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Water quality — Guidance standard for the sampling of zooplankton from standing waters

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British Standards

National foreword

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— aid enquirers to understand the text;

— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep UK interests informed;

— monitor related international and European developments and promulgate them in the UK.

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Qualité de l'eau - Guide pour l'échantillonnage du zooplancton dans les eaux stagnantes

Wasserbeschaffenheit - Anleitung zur Probenahme von Zooplankton aus stehenden Gewässern

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Foreword

This document (EN 15110:2006) 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of an identical text or by endorsement, at the latest by November 2006, and conflicting national standards shall be withdrawn at the latest by November 2006.

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.

Introduction

Zooplankton community structure provides information on a range of physico-chemical and biotic causative variables. These include pH- and acidification-related variables, toxic chemicals, phytoplankton structure and abundance (i.e. lake productivity), and intensity of fish predation. The effects of size-selective predation are well known and the size-structure of zooplankton communities can give valuable information of the fish community.

Metazoan zooplankton (metazooplankton) constitute a large number of species within a range of total lengths of about 0,05 mm to 20 mm, but mostly < 2 mm. The main groups are the rotifers (Rotatoria), the cladocerans (Cladocera) and the copepods (Copepoda). Some shrimps (Natantia; e.g. Mysidae) and larvae of dipterans (Diptera, e.g. *Chaoborus*) may also be considered as part of the zooplankton fauna. Rotifers and crustaceans inhabiting the littoral of standing waters can also be grouped with the more strictly planktonic forms. Fish larvae, hemipterans (Heteroptera, e.g. Corixidae) and coleopterans (Coleoptera) are occasionally recorded in the plankton samples but are not considered as part of the zooplankton fauna. Procedures for sampling of protozooplankton (Protozoa) are not included in this standard.

Surveys of zooplankton have provided valuable information for the environmental monitoring of standing waters, because this group includes species which:

a) occur in a wide range of standing waters over a large geographical area and at the same time have specific environmental requirements;

b) are well known with regard to their geographical distribution and environmental requirements;

c) have a generally high capacity for dispersal enabling them to respond rapidly to remedial actions; while

d) sampling requires only a modest expenditure of time and equipment.

WARNING — Working in or around water is inherently dangerous. 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 conditions.

NOTE According to the classification by Fryer [5] the assemblage long known as the Cladocera is split into four orders; Ctenopoda, Anomopoda, Onychopoda and Haplopoda. Cladocera is however used in this standard as a general descriptive term.

1 Scope

This guidance standard describes general procedures for surveying zooplankton in standing waters for the purposes of water quality assessment and determination of ecological status.

Guidance on sampling procedures and the subsequent steps for preservation and storage are given. The sampling procedures provide estimate for species occurrence and their abundance (relative or absolute), including spatial distribution and temporal trends, for a given body of water. Calculation of biomass and production is made possible.

This method is restricted to the sampling of multicellular zooplankton that inhabit the pelagic and littoral regions of lakes, reservoirs and ponds. The sampling procedure may be also employed in slow running waters and canals.

NOTE The field methods described are suitable for the collection of open-water plankton and littoral plankton species. They are inappropriate for the collection of littoral species that primarily live on or in the surface of sediments and on the surface of aquatic plants.

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.

prEN 14996, *Water quality — Guidance on assuring the quality of biological and ecological assessments in the aquatic environment.*

EN 25667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes (ISO 5667-1:1980).*

EN ISO 5667-3, *Water quality - Sampling - Part 3: Guidance on the preservation and handling of water samples (ISO 5667-3:2003)*

3 Terms and definitions

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

3.1
anoxic
condition in which the concentration of dissolved oxygen is so low that certain groups of micro-organisms prefer oxidized forms of nitrogen, sulphur, or carbon as an electron acceptor

NOTE As the oxygen concentration approach zero the concentration of hydrogensulfide (H_2S), released by bacterial anaerobic (no oxygen present) activity, is increasing. The anoxic conditions markedly affect the cycling of other nutrients, ecosystem productivity, and the distribution of biota.

3.2
body of surface water
discrete and significant element of surface water such as a lake, reservoir, stream, river or canal, part of a stream, river or canal, a transitional water or a stretch of coastal water [EC Directive 2000/60/EC]

3.3
dimictic lake
lake with spring and autumn turnovers (temperate lake)

3.4
epilimnion
water above the thermocline in a stratified body of water

3.5
fixation
protection from disintegration of the morphological structure of organisms

3.6
impact study
investigation of the physical, physico-chemical and biological consequences of a given intervention in a body of water

NOTE A study of consequences should be capable of forming a basis for the subsequent remedial measures.

3.7
habitat
locality in which a plant or animal naturally grows or lives

NOTE It can be either the geographical area over which it extends, or the particular station in which a specimen is found.

3.8
hypolimnion
water below the thermocline in a stratified body of water

3.9
littoral zone
shallow marginal zone of a body of water within which light penetrates to the bottom; usually colonised by rooted vegetation

3.10
metazoan
multicellular animals that develop from embryos

3.11
metazooplankton
multicellular zooplankton (see 3.21)

3.12
pelagic zone
free body of water beyond the littoral zone

3.13
plankton
organisms drifting or suspended in water, consisting chiefly of minute plants or animals, but including larger forms having only weak powers of locomotion

3.14
preservation
protection from (bio)chemical degradation of organic matter

3.15
sampling site (sampling station)
general area within a body of water from which samples are taken

NOTE A station is defined in terms of its location (geographical position, depth) and invariant conditions (e.g. type of bottom in shallow-water areas) and is delimited on the basis of the accuracy with which these are given. In cases of doubt when sampling stations have to be re-identified, most weight should be placed on depth and type of bottom.

3.16
stratified water
standing water with temperature gradients resulting in an upper, warmer, isothermal layer floating on cooler, denser, usually also isothermal water

NOTE Between the upper layer, the epilimnion, and the lower layer, the hypolimnion, is a transitional zone, the metalimnion (see thermocline). The thermal stratification may be very short-lived or persist for all of the warmer part of the year. Lakes with ice-cover during the cold season may show inverse stratification; an upper, cooler (< 4 °C) layer floating on warmer water. Water has its highest density at 4 °C and during stratification and inverse stratification the deeper water has a temperature of approximately 4 °C.

3.17
subsampling
collection of a sub-sample that consists of a known fraction of the total sample and that is representative of the quantity and species composition of the latter

3.18
thermocline (metalimnion)
layer in a thermally stratified body of water in which the temperature gradient is at a maximum

3.19
trend monitoring
study intended to reveal any changes in the ecological status of a body of water over time

3.20
turbidity
reduction of transparency of water caused by the presence of undissolved matter

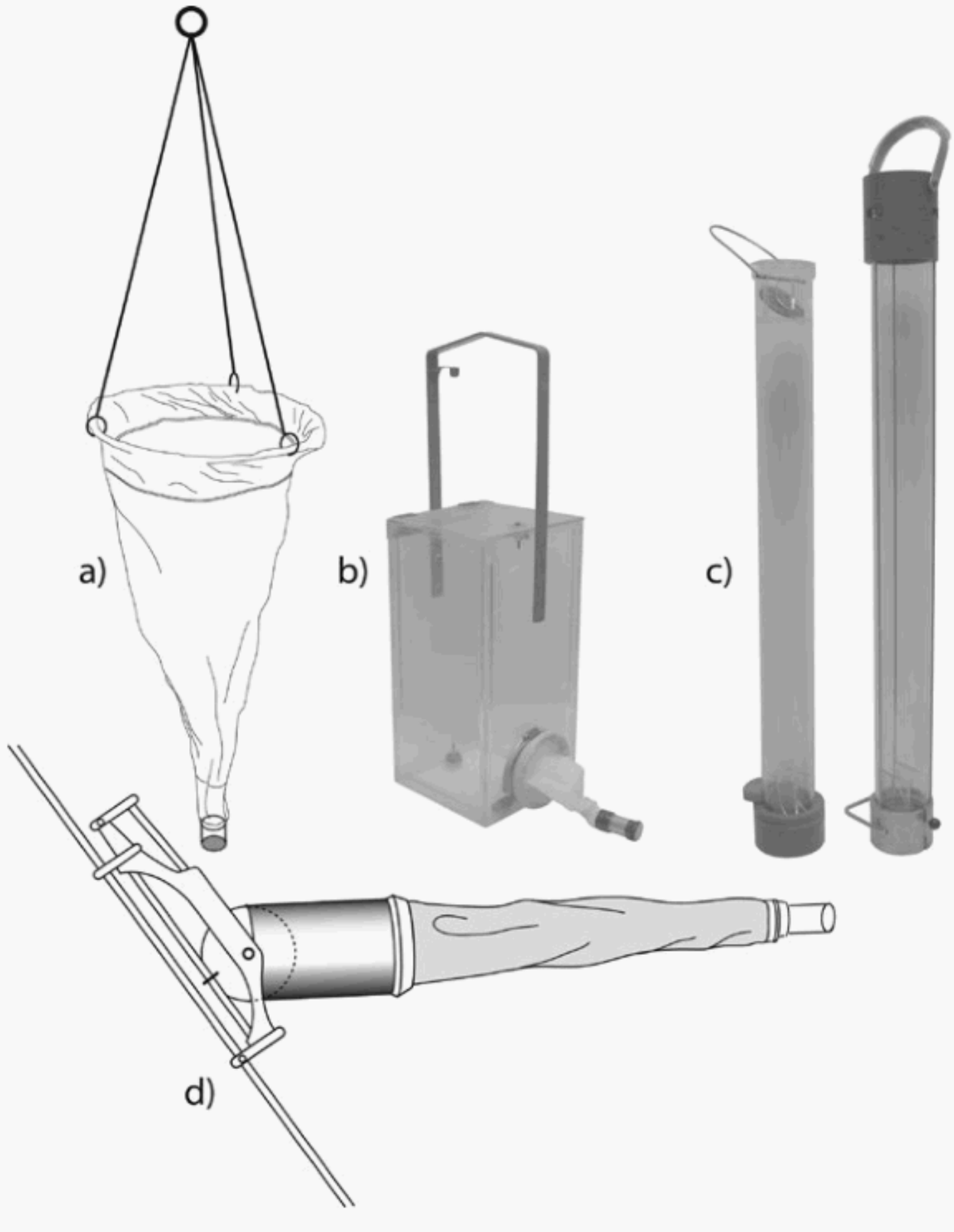
3.21
zooplankton
animals present in plankton

4 Principle

The sampling strategy adopted provides information on the current status of the metazooplankton community. The selection of sampling sites (numbers and location), sampling depth, time and frequency of sampling, size of samples and type of sampling gear is of great importance for the evaluation of the data collected. As a general guidance EN 25667-1 should be consulted.

5 Equipment

There exist several overviews of the most widely used zooplankton sampling techniques and their advantages and drawbacks (e.g. [1], [2], [3], [7], [8] and [13]). This standard provides some general recommendations.



Key

a conical plankton net
b Schindler trap
c modified Ramberg sampler
d Clarke-Bumpus sampler

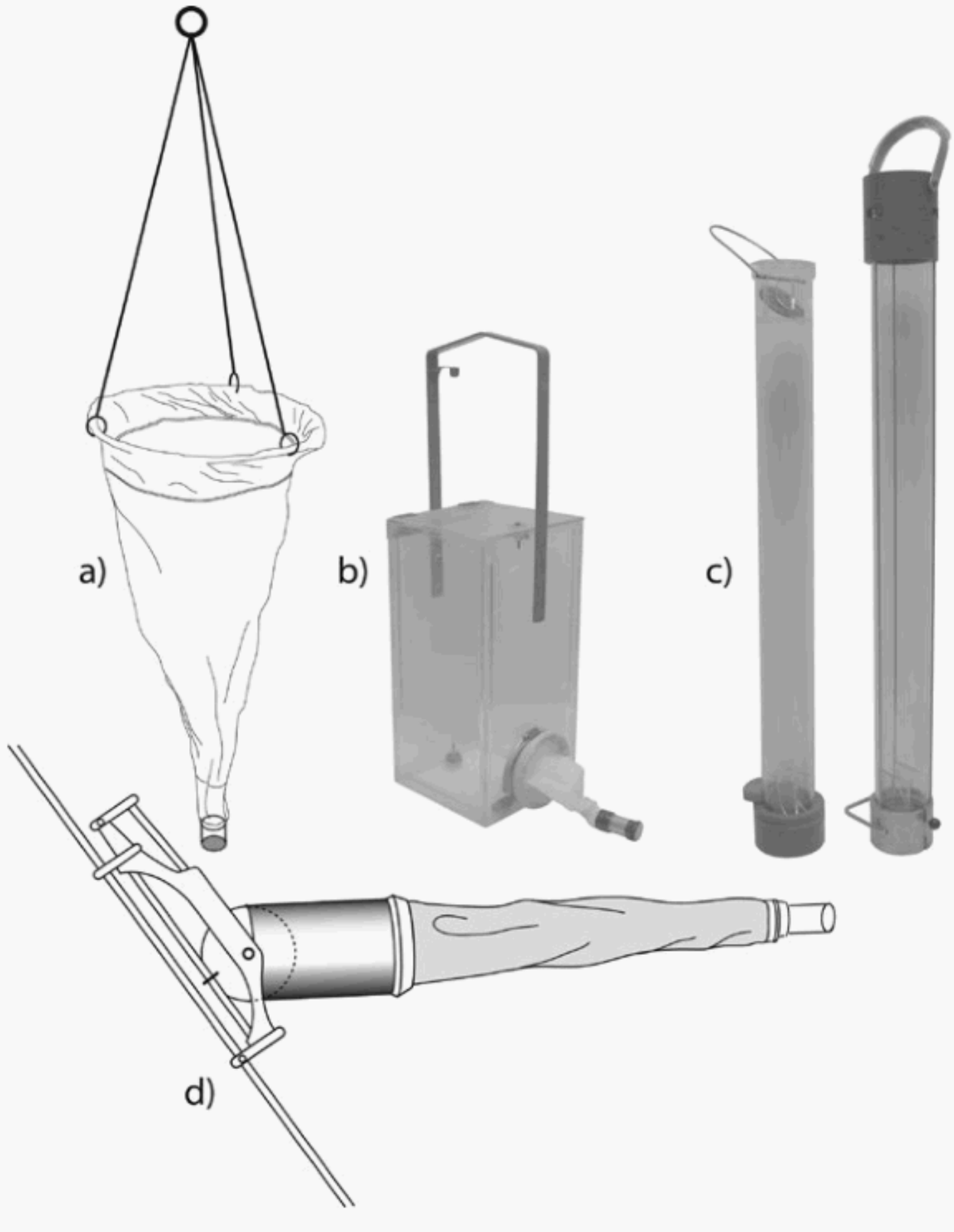
Figure 1 — Examples of different zooplankton sampling equipment

5.1 Qualitative sampling

5.1.1 Nets

Nylon plankton nets of various dimensions and mesh sizes can be used for sampling (Figure 1a). It is important that nets should have a large filtering surface relative to their opening in order to ensure that filtering is as efficient as possible. A net with an opening diameter of 30 cm, for example, should have a length of about one metre as a minimum. A cylindrical net section above the conical part increase the filtering area compared with a conical plankton net with the same opening diameter and length.

If both rotifers and crustaceans are to be analysed, a net with a mesh of about 40 µm to 50 µm should be utilised. Nets with meshes smaller than 40 µm will readily become clogged and their use should normally be avoided, although they may be useful in oligotrophic waters. If only crustacean plankton are to be analysed a mesh of 90 µm (max. 100 µm) can be used. If both rotifers and crustaceans, including large predatory species, are to be sampled with a reasonable degree of efficiency, the use of three nets with different mesh sizes are



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When shallow lakes or littoral areas with a great deal of vegetation are being quantitatively sampled the use of a volume sampler, plankton pump or flexible tube is recommended.

5.2.2 Other field equipment

The equipment listed in 5.1.2 (excluding the lead weight) and in addition a mixing vessel (e.g. plastic bucket or similar) to combine a number of individual samples into a single sample in the field. Conducting mixed samples may be necessary to reducing analysis times and costs.

If a volume sampler is being used (with the exception of a Schindler-Patalas trap) filtration equipment is also required to concentrate the samples. This may take the form of either a plankton net (mesh size 45 µm, or 90 µm if only crustacean plankton are being collected) or a large funnel with draining cup fitted with a netting.

5.3 Storage

Bottles (100 ml, 200 ml or 250 ml brown bottles with screw-tops) or glass vials for storing samples.

Labels or tape to attach to the outside of the sample bottles. Waterproof paper for labels to put inside the sample bottles.

Marker pen. If ethanol is being used, an alcohol-proof pen or pencil is recommended for both internal and external marking.

NOTE Plastic vials are not suitable for storing samples if Lugol's Iodine is used as preserving solution.

6 Preserving solutions

6.1 General

A number of different preserving solutions with different areas of application are available. The advantages and disadvantages of each of these solutions are defined in Annex A.

6.1.1 **Formaldehyde**, 37 % by volume.

This is neutralised, e.g. with hexamethyl tetramine ($C_6H_{12}N_4$). Dilute the formaldehyde with water to 20 % (v/v) to avoid precipitation, and then add 100 g of hexamethyl tetramine and 40 g to 80 g sucrose ([6]) per litre of 20 % formaldehyde.

WARNING — Formaldehyde may trigger allergies or cancers, and should therefore be handled with care.

6.1.2 **Ethanol**, 96 % or 99 % C_2H_5OH.

6.1.3 Lugol's Iondine

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

Most preservatives are also commercially available. For more details on the use of different preservatives the reader is referred to Annex A.

Preserving solutions for field use should be kept in small stoppered bottles and should be accompanied by a pipette for transferring the solution to the plankton samples. The bottles should be kept in a plastic box or container with lid during transportation.

7 Preliminary stages

7.1 Documentation of strategies and methods

The following documentation should be available before the start of field work:

— description of objectives and strategy;

— description of methods;

— safety instructions;

— personnel plan;

— overview of equipment and instruments;

— registration forms;

— procedures for the maintenance of records and samples;

— quality assurance requirements according to prEN 14996.

7.2 Preparation of sampling equipment

After each day of sampling, the net should be washed in warm freshwater with detergent or in an ultrasonic water-bath in order to reduce clogging and ensure optimum filtration capacity.

Check that the netting in the plankton net and draining cups is completely free of holes and tears.

Check that the line is securely attached to the plankton net/volume sampler.

Check that the plankton sampler's closing mechanism is functioning well and that any seals are in order.

It may be advantageous to label the sample bottles and to add the requisite amounts of preserving solution to them before the start of fieldwork.

In order to prevent spreading of flora and fauna between water bodies, the sampling equipment should be disinfected between uses in the different waters.

7.3 Safety instructions

Before initiating the survey notify a contact of which localities and areas are to be surveyed on a specific day. If the samples are being collected from a boat, always have a shore-based contact in case of emergencies. Check the weather forecast in order to ensure safe and effective surveying conditions.

For safety reasons, it is recommended that surveys should not be undertaken by lone workers but by a minimum of two people.

8 Sampling procedure

8.1 Investigation program

An investigation programme shall be developed according to the investigation aims, required precision of results, hydromorphological conditions in the area, prior knowledge of local pollution sources, results of

previous investigations and any other factors that may be of significance. The choice between qualitative and quantitative methods is made according to the aims of the investigation and the required precision of results.

Qualitative samples (net hauls) provide information about the species composition, number of species, size distribution and relative dominance of species and groups of zooplankton.

Quantitative samples also provide information regarding the quantity of zooplankton (individual density) per unit volume. Quantitative samples also allow calculation of biomass and production for the zooplankton assemblage as a whole as well as for the individual species.

In production studies (estimates of biomass and secondary productivity) it is essential to obtain quantitative samples. If the objective is to give a species list as complete as possible, net hauls are also recommended.

The vertical and horizontal distribution of zooplankton is uneven. Some species are found regularly in the shallow-water regions with stands of aquatic vegetation whereas others perform horizontal migration in the course of the day. Vertical patterns and vertical migration is also common among zooplankton species. To obtain whole-lake estimates of species composition and abundances, samples should be taken from both pelagic and littoral areas as well as from different depths.

NOTE Standard plankton nets are not suitable for quantitative sampling and usually give a less accurate estimate on species composition than quantitative zooplankton sampler.

8.2 Number and location of sampling sites

8.2.1 General consideration

The number and location of the sampling sites should be determined according to the aim of the study, the morphology of the water body and the level of accuracy in the provided estimates. In general, sites selected should be representative of the area under consideration.

8.2.2 Pelagic samples

The samples are normally collected at the same site as used for other observations (temperature, Secchi depth, water chemistry, phytoplankton, etc.).

The deepest area near the centre of the main basin of the lake is usually preferred as the sampling location, if a single location is regarded as sufficient for the purposes of the study. If this is not known, the maximum depth of the lake should be estimated, by use of a portable echo sounder or a depth-meter, prior to the sampling.

In large lakes, and lakes with several more or less separate basins or with a complex morphology, it will often be desirable to have several sampling locations in order to obtain an impression of any intra- or inter-basin differences. It is recommended that a minimum of one station should be established in each basin.

If, for example, the effects of point discharges are to be assessed, it may also be appropriate to select a location, which is not near the centre of the main basin of the lake, and/or to set up more than one sampling station. Generally, samples of strictly pelagic species should be collected at a good distance from the shore in order to avoid as far as possible any influence from the littoral fauna.

Zooplankton are often irregularly distributed in lakes, i.e. there is often a horizontal and a vertical variation. If it is important, to the aims of the study, to obtain information regarding the horizontal distribution of the zooplankton, samples should be obtained from several locations along a gradient that represents the dominant wind direction. Other horizontal gradients may also be considered. The vertical variation is discussed in 8.3. If information regarding spatial variation or a high level of precision in the estimates is required, it may be necessary to draw up a sampling programme adapted to the lake morphology and vertical stratification (see 8.4).

8.2.3 Littoral samples

Such samples are collected from the lake's shallow-water regions (usually at depths of < 1 m). Samples are taken from areas containing aquatic vegetation (protected shores) and from areas with little vegetation (exposed shores). In large lakes with a wider range of aquatic vegetation, it is recommended that separate samples should be collected from areas with different types of aquatic vegetation, prioritising the dominant types.

8.3 Sampling depth

8.3.1 General consideration

Samples may be collected individually from fixed depths, or as combined samples where samples from different depths are mixed together to form a single sample. A third option is to use integrated samples, where sampling encompasses the entire water column within fixed depth intervals.

It can be difficult to decide upon the appropriate sampling approach or exact sampling depths in each individual case. Zooplankton usually are distributed unevenly. More information on how to deal with spatial variation is given in 8.4.

8.3.2 Pelagic samples

The samples are collected from specific depths or from the whole watercolumn, depending on the objectives of the study and the depth of the sampling station. In general, it is most important to have a relatively dense network of observations from the surface and down to the thermocline, as the majority of the plankton is usually to be found at these depths in the water column (epilimnion). In clear lakes, zooplankton is usually to be found at greater depths than in humic ("brown-water") lakes or water with high turbidity.

Vertical net hauls should cover all depths in order to include deepwater species. In locations with large quantities of algae and other particles the net will easily become clogged and an additional sample should therefore be taken from the uppermost 1 m to 5 m.

In order to avoid stirring up sediments and contaminating the samples, the deepest samples should be taken no lower than 0,5 m from the sediment surface. If the greatest depths of the lake are anoxic, the samples should be collected down to this zone.

If the objectives of the study make it important to obtain information regarding the vertical distribution of the zooplankton, it is recommended to collect samples at a number of depths. The samples should be taken at intervals of no more than 2 m in the epilimnion and metalimnion. In the hypolimnion samples should be taken at less frequent intervals. In deep lakes the samples taken should cover at least the 0 m to 20 m stratum.

If mixed samples (i.e. integrated samples taken from a given range of depths) are to be taken, separate mixed samples should also be taken from the epilimnion and the hypolimnion.

8.3.3 Littoral samples

Samples are collected from the shallow-water region of the lake at depths of between 0,2 m and maximum depth of rooted vegetation. These samples should be collected just above the sediment surface within areas of vegetation and areas with little vegetation.

8.4 How to deal with spatial variation

The spatial distribution of zooplankton in a lake is uneven and patchy. Both systematic and random variations are common.

Systematic variations often take the form of sharp gradients or patterns (vertical and horizontal) and are best mapped by sampling from a large number of depths and stations along dominant gradients (see 8.2 and 8.3).

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Vertical net hauls should cover all depths in order to include deepwater species. In locations with large quantities of algae and other particles the net will easily become clogged and an additional sample should therefore be taken from the uppermost 1 m to 5 m.

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8.3.3 Littoral samples

Samples are collected from the shallow-water region of the lake at depths of between 0,2 m and maximum depth of rooted vegetation. These samples should be collected just above the sediment surface within areas of vegetation and areas with little vegetation.

8.4 How to deal with spatial variation

The spatial distribution of zooplankton in a lake is uneven and patchy. Both systematic and random variations are common.

Systematic variations often take the form of sharp gradients or patterns (vertical and horizontal) and are best mapped by sampling from a large number of depths and stations along dominant gradients (see 8.2 and 8.3).

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Such samples are collected from the lake's shallow-water regions (usually at depths of < 1 m). Samples are taken from areas containing aquatic vegetation (protected shores) and from areas with little vegetation (exposed shores). In large lakes with a wider range of aquatic vegetation, it is recommended that separate samples should be collected from areas with different types of aquatic vegetation, prioritising the dominant types.

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— Samples preserved in ethanol retain their quality for long periods of time if they have been stored correctly. Such samples thus require little attention;

— The use of 96 % ethanol prevents carapace distortion and loss of eggs and embryos due to ballooning (cladocerans);

— DNA is retained in a form which can subsequently be extracted for genetic analysis (for this purpose, should be stored in fridge or freezer).

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— Can cause cell shrinkage, which will result in underestimation of dimensions;

— May be unpleasant for laboratory personnel (dizziness, headaches). For this reason, this preserving agent should be diluted before samples are analysed.

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