

Procedure 001: Collection and sampling of freshwater fish

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1. Aim of procedure

This procedure describes all the various steps of collection and sampling of freshwater fish for the Environmental Specimen Bank for environmental pollutants. The procedure is adapted to the following species: trout (*Salmo trutta*), Char (*Salvelinus alpinus*), vendace (*Coregonus albula*) and perch (*Perca fluviatilis*). This procedure describes every step of the sampling and handling of samples to ensure that the procedure can be used in a clear way. The procedure should cover all aspects of reproducibility, quality and registration of data for the sampling material.

The number of individuals of each species that should be collected from each location is provided in the relevant annual monitoring programmes (årsprogrammene).

Within this procedure. The terms “collection” and/or “catching” are used for any kind of catching of fish that provides a whole fish, dead or alive. The term “sampling” is used for dissection of the fish and sampling of tissues or organs, as well as registration of individual data (length, weight, sex, etc.). The procedure is partially based on the ICP Waters Program Center (2010) manual and the illustrations there are representative of the sampling that is described in the present procedure.

2. Field sampling

2.1. Trapping methods and handling of the animals

Collection and handling of samples and material should be carried out in order to avoid contamination of potential environmental pollutants and protect the samples from any kind of impact that will affect their usages as research and reference materials.

The timing of the collection should take place in accordance with each programme to avoid any seasonal variations. If no other information or guidelines are given for the fish species and location, the collection should be done during the autumn and preferably in August-September.

Collection, transport and handling of living fishes should be done in accordance with the Norwegian Animal Welfare Act (Dyrevelferdsloven, 2009). The collection should preferably be done with gill nets, cages, lines or with electric fishing apparatus.

Killing of the fish should be done immediately unless the fish can be stored in tubs, big buckets or similar. The killing should be done by a stroke against the head. and not by cutting of the aorta or cutting of the gills. Anesthetic or other chemical/pharmaceutical compounds should not be used in order to avoid contamination. The abdominal cavity should not be opened and the fish should not be slaughtered in the field unless sampling will take place in the field.

Catching and handling of samples and material should be carried out in order to avoid contamination of potential environmental pollutants and protect the samples from any kind of impact that will affect their usages as research and reference materials. Avoid contact between boat decks and fish due to the risk of contact with contaminated water. Nitrile-gloves should be used as long as it is feasible. The fishes should be kept in boxes that are wrapped with clean aluminium foil. The traditional boxes used by fishermen (expanded polystyrene; EPS) should be avoided due to the risk of contamination of flame retardants.



If no other guidelines are provided in the annual monitoring programme (årsprogram), 25 individuals of the species of interest should be collected from each location. Each fish should be wrapped as soon as possible in 3 layers of clean aluminium foil and will thereafter be put in a clean bag of the same type as the ones used for freezing and storage in the environmental specimen bank (MAGIC VAC®; polyethylene with an external reinforcement by a nylon membrane). If not available, clean bags made by non-dyed polyethylene (PE) can be used. A label with sample identification should be put into the bag and the bag is sealed/closed. The material of the label should be resistant to moist and the information should be written with a pencil.

Any deviations from the collection procedure should be written on the field sheet and be written into the table "Fisk" ("Fish") in the database.

2.2. Registration of field data

For each group of fishes, or for every single fish (when needed), a collection sheet with information regarding location, time, method for collection and person responsible for collection should be filled out. Any deviations from procedures should also be written here.

The name of the lake where the fishes was collected from should be recorded with "Navn" (name) and "Vatn løpenummer" (lake running number) in the same way as defined in the lake database of Norwegian Water Resources and Energy Directorate (NVE). If there is no name in this database stated for the present lake, the name of the lake as stated in the national maps (nasjonalt kartverk) M711 should be stated. If the fish was caught in rivers or creeks, the river database of NVE (ELVIS elvenett) should be used for name of the location. "Strekning løpenummer" (distance running numbers) are then used as identification codes and the name should be the same as in the national maps (nasjonalt kartverk) M711.

Running numbers for the locations can be found in the digital atlas of NVE (atlas.nve.no).

The coordinates for the position of the collection site should be written preferably as UTM/EUREF89 (Universal Transverse Mercator) with the zone 33N (epsg projection 32633), although as an alternative, WGS84 (World Geodetic System 1984) in decimal degrees can be used (Statens kartverk, 2009). The WGS84 system is the reference coordinate system used by GPS.

2.3. Storage before shipping

The samples should be frozen (or kept cool) as soon as possible after the collection, preferably no later than 4h after collection and be kept like that until the samples are being sent to the Environmental Specimen Bank. Location of storage and temperatures should be registered at the collection sheet.

3. Sampling in field, freezing of blood and liver

For the locations where blood and/maybe liver are sampled for toxicological or genetic experiments/analyses, these samples should be sampled in field and should be frozen immediately in liquid nitrogen until they are transported to a laboratory.

The following equipment is needed for sampling of organs:

- Tweezers



- Scalpel and extra scalpel blades
- Paper tissues (free from chlorine)
- Clean aluminium foil
- Markers (pens)
- Cryo-tubes (can withstand centrifugation and freezing at temperatures down to -196°C)
- Heparinized syringes and cannulas (5-10 mL 0.6-1.2 mm)
- Pasteur-pipettes
- Micro-centrifuge (2000 g)
- Heparin-solution (1000 IU/mL)
- Nitrogen-tank/transport-tank with liquid N₂

For sampling, the same procedures apply to clean sampling equipment and handling as given in section 4.1. Sampling tubes are marked with the agreed unique code for each individual and matrix. Syringes with cannulas are prepared with a heparin solution by filling the syringe fully with the solution and thereafter empty the syringe. If not used immediately, the syringes should be stored cold (preferably 4°C) until sampling.

The fish must be alive until sampling. They can be stored in a suitable container, cages or similar, but only for a short duration of time. Ensure that there is adequate water exchange and/or oxygenation of the water to not stress the fish by oxygen deficiency. Before sampling, the fish is killed with a stroke against the head. Thereafter, a blood sample is taken from the caudal vein situated directly in front of the tail fin with a heparinized syringe.

Depending on logistics and practicalities, if possible, the blood sample should be centrifuged in the field and separated into a one plasma fraction and one blood cell fraction. This should be done by transferring the blood sample to micro-centrifuge tubes and centrifuging for 5 minutes at 2000 g. The supernatant or plasma is transferred with a Pasteur pipette to a cryo tube, which is frozen immediately in the liquid nitrogen tank, while the fraction with the blood cells is frozen as it is in the centrifuge tube. The blood samples can be stored cold (4°C) for up to 30 minutes before centrifugation.

If it is not practical to centrifuge the samples in the field, the blood sample should be transferred to a labelled cryo tube, which is immediately frozen in the liquid nitrogen tank.

After blood sampling, the abdominal cavity of the fish is opened by scalpel by a ventral cut between the abdominal fins and a piece of the liver (0.5-1.5 g) is cut out from the mid-part of the liver. The liver sample is put into a labelled cryo tube which is immediately frozen in the liquid nitrogen tank. The cut in the abdominal cavity is thereafter closed and the fish is wrapped in clean aluminium foil and labelled with a unique code that corresponds to the tissue samples and is further processed as in chapter 2.

Delivery of the liquid nitrogen container is agreed upon with the Environmental Specimen Bank.

4. Transport of samples

4.1. Packing

The samples should be transported frozen or cooled and be wrapped in aluminium foil and MAGIC VAC® or PE-bags in transport boxes that are approved for food industry, preferably made by polypropylene (PP) or high density polyethylene (HD-PE). The way of packing should ensure that the fishes are transported cold and safe for any kind of damages of the material, and that the fishes are not getting in contact with any areas or compounds that may contaminate the samples. The parcels should be marked clearly with sender's name and address, sent to Environmental Specimen Bank and addressed to a contact person there. The parcel should be marked with a text which states that the parcel contains biological material that needs to be kept cool.



4.2. Transport routines

Unless the samples cannot be kept frozen after sampling, they should be transported as quickly as possible to the Environmental Specimen Bank, e.g. as a “express over-night”-parcel. A contact person at ESB should be informed on forehand regarding time of delivery to ensure that the parcel is received in a proper way. If post or shipping companies are used, the parcel must be sent in a traceable manner. Any deviations from transport routines or damages on the sampling material should be noted on the field sheet attached to the parcel and the information should also be noted in the table “Fisk” (fish) in the database.

5. Sampling within the lab

The procedures for dissection, sampling and storage of tissue sample, scales and otoliths, the registration of individual data (4.1.-4.4) and freezing of whole fish (4.5) are described here. After dissection and sampling of tissues, the rest of the fishes can be thrown away as biologic waste at the Environmental Specimen Bank. Any deviations from the procedures regarding sampling should be noted in the table “Prøver” (samples) in the ESB database.

5.1. Equipment and cleaning procedures

Before sampling, the staff in the lab should prepare the necessary clean dissection equipment, a working space covered with clean aluminium foil, pre-labelled sampling glasses, envelopes for scales and a registration sheet. The staff should use clean gloves (nitrile-gloves) and only touch the outside of the fish.

The following equipment is needed for sampling of char and other freshwater fish:

- Tweezers
- Knife
- Scissors
- Scalpel and extra scalpel blades
- Measurement tape or -board
- Scales (precision: 0.01 g)
- Paper tissues (free from chlorine)
- Clean aluminium foil
- Burned aluminium foil
- Sampling glasses (can withstand freezing down to -25°C)
- Sampling tubes (can withstand freezing down to -25°C)
- Envelopes for scales
- Paper for packing of scales and otoliths
- Solvents (HPLC grade; acetone, cyclo-hexane)
- Glass for washing of equipment
- Nitrile-gloves
- Parafilm™, sealing tape
- Vacuum packing machine with approved bags (MAGIC VAC®)
- Labels (can withstand freezing down to -25°C)



All surfaces that will be in contact with the fish must be covered with clean aluminium foil. The tissue samples should not be in contact with this foil. Any areas that will get in contact with the tissue samples must be covered with burned aluminium foil. The sample tubes must be labelled (freeze-proof labels) with a unique sample number (P_ID) generated from the database of the environmental Specimen Bank. All tools used for sampling should be of either stainless steel or of glass, quartz or other in-organic ceramic materials. The equipment should be cleaned in accordance with the procedure below.

Cleaning of equipment:

Washing with in-organic (base) soap (Neodisher UW) by the washing machine in the Environmental Specimen Bank (Miele G4230, 75 °C, 2h washing). The equipment is thereafter rinsed three times with distilled water or ion-changed MilliQ-water and then washed with solvents (HPLC grade). First, 5 minutes in acetone and then 5 minutes in cyclo-hexane. This rinsing is done twice. Cleaned equipment can be stored wrapped in clean aluminium foil.

Sample glass cleaning: The sample glasses should be un-used and any organic residues should be burned by heating the glasses for 2 hours at 500 °C. The glasses are sealed with burned aluminium foil under the lids. Small vials that cannot withstand the heating procedure should be washed with solvents in the same way as the equipment is being washed (see the section above).

During sampling, the staff should use lab coats and shoes, and if possible, shoe covers. Long hair should be covered with plastic caps. Use of personal care products, with the exception of soaps and shampoos approved by ESB, should be avoided one day before the sampling.

5.2. Individual data

The fish is thawed and the following individual data is registered: length measured from nose to outer edge of the tail fin when it is naturally spread (0.1 cm), total weight (g, minimum 1% precision), weight of liver (0.1 g), gender, maturation stage (Dahl, 1917), colour of the flesh (white, light red, red), any visible parasites in the abdominal cavity and any sores or injuries. A unique number is given to the individual fish and is generated from the database (Fisk_ID).

Data should be written on a separate sheet and then transferred to the database. The scale envelope is labelled with the fish identification number and other individual data. Registration sheet and scale envelopes will be stored in an archive.

5.3. Otoliths, scale and operculum

These are structures that are used for age determination, but otoliths can also be used for analyses of stable isotopes and trace elements. Hence, the otoliths must be treated in a way that avoids contamination or would make them unsuitable for such analyses.

Otoliths (sagittae) are dissected by loosening the bone structure in the head of the fish with a knife or scalpel, and then pick them out with thin tweezers. Be careful not to damage the brain if it will be sampled as well. The otoliths should be wiped dry for slime with clean tissues. They should be stored in a folded, soft, un-coloured paper-tissue placed in a zipper bag in the scale envelope.

Scales are taken from the area between the spine and the adipose fin, near the lateral line of the fish. Slime and skin should be scraped off the surface of the fish before the scales are scraped off with a sharp knife. The scales should be wrapped in a piece clean paper and be placed in the scale envelope.



From perch, operculum are taken instead of scales. The operculum should be cooked for about one minute before loose skin residues are removed by wiping the operculum with paper tissues. The operculum are stored in the scale envelope.

5.4. Tissue samples

The sample should not be touched by anything else than clean equipment. Hands (with or without gloves) shall not touch the sample. The sampling should preferably be made in a clean room. All equipment should be cleaned between sampling of different organs. Dry it carefully with paper tissues and distilled water before cleaning with solvents (rinsing twice with acetone and cyclo-hexane). Scalpel blades are changed between each sample. Both the weight of the sample material and the weight of the sample glass including the lid and its aluminium foil cover should be registered. With these two weights registered, any loss due to drying during the freezing storage can be calculated. All sample glasses should be sealed by Parafilm™ where the glass meets the lid. Vacuum packing in MAGIC VAC®-bags can be used as an alternative sealing.

5.4.1. Brain

The brain, from the frontal nerve of the olfactory bulbs (bulbus olfactorius) and until the extended marrow (medulla oblongata), is carefully cut out with tweezers and scalpel. The entire organ is transferred to a suitable sized glass tube (about 1-3 mL) sealed with burned foil and screw lid.

5.4.2. Liver

The liver is cut out by tweezers and scalpel, or scissors if needed. Make sure that the gall bladder is not punctured. Check the liver for visible deformities / abnormalities and discolouration, and note down the information. The total weight of the liver is registered. Each sample should contain minimum 5 g and up to 6 replicates should be taken from each individual fish. To avoid any effects of local concentration differences in the liver, cut the liver into several pieces and combine the different pieces to replicate samples. If there is enough material, a mixed sample consisting of samples from each fish from the location is also made. The samples are put on 10 mL glasses, possibly larger glasses if there is plenty of material. The glasses are sealed with burned Al-foil under the lid and thereafter frozen.

5.4.3. Gall bladder

If the gall bladder is intact and still got a content, it should be cut loose from the bile duct. Hold the gall bladder above the bile duct with a pair of thin tweezers to avoid leakage of the fluid from the gall bladder. Transfer the content to a small glass vial (ca 1-3 mL) and seal it with burned aluminium foil and a screw cap.

5.4.4. Dorsal muscle

A sample from the dorsal muscle is cut out (without skin or bones), preferably from above the lateral line. The skin is opened by tweezers and scalpel, and the muscle is cut out. Avoid to include the line/band of red muscle type closest to the skin. Cut the muscle into several pieces and combine the different pieces of muscle in up to six replicate samples. If there is enough material, a mixed sample consisting of samples from each fish from the location is also made. The samples are put on glasses (ca 45 mL). The glasses are sealed with burned Al-foil under the lid.

5.5. Whole fish

Small species, such as vendace or small schools of perches (individuals often <100 g) are often better to freeze as a whole fish instead of taking samples before freezing.

The bench is prepared in the same way as described in 4.1, length and weight are recorded as described in 5.2. All handling of the fish should be done wearing clean nitrile-gloves and using clean equipment.



The whole fish should be wrapped in three layers of aluminium foil and vacuum packed in MAGIC VAC®-bags.

6. Registration of data, marking and freezing samples

Data from the field and sample schemes are transferred to electronic format at the Environmental Specimen Bank. Every fish has a unique identification number (Fisk_ID) and are also give a unique sample number (P_ID). In the data base, information about where the samples are stored will be included. This information should include which rack and section of it, which shelf and box where the sample is stored.

The sample glasses (or bags; MAGIC VAC®) with whole fish should be labelled (use labels that can withstand freezing) with a unique sample number. After transfer of sample material to the glasses/bags, the glasses/bags should be closed and frozen at -25 °C in the freezer of the Environmental Specimen Bank.

7. References

Dahl, K. 1917. Studier og forsøk over ørret og ørretvann. Centraltrykkeriet, Kristiania.

Dyrevelferdsloven. 2009. Lov om dyrevelferd. Lovdata. URL: <http://www.lovdata.no/all/nl-20090619-097.html> (04.01.2013).

ICP Waters Programme Centre (2010). ICP Waters Programme Manual 2010. ICP Waters Report 105/2010. 91 pp. URL: <http://www.icp-waters.no/LinkClick.aspx?fileticket=Sk4xcfQaPGo%3D&tabid=61> (04.01.2013)

Statens kartverk. 2009. Koordinatbaserte referansesystemer. versjon 2.1 – Desember 2009. 48 s. URL: http://www.statkart.no/filestore/Standardisering/docs/koo_referansesyst.pdf. (04.01.2013)

8. Appendices

Appendix 1. Field sheet for catching fishes

Appendix 2. Sampling sheet with individual data for each fish (optional)

Field sheet for catching fishes, freshwater fish

This sheet must follow the fish from when it was caught until it is delivered to the Environmental Specimen Bank (MPB). Information on catching, storage and transport should be noted on the sheet. The sheet should be included in the parcel during shipping. The person who receives the parcel at MPB should sign the sheet and register the condition of the parcel upon arrival. Any additional information is noted on the back of the sheet. The sheet should be filed in the Environmental Specimen Bank.

Collection information

Location, name:

Location, type and NVE number

Lake

River/creek

NVE running number:



Location, coordinates
UTM zone 33(EUREF89) or decimal degrees (WGS84) UTM Degrees N:
E:

Date of catching: **Number of fish:** **Species:**

Method of catching: Time and temp. before the fish was transported to storage place:
Time (hours): Was the fish put on ice or kept cool after catchment:

Fisherman:
Name, address, etc.

Method of packing: Alu-foil Magic Vac
Polyethylene-bag Other:

Storage before shipment:
Temp. °C: Place of storage:

Shipping to MPB

Date of sending: **Means of transportation:**

Person responsible for transport:
Name, address etc.

Date of receiving: **Receiver at MPB, name:**

Temp. of fish at arrival: Cooled at ice Frozen Other:

Was the parcel and material un-damaged? Yes Comment:
No
