3. Chemical marker identification to study exposure of biota to common plastics

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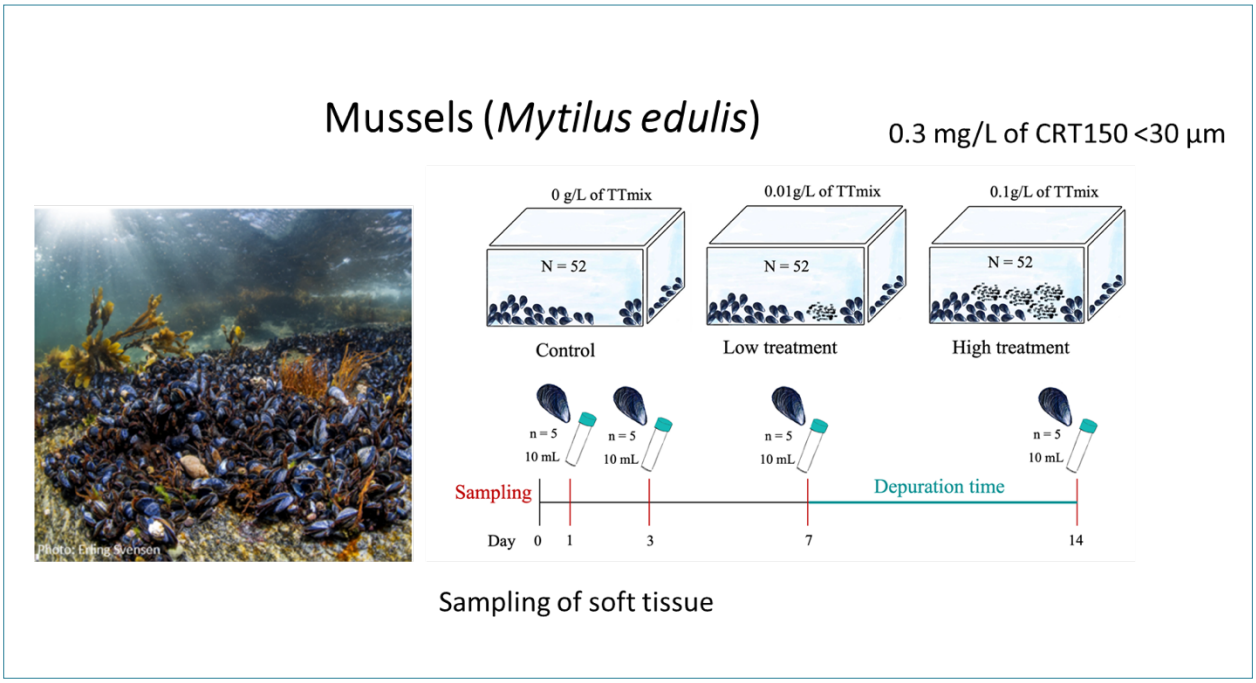
Deliverable 2.5 Analysis techniques for quantifying nano-and microplastic particles and their degradation in the marine environment, as part of the ANDROMEDA project, 2023.



Sampling of soft tissue

3.1 Scope of Protocol

Large knowledge gaps remain regarding the distribution and presence of MPs in the marine environment and their interaction with marine organisms. The determination of the occurrence of MPs of especially very small size ranges (<20 µm) in environmental samples as for instance biota is very challenging and time consuming. Chemical tracers of MP specific additives could be a tool to determine the presence of MP. Further, some plastic related additives have been recognized as persistent organic pollutants (UV 328), requiring further monitoring capabilities. The aim of this protocol is to investigate the occurrence and uptake of MP related additives into organisms in the field and under controlled laboratory conditions. To do so, we used blue mussels (*Mytilus edulis*) exposed to MPs. First, blue mussels (n = 60) were experimentally exposed for 7 days to low or high concentrations of a HDPE mix (CRT 150) (0.0003 g/L and 0.003 g/L, of 10-30 µm size range respectively), followed by a depuration period of 7 days. Individual mussels were collected on day 1, 3, 7, and 14 after initial exposure. Due to their presence in a broad variety of plastics, UV 320, 326, 327, 328, 329 were selected for analyses.

3.1.1 Sample Preparation

Frozen mussels are homogenised using a spatula and scalpel and tissue (1 gram) is transferred to glass centrifuge tubes (15 mL). Further, the samples are spiked with internal standard before adding ceramic beads, acetonitrile n-Hexane and Na_2SO_4. Homogenization is performed with a vortex shaker for 15-20 seconds before the samples are transferred to a sonication bath for 15 minutes (temperature: < 30°C). The vortex- and sonication steps are repeated once. Afterwards, the tubes are shaken in a horizontal shaker for 25 minutes, with a turning of the tubes halfway during the process (12.5 minutes), before centrifugation (1500 rpm, 10 minutes). The hexane- and acetonitrile layers are separated and stored in glass vials (2 mL) at -20°C (Galtung, 2023).

3.1.2 Determination of Chemicals

100 µL of hexane layer from extracted samples are transferred to GC/MS vials (0.3 mL) and spiked with recovery standard (13C6 – 6PPD-Quinone). Each vial is vortexed for 5 seconds before they are run on the gas chromatography mass spectroscopy (GC/MS, Orbitrap). The organic chemical extracts are analysed by a Q Exactive GC (Orbitrap GC/MS) at the NILU laboratory.

3.2 Quality Control Measures

To remove any contamination with the target compounds, Na_2SO_4 is burned at 600°C for 8 hours and all glass wear and ceramic beads are rinsed and burned at 450°C for 8 hours and covered with aluminium foil prior to laboratory work to avoid contamination. Ceramic beads are rinsed with tap water and an alkaline rinse before burning. Metal equipment is rinsed according to the standard of the laboratory (NILU), followed by an ultrasonic bath in n-hexane for 10 minutes. The 100 µL glass pipettes used are not burned due to the risk of changing their accuracy by shape changes caused by heat damage. No plastic equipment is used during sample handling.

To ensure extraction of both polar and non-polar organic chemicals from the tissues, solvents such as acetonitrile (non-polar) and n-hexane (polar) are utilized. An internal standard is added to the samples to detect and quantify organic chemicals related to MP, combined with a recovery standard prior to GC/MS analyses, to control potential losses of target compounds during the laboratory procedure.

References

Galtung, K. (2023). *Exposure of marine invertebrates to car tire rubber: Uptake of rubber particles and related organic chemicals*. Master's Thesis. Faculty of Bioscience, Fisheries and Economics. Department of Arctic and Marine Biology. University of Tromsø, Norway.

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