METHODOLOGY FOR COLLECTION, ANALYSIS AND DATA PROCESSING OF ZOOBENTHOS SAMPLES

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Based on HELCOM (2015), BALSAM Project 2013-2015: Recommendations and Guidelines for Benthic Habitat Monitoring with Method Descriptions for Two Methods for Monitoring of Biotope and Habitat Extent and Manual for Marine Monitoring in the Combine Programme of HELCOM Part C Programme for Monitoring of Eutrophication and Its Effects.

Benthic organisms are all organisms living on the seabed of freshwater and marine ecosystems, on the hard surface or on the bed of another substrate. Benthic organisms can be classified mainly into phytobenthos and zoobenthos and by dimensions into microbenthos (sizes up to 100 µm), meyobenthos (100-500 µm) and macrobenthos (larger than 500 µm) (Stites 1999). Microbenthos consists of bacteria and protists, meyobenthos consists of aquatic animals and larger protists, macrobenthos - aquatic animals and macrophytic algae (Barnes et al. 1982).

The collection of zoobenthos samples is carried out in accordance with HELCOM guidelines and recommendations for the monitoring of benthic habitats in the Baltic Sea.

1. Required inventory

Zoobenthic samples are collected using Van Veen type grab. The following equipment is required for the collection of samples:

- Van Veen type grab (with a sample area of 0.1 m^2);
- A larger container in which to place the grab and rinse it;
- Sieve with a mesh size of 1 mm for rinsing the sample;
- A plastic bucket with a lid in which to insert the sample (1 litre capacity);
- 96% ethanol for sample fixation (~ 300 ml per sample);
- Gloves;
- Sprinklers or hand-operated douches for sample suspension.

Laboratory equipment necessary for the processing of samples:

- Stereomicroscope;
- Tweezers;
- Glass Petri dish;
- Rinse bottle;
- Dissection needle;
- Scales;
- Filter paper;
- Folly;
- Dryer.

2. Collection of samples

Collecting samples at stations with a depth of less than 70 metres should be carried out during the day as certain benthic species are semi-pelagic at night. At least 3 samples should be collected in one station. The date, time of sampling, number, coordinates, depth and ground grading of each sample station should be recorded in the sampling protocol. The granulometric composition should also be noted in the protocol, according to the European Nature Information System littoral sediment classifier, which divides the littoral sediment into the following groups: rocky sediment (large boulders and pebbles), gravel, sand, and mud. Furthermore, sediments can be broken down into gravel (16 - 4 mm), coarse sand (4 - 1 mm),

medium coarse sand (1 – 0.25 mm), fine sand (0.25 – 0.063 mm) and mud (< 0.063 mm) and various combinations of these (and coarser) fractions - muddy sand, sandy sludge and mixed sediments (stones, pebbles, gravel, sand and mud together or in any combination).

Before the start of the sampling, the width and length of the grab should be measured to calculate the sample area from which the sample is collected. The most commonly used grab is a Van Veen grab with a sample size of 0.1 m^2. The lifting and lowering of the grab should be carried out as carefully as possible using the vessel's winch. It is necessary to control that the angle of the hoist band is kept as small as possible when the grab is lowered, thereby ensuring that the grab is placed vertically on the ground. If the immersed sample in the grab is less than 5 litres, it should be considered improperly collected, and the sample should be re-collected by adding weights to the grab. If a sample of less than 5 litres is collected afterwards, this sample may be used, but it should be noted that a smaller sample of less than 5 litres was collected.

Once the sample is collected, the grab is emptied into a larger container and the inside of the grab is washed out with water to ensure that all organisms are collected into the sample. The drained contents of the grab should be transferred from the larger container to the sieve as a sediment and water suspension. Different volume rinse bottles can be used to create this suspension. Further, using a stainless steel mesh of 1 mm mesh size, the suspension is flushed to remove sediment. This step should be done with great care as rinsing the sample through a sieve poses the highest risk of losing and damaging certain benthic organisms, especially polychaetes and oligochaetes, as well as other fragile organisms. In order to collect undeveloped or smaller organisms in the sample, it is suggested to additionally use a sieve of the same material with a mesh size of 0.5 mm. Each sample should be rinsed through the sieve, stored and documented separately.

After rinsing, the samples should be placed in storage buckets and fixed in 96% ethanol, for further analysis of species composition and biomass in the laboratory. Each bucket should be marked with the date, time, number and depth of collection of the sample or station. Approximately 300 ml of ethanol is used to fix each sample.

3. Processing and analysis of samples

The collected organisms in the sample are sorted by taxon, taking them to the lowest possible taxonomic level—species, genus, or family. The number of organisms is counted and weighed, resulting in a wet biomass weight, then dried and weighed again, resulting in dry biomass. Different benthic invertebrate identifying materials are used to identify macrozoobenthos (greater than 500 μm) organisms.

First, the samples are sorted using tweezers, and a Petri dish, in which the entire sample is gradually analyzed. A stereo microscope should be used for sample analysis. Because many species of benthic organisms are very fragile and can break down into several parts during sampling, to determine the number of individuals per square metre, one individual is considered a part of the organism that is easily identifiable (most often, the head).

After the taxon is identified, organisms are weighed for biomass. To obtain the wet biomass of organisms, all individuals of the same taxon are placed on filter paper, drained, placed in the “pocket” of pre-weighed foil bearing the sample number and taxon, and placed further on the scales. The resulting weight is recorded in the protocol. The wet biomass of the organisms is calculated. Further, the foil “pocket” with organisms is placed in a dryer and dried at 60^{o}C for 12 - 24 hours or more, depending on the amount and thickness of organisms. After drying the organisms in the dryer, they are weighed repeatedly and their weight is recorded in the protocol. The dry biomass is calculated.

4. Data processing and analysis

All information obtained in the processing and analysis of the sample shall be recorded in a protocol containing the following information: sample number, date and time of collection, the depth of sampling station, taxa, number of individuals identified by each taxon, foil weight, wet and dry biomass, as well as stereo microscope model with which the sample has been analyzed, as well as the person who has analyzed the particular sample. Sample analysis protocols are collected electronically in the MS EXCEL table, which is also entered in the BIODATA database where the data is checked.

5. Storage of data

Data is stored in protocols, as well as electronically in MS Excel format, on the BIOR server, and in the BIODATA database.

6. Sources

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