BS EN 16698:2015

BSI Standards Publication

Water quality — Guidance on quantitative and qualitative sampling of phytoplankton from inland waters

bsi.

...making excellence a habit.™

National foreword

This British Standard is the UK implementation of EN 16698:2015.

The UK participation in its preparation was entrusted to Technical Committee EH/3/5, Biological Methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Published by BSI Standards Limited 2015

ISBN 978 0 580 84665 6

ICS 13.060.70

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 October 2015.

Amendments/corrigenda issued since publication

Date	Text affected

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16698

October 2015

ICS 13.060.70

English Version

Water quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters

Qualité de l'eau - Lignes directrices sur l'échantillonnage quantitatif et qualitatif du phytoplancton dans les eaux intérieures

Wasserbeschaffenheit - Anleitung für die quantitative und qualitative - Probenahme von Phytoplankton aus Binnengewässern

This European Standard was approved by CEN on 8 August 2015.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 16698:2015 E

Contents

Page

European foreword

This document (EN 16698:2015) 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of an identical text or by endorsement, at the latest by April 2016, and conflicting national standards shall be withdrawn at the latest by April 2016.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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

Introduction

WARNING —Working in or around water is inherently dangerous, Persons using this European standard should be familiar with usual field and 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.

IMPORTANT — It is absolutely essential that tests conducted according to this European Standard be carried out by suitably trained staff.

Series of phytoplankton samples provide information on the taxonomic composition as well as the spatial occurrence of the individual taxa and their relative abundances. They allow the calculation of the biomass of the individual taxa as well as for the phytoplankton assemblage as a whole.

For the purpose of limnological investigations like monitoring and status assessment of surface waters representative phytoplankton samples are necessary. Therefore suitable sampling methods and monitoring strategies are needed depending on the objectives of the investigation and the given natural conditions.

1 Scope

This European Standard specifies procedures for phytoplankton sampling in inland waters and describes methods of sampling techniques for phytoplankton in inland waters (e.g. rivers and channels, or lakes, ponds, reservoirs and other artificial water bodies, respectively).

This European Standard gives guidance for sampling of phytoplankton for qualitative and quantitative limnological investigations and monitoring of water quality, e.g. ecological status.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15204, *Water quality — Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)*

EN ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method (ISO 5814)*

EN ISO 7027, *Water quality — Determination of turbidity (ISO 7027)*

EN ISO 10523, *Water quality — Determination of pH (ISO 10523)*

ISO 17289, *Water quality — Determination of dissolved oxygen — Optical sensor method*

3 Terms and definitions

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

3.1
deep chlorophyll maximum
DCM
local chlorophyll maximum below the epilimnion where the maximum chlorophyll concentration is at least 1,5 times higher than the average chlorophyll concentration measured in the epilimnion

3.2
depth gradient
parameter to distinguish between polymictic and di- or monomictic lakes as a measure for the mixing intensity of a water body calculated as the quotient of maximum depth and theoretical epilimnion depth

Note 1 to entry: See Annex C.

Note 2 to entry: Values > 1,5 indicate a thermally stable stratified lake. For further details see Annex C and [18].

3.3
dimictic lake
lake with uniform water temperature and holomictic conditions twice a year: during autumn (before ice cover) and during spring (after ice cover)

3.4
epilimnion
zone of the water body between surface and thermocline in which the water temperature and density is approximately uniform, showing a temperature gradient of < 1 °C/m

3.5
euphotic zone
zone of the water body where light intensity is sufficient for photosynthetic production

Note 1 to entry: The euphotic zone is the layer with more than 1 % of incident light intensity of subsurface light (see [13]). The euphotic depth is approximately 2,5 times Secchi depth [1], with exception of humic lakes (0,8 to 2,1 times Secchi depth) [13].

3.6
holomictic lake
lake that shows complete mixing of the water body every year

3.7
integral sampler
water sampler which provides a representative sample of a predefined continuous water column

3.8
mean depth
depth value obtained from dividing a lake's volume by its surface area

Note 1 to entry: To obtain the mean depth in metres (m), the lake's volume should be specified in cubic metres (m^3) and the surface area in square meters (m^2).

3.9
metalimnion
thermocline
water zone between epi- and hypolimnion with the greatest vertical density gradient caused by temperature gradients > 1 °C/m

3.10
complete mixing
full mixing of the whole water body, indicated by uniform temperature along the vertical axis

Note 1 to entry: Complete mixing of water is only possible when the density in the whole water column is equal which usually means the temperature is the same in every depth.

3.11
monomictic lake
lake with uniform water temperature and holomictic conditions from autumn to spring (no ice cover in winter) during which the water body is completely mixed

3.12
polymictic lake
lake with more than two circulation phases per year comprising the whole water body (depth gradient ≤ 1,5)

Note 1 to entry: See also [17].

3.13
shallow lake
in this European Standard shallow lakes are defined as polymictic lakes (3.12) – based on the depth gradient - independent of their mean or maximum depth

3.14
secchi depth
visibility through the water column (transparency measured using a white plate lowered into the water)

3.15
stratification
state of a water body during which a vertical density gradient in the water column (caused by a gradient of temperature or dissolved matter concentration) prevents its complete mixing

4 Principles of phytoplankton sampling

4.1 General

Phytoplankton samples are collected and preserved for later microscopic investigation. Phytoplankton samples are most often collected in lakes, reservoirs or ponds and in larger rivers where residence time and light conditions enable phytoplankton growth. The following sections provide detailed recommendations on when and where samples should be taken to enable a more or less detailed study of phytoplankton in any location.

Species composition, number of individuals and biomass of phytoplankton vary throughout the seasons as well as spatially across water bodies and within the water column. When setting up a sampling program it is important to be aware of this variability and adjust the sampling programmes accordingly. There is a range of recognized equipment and procedures used for the collection of phytoplankton samples and these can be selected according to the precise requirements of any particular study or sampling programme.

Most sampling procedures are based on the collection of water samples which contain phytoplankton in its natural composition and abundances. Preservation and subsequent sedimentation of the phytoplankton enable the investigation according to Utermöhl (EN 15204). This allows taxa identification and quantitative assessment of abundance and biomass. Samples taken using a plankton net concentrate the phytoplankton, increasing the likelihood of picking up rare taxa, but cannot be used for quantitative assessment of abundance.

Subsidiary information could include physical data such as water depth, temperature and oxygen profiles, assessment of euphotic zone and chemical determinants such as chlorophyll and nutrient concentrations. The frequency, location and type of samples collected to analyse phytoplankton in lakes and rivers should be determined by the requirements of the monitoring programme or study for which they are required.

Generally, it is distinguished between sampling in lakes and sampling in rivers. In rivers which are assumed to be vertically completely mixed, it is sufficient to take a sample from the main flow.

In lakes the mixed water layer or the zone in which phytoplankton is produced (euphotic zone) should be sampled. Generally, a vertical mixed sample should be taken. This is possible either with an integral sampler or by mixing of sub-samples from all depths of the mixed or euphotic water column.

In clear lakes (euphotic depth > epilimnetic depth) a mixed sample should be taken from the euphotic zone. In turbid and humic lakes (euphotic depth < epilimnetic depth) it is adequate to take the sample from the mixed zone (epilimnion in stable stratified lakes, whole water column in polymictic lakes). The spatial extent of these zones is determined by recording Secchi depth and depth profiles of water temperature. For ambiguous temperature-depth profiles also other parameters such as dissolved oxygen and pH should be measured. It is recommended to determine the vertical distribution of the phytoplankton by measuring chlorophyll-a using a fluorescence probe in order to define the sampling zone.

Samples for phytoplankton and other parameters (e.g. chlorophyll-a and nutrients) should be taken at the same time.

The number of samples, sampling depth range and location of sample sites should be determined by the purpose of the study or sampling programme.

4.2 Selection of sampling sites

4.2.1 General

The location(s) where samples are taken should take into account the spatial variability of phytoplankton.

4.2.2 Sampling sites in rivers and streams

Sampling should ideally take place in the main flow of the river. Samples may be taken from a bridge or boat if it is not safe to enter the river or obtain access to the appropriate location from the river bank.

If samples are collected from engineered stretches, for example those with reinforced banking, they should be representative of the river section as a whole, including the more natural areas. For this reason it is recommended that the width of the river at the sampling point is not larger than twice the mean width in more natural sections, and that the average cross-sectional depth does not exceed the depth of the natural section by more than one third. If lateral heterogeneity is known or expected, several samples along a cross section should be taken and either be mixed or analysed separately.

Samples should ideally be collected from a completely mixed water column. This can be confirmed by vertical measurements of temperature and, whenever possible, chlorophyll fluorescence. River sections which are not completely mixed, such as harbors and impoundments, should be sampled using methods suitable for lakes (see 4.2.3 and 6.3).

4.2.3 Sampling sites in lakes

The spatial distribution of phytoplankton in lakes can be very variable, with vertical variation typically greater than horizontal variation. Wherever possible, phytoplankton samples should be taken from the open water as this enables consideration to be given to the variation in abundance and species composition within the water column; integrated samples can be taken through a known water depth or discrete samples can be taken at intervals through the water column. Only open water sampling locations allow to gather temperature and oxygen profile data to determine the degree of stratification and position of the thermocline. Additionally, to indicate variability in nutrient or chlorophyll concentrations in the whole water column, samples for chemical analysis can be collected.

The location of open water sampling sites are depending on the bathymetry of the lake. Generally, more sampling stations are recommended for elongated fjord lakes and reservoirs (riverine/transitional/lacustrine zone [4]), for lakes with several basins separated by shallow sills, and for lakes with isolated large bays [2], [13], [14]. For lakes with a homogenous morphology, at least one sample should normally be taken at the bathymetric deepest point (z_{max}) as this allows the most complete profile of probe data to be gathered; the centre of the lake may also be used, although this may not be the deepest point. The position of sampling points should be recorded (e.g. by GPS) and should preferably be used again for successive surveys.

NOTE It is well known that in lakes patchiness (small-scale spatial variability of the phytoplankton abundance and/or species composition) can occur. For some monitoring purposes it is advisable to integrate this variability by pooling samples taken from different points. Pooling reduces error in the characterization of the lake phytoplankton community without increasing the number of samples that need to be processed; however information about the horizontal patchiness is necessarily lost [4].

In lakes that are segmented by a distinctive ground topography, samples generally should be taken in each basin and treated separately, i.e. they should not be pooled. If the objective is to investigate the patchiness, all samples should be taken using the same sampling method.

Open water sampling usually requires the use of a boat. Provided that the potential limitations of shore sampling are considered, samples can also be collected from the lake shore, ideally at or near the outflow, or from a jetty or promontory. These can be collected using a bottle fixed to a long pole or a weighted bottle with float, on the end of a long rope thrown out into the lake, away from the immediate influence of the shore. Samples should not be collected near the inflow, or in areas likely to be influenced by hotspots of pollution, such as effluent discharges. Shore/outflow samples collect only the subsurface phytoplankton, and results can differ from open water sampling. Furthermore this method of sampling does not allow collection of information on stratification, or spatial distribution of phytoplankton. Samples collected from the shore/outflow provide an indication of the lake phytoplankton community and biomass and can be used in the context of large-scale monitoring programmes where some data from many lakes covering a wide geographic area is required rather than more detailed information from relatively few lakes.

4.3 Sampling frequency and replicates

The sampling frequency is very important. The required number of samples per year depends on the objectives of the investigation and the intended type of data evaluation. The period without ice cover (which may be all year depending upon local climatic conditions) is also important. At least monthly sampling during the growing season is recommended; the growing season is often March/April to October/November, but can be shorter or longer depending upon local weather patterns. For a reliable assessment of a water body's ecological quality, an investigation period of at least three years is recommended. In particular cases the sampling frequency may be reduced in accordance with the details given in Table A.2.

Phytoplankton species composition and biomass varies strongly through the seasons (associated with, for example, spring peak, clear water phase, stratification period, circulation period). Thus, the more samples are collected, the more representative the results will be for the actual status of the water body. Infrequent sampling increases the likelihood of misrepresenting the actual conditions (see A.3, [13]).

Replicates (multiple samples taken at the same location and same time) are only required when it is an objective of the investigation to get information on the spatial variability of phytoplankton biomass (or chlorophyll concentration) results received from the individual samples taken from one sampling point.

5 Equipment and preservatives

5.1 Equipment for sampling:

5.1.1 Suitable water sampler.

See Annex B for examples of suitable samplers; B.1 for samplers for rivers and B.2 for samplers for lakes.

5.1.2 Electrochemical/photo-optical probe with depth sensor to measure water temperature and dissolved oxygen according to EN ISO 5814 or ISO 17289.

5.1.3 pH probe (optional), e.g. according to EN ISO 10523.

5.1.4 Secchi disk according to EN ISO 7027 (see A.2 for further information).

5.1.5 Chlorophyll fluorescent probe with depth sensor (optional). A probe for total chlorophyll measurement is sufficient.

5.1.6 Boat (optional), suitable for local conditions with appropriate safety equipment.

5.1.7 Detailed bathymetric maps of the area of interest for preparation of the sampling design and localization of the sampling points.

5.1.8 Echo sounder and GPS, to localize the deepest point and the sampling stations on a water body.

5.1.9 Winch, with counter or rope with metre mark.

5.1.10 Mixing container, for example a plastic cask or bucket with lid (to protect the samples from sun light), for homogenizing integrated samples.

The size of the container depends on the sampled volumes.

5.1.11 Plankton net, generally for qualitative sampling only; mesh width from 5 µm to 25 µm, depending on the taxonomic target.

5.1.12 Sample bottles, according to EN 15204.

5.1.13 Lightproof closed containers, suitable for the transport of the sample bottles (e.g. cooling boxes).

5.2 Fixatives and preservatives:

Lugol's reagent is suitable for Utermöhl phytoplankton analysis, depending on pH (see EN 15204). See 5.2.1 and 5.2.2. for further specifications.

5.2.1 Alkaline Lugol's iodine, with sodium acetate for preservation of plankton from neutral or alkaline waters.

5.2.2 Acidic Lugol's iodine, with acetic acid for preservation of plankton from acidic waters.

5.2.3 Ethanol, C_2H_5OH, volume fraction 90 % to 96 %, for preservation of diatom or picoplankton samples.

5.2.4 Formaldehyde (optional).

Formaldehyde is appropriate for preservation of diatom or picoplankton samples.

6 Procedure

6.1 General requirements for phytoplankton sampling

A representative phytoplankton sample should be taken with a suitable water sampler (see Annex B for examples). The contact of the water sampler with the lake/river bed, macrophyte stands, the floor of the boat, mooring buoys or any surface from which sample contamination could occur, particularly benthic algae or sediment, should be avoided.

The phytoplankton sample should be filled immediately or after mixing in sampling bottles and preserved for later investigation according to EN 15204.

Sample bottles should be marked before sampling to avoid interchanging the samples. Waterproof marker pen or pencil should be used (ballpoint pens or regular marker pens are not appropriate). Writing directly on the glass is not recommended. Instead, water-resistant adhesive tape that can be labelled or water-resistant printed labels are recommended.

As a minimum, the following information should be recorded for each sample (included on the label (+) and in the sampling protocol, see Annex D):

— name of the water body (+);

— name of person(s) conducting the sampling;

— sampling date (+);

— sampling time;

— sampling position if appropriate (+);

— sampling depth (mixed, epilimnetic or euphotic depth) (+);

— type of sample (e.g. phytoplankton or pelagic diatoms; mesh width in case of net samples) (+);

— weather conditions;

— water level (rivers and reservoirs);

The sample should be labelled in a way which ensures that it can be clearly identified using the information given in the sampling protocol.

The sample bottles should be transported to the laboratory in lightproof, and if necessary cooled, containers.

If insufficient volume is collected with one fill of the sampling device, additional samples should be collected and pooled into a larger container prior to subsampling. Subsamples for phytoplankton and other parameters (e.g. chlorophyll-a and nutrients) should be taken from the same mixed sample.

Before using a water sampler it should be checked that the closing mechanism is working properly to ensure that unhindered water flow-through is achieved and the valve is closed.

When sampling open water, it should be ensured that the water sampler is lowered vertically to the required depth. When integrating samplers or hose samplers are used, it is important that the device is lowered slowly to the required depth.

The position of sampling points should be recorded by GPS to be found and used for each survey.

6.2 Sampling in rivers

The samples should be taken with a suitable sampler (e.g. a bucket, see B.1) from the main flow of the river.

Since phytoplankton organisms (especially diatoms) of rivers usually settle very rapidly, samples should be transferred quickly into the sampling bottles and only after prior homogenization [5].

6.3 Sampling in lakes

6.3.1 General

This section specifies the sampling in lakes of different depths, turbidity and phases of circulation or stratification and describes the sampling of the full vertical extent of the euphotic or epilimnetic zone under different conditions. For special objectives samples may be collected individually from predetermined depths (e.g. drinking water intake). When sampling from a boat, it is important to prevent drift during sampling.

If a nutrient analysis of the euphotic zone is required and the euphotic zone extends into the anoxic hypolimnion, care should be taken to take separate samples from the epilimnion and the meta- to hypolimnion down to the euphotic depth, because nutrient concentrations may be substantially higher in the meta- to hypolimnion.

6.3.2 Sampling in polymictic lakes

In polymictic lakes the whole water column should be sampled regardless of temporary stratification phases. The sampling zone comprises the water column down to a depth of 6 m, or 0,5 m to 1 m above sediment surface if z_{max} < 6,5 m to 7 m.

6.3.3 Sampling in stratified lakes during circulation

During phases of mixing, sampling in di- or monomictic lakes should take place to a maximum depth of 20 m or 1 m above the sediment surface if z_{max} is < 21 m.

6.3.4 Sampling in stratified lakes during phase of summer stagnation

In stable stratified lakes during summer stagnation the rationale is to sample down to whichever is deeper, euphotic depth or epilimnetic depth. A distinction is drawn between two different states (see Figure 1):

1) **Turbid** or **humic lakes** with euphotic depth < epilimnetic depth ($z_{eu} < z_{epi}$): Take an integrated sample from the epilimnetic zone.

2) **Clear lakes** with euphotic depth > epilimnetic depth ($z_{eu} > z_{epi}$): Take an integrated sample from the euphotic zone.

If a chlorophyll probe is available, the vertical extension of the water column to be sampled can be determined exactly, so that a deep chlorophyll maximum (DCM) will not be missed. The sample should be taken down to a depth including the DCM. See also 6.3 concerning a euphotic zone reaching into the hypolimnion.

If no chlorophyll probe is available, the euphotic depth can be determined using Formula (1):

$$z_{eu} = t \cdot z_{secc} \quad (1)$$

where

z_{eu} is the euphotic depth in metres (m);

z_{secc} is the Secchi depth in metres (m);

t is an empiric constant ranging from 0,8 to 2,5, depending on the turbidity of the lake [12]:

- under usual conditions in clear and turbid lakes: t = 2,5;
- in humic lakes usually: t = 1,0 (see Annex A, Figure A1).

NOTE Humic lakes have a colouration value ≥ 30 mg Pt/l.

Figure 1 provides a decision scheme for sampling in lakes and reservoirs.

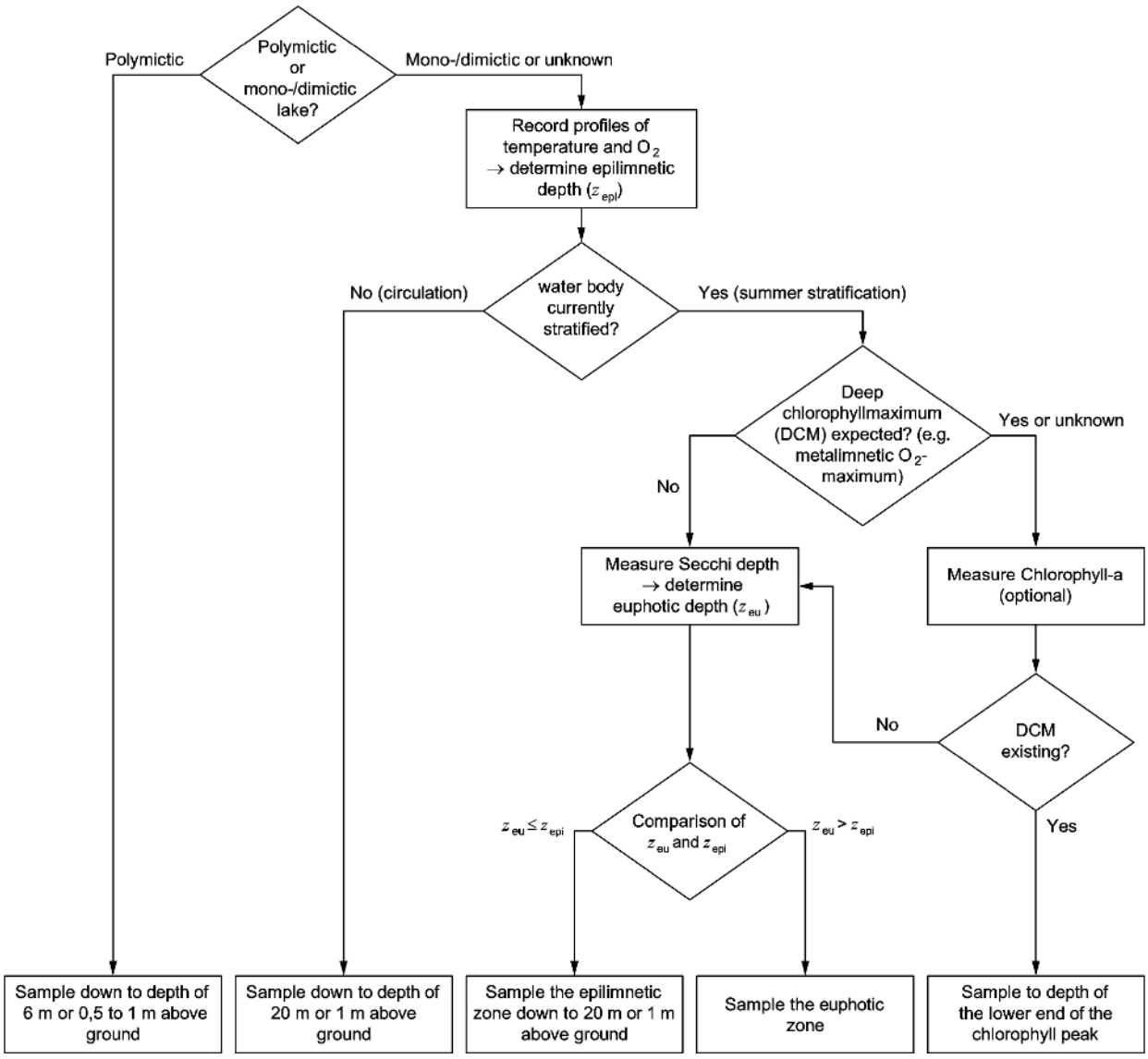


Figure 1 — Decision scheme for sampling in lakes and reservoirs

6.4 Preparation of mixed samples of a water column

6.4.1 Preparation of mixed samples using an integral water sampler

With an integral water sampler a sample of the complete water column can be taken continuously and in a time-saving manner. Prior to bottling the samples, the whole content of the integral water sampler should be filled into a mixing container (5.1.9) to eliminate any layering in the sampler. For sampling very small depth ranges integral water samplers are not recommended due to the very low lowering velocity required or the small volumes obtained, respectively.

6.4.2 Preparation of mixed samples using other water samplers

To collect a complete representative sample of the whole water column, universal water samplers or tube samplers with a length of 0,5 m to 2 m (5.1.1) should be used (see Annex B for examples). Samplers with a closing lid which does not uncover the complete cross-section area of the sampling tube while lowered in the water (e.g. Ruttnersampler) are not appropriate. A depth profile should be produced from sub-samples covering equidistantly the water column. The distance between the sampling depths should not exceed 1 m in shallow (i.e. polymictic) lakes and 2 m in deep lakes.

In cases where a deep chlorophyll maximum (DCM) occurs (recognized by detection of a distinctive maximum of oxygen, chlorophyll-a or pH in the depth profile), it is essential to take a sample from the corresponding depth too. With regard to chlorophyll-a, the conditions for a DCM are met when the maximum value is at least 1,5 times the average measured in the epilimnion. For the parameters oxygen and pH, no quantitative criterion is available.

Sub-samples collected from the different depths should be combined in a mixing container (see 5.1.10). The mixed sample should be protected from direct sunlight until it is filled into the sample bottle(s). The total volume of the mixed sample should be sufficient for the survey objectives. This should be observed especially when using an integrating sampler, where the sample volume can be very limited. The mixed sample should be homogenized by stirring carefully, but avoiding vortex formation. Do not shake the container to avoid destruction of sensitive phytoplankton organisms.

When samples are collected from the outflow or edge of lakes using a bottle on a long pole or weighted bottle on a long rope (see 4.2.3), it is assumed that the collected sample is fully mixed and sufficiently representative of the lake for the purposes of the sampling program.

6.5 Bottling and fixation of samples

The bottling of the sub-sample should be carried out using a clean bailer. The first part of the content of the cup is used for rinsing the sample bottle and is discarded afterwards. The bottles should be filled up to not more than 90 % of their volume or 2 cm to 3 cm below the opening. This is necessary to allow shaking and homogenization of the samples for withdrawal of sub-samples (see EN 15204).

For eutrophic to hypertrophic lakes 100 ml bottles and for oligotrophic to eutrophic lakes 250 ml bottles should be used. For lakes with a Secchi depth of more than 10 m a 500 ml sample should be bottled. 250 ml bottles are required when 50 ml sedimentation tubes are used so that more than one replicate filling of a counting chamber is possible. 500 ml bottles allow pre-sedimentation procedure (see EN 15204), but this step should be avoided if possible because this may introduce another source of error into the process.

The fixation using Lugol's solution (5.2.1, 5.2.2) should lead to a cognac coloured or light brown colour of the sample. For orientation: a 225 ml sample (using a 250 ml bottle) should be fixed with 8 to 10 drops of Lugol's solution (about 0,5 ml). After adding the solution, mix the sample by slowly tilting the closed bottle. The fixed sample should not be heated or exposed to direct sunlight during transport. It is recommended to use backup bottles which should be fixed in the same way.

6.6 Storage and transport of the samples

Preserved samples should be stored in clear narrow-necked glass bottles (not brown glass) in the dark at 4°C to 10 °C for not longer than 6 months prior to analysis (for details see EN 15204). The narrow neck minimizes the loss of iodine (and thus loss of preservative effect) by diffusing through the screw cap of the bottle. Transparent, clear glass bottles should be used to allow a check for colouration (cognac colour) of the samples at regular intervals. Plastic bottles are not suitable, because they do not allow for a visual control of the colouration as the plastic itself becomes tainted by the preservative. Another disadvantage is the loss of iodine diffusing through the plastic material. Beware of over-fixation (dark brown colour) and freezing of samples. The addition of formaldehyde as described by EN 15204 is only necessary for long term fixation and should be avoided. The samples should be stored preferably vibration free and necessarily standing upright (to avoid losses by leakage) in a dark place.

If the samples are to be stored before transport, they should be stored in a dark and cool place. The samples should be packed and sent to the laboratory conducting the analyses within 6 months. A sampling form listing sampling position, date and type of sample and additional sampling information (see 6.1) should be given together with the samples.

6.7 Additional samples for analysis of diatoms

If pelagic diatoms are to be identified to species level, an additional sample is necessary: in rivers at least 0,5 l and in lakes at least 1,0 l additional sample volume. The preservation should be carried out with ethanol (5.2.3) and should yield an ethanol concentration of 10 % or higher. Attention should be paid that the storage time after fixation with ethanol does not exceed 3 months. Preservation with formaldehyde may keep the samples longer but may also cause loss of fine structure elements of the valvae.

Another method for diatom preservation is the storage of a filtered unfixed sample. An unfixed sample should be filtered using a filter with smooth surface and a maximum pore diameter of 2 µm. In case of filter clogging, more than one filter for one sample should be used. After having filtered the sample, the filter should be stored air-dried and at room temperature [5], [6], [7].

6.8 Qualitative sampling

In some cases it is necessary to determine phytoplankton organisms alive (e.g. see EN 15204, [8]). In these cases a separate sample, taken according to 6.2 and 6.3, with a minimum volume of 500 ml and without any fixation, should be stored dark and cool for subsequent sedimentation in an Utermöhl chamber.

Alternatively a sample can be taken using a plankton net (5.1.12) to determine living phytoplankton.

Samples with living plankton organisms should be analysed within 24 h.

In case of studying ontogenetic stages and to enrich rare forms, net samples should be fixed using Lugol's solution (5.2.1, 5.2.2).

7 Measurements of accompanying parameters

7.1 General

Prior to sampling of phytoplankton in lakes, the sampling depth (epilimnion/euphotic zone) should be determined by measuring the following parameters:

— Secchi depth;

— water temperature;

— dissolved oxygen;

— chlorophyll-a, using a fluorescence probe (optional);

— pH (optional).

The results (especially for temperature and oxygen) should be recorded in a depth profile table (see Annex D). The relevant depth range for sampling should be measured using depth intervals of ≤ 1 m.

7.2 Secchi depth

Use a white disk attached to a measuring tape with a length of 20 m; the accuracy of the Secchi depth information should be 2 % of the mean reading value. Lower the disk, on its measuring tape, into the water until the disk is barely visible. Read the length of immersed measuring tape. Then lift the disk until it becomes visible again. Repeat the test several times. Calculate the average of the two readings. If no viewscope is used, choose the shaded side of the boat.

7.3 Water temperature

Use a device equipped with depth probe and a measurement range of 0 °C to 35 °C; accuracy 0,1 °C (see EN ISO 5814).

7.4 Dissolved oxygen

Use a device equipped with depth probe and a detection limit of 0,1 mg/l; accuracy 0,1 mg/l (see EN ISO 5814 or ISO 17289).

7.5 pH

Use a device equipped with depth probe with pH range from 3 to 12 and accuracy 0,2 pH units (see EN ISO 10523).

7.6 Chlorophyll-a

Use a device equipped with depth probe and a fluorescent sensor featuring a detection limit of 0,5 µg/l; accuracy 0,1 µg/l.

8 Quality Assurance

The quality assurance should be in accordance with EN 14996.

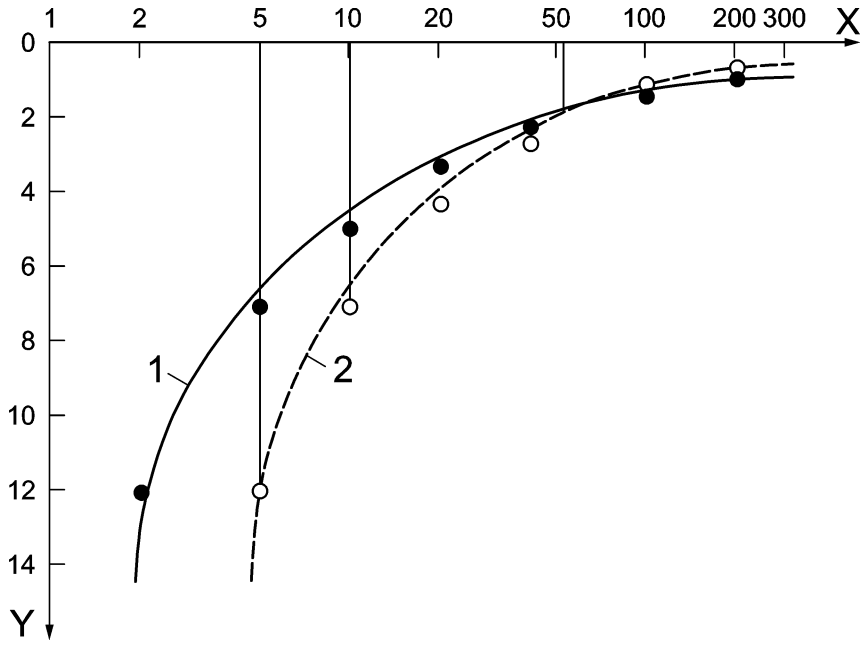
An example for a sampling protocol is given in Annex D.

Annex A
(informative)

Description of methodology

A.1 Water colour, Secchi-depth and euphotic depth

Figure A.1 displays the dependency of Secchi-depth and the euphotic depth (depth featuring 1 % of subsurface illumination) from water colour in humic lakes [13].



Key

Y depth in meters (m)

X water colour (g Pt/m^3)

1 Secchi depth visibility: sdv = 17,54 colour $^{-0,57}$

2 1 % of subsurface illumination: $z_{1\ \%}$ = 41,85 colour$^{-0,77}$

Figure A.1 — Relations between water colour and Secchi disk visibility and water colour and the depth with 1 % of subsurface illumination [13]

Table A.1 — Relations between Colour, absorption coefficient at 430 nm (g_{430}), Secchi depth (sdv) and the depth with 1 % of subsurface illumination ($z_{1\ \%}$)

Colour g Pt/m^3	**Absorption coefficient at 430 nm** m^{-1}	**sdv** m	$z_{1\ \%}$ m	**Ratio $z_{1\ \%}$/sdv**
2	0,150	11,8	24,3	2,1
5	0,376	7,0	12,1	1,7
20	1,502	3,2	4,2	1,3
40	3,004	2,1	2,4	1,1
100	7,511	1,3	1,2	0,9
200	15,022	0,9	0,7	0,8
colour (g Pt/m^3) = $5{,}19 \times 10^{-5} \times g_{430} \times e^{(0{,}415 + 0{,}028 \times 430)}$ [16]				

A.2 Secchi depth – practical hints

The minimum diameter of the disc is 20 cm for Secchi depths < 8 m. For Secchi depths ≥ 8 m larger discs are recommended. The disk attached to a measuring tape is lowered into the water until it is barely visible. The length of the immersed measuring tape is read. Then the disk is lifted until it becomes visible again. The test is repeated several times and the average of the two readings is calculated.

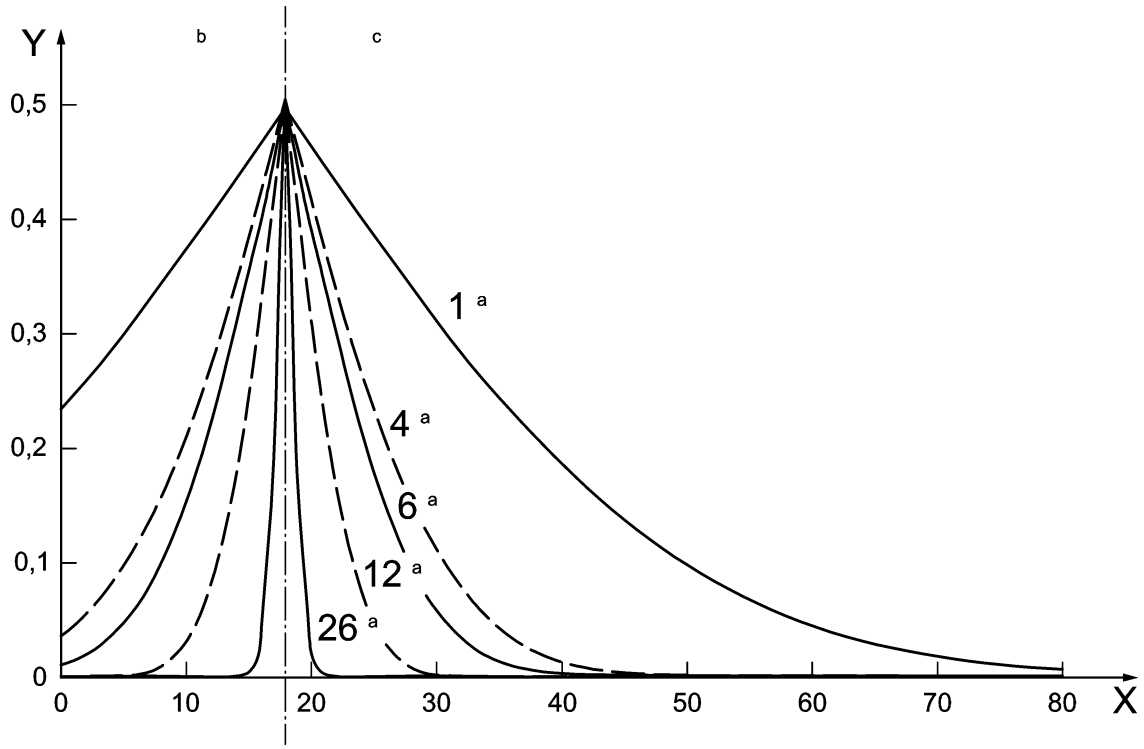
Usually a boat is necessary to measure the Secchi depth. Secchi depth can most easily and reliably be recorded by looking from a small distance above the water surface into the water. The best to avoid reflections from the water surface is to use a viewscope. This is a tube of ca. 18 cm diameter (head size), one end is closed leak-proof with transparent acrylic glass, and the shape of human face is moulded at the other end to avoid reflections by light beside the head of the observer. Very long tubes (>2 m) shall be conical, enlarged at the end with transparent acrylic glass. On ships with high vessel sides a viewscope is strongly recommended. If no viewscope is available the shaded side of the boat should be used. From bridges, the distance to the water surface usually will be too far for exact measurements. In this case, the use of a viewscope of appropriate length is required (practicable for distances up to approximately 3 m).

A.3 Sampling frequency – examples

The frequency and time of sampling for phytoplankton will be determined by the purpose of the study. For a general investigation of phytoplankton in lakes, taking a sample in the spring, summer and late summer will give an impression of the phytoplankton community with minimal resource requirements. Further samples may provide additional information. For some specific sites and objectives it may be useful to survey during the winter period.

For the purposes of phytoplankton monitoring for the Water Framework Directive (WFD, 2000/60/EC), the seasons and frequency of sampling which accords to the specific WFD method developed by each Member State should be used [9], [13]. For a monitoring program in rivers, according to the Water framework directive (WFD) six samplings per year are the minimum [5].

Figure A.2 provides an insight into the effect of sampling frequency on the risk of trophic mis-classification of a lake using chlorophyll (as a substitute parameter for phytoplankton biomass). It shows that when less than 4 samples per year from a lake were taken, the probability of trophic mis-classification was substantially higher than when more samples were collected [10].



Key

Y probability of trophic mis-classification (ecological quality class in accordance with WFD)

X estimate of annual mean chlorophyll-a concentration

a number of samples per year

b good ecological quality

c moderate ecological quality

Figure A.2 — Example showing the effect of within year sampling frequency on the probability of ecological status misclassification at a Good/Moderate boundary value (18 µg/l) for chlorophyll-a concentrations [10]

Table A.2 — Minimum proposed sampling frequencies for three phytoplankton metrics in three GIGs based on analysis of variability for chlorophyll-a within 6 months (April – September) and Phytoplankton Trophic Index (PTI) metrics and cyanobacteria biovolume within three summer months (July – September) [15]

	CB-GIG	M-GIG	N-GIG
Chlorophyll-a	3 months for 4 years	3 months for 3 years	2 months for 3 years or 3 months for 2 years
PTI	2 months for 4 years or 1 month for 6 years	3 months for 3 years or 1 month for 6 years	3 months for 3 years or 1 month for 6 years
Cyanobacteria biovolume	1 month for 6 years	1 month for 6 years	1 month for 6 years

Key
CB-GIG Central-Baltic Geographical Intercalibration Group lakes
M-GIG Mediterranean Geographical Intercalibration Group lakes
N-GIG Northern Geographical Intercalibration Group lakes
PTI Phytoplankton Trophic Index

For example for N-GIG chlorophyll-a should be sampled at least once in two different months (April to September) in each of three different years, or, alternatively, once in three different months (April to September) in each of two different years, meaning six samples together (see [13]). Where alternatives are given, these yield similar levels of uncertainty and the first alternative should not be considered optimal compared to the second.

Annex B
(informative)

Examples for suitable water samplers

B.1 Examples for sampling devices in rivers

B.1.1 General requirements

In rivers with a vertically fully mixed water column, sturdy buckets attached to a rope or to a pole are cheap and simple sampling devices that can provide sufficiently representative samples [19]. Care shall be taken not to sample surface scum or stir up benthic matter. Horizontal water samplers can be used in flowing waters for sampling matter transported in a specified depth, even close to the bottom.

B.1.2 Horizontal sampler

Horizontal water samplers (also: horizontal van Doorn samplers) have been developed to sample flowing waters for matter transported in a specified depth, even close to the bottom. The cylinder of this sampler is oriented horizontally, while its lids (or sealing balls) are kept open with elastics or springs and are closed by a messenger. Horizontal water samplers for use in flowing water should be equipped with steering fins to stabilize in flow direction. They also should have additional weight and reinforced spacers on the bottom of the cylinder for a stable position in the flow and in order to keep a certain distance to the river bottom. A horizontal water sampler with these features is shown in Figure B.1.

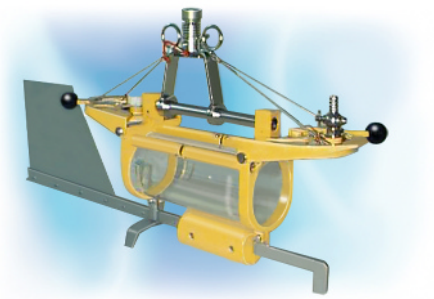


Figure B.1 — Horizontal sampler (Photo ©Hydro-Bios Kiel, Germany)

B.2 Examples for sampling devices in lakes

B.2.1 General requirements

Table B.1 describes exemplary the scope of application and advantages and disadvantages of some popular sampling devices for lakes [19]. Some of them are not commercially produced or out of production.

Table B.1 — Examples for phytoplankton sampling devices suitable for sampling in lakes

Device	Range of Application	Advantages	Disadvantages
Universal Sampler (Friedinger Sampler)	discontiguous depth profile	widespread, thus easily available	no continuous depth profile attainable
Tube Sampler	Discrete samples, but a contiguous set of samples for the whole depth profile can be obtained	big sample volume attainable (if desired)	bulky due to overall length of approximately 1 m to > 2 m
Integral Sampler	continuous depth profile	small sample volume attainable (if desired), rapid sampling	expensive, not suitable for small depth ranges
Hose	continuous depth profile	low-priced	small sample volume, prone to fouling (increased effort for cleaning and drying), prone to drift
Flask	surface sampling	no boat required, low-priced, rapid sampling	no depth profile attainable

B.2.2 Hose sampler

Components:

— silicone hose with an inner diameter of at least 1,6 cm (not a rubber hose);

— rope;

— weight (nozzle made of stainless steel at the intake end of the hose).

The hose is equipped with a nozzle made of stainless steel serving as a weight at one end, such that it hangs vertically in the water column. The weight at the end of the hose should be mounted above the hose opening to ensure proper functioning. A rope should be attached to the weighted lower part (see Figure B.2).

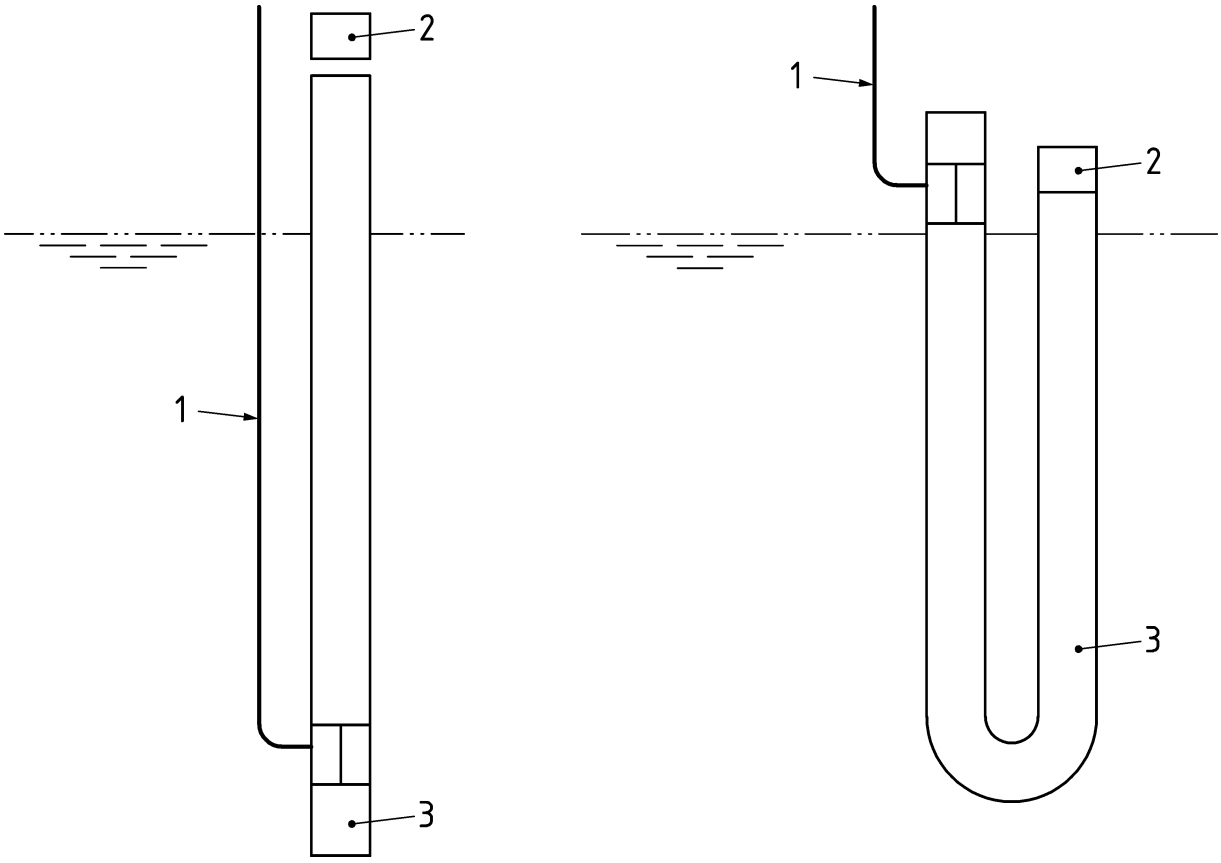
The weighted and open end of the hose is lowered slowly down into the water to the desired sampling depth such that it starts sampling from the surface. Hoses should only be used if they can be lowered exactly perpendicular to the water surface.

The open, upper end of the hose is tightly closed then with a cork and the lower end of the hose is slowly hauled up using the rope.

The hose should be raised slowly and water in it should be decanted into the mixing container.

After each sampling session the hose and the mixing container should be thoroughly washed with tap water. All equipment should be thoroughly dried before being stored for future use. The hose should be hung up to dry. It is important to dry the equipment quickly to prevent algal and bacterial growth within the hose.

It should not be used for any other purpose than sampling phytoplankton and accompanying parameters (e.g. nutrients and chlorophyll-a).



a) lowering of the hose **b) lifting of the hose to collect the sample**

Key

1 rope attached to the weighted lower end of the hose

2 cork

3 hose with weighted lower end

The weight on the hose shall be attached above the hose opening to work properly.

Figure B.2 — Principle of integrated sampling using a hose

Figure B.3 —Integrating sampling using a hose developed by Brandenburg University of Technology Cottbus–Senftenberg, Germany (Photos ©Hoehn, LBH, Germany)

B.2.3 Tube integrating sampler

The Pauli sampling system (Figure B.4) was developed as a simple approach to get large sampling volumes. It samples a complete water column in steps corresponding to the length of the tube (1 m or 2 m). The depth intervals are taken from the surface to the bottom of the lake. All aliquots are collected in a mixing container (5.1.10) (the volume depending on the depth to be sampled) on the boat. Two solid rubber balls close the ends of the tube by a elastic band. The rubber balls are drawn from the tube ends and hang to the mounting clamps. In the desired sampling depth the clamps are released by a messenger. In contrast, the Limnos sampler (Figure B.5) has closing flaps which are released by a messenger.

The content of the tube depends on the length and diameter of the tube.

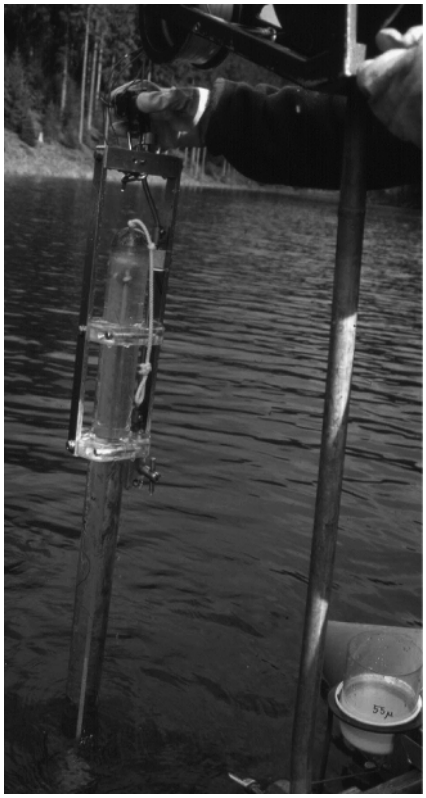


Figure B.4 — Tube integrating sampler as originally developed by Pauli (University of Konstanz, Limnological Institute, Germany, Photo ©Schmidt-Halewicz)

Figure B.5 — Tube integrating sampler by Limnos (Photo ©SYKE, Finland)

B.2.4 Mechanical integrating water sampler

This system was developed by Uwitec (Mondsee, Austria). The sampler takes integral samples of vertical water columns with 10 m or 20 m length. It samples 500 ml per metre of a water column of 10 m and 250 ml per metre of a water column of 20 m.

Operating mode: From its own weight a small chain turns a cogwheel and this raises a piston inside the 5 l Plexiglas tube. The rising of the piston is proportional to the lowering of the sampler and therefore water continuously enters the sampler. Sampling can start at the surface or from any required depth operated from a winch and started by a messenger (see Figure B.6).

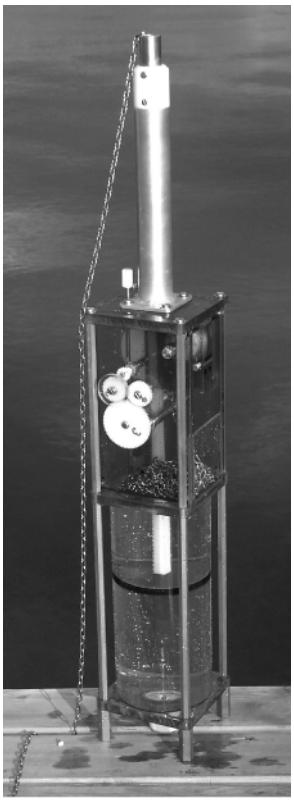
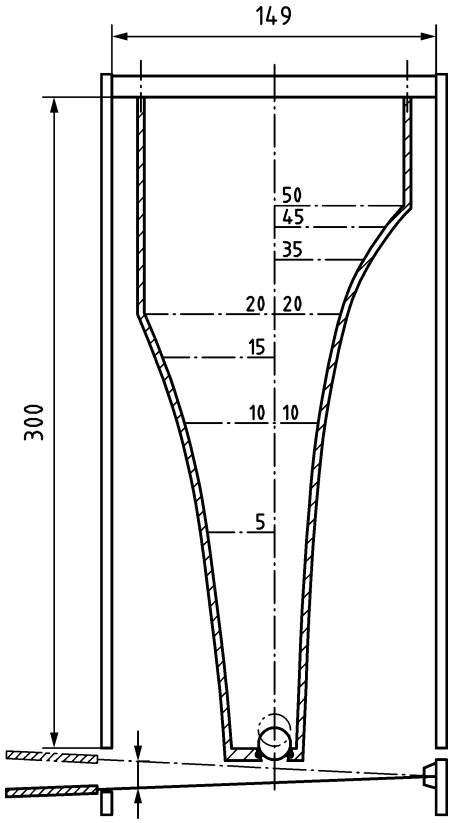


Figure B.6 — Mechanic integrating water sampler (Photo ©Uwitec Mondsee, Austria)

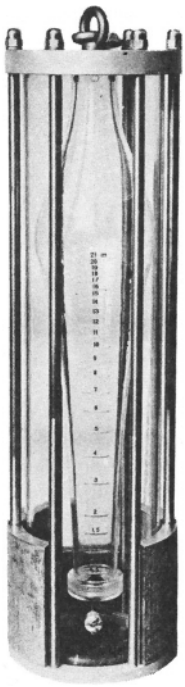
B.2.5 Hydrostatic integrating water sampler

The sampler was developed by Schröder [11] (see Figure B.7 a) and produced by Züllig (Rheineck, Swizerland). A continuous water column from the surface to a maximum depth of 21 m can be sampled. The sampler consists of a glass cylinder, which is open on the lower end and closed on the upper end. Inside the cylinder is a glass funnel with a hyperbolic shape. When the sampler is lowered (< 1 m/s) water enters the funnel through an open valve. Due to the hyperbolic shape of the funnel the water enters commensurately to the depth, the air inside the cylinder is compressed depending on the depth. When lifted quickly the outside flowing water closes the valve and the sampled water remains in the funnel. To compensate for the buoyancy the cylinder is mounted in a metal frame with a weight of 7 kg. The device samples 40 ml per metre (see Figure B.7 b).

Dimensions in millimetres



a)



b)

Figure B.7 — Hydrostatic integrated water sampler (Photo ©Züllig Rheineck, Switzerland)

B.2.6 Electronic integrating water sampler

The electronic integrating water sampler (IWS) has been developed by Hydro-Bios (Kiel, Germany). The desired depth range (start and final depth) is entered and stored in the sampler via a hand-held terminal. The recommended speed for lowering (slowly) is shown in the display. The electronics will regulate if lowering is not constant – e.g. due to moving of the boat or swell. After reaching the final depth the sampler can be lifted with a total content of 2,5 l or 5 l. Due to the freely selectable depth range it is possible to get samples from a specific segment of the water column (see Figure B.8).

Figure B.8 — Electronic integrating water sampler (Photo ©Hydro-Bios Kiel, Germany)

B.3 Sampling equipment cleaning

To prevent spreading of flora and fauna or fish diseases between water bodies, the sampling equipment should be cleaned between use in different water bodies. After each day of sampling, especially with trophically different water bodies, plankton nets should be washed in - preferably warm - pure tap water with detergent or in an ultrasonic water-bath in order to reduce clogging and ensure optimum filtration capacity. Equipment such as water samplers, hoses, plankton nets and mixing containers should be rinsed in pure tap water and dried before they are stored for future use. Hoses should be hung up to dry. Rapid drying is essential to prevent growth of algae and bacteria within hoses. If the water sampler is used also for nutrient samples beware of contamination with phosphates from the tap water.

Annex C
(informative)

Determination of the depth gradient

Patalas [18] analysed mid-summer mixing depth of lakes of different latitudes (Poland, North America, Central Canada, Japan). It was found that the mixis regime can be derived from the maximum depth (z_{max}) and the theoretical epilimnion depth (z_{epi}). The theoretical epilimnion depth can be calculatetd from the fetch. These parameters are the base for the calculation of the depth gradient F [17], which allows to distinguish between polymictic and stable stratified lakes during summer stagnation in the temperate region.

$$F = z_{max} / z_{epi} \tag{C.1}$$

The theoretical depth of the epilimnion (z_{epi}) can be calculated as follows:

$$z_{epi} = 5,81 \cdot D_a^{0,28} \tag{C.2}$$

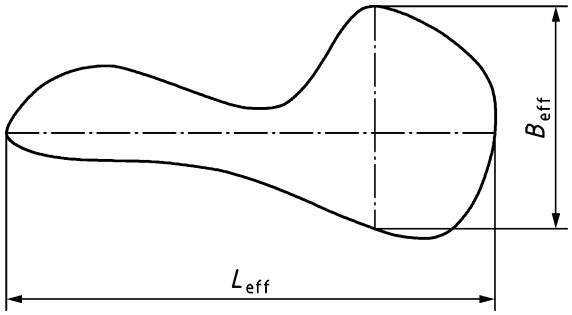
The effective axis length (D_a) can be seen as a measure for the possible effect the wind has on the surface water (fetch). It can be calculated according to:

$$D_a = \frac{L_{eff} + B_{eff}}{2} \tag{C.3}$$

where

L_{eff} is the effective length;

B_{eff} is the effective width (see Figure C.1).



Key

L_{eff} effective length

B_{eff} effective width

Figure C.1 — Effective length and width of a lake [17]

The effective length and width result from the maximum extent of the lake - whereupon the axes shall be perpendicular to each other.

Now, the depth gradient can be calculated with:

$$F = z_{\max} / 4,785 \cdot (L_{\text{eff}} + B_{\text{eff}})^{0,28} \quad \text{(C.4)}$$

Values of $F > 1,5$ indicate a thermally stable stratified lake.

Annex D
(informative)

Example for a sampling protocol

This sampling protocol is applicable for lakes and rivers. Some of the headings specific for lakes can be neglected for rivers.

<table>
<tr><th colspan="3">Sampling protocol</th></tr>
<tr><td colspan="3">Water body</td></tr>
<tr><td colspan="2">Sampling site</td><td>Sampling site coordinates</td></tr>
<tr><td colspan="2">Gauge height [m a. s. l. or cm]</td><td>Person(s) sampling</td></tr>
<tr><td colspan="2">Date</td><td>Time (daylight-saving time?)</td></tr>
<tr><td colspan="3">Weather (degree of cloudiness, wind intensity and direction, air temperature [°C])</td></tr>
<tr><td colspan="3">Water body peculiarities</td></tr>
<tr><td>Secchi depth [m] when disk is lowered / lifted</td><td rowspan="3">Sampling device</td><td>Sampling depth range [m]
(water chemistry epilimnion)
0 -</td></tr>
<tr><td>Ground visible:
yes / no</td><td>Sampling depth range [m] (water chemistry meta-/hypolimnion)</td></tr>
<tr><td>Depth at sampling site [m]</td><td>Sampling depth [m]
(water chemistry above ground)</td></tr>
<tr><td>Euphotic depth [m]</td><td rowspan="3">Depth profile probe</td><td rowspan="3">Depth profile for nutrients taken: yes / no</td></tr>
<tr><td>Epilimnion depth [m]</td></tr>
<tr><td>Sampling depth range [m]
(phytoplankton and chlorophyll)
0 -</td></tr>
<tr><td colspan="3">Comments</td></tr>
</table>

Depth profile						
Depth m	**Temperature** °C	**pH**	**Oxygen concentration** mg/l	**Oxygen saturation** %	**Chlorophyll-a concentration** µg/l	**Other**

Bibliography

[1] SCHWOERBEL J. *Methods of hydrobiology (freshwater biology)*. Pergamon Press, Oxford, 1970, pp. 200

[2] OLRIK K., BLOMQVIST P., BRETTUM P., CRONBERG C., ELORANTA P. (1998): Methods for quantitative assessment of phytoplankton in freshwaters, part 1, Swedish Environmental Protection Agency, report 4860. pp. 85

[3] SOURNIA A., ed. *Phytoplankton manual. Monographs on Oceanographic Methodology 6*. UNESCO, Paris, 1978

[4] KIMMEL B.L., LIND O.T., PAULSON L.J. (1990): Reservoir primary production. In: Thornton, K.W., Kimmel, B.L. & Payne, F.E. [Eds.]: Reservoir limnology. John Wiley & Sons, New York pp. 133-192

[5] MISCHKE U., BEHRENDT H. (2007): Handbuch zum Bewertungsverfahren von Fließgewässern mittels Phytoplankton zur Umsetzung der EU-Wasserrahmenrichtlinie in Deutschland. WeißenseeVerlag. pp. 88 ISBN 978-3-89998-105-6. LAWA-Projekt 6.03. http://unio.igb-berlin.de/abt2/mitarbeiter/mischke/

[6] NIXDORF B., HOEHN E., RIEDMÜLLER U., MISCHKE U., SCHÖNFELDER I. Probenahme und Analyse des Phytoplanktons in Seen und Flüssen zur ökologischen Bewertung gemäß der EU-WRRL (III-4.3.1). Handbuch Angewandte Limnologie - 27. Erg. *Lfg.* 2010, **4/10** pp. 1–24

[7] NIXDORF B., HOEHN E. Riedmüller, Mischke, U., Schönfelder, I. & Bahnwart, M. (2008): Anforderungen an Probenahme und Analyse der Phytoplanktonbiozönosen in Seen zur ökologischen Bewertung gemäß der EU-WRRL. In: Mischke, U. & Nixdorf, B. (Eds.), Gewässerreport (Nr. 10), BTUC-AR 2/2008, University Cottbus: pp. 147-183. http://www-docs.tu-cottbus.de/gewaesserschutz/public/aktuelle_reihe/2008_ar_10.pdf

[8] Hoehn, E., Clasen, J., Scharf, W., Ketelaars, H.A.M., Nienhüser, A.E., Horn, H., Kersken, H. & Ewig, B. (1998): Erfassung und Bewertung von Planktonorganismen. ATT-Technische Information Nr. 7., 2. völlig neu bearbeitete Aufl. Arbeitsgemeinschaft Trinkwassertalsperren e.V., AK-Biologie, Gummersbach Kommisionsverlag Oldenbourg. pp. 151

[9] MISCHKE U., RIEDMÜLLER U., HOEHN E., SCHÖNFELDER I., NIXDORF B. (2008): Description of the German system for phytoplankton-based assessment of lakes for implementation of the EU Water Framework Directive (WFD). In: Mischke, U. & Nixdorf, B. (Eds.), Gewässerreport (Nr. 10), BTUC-AR 2/2008, University Cottbus: pp. 117-146 http://www-docs.tu-cottbus.de/gewaesserschutz/public/aktuelle_reihe/2008_ar_10.pdf

[10] CARVALHO L., PHILLIPS G., MABERLY S.C., CLARKE R. (2006): Chlorophyll and Phosphorus Classifications for UK Lakes, SNIFFER, Edinburgh, Environment Agency, Project WFD38, Final report, pp. 81

[11] SCHRÖDER R. Ein summierender Wasserschöpfer. *Arch. Hydrobiol.* 1969, **66** pp. 241–243

[12] ELORANTA P. (1999): Water colour and Secchi disc visibility. In Keskitalo, J. & Eloranta, P. [eds.], Limnology of Humic Waters. Backhuys Pulishers, Leiden. pp. 70

[13] CARVALHO L., POIKANE S., LYCHE SOLHEIM A., PHILLIPS G., BORICS G., CATALAN J. et al. Strength and uncertainty of lake phytoplankton metrics for assessing eutrophication impacts in lakes. *Hydrobiologia*. 2013, **704** pp. 127–140. DOI:10.1007/s10750-012-1344-1

[14] THACKERAY S.J., NÕGES P., DUNBAR M., DUDLEY B.J., SKJELBRED B., MORABITO G. et al. Quantifying uncertainties in biologically-based water quality assessment: a pan-European analysis of phytoplankton community metrics. *Ecol. Indic.* 2013, **29** pp. 34–47

[15] Commission decision on intercalibration: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:266:0001:0047:EN:PDF

[16] CUTHBERT I.D. & DEL GIORGIO P. (1992): Towards a standard method of measuring color in freshwater. Limnol. Oceanogr. 37 (6) pp. 1319–1326

[17] LAWA (Länderarbeitsgemeinschaft Wasser) (1999): Gewässerbewertung - Stehende Gewässer. Vorläufige Richtlinie für eine Erstbewertung von natürlich entstandenen Seen nach trophischen Kriterien. Kulturbuchverlag, Berlin, pp. 74

[18] PATALAS K. Mid-summer mixing depths of lakes of different latitudes. *Verh. Internat. Verein. Limnol.* 1984, **23** pp. 97–102

[19] EN 14996, *Water quality - Guidance on assuring the quality of biological and ecological assessments in the aquatic environment*

This page deliberately left blank

This page deliberately left blank

British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards -based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at bsigroup.com/standards or contacting our Customer Services team or Knowledge Centre.

Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at bsigroup.com/shop, where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to bsigroup.com/subscriptions.

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

PLUS is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit bsigroup.com/shop.

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email bsmusales@bsigroup.com.

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK

Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

Copyright

Useful Contacts:

Customer Services
Tel: +44 845 086 9001
Email (orders): orders@bsigroup.com
Email (enquiries): cservices@bsigroup.com

Subscriptions
Tel: +44 845 086 9001
Email: subscriptions@bsigroup.com

Knowledge Centre
Tel: +44 20 8996 7004
Email: knowledgecentre@bsigroup.com

Copyright & Licensing
Tel: +44 20 8996 7070
Email: copyright@bsigroup.com

bsi.

...making excellence a habit.™