DAIMON Toolbox Fact Sheets:

Methods to Study the Impact of Dumped Munitions on Marine Biota

Assessment category: Other approaches

Toolbox component: *In situ* exposure experiments

Fact Sheet 4.1: The mussel caging approach

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What is it?

In situ transplantation of mussels has been widely used as a bioeffect monitoring and assessment method in marine environments. In the DAIMON project we used caging of Baltic mussels (*Mytilus trossulus*), which has been demonstrated to be a useful tool in coastal environment of the northern Baltic Sea as well (e.g., Turja et al., 2013, 2014). Mussels are suitable for caging experiments as they filter large amounts of water, accumulate many types of contaminants, and are relatively tolerant to pollution.

In caging experiments, mussels collected from a clean area are transplanted in cages close to a suspected contamination source for a certain period of time, usually 1-2 months. Accumulation and possible effects of contaminants are then measured from the mussel tissue samples.

What does it tell you?

Caged mussels act as sentinels to observe if the surrounding waterbody contains toxic or harmful substances and if they potentially cause biological effects in local organisms.

How to do it?

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. 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 sample subsample is taken to record the measured parameters and tissue contaminant concentrations prior to exposure.

Preferably, samples should be taken also from the natural population at the clean 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 far away from the expected contamination sources and using the same depth range.

***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.

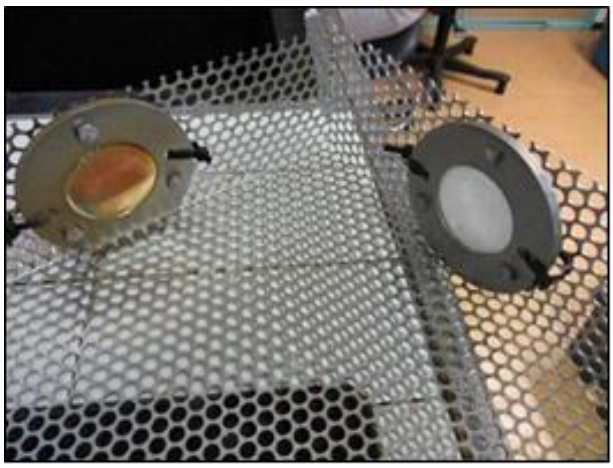

Fig. 3. POCIS passive samplers attached to a box.

After the exposure period the mussels are retrieved and tissue samples should be dissected immediately. Different organs are dissected for the selected biomarker analyses and snap-frozen in liquid nitrogen and subsequently 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 analysed by using a morphometric condition index derived from shell length and dry weight of the mussels.

How to analyse and assess the data?

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 (Leiniö and Lehtonen, 2005).
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.

References

Leiniö S., Lehtonen K.K. 2005. Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and *Macoma balthica* from the northern Baltic Sea. *Comparative Biochemistry and Physiology C: Toxicology and Pharmacology*, 140:408-21.

Turja R., Höher N., Snoeijs P., Baršienė J., Butrimavičienė L., Kuznetsova, T., Kholodkevich S. V., Devier M.-H., Budzinski H. and Lehtonen K. K. 2014. A multibiomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (*Mytilus trossulus*). *Science of the Total Environment* 473-474: 398–409.

Turja R., Soirinsuo A., Budzinski H., Devier M.H., Lehtonen K.K. 2013. Biomarker responses and accumulation of hazardous substances in mussels (*Mytilus trossulus*) transplanted along a pollution gradient close to an oil terminal in the Gulf of Finland (Baltic Sea). *Comparative Biochemistry and Physiology C: Toxicology and Pharmacol ogy*, 157:80-92.