UMWELTPROBENBANK DES BUNDES

Guideline for Sampling and Sample Treatment

Eelpout (*Zoarces viviparus*)

Roland Klein, Martina Bartel, Martin Paulus, Markus Quack, Kathrin Tarricone, Diana Teubner, Gerhard Wagner

Trier University, FB VI – Biogeography
Wissenschaftspark Trier-Petrisberg, D-54286 Trier

Umwelt Bundes Amt
Für Mensch und Umwelt

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 of environmental monitoring for the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) underlying specialized and administrative co-ordination of the Federal Environmental Agency (Umweltbundesamt, UBA). The ESB collects ecologically representative environmental specimen in addition to human samples, maintains and examines them concerning relevant environmental substances (BMU 2008).

Long term storage is accomplished under conditions, which exclude condition change or loss of chemical characteristics, over a period of numerous decades. The archive stores samples for retrospective examination of such substances whose danger potential for the environment or for human health is today unknown.

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

2 Guideline Objective

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based and standardized methods, to avoid contamination and inhibit loss of chemical information. The exceptionally high demand of true quality results derives 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 BACKHAUS et al. (1995) version.

Transport, further sample treatment and storage as well as chemical analysis have to be done following the actual guidelines of the ESB.

3 Function of the Specimen Type

In marine ecosystems Eelpouts 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 (ZANDER & HARTWIG 1982).

In numerous monitoring studies the eelpout proved as good accumulation-, and primarily, as effect-indicator for coastal marine ecosystems (ELLIOTT & GRIFFITHS 1986; JACOBSSON et al. 1993; MATTSON et al. 2001, LARSSON et al. 2002, HANSON, ABERG ET AL. 2005; RASMUSSEN, TEH ET AL. 2005; RONISZ ET AL. 2005, GERCKEN, FORLIN ET AL. 2006; KORSGAARD 2006; LIND, BIGNERT ET AL. 2006; RASMUSSEN, JESPERSEN ET AL. 2006; STURVE, BALK ET AL. 2006; VETEMAA, ESCHBAUM ET AL. 2006; VUORINEN, KEINANEN ET AL. 2006; ABELE, PHILIPP ET AL. 2007; ALBERS, BJORN-MORTENSEN ET AL. 2007; HEISE, ESTEVEZ ET AL. 2007; SKOV, SORENSEN ET AL. 2007). It was proposed 1991 by Sweden as bioindicator for the Baltic see coastline to the Baltic Marine Environmental Protection Commission (HELCOM) and it is accounted as indicator for the BMP (Baltic Monitoring Programme) and the JMP (Joint Monitoring Programme) (THORESSON 1993). Currently the eelpout is used in several national monitoring programs, mainly in countries bordering the Baltic Sea (i.e. Sweden, Finland, Denmark, 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).

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

- wide distribution within Europe from the northern coast of Spain to the White Sea and into the Baltic Sea,
- the juvenile as well as the adult fish primarily live in coastal shallow water regions and are predominantly sedentary,

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

- great ecological valence as for temperature and salt content of the water; it tolerates even the low salt content in the particularly polluted estuary regions,
- it is the only viviparous fish species which occurs in our coastal waters; hence it is particularly suited as bioindicator for the detection of adverse effects on the reproduction,
- no special restrictions by nature- or species protection laws,
- the species is easy to identify.

Problems were experienced during the last years regarding the availability of larger specimen. At the current state of knowledge, the reason is a oxygen deficiency caused by increased water temperatures (PÖRTNER & KNUST 2007).

4 Target Compartments

Because a sufficient homogenization of whole fish is not possible (PAULUS & 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 the liver as the body's main metabolic organ is additionally collected.

5 Predefinitions for the Sampling

5.1 Species Determination

In taxonomy the viviparous eelpout (Fig. 1) 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 incessant 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 approximate the size of the head. The ventral fins in contrast are highly atrophied.

The body color considerably varies and is adapted to the particular surroundings. Fish from the sandy sea grass region are predominately yellow green to yellow brown in color. Specimens from mud or dulse stands are in contrary a grey brown to black brown in color. The dorsal fin and the back are traversed by more or less irregular 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 as with 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 (WIECASZEK 1992).

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, e.g.:

- water depth,
- water quality,
- wind direction,
- wind strength,
- tidal current situation.

Thus the distance to the nearest emission source has to be individually determined for each sampling site and documented in the area related sampling scheme.

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 tide way 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. During sampling an age determination of the individuals is merely estimated according to length and weight (PETERSEN 1982). The exact age analysis is determined through an otolith examination carried out in the laboratory. A balanced age distribution during sampling cannot be guaranteed because younger individuals are naturally more frequent.

Depending on the eelpout's sizes, between 20 to 300 specimen have to be sampled to reach the ESB required quantity of 2.200 g muscle tissue. For statistical purposes a random sample size of at least 20 eelpouts per sampling site is required.

5.4 Sampling Period and Frequency

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

The sampling is carried out prior the mating season which occurs between August and September (GÖTTING 1976). Depending on the weather conditions, sampling should start at early May and be finished at the end of June.
Under problematic conditions this time frame can be extended, but sampling must be completed by 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. This includes amongst others:

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

Describing the characteristic elements of the sampling sites within the area related sampling scheme secures long-term continuous sampling. In the case of changes within the sampling site or the sampled population the document has to be updated.

In case of major changes, so that comparability of the samples could not be guaranteed anymore, a new site has to be selected.

6 Sampling Procedure

Normally the catch of the eelpouts is performed by local professional fishermen, having the proper equipment required and the necessary knowledge of the place. To this, several weeks preceding the sampling, the respective arrangements must be made. In conservation areas additional approvals for the removal of eelpouts may be required, for these permissions must be duly applied at the appropriate authorities.

All data collected in the course of sampling and through the biometric sample description are documented in the respective specimen data sheets (see appendix). Furthermore, for each sampling a record with the following content must be created:

- all persons involved in the sampling,
- chronological procedure of the sampling,
- the underlying version of the sampling guideline and the area related sampling scheme for the current sampling,
- alterations to 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
- freezer bags
- PTFE club and electrical system to anaesthetize the fish
- photographic equipment
- measuring board (reading accuracy 0.5 cm)
- laboratory scales (reading accuracy 1 g)
- laboratory scales (reading accuracy 0.01 g)
- 2 stainless steel scalpel holders and blades
- 3 stainless steel scissors
- 2 stainless steel pliers
- 2 stainless steel tweezers
- 2 stainless steel beakers for deionized water
- deionized water
- PTFE dish
- stainless steel tray
- magnifying glass or (binocular) microscope
- disposable gloves
- laboratory clothing
- tissues
- stainless steel containers (5.5 l and 3.5 l) with lids and fasteners
- insulated containers for stainless steel containers
- liquid nitrogen
- tools and protective clothing for liquid nitrogen handling
- cooling device (dewar vessel) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN), corresponding to the number of required stainless steel containers

Cleaning procedures:

Sample containers and all equipment is 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°) for a minimum of an hour (sterilization). The containers remain closed 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 at this as by-catch of the prawn fishing and have to be sorted out of the catch, conditioned on board in suitable basins, and transferred into stationary conditioning basins (i.e. fish transport container) on shore close to the mobile laboratory.

Fish pots are mostly used in the shallow water areas of the Baltic Sea. They are positioned by local fishermen and controlled daily. The fish caught are conditioned until take over in net cages in the habitat water, and then transferred in a suitable conditioning basin (i.e. fish transport container) close to the mobile laboratory.

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 to transfer the eelpouts 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 it is not allowed to keep an individual in conditioning for more than four days.

For further processing each fish will be taken out individually by means of a landing net and killed either by a knock on the forehead or by an electric shock in a separate water basin. Because the liver must not be damaged the fish can not be killed by directly stabbing the heart.

The dead fish is immediately transferred to the mobile laboratory installed near the waterside, equipped with a clean bench with particles- and activated carbon filtration

Thereafter, the following work steps are to be performed consecutively:

- weighing (0.1 g reading accuracy),
- measuring of the length (0.5 cm reading accuracy) 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 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); they are put aside and further processed after removal of the muscle tissues (described below),
- incision of the skin along the dorsal-ventral line and the operculum on the left body side using a pair of stainless steel scissors to avoid injury of the organs, care must be taken that the incisions do not deeply penetrate the muscle tissue or in the abdominal cavity,
- stripping off the skin from the head to the tail using stainless steel tweezers or stainless steel pliers,
- incision of the muscle tissue with a scalpel along the dorsal line to the end of the tail, removal of the muscle tissue from head to tail by means of stainless steel tweezers and further cutting with a scalpel,
- cutting the remaining muscle tissue with a scalpel,
- weighing of the muscle tissue on a PTFE tray (0.1 g reading accuracy) 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,
- determination of the sex: male gonads are in pairs, female gonads are single,
- dissection of the inner organs: removal of the liver with stainless steel tweezers and stainless steel scissors without injuring other organs. If the gall bladder is injured, the liver will be dismissed after weighing, because it could be contaminated by the leaking biles,
- weighing of the liver (0.1 g reading accuracy) and shock-freezing in liquid nitrogen in a stainless steel container (the livers of all eelpouts are deep-frozen together),
- weighing of the remaining innards (0.1 g reading accuracy); the innards will be dismissed after weighing,
- documentation of all conspicuous features at the viscera,
- resection of the head and packing in labeled freezer bags.

To allow for age determination the otoliths must be dissected from the head at a later date. Till that, the heads will be kept in a deep freezer

7 Biometric 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 the otoliths (e.g. SVEDÄNG et al. 1997).

Further, weight-length-relationship has proved to be trustworthy for the degree of the nutritional status of the fish. It is calculated as follows:

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

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

The hepatosomatic index is used to identify influences of environmental pollutants which lead to an enlargement of the liver. It is calculated as follows:

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

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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:	one to four-year-old individuals
Random Sample Number:	at least 20 individuals
Sample Quantity for the ESB:	depending on the eelpouts sizes, between 20 to 300 specimen per sampling site have to be sampled to reach the ESB required quantity of 2.200 g muscle tissue.
Sampling Period:	optimum is from early May until end of June, not later than the end of July
Sampling Frequency:	1 sampling per annum
Equipment Required 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 • freezer bags for eelpout heads
Transport and Interim Storage:	cooling device (dewar vessel) 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 particles- and activated carbon filtration • waterproof pen • freezer bags • PTFE club and electrical system to anaesthetize the fish • photographic equipment • measuring board (reading accuracy 0.5 cm) • laboratory scales (reading accuracy 1 g) • laboratory scales (reading accuracy 0.01 g) • 2 stainless steel scalpel holders and blades • 3 stainless steel scissors • 2 stainless steel pliers • 2 stainless steel tweezers • 2 stainless steel beakers with deionized water • deionized water • PTFE or stainless steel dish • magnifying glass or (binocular) microscope • disposable gloves • laboratory clothing • tissues • stainless steel containers (5.5 l and 3.5 l) with lids and fasteners • insulated containers for stainless steel containers • liquid nitrogen • tools and protective clothing for liquid nitrogen handling
Biometric Sample Characterization:	for at least 20 individuals: • body weight (1 g reading accuracy) • complete length and total length (0.5 cm reading accuracy)

	• weight of muscle tissue, liver, kidneys, spleen and innards (0.1 g reading accuracy) • age and sex • weight-length-relationship and hepatosomatic index

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

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 Location: __ __ __

Gauß-Krüger-Coordinates:

Easting: __ __ __ __ __ __ __ Northing: __ __ __ __ __ __ __

Datum: _____ Ellipsoid: _____

Size of the Sampling Location: __ __ km² __ __ ha __ __ a __ __ m²

Kind of Use: _____

Remarks: _____

_____

Person(s) in Charge:

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: _____

Conditioning:

Maximum duration of conditioning in fish pots: __ __ d __ __ h

Maximum duration of conditioning in PE-boxes on the ship: __ __ h

Maximum duration of conditioning in the fish transport container: __ __ h

Other method of conditioning: _____ __ __ d __ __ h

Over-all duration of conditioning until further processing: __ __ d __ __ h

Storage:

Number of the stainless steel container	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: Sample Description – Eelpout (*Zoarces viviparus*)

Identification:

__ __ __ __ / X / __ __ __ __ / __ __ __ __ __ / __

Sampling Location: __ __ __

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

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Record

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:

Date	Time		Sample No.		Remarks
	from	to	from	to	

3. Participants: Conductor/Recorder: _____

Other: _____

_____

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

- ☐ 4.1 Sampling Period
- ☐ 4.2 Sampling Site and Sampling Location (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 of, and Reason for Possible Variations (Clear Text):

_____

_____

_____

_____

Remarks:: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature