SLOVENSKI STANDARD
SIST EN 17204:2019

01-julij-2019

Kakovost vode - Navodilo za analizo mezozooplanktona v morskih in brakičnih vodah

Water quality - Guidance on analysis of mesozooplankton from marine and brackish water

Wasserbeschaffenheit - Anleitung zur Analyse von Zooplankton aus marinen und brackigen Gewässern

Qualité de l'eau - Document d'orientation sur l'analyse du mésozooplancton dans les eaux marines et saumâtres

Ta slovenski standard je istoveten z: **EN 17204:2019**

ICS:

13.060.10	Voda iz naravnih virov	Water of natural resources
13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

SIST EN 17204:2019 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 17204

April 2019

ICS 13.060.70

English Version

Water quality - Guidance on analysis of mesozooplankton from marine and brackish waters

Qualité de l'eau - Document d'orientation sur l'analyse du mésozooplancton dans les eaux marines et saumâtres

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 17204:2019) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2019, and conflicting national standards shall be withdrawn at the latest by October 2019.

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Introduction

WARNING — Person using this European Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national and international regulatory conditions.

Mesozooplankton constitute an important part of zooplankton in the pelagic food webs, since these are the organisms representing the link between primary producers and higher trophic levels. 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. [11].

Mesozooplankton comprise a large number of species within a range of total lengths of about 0,2 mm to 20 mm. The main groups are rotifers (Rotatoria), crustacean holozooplankton and merozooplanktonic larvae of other taxa such as echinoderms, bivalves and crustaceans. Small hydromedusae, ctenophores, chaetognaths, appendicularians, doliolids, fish eggs and larvae are also considered as part of the mesozooplanktonic fauna in marine waters. As most protozooplankton species are smaller than 0,2 mm these are not considered part of the mesozooplankton and hence procedures for sampling and enumeration of these species are not included in this standard.

For sampling, preservation and storage of mesozooplankton see EN 17218:2019

1 Scope

This document specifies a procedure for analysing mesozooplankton in marine and brackish waters. The procedure comprises how to identify and enumerate mesozooplankton to estimate quantitative information on diversity, abundance and biomass with regard to spatial distribution and long-term temporal trends for a given body of water.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 17218:2019, *Water quality — Guidance for the sampling of mesozooplankton from marine and brackish waters using mesh*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
biomass
total mass of living organic material in a given body of water

Note 1 to entry: Unit: $g\ m^{-3}$.

[SOURCE: ISO 6107-3:1993, definition 12, modified – Added note1 to entry]

3.2
metazoan
multicellular animal that develops from embryos

3.3
plankton
organisms drifting or suspended in water, consisting chiefly of minute plants or animals, but including larger forms having only weak powers of locomotion

[SOURCE: ISO 6107-5:2004, definition 41]

3.4
zooplankton
animals present in plankton

[SOURCE: ISO 6107-5:2004, definition 49]

3.5
merozooplankton
zooplankton that occurs in the plankton for only a part of their life cycle, usually the larvae stage

3.6
mesozooplankton
zooplankton of 0,2 mm to 20 mm size

3.7
holozooplankton
zooplankton spending their whole life in the pelagic realm

3.8
sampling station
precise location where samples are collected

Note 1 to entry: A sampling station is defined by its geographical position (latitude, longitude), its depth (relative to chart datum and normalized to mean low water as given in tide tables), the sampling depth and any other invariant or physical condition. The station is delineated using the given level of precision. In case of doubts when sampling stations have to be re-identified, most weight should be placed on depth and type of bottom, if known.

[SOURCE: EN ISO 16665:2013, definition 2.2.5, modified - Added note 1 to entry]

3.9
sub-sample
portion removed from a sample and intended to be representative of that sample

[SOURCE: EN ISO 5667-6:2016, definition 3.13]

3.10
semi-quantitative analysis
analysis of relative abundance of a taxon or group of organisms in a sample

3.11
quantitative analysis
analysis of absolute number per volume of a taxon or group of organisms in a sample

4 Principle

The determination of the abundance and biomass of zooplankton in samples of natural communities is based on microscopic counting and measuring of individuals of a representative sample, see Annex A for examples of length measurements of some selected zooplankton taxa. The total biomass of each taxon in the sample is determined by multiplying abundance with established length-weight conversion factors (see Annex B), with species-specific carbon conversion factors (see Annex C) or species-specific weight conversion factors.

5 Equipment

The following equipment is required for the analysis of zooplankton samples.

5.1 Stereoscopic microscope, objective and oculars with sufficient resolving power and magnification for the purpose of the analysis, lighting: incident and transmitted light, preferably cold-light source.

In some cases, the use of a bright-field or inverted compound microscope equipped with a condenser featuring a numeric aperture (NA) of at least 0,5 and plan objectives with a NA of 0,9 or more allowing

for total magnification to 125 × at a minimum is recommended. The microscopes should have binocular, bright field (additional phase contrast is helpful), 10 × or 12,5 × eyepieces.

5.2 Calibrated object micrometre.

5.3 Eyepiece (ocular) micrometre.

5.4 Counting chambers, for example Dolfuss chambers, Bogorov-chambers or Mini-Bogorov-chambers and when using an inverted microscope: Utermöhl chambers, see E.1 or Petri dishes with marked fields.

5.5 Sub-sample equipment, sample splitter for example Plunger sampling pipette according to Hensen, Folsom plankton sample divider, Motoda box splitter or Kott splitter, see E.2.

5.6 Sieves, of a mesh size approximately half of the mesh size of the sampling net.

5.7 Microprobes, microforceps.

5.8 Dispenser.

5.9 Refrigerator.

5.10 Counting devices and/or image analysis software.

5.11 Fume hood, for the work with formaldehyde.

6 Preservatives and other chemicals

The following preservatives are required for analysis of zooplankton samples:

6.1 4 % formaldehyde (CH_2O) solution. 1 part 40 % formaldehyde solution and 9 parts water. The formaldehyde solution has to be buffered to pH 8 to pH 8,2 with disodiumtetraborate (borax, $Na_2B_4O_3 \cdot 10H_2O$).

NOTE Formaldehyde is an organic compound which is available in liquid form. Formalin is a commercially sold aqueous saturated solution of formaldehyde at ∽40 % volume fraction or ∽37 % mass fraction. However, the precise contents may vary slightly between producers. A small amount of stabilizer, such as methanol, is usually added to limit oxidation and polymerization. A typical commercial-grade formaldehyde solution may contain 10 % to 12 % of methanol by volume.

WARNING — Beware of formaldehyde vapours. Do not store large numbers of samples in small work areas.

6.2 Ethanol. 96 % or 99 % C_2H_5OH.

6.3 Lugol's Iodine. Acidified Lugol's Iodine: Dissolve 100 g KI (potassium iodide) in 1 l of demineralized water; then add 50 g iodine (crystalline), shake until it is dissolved and add 100 ml of anhydrous acetic acid. As this solution is close to saturation, any precipitate should be removed by decanting the solution before use.

Calcareous structures of organisms are damaged by acidified Lugol's Iodine; therefore these organisms should not be preserved with acidified Lugol's Iodine. Acidified Lugol's Iodine is mostly used for protozooplankton and microzooplankton, for mesozooplankton samples are usually fixed with formaldehyde or ethanol.

6.4 Mastail and Battaglia solution. Prepare separate solutions by dissolving 8 g buthylhydroxyanisol (BHA, $C_{22}H_{32}O_4$) in 500 ml propane-1-2-diol ($C_3H_8O_2$) and 20 g ethylenediaminetetraacetic acid (EDTA, $C_{10}H_{14}N_2O_8Na_2{\cdot}2H_2O$) in 500 ml demineralized water. Add both solutions to 2 l of 40 % formaldehyde solution while stirring and buffer to pH 8 with sodium glycerophosphate ($C_3H_7Na_2O_6P{\cdot}H_2O$). After buffering add 2 g ascorbic acid ($C_6H_8O_6$) and demineralized water up to 5 l. Samples are preserved by adding 6 ml of the stock solution per 100 ml of sample in sea water.

NOTE This solution improves the preservation of chromatophores, which are key features for identification of decapod larvae or fish eggs and larvae.

The following chemicals may be useful for analysis of zooplankton samples:

6.5 1-Hexadecanol (Cetyl alcohol, $CH_3(CH_2)_{14}CH_2OH$) to reduce the surface tension (few drops per 100 ml).

6.6 Eosin Y, for staining of animals in phytoplankton rich samples (few drops per 100 ml).

6.7 Observation fluid, for example Steedmans observation fluid; 5 ml l^{-1} 1-phenoxypropan-2-ol ($C_9H_{12}O_2$) and 45 ml l-1 propane-1-2-diol ($C_3H_8O_2$) in demineralized water.

7 Procedure

7.1 Sample and sub-sample preparation

For sampling and storage of zooplankton samples from marine waters, see EN 17218:2019. All samples should be retained in storage until the subsequent investigation is completed.

To remove the formaldehyde from the sample before the microscopic investigation the sample should be filtered through a sieve and rinsed with filtered or sieved tap water. The mesh size of the sieves shall be considerably smaller than the mesh size of the plankton net used for sampling. All activities should occur under a fume hood to deflect the formaldehyde fume.

The formaldehyde should be collected in the sample vessel and reused to preserve the zooplankton organisms after completion of the microscopic analysis.

The zooplankton should be transferred under careful rinsing from the sieve with filtered tap water into a glass vessel and refilled to a certain volume depending of the density of organisms. The whole zooplankton sample should be filled in a counting chamber or divided in case organisms are too densely concentrated. Depending on the splitting device, the sample should be concentrated by sieving or diluted with tap water as necessary.

Before filling the splitter the volume of the total sample shall be measured in a graduated glass or plastic cylinder. The volume shall be noted in the protocol.

If the sample contains large clumps of plankton (e.g. by gelatinous organisms or Cercopagis) or macroalgae, these shall be carefully removed and placed on a very large mesh. The clump should be gently rinsed with water or seawater while pulling at the clumps with forceps to free trapped organisms, which are returned to the sample before splitting or analysis. All the organisms still attached to the clump shall be identified, counted and recorded.

To divide the sample into defined subsamples a calibrated Plunger sampling pipette according to Hensen, a Folsom plankton sample divider (splits into two subsamples), a Motoda box splitter or a Kott splitter (splits into eight subsamples) is recommended, see E.2.

The splitter shall be placed on a level surface and operated in a way that ensures a homogenous split of the sample.

After that, the sample shall be mixed intensively until all organisms are distributed homogenous in the sample volume. A few drops of a detergent should be added to allow the cladocerans to mix in the sample. The mixed sample should be splitted. The procedure can be repeated with an aliquot in case the sample is still too dense. A known aliquot or the whole sub sample shall be filled in the counting chambers.

For the work with Stempel-pipette mix the sample thoroughly and remove a 1 ml subsample immediately. Make sure no air bubbles or large lumps of detritus are in the sample chamber. Rinse the content of the pipette into a counting chamber.

The sub sample volumes have also to be recorded for calculating the abundance per sample. The count multiplied by the inverse of the split is the estimated number of organism in the sample.

NOTE 1 Non-random distribution in the sample during sub-sampling is the most important source of error.

NOTE 2 The Kott Splitter, which produces eight sub samples, is somewhat better in precision, but is more time-consuming to handle, while the Folsom sample divider splits samples into halves.

The counting chambers should be cleaned with tap water immediately after finishing the analysis to avoid adhesion of dried organisms.

For routine counting of larger zooplankton taxa a stereomicroscope should be used, which allows manipulation of the specimens during identification. For smaller organisms, an inverted microscope should be used. For special investigations during identification, a compound microscope should be used.

7.2 Species identification and counting

7.2.1 General

Species identification and counting are the basis of all zooplankton community analysis. Depending on the aim of the investigation all taxa appearing in the sample are to be determined or only dominant organisms and groups.

If possible, the complete sample shall be analysed. At least 100 individuals shall be counted. With higher abundance, representative subsamples shall be analysed.

A hierarchical counting technique should be used to obtain density estimates for all taxa. This procedure consists of first identifying all specimens (adults and development stages) and counting at least 100 individuals of the occurring dominating taxonomic groups, excluding nauplii, rotifers and tintinnids. If these minimum counts are not achieved in one subsample, additional subsamples shall be counted. The taxonomic group(s) that reached 100 individuals in the previous subsample(s), do not need to 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 % [11]. The estimation of abundance for other groups (“tail”) will be less precise [10].

All individuals should be counted to avoid heterogeneities due to splitting. While counting the settlement of all organisms to the bottom shall be ensured. It is possible to sink floating Microcrustacea by gently pressing them down using the microprobe or by adding a drop of dilute laboratory detergent (e.g. Cetyl alcohol). If a sample cannot be completely analysed and archived within 2 days, the sample should be kept in the refrigerator and preservative added to prevent the degradation of the sample.

For the quantitative analysis of microzooplankton organisms of particular interest (e.g. tintinnids or naupliar stages) abundance can be estimated semiquantitatively from the first subsample. For the quantitative analysis of macrozooplankton organisms and rare species of particular interest (e.g. non-indigenous species) the whole sample shall be scanned through.