



Guideline for Sampling and Sample Processing



Zebra Mussel (*Dreissena polymorpha*)

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Contents

1	German Environmental Specimen Bank	2
2	Objective of this Guideline	2
3	Function of the Specimen Type	2
4	Target Compartments	3
5	Predefinitions for the Sampling	3
5.1	Species Determination	3
5.2	Selection and Definition of Sampling Sites	4
5.3	Selection of Individuals and Sample Size	4
5.4	Sampling Period and Frequency	4
5.5	Area-Related Sampling Scheme	5
6	Sampling Procedure	5
6.1	Required Equipment and Cleaning Procedures	5
6.2	Sampling Technique	6
7	Biometric Sample Characterization	8
8	References	9

**Appendices: Checklist to Prepare and Conduct the Sampling
Specimen Data Sheets**

**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, stores and investigates them for environmentally relevant substances.

Specific operating procedures and the concept of the ESB are the basics for the ESB operations (Environment Agency 2008, 2014).

The long-term storage is carried out under conditions which, as far 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 yet unknown.

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 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 Wagner *et al.* (2003) 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

The zebra mussel *Dreissena polymorpha* (Pallas, 1771) feeds mainly on plant and animal plankton organisms as well as bacteria and detrital particles, which they filter out of the water in a size range between 1 and 40 µm. Due to the continuous flow of water through the mantle cavity as well as the large surface of the gills, zebra mussels are in close contact with their environment. As a result, *D. polymorpha* is able to absorb and accumulate a wide range of organic and inorganic substances in both particulate and dissolved form from the water. Therefore, it can be used to demonstrate the bioavailability of substances in the environment. In addition, due to its expansive behavior, the species, which originates from the Pontic-Caspian region, is now available in large parts of Europe and North America, mostly in large population densities. Therefore it plays an important role in numerous monitoring studies outside its home territory, e.g. in Belgium (Bervoets *et al.* 2005, Voets *et al.* 2009, de Jonge *et al.* 2012), France (Bourgeault and Gourlay-France 2013, Chatel *et al.* 2015, Kerambrun *et al.* 2016), Italy (Riva *et al.* 2010, Parolini *et al.* 2013, Poma *et al.* 2014), Spain (Alcaraz *et al.* 2011, Benito *et al.* 2017) and the U.S. (Blackwell *et al.* 2013, Kimbrough *et al.* 2014, Shoults-Wilson *et al.* 2015).

Within the scope of the Environmental Specimen Bank, the zebra mussel represents the level of the first order limnic consumers.

The following criteria underline the use of the *D. polymorpha* as an indicator organism:

- It is widely spread.
- Due to the sessile lifestyle of the adult mussels, they guarantee location loyalty for their multi-year lifetime.
- It often occurs in high population densities and biomasses.
- It accumulates dissolved and particulate substances through filtration from the surrounding medium.
- It has a high ecological valence: it can be found in still and running waters of different trophic levels, tolerates brackish water and survives short periods in which the surrounding water dries up.

- It is easy to manipulate, i.e. suitable for active monitoring (exposure of substrates colonized by young mussels) as well as for toxicity and effect tests.
- It is used as food for a number of partly commercially exploited fish species, including the bream, which is also a sample species of the ESB.

4 Target Compartments

The soft body of the zebra mussel, including respiratory water and the intestinal contents it contains, is used as a sample. Respiratory water and intestinal contents remain in the mussels as sample components, as it is not practicable to store the mussels in order to remove the respiratory water or to evacuate the intestines and would be associated with a risk of contamination for the samples.

5 Predefinitions for the Sampling

5.1 Species Determination

In the sampling areas of the ESB, the zebra mussels reach lengths of up to 40 mm. One side is strongly arched and the other almost flat. The umbo is very prominent. The zebra mussel has a wide variety of colors and patterns, which can vary greatly depending on the location. The color ranges from a light yellow-brown to dark brown. The pattern may consist of dark brown bows or zigzag curves that vary from close-meshed to wide-meshed or hardly recognizable. The mussels live up to 4 or 5 years.

The species can be easily confused with the quagga mussel *Dreissena rostriformis* (Deshayes, 1838), formerly also known as *D. bugensis* or *D. rostriformis bugensis* (name changed by Stepien *et al.* 2014), with which it is sympatrically distributed in its Pontic-Caspian home area. The quagga mussel first became invasive many years after the zebra mussel; the first evidence in North America dates back to 1989 (May and Marsden 1992), in Central Europe to 2004 (Paulus *et al.* 2014). In Germany, numerous rivers are now populated, and the quagga mussels often reached

high population densities shortly after their arrival, displacing the stocks of the zebra mussel due to their competitive advantages (bij de Vaate 2010, Heiler *et al.* 2012, 2013, Paulus *et al.* 2014, Stewart 2014, Marescaux *et al.* 2015).

Since the two species have a different accumulation potential (Rutzke *et al.* 2000, Richman and Somers 2005, Schäfer *et al.* 2012, Matthews *et al.* 2015), determining the species with certainty in monitoring studies is important. Although species identification using genetic markers can be done easily and clearly, in monitoring studies both species must be effectively and clearly distinguished in large quantities in the field. For this purpose, external shell features are used.

As a key feature for species differentiation, according to Teubner *et al.* 2016, the transition of the ventral and dorsal shell surfaces can be used, which is angled in zebra mussels and rounded in quagga mussels. In zebra mussels, a visible and palpable anterior-posterior longitudinal ridge is formed here (Teubner *et al.* 2016, s. also Martens *et al.* 2007, Ram *et al.* 2012). Figure 1 shows the main outer shell features for distinguishing zebra and quagga mussels.

In small mussels there is a possibility of confusion with the dark false mussel *Mytilopsis leucophaeata* (Conrad, 1831) (syn. *Congerina cochleata*), which originates from North America and lives in brackish waters. It mainly colonizes estuaries, e.g. the Rhine delta (van der Velde *et al.* 1992), and rivers with high salt load, such as the Weser (Busch 1991). External distinguishing features of the shell are:

D. polymorpha: extremely sharp umbo, transition between the anterior and the dorsal shell surfaces angled, arched flattened ventral side.

M. leucophaeata: shell oblong, ventroposterior rounded, rounded umbo, ventral side not arched, ventrolateral shoulder region missing.

The most definite morphological distinguishing feature is a triangular to rounded tooth (apophysis) in the interior of the valves below the umbo in *M. leucophaeata* (see Fig. 2), which is missing in *D. polymorpha* (according to Pathy and Mackie (1993).

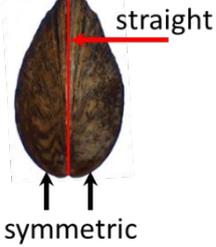
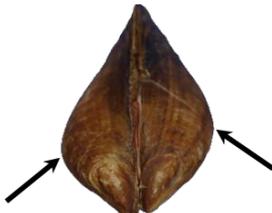
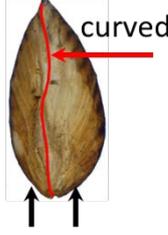
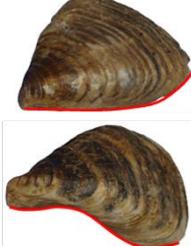
	Key feature	Further features	
	anterior-posterior	dorsal	lateral
Zebra mussel	 acute ridge	 straight symmetric	 rather straight
Quagga mussel	 no acute ridge	 curved asymmetric	 curved

Fig. 1: Important distinguishing features of zebra and quagga mussel

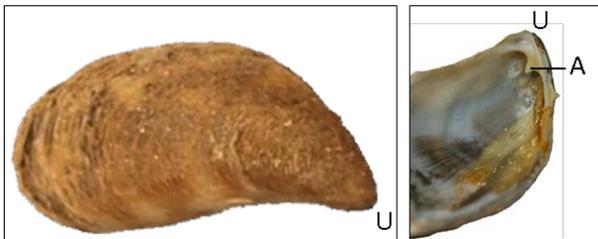


Fig. 2: Outer view (left) and inner view (right) of the right valve of *M. leucophaeata* from the Kiel Canal (U = umbo, A = apophysis)

5.2 Selection and Definition of Sampling Sites

The sampling sites must be representative of the ecosystem or sampling region. This means that they should not be in the immediate vicinity of local emitters. Possible effects of emissions are influenced by the type, amount and distribution of the substances as well as numerous hydrological and hydrographic factors of the body of water.

When selecting the exposure sites for colonized and uncolonized substrates (chapter 6.1), special attention should be paid to a safe and interference-free location. Natural interference factors, such as excessive current or silting up, should be avoided

as well as possible interference caused by shipping, boat traffic or vandalism. Furthermore, good water exchange and guaranteed accessibility of the exposure site even under unfavorable weather and water-level conditions is important.

In selecting and delimiting sampling sites for sampling of the bank substrate, sufficient size, density and stability of the population are also important for long-term sampling assurance.

Where possible, the long-term use of sampling sites as well as access to exposure points should be contractually secured, depending on the protection status and ownership of the sampling sites.

5.3 Selection of Individuals and Sample Size

For reasons of sampling comparability, a uniform target group must be defined for the sample collective, which guarantees not only the availability of the zebra mussels but also a sufficiently high sample volume. When colonized and uncolonized substrates are used (chapter 6.1), the age of the sample collective is determined by the given time periods of colonization and exposure. In the case of zebra mussel populations, which are sampled from

unexposed substrates, age is not used as a selection criterion, as it cannot be determined with sufficient accuracy.

A suitable criterion for reducing the natural variability is the shell length of the mussels. It should be kept in mind that the growth of the zebra mussels differs according to the waters and therefore information on the length structure of the target population must always be determined based on the area. For biological reasons, adult mussels should be sampled starting at the age of 2 years. For practical reasons, relatively large mussels are sought. In order to be able to separate the soft body from the shell when it is at a very low temperature (see chapter 6.2), the shell length should be at least 12 mm.

There is no minimum sample size valid for all substances for the determination of temporal and spatial concentration differences. The minimum sample size can be estimated statistically (e.g. by power analysis) for a specific substance. Due to the low soft body weights, a sample size of at least 100 individuals is recommended for the ESB. In order to achieve the required sample volume specified in the ESB, a significantly higher number of mussels is required. Depending on the size of the mussels, for example, to obtain 1,000 g soft body weight, about 2,000 to 5,000 mussels are required. This is equivalent to about 3 to 4 kg of raw mussels.

5.4 Sampling Period and Frequency

Since the spawning time is characterized by strong physiological dynamics and fluctuations of the biomass, it is not suitable for a reproducible sampling. Depending on the water and climatic conditions, it lasts from May until the end of August. Sampling should therefore be carried out after the spawning period, from mid-September to the end of December (Klein *et al.* 1995).

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,
- size class to be collected,
- required sample size,
- sampling period,
- appropriate authorities.

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

6 Sampling Procedure

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 for the current sampling as well as
- deviations from the sampling guideline and the area-related sampling scheme.

For sample collection for the ESB the use of plate racks that are colonized with young zebra mussels and exposed in the sampling area is preferable. If this is not possible because, for example, there is no suitable water for colonization, resident zebra mussel populations are sampled. If possible, uncolonized substrate is put out in the sampling area where the resident mussels can settle. If there are no suitable exposure sites or not enough mussels can be recovered from the exposed substrate, resident mussels are collected from the bank substrate.

6.1 Required Equipment and Cleaning Procedures

Field Work:

Colonized Substrate:

- plates made of additive-free polyethylene (PE), 30 x 30 cm, screwed to racks,
- stainless steel screw rods (12 mm) with stainless steel screw nuts,
- spanner,

- tube sleeves made of polytetrafluoroethylene (PTFE), PE or stainless steel as spacers,
- stainless steel wire rope,
- stainless steel wire rope clips,
- special wire cutters,
- PE boxes with lids for the transport of colonized plate racks,
- where necessary, nets (mesh size about 10 mm) to protect the mussels from predators.

In addition to PE and PTFE, other substrates can also be used. What is important is that no components of the substrate are released and accumulated in the mussels' soft bodies.

Sampling:

- scale (effective range up to 5 kg, reading 1 g),
- stainless steel wire baskets with maximum mesh size of 8 mm,
- stainless steel containers with lids and fasteners,
- small pads or foils of PTFE or fluorinated ethylene propylene (FEP),
- cooling device for immediate deep-freezing and transport of the samples in the gas phase above liquid nitrogen (LIN),
- specimen data sheets,
- protective clothing for handling liquid nitrogen,
- sampling of exposed substrate:
 - stainless steel or PTFE spatula,
- sampling of bank substrate
 - protective clothing for working in the water
 - safety ropes / lifejackets

Laboratory:

- clean bench with particle and activated carbon filtration,
- protective clothing for handling liquid nitrogen,
- stainless steel containers,
- scales (reading 1 g) to determine soft body weighted sample,
- scales (reading 0.001 g) to determine biometric parameters,
- caliper (reading 0.1 mm),
- liquid nitrogen,
- insulated container
- absorbent laboratory paper,
- stainless steel pincers and scalpels with rounded blades,

- powder-free disposal gloves.

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°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

Use of Colonized Substrate

The polyethylene plates are bolted to a stack of plates at intervals of about 7 cm between the individual plates.

For colonization, the clean plate racks are exposed at the beginning of the spawning season in an unburdened and well monitored water body with a stable population of the zebra mussel (Fig. 3).

The plate racks dangle free in the water at a depth of 2-3 m and have no contact with the bottom of the lake or with possibly contaminated materials or surfaces. The plates are fixed with stainless steel wire ropes, which are anchored at adequate fastening spots by means of screws and stainless steel rope clips.

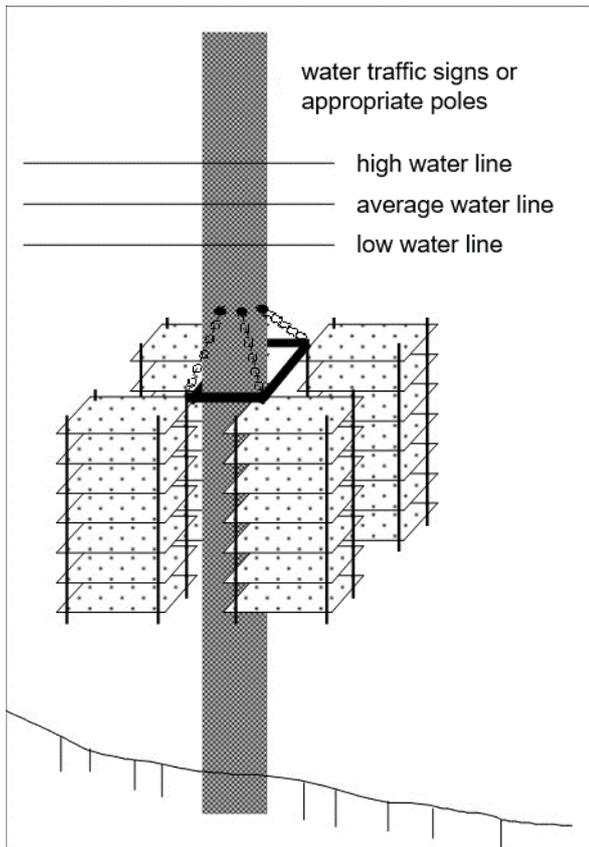


Fig. 3 Example for the attachment of the plate racks in the water

The colonization by free drifting Veliger-larvae takes place in springtime, beginning at a water temperature of approx. 15°C (Boeckman and Bidwell 2014, Kashian and Ram 2014). In large water bodies of Central Europe, this temperature is usually reached in May or June. In autumn, the plate racks are densely populated by juvenile mussels. Then the plate racks are removed and brought to the projected sampling sites. To avoid losses as a result of feeding on by water birds and fish, the plate racks can be spanned with nets that have a mesh size of approx. 10 mm before they are fastened in suitable places. The plate racks must be exposed in the sampling sites for at least one year before sampling. This ensures that the mussels in the waters to be examined pass through their main growth phase and thus reflect their living conditions and pollution levels.

The transport is carried out in boxes made of polyethylene with lids (high air moisture). Thereby, the temperature may not drop to less than 0°C and not significantly higher than 20°C. The higher the temperatures, the shorter the transport time should be and generally should not exceed 2 days.

For exposure in the waters to be examined, the colonized plate racks are attached to piles, piers or suitable floating buoys. The plate racks can be put out either free standing or connected to each other. Care must be taken to ensure that the racks do not hang in the anaerobic area and have no contact with the lake, river or sea floor, or with potentially contaminated materials or surfaces. In streams, the exposed substrates should not be suspended in full flow.

For the sampling, the exposed racks are salvaged and unscrewed. The zebra mussels, which have attached themselves to the plates with their byssus filaments, are carefully removed using a spatula (made of PTFE or stainless steel) and collected in a stainless steel wire basket. The mesh size of the basket should not exceed 8 mm.

In the stainless steel basket, the mussels are manually cleaned with habitat water. Empty shells and injured mussels are sorted out by hand as much as possible. The water adhered to the shells and released by the mussels is removed as much as possible before freezing. Then the mussels are transferred to previously weighed stainless steel containers. After each layer of about 2 cm in height, a thin pad or foil of PTFE or FEP is placed in between. This simplifies the subsequent removal of the frozen zebra mussels in the laboratory. After filling, the stainless steel containers are weighed, the total zebra mussel weight determined and then immediately frozen in the gas phase over liquid nitrogen to quickly kill the mussels and store them without alteration.

Sampling of Resident Zebra Mussels

If no suitable colonization waters are available or if sufficient sample quantities cannot be obtained by using colonized plate racks, resident zebra mussel populations are sampled. In order to be able to better estimate the age of the mussels during the sampling, colonization substrate is actively put out at suitable exposure sites in the spring. As a colonization substrate, the previously described racks of polyethylene plates can be used. The sampling takes place after one and a half years, so that the mussels have passed through their main growth phase and thus reflect the living conditions and pollution levels.

If there are no suitable exposure sites for the colonization substrate or if not enough mussels can be harvested from this, zebra mussels should be sampled from naturally occurring or other hard substrates that have not been contaminated through surface treatment and that are located below the low water line (e.g. stone beds, rock, untreated wood or agglomerations of zebra mussels, known as druses). Surfaces with protective coatings, such as steel, iron, plastic, asphalt or impregnated wood are not suitable. The type of substrate colonized by the mussels sampled must be documented. The size of the surfaces depends on the density in which the zebra mussels appear.

The mussels of the target population are obtained manually from suitable areas, collected in stainless steel wire baskets and cleaned in habitat water. Further processing is analogous to the mussels collected from the racks.

Work-Up in the Laboratory

The stainless steel container designated for the soft bodies is pre-weighed, marked with the associated sample identification and pre-cooled in a dewar vessel above liquid nitrogen.

Since the respiratory water could only be removed by thawing the mussels, which would be contrary to maintaining an uninterrupted cryogenic storage in accordance with ESB requirements, the entire contents of the mussel shells are sampled.

The separation of shell and soft body including respiratory water is done in the laboratory at a pure-air workstation with particle and activated carbon filtration (clean bench) in the frozen state, without thawing the soft bodies during dissection. For this purpose, a part of each of the raw mussels is carefully removed from the sample container and transferred to a stainless steel container cooled above liquid nitrogen.

Then about 10 raw mussels are laid on the work surface for superficial thawing. When the frost formed on the shells begins to thaw, the shells are opened with a scalpel. Frozen solid soft bodies are removed with tweezers; thawed or damaged soft bodies are discarded. The dissected soft bodies are interim-stored in a stainless steel container

filled with liquid nitrogen for the duration of the dissection of all mussels.

After the dissection is completed, the soft bodies are transferred to the pre-cooled sample container without liquid nitrogen and the sample weight is determined.

7 Biometric Sample Characterization

The biometric sample characterization is carried out for each sampling technique (see sample data sheets 2.1 and 2.2) on 50 frozen mussels in the laboratory. The length, width and height of the shells as well as the fresh weight of the entire mussel with respiratory water, the fresh weight of the soft body and that of the shell are determined.

Since the determination of the soft body fresh weight of frozen mussels is subject to significant error risks, a precise standardization of the determination method must be followed to avoid systematic random errors and to minimize them, as described below:

To determine the fresh weight with respiratory water, mussels are individually removed in the frozen state from the sample container, freed of adhering frost and, if necessary, adhering impurities with absorbent laboratory paper and immediately weighed (reading to 0.001 g).

The mussels are laid out on laboratory paper with the shell opening facing downwards. The thawing time for determining the soft body weight is taken from Tab. 1. During thawing, the length, width and height of the shells are measured with a caliper (reading 0.1 mm).

Soft body weight is defined as the weight of the soft body at the point in time that the mussel body is completely thawed and the respiratory fluid fully emitted, but the loss of tissue fluids is minimal.

The time from removal from the storage container up until complete thawing depends on the size of the mussel and the ambient temperature. The thawing time is reached when rapid weight loss due to leaking of the thawed respiratory water changes to a much slower weight loss caused by evaporation of the tissue water. At a room temperature of

20 – 22°C, the following thawing times were empirically determined in relation to the fresh weight with respiratory water (Tab. 1).

Tab. 1: Thawing times as a function of the fresh weight with respiratory water for the determination of the soft body weight

Fresh weight with respiratory water [g]	Thawing time [min]
0.4	22
0.6	28
0.8	30
1.0	34
1.4	38
1.6	40
2.0	42
2.5	45
3.0	48
3.5	50
4.0	54
4.5	55
5.0	56
6.0	60

The soft-body weight is determined according to the weight-dependent thawing time (Tab. 1). For this, the soft body is removed from the mussel shell by means of a scalpel and tweezers, collected in a pre-weighed dish and weighed immediately to avoid evaporation losses (reading 0.001 g). After that, the shell is also weighed (reading 0.01 g).

It should be noted that the stored mussel samples provided for chemical characterization contain the respiratory water. Therefore, the levels of ingredients of the ESB samples are diluted compared to mussels that are often dissected in fresh condition in other studies. With the parameters collected here, the average respiratory water content of each sample can be determined and the results converted accordingly.

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Checklist to Prepare and Conduct the Sampling

Specimen Type	Zebra mussel (<i>Dreissena polymorpha</i>)
Target compartment	soft body (deep-frozen, dissected, including respiratory water and gut content)
Individual specimens	mussels of the size class specified in the area-related sampling scheme (min. shell length of 12 mm)
Sample number	at least 100 individuals
Sample quantity for the ESB	for a sample volume of 1,000 g of soft bodies, it is necessary to collect approximately 2,000 to 5,000 (3 to 4 kg) of raw mussels
Sampling period	mid-September until end of December
Sampling frequency	1 sampling per year
Required equipment for the use of colonized / uncolonized PE plate racks:	<ul style="list-style-type: none"> • plates made of additive-free polyethylene (PE), 30 x 30 cm • stainless steel screw rods (12 mm) and nuts, spanner • tube sleeves made of PTFE, PE or stainless steel as spacers, 7 cm • stainless steel wire rope for attaching • screws, wire rope clips, special wire cutters, • nets with mesh sizes of about 10 mm to protect the colonized racks from mussel predators • PE boxes with lids for transporting colonized substrate
Required equipment for sampling	<ul style="list-style-type: none"> • scale (effective range up to 5 kg, reading 1 g), • stainless steel wire baskets with maximum 8 mm mesh size, • pads / foils made of PTFE or FEP, approx. 15x15 cm, • specimen data sheets, • protective clothing for handling liquid nitrogen, • sampling of plate racks: spatula made of stainless steel or PTFE • sampling of bank substrate: protective clothing for working in the water, safety ropes / life jackets
Sampling packing	<ul style="list-style-type: none"> • stainless steel containers with lids and fasteners
Transport and interim storage	<ul style="list-style-type: none"> • cooling device for immediate deep freezing and transport of the samples in the gas phase above liquid nitrogen (LIN)
Required equipment for laboratory work	<ul style="list-style-type: none"> • clean bench with particles- and activated carbon filtration, • protective clothing for handling liquid nitrogen, • stainless steel containers with lids and fasteners, • scale (reading 1 g), • scale (reading 0.001 g), • caliper (reading 0.1 mm), • insulated container for stainless steel containers with liquid nitrogen, • stainless steel pincers, stainless steel scalpels, • absorbent laboratory paper, powder-free disposable gloves • liquid nitrogen
Biometric sample characterization of 50 mussels per sampling technique	<ul style="list-style-type: none"> • length, width, and height of the shell (reading 0.1 mm) • fresh weight including respiratory water (reading 0.001 g) • weight of the soft body (reading 0.001 g) • weight of the shell (reading 0.001 g)

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

Zebra mussel (*Dreissena polymorpha*)

Identification:

____ / X / ____ / ____ / ____

_____	Specimen Type
_____	Specimen Condition
_____	Collection Date (MM/JJ)
_____	Sampling Area (SA)
_____	Sampling Region (SR)
_____	Sampling Site (SS)
_____	Additional Information

Sampling Site (plain text): _____

Sampling Point (SP) (number of SP and plain text):

Remarks: _____

Sampling Leader: _____

Notes: _____

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 2.1: Sample Composition Raw Mussels

Zebra mussel (*Dreissena polymorpha*)

Identification: _____ / X / _____ / _____ / _____

Sampling technique	Substrate	Number SP	Date of sampling	Number stainless-steel container (SSC)	Weight [g]	Processing priority
<input type="checkbox"/> Use of colonized substrate _____ date duration [months]	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ raw mussels _____	
<input type="checkbox"/> Use of colonized substrate _____ date duration [months]	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ raw mussels _____	
Sampling of resident mussels from <input type="checkbox"/> exposed substrate <input type="checkbox"/> bank substrate	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> rocky natural substrate <input type="checkbox"/> rocky artificial substrate <input type="checkbox"/> wood <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ raw mussels _____	
Sampling of resident mussels from <input type="checkbox"/> exposed substrate <input type="checkbox"/> bank substrate	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> rocky natural substrate <input type="checkbox"/> rocky artificial substrate <input type="checkbox"/> wood <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ raw mussels _____	

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 2.2: Sample Composition

Zebra mussel (*Dreissena polymorpha*)

Identification:

_____ / X / _____ / _____ / _____

Sampling technique	Substrate	Number SP	Date of sampling	Number stainless-steel container (SSC)	Weight [g]	Number of biometry
<input type="checkbox"/> Use of colonized substrate _____ date duration [months]	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ soft tissue _____	
<input type="checkbox"/> Use of colonized substrate _____ date duration [months]	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ soft tissue _____	
Sampling of resident mussels from <input type="checkbox"/> exposed substrate <input type="checkbox"/> bank substrate	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> rocky natural substrate <input type="checkbox"/> rocky artificial substrate <input type="checkbox"/> wood <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ soft tissue _____	
Sampling of resident mussels from <input type="checkbox"/> exposed substrate <input type="checkbox"/> bank substrate	<input type="checkbox"/> polyethylene racks <input type="checkbox"/> rocky natural substrate <input type="checkbox"/> rocky artificial substrate <input type="checkbox"/> wood <input type="checkbox"/> other: _____				SSC empty _____ SSC filled _____ soft tissue _____	

