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 4.2: The fish caging approach

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What is it?

In situ transplantation of fish has been used as a bioeffect monitoring and assessment tool in marine environments (Hall et al. 1990, Beyer at al. 1996, Burton et al. 2005, Ek et al. 2006, Oikari 2006). In the DAIMON project, an approach for exposure of the flatfish species common dab (*Limanda limanda*) to dumped conventional munitions was developed and tested in the dumpsite Kolberger Heide (Kiel Bight, Germany).

In caging experiments, fish collected from a clean area are transplanted in cages close to a suspected contamination source for a certain period of time. Accumulation and possible effects of contaminants are then measured by targeted chemical analysis of appropriate tissue samples and by applying a range of biological effects techniques selecting on basis of the contaminant characteristics.

What does it tell you?

Caged fish act as sentinels to observe if the surrounding environment (water, sediment, biota as food) contains toxic or harmful substances and if they potentially cause biological effects in the test organisms.

How to do it?

Fish 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. The sampling of control fish should not be done too far away from the study site in order to minimise the risk that fish are from different stocks with possible different characteristics.

The collection of fish is usually done by trawling (bottom trawling for demersal/benthic species; pelagic trawling for shoaling fish) on board research vessels. The duration of the hauls should be as short as feasible (e.g., 10-15 min) in order to minimise stress to the fish. If feasible, sampling of fish can also be done with fike nets or fish traps, both less stressful methods than trawling. Once the fish are on deck, they need to be transferred as soon as possible to tanks with aerated water from the collection site. The water temperature during maintenance should be more or less the same as in the sea.

Before deployment, a sample subsample is taken for the analysis of the parameters to be measured and tissue contaminant concentrations prior to exposure. If the aim of the experiment is to trace changes at the individual levels, sex, length, weight and externally visible diseases of fish should be recorded prior to the experiment. Also, non-destructive tissue sampling (e.g. blood) and individual labelling (e.g. by using PIT tags) of the fish can be done. However, care must be taken that stressing of the fish is kept to the minimum possible.

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 away from the expected contamination sources and using the same depth range.

***Equipment*:** The fish cages can be designed as shown in Figures 1 and 2. Such cages were used in the DAIMON project to expose dab in a conventional munitions dumpsite. The cage frame is made of stable, but flexible plastic tubes in a way that the cages can be folded when not used or during transport. The size of the cages can be variable, depending on the possibilities to handle them easily. If flatfish are to be exposed, the bottom area should be large while the vertical dimension could be kept to the minimum feasible. The diameter of the cages used in DAIMON was 1.4 m and the height was 0.7 m. The meshes of the net material allow the water to enter the cage and avoid the fish from escaping. The mesh size and the material of the net should be chosen in a way that fish cannot hurt themselves by squeezing through the meshes or when getting in close contact with the net (e.g., by using net material without knots). In DAIMON, the mesh size of the bottom was 20 mm and 30 mm at the side and top parts (suitable for flatfish ≥20 cm).

For removal of the fish from the cages after exposure, a zipper opening can be installed at the bottom part or at the side part, and there should be a large closable opening on top.

Different kinds of data loggers, measuring, e.g., oxygen, temperature and salinity, or passive samplers or video cameras can be attached to the cage frame. Water surface buoys can be attached for easier spotting of the cages.

***Deployment and retrieval of cages*:** When using flatfish, the cages are directly placed on the sea floor and are anchored, e.g., by attaching 3-4 stones or other suitable weights. When using other fish species, an option may be to place the cages in the water column at a suitable water depth.

Depending on the characteristics of the exposure sites, the cages can either be deployed or retrieved directly from a vessel or with the help of divers. Divers are recommended particularly when cages have to be deployed at very specific spots, e.g., in direct vicinity to dumped munitions.

The duration of the caging experiments largely depends on local conditions and on the condition of fish used. Local conditions (water temperature, oxygen concentration, salinity) have to checked prior to the experiment to make sure that fish will survive. Food availability is an issue: If sufficient food organisms enter the cages from the outside or are abundant in the sediment on top of which the cages are located, no extra feeding needs to be considered. However, is food availability is limited, extra feeding may be required or the duration of the experiments is kept short to avoid starvation or mortality due to starvation. Preferably, experiments are carried out when water temperature is low, because low water temperatures reduce stress in fish adapted to cold water and slow down metabolism so that fish can do well without or with only little food for longer periods of time.

In any case, it is recommended that cages are controlled regularly (e.g., by divers) in order to check the condition of the cages and of the fishes inside the cages. If mortality increases significantly, the experiment has to be terminated and the cages have to be retrieved.

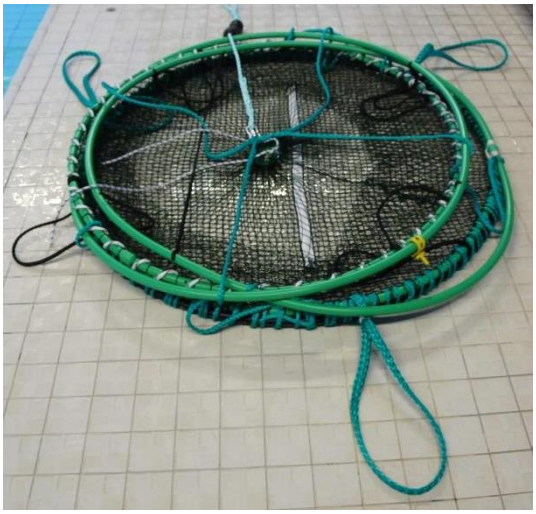

Fig 1: Fish cages used for exposure experiments with the flatfish species common dab (*L. limanda*) in munitions dumpsites in shallow waters (10-15 m) of the western Baltic Sea (photos: T. Lang, Thünen-Institut)

After the exposure period, the cages are retrieved and the fish are transferred into tanks with aerated water of ambient temperature (normally onboard suitable vessels). Individual fish are measured (sex, length weight) and examined externally. The PIT tag signal is read and recorded.

All tissue samples should be dissected immediately. Different organs are dissected for the chemical analysis and for selected biomarker analyses. Depending on the type of samples, they are either snap-frozen in liquid nitrogen or subsequently stored at -80 °C or deep frozen and stored at -20 °C. Tissue samples for histology are preferably fixed in 10 % neutral buffered formalin and transferred to 70 % ethanol after 24-48 hours.

How to analyse and assess the data?

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

- Seasonal variability in the physiology of fish, including nutritional and reproductive status: this may have marked effects on the condition of the fish and to the natural levels of many biomarkers.
- Environmental conditions in the caging area: temperature and salinity affect many biological functions and together with the oxygen level should be measured prior to the experiment and monitored continuously or in intervals during the experiment.

- Sample size: the number of fish per cage should be determined according to statistical requirements.
- For statistical analyses and for assessment of data, the same methods and criteria can be used as for studies in wild fish (see, e.g., DAIMON Fact Sheets 3.16, Lang & Straumer 2019).

References

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