

# Chronic Boat Noise Impacts on the Early Life Stages of Cichlids (Maylandia zebra): Preliminary Findings

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- 1 Preliminary findings of chronic boat noise impacts on the early
- 2 life stage of cichlids (Maylandia zebra)
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## 1. Abstract

- 39 Anthropogenic noise is globally recognized as a stressor for animals. However, despite
- 40 evidence of detrimental effects of noise exposure on fish, knowledge about chronic effects on
- 41 critical early life stages is still scarce. Using a split-brood design to exclude the genetic effect,
- 42 we exposed African cichlids (Maylandia zebra) from the same brood to boat noises (~120dB)
- and to control condition (~100dB) for 12 weeks, starting when mouthbrooding females

released their young. Treatment fish were exposed to motorboat noises from 9 am to 6 pm to mimic a typical daily boating activity. Larvae total length and foraging activity was measured on week 1 and week 12. We did not find a significant effect of chronic boat noise exposure on these variables. However, whether noise has any impact within the study period or after week 12 is not clear. Future work will focus on analyzing the data weekly over the 12 weeks exposure period to further substantiate the current results. Addressing the effect of anthropogenic noise on early life stages have implications for our understanding of the effect of this pervasive stressor on aquatic organisms.

# 2. Keywords

Anthropogenic noise, Long-term noise, Cichlidae, Early-life stage, Growth, Foraging

# 3. Introduction

## 3.1 Anthropogenic sound effect

Anthropogenic underwater noise from commercial shipping, oil and gas exploration, naval operations, fishing, and construction work, has been shown to have increased low-frequency ambient noise at an average rate of 2.5–3dB per decade since the 1960s (McDonald et al., 2006; Miksis-Olds and Nichols, 2016). The higher concentration of human activities in some areas increases the noise above the ambient level not only locally but also away from the source. As many anthropogenic activities generate low-frequency sound, it propagates long-range making it a particularly pervasive pollutant (Miksis-Olds and Nichols, 2016).

### 3.2 Anthropogenic sound effect on adult aquatic animals

Underwater sounds carry important information such as cues from the surrounding environment or from other animals. Aquatic animals use these acoustic information for communication, navigation, mating, foraging, agonistic displays, territorial defence, and reproduction (Kasumyan, 2009). However, the hearing frequency range of most marine animals overlaps with anthropogenic noise, such as boat noise (Erbe et al., 2014; Merchant et al., 2020; Nikolopoulos et al., 2016). Underwater anthropogenic noise negatively affects aquatic animals, for example by masking important acoustic information (Clark, 2009; Clark et al., 2009; Codarin et al., 2009; McCauley et al., 2003) impairing hearing by causing permanent and/or temporary hearing threshold shifts (Kastak et al., 2008; McCauley et al., 2003) and inner ear hair cell loss (Lara et al., 2022). Among other consequences, one may quote changing in hormone levels and other stress responses (Kusku, 2020; Kusku et al., 2020; Wysocki et al., 2006), inducing avoidance response (Fewtrell and McCauley, 2012; Pearson et al., 1992), increasing predation risk (Voellmy, Purser, Simpson, et al., 2014) and metabolism (Lagardère, 1982), decreasing growth performance (Kusku et al., 2020) and foraging performance (Purser and Radford, 2011).

# 3.3 Importance of early life stages

There are few studies focused on the effect of chronic noise on developmental period of fish (Faria et al. 2022). Early life experiences has been shown to have impacts on adult behaviour and the effects could be complex and long-lasting (Carlson, 2017). However, as increasing noise made by humans alters the underwater soundscape, it is expected that it will affect the early life stages of aquatic organisms, which are generally regarded as the most sensitive period in the whole life span. Fish during early life stages suffer high mortality even under normal environmental fluctuations and challenges (Catalán et al., 2020) and increased mortality could have profound consequences on populations (Slabbekoorn, 2019).

### 3.4 Anthropogenic sound effects on early life stages

The limited studies on the impact of anthropogenic noise during the developmental period in aquatic animals point to species-specific effects. For example, noise was shown to decrease growth rate and increase overall stress responses in Lusitanian toadfish embryos (Faria et al.,

2022), increase the heart rate of damselfish embryos (Fakan and McCormick 2019; Jain-Schlaepfer et al. 2018), increase heart rate, yolk sac use and cortisol levels of the zebrafish larvae (Lara and Vasconcelos, 2021), reduce feeding success in winter flounder larvae (*Pseudopleuronectes americanus*) (Gendron et al., 2020), and reduce the settlement of coral reed fish larvae to reefs (Simpson et al., 2016).

#### 3.5 Effect of chronic Anthropogenic sound

Noise effects are also dependent on exposure duration. It is notable that most of the studies about the anthropogenic noise effects are focused on the acute or relatively short-term exposure, likely due to long-term experiments being harder to carry out. There is evidence that long-term anthropogenic noise negatively affects animals (Barber et al., 2010; Crino et al., 2013; Kusku, 2020) but knowledge about the chronic effects of noise on critical early life stages (larvae and/or juvenile period) is still scarce.

# 3.6 Animals for the present study

Maylandia zebra is an African cichlid living on the sediment-free rocky coast in Lake Malawi. This species is able to produce sounds during social interactions, such as territory defense (Bertucci et al., 2010; Chabrolles et al., 2017) or mating (Simões et al., 2008). This is an aggressive species with well-established dominance hierarchies. Maternal mouth-brooding is also an important feature of the species, lasting for three weeks before the female releases the larvae. Due to increased human activities and development of fishing and tourism industries (Allison and Mvula, 2002; Makochekanwa, 2013) in lake Malawi man-made noise has become a pollutant of concern. As *M. zebra* is an established study model in behaviour and bioacoustics (Amorim et al., 2019; Bertucci et al., 2010; Chabrolles et al., 2017; Mellor et al., 2011), it provides an excellent opportunity to assess the effects of long-term anthropogenic noise on growth and behaviour during the early life stages.

Here, we investigate the impacts of chronic anthropogenic noise on growth and foraging during a critical developmental period. We hypothesized that long exposure to boat noise acts as a stressor on the early life stages having the potential to affect growth and foraging.

# 4. Materials & Methods

# 4.1 Females and juveniles rearing conditions

We purchased *Maylandia zebra* from Oxyfish (Verlinghem, France) and raised fish in stock tanks ( $1 \times w \times h$ :  $120 \times 60 \times 50$  cm) at ENES laboratory (Saint-Etienne, France). Each holding tank were fed daily with commercial cichlid food (JBL NovoRift sticks and Tetra flakes). All tanks were equipped with an external filter (Tetra EX filter, Tetra GmbH, Melle, Germany), aeration and PVC tubes as shelters. The water temperature was maintained at  $25 \pm 1^{\circ}$ C with a pH of 8.0 and a 12:12h light: dark cycle.

Stock tanks were checked daily to find the females at the beginning of the mouth-brooding period, which were then isolated individually. The mouth-brooding period lasted for 3 weeks after which we used round-head tweezers to open the mouth of the female and help them release all larvae. These were transferred immediately into experimental aquaria ( $L \times 1 \times h$ : 30 x 20 x 20 cm). The aquaria were equipped with PVC tubes as shelters, sand as substrate (Aquasand Nature, Zolux déco), and aeration. Each aquarium was equipped with an underwater loudspeaker developed by Fonseca & Maia Alves (2012) and placed in the middle of the aquarium (see below). Fish were fed daily with commercial food (JBL NovoMalawi sticks for Malawi cichlids, JBL NovoRift, and cichlid flakes, Tetra).

### 4.2 Noise playback

We used motorboat sounds that were originally recorded by V. Médoc in the Grangent lake (45°45′ 07.54″ N, 4°25′ 56.47″ E, Loire, France) at 1-m depth (hydrophone Aquarian Audio Products H2a-XLR, AFAB Enterprises, Anacortes, WA, USA; sensitivity: –180 dB re.

140 1 V μPa-1, frequency response within ±4 dB in the range 20 Hz-4.5 kHz, connected to a 141 ZOOM H4 Handy recorder). Twenty-five original boat sounds were excerpted and linearly 142 faded at both ends using Audacity 3.1.3 (http://audacity.sourceforge.net) to make them 143 emerge from silence, then they were used in the playbacks. 144 The aquaria background noise was around 100 dB re. 1µPa and was used as the control condition in our experiment. To keep it around 100 dB re. 1 µPa, we changed one-third of the 145 146 water every 2 days instead of installing underwater filters in the experimental or control aquaria. 147 148 Boat noise was played back through a DENON PMA-100M amplifier connected to a 149 TASCAM US-366 preamplifier and controlled by a laptop running Audacity, and was 150 delivered through the above mentioned underwater loudspeaker. The loudspeaker was placed 151 in the middle of each experimental aquarium, including control aquaria but without 152 connecting to the laptop. The excerpts of boat noise playbacks were adjusted to 120 dB re. 1μPa before broadcasting to the fish. All modified boat noise was played back and then 153 recorded by a pre-calibrated chain composed of a H2A-HLR hydrophone placed 3 cm away 154 155 from the speaker and connected to a ZOOM H4 recorder. Sound pressure levels of playbacks 156 were calculated by integrating the average power spectra of the recorded sound files. 157 Boat noise after adjusting the sound level was used to generate the audio tracks for the 158 experiment. Different boat sounds were randomly positioned along nine 1-hour audio to 159 mimic the variability in the daily activity of small leisure boats, and in the remaining time

they were replaced by silent tracks. The number of boats varied from 6 to 15 boats per track.

The boat noise was broadcast to the fish from 9 a.m. to 6 p.m. Boat noise was broadcast to

the boat noise fish treatment every day during 12 weeks.

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## 4.3 Experimental design

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Five different pregnant females were used for the experiment and each female gave one clutch (n = 12 to 21 larvae). Following a split-brood design, larvae from each clutch was divided randomly into two groups, and transferred directly into the aquarium assigned to either the boat noise or the control treatment, where they stayed for 12 weeks.

During the experimental period, we took a 10 min video of foraging activity in the daytime

once a week using a video camera (HD 1080p C920; Logitech International SA, Lausanne, Switzerland) positioned in front of the aquarium. The frame rate of the camera was set at 120 fps (frames per second). Fish were fed with commercial food pellets every day. During filming of the foraging activity, fish were fed to statiation. All the videos were analysed with Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba 2016) to extract the foraging behaviour events. Each time one fish opened its mouth to catch the food pellet was counted as a proxy for eating. The total number of foraging events was divided by the number of fish in the aquarium to get the average foraging activity per fish. Behaviours were counted at the group level (the sum of the numbers of the behaviours of all fish in one aquarium) with the software because the larvae were too small to be labelled or identified. We measured the total length (TL) of each individual every week. A transparent box with 0.5 cm grid paper on the bottom was prepared for measuring the total length. To disturb the fish as little as possible, all fish in one aquarium were taken out simultaneously with a net that spanned the width of the aquarium, and fish were then placed together in the measuring box. The water in the box was changed between measurements from different aquaria. We took a photo from the top of the box to later measure the fish length. This was possible as the depth of the water was no more than the body depth of the fish inside. After taking the photo we immediately placed them back in the aquarium. With the software ImageJ (Rueden et al., 2017), we used the grid paper as the scale and measured the total length of each fish. From the total length of each fish, we calculated the average TL of each group under the two different conditions at week 1 and at week 12, representing the start and the end of the experiment, respectively.

## 4.4 Statistical Analyses

Statistical analyses were conducted using R (version 4.2.1). According to the Shapiro–Wilk test, only the foraging per fish at week 12 was non-normal. Therefore we used a Wilcoxon signed-rank test to test treatment effect on foraging at week 12, and we used t-test on total length (week 1 and 12) and foraging at week 1.

#### 4.5 Ethics notes

The research carried out comply with relevant guidelines and regulations including French national guidelines, permits, and regulations regarding animal care and experimental use (approval no. D42-218-0901, ENES lab agreement, Direction Départementale de la Protection des Populations, Préfecture du Rhône).

# 5. Results

#### 5.1 Total length

At the beginning of the experiment, the average total length (TL) of the larvae from the boat noise condition was 1.39 ( $\pm$ SD, range:  $\pm$ 0.07, 1.33-1.50 cm), and the average TL of the larvae for the control condition was 1.39 ( $\pm$ 0.07, 1.32-1.52 cm). There was no difference in the TL at the beginning of the experiment (p-value = 0.97) (Fig. 1A). After 12 weeks of exposure to the boat noise, the average total length (TL) of the fish under boat noise condition was 3.02 ( $\pm$ 0.74, 2.11-3.86cm), and the average TL of the control fish was 3.06 ( $\pm$ 0.56, 2.52-3.69cm). Total length of fish after 12 weeks of exposure to boat noise did not differ from the fish under control condition (p-value = 0.92, Fig. 1A).

# 5.2 Foraging

The result of foraging activity was shown by times of eating per fish during 10 minutes of filming. In the first week, the foraging activity per fish under the boat noise condition was  $4.94 \pm 3.72$ , 0-8.33, and the foraging per fish under the control condition was  $4.44 \pm 2.47$ , 2.63-8.7). There was no difference in the foraging per fish at the beginning of the experiment (p-value = 0.81, Fig. 1B). After 12 weeks of exposure to the boat noise, the foraging per fish under boat noise condition was  $25.21 \pm 5.84$ , 16.17-30.86), and the foraging per fish under the control condition was  $28.83 \pm 14.74$ , 19-54.83). The results showed no difference between the fish under 12 weeks of boat noise exposure and under control condition (p-value = 0.84) (Fig.1B).

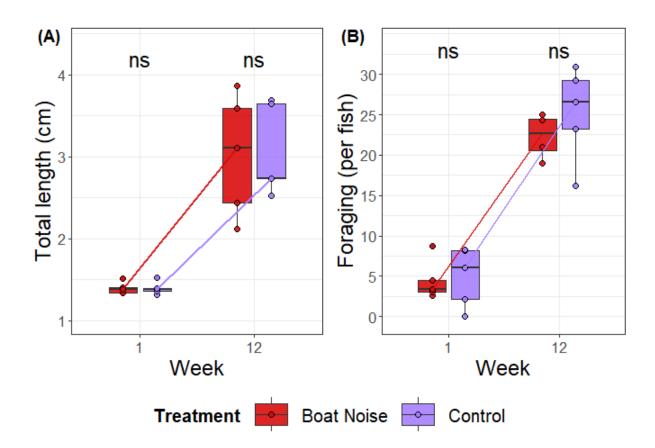


Fig 1. Effects of boat noise on Total Length (A) and Foraging (per fish) (B) at week 1 and at week 12 of the experiment. Red represents fish exposed to boat noise condition, and blue

represents fish exposed to control condition. Dots represent 5 different groups, and the boxes represent the 25th to 75th percentiles.

# 6. Discussion

### 6.1 Growth

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In this study, we did not find significant effects of 12 weeks of boat noise exposure on the total length and foraging behaviour of M. zebra. The lack of effects on growth is consistent with the findings for another cichlid (*Neolamprologus pulcher*) (Bruintjes and Radford, 2014) in which 4 weeks of noise exposure did not affect hatching success or post-hatching larvae growth and survival. A study on rainbow trout (*Oncorhynchus mykiss*) juveniles reared under the noise made by the intensive aquaculture systems for 5 months (Davidson et al., 2009) also suggested no impact in the growth and survival of the noise-exposed fish when compared to control. However, some other studies have shown that long-term noise exposure has detrimental effects on growth. For example, the total length of Lusitanian toadfish larvae (Halobatrachus didactylus) after 2 weeks of boat noise exposure was smaller than in the control condition (Faria et al., 2022). The larvae of freshwater sheepshead minnow (Cyprinodon variegatus) and killifish (Fundulus similis) exposed to high noise for 12 days were also smaller than those under quiet conditions (Banner and Hyatt, 1973). Studies on larvae of Atlantic cod (Gadus morhua) showed that even larvae 2 days after hatching were smaller under noise than under control conditions, although there was growth compensation at later stages (Nedelec et al., 2015). Different effects on the total length under noise exposure might be species-specific, namely due to interspecific differences on fish hearing and the presence of a swim bladder or other resonant structures, which can cause different vulnerability to anthropogenic noise (R. Fay and Popper, 2012; R. R. Fay et al., 2008). Differences in experimental procedures, including whether they are field- or lab-based (Popper and Hawkins, 2019), the sound type (Bruintjes and Radford, 2014; Kusku et al., 2020), boat type (Jain-Schlaepfer et al., 2018; McCormick et al., 2019), amplitude levels (Lara and Vasconcelos, 2021) and exposure duration (Popper and Hawkins, 2019) could also have an impact on the fish's response to the noise.

### 6.2 Foraging

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We also did not find evidence that foraging activity was affected by chronic noise exposure during early life stages, when comparing that behavior on weeks 1 vs 12. Although other studies have shown that foraging can be affected by additional man-made noise (Bracciali et al., 2012; Purser and Radford, 2011; Voellmy et al., 2014), habituation, sensitization, and tolerance can occur in most animals after repeated and/or chronic noise exposure (Bejder et al., 2009). This suggests that young M. zebra fish may have habituated or gained tolerance to the added noise. However, as habituation should be the result of a longitudinal process, the comparison at one-time point (week 12) cannot provide strong evidence for distinguishing between habituation and tolerance (Bejder et al., 2009). Magnhagen et al. (2017) examined the effect of noise on foraging behavior in both roach and the Eurasian perch in the field. These authors found that perch can habituate to noise exposure as their feeding attempts first decreased and then increased gradually over the five days of the experiment (Magnhagen et al., 2017). However the roach, which has higher hearing sensitivity, presented fewer feeding attempts than the perch and did not habituate to the noise during the experimental period (Magnhagen et al., 2017). This study on perch and roach suggests that fish are able to habituate to anthropogenic noise or that the time needed for habituation has interspecificdifferences.

As hearing sensitivity in larvae and juveniles has not been studied in our study species, it is possible that the 120 dB boat noise level fell below hearing thresholds, thus not causing an effect. Noise is known to affect foraging behaviour by directly reducing appetite

273 (Charmandari et al., 2005), or indirectly through a reduction in activity and locomotion 274 (Mendl, 1999). It is also possible that it alters cognitive processes involved in food detection, 275 or distracts the attention of the fish (Chan and Blumstein, 2011; Mendl, 1999). 276 The current study considered only two time points, the start and the end of the experiment, 277 preventing from acknowledging possible effects of noise over the full 12 weeks period. 278 Future analyses should focus on more time points within the long-term noise exposure. This 279 will give a better insight regarding the effects of noise along the development of fish, and on 280 whether these effects are reversable or not. 7. References 281 282 Allison, E. H., Mvula, P. M. (2002). Fishing Livelihoods and Fisheries Management in 283 Malawi. 284 Amorim, M. C. P., Fonseca, P. J., Mathevon, N., Beauchaud, M. (2019). Assessment of 285 fighting ability in the vocal cichlid Metriaclima zebra in face of incongruent 286 audiovisual information. Biology Open, 8(12), bio043356. 287 https://doi.org/10.1242/bio.043356 288 Banner, A., Hyatt, M. (1973). Effects of Noise on Eggs and Larvae of Two Estuarine Fishes. *Transactions of the American Fisheries Society*, 102(1), 134–136. 289 290 https://doi.org/10.1577/1548-8659(1973)102<134:EONOEA>2.0.CO;2 291 Barber, J. R., Crooks, K. R., Fristrup, K. M. (2010). The costs of chronic noise exposure for 292 terrestrial organisms. Trends in Ecology Evolution, 25(3), 180–189. 293 https://doi.org/10.1016/j.tree.2009.08.002 294 Bertucci, F., Beauchaud, M., Attia, J., Mathevon, N. (2010). Sounds Modulate Males' 295 Aggressiveness in a Cichlid Fish. *Ethology*, 116(12), 1179–1188.

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