HELCOM

Guidelines for monitoring of mesozooplankton (2021)

Updated 13 Sep 2021 (Chapter 3)

1. Background

Zooplankton includes an array of macro and microscopic animals. They play a vital role in the marine food webs. The herbivorous zooplankton feed on phytoplankton and in turn constitute prey to animals at higher trophic levels, including fish. Therefore, ecological roles of zooplankton are integral to food web functioning, ecosystem productivity, nutrient and carbon cycling. Because of these critical roles, the main functional traits of zooplankton populations, i.e., their body size distribution, reproductive cycles, growth, reproduction, and survival rates, are important factors influencing energy transfer in the pelagic food webs and recruitment to fish stocks (Harris at al. 2000).

In the environmental assessment, zooplankton data provide fundamental information on the dynamics and functioning of the pelagic ecosystem; therefore, understanding zooplankton responses to natural and anthropogenic pressures is a prerequisite for an ecosystem approach to management. Obviously, the success of zooplankton dynamics analysis and productivity estimation largely depend upon the use of correct methodology that involves collection of samples, fixation, preservation, analysis and computation of data.

1.1 Introduction

The mesozooplankton is a size fraction of zooplankton community (0.2 to 20 mm) that is addressed in the HELCOM- monitoring guidelines. Mesozooplankton constitute an important part of zooplankton in the pelagic food webs, since these are the organisms representing the link between primary producers (phytoplankton) and higher trophic levels (zooplanktivorous fish and invertebrates). Mesozooplankton community structure and productivity can be affected by changes in phytoplankton stocks, species/size composition and phenology. Further, alterations in mesozooplankton can influence prey availability for zooplanktivores and, thus, fish stock recruitment, as well as sedimentation of the primary production, which, in turn, may affect food supply to benthic animals and oxygen levels in the bottom water.

1.2 Purpose and aims

The sampling of mesozooplankton serves, *inter alia*, the following purposes:

- To describe the species composition and the spatial distribution of mesozooplankton abundance and biomass;
- To derive zooplankton-based indicator metrics for the HELCOM core indicator 'Zooplankton mean size and total stock (MSTS): mean body weight of a zooplankter (MeanSize, µg wet weight ind.$^{-1}$) and total biomass of herbivorous holoplankton (Total Biomass, mg m^{-3});
- To describe temporal trends (i.e. over several years) in mesozooplankton biomass and community structure.

2. Monitoring methods

2.1 Monitoring features

Mesozooplankton is a size-based fraction of zooplankton community. This fraction includes all taxa and stages with body length that is between 0.2 and 20 mm in any dimension, which dictates using plankton nets of certain mesh size as sampling gears for quantitative collections in the field and microscopic analysis for taxonomic identification and enumeration as laboratory methods.

2.2 Time and area

Sampling frequency varied in 2017 from 1-2 to 24 samples/station/year, depending on the country.

[*to be added: link to HELCOM data and map service can be provided to show sampling locations and frequency of sampling. The information provided in the map however needs to be reviewed*].

2.3 Monitoring procedure

2.3.1 Monitoring strategy

The approach involves (1) sampling with a WP-2 net towed vertically through a water column and conducted at selected monitoring stations on a regular basis; (2) microscopic examination of preserved zooplankton samples, including taxonomic identification and specimen counting; and (3) calculating abundance and biomass values for mesozooplankton and reporting these values to a data host.

2.3.2 Sampling method(s) and equipment

- Mesozooplankton should be sampled by means of vertical hauls using a WP-2 net with 100 µm mesh size. The WP-2 net should be hauled vertically with a speed of about 0.5 m/s. The nets should be equipped with flowmeters mounted at 1/4 of the diameter of the ring (UNESCO 1968, BS EN 15110:2006, BS 6068-5.41:2006).

- If the filtration capacity of the net is lower than 70%, the sample should be discarded and a new sample taken after the rinsing of the net.

- The weight to keep the wire vertical should be 25 kg (40 kg when the wire angle tends to exceed 25°, UNESCO, 1968, BS EN 15110:2006, BS 6068-5.41:2006). The wire angle should always be reported. A correction table for sampling depth is given in Table 1. If the wire angle exceeds 40°, the sample should be discarded. Records of wind speed should be kept.

- If there is no thermocline, a standard haul of 25-0 m should be made. If there is no halocline, there should be a standard haul of 75 m to the thermocline (included) or to 25 m in case there is no thermocline. For the stratified hauls, the following intervals should be considered (Fig. C.7.1):

    o From bottom (3-5 m above) to halocline (included);

    o From top of halocline to thermocline (included);

    o From top of thermocline to surface.

- If an anoxic bottom layer is present, sampling should be conducted above the anoxic zone.

- No hauls shorter than 5 m should be made.

- In the Kattegatt and the Belt Sea, a standard haul from the 25 m depth to the surface should be made;

- After collection of each sample, the net shall be rinsed by use of a gentle flow from a hose. When stratified hauls are taken, only the part below the net strap should be rinsed. After emptying, the whole net shall be rinsed with the cod-end open.

- After each cruise, the net shall be washed in warm freshwater with a detergent to maintain optimum filtration capacity (BS EN 15110:2006, BS 6068-5.41:2006).

- When jellyfish appear in the sample, it is recommended to discard the sample and repeat the sampling. When it is impossible to avoid jellyfish (due to e.g., jellyfish bloom), they should be rinsed from other mesozooplankton, the zooplankton occurring in the rinse water should be returned to the sample. The jellyfish can be discarded; if possible, an effort should be made to record its taxonomic identity and the bell diameter. When applicable, these procedures should be recorded.

2.3.3 Sample handling and analysis

2.3.3.1 Preservation and storage

- Zooplankton samples should be preserved in ~4% formaldehyde solution (1 part 37% formaldehyde solution and 9 parts water; ASTM E1200 – 87, 2012) buffered to pH 8-8.2 with disodiumtetraborate (borax; $Na_2B_4O_3 \times 10\ H_2O$). The samples should be stored until the subsequent assessment is

completed. It is also advisable to store analyzed samples for some period after the analysis was completed, because it would provide an opportunity to confirm the analysis result in case of a doubt regarding the sample composition and abundance.

2.3.3.2 Subsampling

- Each sample should be thoroughly mixed before being subsampled. Non-random distribution of the organisms in the sample is the most important source of subsampling error. Large aggregates (e.g., *Cercopagis*) should be taken out of the sample, examined, and any organisms occurring within these aggregates should be counted.

- A few drops of a detergent should be added to a sample before the subsampling to prevent entrapment of small crustaceans to the liquid surface.

- A calibrated Stempel-pipette or a Kott splitter are recommended. Repeated sub-sampling by Kott splitter (Kott 1953) and Stempel pipette (Hensen, 1887) produces a coefficient of variation of <5% and 7-9%, respectively (Kott 1953; Guelpen et al. 1982).

- For the work with Stempel-pipette, the sample should be concentrated by sieving or diluted with tap water as necessary. The volume of the sample is measured in a graduated glass or plastic ware.

2.3.3.3 Taxonomic identification and counting procedure

- For species identification, research quality compound microscopes equipped with at least 100× magnification optics should be used.

- All specimens should be identified to the lowest possible taxonomic level. The taxonomic categories should be defined in accordance with the Baltic zooplankton checklist compiled by the HELCOM Zooplankton Expert Network (ZEN). The term *taxonomic categories* includes species, genera, families and different developmental stages of copepods.

- When discovering a non-native species that is not included in the Baltic zooplankton checklist and has not been reported in the area, the record should be documented and ZEN members should be informed. It is also recommended to involve taxonomic experts in evaluations of such findings.

- At least 100 individuals of each of three dominant taxonomic categories (excluding nauplii and tintinnids) should be counted. If this figure is not reached in the first subsample, additional subsamples must be counted. When this number is reached for a specific category, the counting for this group is discontinued in the next subsample(s). See Tables 1 and 2 for the precision estimate as a function of the number of specimens counted (Cassie 1971, HELCOM 1988).

- Although macrozooplankton, nauplii, tintinnids, some rotifers and some meroplankton fall outside mesozooplankton size range, these zooplankters are ecologically important, there is a considerable amount of historical data on these groups, and some of them are used for the indicator-based assessment. Therefore, they should be reported quantitatively.

- The whole sample should be examined for any macrozooplankton and rare species; their presence and abundance (if determined) should be noted.

2.4 Data analysis

2.4.1 Abundance calculations

- The number of counted specimens should be converted to abundance (ind. m^{-3}) values using estimated volume of water that was filtered through the net during sampling and any coefficients for dilution (split factors) derived from the subsampling procedure.

2.4.2 Biomass calculations

- For individual weight (wet weight) values of different taxonomic groups and developmental stages, the established biomass factors should be used (Hernroth, 1985).

- When a standard factor for a particular taxa/stage is not provided, size measurements of the organism in question should be used to calculate individual mass according to the method of Standard Size Classes (SSC) (Witek et al. 1996); the in-house documentation for individual weight calculations should be available on request for interlaboratory comparisons.

- The biomass (mg m^{-3}) calculations are based on the abundance values for counted taxa and developmental stages multiplied by the corresponding individual weight factors.

3. Data reporting and storage

Zooplankton data at sample level should be stored in national database and reported in accordance with HELCOM/ICES Biological Data Reporting Formats (https://www.ices.dk/data/Documents/ENV/Environment_Formats.zip) to HELCOM data host ICES to be included in HELCOM assessments.

For mesozooplankton biomass, revised individual biomass factors should be used. Revised biomass factors are available at HELCOM workspace for ZEN. If some species/stages are missing, the list must be complemented.

4. Quality control

Basic principles of quality assurance follow those for other components of the monitoring, so only the main issues in zooplankton identification and enumeration are addressed here. All laboratories should develop in-house QA routines and adopt internal and external quality control.

4.1 Quality control of methods

4.1.1 Internal quality control

Internal quality control of methods and skills (Figure 2) in zooplankton identification and enumeration involves:

- Documenting all procedures employed and making the documentation readily accessible to all staff concerned. The documentation includes acceptable error sizes and confidence limits and calibration protocols for subsampling equipment. Quality of counting results is enhanced by regularly revising the procedures with staff to ensure creates consistency amongst operators in terms of procedures and taxonomic identification.

- Ensuring operator's competence. This includes in-house training of personnel to maintain consistent and correct sample collection and analysis procedures.

- Ensuring validity of counting results and assessment of their accuracy. This includes the following procedures:

  o testing individual operator precision (operator error) a count on triplicate subsamples of one sample (single-species artificial sample) is conducted every year and related errors and confidence limits calculated;

  o testing precision within the laboratory, triplicate subsamples of the same sample (single-species artificial sample) is counted by each operator and the error and associated confidence limits calculated;

- o performing day-to-day verification, every 20th sample is counted by at least two operators and the results are presented and evaluated as R-charts;

- o testing consistency of species identification among operators and ensuring that all operators reach the predetermined level of precision, once a year a taxonomy proficiency check is performed. For example, all operators in turn examine the same fields of vision (FoV) of a selected sample containing representative species. The results are compared and discussed; the examination continues until 100% agreement between the operators is reached. The precision and accuracy of counting results always need to be stated in the final report.

4.1.2 External quality control

External quality assessment (Figure 2), is the implementation of external checks to ensure adherence to the documented procedures. This includes interlaboratory tests (ring tests), follow-up in-house evaluations and feedbacks to the internal quality assurance measures (4.1.1).

4.2 Quality control of data and reporting

- All data should be proof-read after input to the computer. It is desirable that data is entered once by one person and then either re-entered or rechecked by a different person, since people can make systematic errors and therefore repeat the same mistakes every time. If significant numbers of similar errors are discovered regularly when a form's data is being recorded, it may be that the design of the form is at fault. Redesigning the form may help to lower error rates in this case.

- Similarly, arithmetic checks and logic tests can be built into data-entry systems involving entry of numbers so that the data entry operator is prompted to correct the data, the entry is cancelled, an error log is written or any another relevant action is taken. For example, if an operator is entering a number with too high number of digits, the system can be programmed to query this result. Trends and/or variance can be also calculated by computer systems and any results that vary from the trend by an unusual amount can be identified and queried.

- When transferring data to and between databases, control procedures should be established in order to ensure that data format is correct and to obviate calculation errors. Data stored in databases should be checked and validated.

5. Contacts and references

5.1 Contact persons

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5.2 References

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Table 1. Correction of sampling depth (z_1, m) based on wire angle (α, expressed in radians) recorded when taking a zooplankton sample. The corrected depth (z, m) is calculated as: $z = z_1/\cos(\alpha)$.

depth z_1 (m)	wire angle 5°	10°	15°	20°	25°	30°
5	5	5	5	5	6	6
10	10	10	10	11	11	12
15	15	15	16	16	17	17
20	20	20	21	21	22	23
25	25	25	26	27	28	29
30	30	30	31	32	33	35
35	35	36	36	37	39	40
40	40	41	41	43	44	46
45	45	46	47	48	50	52
50	50	51	52	53	55	58
55	55	56	57	59	61	64
60	60	61	62	64	66	69
65	65	66	67	69	72	75
70	70	71	72	74	77	81
75	75	76	78	80	83	87
80	80	81	83	85	88	92
85	85	86	88	90	94	98
90	90	91	93	96	99	104
95	95	96	98	101	105	110
100	100	102	104	106	110	115
110	110	112	114	117	121	127
120	120	122	124	128	132	139
130	130	132	135	138	143	150
140	141	142	145	149	154	162
150	151	152	155	160	166	173
160	161	162	166	170	177	185
170	171	173	176	181	188	196
180	181	183	186	192	199	208
190	191	193	197	202	210	219
200	202	203	207	213	221	231

Table 2. Lower and upper 95% confidence limits (as counting units and as a percentage) when the number of counted specimens is lower than 17.

N ind counted	Lower limit	Upper limit	Lower limit (%)	Upper limit (%)
0	0	3.7	!!!	!!!
1	0.03	5.6	97	460
2	0.2	7.2	90	260
3	0.6	8.7	80	190
4	1.1	10.2	72.5	155
5	1.6	11.8	68	136
6	2.2	13	63.3	116.7
7	2.8	14.4	60	105.7
8	3.4	15.7	57.5	96.3
9	4.1	17	54.4	88.9
10	4.8	18.3	52	83
11	5.5	19.6	50	78.2
12	6.2	21	48.3	75
13	6.9	22.2	46.9	70.8
14	7.6	23	45.7	64.3
15	8.4	24.7	44	64.7
16	9.1	25.3	43.1	58.1

Table 3. Lower and upper 95% confidence limits (as counting units and as a percentage) when the number of counted specimens is greater than 17.

N ind counted	Lower limit	Upper limit	Lower limit (%)	Upper limit (%)
17	8.9	25.1	47.5	47.5
18	9.7	26.3	46.2	46.2
19	10.5	27.5	45	45
20	11.2	28.8	43.8	43.8
25	15.2	34.8	39.2	39.2
30	19.3	40.7	35.8	35.8
35	23.4	46.6	33.1	33.1
40	27.6	52.4	31	31
45	31.9	58.1	29.2	29.2
50	36.1	63.9	27.7	27.7
60	44.8	75.2	25.3	25.3
70	53.6	86.4	23.4	23.4
80	62.5	97.5	21.9	21.9
90	71.4	108.6	20.7	20.7
100	80.4	119.6	19.6	19.6
110	89.4	130.6	18.7	18.7
120	98.5	141.5	17.9	17.9
130	107.7	152.3	17.2	17.2
140	116.8	163.2	16.6	16.6
150	126	174	16	16
275	242.5	307.5	11.8	11.8
300	266.1	333.9	11.3	11.3
350	313.3	386.7	10.5	10.5
400	360.8	439.2	9.8	9.8
450	408.4	491.6	9.2	9.2
500	456.2	543.8	8.8	8.8
600	552	648	8	8
700	648.1	751.9	7.4	7.4
800	744.6	855.4	6.9	6.9
900	841.2	958.8	6.5	6.5
1000	938	1062	6.2	6.2
1500	1424.1	1575.9	5.1	5.1

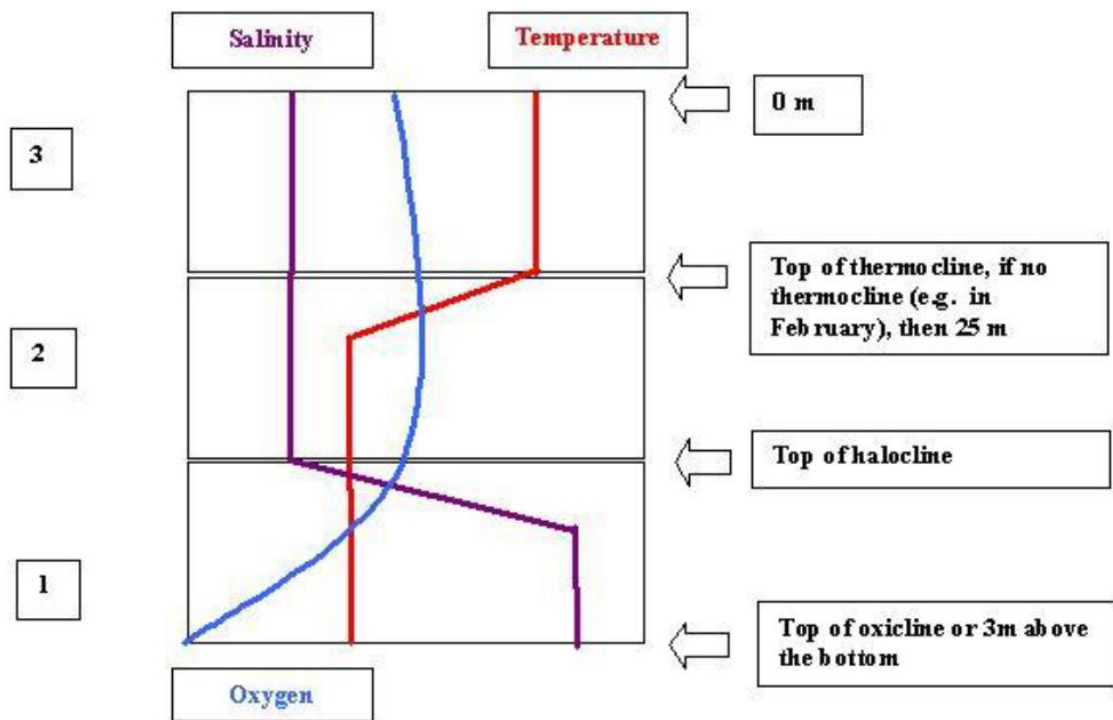**Figure 1.** Fractionated hauls based on vertical profiles of salinity, temperature and oxygen.

Figure 2. Quality assurance (QA) in zooplankton analysis (species identification and enumeration)