Standard Operating Procedure (SOP)

Aim of SOP (tick box)

☐ Munition detection or identification	☐ Toxicity
☒ Sampling	☐ In situ exposure studies
☐ Chemical analysis	☐ Bioassays
☐ Bioindicators/biomarkers	

14. Sampling of mussels

version 1.1

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Scope

This SOP describes the method of sampling Baltic Sea blue mussels (*Mytilus trossulus*) for biological and chemical analysis of effects potentially caused by dumped munitions. The method is based on Turja et al. 2014[1] and Leiniö & Lehtonen, 2005[2], and has been modified by SYKE. It has previously been used in two EU-funded projects dealing with chemical warfare agents: Chemical Munitions, Search and Assessment (CHEMSEA) and Decision Aid for Marine Munitions (DAIMON). These instructions also apply to the procedure of caging mussels (see separate SOP).

Summary of the method/SOP

Mussels can act as sentinels to observe if the surrounding waterbody contains toxic or harmful substances and if they potentially cause biological effects in local organisms. Sampling them correctly will ensure the validity of the chemical and biological effects analyses.

Safety aspects

Normal scuba diving safety rules should be applied. If sampling is close to old munition, special restrictions on who can perform the diving may apply, and special permissions may be required

Documentation

The sampling coordinates should be recorded, as well as ambient temperature, salinity, oxygen and Secchi depth at the sampling location. Sampling site contamination history should be known.

Methods

In the non-tidal Baltic Sea the collection is usually done by scuba diving. The mussels are scraped from the surface they are attached to (rocks, munitions) with a plastic or metal tool such as a kitchen spatula into a scuba mesh bag. After collecting enough samples, the mussels are kept in aerated water from the collection site.

Samples for the laboratory analyses should be dissected from the contaminated area, but also

from a natural population in a clean area, from the same depth as the study area mussels.

Taking samples for biological analysis: Cut the mussel open and remove any debris inside it by gently rinsing with seawater from the sampling location. Dissect the mussel and remove the gills into a cryovial. Dissect the digestive gland into a separate cryovial. Freeze the samples in the cryovials immediately in liquid nitrogen. For long-term storage, the samples should be kept in -80°C. Gills and digestive glands from different individual should be placed in separate cryovials.

Taking samples for chemical analysis: Cut the mussel open and remove any debris inside by gently rinsing with seawater from the sampling location. Dry the mussel by dabbing it into tissue paper. Scrape the soft tissue of the mussel into a glass vial. Soft tissues from 20-40 individual mussels can be pooled into the same glass vial in order to obtain the required sample mass for chemical analysis (ca. 20 g). Keep the glass vial on ice if possible and freeze at -20°C when the needed mass is obtained. Dissecting equipment should be changed or cleaned between mussels from different sampling locations in order to avoid cross-contamination. If possible, start the sampling with reference area mussels that are expected to be the cleanest.

Equipment: A boat, scuba diving gear, scuba mesh bags, scraping tools, cooler boxes, aeration (e.g. aquarium aereators), equipment to measure water temperature, salinity, oxygen, Secchi plate, glass cutting boards, disposable scalpels, forceps, cryovials, glass vials, marking equipment, liquid nitrogen, -80°C freezer, -20°C freezer.

Conclusions (if applicable)

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References

[1] Turja, R., Höher, N., Snoeijs, P., Baršienė, J., Butriavičienė, L., Kuznetsova, T., Kholodkevich, S.V., Devier, M.H., Budzinski, H., Lehtonen., K.K., *Sci Total Environ 473-474,* **2014**, 398-409.

[2] Leiniö S., Lehtonen K.K., *Comp Biochem Phys C 140,* **2005,** 408-21.

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