SLOVENSKI STANDARD
oSIST prEN 17136:2017

01-julij-2017

Kakovost vode - Navodilo za terenske in laboratorijske postopke kvantitativne analize in identifikacije velikih nevretenčarjev v celinskih površinskih vodah

Water quality - Guidance on field and laboratory procedures for quantitative analysis and identification of macro-invertebrates from inland surface waters

Wasserbeschaffenheit - Anleitung zu Feld- und Laborverfahren zur quantitativen Analyse und Identifizierung von Makroinvertebraten aus Inland-Oberflächengewässern

Ta slovenski standard je istoveten z: **prEN 17136**

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

oSIST prEN 17136:2017 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 17136

June 2017

ICS 13.060.10

English Version

Water quality - Guidance on field and laboratory procedures for quantitative analysis and identification of macro-invertebrates from inland surface waters

Wasserbeschaffenheit - Anleitung zu Feld- und Laborverfahren zur quantitativen Analyse und Identifizierung von Makroinvertebraten aus Inland-Oberflächengewässern

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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Ref. No. prEN 17136:2017 E

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European foreword

This document (prEN 17136:2017) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

WARNING — Working in or around water is inherently dangerous. Care should be taken when working with different and generally toxic preservatives. This standard does not purport to address the safety problems associated with its use. It is the responsibility of the user to adopt appropriate health and safety practices in compliance with national regulatory conditions.

Introduction

Macroinvertebrates are important in water quality assessment and form one of the biological quality element in the EC Water Framework Directive (WFD 2000/60/EC). Macroinvertebrates are a very diverse group of organisms for which an efficient and effective procedure for analysis is not always obvious. Their large size range, very different appearances and the diverse range of habitats they live in produce specific problems for collecting and analysis. This guidance aims to provide general guidance for the quantitative processing of macroinvertebrate samples to reduce sample processing related variation for WFD in particular and scientific studies in general. It is not intended to give a full and exact overview of all possible methods. Specific situations and investigation purposes may require expert judgement based deviations from the outlined procedure but the general principles given in this guideline should always apply.

1 Scope

This European Standard gives guidance on the estimation of abundance and identification of macroinvertebrates in samples taken from inland waters. The procedure deals with pre-treatment (cleaning), sub-sampling, sorting, and final identification of organisms from preserved and live samples originating from natural habitats or artificial substrates. Specific guidance is given for preservation for DNA-analysis.

2 Normative references

Not applicable.

3 Terms and definitions

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

3.1
macro invertebrate
Invertebrate that is easily visible without magnification (> 0,5 mm) and generally belongs to the group of organisms that live for at least one life stage on or in the bottom substrate or vegetation of inland surface waters

3.2
matrix
components of a sample other than the macroinvertebrates

4 Principle

The process for the identification and estimation of the abundance of macroinvertebrates comprises both field and laboratory procedures. A pre-treatment of a fresh sample should be performed in the field or upon arrival in the laboratory to allow for efficient analysis. Pre-treatment consists of rinsing and sieving to remove as much matrix as possible. If needed, specific groups (Table 1) can directly be sorted, removed and identified. After pre-treatment the sample should be immediately cooled and/or preserved by addition of preservative. As alternative to cooling or preservation samples can also be frozen at ≤ −18°C. Freezing is not suitable for Platyhelminthes and Annelida.

In the laboratory the need and selection of a subsequent sorting step should be determined by visual inspection of the composition of matrix material (silt, clay, sand, macrophytes) and number of organisms. Such a procedure may comprise further rinsing and cleaning and/or sub-dividing of the sample. In case of preserved samples small trays are used in combination with a stereo-zoom microscope. For unpreserved samples macroinvertebrates are sorted out visually (naked eye or magnifying glass) on a tray. Organisms are immediately identified to the main taxonomic groups. Then, individuals from each group are identified to the desired taxonomic level.

Generally the smallest mesh size for collecting the macroinvertebrates during the analysis is 500 µm (e.g. sampling EN ISO 10870) as for most waters it is the best combination for trapping the macroinvertebrates and at the same time allowing effective rinsing. In specific situations (e.g. clear stony rivers) a smaller mesh size for maximum trapping of small macroinvertebrates can be selected.

For samples intended for DNA analysis, special emphasis should be put on working with clean equipment during the whole process to minimize the risk of cross-contamination during both field and laboratory procedures.

Table 1 — Typical taxonomic groups of macroinvertebrates

Bryozoa, Cnidaria, PoriferaF
Platyhelminthes
Annelida (Hirudinea, Oligochaeta, Polychaeta)
Crustacea-(Decapoda, Isopoda, Amphipoda, Mysida, remaining)
Diptera- (Chironomidae, remaining)
Arachnida
Coleoptera
Ephemeroptera
Heteroptera
Lepidoptera
Odonata
Trichoptera
Insecta-remaining
Bivalvia
Gastropoda

5 Equipment, preservatives and reagents

5.1 Sieves

Each new sieve shall be carefully checked for the exact mesh size before being put into service. If the mesh size does not fulfil the desired quality grade the sieve shall be excluded from use. Subsequently, before every use a sieve should be inspected for damage. The critical and smallest (typically 500 µm) sieves (having a significant effect on the validity of the analysis) should be fully traceable calibrated.

For DNA-based studies the removal of larger, visible contaminants by washing the equipment in water only is not sufficient. Instead sieves should be cleaned preferably with an appropriate bleach solution for each consecutive sample location.

5.2 Microscope

For accurate quantitative sorting and most identifications a low-power stereo-zoom microscope (typical magnification ~10x-60x) is needed. For the species identification of specific groups like Chironomidae and Oligochaeta a compound microscope (typical magnification 40x-400x) should be used.

5.3 Preservatives and fixatives

5.3.1 General

There is a distinct difference between fixation and preservation. In general fixation is the most desirable for morphological analysis but specimens should never be stored in any fixative for long or even short periods of time. Within the context of macro-invertebrate analysis ethanol as a preservative and formaldehyde as a fixative are by tradition the most often used chemicals.

5.3.2 Ethanol, C_2H_5OH

The final ethanol concentration in the sample should be about 70 % (≥80 % for samples intended for DNA extraction) to ensure preservation. Therefore ethanol 96 % should be added to the sample in most cases to compensate for dilution by sample water. Ethanol concentrations well above 70 % will harden many organisms rendering them difficult to manipulate and identify. Ethanol concentrations below 70 % will lead to tissue degradation.

Macroinvertebrate samples used for DNA studies or DNA-based identification that need to be stored for extended times before analysis (e.g. DNA barcoding) should be placed within two days of sampling in ≥ 90 % ethanol and preferably stored in a cool and dark place. At concentrations below 80 % DNA will degenerate.

5.3.3 Formaldehyde, 37 % (volume fraction)

Formaldehyde should be added to the sample to give a final concentration of 4 %–6 %. It is most suitable for fixation of organisms (avoiding damaging and shrinking). Formaldehyde is not suitable for DNA analysis and should be avoided as much as possible at it is hazardous to humans. Formaldehyde fixation should be limited to 24 h after which the organisms should be rinsed thoroughly and transferred to 70 % ethanol for (long-term) storage.

Preservation by ethanol and formalin fixation affects the estimation of biomass. Although weight loss by ethanol is considered to be more significant than by formalin no unambiguous scientific data are available on the exact effect of either preservative. Therefore, prior to estimating biomass on the basis of preserved organisms the bias by using preserved material should be assessed for the specific taxonomic groups under investigation and the relevant conditions of sampling and preservation.

5.4 Reagents for examination using compound microscopes

5.4.1 General

Many chemicals are known to enhance the microscope image quality in order to be able to accurately view diagnostic features. Koenike and Laevulose syrup are the most often used reagents. Both solution can be stored at room temperature for 1 year.

5.4.2 Koenike

Mix 50 ml of glycerol with 30 ml of water and add 20 ml of acetic acid. This solution can be stored at room temperature for an unlimited period of time. “Koenike’s Gemisch” is used for identification and long-term storage of Acari.

5.4.3 Laevulose syrup

Dilute 25 g D(-)fructose in 25 ml water by proper stirring (magnetic stirring bar). The solution can be slightly heated. Thereafter add 25 mL of lactic acid and stir again. Laevulose is used for the identification of Oligochaeta and Chironomidae.

6 Procedure

6.1 General

If live sample sorting is used samples should be sorted preferably within 24 h (max 48 h) and measures should be taken to prevent predation [1] and dying of organisms by oxygen depletion. Generally logistic considerations and negative effects of predation necessitate the preservation of samples before sorting. In case of preserved samples it can be advantageous to select specific groups (e.g. Platyhelminthes) from the live sample in the field for identification to anticipate the negative effects of nonspecific preservation for some organism groups.

6.2 Selection of preservatives

Ethanol is the best general preservative. Before adding ethanol the sample should be drained to minimize the dilution with excess sample water. In samples with absorbing material and/or relatively large organisms (e.g. numerous Molluscs) the initial 96 % ethanol should be replaced once to ensure a final concentration (approximately 70 %) in the sample. The sample container should be filled not more than one third with material. Ethanol should be added until the sample is completely immersed and the container is filled to achieve a concentration of 75 %. Ethanol concentration should be checked on a regular basis with an ethanol meter. This check can best be carried out in a graduated cylinder.

If formaldehyde is used for preservation (not recommended) samples should not be drained. Usually 37 % formaldehyde is used to obtain a final concentration of 4 %–6 %.

After the preservative has been added the sample should be gently turned to disperse the preservative.

Ethanol preserved samples can be stored for a long time (several months). Formaldehyde fixed samples should be transferred to ethanol if they are kept for more than a few months before sorting. Formaldehyde samples should be rinsed with water on a sieve prior to processing. Long-term storage in formaldehyde can seriously affect the colour of specimens and dissolves the shells of Mollusca (if not buffered).

6.3 Sample processing in the field

The fresh sample should first be put on a large sieve (typically 500 µm mesh size) to rinse with water or if clean enough directly in a white tray. However, dependent on logistics and available time it can be decided to process the sample completely in the laboratory.

Typical initial activities are:

— removing of large wood, plant particles and stones after being accurately inspected for clinging or sessile organisms;

— preservation of specific taxa in appropriate preservatives;

— sorting and identification of vulnerable, large, easy recognizable and rare taxa which can be released again in the field;

— gently rinsing the sample on a sieve to remove fine material like silt and sand.

As organisms (not only small) can actively pass through a 500 µm sieve speed in processing is required.