



Naturhistoriska  
riksmuseet

## Manual for collection, preparation and storage of fish

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## Collection of fish

To get comparable data in time series for environmental contaminants and endogenous compounds studies it is important to consider time, locality and sex etc. For other studies the selection of which sex to use is determined according to the design of the study. Both sexes are collected within the Swedish monitoring programme.

To get comparable data between years and locations it is important that fish of comparable ages are collected. Young fish are often more stationary compared to older ones and because of this, young fish are preferred in monitoring. Size at different ages can vary between different collection sites and it is also important to get specimens that are large enough to allow individual chemical analyses.

### *Area for collection*

The sampling localities have been selected in order to represent the condition in coastal marine waters and in lakes throughout Sweden. The localities are regarded as not polluted from any local sources and are chosen in a way that influences from river outlets are avoided.

The fish collected in these areas is used to describe the background contamination and also serve as reference material for studies in more polluted waters. Sampling procedures for each species have been designed to obtain samples that are as stable as possible from an ecological and physiological point of view. Most sampling programs are designed in a way that requires annual sampling to detect changes of concentrations of contaminants.

### *Time for collection*

The season of collection is important as the level of many contaminants fluctuate over the year in fish. Certain substances show a maximum during late spring in both marine and fresh water species.

Most sampling takes place during late summer and early autumn and collection at periods of reproduction are generally avoided.

Species	Time	Size (cm) Total length	Method
Herring	August-September	15-20	Trawl, net
Herring	May-June	15-20	Trawl, net
Cod	August-September	25-40	Trawl, net
Flounder	August-September	20-30	Trawl, net
Dab	August-September	20-25	Trawl
Eelpout	August	15-30	Fyke nets
Perch	August-September	15-20	Net, fyke nets
Roach	August-September	15-20	Net
Pike	April-May	40-60	Net, fyke nets
Char	August-September	20-30	Net

Table 1. Species in the Swedish marine and fresh water monitoring programmes, time of capture, size of fish and method of capture.

## Species

### Herring (*Clupea harengus*)

When collection of herring started in the beginning of the 1970s, the collection took place during the spring. Herring collected during this period are less suitable for monitoring of contaminants, because herring generally spawn during late spring to early summer. Due to this, the collection time was changed to autumn. In order to maintain some of the long time series, herring is also collected during spring at Utlången and Ångskärsklubb.

### Cod (*Gadus morhua*)

Cod is collected in the autumn in the southern parts of the Baltic and at the Swedish West coast.

### Flounder (*Platichthys flesus*) and dab (*Pleuronectes limanda*)

Flounder and dab are not analysed for environmental contaminants because herring are the priority in the present. Flounder and dab are still collected and stored in SESB to keep the long time series. They have proven to be very suitable to use in research about physiological changes.

### Eelpout (*Zoarces viviparus*)

Eelpout are stationary and has been found to be a very suitable species for long term monitoring studies. As eelpout are viviparous (give birth to living offspring), females are used in monitoring studies concerning reproductive outcome. For monitoring of environmental contaminants, males are collected in late autumn.

### **Perch (*Perca fluviatilis*)**

Perch is collected for coastal monitoring of the Baltic as well as for freshwater monitoring.

### **Pike (*Esox lucius*)**

Pike has been collected for freshwater monitoring since the late 1960s. Pike is collected in the spring which is not ideal as pike spawn in the spring. In order to maintain the long time series, this sampling time is still used. Newly started monitoring programs where pike is used are recommended to run with a collection time in late August to early September.

### **Arctic char (*Salvelinus alpinus*)**

Arctic char is collected for freshwater monitoring at localities near the northern Scandinavian mountain range, outside the distribution for pike and perch.

#### *Equipment for collection of fish*

- Net
- Thin plastic bags
- Labels
- Cool box
- Dry ice

### **Transportation**

It is important to deep-freeze the fish as soon as possible after capture preferably with dry ice in a cool box. Each specimen should be put in a separate plastic bag and frozen with a straight body. The fish should remain frozen until they are prepared for permanent storage in SESB or analysis. If any procedure other than fairly immediate deep freezing is used, this should be noted on the data sheet.

### **Handling of arriving fish**

#### *Small fishes*

##### **Registration**

Small fish do not get individual acquisition forms instead a whole “batch” of fish is registered on a sequence acquisition- form. Only fish of the same species, caught at the same occasion is registered together.

On the acquisition form the following data are compiled

- Acquisition number. Here the number of fish that are collectively registered on the form should be disclosed (I. e. 2011/01000-01049 shows that there is 50 individual fish registered on the form).
- Species
- Locality
- Date of capture
- Gear
- Depth
- Coordinates alt. station name
- Name of collector
- Remarks

The following section applies for fish that are to be stored in SESB without prior sampling for analysis

### **Packaging**

Each fish is put a thin plastic bag, and frozen in a straight mode.

-Small fish from the same locality, caught at the same time are packed together in a laminate plastic bag. The bag is labeled with acquisition number, species, locality and number of specimens.

-The laminated plastic bag is vacuum-packed

### **Large fishes**

#### **Registration**

Each specimen is given an individual acquisition number and an acquisition form for each fish is prepared. The data that is compiled is the same as for small fishes (see under registration of small fishes).

Following measurements are taken for large specimens:

- Body weight (g)
- Total length, length from nose tip and to tip of the caudal fin (cm).
- Standard length, length from nose tip to end of body (cm).
- Sex
- Weight of liver (g)
- Weight of gonads (g)
- Age – annual rings on scales, otolithes,

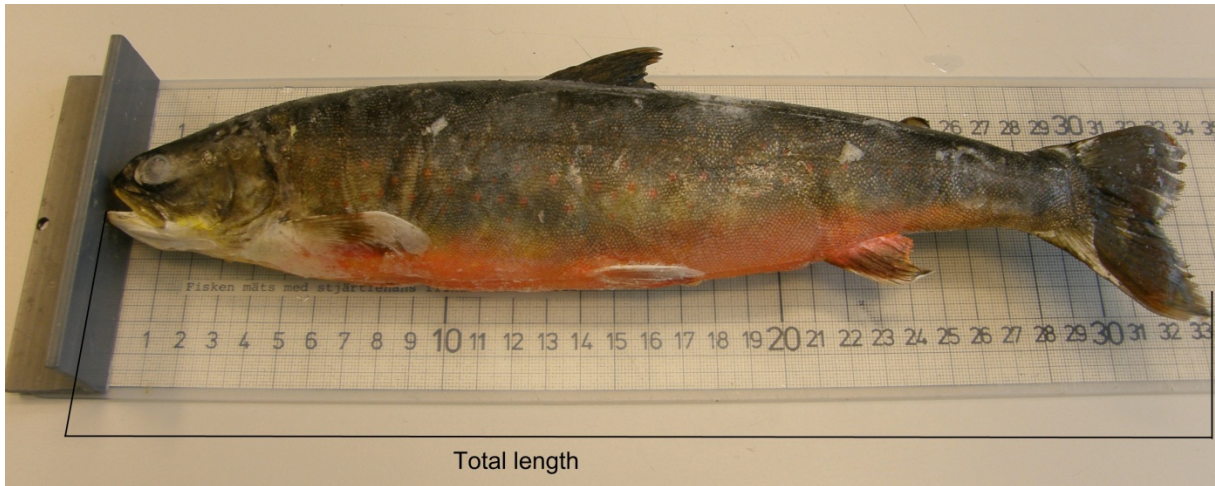


Photo 1. Measurement of total length. Photo: Henrik Dahlgren

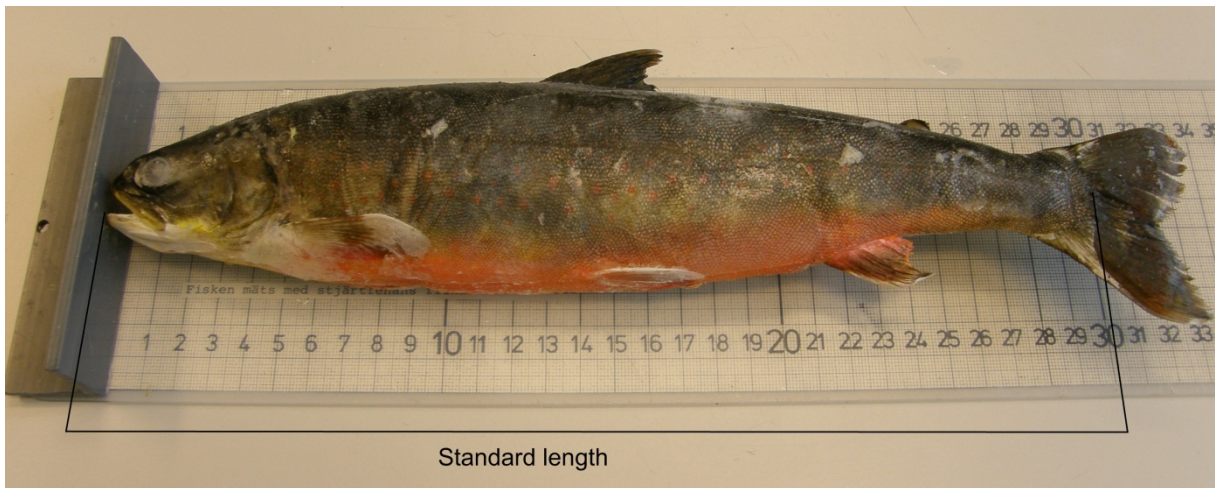


Photo 2. Measurement of standard length. Photo: Henrik Dahlgren

After registration, measuring and weighing, large fish are handled in the following way:

- Cut out the middle part of the fish, the liver, gonads and the head
- Weigh the liver and the gonads.
- Pack the middle part and the liver separately in aluminum foil and put in labeled, laminated plastic bags, one for each fish.
- Pack all samples from the same locality together in a laminate plastic bag and labeled with locality, content and identification number. The bags are sealed in a chamber vacuum machine.
- The otoliths or cleithrum are stored in acid free paper bags. For removal and handling of otholites and cleithrum a see section "Preparation for analyses".

## Preparation for analyses

When fish are sampled for analysis, fish that does not already have individual identification number (i.e. small fish) get an individual acquisition number.

Following measurements are taken for each specimen:

- Body weight (g)
- Total length (cm)
- Standard length (cm)
- Sex
- Weight of liver (g)
- Weight of gonads (g)
- Maturity of the gonads (herring)
- Age – annual rings on scales, otolithes,
  
- Extracted organs, weight of the organs (g)
- Name of the person responsible for preparation
- Remarks if any

The dissection of fish is most easy when the surface layer of the muscle tissue is half frozen.

## Muscle

- Cut of the tail.



Photo 3. The tail is cut off.



- Open the fish along its dorsal side and remove the skin and subcutaneous fat from the fish.



Photo 4. The fish is opened along its dorsal side.

- In order to ensure uniformity use the muscle tissue on the left dorsal side.

- It is important to use the same amount of muscle tissue for each sample to ensure comparability.



Photo 5. Preparation of muscle tissue on the left dorsal side.

### Liver

-Open the abdomen and remove the complete liver while it still is frozen to avoid water and fat loss.

## Otolithes

This applies to species as perch, flatfish, cod, eel, herring, eelpout, and the arctic char.

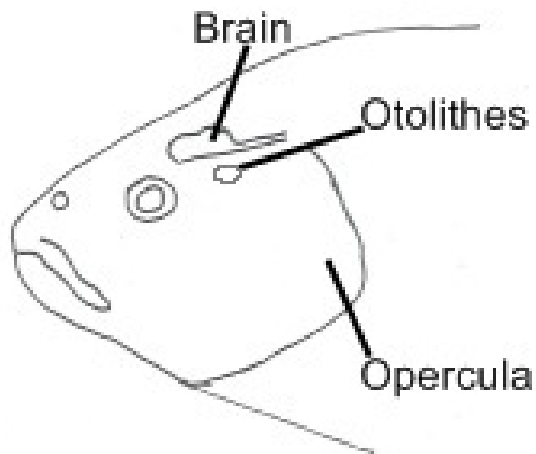


Figure 1. Shows the position of the otolithes, opercula and brain.



Photo 6. Open the head above the eye to reach the otolithes



Photo 7. The otoliths are removed

- The head is allowed to thaw to facilitate further treatment. The head is opened behind the eyes and the otoliths are removed. The otoliths are rinsed in water and dried before they are stored.
- They should be stored in acid free paper bags, in a dry place.

### **Cleithrum**

- Cut out the cleithrum.
- Pour boiling water over cleithrum and let them stay in the water for a few minutes.
- Clean the cleithrum by rubbing them with kitchen paper, between your fingertips.
- Repeat the boiling and rubbing, and let the cleithrum dry.

### **Scales**

- Scales are collected using a forceps, and cleaned in lukewarm water.
- Make sure only to use scales attached to the fish body
- The scales are mounted on microscope slides with the underneath down.
- Let the slides and scales dry and keep them in a dry place.
- Scales are preferably taken from herring and roach.

When the appropriate samples have been taken the remaining material are packed and stored according to the procedures described in the chapter "Storage in ESB".

### *Equipment for preparation*

- Ruler
- Scale
- Scalpel (one piece, blade and handle)
- Ceramic knife (for tissue prepared for metals analysis)
- Forceps (stainless)
- Forceps, plastic (for tissue prepared for metals analysis)
- Glass jars
- Acid washed plastic jars (for tissue prepared for metals analysis)
- Aluminium foil
- Laboratory blotting paper

### **Storage**

For fish that arrive at the museum and are stored in the SESB without prior sampling for analyses, see under chapter "Handling of arriving fish"

The following applies to fish that have been sampled for analysis.

### **Organs and specimens**

All organs or tissues are packed separately in aluminium foil and marked with acquisition number and content. The packages are placed in thin plastic bags and sealed in a chamber vacuum machine.

All samples from one organism are packed together in a laminate bag along with a label with necessary information such as acquisition number. The bag is sealed in a chamber vacuum packaging machine.



Photo 8. All specimens from one individual packed together and sealed in a chamber vacuum machine

## Cleithrum

The cleithrum should be kept in acid free paper bags, in a dry place. The bag is marked with acquisition number, species and locality.

## Scales

See section Preparation for analysis, scale page 12.

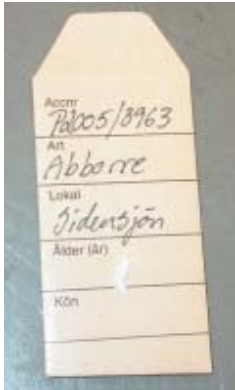


Photo 9. Acid free paper bag

## Code list

<b>Maturity of the gonads (herring)</b>	<b>Code</b>
Recently spent	1
Juvenile	2
Maturing	3
Mature	4
Ripe	5

<b>Degree of putrefaction</b>	<b>Code</b>
Fresh	1
Slightly cadaverous	2
Cadaverous	3
Strongly cadaverous	4
Dried	5

<b>Sex</b>	<b>Code</b>
Not determined	0
Male	1
Female	2
Not determinable	3
Hermaphrodite	4

<b>Health condition</b>	<b>Code</b>
Fat	1
Normal	2
Thin	3
Emaciated	4