DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category 4: Other approaches

Toolbox component: Lab toxicity studies

Fact Sheet 4.5: Mussels lab exposure to warfare agents

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What is it?

Lab exposure studies are developed to establish threshold values (e.g. lethal concentrations, "No-effect"-concentration, etc.) for a certain warfare agent.

What does it tell you?

Evaluations of effects of warfare agents produce a basic understanding of the impacts of these compounds on biota and help to estimate environmental risks.

How to measure it?

Mussel individuals are collected at a pristine site. Ambient conditions, such as temperature and salinity are measured at the sampling site. Mussels are transported in aerated, cooled ambient water to the lab. In the lab ambient conditions are imitated using temperature and light controlled labs. Mussel shells are carefully cleaned and subsequently placed in big glass aquaria using the ambient seawater from the transport. After 48 hours of acclimatization ambient water was partly removed by exchanging half of the volume every 24 hours using premixed artificial sea water with ambient salinity. Artificial seawater consists of deionised water to which the appropriate amount of aquarium salt was added. The light regime is adjusted to-the day/night-rhythm at the time of sampling. During that time of acclimatisation the mussels are fed twice a week using live algae suspensions.

The experimental setup encompassed 15-litre glass aquaria filled with a volume of ten litres each for each treatments and control. Each treatment is carried out in triplicates. Due to the large filter volume of mussels we recommend to use only low number (5-10) mussels per aquaria. Oxygen is supplied by air pumps.

Water exchange and re-dosing of the pollutant is done every 24 hours. In the course of the water exchange the mussels were removed from the basins. After that they were returned to the aquaria. This procedure was carried out for the treatment mussels as well as for the control mussels. Having finished the water exchange for all three replicates of a treatment, the warfare agent is added to the basins.

To test for the toxicity of dissolved warfare agents an acute toxicity test of 96 hours and a test on chronic exposure is performed following OECD guidelines (OECD, 2009; 2014).

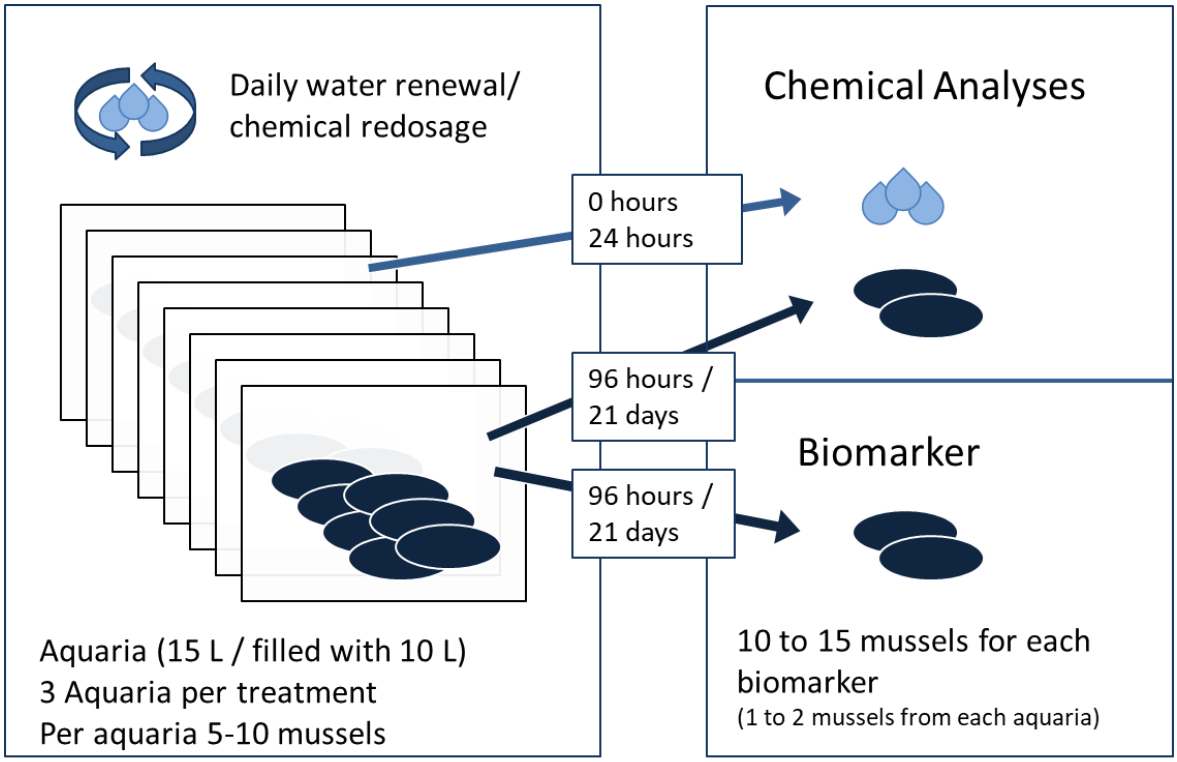

Fig. 1: Experimental design for a lab exposure study using blue mussels as target organism.

How to analyse and assess the data?

A wide battery of chemical and biomarker analyses can be performed on the mussel samples. Mussel should be supervised and monitored during the entire experiment. Mussels which spawned spontaneously should be excluded from biomarker analysis. Chemical analysis for both water and tissue concentrations should be performed.

References

OECD (2009). Guideline 230: OECD Guideline for the Testing of Chemicals; 21-day Fish Assay: A Short-Term Screening for Oestrogenic and Androgenic Activity, and Aromatase Inhibition

OECD (2014). Guideline 203: OECD Guideline for the Testing of Chemicals; Fish, Acute Toxicity Test