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ORIGINAL PAPER

# Auditory saccular sensitivity of the vocal Lusitanian toadfish: low frequency tuning allows acoustic communication throughout the year

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**Abstract** A novel form of auditory plasticity for enhanced detection of social signals was described in a teleost fish, *Porichthys notatus* (Batrachoididae, Porichthyinae). The seasonal onset of male calling coincides with inshore migration from deep waters by both sexes and increased female sensitivity to dominant frequencies of male calls. The closely related Lusitanian toadfish, *Halobatrachus didactylus*, (Batrachoididae, Halophryninae) also breeds seasonally and relies on acoustic communication to find mates but, instead, both sexes stay in estuaries and show vocal activity throughout the year. We investigated whether the sensitivity of the inner ear saccule of *H. didactylus* is seasonally plastic and sexually dimorphic. We recorded evoked potentials from populations of saccular hair cells from non-reproductive and reproductive males and females in response to 15–945 Hz tones. Saccular hair cells were most sensitive at 15–205 Hz (thresholds between 111 and 118 dB re. 1 μPa). Both sexes showed identical hearing sensitivity and no differences were found across seasons. The saccule was well suited to detect conspecific vocalizations and low frequencies that overlapped with lateral line sensitivity. We showed that the saccule in *H. didactylus* has major importance in acoustic communication throughout the year and that significant sensory differences may exist between the two batrachoidid subfamilies.

**Keywords** Hearing · Tuning · Saccule · Acoustic communication · Batrachoididae

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## Introduction

There is growing evidence that the auditory system of adult vertebrates is dynamic in the way it encodes sound and it is often optimized to detect behaviourally relevant signals that couple sender and receiver in acoustic communication as proposed by the matched filter hypothesis (Capranica and Moffat 1983; Kostarakos et al. 2008). The physiological state of the receiver, namely the hormonal status, can shape the "matched filter" of auditory receivers affecting the response properties of peripheral and/or central auditory systems as reported in anurans, birds and mammals, including humans (Guimarães et al. 2006; Lucas et al. 2007; Miranda and Wilczynski 2009). However, other examples which are not mutually exclusive support the alternative sensory exploitation hypothesis that can explain the mismatch in some Tungara frogs in which the auditory tuning of female receivers does not match the dominant frequencies of mate calls produced by male senders (Ryan et al. 1990; Ryan and Rand 1993). Comparative studies that examine mechanisms of vocal production and auditory reception across closely related species can ultimately provide valuable insight into the diversity and evolution of communication systems.

A novel form of auditory plasticity was demonstrated in a teleost fish, the plainfin midshipman fish *Porichthys*

*notatus* (Batrachoididae, subfamily Porichthyinae). During the reproductive season, females migrate from deep offshore and become more sensitive, with increased accuracy of saccular afferent phase-locking, to higher dominant frequencies of male advertising calls that propagate better in the shallow nesting areas (Sisneros and Bass 2003). This seasonal plasticity of the females' auditory sense occurs at the level of the sensory hair-cell receptors in the saccule, the main auditory endorgan, and is sex steroid-dependent (Sisneros et al. 2004a, b, Sisneros 2009). This adaptive mechanism enhances the coupling between the sender and the receiver, promoting the acquisition of acoustic information that is likely needed and probably critical for mate detection, localization and selection (Sisneros et al. 2004a).

The closely related Lusitanian toadfish, *Halobatrachus didactylus*, (Batrachoididae, subfamily Halophryninae) is also a seasonally breeding species (Modesto and Canário 2003) but, contrary to *P. notatus*, may show vocal activity throughout the year depending on the temperature (Amorim et al. 2006) and both sexes stay in shallow estuary areas even during the winter non-reproductive season (Pereira 2006; Amorim et al. 2010). Males of this species exhibit an unusually rich vocal repertoire, producing acoustic signals to attract mates, defend territories and during other agonistic contexts (Amorim and Vasconcelos 2008; Amorim et al. 2008; Vasconcelos et al. 2010). The onset of sound production starts early in life, along with the development of agonistic behaviours and territorial activity (Vasconcelos and Ladich 2008). A recent study shows that both sexes can accurately perceive fine temporal, spectral and amplitude features of conspecific vocalizations (Vasconcelos et al. 2011).

Here, we investigated whether auditory saccular sensitivity of *H. didactylus* varies seasonally and if saccular sensitivity is sexually dimorphic at the level of the hair-cells receptors. We tested the hypothesis that saccular sensitivity does not change across seasons or sexes since both male and female of this species maintain vocal communication all year round despite seasonal variation of circulating sex steroid levels (Modesto and Canário 2003). We provide detailed data on the auditory sensitivity of the saccule, the main auditory endorgan in *H. didactylus* and in most teleosts (reviewed in Wysocki 2006), across a wide range of behaviourally relevant frequencies from 15 Hz to 945 Hz.

## Methods

### Test subjects

The test subjects were 93 Lusitanian toadfish adults caught in the Tagus estuary (Portugal) during the breeding season at the end of April–June (32 females: 25–35 cm total length (TL), 279–651 g total body mass (BM); 22 type I males: 23–43 cm TL, 197–1,267 mg BM), and winter, December-February (23 females: 23–41 cm TL, 188–655 g BM; 16 type I males: 24–38 cm TL, 274–896 g BM) by local fishermen (trawl) or collected directly from nests in intertidal areas during low tide. Fish were transported during the same day to the stock tanks (80 l) and maintained at 21 ± 1°C up to 15 days prior to the saccular potential recordings, in order to avoid effects of stress and captivity that may affect saccular sensitivity, namely possible reduction in sensitivity (Sisneros and Bass 2003). Females were first identified by their larger abdomens and/or their wider genital papilla with a genital pore. Type I males or parental nesting males were typically bigger than females, presented elongated genital papilla and generally released a secretion of their larger accessory glands when gently pressed near the anus (Modesto and Canário 2003). Type I males differed in size from type II males (sneaker males that attempt opportunistic fertilizations), which are similar in size to females but with more elongated genital papilla. Both sex and male type (type I vs. type II) were always confirmed by the dissection of the specimen after each experiment when the fish were sacrificed by immersion in a 0.025% ethyl *p*-aminobenzoate saltwater bath. The total length (TL), total (TW) and eviscerated (EW) weights, gonad (GW) and accessory testicular glands (AGW) weights were measured for each individual. The reproductive state of the specimens and the male type was verified according to Modesto and Canário (2003), by calculating the gonadosomatic index (GSI) as 100*GW/EW, as well as the accessory glands index (AGI) as 1,000*AGW/EW.

During the breeding season, GSI varied between 14 and 37% in non-spent females (before releasing the eggs) and 0.02–4% for type I males; during the winter, GSI was 0.7–10% in females and 0.06–4% in type I males. Consistent with Modesto and Canário (2003), a marked seasonal difference in GSI was found among females, but not in type I males. Spent females (GSI: 1–6%) collected from the nests during the breeding season were considered reproductive or breeding fish since they were found in the nest with type I males. No differences in saccular sensitivity were observed between spent and non-spent breeding female *P. notatus* collected during the breeding season (Sisneros, personal observation). Only four type II males were collected during the whole year and they were identified by their larger gonads and generally smaller accessory testicular glands (Apr–Jun: 3 fish, GSI: 8–9%, AGI: 1.1–1.2‰; Dec–Feb: 1 fish, GSI: 5%, AGI: 0.1‰), which was in contrast to type I males (Apr–Jun: AGI: 0.7–5‰; Dec–Feb: 0.6–4‰).

Saccular potential recording setup

The method for recording the saccular potential in the Lusitanan toadfish was based on the experimental procedure adopted by Sisneros (2007, 2009). Surgical procedures for exposing the inner ear saccule followed those in previous studies (Sisneros and Bass 2003; Sisneros 2007). Briefly, fish were first anesthetized in a 0.025% ethyl *p*-aminobenzoate saltwater bath and then immobilized by an intramuscular injection of pancuronium bromide (circa 2-4 mg/kg). The saccule was then exposed by dorsal craniotomy (see Fig. 1) and a barrier of denture cream was built up around the cranial cavity to allow the fish to be lowered below the water surface. A saline solution was used to prevent the cranial cavity from drying out and to clean eventual bleeding.

Test subjects were positioned below the water surface in the middle of a round plastic experimental tank (diameter: 36 cm, water depth: circa 18 cm), with the saccule kept at about 7 cm above the vibrating disc of the sound generating device. The tank was placed on a vibration isolated table inside a Faraday cage. All recording and sound generating equipment was located outside the recording room. Fish gills were perfused with saltwater through the mouth using a simple temperature-controlled (21 ± 2°C) gravity-fed water system.

Acoustic stimuli generated via the reference output signal of a lock-in amplifier (SR830, Stanford Research Systems, Sunnyvale, CA, USA) was delivered to the underwater sound generating device through a custom built amplifier. The sound generating device was composed of an immersed plexiglass disc (diameter: 8 cm) driven by a mechanical wave driver (SF9324, PASCO, Roseville, CA, USA) kept below the experimental tank. The disc was attached to the wave driver by a stainless steel rod which crossed the tank bottom through a water restraining flexible device, which not only prevented water drainage but also kept the rod vertically positioned. The sound stimuli were delivered via the vibrating disc placed at the midpoint of the tank. Auditory stimuli were calibrated before each experiment. Sound measurements were performed using a calibrated mini-hydrophone (8103 Brüel and Kjaer, Naerum, Denmark) positioned 7 cm above the disc, a position normally occupied by the fish's head during the recordings. The hydrophone was connected to an amplifier (2692 Nexus, Naerum, Denmark) and the acoustic signal recorded was digitized (Edirol UA-25, Roland Corporation, Tokyo, Japan) and monitored by a laptop running Audition 2.0 (Adobe Systems Inc., CA, USA), which was used to verify stimuli spectra and control the relative amplitudes of the auditory stimuli. Sound pressure levels (SPL) were measured using a sound level meter (Mediator, 2238 Brüel and Kjaer, Naerum, Denmark) connected to the mini-hydrophone. Stimuli amplitudes were equalized (130 ± 1 dB re 1 μPa) at all tested frequencies by a custom MatLab script (MathWorks, Inc., Natick, MA, USA) that controlled the signal output of the lock-in amplifier. Auditory stimuli consisted of eight repetitions of single 500 ms tones from 15 to 945 Hz (in 5–80 Hz increments) presented randomly at a rate of one every 1.5 s.

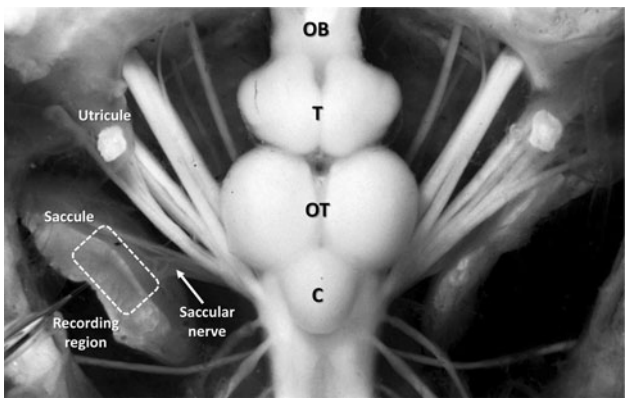


**Fig. 1** Dorsal view of the brain and inner ears of the Lusitanian toadfish, *Halobatrachus didactylus*, obtained from a perfusion fixed specimen with paraformaldehyde. The recording region is indicated in the saccule, which was slightly deflected to improve visibility of the saccular nerve. *OB* olfactory bulb, *T* Telencephalon, *OT* optic tectum, *C* cerebellum

Although toadfishes possess no hearing specializations and thus are primarily sensitive to particle motion (Fay and Edds-Walton 1997), we report in this study hearing thresholds based on pressure measurements for both technical reasons and comparison purposes with previous studies using batrachoidid fish (e.g. Sisneros 2007, 2009). The intention of this study was to compare the saccular sensitivity of *H. didactylus* between sexes (male vs. female) and across seasons (non-reproductive vs. reproductive state) under identical experimental conditions. The data presented in sound pressure levels (SPL) to describe hearing sensitivities (i.e., thresholds based on saccular potential measurements) should not be considered in terms of absolute values but instead should be used as a mean to make quantifiable comparisons of relative differences in hearing sensitivity between the different groups. Moreover, the saccular potential recording technique used here and developed by Sisneros (2007) provide comparable data with other recently published studies that also adopted this technique (Sisneros 2007, Sisneros 2009; Rohmann and Bass unpublished). After finishing the data collection, calibration tests were performed using an underwater miniature acoustic pressure–particle acceleration (*p*–*a*) sensor S/N 2007-001 (Applied Physical Sciences Corp., Groton, CT, USA) provided by F. Ladich and showed that pressure and particle acceleration were positively correlated below the water surface in the experimental tank. In the primary

axis of particle motion (the vertical *z* axis), a 3 dB change in SPL was generally accompanied by a 3 dB change in particle acceleration level for all stimuli.

Saccular potentials were recorded with glass electrodes filled with 3 M KCl (0.5–6 MΩ). Electrodes were visually guided and placed in the middle region of the saccular macula (see Fig. 1) in either the left or right saccule. The hair-cell orientation patterns are not known for *H. didactylus* and thus variations in the placement of the recording electrode in the saccular region could have resulted in variability in the auditory responses. In the closely related batrachoidid *Opsanus tau*, hair-cell orientation pattern in the saccule changes from rostrally oriented cells in the rostral region to vertical oriented cells in the middle to caudal regions (Edds-Walton and Popper 1995). Also in *P. notatus*, sensitivity differences were found among the rostral, middle and caudal regions of the saccule (Sisneros 2007, 2009). Hence, all the recordings performed in this study were from the middle recording region of the saccule in order to obtain data that could be comparable between different specimens. Saccular potentials were preamplified 10× (Getting 5A, Getting Instruments, San Diego, CA, USA), band-pass filtered (20–5,000 Hz, further 10× gain, SR650, Stanford Research Systems, Sunnyvale, CA, USA), inputted into the digital signal processor of the Lock-in amplifier (SR830) and stored on a PC computer running the custom MatLab control program. The auditory stimuli, evoked saccular potentials, and background noise were continuously monitored with an oscilloscope. The Lock-in amplifier converts the saccular potential response (RMS) into a DC voltage output signal that is proportional to the component of the signal whose frequency is exactly locked to the reference frequency. The second harmonic of the stimulus frequency was set as the reference frequency because the maximum evoked saccular potentials typically occur at twice the stimulation frequency. This phenomenon results from opposite-oriented hair cells within the saccular macula that respond in opposite phases of the stimulus in teleost fishes (Cohen and Winn 1967; Furukawa and Ishii 1967; Fay 1974).

To estimate auditory thresholds, the saccular potentials were recorded in response to single tone stimuli that were reduced in 3 dB steps until the saccular response (mean voltage of eight evoked saccular potential measurements) was no longer above background noise (mean voltage measured without acoustic stimulation) ± 2 SD (standard deviation). Background noise measurements were made prior to the recording of each threshold tuning curve and were averaged across eight measurements. The background noise levels (recorded with no auditory stimulation present) were consistently between 2 and 5 μV.

Iso-level responses were obtained by presenting all the single-tones at a sound pressure of 130 dB re 1 μPa and plotting the amplitude of the saccular responses. This sound amplitude was selected because it corresponds approximately to the recorded amplitude of a boatwhistle in close proximity (<0.5 m) to a vocalizing nesting fish (personal observations) and it was previously used in similar studies with other batrachoidids (McKibben and Bass 1999; Sisneros 2009). In order to calculate the relative gain of the saccular responses, the iso-level frequency response profiles were first converted to dB and then normalized by subtracting the dB value obtained for the best frequency. Best frequency (BF) was defined as the stimulation frequency that induced the greatest evoked saccular potential at the specific stimulus level of 130 dB re 1 μPa. This procedure allows a comparison of profiles with different absolute values but preserves the relation between frequencies (within a profile), thus cancelling the effect of the distance from the electrode to the macula epithelium. Average relative gain allowed comparing auditory amplitude responses between sexes and seasons.

### Statistical analysis

The overall effects of sex (females vs. type I males), reproductive state (reproductive vs. non-reproductive) and stimulus frequency on the auditory thresholds and relative gain were analyzed with a repeated-measures ANOVA. This test analyzed responses (auditory thresholds or relative gain) to several frequencies in each subject fish (within-subject factor) of different sex and reproductive state (two between-subject factors). For the auditory threshold data analysis, only the frequency range up to 625 Hz was considered because some thresholds were missing at the higher frequencies. For the relative gain data, the whole frequency range tested (15–945 Hz) was considered for comparison purpose between groups. Parametric tests were used preferentially since data were normally distributed and variances were homogenous. Statistical tests were performed with Statistica 7.1 for Windows (StatSoft, Inc. 2005).

## Results

### Iso-level frequency responses

The evoked saccular potential response typically occurred at twice the stimulation frequency as expected (Fig. 2). Examples of iso-level response profiles of the evoked saccular potentials recorded in response to frequencies between 15 and 945 Hz at 130 dB re 1 μPa are shown in Fig. 3. The best frequency (BF) varied widely between specimens ranging from 15 to 205 Hz, but the majority or mode of BFs occurred at 15 Hz in both reproductive fish

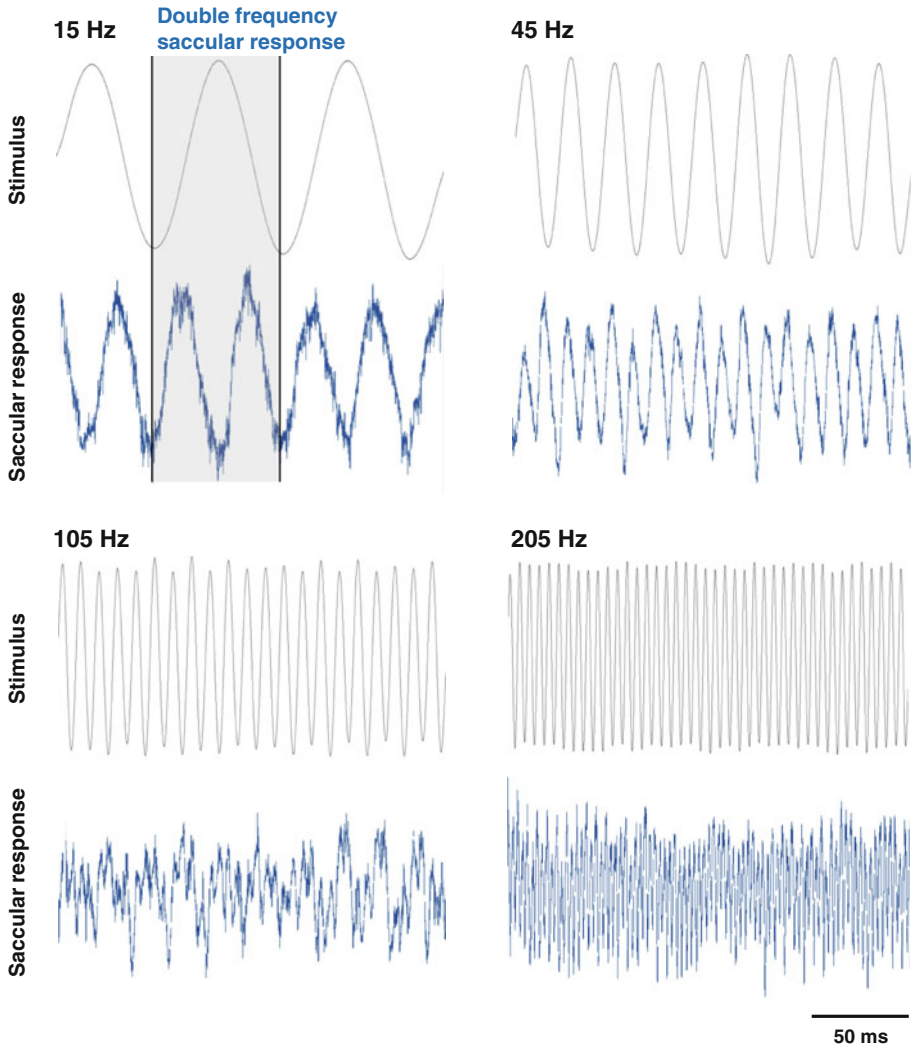


**Fig. 2** Representative examples of the acoustic stimulation and evoked auditory responses obtained from the Lusitanian toadfish saccule under 130 dB re 1 μPa single tones. Note the frequency doubling effect present in the saccular responses most likely resulting from opposite oriented hair cells. Sound waveform and saccular response amplitudes were adjusted to enhance visibility

(24% of recordings) and non-reproductive fish (21% of recordings).

The relative gain (iso-level saccular potential data obtained at 130 dB re 1 μPa normalized to BF in each recording) varied on average between −7.2 dB (15 Hz) and −39.7 dB (865 Hz). The relative gain did not reveal overall significant differences between sexes or seasons (repeated measures ANOVA, $F_{1,159} = 0.2–2.1$, $P > 0.05$), with no interaction between the two variables (repeated measures ANOVA, $F_{1,159} = 1.1$, $P > 0.05$) as shown in Fig. 4.

## Auditory saccular sensitivity

The auditory thresholds determined from populations of hair-cells located in the middle region of the saccule were on average between 111 and 118 dB re 1 μPa for the lowest frequencies (15–205 Hz) and increased gradually to thresholds of 145 dB re 1 μPa (the maximum amplitude tested) at the highest frequencies (Fig. 5). There were no significant differences in auditory thresholds between the sexes or seasons (repeated measures ANOVA, $F_{1,150} = 0–2.5$, $P > 0.05$, see Fig. 5) as well as no interaction between the two variables (repeated measures ANOVA, $F_{1,150} = 0.1$, $P > 0.05$).

The comparison between both sexes audiograms with the power spectra of conspecific vocalizations, recorded previously at 10–20 cm from calling adult toadfish (Vasconcelos et al. 2010), revealed that the main energy of boatwhistles and grunts coincided with the greatest saccular sensitivity range, i.e. frequencies lower than 205 Hz. Most of the energy associated with typical boatwhistles and grunts are well above the hearing thresholds for saccule hair cells reported here, up to 20 and 10 dB for peak frequencies contained in the boatwhistle and grunt, respectively (Fig. 6). In addition, saccular hair cells showed a remarkable sensitivity to frequencies as low as 15 Hz, where sound energy of conspecific calls is either greatly reduced or absent.

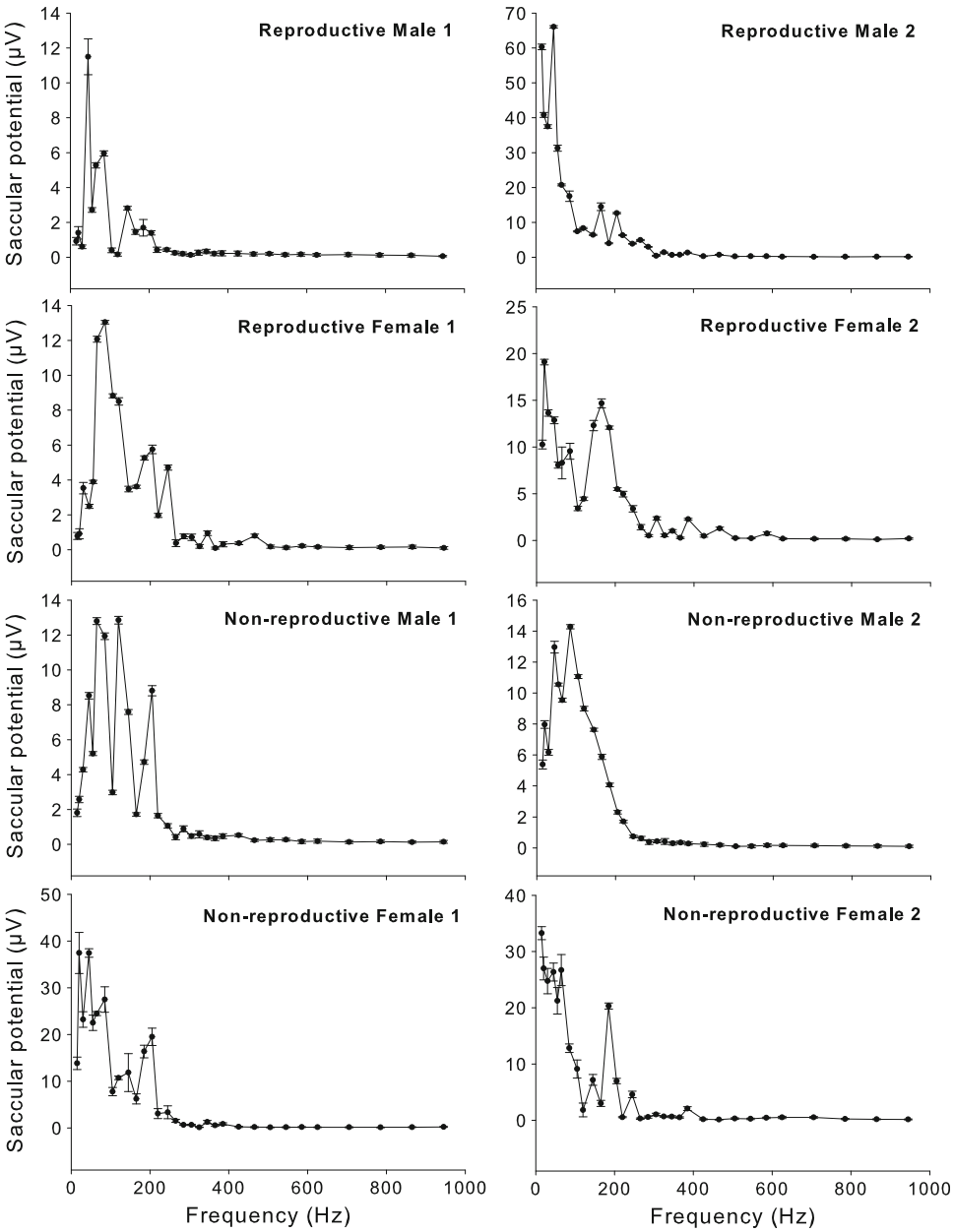


**Fig. 3** Representative examples of iso-level curves (mean ± SD) of evoked saccular potentials recorded from the middle region of the saccule in different Lusitanian toadfish. Auditory responses shown were obtained at 130 dB re 1 μPa from both males and females, during reproductive and non-reproductive seasons

## Discussion

The physiological state of the receiver is known to shape the auditory response properties in several vertebrates such that behaviourally relevant signals can be better detected and encoded by the auditory system (matched filter hypothesis, Capranica and Moffat 1983; Kostarakos et al. 2008). A novel form of auditory plasticity that enhances the coupling between sender and receiver has been reported in the teleost fish, *P. notatus* (Batrachoididae), and revealed that females become more sensitive to the dominant frequency components of male advertising calls during the breeding season (Sisneros et al. 2004a; Sisneros 2009). However, it is not known whether other seasonal-reproductive teleost species that rely heavily on acoustic signalling during social life show similar auditory plasticity to optimize vocal communication.

The closely related Lusitanian toadfish *H. didactylus*, although from a different subfamily, is also a seasonal breeding species where mate finding is mediated by acoustic communication. Since vocal communication is maintained throughout the year in *H. didactylus* (Amorim et al. 2006, 2010), contrary to *P. notatus*, we hypothesised that saccular sensitivity does not change

seasonally, despite the seasonal variation of sex steroids levels related to the reproductive state (Modesto and Canário 2003).

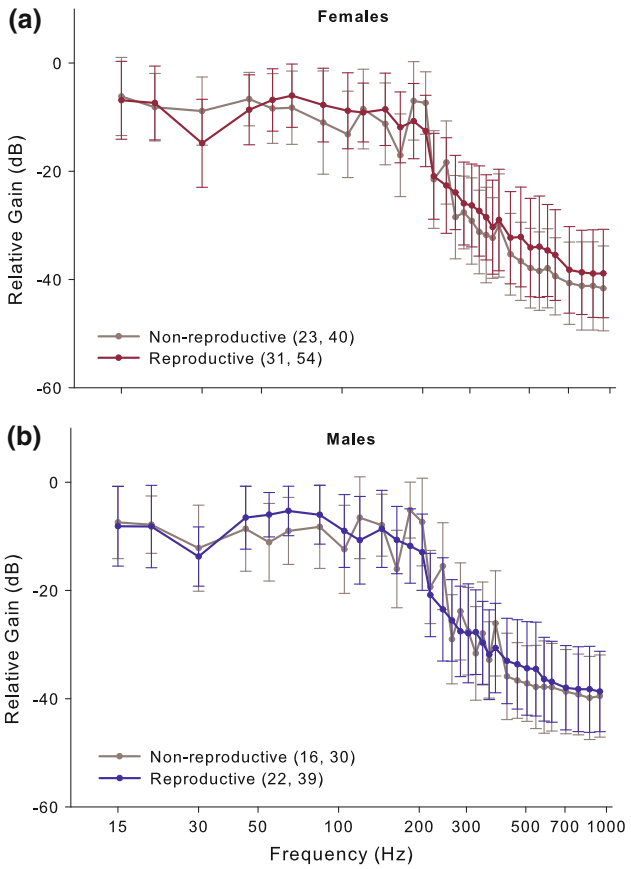


**Fig. 4** Mean (±SD) iso-level response curves of saccular evoked potentials obtained at 130 dB re 1 µPa in Lusitanian toadfish females (**a**) and males (**b**) during reproductive and non-reproductive seasons. Iso-level response data were normalized to a relative value of 0 dB assigned to the peak response for each record and then expressed in relative dB, i.e. relative to the Best Frequency sensitivity for that record. Number of animals and records per group are indicated in *parentheses*

## Toadfish auditory sensitivity: seasonal plasticity or sensory stability?

Our data indicated that the saccular sensitivity of the Lusitanian toadfish does not change seasonally between end of April-June (breeding season) and December–February (non-breeding season). Similarly to other species that display conspicuous reproductive behaviour, the Lusitanian toadfish undergoes marked seasonal changes in circulating levels of sex steroids. In females, circulating plasma levels of estradiol ($E_2$) increases considerably prior to the breeding season (around May) during vitellogenesis, whereas in nesting males testosterone (T) and 11-KT (11-ketotestosterone) levels peak in June during spermatogenesis (Modesto and Canário 2003). Our results suggest that the seasonal variations of circulating sex steroids levels in this species (Modesto and Canário 2003) are not correlated with any changes in auditory sensitivity throughout the annual reproductive cycle. Nevertheless, future studies that examine both auditory saccular sensitivity and hormonal levels would be useful to resolve whether there are any slight effects of sex steroids on auditory hair cell tuning or phase-locking accuracy in this teleost species.

The unchanging perception of acoustic communication signals, independent of the reproductive state, in the Lusitanian toadfish is consistent with behavioural and ecological observations. Lusitanian toadfish exhibits an unusually large vocal repertoire composed of at least five distinct vocalisations produced in different social contexts, such as mate attraction, establishment of territories and other agonistic interactions (Amorim and Vasconcelos 2008; Vasconcelos et al. 2010) and the full acoustic repertoire can be detected all year round (Amorim et al. 2006, 2010; Vasconcelos and Ladich 2008). This species breeds in estuarine shallow waters and remains in the estuaries (Pereira 2006), including in subtidal areas (Amorim et al. 2010), during the winter non-breeding season. Vocal behaviour outside the breeding season is most likely

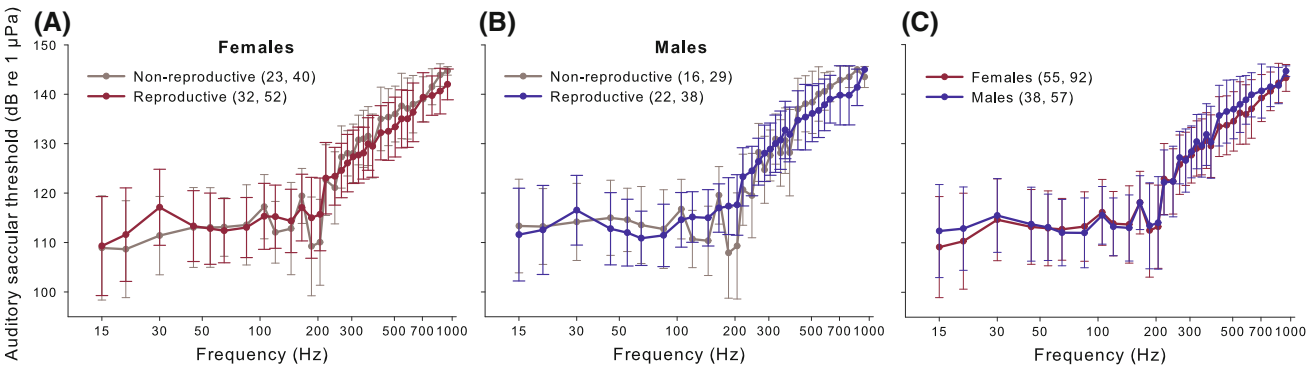


**Fig. 5** Comparison between mean (±SD) auditory threshold curves from reproductive and non-reproductive Lusitanian toadfish females (**a**) and males (**b**). All data obtained from both females and males were plotted together to compare threshold curves between sexes (**c**). Number of animals and records per group are indicated in *parentheses*

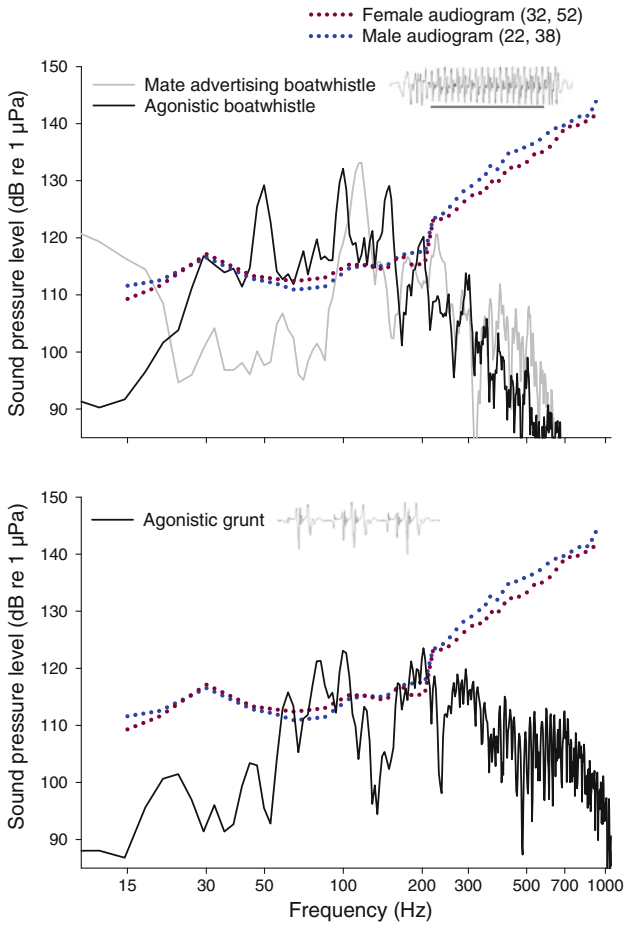


**Fig. 6** Comparison between the saccular sensitivity of reproductive toadfish and the power spectra of conspecific mate advertising boatwhistle and agonistic grunt recorded at 10–20 cm from a calling adult toadfish. Sampling frequency 6 kHz, filter bandwidth 10 Hz, 75% overlap, Hamming window. Number of animals and records per group are indicated in *parentheses*

associated with disputes for the access of suitable shelters and feeding sites. In addition, sound production seems remarkably important throughout life, starting in early developmental stages when it is used during food and space competition (personal observations). Moreover, *H. didactylus* can only be found along the Eastern Atlantic, from the Tagus estuary (Portugal) down to the Guinea Gulf, and in the Mediterranean (Roux 1986). Such geographical distribution suggests that this species probably evolved to communicate also in tropical areas where temperature remains more stable and thus a seasonal reproduction and a plastic auditory sensitivity are not required.

Conversely, *P. notatus* females inhabit deeper low-temperature waters during most of the year along the Pacific coast of North America and experience a dramatic change in their environment when they migrate to the intertidal nesting areas to spawn (Sisneros et al. 2004a). Females undergo seasonal sex steroid-dependent changes in the hearing thresholds at the level of hair-cell receptors within the saccule macula (Sisneros 2009). Estrogen receptor alpha was found in the inner ear's saccular epithelium in females and the annual spike in estrogen levels prior to the breeding season appears to be responsible for the induced changes that enhance the encoding of the dominant harmonics of male advertising calls (Sisneros and Bass 2003; Sisneros 2004a, b). Moreover, nothing has been described in terms of vocal activity in adult *P. notatus* during the non-breeding season or in juveniles that are clearly non-territorial. Vocal activity in this species seems to start later in life probably associated with sexual maturity and reproduction behaviours (personal observations).

Although *H. didactylus* and *P. notatus* belong to the same family Batrachoididae, they are classified in different subfamilies (Halophryninae and Porichthyinae, revised by Greenfield et al. 2008) and nothing is known about when these subfamilies diverged and how closely related they are. In fact, previous work showed that there is a notable difference in the forebrain nuclei responsible for generating vocalizations in species from two different subfamilies within Batrachoididae (Batrachoidinae and Porichthinae, Fine and Perini 1994). Such differences in the vocal motor now combined with our auditory sensitivity data suggest that there may be major differences in the nervous system of the subfamilies within the Batrachoididae.

The auditory plasticity and the influence of the hormonal or reproductive state (and sex) on the auditory receptors and processing in acoustic communication systems remains poorly understood. The influence of seasonal changes in the response properties of the peripheral and/or central auditory system have been described in other vertebrate taxa. For example, Lucas et al. (2002, 2007) measured auditory evoked potentials in several bird species and described seasonal changes in the response amplitude and latency correlated with their vocal complexity. Goense and Feng (2005) reported seasonal changes in frequency tuning and temporal processing in the midbrain torus semicircularis of the Northern leopard frog where a smaller number of neurons sensitive to low-frequencies (100–500 Hz) in winter show increased phase locking in late spring. Although no differences were found in the auditory thresholds, these seasonal changes in phase locking accuracy may facilitate call recognition and/or localization of conspecific calling males. Recently, Gordon and Gerhardt (2009) showed hormonal-induced phonotaxis in green treefrog females treated with estradiol and progesterone. Similar hormone-induced changes in hearing sensitivity have been reported in humans. Psychoacoustic experiments in women showed differences in the auditory perception depending on the phase of the menstrual cycle (e.g. Swanson and Dengerink 1988). Conversely, other examples in vertebrates, namely in frogs, point to a divergence

between the auditory system sensitivity and the frequency content of the vocal output that results in the mismatch between the auditory tuning of the receiver and the dominant frequencies of the mate call produced by the sender. These types of mismatches have been used to support the sensory exploitation model for sexual selection and evolution of communication signals (Ryan et al. 1990). However, remains unclear whether there is a hormonal influence in the frequency tuning of the two sexes (eg. Narins and Capranica 1976), as well as the presence of the estrogen receptor in the frog's inner ear.

### Auditory sensitivity among different sexes

Our results also point to identical saccular sensitivity between nesting type I males and females. The lack of sexual dimorphism in the auditory sense of *H. didactylus* suggests that the perception of acoustic communication signals is potentially important for both sexes. Parental males nest in aggregations and vocalize in choruses to attract females and behavioural observations suggest that nesting males interact acoustically altering their own boatwhistle calling rate in response to other calling males (Amorim et al. 2011). Moreover, nesting males produce boatwhistles as well as other sound types during territorial intrusions indicating that acoustic signals also have an important role in male–male assessment and territorial defense (Vasconcelos et al. 2010). These facts suggest that the auditory system of nesting males must be adapted to detect and resolve acoustic parameters of boatwhistles (and other sound types) similar to females, which probably select mates based on acoustic cues (Amorim and Vasconcelos 2008; Amorim et al. 2010). Moreover, Vasconcelos et al. (2011) measured auditory evoked potentials (AEP), i.e. overall synchronous neural electric activity from the endorgans, VIII nerve and CNS auditory nuclei induced by acoustic stimulation, and demonstrated that both females and males can accurately resolve fine temporal features of both pulsed grunts and tonal boatwhistles and spectral content and amplitude modulation of boatwhistles.

Sisneros (2009), based on recordings of saccular potentials, showed that reproductive *P. notatus* females had saccular thresholds 8–13 dB lower than non-reproductive females across a wide frequency range, which included the dominant higher harmonics of the male advertising call. This most likely enhances mate detection, recognition, and localization during the breeding season. Recent work by Rohmann and Bass (unpublished) suggest that *P. notatus* nesting (type I) males also undergo reproductive-dependent changes in auditory saccular sensitivity. Here, we provide the first data on the saccular tuning in the Lusitanian toadfish and show that similar saccular sensitivity is found in both sexes.

### Functional significance of the saccule's frequency tuning

The iso-intensity potential recordings indicated that saccular hair-cells of the Lusitanian toadfish were most sensitive to frequencies below 205 Hz, at a behaviourally relevant sound level of 130 dB re 1 μPa. We demonstrated not only that saccular sensitivity matches the frequencies with greatest energy in agonistic and advertising vocalizations, but also that hearing thresholds were well below (circa 10–20 dB) the peak amplitudes of the dominant frequencies that compose the conspecific vocalizations. A similar match between audiograms and conspecific sound spectra of the Lusitanian toadfish, but based on AEPs, was also described in Vasconcelos et al. (2007) and Vasconcelos and Ladich (2008).

Correlation between saccular sensitivity and the main energy of conspecific sounds was also found in *P. notatus* (Sisneros 2007). Similarly, Fay and Edds-Walton (1997) reported an identical match between saccular afferent sensitivity and the dominant (fundamental) frequency of vocal signals in the batrachoidid *O. tau*. Other studies in batrachoidids, based on extracellular recordings of saccular afferents or auditory brainstem units described similar observations (e.g. *O. tau*, Yan et al. 2000; Fay and Edds-Walton 2000; *P. notatus,* McKibben and Bass 1999; Weeg et al. 2002; Sisneros and Bass 2005). Such correspondence between the spectral content of vocalizations and hearing sensitivity has been reported not only in other fish taxa (e.g. Stabentheiner 1988; Ladich and Yan 1998), but also in other animal groups (e.g. insects, anurans, birds, Dooling et al. 2000; Gerhardt and Huber 2002).

Additionally, we provide evidence that the saccule can detect very low frequencies outside the frequency range of conspecific vocalizations, potentially overlapping with the lateral line sensitivity (e.g. Coombs and Janssen 1990; Weeg and Bass 2002). Prior to these experiments, saccular sensitivity of batrachoidid fish has only been investigated at frequencies as low as 30–75 Hz (e.g. Cohen and Winn 1967; Fay and Edds-Walton 1997; Sisneros 2007). Here, we present data on the saccular sensitivity as low as 15 Hz, which corresponded to the best stimulation frequency that induced the greatest evoked saccular potential (BF) in most specimens. Cohen and Winn (1967), using the saccular potential recording technique, also reported best auditory sensitivity at frequencies as low as 30 Hz in *P. notatus*. Although lateral line sensitivity has never been determined in *H. didactylus*, we predict an overlap in sensitivity between auditory and this other mechanosensory sense. Our finding suggests a potential adaptation of this species to detect low frequencies potentially important to enhance detection of conspecifics and/or predator/prey items when close to the biologically relevant sound source.

Phylogenetic analysis indicated that *H. didactylus* probably represents a basal lineage in the Batrachoididae (Rice and Bass 2009), providing an excellent model for understanding integrated mechanisms underlying the evolution of hearing and acoustic communication in fishes. Future studies should evaluate the contribution of the different otolithic endorgans, also utricle and lagena, for the auditory sensory coding; as well as verify the specific role of the inner ear and lateral line for sound detection.

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