Method for Sampling, Analysis, and Data Processing of Zooplankton Samples

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Purpose and Application of the Method Description

This description is created with the aim of providing a detailed overview of the quantitative and qualitative determination methods for zooplankton in the Gulf of Riga and the Baltic Sea. The described method is developed for the Zoological Institute's BIOR Fish Resources Research Department to characterize the data acquisition and data storage stages of zooplankton.

Collection of Zooplankton Samples in the Gulf of Riga and the Baltic Sea

2.1. Required Equipment:

Juday-type plankton net (upper ring diameter 36 cm, middle ring diameter 50 cm, mesh size 160 µm)

Water flow meter attached to the upper ring of the net

Formalin or ethanol for sample fixation

Rinsing container (for ethanol)

Bottles for sample collection

Measuring cylinder (1000 ml)

Sieve with mesh (100 µm) for sample concentration

Funnel

2.2. Sample Collection Procedure

Samples are collected using the Juday-type net: it is first lowered to the desired depth and vertically pulled up to the water surface. The net is rinsed, and its contents are poured into a measuring cylinder. If the sample contains a large amount of water, it is filtered through the sieve. The obtained sample is rinsed into a storage bottle using the rinsing container, in which ethanol has been previously poured.

In the Gulf of Riga, samples are collected in two water layers - throughout the entire depth from the bottom to the water surface and in the upper 0-20 m water layer. In shallow stations (depth around 20 m), samples are collected only in the entire water layer.

In the Baltic Sea, the sampling depth depends on the station's depth. In the shallowest stations with a depth not exceeding 100 meters, samples are collected throughout the entire water layer from the bottom to the water surface. In deeper stations (depth > 100 m), samples are collected in the 0-50 m and 0-100 m water layers.

Zooplankton samples are collected at 5 standard stations in the open part of the Baltic Sea and 12 stations in the Gulf of Riga (Figure 1). Depending on the research objectives, zooplankton samples can be collected in predetermined trawling locations before or after trawling.

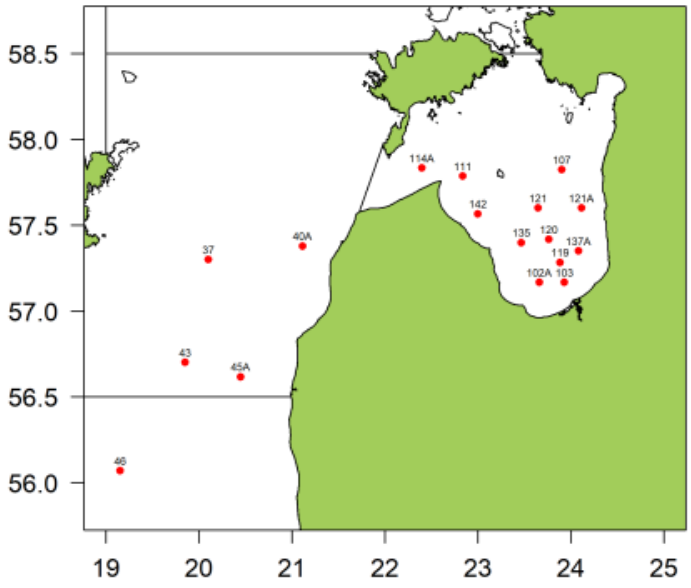

Figure 1. Placement of the standard zooplankton station.

The following information is recorded in the sample collection protocol: date, month, year, time, station, station coordinates, depth (m), sampling layer, flow meter reading before sampling, flow meter reading after sampling, bottle number.

Processing and analysis of zooplankton samples.

3.1. Necessary materials:

- Beakers with volumes of 100ml, 250ml, 500ml, and 1000ml;
- Calibrated Pasteur pipette (2ml, 2.25ml, or 2.5ml);
- Plankton counting chamber;
- Binocular microscope equipped with a measuring scale;
- Sieve for rinsing samples, equipped with a mesh size of 100 µm or smaller;
- Rinse bottle;
- Sample counting journal.

3.2. Preparation of samples for analysis

Rinse the fixed zooplankton samples through a rinsing sieve to separate formalin or ethanol from the sample.

The rinsed sample is collected in a beaker using a rinse bottle and concentrated or diluted to a precisely determined volume. The prepared sample volume can be 50ml, 100ml, 200ml, 300ml, or larger. The dilution or concentration of the sample depends on the total amount of zooplankton or the quantity of individual organisms to be counted in the respective sample. For example, to accurately assess the quantity of individual rarer species (*Limnocalanus macrurus, Cercopagis pengoi*) in the sample, they sometimes need to be counted throughout the sample. In this case, the sample is concentrated to 100ml or a smaller volume and analysed in parts in the plankton chamber (see 3.2).

3.3. Sample analysis

1. The following information about the analysed sample is recorded in the sample counting journal:
    - Bottle number;
    - Date of sample collection;
    - Time of sample collection;
    - Station number (if applicable);
    - Station coordinates;
    - Sampling layer;
    - Station depth;
    - Initial and final readings of the water flow meter;
    - Volume of sample dilution;
    - Volume of the Pasteur pipette.
2. Thoroughly mix the sample so that zooplankton organisms are evenly distributed throughout the sample. Immediately take a subsample using the Pasteur pipette and pour it into the plankton counting chamber. The number of transferred subsamples depends on the quantity of zooplankton present in them. The data for each subsample are recorded separately.
3. Analyse the subsamples by identifying the species or genus and counting their numbers. Counting is performed until 100 individuals are reached within the three dominant groups.
4. For copepod species (*Acartia spp., Eurytemora affinis, Limnocalanus macrurus, Pseudocalanus sp., Temora longicornis, Centropages hamatus, Cyclops spp.*), determine the developmental stages (6 copepodite stages and nauplii). Count *Limnocalanus macrurus* in a small volume throughout the sample if the number of individuals does not exceed 100. In cases where the number of individuals of a species in the sample exceeds 100, count up to 100 individuals.
5. For the cladoceran group (Cladocera), identify the species (*Evadne nordmanni, Cercopagis pengoi) or genus (Podon/Pleopis spp., Bosmina spp.*). *Evadne nordmanni, Bosmina spp., and Podon/Pleopis spp.* are measured for length, and individuals are counted for each size group separately. The size groups in millimetres are provided in Table 1. Before starting work with the microscope, it is necessary to calculate the number of divisions on the scale per 1 mm for the specific microscope. After taking the measurements, determine the number of divisions that correspond to the size group in millimetres. Measurements are not conducted for the cladoceran *Cercopagis pengoi*; their count is determined for the entire sample.
6. For the rotifer group (*Rotatoria*), identify the species (*Synchaeta baltica, Synchaeta monopus, Keratella quadrata, Keratella cochlearis*) and count their numbers.
7. Other zooplankton forms, classified as Varia, are identified to the species/genus level and counted.

4. Data processing and analysis

4.1. Electronic spreadsheet

Information about the samples and the obtained sample counting data are compiled in separate MS Excel spreadsheets for each month. The following information is recorded in the data spreadsheet:

1. Date of sample collection;
2. Month of sample collection;

3. Year of sample collection;
4. Time of sample collection;
5. Station number (if applicable);
6. Station coordinates (Latitude/Longitude degrees and minutes);
7. Station depth;
8. Upper and lower sampling layers;
9. Initial and final readings of the water flow meter;
10. Species name;
11. Developmental stages/size groups.

Perform calculations and update the table with the following data:

12. Number of individuals in the sample (N/x m3);
13. Number of individuals per cubic meter (N/m3);
14. Biomass of taxonomic groups (mg/m3).

The data are also imported into the national database BIODATA, where data validation is performed.

4.2. Calculation of zooplankton abundance and biomass:

The total number of individuals in the sample (N):

$$\mathbf{N = n\ (Vp/mVs)},$$

where n is the number of individuals in the sub-sample(s), Vp is the total volume of the sample (dilution), m is the number of sub-samples, and Vs is the volume of each sub-sample (pipette volume).

The number of individuals per cubic meter (N/m3):

$$\mathbf{(N/m3) = N / Vm},$$

where Vm is the volume of filtered water (m3).

The number of filtered cubic meters (Vm) is determined based on the readings of the water flow meter and the area of the net opening:

$$\mathbf{Vm = S\ (F2 – F1)\ 0.3},$$

where S is the area of the net opening (0.1 square meters for a Juday net), and F2 and F1 are the final and initial readings of the water flow meter.

If the net is not equipped with a water flow meter, the volume of filtered water (Vm, m3) is calculated using the formula:

$$\mathbf{Vm = (H0\text{-}H1)\ S},$$

where H0 and H1 are the readings of the length counter of the towing cable (m) at the start and end of filtration.

The biomass of species/genus is calculated using the individual mass factors for each species/genus (provided in Table 1 and Table 2):

$$\mathbf{B = (N/m3)\ m},$$

where m is the individual mass factor for the species/genus.

The individual mass factors for zooplankton in the Baltic Sea are documented by Hernroth (1985).

4.3. Calculation of mean values:

The mean abundance (ind/m3) and biomass (mg/m3) are calculated separately for each zooplankton species/genus. For the Riga Bay, the mean values need to be calculated for two water layers:

- Surface layer (0-20m);
- Full water column from the surface to the seabed.

Baltic Sea stations are divided into several groups based on their depth and sampling layer. The mean biomass and abundance are calculated for each group separately. The group divisions are as follows:

- Entire central part of the Baltic Sea (all stations regardless of depth);
- Shallow zone (station depth <100m);
- Deep zone (station depth >100m);
- Surface layer (0-50m).

4.4. Accuracy verification of results:

For the accuracy verification of the results, 10% of the samples need to be retested.

5. Data storage:

The data are stored in MS Excel format on the BIOR server and the national database BIODATA.

6. References:

Hernroth L. 1985. Recommendations on methods for marine biological studies in the Baltic Sea Mesozooplankton Biomass Assessment, vol. 10, Baltic Marine Biological Publications.

Table 1: Individual Mass Factors of Cladocera in the Baltic Sea (Hernroth, 1985)

Mikroskopa palielinājums	Mērskalas iedaļu skaits	Milimetri	Bosmina spp.	Podon/Pleopis spp.	Evadne nordmanni
2	<1,2	<0,3	0,0025	0,002	0,002
2	1,2-1,6	0,3-0,4	0,007	0,006	0,006
2	1,6-2,0	0,4-0,5	0,015	0,013	0,01
2	2,0-2,4	0,5-0,6	0,025	0,025	0,02
2	2,4-2,8	0,6-0,7	0,045	0,04	0,03
2	2,8-3,6	0,7-0,9	0,08	0,07	0,05
2	3,6-4,4	0,9-1,1	-	0,14	0,09
2	>4,4	>1,1	-	0,2	0,14

Table 2. Individual mass factors of zooplankton species for the central part of the Baltic Sea in different seasons according to Hernroth (1985).

Sugas/ģints nosaukums	Attīstības stadija	Individuālā masa (mg)			
		jan-mar	apr-jun	jul-sep	okt-dec
Acartia spp.	f	0,020	0,020	0,020	0,020
	m	0,018	0,015	0,015	0,015
	5	0,012	0,012	0,011	0,009
	4	0,012	0,012	0,011	0,009
	3	0,005	0,005	0,005	0,005
	2	0,005	0,005	0,005	0,005
	1	0,005	0,005	0,005	0,005
	0	0,002	0,002	0,002	0,002
Temora longicornis	f	0,060	0,065	0,060	0,050
	m	0,055	0,040	0,045	0,050
	5	0,018	0,015	0,015	0,014
	4	0,018	0,015	0,015	0,014
	3	0,009	0,006	0,006	0,004
	2	0,009	0,006	0,006	0,004
	1	0,009	0,006	0,006	0,004
	0	0,003	0,003	0,003	0,003
Pseudocalanus sp.	f	0,050	0,045	0,050	0,055
	m	0,035	0,030	0,035	0,050
	5	0,020	0,020	0,020	0,020
	4	0,020	0,020	0,020	0,020
	3	0,007	0,008	0,010	0,010
	2	0,007	0,008	0,010	0,010
	1	0,007	0,008	0,010	0,010
	0	0,003	0,003	0,003	0,003
Centropages hamatus	f	0,055	0,050	0,045	0,050
	m	0,045	0,040	0,040	0,045
	5	0,017	0,015	0,015	0,017
	4	0,017	0,015	0,015	0,017
	3	0,006	0,006	0,006	0,006
	2	0,006	0,006	0,006	0,006
	1	0,006	0,006	0,006	0,006

	0	0,003	0,003	0,003	0,003
Eurytemora affinis	f	0,025	0,050	0,025	0,025
	m	0,020	0,040	0,020	0,020
	5	0,013	0,014	0,014	0,014
	4	0,013	0,014	0,014	0,014
	3	0,005	0,005	0,005	0,005
	2	0,005	0,005	0,005	0,005
	1	0,005	0,005	0,005	0,005
	0	0,002	0,002	0,002	0,002
Limnocalanus macrurus	f	0,700	0,700	0,700	0,700
	m	0,500	0,500	0,500	0,500
	5	0,013	0,200	0,200	0,200
	4	0,013	0,200	0,200	0,200
	3	0,028	0,028	0,028	0,028
	2	0,028	0,028	0,028	0,028
	1	0,028	0,028	0,028	0,028
	0	0,003	0,003	0,003	0,003
Cyclops spp	f	0,025	0,025	0,025	0,025
	m	0,020	0,020	0,020	0,020
	5	0,013	0,013	0,013	0,013
	4	0,013	0,013	0,013	0,013
	3	0,0035	0,0035	0,0035	0,0035
	2	0,0035	0,0035	0,0035	0,0035
	1	0,0035	0,0035	0,0035	0,0035
	0	0,0025	0,0025	0,0025	0,0025
Bosmina spp. (ja netiek mērīta)		0,015	0,015	0,015	0,015
Podon/Pleopis spp. (ja netiek mērīta)		0,050	0,050	0,050	0,050
Evadne nordmanni (ja netiek mērīta)		0,040	0,040	0,040	0,040
Synchaeta spp.		0,006	0,006	0,006	0,006
Keratella spp		0,001	0,001	0,001	0,001
Bivalvia larvae		0,001	0,001	0,001	0,001
Fritillaria borealis		0,010	0,010	0,010	0,010
Polychaeta larvae		0,030	0,030	0,030	0,030
Pleurobrachia pileus		0,010	0,010	0,010	0,010
Sagitta elegans baltica		0,250	0,250	0,250	0,250
Oithona similis		0,006	0,006	0,006	0,006
Amphibalanus improvisus larvae		0,010	0,010	0,010	0,010
Cercopagis pengoi		0,400	0,400	0,400	0,400
Harpacticoida (Mesochra spp.)		0,010	0,010	0,010	0,010