UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Processing

Zebra Mussel (*Dreissena polymorpha*) Quagga mussel (Dreissena rostriformis bugensis)

Diana Teubner, Julian Hans, Isabelle Junk, Roland Klein, Martin Paulus

Trier University, FB VI – Biogeography
Universitätsring 15, D-54286 Trier

Umwelt Bundesamt

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 5
5.3 Selection of Individuals and Sample Size 5
5.4 Sampling Period and Frequency 5
5.5 Area-Related Sampling Scheme 5

6 Sampling Procedure 6

6.1 Required Equipment and Cleaning Procedures 6
6.2 Sampling Technique 7

7 Biometric Sample Characterization 9

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

Status: July 2024, V 3.0.0

1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an environmental monitoring instrument of the German Federal Ministry for the Environment, which is under the technical and administrative coordination of the Umweltbundesamt (UBA), Germany's environmental protection agency. 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, 2023).

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 Teubner *et al*. (2018) 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) and the quagga mussel *Dreissena rostriformis bugensis* (Andrusov, 1897) are invasive species that originate from the Ponto-Caspian region (Son 2007). The taxonomic status of the quagga mussel has been much discussed and has changed many times (see Rosenberg and Ludyankskiy 1994, Stepien *et al.* 2014). Following the suggestion of Karatayev and Burlakova (2022), the name accepted by most scientists, *Dreissena rostriformis bugensis*, will be used in this guideline for the quagga mussel.

While the zebra mussel had already expanded its range by the late 18th century (Kinzelbach 1992), the quagga mussel's expansion only began in the 1940s (Orlova 2014). The first evidence of the quagga mussel in Western Europe came from an ESB sampling site in 2004 (Paulus *et al*. 2014). The introduction of the quagga mussel into waters that are already populated by zebra mussels often leads to a sharp decline in the population of zebra mussels (Ram *et al*. 2012, Strayer *et al*. 2019) within four to twelve years, without completely displacing the zebra mussel (Karatayev and Burlakova 2022).

Zebra and quagga mussels filter their food out of the water, which consists primarily of phytoplankton and zooplankton (Kissman *et al.* 2010) as well as planktonic bacteria (Cotner *et al.* 1995, Makhutova *et al*. 2012) and detrital particles (Makhutova *et al*. 2012). Quagga mussels have a higher filtration rate than zebra mussels (*Haltinger et al*. 2023); according to Diggins (2001), they filter up to 37% faster.

Due to the continuous flow of water through the mantle cavity and the large surface area of the gills, both *Dreissena* species are able to absorb and accumulate a wide range of organic and inorganic substances from the water in both particulate and dissolved form. Therefore, they can be used to demonstrate the bioavailability of substances in the environment. In addition, *Dreissena* mussels can also be used as natural eDNA (environmental deoxyribonucleic acid) filters, e.g. for biodiversity monitoring (Weber *et al.* 2022).

Due to high population densities, the zebra mussel is used as a monitoring organism in various countries (Balogh *et al.* 2022, Bashnin 2019, Benito *et al*. 2017, Kerambrun *et al*. 2016, Pastorino *et al*. 2021, Shoults-Wilson *et al*. 2015). The quagga mussel is also used as a monitoring organism (Bai and Acharya 2018, Baldwin *et al*. 2020, Evariste *et al.* 2018), but is not yet established as such due to the fact that its expansion period took place quite recently, especially in Western Europe. The availability of each species ultimately determines which of the two species will be used in a monitoring study.

In the German Environmental Specimen Bank, zebra and quagga mussels represent the level of first-order limnic consumers.

The species are well suited to be bioindicators due to the following reasons:

- They are widely spread.
- Due to the sessile lifestyle of the adult mussels, they guarantee location loyalty for their multi-year lifetime.
- They often occur in high population densities and biomasses.
- They accumulate dissolved and particulate substances through filtration from the surrounding medium.
- They have a high ecological valence: they can be found in still and running waters of different trophic levels, tolerate brackish water, and survive short periods in which the surrounding water dries up.
- They are easy to manipulate, i.e. suitable for active monitoring (exposure of substrates colonized by young mussels) as well as for toxicity and effect tests.
- They are 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/quagga 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 ESB sampling sites, the zebra mussels reach lengths of up to 40 mm and quagga mussels up to 35 mm. The typical lifespan of zebra and quagga mussels is three to five years and four to five years, respectively (Karatayev and Burlakova 2022).

Zebra and quagga mussels are very similar, so there is a great risk of confusing the two species. Both species vary greatly in the shape, color, and pattern of the shells. The colors range from white to brown to almost black. The shells may be monochrome and have more or less pronounced stripes or zigzag lines.

Various studies show that the accumulation behavior of zebra and quagga mussels varies and that there is also no clear pattern for certain pollutant groups (Evariste *et al.* 2018, Kerambrun *et al*. 2018, Potet *et al*. 2016, Mathews *et al*. 2015, Schäfer et al. 2012, Rishman and Somers 2005). Therefore, it is necessary to be able to reliably distinguish between the two species and separate them for pollutant testing.

According to Teubner *et al*. 2016, a key feature used for species differentiation is the junction of the ventral to the dorsal shell side, which is angled in zebra mussels and rounded in quagga mussels. In zebra mussels, a visible and palpable anterior-posterior longitudinal edge is formed here (Teubner *et al*. 2016, see 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 also a possibility of confusion with the dark false mussel or Conrad's false mussel *Mytilopsis leucophaeata* (Conrad, 1831), which originates from North America and can be found in brackish to fresh water. *M. leucophaeata* mainly colonizes estuaries (Kennedy 2011). The

dark false mussel appears more elongated than the zebra and quagga shells and its shell ridge (umbo) is rounded, whereas the shell ridge is tapered in the other two species (Pathy and Mackie 1993). It is dark brown, but can also have stripes (Verween *et al*. 2010). The most definite morphological distinguishing feature however is a triangular to rounded tooth (apophysis) in the interior of the shell below the shell ridge in *M. leucophaeata* (see Fig. 2), which is missing in *D. polymopha* and *D.r. bugensis* (Pathy and Mackie 1993)

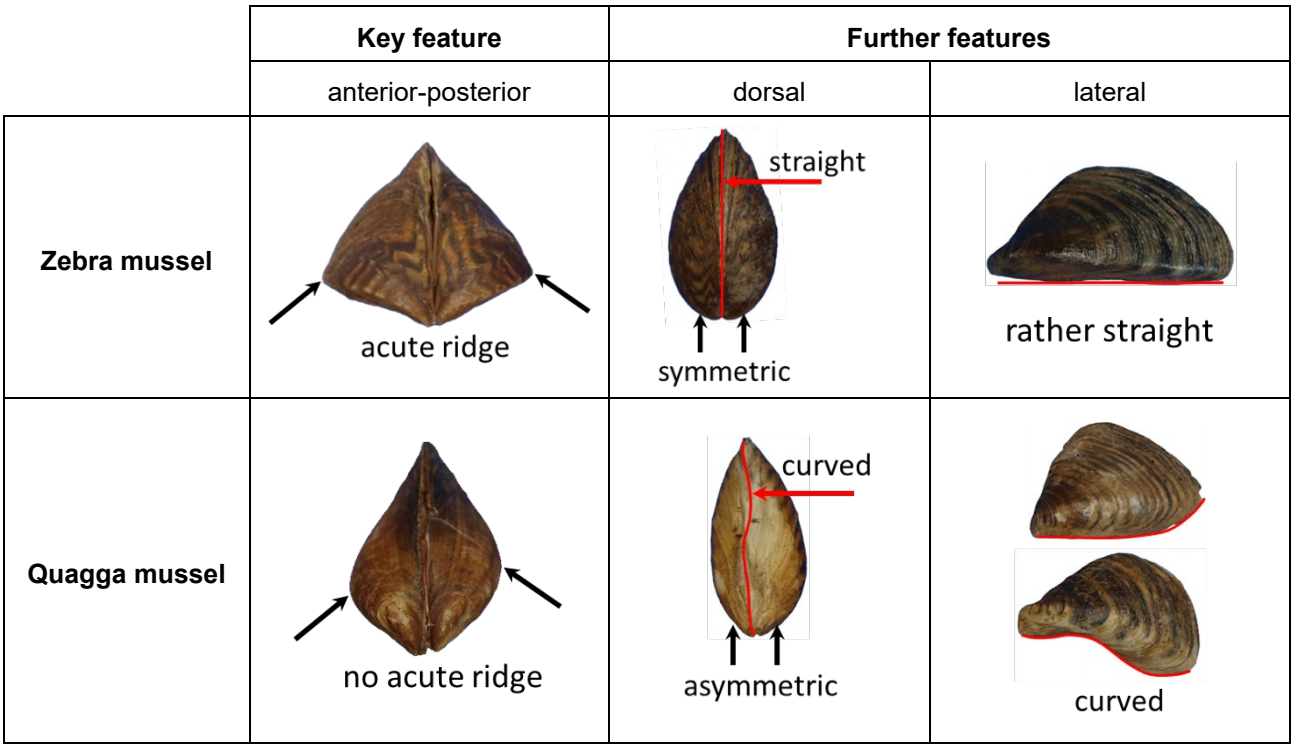

Fig. 1: Important distinguishing features of zebra and quagga mussel

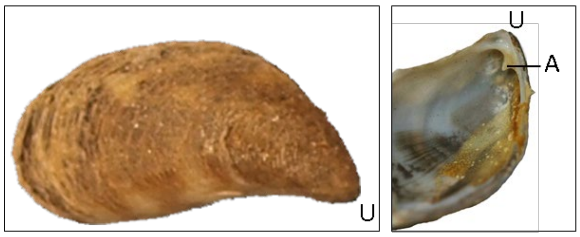

Fig. 2: Outer view (left) and inner view (right) of the right valve of *M. leucophaeata* from the Kiel Canal (U = umbo, A = apophysis)

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.

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 (species, age, size) 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, an average of 2,000 zebra mussels or 1,500 quagga mussels are required to obtain 1,000 g of soft body weight. In order to also be able to obtain the necessary quantity of soft bodies from small mussels, a total of about 3 to 4 kg of raw mussels must be sampled.

5.4 Sampling Period and Frequency

The spawning period of the mussels is characterized by strong physiological dynamics and fluctuations of the biomass and is therefore not suitable for a reproducible sampling. In warm, polymictic waters, the spawning period lasts from about May/June to August/September (Karatayev and Burlakova 2022). 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.

The objective of long-term and continuous sampling should be taken into account when drawing up and updating the area-related sampling schemes.

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 or quagga 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, 7 cm
- stainless steel wire rope,
- stainless steel wire rope clips and screws,
- 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:

- specimen data sheets,
- 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 clips,
- 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),
- protective clothing for handling liquid nitrogen,
- sampling of exposed substrate:
    - stainless steel or PTFE spattle,
- sampling of bank substrate
    - protective clothing for working in the water
    - safety ropes / lifejackets

Laboratory:

- specimen data sheets,
- pure-air workstation with particle and activated carbon filtration,
- protective clothing for handling liquid nitrogen,
- stainless steel containers with lids and clips,
- scale (reading 1 g) to determine soft body weighted sample,
- scale (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 a temperature of at least 120°C for a minimum of one hour (sterilization). Subsequently, the vessels cool in a closed drying oven. 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.

The colonization by free drifting veliger larvae takes place in spring. Male and female gametes are released by the zebra mussels at water temperatures of 12°C to 15°C (Borcherding 1991, Boeckman and Bidwell 2014, Kashian and Ram 2014). In polymictic Central European waters, this temperature is usually reached in May or June. According to Karatayev and Burlakova (2022), the quagga mussels probably reproduce in polymictic waters at the same time.

In autumn, the plate racks are 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.

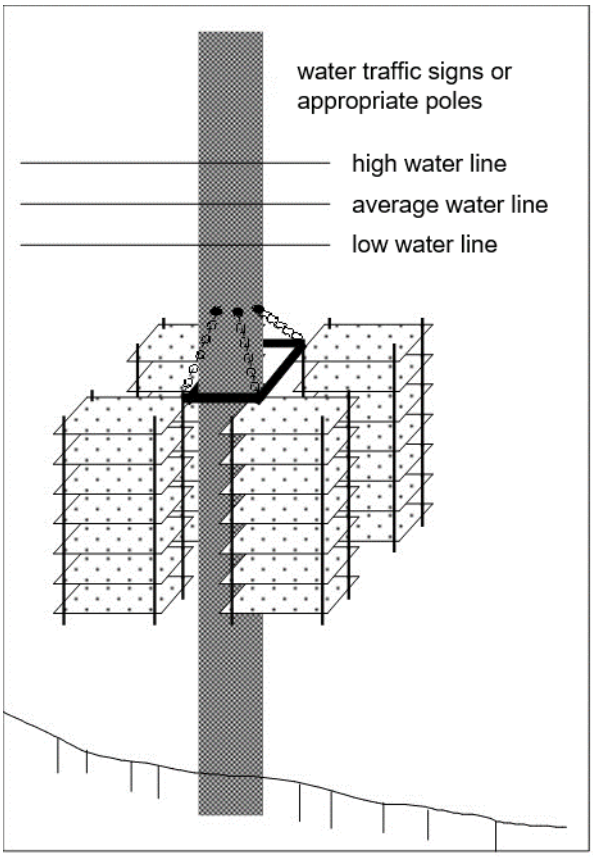

Fig. 3 Example for the attachment of the plate racks in the water

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 spattle (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 – 3 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). Iron, steel, plastic, asphalt, and impregnated wood are not suitable substrates. 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.

The entire contents of the mussel shells, including respiratory water, are collected as a sample. The respiratory water could only be removed by thawing the entire mussel, which is contrary to the requirements of the ESB.

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 or a sharp tweezers. Frozen solid soft bodies including respiratory water are removed with a 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]
0.2	22
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 0.001 g).

After the biometric characterization has been completed, these mussels are discarded.

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.

8 References

Bai X.L. und Acharya K. (2019): Uptake of endocrine-disrupting chemicals by quagga mussels (*Dreissena bugensis*) in an urban-impacted aquatic ecosystem. *Environmental Science and Pollution Research*, 26(1), 250-258

Baldwin A.K., Spanjer A.R., Rosen M.R. und Thom T. (2020): Microplastic in Lake Mead National Recreation Area, USA: Occurrence and biological uptake. *Plos One*, 15(5), e0228896

Balogh C., Kobak J., Kovacs Z., Serfozo J., Farago N. und Serfozo Z. (2022): Contribution of invasive bivalves (*Dreissena* spp.) to element distribution: phase interaction, regional and seasonal comparison in a large shallow lake. *Biogeochemistry*, 158(1), 91-111

Bashnin T., V. Verhaert M., De Jonge L., Vanhaecke, J. Teuchies und Bervoets I. (2019): Relationship between pesticide accumulation in transplanted zebra mussel (*Dreissena polymorpha*) and community structure of aquatic macroinvertebrates. *Environmental Pollution*, 252, 591–598

Benito M., Mosteo R., Rubio E., LaPlante D., Ormad M.P. und Goni P. (2017): Bioaccumulation of inorganic elements in *Dreissena polymorpha* from the Ebro River, Spain: Could zebra mussels be used as a bioindicator of the impact of human activities? *River Research and Applications*, 33(5), 718-728

Boeckman C.J. und Bidwell J.R. (2014): Density, growth, and reproduction of zebra mussels (*Dreissena polymorpha*) in two Oklahoma reservoirs. In: Nalepa T.F. and Schloesser D.W. (Hrsg.): *Quagga and zebra mussels: biology, impacts, and control*, S. 369-382. CRC Press, Boca Raton

Bervoets L., Voets J., Covaci A., Chu S.G., Qadah D., Smolders R., Schepens P. and Blust R. (2005): Use of transplanted zebra mussels (*Dreissena polymorpha*) to assess the bioavailability of microcontaminants in Flemish surface waters. *Environmental Science & Technology*, 39(6), 1492-1505

Borcherding J. (1991): The annual reproductive cycle of the freshwater mussel *Dreissena polymorpha* Pallas in lakes. Oecologica, 87, 208-218

Cotner J.B., Gardner W.S., Johnson J.R., Sada R.H., Cavaletto J.F. und Heath R.T. (1995): Effects of zebra mussels (*Dreissena polymorpha*) on bacterioplankton: Evidence for both size-selective consumption and growth stimulation. *Journal of Great Lakes Research*, 21(4), 517-528

Diggins T.P. (2001): A seasonal comparison of suspended sediment filtration by quagga (*Dreissena bugensis*) and zebra (*D. polymorpha*) mussels. *Journal of Great Lakes Research*, 27(4), 457-466

Evariste L., David E., Cloutier P.-L., Brousseau P., Auffret M., Desrosiers M., Groleau P.E., Fournier M. und Betoulle S. (2018): Field biomonitoring using the zebra mussel *Dreissena polymorpha* and the quagga mussel *Dreissena bugensis* following immunotoxic reponses. Is there a need to separate the two species? *Environmental Pollution*, 238, 706-716

Haltinger L., Rossbacher S., Alexander J., Dennis S.R. und Spaak P. (2023): Life in a changing environment: dreissenids' feeding response to diferenent temperature. *Hydrobiologia*, 850, 4879-4890

Karatayev A. Y. und Burlakova L.E. (2022): What we know and don't know about the invasive zebra (Dreissena polymorpha) and quagga (Dreissena rostriformis bugensis) mussels. Hydrobiologia, https://doi.org/10.1007/s10750-022-04950-5

Kashian D.R. und Ram J.L. (2014): Chemical regulation of dreissenid reproduction. In: Nalepa T.F. and Schloesser D.W. (Hrsg.): *Quagga and zebra mussels: biology, impacts, and control*, S. 461-469. CRC Press, Boca Raton

Kennedy V.S. (2011): The invasive dark falsemussel *Mytilopsis leucophaeata* (Bivalvia: Dreissenidae): a literature review. *Aquatic Ecology*, 45, 163-183

Kerambrun E., Delahaut L., Geffard A. und David E. (2018): Differentiation of sympatric zebra and quagga mussels in ecotoxicological studies: A comparison of morphometric data, gene expression, and body metal concentrations. *Ecotoxicology and Environmental Safety*, 154, 321-328

Kerambrun E., Ladeiro M., Palos M., Bigot-Clivot A., Dedourge-Geffard O., Dupuis E., Villena I., Aubert D. und Geffard A. (2016): Zebra mussel as a new tool to show evidence of freshwater contamination by waterborne *Toxoplasma gondii*. *Journal of Applied Microbiology*, 120(2), 498-508

Kinzelbach R. (1992): The main features of the phylogeny and dispersal of the zebra mussel *Dreissena polymorpha*. In: Neumann D. and Jenner H.A. (Hrsg.): *The zebra mussel Dreissena polymorpha: ecology, biological, monitoring and first applications in the water quality management*. *Limnologie Aktuell*, Band 4, S. 5-17. Gustav Fischer Verlag, Stuttgart Jena New York

Kissman C.E.H., Knoll L.B. und Sarnelle O. (2010): Dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) reduce microzooplankton biomass in thermally stratified lakes. *Limnology and Oceanography*, 55(5), 1851-1859

Klein R., Krotten J., Marthaler L., Sinnewe C. und Dittmann J. (1995): Biomonitoring und Umweltprobenbank - III. Die Abhängigkeit des Informationsgehaltes limnischer Akkumulationsindikatoren vom Zeitpunkt der Probenahme. *Umweltwissenschaften und Schadstoff-Forschung*, 7(2), 115-126

Makhutova O.N., Pryanichnikova E.G. und Lebedeva I.M. (2012): Comparison of nutrition range in *Dreissena polymorpha* and *Dreissena bugensis* mussels by biochemical markers. C*ontemporary Problems of Ecology*, 5(4), 459-469

Matthews J., Schipper A.M., Hendriks A.J., Le T.T.Y., de Vaate A.B., van der Velde G. und Leuven R.S.E.W. (2015): A dominance shift from the zebra mussel to the invasive quagga mussel may alter the trophic transfer of metals. *Environmental Pollution*, 203, 183-190

Martens A., Grabow K. und Schoolmann G. (2007): Die Quagga-Muschel *Dreissena rostriformis bugensis* (Andrusov, 1897) am Oberrhein (Bivalvia: Dreissenidae). *Lauterbornia*, 61, 145-152

Orlova M.I. (2014): Origin and spread of quagga mussels (*Dreissena rostriformis bugensis*) in eastern Europe with notes on size structure of populations. In: Nalepa T.F. and Schloesser D.W. (Hrsg.): *Quagga and zebra mussels: biology, impacts, and control*, S. 93-102. CRC Press, Boca Raton

Pathy D.A. und Mackie G.L. (1993): Comparative shell morphology of *Dreissena polymorpha*, *Mytilopsis leucophaeata*, and the “quagga” mussel (Bivalvia: Dreissenidae) in North America. *Canadian Journal of Zoology*, 71, 1012-1023

Paulus M., Teubner D., Hochkirch A. und Veith M. (2014): Journey into the past: Using cryogenically stored samples to reconstruct the invasion history of the quagga mussel (*Dreissena rostriformis*) in German river systems. *Biological Invasions*, 16, 2591–2597

Pastorino P., Prearo M., Anselmi, S., Menconi V., Bertoli M., Dondo A., Pizzful E. und Renzi M. (2021): Use of zebra mussel *Dreissena polymorpha* (Mollusca, Bivalva) as a bioindicator of microplastic pollution in freshwater ecosystems: A case study from Lake Iseo (North Italy). *Water*, 13, 434

Potet M., Devin S., Pain-Devin S., Rousselle P. und Giamberini L. (2016): Integrated multi-biomarker responses in two dreissenid species following metal and thermal cross-stress. *Environmental Pollution*, 218, 39-49

Ram J.L., Karim A.S., Banno F. und Kashian D.R. (2012): Invading the invaders: reproductive and other mechanisms mediating the displacement of zebra mussels by quagga mussels. *Invertebrate Reproduction & Development*, 56(1), 21-32

Richman L. und Somers K. (2005): Can we use zebra and quagga mussels for biomonitoring contaminants in the Niagara River? *Water Air and Soil Pollution*, 167(1-4), 155-178

Rosenberg G. und Ludyanskiy M.L. (1994): A nomenclatur review of *Dreissena* (Bivalvia: Dressenidae), with identification of the quagga mussel as *Dreissena bugensis*. *Canadian Journal of Fisheries and Aquatic Sciences*, 51, 1474-1484

Schäfer S., Hamer B., Treursic B., Mohlenkamp C., Spira D., Korlevic M., Reifferscheid G. und Claus E. (2012): Comparison of bioaccumulation and biomarker responses in *Dreissena polymorpha* and *D. bugensis* after exposure to resuspended sediments. *Archives of Environmental Contamination and Toxicology*, 62(4), 614-627

Shoults-Wilson W.A., Elsayed N., Leckrone K. und Unrine J. (2015): Zebra mussels (*Dreissena polymorpha*) as a biomonitor of trace elements along the southern shoreline of Lake Michigan. *Environmental Toxicology and Chemistry*, 34(2), 412-419

Son M.O. (2007): Native range of the zebra mussel and quagga mussel and new data on their invasion within the Ponto-Caspian Region. Aquatic Invasions, 2(3), 174-184

Stepien C.A., Grigorovich I.A., Gray M.A., Sullivan T.J., Yerga-Woolwine S. und Kalayci G. (2014): Evolutionary, biogeographic, and population genetic relationships of *Dreissenid* mussels, with revision of component taxa. In: Nalepa T. and Schloesser D.W. (Hrsg.): *Quagga and zebra mussels: biology, impacts, and control*, S. 403-444. CRC Press, Boca Raton

Strayer D.L., Adamovich B.V., Adrian R., Aldridge D.C., Balogh C., Burlakova L.E., Fried-Petersen H.B., Tóth L.G., Hetherington A. L., Jones T.S., Karatayev A.Y., Madill J.B., Makarevich O.A., Marsden J.E., Martel A.L., Minchin D., Nalepa T.F., Noordhuis R., Robinson T.J., Rudstam L.G., Schwalb A.N., Smith D.R., Steinman A.D. und Jeschke j.M. (2019): Long-term population dynamics of dreissenid mussels (*Dreissena polymorpha* and *D. rostriformis*): a cross-system analysis. Ecosphere 10(4): e02701. 10.1002/ecs2.2701:

Teubner D., Klein R., Tarricone K. und Paulus M. (2018): Richtlinie zur Probenahme und Probenbearbeitung Dreikantmuschel *(Dreissena polymorpha*). www.umweltbrobenbank.de

Teubner D., Wesslein A.K., Browne Rønne P., Veith M., Frings C. und Paulus M. (2016): Is a visuo-haptic differentiation of zebra mussel and quagga mussel based on a single external morphometric shell character possible? *Aquatic Invasions*, 11(2), 145-154

Umweltbundesamt (Hrsg.) (2008): Umweltprobenbank des Bundes – Konzeption (Stand: Oktober 2008); www.umweltprobenbank.de

Umweltbundesamt (Hrsg.) (2014): Umweltprobenbank des Bundes – Konzeption (Stand: Oktober 2014); www.umweltprobenbank.de

Umweltbundesamt (Hrsg.) (2023): Umweltprobenbank des Bundes – Konzeption (Stand: Juli 2023); www.umweltprobenbank.de

Verweem A., Vincx M.und Degraer S. (2010): *Mytilopsis leucophaeta*: the brackish water equivalent of *Dreissena polymorpha*? A review. In: Van der Velde G., Ragagopal S und Bij de Vaate A (Hrsg.): T*he zebra mussel in Europe*. S. 29-43. Backhuys Publishers, Leiden

Weber S., Junk I., Brink L., Wörner M., Künzel S., Veith M., Teubner D., Klein R., Paulus M. und Krehenwinkel H. (2023): Molecular diet analysis in mussels and other metazoan filter feeders and an assessment of their utility as natural eDNA samplers. *Molecular Ecology Resources*, 23, 71-485

Checklist to Prepare and Conduct the Sampling

Specimen Type	**Zebra mussel (*Dreissena polymorpha*) / Quagga mussel (*D. rostriformis bugensis*)**
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 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:	• 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	• specimen data sheets, • scale (effective range up to 5 kg, reading 1 g), • stainless steel wire baskets with maximum 8 mm mesh size, • protective clothing for handling liquid nitrogen, • sampling of plate racks: spattle made of stainless steel or PTFE • sampling of bank substrate: protective clothing for working in the water, safety ropes / life jackets
Sampling packing	• stainless steel containers with lids and clips, • small pads / foils made of PTFE or FEP
Transport and interim storage	• cooling device for immediate deep freezing and transport of the samples in the gas phase above liquid nitrogen (LIN)
Required equipment for laboratory work	• specimen data sheets, • a pure-air workstation with particles- and activated carbon filtration, • protective clothing for handling liquid nitrogen, • stainless steel containers with lids and clips, • 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 scalpel holder with blades, • absorbent laboratory paper, powder-free disposable gloves • liquid nitrogen

Biometric sample characterization of 50 mussels per sampling technique	• 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*) / Quagga mussel (D. rostriformis bugensis)

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*) / Quagga mussel (D. rostriformis bugensis)

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

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

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 2.2: Sample Composition

Zebra mussel (*Dreissena polymorpha*) / Quagga mussel (D. rostriformis bugensis)

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

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

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 3: Sample Description

Zebra mussel (*Dreissena polymorpha*) / Quagga mussel (D. rostriformis bugensis)

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

Sampling technique: **Exposure duration:**

No.	Time weight soft body _ _ : _ _ (24 hr)	Fresh weight (resp. water incl.) _ _ , _ _ _ g	Length _ _ , _ mm	Width _ _ , _ mm	Height _ _ , _ mm	Weight of soft body _ _ , _ _ _ g	Weight of shell _ _ , _ _ _ g

from No.	to No.	Date: dd.mm.yy	Reviser	Signature

German Environmental Specimen Bank

Sampling Protocol

Zebra mussel (*Dreissena polymorpha*) / Quagga mussel (D. rostriformis bugensis)

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		Sampling Leader	Remarks
date	time	date	time		

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 procedures for the packages
- ☐ 4.6 Sampling technique / method of capture
- ☐ 4.7 Sampling amount
- ☐ 4.8 Data collection
- ☐ 4.9. Transport and interim storage

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

_____

_____

_____

Remarks: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature