Standard Operating Procedure (SOP)

Aim of SOP (tick box)

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

28. Fish caging approach

version 1.1

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Scope

This Standard Operating Procedure describes an approach on *in situ* exposure of caged fish to monitor and assess bioeffects in the marine environment. *In situ* exposure of fish has also been used in several studies to achieve these objectives[1–4]. Within the DAIMON project, an approach for the exposure of the flatfish species dab (*Limanda limanda*) to dumped conventional munitions was developed and tested in Kolberger Heide (Kiel Bight, Germany). The experience gained from this experiment is described in this SOP.

Summary of the method

In caging experiments, fish taken from a clean area are placed in cages near a suspected source of contamination for a certain period of time. The accumulation and possible effects of contaminants are then measured by targeted chemical analysis from appropriate tissue samples and by applying a range of bioassays selected based on the contaminant characteristics. In this context, caged fish act as sentinels to observe if the surrounding ecosystem (water, sediment, biota) contains toxic or hazardous substances and if they cause biological effects in the caged organism.

Safety aspects

When working onboard research vessels and with pointed or sharp objects, the safety instructions of the responsible safety officer must be followed.

When using vertebrates (fish) as test organisms, the animal testing regulations and laws must be followed, and suitable permits applied in advance.

Documentation

Geographical coordinates must be logged for each sampling site and hydrographic data such as temperature and salinity must be measured so that the sampling site can be clearly identified afterwards. Individual samples are marked and documented by the sampling plan.

Methods

Equipment:

The fish cages can be designed as shown in Figure 1. 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 fish from the cages after exposure, a zipper opening may be fitted to the bottom or side of the cage and a large lockable opening should be provided at the top. Various types 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 installed to facilitate the location of the cages.

Figure 1: Fish cages used for exposure experiments with the flatfish species common dab (*Limanda limanda*) in munition dumpsites in shallow water (10-15 m) of the western Baltic Sea (photos: T. Lang, Thünen-Institute).

Sample size:

The number of fish per cage should be determined according to statistical requirements and need to be in accordance to a possible animal test proposal.

Procedure:

The fish for the caging experiment should be collected from a clean area with environmental conditions and depth as similar as possible to the intended caging area. Sampling of control fish should not be carried out too far from the study site in order to minimize the risk that fish are

from different stocks with possibly 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 minimize 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 to tanks with aerated water from the collection site as soon as possible. The water temperature during maintenance should be more or less the same as in the surrounding sea.

Before the cages are installed, a sub sample is taken to analyze the parameters to be measured and the tissue contaminant concentrations before exposure. If the aim of the experiment is to monitor changes at individual levels, the sex, length, weight and externally visible diseases of the fish should be recorded before the experiment. Non-destructive tissue sampling (e.g. blood) and individual identification (e.g. by PIT tags) of the fish is also possible. However, care must be taken to keep the stress on the fish as low as possible.

Preferably, after the exposure period, samples from the natural population should also be taken at the clean sampling site in order to investigate the natural seasonal variability and the effect of the husbandry method on the measured parameters. At least one reference cage should be moored in the same sea area, away from the expected sources of contamination and using the same depth range.

Deployment and exposure of the cages:

When using flatfish, the cages are placed directly on the seabed and anchored, e.g. with 3-4 stones or other suitable weights. If other fish species are used, it is possible to place the cages at a suitable water depth in the water column.

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

The duration of cage experiments depends largely on local conditions and the condition of the fish used. The local conditions (water temperature, oxygen concentration, salinity) must be checked before the experiment to ensure that the fish survive. The availability of food is a problem: If sufficient food organisms enter the cages from outside or are abundant in the sediment on which the cages are placed, no additional feeding needs to be considered. However, if feed availability is limited, additional feeding may be necessary, or the duration of experiments may be kept short to avoid starvation or mortality from starvation. It is preferable to conduct the experiments at low water temperatures, as low water temperatures reduce stress in fish adapted to cold water and slow down the metabolism so that the fish can survive for longer periods without or with little food.

In any case, it is recommended to check the cages regularly (e.g. by divers) to check the condition of the cages and the fish in the cages. If mortality increases significantly, the experiment must be terminated and the cages must be recovered. In doing so, the specific requirements of an animal test proposal must also be taken into account.

Retrieval of the cages:

After the exposure period, the cages are retrieved and the fish are transferred to tanks with aerated water at ambient temperature (normally on board suitable vessels). The individual fish are measured (sex, length weight) and examined externally. The PIT-tag signal for identification is read

and recorded.

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

Further remarks:

A wide range of chemical and biomarker analyses can be performed on the fish samples. Important points to consider when evaluating the data are:

- Seasonal variability in fish physiology, including nutritional and reproductive status: This can have a significant impact on the condition of the fish and on the natural variability of many biomarkers.
- Environmental conditions in the caging area: Temperature and salinity affect many biological functions and should be measured together with oxygen levels before the experiment and monitored continuously or at intervals during the experiment.

References

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Change history

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