Instituto Universitário
de Ciências Psicológicas,
Sociais e da Vida

IMPACT OF ANTHROPOGENIC NOISE IN THE DEVELOPMENT, SURVIVAL AND BEHAVIOUR OF *ARGYROSUMUS REGIUS* EARLY LIFE STAGES

Rita João Trabulo Silva

Orientador de Dissertação:

DOUTORA ANA FARIA

Co- Orientador de Dissertação:

DOUTORA MARIA CLARA AMORIM

Professor de Seminário de Dissertação:

DOUTOR EMANUEL GONÇALVES

Dissertação submetida como requisito parcial para a obtenção do grau de:

MESTRE EM BIOLOGIA MARINHA E CONSERVAÇÃO

2021

Dissertação de Mestrado realizada sob a
orientação da Doutora Ana Faria e da
Doutora Maria Clara Amorim,
apresentada no ISPA – Instituto
Universitário para obtenção de grau de
Mestre na especialidade de Biologia
Marinha e Conservação

Acknowledgements

I would like to start by thanking Professor Ana Faria, Professor Clara Amorim and Professor Paulo Fonseca for trusting me from the beginning and for giving me the chance of participating in this project. Their “door” was always open whenever I had troubles or had a question about my research or writing. Their knowledge and experience steered me in the right direction allowing for the success of this project.

To my colleague, Manuel Vieira, my sincere gratitude. Without his patience and tranquility my adaptation to this project would have been much harder. He was always available to answer any of my sometimes-silly questions and his knowledge, guidance and support were a vital part of the success of this project. Without him, I wouldn’t have made it.

I would also like to thank André and Thibaut, for their readiness to help in the practical work. They were fundamental in the flawless execution of our research tasks.

Additionally, I would like to acknowledge the people at Estação Piloto de Piscicultura de Olhão – Instituto Português do Mar e da Atmosfera (EPPO-IPMA), specially Pedro Pousão-Ferreira, Ana Mendes and Marisa Barata. Thank you.

Finally, I must express my very profound gratitude to my family and friends, for providing me with unfailing support and continuous encouragement through the process of researching and writing this thesis. This accomplishment would not have been possible without them.

This thesis had the support of Fundação para a Ciência e Tecnologia through the project FishNoise (PTDC/BIA-BMA/29662/2017).

Resumo

A corvina (*Argyrosumus regius*) é um peixe vocal que utiliza o Estuário do Tejo, área com tráfego marinho intenso, como zona reprodutiva. Procurámos estudar o efeito da exposição a ruído de barco na sobrevivência e desenvolvimento de larvas, e no comportamento – nomeadamente aproximação e perseguição – de juvenis de corvina.

Para tal, expusemos ovos e larvas a tratamentos de ruído (*playback* de ruído de barco) ou de controlo (bobines produzindo um campo eletromagnético semelhante ao gerado pelas colunas) e analisámos as suas características morfométricas à eclosão e dois dias depois da eclosão (dph). Os resultados mostraram que existe impacto do ruído na gestão das reservas energéticas das larvas, que aumentaram o consumo da gota lipídica sob este *stressor*. Verificou-se, ainda, um condicionamento do desenvolvimento das larvas apresentando, aos 2dph, uma altura máxima de corpo inferior quando expostas a ruído de embarcação. Expusemos, ainda, juvenis a condições de tratamento e de controlo semelhantes, durante 2 ou 7 dias, analisando o seu comportamento antes e depois da alimentação. O rácio de aproximação pós/pré alimentação diminuiu do dia 2 ao 7 no grupo de ruído, mas aumentou no controlo, sugerindo uma influência negativa do ruído na sua estimulação social. Implicações destes resultados em estádios mais avançados ainda não foram investigados. Importa salientar que estes peixes provêm de ambiente de aquacultura onde o ruído é superior ao do ambiente natural. Estudos complementares com corvina selvagem podem contribuir para aprofundar o conhecimento atual, ajudando a compreender o impacto do ruído antropogénico nos estádios inicias da corvina.

Corvina + ruído antropogénico + estádios iniciais + morfologia + comportamento

Abstract

Meagre (*Argyrosumus regius*) is a vocal fish that uses the Tagus estuary, an area with intense marine traffic, as breeding grounds. Here, we studied the effects of boat noise exposure on the development and survival of larvae, and on the behaviour – namely approach and pursuit – of meagre juveniles .

We exposed eggs and larvae to either noise (boat noise playback) or control treatments (coils producing a similar electric field to the speakers) and analysed their morphometric characteristics at hatching and 2 days-post-hatching (dph). Our results showed an impact of boat noise playback on the management of energy reserves by meagre larvae, which increased their lipid droplet consumption when under this stressor. There was also a conditioning of their development illustrated by the smaller body depth achieved by larvae exposed to boat noise at 2 dph. We further exposed juveniles to similar treatment and control conditions and recorded their pre- and post-feeding behaviour after 2 and 7 days of exposure. Post/pre-feeding approach ratio decreased from day 2 to day 7 in the noise group but increased in the control, suggesting that the arousal or search behaviour with feeding is being affected by prolonged noise exposure. Whether these results have implications in later stages remains to be investigated. Also, tested fish were reared in an aquaculture facility, i.e. in a much noisier environment than the natural one. Further studies with wild meagre could help deepen this knowledge and give us a better understanding of how anthropogenic noise can impact meagre early stages.

Meagre + anthropogenic noise + early life + morphology + behaviour

Table of contents

List of Tables VII

List of Figures VIII

List of Appendices X

1. Introduction 11

2. Methodology 13

   2.1. Experimental set up 13

   2.2. Eggs and larvae experimental procedure 18

   2.3. Juveniles' experimental procedure 21

   2.4. Statistical analysis 23

   2.5. Ethics 23

3. Results 24

   3.1. Effects of boat noise exposure on larvae development 24

   3.2. Effect of boat noise exposure on juvenile behaviour 25

4. Discussion 27

   4.1. Effect on larvae development 27

   4.2. Effect on juvenile behaviour 29

   4.3. Final remarks 31

6. References 33

List of Tables

Table 1 - Number of meagre larvae sampled per batch, tank and rearing box. 19

Table 2 - Description of the morphometric traits of meagre larvae measured in this study (adapted from Klimogianni et al., 2013). 20

Table 3 - Ethogram of meagre juveniles 22

Table 4 - Measurements of larvae under boat noise treatment or control, at 0dph and 2dph (values are represented in mean ± SE format). 24

Table 5 - Definition of effects of anthropogenic noise, used in Guidelines. Adapted from Popper et al. (2014)... 44

Table 6 - Groups of fish by hearing apparatus morphology and criteria for mortality and recoverable injury. Sound pressure in used even for fishes without swimbladders since no data for particle motion existed. Relative risk (high, moderate, low) is given for individuals at three distances from the source defined in relative terms: SB- swimbladder; PM – particle motion; SP – sound pressure; SELcum (cumulative Sound Exposure Level); N - near; I - intermediate; F – far. Adapted from Popper et al., (2014). 44

Table 7 - Division of fishes into groups. SB – Swimbladder; PM – Particle motion; SP – Sound pressure. Adapted from Popper and Hawkins (2019)... 52

Table S 1 - Results of Post-hoc LSD test for Lipid Droplet Area 72

Table S 2 - Results of Post-hoc LSD test for Body Depth 72

Table S 3 - Results of Post-hoc LSD test for pre-feeding approach.. 72

Table S 4 - Results of Post-hoc LSD test for ratio approach post/pre feeding 72

Table S 5 - Results of Post-hoc LSD test for aggressiveness score post/pre feeding ratio 73

List of Figures

Figure 1- Schematic depicting of the experimental laboratorial setup to expose meagre eggs and larvae (A), and juveniles (B) to boat noise playback (Spk: Speaker) or control (Coil: electromagnetic coil). On (A) 3 small rearing boxes were used to distribute the eggs (also depicted). On (B) juveniles were free to swim on 2/3 of the tank. 14

Figure 2 – A – Speaker (Playback condition) in a waterproof container; B – Coil (Control condition) in a container filled with freshwater. 15

Figure 3 - Characterization of boat noise treatment. (A,B) playback spectra for the ferry and small boat noise, and silence for larvae experiment (A) and juveniles experiment (B). (C) The original spectra of different ferry and small boat sounds recorded at Air Force Base no. 6 in Tagus estuary. The frequencies of the sounds, in Hertz (Hz), are represented in the X-axis. The amplitude of the sounds, in decibels (dB, SPL) are represented in the Y-axis. (D) Sound pressure level characterization in 9 positions of one tank, while boat noise was played and when the playback was halted. The values are presented in dB re. 1µPa. Note that for (D), only one ferry passage was used for this characterization. 17

Figure 4 - Representation of the morphometric traits measured in meagre larvae. For abbreviations description view Table 2. 21

Figure 5 - Comparison of mean yolk sac area (A), lipid droplet area (B) body depth (C) and standard length (D) of larval meagre with 0dph (grey) and 2dph (black), exposed either to control or boat noise treatment. Each plot represents mean ± SE. Statistical results for treatment effect - * $p < 0.05$; $^{(*)}$ $p = 0.07$. 25

Figure 6 - Frequency of juveniles' behaviour exposed either to control or boat noise treatment, at day 2 (grey) and day 7 (black) of exposure. (A) pre-feeding approach; (B) pre-feeding pursuit. Furthermore, ratios of approach post/pre-feeding (C); pursuit post/pre-feeding (D) and aggressiveness score post/pre feeding ratio (G) are also shown. Each plot represents mean ± SE. * $p < 0.05$; (*) $p = 0.07$. Events are normalized per number of juveniles and per minute. 27

Figure 7 - Different types of intrinsic sonic muscles in teleost fishes. A - *Dactylopterus* sp. (family Dactylopteridae). B - *Lota lota* (family Gadidae). C - *Porichthys notatus* (family Batrachoididae). D - Toadfish, *Opsanus tau* (family Batrachoididae); E: Norther Searobin, *Prionotus carolinus* (family Triglidae). F- European gurnard (family Triglidae). Sb— swimbladder, SM—sonic muscle. Adapted from Ladich and Fine (2006). 54

Figure 8 - Two examples of extrinsic swimbladder types in catfishes (Siluriformes). On the left: The sonic muscle (SM) is in connection to the ramus Mülleri (RM) shown, that connects to the swimbladder (SB). On the right: The sonic muscle (SM) is inserted in the transverse process of the 4th vertebra (TVP4) and then directly attached to the swimbladder (SB). Adapted from Ladich and Fine (2006). 55

Figure 9 - Agonistic sounds produced by different members of the family Gadidae. The black bar is the time scale of 100 ms for each fish. The sounds represented are made up of low frequency rapidly repeated pulses emitted at different rates. Adapted from Amorim (2006)........................ 56

Figure 10 - Body cavity of 3 meagres, where we can see the differences in the sonic muscles shape and color: on the upper left-corner the body cavity of a sperm-producing male with large red sonic muscles; on the upper right corner a non-sperm producing male where we can see a change of color in the sonic muscles; on the bottom a pre-spawning female with less developed pink sonic muscles. SM – sonic muscle; T - testicle; L – liver; SW – swimbladder. Adapted from Lagardère and Mariani, (2006).. 59

Figure 11- Juveline male meagre (9cm) without sonic muscles yet present. T – testicles; SB – swimbladder; BW – body wall. Retrieved from Pereira et al. (2020). 60

Figure S 1 - Frequency spectrum of the electrical signal produced by the speakers and the reels for the eggs and larvae experiment (left) and for the juveniles' experiment (right). 71

Figure S 2 - Propagation of boat noise to control tanks. (A, C) Spectra of the recorded sound on the control tanks; and (B, D) on the tanks with the boat noise treatment during boat noise playback, and silence. Similar results were obtained on both setups: A,B represent larvae setup and C,D juveniles setup. Only one ferry passage was used for this characterization. 71

List of Appendices

Appendix I – STATE OF THE ART 39

1. Anthropogenic Noise 39

  1.1 Types of anthropogenic noise 39

    1.1.1. Transient/impulsive anthropogenic sounds 39

    1.1.2. Continuous anthropogenic sounds 41

  1.2 Known effects in: 41

    1.2.1. Different species 41

    1.2.2. Effects in fish 43

      1.2.2.1. Early life stages 45

      1.2.2.2. Adults 47

2. Acoustic communication in fish 48

  2.1. Context and function of acoustic communication/signals/cues 48

  2.2. Ontogeny of hearing abilities 50

    2.2.1. Larvae hearing abilities, acoustic 50

  2.3. Sound reception 51

    2.3.1 Mechanisms of sound production 52

    2.3.2 Diversity of sounds (time and frequency domains) 55

3. *ARGYROSUMUS REGIUS* 57

  3.1. Life cycle 57

  3.2. Acoustic communication in meagre 58

    .3.2.1. Sound generating apparatus 58

    3.2.2. Acoustic repertoire 60

4. Knowledge gaps in research on the impacts of anthropogenic noise on fish 61

Appendix II – Supplementary Material 71

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). All the 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; Hildebrand, 2009), 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).

Marine traffic produces pervasive noise that causes a widespread impact on marine life and can overlap with communication and hearing ranges of aquatic animals (Hildebrand, 2009; Williams at al., 2015). In addition, the growth of human populations has been driving such a huge and widespread increase in marine traffic that it poses a real threat to marine biodiversity (Frisk, 2012; de Jong 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 (Payne & Webb, 1971; Cox et al., 2006; Peng et al., 2015; Middel & Verones, 2017), but nowadays the relevance of its effects on fish is well established (Popper & Hastings 2009; Radford et al., 2014; Popper & Hawkins, 2019; de Jong et al. 2020). The use of sound by fish varies along different taxa 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; Amorim et al., 2008). 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 & Popper, 2018). However, impacts on population dynamics of species 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, compromising different aspects of its life and interfering with conspecific and heterospecific interactions (Popper et al., 2014; Hawkins et al., 2015; 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, 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. 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 can affect their overall fitness (Bolle et al., 2012; Debusschere, 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 shoal organization, movement direction and speed (Herbert-Read et al., 2017), slow or inappropriate responses to predators (Simpson et al, 2016), or stress increase and behaviour alteration (Lara & Vasconcelos, 2021) are some examples of the impact of anthropogenic noise in these early life stages. Likewise, Nedelec and colleagues (2015) showed that, for atlantic cod larvae (*Gadus morhua*), two days of exposure to noise (either regular or random) was enough to reduce growth and, for regular sound, to increase yolk sac consumption (Nedelec et al., 2015). For winter flounder larvae (*Pseudopleuronectes americanus*), 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 (Gendron et al., 2020).

The meagre (*Argyrosumus regius*) is a highly vocal sciaenid (Lagardère & Mariani, 2006) that inhabits the subtropical waters of the European and African coast of the Atlantic, and the Black Sea and the Mediterranean (Cabral & Ohmert, 2001; González-Quirós et al., 2011), and can reach up to two meters 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 (>30cm; Pereira et al., 2020). Hence, in addition to overfishing and climate change, noise is likely a pressing stressor for meagre populations (González-Quirós et al., 2011; Vieira et al., 2021) as this species relies on vocal communication for reproduction (Pereira et al. 2020). Even though meagre has a Least Concern status on the IUCN list, its current population trend is unknow (Pollard & Bizsel, 2020) and since it is a long-lived heavily fished species, specially the spawning adults, this practice can heavily compromise the species survival (Lagardère & Mariani, 2006; González-Quirós et al., 2011). Therefore, properly protecting its different life stages is of great importance.

To date, we know 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 hearing masking by boat noise was observed in juveniles (Vieira et al., 2021). Impacts of noise on meagre early life stages, namely in egg/larval stages and in small juveniles, have not thus far been addressed.

In the current study we aim to examine how exposure to boat noise playbacks during early life stages can affect 1) survival and development of eggs and larvae, and 2) juveniles' behaviour, using meagre as the study species. For this purpose, we exposed embryonic stage meagre to either boat or ambient noise and assessed hatching success and larvae development at two days post-hatching. Furthermore, on a separate experiment, we also addressed meagre's juvenile behaviour, namely approach and pursuit, after two and seven days of exposure to either boat or ambient noise. 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. Methodology

2.1. Experimental set up

With laboratory experiments we tested the effects of exposing meagre eggs, larvae, and juveniles to boat noise playbacks.

Experimental tanks and equipment

Experiments took place in 6 tanks (35 L; 25 × 49 × 29.2 cm). During the experiments, the eggs, larvae or juvenile fish were exposed to either boat noise (treatment, 3 tanks) or silence, i.e. no added noise (control, 3 tanks). Control aquaria were equipped with a copper coil to control for possible effects of the electromagnetic field generated by the speaker (see below) (Figure 1). Temperatures were kept stable at 18ºC 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).

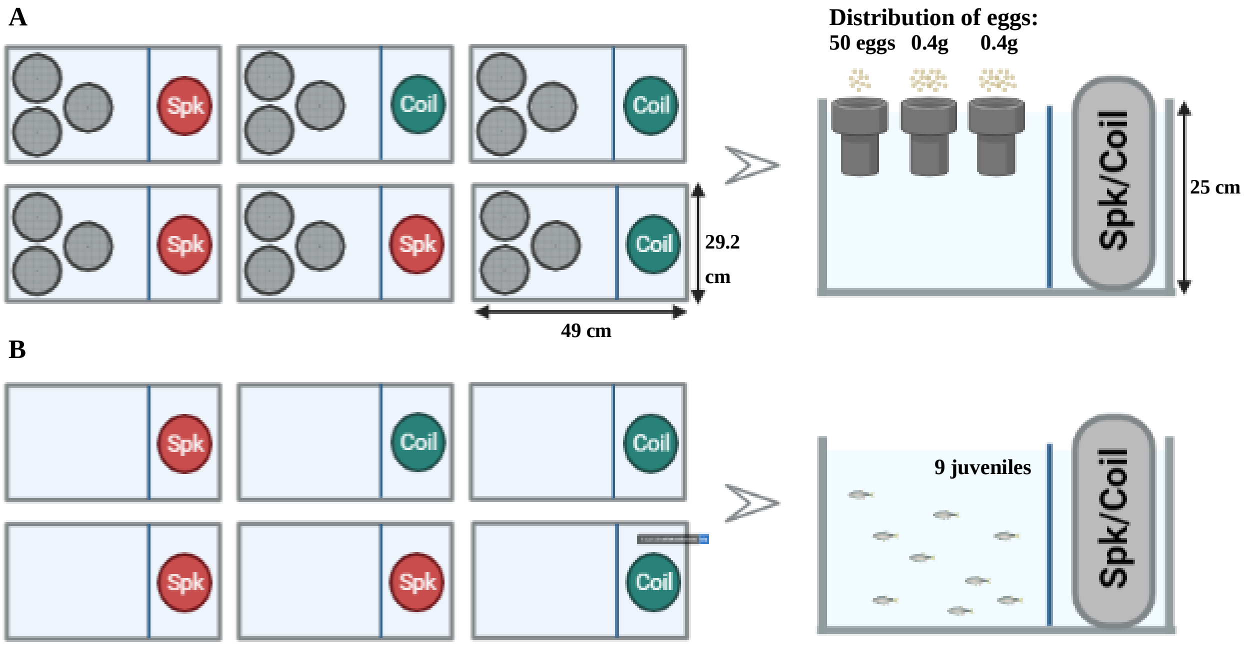

Figure 1- Schematic depicting of the experimental laboratorial setup to expose meagre eggs and larvae (A), and juveniles (B) to boat noise playback (Spk: Speaker) or control (Coil: electromagnetic coil). On (A) 3 small rearing boxes were used to distribute the eggs (also depicted). On (B) juveniles were free to swim on 2/3 of the tank.

For the eggs and larvae experiments, 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 (Figure 1A). 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 and the lights had to remain on to ensure that conditions were maintained.

For the juveniles' experiments, fish could move freely in 2/3 of the tank (Figure 1B). One camera (Nedis, ACAM40BK, UHD 4K) on top of each tank was also deployed to film the juveniles from above. In this case, the photoperiod was set under a 12h:12h light:dark cycle.

Sound stimuli

Boat noise playbacks were carried out using three synchronized JBL speakers (eggs and larvae experiments: mp3 device connected to FLIP Essential - 80 Hz-20 KHz, HARMAN; juveniles' experiment: computer connected to FLIP 5 - 65 Hz-20 KHz HARMAN) (Figures 1 and 2). Each speaker was placed inside a waterproof container, which in turn was vertically placed inside each treatment tank (3 speakers in total, see Figure 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 mimicked electromagnetic fields present during noise playback to control for their possible effects.

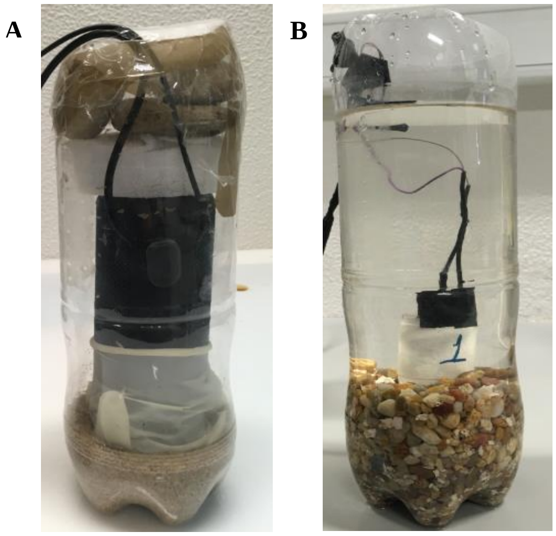

Figure 2 – A – Speaker (Playback condition) in a waterproof container; B – Coil (Control condition) in a container filled with freshwater.

To perform this silence treatment, we made copper coils with an impedance roughly similar to that of a loudspeaker, that were later positioned in the tanks (Figure 2B) similarly to 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 one control tank (3 in total) and kept inside a container with fresh water to guarantee no overheating would occur. 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 (also using Adobe Audition) 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 deployed in the treatment tanks (Figure S1). Note that operating coils did not produce measurable acoustic noise (see Figure 3).

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 might experience in Tagus estuary. To achieve this, a 1-hour file composed of these boat recordings interspersed with random silence periods was played on loop. The same file was used for control so that the electromagnetic coils would produce an electromagnetic field similar to the one of speakers. For the larvae experiments, continuous noise was played for the duration of the experiment, since the testing period was very short (4 days). On the juveniles' experiments, each day was composed of 6 hours of silence and 18 hours of playback in order to mimic the boat traffic of the Tagus estuary, considering, however, that the recording was made in the area around the pier of Base Aérea n.6 in Montijo.

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). Figure 3 compares the spectra of the boat noises recorded in the Tagus estuary and used to create the playbacks with the boat noise emitted by the speakers. Some differences were observed between speaker models (cf. Figure 3A and 3B), and both models were unable to produce the low frequencies present on the original recordings (< 100 Hz; cf. Figure 3C).

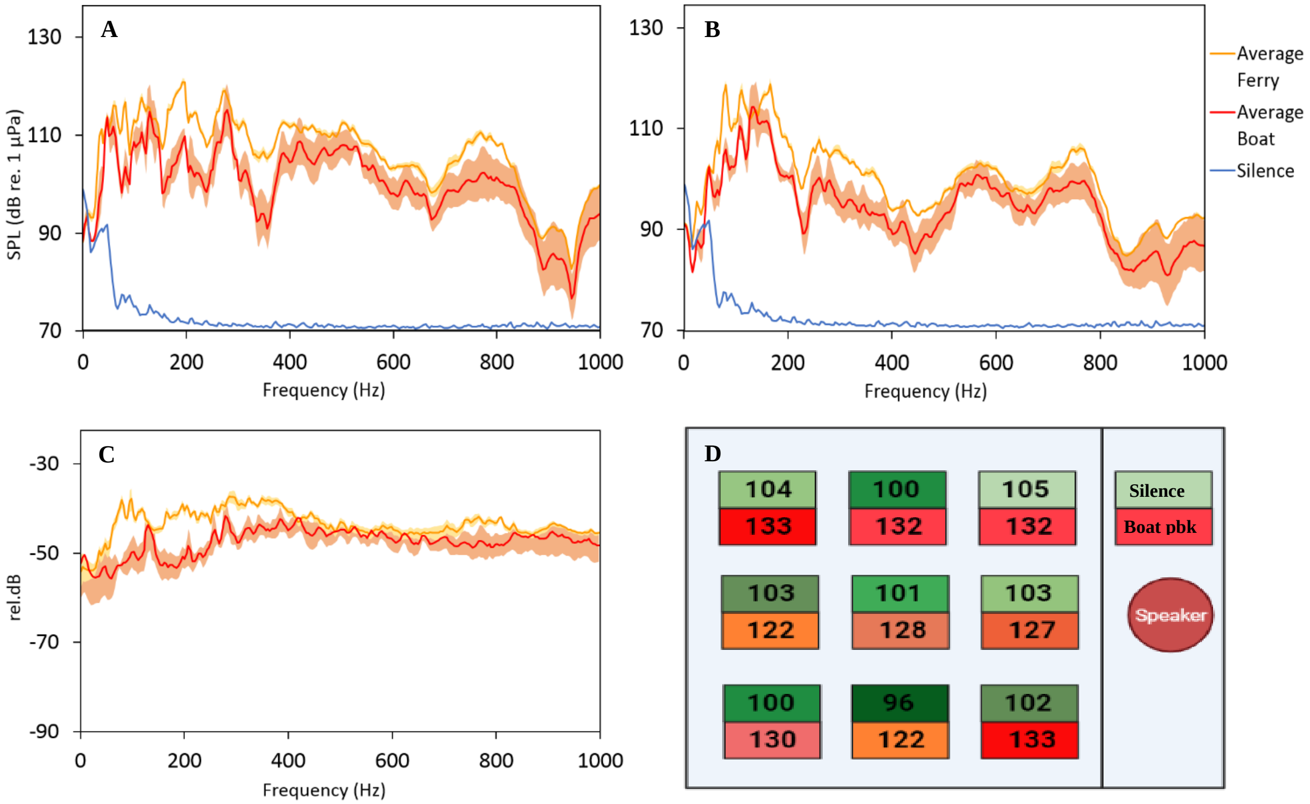

Figure 3 - Characterization of boat noise treatment. (A,B) playback spectra for the ferry and small boat noise, and silence for larvae experiment (A) and juveniles experiment (B). (C) The original spectra of different ferry and small boat sounds recorded at Air Force Base no. 6 in Tagus estuary. The frequencies of the sounds, in Hertz (Hz), are represented in the X-axis. The amplitude of the sounds, in decibels (dB, SPL) are represented in the Y-axis. (D) Sound pressure level characterization in 9 positions of one tank, while boat noise was played and when the playback was

halted. The values are presented in dB re. 1μPa. Note that for (D), only one ferry passage was used for this characterization.

For the eggs and larvae experimental set-up, noise levels varied from 126 dB (average noise level calculated in the 0-22 kHz bandwith; sd = 5.93) to 129 dB (sd = 1.27). Background noise level (control) was ca. 101 dB re. 1 μPa. (sd = 0.81), with the water filtering system turned off (Figure 3). For the juveniles' experiment it varied from an average of 122 dB (sd = 6.68) to 125 dB (sd = 1.15) for boat and ferry playback respectively. We chose a noise level average around 25 dB (sd = 2.94) above background noise since it corresponds approximately to the increase caused by a ferry boat, recorded around 50 to 100 meters 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).

As noise and control tanks were in close vicinity of each other, we also measured the propagation of the boat noise playbacks to the control tanks on both experiments. A maximum increase of ca. 5 dB above the background noise was observed (Figure S2). Most noise transmission from the treatment tanks to the control ones occurred in the 300-500 Hz bandwidth. Additionally, the sound pressure level characterization of the tanks exposed to boat noise playback was made by recording 9 points evenly disposed throughout the tank at a depth of 9 cm. Using the same boat noise playback, the noise level varied from 122 to 133 dB re. 1 μPa while background noise ranged from 96 to 105 dB re. 1 μPa (water filter system turned off) (Figure 3D).

2.2. Eggs and larvae experimental procedure

Eggs and larvae origin and maintenance

Experiments took place from March to July 2021. 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 (37º02′ N, 7º49′ W). IPMA is certified to perform experimental work with animals. The organization has DGAV—the Portuguese National Authority for Animal Health—authorization according to EU legislation for EPPO to breed, use and supply aquatic animals for scientific experimental work (DGAV reference 0421/000/000/2018).

Eggs were obtained from naturally spawning meagre (maturation and spawning were performed spontaneously under natural photoperiod and temperature conditions), were collected at the beginning of the night just after spawning (note that sciaenids are known to spawn at dusk (Holt et al., 1985)) and transferred to the experimental facilities at Faculdade de Ciências da Universidade de Lisboa (FCUL) in Lisbon, Portugal, under controlled temperature conditions (around 3h transport). At arrival, the eggs were immediately distributed by the experimental tanks. The following experimental protocol was repeated for the three batches that were used in this experiment.

Experimental protocol

At arrival, eggs were distributed by three small rearing boxes in each tank (six tanks in total, 3 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) (cf. Figure 1A).

Hatching success, based on the number of surviving larvae at hatching, was measured from the group of 50 eggs (box 1). From the larvae alive in box 2, 15 (45 per treatment) were sampled and fixed in 80% ethanol for morphometric measurements. We were not able to sample this number of larvae in every box (Table 1). At 2dph (days post-hatching), corresponding to the end of the experimental period, 15 larvae from box 3 (45 per treatment) were sampled and fixed in 80% ethanol for morphometric measurements, again we were not able to sample this number of larvae in every box (Table 1).

This process was repeated for the 6 tanks.

Table 1 - Number of meagre larvae sampled per batch, tank and rearing box.

Batch	**Tank**	***N* of larvae sampled (box 2/box 3)**
1	1	15 / 3
	2	15 / 3
	3	15 / 8
	4	16 / 8
	5	14 / -
	6	15 / -
2	1	14 / 4
	2	16 / 4
	3	13 / 2
	4	13 / 16
	5	16 / 11
	6	13 / 4
3	1	16 / 15
	2	12 / 13
	3	- / -

	4	15 / 15
	5	16 / 5
	6	15 / 4

Morphometry

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

Photographs were obtained with a digital camera (Moticam 10 10.0MP) 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) (Table 2), following the methodology used by Klimogianni et al. (2013). The length was measured parallel to the longitudinal axis and the depth was obtained perpendicularly (Table 2, Figure 4). Measurements were done using *ImageJ* (Schindelin et al., 2012).

Table 2 - Description of the morphometric traits of meagre larvae measured in this study (adapted from Klimogianni et al., 2013).

Abbreviation	**Character**	**Description**
SL	Standard Length	From tip of snout to the posterior margin of the spine
YSA	Yolk Sac Area	Maximum area of the yolk sac including the lipid globule
BD	Body Depth	Body height just posterior to anus
LDA	Lipid Droplet Area	Maximum area of the lipid droplet

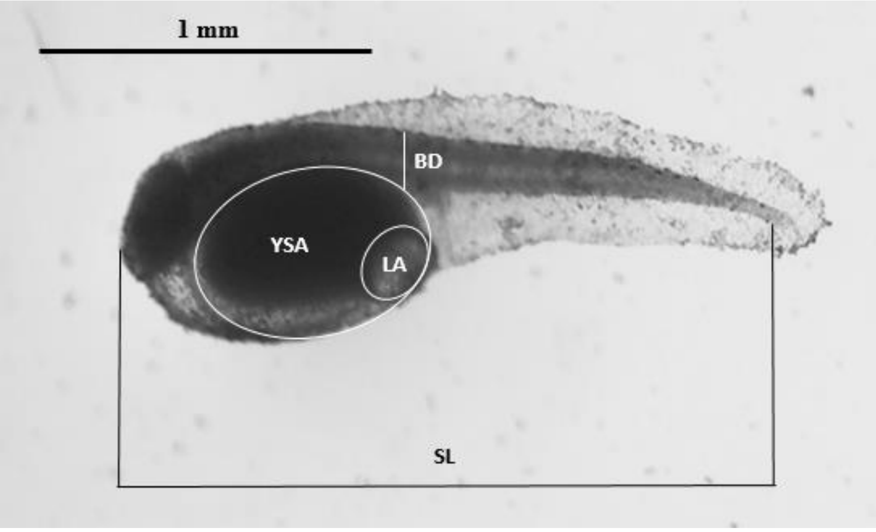

Figure 4 - Representation of the morphometric traits measured in meagre larvae. For abbreviations description view Table 2.

2.3. Juveniles' experimental procedure

Juvenile origin and maintenance

Experiments took place in July and August 2020. Meagre juveniles were also obtained from the aquaculture facilities of IPMA – EPPO and transported to the experimental facilities at Faculdade de Ciências da Universidade de Lisboa (FCUL). Fish were kept in 200L stock tanks equipped with a circulation pump and cooler (Hailea HC300A), and an external filter (Eheim 150), under a 12h:12h light:dark cycle, and fed twice a day with the same inert semi-moist feeds used in the aquaculture facility. Water temperature was kept at 18±1 ºC and salinity at 37±1.0‰.

For each experiment, juveniles were transferred to the experimental tanks to be exposed to: (1) playback of noise from ferries and boats (treatment) or (2) a coil with no added noise to recreate a similar electromagnetic field as that of the speakers (control). We used a total of 108 juvenile fish (sex undefined), with total length ranging from 1.4 to 2.7 cm (1.9 ± 0.3 cm; Mean ± SD). In the experimental tanks, fish were also fed twice a day. At each feeding occasion fish were counted and dead fish, if present, were annotated and removed from the tanks. The experiment was performed twice.

Experimental protocol

At the beginning of the experiment, nine fish were distributed per tank, and were immediately exposed to the sound stimulus as described above. Tanks were surrounded by opaque plastic foil to reduce possible stress caused by human presence.

Data was collected in two timepoints, at 2 and 7 days of exposure. At each timepoint, one feeding event was filmed, in the evening. Each tank was filmed for 10 minutes prior to the feeding event and for 5 min after.

Behaviour analysis

The videos obtained from days 2 and 7 of exposure were analyzed with the Behavioural Observation Research Interactive Software (BORIS) (Friard & Gamba, 2016), using the behaviour catalogue described in Table 3.

Table 3 - Ethogram of meagre juveniles

CODE	DESCRIPTION
ESCAPE	Moving away rapidly from other individuals
PURSUIT	Moving rapidly, accelerating, towards other individuals
APPROACH	Approaching other individuals at regular moving speed
WITHDRAWAL	Swimming away from other individuals at regular moving speed

The behaviours were registered starting ten minutes before the feeding event and continuing to five minutes after. However, for data analysis purposes, the three minutes prior and after feeding were excluded, in order to avoid the effect of arousal of the feeding on the juveniles' behaviour. Since approach and withdrawal occurred paired, as well as escape and pursuit, we focused the analysis on Approaches and Pursuits, standardizing the counts according to minutes and number of individuals.

Videos from each experiment were analysed with the sound muted and by an individual blind to treatment to avoid potential bias.

2.4. Statistical analysis

Eggs and larvae

We tested the effect of noise exposure on larvae morphometric traits with a Block Analysis of Variance, with treatment, timepoint and treatment/timepoint interaction as fixed factors and batch as the block effect, also a fixed factor. This was followed by LSD multiple comparison post hoc tests to verify pairwise differences. Additionally, a Mann-Whitney non-parametric U Test was used to compare the hatching rate of meagre eggs between treatments.

Juveniles

We tested the effect of noise exposure on juveniles' approach and pursuit behaviours, before feeding, with a General Linear Model (GLM) using treatment, timepoint and treatment/timepoint interaction as fixed factors and tank and batch as a random factor. However, batch and tank were removed from the model when they showed no significant results. This was followed by LSD multiple comparison post hoc tests to verify pairwise differences. Because feeding can increase juvenile interactions, we also tested the effect of treatment on behavioural changes caused by feeding. We carried out similar GLMs as above but using as dependent variables the ratios of post-feeding with the pre-feeding levels (i.e. the basal activity) for Approach and Pursuit, and for an aggressiveness score (Pursuit/(Pursuit+Approach)) post/pre feeding ratio, the latter allowed to examine the effect of noise and exposure duration on the proportion of pursuit behaviour in the interactions among juveniles.

All tests were done with Statistica 15.0 (Dell Software, Inc., Round Rock, T, USA)). All test assumptions were met.

2.5. Ethics

All experimental procedures were authorized by the Portuguese National Authority for Animal Health (Direção Geral de Alimentação e Veterinária), and were performed in line 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.

3. Results

3.1. Effects of boat noise exposure on larvae development

A period of approximately 48h elapsed from spawning to hatching. Hatching rate was not affected by the boat noise exposure (U = 8; p = 0.71), averaging 23% (± 3% SE) in the control group, and 28% (± 4%) in the boat noise exposure group.

In general, exposure to boat noise did not affect larval size (standard length (SL)) ($F_{(1,175)} = 0.88$; $p = 0.35$) or yolk sac area (YSA) ($F_{(1,176)} = 1.63$; $p = 0.20$), but it affected lipid droplet area (LDA), which was significantly smaller compared to larvae from control treatment ($F_{(1,175)} = 6.20$; $p = 0.01$) (Figure 5A, 5B and 5D). A marginally non-significant effect on body depth (BD) ($F_{(1,175)} = 3.25$; $p = 0.07$) was observed for specimens under boat noise (Figure 5C).

Table 4 - Measurements of larvae under boat noise treatment or control, at 0dph and 2dph (values are represented in mean ± SE format).

	Standard length (mm)		Yolk Sac Area (mm^2)		Lipid Droplet Area (mm^2)		Body Depth (mm)	
	Boat	Control	Boat	Control	Boat	Control	Boat	Control
0 dph	**1.929 ± 0.032**	**1.888 ± 0.032**	**0.326 ± 0.012**	**0.302 ± 0.015**	**0.031 ± 0.002**	**0.035 ± 0.001**	**0.174 ± 0.003**	**0.176 ± 0.002**
2 dph	**2.317 ± 0.092**	**2.246 ± 0.075**	**0.089 ± 0.007**	**0.088 ± 0.006**	**0.002 ± 0.001**	**0.008 ± 0.002**	**0.209 ± 0.006**	**0.221 ± 0.003**

Post-hoc comparisons indicated that, at hatch, exposure to boat noise did neither affect LDA nor BD. After 2 days of exposure, LDA was significantly smaller in boat noise exposed larvae (LSD; $p < 0.05$; Table S1) (0.002 ± 0.001 mm^2; mean ± SE; Table 4)), whereas larvae from the control group had an average 0.008 ± 0.002 mm^2 (Table 4). Even though boat noise did not affect BD at hatch, larvae exposed for 2 days presented a smaller BD (LSD; $p<0.05$; Table S2) (0.209 ± 0.006 mm; Table 4) than the controls (0.221 ± 0.003 mm; Table 4).

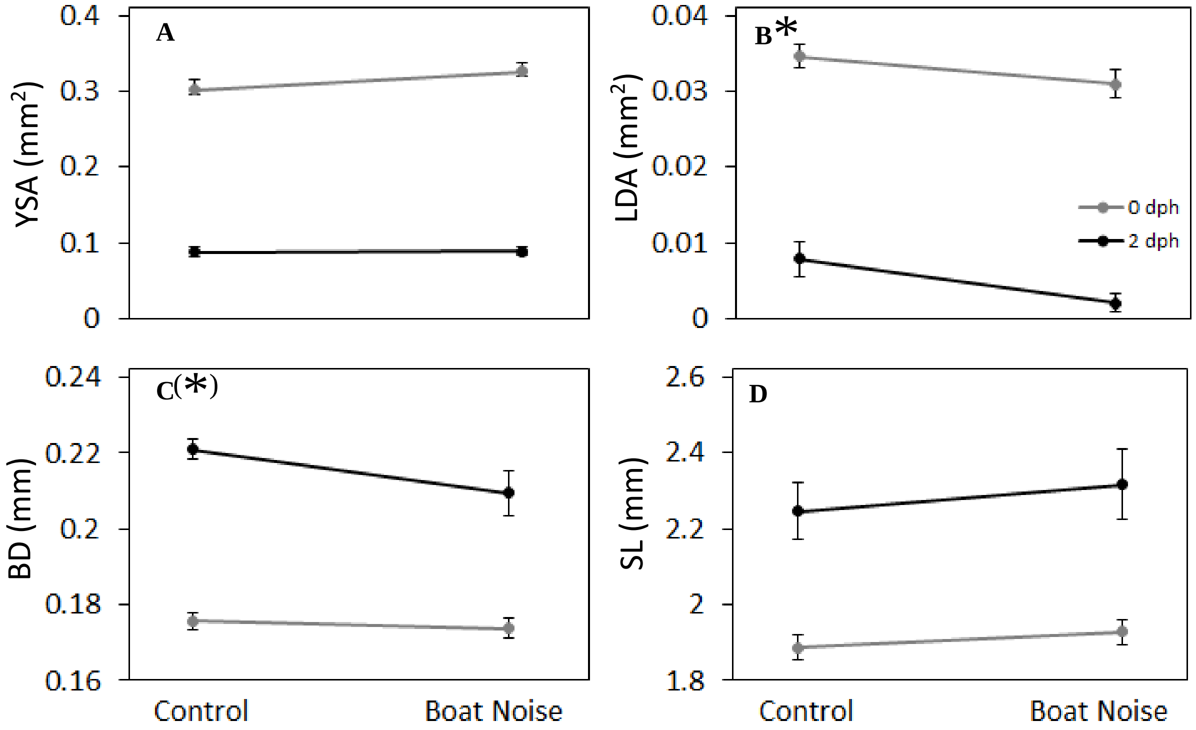

Figure 5 - Comparison of mean yolk sac area (A), lipid droplet area (B) body depth (C) and standard length (D) of larval meagre with 0dph (grey) and 2dph (black), exposed either to control or boat noise treatment. Each plot represents mean ± SE. Statistical results for treatment effect - * $p < 0.05$; $^{(*)}$ $p = 0.07$.

3.2. Effect of boat noise exposure on juvenile behaviour

The effect of noise exposure on approach and pursuit levels in the pre-feeding period (representing the basal activity level) was examined. For approach behaviour there was no significant effect of the noise treatment ($F_{(1,14)} = 2.92$; $p = 0.10$) and no effect of exposure duration ($F_{(1,14)} = 2.71$; $p = 0.12$). Post-hoc tests showed that there was a significant difference in the approach levels between treatments (LSD, $p < 0.5$) at 2 but not at 7 days of treatment (Figure 6A; Table S3). The interaction between treatment and exposure duration was significant ($F_{(1,14)} = 4.80$; $p = 0.04$) - there was a large decrease in approach levels between timepoints for control fish, but not for fish exposed to the boat noise treatment (Figure 6A).

Pursuit levels were not affected by boat noise ($F_{(1,14)} = 0.86$; $p = 0.37$), exposure duration (day 2 and day 7 of exposure) ($F_{(1,14)} = 2.84$; $p = 0.11$), or the interaction of noise treatment and exposure ($F_{(1,14)} = 0.03$; $p = 0.87$) (Figure 6B).

For the approach post-feeding/pre-feeding ratio we did not find either a treatment effect ($F_{(1,19)} = 0.77$; $p = 0.39$), nor an exposure duration effect ($F_{(1,19)} = 0.14$; $p = 0.72$). However, there was significant interaction between treatment and exposure duration ($F_{(1,19)} = 5.3$; $p = 0.03$) (Figure 6C; Table S4), indicating an increase in approach between timepoints in the control group, and a decrease in the treatment group. For the pursuit post-feeding/pre-feeding ratio no effect of treatment ($F_{(1,19)} = 1.82$; $p = 0.19$), exposure duration ($F_{(1,19)} = 0.31$; $p = 0.58$) or the interaction between the two ($F_{(1,19)} = 0.55$; $p = 0.47$) was found (Figure 6D).

Considering the aggressiveness score post-feeding/pre-feeding ratio, there was no treatment ($F_{(1,20)} = 0.04$; $p = 0.85$) or exposure duration ($F_{(1,20)} = 0.23$; $p = 0.64$) effect on juveniles' behaviour, but there was a marginal effect of the interaction of these two variables ($F_{(1,20)} = 3.75$; $p = 0.07$), with the levels of this ratio decreasing for the control and increasing for the noise group, from day 2 to 7 (Figure 6E; Table S5).

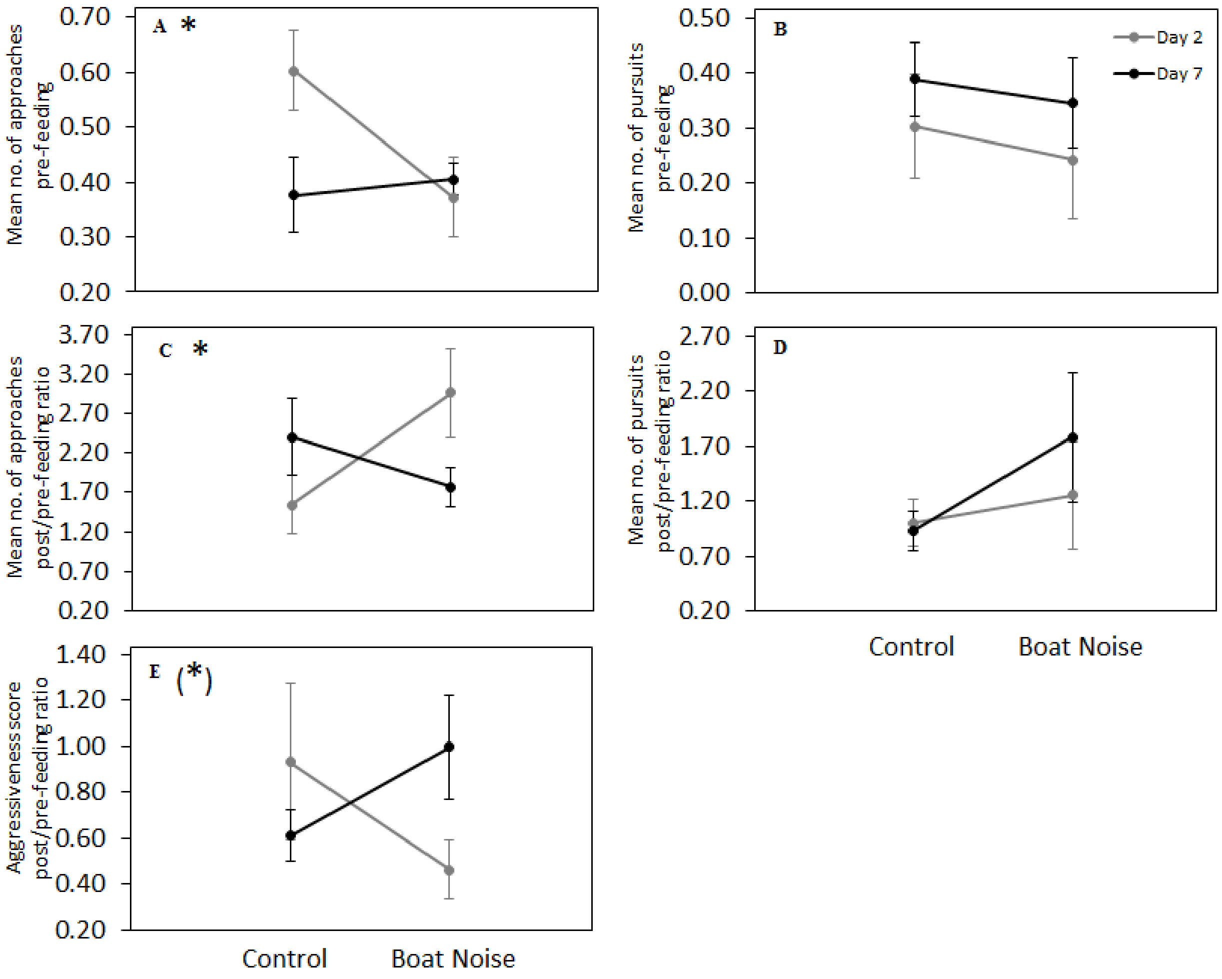

Figure 6 - Frequency of juveniles' behaviour exposed either to control or boat noise treatment, at day 2 (grey) and day 7 (black) of exposure. (A) pre-feeding approach; (B) pre-feeding pursuit. Furthermore, ratios of approach post/pre-feeding (C); pursuit post/pre-feeding (D) and aggressiveness score post/pre feeding ratio (G) are also shown. Each plot represents mean ± SE. * $p < 0.05$; (*) $p = 0.07$. Events are normalized per number of juveniles and per minute.

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 in early life stages and in different species. In the present study, we assessed the effects of boat noise in larval development and on juvenile behaviour in captive fish, 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 significant effects on morphometric traits related to energy use (lipid droplet area) and growth (body depth) in recently hatched larvae, suggesting an increased energy use under boat noise conditions. They also suggest a tendency for lower activity levels and increased aggressiveness in juveniles under boat noise treatments.

4.1. Effect on larvae development

We found no evidence of detrimental effects of exposure to boat noise playback on the hatching rate of meagre. This was an expected result considering the time of exposure. The time to hatch is quite short (around 48 hours), and exposure to treatments only started approximately 6 hours 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 & 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 (2dph). 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).

There were no significant differences in the yolk sac area between the group exposed to boat noise playback and the control group. These results contrast with the ones found for 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 & McCormick, 2019). Also, Lara and Vasconcelos (2021) have shown a similar impact of boat noise on zebrafish larvae (at 3dph and 5dph, 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 & McCormick, 2007). The impact this stressor might have on yolk sac consumption could be related to both physiological responses, or with the hearing range of the fishes tested, since different species can have different hearing ranges, which can mean different perception of sound and impact of the noise on the species.

Despite the lack of differences in yolk sac area, and interestingly, at 2dph, lipid droplet area was significantly smaller in larvae exposed to boat noise playback than in larvae exposed to the control treatment. 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 & 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*) 3dph 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). These results suggest that different stressors can lead to a higher usage of energy reserves by larvae.

A word of caution is needed when addressing the smaller size of lipid droplet at 2dph, since in several of our 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 smaller in larvae exposed to boat noise comparing to the control group. Despite the lack of differences on standard length at hatch and at 2dph, we did observe larvae with smaller body depth under boat noise, suggesting compromised growth and condition under more stressful circumstances. 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 and co-workers (2014) found that summer flounder larvae (*Paralichthys dentatus*) after 21dph were smaller in terms of length and body depth, when exposed high CO2 environments (pCO_2 mean of 4714 µatm and pH = 7.06).

In our study, the period of exposure to boat noise was short (4 days, i.e. an average of 2 days until hatching plus 2 more days post-hatching) and reflects the constraints of rearing larval fish after their exogenous feeding starts, in laboratory conditions. We should keep in mind that the effects of noise for longer exposures could be different, since species can adapt to the stressor, their response can change with time and/or there can be cumulative effects. Hence the importance of further investigation, with longer periods of exposure, to complement these findings.

Overall, our results point to some development effects of noise on larvae of meagre, particularly in terms of body depth and lipid reserves.

4.2. Effect on juvenile behaviour

Juvenile behaviour was influenced by feeding time. Before feeding, juveniles showed mainly behaviours such as approach, withdrawal, escape and pursuit, which we considered their basal activity. With the approach of feeding time there was an increase in their activity and a variation in their distribution in the aquarium, probably motivated by the presence of people. During feeding we could see an increase in pursuits and escapes probably related to competition for food (not measured). Just after feeding there was still a higher activity level in general, although food had already been consumed.

Social interactions (approaches) during basal activity (in the pre-feeding period) in juveniles exposed to boat noise were, at 2 days of exposure, less frequent than interactions in fish from the control group.

However, after 7 days of exposure, there were no detectable differences between groups. In the control group, these interactions decreased with time, which can indicate the establishment of social hierarchies, consistent with the observed decrease in the aggressive score in this group.

The establishment of social hierarchies has been shown to happen in different fish species, such as gilthead sea bream (*Sparus aurata*) juveniles (Montero et al. 2009). Still, in the boat noise exposed juveniles, the frequency of the approach behaviour was low throughout the exposure period, suggesting a depressed level of activity.

The ratio of approaches between post- and pre-feeding periods was >1 in both groups, suggesting feeding arousal or increased food search. Interestingly, the approach ratio increased from day 2 to day 7 in the control group after feeding, which suggests increase in arousal or exploratory behaviour with feeding. A general arousal would result in an increase of random encounters, translated by more approaches between individuals. Alternately, the increase in approaches could have been caused by an augment in exploratory behaviour to gain information on the feeding behaviour of other animals (to obtain information, for example, about the availability and location of food). Interestingly, the opposite trend was observed in the juveniles submitted to the noise treatment, suggesting that this post-feeding increase in activity is being affected by prolonged noise exposure. The suppressed levels of post feeding activity in the noise group are consistent with a tendency to increase the aggressive score after feeding, suggesting increased stress levels. In line with these observations, Pieniazek et al. (2020) registered a reduction in foraging in black bullhead (*Ameiurus melas*) when exposed to both boat noise and white noise, and Voellmy et al. (2014) observed a significant decrease in foraging activity in two sympatric species three-spined stickleback, *Gasterosteus aculeatus*, and the European minnow, *Phoxinus phoxinus*, when exposed to additional noise created from the recording of different cargo-ships.

Gendron et al. (2020) found that, when exposed to boat noise playback, winter flounder larvae (*Pseudopleuronectes americanus*) end up spending less of their time hunting, decreasing the number of attacks made. Noise induced stress can also increase the latency of response to predators' attacks, as was seen for whitetail damselfish (*Pomacentrus chrysuru*) at the end of the larval stage, when exposed to the playback of 2-stroke engine noise or 4-stroke engine noise, comparing to the ones exposed to playback of ambient reef sound (McCormick et al., 2019).

Aggressiveness score post-feeding/pre-feeding ratio tended to increase in fish exposed to boat noise and to decrease in fish exposed to the control treatment, over time. This goes in line with the social hierarchy establishment hypothesis, prevailing a reduction of aggressiveness in stable groups (Earley & Dugatkin, 2006) and an increase in social interactions with the arousal caused by food exploration. For juveniles exposed to boat noise, the opposite was observed – social interactions were reduced after feeding and the aggressiveness score increased. This increase in aggressiveness agrees with Bruintjes and Radford's findings (2013), who observed increased aggressive/submissive behaviour in the presence of boat noise playback in the cichlid *Neolamprologus pulcher*.

Altogether, despite the lack of a significant treatment effect, our results still suggest the existence of behavioural adjustments under boat noise, namely related to social interactions and aggressiveness events.

4.3. Final remarks

Our results showed an impact of boat noise playback on the management of energy reserves by meagre larvae, which increased their lipid droplet consumption when under this stressor. There was also a conditioning of their development illustrated by the smaller body depth achieved by larvae exposed to boat noise at 2dph. For juveniles, we observed an impact of boat noise on their social behaviour, reducing the number of approaches between day 2 and day 7 of exposure as well as a marginally significant increase on the aggressiveness score post- feeding/pre-feeding ratio on the noise exposed group from the day 2 to the day 7.

Results from the current experiments should be interpreted with caution as these are fish of aquaculture origin, chronically exposed to elevated noise caused by the use of water circulation, machinery and aeriation systems, that is different from the noise they would encounter in the wild, and could be around 20 to 50 dB above to their natural habitats (Bart et al., 2001; Wysocki et al., 2007; Caiger et al., 2012). In particular, lower frequencies between 25 and 1000 Hz are the loudest in aquaculture systems, with sound pressure levels ranging from 125 to 135 dB re 1 μPa (Bart el at., 2001), which overlaps with the hearing range of meagre (Vieira et al. 2021). Moreover, the sample size used was low, which might have prevented the detection of a treatment effect in some variables.

Acoustic analyses of the audio files showed that the boat playbacks were not an exact match to real boat noise (Figure 3A, 3B and 3C), which alerts us for the importance of taking the results obtained in a conservative way. Also, the acoustic field characteristics, namely particle motion patterns, are different in restricted environments compared to the open sea, and hardly replicable in aquaria, which could also influence our results (Popper et al., 2020). Moreover, the speakers were limited to reproducing boat noise in the low frequencies, both in terms of sound pressure and particle motion (Figure 3A and 3B).

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). Additionally, it would be highly relevant to use wild meagre larvae, in order to prevent the noise influence that aquaculture raised larvae are already subjected to. Combining both lab and field designs when possible could be the best approach, as made by Simpson and colleagues (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,

Overall, the current study adds to the growing body of literature addressing anthropogenic noise effects on fish, and further points to a species-specific response. Early development stages are a critical phase for fish and anthropogenic noise can severely affect these stages, potentially influencing survival and population dynamics. More studies are needed in order to provide accurate information for policy makers, so the impacts of this widespread stressor can be reduced.

6. References

Amorim, M. C. P. (2006). Diversity of Sound Production in Fish. In F. Ladich (Ed.), *Communication in Fishes* (Vol. 1, pp. 71–105). Science Publishers.

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. *Journal of the Marine Biological Association of the United Kingdom*, *88*(5), 1069–1073. https://doi.org/10.1017/S0025315408001677

Andersson, M. H., Andersson, S., Ahlsén, J., Andersson, L., Hammar, J., Persson, L. K., … Wikström, A. (2017). A framework for regulating underwater noise during pile driving. In *A technical Vindval report*. Stockholm, Sweden.

Bart, A. N., Clark, J., Young, J., & Zohar, Y. (2001). Underwater ambient noise measurements in aquaculture systems: A survey. *Aquacultural Engineering*, *25*(2), 99–110. https://doi.org/10.1016/S0144-8609(01)00074-7

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. *Transactions of the American Fisheries Society*, *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. *Scientific Reports*, *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., … Dekeling, R. P. A. (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

Bruintjes, R., & Radford, A. N. (2013). Context-dependent impacts of anthropogenic noise on individual and social behaviour in a cooperatively breeding fish. *Animal Behaviour*, *85*(6), 1343–1349. https://doi.org/10.1016/j.anbehav.2013.03.025

Cabral, H. N., & Ohmert, B. (2001). Diet of juvenile meagre, argyrosomus regius, within the tagus estuary. *Cahiers de Biologie Marine*, *42*(3), 289–293.

Caiger, P. E., Montgomery, J. C., & Radford, C. A. (2012). Chronic low-intensity noise exposure affects the hearing thresholds of juvenile snapper. *Marine Ecology Progress Series*, *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., … Phelan, B. A. (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

Cox, T. M., Ragen, T. J., Read, A. J., Vos, E., Baird, R. W., Balcomb, K., … Crum, L. (2006). Understanding the impacts of anthropogenic sound on beaked whales. *J. CETACEAN RES. MANAGE*, *7*(3), 177–187.

de Jong, K., Amorim, M. C. P., Fonseca, P. J., & Heubel, K. U. (2018). Noise Affects Multimodal Communication During Courtship in a Marine Fish. *Frontiers in Ecology and Evolution*, *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. *Reviews in Fish Biology and Fisheries*, *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., … Degraer, S. (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

Earley, R. L., & Dugatkin, L. A. (2006). Merging social hierarchies: Effects on dominance rank in male green swordtail fish (Xiphophorus helleri). *Behavioural Processes*, *73*(3), 290–298. https://doi.org/10.1016/j.beproc.2006.07.001

Fakan, E. P., & McCormick, M. I. (2019). Boat noise affects the early life history of two damselfishes. *Marine Pollution Bulletin*, *141*(January), 493–500. https://doi.org/10.1016/j.marpolbul.2019.02.054

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

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

Gagliano, Monica & Mccormick, Mark. (2007). Maternal condition influences phenotypic selection on offspring. *The Journal of animal ecology*, *76*, 174-82. 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). *Environmental Biology of Fishes*, *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). *Fisheries Research*, *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., … Peters, D. P. C. (2008). The changing landscape: Ecosystem responses to urbanization and pollution across climatic and societal gradients. *Frontiers in Ecology and the Environment*, *6*(5), 264–272. https://doi.org/10.1890/070147

Guevara-Fletcher, C., Alvarez, P., Sanchez, J. and Iglesias, J. (2017), The effect of temperature on the development of yolk-sac larvae of European hake (*Merluccius merluccius* L.) under laboratory conditions. *Aquaculture Research*, *48*, 1392-1405. https://doi.org/10.1111/are.12975

Hansen, B. H., Salaberria, I., Read, K. E., Wold, P. A., Hammer, K. M., Olsen, A. J., … Kjørsvik, E. (2019). Developmental effects in fish embryos exposed to oil dispersions – The impact of crude oil micro-droplets. *Marine Environmental Research*, *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. *Behavioural Ecology*, *30*(6), 1501–1511. https://doi.org/10.1093/beheco/arz114

Hawkins, A. D., Pembroke, A. E., & Popper, A. N. (2015). Information gaps in understanding the effects of noise on fishes and invertebrates. *Reviews in Fish Biology and Fisheries*, *25*(1), 39–64. https://doi.org/10.1007/s11160-014-9369-3

Hawkins, A. D., & Popper, A. N. (2018). Effects of Man-Made Sound on Fishes. In H. Slabbekoorn (Ed.), *Effects of Anthropogenic Noise on Animals* (Vol. 66, pp. 415–429). https://doi.org/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. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1863). https://doi.org/10.1098/rspb.2017.1627

Hildebrand, J. A. (2009). Anthropogenic and natural sources of ambient noise in the ocean. *Marine Ecology Progress Series*, *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. *Marine Ecology Progress Series*, *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. *Marine Ecology Progress Series*, *27*(1), 7.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium*, *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

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). *Journal of Aquatic Science*, *1*(1), 1–6. https://doi.org/10.12691/jas-1-1-1

Lagardère, J. P., & Mariani, A. (2006). Spawning sounds in meagre Argyrosomus regius recorded in the Gironde estuary, France. *Journal of Fish Biology*, *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. *Scientific Reports*, 33.

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*. 1–25.

Magnhagen, C., Johansson, K., & Sigray, P. (2017). Effects of motorboat noise on foraging behaviour in Eurasian perch and roach: A field experiment. *Marine Ecology Progress Series*, *564*(February), 115–125. https://doi.org/10.3354/meps11997

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. *Scientific Reports*, *9*(1), 1–10. https://doi.org/10.1038/s41598-019-43099-5

Middel, H., & Verones, F. (2017). Making marine noise pollution impacts heard: The case of cetaceans in the North Sea within life cycle impact assessment. *Sustainability (Switzerland)*, *9*(7). https://doi.org/10.3390/su9071138

Montero, D., Lalumera, G., Izquierdo, M. S., Caballero, M. J., Saroglia, M., & Tort, L. (2009). Establishment of dominance relationships in gilthead sea bream Sparus aurata juveniles during feeding: Effects on feeding behaviour, feed utilization and fish health. *Journal of Fish Biology*, *74*(4), 790–805. https://doi.org/10.1111/j.1095-8649.2008.02161.x

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. *Advances in Marine Biology*, *51*(06), 143–196. https://doi.org/10.1016/S0065-2881(06)51003-X

Munday, P. L., Watson, S. A., Parsons, D. M., King, A., Barr, N. G., McLeod, I. M., … Pether, S. M. J. (2016). Effects of elevated CO2 on early life history development of the yellowtail kingfish, Seriola lalandi, a large pelagic fish. *ICES Journal of Marine Science*, *73*(3). https://doi.org/10.1093/icesjms/fsv210

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. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1856). https://doi.org/10.1098/rspb.2017.0143

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). *Proceedings of the Royal Society B: Biological Sciences*, *282*(1817). https://doi.org/10.1098/rspb.2015.1943

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.

Payne, R., & Webb, D. (1971). Orientation By Means of Long Range Acoustic Signaling in Baleen Whales. *Annals of the New York Academy of Sciences*, *188*(1), 110–141. https://doi.org/10.1111/j.1749-6632.1971.tb13093.x

Peng, C., Zhao, X., & Liu, G. (2015). Noise in the sea and its impacts on marine organisms. *International Journal of Environmental Research and Public 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

Pieniazek, R. H., Mickle, M. F., & Higgs, D. M. (2020). Comparative analysis of noise effects on wild and captive freshwater fish behaviour. *Animal Behaviour*, *168*(September), 129–135. https://doi.org/10.1016/j.anbehav.2020.08.004

Pollard, D. A., & Bizsel, K. (2020). Argyrosomus regius. The IUCN Red List of Threatened Species 2020: *e.T198706A*.https://doi.org/https://dx.doi.org/10.2305/IUCN.UK.2020-.RLTS.T198706A130099146.en. Downloaded on 08 April 2021

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

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., & Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. *Journal of Fish Biology*, *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., … Tavolga, W. N. (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? *Behavioural Ecology*, *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. *Journal of Experimental Biology*, *209*(22), 4444–4451. https://doi.org/10.1242/jeb.02525

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

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

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. *Nature Communications*, *7*(1), 1–7. https://doi.org/10.1038/ncomms10544

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. *Fisheries Science*, *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. *Marine Ecology Progress Series*, *207*, 219–224. https://doi.org/10.3354/meps207219

Vieira, M., Beauchaud, M., Amorim, M. C. P., & Fonseca, P. J. (2021). Boat noise affects meagre (Argyrosomus regius) hearing and vocal behaviour. *Marine Pollution Bulletin*, *172*, 112824.

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*(7), 1–8. https://doi.org/10.1371/journal.pone.0102946

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

Wysocki, L. E., Amoser, S., & Ladich, F. (2007). Diversity in ambient noise in European freshwater habitats: Noise levels, spectral profiles, and impact on fishes. *The Journal of the Acoustical Society of America*, *121*(5), 2559–2566. https://doi.org/10.1121/1.2713661

Appendices

Appendix I – STATE OF THE ART

1. Anthropogenic Noise

The industrial revolution changed the way humans interacted with the natural world and, since then, the impact on terrestrial and aquatic environments has been growing exponentially (Grimm et al., 2008). Shannon et al., (2016) reviewed the impact that all the human sound producing activities, containing no useful information (Hildebrand, 2009), have been creating on wildlife. Before the 90's, there is not a lot of records of anthropogenic noise in the marine soundscape, however we can see that there has been an increase of at least 12 dB in the last thirty or so years in some locations (de Jong et al., 2020).

1.1. Types of anthropogenic noise

Anthropogenic noise sources that are present in the sea, lakes or rivers, have quite different acoustic characteristics, and can be generated by a diverse number of activities and processes (Popper & Hawkins, 2019). Anthropogenic sound can thus be divided in transient/impulsive or continuous sounds (Popper & Hawkins, 2019). Impulsive sounds are provoked mainly by explosions, seismic surveys, sonar or pile driving, are usually present for a limited period of time, during a specific project or intervention, are short (less than a second), can have big amplitude shifts, and can be repetitive or isolated (Hildebrand, 2009; Williams at al., 2015; Popper & Hawkins, 2019). Commercial and recreational boats or wind turbines are responsible for more continuous sounds, since they can last for an indefinite period (Hildebrand, 2009; Popper et al., 2014; Williams at al., 2015; Hawkins et al., 2015).

1.1.1. Transient/impulsive anthropogenic sounds

Explosions are mainly used in construction or removal of underwater infrastructure, such as oil platforms or in military exercises (Popper et al., 2014; Hawkins et al., 2015). They cause a spherical shock wave that travels faster than sound in water, and a gas bubble that radiates sound (Popper et al., 2014; Hawkins et al., 2015). Near the epicenter, the pressure rise is practically instantaneous

(Popper et al., 2014; Hawkins et al., 2015). These acute conditions can injure aquatic life even at a considerable distance (Popper et al., 2014; Hawkins et al., 2015).

Airguns are used to survey the substrate for seismic exploration, especially for oil and natural gas and, using a compressed air supply, they can produce an air bubble that expands fast creating an impulsive signal with pressure rise being considerate but slower than in explosions (Hildebrand, 2009; Popper et al., 2014; Hawkins et al., 2015). One airgun can generate an omnidirectional sound impulse with energy in the order of 20-50Hz (low frequencies) (Hawkins et al., 2015). 12 to 48 airguns can be shot at the same time, while they are towed behind the survey vessel, these shots happening regularly every 10 to 16 seconds and lasting from hours to several days (Popper et al., 2014; Hawkins et al., 2015). When all this acoustic energy reaches the bottom of the ocean, large interface waves can generate intense vertical and horizontal particle motion inside the substrate, detectable by marine life (Hawkins et al., 2015).

Pile driving is a method used to put foundations into the ocean floor, in the building of bridges, buildings, walls, wind turbines or oil and gas industry structures (Popper et al., 2014). It usually consists of multiple strikes with short intervals (one second or two) over a usually prolonged time period, consisting thus in an important source of underwater noise (Hildebrand, 2009; Hawkins et al., 2015). This activity can be divided into vibrating pile driving (which is considered a continuous sound source) and impact pile driving (Popper & Hawkins, 2019). Impact pile driving generates high peak pressures and can affect nearby animals since the strikes of a hydraulic hammer cause vibration of the pile in the water and substrate, generating sound (Popper & Hawkins, 2019).

Sonar is broadly used for multiple activities such as fishing (to find fish or monitor fishing nets), mapping the seabed topographically, depth sounding or military surveillance (detection of submarines or other systems of importance) (Hildebrand, 2009; Hawkins et al., 2015). Although not all types of sonar are prejudicial, if operating above hearing ranges of fish and invertebrates, between 10 and 800 kHz (mostly ultrasonic frequencies), there is a significant influence on ambient noise since there are so many active throughout the world at the same time (Hawkins et al., 2015). Low-frequency active (LFA) sonars are, however, used for military purposes, such as long-range detection of submarines (hundreds of kilometers away) and operate in a frequency range of 100 to 500 Hz (Hildebrand, 2009; Hawkins et al., 2015). Their signals can be very intense and can cause temporary hearing loss in some species (Popper et al., 2007).

1.1.2. Continuous anthropogenic sounds

Vibration pile driving consists of rotation of masses that create a vertical vibratory force that produces a continuous sound with low fundamental frequency dependent on the speed of rotation of the vibratory hammer, usually around 30 Hz (Popper et al., 2014; Hawkins et al., 2015).

Off-shore wind farms have been on the rising since the beginning of the century, especially in the European coasts, which gave rise to concern about the impact of the noise they generate on sea life (Popper & Hawkins, 2019). However, information on the acoustics of these turbines is still very limited (Popper & Hawkins, 2019). The turbine noise of wind farms propagates into the tower and foundations being transmitted to the water and to the seabed, with frequencies usually bellow 700Hz (Hawkins et al., 2015). During construction, sound is high and can acutely impact marine life and, during its functioning, the sound produced is low, but its continuity has the potential to generate chronic impacts on fish since they can detect the particle motion generated over long periods (Hawkins et al., 2015; Williams et al., 2015; Popper & Hawkins, 2019).

Growth of the human population drove a huge increase in marine traffic, contributing to the mixture and even replacement of biological noise in some areas with mainly low-frequency (<1kHz) shipping noise. (Frisk, 2012; de Jong et al., 2020). The noise created by boats can derive from boat machinery, propeller blade cavitation or hydrodynamic flow at the hull (Popper & Hawkins, 2019). Large shipping vessels, operating over wide geographical areas, emit low frequency sounds that can be heard over hundreds of kilometers, resulting in chronic exposure and potentially interfering with species communication in the same frequency range (Hawkins et al., 2015; Popper & Hawkins, 2019). Recreational boats tend to produce mid-frequency sounds, from 1kHz to 5 kHz, producing lower sound levels than ships, due to their reduced size and horsepower, however, speed increase can contribute to the raising of the sound level (Hildebrand, 2009). These noise sources have serious implications in the masking of important biological sounds, which should be detected, for example, by fish (Popper et al., 2014).

1.2. Known effects in:

1.2.1. Different species

The impact of anthropogenic noise on marine ecosystems has become a very important research topic, since pollution and destruction of habitats have been increasing due to the continuous growth of the human population (Middel & Verones, 2017; Popper & Hawkins, 2019). Thus, it is important to explore how this stressor affects different species in a way that allows the proper implementation of regulation to mitigate the impacts (Middel & Verones, 2017).

Peng and colleagues (2015) did an extensive review on the impact of underwater noise in different species, from marine mammals and marine reptiles to invertebrates.

Considering marine mammals, there is a variation of sensitivity and range of hearing. Fin whales (*Balaenoptera physalus*) has a wider hearing range (0,01 kHz to 10 kHz) than sea lions (*Zalophus californianus*) which can hear between 1kHz and 10kHz (Slabbekoorn et al., 2010; Peng et al., 2015). Cetaceans depend on sound for most of their tasks, from communication, navigation, hunting, foraging to protection (Mooney et al., 2012). So, being hearing specialists, their survival can be compromised if anthropogenic noises impact any of these abilities (Mooney et al., 2012). Blainville's beaked whale (*Mesoplodon densirostris*) were found to have an interruption and alteration of its feeding behaviour and an avoidance behaviour when in the presence of simulated and actual mid-frequency sonar exercises (Tyack et al., 2011). Several stranding events of beaked whales have been reported and associated with naval exercises occurring in the areas of stranding (with high intensity sources mainly) and some of these animals presented internal hemorrhages at necropsy, but other disruptions in orientation or behaviour could be also responsible for these situations (Cox et al., 2006). Masking is another important effect and some cetaceans have been altering their communication behaviour in order to beat this problem. Male humpback whales (*Megaptera novaeangliae*) can change their sexual displays when in the presence of sonar sounds (Miller et al., 2000) and bottlenose dolphins (*Tursiops truncatus*) produce whistles with higher frequencies when in the presence of elevated low frequency noise, from high boat traffic for example (Gospic'& Picciulin, 2016).

Marine reptiles such as marine turtles also suffer impacts of anthropogenic noise. Loggerhead turtles (*Caretta caretta*) show avoidance behaviour in the presence of seismic airguns' sounds (DeRuiter & Larbi Doukara, 2012) and comparison of stranding data of Kemp's ridley sea turtle (*Lepidochelys kempi*) has showed that there might be a relation between these events and offshore prospection explosions (Klima et al., 1988; Peng et al., 2015). However, data for marine reptiles on the impact of anthropogenic noise is still very much lacking (Peng et al., 2015).

Information on effect of noise on invertebrates usually comes in industry or government reports, being peer-review papers not very common (Peng et al., 2015; Costa et al., 2016). Studying the impact of anthropogenic noise on invertebrates is hard, since there are a lot of different species from quite a few phyla, conversely, with different behavioural and physiological responses (Costa et al., 2016). Tidau and Briffa (2019) showed that European hermit crabs (*Pagurus bernhardus*) can suffer from disruption of their social behaviour in the presence of boat noise, when compared to exposition to ambient sound. In a different study, it was shown that Caribbean hermit crabs (*Coenobita clypeatus*), when exposed to boat noise playback, allowed predators (simulated) to approach closer before hiding (Chan et al., 2010). Low-frequency noise has also been shown to induce massive acoustic trauma in at least four cephalopod species, in laboratory conditions, due to alteration of the sensorial hair cells of statocysts (balance and orientation receptor present in some aquatic invertebrates) (André et al., 2011; Costa et al. 2016). Considering high intensity sounds, scallop larvae when exposed to seismic pulses in the laboratory, showed development delays and almost half of the individuals developed body malformations (De Soto et al., 2013). Likewise, giant squids (*Architeuthis* spp.) were found to have atypical mass stranding events due to the noise created by seismic surveys in the area and in the necropsy, they were found to have lesions of the statocysts and other internal tissues (Guerra et al., 2011).

1.2.2. Effects in fish:

The impact of anthropogenic noise has been mostly studied on marine mammals (Payne & Webb, 1971; Cox et al., 2006; Peng et al., 2015; Middel & Verones, 2017) but, nowadays, the relevance of its effects on fish is well established (Popper & Hastings 2009; Radford et al., 2014; Popper & Hawkins, 2019). Sound dependence varies along different fish taxa and can occur in detection of prey or predators, communication between individuals (Amorim, 2006; Amorim et al., 2008) or navigation and selection of reefs or other habitats to live or settle in (Tolimieri et al., 2000; Montgomery et al., 2006). Even when not directly used, sound has proven to be important to acquire information about the environment and its soundscape, allowing for an integrative approach to the environment when combined with other cues (Popper, 2003; Hawkins & Popper, 2018). Accordingly, anthropogenic noise will inevitably have an impact in the different stages of the life cycle, compromising different aspects of the animal’s life and interfering with conspecific and heterospecific interactions.

There are different effects (Table 5) that anthropogenic noise can have in fish and a set criteria and guidelines were established in the USA by Popper et al. (2014). These guidelines are only provided for the sound sources for which there is enough information, and the levels of noise might vary considering the species, the sound source and the circumstances (Popper et al., 2014).

Table 5 - Definition of effects of anthropogenic noise, used in Guidelines. Adapted from Popper et al. (2014).

Effect	Definition
Mortality or mortal injury	Immediate or delayed death.
Recoverable Injury	Hair cell damage, internal or external minor hematoma (injuries that don’t result in death).
Temporary Threshold Shift (TTS)	Any change in hearing of more than 6 dB that persists (may or may not reduce fitness) for a short or long period.
Masking	Impairment of hearing sensitivity by more than 6dB due to noise presence.
Behavioural changes	Substantial change in behaviour. Long-term change in behaviour and distribution, altering migration patterns, changing feeding or reproduction sites. Startle response or small movements not included.

Popper and his colleagues (2014) focused on the morphology of the auditory apparatus of different fish groups (Table 6), on the principal sound types and on the major effects in order to create the criteria in which to base the guidelines, considering the available information.

Table 6 - Groups of fish by hearing apparatus morphology and criteria for mortality and recoverable injury. Sound pressure in used even for fishes without swimbladders since no data for particle motion existed. Relative risk (high, moderate, low) is given for individuals at three distances from the source defined in relative terms: SB- swimbladder; PM – particle motion; SP – sound pressure; SELcum (cumulative Sound Exposure Level); N - near; I - intermediate; F – far. Adapted from Popper et al., (2014).

Morphology of the fish	**Mortality/Mortal Injury**	**Recoverable Injury**	**TTS**	**Masking**	**Behaviour**
No SB (PM detection)	> 219 dB SEL$_{cum}$ or > 213 dB peak	> 216 dB SEL$_{cum}$ or > 213 dB peak	>>> 186 dB SEL$_{cum}$	(N) Moderate (I) Low (F) Low	(N) High (I) Moderate (F) Low
SB not involved in hearing (PM detection)	210 dB SEL$_{cum}$ or > 207 dB peak	203 dB SEL$_{cum}$ or > 207 dB peak	> 186 dB SEL$_{cum}$	(N) Moderate (I) Low (F) Low	(N) High (I) Moderate (F) Low
SB involved in hearing (SP detection)	207 dB SEL$_{cum}$ or > 207 dB peak 210	203 dB SEL$_{cum}$ or > 207 dB peak	186 dB SEL$_{cum}$	(N) High (I) High (F) Moderate	(N) High (I) High (F) Moderate
Eggs & Larvae	> 210 dB SELcum or >207 dB peak 203	(N) Moderate (I) Low (F) Low	(N) Moderate (I) Low (F) Low	(N) Moderate (I) Low (F) Low	(N) Moderate (I) Low (F) Low

However, there are still major gaps in research that need to be filled before we can effectively understand the impact of anthropogenic noise on fish. One of the main concerns demonstrated by Popper and Hawkins (2019) is the lack of quality studies, considering the amount of experiments done on captive fish, which can lack representation in the natural worlds. Additionally, there is almost no information on the influence on particle motion of the diversity of anthropogenic noises, which is important considering that a lot of fish species don't detect sound pressure (Popper & Hawkins, 2019). In Europe, there are no specific criteria or guidelines for sound exposure on the EU Marine Strategy Framework Directive (MSFD) of 2008 (Popper & Hawkins, 2019), however, a report by Andersson and colleagues (2017) was published about the regulation of pile drive underwater sounds.

1.2.2.1. Early life stages;

Currently, not a lot of studies on eggs, larvae and juveniles have been done, most studies on fish have focused on adults which compromises our knowledge on the real anthropogenic noise impact of these early life stages.

Otoliths are fully developed one or two days post-hatching (Carroll et al., 2017), however, hearing in smaller larvae is less sensitive than larger ones, depending on the species of fish analyzed, especially for higher frequencies, as shown by Wright and colleagues (2011), for *Epinephelus coioides*, a serranid that, using an electrophysiological technique called auditory brainstem response (ABR) could respond to 600Hz sounds when larvae were 9 to 13 mm, increasing to 2000Hz in larvae of 24 to 28 mm.

Impulsive sounds appear to have the most impact on these early life stages (though they could also be the most tested ones). Payne et al. (2009) showed that there was significantly more mortality between capelin eggs (*Mallotus villosus*) three days after being exposed to seismic energy (around 199 dB peak to peak) and control, but monkfish larvae (*Lophius americanus*) exposed to the same conditions were found to have no significance difference in mortality or morbidity 48 hours after exposure. Bolle and co-workers (2012) showed that for pile driving sounds with SEL_{cum} of 206 dB re 1 mPa2s, no significance effect in survival of different stages of common sole larvae (*Solea solea*) was found, but no effects other than survival were tested. These results were supported by Hammar and his team (2014) who also showed that the common sole larvae where tolerant to pile driving noise levels correspondent of a 100m distance from the sound source.

Herbert-Read and colleagues (2017) showed that the playback of pile driving sound on juvenile sea bass (*Dicentrarchus labrax*) compared with the playback of ambient noise induced a decrease in directional and speed organization of the shoals and increase the distance between fish. Another interesting find was of Simpson and co-workers (2016) that show that post-settling young damselfish (*Pomacentrus amboinensis*), which are usually exposed to ambush and pursuit techniques from predators, were more likely to be killed by predators when exposed to motorboat noise playback and direct disturbance by motor boat, since they responded fewer times and slower to simulated predatory strikes in experimental reefs.

Andersson and colleagues (2017) produced a report to help regulate underwater noise during pile driving, summarizing the existing literature on the effect of impulsive sound on survival during early stages of life

Besides sound level mortality, behaviour and development can also be important to study as it was done in a study comparing the effects of regular and random noise, such as ship recordings, to control ambient noises with atlantic cod larvae (*Gadus morhua*): it showed that two days of noise (either regular or random) was enough to reduce growth and, for regular sound, to increase yolk sac consumption (Nedelec et al., 2015). After two weeks the growth ended up converging, however, larvae with lower width-length ratios were more easily predated (Nedelec et al., 2015).

Eggs, larvae and juveniles should benefit from a more specific approach in matters of anthropogenic noise impact since they are vulnerable, have a small size and considerably reduced mobility (Popper et al., 2014). Additionally, the different early life stages should be examined separately since there could be differences in physical damage, survival or settlement behaviour and habitat choice, depending on the development (Popper et al., 2014; Hawkins et al., 2015).

1.2.2.2. Adults;

Species-specific fitness impact and population dynamics alterations are hard to predict since fish may swim away from noise sources, can get used to them or can even compensate this disturbance in some way to avoid its long-term negative effects (Bolle et al., 2012; Harding et al., 2019). However, we know that different taxa are suffering effects such as shifts in hearing thresholds, physical and physiological damages, behavioural alterations and the masking of acoustic cues and signals (Caiger et al., 2012; Holles et al., 2013; Peng et al., 2015; de Jong et al., 2020).

Adult rainbow trouts (*Oncorhynchus mykiss*) were exposed to sound pressure levels from low-frequency sonar higher than they would probably be in the wild which caused a maximum of a 20 dB shift in auditory threshold, which changed from group to group, suggesting individual specific tolerance, and there was no mortality registered immediately after exposure or until one week after (Popper et al., 2007). This TTS has, however, the potential to, in the wild, compromise fish survival, since they could have difficulties hearing predators, mates and their surroundings (Popper et al., 2005). Other salmonids have been tested for anthropogenic noise impacts, such as the broad whitefish (*Coregonus nasus*), that when exposed to a seismic airgun at a higher exposure sound level, showed small to no physiological effects (Popper et al., 2005). McCauley and colleagues (2003) had previously tested the effect of airgun impulsive sound on caged pink snappers (*Pagrus auratus*), showing that damage in the structure of the ears was present 18 hours after testing until 58 days after exposure, still, there was no information about the concrete effect on the fishes' hearing.

Anthropogenic noise can create different behavioural responses in marine fish, which can have a deleterious effect on population and individual survival, even though these are difficult to monitor *in situ* since it is hard to observe behaviour changes in a large unlimited area (Carroll et al., 2017). Therefore, most are done in labs or in cages, compromising the illation of results to the wild, since they often do non exhibit the whole range of wild behaviours (Carroll et al., 2017; Popper & Hawkins, 2019).

Nevertheless, Shafiei Sabet and collaborators (2016) compared zebrafish (*Danio rerio*) and Lake Victoria cichlids (*Haplochromis piceatus*), two species with different hearing sensitivities, and discovered that, for both, experimental sound exposure induced a reduction in the swimming speed in the first minute of exposure, and zebrafish also presented a startle response, which was not found in the cichlids, that changed position in the tank, moving downwards. Similar and species-specific behavioural shifts were shown in this study, independent of the hearing sensitivity (Shafiei Sabet et al., 2016).

Vasconcelos and co-workers (2007) showed that the presence of ferryboats slowly approaching the nesting area of Lusitanian toadfishes (*Halobatrachus didactylus*), can also affect hearing sensitivity, but through substantial masking at almost all frequencies, compromising the detection of conspecific sounds. This ship noise was calculated from a 20m distance, which highlights the higher impact at a closer range from the ship (Vasconcelos et al., 2007).

These studies showed above are just some examples of all the research that has been exponentially increasing in the last decades, in accordance with the rising concern of the impacts of anthropogenic noise on fish.

2. Acoustic communication in fish

2.1. Context and function of acoustic communication/signals/cues

For a long time, fish acoustic sensibility has been a topic of interest for researchers, and it is now well established that probably the totality of fish are able to hear and use sound to learn about and interact with their environment (Simpson et al., 2004; Popper & Hawkins, 2019). Different acoustic sources, from biotic to abiotic, constitute the orchestra that plays the soundscape of the sea. Marine life produces sounds in different contexts, intentionally, such as courtship displays, attraction of mates, agonistic behaviour, territorial protection, distress, presence of predators or passively, derived from mechanical movements such as feeding or moving (Hawkins & Amorim, 2000; Cox et al., 2006; Amorim et al., 2008; Coquereau et al., 2016; Pereira et al., 2020). Additionally, wind, rain and waves contribute by producing noise from 150-200Hz up (Montgomery et al., 2006; Radford et al., 2010).

Calls related with reproduction can have specific functions as it's been showed by multiple researchers. Thus, sound can be produced during courtship as we can see in the male haddock

(*Melanogrammus Aeglefinus*), that does so throughout its courtship behaviours (Hawkins & Amorim, 2000); as a facilitator of aggregation of spawning groups as male and female meagers (*Argyrosomus Regius*) do, producing great chorus formed by several individuals (Vieira et al., 2019; Bolgan et al., 2020) or as a mediator of male versus male interactions such as in Lusitanian toadfish (*Halobatrachus didactylus*)(Amorim & Vasconcelos, 2008; Hawkins & Amorim, 2000). These sounds produced in the reproduction context can carry important information on species, location, individual characteristics and motivation (Amorim et al., 2015).

Acoustic communication is also used in agonistic behaviour and territorial protection. This type of behaviour is an important part of social communication among individuals, intra and interspecifically, allowing for a good regulation of the access to food, space or reproductive mates, independent of season, age or sex (Ladich, 1997). Marine and freshwater fish produce these sounds when disturbed, handled or during territorial or male-male conflicts in an aggressive way, typically when an opponent is seen by another fish and are usually accompanied by other displays like color change, fin spreading or erection of dorsal fins, as it happens with *Polypterus retropinnis*, or in a submissive way, by the fleeing fish in a fight (Ladich, 1997). Another example of this behaviour is the Lusitanian toadfish males, that usually defend their nests from other male intruders, by producing sequences of boatwhistles (Amorim et al., 2015) or the male haddock that usually produce sounds during disputes for territory or females, with other males (Hawkins & Amorim, 2000). Related with agonistic behaviour, it is also known that distress is another situation in which fish produce sound, either when approached by predators or when caught or manipulated by humans (Ladich, 1997).

Different habitats have, therefore, distinct soundscapes, presenting different sound signatures that can be helpful for larvae to locate their preferred settlement habitat more effectively (Radford et al., 2010; Salas et al., 2019). The rasping feeding activity of sea urchins (*Evechinus chloroticus*) is thought to be the main sound source of the temperate reefs of New Zealand and in Australian waters the snapping shrimp plays a major role in the evening reef noise, that can be heard for more than 10 kilometers away (McCauley & Cato, 2000; Montgomery et al., 2006). Larvae can use this soundscape to navigate at a long range or select habitats at a close range, so the distance at which they can detect sound is important for their success, and it can be compromised by noise (without useful directional information) from abiotic or anthropogenic sources that mask relevant data

(Tolimieri et al., 2000; Montgomery et al., 2006; Salas et al., 2019). However, waves breaking on coast has been showed to provide a shoreward orientation cue for some fish or crustacean larvae (Montgomery et al., 2006). Identifying the noises that attract larvae can help create measures to improve recruitment in places where pollution or overfishing has impacted the habitat (Simpson et al., 2004).

2.2. Ontogeny of hearing abilities

2.2.1. Larvae hearing abilities, acoustic signals/cues dependence

Until not long ago it was thought that larvae would change position only due to physical processes (Roberts, 1997), as inert units. However, we now know that fish larvae are not passive particles and actively contribute to their dispersal and settlement processes, navigating with the help of swimming abilities and sensory cues that are available, contributing for the maintenance of balance in their population dynamic (Montgomery et al., 2006; Caiger et al. 2012). These cues are very diverse and range from chemical or hydrodynamic cues, magnetic or electrical fields and polarized light to sound (Montgomery et al., 2006).

Underwater sound travels in all directions, is current independent and is transmitted fast, achieving long distances without great attenuation, which shows its potential carrying directional habitat information that can be used by fish (Tolimieri et al., 2000; Leis & McCormick, 2002; Montgomery et al., 2006; Caiger et al., 2012). A consistent amount of studies has shown that the pelagic larval stage of some fish and invertebrates is able to navigate towards biological underwater noise (Tolimieri et al., 2000; Montgomery et al. 2006, Radford et al. 2010). For example, Simpson and colleagues (2004) showed that light traps deployed with speakers playing biological noise recorded at dusk in reef habitat had significantly more larvae catches, than light traps without this record being played.

These sound cues can be heard by larvae since sensory organs begin to form during larval development (Leis & McCormick, 2002). Morphologic changes in size or shape of the swimbladder or in its distance to the inner ear can affect the pressure detection sensitivity, as showed by Salas and co-workers' (2019) predictions, in which we can see that pressure sensitivity is better in smaller fish (shorter distance between the bladder and the inner ear), but compensated by the enlargement of the swimbladder with growth, suggesting the availability of this system throughout the larval stage. Hence, larvae of different species of fish that lack adaptations such as extensions of the bladder may still be prepared to sense the pressure component of sound besides the particle motion, when the bladder inflates (Salas et al., 2019). This sensitivity to pressure might be very important to settlement of fish larvae that depend on the acoustic cues for that.

2.3. Sound reception

There is great diversity in ear morphology in fish, suggesting diverse hearing mechanisms. (Popper & Hawkins, 2019). However, in general, fish detect sound using a pair of inner ears that are constituted of three semi-circular channels in relation with sensory regions, as the ampullae, responsible for the detection of angular acceleration, and the saccule, lagena and utricle (3 otoliths) involved in hearing and with positional senses (Popper & Hawkins, 2019).

Orientation, balance and hearing are therefore controlled by a membranous labyrinth system where dense calcium carbonate structures, the otoliths, are in close relation with the macula (sensory epithelium) that has hair cells that trigger nerve impulses when neural transmitters are released (Leis & McCormick, 2002; Wright et al., 2011; Salas et al., 2019). This release occurs with particle motion, when the macula moves associated to the otolith (Wright et al., 2011; Salas et al., 2019). Interestingly, these hair cells allow for determination of the direction of sound, since they are polarized and organized in groups that bend in the same direction, depending on the particle motion perceived, aiding fish in the determination of direction by comparison with the information provided by the different groups (Popper & Hawkins, 2019).

Fish primarily detect sound by using the particle motion component of a longitudinal acoustic wave (Popper & Hawkins, 2019). Fish tissue moves in accordance to the compression and rarefaction of water particles in response to an acoustic stimulus, allowing for sound detection through external particle motion and it is the retarded motion of the otoliths (which are denser) that allows for the deflection of the maculae hair cells, as seen above (Salas et al., 2019).

Furthermore, in some species, otoliths also interact with the swimbladder when inflated allowing for the detection of the pressure component of sound, contributing to auditory cue detection at longer distances than the ones predicted for fish that use only particle motion, likely contributing to survival (Wright et al., 2011; Salas et al., 2019). This can happen when there is a mechanical coupling of the bladder with the otoliths, through Webber’s ossicles or swimbladder extensions, that can position the swimmbladder close enough to the ear so that the particle motion created by it can get to the otoliths (Salas et al., 2019).

There is great diversity in ways of sound perception for fish, being particle motion and pressure the components that they can detect (Popper & Hawkins, 2019). Some fish are only able to use the particle motion, having no swimmbladder, and they are called generalists being able to hear up to 1 kHz; some can use both, the specialists, which can hear up to several kHz, even though the pressure component can contribute in different degrees to the hearing process, depending on the distance between the bladder and the otoliths, however the hearing process is not completely understood yet, so morphology and earing sensibility are not yet completely relatable (Ramcharitar et al., 2006; Wright et al., 2011; Salas et al., 2019).

Popper and colleagues (2014) divided fish into different categories considering their hearing apparatus morphology, while designing the guidelines for sound exposure, as we can see summarized in the Table 7 bellow:

Table 7 - Division of fishes into groups. SB – Swimbladder; PM – Particle motion; SP – Sound pressure. Adapted from Popper and Hawkins (2019).

Group	Characteristics	Frequency range	Some examples
1	No SB + Only PM	Narrow band	Flatfishes, rays and sharks
2	SB (not used for hearing) + sensitive to PM	Narrow band	Salmonids, some tunas and mackerels
3	SB (close to the ear but not connected) + sensitive to SP and PM	<500 Hz	Cod fishes, eels, Sciaenids,
4	SB (Structures linking to the ear) + sensitive SP mainly (also detect PM)	Until several kHz	Squirrelfishes, Sciaenids, herrings, otophysan fishes

2.3.1. Mechanisms of sound production

Fish have different sound producing mechanisms depending on the species, using different organs and relying mainly on two major strategies, making the swimbladder vibrate with specialized muscles or provoking the stridulation of bony structures (Fine & Parmentier, 2015; Parmentier et al., 2017). These mechanisms evolved in an independent and occasional way, because of similar environmental constraints, and therefore tend, due to mechanical and functional reasons, to need the same organs, like the swimbladder, bones or teeth and can be found in closely related or far apart species (Kasumyan, 2008; Parmentier et al., 2017).

Stridulation mechanisms can be produced by rubbing together different body parts such as teeth, bones or fin rays and are often made of a series of irregular temporary pulses with a great frequency range, usually higher than swimbladder produced sounds (Kasumyan, 2008; Fine & Parmentier, 2015). Fish in many lineages possess developed pharyngeal teeth and use pharyngeal movements to process and chew the food they ingest creating stridulation sounds during this feeding process but also in distress situations, when held by humans for example. (Rachmaritar et al., 2006; Kasumyan, 2008; Parmentier et al., 2017). Buccal teeth can be used for the same purpose, as damselfish do in different contexts, by snapping their jaw they provoke sound onset and create vibration waves that are transmitted to the ribcage and the swimbladder (Parmentier et al., 2017). Seahorses and pipefish produce sound rubbing the bones of their skull during feeding and competition or distress situations (Parmentier et al., 2017). Sound is produced by the rapid backward movement of the head that causes a strike between two unpaired cranial plates, the supraoccipital and the postcranial (Kasumyan, 2008; Parmentier et al., 2017). Finally, another way of producing stridulation sounds is by using pectoral structures, as fin rays and catfishes do (Kasumyan, 2008; Parmentier et al., 2017).

Some fishes, like the sciaenids, produce drumming sounds by rapidly contracting sonic muscles, that are in close relation with the swimbladder, making its wall vibrate producing audible sounds, each pulse of sound resulting from a single contraction of these muscles (Ramcharitar et al., 2006). There are, however, different structures of this apparatus, the swimmbladders can vary in size and shape and there is a great variability in the degree of development of these sonic muscles, even in the same family, as it is seen in Sciaenids (Kasumyan, 2008; Popper & Hawkins, 2019). Also, the sonic muscles can be present in only males or in both male and female individuals, depending on the species (Kasumyan, 2008).

These muscles can be extrinsic, originating from different parts of the skull, ribs or vertebral bodies and inserting on the swimbladder directly or on a bone/tendon connected to it, or intrinsic, that are exclusively attached to the wall of the bladder, not having other origins or insertions (Fine & Parmentier, 2015) Some fish can have both extrinsic and intrinsic muscles together, such has the northern sea robin (*Prionotus carolinus*) (Kasumyan, 2008).

The intrinsic muscles can be of different types (Figure 7) and are present in different toadfishes, sea robins and several other families such as Dactylopteridae or Scorpaenidae, however, more seldomly than extrinsic muscles (Kasumyan, 2008). Intrinsic muscles form a strong muscle envelope, usually with fibers attached with a dorso-ventral orientation on the swimbladder, being superfast, producing high frequency contractions (Kasumyan, 2008; Fine & Parmentier, 2015).

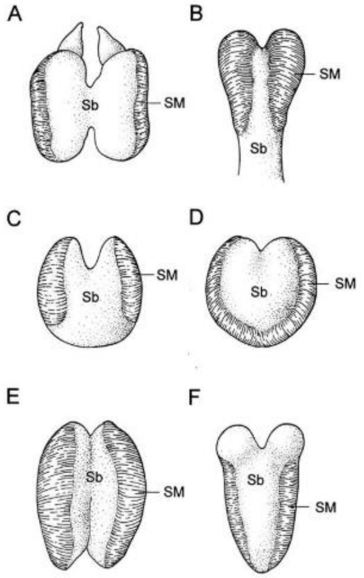

Figure 7 - Different types of intrinsic sonic muscles in teleost fishes. A - *Dactylopterus* sp. (family Dactylopteridae). B - *Lota lota* (family Gadidae). C - *Porichthys notatus* (family Batrachoididae). D - Toadfish, *Opsanus tau* (family Batrachoididae); E: Norther Searobin, *Prionotus carolinus* (family Triglidae). F- European gurnard (family Triglidae). Sb— swimbladder, SM—sonic muscle. Adapted from Ladich and Fine (2006).

Extrinsic muscles (Figure 8) can have a direct or indirect action, depending on whether their end is directly attached to the swimmbladder or attached to another structure, like connective tissue or a narrow and flexible protrusion like the ramus Mülleri, which in turn, is connected to the swimmbladder (Ladich & Fine, 2006). Some fish have both types of extrinsic sonic muscles which allows them to produce more complex sounds (Kasumyan, 2008).

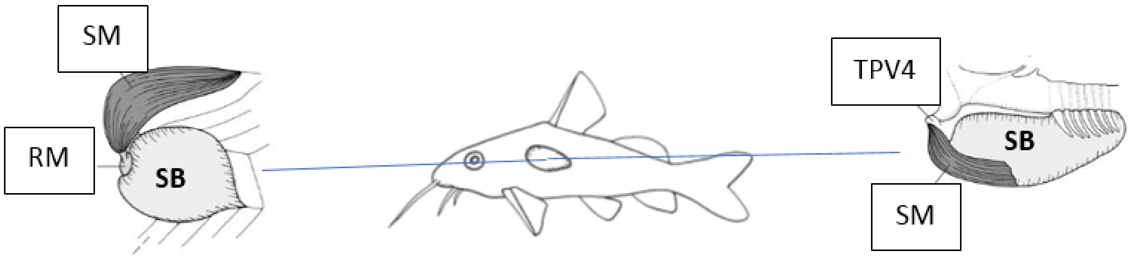

Figure 8 - Two examples of extrinsic swimbladder types in catfishes (Siluriformes). On the left: The sonic muscle (SM) is in connection to the ramus Mülleri (RM) shown, that connects to the swimbladder (SB). On the right: The sonic muscle (SM) is inserted in the transverse process of the 4th vertebra (TVP4) and then directly attached to the swimbladder (SB). Adapted from Ladich and Fine (2006).

In addition to these two main mechanisms, there are other ways that fish produce sound, usually in an unintentional way. Cavitation sounds are produced during feeding and swimming causes hydrodynamic sounds provoked by water movements (Kasamyan, 2008; Parmentier et al., 2017). These sounds can inform other fishes but it unclear whether they can be considered a proper form of communication (Kasamyan, 2008; Parmentier et al., 2017).

2.3.2. Diversity of sounds (time and frequency domains)

Communication depends on the capacity of the receiver to retrieve information from a sound wave produced by another individual (conspecific or not), in the presence of other signals, and its efficacy can be assessed by a behaviour alteration of the recipient (Ladich, 1997; Ramcharitar et al., 2006).

The main features of acoustic signals are frequency and time and they can give cues and inform about the characteristics of the emitter, however, comparatively to birds and mammals, which produce frequencies higher than 1kHz, vocal fish emit acoustic signals which are usually simpler, comprising a slight modulated frequency that usually ranges from 100-1000Hz, and that varies in number, repetition rate and duration (Amorim, 2006; Ramcharitar et al., 2006). This appears to happen due to the physical constraints of the water medium (compared with air) and also due to physiological constraints related to the sonic muscles physiology which are limited by their contraction rate and the acoustic characteristics of the swimbladder, which if moved at low speed won’t produce sound (Ladich & Fine, 2006).

In general, fish have a great range of sound producing mechanisms and differences in frequency, pulse duration, repetition rate and number of pulses per call can be found among members of the same family, individuals of the same species (large or small individuals) and even in the same individual (function of the call or temperature regimes during the same call), especially in the time domain, which can provide sufficient information for communication (Amorim, 2006; Ladich & Fine, 2006; Ramcharitar et al., 2006).

Hawkins and Rasmussen (1978) studied the agonistic sounds of different species of the family Gadidae and showed that all the sounds had low-frequency and could be distinguished mainly by the time domain structure (Figure 9). Additionally, closely related species can have distinct courtship sounds, with different patterns and pulse repetition rates which could aid in mate choice and in competition situations. In *Pollimyrus* spp., grunts vary in the pulse repetition rate and moans differ in duration and fundamental frequency, which should allow for species recognition (Crawford et al., 1997).

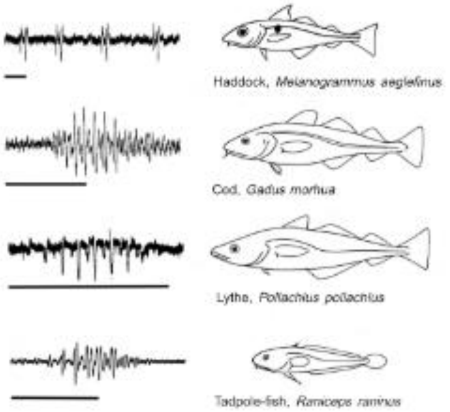

Figure 9 - Agonistic sounds produced by different members of the family Gadidae. The black bar is the time scale of 100 ms for each fish. The sounds represented are made up of low frequency rapidly repeated pulses emitted at different rates. Adapted from Amorim (2006).

Courtship sounds of different individuals from the same species have a clear distinction which indicates that the acoustic displays themselves can help with the mate choice, by encoding relevant information about the individual which is vocalizing, as it has been shown for the gulf toadfish (*Opsanus beta*) (Thorson & Fine, 2003). Additionally, the female Lusitanian toadfish can obtain information about the male condition based on the number of pulses and consequently the mating sound duration, and the higher the duration, the better the condition of the individual (Amorim et al., 2010). This is especially important considering that this species' females only mate with one male (Amorim, 2006; Amorim et al., 2010).

Acoustic communication in vocal species of fishes has been broadly studied, however, it is not always easy to establish comparisons between sound characteristics. The naming of sound types, the choice of parameters measured, or the precision of data has not always been coincident which creates confusion and compromises future studies (Popper & Hawkins, 2019).

3. *ARGYROSUMUS REGIUS*

3.1. Life cycle

Belonging to the Sciaenidae family, the meagre (*Argyrosumus regius*, Asso 1801) is a teleost that inhabits the subtropical waters of the European coast, including the Black Sea and the Mediterranean, and the African coast of the Atlantic ocean (Cabral & Ohmert, 2001; González-Quirós et al., 2011). This species can reach up to two meters in length, growing rapidly during the first years of life, reducing its growth rate after maturation (González-Quirós et al., 2011).

Spawning usually occurs from March to August inside estuaries or saltmarshes, considering the presence of big and mature specimens observed is these habitats alongside early life stages such as larvae and juveniles. Interestingly, the *Argyrosumus japonicus* also reproduces in association with these habitats (González-Quirós et al., 2011). During the reproduction season, meagre forms aggregations of large numbers using vocalizations, such has long grunts, to facilitate successful mating process in the highly turbid waters of these habitats, which leads to fertilization in courtship made pairs (Largardère & Mariani, 2006).

After that, juveniles usually recruit to the coastal areas, probably around their birth place, progressively expand their habitat, migrating or changing behaviour until they reach maturity and return to one of those habitats to reproduce, however, there isn't still certainty about whether they migrate back to the place they were born in to reproduce or even if they use the same habitat every year (González-Quirós et al., 2011).

In the last decades, concerns about the conservation status of meagre have been raised (Prista el at., 2009). Despite their high fertility rate, the spawning and reproduction sites are usually damaged by pollution and degradation which compromises the populations’ stability (Jiménez et al., 2005). Moreover, meagre suffers a great pressure from fishing, being the mature big individuals the targeted ones, since fishermen can identify their aggregative calls which helps them place the gillnets as close as possible (Largardère & Mariani, 2006). However, meagre is a promising species for aquaculture which has also helped increase the number of studies addressing the biology of the wild populations in order to improve aquaculture production (Jiménez et al., 2005) These mature reproducing adults are very important for the continuity of the population, since they can produce the greatest quantity of viable eggs.

3.2. Acoustic communication in meagre

3.2.1. Sound generating apparatus

The Sciaenidae family, commonly called croakers or drummers, has some members that produce sounds by contracting a pair of extrinsic sonic muscles against the swimbladder wall, causing its vibration, each muscle twist being able to originate multiple vibrations (Ramcharitar et al., 2006; Largardère & Mariani, 2006; Vieira et al., 2019; Pereira et al., 2020). This ability is usually a male characteristic, however, in meagre, both male and female can produce sounds, being these specific muscles present in both genders (Ramcharitar, et al., 2006; Vieira et al., 2019; Pereira et al., 2020).

Sexual dimorphism is found in what concerns this sound producing apparatus: in males, we can find two red bands formed by the sonic muscles, that are symmetrical and run along the posterior part of the body cavity, closely related to the swimbladder (in meagre an oval chamber with walls 1cm thick), which has paired lateral diverticula, and that experience a seasonal cycle, being enlarged during the spawning period (Figure 10) (Largardère & Mariani, 2006; Fine & Parmentier, 2015). On the other hand, female have smaller sonic muscles, occupying the same position in the body cavity in relation to the swimbladder, located between the vertebral column and the viscera and, if they are gravid, the muscles have a pink color and are thinner (Largardère & Mariani, 2006).

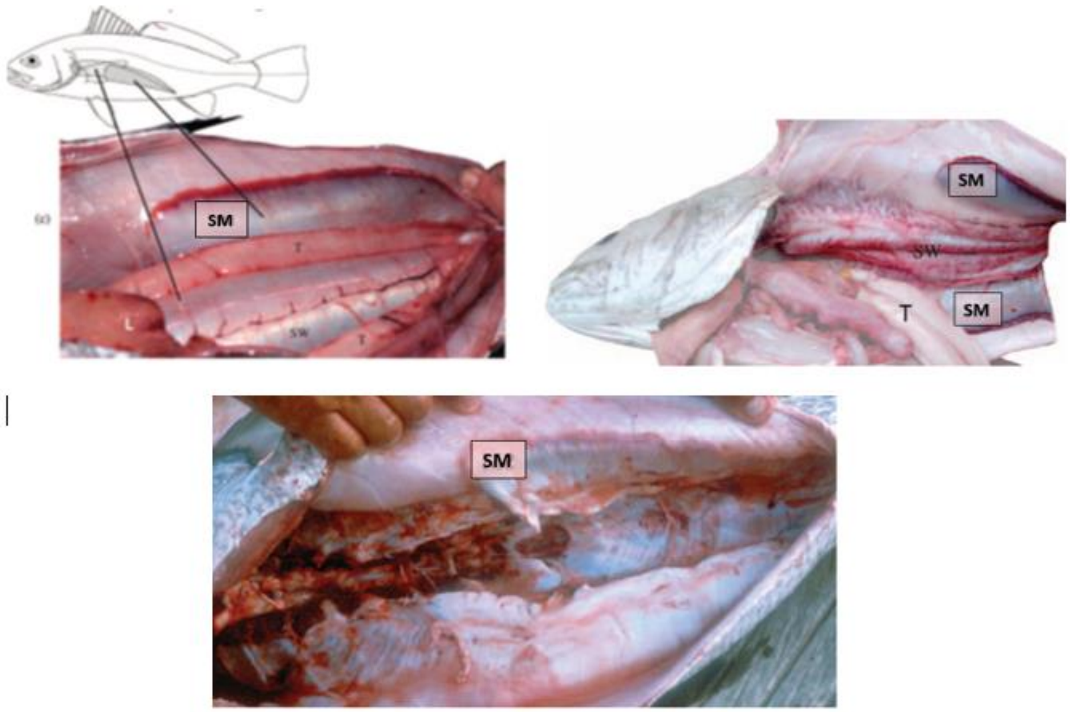

Figure 10 - Body cavity of 3 meagres, where we can see the differences in the sonic muscles shape and color: on the upper left-corner the body cavity of a sperm-producing male with large red sonic muscles; on the upper right corner a non-sperm producing male where we can see a change of color in the sonic muscles; on the bottom a pre-spawning female with less developed pink sonic muscles. SM – sonic muscle; T - testicle; L – liver; SW – swimbladder. Adapted from Lagardère and Mariani, (2006).

Sonic muscles are usually dark red since they are well vascularized, and are formed by very thin fibers, perpendicular to the swimbladder longer axis, and are around 30 µm in diameter (Ono & Poss, 1982). Several motor neurons innervate these mitochondrial rich fibers which allows for typical rapid contractions of the sonic muscles without being subject to tetany or spasms (Ramcharitar et al., 2006; Fine & Parmentier, 2015). Additionally, even though there aren’t antagonistic muscles present, these muscles are able to return to their shape immediately after contraction due to the pressure of the swimbladder or even to the elasticity of the lateral body musculature (Ramcharitar et al., 2006).

Usually the sonic muscles develop during the puberty and continue to grow, allowing for larger fish, with longer muscles and fibers, to produce longer pulses with lower peak frequencies, since they will take longer to complete a twitch (Tellechea et al., 2010; Fine et Parmentier, 2015; Pereira et al., 2020). It is not yet known from which age meagre begin to produce sound, nevertheless Pereira et al., (2020) found that juveniles of a total length of 9 cm didn't produce distress sounds (Figure 11), however, bigger studied juvenile fish (>30cm) were able to produce distress and social sounds.

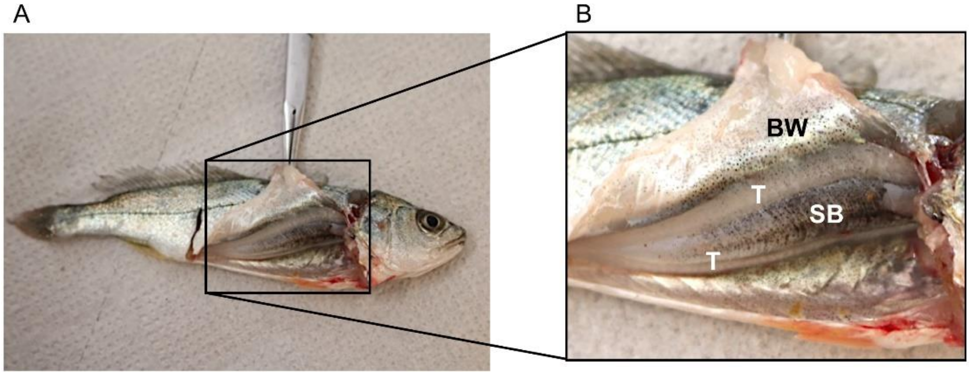

Figure 11- Juveline male meagre (9cm) without sonic muscles yet present. T – testicles; SB – swimbladder; BW – body wall. Retrieved from Pereira et al. (2020).

3.2.2. Acoustic repertoire

Acoustic communication in meagre is used in spawning aggregations, typically in an advertisement chorus, as described by Vieira et al. (2019), but also in disturbance contexts and voluntary situations (Pereira et al., 2020). Pereira and colleagues (2020) showed that there is variability in the sounds produced and that it can be associated with fish size, sex, social context or ontogenetic phase. For example, adults and juveniles produced very different disturbance sounds when handled, depending on their size (Pereira et al., 2020). This event can be related with the fact that longer muscles (present in larger individuals), need more time to twitch, consequently originating longer pulses (Pereira et al., 2020). Male and female meagre produce different sounds, being the acoustic parameters of sound studied (duration, period, peak frequency) higher in males (Pereira et al., 2020).

These calls can have different characteristics depending on the context they are used in: on one hand, in a voluntary context they are long and have a big number of pulses per call, on the other hand, disturbance sounds are short and have less pulses (Pereira et al., 2020; Bolgan et al., 2020). For juveniles and adults, differences are found in both contexts, mostly related to fish size (Pereira et al., 2020).

To date, different types of sounds have been identified such as knocks (1-3 pulses) and grunts (short, intermediate and long), which are formed by a different number of pulses, the basic unit of the acoustic structure of each sound produced, generated by each muscle twitch (Vieira et al., 2019; Bolgan et al., 2020). Even though knocks and grunts sound different, they have the same structure which suggests that they are produced similarly (Bolgan et al., 2020).

So far, it is not possible to associate behaviour significance to all the sounds produced by meagre, since this lacks association testing (Bolgan et al.,2020) and the ability of meagre to extract information based on these sound differences still lacks testing (Pereira et al., 2020). However, the wide range of sounds produced suggests the importance of this communication mean for the species.

4. Knowledge gaps in research on the impacts of anthropogenic noise on fish

Further appropriate research on the impacts of anthropogenic noise on fish can help scientists, policy makers and industries in implementing serious guidelines considering sound thresholds and mitigation measures (Shannon et al., 2016).

There are so many fish species that it is hard to have proper information that will steer guidelines and orientations that will protect them all equally. Accordingly, the division of fish in groups for which we can find good representatives must be a priority. Popper and co-workers (2014) suggested a division based on the swimbladder morphology, its presence or absence, and on the life stage of the fish, that has been discussed above, and it could be a good starting point. This way, emphasis could be on the representatives of each group rather than on the species selected for other different reasons, reducing the confusion and dispersal of information. Furthermore, investigating the effects of noise on a broader range of habitats would also be useful to explain variations observed, for example, propagation of sound and vibration in the seabed has surely different characteristics and can have unknown impacts on benthic fishes (Shannon et al., 2016; Popper & Hawkins, 2019).

We know now that physical harm to fish from anthropogenic sound sources happens in a much smaller area (Andersson et al., 2017) than the broad one through which the sound can be heard and influence behaviour responses, so, investing in behavioural studies is an important step further in research of anthropogenic noise impact (Popper et al., 2019). Proper data about effects on behaviour is still scarce and little uniformized, and there are still some issues that can be examined such as the reaction to sound pressure or particle motion or even the general behavioural response to different sound levels and not only high intensity ones (Popper et al., 2014; Popper & Hawkins, 2019; Popper et al., 2019). Moreover, under high intensity noise, fish can have temporary hearing loss, can get used to sounds or even change place, which brings up the question on how this and stress physiological changes can affect their populations over time (Slabbekoorn et al., 2019). Also, it would be interesting to understand the role of noise chronicity and its relevance in management and mitigation measures, since most likely, noise won't stop to be produced and its frequency will continue to increase in the future (Nedelec et al., 2015).

Fish population studies that explore the impacts of anthropogenic noise are still very few and end up being more relevant than individual ones considering that behavioural and physiological stress effects imply a larger magnitude impact (Slabbekoorn et al., 2019). Besides, population will influence the ecosystems they are inserted in, so the exposure to anthropogenic sound can also bring consequences on this level, making it important to broaden the spectra of research, considering prey-predator interactions, other biotic connections in the community and consequently in the broader ecosystem, over long periods of time (Shannon et al., 2016; Popper & Hawkins, 2019; Slabbekoorn et al., 2019). Describing soundscapes in a long-term way and how they change over time and space might also be useful, since there is not much data on the variation of natural patterns of particle motion long-term and it could be interesting to have a good baseline to compare different circumstances to (Slabbekoorn et al., 2019).

Another issue that can contribute to current knowledge gaps is the lack of consensus in the metrics, nomenclature and methodology applied in anthropogenic noise impact studies (Hawkins, et al., 2015). Relevant and universal metrics that allow the understanding and, most of all, the comparison of sound types of different taxa should be chosen in a consensual decision, focusing not only on sound pressure but urgently on particle motion too (Hawkins et al., 2015). Moreover, a uniformization in audiogram measurement methodology is also important, since it gives a good base for quantitative comparisons of hearing sensitivity (Slabbekoorn et al., 2019). Terminology, or the lack of consensual usage of it, has been also one of the main problems in comparing the results of different studies, and it should be used in a way that allows the whole community to understand it (Hawkins et al., 2015; Bolgan et al., 2020).

Ideally, experiments should be set where full calibration of sound is possible, using acoustic environments such as open water or specifically designed tanks, that allow for non or minimal reflection of sound (Popper & Hawkins, 2019). However, this is not always conceivable, so other cautions should be taken, in order to present sounds to aquatic organisms, in the lab or in the field, such as a complete specification of the signals that are being presented to the treatment groups, in sound pressure and in particle motion and the creation of adequate controls in order to exclude confounding factors (Bruintjes & Radford, 2014; Hawkins et al., 2015). Thus, suitable, easy to use and cheap instruments should be available to measure with precision all the needed characteristics of sound (Popper & Hawkins, 2019).

Finally, the amount of data generated in such experiments can sometimes be quite extensive and it is not always possible to analyze it in one go, so it becomes quite important to correctly save and archive the raw or processed data for future usage (Hawkins et al., 2015).

Our work will focus mainly on early life stages of fish, and we will try to contribute to the research gap that exists on how anthropogenic noise will affect survival, development and behaviour, since studies with this aim, even though they exist (Bruintjes & Radford, 2014; Caiger et al., 2012; Nedelec et al., 2015) are still rare. We will specifically examine how exposure to boat noise during the early life stages affects development and survival of larvae and eggs, and the behaviour of juveniles of meagre. We know that adult meagre is affected by boat noise passage, since they show a great decrease in the intensity of reproductive chorus, which could either mean a decrease in the number of fish that are producing the sounds or avoidance behaviour, with fish moving away from the area and thus from the hydrophones (Fonseca & Amorim, 2019). Even though the accurate reason for this situation is not yet known, it clearly shows an impact in their behaviour by the boat noise (Fonseca & Amorim, 2019).

Complementing the information we already have on meagre's reaction to boat noise will allows us to have a better understanding on how anthropogenic noise can impact the different life stages of this fish and to realize how we can suggest measures that will ensure the protection of the population of this species. However, even though it has a Least Concerned status on the IUCN list its current population trend is unknown (Pollard & Bizsel, 2020), and it is a long lived species that is heavily fished, causing the removal of spawning adults, that will heavily compromise the continuity of the species (Lagardère & Mariani, 2006; González-Quirós et al., 2011), so properly protecting its different life stages is of great interest. Also, meagre can serve as a representative of a group of fish as suggested by Popper and co-workers (2014), allowing for a more complete overview of the impacts of anthropogenic noise in at least a group of different fish species, reducing the dispersal of information.

5. References

Amorim, M. C. P. (2006). Diversity of Sound Production in Fish. *Communication in Fishes.*, *1*(December), 71–105.

Amorim, M. C. P., & Vasconcelos, R. O. (2008). Variability in the mating calls of the Lusitanian toadfish Halobatrachus didactylus: Cues for potential individual recognition. *Journal of Fish Biology*, *73*(6), 1267–1283. https://doi.org/10.1111/j.1095-8649.2008.01974.x

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*. *88*(5), 1069–1073. https://doi.org/10.1017/S0025315408001677

Amorim, M. C. P., Simões, J. M., Mendonça, N., Bandarra, N. M., Almada, V. C., & Fonseca, P. J. (2010). Lusitanian toadfish song reflects male quality. *Journal of Experimental Biology*, *213*(17), 2997–3004. https://doi.org/10.1242/jeb.044586

Amorim, M. C. P., Vasconcelos, R. O., & Fonseca, P. J. (2015). *Fish Sounds and Mate Choice*. https://doi.org/10.1007/978-3-7091-1846-7_1

Andersson, M. H., Andersson, S., Ahlsén, J., Andersson, L., Hammar, J., Persson, L. K., … Wikström, A. (2017). A framework for regulating underwater noise during pile driving. A technical Vindval report, ISBN 978-91-620-6775-5. Swedish Environmental Protection Agency. In *Report*.

André, M., Solé, M., Lenoir, M., Durfort, M., Quero, C., Mas, A., … Houégnigan, L. (2011). Low-frequency sounds induce acoustic trauma in cephalopods. *Frontiers in Ecology and the Environment*, *9*(9), 489–493. https://doi.org/10.1890/100124

Bolgan, M., Pereira, B. P., Id, A. C., Mylonas, C., Pous, P., Parmentier, E., … Amorim, P. (2020). *Vocal repertoire and consistency of call features in the meagre Argyrosomous regius*. 1–20. https://doi.org/10.1371/journal.pone.0241792

Bolle, L. J., de Jong, C. A. F., Bierman, S. M., van Beek, P. J. G., van Keeken, O. A., Wessels, P. W., … Dekeling, R. P. A. (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*, *2014*(1), 1–12. https://doi.org/10.7717/peerj.594

Cabral, H. N., & Ohmert, B. (2001). Diet of juvenile meagre, argyrosomus regius, within the tagus estuary. *Cahiers de Biologie Marine*, *42*(3), 289–293.

Caiger, P. E., Montgomery, J. C., & Radford, C. A. (2012). Chronic low-intensity noise exposure affects the hearing thresholds of juvenile snapper. *Marine Ecology Progress Series*, *466*, 225–232. https://doi.org/10.3354/meps09933

Carroll, A. G., Przeslawski, R., Duncan, A., Gunning, M., & Bruce, B. (2017). A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. *Marine Pollution Bulletin*, *114*(1), 9–24. https://doi.org/10.1016/j.marpolbul.2016.11.038

Chan, A. A. Y. H., Giraldo-Perez, P., Smith, S., & Blumstein, D. T. (2010). Anthropogenic noise affects risk assessment and attention: The distracted prey hypothesis. *Biology Letters*, *6*(4), 458–461. https://doi.org/10.1098/rsbl.2009.1081

Coquereau, L., Grall, J., Chauvaud, L., Gervaise, C., Clavier, J., Jolivet, A., & Di Iorio, L. (2016). Sound production and associated behaviours of benthic invertebrates from a coastal habitat in the north-east Atlantic. *Marine Biology*, *163*(5), 1–13. https://doi.org/10.1007/s00227-016-2902-2

Costa, D. P., Schwarz, L., Robinson, P., Schick, R. S., Morris, P. A., Condit, R., … Kilpatrick, A. M. (2016). The Effects of Noise on Aquatic Life II. In A. N. Popper & A. Hawkins (Eds.), *Advances in Experimental Medicine and Biology* (Vol. 875). https://doi.org/10.1007/978-1-4939-2981-8_19

Cox, T. M., Ragen, T. J., Read, A. J., Vos, E., Baird, R. W., Balcomb, K., … Crum, L. (2006). Understanding the impacts of anthropogenic sound on beaked whales. *J. CETACEAN RES. MANAGE*, *7*(3), 177–187. Retrieved from http://www.dtic.mil/docs/citations/ADA593622%0Ahttp://www.dtic.mil/dtic/tr/fulltext/u2/a593622.pdf

Crawford, J. D., Cook, A. P., & Heberlein, A. S. (1997). Bioacoustic behaviour of African fishes (Mormyridae): Potential cues for species and individual recognition in Pollimyrus . *The Journal of the Acoustical Society of America*, *102*(2), 1200–1212. https://doi.org/10.1121/1.419923

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. *Reviews in Fish Biology and Fisheries*, *30*(2), 245–268. https://doi.org/10.1007/s11160-020-09598-9

De Soto, N. A., Delorme, N., Atkins, J., Howard, S., Williams, J., & Johnson, M. (2013). Anthropogenic noise causes body malformations and delays development in marine larvae. *Scientific Reports*, *3*, 1–5. https://doi.org/10.1038/srep02831

DeRuiter, S., & Larbi Doukara, K. (2012). Loggerhead turtles dive in response to airgun sound exposure. *Endangered Species Research*, *16*(1), 55–63. https://doi.org/10.3354/esr00396

Fine, M. L., & Parmentier, E. (2015). *Mechanisms of Fish Sound Production*. https://doi.org/10.1007/978-3-7091-1846-7_3

Fonseca, P., & Amorim, M. (2019). Impacto do ruído de barcos em peixes. *Revista de Ciência Elementar*, *7*(2), 1–3. https://doi.org/10.24927/rce2019.019

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

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). *Fisheries Research*, *109*(1), 140–149. https://doi.org/10.1016/j.fishres.2011.01.031

Gospić, N. R., & Picciulin, M. (2016). Changes in whistle structure of resident bottlenose dolphins in relation to underwater noise and boat traffic. *Marine Pollution Bulletin*, *105*(1), 193–198. https://doi.org/10.1016/j.marpolbul.2016.02.030

Grimm, N. B., Foster, D., Groffman, P., Grove, J. M., Hopkinson, C. S., Nadelhoffer, K. J., … Peters, D. P. C. (2008). The changing landscape: Ecosystem responses to urbanization and pollution across climatic and societal gradients. *Frontiers in Ecology and the Environment*, *6*(5), 264–272. https://doi.org/10.1890/070147

Guerra, Á., González, Á. F., Pascual, S., & Dawe, E. G. (2011). The giant squid Architeuthis: An emblematic invertebrate that can represent concern for the conservation of marine biodiversity. *Biological Conservation*, *144*(7), 1989–1997. https://doi.org/10.1016/j.biocon.2011.04.021

Hammar, L., Wikström, A., & Molander, S. (2014). Assessing ecological risks of offshore wind power on Kattegat cod. *Renewable Energy*, *66*, 414–424. https://doi.org/10.1016/j.renene.2013.12.024

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. *Behavioural Ecology*, *30*(6), 1501–1511. https://doi.org/10.1093/beheco/arz114

Hawkins, A. D., & Rasmussen, K. J. (1978). The calls of gadoid fish. *Journal of the Marine Biological Association of the United Kingdom*, *58*(4), 891–911. https://doi.org/10.1017/S0025315400056848

Hawkins, A. D., & Amorim, M. C. P. (2000). Spawning sounds of the male haddock, Melanogrammus aeglefinus. *Environmental Biology of Fishes*, *59*(1), 29–41. https://doi.org/10.1023/A:1007615517287

Hawkins, A. D., Pembroke, A. E., & Popper, A. N. (2015). Information gaps in understanding the effects of noise on fishes and invertebrates. *Reviews in Fish Biology and Fisheries*, *25*(1), 39–64. https://doi.org/10.1007/s11160-014-9369-3

Hawkins, A. D., & Popper, A. N. (2018). Effects of Man-Made Sound on Fishes. In H. Slabbekoorn (Ed.), *Effects of Anthropogenic Noise on Animals* (Vol. 66, pp. 415–429). Springer Science.

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. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1863). https://doi.org/10.1098/rspb.2017.1627

Hildebrand, J. A. (2009). Anthropogenic and natural sources of ambient noise in the ocean. *Marine Ecology Progress Series*, *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. *Marine Ecology Progress Series*, *485*, 295–300. https://doi.org/10.3354/meps10346

Jiménez, M. T., Pastor, E., Grau, A., Alconchel, J. I., Sánchez, R., & Cárdenas, S. (2005). Revisión del cultivo de esciénidos en el mundo, con especial atención a la corvina Argyrosomus regius (Asso, 1801). *Boletin - Instituto Espanol de Oceanografia*, *21*(1–4), 169–175.

Kasumyan, A. O. (2008). Sounds and sound production in fishes. *Journal of Ichthyology*, *48*(11), 981–1030. https://doi.org/10.1134/s0032945208110039

Klima, E. F., Gitschlag, G. R., & Renaud, M. L. (1988). Impacts of the explosive removal of offshore petroleum platforms on sea turtles and dolphins. *Marine Fisheries Review*, *50*(3), 33–42.

Ladich, F., & Fine, M. L. (2006). Sound-generating mechanisms in fishes: a unique diversity in vertebrates. *Communication in Fishes.*, *1*(January), 3–34.

Ladich, F. (1997). Agonistic behaviour and significance of sounds in vocalizing fish. *Marine and Freshwater Behaviour and Physiology*, *29*(1–4), 87–108. https://doi.org/10.1080/10236249709379002

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

Leis, J. M., & McCormick, M. I. (2002). The Biology, Behaviour, and Ecology of the Pelagic, Larval Stage of Coral Reef Fishes. *Coral Reef Fishes*, 171–199. https://doi.org/10.1016/b978-012615185-5/50011-6

McCauley, R. D., & Cato, D. H. (2000). Patterns of fish calling in a nearshore environment in the Great Barrier Reef. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *355*(1401), 1289–1293. https://doi.org/10.1098/rstb.2000.0686

McCauley, R. D., Fewtrell, J., & Popper, A. N. (2003). High intensity anthropogenic sound damages fish ears. *The Journal of the Acoustical Society of America*, *113*(1), 638–642. https://doi.org/10.1121/1.1527962

Middel, H., & Verones, F. (2017). Making marine noise pollution impacts heard: The case of cetaceans in the North Sea within life cycle impact assessment. *Sustainability (Switzerland)*, *9*(7). https://doi.org/10.3390/su9071138

Miller, P. J. O., Biassoni, N., Samuels, A., & Tyack, P. L. (2000). Whale songs lengthen in response to sonar. *Nature*, *405*(6789), 903. https://doi.org/10.1038/35016148

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. *Advances in Marine Biology*, *51*(06), 143–196. https://doi.org/10.1016/S0065-2881(06)51003-X

Mooney, T. A., Yamato, M., & Branstetter, B. K. (2012). Hearing in Cetaceans: From Natural History to Experimental Biology. In *Advances in Marine Biology* (1st ed., Vol. 63). https://doi.org/10.1016/B978-0-12-394282-1.00004-1

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). *Proceedings of the Royal Society B: Biological Sciences*, *282*(1817). https://doi.org/10.1098/rspb.2015.1943

Ono, R. D., & Poss, S. G. (1982). Structure and innervation of the swim bladder musculature in the weakfish, Cynoscion regalis (Teleostei: Sciaenidae) . *Canadian Journal of Zoology*, *60*(8), 1955–1967. https://doi.org/10.1139/z82-253

Parmentier, E., Diogo, R., & Fine, M. L. (2017). Multiple exaptations leading to fish sound production. *Fish and Fisheries*, *18*(5), 958–966. https://doi.org/10.1111/faf.12217

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*.

Payne, R., & Webb, D. (1971). Orientation By Means of Long Range Acoustic Signaling in Baleen Whales. *Annals of the New York Academy of Sciences*, *188*(1), 110–141. https://doi.org/10.1111/j.1749-6632.1971.tb13093.x

Peng, C., Zhao, X., & Liu, G. (2015). Noise in the sea and its impacts on marine organisms. *International Journal of Environmental Research and Public 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

Pollard, D.A. & Bizsel, K. 2020. *Argyrosomus regius*. *The IUCN Red List of Threatened Species* 2020:.T198706A130099146. https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T198706A130099146.en. Downloaded on 20 January 2021.

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

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., Halvorsen, M. B., Kane, A., Miller, D. L., Smith, M. E., Song, J., … Wysocki, L. E. (2007). The effects of high-intensity, low-frequency active sonar on rainbow trout. *The Journal of the Acoustical Society of America*, *122*(1), 623–635. https://doi.org/10.1121/1.2735115

Popper, A. N., & Hawkins, A. D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. *Journal of Fish Biology*, *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., … Tavolga, W. N. (2014). Sound Exposure Guidelines for Fishes and Sea Turtles: A Technical Report prepared by ANSI-Accredited Standards Committee S3/SC1 and registered with ANSI. In *Springer*. https://doi.org/10.1007/978-3-319-06659-2

Popper, A. N., Smith, M. E., Cott, P. A., Hanna, B. W., MacGillivray, A. O., Austin, M. E., & Mann, D. A. (2005). Effects of exposure to seismic airgun use on hearing of three fish species. *The Journal of the Acoustical Society of America*, *117*(6), 3958–3971. https://doi.org/10.1121/1.1904386

Popper, A. N., Hawkins, A. D., & Sand, O. (2019). Examining the hearing abilities of fishes. The Journal of the Acoustical Society of America, 948(2019), 146. https://doi.org/10.1121/1.5120185

Prista, N., Costa, J. L., Costa, M. J., & Jones, C. M. (2009). *Age determination in meagre Argyrosomus regius* (Vol. 49).

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

Radford, C. A., Stanley, J. A., Tindle, C. T., Montgomery, J. C., & Jeffs, A. G. (2010). Localised coastal habitats have distinct underwater sound signatures. *Marine Ecology Progress Series*, *401*(July 2014), 21–29. https://doi.org/10.3354/meps08451

Ramcharitar, J., Gannon, D. P., & Popper, A. N. (2006). Bioacoustics of Fishes of the Family Sciaenidae (Croakers and Drums). *Transactions of the American Fisheries Society*, *135*(5), 1409–1431. https://doi.org/10.1577/t05-207.1

Roberts, C. (1997). Connectivity and management of caribbean coral reefs. *Science*, *278*(5342), 1454–1457. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9367956

Salas, A. K., Wilson, P. S., & Fuiman, L. A. (2019). Ontogenetic change in predicted acoustic pressure sensitivity in larval red drum (Sciaenops ocellatus). *Journal of Experimental Biology*, *222*(16). https://doi.org/10.1242/jeb.201962

Shafiei Sabet, S., Wesdorp, K., Campbell, J., Snelderwaard, P., & Slabbekoorn, H. (2016). Behavioural responses to sound exposure in captivity by two fish species with different hearing ability. *Animal Behaviour*, *116*(May), 1–11. https://doi.org/10.1016/j.anbehav.2016.03.027

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

Simpson, S. D., Meekan, M. G., McCauley, R. D., & Jeffs, A. (2004). Attraction of settlement-stage coral reef fishes to reef noise. *Marine Ecology Progress Series*, *276*(1), 263–268. https://doi.org/10.3354/meps276263

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. *Nature Communications*, *7*, 1–7. https://doi.org/10.1038/ncomms10544

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 in Ecology and Evolution*, *25*(7), 419–427. https://doi.org/10.1016/j.tree.2010.04.005

Slabbekoorn, H., Dalen, J., de Haan, D., Winter, H. V., Radford, C., Ainslie, M. A., … Harwood, J. (2019). Population-level consequences of seismic surveys on fishes: An interdisciplinary challenge. *Fish and Fisheries*, *20*(4), 653–685. https://doi.org/10.1111/faf.12367

Tellechea, J. S., Martinez, C., Fine, M. L., & Norbis, W. (2010). Sound production in the whitemouth croaker and relationship between fish size and disturbance call characteristics. *Environmental Biology of Fishes*, *89*(2), 163–172. https://doi.org/10.1007/s10641-010-9709-7

Thorson, R. F., & Fine, M. L. (2002). Crepuscular changes in emission rate and parameters of the boatwhistle advertisement call of the gulf toadfish, Opsanus beta. *Environmental Biology of Fishes*, *63*(3), 321–331. https://doi.org/10.1023/A:1014334425821

Tidau, S., & Briffa, M. (2019). Anthropogenic noise pollution reverses grouping behaviour in hermit crabs. *Animal Behaviour*, *151*, 113–120. https://doi.org/10.1016/j.anbehav.2019.03.010

Tolimieri, N., Jeffs, A., & Montgomery, J. C. (2000). Ambient sound as a cue for navigation by the pelagic larvae of reel fishes. *Marine Ecology Progress Series*, *207*(December 2013), 219–224. https://doi.org/10.3354/meps207219

Tyack, P. L., Zimmer, W. M. X., Moretti, D., Southall, B. L., Claridge, D. E., Durban, J. W., … Boyd, I. L. (2011). Beaked whales respond to simulated and actual navy sonar. *PLoS ONE*, *6*(3). https://doi.org/10.1371/journal.pone.0017009

Vasconcelos, R. O., Amorim, M. C. P., & Ladich, F. (2007). Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. *Journal of Experimental Biology*, *210*(12), 2104–2112. https://doi.org/10.1242/jeb.004317

Vieira, M., Pereira, B. P., Pousão-Ferreira, P., Fonseca, P. J., & Amorim, M. C. P. (2019). Seasonal variation of captive meagre acoustic signalling: A manual and automatic recognition approach. *Fishes*, *4*(2), 1–19. https://doi.org/10.3390/fishes4020028

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

Wright, K. J., Higgs, D. M., & Leis, J. M. (2011). Ontogenetic and interspecific variation in hearing ability in marine fish larvae. *Marine Ecology Progress Series*, *424*, 1–13. https://doi.org/10.3354/meps09004

Appendix II – Supplementary Material

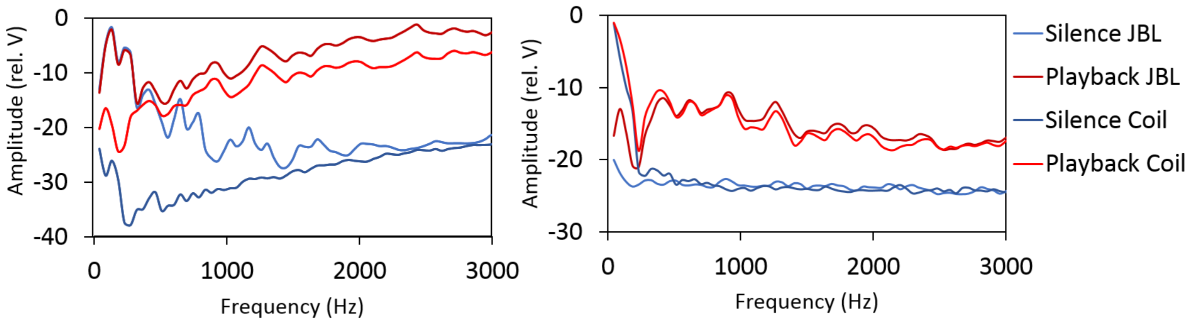

Figure S 1 - Frequency spectrum of the electrical signal produced by the speakers and the reels for the eggs and larvae experiment (left) and for the juveniles' experiment (right).

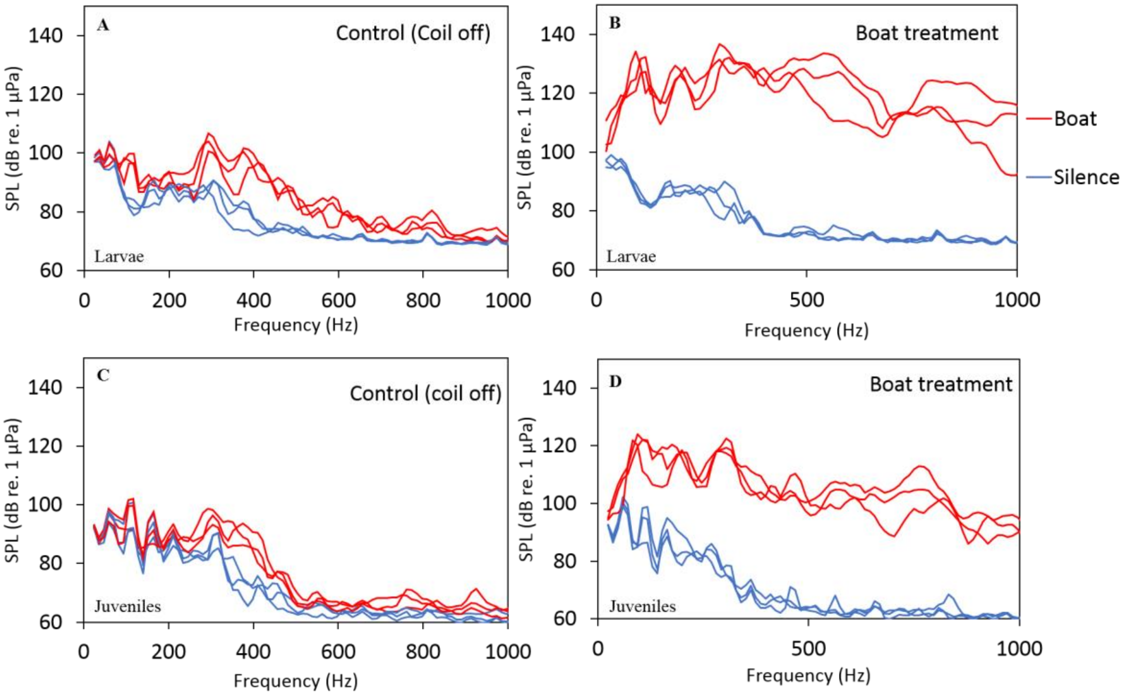

Figure S 2 - Propagation of boat noise to control tanks. (A, C) Spectra of the recorded sound on the control tanks; and (B, D) on the tanks with the boat noise treatment during boat noise playback, and silence. Similar results were obtained on both setups: A,B represent larvae setup and C,D juveniles setup. Only one ferry passage was used for this characterization.

Table S 1 - Results of Post-hoc LSD test for Lipid Droplet Area

	Error: Between MSE = ,00012, df = 175,00					
Comparisons	Treatment	Timepoint	**1**	**2**	**3**	**4**
1	Boat noise	0dph		0.000000	0.117807	0.000000
2	Boat noise	2dph	0.000000		0.000000	0.041574
3	Control	0dph	0.117807	0.000000		0.000000
4	Control	2dph	0.000000	0.041574	0.000000	

Table S 2 - Results of Post-hoc LSD test for Body Depth

	Error: Between MSE = ,00050, df = 175,00					
Comparisons	Treatment	Timepoint	**1**	**2**	**3**	**4**
1	Boat noise	0dph		0.000000	0.834803	0.000000
2	Boat noise	2dph	0.000000		0.000000	0.045861
3	Control	0dph	0.834803	0.000000		0.000000
4	Control	2dph	0.000000	0.045861	0.000000	

Table S 3 - Results of Post-hoc LSD test for pre-feeding approach

	Error: Between MSE = ,02092, df = 19,000					
Comparisons	Treatment	Timepoint	**1**	**2**	**3**	**4**
1	Control	day 2		0.013777	0.012504	0.028346
2	Control	day 7	0.013777		0.964772	0.737146
3	Boat noise	day 2	0.012504	0.964772		0.704260
4	Boat noise	day 7	0.028346	0.737146	0.704260	

Table S 4 - Results of Post-hoc LSD test for ratio approach post/pre feeding

	Error: Between MSE = 1,1364, df = 20,000					
Comparisons	Treatment	Timepoint	**1**	**2**	**3**	**4**
1	Control	day 2		0.177626	0.032297	0.715406
2	Control	day 7	0.177626		0.377054	0.316448
3	Boat noise	day 2	0.032297	0.377054		0.067790
4	Boat noise	day 7	0.715406	0.316448	0.067790	

Table S 5 - Results of Post-hoc LSD test for aggressiveness score post/pre feeding ratio

	Error: Between MSE = ,29174, df = 20,000					
Comparisons	Treatment	Timepoint	**1**	**2**	**3**	**4**
1	Control	day 2		0.315250	0.148742	0.839230
2	Control	day 7	0.315250		0.642175	0.230904
3	Boat noise	day 2	0.148742	0.642175		0.103215
4	Boat noise	day 7	0.839230	0.230904	0.103215	