# Standard Operating Procedure (SOP)

**Aim of SOP** (tick box)

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

## 14. Mussel caging approach

**version 1.1**

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### Scope

This SOP describes the method of caging Baltic Sea blue mussels (*Mytilus trossulus*) to identify the possible effects of dumped munitions. In this method, the mussels are transplanted in cages for examination of the *in situ* exposure of chemical warfare agents and their biological effects. The combination of chemical analysis and biological effects methods (biomarkers), provides essential information for establishment of link between contaminant concentrations and stress responses in the target organism. The method is based on Turja et al. 2014[1] and Leiniö & Lehtonen, 2005[2] and has been modified by SYKE. It has been used worldwide for studying of different kind of contamination in the marine areas. For contamination of chemical warfare agents, the caging method has been used in two EU-funded projects: Chemical Munitions, Search and Assessment (CHEMSEA) and Decision Aid for Marine Munitions (DAIMON)[3].

### Summary of the method/SOP

Mussels collected from a clean, uncontaminated area are transplanted in specially designed cages close to a suspected source of dumped munitions for a certain period of time, usually 1-2 months. Accumulation and possible effects of contaminants are then measured in the mussel tissue samples.

### Safety aspects

For sampling, 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. Permissions from the military etc. may be required for caging next to munitions. The positions of the studied munitions should be known exactly to avoid dropping the anchor of the cages on them.

### Documentation

Ambient temperature, salinity, oxygen and Secchi depth at the caging location should be monitored by loggers. Passive samplers can be used to detect chemical pollution pulses in the area.
If mussels are caged for more than a month, effects of seasonality should be controlled with sampling mussels again from the uncontaminated area at the same time as sampling from the

cages.

**Methods**

Mussels for the caging experiment should be collected from a clean area, with as similar as possible environmental conditions and depth as the planned caging area. In the non-tidal Baltic Sea the collection is usually done by scuba diving. The mussels are kept in aerated water from the collection site. Only mussels between the size of 2 and 3 cm (in the Northern Baltic) or between 3 and 4 cm (in the Southern Baltic) and are used in caging to have individuals of approximately the same age. The amount of mussels used in caging varies based on what measurements will be studied, but 300-400 individuals per cage has been enough for both biological and chemical analyses and allows for some mortality during the caging period. Before deployment, all epibionts are removed from the shells surfaces and the individuals are counted for the estimation of mortality rate during the exposure period. A subsample is taken to record the measured parameters and tissue contaminant concentrations prior to exposure. The size of the initial subsample should be the same as the expected sample after the caging period (e.g. 40 mussels for biological biomarkers and 80 mussels for chemical analysis).

Preferably, samples should be taken also from the same natural population at the clean, uncontaminated sampling area after the exposure time to examine natural seasonal variability and the effect of the caging procedure on the measured parameters. At least one reference cage should be anchored to the same sea area for example 2-10 km away from the dumped munition site, far enough from the expected contamination sources and using the same depth range and other physical conditions (salinity, oxygen, Secchi depth). The exact distance of the reference cage(s) from the “hotspot” cage depends on the sea area (currents, bottom geology) and degree of contamination (point source or contaminated sediment spread to a larger area).

*Equipment*: The cages should have boxes, bags or equivalent containers where the mussels are placed in. A mesh-like structure allows the water to enter the box and avoids the mussels from dropping out. In the DAIMON project SYKE applied specially designed metal cages (Fig. 1). To avoid any harmful chemicals leaking from the cage itself they were manufactured using AISI 316 stainless steel. The cages are anchored to the bottom with a rope attached to a weight of approx. 350 kg and they are held in a stable vertical position by submerged buoys (Figs. 1 and 2). Different kinds of data loggers measuring, e.g., oxygen, temperature and salinity, or passive samplers can be attached to the metal caging frame or to the boxes (Fig. 3).


Fig. 1. Metal caging frame with boxes.

Fig. 2. Anchoring set-up.

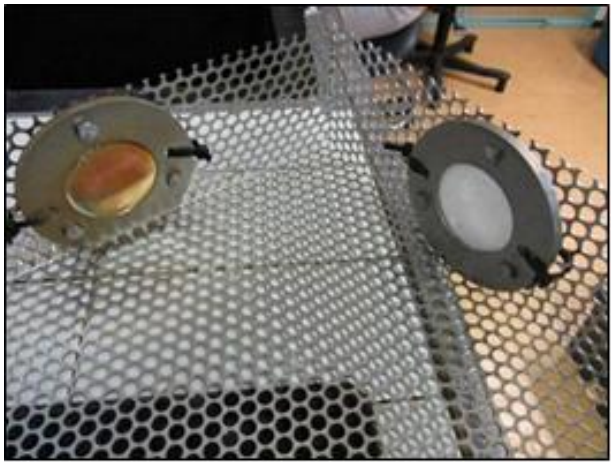

Fig. 3. POCIS passive samplers attached to a box.

After the exposure period the mussels are retrieved and tissue samples should be dissected immediately (see separate SOP “Sampling of mussels” for details). Different organs are dissected for the selected biomarker analyses and snap-frozen in liquid nitrogen and subsequently stored at -80°C, and whole soft tissue samples from separate individuals are dissected and stored at -20°C for chemical analyses. The general condition of the population is recommended to be analyzed by using a morphometric condition index derived from shell length and dry weight of the mussels.

**Conclusions (if applicable)**

A wide battery of chemical and biomarker analyses can be performed on the mussel samples. Important issues to be taken account when assessing the data are:

1. Seasonal variability in the physiology of mussels, including nutritional and reproductive status: this has marked effects on the condition of the mussels and to the natural levels of many biomarkers[2].
2. Environmental conditions in the caging area: temperature and salinity affect many biological functions and together with the oxygen level should be measured and monitored during the experiment.
3. Measurements from the "hotspot" cage should be compared (statistically) to samples from a reference cage. Increasing the number of measurements from each cage increases the statistical power.

## References

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[3] Bełdowski, J., Fabisiak, J., Popiel, S., Östin, A., Olsson, U., Vanninen, P., Lastumaki, A., Lang, T., Fricke, N., Brenner, M., Berglind, R., Baršiene, J., Klusek, Z., Pączek, B., Söderström, M., Lehtonen, K., Szubska, M., Malejevas, V., Koskela, H., Michalak, J., Turja, R., Bickmeyer, U., Broeg, K., Olejnik, A. and Fidler, J. *Institute of Oceanology Polish Academy of Sciences, Gdańsk, Poland,* **2014**, ISBN: 978-83-936609-1-9, 86 pp

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