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Water quality — Biochemical and physiological measurements on fish —

Part 1: Sampling of fish, handling and preservation of samples

Qualité de l'eau — Mesurages biochimiques et physiologiques sur poisson —

Partie 1: Échantillonnage des poissons, manipulation et conservation des échantillons

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 23893-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

ISO 23893 consists of the following parts, under the general title *Water quality — Biochemical and physiological measurements on fish*:

— *Part 1: Sampling of fish, handling and preservation of samples*

— *Part 2: Determination of ethoxyresorufin-O-deethylase (EROD)* [Technical Specification]

Introduction

Determination of biomarker responses can be used to detect toxicity of known as well as unknown pollutants, when they occur singly or in combination. Therefore, measurement of biomarkers is a cost-effective way to assess ecosystem health. In combination with determinations of occurring and suspected pollutants, determinations of biomarkers can facilitate the interpretation of cause-effect relationships in the environment, as well as in laboratory toxicity tests. Information on commonly used biomarkers and the interpretation of biomarker responses is given in Annexes A and B, respectively.

Biomarkers like ethoxyresorufin-*O*-deethylase (EROD), metallothionein and vitellogenin are used to detect and quantify sublethal effects of pollutants, especially in fish. However, many of the biochemical and physiological variables that are used as biomarkers are sensitive not only to disturbances by the pollutants of concern, but also by the normal biochemical and physiological adjustments made by the fish in response to seasonal variation, its normal development and sexual maturation. Some variables can also be affected by general stress to disturbances caused by the handling during fish and fish tissue sampling. Therefore, standardisation of procedures used for sampling and handling of samples prior to determination of the biochemical and physiological variables is important.

Sublethal responses at the individual level usually occur before effects are seen at the population and community level. In the aquatic environment, fish are suitable for detection of physiological effects of pollutants, because they are exposed both through the water and through their food organisms. Also, the physiology and biochemistry of fishes is rather similar to that of humans and other vertebrates, making comparisons with studies on mammals easier than for those with crustaceans and other invertebrates.

This part of ISO 23893 serves as guidance for sampling and a platform for determination of biomarkers in fish, making it possible to use the measurements to:

- describe the state of the environment regarding effects of anthropogenic compounds on the health of fish;
- perform time-trend surveillance (monitoring);
- provide reference data and material for assessment of effects from point sources;
- evaluate and assess environmental threats;
- provide background information for environmental measures;
- follow up and assess effects of environmental corrective measures;
- integrate the biomarker responses with other measurements (e.g. fish abundance, recruitment and pollutant residues) in order to facilitate the interpretation of environmental status or impact.

Water quality — Biochemical and physiological measurements on fish —

Part 1: Sampling of fish, handling and preservation of samples

1 Scope

This part of ISO 23893 provides guidance on how to sample fish for determination of biochemical and physiological characteristics, such as the composition and enzyme activities of blood, liver, muscle and other tissues in order to asses the health of fish in the field as well as in the laboratory. The biochemical and physiological variables used for this purpose are often called biomarkers. This part of ISO 23893 includes recommendations and methods for:

obtaining a site-specific sample of a representative number of fish;

sampling fish tissues in the field and in the laboratory; and

handling and preservation of samples prior to analysis of biochemical and physiological variables.

2 Principle

Fish of a suitable species, age (size), and sex are sampled at selected sites at a suitable time of the year in order to reduce variability due to biological, geographical, and seasonal influences. Standardised sampling and measurement procedures, and qualified staff are used for collection of samples, transport, storage, and analysis. By these means, the results from time series of comparable data can be used to detect changes in the environment that are caused by anthropogenic compounds.

Necessary permits for fish and fish tissue sampling shall be obtained to comply with national legislation. This may include permits from the (land) owner of the fishing rights, regional environmental and fishery authorities, and ethical (animal rights) authorities.

The health of fish can be assessed by determination of biochemical, physiological, histological, and pathological methods. The subcellular and cellular variables are often called biomarkers. The primary toxic effect of pollutants, which occurs at the subcellular level, results in a biochemical or physiological change. This reaction is usually fast, and it can progress further and cause disturbances at higher levels of biological organisation within the organism, resulting in changes at the cellular and tissue (organ) level (histological changes). These can lead to disturbances of reproduction and growth, and can eventually cause death of the organism. Monitoring of fish health can, therefore, serve as an early warning system for anthropogenic disturbance. Through a combination with other measurements (integrated monitoring), it may be possible to correlate biomarker responses with for instance pollutant residues, distance from point sources, and ecological variables such as reproductive recruitment, which are known to be sensitive to pollutants.

In principle, this method can be applied to all species of fish from all types of environments (fresh, salt, brackish, cold, and warm water) and in shallow as well as reasonably deep water habitats. However, it is usually advantageous to restrict these methods to certain species of fish, which can be used as indicator species for fish health. These species shall be stationary, readily available (catchable in most locations) and reasonably resistant to handling stress. Their biology and physiology should be well known in order to make the interpretation of data easier. Examples of such species are the perch (*Perca fluviatilis*) and the eelpout (viviparous blenny, *Zoarces viviparus*), which are used for monitoring along the Swedish coast.

Preferably the fish species used in the field should be suitable for keeping in the laboratory for toxicological studies to investigate and confirm cause-effect relationships detected or suspected to take place in the field. Procedures for organ and tissue sampling are essentially identical in both field and laboratory studies. Procedures for collection of fish for field studies and for collection of organs are, therefore, described in separate sections.

3 Equipment

3.1 Fish sampling equipment

3.1.1 Fishing boat, suitable for the area.

3.1.2 Clothing for outdoor work.

3.1.3 Lifejacket, of suitable size and buoyancy for each crew member.

3.1.4 Gill nets, made from textile or nylon fibres and of specified and suitable size for catching the desired species and size and their gentle release into the fish chest used for storage.

3.1.5 Other equipment for fish capture, e.g. electroshocker and fyke nets, shall be described in enough detail to allow interpretation and repeated sampling.

3.1.6 Global positioning system (GPS) instrument, for exact location of sampling sites.

3.1.7 Nautical map, for marking of sampling sites.

3.1.8 Knife and **pair of scissors**, for gentle removal of fish from gill nets.

3.1.9 Fish chest, made from wood or other inert material for storage of fish before tissue sampling.

3.1.10 Instruments for measurement of physical and chemical characteristics of water, e.g. **thermometer, pH meter, conductivity meter**.

3.1.11 Equipment for determination of water depth, an echo-sounder or a calibrated line can be used to determine the depth.

3.2 Tissue sampling equipment

3.2.1 Jetty, with easy access to fish chest and within 100 m of the field laboratory.

3.2.2 Landing net, suitable for the fish species and size.

3.2.3 Field laboratory, boathouse, garage or mobile laboratory supplied with electricity.

3.2.4 Stick (baton), for stunning the fish prior to sampling of blood.

3.2.5 Anaesthetic, to anaesthetise the fish (details on usage are given in 4.4.3).

3.2.6 Dissection equipment: forceps, scissors, scalpel, syringes, needles.

3.2.7 Ruler, for determination of body length.

3.2.8 Balance, for determination of body mass and tissue (liver, gonad, spleen) mass.

3.2.9 Centrifuge and **tubes**, for blood plasma.

3.2.10 Microscope slides, for preparation of blood smears.

3.2.11 Sample containers, of suitable sizes for tissue samples (e.g. of plastic with snap locks).

3.2.12 Marking pen, waterproof and freezeproof.

3.2.13 Vacuum flask with liquid nitrogen, for rapid freezing and temporary storage of samples.

3.2.14 Container with solid carbon dioxide, for transport of deep-frozen tissue samples between field laboratory and analytical laboratory.

3.3 Biomarker determination equipment for the field laboratory

3.3.1 Haematocrit tubes and **centrifuge**, if required.

3.3.2 Blood glucose meter, if required.

3.3.3 Haemoglobin meter, if required.

4 Fish sampling

4.1 Statistical aspects

Feral fish, like other wild animals, are affected by a number of natural factors besides those caused by anthropogenic load. Important natural factors for fish are climate, hydrology, oxygen and salinity (abiotic factors), as well as age, size, sex, maturation, nutritional status, parasites and diseases (biotic factors). All these factors can contribute to the overall variability of the measured response variables. In order to detect temporal changes in trend monitoring and geographical variation in mapping of potential disturbance, all the abiotic and biotic factors mentioned above shall be reduced in importance as much as possible.

4.2 Frequency and season for sampling

Fish should be sampled once a year during the autumn period in order to avoid the effects of rapid changes in physiological conditions due to the reproduction season. During the autumn, most species of fish are not reproducing, and the conditions to get enough fish by stationary gear like gill nets (3.1.4) and fyke nets (3.1.5) are still good because the fish are still active. More frequent sampling at other times of the year does generally not provide any new information in trend monitoring.

In Sweden, perch for fish-health monitoring is sampled by gill nets in September, and eelpout by fyke nets in November. The most suitable period differs between countries and regions due to differences in climate. Often only sexually mature fish of one sex (e.g. females for perch and eelpout, and males for chub and zebrafish) within a certain size interval are used for each species in order to minimise the influence of sex and size.

4.3 Selection of sampling sites

In fish-health monitoring, it is of utmost importance to have as much detailed information as possible about the anthropogenic load on sites to be used as reference locations. These sites should be monitored regularly, preferably each year, in order to detect any large-scale impact from diffuse sources of pollution.

Fish-health monitoring can also be applied on a local scale. The locations of the sampling sites should then be determined by the objectives, which are usually related to the location of point sources of pollution. A suitable number of sites should be placed in a gradient from the local discharge point, or at sites which should be protected from disturbances. A reference site with a biotope, which is as similar as possible to the recipient, should also be selected.

Another aspect to be considered in the selection of sampling sites is availability of fish and reasonably easy access to the sampling site, or at least to the site where the fish is to be killed for taking the samples [the fish chest site (3.1.9)].

4.4 Sampling procedures

4.4.1 General

The number of fish should be sufficient in order to detect a predetermined change in the response variable within a certain number of years. An experienced statistician can give advice on this. It should also be considered that an additional number of fish to be sampled does not necessarily add much to the total cost of the monitoring programme. For example for perch and eelpout, 25 females each, with a total length of 20 cm to 30 cm, are sampled at each station in the Swedish monitoring programme. This number fulfils the statistical need for determination of differences between stations for all the monitoring response variables used in that programme. These are presented in Annexes A and B. If more stations are used, as in mapping of disturbance from a point source, a lower number (10 to 20) should be used at each station. By these means, more sites can be included at the same overall cost. The sex of the fish shall be determined and recorded, and a sufficient number of the sex to be used shall be sampled. For most variables, females are the preferred sex, but in some studies males should be used (e.g. for determination of vitellogenin in blood plasma).

4.4.2 Fish sampling

Fish can be caught by several methods (reviewed in Reference [3]) like angling and electric fishing gear (Reference [1]), if they are killed immediately on site, sampled directly and samples are handled appropriately. However, in most long-term monitoring programmes, adult fish are captured by gill nets (3.1.4), traps or fyke nets (3.1.5) in order to get a sufficient sample of fish of suitable size and sex.

In order to avoid unnecessary stress on the fish when they are caught and killed for tissue sampling, they should first be brought to a fish chest (3.1.9) and kept there for 2 days to 4 days before they are killed. This stabilises stress-sensitive response variables like blood glucose, blood lactate and haematocrit.

In the field, fishes should preferably be caught by gill nets or fyke nets and kept alive through frequent sampling carried out with the fishing equipment. However, other fishing techniques may also be used to collect fish. Reference to the method used or a detailed description shall be given in a report in such cases. The intention is that the fish are sampled from predetermined sampling sites by suitable fishing gear, e.g. gill nets for perch and fyke nets for eelpout. Gill nets shall be made from suitable material that facilitates the removal of fish with a minimum of damage. The mesh shall be adjusted to the species and size of the fish to be used in the study. For perch of 20 cm to 30 cm body length, a mesh size of 30 mm to 33 mm is suitable. The gill nets used for sampling of fish for population studies, as described in Reference [2], are multi-mesh gill nets, and these are not the same as the nets used in this part of ISO 23893. Ordinary fyke nets can be used to catch eelpout.

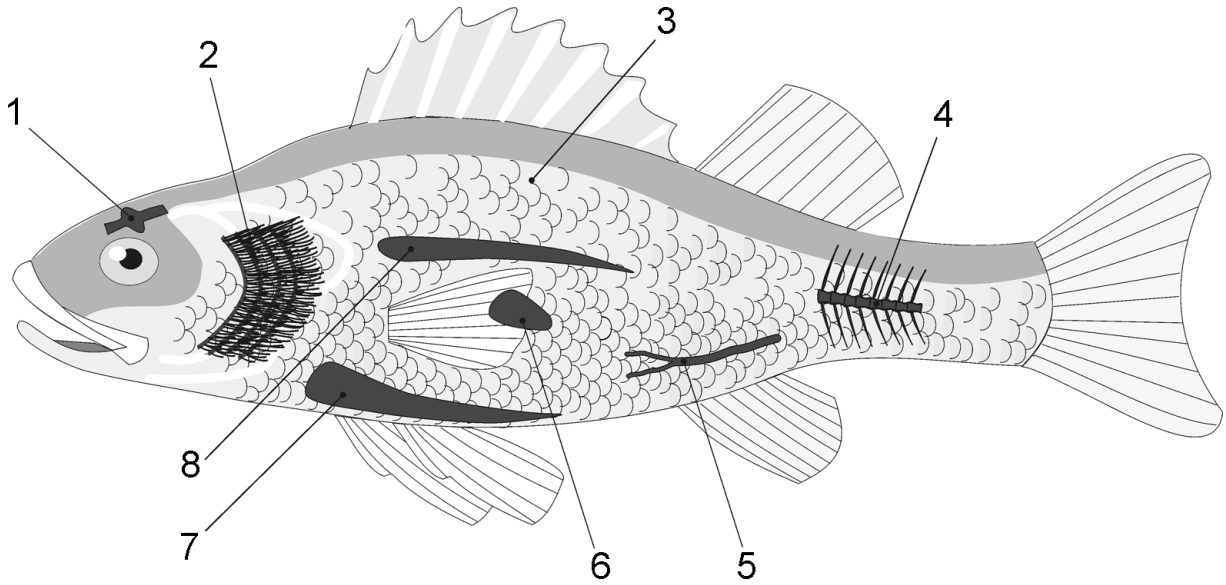
The nets should be set 3 days to 5 days before the fish tissues are to be sampled, in order to keep the fish in the fish chest for 2 days to 4 days prior to sampling the tissues. The gill nets shall be sampled frequently, and at least every 12 h, in order to collect as many live fishes as possible. They should be set at sunset and collected during sunrise. This also means that the laboratory staff sampling the tissues shall maintain contact with the local fishermen involved in the fishery to check that enough fish of suitable size is available before they arrive.

An example of a sampling form is given in Annex C.

4.4.3 Fish tissue sampling

The fish shall be collected from the fish chest (3.1.9) one by one by a landing net (3.2.2), taking care to disturb the remaining fish as little as possible. Tissue sampling shall be performed in a locality that is less than 100 m from the fish chest. The sampling locality (3.2.3) shall have electricity and adequate lighting, and be reasonably comfortable, so that the staff can operate safely and under suitable working conditions. A boathouse, a garage or a caravan is a suitable locality.

After its capture, the fish is either stunned by a blow on the back of the head with a wooden rod or a rubber baton (3.2.4) or anaesthetised with a suitable anaesthetic (3.2.5) such as MS-222 (tricaine methanesulfonate or ethyl 3-aminobenzoate methanesulfonate)[1)]. Then the samples should be taken in the following order: blood, bile, liver, spleen, muscle, gonads, and other tissues (see Figure 1).



Key

1 brain
2 gills
3 muscle
4 backbone
5 blood
6 spleen
7 liver
8 kidney

Figure 1 — Sampling of blood from caudal blood vessels using a heparinised syringe

If some tissues are not needed then just proceed to the next item.

1) The body mass is determined to the nearest gram and the total body length is determined to the nearest millimeter.

2) Blood is taken by a heparinised syringe from the caudal vessels (see Figure 1).

3) The fish is decapitated.

4) The body cavity is cut open, taking care not to damage the gall bladder.

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1) MS-222 is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 23893, and does not constitute an endorsement of this product by ISO.

Tricaine methanesulfonate is probably the most widely used fish anaesthetic, even if it is rather expensive. A dilution of 1:1 000 is lethal in 5 min to 10 min. Other commonly used anaesthetics for fish are quinaldine (2-methylquinoline), for which a dilution of 1:20 000 is lethal in 5 min to 10 min, and benzocaine (ethyl 4-aminobenzoate) which can be used in the field by dissolving 0,2 g in 5 ml acetone (to facilitate solubility) and adding it to 8 l of water (Reference [11]). It should be noted that the use of anaesthetics may affect certain biomarkers.