SLOVENSKI STANDARD
SIST EN 15196:2007

01-februar-2007

Kakovost vode ï Navodilo za vzorļ enje in obdelavo levov bub trzaļ (Diptera: Chironomidae) za ekoloģko ocenjevanje

Water quality - Guidance on sampling and processing of the pupal exuviae of Chironomidae (Order Diptera) for ecological assessment

Wasserbeschaffenheit - Anleitung zur Probenahme und Behandlung von Exuvien von Chironomidae-Larven (Diptera) zur ökologischen Untersuchung

Qualité de l'eau - Guide d'échantillonnage et de traitement d'exuvies nymphales de Chironomidae (Ordre des Dipteres) pour l'évaluation écologique

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This European Standard was approved by CEN on 26 June 2006.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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Foreword

This document (EN 15196:2006) 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of an identical text or by endorsement, at the latest by February 2007, and conflicting national standards shall be withdrawn at the latest by February 2007.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

WARNING — Working in or around water is inherently dangerous. Persons using this 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 regulatory guidelines.

1 Scope

This guidance standard specifies equipment and procedures for collecting floating pupal exuviae of Chironomidae from aquatic habitats; rivers from source to estuary, canals, ponds, lakes and sea coasts. Guidance in preparing specimens for subsequent identification is provided. These samples provide representative data on relative species abundance, suitable for numerical analysis, classification and monitoring of environmental conditions.

2 Normative references

The following referenced documents are indispensable for the application 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.

No normative references.

3 Terms and definitions

For the purpose of this European Standard, the following terms and definitions apply

3.1
adult
terrestrial, reproductive phase of the life history

3.2
Chironomidae
family of true flies (Diptera) known in the adult stage as non-biting midges

3.3
exuviae
cast skin of an arthropod developing to a subsequent life stage

NOTE From the Greek word meaning "cast clothes", there is no singular term.

3.4
larva
among aquatic species of Chironomidae this is the aquatic, juvenile phase of their life history

3.5
leeward shore
lake edge to which the wind is blowing

3.6
pupa
intermediate stage linking the larval and adult phases of a metamorphosing insect

4 Principle

A collection of floating chironomid pupal exuviae can be used for assessing and classifying most water bodies, without changing the equipment or procedure used (see Clause 6). The technique was first used by Thienemann in 1910 [12]. Chironomidae have exceptional species richness and ecological diversity, with an estimated 2 000 to 3 000 species per biogeographical region [3].

Pupae of all aquatic species of Chironomidae rise to the water surface for adult emergence [6]. Discarded pupal exuviae float on the water surface due to trapped air and a wax-layered cuticle. After about two days, bacterial attack of the wax layer causes the exuviae to sink [2], [7], [14]. Wind and water currents will cause floating exuviae to drift. In canals and rivers up to 10 m wide and with a mean velocity up to 50 cm sec^{-1}, 90 % of pupal exuviae become trapped behind vegetation or artefacts within 100 m of where the adult emerged and none normally drift further than 500 m [7], [8], [14]. A sample of floating debris from a single point along a river or canal will therefore contain an integrated collection of pupal exuviae accumulated over the previous couple of days. These exuviae are associated with individuals, which have colonised available habitats a short distance upstream of the sampling point. In ponds and lakes, pupal exuviae are assumed to drift without hindrance until they reach the shore or sink. A collection of chironomid pupal exuviae from standing water will be representative of recent adult emergence over a wide expanse of water.

Owing to the passive collection of discarded pupal exuviae there is little opportunity for the operator to influence the contents of the sample. In a comparison of a number of 10 minute collections of surface-floating chironomid exuviae with handnet samples of benthic (bottom-dwelling) larvae and pupae, twice as many species were obtained with exuviae, and these could be processed in one quarter to one third of the time needed to process benthic samples [4]. The sample will often contain exuviae from other insect groups and it is therefore important to distinguish them from Chironomidae. It may be possible to use these non-chironomid exuviae for water quality assessment but little is known on their reliability in representing species inhabiting the waterbody sampled. Mayfly exuviae break up rapidly in turbulent water while some caddisfly species leave their pupal exuviae in the larval case and this would bias any sample of floating exuviae.

5 Sampling equipment

5.1 Handnet, consisting of an extendable handle and frame supporting a net of mesh, sized no greater than 250 µm. Although an extendable handle and frame is not essential, it enables the operator to collect a sample without entering the water body.

5.2 Coarse mesh sieve, 30 cm diameter, 4 mm mesh or greater.

5.3 Fine mesh sieve, 30 cm diameter, 250 µm mesh or smaller.

5.4 Bucket and/or bowl, 30 cm diameter mouth, approx 10 l.

5.5 Pot with water-tight lid, 100 ml to 250 ml.

5.6 Low-power microscope, x15 to x100.

5.7 High power microscope, x100 to x400.

5.8 Fine mesh net or sieve, for sub-sampling.

6 Sampling procedure

6.1 Sampling season and frequency

In northern temperate regions, adult emergence largely occurs during the warmest 5 to 10 months of the year [1].

Any combination of three monthly river samples between April and September has been found to yield at least 80 % of the available genera of chironomids found from 12 consecutive monthly samples [11]. For lakes, 80 % to 90 % of species obtained from monthly samples during April to October have been obtained by combining data from any four of the samples, the lower figure of 80 % being obtained by permutations of four consecutive months [10]. To verify these data for other regions it will be necessary to collect pupal skin samples monthly, as detailed in paragraph 6.2, and subsample according to 6.3. The period of emergence for most species can be determined from a histogram of species richness for each month. The minimum number of samples to obtain 80 % to 90 % of species during the emergence season can then be determined by calculating the percentage of species obtained for all permutations of 1, 2, 3, 4 or more samples during that period.

6.2 Sample collection

The same procedure is applicable to all types of water body that are sufficiently large to insert a net. With an extendable handnet the operator can skim the surface of the water from the bank or shore without entering the water. In flowing water, the number of floating pupal exuviae will be greater upstream of obstructions at the water surface; overhanging bankside vegetation, emergent vegetation, weirs, navigation locks, moored boats etc. In standing water, the greatest collection of pupal exuviae will be found on the leeward shore.

The net will fill with debris, including exuviae, as it is dragged through the water surface. If necessary, the debris can be regularly emptied into a bucket containing water from the same site. In practice, sufficient pupal exuviae for the recommended method (see 6.3) are likely to be obtained before the net is full of debris. There is no need to time the sample, it is only necessary to obtain a sufficient number of exuviae from which a random sub-sample will be extracted.

After sampling has finished, the contents of the bucket can be poured through the coarse sieve stacked on top of the fine sieve (Figure 1). The debris retained within the coarse sieve is emptied back into the bucket containing a fresh supply of source water. The coarse sieve is restacked above the fine sieve and the contents of the bucket poured through the sieve stack a second time. This procedure is repeated at least once more. The contents of the coarse sieve are now discarded and the contents of the fine sieve gathered up and transferred to the pot. Sufficient absolute alcohol or industrial methylated spirit is added to the pot to cover the contents. If desired, the sieving may be performed in the laboratory. In this case the contents of the bucket can be strained back through the handnet, emptied into a plastic bag and sealed without the addition of any liquid.

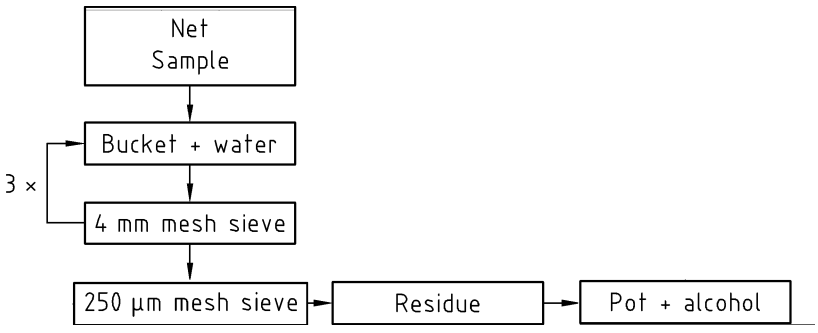

Figure 1

6.3 Sample processing

6.3.1 Quantitative

In the laboratory the sample should be floated in a bucket or bowl of water. After stirring the contents of the bowl, an aliquot of sample can be sub-sampled with a tea strainer or small fine mesh sieve. The sub-sample is emptied onto a petri dish (approximately 9 cm diameter) and sufficient water added to wet the whole inside base of the dish and float the exuviae. While viewing the dish contents through a low-power binocular microscope all chironomid pupal exuviae should be picked out from the petri dish with fine forceps and placed in 70 % alcohol. It should be ensured to pick out all chironomid pupal exuviae from the petri dish since the larger, darker species are likely to be selected first. A sub-sample of 200 exuviae using this technique provides an unbiased representation of the species present in the original sample [8], [15]. Consequently, the sub-sample should be checked for exuviae abundance before any are picked-out. If there are clearly too many exuviae a smaller sub-sample should be taken.

6.3.2 Qualitative

If it is only possible to collect a single sample from a site, rather than the recommended three (rivers) or four (lakes) monthly visits, then it is recommended that at least 500 exuviae are sub-sampled. Owing to the broad adult emergence periods of northern temperate chironomids [9], [10], [11] many species will be represented in this sub-sample, despite the sample occurring outside of their peak emergence period. For this reason, data for a single sample should be used only for qualitative analysis (presence/absence).

6.3.3 Slide preparation

Many genera can be identified while observed under a low-power microscope. To identify a species, a temporary mount of sub-sampled exuviae placed in 70 % alcohol on a glass slide under a cover slip will allow subsequent manipulation of the specimen if any morphological structure is obscured. A permanent slide is prepared by transferring exuviae from 70 % to 100 % alcohol for at least a minute before mounting in Euparal (Asco Laboratories, 52 Levenshulme Road, Manchester M18 7NN, England). Identification of a species level will require a high-powered microscope. Species of the West Palaearctic can be identified using reference [5] while identification of genera from the Holarctic is possible using reference [13], and for Britain and Ireland using reference [16].

7 Quality assurance

The quality assurance should be in accordance with EN 14996.