Baltic Marine Environment Protection Commission

Working Group on the State of the Environment and Nature Conservation

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Document title	Zooplankton species composition, abundance and biomass – proposed changes to monitoring guidelines
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Background

The review of HELCOM monitoring guidelines on zooplankton species composition, abundance and biomass has been undertaken by the ZEN-ZIIM project. This document contains proposed changes to the current version of the COMBINE guidelines for zooplankton monitoring (Annex C-7). The proposed revisions are based on discussion by ZEN-QAI (2011-2014) and ZEN-ZIIM meetings (2015-2016). The updated guidelines will be transferred to the template for HELCOM monitoring guidelines and finalized at the ZEN-ZIIM 2016 meeting to be held in November 2016.

Action requested

The Meeting is invited to:

- consider and endorse the proposed changes to the monitoring guidelines of zooplankton,
- mandate the ZEN-ZIIM project of finalize the revised monitoring guidelines at their annual meeting in November 2016.

Changes proposed to the current version of HELCOM zooplankton monitoring guidelines

The proposed changes are summarized by ZEN chair E. Gorokhova based on the discussions within ZEN QAI and ZEN ZIIM projects. Page numbers refer to Annex C-7 of the current version of the Manual for Marine Monitoring in the COMBINE Programme of HELCOM.

Page	Original text	Suggested correction
326	When jelly-fish appear in the sample, it is recommended to discard the sample and take a new. When it is impossible to avoid jelly-fish, they should be rinsed from other mesozooplankton and then discarded. When applicable, these procedures should be recorded.	When jellyfish appear in the sample, it is recommended to discard the sample and take a new. When it is impossible to avoid jellyfish, they should be rinsed from other mesozooplankton, the zooplankton occurring in the rinse water should be returned to the sample, and jellyfish discarded. When applicable, these procedures should be recorded.
328	A calibrated Stempel-pipette or a Kott Splitter is recommended. The Kott Splitter is somewhat better in precision but is more time-consuming to handle (G. Behrends, A. Korshenko, pers.comm.).	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).
328	A few drops of a detergent should be added to allow the cladocerans to mix in the sample	A few drops of a detergent should be added to a sample before the subsampling to prevent entrapment of small crustaceans to the water surface.
329	The sample should be mixed intensively until all organisms are distributed randomly in the sample volume. Non-random distribution in the sample during sub-sampling is the most important source of errors. Aggregations of organisms should be taken out of the sample and the organisms counted.	Non-random distribution of organisms in the sample is the most important source of subsampling error. Each sample should be thoroughly mixed before the subsampling. Large aggregates should be taken out of the sample, examined, and any organisms occurring within these aggregates should be counted.
329	The microscopes used should have magnifications to at least 125 X.	Research quality compound microscopes equipped with at least 100× magnification optics should be used.
329	All specimen should be identified and counted until one has reached 100 individuals of each of the three dominating taxonomic groups (excluding nauplii, rotifers and tintinnids). If this figure is not reached in one subsample, additional subsamples must be counted. The taxonomic group(-s) that reached 100 individuals in the previous subsamples, need not be counted in the next subsample(-s). The precision of calculated abundance for organisms of the first three groups, that will be counted up to 100 specimens, amounts to 20% (Tables C-7.1 and C-7.2). The estimation of abundance for other groups ("tail") will be less precise (Cassie 1971, HELCOM 1988).	All specimens should be identified to the lowest possible taxonomic level. All taxonomic categories should be defined in accordance with the Baltic zooplankton checklist compiled by ZEN. The term taxonomic categories includes species, genera, families and different developmental stages of copepods. 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 term "taxonomic groups" includes species, genera, families and different developmental stages of copepods.	the next subsample(s). See Tables C-7.1 and C-7.2 for a precision estimate as a function of the specimens counted (Cassie 1971, HELCOM 1988).
329	The abundance of nauplii, rotifers, tintinnids and meroplankton larvae can be estimated semi-quantitatively from the first subsample. The presence of macrozooplankton organisms and rare species can be noted after an overview of the whole sample. Although macrozooplankton, nauplii, rotifers and tintinnids fall outside the size range of mesozooplankton, as do many of the meroplankton, there is a considerable amount of historic data on these groups. Thus they could be reported.	The abundance of nauplii, tintinnids and meroplankton can be estimated semi-quantitatively from the first subsample. The whole sample can be examined for any macrozooplankton and rare species; their presence can be noted. Although macrozooplankton, nauplii, tintinnids, some rotifers and some meroplankton fall outside mesozooplankton size range, there is a considerable amount of historical data on these groups. Therefore, they could be reported.
329	Table C-7.2. Lower and upper 95% confidence limits (in units and as a percentage) for number of counting specimens lower than 17	**Table C-7.2.** Lower and upper 95% confidence limits (in units and as a percentage) when number of counted specimens is lower than 17
330	**Table C-7.3.** Lower and upper 95% confidence limits (in units and as a percentage) for number of counting specimens more than 17	**Table C-7.3.** Lower and upper 95% confidence limits (in units and as a percentage) when number of counted specimens is larger than 17 *NB: The content of these tables will be discussed in the next ZEN meeting and will most probably be recommended for revision.*
331	The biomass factors for the different taxonomic groups and developmental stages should be used (Hernroth, 1985). The method of Standard Size Classes (Witek Z., G. Breuel, M. Wolska-Pyś, P. Gruszka, A. Krajewska-Sołtys, L. Ejsymont, D. Sujak 1996. Comparison of different methods of Baltic zooplankton biomass estimations. Proceedings of the XII BMB Sympozjum, Institute of Aquatic Ecology, University of Latvia: 87-92)** should be used if appropriate factor is missing. The improvement of present factors taking into account the seasonal and geographical differences in individual volume is an urgent QA task. Direct measurements of ash free dry weight (AFDW) of ½ sample should be used. Samples, which have been deep frozen (- 18 C) on pre-weighted glass fibre filters (Whatman GF / C, d = 47 mm), should be dried at 60 C in an oven (Lovegrove, 1962, 1966) and ashed at 500 C. **Reference to Standard Size Classess has been added instead of reference to table C.7.2. which is not available.	For the 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). *NB: The rest should be deleted. More will be added when the on-going intercalibration is completed (end of 2016).*

Section 7. Should be re-written as follows:

Reporting should be in accordance with HELCOM/ICES Biological Data Reporting Formats (http://www.ices.dk/env/index.htm) and the taxonomic categories should follow the Baltic zooplankton checklist compiled by ZEN (the list can be attached as Annex table, for example, or placed on ZEN website at HELCOM portal).

Section 8. Should be re-written as follows:

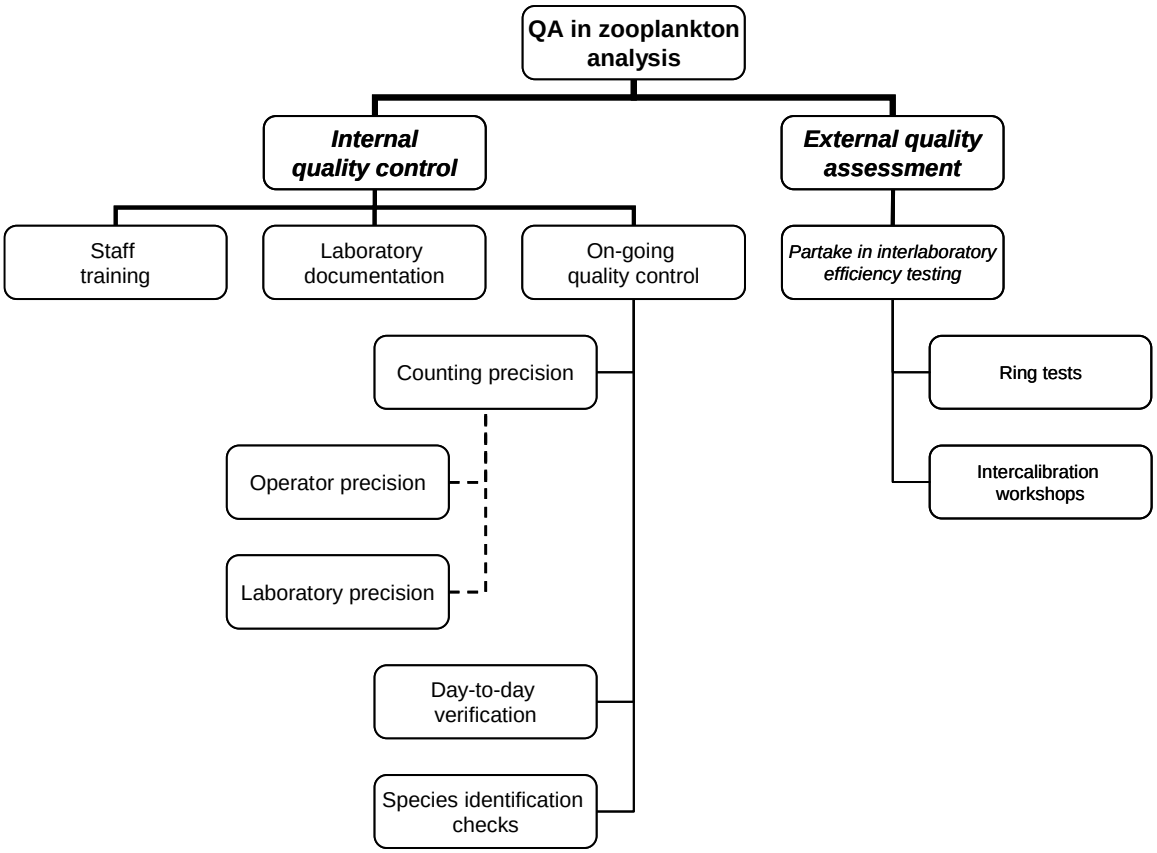
Basic principles of quality assurance should follow those for other components of the monitoring, so only the main issues in zooplankton identification and enumeration are addressed here. All laboratories should develop in-house QA routines and adopt internal and external quality control.

Internal quality control (Fig. 1) 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 consistency amongst operators in terms of procedures and taxonomic identification as well as day-to-day variation in performance.
- *Ensuring operator's competence*. This includes in-house training of personnel to maintain consistent and correct sample collection and analysis procedures.
- *Assessment of precision and accuracy of counting results*. This includes the following procedures:
    - testing *individual operator precision* (operator error). A count on triplicate subsamples of one sample (single-species artificial sample) should be conducted every year and related errors and confidence limits calculated;
    - testing *precision within the laboratory* (when more than one person is involved in zooplankton analysis). Triplicate subsamples of the same sample (single-species artificial sample) are counted by each operator and the error and associated confidence limits calculated;
    - performing *day-to-day verification*, every 20th sample is counted twice by each operator and by all operators involved and the results are presented and evaluated as R-charts;
    - 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.*

External quality assessment (Fig. 1), is the implementation of external checks to ensure adherence to the documented procedures. This includes interlaboratory tests (ring tests) and follow-up in-house evaluations.

Figure 1. Quality assurance (QA) in zooplankton analysis (species identification and enumeration).



References added

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