Standard Operating Procedure (SOP)

Aim of SOP (tick box)

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

19. Sampling of wild fish

version 1.1

Thomas Lang and Katharina Straumer

Thünen Institute of Fisheries Ecology

Scope

Sampling of wild fish is a prerequisite of studies addressing the uptake and effects of munitions-related compounds in fish under in situ conditions. The way sampling is carried out may have a significant influence on the analytical results obtained and, therefore, some general principles need to be applied that are outlined in the present SOP. Some of these have already been published elsewhere and are summarized in here. References to relevant published guidelines are provided.

Wild fish act as sentinels to analyse if the surrounding environment (water, sediment, biota as food) contains toxic or harmful substances and if these are taken up by the fish and potentially cause biological effects. Preferably, chemical and biological analyses should be carried out in an integrated manner [1], so that results can directly be compared and assessed in an integrated way.

Summary of the method/SOP

A standardized sampling of fish for taking samples for the examination of biological, chemical and other parameters of fish. Therefore, a comparable analysis of samples for seasons, area, fishing methods and so on are possible.

Safety aspects

Since the work is largely carried out onboard of unstable platforms (e.g., research vessels) under partly bad weather conditions, specific safety regulations apply and relevant staff training needs to be carried out. Equipment and fish tanks etc. need to be fixed properly. Dissecting fish and taking tissue samples involves handling of sharp knives and scalpels and these should be used carefully in order to avoid injuries. Gloves should be worn to protect the observer's skin. Protective clothes (rubber boots, oil skin trousers, gloves) should also be worn. If some samples should be taken, safety aspect depends on used chemicals. Depending on the chemicals to be tested, the corresponding risk assessments have to be considered before starting work. If further instructions are required, contact the local safety officer or the laboratory manager.

Documentation

Equipment:

- Information on the research vessel, commercial fishing boat or sampling platforms
- Information on nets: gill net, pelagic or standard gear for trawling, trap
- PC, suitable software and other electronically equipment for documentation
- Electronic balances, which are suitable for unsteady conditions at sea.

Sampling procedure:

- Information on:
    - geographical coordinates
    - season, weather
    - sampling procedure and sampling strategy
    - towing time, towing speed
    - sampling condition and water conditions (e.g. temperature, depth, oxygen)

Results:

- detailed documentation of fish species, total weight (per haul)
- Detailed listing of focused fish species including their total weight
- All further documentation depends on the sample collection and the scientific goal

Methods

Equipment:

- Research vessel, commercial fishing boat or sampling platforms
- gill net, pelagic or standard gear for trawling
- PC, suitable software and other electronically equipment for documentation
- Paper protocols for recording data
- Pencils for writing protocols and for labelling
- Electronic balances
- Buckets for transporting fish
- Plastic bags for freezing of fish for subsequent processing in the laboratory
- Insulated boxes for transport of frozen samples
- Protective clothes (rubber boots, oil skin trousers, gloves)
- If applicable, a camera for documentation

***Sampling wild fish*:** Ideally, sampling of wild fish in offshore regions is carried out under fully controlled conditions onboard research vessels or, if not feasible, onboard commercial fishing vessels with scientific staff doing the sampling. For sampling in areas close to the coast, other sampling platforms (e.g., smaller boats) may be appropriate.
Sampling on a long-term basis should preferably be conducted using identical equipment (ship, gear) and conditions (e.g., towing time and speed in case of trawling) in order to reduce sampling variation. Changes in sampling gear and sampling conditions might change the catch composition and may even affect the general condition and biological characteristics of the target fish because there might be differences in behaviour and catchability of the fish.
Appropriate fishing gear is required for sampling. In offshore regions free of dumped munitions, bottom trawling with standard gears (e.g., those used in internationally coordinated stock assessment surveys) is the method of choice, particularly if flatfish or other demersal fish species (e.g., cod) are targeted. For pelagic species or in areas with low oxygen contents in the bottom water (such the deep basins of the Baltic Sea), pelagic trawls may be selected, because

fish avoid the hypoxic or anoxic bottom zones and are gathering higher in the water column. In coastal or shallow waters or in munitions dumpsites not suitable for trawling (e.g., because of a risk to get in contact with dumped munitions), fike nets, entangling gill nets or long lines may be more appropriate. Gill nets or longlines should only be used if fish are examined very rapidly after catching. For collecting fish from shallow conventional munitions dumpsites in the DAIMON project, gill nets were used successfully. For hagfish, plastic bottles prepared as baited traps were used successfully in deep waters.
For trawling, the towing time should be as long as necessary, but as short as possible, depending on the abundance of fish. Prolonged trawling would lead to too much superficial damage of the fish and, thus, problems to identify externally visible diseases/parasites. It, furthermore, would increase stress in fish caught which should be avoided if, e.g., biomarker samples (e.g., for enzymatic or immunological measurements) are taken in addition to disease examination. When using a standard bottom trawl, the average trawling speed is 3-4 knots.
If the number of specimens from one catch is not sufficient to yield a proper sample, repeated catches should be made in a narrow time window.

***Sampling strategy*:** Sampling of fish should be carried out in a period of the year when the fish species to be examined is in its stationary phase. The spawning season is not advisable because of partly considerable geographical migrations between spawning and feeding grounds and because of potential interference with spawning stress. If sampling has to be carried out in different seasons of the year for logistic reasons, possible effects of the season on the chemical and biological characteristics need to be taken into account. The frequency of sampling depends on the objectives of the study/monitoring and on resources available. However, for regular long-term monitoring it is recommended to sample (at least) once a year, always in the same narrow time window to avoid seasonal variation.
The selection of sampling sites has to be based on the objectives of the study/monitoring. For instance, if environmental effects of a known munitions point source are to be studied/monitored and assessed, sampling sites may be arranged on a contaminant gradient. If the impact on ecosystem health of a larger region known or suspected to be affected by dumped munitions is studied/monitored and assessed, a different strategy with a number of sampling sites at places representing different habitats may be considered feasible. Basically, there are two strategies for sampling: either sampling is carried out on a fixed nominated latitude and longitude or in a box with a nominated latitude and longitude at each corner of the box. Within the box, sampling positions may be randomised. Sampling should preferably be based on multiple samples in order to reduce sampling variation (haul-to-haul variation, patchiness).
Selection of sampling sites should in any case take into account information on species availability, age/length structure of the population, temporal and spatial migration patterns, general fish condition (e.g., health status) and other stressors potentially affecting fish health. Areas with mixed stocks of the same species should be avoided, because there might be genetic differences in disease susceptibility or stress reactions.

***Fish species*:** Suitable fish species are those that are abundant in the study area and are largely stationary (otherwise they might possibly not be representative of the study area). Their biology, demography and behaviour should preferably be known (e.g., from stock assessment studies). It is also helpful for the assessment of data if data on chemical contaminants and on biological effects (incl. assessment criteria) are available for these species from other studies or from monitoring for other purposes. In waters of the North Sea and Baltic Sea as well as in adjacent waters, major sampling programmes have focused on flatfish species such as the common dab (*Limanda limanda*) and the European flounder (*Platichthys flesus*) and also on bottom-dwelling roundfish species, such as Atlantic cod (*Gadus morhua*)[2] or eelpout (*Zoarces viviparus*) [3, 4]. In deep areas, the Atlantic hagfish (*Myxine glutinosa*)[5] may be a promising target

species as recent studies have shown. However, other species may also be appropriate, depending on the local conditions and fauna composition in the study areas. The main target species in the DAIMON project were Atlantic cod, common dab and Atlantic hagfish.

***Sample size*:** The sample size (number of fish sampled) to be selected largely depends on the indicator to be measured. For instance, if fish are to be examined for the occurrence of externally visible diseases, the sample size can be large since the inspection of fish only requires a short period of time (minutes) and does not involve time-consuming processing or following analytical lab work. In contrast, if the measurement of the indicator requires sophisticated sample processing and/or subsequent time-consuming and expensive analytical procedures and infrastructure, the number of samples to be analysed will have to be much smaller. However, the decision about the sample size cannot only be made on the basis of capacities, but needs primarily to be based on statistical requirements that have to be identified in the planning of the sampling. As a rule of thumb for chemical and biomarker indicators, a sample size of 20 specimens per study area/site and point in time for sampling is considered feasible and has been used in many other studies for the integrated analysis and assessment of contaminants and biological effects in wild fish.
Often, the specimens sampled belonged to certain size groups of a given fish species, e.g., for the common dab, the size group 20-24 cm total length has been used frequently. For indicators that are known or suspected to respond in a sex-specific way, sampling is often restricted to either male or female fish. Standardised size-stratified and sex-specific sampling is made to reduce the indicator response variability and to facilitate comparison between sampling points (in terms of time and space).

***Sampling strategy and processing for chemical and biomarker analysis*:** Strict sampling schemes based on the requirements of the programme/project and the local conditions at the sampling sites are needed for research and monitoring programmes, in particular if they involve a number of different labs that carry out analyses of samples from a common sample pool. Annex1 gives an overview of a stepwise strategy to take samples from fish for the measurement of indicator.
However, experience has shown that it is not always possible to follow such strict schemes. This may partly be due to practical reasons (e.g., sampling not possible because of technical problems or bad weather conditions, for instance onboard RVs) or because of the variability in environmental conditions in the sampling area (e.g., in terms of species distribution, abundance and size ranges). Thus, sampling schemes have to be developed in a way that they can be applied in a flexible way in order to meet at least the minimum requirements defined. It is important that any deviations from the original sampling schemes are properly documented.
The way the samples are processed depends on the type of biomarker/chemical analysis performed. However, some general rules apply to all samples:

- Organisms should be alive and, to the extent possible, non-stressed.
- If the impact of stressors cannot be avoided, their effects should be minimised and should always be kept at the same level (e.g., constant towing time if fish/bivalves are collected with a bottom gear).
- The number, size range and sex of specimens used for sampling should be according to the defined standard operating procedures (SOPs).
- Tissue sampling for the various purposes should follow the relevant SOPs (see Annex 1); any deviations from the standard procedures should be noted.
- Fixation and storage of samples in appropriate containers are important issues and the relevant SOPs should be closely followed.

After each haul/catch, the fish species to be examined/sampled should immediately be sorted from the catches (either from the total catch or from representative sub-samples). All specimens to be processed should be alive and in good condition. Therefore, tanks/containers with seawater of ambient water temperature need to be prepared to maintain specimens sorted from the samples/catches. It is advisable to supply tanks with running and oxygen-rich seawater. Particularly in summer, care must be taken that the water temperature is not too high. The fish are kept alive in the containers until dissecting. The time span spent in the containers/cages should be as short as possible in order to avoid effects of additional stress. If the water temperature is too high, cooling with blocks of frozen seawater is recommended.
For dissection, an area for working should be cleared, preferably a bench or table at standing height with good lighting and running water. Instruments required (measure boards, scissors, forceps, scalpels with disposable blades etc.) should be available in sufficient numbers and should be cleaned. Sample containers and fixatives (ethanol, formalin etc.) should be prepared. Suitable cloths for cleaning should be available. Paper protocols, boards and suitable pens (waterproof) for hand-written protocols and for labelling sample containers should be available.

If computer data entry software is used instead of hand-written protocols, a PC with the software installed is required and should have been checked. PC and other electronical equipment used in the lab should be placed in a clean and dry environment.

If individual weights (total weight and/or organ weight) are recorded at sea (onboard research vessels), an appropriate balance is required that is suitable for operation under instable conditions. The balance needs to be calibrated regularly at least once a day. A camera (preferably digital) is useful for documentation of all peculiarities observed.

Measurements and units: Depending on the type of indicator, various parameter measurements can be made. However, there are some supporting variables that should be recorded in addition to the indicator itself [6], preferably on a mandatory basis. These include body weight, body length, condition, gonad maturation status, gonadosomatic index (GSI), hepatosomatic index, age (usually by otolith reading) and growth (by combining information on age and size). Also, grossly visible anomalies, lesions and parasites should be recorded.

References

1 Davies, I. and Vethaak, A. Integrated Marine Environmental Monitoring of Chemicals and their Effects *ICES Cooperative Research Report* **2012,** *No. 315*, 277 pp.

2 Niemikoski, H., Straumer, K., Ahvo, A., Turja, R., Brenner, M., Rautanen, T., . . . Vanninen, P. Detection of chemical warfare agent related phenylarsenic compounds and multi-biomarker responses in cod (Gadus morhua) from munition dumpsites. *Marine environmental research* **2020,** *under review*.

3 Fricke, N. F., Stentiford, G. D., Feist, S. W. and Lang, T. Liver histopathology in Baltic eelpout (Zoarces viviparus) - A baseline study for use in marine environmental monitoring. *Mar Environ Res* **2012,** *82*, 1-14.

4 Lang, T. Fish disease surveys in environmental monitoring: the role of ICES. *ICES Marine Science Symposia* **2002,** *215*, 202-212.

5 Straumer, K., Kraugerud, M., Feist, S., Ahvo, A., Lehtonen, K., Lastumäki, A., . . . Lang, T. The use of Atlantic hagfish (Myxine glutinosa) as a bioindicator species for studies on effects of dumped chemical warfare agents in the Skagerrak. 1: Liver histopathology.

Marine environmental research **2020**, 105046.

6 Hansson, T., Thain, J., Martínez-Gómez, C., Hylland, K., Gubbins, M. J. and Balk, L. Supporting variables for biological effects measurements in fish and blue mussel. *ICES Techniques in Marine Environmental Science* **2017,** *60*.

Change history

1.0	27.06.2020	First edition
1.1	24.10.2020	Revised edition

List of authors

Thomas Lang, Katharina Straumer (1.0)

Katharina Straumer (1.1)

List of Reviewers

Anu Lastumäki (1.0)

Annex 1: Sampling scheme for the integrated chemical and biological analysis of effects of munitions compounds on fish, based on sequential steps (from step 1 to step 11)

Step	Sample/Matrix	Parameter (per individual)	Storage/Measurement Technique	SOP
1	Whole fish	Total length	Directly after catch Measure board, unit: cm below	
		Sex	Directly after catch Naked eye/dissection	
		Total weight	Directly after catch Balance, appropriate for work at sea	
2	Whole fish	Externally visible diseases/parasites	Directly after catch Naked eye	
3	Blood	Glucose	Directly after catch Medical glucose assay kit	
		Haematocrit	Directly/shortly after catch Store blood on ice in appropriate sample tubes, preferably using an anticoagulant (Li Heparin) Quantification in micro-haematocrit capillaries after centrifugation	
		Leucocrit	“	
		Erythrocyte number	Directly/Shortly after catch Store blood on ice in appropriate sample tubes, preferably using an anticoagulant (Li Heparin) Photometric detection, commercial kit	
		Haemoglobin concentration	“	
4	Blood smear	Differential leucocyte count	Air-dried, fixed (methanol) and stained (e.g., Giemsa) blood smears Microscopic quantification	
		Micronucleus assay	“	
5	Bile	Chemical analysis CWA/explosives/ metabolites/degradation products	Puncture of gall bladder Storage at -20 °C or Snap-freezing in liquid nitrogen and storage at -80 °C Analytical instruments depending on chemical compound	

6	Urine	Chemical analysis CWA/explosives/ metabolites/degradation products	Puncture of bladder Storage at -20 °C or Snap-freezing in liquid nitrogen and storage at -80 °C Analytical instruments depending on chemical compound	-
7	Liver	Liver weight (for HSI)	Balance, appropriate for work at sea	
		Macroscopic liver neoplasms	Visual inspection Fixation of liver nodules >2 mm, preferably in 10 % neutral buffered formalin Storage in 70 % ethanol Histology, light microscopy	
		Liver histopathology	Fixation of tissue sample, preferably in 10 % neutral buffered formalin Storage in 70 % ethanol Histology, Light microscopy	
		Lysosomal membrane stability	Snap-freezing of tissue sample in liquid nitrogen Storage at -80 °C Histochemistry, Light microscopy	
		Catalase	Snap-freezing of tissue sample in liquid nitrogen Storage at -80 °C Photometric measurement	
		Glutathione peroxidase	“	
		Glutathione reductase	“	
		Glutathione S-transferase	“	
		Lipid peroxidase	“	
		Superoxide dismutase	“	
		Gene transcription	Snap-freezing of tissue sample in liquid nitrogen Storage at -80 °C Molecular/genetic equipment	
		Chemical analysis CWA/explosives/ metabolites/degradation products	Deep-freezing of sample/s at -20 °C Analytical instruments depending on chemical compound	
		Macrophage aggregates	Fixation of tissue sample, preferably in 10 % neutral buffered formalin Storage in 70 % ethanol Histology, Light microscopy	
8	Head kidney	Histopathology	Fixation of tissue sample, preferably in 10 % neutral buffered formalin or in Baker’s formalin/gum sucrose	

			Storage in 70 % ethanol (for formalin fixation) or in gum sucrose (for fixation in Baker's formalin) Histology, Light microscopy	
		Lipofuscinosis	"	
		Lysosomal membrane stability	Snap-freezing of tissue sample in liquid nitrogen Storage at -80 °C Histochemistry, Light microscopy	
9	Muscle	Acetylcholinesterase inhibition	Snap-freezing of tissue sample in liquid nitrogen Storage at -80 °C Photometric measurement	
		Chemical analysis CWA/explosives/ metabolites/degradation products	Deep-freezing and storage of sample/s at -20 °C Analytical instruments depending on chemical compound	
10	Whole fish	Somatic weight (weight without inner organs) (for CF)	Directly after catch and after tissue sampling	