Guideline for Sampling and Sample Processing

Eelpout (*Zoarces viviparus*)

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Contents

Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

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1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) subject to specialist and administrative coordination by the Federal Environment Agency (UBA). The ESB collects ecologically representative environmental and human samples and stores and investigates them for environmentally relevant substances.

Specific operating procedures as well as the conception of the ESB are the basis of the program. (Umweltbundesamt 2008, 2014)

The long-term storage is carried out under conditions which, as much as possible, exclude a change in state or a loss of chemical characteristics over a period of several decades. The archive therefore provides samples for retrospective investigations of substances for which the potential risk for the environment or human health is not yet known.

Comprehensive information on the ESB is available at www.umweltprobenbank.de.

2 Objective of this Guideline

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based, standardized methods to avoid contamination and inhibit loss of chemical information. The need for an exceptionally high level of quality assurance results from the extraordinary value of the samples as archive material. Representativeness and reproducibility of the samples are the basis for spatial and temporal comparison.

The current guideline is an update of the Klein *et al.* (2010) version.

Transport, further sample treatment and storage as well as chemical analysis have to be carried out according to the current guidelines of the ESB.

3 Function of the Specimen Type

In marine ecosystems eelpouts (*Zoarces viviparous*) occupy the trophic level of carnivorous consumers. They mainly feed on benthic invertebrates, but their food strongly varies due to seasonal fluctuations. Especially in summer, pelagic organisms like cladocerans or copepods are consumed.

In numerous monitoring studies the eelpout proved to be a good accumulation indicator, and, primarily, a good effect indicator for coastal marine ecosystems (Skov *et al*. 2010, Theobald *et al.* 2011, Rüdel *et al.* 2011, Bignert *et al.* 2011, Albertsson *et al.* 2012, Barsiene *et al.* 2012, Bergek *et al.* 2012, Kreitsberg *et al*. 2012, Tairova *et al*. 2012, Asker *et al*. 2013, Velasco-Santamaria *et al*. 2013, Sturve *et al.* 2014). It was proposed in 1991 by Sweden as bio-indicator for the Baltic Sea coastline to the Baltic Marine Environmental Protection Commission (HELCOM) and it is used as an indicator for the BMP (Baltic Monitoring Programme) and the JMP (Joint Monitoring Programme) (Thoresson 1993). The eelpout is used in several national monitoring programs, mainly in countries bordering the Baltic Sea (i.e. Sweden, Finland, Denmark, and Germany). Additionally, it is part of international programs like BALCOFISH (Integration of pollutant gene responses and fish ecology in Baltic coastal fisheries and management) and BEAST (Biological Effects of Anthropogenic Chemical Stress). Furthermore, the eelpout has been suggested as a key indicator organism for the European Marine Strategy Framework Directive (Hedman *et al.* 2011).

The reasons for the particular suitability of the eelpout as an accumulation and effect indicator are summarized as follows:

- wide distribution within Europe from the northern coast of Spain to the White Sea and into the Baltic Sea,
- primarily lives in coastal shallow water regions but migrates more than formerly assumed (Bergek *et al*. 2012, Kinitz *et al*. 2013),
- great ecological valence regarding temperature and salt content of the water; it tolerates even the low salt content in the particularly polluted estuary regions,

Fig. 1: ***Zoarces viviparus*** **(www.fishermix.de)**

- it is the only viviparous fish species which occurs in our coastal waters; hence it is particularly suited as a bio-indicator for the detection of adverse effects on the reproduction,
- no special restrictions by nature or species protection laws,
- the species is easy to identify.

4 Target Compartments

Because a sufficient homogenization of whole fish is not possible (Paulus and Klein 1995), specific suitable organs have to be selected for the purposes of the ESB.

The muscle and liver tissue are chosen for the examination of chemical substances. The former is edible and therefore a link to the human food chain. Further it is simple to dissect and has a large biomass, allowing a multitude of chemical analyses even for single specimens. On the basis of the muscle tissue, only a part of the eco-toxicological relevant substances can be represented. Thus, as the body's main metabolic organ, the liver is also collected.

5 Predefinitions for the Sampling

5.1 Species Determination

In taxonomy, the viviparous eelpout is a member of the perch fish genus (Perciformes), sub genus blennies of the *Zoarcicidea* family. Its elongated, stretched body is rounded and has a long dorsal fin from the vertex to its tail and an continuous caudal fin from the anus to the end of its body. Both fins merge at the pointed tail, the typical caudal fin does not exist. Characteristic is an indentation of the dorsal fin close to its end. The pectoral fins are especially well developed and reach approximately the size of the head. The ventral fins, in contrast, are highly atrophied (Fig. 1).

The body color varies considerably and is adapted to its particular surroundings. Fish from the sandy sea grass region are predominately yellow green to yellow brown in color. Specimens from mud or dulse stands, in contrast, are grey brown to black brown in color. The dorsal fin and the back are traversed by more or less irregularly shaped darkish diagonal bands. The flanks show 13 to 15 dark spots on both sides. The ventral side is yellowish white. The smooth-edged, small scales are concealed in the depth of the mucus layer. A papilla is situated rear of the anus, which is exceptionally well developed, similar to the milter. Further peculiarities are the absence of an air bladder and a phosphate compound embedded in the skeleton (Vivianit), which leads to a green discoloration of the bones when cooked.

5.2 Selection and Definition of Sampling Sites

The sampling sites must represent the respective ecosystem, meaning that they must not be located close to local emission sources. The distance to pollution sources depends on the type of emissions and on numerous hydrologic and hydro-geographic factors.

The size of the sampling site is based on the habitat structures and the population densities of the eelpouts. In Wadden Sea ecosystems it mostly comprises the main tideway systems of the entire sampling region.

5.3 Selection of Individuals and Sample Size

According to Backhaus *et al.* (1995) all occurring ages of the eelpout, i.e. fish between one and four years of age with a minimal length of 15 cm should be dissected and stored. A balanced age distribution during sampling cannot be guaranteed because younger individuals are naturally more frequent. The exact age analysis is determined through an otolith examination carried out in the laboratory.

The minimum sample size is 20 eelpouts for representatively characterizing a sampling site.

In order to get 1,100 g muscle tissue, as requested for the German ESB, up to 200 eelpouts have to be sampled, depending on the fish size.

The minimal sampling size for a specific chemical substance can be estimated statistically (e.g. by power analysis).

5.4 Sampling Period and Frequency

In long-term programs, such as that of the ESB, sampling should be carried out annually.

The sampling is carried out prior to the mating season, which occurs in August and September. Depending on the weather conditions, sampling should start in early May and be finished by the end of June. If not enough individuals were caught during this period, it can be extended through the end of July.

5.5 Area-Related Sampling Scheme

Based on the sampling guidelines, specific definitions for the individual sampling areas and sites must be made and documented in an area-related sampling scheme. These include, but are not limited to:

- location and demarcation of the sampling sites,
- required sample size,
- time frame for sampling,
- appropriate authorities.

Here it is important to consider how to ensure a long-term sampling continuity. If changes are made, the document must be updated.

6 Sampling Procedure

Normally the catch of the eelpouts is performed by local professional fishermen, who have the proper equipment required and the necessary knowledge of the place. For this, the respective arrangements must be made. In conservation areas, additional approval for the removal of eelpouts may be required, and these permissions must be applied for at the appropriate authorities.

All data collected during sampling and biometric sample characterization must be documented in the corresponding specimen data sheets (see appendix). In addition, a protocol must be prepared for each sampling with the following information:

- persons that participated in the sampling,
- chronological sequence of the sampling,
- the underlying version of the sampling guideline and the area-related sampling scheme for the current sampling as well as,
- deviations from the sampling guideline and the area-related sampling scheme.

6.1 Required Equipment and Cleaning Procedures

Field work:

- specimen data sheets for documentation during the sampling,
- species-appropriate net cage or transport container with ventilation equipment,
- landing net.

Laboratory:

- specimen data sheets for the biometric sample description,
- club and electrical system to anaesthetize the fish,
- measuring board (reading 0.5 cm),
- 2 x laboratory scales (reading 1 g and 0.01 g),

- scale bowl for whole fish,
- 2 stainless steel beakers for dissecting instruments,
- stainless steel tweezers,
- stainless steel scalpel holders and blades,
- stainless steel pliers,
- stainless steel scissors,
- PTFE pad,
- zip lock bags to freeze the heads or the dissected fish,
- stainless steel containers (5.5 l and 3.5 l),
- insulated container to hold stainless steel containers,
- liquid nitrogen,
- protective clothing for liquid nitrogen handling,
- paper towels.

Sample containers and all equipment are cleaned in a laboratory washer using a chlorine-free powerful washing agent in a first step. After cold and hot (90 – 95°C) rinsing, neutralization using 30 % phosphorus acid in warm water is performed, followed by hot and cold rinsing with deionized water. After this procedure the containers are dried in a cabinet dryer at 130°C (± 10°C) for a minimum of an hour (sterilization). The containers remain in the closed cabinet dryer while they are left to cool. Sterilization is not applied to synthetic materials

6.2 Sampling Technique

Trawl nets and outrigger trawler, respectively, are the method of capture usually applied in the main tideway systems of the Wadden Sea. The eelpouts accrue through this as by-catch of prawn fishing operations and have to be sorted out of the catch, conditioned on board in suitable transport basins.

Fish pots are mostly used in the shallow water areas of the Baltic Sea. They are positioned by local fishermen. The fish caught are conditioned until take over in net cages in the habitat water.

Using **push nets** is only possible during low tide along the shallow margins of the tideway systems. Because this method is very time consuming to reach the quantities demanded, it should only be applied in exceptional cases.

For all methods of capture it is imperative that the eelpouts are transferred immediately after the catch into a species-appropriate net cage, which is floating in habitat water. Alternatively, the fish can be transferred to a species-appropriate fish transport container filled with habitat water, where they are provided with fresh air through a ventilation system. It is important that keeping an individual fish in conditioning for more than four days is not allowed.

For further processing each fish is taken out individually by means of a landing net and is anesthetizedby an electric shock in a separate water basin. After that, the fish is killed by a knock on the forehead. Because the liver must not be damaged, the fish cannot be killed by directly stabbing the heart.

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The following work steps are chronologically processed:

- weighing (reading 0.1 g),
- measuring of the length (reading 0.5 cm) from tip of the mouth to end of caudal tip fins (complete length = LC),
- recording of all conspicuous skin features.

The subsequent dissection is performed on a clean bench with particles- and activated carbon filtration. The required instruments are kept in stainless steel receptacles filled with deionized water. One contains the instruments required for stripping the skin, and the other one the instruments required for the removal of organs, which are then stored. The following work steps are carried out:

- opening of the abdominal cavity by means of stainless steel scissors and removal of the innards (except kidneys)
- determining the sex: male gonads always appear in pairs, female are single.
- dissecting the organs on a PTFE pad: separating the liver with a stainless steel tweezers and a stainless steel scissors without damaging other organs; if the gallbladder is damaged, the liver has to be discarded after weighing, as it can be contaminated with leaking bile,
- weighing of the liver (reading 0.1 g) shockfreezing in liquid nitrogen in a stainless steel container (the livers of all eelpout are frozen together),
- weighing of the rest of the innards (reading 0.1 g), innards are discarded after weighing,

- incision in the skin along the back line, abdominal line and the gill covers on one side of the body with a scalpel or a stainless steel scissors; it is important to ensure that no deep cuts are made into muscle,
- stripping off the skin from head to the tail using strong stainless tweezers or pliers,
- separating the musculature from the spine with a scalpel,
- cutting away the rest of the musculature with a scalpel,
- weighing of the muscle tissue on a PTFE pad (reading 0.1 g) and shock-freezing in liquid nitrogen in a stainless steel container (the muscle tissue of all dissected eelpouts is deep-frozen together).
- repetition of the procedure described above with the other body side.
- recording all abnormal features of the inner organs and musculature.
- resection of the head and packing in labeled freezer bags.

At a later time, the otoliths, gill covers and/or hard structures (e.g. neck bones) are prepared for age determination. Until then the heads and dissected fish are kept frozen.

7 Biometric Sample Characterization

Most of the biometric parameters are ascertained during the sampling (chap. 6.2). Solely the exact determination of the age is carried out in the laboratory subsequent to the sample collection on the basis of bony structures e.g. otoliths (Svedäng *et al.* 1997).

Furthermore, the condition index (K) has proved to be trustworthy for the degree of the nutritional status of the fish. It is calculated as follows:

$$K = \frac{100 \times \text{body weight[g]}}{(\text{total length[cm]})^3}$$

In general, a reduced condition index indicates degraded living conditions, possibly caused by e.g. adverse water temperatures, chronic oxygen deficiency, or symptoms of poisoning.

The hepatosomatic index (*HSI*) is used to identify influences of environmental pollutants that lead to an enlargement of the liver. It is calculated as follows:

$$HSI = \frac{100 \times \text{liver weight[g]}}{\text{total body weight[g]}}$$

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Checklist to Prepare and Conduct the Sampling

Specimen Type	**Eelpout (*Zoarces viviparus*)**
Target Compartments	muscle tissue from both sides of the body and liver
Individual Specimens	all occurring ages
Random Sample Number	at least 20 individuals
Sample Quantity for the ESB	for a sample quantity of 1,100 g musculature, 20 – 200 eelpout must be collected, depending on the sample area
Sampling Period	optimum is from early May until end of June, not later than the end of July
Sampling Frequency	1 sampling per annum
Required Equipment for Field Work	• specimen data sheets for documentation during the sampling • species-appropriate net cage or transport container with ventilation equipment • landing net
Sample Packing until Further Processing	• stainless steel containers (3.5 or 5.5 l) with lids and fasteners • zip lock bags to freeze the heads or the dissected fish
Transport and Interim Storage	cooling device (dewar) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN)
Required Equipment for Laboratory Work	• specimen data sheets for the biometric sample description • clean bench with particle and activated carbon filtration • club and electrical system to anaesthetize the fish • measuring board (reading 0.5 cm) • 2 laboratory scales (reading 1 g and 0.01 g) and standard weights • deionized water • stainless steel scalpel holders and blades • stainless steel scissors • stainless steel pliers • stainless steel tweezers • 2 stainless steel beaker with deionized water • teflon dissecting pad, • sealable plastic bags to freeze the heads or the dissected fish, • stainless steel containers (5.5 l and 3.5 l) with lids and fasteners • insulated container to hold stainless steel containers, • liquid nitrogen • disposable gloves and laboratory clothing • protective clothing for liquid nitrogen handling • towel tissues
Biometric Sample Characterization	• body weight (reading 0.1 g) • complete length and total length (reading 0.5 cm) • weight of muscle tissue, liver, and innards (reading 0.1 g) • age and sex • condition index and hepatosomatic index

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location(s)

Eelpout (*Zoarces viviparus*)

Identification:

__ __ __ __ / x / __ __ __ __ / __ __ __ __ __ / __

- Specimen Type
- Specimen Condition
- Collection Date (MM/YY)
- Sampling Area (SA)
- Sampling Region (SR)
- Sampling Site (SS)
- Additional information

Sampling Site (plaintext) _____

Sampling Point (number) _____

Sampling Point (plaintext) _____

Sampling Leader _____

Remarks _____

_____

_____

_____

_____

_____

Notes _____

_____

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_____

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GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 2: Sampling Method

Eelpout (*Zoarces viviparus*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

From: __ __ . __ __ . __ __ Sampling date To: __ __ . __ __ . __

Start: __ __ : __ __ Time End: __ __ : __ __

Method of Capture:

☐ Trawl nets

☐ Push nets / Shore seine

☐ Fish pots

☐ Other: _____

Caging:

Maximum duration of the storage period until sample preparation Action 1: __ __ h

Maximum duration of the storage period until sample preparation Action 1: __ __ h

Maximum duration of the storage period until sample preparation Action 1: __ __ h

Maximum duration of the storage period until sample preparation Action 1: __ __ h

Maximum duration of the storage period until sample preparation Action 1: __ __ h

Maximum duration of the storage period until sample preparation Action 1: __ __ h

Storage

Number of the stainless steel containers	Weight empty [g]	Weight filled [g]	Weighted sample [g]	
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Muscle tissue
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Muscle tissue
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Muscle tissue
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Liver
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Liver

Remarks: _____

_____

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 3.1: Sample Description – Eelpout (*Zoarces viviparus*)

Identification: __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ **/** __ __

No.	**Complete length** _ _ _ . _ cm	**Weight** _ _ _ . _ g	**Remarks**

No. (from to), Date, Signature:

No. (from to), Date, Signature:

GERMAN ENVIRONMENTAL SPECIMEN BANK **Specimen Data Sheet 3.2: Sample Description – Eelpout (*Zoarces viviparus*)**

Identification: __ __ __ __ / X / __ __ __ __ / __ __ __ __ __ __ / __

No.	Muscle tissue _ _ _ . _ g	Liver _ _ . _ g	Innards _ _ . _ g	Sex (mark w. cross)			Age [a]	Remarks
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
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				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		
				♂	♀	?		

No. (from to), Date, Signature:

No. (from to), Date, Signature:

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Protocol

Eelpout (*Zoarces viviparus*)

Sampling Area: _____ Identification: __ __ __ __ __

Underlying Version of the Sampling Guideline __ __ . __ __ . __ __

Underlying Version of the Sampling Scheme __ __ . __ __ . __ __

1. Objective of the Sampling: _____

_____

2. Actual Timeframe of the Sampling:

Start		End		Sample no.		Sampling Leader	Remarks
date	time	date	time	from	to		

3. Participants: internal _____

_____

external _____

4. Checklist Referring to Sampling Scheme and Sampling Guideline: ☒ as prescribed

- ☐ 4.1 Sampling Period
- ☐ 4.2 Sampling Site and Sampling Point (selection/definition)
- ☐ 4.3 Selection of the Individual Specimens
- ☐ 4.4 Technical Preparations
- ☐ 4.5 Cleaning Procedure for the Packages
- ☐ 4.6 Sampling Technique/Method of Capture
- ☐ 4.7 Sample Amount
- ☐ 4.8 Data Collection
- ☐ 4.9 Transport and Interim Storage

Number, kind and reason for deviation (clear text):

_____

_____

_____

_____

Remarks: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature