsis vitatta*. Sound duration, number of pulses, pulse period and sound level increased, while dominant frequency decreased with age (Henglmüller and Ladich, 1999; Wysocki and Ladich, 2001). Such a negative correlation between dominant frequency and size was also found in other fish species (e.g. Ladich et al., 1992; Myrberg et al., 1993; Crawford, 1997; Amorim and Hawkins, 2005).

Whereas sound characteristics change with age and size in all fishes investigated, no clear picture exists on whether auditory sensitivity changes during development. Using whole nerve action potential recordings, Corwin (Corwin, 1983) first described an increment in vibrational sensitivity with growth for the elasmobranch *Raja clavata*. Improved hearing with increasing size was reported in the damselfish *Stegastes partitus*, the labyrinth fish *T. vittata* and the batrachoidid *Porichthys notatus* (Kenyon, 1996; Wysocki and Ladich, 2001; Sisneros and Bass, 2005), whereas no improvement was observed in the otophysines *Carassius auratus* and *Danio rerio* (Popper, 1971; Higgs et al., 2002; Higgs et al., 2003) or in the damselfish *Abudefduf saxatilis* (Egner and Mann, 2005).

Furthermore, the relationship between development of hearing and sound production is almost unknown in fishes. The only study correlating both processes was in *T. vitatta* (Wysocki and Ladich, 2001), where auditory sensitivity develops prior to the ability to vocalize and sound production occurs prior to the ability to communicate acoustically.

The aims of the present study were to (1) describe the developmental changes of temporal, spectral and intensity characteristics of agonistic grunt sounds emitted by the Lusitanian toadfish, *Halobatrachus didactylus* (Bloch and Schneider 1801), in a distress situation; (2) analyze the development of auditory sensitivity with growth; and (3) determine whether the ability to communicate acoustically changes across the life history in this species.

The Lusitanian toadfish (Batrachoididae) possesses a relatively complex acoustic repertoire of different low-frequency vocalizations, i.e. at least three sounds likely used in agonistic contexts (grunt call, croak and double-croak), and one for mate attraction (boatwhistle) (Dos Santos et al., 2000). Males are territorial and defend nests under rocks in shallow waters during the breeding season, from May to July (Dos Santos et al., 2000; Palazón-Fernández et al., 2001; Modesto and Canário, 2003a). Grunt calls (or trains of grunts) are detectable almost the year round but are more frequent early in the reproductive season, and are therefore thought to be important for occupation of territories and nest defence (Amorim et al., 2006).

MATERIALS AND METHODS

Animals

The test subjects were 79 Lusitanian toadfish, *H. didactylus*, caught by local fishermen in the estuaries of the Mira and Tagus (only the largest fish size group) Rivers (Portugal). Fish were kept in 250 l tanks separately according to their size for at least 2 weeks before starting the auditory experiments. The bottoms of aquaria were covered with sand and equipped with several half flowerpots and plastic shelters (for larger specimens). The aquaria were filtered by external filters and protein skimmers and a 12 h:12 h L:D cycle was maintained. Animals were fed every second or third day with cod and occasionally shellfish.

Sound recordings were obtained in 73 fish (standard length, *SL*=3.8–31.8 cm; body mass=2.14–800 g), whereas sound pressure levels (SPL) were measured from 38 calling specimens (*SL*=3.8–23.8 cm; body mass=2.14–323 g).

For auditory sensitivity measurements and comparison with sound spectra, tested animals were classified by size into five different groups (G); G1: *SL*=2.8–3.8 cm, body mass=0.60–2.14 g (*N*=6); G2: *SL*=5.4–6.6 cm, body mass=4.2–7.0 g (*N*=6); G3: *SL*=8.0–10.2 cm, body mass=11–27 g (*N*=7); G4: *SL*=12.4–15.3 cm, body mass=43–84 g (*N*=6); and G5: *SL*=20.2–31.8 cm, body mass=221–800 g (*N*=9). Individuals of these groups were probably just a few months, 1 year, 1–2 years, 2–3 years and 5–8 years old, respectively (based on J. L. Costa, unpublished). Hearing thresholds from the largest size group (G5) are reported elsewhere (Vasconcelos et al., 2007).

All experiments were performed with the permission of the Austrian Commission on Experiments in Animals (GZ 68.10/50-Pr/4/2002 and GZ 66.006/2-BrGT/2006).

Sound recordings and sound pressure level measurements

Test subjects were handheld by the investigator and positioned inside an oval plastic tub (diameters: 45×30 cm, water depth: 12 cm) covered with sand on the bottom and lined on the inside with acoustically absorbent material (air-filled packing wrap) to reduce resonances and reflections. Fish were positioned underwater in the center of the experimental tub at a distance of 10 cm from the hydrophone fixed at the right side of the animal. We chose this recording procedure because agonistic fish–fish interactions typically take place at roughly this distance, in particular during nest defense in aquaria (R.O.V. and F.L., personal observations).

Most of sound recordings were performed in the laboratory (*N*=44 fish, *SL*=3.8–27.0 cm, body mass=2.14–579 g). However, in order to avoid any lab artifacts in terms of frequency content of sounds from larger specimens, vocalizations from 29 fish (*SL*=8.0–31.8 cm, body mass=11–800 g) were also recorded at the field near an intertidal toadfish nesting area inside the experimental tub over the sand substrate. These field recordings were used for dominant frequency determinations and spectral analysis (groups 3–5).

Fish sounds were recorded for over 1–4 min (at least 10 sounds) per specimen using a hydrophone (Brüel and Kjaer 8101, Naerum, Denmark; frequency range: 1 Hz–80 kHz, ±2 dB; voltage sensitivity: –184 dB re. 1 V/μPa) connected to a Brüel and Kjaer 2804 power supply and a DAT recorder (Sony TCD-D100, Sony Corporation, Tokyo, Japan) or a flashcard recorder (Marantz PMD 660, Eindhoven, The Netherlands). Field recordings were performed with a hydrophone (High Tech 94 SSQ, Gulfport, MS, USA; frequency range: 30 Hz–6 kHz, ±1 dB; voltage sensitivity: –165 dB re. 1 V/μPa) connected to an amplifier (Edirol UA-25, Roland Corporation, Tokyo, Japan) and a portable computer. Instantaneous SPL values, i.e. L_{LFP} (linear frequency weighting, RMS fast time weighting), were measured for 10 sounds per fish using a sound level meter (Brüel and Kjaer 2804 Mediator) connected to the power supply.

Sound analysis

Sound recordings (sampling frequency 6 kHz) were analyzed using Raven 1.2 for Windows (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA). The following sound characteristics (see Fig. 1) were determined from 10 grunts per fish: total duration of single grunts (ms), from the start of the first pulse to the end of the last pulse; number of pulses within a single grunt; pulse period (ms), as the average time period between two up to six consecutive peaks (depending on number of pulses within a grunt); dominant frequency (Hz), as the highest amplitude within the sound power spectrum (Blackman-Harris window, filter bandwidth 10 Hz).

Cepstrum-smoothed sound power spectra (Noll, 1967) were calculated for each size group. A sound file composed of vocalizations emitted by different specimens (10 sounds per individual) was created separately for each size groups (number of fish per group: G1, *N*=1; G2, *N*=5; G3, *N*=9; G4, *N*=6; G5, *N*=8) and used to create group-specific sound spectra. These were determined using the acoustic analysis software S_TOOLS-STx 3.7 (Acoustics Research Institute, Austrian Academy of Sciences, Vienna, Austria). Absolute sound spectra of the recordings were calculated as described previously (Amoser et al., 2004; Wysocki and Ladich, 2005a).

Auditory sensitivity measurements

The auditory evoked potential recording protocol was based on that originally reported and evaluated (Kenyon et al., 1998) and subsequently modified (Wysocki and Ladich, 2005a; Wysocki and Ladich, 2005b). Hence, just a shortened description of the experimental procedure will be given.

In order to immobilize fish, Flaxedil (gallamine triethiodide; Sigma-Aldrich, Vienna, Austria) diluted in a Ringer solution (see Walsh, 1987) was administered intramuscularly, i.e. 5–6 μg g^{-1} body mass for groups 1–4 and 10–15 μg g^{-1} body mass for group 5. This still enabled the fish to produce slight opercular movements. The subjects were positioned below the water surface in the center of an oval plastic tub (diameters: 45×30 cm, water depth: 12 cm, 1.5 cm layer of sand) lined on the inside with air-filled packing wrap. The contacting points of the electrodes were maximally 1–2 mm above the water surface. A small piece of Kimwipes™ tissue paper was placed on the fish head to keep it moist and ensure proper contact of electrodes. Respiration pipettes with different dimensions were inserted into the subjects' mouth

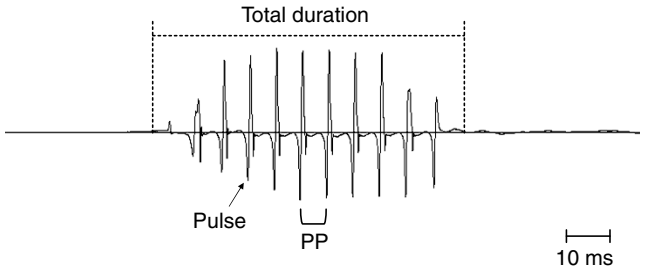

Fig. 1. Oscillogram of a single grunt of a juvenile *H. didactylus* showing temporal sound characteristics analyzed (PP, pulse period).

according to their size. Respiration was achieved through a simple temperature-controlled (22±1°C), gravity-fed water system. The recording electrode was placed at the brainstem region and the reference electrode cranially close to the nares (silver wire, 0.25 mm diameter), pressed firmly against the subject's skin. Shielded electrode leads were attached to the differential input of an a.c. preamplifier (Grass P-55, Grass Instruments, West Warwick, RI, USA; gain 100×, high-pass at 30 Hz, low-pass at 1 kHz). A grounding electrode was placed underwater near the fish body. A hydrophone (Brüel and Kjaer 8101) was placed on the right side of the fish (circa 1 cm away) near the inner ear in order to determine absolute stimulus SPL values underwater in close proximity to the subjects. The experimental tub was positioned on an air table (TMC Micro-g 63–540, Technical Manufacturing Corporation, Peabody, MA, USA), which rested on a vibration-isolated concrete plate. The entire experimental setup was enclosed in a walk-in soundproof room (interior dimensions, 3.2 m× 3.2 m×2.4 m), which was constructed as a Faraday cage.

Acoustic stimuli consisted of tone bursts presented at a repetition rate of 21 s^{-1}. The hearing thresholds were determined at the following frequencies: 50, 100, 200, 300, 500, 800 and 1000 Hz, always presented at random. Duration of sound stimuli increased from 2 cycles at 50 Hz (40 ms) up to 5 cycles at 1000 Hz (5 ms). All bursts were gated using a Blackman window. For each test condition, one thousand stimuli were presented at opposite polarities (180° phase shifted) and were averaged together by the BioSig RP Software, yielding a 2000-stimulus trace to eliminate any stimulus artifact. At frequencies close to the threshold, this procedure was performed at least twice and the AEP traces were overlaid to examine if they were repeatable. SPL values of tone burst stimuli were reduced in 4 dB steps. The lowest SPL where a recognizable and repeatable AEP trace could be obtained was considered the hearing threshold.

Sound stimuli presentation and AEP waveform recording were accomplished using a Tucker-Davis Technologies (Gainesville, FL, USA) modular rack-mount system (TDT System 3) controlled by Pentium 4 PC containing a TDT digital processing board and running TDT BioSig RP Software. A dual-cone speaker (Wharfedale Pro Twin 8, frequency response: 65 Hz–20 kHz ±3 dB), mounted 1 m above subjects in the air, was used to present tone stimuli during testing.

Hearing thresholds were obtained using the auditory evoked potentials (AEP) recording technique. Although hearing generalists, such as batrachoidids, primarily detect particle motion of sounds (Fay and Edds-Walton, 1997; Weeg et al., 2002), for technical reasons we determined hearing thresholds of the Lusitanian toadfish in pressure units. This experimental procedure is acceptable because our study emphasized a comparison of hearing abilities of different-sized fish with their corresponding absolute sound power spectra of agonistic vocalizations, which are also given in pressure units. Moreover, this approach with hearing generalists has frequently been adopted in similar studies, e.g. the Lusitanian toadfish *Halobatrachus didactylus* (Vasconcelos et al., 2007), the oyster toadfish *Opsanus tau* (Yan et al., 2000), the bluegill sunfish *Lepomis macrochirus* (Scholik and Yan, 2002), the gobies *Padogobius martensii* and *Gobius nigricans* (Lugli et al., 2003), the European perch *Perca fluviatilis* (Amoser et al., 2004; Amoser and Ladich, 2005) and the damselfish *Abudefduf saxatilis* (Egner and Mann, 2005). Even so, the hearing thresholds should not be considered as absolute values. Calibration tests were performed later on using an uniaxial pressure acceleration sensor (p-a probe, Applied Physical Sciences Corporation, Groton, CT, USA) and showed that pressure and particle velocity were positively correlated to each other below the water surface in our experimental tub. Any 4 dB change in SPL was accompanied by a 4 dB change in particle acceleration at any frequency (re. 1 μm s^{-2}).

Statistical analysis

Means of sound characteristics were calculated for each fish (based on 10 sounds per individual) and used for further analyses. Relationships between fish size (*SL* or log*SL*) and sound characteristics (or log of the measured variables) were determined by Pearson's correlation coefficients and linear regressions.

Audiograms from different fish groups were compared by a repeated-measures ANOVA, which analyzed responses (hearing thresholds) to several frequencies in each subject fish (within-subject factor) of different size groups (between-subject factor).

In addition, a one-way ANOVA was performed separately at each test frequency, followed by a Bonferroni *post-hoc* test, in order to verify group-specific differences.

Parametric tests were used preferentially since data were normally distributed and variances homogeneous. All SPL values obtained (in dB) were converted to sound pressure (μPa), used for calculations, and then converted back to dB. Therefore, two different values for s.e.m. are given (see Table 1). The statistical tests were performed with Statistica 7.1 for Windows (StatSoft, Inc., 2005).

RESULTS

Sound production

Lusitanian toadfish were territorial at early stages of development. Small specimens from G3 exhibited several agonistic displays during shelter occupation and feeding, such as opening the mouth and spreading of pectoral fins and opercula during confrontation in aquaria. Sounds were produced in all groups tested and started almost immediately when handling the specimens. However, within the G1 size range, only one specimen measuring 3.8 cm *SL* (body mass=2.14 g) showed vocal activity, whereas the others did not utter sounds during the experimental procedure (*SL*=2.8–3.8 cm, body mass=0.60–1.80 g, *N*=6).

Agonistic vocalizations in groups G1 and G2 consisted primarily of single grunts, whereas in groups G4 and G5 they were often produced in series with shorter intervals between consecutive grunts (Fig. 2).

The total duration of single grunts (r=–0.469, N=44, P=0.001) and the number of pulses within grunts (r=–0.761, N=44, P<0.001, Fig. 3) decreased with growth, in contrast to pulse period (r=0.693, N=44, P<0.001, Fig. 4). Sound pressure levels were positively correlated with fish size (r=0.944, N=38, P<0.001, Fig. 5).

Sound spectra showed that in G1 sound energy was concentrated at the third and fourth harmonics (420–570 Hz), while in G5, the main energy was mostly found at the first harmonic at about 110 Hz. Intermediate groups showed a gradual change as fish grew (Fig. 6).

Auditory sensitivity

Auditory evoked potentials were recorded in all test groups between 50 and 1000 Hz, with the exception of G1 and G2, where a recognizable and repeatable AEP trace could not be obtained at 1000 Hz (Table 1, Fig. 7). All size groups revealed best hearing at 50 Hz and a sensitivity decrease towards 1000 Hz. The mean hearing thresholds increased from about 77 dB re. 1 μPa at 50 Hz (G3–G5) up to 132 dB at 1000 Hz (G3).

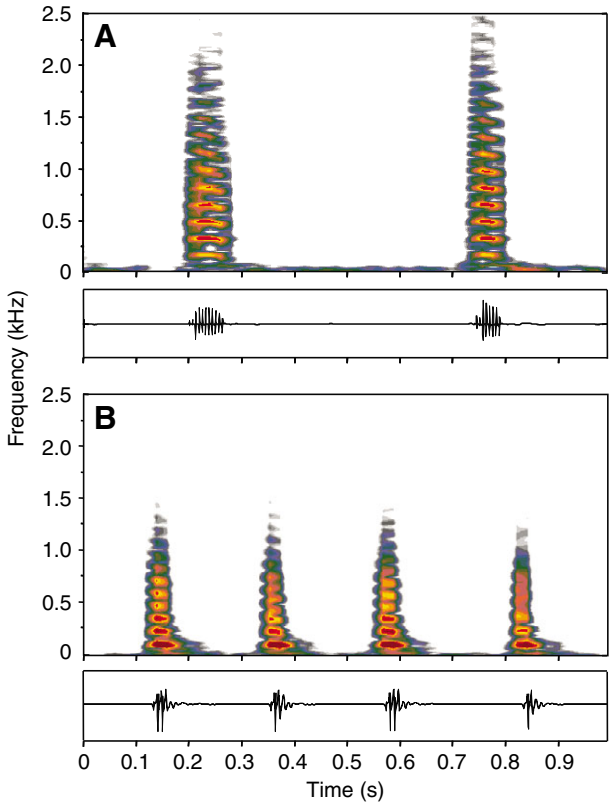

Fig. 2. Sonogram and oscillogram of a grunt call produced by a representative *H. didactylus* of (A) group G2 (6.1 cm *SL*) and (B) group G5 (28.5 cm *SL*), showing two single grunts (A) and a part of a grunt train (B). Note the shorter grunt duration and lower dominant frequency in B. Sampling frequency 6 kHz, filter bandwidth 15 Hz, 70% overlap, Blackman–Harris window.

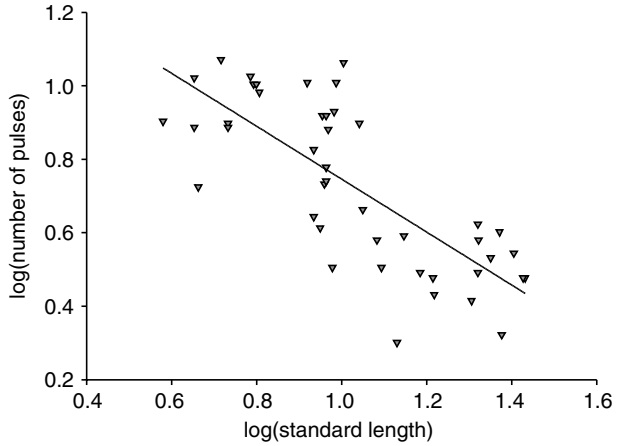

Fig. 3. Log–log plot of mean number of pulses against standard length (*SL*, in cm). Regression equation: log number of pulses=–0.72×log(*SL*)+1.47. N=44, P<0.001, r^2=0.579.

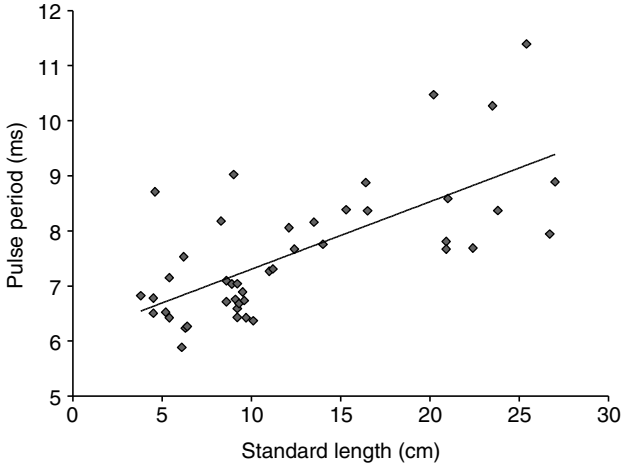

Fig. 4. Correlation between mean pulse period and standard length (*SL*). Regression equation: pulse period=0.12×*SL*+6.08. N=44, P<0.001, r^2=0.480.

Comparisons between audiograms obtained from all size groups (at the frequency range 50–800 Hz) showed significant overall differences (repeated-measures ANOVA, $F_{4,27}$=9.01, P<0.001) and significant interactions between size and frequency ($F_{20,135}$=8.99, P<0.001). Namely, the audiogram of the smallest size group (G1) differed significantly from those of G4 (repeated measures ANOVA, $F_{1,10}$=9.77, P=0.011) and G5 (repeated measures ANOVA, $F_{1,12}$=21.58, P<0.001).

Comparing groups at each frequency separately revealed significant differences at 100 Hz (one-way ANOVA, $F_{4,28}$=11.85, P<0.001) and at the highest test frequencies, 800 Hz (one-way ANOVA, $F_{4,29}$=9.80, P<0.001) and 1000 Hz (one-way ANOVA, $F_{2,19}$=27.58, P<0.001) (Fig. 7). Bonferroni *post-hoc* tests revealed significant group-specific differences, namely: at 100 Hz, between G1 and all the others; at 800 Hz, between groups G1 and G3 and groups G4 and G5; and at 1000 Hz, between G3 and groups G4 and G5. At 50 Hz, inter-group differences were close to significance (one-way ANOVA, $F_{4,28}$=2.98, P=0.036; Bonferroni *post-hoc* test: between G1 and G5: P=0.061; between G1 and G3: P=0.073).

Comparison between sound spectra and audiograms

Comparison between audiograms and sound power spectra within the same size group (Fig. 8), calculated for a distance of 10 cm, showed that the agonistic vocalizations were clearly detectable in groups G4 and G5. Sound spectra were considerably above hearing thresholds in the frequency range below 200 Hz (up to circa 20–30 dB re. 1 μPa at 100 Hz), where the main energy of agonistic vocalizations was concentrated. In G3, sound energy was up to about 5 dB re. 1 μPa above hearing thresholds, at approx. 160 Hz. However, within G2 and G1 juveniles, the sound spectrum was more than 5 and 15 dB re. 1 μPa below the auditory curve, respectively.

DISCUSSION

Development of sound production

Agonistic vocalizations are produced in numerous contexts, such as distress or disturbance situations (e.g. while being attacked or grabbed by potential predators), competitive feeding and competition for space (Ladich and Myrberg, 2006). Competition for food and space is important for both adults and all juveniles, and sound production during agonistic contexts has been reported in juvenile stages of several non-related families such as tigerperches, cobitids, gouramis and gurnards (Schneider, 1964; Valinski and Rigley, 1981; Henglmüller and Ladich, 1999; Wysocki and Ladich, 2001; Amorim and Hawkins, 2005).

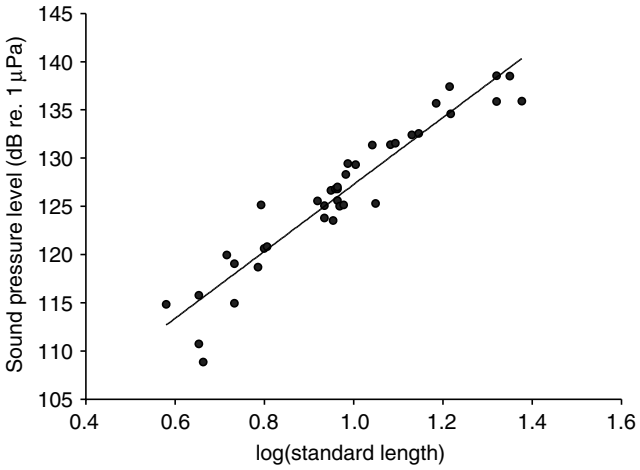

Fig. 5. Correlation between mean sound pressure level and log standard length (*SL*, in cm). Regression equation: sound pressure level=34.70×log(*SL*)+92.56. *N*=38, *P*<0.001, r^2=0.903.

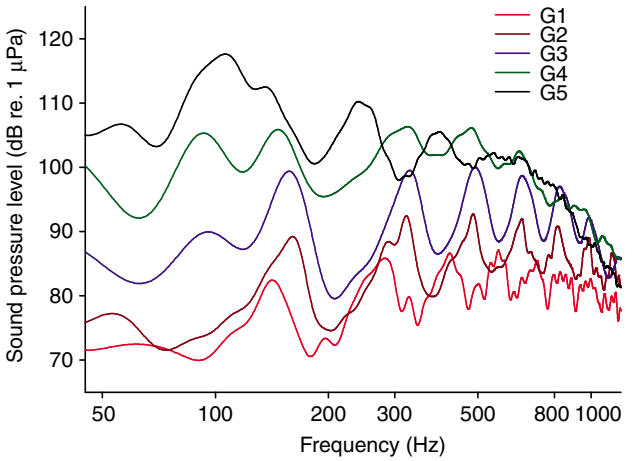

Fig. 6. Cepstrum-smoothed sound power spectra (mean) of the Lusitanian toadfish grunt call from groups G1 (3.8 cm *SL*, *N*=1); G2 (5.4–6.6 cm *SL*, *N*=5); G3 (8.0–10.2 cm *SL*, *N*=9); G4 (12.4–15.3 cm *SL*, *N*=6); and G5 (20.2–31.8 cm *SL*, *N*=8). Sampling frequency 6 kHz, filter bandwidth 1 Hz, 75% overlap, Blackman–Harris window.

Lusitanian toadfish juveniles were extremely territorial and exhibited agonistic displays (at least starting at *SL*=8 cm, probably 1–2 years old), including opening the mouth and extension of pectoral fins during confrontation with similar-sized conspecifics. When handling the fish, agonistic vocalizations were uttered in all different size/age classes studied (from *SL* 4–32 cm, a few months up to *circa* 5–8 years old). However, in the smallest size group (*SL*=2.8–3.8 cm), most of the tested animals did not exhibit vocal activity and only the heaviest specimen uttered sounds during the experimental proceeding. These data suggest that either in this early stage the sound-producing apparatus was not sufficiently developed to produce sounds or it could be too risky demonstrate toughness when the fish are too small and vulnerable to potential predators.

In general, sounds consisted mostly of single grunts in juveniles (groups G1–3), whereas in sexually mature specimens, i.e. G5 and probably G4 (total length more than 15 cm), were often produced series or trains of grunts. The minimum maturity sizes are 16 cm and 19 cm total length for males and females, respectively (Palazón-Fernández et al., 2001).

Agonistic calls of adults recorded in the laboratory by handling the specimens were similar to those obtained from field recordings at the nesting places of *H. didactylus*, which are important during agonistic contexts and for territorial occupation (see Dos Santos et al., 2000; Amorim et al., 2006). This similarity in terms of temporal and spectral characteristics between handheld fish calls underwater and field-recorded grunt trains has also been described in other batrachoidids, e.g. *Opsanus tau* (Cohen and Winn, 1967). In addition, through brain stimulation in *Opsanus beta* (Demski and Gerald, 1972; Demski and Gerald, 1974) and in *O. tau* (Fine, 1979; Fine and Perini, 1994), grunts were produced in the laboratory and shown to be similar to field-recorded calls of the species. Interestingly, the other agonistic vocalizations of the Lusitanian toadfish, such as croak and double-croak, were not emitted during sound recordings, because they are probably related to spacing functions and not distress.

The vocalizations produced during different developmental stages showed clear changes in temporal characteristics, spectral content and intensities. These changes are perhaps associated with

Table 1. Auditory thresholds in the five different test groups

	Test frequency (Hz)													
Group*	50		100		200		300		500		800		1000	
G1	85	+2.22 –2.99	98	+1.19 –1.37	97	+1.37 –1.63	102	+1.13 –1.31	114	+0.72 –0.79	132	+1.74 –2.18	NR	
G2	80	+1.56 –1.90	91	+1.34 –1.59	97	+1.83 –2.33	97	+1.10 –1.26	115	+2.20 –2.95	126	+0.86 –0.96	NR	
G3	77	+1.24 –1.44	89	+1.24 –1.45	97	+2.52 –3.56	101	+1.49 –1.79	117	+1.99 –2.59	130	+1.48 –1.78	132	+1.03 –1.17
G4	77	+2.07 –2.72	87	+0.78 –0.85	98	+1.29 –1.52	100	+0.19 –0.19	115	+0.08 –0.09	120	+0.12 –0.12	120	+0.41 –0.43
G5	77	+1.96 –2.53	91	+1.03 –1.17	98	+0.11 –0.11	102	+0.25 –0.25	111	+0.24 –0.24	117	+0.24 –0.25	121	+0.61 –0.65

Values are mean ± s.e.m.

*Group G1, *SL*=2.8–3.8 cm (*N*=6); G2, *SL*=5.4–6.6 cm (*N*=6); G3, *SL*=8.0–10.2 cm (*N*=7); G4, *SL*=12.4–15.3 cm (*N*=6); G5, *SL*=20.2–31.8 cm (*N*=9). NR, no response.

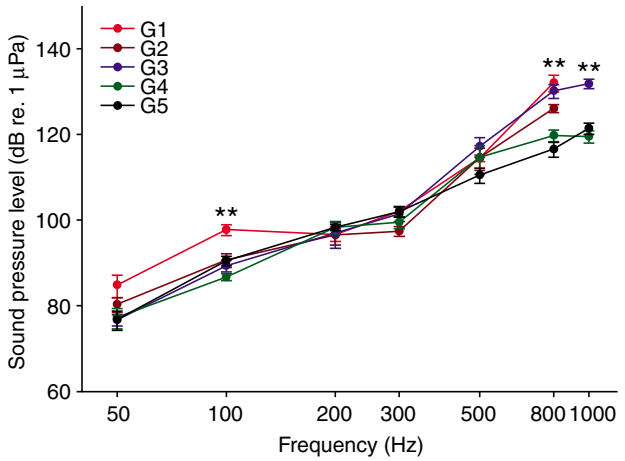

Fig. 7. Auditory thresholds of juveniles from the five different size groups. G1, 2.8–3.8 cm *SL* (*N*=6); G2, 5.4–6.6 cm *SL* (*N*=6); G3, 8.0–10.2 cm *SL* (*N*=7); G4, 12.4–15.3 cm *SL* (*N*=6); and G5, 20.2–31.8 cm *SL* (*N*=9). Values are mean ± s.e.m. Asterisks indicate highly statistically significant differences between groups (one-way ANOVA); $^{**}P<0.001$.

the swimbladder and intrinsic sonic muscles, which both increase in size throughout life in *H. didactylus* (Modesto and Canário, 2003b).

The duration and therefore number of pulses within a grunt diminished with toadfish growth, contrary to other fish species such as the croaking gourami *T. vittata* and the grey gurnard *Eutrigla gurnardus*, where these parameters increased with size (Henglmüller and Ladich, 1999; Amorim and Hawkins, 2005). This difference is probably because larger toadfish emitted long trains of grunts with shorter intervals between consecutive grunts. These trains may indicate elevated aggression but also higher development of the sonic neuromuscular system, i.e. sonic motor nucleus (SMN) and intrinsic swimbladder sonic muscles (Fine et al., 1984; Fine, 1989).

On the other hand, pulse period within a grunt increased with size in our study species, similar to the gourami (Henglmüller and Ladich, 1999); this points to a lower sonic muscle contraction rate in larger toadfish (Fine et al., 2001) (for a review, see Ladich and Fine, 2006).

The dominant frequency of sounds decreased with increasing fish size. Comparing sound spectra of agonistic vocalizations obtained at different stages of development indicated a clear gradual shift in main energies of sounds from higher harmonics (between 420 and 570 Hz, groups G1–3, <10 cm *SL*) down to the first harmonic (at approx. 110 Hz) with increasing size (G5, >20 cm *SL*). Correlations between dominant frequencies of sounds and size are also known in other fish species, e.g. bicolor damselfish (Myrberg et al., 1993), croaking gouramis (Ladich et al., 1992), mormyrids (Crawford, 1997) and grey gurnard (Amorim and Hawkins, 2005). However, a decrease in dominant frequency during ontogeny since early developmental stages has only been reported in the croaking gourami (Henglmüller and Ladich, 1999; Wysocki and Ladich, 2001).

SPL values increased significantly during growth. This allowed larger fish to produce louder signals to deter opponents. A similar positive relationship between size and sound amplitude was reported for the croaking gourami *T. vittata* (Wysocki and Ladich, 2001), as well as for the weakfish *Cynoscion regalis* (Connaughton et al., 2002).

Our data suggest that sound characteristics may inform conspecifics about the size of sound producers. In addition to visual cues, this information can be valuable for assessing the fighting ability of opponents and thus to decide contests before they escalate to more costly phases, i.e. damaging combat (Ladich, 1998).

Development of hearing

Auditory evoked potentials could be obtained in all size groups, including the smallest juveniles with, for instance, 2.8 cm *SL* (the maximum size of *H. didactylus* exceeds 50 cm). In general, this species revealed best auditory sensitivity at low frequencies in all stages of development, namely below 300 Hz (with hearing

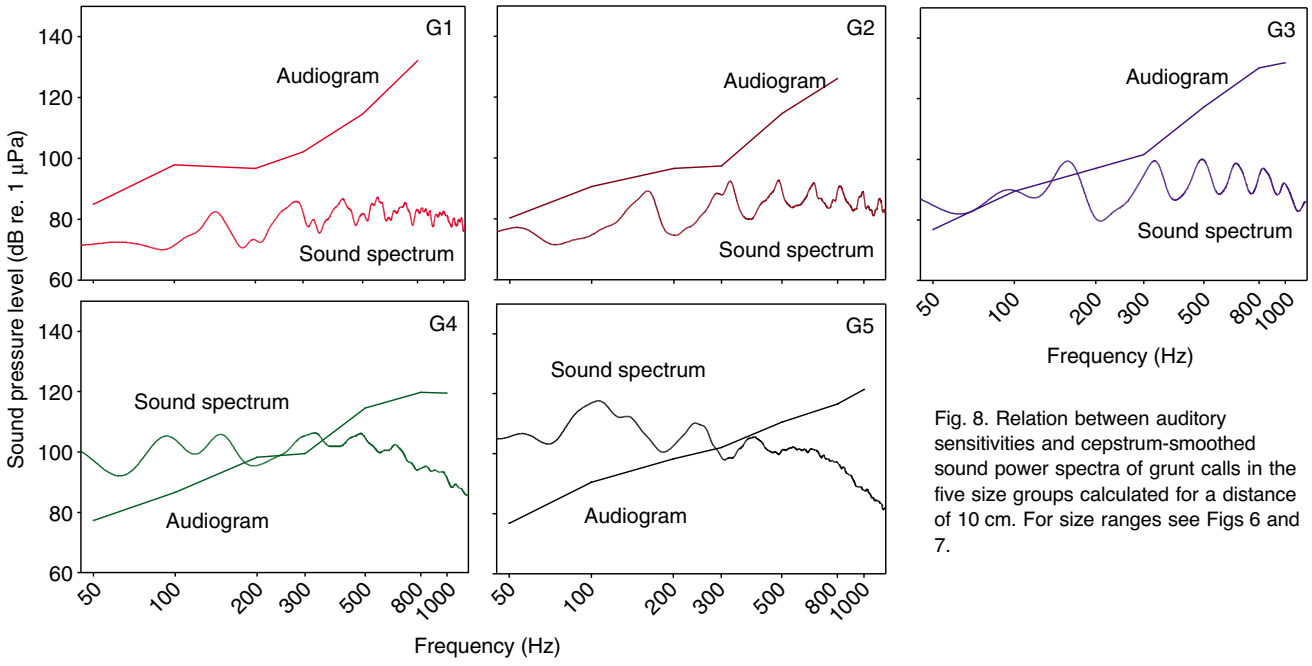

Fig. 8. Relation between auditory sensitivities and cepstrum-smoothed sound power spectra of grunt calls in the five size groups calculated for a distance of 10 cm. For size ranges see Figs 6 and 7.

thresholds under 100 dB re. 1 μPa), with a decrease in sensitivity by up to 55 dB re. 1 μPa observed towards 1000 Hz. Although earlier stages were not investigated (the fish did not hatch in the laboratory), our data indicated that hearing sensitivity changes only slightly during growth. Only the smallest toadfish group revealed higher hearing thresholds within the best hearing range (100 Hz). Moreover, at higher frequencies (i.e. 800 and 1000 Hz) younger fish demonstrated either absence of auditory response or lower sensitivity.

Batrachoidids are classified as hearing non-specialists or generalists (Fish and Offutt, 1972; McKibben and Bass, 1999; Weeg et al., 2002; Sisneros and Bass, 2005); they lack accessory hearing structures to enhance auditory abilities and therefore likely respond to the particle motion component of low frequency sounds at relatively high sound intensities (Hawkins and Myrberg, 1983; Ladich and Popper, 2004). The Lusitanian toadfish, similar to other generalists, possesses limited auditory abilities and, as a consequence, probably does not show considerable sensitivity changes during life history. According to the calibration tests carried out using a particle acceleration sensor it can be assumed that the slight changes in pressure thresholds observed during ontogeny are paralleled by particle acceleration changes of the same degree. In an ontogenetic study, Sisneros and Bass (Sisneros and Bass, 2005) investigated the response properties of individual primary auditory afferents in the plainfin midshipman fish *P. notatus* (Batrachoididae) and showed that the best hearing range was between 60 and 200 Hz in small juveniles and large juveniles as well as adults. Similar to our results in the Lusitanian toadfish, the most sensitive frequencies did not change during ontogeny. The same authors reported an increment in auditory sensitivity in *P. notatus* at the most sensitive frequency (from 118 to 104 dB re. 1 μPa) from small to large juveniles. No difference was found between large juveniles and adults. Congruently, our study revealed significant hearing differences between size groups, i.e. *circa* 7 dB re. 1 μPa at 100 Hz (and 8 dB at 50 Hz close to significance) between the smallest and largest fish. This smaller hearing difference during growth of the European toadfish relative to the Californian batrachoidid might reflect genus-specific differences or the different age groups chosen.

Studies on other species, including hearing specialists, are contradictory, with no straightforward conclusions. Auditory sensitivity increases dramatically during development, by about 50 dB re. 1 μPa in the bicolor damselfish *S. partitus* (Kenyon, 1996), whereas the opposite was found in another damselfish, the sergeant major *Abudefduf saxatilis* (Egner and Mann, 2005). Egner and Mann revealed that sensitivity decreases at low frequencies in larger fish. Different developmental tendencies were also reported among non-related hearing specialists, namely improvements as well as no changes in hearing sensitivity. Hearing sensitivity improves by about 14 dB re. 1 μPa in croaking gourami and the most sensitive frequency drops from 2.5 kHz to 1.5 kHz (Wysocki and Ladich, 2001). In contrast, no changes were observed in differently sized cyprinids. Neither the goldfish *Carassius auratus* nor the zebra fish *Danio rerio* exhibited improved hearing during growth (Popper, 1971; Higgs et al., 2002; Higgs et al., 2003).

Relationship between development of hearing and sound production: onset of acoustic communication

Comparing audiograms and sound spectra in larger size groups (G4 and G5) revealed that the main energy of sounds was located within their most sensitive frequencies, i.e. below 300 Hz. In small juveniles (groups G1–2), however, dominant frequencies were found between 420–570 Hz and did not match as well with their best hearing range.

According to our results, adults were able to detect vocal agonistic signals of same-sized conspecifics, as sound energies were up to 30 dB re. 1 μPa (at about 110 Hz) above hearing thresholds. In the smallest juveniles analyzed (<4 cm *SL* and just a few months old) the sound spectrum was somewhat below the auditory curve, suggesting that the ability to perceive sounds and therefore to communicate acoustically with same-sized conspecifics is lacking or only possible at very short distances. This is due to the low SPL values of vocalizations and to the high dominant frequency. Although we determined sound pressure levels in our ontogenetic study we assume that our conclusion also hold for particle acceleration levels because these two acoustical parameters were proportional in our tanks according to calibration tests. Additionally, pressure and particle velocity spectra of ambient noise and vocalizations of the goby *Padogobius bonelli* are relatively similar in terms of main energy distribution (Lugli and Fine, 2007).

The onset of the development of acoustic communication is still poorly investigated in fishes. Hearing develops prior to the onset of sound production in the croaking gourami and the ability of juveniles to communicate acoustically starts gradually when thresholds decrease and sound intensities increase (Wysocki and Ladich, 2001). The species investigated so far (croaking gouramis and Lusitanian toadfish) reveal similar developmental trends. The results suggest that, in both cases, sound detection develops prior to the ability to generate sounds and that acoustic communication might be absent in earliest developmental stage because of low hearing sensitivities or low sound levels. Nevertheless, juveniles of both hearing specialist and generalist start early to communicate acoustically during agonistic interactions.

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