Marine Environmental Research 185 (2023) 105894

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Impact of anthropogenic noise on the survival and development of meagre (*Argyrosomus regius*) early life stages

Rita Trabulo [a,b,*], M. Clara P. Amorim [a,b], Paulo J. Fonseca [a,c], Manuel Vieira [a,b,c], André B. Matos [a,b], Thibaut Marin-Cudraz [a], Marco F.L. Lemos [d], Ariana B. Moutinho [d], Sara C. Novais [d], Pedro Pousão-Ferreira [e], Ana Candeias-Mendes [e], Ana M. Faria [b]

[a] *Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal*
[b] *MARE – Marine and Environmental Sciences Centre, ARNET - Aquatic Research Network, ISPA – Instituto Universitário, Lisbon, Portugal*
[c] *cE3c - Center for Ecology, Evolution and Environmental Changes & CHANGE - Global Change and Sustainability Institute, Lisbon, Portugal*
[d] *MARE–Marine and Environmental Sciences Centre, ARNET - Aquatic Research Network, ESTM, Politécnico de Leiria, Peniche, Portugal*
[e] *Instituto Português do Mar e da Atmosfera, Av. 5 de Outubro, 8700-305, Olhão, Portugal*

A B S T R A C T

The growth of human populations has been driving an unprecedent and widespread increase in marine traffic, posing a real threat to marine biodiversity. Even though we are now aware of the negative effects of shipping noise exposure on fish, information about the impact on their early life stages continues to lack. Meagre (*Argyrosomus regius*) is a vocal fish that uses estuaries with high levels of anthropogenic noise pollution as both breeding areas and nurseries. Here, the effects of boat noise exposure on the development and survival of meagre larvae were studied.

Embryos and larvae were exposed to either noise (boat noise playback) or control treatments (coils producing a similar electric field to the speakers) and hatching rate, survival rate, morphometric traits and stress-related biomarkers, at hatching and at 2 days-post-hatching (dph) were analyzed. Results showed no conclusive effects of the impact of boat noise playback, even though there was an increased lipid droplet consumption and a decrease in body depth at 2dph larvae under this stressor. The assessment of oxidative stress and energy metabolism-related biomarkers at hatching showed a marginal decrease in superoxide dismutase (SOD) activity and no changes in DNA damage or electron transport system activity (ETS), although it cannot be disregarded that those effects could only be visible at later stages of larval development. Whether these morphological and developmental results have implications in later stages remains to be investigated. Further studies with longer exposure and wild meagre could help deepen this knowledge and provide a better understanding of how anthropogenic noise can impact meagre early stages.

1. Introduction

Since the industrial revolution, the impact of humans on earth has been growing exponentially, generating different and diverse impacts on terrestrial and aquatic environments (Grimm et al., 2008). Human-sound-producing-activities have been altering the world's soundscape, which can be defined as "ambient sound in terms of its spatial, temporal, and frequency attributes, and the types of sources contributing to the sound field" (ISO, 2017), consequently impacting wildlife (for a review see Shannon et al., 2016). In the marine environment, anthropogenic noise is generated mostly by 1) seismic surveys, sonar and pile driving, which can have an acute and local impact, and 2) recreational and commercial boating and renewable energy structures, which produce lower noise levels for longer periods of time (Hildebrand, 2009; Williams et al., 2015; ISO, 2017). Marine traffic produces low (<1 kHz) and mid-frequency (1 kHz–5 kHz) pervasive noise that causes a widespread impact on marine life and can overlap with communication and hearing ranges of aquatic animals, including fish (Williams et al., 2015; de Jong et al., 2020; Duarte et al., 2021). In addition, the growth of human populations has been driving an unprecedent and widespread increase in marine traffic, posing a real threat to marine biodiversity (Frisk, 2012, 2012de Jong et al., 2020; Sordello et al., 2020). It is estimated that, since pre-industrial times, low-frequency boat and shipping traffic have been responsible for an increase of approximately 20 dB in low-frequency ambient noise (Hildebrand, 2009; Frisk, 2012).

The impact of anthropogenic noise has been mostly studied on marine mammals (Popper and Hawkins, 2016), but nowadays the relevance of its effects on fish is well established (Popper and Hastings 2009; Radford, Kerridge & Simpson, 2014; Popper and Hawkins, 2019; de Jong et al., 2020). The use of sound by fish varies along different taxa

* Corresponding author. Campo Grande 016, 1749-016, Lisbon, Portugal.
E-mail address: trabulo.rita@gmail.com (R. Trabulo).

https://doi.org/10.1016/j.marenvres.2023.105894
Received 21 November 2022; Received in revised form 17 January 2023; Accepted 19 January 2023
Available online 21 January 2023
0141-1136/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

and can occur for prey or predators' detection (Remage-Healey et al., 2006), orientation, navigation, habitat selection (Tolimieri et al., 2000; Montgomery et al., 2006), or for communication between individuals (Amorim, 2006; Ladich, 2015). Even when not produced by the animal itself, sound has proven to be important to acquire information about the environment, especially when integrated with other environmental cues, such as chemical, visual or electromagnetic (Popper, 2003; Hawkins and Popper, 2018). However, impacts on population dynamics are hard to predict since fish may swim away from noise sources, show habituation, or even exhibit compensation mechanisms to avoid its long-term negative effects (Bolle et al., 2012; Harding et al., 2019).

Anthropogenic noise can impact different stages of the fish life cycle, since it overlaps with the hearing range of several species (Kunc et al., 2016) compromising different aspects of its life and interfering with conspecific and heterospecific interactions (Popper et al., 2014; Hawkins and Popper, 2018; Nedelec et al., 2015).

It is currently known that adults from different fish taxa may suffer masking of acoustic cues and signals, shifts in hearing thresholds, increase stress and other physical and physiological impacts which can influence behaviour and impact reproduction (Caiger et al., 2012; Holles et al., 2013; Peng et al., 2015; de Jong et al., 2020). Less known are the effects of anthropogenic noise on the highly vulnerable early life stages (Faria et al., 2022). A few studies have shown that anthropogenic noise can detrimentally impact fish larvae response to ambient sound, compromising settlement and influencing population dynamics (Montgomery et al., 2006; Holles et al., 2013). Since larvae cannot escape from noise sources and need to recognize certain cues for adequate settlement, it is important to understand the mechanisms behind this disruption and how this stressor may affect their overall fitness (Bolle et al., 2012; Debusschere et al., 2014).

Impulsive sounds appear to have the most impact on early life stages in terms of survival, but continuous sounds are also likely to influence behaviour and development, and therefore impact fitness (Payne et al., 2009; Andersson et al., 2017). Changes in behaviour (Lara and Vasconcelos, 2021) and shoal organization, movement direction and speed (Herbert-Read et al., 2017), slow or inappropriate responses to predators (Simpson et al., 2016), changes in development and growth patterns (Nedelec et al., 2015) or altered stress responses (Smith et al., 2004; Sierra-Flores et al., 2015; Faria et al., 2022), are some examples of the impact of anthropogenic noise in these early life stages. Also, Gendron et al. (2020) found that feeding is reduced in the presence of anthropogenic boat noise, showing, for the first time, the importance of this effect in larvae that are born in estuaries.

The meagre (*Argyrosomus regius*) is a highly vocal sciaenid (Lagardère and Mariani, 2006) that inhabits the subtropical waters of the European and African coast of the Atlantic, the Black Sea and the Mediterranean (González-Quirós et al., 2011), and can reach up to 2 m in length (González-Quirós et al., 2011). Meagre has good hearing abilities (Vieira et al., 2021), and is known to produce sounds as early as the juvenile stage (>30 cm in total length; Pereira et al., 2020). To date, it is known that adult meagre is affected by boat noise passages, decreasing the intensity of the reproductive chorus, which could either mean a decrease in the number of fish that are producing the sounds, or avoidance behaviour, effects that might ultimately impact spawning (Vieira et al., 2021). Furthermore, significant auditory masking by boat noise was observed in juvenile meagre (Vieira et al., 2021). Impacts of noise on meagre early life stages, namely in egg/larval stages, have thus far not been addressed.

In the current study we aim to examine how exposure to boat noise playbacks during early life stages can affect survival and development of meagre eggs and larvae. For this purpose, meagre's embryonic stages (from egg to 2 days post-hatching larvae, i.e. until depletion of the yolk sac) were exposed to either boat noise, mimicking typical traffic noise in the Tagus estuary, or a control; for the control we used 'silent' coils producing a similar electric field to the speakers, thus controlling for electromagnetic fields present during noise playback. We measured hatching success, larval development, and oxidative stress and energy metabolism-related biomarkers (both proxies for physiological stress) to assess treatment effect. This information will allow a better understanding on how anthropogenic noise, namely boat noise, can impact the different life stages of this species, which could be relevant to devise conservation measures.

2. Materials & methods

2.1. Ethical note

Meagre eggs were obtained from the aquaculture facilities of Instituto Português do Mar e da Atmosfera (IPMA) – Estação Piloto de Piscicultura de Olhão (IPMA – EPPO), Portugal (37°02′ N, 7°49′ W). IPMA is authorized by the Portuguese National Authority for Animal Health (Direção Geral de Alimentação e Veterinária, DGAV)— in accordance to EU legislation for EPPO to breed, use and supply aquatic animals for scientific experimental work (DGAV reference 0421/000/000/2018). Experiments took place at Faculty of Sciences, University of Lisbon (FCUL), authorized by DGAV (DGAV reference 0421/000/000/2021) and performed in strict accordance with the EU Directive 2010/63/EU for animal experiments. The study also followed the recommendations of the Animal Care and Use Committee of FCUL.

2.2. Experimental setup and procedure

Using laboratory-controlled experiments, the effects of exposing meagre eggs/larvae to boat noise playbacks were tested. Experiments took place in 6 tanks (35 L; 49 × 29 × 25 cm). During the experiments, the eggs and larvae were exposed to either boat noise (treatment, 3 replicate tanks) or silence, i.e. no added noise (control, 3 replicate tanks). Control aquaria were equipped with a copper coil to control for possible effects of the electromagnetic field generated by the speaker (see below) (Fig. 1). Temperatures were kept stable at 18 °C using chiller systems (Hailea HC100A) and circulation pumps kept outside the tanks. All rearing systems were filled with artificial seawater adjusted to a salinity of 37 ± 1.0‰, in a closed-circulation system with an external filter (Eheim 150).

Each tank held three small rearing boxes (18 rearing boxes in total), made of PVC tubes with a fine net at the bottom, to avoid the loss of eggs to the aquaria (Fig. 1). A system delivering air bubbles oxygenated and kept the water moving inside every rearing box to avoid deposition of the eggs at the bottom of the container. Artificial light was provided by overhead fluorescent lights for 24h. Since the experimental protocols occurred mostly during night-time, lights were needed to execute the experiments. This condition was maintained throughout the experiments.

2.2.1. Sound stimuli

Boat noise playbacks were carried out using three synchronized JBL speakers (mp3 device connected to FLIP Essential - 80 Hz-20 KHz, HARMAN) (Fig. 1). Each speaker was placed inside a waterproof container, which in turn was vertically placed inside each treatment tank (3 speakers in total, see Fig. 1), separated from the small rearing boxes by a plastic barrier.

During sound playback, speakers (which are composed of a coil and a magnet) create an electromagnetic field proportional to the current that causes the magnet and the sound radiating membrane to move. Since electromagnetic fields might influence fish (Bevelhimer et al., 2013), control treatment also controlled for electromagnetic fields present during noise playback.

For this purpose, copper coils were made with an impedance similar to that of the loudspeakers. The coils were fed with the same boat playbacks, which were produced by an MP3 Player (A730 – HOTT) and delivered through an amplifier (Sony XM-N1004). Each coil was placed in a control tank (3 in total) and kept inside a container with fresh water

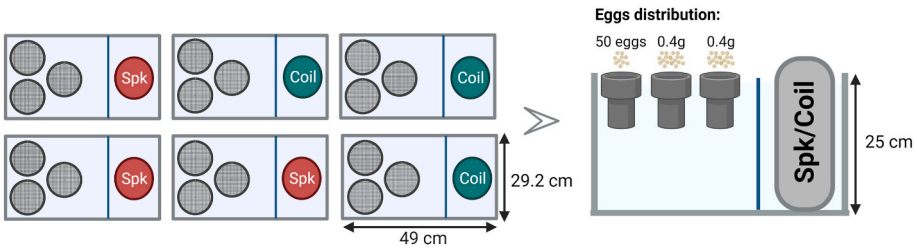

Fig. 1. Schematic depicting of the experimental laboratorial setup to expose meagre eggs and larvae to boat noise playback (Spk: Speaker) or control (Coil: electromagnetic coil). 3 small rearing boxes were used to distribute the eggs (also depicted). The distribution of the speakers and coils was randomly altered between experiments. Created with BioRender.com.

to avoid overheating. The coil was separated from the small rearing boxes by a plastic barrier, similarly to the treatment set up. The electric fields generated by loudspeakers and coils were measured with a pair of platinum electrodes placed in the water always at the same distance from the playback device under test. The electrodes were connected to an AC amplifier (Grass CP511, Grass Instruments, USA, gain 500 ×) whose output was monitored with an oscilloscope and digitized (Edirol UA-25, 16 bit, 48 kHz) and recorded on a PC running Adobe Audition (3.0) for graphical comparisons. The amplitude and spectra of the boat noise signals were adjusted so that the electromagnetic field of the coils mimicked as closely as possible the electromagnetic field generated by the speakers during boat noise playback (Fig. S1). Note that operating coils did not produce measurable acoustic noise (see Fig. 2).

The exposure to boat noise treatment mimicked the passages of 10 ferries (8 original passages + 2 repeated ones) and 4 small boats per hour, representative of the traffic that fish experience on average in the Tagus estuary (Faria et al., 2022). The original boat recordings were made in the area around the pier of the Air Force Base no. 6 in Montijo, Portugal (38°42′N, 8°58′W). A 1-h file composed of these boat recordings interspersed with random silence periods was played on loop continuously for the duration of the experiment. The same file was also used in the control treatment so that the electromagnetic coils would produce an electromagnetic field similar to the one of the speakers, as previously mentioned.

The sound played by the speaker was characterized and recorded by a hydrophone (8104, Brüel and Kjær, Naerum, Denmark; sensitivity −205 dB re. 1 V µPa−1; frequency response from 0.1 Hz to 180 kHz) located in the center of the three small rearing boxes, at a depth of 9 cm, coupled to a sound level meter (Bruël & Kjaer 2238 Mediator, Naerum, Denmark) and connected to a recording device (Tascam DR-40, TEAC American Inc., CA, USA). Fig. 2 compares the spectra of the boat noises recorded in the Tagus estuary and used in the playbacks with the boat noise emitted by the speakers, Note that the speakers were unable to produce the low frequencies present on the original recordings (<100 Hz; Fig. 2A).

In the experimental set-up, noise levels varied from 126 dB (average noise level calculated in the 0–22 kHz bandwith; sd = 5.93) for small boats' passages to 129 dB (sd = 1.27) re. 1 µPa when ferries were passing. Background noise level (control) was ca. 101 dB re. 1 µPa (sd = 0.81), with the water filtering system turned off (Fig. 2). A noise level average around 25 dB (sd = 2.94) above control background noise was chosen since it corresponds approximately to the increase caused by a ferry boat, recorded around 50–100 m away from the pier of Base Aérea n.6 in Montijo and it is comparable to the increase reported in different studies (Magnhagen et al., 2017; Nedelec et al., 2017; de Jong et al., 2018; Blom et al., 2019; Faria et al., 2022).

2.2.2. Eggs and larvae origin and maintenance

Experiments took place from March to July 2021. Eggs were obtained from naturally spawning meagre (maturation and spawning were performed spontaneously under natural photoperiod and temperature conditions) and collected at the beginning of the night just after spawning (note that sciaenids are known to spawn at dusk (Holt et al., 1985; Vieira et al., 2022) and transferred to the experimental facilities at FCUL, in Lisbon, Portugal, under controlled temperature conditions (around 3h transport). At arrival, the eggs were immediately distributed over the experimental tanks. Later on, larvae were only dealt with until they were at 2 dph (days post-hatching), so only during the period they had yolk sac to consume. The following experimental protocol was repeated for three batches that were used in this experiment.

2.2.3. Experimental protocol

This experimental protocol was prepared before the study. At arrival, eggs were distributed among three small rearing boxes in each tank (six tanks in total, three tanks per treatment, thus 18 rearing boxes). Box 1 received 50 eggs while boxes 2 and 3 received ca. 0.4 g each (around 660 eggs per box) (Fig. 1).

In every tank, hatching success, based on the number of surviving larvae at hatching, was measured from the group of 50 eggs (box 1). At hatching (0 dph), 15 larvae from box 2 (45 per treatment) were sampled and fixed in 80% ethanol for morphometric measurements. It was not always possible to sample this exact number of larvae in every box, in

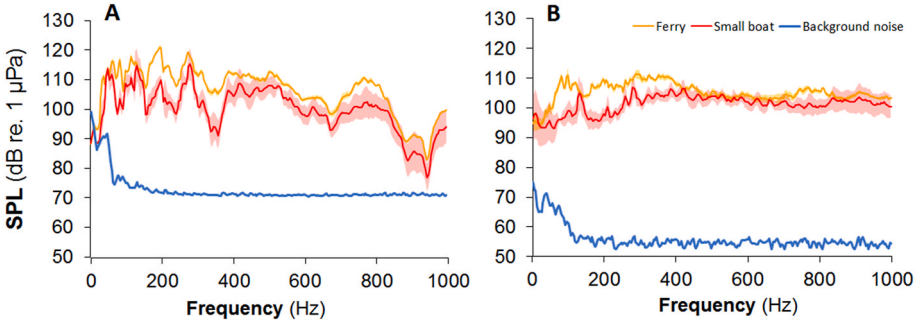

Fig. 2. (A) Characterization of boat noise playback spectra for the ferry and small boat noise (treatment), and background noise with operating coils (control). (B) The original spectra of different ferries and small boat sounds recorded at Air Force Base no. 6 in Tagus estuary and background noise recorded in the same location. Spectra settings: sampling frequency, 16 kHz; FFT size, 4096; window type, Hanning; window overlap, 50%.

cases of lower hatching rates (Table S1). For biochemical analysis, 3 × 15 larvae (3 samples per tank) were sampled from the same box, immediately placed inside microtubes in dry ice and then stored at −80 °C until biochemical analysis. At 2 dph, corresponding to the end of the experimental period, and coincident with the depletion of yolk sac, the remaining hatched larvae from box 3 were sampled until a maximum of 15 (45 per treatment) and fixed in 80% ethanol for morphometric measurements. There were not enough larvae to provide sufficient replicates for biochemical analysis at 2 dph and therefore these biomarkers were only assessed at 0 dph (Table S1).

2.3. Morphometric measurements

The larvae preserved in ethanol underwent morphological observations under a stereo binocular microscope. Larvae from the different sampling time points were used for comparative analyses, in a total of 249 larvae at hatching and 115 larvae at 2 dph.

Photographs were obtained with a digital camera (Moticam 10 10.0 MP) adapted to a stereomicroscope, using the Motic Images Plus software (Motic Asia, Hong Kong).

The morphometric measurements included Standard Length (SL), Yolk Sac Area (YSA), Body Depth (BD) and Lipid Droplet Area (LDA) (Fig. 3), following the methodology used by Klimogianni et al. (2013). The length was measured parallel to the longitudinal axis and the depth was obtained perpendicularly (Fig. 3). Measurements were done using ImageJ (Schindelin et al., 2012).

2.4. Biochemical analyses

2.4.1. Sample preparation

Pools of 15 meagre larvae were homogenized in 300 μL of ice-cold K-phosphate buffer (0.1 M, pH 7.4) with a motor-driven plastic pestle (VWR International, USA). Then, 50 μL of the obtained homogenate was separated for analysis of DNA damage (DNAd). For the electron transport system (ETS) activity, 125 μL of homogenate was centrifuged (1000 g, 10 min, 4 °C) with 50 μL ETS buffer (0.1 M Tris-HCl, 15% (w/v) Poly Vinyl Pyrrolidone, 153 M MgSO4, 0.2% (w/v) Triton X-100, and pH 8.5; De Coen and Janssen, 1997) and the resulting supernatant was kept for the analysis. The remaining homogenate volume was centrifuged (10 000 g, 20 min, 4 °C) and the supernatant collected for superoxide dismutase (SOD) activity, and for protein measurement (normalization of biochemical analyses). Aliquots were stored at −80 °C until processing.

All biochemical measurements were carried out spectrophotometrically in a Synergy H1 Hybrid Multi-Mode Microplate Reader (BioTek Instruments, Vermont, USA). To determine the concentration of total protein, Bradford's method (1976) was adjusted to microplate, read at 600 nm, and with a standard of ɣ-globulin from bovine blood (Sigma-Aldrich). The biomarkers used were chosen considering a recent study by Faria et al. (2022).

Fig. 3. Representation of the morphometric traits measured in meagre larvae. SL - Standard Length; YSA - Yolk Sac Area; LA – Lipid droplet Area; BD – BodyDepth.

2.4.2. Oxidative stress and damage

The DNA damage (double-strand breaks) was assayed according to Olive (1988) and adapted from De Lafontaine et al. (2000), where damaged DNA is separated from remaining nucleic acids and stained with a fluorescent DNA-specific dye, Hoechst 33258 (1 μg/mL, Sigma-Aldrich) and measured at 360:460 nm excitation:emission wavelengths. Results were expressed as μg DNAd/mg protein, using DNA from calf thymus (Sigma-Aldrich) as standard.

Superoxide Dismutase (SOD) activity was determined by the method of McCord and Fridovich (1969), converted to microplate (Lima et al., 2007), in which SOD competes for the superoxide radicals generated by xanthine/xanthine oxidase, resulting in the inhibition of cytochrome *c* reduction. The reaction was followed at 550 nm over 10 min, with a SOD standard (Sigma-Aldrich). Activity was expressed as U SOD/mg protein, being one U the amount of enzyme that inhibits 50% of cytochrome *c* reduction.

2.4.3. Energy metabolism

The ETS activity was measured according to King and Packard (1975), with modifications from De Coen and Janssen (1997, 2003), and is based on the reduction of INT (ρ-iodonitrotetrazolium violet, MP Biomedicals, 2 mM) to INT-formazan by the cellular respiration, replacing O_2 as final electron acceptor. The reaction was followed at 490 nm over 5 min, in the presence of NADPH. The O_2 consumption rate was calculated using $\varepsilon = 15\ 900\ M^{-1}cm^{-1}$ for INT-formazan, and the stoichiometric relationship that for each 2 μmol of INT-formazan formed, 1 μmol of O_2 is being consumed in the ETS (De Coen and Janssen, 1997). Results were expressed as μmol O2/h/mg protein.

2.5. Statistical analysis

The effect of noise exposure on the various variables was tested with Analysis of Variance (ANOVA) with a Blocked design, using batch as the block with fixed effects (Dixon, 2016). We tested the effect of noise exposure on hatching rate of meagre eggs with a Blocked One-way ANOVA, with treatment as a fixed factor. Hatching rate was log-transformed to meet the ANOVA assumptions. Additionally, the effect of treatment on larvae morphometric traits was tested with a Block Two-way ANOVA, with treatment, timepoint (0 and 2 dph) and treatment/timepoint interaction as fixed factors. In these analyses, data concerning larvae morphometric were restricted to a random selection from the whole data set to avoid large imbalances in sample sizes between timepoints. Samples sizes in this random selection were: 0 dph – boat noise (N = 60), Control (N = 60); 2 dph – boat noise (N = 31), Control (N = 31). ANOVAs were followed by contrast analysis (planed comparisons for least square means) to test for statistical significance between treatment levels at 0 dph and at 2 dph.

The effect of noise exposure was tested on biomarker activity levels on 0 dph larvae with a Block One-way ANOVA, including the factor treatment. Activity of ETS in larvae were reciprocal-transformed to meet the test assumptions. All test assumptions were met and statistical significance was set at $p < 0.05$. Tests were done with Statistica 15.0 (Dell Software, Inc., Round Rock, T, USA).

3. Results

3.1. Effects of boat noise exposure on larvae development

A period of approximately 48 h elapsed from spawning to hatching. Hatching rate was not affected by boat noise exposure ($F_{(1,6)} = 0.49$; $p = 0.51$), averaging 23 % (±3 % SE) in the control and 28 % (±4 %) in the boat noise group. Likewise we did not find a batch effect on hatching rate ($F_{(1,6)} = 0.79$; $p = 0.41$).

Exposure to boat noise did not affect overall larval size (SL, $F_{(1,175)} = 0.88$; $p = 0.35$) or yolk sac area (YSA, $F_{(1,176)} = 1.63$; $p = 0.20$), but affected lipid droplet area (LDA, $F_{(1,175)} = 6.20$; $p = 0.01$) (Table 1).

Table 1
ANOVA results comparing morphometric characteristics of larvae exposed to either control or boat noise: Lipid Droplet Area, Yolk Sac Area, Standard Length and Body Depth (N = 182).

Dependent variable	Effect	df	F	*p*
Lipid Droplet Area	Treatment	1	6.20	0.01
	Timepoint	1	267.83	<0.001
	Treatment*Timepoint	1	0.60	0.44
	Batch	2	12.11	<0.001
Yolk Sac Area	Treatment	1	1.63	0.20
	Timepoint	1	202.98	<0.001
	Treatment*Timepoint	1	0.52	0.47
	Batch	2	24.28	<0.001
Standard Length	Treatment	1	5966.02	0.35
	Timepoint	1	0.89	<0.001
	Treatment*Timepoint	1	60.52	1.00
	Batch	2	4.004	0.02
Body Depth	Treatment	1	3.25	0.07
	Timepoint	1	94.89	<0.001
	Treatment*Timepoint	1	2.66	0.10
	Batch	2	2.10	0.12

Contrast analysis showed that LDA at 2 dph was significantly smaller in boat noise exposed larvae when compared to control (p = 0.04; Table 1; Fig. 4B) (noise: 0.002 ± 0.001 mm2; control: 0.011 ± 0.002 mm2; Table 2A) but not at 0 dph (p = 0.12; Table 1; Fig. 4B).

ANOVA showed a marginally non-significant effect of treatment for body depth (BD, $F_{(1,175)} = 3.25$; p = 0.07; Table 1); contrast analysis indicated that 2 dph larvae exposed to noise presented a smaller BD than the controls (p = 0.04; noise: 0.209 ± 0.006 mm; control: 0.221 ± 0.003 mm; Table 2A; Fig. 4C) but not 0 dph larvae (p = 0.83; Table 2A; Fig. 4C).

3.2. Effects of boat noise exposure on larvae stress response

The effect of boat noise exposure was assessed on biomarkers activity levels in larvae at hatching (Fig. 5). We found marginally non-significant lower levels of SOD activity in larvae exposed to boat noise ($F_{(1,29)} = 3.18$; p = 0.085; Table 3; Fig. 5A). However, we did not find a significant treatment effect either in the oxidative stress biomarker DNA damage ($F_{(1,28)} = 1.00$; p = 0.33; Table 3; Fig. 5B) or in the energy related biomarker ETS ($F_{(1,29)} = 0.04$; p = 0.85; Table 3; Fig. 5C).

4. Discussion

Early life stages of fish development are known to critically influence population dynamics (Houde, 1987). However, there is a lack of studies testing the effects of noise on these life stages. In the present study, the effects of exposure to boat noise on larval development and physiologic stress were assessed in a laboratory-controlled environment. To our knowledge, this is the first study assessing the effects of boat noise exposure on early life stages of meagre.

Results point to no significant effects of boat noise playback on morphometric traits and on stress response in recently hatched larvae, but at 2 dph, larvae presented smaller body depth and higher energy consumption (smaller lipid droplet area) under boat-noise exposure.

4.1. Effect of boat noise exposure on larvae development

No evidence of detrimental effects of exposure to boat noise playback on hatching rate of meagre was found. This was an expected result considering the time of exposure. The time to hatch is quite short (around 48 h), and exposure to treatments only started approximately 6 h after spawning. These results are in line with those of Bruintjes and Radford (2014) who did not find a significant impact of small boat noise (127 dB re 1 μPa RMS) on hatching success of the cichlid *Neolamprologus pulcher*, and with those of Lara and Vasconcelos (2021) who also failed to detect a significant impact of noise treatments on the hatching success of zebrafish larvae (*Danio rerio*).

Size at hatch was not affected by boat noise exposure, neither was size at the end of yolk sac consumption (2 dph). The literature reports mixed results regarding the effects of boat noise exposure on larval length. Bruintjes and Radford (2014), for example, did not report significant changes on the body length of the cichlid fish exposed to four weeks of playback of small boat noise, but Nedelec et al. (2015) showed that atlantic cod (*Gadus morhua*) had a significant reduction in development when exposed to increased noise levels (even though catch-up growth happened by day 16 of the experiment). Also, Faria et al. (2022) showed that *ca.* 2 weeks of boat noise exposure had a detrimental effect on Lusitanian toadfish (*Halobatrachus didactylus*) larval growth, the noise exposed larvae being 8 % smaller than the ones under control conditions.

Similarly, there were no significant differences in the yolk sac area between the group exposed to boat noise playback and the control group at 0 dph or 2 dph. These results contrast with the ones found for

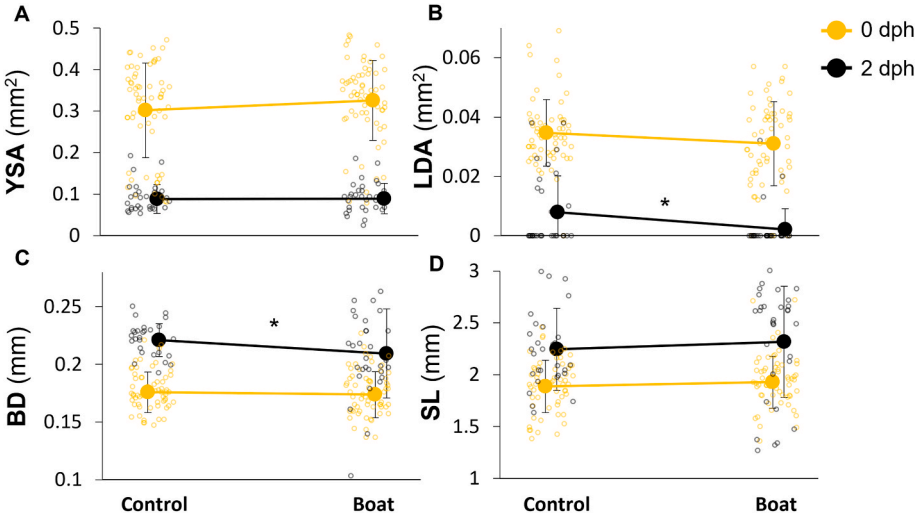

Fig. 4. Comparison of mean yolk sac area (A), lipid droplet area (B) body depth (C) and standard length (D) of larval meagre with 0 dph (yellow) and 2 dph (black), exposed either to control or boat noise treatment. Each plot represents mean ± SD. Statistical results for contrast analysis (planned comparisons for treatment effect at 0 dph and 2 dph. -* p < 0.05. N = 182.

Table 2
Measurements made for larvae under boat noise treatment or control, at 0 dph and 2 dph. A – Descriptive statistics for morphometric measurements. B – Descriptive statistics for biochemical analyses. Note that for biochemical markers each value measured represents 15 larvae while morphometric measurements were made for each larva individually. SD = Standard deviation; Min = minimum value; Max = maximum.

A	Control					Boat noise				
	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N
0 dph										
Standard length (mm)	1.88	0.24	1.37	2.45	128	1.96	0.23	1.19	2.70	119
Yolk Sac Area (mm^2)	0.30	0.11	0.08	0.50	128	0.33	0.08	0.05	0.48	119
Lipid Droplet Area (mm^2)	0.03	0.01	0	0.07	128	0.03	0.01	0	0.06	118
Body Depth (mm)	0.17	0.02	0.12	0.24	128	0.18	0.02	0.12	0.23	119
2 dph										
Standard length (mm)	2.28	0.39	1.55	3.23	75	2.31	0.50	1.25	3.00	38
Yolk Sac Area (mm^2)	0.088	0.034	0.05	0.20	76	0.090	0.035	0.02	0.18	38
Lipid Droplet Area (mm^2)	0.011	0.015	0	0.09	76	0.002	0.007	0	0.03	38
Body Depth (mm)	0.213	0.019	0.16	0.10	75	0.206	0.036	0.25	0.30	38
B	Control					Boat noise				
	Mean	**SD**	**Min**	**Max**	**N**	**Mean**	**SD**	**Min**	**Max**	**N**
0 dph										
Damaged DNA (µg DNA/mg protein)	42.9	10.7	24.9	64.8	16	45.3	12.3	28.0	66.0	15
ETS (µmol O2/h/mg protein)	0.415	0.463	0.008	1.265	17	0.244	0.338	0.014	0.860	15
SOD (U/mg protein)	3.53	1.14	2.28	6.99	17	2.92	0.68	1.61	4.41	15

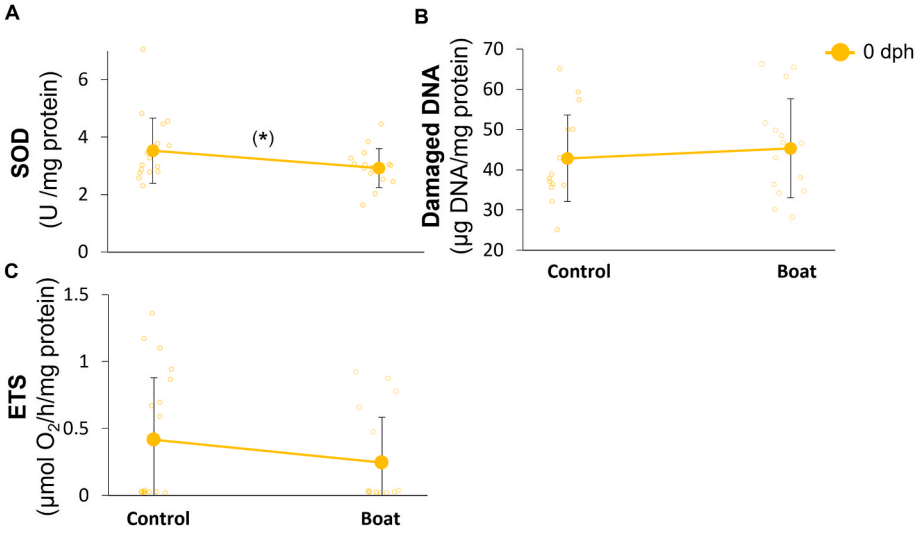

Fig. 5. Changes in Biomarkers (A) SOD (N = 32), (B) Damaged DNA (N = 31) and (C) ETS (N = 32) at 0 dph (yellow), for larvae exposed either to control or boat noise treatment. Each plot represents mean ± SD. (*) p = 0.085.

Table 3
ANOVA results comparing levels of oxidative stress - DNA damage (µg DNA/mg prot; N = 31), Energy metabolism- ETS (µmol O2/h/mg prot; N = 32) biomarker, SOD activity (U SOD/mg prot; N = 32) of larvae exposed to either control or boat noise.

Dependent variable	Effect	df	F	*p*
DNA damage	Treatment	1,28	1.00	0.33
	Batch	1,28	10.16	0.003
ETS[a]	Treatment	1,29	0.04	0.85
	Batch	1,29	48.43	<0.001
SOD[b]	Treatment	1,29	3.18	0.085
	Batch	1,29	0.56	0.46

[a] Reciprocal transformation 1/ETS.
[b] Log-transformed LogSOD.

damselfish larvae (*Acanthochromis polyacanthus*), which hatched with significantly smaller yolk sacs when exposed to boat noise playback, suggesting a higher yolk consumption under stressful conditions (Fakan and McCormick, 2019). Also, Lara and Vasconcelos (2021) have shown a similar impact of boat noise on zebrafish larvae (at 3 dph and 5 dph, when exposed to noise from the embryonic stage). This impact is relevant for larvae survival since yolk sacs are the energy reserves that allow the fish to develop until they begin exogenous feeding. This faster consumption could lead to unprepared larvae that will struggle to find food, potentially compromising their survival (Gagliano and McCormick, 2007). The impact this stressor might have on yolk sac consumption could be related to physiological responses or with the sensitivity to sound of the tested fishes, which varies among species.

Despite the lack of differences in yolk sac area, at 2 dph the lipid droplet area was significantly smaller in larvae exposed to boat noise playback than in larvae under control treatment. However, with only 2 days of life these larvae might not yet show the effects of the stressor, even though that has been shown for other fish larvae (Toledo et al., 2002; Lara and Vasconcelos, 2021). Lipid droplets are important energy reservoirs both during the embryonic stage and after hatching, when they supply energy for development, until they are completely absorbed (Iwamatsu and Muramatsu, 2008). For example, grouper larvae (*Epinephelus coioides*) had a smaller lipid droplet volume and a smaller total

length after 2 days of rearing in high aeration levels (2.50 mL/min per L and 3.75 mL/min per L) when compared to larvae reared at gentler aeration rates (0.62 mL/min per L and 1.25 mL/min per L) (Toledo et al., 2002). Moreover, Munday and coworkers (2016) showed that yellowtail kingfish (*Seriola lalandi*) 3 dph larvae varied their oil globule diameter depending on the CO_2 level they were exposed to, when exposed to high CO_2 levels (~1700 μatm) their lipid droplet diameter was significantly lower than ambient control CO_2 levels (~450 μatm). Together, these results suggest that different stressors can lead to a higher usage of energy reserves by newly hatched larvae.

A word of caution is needed when addressing the smaller size of lipid droplet at 2 dph, since in several of the sampled larvae we were not able to measure it, as it was not observable under the microscope. This could be because it was not present anymore, or because the position of the fish prevented us from seeing it. Nevertheless, measurements were made blind to treatment, and we do not expect results to be biased. Adding lipophilic dies at the time of sampling, such as the ones used for zebrafish, should be considered in the future, since they can stain the lipid-rich core of the lipid droplet, facilitating its identification (Lumaquin et al., 2021).

Body depth was also smaller in larvae exposed to boat noise comparing to the control group at 2 dph, which could suggest compromised growth and condition under more stressful circumstances. However, longer exposure is needed to verify this finding. Body depth is usually related with growth and condition and could be used as a proxy for these two factors. Hansen et al. (2019) found that Atlantic cod larvae when exposed to crude oil medium (0.67–0.85 mg oil/L) and high concentrations (3.53–4.34 mg oil/L) had a significantly increased myotome height compared to control larvae, but shorter mean larval length, which could be related with the development of spinal deformations induced by the toxicity of the crude oil. Also, Chambers et al. (2014) found that summer flounder larvae (*Paralichthys dentatus*) after 21 dph were smaller in terms of length and body depth, when exposed to high CO_2 environments (pCO_2 mean of 4714 μatm and pH = 7.06).

4.2. Effects of boat noise exposure on larvae stress response

Environmental stress impact on fish can be studied for instance by looking at antioxidant enzyme activity and the formation of free radicals (like ROS); in teleosts, these antioxidant defence mechanisms are mainly present in essential organs such as the brain, the liver or the kidney (Basha and Rani, 2003; Guh et al., 2021). Oxidative stress responses can also lead to higher energy expenditure associated with these coping mechanisms. In the present study, superoxide dismutase (SOD) activity was marginally lower in larvae at hatch when exposed to boat noise. These results contrast with other studies, that found SOD activity to be higher in fish larvae exposed to stressful conditions. For example, Faria et al. (2022) found higher SOD activity in the Lusitanian toadfish larvae under boat noise exposure. Other stressors, such as water pollution, were shown to increase the SOD concentration in wallago catfish (*Wallago attu*) as well (Pandey et al., 2003).

We found no evidence of treatment effect on the levels of ETS activity (mirrors energy consumption and cellular respiration; De Coen and Janssen, 1997) and DNA damage (expected if antioxidant defenses are not effective) in larvae at hatch. However, in our experiment, time to hatch is quite short (around 48 h) and the biomarker endpoints were also only possible to measure at hatch (0 dph), which could explain the lack of measurable effects. Consistently, Faria et al. (2022) found a significantly higher overall stress response related to DNA damage, SOD and ETS levels in Lusitanian toadfish larvae exposed to boat noise playback during a fortnight but not in eggs, that showed depressed levels of ETS instead. Additionally, it could be the case that the selected biomarkers may not be relevant for the studied mechanisms or are not sensitive enough. Further experiments with a longer boat noise exposure period should be performed to evaluate possible changes at the biochemical level in meagre larvae.

Laboratory experiments allow for a better control and manipulation of experimental conditions, facilitating the exclusion of confounding factors, which is challenging to do in the wild. However, future studies should aim at running experiments in the field with a large-scale mesocosm using real boat passages. This would be helpful in avoiding some limitations caused by laboratory settings, such as the differences in the distance of fish to the noise source that in the aquaria varies much less than in the natural habitat, the different particle motion patterns, and the complexity of the acoustic fields in restricted environments comparing to the open sea (Popper et al., 2020). Combining both lab and field designs, when possible, could be the best approach, as made by Simpson et al. (2016), who demonstrated a detrimental impact of anthropogenic noise on anti-predator behaviour in Ambon damselfish (*Pomacentrus amboinensis*) both in laboratory and in a natural setting.

5. Conclusions

Our study did not find marked effects of boat noise playback on physiological stress at hatch or on the development of meagre larvae until 2 dph, i.e. during the yolk sac consumption period. Future work will need to examine the effect of boat noise exposure in older larvae with a more developed nervous and sensory system, which develop fast after the onset of exogenous feeding (Yúfera and Darias, 2007). Also, using wild fish exposed to real boat noise for longer periods and in a natural set-up could be very interesting. Early development stages are a critical phase for fish and anthropogenic noise can severely affect these stages, by inducing stress and altering the homeostasis of the individuals, potentially influencing survival and population dynamics. More studies are needed to provide accurate information for policy makers, so the impacts of this widespread stressor can be reduced.

Author statement

Rita Trabulo: Methodology, Validation, Investigation, Data Curation, Writing – Original draft. **M. Clara P. Amorim:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – Review&Editing, Supervision. **Paulo J. Fonseca:** Conceptualization, Resources, Writing – Review&Editing, Supervision. **Manuel Vieira:** Investigation, Methodology, Validation, Formal Analysis, Writing – Review&Editing. **André B. Matos:** Methodology, Investigation, Software. **Thibaut Marin-Cudraz**: Investigation, Validation. **Marco F.L. Lemos:** Resources, Validation. **Ariana B. Moutinho:** Investigation, Writing – Original draft. **Sara C. Novais:** Methodology, Formal analysis, Validation, Resources, Writing – Review&Editing. **Pedro Pousão-Ferreira:** Resources. **Ana Candeias-Mendes**: Resources. **Ana M. Faria**: Conceptualization, Methodology, Validation, Investigation, Writing – Review&Editing, Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to IPMA-EPPO for the test subjects. We also thank Joaquim Tapisso for the assistance in the bioterium and Marta Bolgan for helping with the preliminary work. This study had the support of FCT through the strategic project UIDB/04292/2020 awarded to MARE, and through project LA/P/0069/2020 granted to the Associate Laboratory ARNET, through the strategic project UIDB/00006/2020

awarded to CE3C; and through project PTDC/BIA-BMA/29662/2017. FCT also attributed individual PhD grant to M.V (SFRH/BD/115562/2016).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2023.105894.

References

Amorim, M.C.P., 2006. Diversity of sound production in fish. In: Ladich, F. (Ed.), Communication in Fishes, vol. 1. Science Publishers, pp. 71–105.

Andersson, M.H., Andersson, S., Ahlsén, J., Andersson, L., Hammar, J., Persson, L.K., et al., 2017. A framework for regulating underwater noise during pile driving. In: A Technical Vindval Report (Stockholm, Sweden).

Basha, P.S., Rani, A.U., 2003. Cadmium-induced antioxidant defense mechanism in freshwater teleost Oreochromis mossambicus (Tilapia). Ecotoxicol. Environ. Saf. 56 (2), 218–221. https://doi.org/10.1016/S0147-6513(03)00028-9.

Bevelhimer, M.S., Cada, G.F., Fortner, A.M., Schweizer, P.E., Riemer, K., 2013. Behavioural responses of representative freshwater fish species to electromagnetic fields. Trans. Am. Fish. Soc. 142 (3), 802–813. https://doi.org/10.1080/00028487.2013.778901.

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 (1), 1–9. 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., et al., 2012. Common sole larvae survive high levels of pile-driving sound in controlled exposure experiments. PLoS One 7 (3). https://doi.org/10.1371/journal.pone.0033052.

Bruintjes, R., Radford, A.N., 2014. Chronic playback of boat 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.

Caiger, P.E., Montgomery, J.C., Radford, C.A., 2012. Chronic low-intensity noise exposure affects the hearing thresholds of juvenile snapper. Mar. Ecol. Prog. Ser. 466, 225–232. https://doi.org/10.3354/meps09933.

Chambers, R.C., Candelmo, A.C., Habeck, E.A., Poach, M.E., Wieczorek, D., Cooper, K.R., et al., 2014. Effects of elevated CO2 in the early life stages of summer flounder, Paralichthys dentatus, and potential consequences of ocean acidification. Biogeosciences 11 (6), 1613–1626. https://doi.org/10.5194/bg-11-1613-2014.

De Coen, W.M., 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. Aquatic Ecosyst. Stress Recovery (6), 43–55.

De Coen, W.M., Janssen, C.R., 2003. The missing biomarker link: relationships between effects on the cellular energy allocation biomarker of toxicant-stressed Daphnia magna and corresponding population characteristics. Environ. Toxicol. Chem. 22 (7), 1632–1641.

de Jong, K., Amorim, M.C.P., Fonseca, P.J., Heubel, K.U., 2018. Noise affects multimodal communication during courtship in a marine fish. Front. Ecol. Evol. 6 (July), 1–8. https://doi.org/10.3389/fevo.2018.00113.

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. Fish. 30 (2), 245–268. https://doi.org/10.1007/s11160-020-09598-9.

Debusschere, E., De Coensel, B., Bajek, A., Botteldooren, D., Hostens, K., Vanaverbeke, J., et al., 2014. In situ mortality experiments with juvenile sea bass (dicentrarchus labrax) in relation to impulsive sound levels caused by pile driving of windmill foundations. PLoS One 9 (10), 1–9. https://doi.org/10.1371/journal.pone.0109280.

Dixon, P., 2016. Should blocks be fixed or random?. In: Conference on Applied Statistics in Agriculture https://doi.org/10.4148/2475-7772.1474.

Duarte, C.M., Chapuis, L., Collin, S.P., Costa, D.P., Devassy, R.P., Eguiluz, V.M., 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 (January), 493–500. https://doi.org/10.1016/j.marpolbul.2019.02.054.

Faria, A., Fonseca, P.J., Vieira, M., Alves, L.M.F., Lemos, M.F.L., Novais, S.C., Matos, A. B., Vieira, D., Amorim, M.C.P., 2022. Boat noise impacts early life stages in the Lusitanian toadfish: a field experiment. Sci. Total Environ. 811 https://doi.org/10.1016/j.scitotenv.2021.151367.

Frisk, G.V., 2012. Noiseonomics: the relationship between ambient noise levels in the sea and global economic trends. Sci. Rep. 2 (1), 2–5. https://doi.org/10.1038/srep00437.

Gagliano, Monica, McCormick, Mark, 2007. Maternal condition influences phenotypic selection on offspring. J. Anim. Ecol. 76, 174–182. https://doi.org/10.1111/j.1365-2656.2006.01187.

Gendron, G., Tremblay, R., Jolivet, A., Olivier, F., Chauvaud, L., Winkler, G., Audet, C., 2020. Anthropogenic boat noise reduces feeding success in winter flounder larvae (Pseudopleuronectes americanus). Environ. Biol. Fish. 103 (9), 1079–1090. https://doi.org/10.1007/s10641-020-01005-3.

González-Quirós, R., Del Árbol, J., García-Pacheco, M., del, M., Silva-García, A.J., Naranjo, J.M., Morales-Nin, B., 2011. Life-history of the meagre Argyrosomus regius in the gulf of cádiz (SW Iberian Peninsula). Fish. Res. 109 (1), 140–149. https://doi.org/10.1016/j.fishres.2011.01.031.

Grimm, N.B., Foster, D., Groffman, P., Grove, J.M., Hopkinson, C.S., Nadelhoffer, K.J., et al., 2008. The changing landscape: Ecosystem responses to urbanization and pollution across climatic and societal gradients. Front. Ecol. Environ. 6 (5), 264–272. https://doi.org/10.1890/070147.

Guh, Y.J., Tseng, Y.C., Shao, Y.T., 2021. To cope with a changing aquatic soundscape: neuroendocrine and antioxidant responses to chronic noise stress in fish. Gen. Comp. Endocrinol. 314 (March), 113918 https://doi.org/10.1016/j.ygcen.2021.113918.

Hansen, B.H., Salaberria, I., Read, K.E., Wold, P.A., Hammer, K.M., Olsen, A.J., et al., 2019. Developmental effects in fish embryos exposed to oil dispersions – the impact of crude oil micro-droplets. Mar. Environ. Res. 150 (June), 104753 https://doi.org/10.1016/j.marenvres.2019.104753.

Harding, H.R., Gordon, T.A.C., Eastcott, E., Simpson, S.D., Radford, A.N., 2019. Causes and consequences of intraspecific variation in animal responses to anthropogenic noise. Behave. Ecol. 30 (6), 1501–1511. https://doi.org/10.1093/beheco/arz114.

Hawkins, A.D., Popper, A.N., 2018. Effects of man-made sound on fishes. In: Slabbekoorn, H. (Ed.), Effects of Anthropogenic Noise on Animals, vol. 66, pp. 415–429. https://doi.org/10.1007/978-1-4939-8574-6_6.

Herbert-Read, J.E., Kremer, L., Bruintjes, R., Radford, A.N., Ioannou, C.C., 2017. Anthropogenic noise pollution from pile-driving disrupts the structure and dynamics of fish shoals. Proc. Biol. Sci. 284 https://doi.org/10.1098/rspb.2017.1627, 1863.

Hildebrand, J.A., 2009. Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5–20. https://doi.org/10.3354/meps08353.

Holles, S.H., Simpson, S.D., Radford, A.N., Berten, L., Lecchini, D., 2013. Boat noise disrupts orientation behaviour n a coral reef fish. Mar. Ecol. Prog. Ser. 485, 295–300. https://doi.org/10.3354/meps10346.

Holt, G.J., Holt, S.A., Arnold, C.R., 1985. Diel periodicity of spawning in sciaenids. Mar. Ecol. Prog. Ser. 27 (1), 7.

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

International Organization for Standardization (ISO) "Underwater Acoustics—Terminology" (ISO 18405:2017, ISO, 2017).

Iwamatsu, T., Muramatsu, Æ.T., 2008. Oil droplets and yolk spheres during development of Medaka embryos, 22, 344–348. https://doi.org/10.1007/s10228-008-0048-z.

King, F.D., Packard, T.T., 1975. Respiration and the activity of the respiratory electron transport system in marine zooplankton. Limnol. Oceanogr. 20 (5), 849–854. https://doi.org/10.4319/lo.1975.20.5.0849.

Klimogianni, A., Pagoulatou, M., Trageli, M., Hotos, G.N., 2013. Investigation on early development, the feeding ability and larval survival under starvation in common meagre, Argyrosomus regius (asso 1801). J. Aquat. Sci. 1 (1), 1–6. https://doi.org/10.12691/jas-1-1-1.

Kunc, H.P., McLaughlin, K.E., Schmidt, R., 2016. Aquatic noise pollution: implications for individuals, populations, and ecosystems. Proc. Biol. Sci. 283 https://doi.org/10.1098/rspb.2016.0839, 1836.

Ladich, F. (Ed.), 2015. Acoustic Signaling in Female Fish. Sound Communication in Fishes. Springer-Verlag, Wien, pp. 149–173.

Lafontaine, Y. De, Blaise, C., Costan, G., Gagnon, P., Chan, H.M., 2000. Biomarkers in zebra mussels (Dreissena polymorpha) for the assessment and monitoring of water quality of the St Lawrence River (Canada). Aquat. Toxicol. 50, 51–71.

Lagardère, J.P., Mariani, A., 2006. Spawning sounds in meagre Argyrosomus regius recorded in the Gironde estuary, France. J. Fish. Biol. 69 (6), 1697–1708. https://doi.org/10.1111/j.1095-8649.2006.01237.x.

Lara, R.A., Vasconcelos, R.O., 2021. Impact of noise exposure on development, physiological stress and behavioural patterns in larval zebrafish. Sci. Rep. 33.

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

Lumaquin, D., Johns, E., Montal, E., Weiss, J.M., Ola, D., Abuhashem, A., White, R.M., 2021. An in Vivo Reporter for Tracking Lipid Droplet Dynamics in Transparent Zebrafish, vols. 1–25.

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 (February), 115–125. https://doi.org/10.3354/meps11997.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for 772 erythrocuprein (hemocuprein). J. Biol. Chem. 44, 6049–6055.

Montgomery, J.C., Jeffs, A., Simpson, S.D., Meekan, M., Tindle, C., 2006. Sound as an orientation cue for the pelagic larvae of reef fishes and decapod Crustaceans. Adv. Mar. Biol. 51, 143–196. https://doi.org/10.1016/S0065-2881(06)51003-X, 06.

Munday, P.L., Watson, S.A., Parsons, D.M., King, A., Barr, N.G., McLeod, I.M., et al., 2016. Effects of elevated CO2 on early life history development of the yellowtail kingfish, Seriola lalandi, a large pelagic fish. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 73 (3) https://doi.org/10.1093/icesjms/fsv210.

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. Biol. Sci. 282 https://doi.org/10.1098/rspb.2015.1943, 1817.

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. Biol. Sci. 284 https://doi.org/10.1098/rspb.2017.0143, 1856.

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.

Pandey, S., Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B., Raisuddin, S., 2003. Biomarkers of oxidative stress: a comparative study of river Yamuna fish Wallago attu (Bl. & Schn.). Sci. Total Environ. 309 (1–3), 105–115. https://doi.org/10.1016/S0048-9697(03)00006-8.

Payne, J.F., Coady, J., White, D., 2009. Potential effects of seismic airgun discharges on monkfish eggs (Lophius americanus) and larvae. In: Environmental Studies Research Funds (St. John's).

Peng, C., Zhao, X., Liu, G., 2015. Noise in the sea and its impacts on marine organisms. Int. J. Environ. Res. Publ. Health 12 (10), 12304–12323. https://doi.org/10.3390/ijerph121012304.

Pereira, B.P., Vieira, M., Pousão-Ferreira, P., Candeias-Mendes, A., Barata, M., Fonseca, P.J., Amorim, M.C.P., 2020. Sound production in the Meagre, Argyrosomus regius (Asso, 1801): intraspecific variability associated with size, sex and context. PeerJ 2020 (2). https://doi.org/10.7717/peerj.8559.

Popper, A.N., 2003. Effects of anthropogenic sounds on fish. Fisheries 28 (10), 24–31. https://doi.org/10.1080/09524622.2008.9753822.

Popper, A.N., Hastings, M.C., 2009. The effects of anthropogenic sources of sound on fishes. J. Fish. Biol. 75 (3), 455–489. https://doi.org/10.1111/j.1095-8649.2009.02319.x.

The effects of noise on aquatic life II. In: Popper, A.N., Hawkins, A. (Eds.), 2016. Advances in Experimental Medicine and Biology, vol. 875. https://doi.org/10.1007/978-1-4939-2981-8_19.

Popper, A.N., Hawkins, A.D., 2019. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. Fish. Biol. 94 (5), 692–713. https://doi.org/10.1111/jfb.13948.

Popper, A.N., Hawkins, A.D., Fay, R.R., Mann, D.A., Bartol, S., Carlson, T.J., et al., 2014. Sound exposure guidelines for fishes and sea turtles: a technical report prepared by ANSI-accredited standards committee S3/SC1 and registered with ANSI. https://doi.org/10.1007/978-3-319-06659-2.

Popper, A.N., Hawkins, A.D., Sand, O., 2020. Examining the hearing abilities of fishes, 948(2019. https://doi.org/10.1121/1.5120185.

Radford, A.N., Kerridge, E., Simpson, S.D., 2014. Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behave. Ecol. 25 (5), 1022–1030. https://doi.org/10.1093/beheco/aru029.

Remage-Healey, L., Nowacek, D.P., Bass, A.H., 2006. Dolphin foraging sounds suppress calling and elevate stress hormone levels in a prey species, the Gulf toadfish. J. Exp. Biol. 209 (22), 4444–4451. https://doi.org/10.1242/jeb.02525.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9 (7), 676–682. https://doi.org/10.1038/nmeth.2019.

Shannon, G., McKenna, M.F., Angeloni, L.M., Crooks, K.R., Fristrup, K.M., Brown, E., et al., 2016. A synthesis of two decades of research documenting the effects of noise on wildlife. Biol. Rev. 91 (4), 982–1005. https://doi.org/10.1111/brv.12207.

Sierra-Flores, R., Atack, T., Migaud, H., Davie, A., 2015. Stress response to anthropogenic noise in Atlantic cod Gadus morhua L. Aquacult. Eng. 67, 67–76. https://doi.org/10.1016/j.aquaeng.2015.06.003.

Simpson, S.D., Radford, A.N., Nedelec, S.L., Ferrari, M.C.O., Chivers, D.P., McCormick, M.I., Meekan, M.G., 2016. Anthropogenic noise increases fish mortality by predation. Nat. Commun. 7 (1), 1–7. https://doi.org/10.1038/ncomms10544.

Smith, M.E., Kane, A.S., Popper, A.N., 2004. Noise-induced stress response and hearing loss in goldfish (Carassius auratus). J. Exp. Biol. 207 (3), 427–435. https://doi.org/10.1242/jeb.00755.

Sordello, R., Ratel, O., de Lachapelle, F.F., Leger, C., Dambry, A., Vanpeene, S., 2020. Evidence of the impact of noise pollution on biodiversity: a systematic map. Environ. Evid. 9 (1), 1–27. https://doi.org/10.1186/s13750-020-00202-y.

Toledo, J.D., Caberoy, N.B., Quinitio, G.F., Choresca, C.H., Nakagawa, H., 2002. Effects of salinity, aeration and light intensity on oil globule absorption, feeding incidence, growth and survival of early-stage grouper Epinephelus coioides larvae. Fish. Sci. 68 (3), 478–483. https://doi.org/10.1046/j.1444-2906.2002.00451.x.

Tolimieri, N., Jeffs, A., Montgomery, J.C., 2000. Ambient sound as a cue for navigation by the pelagic larvae of reel fishes. Mar. Ecol. Prog. Ser. 207, 219–224. https://doi.org/10.3354/meps207219.

Vieira, M., Amorim, M.C.P., Marques, T.A., Fonseca, P.J., 2022. Temperature mediates chorusing behaviour associated with spawning in the sciaenid Argyrosomus regius. Mar. Ecol. Prog. Ser. 697, 109–124.

Vieira, M., Beauchaud, M., Amorim, M.C.P., Fonseca, P.J., 2021. Boat noise affects meagre (Argyrosomus regius) hearing and vocal behaviour. Mar. Pollut. Bull. 172, 112824.

Williams, R., Wright, A.J., Ashe, E., Blight, L.K., Bruintjes, R., Canessa, R., 2015. Impacts of anthropogenic noise on marine life: Publication patterns, new discoveries, and future directions in research and management. Ocean Coast Manag. 115, 17–24. https://doi.org/10.1016/j.ocecoaman.2015.05.021.

Yúfera, M., Darias, M.J., 2007. The onset of exogenous feeding in marine fish larvae. Aquaculture 268 (1–4), 53–63. https://doi.org/10.1016/j.aquaculture.2007.04.050.