Guideline for Sampling and Sample Treatment

Bream (*Abramis brama*)

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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 Klein & Paulus (1993) 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 freshwater ecosystems breams occupy the trophic level of carnivorous consumers. Though as non-predatory fish they do not rank at the end of the food chain (Müller & Wagner 1985, 1988). Breams mainly feed on benthic organisms, particularly on the benthos larvae, which rate as decomposers, carnivorous dragonfly larvae and mollusks (Löffler 1982, 1984, Winfield & Townsend 1988, Abdullaev 1980, Persson & Brönmark 2002). Thus their close contact to the river bed underlines the relation to the sediment and not only to the limnetic zone, as e.g. that of the roach (*Rutilus rutilus*).

For a long time, the bream has successfully been used in passive biomonitoring as an accumulation indicator (e.g. Kittelberger 1973; Appel 1980; Wachs 1980; Krüger & Kruse 1981, 1984; Lindstrom-Seppa & Oikari 1990; Paulus & Klein 1995). Further in some recent field studies, they are used as effect indicators, especially for endocrine effects. (Tyler et al. 1996; Lehmann et al. 2000; Hecker 2001).

The following criteria underline its use as an accumulation indicator in the scope of the ESB:

- wide distribution in Europe, except the extreme north and south (e.g. Ladiges 1979; Lelek & Buhse 1992),
- one of the most common fish species in Central Europe (Müller 1983), therefore available for sustainable and repeatable long term sampling,
- largest species among the most frequently found fish in Central Europe, making it especially convenient for organ dissection,
- long life span (up to 25 years),
- wide ecological amplitude: occurs in (slow) moving and stagnant waters with differential pollution, and degree of waterway construction and even in brackish water of the Baltic Sea (Müller 1983; Lelek & Buhse 1992),

- "conservative" reacting fish (HARTMAN 1978, 1979), i.e. even in varying eutrophic water conditions, its populations remain stable; only slightly altering weight and elongation (GOLDSPINK 1978; DEUFEL 1978; LÖFFLER 1982),
- resistance to high pollution (MÜLLER & MENG 1990),
- relatively sedentary, it is not known whether the habitat specificity is the same in all water bodies and what part of a given population leaves their place of birth and by which distance (HARTMAN & LÖFFLER 1977, 1978; SCHULZ & BERG 1987; DONNELLY et al. 1998),
- mirrors the pollution of the water bed (including the sediment) and the limnetic zone,
- regionally used for human consumption (e.g. LADIGES & VOGT 1979; MÜLLER 1983), which constitutes a direct relation to the human food chain.

Problems arise with the bream in captivity, because they are difficult to rear. However this is necessary for toxicity and impact tests.

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 loading site is additionally collected.

Blood plasma is primarily used for biomarker impact studies (e.g. immunological, hormonal, genotoxic). Due to its lipid content and homoeostatic capacity it is also ideal for the quantitative analysis of organic, lipophilic xenobiotics and elements. In clinical diagnostics blood plasma is the most important body fluid.

5 Predefinitions for the Sampling

5.1 Species Determination

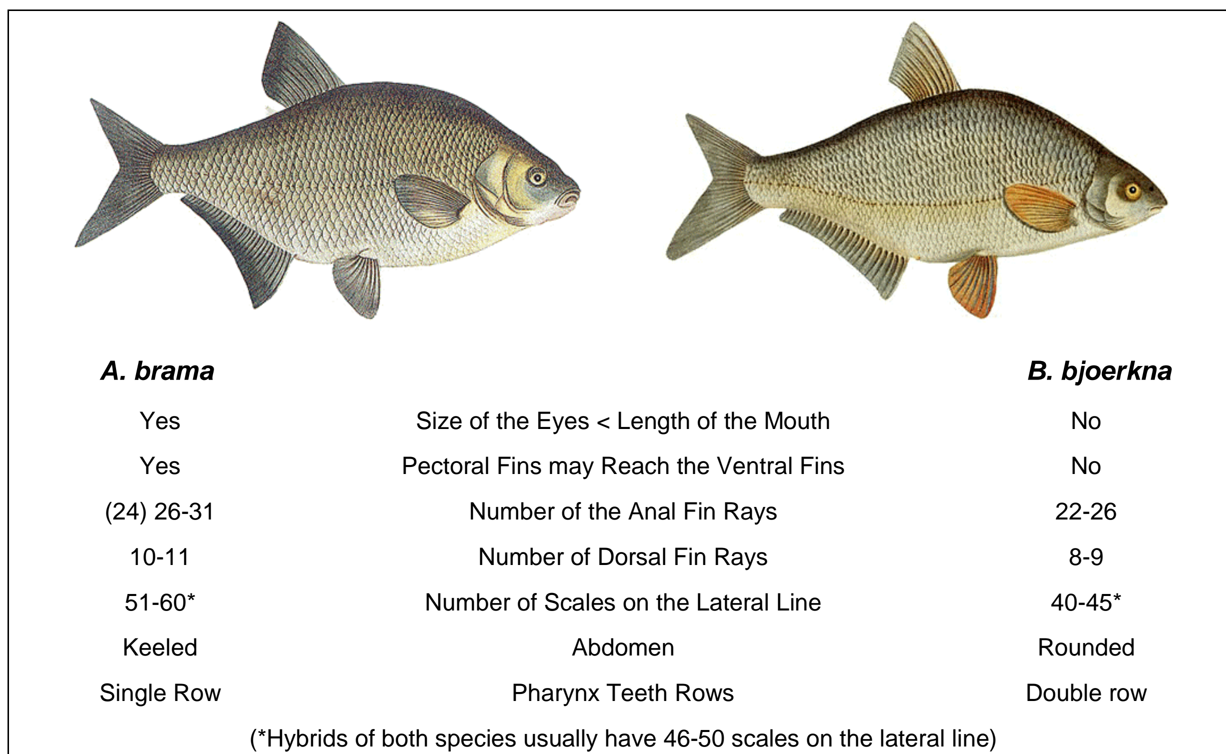
The bream is easily confused with *Blicca björkna* (white bream, silver bream, etc.). Basic distinctive features are shown in Fig. 1. In addition, the bream tends to hybridize with this species and other members of the Cyprinids (WOOD & JORDAN 1987).

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, -width, -surface, -volume,
- degree of mixing,
- pH-value,
- oxygen content,
- water hardness,
- conductivity,
- trophic level,
- flow rate,
- wind direction,
- wind strength,
- character of the banks,
- exposure, etc..

Thus the distance to the nearest emission source has to be individually determined for each sampling site (chap. 5.5).



A. brama		*B. bjoerkna*
Yes	Size of the Eyes < Length of the Mouth	No
Yes	Pectoral Fins may Reach the Ventral Fins	No
(24) 26-31	Number of the Anal Fin Rays	22-26
10-11	Number of Dorsal Fin Rays	8-9
51-60*	Number of Scales on the Lateral Line	40-45*
Keeled	Abdomen	Rounded
Single Row	Pharynx Teeth Rows	Double row

(*Hybrids of both species usually have 46-50 scales on the lateral line)

Fig. 1: Distinctive Features of *Abramis brama* and *Blicca björkna*

5.3 Selection of Individuals and Sample Size

To ensure the comparability of homogenates the breams have to be within a defined age class. This guarantees not only a sufficient availability of samples but also adequate body and organ weight. In the extremely varying waters the listed criteria is most suitable for the eight- to twelve-year-old breams. Screenings have proved that metals accumulate independent of age within this group. There is as yet no knowledge for age-related accumulation of organic pollutants in the target group (KLEIN & PAULUS 1995).

While performing the sampling the age of individuals is estimated by their length and weight. Because the growth of the bream depends on the specific water conditions and varies greatly, there is no thumb rule of length and weight. Prior to the first sampling, a screening is carried out to investigate availability and bream growth. Through this screening a further purpose is met. It enables a survey of at least 20 breams to determine their individual variance of substance concentration.

Weight and length are merely estimations for the sampling. The exact age is determined in the laboratory using scales and opercula for the analysis. (chap. 7). Hence breams outside of the mentioned age group may be present in the random sample. If there is an insufficient quantity of the target age group, younger or elder age groups compliment the sample, where necessary.

For statistical purposes at least 20 breams per sampling are dissected and stored. This minimum quantity is increased in waters accommodating small, scanty breams, to reach the ESB required quantity of 2.200 g muscle tissue.

5.4 Sampling Period and Frequency

In long term programs as that of the ESB sampling should be carried out annually.
The sampling is realized after the spawn period in August and September. Depending on the weather conditions it is possible to perform the sampling as early as the middle of July or, at the latest, mid October. Later sampling dates hamper the catch because breams retreat to deeper water during the cold seasons.

The spawn season (approx. from April to the beginning of July), a period of permanent physiologic changes of the sexually mature individuals, is also excluded due to dynamics which cannot be standardized.

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 (i. e. permission by the respective Water and Shipping Authority to use the waterway rights and the adjacent work paths, approval from the respective Fishing Agency or Nature Conservation Agency, a certificate of exemptions issued by the Nature Conservation Agency for containment of the fish in net cages until dissection).

At least an agreement with the respective owner of the fishing rights is required.

Describing characteristic elements of 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

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
- fishing tackle dependent on the applied method (chap. 6.2)
- species-appropriate net cage
- species-appropriate transport containers (at least 200 l) with ventilation
- landing net

Laboratory:

- specimen data sheets for the biometric sample description
- clean bench with particles- and activated carbon filtration
- waterproof pen
- 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)
- stainless steel scalpel holders and blades
- stainless steel scissors
- stainless steel pliers
- stainless steel tweezers
- bone cutter
- freezer bags for opercula
- 2 stainless steel beakers with deionized water
- deionized water
- PTFE dish
- magnifying glass or (binocular) microscope
- 2.6 ml polypropylene tube with sealing push-in cap, treated with heparin (S-Monovette with 10-30 LE/ml whole blood) (per individual 2 units)
- 0.9 mm hypodermic needle, 38 mm long
- pipettes with appropriate tips (0.1-1000 µl)

- cryo-tubes (PP) (Ø11 mm, L 47 mm, capacity max. 500 µl), labeled for identification
- cooling centrifuge for laboratory use with external rotor motor
- disposable gloves
- laboratory clothing
- tissues
- paper bags
- stainless steel containers (5.5 l and 3.5 l) with lids and fasteners and cardboard tube storage boxes for blood plasma samples
- 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

In addition at screenings:

- 100 ml Schott-Duran-bottles
- 500 ml Schott-Duran-bottles

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 (+/– 5°) 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

The method of catching the eight to twelve-year-old breams generally depends on the type of water under the Water Framework Directive (Directive 2000/60/EC). Thus it is not possible to successfully catch the fish using the same method in every location.

Anchored nets are used in deep, stagnant or slow moving waters and their branches. Gill nets are suitable as ground nets with a mesh gaugage of 70-100 mm depending on the type of the water body and the size of the eight to twelve-year-old breams. The in situ net time should not exceed few hours because otherwise the captured fish could suffer too much stress or injury. The advantage of the relatively large mesh and short duration also insures a minimum impact on the non-target species.

Dragnets are particularly suitable for catching bream in shallow stagnant waters. The actual catching is normally left to professionals.

In major water flows using **scoop nets** or **stow nets** is the most suitable method for catching (MÖLLER 1984).

Additionally electric fishing can be applied in suitable parts of the water body.

Since the mentioned catching equipment and techniques are normally applied by professional fishermen or specially trained persons detailed technical explanations are not necessary.

Only when everything else fails and the catch is insufficient **angling** is considered. In that case the fish usually have to be fed to be successful. The kind of feed and the catch method are noted in the specimen data sheet.

Independent of the catch method, all breams are immediately transferred to a net cage swimming in the habitat water. Per net cage less than 25 fish, depending on the size, should be kept together.

Alternatively, the fish can be transferred to species-appropriate transport containers, provided with a ventilation system for fresh air. That might be necessary in case the mobile laboratory can not be placed next to the waterside.

It is important that no individual remains in captivity for longer than four days, because starvation results physiological changes (KAUSCH 1972).

For further processing each fish is individually removed from the net cage or transport container using a landing net. The exact species determination is done before anesthetization through an electrical system. After this at least 2 x 2.5 ml whole blood is taken directly from the heart. Therefore the opercula is opened and the

Monovette cannula penetrates the skin under the gill directly above the osseous base.

The anesthetized fish is killed according to the animal protection laws and immediately transferred to the mobile laboratory (clean bench with particles- and activated carbon filtration). Here the following steps are chronologically processed:

- weighing (1 g reading accuracy),
- measuring of the length (0.5 cm reading accuracy) from tip of the mouth to end of the compressed tips of the caudal fins (= length complete or LC) and of the length from tip of the mouth to the centre between the ends of the caudal bifurcation (= total length or LT),
- recording of all conspicuous skin features,
- removal of a minimum of six scales (shortly underneath the lateral line between the ventral and the anal fin) with tweezers and transferring to labeled paper bags,
- centrifugation of the blood sample (10 min. at 3000 rpm and 2°C (+/– 3°) or direct storage in a cooling device (refrigerator) at 5°C (+/– 3°) until the centrifugation, which must be carried out not later than four hours after the blood withdrawal,
- afterwards, the blood plasma using pipettes is allocated by quota in PP-cryo-tubes to at least 100 µl per sample-container, and transferred above LIN.,

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:

- 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 flesh or in the abdominal cavity,
- stripping off the skin from the head to the tail using strong stainless steel pliers,
- incision of the muscle tissue along the dorsal line and along the upper edge of the spine and its removal from head to tail using stainless steel tweezers and using a scalpel for further cutting,
- 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 (5.5 l) (the muscle tissue of all dissected breams is deep-frozen together),
- opening of the abdominal cavity using stainless steel scissors; while lifting and stripping the abdominal wall, care must be taken to avoid damage to the organs,
- removal of the liver by using stainless steel tweezers and scissors without damaging other organs (especially the gall bladder),
- weighing of the liver (0.1 g reading accuracy) and shock-freezing in liquid nitrogen in a stainless steel container (3.5 l) (the livers of all breams are deep-frozen together),
- removal of the spleen and weighing (0.1 g reading accuracy),
- removal of the remaining innards (excluding the kidney) and weighing (0.1 g reading accuracy); determination of the sex on the basis of the gonads using a magnifying glass or a (binocular) microscope,
- dissection and weighing of the kidney and weighing (0.1 g reading accuracy),
- resection and packing the opercula for maceration,
- documentation of all conspicuous features at the viscera.

Screenings are carried out on at least 20 breams to determine the individual variation of substance values. The dissection of the muscle tissue as well as the packing of the liver differs as follows:

- the muscle tissue from the right side of the body is additionally dissected and weighed. Depending on the volume the muscle tissue is packed individually (!), in a 100 ml or 500 ml Schott-Duran-bottle,
- each liver is individually packed in a 100 ml Schott-Duran-bottle.

7 Biometric Characterization

Most of the biometric parameters are obtained during the sampling (chap. 6.2). Merely the exact age determination gained from the scales and the opercula is carried out in the laboratory. Therefore the opercula are macerated and then cleaned. On the scales as well as the opercula, the annual growth rings are counted, which develop during winter and are visible as lines. The double age check ensures that even in critical cases the age is determined. Opercula provide a more reliable result than scales, especially in breams older than 10 years.

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:	**Name (*Genus species*)**
Target Compartments:	muscle tissue from the left side of the body and liver
Individual Specimens:	eight to twelve-year-old individuals
Random Sample Number:	at least 20 individuals
Sample Quantity for the ESB:	• 2.200 g of muscle tissue from the left side of the body, • entire available liver
Sampling Period:	August and September, depending on the weather conditions sometimes possible from the middle of July until the middle of October
Sampling Frequency:	1 sampling per annum
Equipment Required for Field Work:	• specimen data sheets for documentation during the sampling • fishing equipment in dependence of the method to be applied (chap 6.2) • net cage or transport containers with ventilation equipment • landing net
Sample Packing until Further Processing:	• stainless steel containers (5.5 l and 3.5 l) • for screenings additional 100 and 500 ml Schott-Duran bottles
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 • PTFE club and electrical system to anaesthetize the fish • measuring board (reading accuracy 0.5 cm) • laboratory scales (1 g reading accuracy and 0.01 g reading accuracy) and standard weights • paper bags and tissues • freezer bags • 2.6 ml polypropylene tube with sealing push-in cap, treated with heparin (S-Monovette with 10-30 LE/ml whole blood) and 0.9 mm cannula, 38 mm length) • pipettes for laboratory use with adequate tips (0.1-1000 µl) • cryo-tubes (PP), labeled for identification • cooling centrifuge for laboratory use with external rotor motor • 2 scalpel holders with stainless steel blades • 2 pairs of stainless steel scissors • 2 pairs of stainless steel pliers • 3 stainless steel tweezers • stainless steel bone cutters • 2 stainless steel beakers for deionized water • deionized water • magnifying glass or (binocular) microscope • PTFE dish • disposable gloves • clothes for laboratory work • liquid nitrogen • tools and protective clothing for liquid nitrogen handling

	• cooling devices for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN), • stainless steel containers (5.5 l and 3.5 l) with lids and fastener and cardboard tube storage boxes for blood plasma samples • insulated containers for stainless steel containers
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

Bream (*Abramis brama*)

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: _________________

River Kilometer: __ __ __ __ to __ __ __ __

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
Bream (*Abramis brama*)

Identification:

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

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

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

Method of Capture:

☐ Electric fishing gear

Type of gear ________________

Current type ________________

Voltage [V]: ________________ Capacity [kW]: __________

Type of Anode: ________________

Type of Cathode: ________________

☐ Anchored gillnet

☐ Dragnet

☐ Scoop net / stow net

Mesh width: __ __ __ mm

Net length: from __ __ __ m to __ __ __ m

Net depth: from __ __ __ m to __ __ __ m

Depth: from __ __ __ m to __ __ __ m

Number of rows: __ __

☐ Fishing rod ☐ Feeding Kind of feed: ________________________________

☐ Other: ________________________

Conditioning:

Maximum duration of the storage period of the breams in the anchored gillnet: __ __ h

Maximum duration of conditioning of the breams in the net cage: __ __ h

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

Overall duration of conditioning until further processing
(Anchored gillnet, net cage and fish transport container) __ __ h

Maximum number of individuals in the net cage: __ __ h

Position of the net cage in the water: ________________________

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 3: Sample Description - Bream (*Abramis brama*)

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

No.	Weight ____ g	Complete length (*) __,_ cm	Total length (**) __,_ cm	Age [a]	Left side Musculature ___,_ g	Right side Musculature ___,_ g	Liver __,_ g	Incom-plete	Kidney __,_ g	Spleen __,_ g	Remaining Innards ___,_ g	Sex (mark with cross)	Remarks
												♂ ♀ ?	
												♂ ♀ ?	
												♂ ♀ ?	
												♂ ♀ ?	
												♂ ♀ ?	
												♂ ♀ ?	
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												♂ ♀ ?	
												♂ ♀ ?	
												♂ ♀ ?	
												♂ ♀ ?	

(*) from the tip of the mouth to the end of the compressed caudal tips (**) from the tip of the mouth to the centre between the ends of the caudal bifurcation

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 4: Storage

Bream (*Abramis brama*)

Identification

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

Number of the stainless steel container	Weight empty [g]	Weight filled [g]	Weighted sample [g]	
__ __ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Left muscle tissue
__ __ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Left muscle tissue
__ __ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Left muscle tissue
__ __ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	Liver
				Blood

Remarks: ____

____

____

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Record

Bream (*Abramis brama*)

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