Cold-water coral larvae noise exposure experiment

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Introduction

The MASTS Aquatic Stressors Forum supported my accommodation, meals and bench fees (use of laboratory space) for ten days (8-17 March 2023) at the Tjarno Marine Laboratory (TML) (University of Gothenburg, Sweden) to allow me to conduct a noise exposure experiment on cold-water coral larvae. Other funding was received from the University of Edinburgh's Small Grant and the H2020 iAtlantic project (Grant agreement No 818123). In-kind contributions (i.e. staff time, larvae, equipment) were provided by Prof Ann Larsson, Dr Susanna Strömberg and Iga-maria Nestorowicz from the University of Gothenburg and by Prof Karen Diele and Dr Matthew Wale from Edinburgh Napier University.

The overarching aim of the conducted experiments was to gain a deeper understanding of how natural and human-induced sounds affect the functioning of cold-water coral habitats. This is important because noise pollution, an anthropogenic stressor, is increasingly recognised as a significant threat affecting the behaviour and health of marine organisms but remains poorly understood (De Clippele and Risch 2021). These experiments will help us understand how field-recorded natural and anthropogenic sounds, present at a Norwegian cold-water coral reef, affects the behaviour of the larvae of the iconic reef-forming coral *Desmophyllum pertusum* (a.k.a. *Lophelia pertusa*). Since larvae attraction is one of the only viable methods for benthic species to maintain healthy populations long-term, it is important that we learn more about the extrinsic cues that deep-sea larvae use to recognise suitable habitats and how noise pollution might affect this (Larsson et al. 2014, Strömberg et al. 2017, De Clippele and Risch

Figure 1 An adult Lophelia pertusa coral

2021). To close this gap in our knowledge, I conducted multidisciplinary experiments in March 2023 at TML to contribute to a better understanding of the cold-water coral *L. pertusa* larvae's settlement behaviour in relation to noise exposure.

Activities

During my ten-day stay at TML I set up and conducted a total of three experiments to study changes in the swimming behaviour of *Lophelia pertusa*'s larvae in response to: healthy reef sounds, healthy reef sounds + coral rubble (see Figure 1), and ship's produced noise pollution. Each experiment lasted 60 minutes and was filmed with Canon EOS 5D and EOS 90D DSLR cameras. The adult coral colonies that produced the larvae used in these experiments were collected ahead of my arrival, in December 2022 at the Tisler cold-water coral reef, by Dr Ann Larsson and her team (TML) as part of the LIFE Lophelia project (Grant agreement no LIFE18 NAT/SE /000959). The corals started spawning in January 2023. After ten days they start swimming and after three weeks they develop functional cnidocysts, which they need to settle (Strömberg et al. 2019). While the larvae can stay alive for up to a year (Strömberg et al. 2017)., the experiments were conducted mid-march when the larvae are at peak health and ready for settlement. The "healthy sound" experiment was conducted on 14 March with 150 (20 larvae per cup) from a spawning event on 7 February. The "healthy + rubble" experiment was conducted on 15 March with 240 (40 larvae per cup) larvae from a spawning event that occurred on 14 February, the "Noise" experiment was conducted on 18 March with 150 (20 larvae per cup) larvae from a spawning event that happened on 7 February. Thanks to the University of Gothenburg's team I gained experience in cold-water coral larvae husbandry.

The experiment was conducted in a climate-controlled room with a temperature of 7 degrees Celsius. An experimental and a control plexiglass aquarium (Dimensions: 27 x 27.5 x 27.9 cm) were used, each with one DNH waterproof speaker (DNG Norway) installed (Figure 2). The speaker was hanging 1 cm above the bottom of the tank to minimise the effect of unwanted vibrations caused by the played sounds. The waterproof speakers are able to emit sounds at a frequency range of 100Hz-20kHz and both had a cable connected to a Pioneer amplifier A-10-S. The tank's walls and bottom were padded with bubble wrap to minimise the 'tank effect', i.e. sounds reflecting off the tank's walls. The speaker is 6 cm in height and has a diameter of 6.5 cm. Three small plastic cups, were installed per tank. The cups were hanging 15 cm above the speaker. The size of the cups was 6.4 x 6 x 6 cm. To minimise the effect of vibrations and non-experimental noise pollution, all other equipment, including the room's cooling systems, were turned off during the duration of the experiment (Figure 1). The temperature of the water within the experimental tanks was measured every 30 minutes. During the experiment, the room's lights were turned off but LED lights were used to illuminate the larvae.

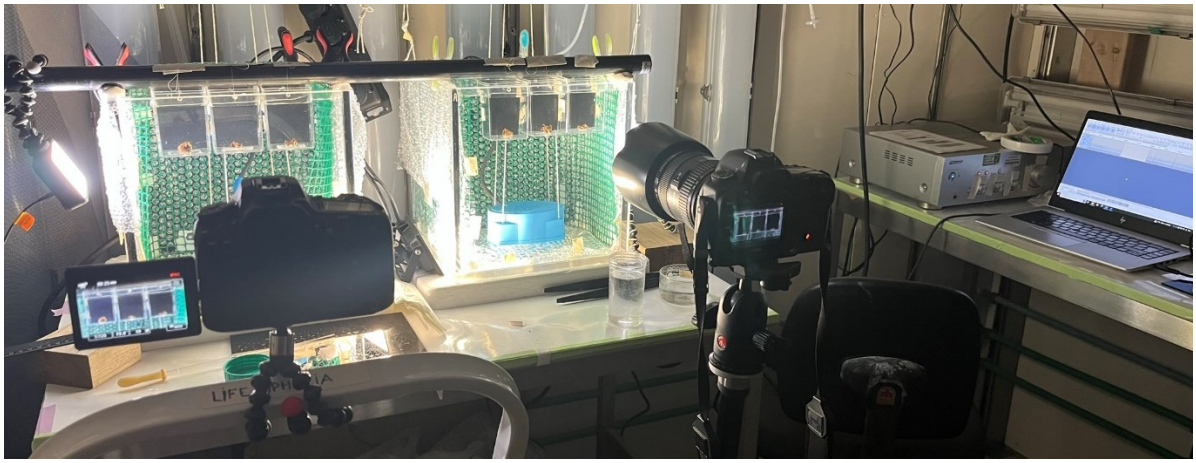

Figure 2 Experimental set-up showing the cameras filming the larvae that were distributed in the small cups (with black background). The blue underwater speakers are connected to an amplifier and a laptop through which the sounds were played. This picture was taken during the second experiment "healthy sounds + rubble".

The experimental sounds were playbacks from data collected in 2020 as part of the ASSSEMBLE Plus AmpLophelia project (Grant Agreement No. 730984) (De Clippele and Risch 2021). These sounds were collected at the same cold-water coral reef the adult *Lophelia pertusa* corals were collected. These recordings were made with a calibrated omnidirectional long-term ST500 recorder (Ocean Instruments). A sample of the playbacks were recorded, characterised and compared with the original recording. This was to make sure the sound pressure levels in the tanks were the same as those recorded in the field. The playbacks in the experimental tanks were recorded using a long-term ST600 recorder(Ocean Instruments). The recorded .wav files were exported and Power Spectral Density plots were created in PAMGuide and visually compared. The amplifier's knobs (bass, treble, balance, volume) and settings in the software Audacity were adjusted to ensure the speaker's sound pressure levels matched those recorded in the field as well as possible (Figure 3).

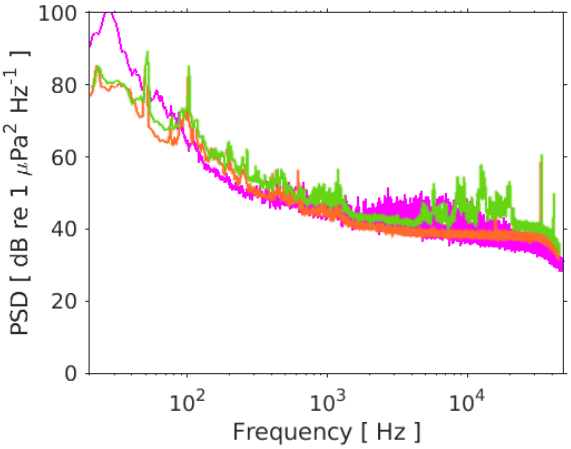

Figure 3 Power Spectral Density plot comparing the natural reef sounds (pink), with the tank's reef sounds (green) and the sound measured in the control tank (orange).

Preliminary results

To analyse the larvae's behaviour in the video recordings, first, the software VLC was used to extract a frame per second. Then pre-processing of the Images was done in the Fiji Image J software. Pre-processing included cropping the images, subtracting the background and removing air bubbles. The Fiji plugin TrackMate (Tinevez et al. 2017, Ershov et al. 2022) was used to automatically detect the larvae. TrackMate can extract information on the location of the larvae in the cups (i.e., top, middle, bottom) using the Y-coordinates and it also allows the visualisation of their swimming tracks (Figure 4,5), measure their track duration and swimming speed. While full analysis will be conducted on the video recordings from all three experiments, here preliminary results are visualised for the first ten minutes of the third noise pollution experiment.

The location of the swimming tracks indicates that the larvae exposed to noise pollution (Figure 4) remain more closely associated with the surface waters, while those not exposed to noise pollution explore the bottom of the cups (Figure 5). This could indicate that the noise pollution present at the Tisler Reef could negatively impact the settlement of larvae, and therefore affect the long-term health and survival of the reef. Further detailed statistical analysis will be conducted to confrim this.

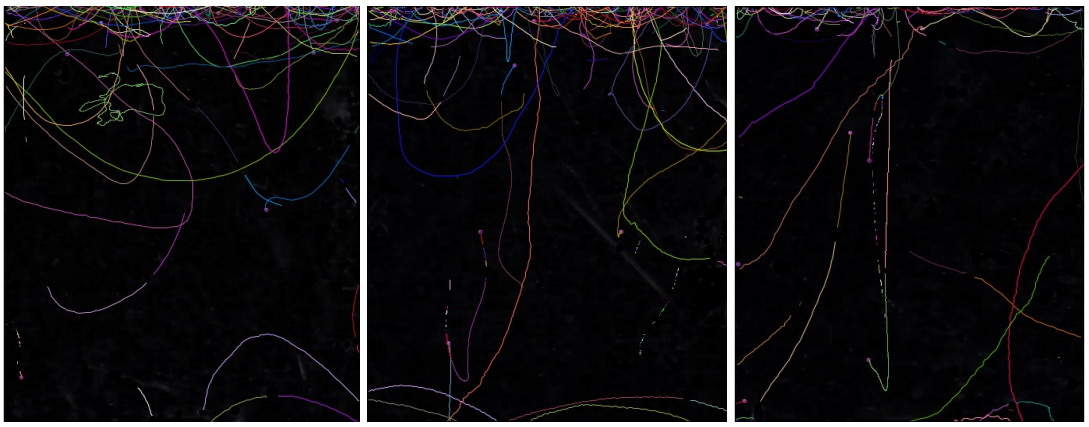

Figure 4. Swimming tracks of the larvae within Tank A (Experimental tank)

Figure 5. Swimming tracks of the larvae within Tank B (Control tank)

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