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Boat noise impacts early life stages in the Lusitanian toadfish: A field experiment

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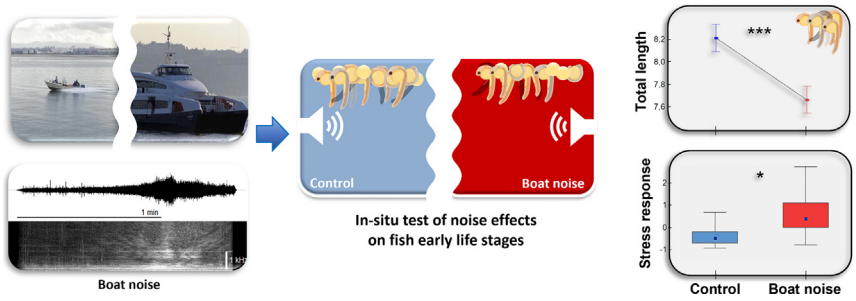
HIGHLIGHTS

- In-situ experiments examined the effect of boat noise on toadfish early life stages.
- The impact of chronic boat noise exposure was evident at the larval stage.
- Larvae exposed to boat noise showed reduced growth.
- Noise exposure affected stress responses assessed by biomarkers.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine traffic is the most common and chronic source of ocean noise pollution. Despite the evidence of detrimental effects of noise exposure on fish, knowledge about the effects on the critical early life stages - embryos and larvae - is still scarce. Here, we take a natural habitat-based approach to examine potential impacts of boat noise exposure in early life stages in a wild fish population of the Lusitanian toadfish (*Halobatrachus didactylus*). In-situ experiments were carried out in the Tagus estuary, an estuary with significant commercial and recreational boat traffic. Nests with eggs were exposed to either ambient (control) or boat noise (treatment), for 1 fortnight. Eggs were photographed before being assigned to each treatment, and after exposure, to count number of eggs and/or larvae to assess survival, and sampled to study development and oxidative stress and energy metabolism-related biomarkers. Data concerns 4 sampling periods (fortnights) from 2 years. Results indicate that offspring survival did not differ between treatments, but boat noise induced a detrimental effect on embryos and larvae stress response, and on larvae development. Embryos showed reduced levels of electron transport system (ETS), an energy metabolism-related biomarker, while larvae showed higher overall stress responses, with increased levels of superoxide dismutase (SOD) and DNA damage (oxidative stress related responses), ETS, and reduced growth. With this study, we provided the first evidence of detrimental effects of boat noise exposure on fish development in the field and on stress biomarker responses. If these critical early stages are not able to compensate and/or acclimate to the noise stress later in the ontogeny, then anthropogenic noise has the potential to severely affect this and likely other marine fishes, with further consequences for populations resilience and dynamics.

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1. Introduction

Aquatic environments are facing unparalleled pressure from anthropogenic noise pollution resulting from different human activities such as seismic surveys, pile driving, coastal development and ship traffic (Slabbekoorn, 2019; Duarte et al., 2021). Long-term measurements of ocean ambient noise indicate that low frequency noise (<1 kHz) has increased in the last decades and is expected to continue to rise dramatically in the near future, particularly in coastal and transitional waters (Kaplan and Susan Solomon, 2016). This low-frequency noise overlaps with the hearing range of several taxa (Slabbekoorn, 2019), and can mask important biological sounds, causing a widespread impact on marine organisms (Kunc et al., 2016).

Attempts to gain an understanding about the effects of anthropogenic noise on fish have been growing in the past decade. Noise can impact fishes by changes in the anatomy, physiology and/or behaviour (Slabbekoorn et al., 2010; Kunc et al., 2016; Kuşku et al., 2018). It is linked to damages to the ears and/or swim bladder (Popper et al., 2019; Breitzler et al., 2020), changes in hearing abilities (by increasing the auditory threshold level, Scholik and Yan, 2001, and/or due to masking, Alves et al., 2021), increased stress response (secretion of cortisol - Wysocki et al., 2006; ventilation rate - Kusku, 2020; Kusku et al., 2020), increased metabolic costs (Buscaino et al., 2010), decreased growth performance (Kusku et al., 2020), reduced foraging performance (Purser and Radford, 2011), increased risk of predation (Voellmy et al., 2014), and changes in reproduction (de Jong et al., 2020). Despite the already well documented impacts on behaviour and physiology of adult fish, the impacts of human-generated noise on the critical early life stages remains understudied. Early life stages are frequently marked by high mortality rates, which can result in population fluctuations and changes in the population dynamics (Houde, 1987). Any additional stressor at this stage can further increase mortality, with far more reaching consequences at the population level. The European Commission Marine Strategy Framework Directive has pinpointed studies addressing anthropogenic noise effects on these life stages as a research priority (Dekeling et al., 2016). The few available studies, to date, suggest that boat noise exposure can increase heart rate in damselfish embryos (Jain-Schlaepfer et al., 2018; Fakan and McCormick, 2019), reduce growth and lead to faster yolk sac consumption in recently hatched Atlantic cod (Nedelec et al., 2015), increase heart rate, yolk sac use and increase cortisol levels on larval zebrafish (Lara and Vasconcelos, 2021), and affect fast-start kinematics and routine swimming in juveniles of a damselfish species (McCormick et al., 2019). However, these studies were held in captive fish, compromising the inference of results to the wild, since the overall environment in laboratory differs from the wild, including in the sound field, especially in terms of the magnitude of particle motion relative to sound pressure (Popper and Hawkins, 2019).

Here we take a natural habitat-based approach to examine potential impacts of boat noise exposure in early life stages in a wild fish population. Specifically, we assessed the effect of common small outboard engine boats and ferry boats on Lusitanian toadfish (*Halobatrachus didactylus*), a species with a rich vocal repertoire (Amorim et al., 2008), that relies on acoustic communication for mate finding (Vasconcelos et al., 2012) and exhibits complex male-male interactions (Vieira et al., 2021a). In-situ experiments were carried out in the Tagus estuary, one of the largest estuaries in Europe with significant incidence of noise from commercial and recreational boat traffic (Vieira et al., 2021b). It has been found in adult toadfish that boat generated noise increase hearing thresholds (Vasconcelos et al., 2007), can affect calling rate in males, and reduce the acoustic active space, thereby affecting chorusing behaviour in this species (Alves et al., 2021). Despite the evidence of detrimental effects of noise on toadfish adults, we know little of the effects of anthropogenic noise on the early life stages. Toadfishes have demersal eggs that are laid in a nest and guarded by the male until the fry are free-swimming. After hatching, larvae do not move up into the water column to disperse (unlike most other demersal spawners) but stay attached to the nest until most of the yolk sac has been absorbed (Collette, 2005). This can take up to 2–3 months, depending on temperature (MCP Amorim, personal observation), meaning that larvae cannot swim away from the noise source.

The present study represents a significant advancement to this field of research as it manipulates the embryos and larvae acoustic environment in the natural breeding habitat, thus allowing to address the effects of boat noise exposure in the wild. We explored how chronic exposure to boat noise (c. 2 weeks) affects survival and growth of toadfish early life stages, and further measured oxidative stress and energy metabolism-related biomarkers as proxies for physiological stress (Van der Oost et al., 2003; Silva et al., 2016). We hypothesized that if boat noise acts as a stressor to these early life stages, changes in growth and biochemical endpoints will be noticeable.

2. Methods

2.1. Field setup and playback design

Two nest-sets, each with 12 concrete hemicylindrical structures (50 cm long, 30 cm wide and 20 cm max height), were deployed c. 30 m apart in an intertidal area of the Tagus estuary (Air Force Base no. 6, Montijo, Portugal; 38°42′N, 8°58′W). Nests were placed every 2 m in 2 rows (6 nests per row) parallel to the shoreline (Fig. 1a). During the breeding season (May to July 2016 and 2017), Lusitanian toadfish males spontaneously occupied these shelters and used them to attract females to spawn. The shelters were internally lined with a removable plastic sheet where the females laid their eggs on. These nests were usually underwater (maximum level of c. 2.8 m) except at spring low tides, when they were exposed to air, allowing periodic access to nests to verify nest occupation and the presence of clutches. Water temperature measured by a temperature and pressure datalogger (HOBO-U20-001-01, Onset Computer Corp., MA, USA) ranged 17–30 °C in both years.

Three UW30 underwater loudspeakers (frequency response 0.1–10 kHz, Electro-Voice, Columbus, USA) were evenly spaced between the two nest rows in each nest-set and fixed to the substrate facing upwards (Fig. 1a). Each speaker was fed by an amplifier (Sony XM-N1004, Tokyo, Japan) and a mp4 device (A730 Music Player, HOTT, Shenzen, China) and played back either ambient sound (control) or boat noise (treatment). Noise treatment consisted in the playback of 4 small outboard engine boats plus 10 ferry boat passages per hour (Transtejo ferry boats) and was on from 6 h00 to 24 h00 (18 h), mimicking what fish experience on average in Tagus estuary. Boat noise playback was set at a level of c. 30 dB (20–40 dB RMS of 5 s centred in the noise of each boat passage) above background corresponding to the increase caused by a ferryboat recorded c. 50–100 m away from the pier near our study site and comparable to the increase in noise level due to boating reported in other studies (e.g. Magnhagen et al., 2017; Nedelec et al., 2017; de Jong et al., 2018; Blom et al., 2019). The ambient sound treatment (control) replaced boat noise by background environmental sound recorded in this area during the toadfish breeding period and was played back above the background noise level with the same amplification used in the boat noise treatment to control for possible electromagnetic effects caused by a working loudspeaker (Fig. 2). Playback treatment was randomly assigned to a nest-set and swapped in the subsequent fortnight. Nest-sets were sufficiently spaced to prevent noise played treatment being received in the other nest-set.

Playbacks were calibrated with a hydrophone (Bruel & Kjaer 8104, Naerum, Denmark; sensitivity −205 dB re. 1 V μPa^{-1}; frequency response from 0.1 Hz to 180 kHz) connected to a sound level meter (Bruël & Kjaer 2238 Mediator, Naerum, Denmark). Particle motion patterns of the boat noise playbacks were measured with a 3-axis accelerometer (M20-040, sensitivity 1–3 kHz, GeoSpectrum Technologies, Dartmouth, Canada). The output of both instruments (pressure and acceleration) was simultaneously logged using a 4-channel digital

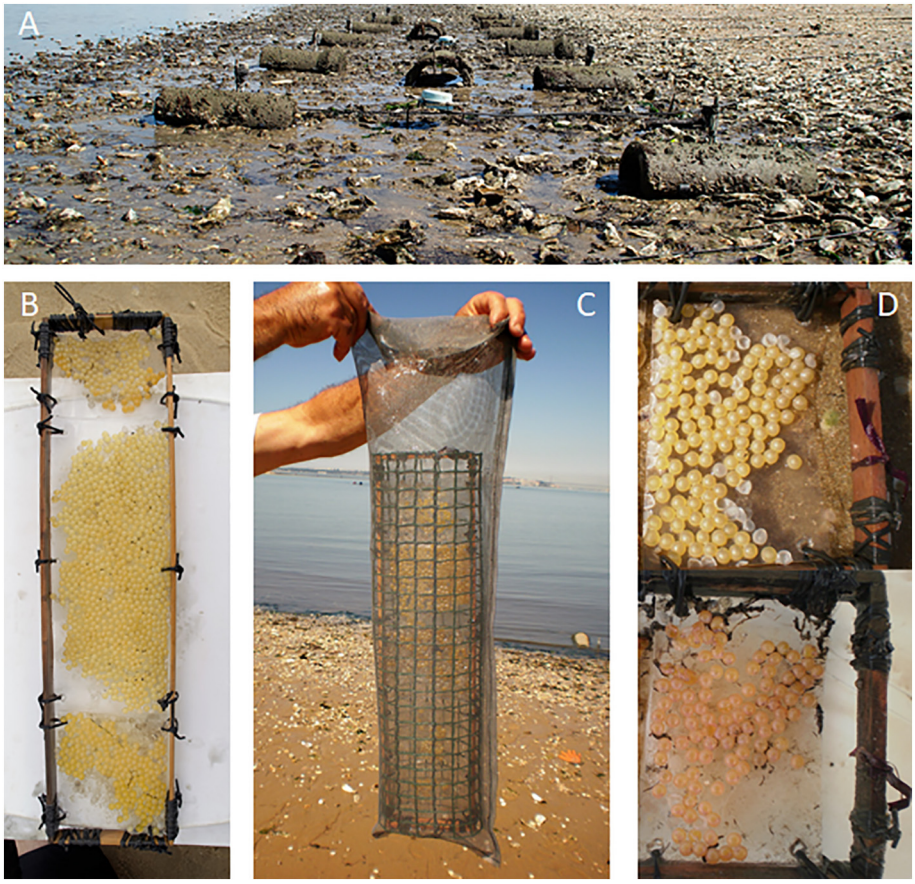

Fig. 1. Field setup depicting (A) a side view of a nest-set with 12 male artificial concrete nests placed every 2 m in 2 rows, three UW30 underwater loudspeakers evenly spaced between the two nest rows, and two larger empty nests positioned parallel to the shoreline, 1 m from the speaker, where the racks with egg samples (B) were placed, after being wrapped with a medium-sized grid plastic mesh surrounded by a fine mesh (C) to prevent egg/larvae loss during the experiment from detachments or predation. Panel D depicts an egg sample before and after treatment exposure. Note that in this example eggs did not develop into larval stage.

recorder (Edirol R4, Roland Corporation, Tokyo, Japan). The analogue-to-digital converter input voltage of each channel used to record the 3 axis accelerometer outputs (xx, yy or zz) and the sound level meter was calibrated by recording sinusoidal waves at pre-set amplitude voltage. Both sensors were located side by side c. 20 cm from the bottom, with c. 1 m water depth. The boat noise sound files used in the playback were recorded nearby at Air Force Base no. 6 pier and included noise produced by two small private open deck boats with an outboard engine at 7–20 m from the hydrophone (rms 120–140 dB re. 1 μPa, calculated in the 0–20 kHz bandwidth or rms 104–133 dB re. 1 μPa, calculated in the 0–2 kHz bandwidth), and two passages of ferryboats that regularly cross the Tagus estuary (50–100 m; rms 122–131 dB re. 1 μPa or rms 117–127 dB re. 1 μPa calculated in the 0–2 kHz bandwidth, Fig. 2). Note that playback levels changed with tide level due to changes in the output of the speakers caused by the changes in water pressure.

2.2. Experimental design

To evaluate the effect of boat noise on toadfish early life stages, during nest inspections we removed the plastic sheets from the nests that received eggs. Each sheet was cut in two pieces (clutch samples) with similar number of eggs and assigned randomly to noise treatment following a split-brood balanced design to control for potential genetic effects on development. Note that each clutch sample could contain more than one brood as males mate with multiple females and may experience cuckoldry from other males (Amorim et al., 2010, 2016). Clutch samples were photographed, identified, and attached to a rack (Fig. 1b). The rack was wrapped with a medium-sized grid plastic mesh and then in a fine plastic mesh allowing water to flow through while preventing egg/larvae loss during the experiment from detachments or predation (Fig. 1c). The racks, with the eggs facing downwards, were placed inside an empty nest with the back concrete removed, and positioned 1 m from the speaker, parallel to the shoreline to allow inner water flow and maximise oxygen availability as eggs could not receive aeration typically given by parental males. Although the nests with eggs were in proximity (c. 1 m) to the nests with the nest-holders (Fig. 1a) they were never occupied by males. In the subsequent fortnight we assessed the racks and removed clutch samples to evaluate treatment effects. Clutches were thus exposed for c. 2 weeks to treatment. Clutch samples were again photographed to count the number of eggs and larvae to assess survival (Fig. 1d). After a fortnight, eggs were sampled to measure stress response as described below, and larvae were sampled to study both development and stress response.

This study was authorized by the Portuguese National Authority for Animal Health (Direção Geral de Alimentação e Veterinária), performed in strict accordance with the EU Directive 2010/63/EU for animal experiments and followed the recommendations of the Animal Care and Use Committee of the Faculty of Sciences, University of Lisbon.

2.3. Morphometric analysis

After playback exposure, an average of 25.0 larvae (±SD, range: ± 1.6, 22–28) per clutch sample were gently detached from the plastic sheet with tweezers, euthanised with an excessive dosage of anaesthetics and immediately placed in 70% ethanol inside Eppendorf tubes. Larvae were only sampled when they were available in sufficient number in both treatments, from the same original clutch sample (i.e. nest). Six nests from two sampling periods (2 fortnights, 2017) met these

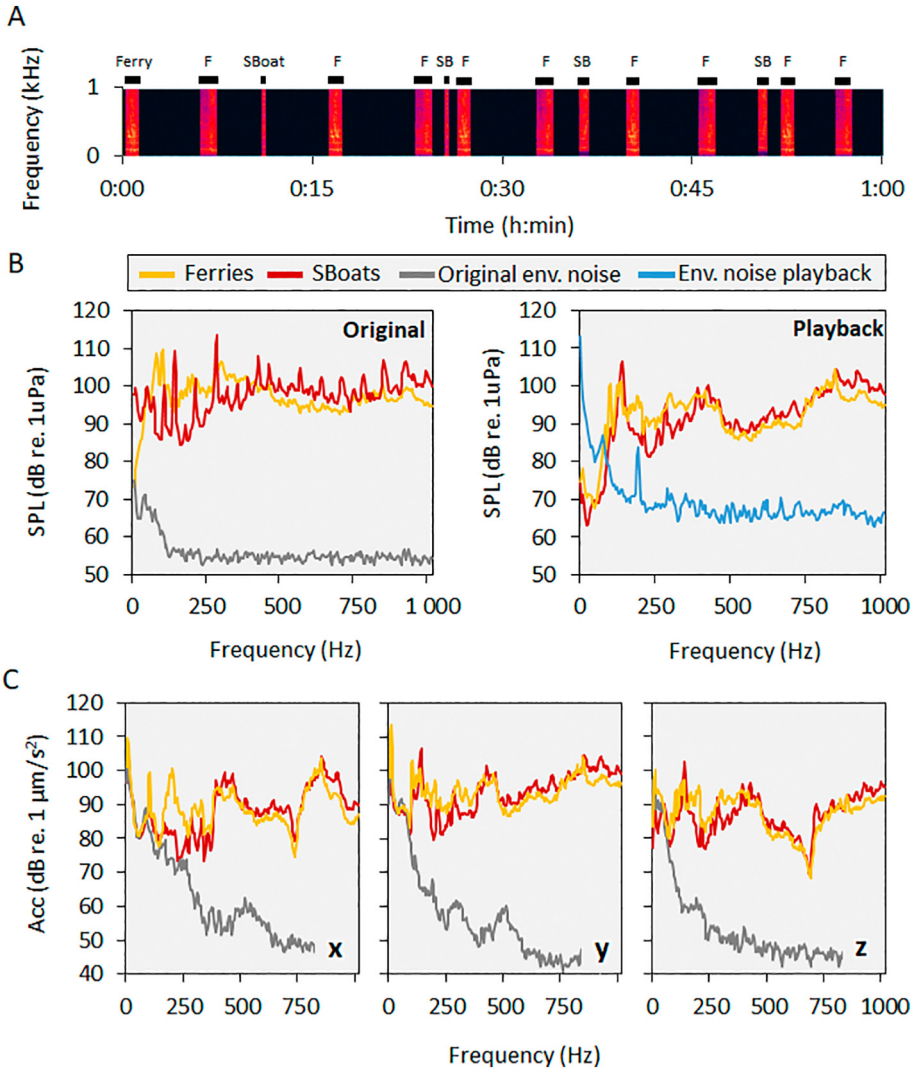

Fig. 2. (A) Spectrogram of the recording used for boat noise playback depicting passages of ferry boats and small boats (sboat). (B) Mean power spectra of different ferries, small boats and environmental sounds recorded at Air Force Base no. 6 pier and respective playbacks used in the field experiments. (C) Acceleration spectra on the three axes (xx, yy, zz) of different ferry and small boat noise playbacks. Acceleration spectrum of environmental noise is shown for reference. Note that spectra represent 5 s centred in the noise of each boat passage. Spectra settings: sampling frequency, 4 kHz; FFT size, 1024; window type, Hanning; window overlap, 50%.

conditions. A total of 148 and 153 larvae exposed to environment and boat noise playback, respectively, were sampled and photographed under a dissecting stereomicroscope for morphometric analysis, using Image-J (v1.48; U. S. National Institutes of Health, Bethesda, Maryland). We measured total length (TL), head height (HH), body height (BH), yolk sac area (YSA) (Fig. 3), which are indicators of development in larval fishes (Chambers et al., 2014).

2.4. Biochemical responses

Biochemical responses were assessed in both eggs and larvae exposed to c. 2 weeks of playback. On site, an average of 14.8 eggs (± 5.0, 10–20) per clutch sample/treatment were gently detached from the plastic sheet with tweezers and immediately placed inside Eppendorf tubes in dry ice and then taken to the laboratory, where they were stored at −80 °C until biochemical analysis. An average of 17.8 (±5.3, 4–20) larvae per clutch sample/treatment were collected with tweezers as described for the morphometric analysis. As above, larvae were only sampled when a clutch sample (i.e., the eggs in one nest) had a sufficient number available in both treatments. A total of 16 nests were sampled in the 2 years (3 sampling periods, 2016 and 2017) for stress response evaluation in eggs, whereas 8 nests sampled

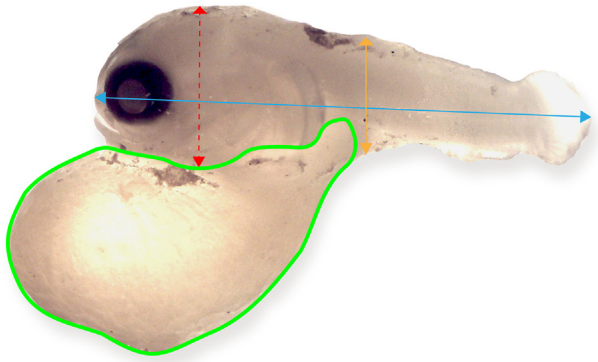

Fig. 3. Morphometric measurements for Lusitanian toadfish larvae. We measured total length (blue horizontal line), head height (red vertical dashed line), body height (yellow vertical solid line), yolk sac area (green contour line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

in 1 year (2 sampling periods, 2017) were considered for larvae. Each group of eggs or larvae collected from each clutch sample/treatment were analysed as one sample.

2.4.1. Tissue preparation

After defrosting, the sampled eggs/larvae were homogenized using an Ystral X10/25 homogenizer (Ystral, Ballrechten-Dottingen, Germany) and separated for different biochemical measurements. Pools of 14.8 (average, range: 10–20) eggs and 17.8 (4–20) larvae from each nest were homogenized using K-phosphate buffer (0.1 M, pH 7.4), with a 1 mL per egg/larvae ratio. To assess lipid peroxidation (LPO), part of the homogenate was transferred to a microtube containing an antioxidant (2.6-dieter-butyl-4-metylphenol 4% in methanol). Second and third portions were separated for the quantification of DNA strand breaks and the evaluation of the electron transport system (ETS), respectively. The rest of the homogenate was centrifuged at 10,000*g* for 20 min (4 °C). The obtained post mitochondrial supernatant (PMS) was then aliquoted into different microtubes for posterior protein quantification, as well as for the activity measurements of superoxide dismutase (SOD). All microtubes were stored at −80 °C until the day of the respective assay. Every spectrophotometric measurement was performed at 25 °C using a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Vermont, USA). Reaction blanks were made for each measurement using homogenization buffer (K-phosphate buffer, 0.1 M, pH 7.4) instead of samples.

2.4.2. Protein quantification

The protein concentration of the PMS, needed for normalization of SOD activity, was quantified as described by Bradford (1976), adapted from BioRad's Bradford microassay set up in 96-well flat bottom plate, using the bovine γ-globulin (BGG, Sigma-Aldrich, USA) as standard. Absorbance was read spectrophotometrically at 600 nm and the results were expressed in mg of protein mL^{-1}.

2.4.3. Oxidative stress parameters

The DNA strand breaks were determined according to the DNA alkaline precipitation assay by Olive (1988) adapted from LaFontaine et al. (2000). The damaged DNA present in the supernatant links to Hoesch dye. The fluorescence was measured with an excitation/emission wavelength of 360/460 nm. Calf thymus DNA was used as standard, and the results were expressed as μg DNA g^{-1} of wet weight (ww). The LPO levels were measured using the method described by Ohkawa et al. (1979) and Bird and Draper (1984), adapted by Filho et al. (2001) and Torres et al. (2002). The LPO products were quantified colorimetrically at 535 nm using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and expressed as nmol of TBARS g^{-1} of wet weight (ww). SOD activity was determined according to McCord and Fridovich (1969), adapted to microplate (Lima et al., 2007). This method is based on the reaction of generated superoxide radicals for xanthine/xanthine oxidase with cytochrome C at 550 nm for 10 min. SOD activity was expressed in U mg^{-1} of protein, being 1 U the amount of enzyme in the sample that causes 50% inhibition of cytochrome C reduction.

2.4.4. Energy metabolism parameters

ETS activity was measured according to De Coen and Janssen (1997), following formazan generation at 490 nm for 3 min. The cellular consumption oxygen rate was determined using a stoichiometric relationship (2 μmol INT-formazan formed to 1 μmol oxygen consumed) and the activity was expressed in nmol O_2 h^{-1} g^{-1} of wet weight (ww).

2.5. Statistical analysis

We assessed the effect of playback treatment on egg survival with paired *t*-tests. We tested the effect of treatment on larvae morphometric traits with Nested ANOVAs using brood ID (i.e. nest ID) as the nested variable. As the morphometric variable TL was correlated with HH (Pearson correlation, $N = 301$, $r = 0.80$, $P < 0.001$) and with BH ($r = 0.82$, $P < 0.001$) but not with YSA ($r = 0.02$, $P = 0.70$), we tested the effect of noise exposure only on TL and YSA.

We investigated playback effects on oxidative stress response and energy metabolism biomarkers in eggs and larvae with paired *t*-tests. Levels of DNA damage, LPO and SOD activity in eggs, as well as the activity of ETS in larvae, were log-transformed to meet the test assumptions. Egg DNA damage levels did not show a normal distribution even after data transformation and so a Wilcoxon non-parametric test was done instead. In addition, we performed a principal component analysis (PCA) to eliminate redundancy caused by intercorrelation among variables and obtained composite scores to further explore treatment effect on stress response on larvae, as several parameters were correlated in larvae but not in eggs (Table S1). PC1 explained 53% and PC2 31% of data variance. DNA damage, SOD and ETS loaded significantly on PC1 with loadings larger than 0.78, and LPO contributed to PC2 (factor loading score of 0.95, Fig. S1). We then performed a paired *t*-test on PC1 and PC2 scores to investigate the effect of treatment on these new composite variables.

Note that, because we used pools of eggs or larvae to assess oxidative stress response and energy metabolism biomarkers, there was one data point per treatment/nest in contrast with morphometric data where there were c. 25 replicates per treatment/nest.

All tests were done with Statistica 14.0.0.15. All test assumptions were met.

3. Results

3.1. Offspring survival

An average of 187.6 (±SD, range: ±136.9, 8–530) and 183.2 (±134.6, 10–499) eggs per clutch sample, from 37 nests, were assigned to boat noise and environmental noise treatments, respectively. After c. 2 weeks of noise exposure, approximately 30% of offspring survived in both treatments: environmental noise 29.5% (±25.3, 0–84.3), boat noise 34.2% (±24.5, 0–90.2). Offspring survival did not differ between treatments (paired *t*-test, $t = 1.07$, $DF = 36$, $P = 0.29$).

3.2. Effect on larvae development

Boat noise exposure showed a detrimental effect on larval growth. After approximately 2 weeks of treatment, larvae exposed to boat noise were about 8% smaller (Nested ANOVA, $F_{1,286} = 40.51$, $P < 0.001$, Fig. 4A) and presented a larger YSA (c. 4% larger; $F_{1,289} = 6.37$, $P = 0.01$, Fig. 5A) than control larvae. Larvae exposed to boat noise playback had an average TL of 7.63 mm (±0.98, 5.0–9.5 mm) and an average YSA of 19.19 mm^2 (±3.30, 10.04–27.08 mm^2) whereas larvae from the environmental noise group had an average TL of 8.22 mm (±0.85, 5.9–9.9 mm) and an average YSA of 18.47 mm^2 (±3.16, 11.62–28.12 mm^2). Brood ID had a significant effect in TL ($F_{10,286} = 16.34$, $P < 0.001$, Fig. 4B) and YSA ($F_{10,289} = 19.07$, $P < 0.001$, Fig. 5B).

3.3. Oxidative stress and energy metabolism biomarkers - eggs

We did not find a significant treatment effect in the oxidative stress biomarkers DNA damage, LPO levels or SOD activity in toadfish eggs exposed to different noises for c. 2 weeks (Tables 1, S2). However, we found significantly lower levels ($P = 0.04$) of ETS activity in eggs exposed to boat noise than in eggs exposed to environmental noise (Fig. 6).

3.4. Oxidative stress and energy metabolism biomarkers - larvae

Playback treatment had a significant effect on SOD activity ($P < 0.001$) and a slight effect on DNA damage ($P = 0.06$), but we

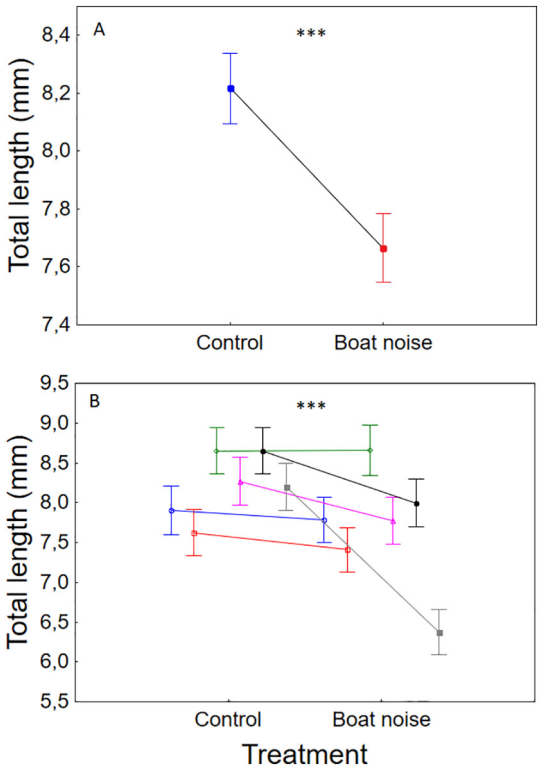

Fig. 4. Effect of playback treatment on larvae total length shown for all broods (A) and per brood (B). Dots are means and error bars are 95% confidence intervals. ***$P < 0.001$.

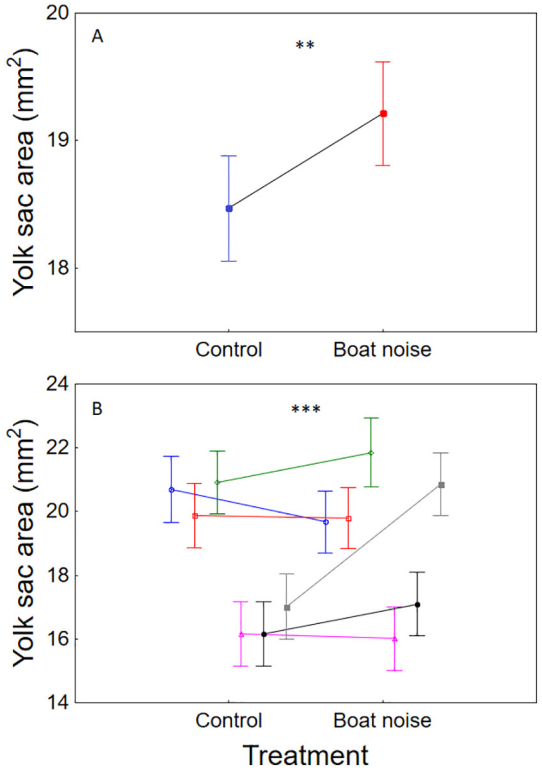

Fig. 5. Effect of playback treatment on larvae yolk sac area shown for all broods (A) and per brood (B). Dots are means and error bars are 95% confidence intervals. ***$P < 0.001$; **$P < 0.01$.

Table 1
Paired *t*-test results comparing levels of oxidative stress - DNA damage (ug DNA/g WW), LPO (TBARS/g WW), SOD (U/mg protein), and energy metabolism - ETS (O2/h/g WW) biomarker activity between eggs and larvae exposed to either environmental or boat noise.

	Eggs			Larvae		
	t	DF	*P*	*t*	DF	*P*
DNA damage	0.67[a]	15	0.50	−2.24	7	0.06
LPO	1.36[b]	14	0.19	0.69	7	0.51
SOD	0.45[b]	14	0.66	−8.35	6	<0.001
ETS	2.25[b]	13	0.04	−2.24[b]	7	0.06

[a] Results from a Wilcoxon test (Z statistics).
[b] Results from paired *t*-tests on log-transformed data.

found no treatment effect on LPO levels ($P = 0.51$) (Tables 1, S2). ETS levels were also slightly affected by playback treatment ($P = 0.06$). DNA damage, SOD and ETS levels were higher or had a tendency to be higher in larvae exposed to noise (Fig. 7). Consistently, noise exposure had a significant effect in PC1 values, i.e., overall stress response related to DNA damage, SOD and ETS levels (paired *t*-test, $t = -3.20$, DF = 6, $P = 0.02$) but not with PC2 which was related with LPO levels ($t = 1.46$, DF = 6, $P = 0.20$) (Figs. 8, S1).

4. Discussion

Our approach of experimental manipulation of toadfish acoustic environment in a natural breeding habitat suggests that anthropogenic noise has detrimental fitness consequences early in life. To our knowledge this is the first study assessing the impact of chronic anthropogenic noise exposure on fish early life stages, using a natural field setting and biochemical stress responses. Exposure of toadfish early life stages to chronic boat noise for a 2-week period had no effect on survival rate, but resulted in changes in larval development, with smaller larvae under chronic noise, consistent with concurrent altered antioxidant responses at the egg and larval stages. Our field experiment suggests that boat noise may impact fish early life stages as the reduction in growth rate can have severe consequences for the larvae. Early life-history stages are subject to very high rates of mortality (Bailey and Houde, 1989), and several studies have provided evidence that fast larval growth will enhance survival, as larvae will be better at foraging, escaping predators and resist starvation (Miller et al., 1988; Bailey and Houde, 1989). Our current study prevents us from inferring further consequences of slower growth rates later in the ontogeny, as there might be growth compensation mechanisms later in life (McCormick and Hoey, 2004; Gagliano and McCormick, 2007). Future studies should aim at following larval growth for a longer period, for example, till larvae detach from the nest and become free swimmers.

Surprisingly, although smaller, larvae under noise stress had greater yolk sac area, which contradicts findings from other studies. Nedelec et al. (2015) reported faster yolk sac consumption in 2-days post hatch codfish (*Gadus morhua*) exposed to regular shipping noise since hatching. This trend was further supported by Fakan and McCormick (2019), who registered higher yolk sac absorption rate of the damselfish (*Acanthochromis polyacanthus*), at hatch, under boat noise. Lara and Vasconcelos (2021) also reported that larval zebrafish (*Danio rerio*), at 3- and 5-days post hatch, and exposed to different acoustic treatments since embryonic stage, consumed their yolk sac faster under noise exposure than under baseline conditions. Overall, these studies predict that increased yolk consumption are related to additional energetical costs under acoustic stress.

Considering that noise-exposed toadfish larvae had larger yolk sac but had also reduced standard length, we hypothesize that chronic exposure to boat noise caused physiological stress (evidenced by the addressed biomarkers – discussed below), ultimately reducing growth rate of these early life stages. There is still limited information available on the effects of anthropogenic noise on fish early life growth, but the current state of the art suggests that responses might be species-

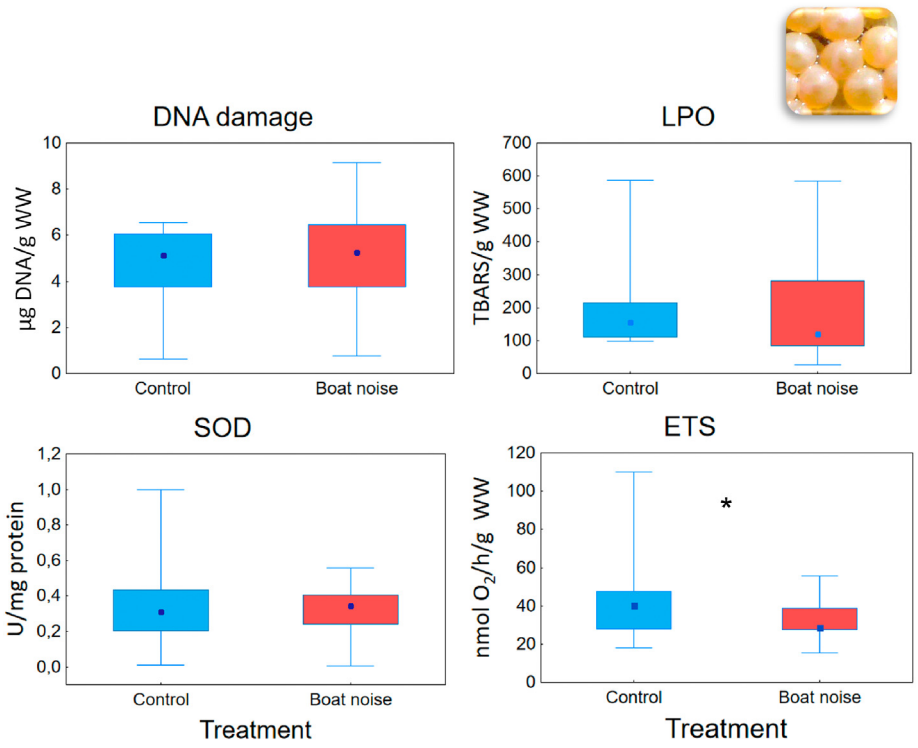

Fig. 6. Effect of playback treatment on levels of oxidative stress (DNA damage, LPO, SOD) and energy metabolism (ETS) biomarker activity in Lusitanian toadfish eggs. The boxplots represent medians (dots), the 25th to 75th percentiles (boxes) and range (whiskers) of raw data. *$P < 0.05$.

specific. Banner and Hyatt (1973) hatched and reared larvae of freshwater sheepshead minnow (*Cyprinodon variegatus*) and killifish (*Fundulus similis*) in noise treatments for 12 days and found smaller larvae in the high-noise treatment. However, longer exposure to acoustic stressful environments showed no effects on growth rates of juvenile rainbow trout (*Oncorhynchus mykiss*), reared since juvenile stage for 5 months under 2 sound treatments (Davidson et al., 2009), neither on the cichlid fish (*Neolamprologus pulcher*) exposed, since the egg stage, to boat noise and reared for 4 weeks (Bruintjes and Radford, 2014). Kusku et al. (2020) exposed juvenile tilapia to underwater shipping noise over 120 days and reported sustained elevated ventilation rates during the first 4 weeks followed by a declining trend suggesting habituation to

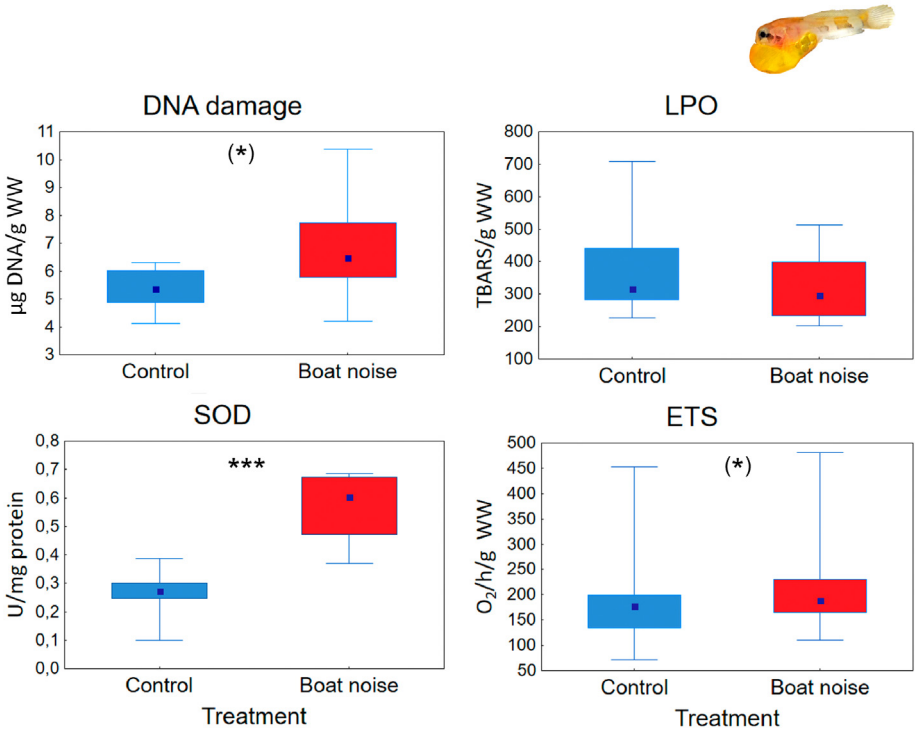

Fig. 7. Effect of playback treatment on levels of oxidative stress (DNA damage, LPO, SOD) and energy metabolism (ETS) biomarker activity in Lusitanian toadfish larvae. The boxplots represent medians (dots), the 25th to 75th percentiles (boxes) and range (whiskers) of raw data. ***$P < 0.001$; (*) $P < 0.1$.

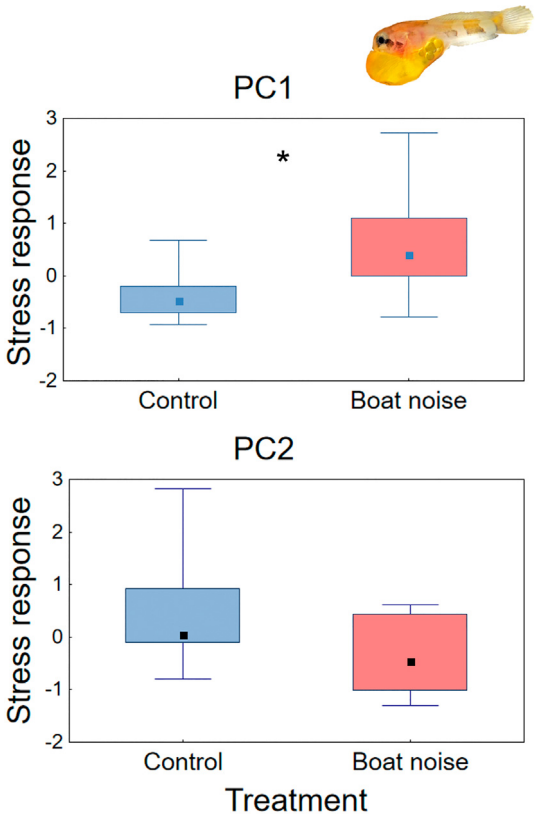

Fig. 8. Effect of playback treatment on overall stress response in Lusitanian toadfish larvae represented by the first two PCA components. The boxplots represent medians (dots), the 25th to 75th percentiles (boxes) and range (whiskers) of raw data. * = $P < 0.05$.

shipping noise. Nedelec et al. (2015) found that increased noise levels led to smaller newly hatched larvae of Atlantic cod (*Gadus morhua*), at 2 days post hatch (dph), however this was followed by growth compensation at later stages. Lara and Vasconcelos (2021) found that the acoustic treatments employed since the egg stage in zebrafish (*Danio rerio*) did not affect general development at 3 and 5 dph.

Differences in species responses to noise exposure might be also related to differences in methodology. Experimental studies differ in duration of exposure (repeated or chronic exposure, e.g. Nedelec et al., 2015; Lara and Vasconcelos, 2021), time of exposure (embryonic stage vs. larval stage, e.g. Fakan and McCormick, 2019; Nedelec et al., 2015), amplitude levels (e.g. Lara and Vasconcelos, 2021), sound type (pile driving, drilling, boat, e.g. Bolle et al., 2012; Bruintjes and Radford, 2014; Kusku et al., 2020), boat type (Jain-Schlaepfer et al., 2018; McCormick et al., 2019), among other experimental aspects, which may ultimately lead to different responses to noise exposure. Furthermore, most studies conducted to date use playback of anthropogenic noise in tanks, which is not representative of noise in natural settings (Popper and Hawkins, 2019).

Studies using other environmental stressors, such as acidification (Pimentel et al., 2016), temperature (Villalobos et al., 2020) and hypoxia (Del Rio et al., 2019), likewise showed reduced larval growth rates, likely associated to increased metabolic costs under stressful conditions, although, as reported for noise stressor, results suggest interspecific differences in vulnerability.

In the present study we found further evidence of noise-induced physiological stress on both embryos and larvae, which showed indications of oxidative stress and unbalanced antioxidant status. Eggs under boat noise exposure showed reduced ETS activity, which relates to cellular respiration and energy consumption. This reduced activity might suggest that embryos were metabolically less active, which in turn could help to explain the observed smaller sizes of larvae at hatch. Exposure to environmental stressors, such as contaminants and high pCO_2 levels, can lead to the formation of free radicals (Lesser, 2011; Lushchak, 2016; Silva et al., 2016), which can contribute to increased damage at the cellular level. As part of the defence mechanisms of the organisms, the antioxidant enzymes, such as superoxide dismutase (SOD), play a major role protecting or delaying the oxidative DNA damage (Abele and Puntarulo, 2004). Here we observed higher SOD activity of larvae under boat noise exposure, suggesting a strong antioxidant response; however, there was also a tendency for higher DNA damage ($P = 0.06$), which suggests that these mechanisms may be failing to prevent further cellular impairment. Furthermore, we also observed a tendency ($P = 0.06$) for higher levels of energy consumption in larvae, given by the ETS marginally higher activity, under boat noise exposure. Being a proxy for the cellular oxygen consumption, ETS is highly indicative of alterations in the cellular metabolism and energy requirements (De Coen and Janssen, 1997). The increased ETS levels with boat noise suggest higher energy requirements for defence mechanisms, namely for coping with oxidative stress by enhancing SOD, which may result in less energy available for other important functions, such as growth, and may contribute to explain the smaller sizes of toadfish larvae under boat noise exposure. These overall results are supported by the observed significant effect of noise exposure in PC1 values, i.e., overall stress response related to DNA damage, SOD and ETS levels. Notably, we observed different ETS profiles in Lusitanian toadfish eggs and larvae. ETS levels may be affected by external stressors but may also highly depend on the development stage of the organisms thus triggering different defence mechanisms with distinct energetic trade-offs along developmental stages. It is likely that in egg stage, boat noise was not yet causing high levels of oxidative stress (seen by the lack of differences in the antioxidant capacity (SOD) or oxidative damage in DNA and lipids) and the effect may be more directly related with mechanisms of developmental arrest and thus lower metabolism. In the larval stage, increased oxidative stress starts to be visible by the increased SOD and DNA damage levels. Therefore, in larvae a higher demand of energy is possibly necessary (higher cellular respiration given by the ETS) and being allocated to deal with this oxidative stress, at the cost of allocating it to growth as seen by the smaller sizes of toadfish larvae. Evidence of increased metabolic costs under stress can be further addressed by assessing heart rates. As a response to a stressful situation, the oxygen demands increase, thus increasing cardiac rates (Atherton and McCormick, 2020; Cooke et al., 2003). Simpson et al. (2005) reported, for the first time, an increase of cardiac activity in embryos of clownfishes *Amphiprion* spp. exposed to sound stimuli ranging from 100 to 1200 Hz. This study provided evidence that the sensory organs responsible for hearing become functional during the embryonic stage, as early as 3 dph (Simpson et al., 2005), thus bringing attention to the fact that fish might be vulnerable to anthropogenic noise since very early in development. Jain-Schlaepfer et al. (2018) and Fakan and McCormick (2019) further reported faster heart rates of embryos of damselfish species (*Amblyglyphidodon curacao*, *Amphiprion melanopus* and *Acanthochromis polyacanthus*) reared under playback of boat noise. Evidence of increased heart rates in fish larvae under increasing noise levels have also been reported by Lara and Vasconcelos (2021). Although easily determined and a reliable indicator of stress, in a natural field setting as the one we used in our current study, measuring embryo or larvae heart rates would be logistically challenging.

Care must be taken when interpreting our findings because we placed toadfish embryos in a rack wrapped with plastic mesh, away from the male responsible for providing parental care (egg aeration, removal of dead eggs, antibacterial protection with mucus, protection from predators). While we ensured that embryos and larvae had flowing water and were protected from predators, we are unable to predict whether the presence of the male in the nest may have changed the results. Moreover, instead of real boats noise we used underwater loudspeakers that cannot fully recreate boat noise both in sound pressure and particle motion, particularly at frequencies lower than 100 Hz

(Fig. 2). As in this study eggs and larvae were c. 1 m away from the source rather than likely further away with a real boat passage, they were exposed to different acoustic fields in terms of sound pressure and particle motion (see Nedelec et al., 2016 for the relevance of differences in particle motion for fishes). Because Lusitanian toadfish from different life stages are more sensitive to these low frequencies and mainly sensitive to particle motion (<100 Hz, Vasconcelos et al., 2015) we anticipate that real boat passages will have a greater impact than the observed in this study. In addition, we only measured larvae from 6 nests to assess larval development and our results concerning this endpoint could be conditioned by the small sample size. Nevertheless, nest-holder males typically mate with several females and male cuckoldry is also known in this population (Amorim et al., 2016). Thus, the studied larvae were offspring from likely much more than 6 males and 6 females. With this study, we have provided first evidence of detrimental effects of chronic boat noise exposure on fish development in the field, and further of increased physiological stress assessed by oxidative stress and energy metabolism biomarkers. If these critical early stages are not able to compensate and/or acclimate to the noise stress later in the ontogeny, then anthropogenic noise has the potential to severely affect this and likely other marine fishes, with further consequences for populations resilience and dynamics. Notably, we lack insight into whether these or other documented noise-induced effects translate into population-level consequences in fishes (Slabbekoorn et al., 2019). Further studies directly addressing consequences of noise at population and community levels are urgent if we want to provide recommendations for policy makers and contribute to diminish the impacts of this global stressor on aquatic environments.

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CRediT authorship contribution statement

A. Faria: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **P.J. Fonseca:** Project administration, Conceptualization, Methodology, Writing – review & editing. **M. Vieira:** Formal analysis, Writing – review & editing. **L.M.F. Alves:** Investigation. **M.F.L. Lemos:** Methodology. **S.C. Novais:** Conceptualization, Methodology, Writing – review & editing. **A.B. Matos:** Investigation. **D. Vieira:** Investigation. **M.C.P. Amorim:** Funding acquisition, Project administration, Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abele, D., Puntarulo, S., 2004. Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. Comp. Biochem. Physiol. - Part A: Mol. Integr. Physiol. 138, 405–415. https://doi.org/10.1016/j.cbpb.2004.05.013.

Alves, D., Vieira, M., Amorim, M.C.P., Fonseca, P.J., 2021. Boat noise interferes with lusitanian toadfish acoustic communication. J. Exp. Biol. 224 (11), jeb234849. https://doi.org/10.1242/jeb.234849.

Amorim, M.C.P., Simões, J.M., Fonseca, P.J., 2008. Acoustic communication in the Lusitanian toadfish, Halobatrachus didactylus: evidence for an unusual large vocal repertoire. J. Mar. Biol. Ass. U.K. 88 (5), 1069–1073. https://doi.org/10.1017/S0025315408001677.

Amorim, M.C.P., Simões, J.M., Fonseca, P.J., Almada, V.C., 2010. Patterns of shelter usage and social aggregation by the vocal lusitanian toadfish. Mar. Biol. 157, 495–503. https://doi.org/10.1007/s00227-009-1335-6.

Amorim, M.C.P., Conti, C., Sousa-Santos, C., Novais, B., Gouveia, M.D., Vicente, J.R., Modesto, T., Gonçalves, A., Fonseca, P.J., 2016. Reproductive success in the Lusitanian toadfish: influence of calling activity, male quality and experimental design. Physiol. Behav. 155, 17–24. https://doi.org/10.1016/j.physbeh.2015.11.033.

Atherton, J., McCormick, M.I., 2020. Parents know best: transgenerational predator recognition through parental effects. PeerJ. 8, e9340. https://doi.org/10.7717/peerj.9340.

Bailey, K.M., Houde, E.D., 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. 25, 1–83. https://doi.org/10.1016/S0065-2881(08)60187-X.

Banner, A., Hyatt, M., 1973. Effects of noise on eggs and larvae of two estuarine fishes. Trans. Am. Fish. Soc. 102, 134–136. https://doi.org/10.1577/1548-8659(1973)102<134:EONOEA>2.0.CO;2.

Bird, R.P., Draper, H.H., 1984. Comparative studies on different methods of malonaldehyde determination. Methods Enzymol. 105, 299–305. https://doi.org/10.1016/s0076-6879(84)05038-2.

Blom, E.L., Kvarnemo, C., Dekhla, I., Schöld, S., Andersson, M.H., Svensson, O., Amorim, M.C.P., 2019. Continuous but not intermittent noise has a negative impact on mating success in a marine fish with paternal care. Sci. Rep. 9, 5494. https://doi.org/10.1038/s41598-019-41786-x.

Bolle, L.J., de Jong, C.A.F., Bierman, S.M., van Beek, P.J.G., van Keeken, O.A., Wessels, P.W., van Damme, C.J.G., Winter, H.V., de Haan, D., Dekeling, R.P.A., 2012. Common sole larvae survive high levels of pile-driving sound in controlled exposure experiments. PLoS ONE. 7 (3), e33052. https://doi.org/10.1371/journal.pone.0033052.

Bradford, M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Breitzler, L., Lau, I.H., Fonseca, P.J., Vasconcelos, R.O., 2020. Noise-induced hearing loss in zebrafish: investigating structural and functional inner ear damage and recovery. Hearing Res. 391, 107952. https://doi.org/10.1016/j.heares.2020.107952.

Bruintjes, R., Radford, A.N., 2014. Chronic noise does not impact hatching success or post-hatching larval growth and survival in a cichlid fish. PeerJ. 2, e594. https://doi.org/10.7717/peerj.594.

Buscaino, G., Filiciotto, F., Buffa, G., Bellante, A., Di Stefano, V., Assenza, A., Fazio, F., Caola, G., Mazzola, S., 2010. Impact of an acoustic stimulus on the motility and blood parameters of European sea bass (Dicentrarchus labrax L.) and gilthead sea bream (Sparus aurata L.). Mar. Environ. Res. 69, 136–142. https://doi.org/10.1016/j.marenvres.2009.09.004.

Chambers, R.C., Candelmo, A.C., Habeck, E.A., Poach, M.E., Wieczorek, D., Cooper, K.R., Greenfiel, C.E., Phelan, B.A., 2014. Effects of elevatedCO2 in the early life stages of summer flounder, Paralichthys dentatus, and potential consequences of ocean acidification. Biogeosciences 11, 1613–1626. https://doi.org/10.5194/BG-11-1613-2014.

Collette, B.B., 2005. Chapter 51. Batrachoidiformes, Batrachoididae: toadfishes. In: Richards, W.J. (Ed.), Early Stages of Atlantic Fishes: An Identification Guide for the Western Central North Atlantic. CRC Press, Boca Raton, Florida, USA, pp. 759–767 2640 pp.

Cooke, S.J., Ostrand, K.G., Bunt, C.M., Schreer, J.F., Wahl, D.H., Philipp, D.P., 2003. Cardiovascular responses of largemouth bass to exhaustive exercise and brief air exposure over a range of water temperatures. Trans. Am. Fish. Soc. 132, 1154–1165. https://doi.org/10.1577/T02-059.

Davidson, J., Babak, J., Mazik, P., 2009. The effects of aquaculture production noise on the growth, condition factor, feed conversion, and survival of rainbow trout (Onocorhynchus mykiss). Aquaculture 288, 337–343. https://doi.org/10.1016/j.aquaculture.2008.11.037.

De Coen, W., Janssen, C.R., 1997. The use of biomarkers in Daphnia magna toxicity testing. IV.Cellular energy allocation: a new methodology to assess the energy budget of toxicant-stressed daphnia populations. J. Aquat. Ecosyst. Stress Recov. 6, 43–55. https://doi.org/10.1023/A:1008228517955.

de Jong, K., Amorim, M.C.P., Fonseca, P.J., Fox, C.J., Heubel, K.U., 2018. Noise can affect acoustic communication and subsequent spawning success in fish. Environ. Pollut. 237, 814–823. https://doi.org/10.1016/j.envpol.2017.11.003.

de Jong, K., Forland, T.N., Amorim, M.C.P., Rieucau, G., Slabbekoorn, H., Sivle, L.D., 2020. Predicting the effects of anthropogenic noise on fish reproduction. Rev. Fish. Biol. Fisheries. 30, 245–268. https://doi.org/10.1007/s11160-020-09598-9.

Dekeling, R., et al., 2016. The European marine strategy: noise monitoring in european marine waters from 2014. In: Popper, A., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life II. Advances in Experimental Medicine and Biology. 875. Springer, New York, NY.. https://doi.org/10.1007/978-1-4939-2981-8_24.

Del Rio, A.M., Davis, B.E., Fangue, N.A., Todgham, A.E., 2019. Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. 7 (1), coy078. https://doi.org/10.1093/conphys/coy078.

Duarte, C.M., Chapuis, L., Collin, S.P., et al., 2021. The soundscape of the Anthropocene ocean. Science 371 (6529), eaba4658. https://doi.org/10.1126/science.aba4658.

Fakan, E.P., McCormick, M.I., 2019. Boat noise affects the early life history of two damselfishes. Mar. Pollut. Bull. 141, 493–500. https://doi.org/10.1016/j.marpolbul.2019.02.054.

Filho, D.W., Tribess, T., Gáspari, C., Claudio, F.D., Torres, M.A., Magalhães, A.R.M., 2001. Seasonal changes in antioxidant defenses of the digestive gland of the brown mussel (Perna perna). Aquaculture 203, 149–158. https://doi.org/10.1016/S0044-8486(01)00599-3.

Gagliano, M., McCormick, M.I., 2007. Compensating in the wild: is flexible growth the key to early juvenile survival? Oikos 116, 111–120. https://doi.org/10.1111/j.2006.0030-1299.15418.x.

Houde, E.D., 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. 2, 17–29.

Jain-Schlaepfer, S., Fakan, E., Rummer, J.L., Simpson, S.D., McCormick, M.I., 2018. Impact of motorboats on fish embryos depends on engine type. Conserv. Physiol. 6 (1), coy014. https://doi.org/10.1093/conphys/coy014.

Kaplan, M.B., Susan Solomon, S., 2016. A coming boom in commercial shipping? The potential for rapid growth of noise from commercial ships by 2030. Mar. Policy 73, 119–121. https://doi.org/10.1016/j.marpol.2016.07.024.

Kunc, H.P., McLaughlin, K.E., Schmidt, R., 2016. Aquatic noise pollution: implications for individuals, populations, and ecosystems. Proc. R. Soc. B 283, 20160839. https://doi.org/10.1098/rspb.2016.0839.

Kusku, H., 2020. Acoustic sound-induced stress response of Nile tilapia (Oreochromis niloticus) to long-term underwater sound transmissions of urban and shipping noises. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-09699-9.

Kuşku, H., Yiğit, M., Ergün, S., Yiğit, Ü., Taylor, N., 2018. Acoustic noise pollution from marine industrial activities: exposure and impacts. Aquat. Res 1 (4), 148–161. https://doi.org/10.3153/AR18017.

Kusku, H., Yigit, Ü., Yilmaz, S., Yigit, M., Ergün, S., 2020. Acoustic effects of underwater drilling and piling noise on growth and physiological response of Nile tilapia (Oreochromis niloticus). Aquac. Res. 2020 (00), 1–9. https://doi.org/10.1111/are.14652.

LaFontaine, Y., Gagné, F., Blaise, C., Costan, G., Gagnon, P., Chan, L.H.M., 2000. Biomarkers in zebra mussels (Dreissena polymorpha) for the assessment and monitoring of water quality of St. Lawrence River (Canada). Aquat. Toxicol. 50 (1–2), 51–71. https://doi.org/10.1016/S0166-445X(99)00094-6.

Lara, R.A., Vasconcelos, R.O., 2021. Impact of noise on development, physiological stress and behavioural patterns in larval zebrafish. Sci. Rep. 11, 6615. https://doi.org/10.1038/s41598-021-85296-1.

Lesser, M.P., 2011. Oxidative stress in tropical marine ecosystems. In: Abele, D., Zenteno-Savín, T., Vazquez-Medina, J. (Eds.), Oxidative Stress in Aquatic Ecosystems. John Wiley & Sons, Ltd., Chichester, UK, pp. 7–19.

Lima, I., Moreira, S.M., Osten, J.R., Soares, A.M.V.M., Guilhermino, L., 2007. Biochemical responses of the marine mussel Mytilus galloprovincialis to petrochemical environmental contamination along the northwestern coast of Portugal. Chemosphere 66, 1230–1242. https://doi.org/10.1016/j.chemosphere.2006.07.057.

Lushchak, V.I., 2016. Contaminant-induced oxidative stress in fish: a mechanistic approach. Fish Physiol. Biochem. 42 (2), 711–747. https://doi.org/10.1007/s10695-015-0171-5.

Magnhagen, C., Johansson, K., Sigray, P., 2017. Effects of motorboat noise on foraging behaviour in eurasian perch and roach: a field experiment. Mar. Ecol. Prog. Ser. 564, 115–125. https://doi.org/10.3354/meps11997.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 44 (22), 6049–6055.

McCormick, M.I., Hoey, A.S., 2004. Larval growth history determines juvenile growth and survival in a tropical marine fish. Oikos 106 (2), 225–242. https://doi.org/10.1111/j.0030-1299.2004.13131.x.

McCormick, M.I., Fakan, E.P., Nedelec, S.L., Allan, B.J.M., 2019. Effects of boat noise on fish fast-start escape response depend on engine type. Sci. Rep. 9, 6554. https://doi.org/10.1038/s41598-019-43099-5.

Miller, T.J., Crowder, L.B., Rice, J.A., Marschall, E.A., 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. 45, 1657–1670. https://doi.org/10.1139/f88-197.

Nedelec, S.L., Simpson, S.D., Morley, E.L., Nedelec, B., Radford, A.N., 2015. Impacts of regular and random noise on the behaviour, growth and development of larval Atlantic cod (Gadus morhua). Proc. R. Soc. B 282, 20151943. https://doi.org/10.1098/rspb.2015.1943.

Nedelec, S.L., Campbell, J., Radford, A.N., Simpson, S.D., Merchant, N.D., 2016. Particle motion: the missing link in underwater acoustic ecology. Methods Ecol. Evol. 7, 836–842. https://doi.org/10.1111/2041-210X.12544.

Nedelec, S.L., Radford, A.N., Pearl, L., Nedelec, B., McCormick, M.I., Meekan, M.G., Simpson, S.D., 2017. Motorboat noise impacts parental behaviour and offspring survival in a reef fish. Proc. R. Soc. B Biol. Sci. 284, 20170143. https://doi.org/10.1098/rspb.2017.0143.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Olive, P.L., 1988. DNA precipitation assay: a rapid and simple method for detecting DNA damage in mammalian cells. Environ. Mol. Mutagen. 11 (4), 487–495. https://doi.org/10.1002/em.2850110409.

Pimentel, M., Faleiro, F., Marques, T., Bispo, R., Dionísio, G., Faria, A.M., Machado, J., Peck, M., Pörtner, H., Pousão-Ferreira, P., Gonçalves, E.J., Rosa, R., 2016. Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming. Clim. Chang. 137, 495–509. https://doi.org/10.1007/s10584-016-1682-5.

Popper, A.N., Hawkins, A.D., 2019. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. Fish Biol. 94, 692–713. https://doi.org/10.1111/jfb.13948.

Popper, A.N., Hawkins, A.D., Halvorsen, M.B., 2019. Anthropogenic sound and fishes. 891, 1.

Purser, J., Radford, A.N., 2011. Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (Gasterosteus aculeatus). PLoS ONE. 6, e17478. https://doi.org/10.1371/journal.pone.0017478.

Scholik, A.R., Yan, H.Y., 2001. Effects of underwater noise on auditory sensitivity of a cyprinid fish. Hear Res. 152, 17–24. https://doi.org/10.1016/S03785955(00)00213-6.

Silva, C.S.E., Novais, S.C., Lemos, M.F.L., Mendes, S., Oliveira, A.P., Gonçalves, E.J., Faria, A.M., 2016. Effects of ocean acidification on the swimming ability, development and biochemical responses of sand smelt larvae. Sci. Total Environ. 563–564, 89–98. https://doi.org/10.1016/j.scitotenv.2016.04.091.

Simpson, S.D., Yan, H.Y., Wittenrich, M.L., Meekan, M.G., 2005. Response of embryonic coral reef fishes (Pomacentridae: amphiprion spp.) to noise. Mar. Ecol. Prog. Ser. 287, 201–208. https://doi.org/10.3354/meps287201.

Slabbekoorn, H., 2019. Noise pollution. Curr. Biol. 29, R942–R995. https://doi.org/10.1016/j.cub.2019.07.018.

Slabbekoorn, H., Bouton, N., Van Opzeeland, I., Coers, A., Ten Cate, C., Popper, A.N., 2010. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends Ecol. Evol. 25, 419–427. https://doi.org/10.1016/j.tree.2010.04.005.

Slabbekoorn, H., Dalen, J., de Haan, D., 2019. Population-level consequences of seismic surveys on fishes: an interdisciplinary challenge. Fish Fish. 20, 653–685. https://doi.org/10.1111/faf.12367.

Torres, M.A., Testa, C.P., Gáspari, C., Masutti, M.B., Panitz, C.M.N., Curi-Pedrosa, R., De Almeida, E.A., Di Mascio, P., Wilhelm Filho, D., 2002. Oxidative stress in the mussel mytella guyanensis from polluted mangroves on Santa Catarina IslandBrazil. 44, 923–932. https://doi.org/10.1016/S0025-326X(02)00142-X.

Van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6.

Vasconcelos, R.O., Amorim, M.C.P., Ladich, F., 2007. Effects of ship noise on the detectability of communication signals in the in the lusitanian toadfish. J. Exp. Biol. 210, 2104–2112. https://doi.org/10.1242/jeb.004317.

Vasconcelos, R.O., Carriço, R., Ramos, A., Modesto, T., Fonseca, P.J., Amorim, M.C.P., 2012. Vocal behaviour predicts reproductive success in a teleost fish. Behav. Ecol. 23, 375–383. https://doi.org/10.1093/beheco/arr199.

Vasconcelos, R.O., Alderks, P.W., Ramos, A., Fonseca, P.J., Amorim, M.C.P., Sisneros, J.A., 2015. Vocal differentiation parallels development of auditory saccular sensitivity in a highly soniferous fish. J. Exp. Biol. 218 (18), 2864–2872. https://doi.org/10.1242/jeb.123059.

Vieira, M., Amorim, M.C.P., Fonseca, P.J., 2021a. Vocal rhythms in nesting lusitanian toadfishHalobatrachus didactylus. 63, 101281. https://doi.org/10.1016/j.ecoinf.2021.101281.

Vieira, M., Fonseca, P.J., Amorim, M.C.P., 2021b. Fish sounds and boat noise are prominent soundscape contributors in an urban european estuary. Mar. Pollut. Bull. 172, 112845. https://doi.org/10.1016/j.marpolbul.2021.112845.

Villalobos, C., Love, B.A., Olson, M.B., 2020. Ocean acidification and ocean warming effects on Pacific herring (Clupea pallasi) early life stages. Front. Mar. Sci. 7, 597899. https://doi.org/10.3389/fmars.2020.597899.

Voellmy, I.K., Purser, J., Simpson, S.D., Radford, A.N., 2014. Increased noise levels have different impacts on the anti-predator behaviour of two sympatric fish species. PLoS ONE 9, e102946. https://doi.org/10.1371/journal.pone.0102946.

Wysocki, L.E., Dittami, J.P., Ladich, F., 2006. Ship noise and cortisol secretion in european freshwater fishes. Biol. Conserv. 128, 501–508. https://doi.org/10.1016/j.biocon.2005.10.020.