Suomen ympäristökeskus
Finlands miljöcentral
Finnish Environment Institute

# Action A8.2 Deliverable – Plan for noise exposure experiments on fish and blue mussels

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LIFE20 IPE/FI/000020 LIFE-IP BIODIVERSEA

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# Summary

Sub-Action A8.2 within the Life-IP Biodiversea project includes laboratory and field studies on three taxa, namely, zebrafish (*Danio rerio*), blue mussels (*Mytilus* spp.) and three-spined stickleback (*Gasterosteus aculeatus*). A team within the Finnish Environment Institute has been conducting pilot testing on *Mytilus* spp. and *D. rerio* to ensure that the experiments successfully assess relevant threshold levels of underwater noise that induce adverse effects on these taxa. The laboratory experiments will be conducted in 2024–2025, whereas the field experiments are preliminarily planned to take place in 2025–2026. Therefore, all details particularly on field experiments are not clear yet, but they will be updated closer to the testing events. The noise exposure tests on *D. rerio* will include only lab tests for various zebra fish life stages, including embryos (maximum 5 days post fertilization, dpf), as well as juvenile and adult life stages. The tests on Mytilus spp. will include laboratory and field studies and the main method of detection adverse effects will be monitoring behaviour via valve gape activity. The team needs to obtain more information on the practicalities how to conduct experiments on *G. aculeatus* in the field and/or laboratory conditions, but these experiments will be conducted for adult life stages. All experiments will aim to utilise realistic underwater noise exposure recorded from the Archipelago Sea. In addition, the underwater noise treatments will include various sound pressure level ranges to determine threshold values that induce adverse effects on the test organisms.

# Contents

# 1. Noise experiments on blue mussels (*Mytilus* spp.)

## 1.1 Laboratory experiments on blue mussels

Laboratory tests will be conducted in Viikki marine research laboratory on blue mussels that have been collected from Southwestern coastal waters of Finland. The size and origin of the tested mussels will be recorded for every test. The mussels will be placed on plastic holders, and 3–12 individuals can be placed simultaneously for testing, as the installation system of valve gape movement sensors includes holders, magnets glued to the mussels, and the sensors that monitor the valve gape movement (behaviour) (Figure 1). A software in the laptop will record the valve gape movement during a selected test period. Approximately 60 litre tanks have been tested for this purpose and they have appeared suitable for testing.

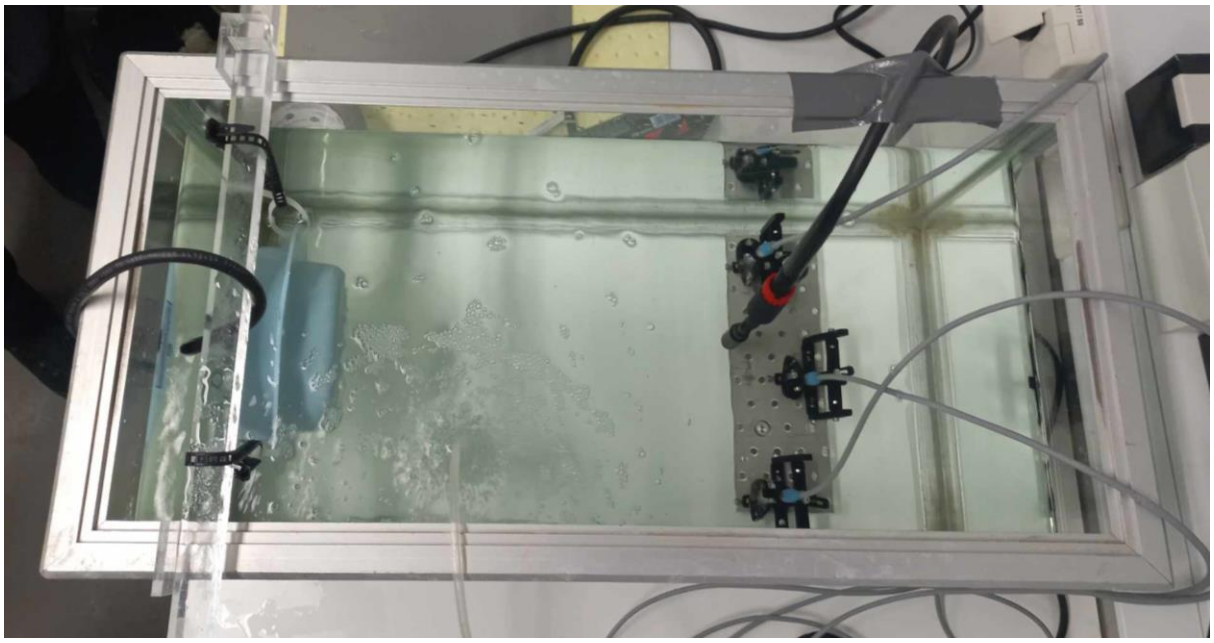

Figure 1. A view above the testing tank for blue mussels from preliminary tests, with an underwater speaker on the left, and 3 test mussels and a hydrophone on the right.

First, all testing equipment and noise exposure files will be tested in the tank filled with sea water to ensure that the sound exposures are suitable for testing. In addition, the team needs to make sure that light conditions and other potential external disturbance factors are minimized during the test period. Temperature and light conditions of the test room can and will be controlled. Once the mussels have been collected from the field, they will be acclimatized to laboratory conditions. Syke has appropriate coolers, tanks and rooms for this purpose and various concentrations of algae will be prepared to feed the mussels throughout the acclimatization period and testing.

Once the mussels have been acclimatized to the laboratory conditions, the testing will include the following steps;

a) Valve gape movement will be monitored within the tanks. This will include gluing magnets and recording the mussels for 1–3 days without any exposure to make sure that the installation has been successful, and the mussel valve gape behaviour is being monitored correctly.
b) 2–4 different noise exposure ranges will be exposed to the tested mussels using an underwater speaker, for example ship and/or boat noise of 100 – 110, 120 – 130 and 140 – 150 dB re 1µPa, to detect their behavioural response to the noise exposure. A hydrophone will be used to record the sound exposure in the tanks. The noise exposure files will include a constant noise exposure to

ensure that a response will be received, as well as more realistic, shorter noise exposures that mimic the duration, sound intensity and spectrum of a ship or a leisure boat passing by.

c) The mussel response to noise exposure will be recorded short term, i.e., how do the mussels initially react to the noise exposure, and how long does it take for them to recover from the exposure and return to their normal behaviour.
d) All laboratory tests will be relatively short term (from hours to days, maximum 1–3 weeks) to ensure that laboratory conditions and other external disturbance factors can be minimised.
e) In addition, certain biomarkers of oxidative stress, growth and energy metabolism will be tested from the individuals exposed to noise, which can be compared to individuals in control tanks.

Background literature

Hubert, J., Booms, E., Witbaard, R. and Slabbekoorn, H., 2022. Responsiveness and habituation to repeated sound exposures and pulse trains in blue mussels. Journal of Experimental Marine Biology and Ecology, 547, 151668. doi: https://doi.org/10.1016/j.jembe.2021.151668

Hubert, J., Moens, R., Witbaard, R. and Slabbekoorn, H., 2022. Acoustic disturbance in blue mussels: sound-induced valve closure varies with pulse train speed but does not affect phytoplankton clearance rate. ICES Journal of Marine Science, 79(9), 2540–2551. doi: https://doi.org/10.1093/icesjms/fsac193

### 1.2 Field experiments on blue mussels

The team discussed various options on how to deploy blue mussel installations to coastal areas that are expected to be noisy and silent and detect changes in mussel behaviour in field conditions. The exact test locations will be selected later from the Archipelago Sea, and the team will aim for longer test duration in comparison to laboratory experiments, from weeks to months. The team will explore different options for blue mussel field installations to be conducted in 2025 and/or 2026, including mussel cages and metal frames (landers) to ensure that the installation remains stable and various sensors in the installation remain operational.

Background literature

Ballesta-Artero, I., Witbaard, R., Carroll, M.L. and van der Meer, J., 2017. Environmental factors regulating gaping activity of the bivalve Arctica islandica in Northern Norway. Marine Biology, 164, 1–15. doi: https://doi.org/10.1007/s00227-017-3144-7

## 2. Noise experiments on zebrafish (*D. rerio*)

All noise exposure experiments on zebrafish will be conducted under laboratory conditions, and Meilahti zebrafish unit is the preferable laboratory, as they have very good facilities for testing zebrafish. Syke team did preliminary underwater noise exposure experiments on zebrafish embryos in November 2023 at Meilahti and were able to utilise underwater speaker and expose sound to embryos placed in approximately 40 litre tanks (Figure 2).

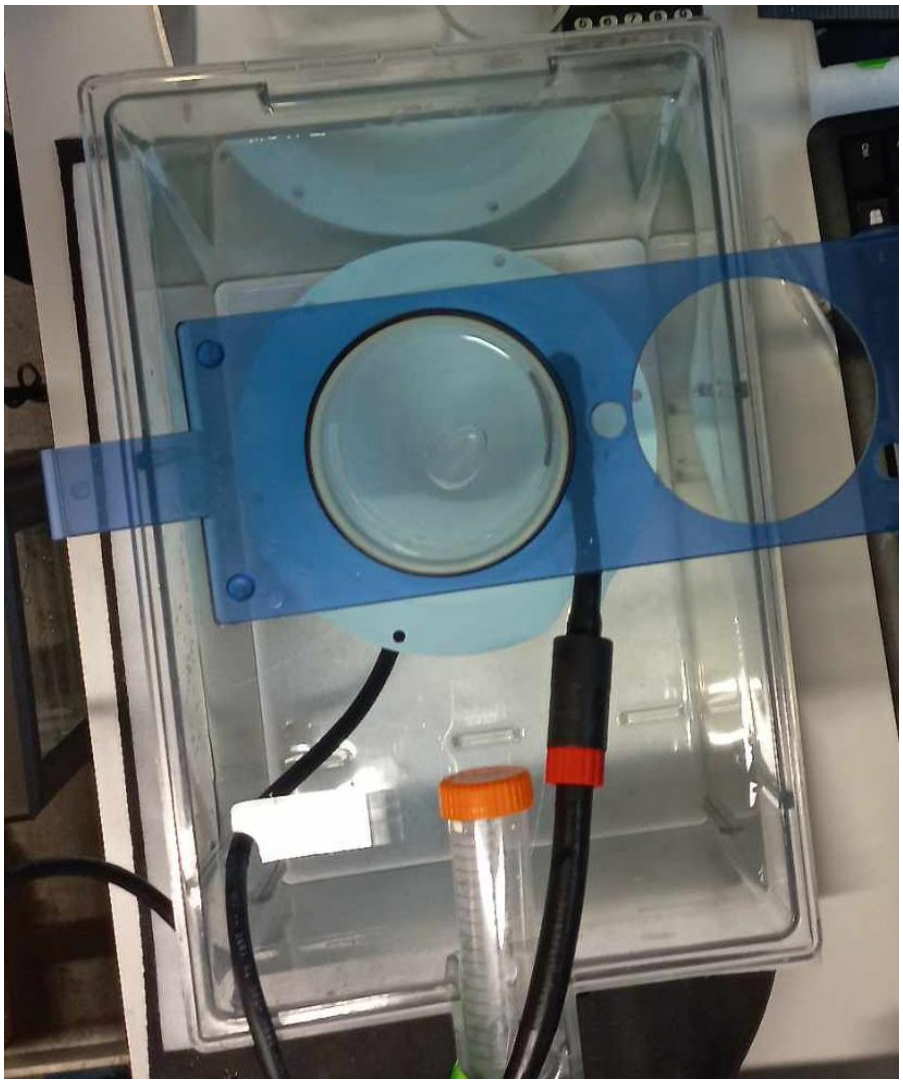

Figure 2. A view above the testing tank for zebrafish embryos from preliminary tests, with an underwater speaker at the bottom of the tank, a hydrophone and zebrafish embryos in a cylinder-shaped basin with a mesh in the bottom.

Similarly to laboratory testing for blue mussels, all testing equipment and noise exposure files will be tested in the test tanks prior to beginning the tests to make sure that the system is operational, and the effect of potential other external factors are minimised. In addition, the team will conduct tests on zebrafish embryos that are 5 days post fertilisation (dpf) at maximum, which does not require applying for permissions to conduct tests on vertebrates. Noise exposure experiments on juveniles and adult zebrafish will require applying for permissions, and they will be applied in early 2024.

Background literature

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

## 2.1 Laboratory experiments for zebrafish embryos

As the testing for zebrafish embryos will last only for 5 days (at maximum), these experiments will have a short noise exposure duration (hours to days). The behavioural response of zebra fish on noise exposure will be monitored with cameras. Furthermore, gene-level responses will be assessed from the zebrafish embryos exposed to noise, and the replicate samples will include individual embryos, and potentially

pooled sample replicates that include tissue from several individuals. Mortality of embryos is a risk, and therefore approximately 48–96 embryos will be used per each noise treatment.

2–4 different noise exposure ranges will be exposed to the zebrafish embryos using an underwater speaker, and they will be recorded with a hydrophone, similarly to the experimental setting for the blue mussel experiments. Key information on the tested embryos, such as origin, age of their parents and embryo development will be recorded and monitored, and the individuals will be tested in temperature-controlled tanks, where the noise exposure will be the only external pressure. Syke team will seek to begin the testing for zebrafish embryos in spring, 2024.

### 2.2 Laboratory experiments on zebrafish (embryos, juveniles, adult life stages)

Additional testing for different life stages of zebra fish will be conducted at the Meilahti zebrafish unit. Details of these experiments will be discussed with the personnel working at Meilahti, but the initial idea is to expose zebrafish embryos to various levels of underwater noise and monitor the effects throughout the first 2–6 months of their life cycle. Gene-level responses and biomarkers for various biochemical responses will be tested within the exposed zebrafish when they are five days, 1–3 weeks old, 1 month-old and older than 3 months to detect whether exposure to noise at an early age affects zebrafish at different stages of their life cycle.

## 3. Noise experiments for three-spined stickleback (*G. aculeatus*)

Plans to conduct testing on three-spined sticklebacks is still at very early stages. Previous experience with colleagues from Tvärminne and University of Turku has shown that three-spined stickleback is a sensitive test species and if they are tested in a laboratory, they need to be treated carefully to ensure that they are acclimatized for laboratory conditions. Another alternative will be to conduct field experiments for three-spined sticklebacks, placing them in noisy and silent areas within the Archipelago Sea. Additional planning will be done with Tvärminne field station to determine the best alternatives to test three-spined sticklebacks' responses to underwater noise. These experiments will be conducted in 2025 and/or 2026.

Background literature

Purser, J. and Radford, A.N., 2011. Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (Gasterosteus aculeatus). PloS one, 6(2), p.e17478. doi: https://doi.org/10.1371/journal.pone.0017478