

Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish

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Summary

Underwater noise pollution is an increasing environmental problem which might affect communication, behaviour, fitness and consequently species' survival. The most common anthropogenic noises in aquatic habitats derive from shipping. In the present study we investigated the implications of noise pollution from a ship on the sound detectability, namely of conspecific vocalizations in the Lusitanian toadfish, *Halobatrachus didactylus*. Ambient and ferry-boat noises were recorded in the Tagus River estuary (Portugal), as well as toadfish sounds, and their sound pressure levels determined. Hearing sensitivities were measured under quiet lab conditions and in the presence of these masking noises at levels encountered in the field, using the auditory evoked potentials (AEP) recording technique. The Lusitanian toadfish is a hearing generalist, with best hearing sensitivity at low frequencies between 50 and 200 Hz (below 100 dB re. 1 μ Pa). Under ambient noise

conditions, hearing was only slightly masked at lower frequencies. In the presence of ship noise, auditory thresholds increased considerably, by up to 36 dB, at most frequencies tested. This is mainly because the main energies of ferry-boat noise were within the most sensitive hearing range of this species. Comparisons between masked audiograms and sound spectra of the toadfish's mating and agonistic vocalizations revealed that ship noise decreased the ability to detect conspecific acoustic signals. This study provides the first evidence that fishes' auditory sensitivity can be impaired by ship noise and that acoustic communication, which is essential during agonistic encounters and mate attraction, might be restricted in coastal environments altered by human activity.

Key words: ship noise pollution, masking, hearing, auditory evoked potential, acoustic communication, fish.

Introduction

Most urban aggregations are confined to coastal zones, which have become strongly affected by human activities. One negative aspect of urban environments is the noise created, which increasingly extends into natural habitats (Katti and Warren, 2004). However, investigators are only just beginning to identify the implications of this negative environmental factor for acoustically communicating species. Anthropogenic noise may affect the behaviour in several taxa, including birds (Slabbekoorn and Peet, 2003), mammals (Myrberg, 1990; Richardson et al., 1995; Lesage et al., 1999; Morton and Symonds, 2002; Gibeau et al., 2002; Rabin et al., 2006), anurans (Sun and Narins, 2005) and fishes (Fernandes et al., 2000), with direct or indirect consequences on their ecology and fitness.

In aquatic environments, noise is produced mainly by shipping, recreational activities, drilling, seismic explorations

and energy production such as hydroelectric power plants or offshore windmills (Richardson et al., 1995; Popper, 2003). Most of these anthropogenic activities generate low frequency noise below 1 kHz (Richardson and Würsig, 1997), matching with the best hearing range and vocalizations of most fish species (Hawkins, 1973; Popper and Fay, 1999; Amorim, 2006). Despite the concerns raised by the increasing underwater noise pollution, little is known about its impacts on marine life. While efforts have been made to document the consequences of such a negative environmental factor on marine mammals, the effects on fishes have been poorly investigated.

Noise exposure has several effects on fishes, among them temporary hearing loss (Scholik and Yan, 2001; Amoser and Ladich, 2003; Smith et al., 2003; Popper et al., 2005), impaired sound detection and temporal resolution ability (Wysocki and Ladich, 2005a), damage to the sensory epithelia of the inner

ear (Hastings et al., 1996; McCauley et al., 2003), and endocrinological stress responses (Smith et al., 2003). The impacts of noise from ships, the most common source of aquatic noise pollution, have been investigated mostly within the framework of population assessments for fisheries in the marine environment, e.g. avoidance reactions (Fernandes et al., 2000; Vabø et al., 2002; Jørgensen et al., 2004). Besides these behavioural effects, few studies have addressed the harmful impacts of this noise source on fishes. According to Scholik and Yan (Scholik and Yan, 2002a), exposure to boat noise significantly reduces the hearing capability of the fathead minnow, *Pimephales promelas* (Cyprinidae). Amoser et al. (Amoser et al., 2004) investigated the effects of high-speed boating on native species in an Alpine lake. Comparisons between power boat noise spectra and audiograms revealed that fishes can perceive boat noise up to 300 m away. Furthermore, Wysocki et al. (Wysocki et al., 2006) showed that ship noise represents a potential stressor which affects both hearing specialists and generalists. All European freshwater species tested responded with increased cortisol secretion when exposed to ship noise.

Fishes depend on their auditory system to obtain information about predators and prey, for acoustic orientation, and to communicate inter- and intra-specifically, i.e. for mate attraction, agonistic encounters and territorial defence (Hawkins and Myrberg, 1983; Hawkins, 1993; Ladich and Myrberg, 2006; Myrberg and Lugli, 2006). The ability to accurately interpret information in the acoustic environment is extremely important for fishes' survival. It is therefore crucial to understand how certain typical noise sources resulting from human-altered habitats influence auditory perception.

The major goals of the present study were (1) to examine the effects of anthropogenic noise generated by a ferry-boat on hearing of the Lusitanian toadfish, *Halobatrachus didactylus* (Bloch and Schneider 1801) and (2) to investigate the degree to which the detection of conspecific sounds (boatwhistle and grunt) is impaired under ship noise conditions. This acoustically communicating species inhabits intertidal zones strongly affected by human activities, particularly shipping (personal observations).

Our study species, *Halobatrachus didactylus* (Batrachoididae), occurs in estuaries and coastal zones of the Eastern Atlantic and in the Mediterranean (Roux, 1986). During the breeding season [usually May to July (Palanzón-Fernández et al., 2001; Modesto and Canário, 2003)], males are territorial, defending their nest sites under rocks in shallow waters. They produce a long and tonal sound, the boatwhistle, which is primarily important for female attraction and mate choice in batrachoidids (Gray and Winn, 1961; Winn, 1967; Fish, 1972; McKibben and Bass, 1998; Dos Santos et al., 2000). Besides this long-distance advertising call, the Lusitanian toadfish frequently produces two other shorter sounds (grunt and double-croak), most likely associated with nest defence and agonistic encounters (Dos Santos et al., 2000; Amorim et al., 2006).

Materials and methods

Animals

The test subjects were 15 Lusitanian toadfish, *H. didactylus*, with a standard length (*SL*) of 14–30 cm and body mass of 77–579 g, which were caught in the estuaries of the Tagus and Mira Rivers (Portugal) by local fishermen and then transported to Vienna. Fish were kept in 250 l tanks for at least 2 weeks before starting the experiments. The bottoms of the aquaria were covered with sand and equipped with several plastic shelters. The aquaria were fitted with external filters and protein skimmers for salt water, and a 12 h:12 h L:D cycle was maintained. Fish were fed every second or third day with cod and occasionally with shellfish.

From this animal sample, two different fish groups were used for testing hearing under quiet laboratory noise ($N=9$) and under ambient and ship noise ($N=6$). One week of recovery was allowed between experiments for subjects that were tested under ambient and ship noise.

All the 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 recording and sound pressure level measurements

Ship and ambient noise were recorded in the Tagus River estuary (Montijo, Portugal; 38°42' N, 8°58' W) from a pier close to a ferry-boat station where the substrate consists of mud, fine sand and loose rock barriers. Lusitanian toadfish males usually establish nests in these rock aggregations during the reproductive season. Noise types were recorded with a DAT recorder (Sony TCD-D8, Sony Corporation, Tokyo, Japan), a hydrophone (Brüel and Kjaer 8101, Naerum, Denmark; frequency range: 1 Hz–80 kHz, ± 2 dB; voltage sensitivity: -184 dB re. 1 V/ μ Pa) positioned near the bottom at approximately 4 m depth, and a Brüel and Kjaer 2804 power supply. The ambient noise was a mixture of biological activity and water current sounds characteristic for this intertidal-nesting fish habitat. For playback of ambient noise during AEP recordings, 27 s from a representative recording were chosen randomly. The ship noise was obtained during the approach of a ferry-boat to a pier (ferry-boat station) located about 20 m away from the recording point (at an abandoned pier from where it was also possible to hear toadfish sounds). In this case, a 4.4 s section including the highest sound amplitudes was used for playbacks (Fig. 1).

Representative sound pressure level (SPL) values of ambient and ship noises were measured in the field using a sound level meter (Brüel and Kjaer 2238 Mediator) and the hydrophone (Brüel and Kjaer 8101) positioned at the same water depth as for the recordings (4 m), both connected to the power supply (Brüel and Kjaer 2804). For that purpose the *L*-weighted (5 Hz–20 kHz) equivalent continuous SPL (L_{Leq}) averaged over 1 min of measuring time was determined for ambient noise. In the case of ship noise, the highest instantaneous SPL values (L_{LSP}) were measured while the ship was approaching the pier (Fig. 1), and later averaged. The whole system was calibrated using a Brüel and Kjaer 4229 calibrator.

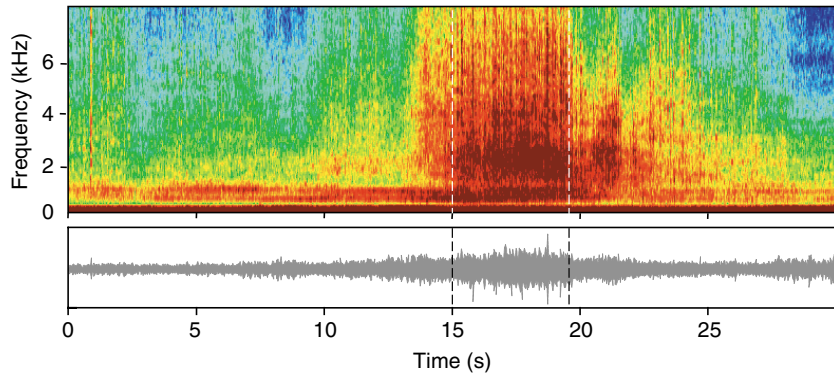


Fig. 1. Sonogram (above) and oscillogram (below) of the ferry-boat noise recorded in the Tagus River estuary (Portugal). The dashed lines indicate the temporal interval of 4.4 s selected for determining ship noise spectra. Sampling frequency 44.1 kHz, filter bandwidth 10 Hz, Blackmann Harris window, 50% overlap.

Amplitude spectra of fish vocalizations were also determined in order to compare conspecific sound spectra with hearing curves. Boatwhistles were recorded in the field and their SPL values (L_{LFP} , linear frequency weighting, RMS fast time weighting) determined. A hydrophone (High Tech 94 SSQ, Gulfport, MS, USA; frequency range: 30 Hz–6 kHz, ± 1 dB, voltage sensitivity: -165 dB re. 1 V/ μ Pa) was placed 20 cm away from the nest site of a vocalizing fish (35.0 cm SL, 1030 g body mass) and the reproductive calls were recorded using a digital portable recorder (Edirol R-4, Roland Corporation, Tokyo, Japan). The SPL values were then determined in the laboratory using the calibrated Brüel and Kjaer equipment (8101 hydrophone, 2804 power supply and sound level meter 2238 Mediator). Grunt trains were recorded in the lab and their SPL values (L_{LFP}) measured from at least 10 sounds per fish. This involved holding four fish (20.9–22.4 cm SL; 230–323 g body mass) underwater inside the experimental tub for auditory evoked potential (AEP) recordings (see next section) and using a DAT recorder (Sony TCD-D100) along with the Brüel and Kjaer equipment.

Noise and fish sound recordings (sampling frequency of 44.1 kHz and 8 kHz, respectively) were analyzed and the absolute sound spectra obtained using the acoustic analysis software S_TOOLS-STx 3.7 (Acoustics Research Institute, Austrian Academy of Sciences, Vienna, Austria). Averaged sound spectra of the recordings were calculated according to Amoser et al. (Amoser et al., 2004) and Wysocki and Ladich (Wysocki and Ladich, 2005b).

Auditory sensitivity measurements

Hearing thresholds were obtained using the AEP recording technique. Although hearing generalists, like the Batrachoididae, detect mostly particle motion of sounds (Fay and Edds-Walton, 1997; Weeg et al., 2002) and sound pressure level is an adequate measure of the degree of auditory stimulation in hearing specialists (Fay and Popper, 1974), the hearing thresholds of the Lusitanian toadfish were determined in SPL values for technical reasons. Fishes are usually able to

detect and process both pressure and vector components (or one of the derivatives, velocity, displacement and acceleration) of sound waves, albeit to different degrees (Popper and Coombs, 1980). Hence, this approach is appropriate as long as the displacement field is proportional to the pressure field, because in masking investigations the ratio of the tone level to the noise level at nearby frequencies is most important (Wysocki and Ladich, 2005b). This is also acceptable because our study emphasized a comparison between the effects of different noise types (lab, ambient and ship noise), the spectra of which are also given in pressure units, on signal detection and relative threshold shifts, always using the same experimental setup. This approach was broadly adopted in other similar studies with hearing generalists, e.g. oyster toadfish, *Opsanus tau* (Yan et al., 2000); bluegill sunfish, *Lepomis macrochirus* (Scholik and Yan, 2002b), gobies, *Padogobius martensii* and *Gobius nigricans* (Lugli et al., 2003) and European perch, *Perca fluviatilis* (Amoser et al., 2004; Amoser and Ladich, 2005). Nevertheless, the hearing thresholds should not be considered as absolute values because of the unknown exact proportional factor between the two sound components: sound pressure and particle motion.

Experimental setup

The AEP protocol was based on that described by Kenyon et al. (Kenyon et al., 1998) and adapted by Wysocki and Ladich (Wysocki and Ladich, 2005a; Wysocki and Ladich, 2005b), therefore only a brief summary of the technique will be given.

Fish were mildly sedated with Flaxedil (gallamine triethiodide; Sigma-Aldrich, Vienna, Austria) diluted in a Ringer solution (see Walsh, 1987). Approximately 4.8 – 15.0 μ g g^{-1} were injected intramuscularly and adjusted so that fish were still able to produce slight opercular movements during the experiments. The subjects were placed below the water surface in an oval-shaped plastic tub (diameter 45×30 cm; water depth 12 cm; 1.5 cm layer of sand) which was lined on the inside with acoustically absorbent material (air-filled packing wrap) to reduce resonances and reflections. Fish were positioned in the centre of the experimental tub and underwater with the exception of the contacting points for the electrodes, which were maximally 1–2 mm above the water surface. Tissue paper (Kimwipes[®]) was placed on the fish head to keep it moist and ensure proper contact of electrodes. Fish respiration was secured through a simple temperature-controlled ($22 \pm 1^\circ$ C), gravity-fed water system using a pipette inserted into the subject's mouth. The recording electrode was placed at the brainstem and the reference electrode approximately 2 cm cranially (silver wire, 0.25 mm diameter), pressed firmly against the subject's skin. Shielded electrode leads were attached to the differential input of an AC preamplifier (Grass P-55, Grass Instruments, West Warwick, RI, USA; gain $100 \times$, high-pass at 30 Hz, low-pass at 1 kHz).

A ground electrode was placed in the water near the fish body. A hydrophone (Brüel and Kjaer 8101) was placed on the right side of the animals (~1 cm away), specifically near the inner ear, in order to determine absolute stimulus SPLs under water 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, which was constructed as a Faraday cage (interior dimensions: 3.2 m×3.2 m×2.4 m).

Sound stimuli and masking noise presentation

Acoustic stimuli consisted of tone bursts that were presented at a repetition rate of 21 s⁻¹. The hearing thresholds were determined at frequencies of 50, 100, 200, 300, 500, 800 and 1000 Hz, which were always presented at random. The number of cycles in a tone burst was adjusted according to frequency in order to obtain the best compromise between stimulus rapidity (greater rapidity of onset means greater efficacy at generating AEPs) and peak frequency bandwidth (longer duration implies sharper spectral peak) (Silman and Silverman, 1991). Duration of sound stimuli increased from 2 cycles at 50 Hz up to 5 cycles at 1000 Hz. All bursts were gated using a Blackman window. For each test condition, one thousand stimuli were presented at opposite polarities, i.e. 90° and 270°, and were averaged together by the BioSig RP Software, resulting in a 2000-stimulus trace to eliminate any stimulus artifact. At each tested frequency, this procedure was performed twice and the AEP traces were overlaid to examine if they were repeatable. The lowest SPL where a repeatable AEP trace could be obtained, as determined by overlaying replicate traces, was considered the threshold. Sound pressure levels were attenuated in 4-dB steps until recognizable and repeatable waveforms could no longer be produced.

Auditory sensitivity was determined under three different conditions: laboratory, ambient noise and ship noise. In order to playback ambient and ship noise in the lab, sound files were prepared with TDT SigGen RP Software, sent to a 30-band equalizer (Alesis MEQ 230, Alesis Corp., Los Angeles, CA, USA) and adjusted to ensure that the noise spectra in the experimental tub were similar in frequency content to those recorded in the field. The noise files were then fed through a power amplifier (Alesis RA 300) to the loudspeaker and presented simultaneously with the tone bursts. Ambient and ship noise levels were also adjusted in the lab to equal those obtained in the field. The background noise in the laboratory inside the tub was also recorded and the equivalent continuous SPL averaged over 1 min (L_{Leq} =84.5 dB) determined.

A dual-cone speaker (Tannoy System 600, Coatbridge, Scotland, UK; frequency response: 50 Hz–15 kHz ±3 dB), mounted 1 m above subjects in the air, was used to present tone stimuli. For playback of ambient and ship noise, two speakers (Fostex PM-0.5 Sub and PM-0.5 MKII, Fostex Corporation, Tokyo, Japan), including a sub-woofer, were positioned 50 cm above the water surface to achieve low frequency noise spectral

amplitudes similar to those in the field recordings. Air speakers offer advantages compared with underwater ones, as they avoid problems related to near field sound propagation.

Both 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 a Pentium 4 PC containing a TDT digital processing board and running TDT BioSig RP Software.

Statistical analysis

Baseline and ambient noise audiograms were compared by a repeated measures ANOVA, which analyzed responses (hearing thresholds) to several frequencies in each subject fish (within-subject factor) under different noise conditions (between-subject factor). Noise condition was considered an independent variable in the ANOVA design because tested fish groups were different in the two experiments (laboratory and ambient noise conditions).

Ambient and ship noise audiograms were compared by a repeated measures ANOVA based on two within-subjects factors (frequency and noise condition), since only one fish group was tested in both noise experiments.

Both statistical analyses were followed by unpaired (baseline *versus* ambient noise audiograms) and paired (ambient *versus* ship noise audiograms) *t*-tests at each frequency separately. According to Bonferroni correction, threshold values were only considered to be significantly different when the level of significance was under 0.007 (0.05/7, as seven frequencies were tested).

Parametric tests were performed since data were normally distributed and variances homogeneous. The mean SPLs were calculated in Pa and converted to dB, therefore two s.e.m. values were given. The statistical tests were run using Statistica 7.1 for Windows (StatSoft, Inc., Tulsa, OK, USA).

Results

Absolute noise spectra

The equivalent continuous SPL (L_{Leq}) of ambient noise was 111.4 dB and the maximum instantaneous SPL of ship noise (L_{LSP}) measured at 20 m distance was 130.8 dB. The sound power spectrum of the ambient noise showed main energies at low frequencies below 300 Hz and was rather flat up to higher frequencies (Fig. 2). Ship noise had a conspicuous peak around 60 Hz followed by a marked drop at about 230 Hz and then a considerable increment at higher frequencies (Fig. 2). Spectral energies of the ship noise were approximately 40 dB above those of ambient noise between 300 Hz and 4 kHz.

Hearing under ambient and ship noise conditions

The baseline audiogram of the Lusitanian toadfish, obtained under quiet laboratory conditions, demonstrated best hearing at 50 Hz and a gradual sensitivity decrease towards 1000 Hz, the highest analyzed frequency (Table 1; Fig. 3). Above 1000 Hz, AEPs were no longer detectable. The hearing thresholds increased from 68 dB at 50 Hz up to 132 dB at 1000 Hz.

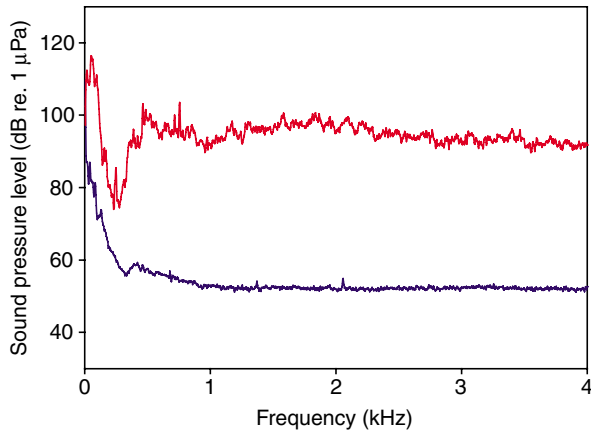


Fig. 2. Sound power spectra of ambient (blue line) and ship (red line) noises recorded in the Tagus River estuary (Portugal), near an intertidal nesting place of the Lusitanian toadfish. Sampling frequency 44.1 kHz, filter bandwidth 5 Hz, Blackmann Harris window, 75% overlap.

When ambient noise was played back, auditory sensitivity decreased by up to 29 dB especially at the lowest frequencies (Table 1). Comparing the baseline and ambient noise hearing thresholds by repeated measures ANOVA revealed overall significant differences between audiograms ($F_{1,12}=34.24$, $P<0.001$) and a significant interaction between noise and frequency ($F_{6,72}=27.93$, $P<0.001$), suggesting that this noise type affects hearing thresholds differently at different frequencies. Unpaired *t*-tests revealed significant decrease in sensitivity at 50 and 100 Hz (Fig. 3).

In the presence of ship noise, hearing sensitivity dropped considerably at all frequencies compared with the baseline audiogram. The shift in sensitivity decreased with increasing frequency from 36 dB at 50 Hz down to 3 dB at 1000 Hz

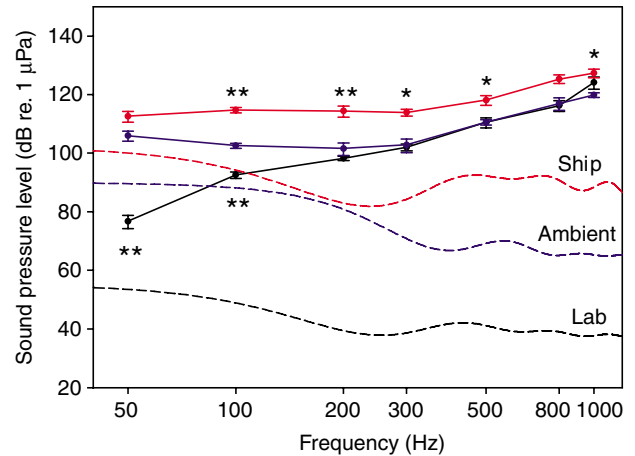


Fig. 3. Mean (\pm s.e.m.) hearing thresholds of the Lusitanian toadfish under laboratory conditions (baseline; black line) and in the presence of ambient noise (blue line) and ship noise (red line). Dashed lines of same colour show the cepstrum-smoothed sound power spectra for the respective noise types used during audiogram determinations. Asterisks indicate statistically significant differences between the ambient noise and baseline (unpaired *t*-tests) as well as ambient and ship noise (paired *t*-tests); * $P<0.007$ and ** $P<0.001$). $N=9$, baseline; $N=6$, ambient and ship noise audiograms.

(Table 1). Ambient and ship noise audiograms showed overall significant differences (repeated measures ANOVA, $F_{1,5}=48.71$, $P<0.001$), as well as significant interaction between noise and frequency ($F_{6,30}=3.11$, $P=0.017$). Paired *t*-tests revealed significant differences at almost all tested frequencies, except for 50 and 800 Hz (Fig. 3).

Threshold-to-noise (or T/N) ratios were also determined (Fig. 4) by subtracting the masking noise spectra (cepstrum-smoothed) levels from the hearing threshold SPL values at a

Table 1. Mean hearing thresholds under different noise conditions and threshold shifts (with the baseline audiogram as reference) of the Lusitanian toadfish

Frequency (Hz)	Baseline		Ambient			Ship		
	Hearing threshold (dB re. 1 µPa)		Hearing threshold (dB re. 1 µPa)	Threshold shift (dB)		Hearing threshold (dB re. 1 µPa)	Threshold shift (dB)	
50	76.77	+1.96 -2.53	105.97	+1.54 -1.87	29.2	112.65	+1.68 -2.08	35.88
100	92.59	+1.03 -1.16	102.56	+0.80 -0.88	9.97	114.73	+0.89 -1.00	22.14
200	98.29	+0.70 -0.77	101.61	+1.91 -2.46	3.32	114.44	+1.70 -2.11	16.15
300	102.01	+1.11 -1.28	102.82	+2.02 -2.63	0.81	113.90	+1.07 -1.23	11.89
500	110.54	+1.58 -1.93	110.57	+0.86 -0.96	0.03	118.12	+1.51 -1.83	7.58
800	115.86	+1.75 -2.19	116.99	+1.88 -2.40	1.13	125.33	+1.33 -1.58	9.47
1000	124.16	+1.81 -2.28	119.84	+0.79 -0.87	-4.32	127.36	+1.31 -1.54	3.20

Threshold hearing values are means \pm s.e.m.; $N=9$, baseline; $N=6$, ambient and ship noise audiograms.

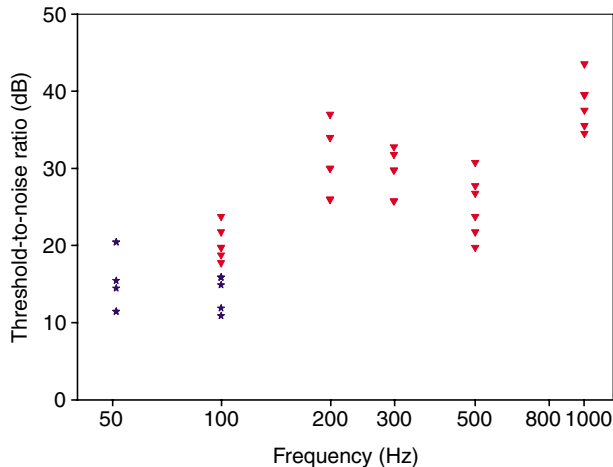


Fig. 4. Threshold-to-noise ratios for masked thresholds for ambient (blue stars) and ship (red triangles) noises. Note that only masked thresholds that were statistically significantly different are represented. ($T/N \text{ ratio} = \text{frequency} \times 21.034 + 18.008$, $r = 0.760$, $P < 0.001$).

specific frequency. This calculation was made solely for masked thresholds that were significantly different (see Fig. 3) according to *t*-tests performed for each frequency. The mean T/N ratios typically increased with increasing frequency, varying from 16.42 (+1.54, -1.87) dB (mean \pm s.e.m.) to 38.91 (+1.31, -1.54; mean \pm s.e.m.).

Detection of conspecific sounds in the presence of noise

The L_{LSP} levels of the two recorded acoustic signals of Lusitanian toadfish – boatwhistle and grunt train – were 140 dB (about 20 cm away) and 137.30 (+3.94, -7.41) dB (10 cm away; mean \pm s.e.m., $N = 4$ fish), respectively.

Comparisons between boatwhistle and grunt train power spectra and the ambient noise audiogram indicated that both sound types were clearly detectable by the Lusitanian toadfish (Fig. 5). The sound energies of both sounds were up to 23 dB above the hearing thresholds in the frequency range below 400 Hz, where the main energies of sounds are concentrated. However, sound detectability decreased dramatically when ship noise was present in the environment. Sound frequencies were only detectable below 300 Hz, and sound energies were merely 12 dB above the hearing curve (Fig. 5).

Discussion

Ambient and ship noise characteristics

Marine habitats are generally thought to be noisier than freshwater habitats, in which spectral levels below 60 dB are found in lakes and backwaters (Wenz, 1962; Urick, 1983; Amoser and Ladich, 2005). Running freshwaters such as rivers and streams, however, are actually as noisy as marine environments (Amoser and Ladich, 2005). In the Tagus River estuary the mean spectral ambient noise levels were roughly 60 dB and thus relatively quiet (compared with some marine habitats) except for considerably higher SPLs at frequencies below 300 Hz. This may be because the study area was near

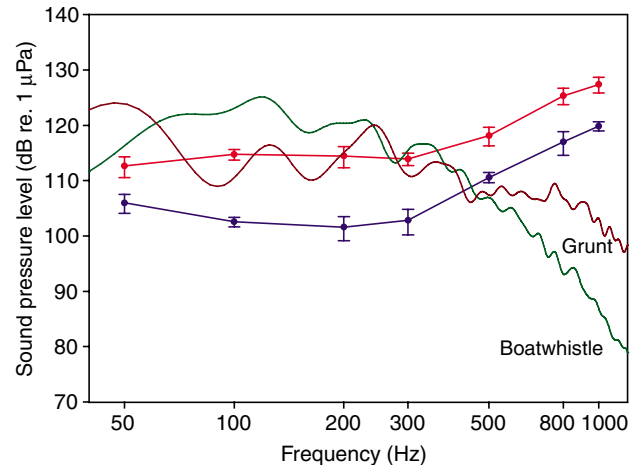


Fig. 5. Mean (\pm s.e.m.) hearing thresholds of the Lusitanian toadfish in the presence of ambient (blue line) and ship (red line) noises as well as cepstrum-smoothed power spectra of two conspecific sounds: boatwhistle (green line) and grunt train (brown line). The boatwhistle spectrum is calculated from a distance of 20 cm, the grunt train 10 cm away from the calling animals.

the coast in shallow waters, where most anthropogenic activity and the presence of concrete structures (piers) generate low frequency noises. Ambient noise in such habitats, however, which generally consists of a mixture of surf, wind, shipping, industrial and biological noises, is extremely variable with time and from place to place (Urick, 1983; Greene, 1995). Thus, only rough information can be given about the spectral levels of noise potentially found in such coastal zones.

In the present study, ferry-boat noise elevated ambient noise spectral pressure levels by about 40 dB in the study area of the Tagus River estuary, presenting a conspicuous pressure peak at lower frequencies (around 60 Hz), which is a characteristic feature of noise generated by ship traffic (e.g. Urick, 1983; Amoser et al., 2004). There has been little focus on SPL measurements and noise spectra of recreational boats or vessels and ferry-boats, in comparison to larger cargo ships for instance (e.g. Arveson and Vendittis, 2000; Amoser et al., 2004). The instantaneous SPL of the ferry-boat was approximately 131 dB re. 1 μ Pa at 20 m distance from the ship at the site where Lusitanian toadfish were calling. Assuming cylindrical spreading this gives a source level at 1 m of approx. 143 dB. Although SPL values were much higher close to the ferry-boat, we concentrated only on SPLs recorded in the nesting place of the study species. Considering the distances to the noise source and consequently signal amplitude attenuation (Fine and Lenhardt, 1983; Mann and Lobel, 1997; Mann, 2006), we find that the level determined was far below other reported SPL values for different ships. According to Greene and Moore (Greene and Moore, 1995), a 70 horsepower outboard motor boat recorded at 50 m distance generated noise at 142 dB re. 1 μ Pa. Amoser et al. (Amoser et al., 2004) reported 124–128 dB re. 1 μ Pa from a high-speed boat during a powerboat race at a distance of 300 m.

Auditory sensitivity and noise masking effects

Batrachoididae (toadfishes) are hearing generalists (Fish and Offutt, 1972; McKibben and Bass, 1999; Weeg et al., 2002; Sisneros and Bass, 2005) that lack accessory hearing structures (air-filled cavities connected to the inner ear) to enhance auditory abilities (Hawkins and Myrberg, 1983; Ladich and Popper, 2004). They therefore essentially respond to the particle motion component of low frequency sounds (and only below 1000 Hz) at relatively high sound intensities. Our data confirmed that *H. didactylus* is a generalist, exhibiting best hearing range at low frequencies between 50 and 200 Hz, with hearing thresholds below 100 dB re. 1 μ Pa. Above this frequency range, the species revealed a gradual decrease in sensitivity up to 1000 Hz (124 dB). Compared to other batrachoidids, the Lusitanian toadfish has somewhat better hearing abilities at low frequencies. Fish and Offutt (Fish and Offutt, 1972), using a conditional heart rate technique, reported a hearing threshold at about 100 dB re. 1 μ Pa just below 150 Hz in the oyster toadfish *O. tau*. Yan et al. (Yan et al., 2000), by utilizing the AEP technique, found threshold values for the same species ranging from 117 (at 100 Hz) up to 134 dB (at 800 Hz). The hearing differences compared with *H. didactylus* may reflect differences in laboratory background noise, methodological differences or higher sensitivity to low frequencies due to the lower fundamental call frequency in this species, which may be around 50–60 Hz. By contrast, the dominant frequencies of *O. tau* are between 90 and 200 Hz (Fine, 1978; Fine, 1981). In *H. didactylus*, the main energies of boatwhistles sometimes lie within the fundamental frequency (Amorim and Vasconcelos, 2006), as was the case in the grunt call presented in this study. We expect that *H. didactylus* accurately detects the first harmonic, as do other toadfish species (Fay and Edds-Walton, 1997; McKibben and Bass, 1999).

The sound detection limit may not be set by auditory sensitivity constraints but instead by the level of background noise in the environment (Hawkins, 1981). Chapman (Chapman, 1973) and Chapman and Hawkins (Chapman and Hawkins, 1973) showed that the hearing in cod is adapted to quiet sea noise conditions and that any increase in prevailing sea noise (e.g. increase in wind speed and surface motion) was accompanied by an upward shift in threshold. The ability to discriminate between conspecific sound stimuli and background noise is essential for a vocal species. Ambient noise from the Tagus River estuary, where the Lusitanian toadfish is abundant and males establish their nests during the reproductive season, partially masks hearing thresholds only at 50 and 100 Hz when compared to quiet laboratory conditions. Clearly, the amount of masking in the toadfish was frequency dependent, as revealed by the significant interactions between noise (baseline *versus* ambient) and frequency. Hence, the Lusitanian toadfish can exploit its hearing abilities at this nesting place, being just slightly affected at very low frequencies where it is most sensitive. Amoser and Ladich (Amoser and Ladich, 2005) also reported that the auditory sensitivity of the European perch *P. fluviatilis*, another hearing generalist, suffered a slight masking effect from the Danube

River noise, predominantly at the low frequencies (200 and 300 Hz), which fall in the best hearing range of the species.

In the presence of a ferry-boat that slowly approached the coast where Lusitanian toadfish were nesting, hearing sensitivity became considerably masked at almost all frequencies, showing threshold shifts ranging from 36 dB down to 3 dB relative to the baseline audiogram. Sound detection deteriorated especially between 100 and 300 Hz. The masking effect was somewhat lower at 50 Hz, despite an energy peak of ship noise at approximately this frequency value, because the fish's hearing was already masked by the ambient noise by approximately 29 dB.

Masking effects increased approximately linearly with the frequency tested, mainly within the frequency range in which the species is most sensitive. Threshold-to-noise ratios increased from 16 to 38 dB. This trend has also been shown in hearing specialists such as the goldfish *Carassius auratus*, the common carp *Cyprinus carpio* and the catfish *Platydoras costatus*, as well as for generalists such as the sunfish *Lepomis gibbosus* and the European perch (Wysocki and Ladich, 2005b; Amoser and Ladich, 2005).

Detectability of vocalizations and the impact of anthropogenic noise on acoustic communication

The Lusitanian toadfish is a notable sound producer, exhibiting a complex acoustic repertoire consisting of various vocalizations used in different contexts (Dos Santos et al., 2000; Amorim et al., 2006). The boatwhistle of toadfishes consists in a multi-harmonic advertising call produced by nesting males to attract females for mating and to compete intra-sexually (Gray and Winn, 1961; Winn, 1967; Fish, 1972; McKibben and Bass, 1998). This sound is relatively long (approximately 800 ms), with a fundamental frequency at about 60 Hz and dominant energies in either the second or fourth harmonic (Amorim and Vasconcelos, 2006; Amorim et al., 2006). Comparing the sound spectrum and ambient noise audiogram revealed that the main sound energies (120 and 240 Hz) were located within the best hearing range of the species (50–300 Hz). The SPL at boatwhistle dominant frequency (at about 120 Hz) was 23 dB above hearing thresholds, indicating that the fish can easily detect this acoustic signal under ambient noise conditions.

The grunt train is composed of several consecutive short broad-band pulsed sounds (single grunts), probably produced during agonistic contexts such as deterring territory intruders or predators (Dos Santos et al., 2000; Amorim et al., 2006). Similarly to boatwhistles, highest spectral levels of the grunt train matched well with the Lusitanian toadfish hearing sensitivity (dominant sound frequency was 19 dB above hearing thresholds).

We demonstrated a correlation between sound production and auditory sensitivity in the Lusitanian toadfish, as was also found in other batrachoidids [e.g. *O. tau* (Fay and Edds-Walton, 1997; Fay and Edds-Walton, 2000) (but see Fine, 1981); *P. notatus* (McKibben and Bass, 1999; Weeg et al., 2002; Sisneros and Bass, 2005)]. Such correlations have been

reported in other fish taxa [e.g. piranha (Stabentheiner, 1988); anabantoids (Ladich and Yan, 1998)].

Ship noise seems to interfere with the detection of conspecific sounds in the Lusitanian toadfish because the difference between peak spectral levels and hearing thresholds decreased from 23 dB at ambient noise conditions to merely 12 dB when ferry-boats arrived. Assuming cylindrical sound spreading in the shallow estuary and that sound levels attenuate by about 3 dB when doubling the distance (10 dB when distance increases tenfold), females, for instance, will not be able to detect boatwhistles much beyond 2 m (see Amoser et al., 2004; Mann, 2006). Hence, the ability of females to detect nesting males is fairly restricted under ship noise conditions.

These results provide the first evidence that ferry-boat noise negatively impacts hearing and communication in the Lusitanian toadfish because it diminishes the distance over which fish can accurately perceive conspecific acoustic signals in the natural habitat. Since we determined ship noise level approximately 20 m away from the ferry-boat, we can predict that the effect on communication would probably be much larger closer to the ship and that toadfishes might be forced to establish their nests farther away from the noise source.

Grunt train SPL (137 dB) was measured 10 cm from the vocalizing fish. As this vocalization seems to be important during agonistic encounters and nest defence, we assumed that it is uttered by nesting males typically at this distance or even closer to nest intruders. This acoustic signal is probably important for assessing the fighting ability of opponents (R.O.V. and F.L., unpublished); thus, deteriorated sound characteristics might result in misleading information and perhaps in more escalated contests.

The boatwhistle SPL value (140 dB) was determined in the field about 20 cm from the calling animal. This corresponds roughly to the closest distance at which male neighbours establish their nests in aggregations in the peak of the breeding season (R.O.V., unpublished). Therefore, masking hearing capacities might influence spacing between males, and even have a major impact on females' attraction to the nests, since they are thought to detect and be attracted to calling males at greater distances. In the reproductive season, batrachoidid females come from deeper waters attracted to vocal nesting males (Gray and Winn, 1961; Brantley and Bass, 1994; Sisneros et al., 2004). Moreover, previous work has shown that nesting males seem to emit individual specific boatwhistles and the differences in these mating vocalizations may provide scope for mate choice (Amorim and Vasconcelos, 2006). Hence, the diminished ability to perceive subtle individual signal differences in the presence of ferry-boat noise could compromise the sexual selection.

Human activities are dramatically transforming natural habitats and creating novel environmental conditions, which animals must either adapt to or abandon (Katti and Warren, 2004). The Lusitanian toadfish, like several other acoustically communicating species, inhabits shallow waters where low frequency sound propagates only over short distances and

where the frequency composition and temporal patterns are lost (Fine and Lenhardt, 1983; Mann and Lobel, 1997; Mann, 2006). Additional signal degradation by anthropogenic noise sources such as recreational and commercial ships may restrict acoustic communication with notable consequences in behaviour and reproduction.

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