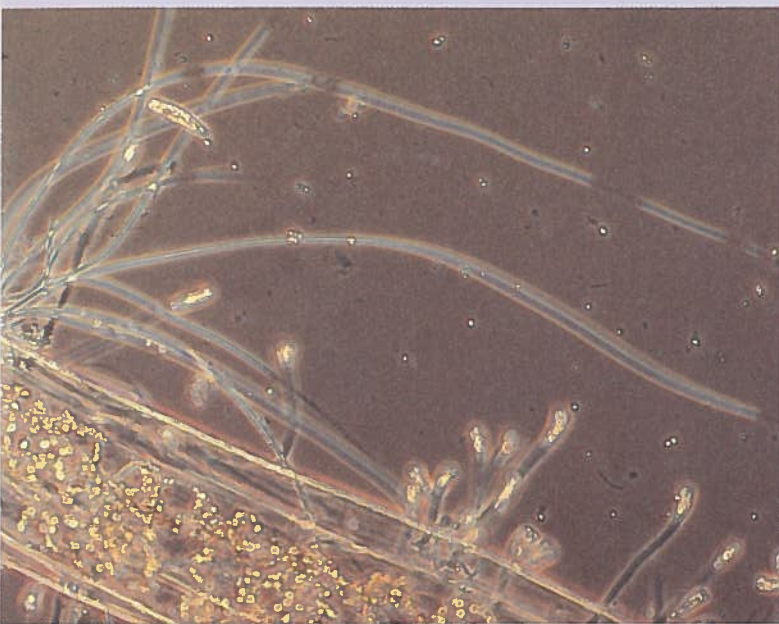
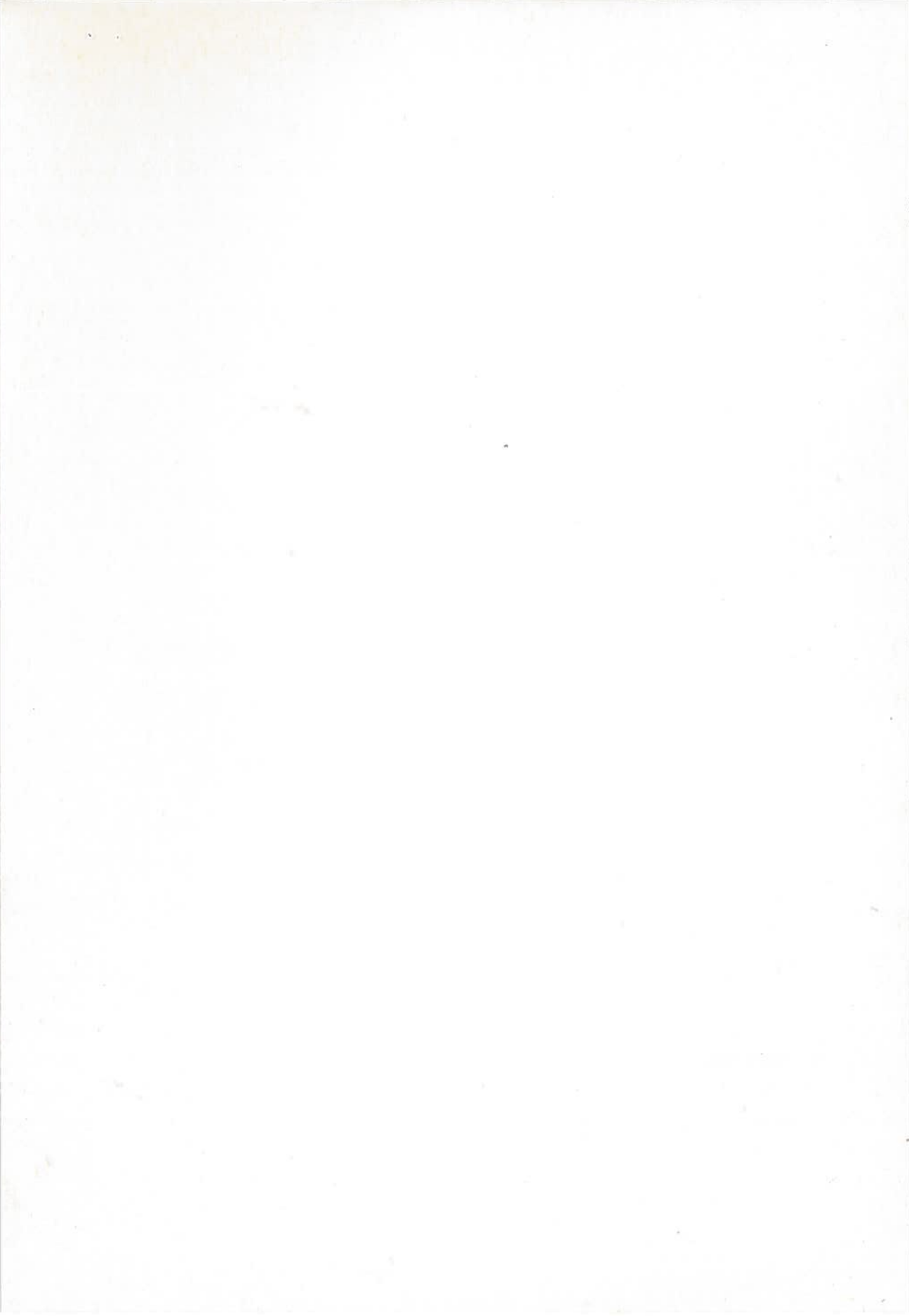


16 Manual for Integrated Monitoring

Jorma Keskitalo & Kalevi Salonen

Subprogramme Hydrobiology of Lakes





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Manual for Integrated Monitoring

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Cover photos

Top left: Blue-green algae and diatoms on a filamentous green alga.

Photo: Jorma Keskitalo

Bottom left: Zooplankton (*cladoceran, rotifer*).

Photo: Kirsi Kuoppamäki

Right: Macroalga (*Chara tomentosa*).

Photo: Jorma Keskitalo

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Manual for Integrated Monitoring. Subprogramme Hydrobiology of Lakes.

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*Commissioned by**Date of assignment**Abstract*

This manual describes hydrobiological field and laboratory methods in use by the International Co-operative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems (ICP/IM Programme). ICP/IM Programme is a multidisciplinary ecosystem monitoring programme for the United Nations Economic Commission of Europe (ECE).

The Minimum programme includes phytoplankton primary production, chlorophyll concentration, respiration of plankton, as well as species composition and biomass of zoobenthos. The Optimum programme further includes sedimented matter, species composition and biomass of phytoplankton, bacterioplankton, zooplankton, aquatic macrophytes and fish. The sampling frequencies are adjusted to match the length of life cycles of organisms thus making it possible to link changes in the environment with changes in biota and to reveal causal relationships between organisms. The rather good coverage of all trophic levels of plankton also makes the programme capable of providing information on unforeseen changes.

No methods are likely to be universal for all situations, but the proposed ones are simple and robust and hence considered reliable in most cases. Attention has been paid to the integration of different procedures so that minimum effort would be needed in the field and in the laboratory.

Continuous quality control of the determinations should be an essential part of Integrated Monitoring methods. Samples should also be documented by photography, and the creation of a digitized computer image library is encouraged. Some original samples should be deposited in a repository for control purposes and retrospective investigations.

Keywords

Environment, environmental monitoring, integrated monitoring, biological methods, water ecosystem

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Julkaisun nimi

Ympäristön yhdenntyn seurannan vesibiologiset tutkimukset.
Menetelmäkäsikirja.

Julkaisun laji

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Tiivistelmä

Käsikirjassa esitetään vesibiologisten tutkimusten kenttä- ja laboratoriomenetelmät ympäristön yhdenntyelle seurannalle, joka on YK:n Euroopan Talouskomission alainen kansainvälinen monitieteellinen yhteistyö-ohjelma. Siinä tutkitaan ilmasta tulevan laskeuman aiheuttamia muutoksia ekosysteemeissä.

Vesibiologisten tutkimusten minimiohjelmaan kuuluvat kasviplanktonin perustuotanto, klorofyllipitoisuus, yhteisöhengitys ja pohjaeläimet. Optimiohjelmaan kuuluvat edellisten lisäksi pohjalle laskeutuva aines, kasviplanktonlajisto ja -biomassa, bakteeriplankton, eläinplankton, suurvesikasvit ja kalasto. Näytteenottotäajuuudet on sovitettu riittävän tiheiksi suhteessa eliöiden elinkiertojen pituuteen. Näin biologiset muutokset voidaan suhteuttaa veden laadun muutoksiin ja eri eliöryhmien väliset vuorovaikutussuhteet voidaan selvittää. Koska valitut muuttujat kattavat kaikki planktonin trofiatasot, ohjelma on mahdollisimman hyvin varautunut myös ennalta arvaamattomien kysymysten selvittämiseen.

Ehdotetut menetelmät eivät todennäköisesti sellaisinaan sovellu kaikkiin mahdollisiin olosuhteisiin, mutta niillä voidaan useimmissa tapauksissa saada luotettavia tuloksia. Kenttä- ja laboratoriomenetelmät on pyritty valitsemaan siten, että koko ohjelman suoritus olisi mahdollisimman vaivatonta ja aikaa kului mahdollisimman vähän.

Jatkuva tutkimustyön ja tulosten laaduntarkkailu kuuluu olennaisena osana yhdenntettyyn seurantaan. Näytteitä dokumentoidaan valokuvaamalla sekä muodostetaan atk-perusteinen kuvantallennus- ja arkistointijärjestelmä. Varsinaisen seurantatutkimuksen lisäksi muodostetaan näytepankki myöhempiä varmistuksia ja mahdollisia jatkotutkimuksia varten.

Asiasanat (avainsanat)

Ympäristö, ympäristönvalvonta, yhdenntetty seuranta, biologiset menetelmät, vesiekosysteemit

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I handboken presenteras de vattenbiologiska undersökningarnas fält- och laboriemetoder, som används i den integrerade miljöövervakningen, som är ett internationellt tvärvetenskapligt projekt, där förändringar i ekosystemen orsakade av luftburet nedfall undersöks. Det är ett av de internationella samarbetsprogrammen inom Förenta Nationernas Europeiska Ekonomiska Kommission.

Objekt för minimumprogrammet är växtplanktons primärproduktion, klorofyllhalt, planktonsamhällets respiration och bottenjur. Till optimiprogrammet hörs dessutom avsättbara ämnen i vattnet, växtplanktonflora och -biomassa, bakterieplankton, djurplankton, makrofyter och fiskbestånd. Provtagningsfrekvensen för variablerna har anpassats till att stå i proportion till längden på organismernas livscyklar så att förändringarna kan kopplas till ändringarna i vattenkvaliteten och så att olika organismsgruppers beroendeförhållanden kan utredas. Då programmet omfattar planktonsamhällets alla trofinivåer, har det god beredskap att lösa oväntade problem.

Fastän de föreslagna metoderna troligen inte lämpar sig till alla tänkbara situationer, kan man med dem i flesta fall relativt lätt uppnå tillförlitliga och jämförbara resultat. Fält- och laboriearbetena har koordinerats så att tidsinbesparingen blir så stor som möjligt.

En kontinuerlig kontroll av metoderna och resultaten är en väsentlig del av övervakningen. Proven dokumenteras genom fotografering samt genom att bygga upp ett adb-baserat bildlagrings- och arkiveringssystem. Förutom den egentliga övervakningsforskningen bildas en provbank för senare kontroller och eventuella fortsatta undersökningar.

Nyckelord

Miljö, miljöövervakning, integrerad miljöövervakning, biologiska metoder, vattensystem

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Contents

PREFACE.....	8
1 INTRODUCTION	9
2 AN OVERVIEW OF THE MONITORING METHODS	11
3 PHYTOPLANKTON	14
3.1 Primary production	14
3.2 Chlorophyll concentration	16
3.3 Phytoplankton flora and biomass	18
4 BACTERIOPLANKTON.....	22
4.1 Bacterial density and biomass	22
4.2 Decomposition	23
5 ZOOPLANKTON	26
5.1 Protozoan plankton	26
5.2 Zooplankton (excluding Protozoa)	26
6 AQUATIC MACROPHYTES	28
6.1 Aerial and field mapping of vegetation	28
6.2 Permanent vegetation transects	29
7 ZOOBENTHOS	31
8 FISH	34
9 SEDIMENTATION	36
10 QUALITY CONTROL	38
11 LITERATURE	39

PREFACE

The International Co-operative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems (ICP/IM Programme) is a multidisciplinary monitoring programme under the auspices of the United Nations Economic Commission for Europe (UN/ECE). The pilot phase of the programme ran from 1989 to 1991 and the ICP/IM Programme was begun in 1992. The programme detects the effects of long-range air pollutants on natural ecosystems. To date, 23 countries have attended the programme.

The subprogramme Hydrobiology of Lakes is one of the 21 subprogrammes of the ICP/IM Programme. The present subprogramme manual was prepared to harmonize the hydrobiological methods used by the participating countries for obtaining comparable data. In addition to the field and laboratory methods included in the Manual for Integrated Monitoring, Programme Phase 1993-1996 (EDC 1993), this subprogramme manual also contains methods of optimum hydrobiological monitoring for those wishing to collect more detailed information for national purposes. On the basis of Finnish experiences, we recommend the use of the additional parameters for obtaining a better understanding of the state of the ecosystem.

The manual was presented in 1992 at the Fourth Task Force Meeting of the Pilot Programme on Integrated Monitoring and the countries were invited to apply it according to national circumstances. The preparation of the manual was funded by grants from the Ministry of the Environment, Finland.

National Board of Waters and the Environment

1 Introduction

The International Co-operative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems (ICP/IM Programme) is a multidisciplinary programme designed to observe the effects of long-range atmospheric pollution on natural ecosystems and to monitor the possible effects of climatic change. The ICP/IM Programme is conducted in specially selected study areas with minimum local human interference. In Finland, all monitoring areas include a headwater lake and its catchment area.

This manual describes detailed hydrobiological field and laboratory methods for the Subprogramme 'Hydrobiology of Lakes' of the ICP/IM Programme. The idea is to focus the monitoring of lake ecosystems on determinations that will register environmental change with a high degree of sensitivity. Extensive monitoring of many biological communities is warranted because the programme must be capable of providing information on unforeseen changes.

The minimum set of hydrobiological variables recommended in the ICP/IM Programme is briefly described in the Manual for Integrated Monitoring (EDC 1993). Here it is referred to as the 'Minimum programme' whereas the more extensive set of variables is called the 'Optimum programme' (Tables 1 and 2).

The organism groups dealt with in this manual are: 1) phytoplankton, 2) bacterioplankton, 3) zooplankton, 4) macrophyte vegetation, 5) zoobenthos and 6) fish. Within the biological

monitoring programme, sedimented matter is also studied. The physical and chemical characteristics of the water (temperature, pH, alkalinity, colour, conductivity, oxygen, inorganic nitrogen (NH_4^+ , NO_2^- , NO_3^{2-}), total nitrogen, inorganic phosphorus and total phosphorus) should be determined together with the biological parameters. These determinations, however, are not dealt with in this manual. Meteorological conditions must also be considered in interpreting the results; thus observation data from a nearby meteorological station should be made available.

In addition to the actual monitoring programme, samples are taken and stored in an environmental sample repository for control purposes, sample comparison and future needs. The biological samples should also be documented as extensively as possible by photographic or equivalent means. In addition, continuous quality control must be carried out as part of the monitoring procedure to ensure the reliability of the data.

The sampling frequency should be adjusted to match the life cycles of the aquatic organisms under study, which are often short; e.g. it is impossible to correlate phytoplankton biomass and population fluctuations to environmental or other biological parameters if the sampling intervals are too long for changes to be accurately detected. If continuous monitoring is impossible, the best alternative is probably to do the

monitoring intensively, but only every second year. For the Optimum programme (Table 1), parameters illustrating phytoplankton assemblages are included because they have been proved to be sensitive indicators of environmental changes (Schindler 1987).

Deviations from the procedures described in this manual should be avoided, although some modifications may be necessary due to local conditions. When a new method is to be adopted for monitoring purposes, the differences between the old and the new methods must be thoroughly tested.

In the compilation of this manual we have received valuable advice from numerous spe-

cialists, of whom Lauri Arvola, Lauri Heitto, Anna-Liisa Holopainen, Maija Huttunen, Timo Kairesalo, Kai Kivi, Esa Koskenniemi, Harri Kuosa, Jorma Kuparinen, Martti Rask, Jouko Sarvala, Matti Sipponen, Heikki Toivonen and Matti Verta deserve special mention. The Finnish Ministry of the Environment circulated draft versions of this manual and received critical and constructive commentaries from the countries participating in the ICP/IM Programme. They have been taken into account in compiling this final version. Heikki Simola translated the manuscript from Finnish to English, and his translation was revised by the English Centre, Helsinki.

2 An overview of the monitoring methods

The multidisciplinary and integrated nature of environmental monitoring sets demanding requirements on the planning, field and laboratory work and data-processing of all the component studies.

In the field, utmost care should be taken to ensure the representativeness of samples. Proper training of the field personnel by the scientists in charge is therefore essential. Unnecessary errors due to unclear instructions or faulty communication should be avoided.

Sampling sites must be well documented. In the pelagial area of each lake, there should be one site for the determination of physico-chemical and metabolic parameters and two sites for plankton assemblage monitoring. The sites should be marked with steadily anchored floats. If damage or displacement of the floats by ice is to be expected, they should be removed for the winter. During each sampling week, the plankton samples and the physical and chemical samples must be taken on the same day. Due to practical factors, the preferred order for the taking of samples (Table 1) is: 1) physical and chemical samples, 2) primary production and respiration samples, 3) other phytoplankton, bacteria and zooplankton samples. The samples in group 1 are to be taken directly from the water sampler and those in group 2 and 3 as subsamples from pooled samples from each depth or depth zone. The sampling depths are given in sections 3.1 and 3.2. The sampling should always be

started in the morning and concluded by noon. The temporal sequence should be kept constant for each sampling occasion - for example: group 1 samples taken from 9 to 10 o'clock; group 2 samples from 10 to 11 o'clock; group 3 samples from 11 to 12 o'clock. However, the group 2 samples can be taken first, if it is otherwise impossible to start the primary production experiment during the forenoon. Once chosen, the sequence should not be varied. More detailed instructions are given in the respective Chapters (3-5). The site selection criteria and methods for the other hydrobiological determinations are given in Chapters 6-9.

Disturbance of water stratification should be avoided when approaching the sampling site and during sampling, especially in the cases of thin epilimnion. During transport, all samples should be protected from light and heat by placing them in an insulated box with crushed ice or cooling elements. Fixatives (as appropriate) should be added to the samples at the first occasion, preferably in the field. Sampling equipment and sample bottles must be clean for the various specific purposes, and sample contamination must be avoided at all stages of the work.

Sampling in ice-covered lakes during the winter will cause some special problems. A hole may be made in the ice with a drill or an ice-saw; chainsaws should not be used because they can cause contamination by oil. Ice fragments must be shovelled out of the hole before sampling,

and not “pumped” up with the ice-drill, as this may destratify the site. The thickness of snow and ice (separately) and the ice quality should be recorded on the sampling form. Freezing of sampling gear and samples may be a problem in winter, and a sampling tent where an above-zero temperature can be maintained in the field

(e.g. with a gas-heater) may be necessary. Glass-stoppered sample bottles break very easily at sub-zero temperatures. Keeping the bottles immersed in lake water in an insulated box is a practical way of preventing samples from freezing.

Table 1. Minimum and optimum sampling protocol and sequence for physical-chemical measurements, plankton and sedimentation in the subprogramme "Hydrobiology of Lakes". Vertical extent: E = samples from epilimnion only, EH = samples from epilimnion and hypolimnion. D = samples from discrete depths, C = column samples.

	Monthly frequency ¹⁾		Sampling sites	Vertical extent	Samples
	Minimum	Optimum			
1. Physical and chemical samples	1	4	1	EH	D
2. Primary production	1	4	1	E	D
Dark fixation	1	4	1	E	D
Respiration	1	4	1	EH	D
3. Chlorophyll concentration	1	4	2	EH	C
Phytoplankton flora and biomass	-	4	2	EH	C
Bacterioplankton ²⁾	-	4	2	EH	C
Protozoan plankton ²⁾	-	4	2	EH	C
Zooplankton (excl. Protozoa)	-	2	2	EH	C
4. Sedimentation ³⁾	-	2	1		

1) During ice cover all samples monthly

2) May be analyzed in the phytoplankton flora and biomass sample

3) Sediment traps continuously in water; sampling day may differ from that of other samples.

Table 2. Field investigation frequency for macrophytes, benthic animals and fish in the subprogramme "Hydrobiology of Lakes".

	Yearly frequency	
	Minimum	Optimum
Macrophytes		
Aerial and field mapping	-	1/6
Vegetation transects	-	1/3
Benthic animals	2	2
Fish		
Test fishing (community structure, growth rates)	-	1/2
Mark-and-recapture ¹⁾	-	1
Spawning success ¹⁾	-	1
Heavy metal analysis	-	1/2

1) Only in small lakes (< 0.1 km²)

3 Phytoplankton

3.1 PRIMARY PRODUCTION

Introduction

Phytoplankton primary production is a measure of the trophic level of the lake ecosystem. Carbon assimilation by phytoplankton algae is measured with radioactive ^{14}C as a marker (Steemann Nielsen 1952). The measurement is carried out *in situ*, by incubating water samples in light and dark bottles at the sampling depths. The unassimilated radiocarbon is expelled from the sample by acid addition and exchange with air (Niemi *et al.* 1983). This simple procedure overcomes the problem of cells breaking during preservation or filtering. The laboratory in which the radiocarbon determinations are carried out must be licensed for treating radioactive materials.

Field work

Primary production is measured at one sampling site. The samples are taken during the forenoon using a water sampler in which the metal parts are coated with teflon. The sampler should be of a design that allows unimpeded water passage through the sampler while it is being lowered. (A good model is the Limnos-sampler, which has the top and bottom lids in a vertical position and entirely out of the water path when the sampler is open; Fig. 1.) The

sampling depth is measured from the middle of the sampler. The surface sample (0 m) is taken directly in a plastic container. The sampling depths are to be selected for each lake according to the average epilimnetic water colour. One of the following series may be chosen:

1. colour $>150 \text{ g Pt m}^{-3}$: 0, 0.25, 0.5, 1 and 2 m;
2. colour 30-150 g Pt m^{-3} : 0, 0.5, 1, 2 and 4 m;
3. colour $<30 \text{ g Pt m}^{-3}$: 0, 1, 2, 4 and 8 m (the lowermost sample 1 m above bottom, if sampling site less than 8 m deep).

The suggested depth series are based on Finnish experience; in lakes with very clear water much deeper sampling may be warranted. The goal of lake-specific design of sample depths, which should not be varied in the course of the study, is to extend the measurement to the entire photic layer and to have the vertical distribution of production optimally determined.

At least three water samples are to be taken from each sampling depth. These should be mixed in a darkened plastic collecting vessel equipped with an outlet tap. The surface sample should be taken in a specific plastic container or directly into the collecting vessel. When skimming the surface water, the vessel should be moved forward slowly, so as to avoid sample contamination from the hands of the person holding the container. The following sample bottles are to be filled from the compound sample for each depth:

- two light bottles for light fixation;
- one dark bottle for inorganic carbon dark fixation;
- two bottles with ground glass stoppers for the measurement of the initial concentration of dissolved inorganic carbon (respiration measurement; see details in section 4.2).

In addition, a dark control bottle should be filled with both the surface and the lowermost depth samples.

The samples for light and dark fixation and dark control are taken in glass vials (vol ca. 25 ml; glass-stoppered bottles or e.g. scintillation vials with tightly sealing caps); about 0.3 ml is taken from each bottle by pipetting, in order to leave an airspace for subsequent radiocarbon and formaldehyde additions. Same volume of carrier free ^{14}C solution (ca. 370 kBq ml $^{-1}$) is added (e.g. 10 μl per 1 g DIC m $^{-3}$) under water to each primary production and dark fixation bottle. To the dark control samples, 0.2 ml of concentrated (ca. 40%) formaldehyde solution is added before adding the radiocarbon solution. Immediately after radiocarbon addition, the dark fixation and dark control bottles are wrapped in aluminium foil or put into aluminium tubes with rubber stoppers, and the bottles are submerged - together with the light ones - in a bottle rack to the sampling depth. Neither the rack nor the dark bottles should shadow the light bottles or reflect excess light onto them. During winter experiments, the incubation rack for the light bottles should not be placed immediately below the drilled hole in the ice; instead it should be shifted, e.g. by means of a rod equipped with floats, at least one metre to the side of the opening. The opening should be covered with snow during incubation.

The sample incubation time is 24 hours (48 hours under the ice), after which the samples (excluding the dark controls) are immediately fixed with 0.2 ml conc. formaldehyde. While being transported to the laboratory, the bottles should be protected from light and immersed in crushed ice (or cooled in insulated boxes with cold elements).

Laboratory procedure

Dissolved inorganic carbon should be determined with a carbon analyzer on the sampling day, as soon as the samples have reached the laboratory (e.g. Salonen 1981). If there is no carbon analyzer available, inorganic carbon concentration can be calculated from alkalinity, pH and temperature (Vollenweider 1974, pp. 54-56, Rebsdorf 1972); however, this method cannot be applied to samples with pH < 5.5. In such cases, CO $_2$ may be determined by distillation (Vollenweider 1974, pp. 57-59) or by titration (Golterman & Clymo 1970, pp. 33-34).

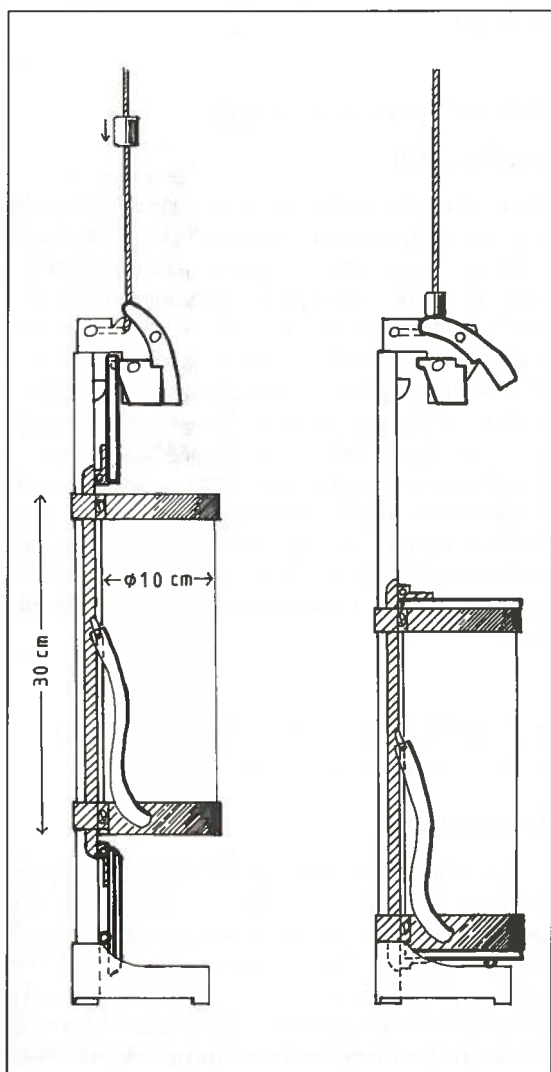


Fig. 1. Limnos sampler; left open, right closed.

The total radioactivity of the added ^{14}C should be measured in at least two incubated sample bottles. For this purpose, 0.5 ml of the CO_2 absorption solvent (1:7 mixture of ethanol amine and ethylene glycol monomethyl ether) is pipetted into an empty scintillation vial, and then 0.5 ml of the sample is injected below the liquid surface in the vial. Next, 7 ml of distilled water is added (making the volume equal to the volume of the sample used to determine assimilated carbon, see below). Finally, 10 ml of Lumagel is mixed with the sample.

The radiocarbon assimilated by algae and other microorganisms is measured from the light and dark samples as well as the dark control samples by transferring a standard volume (8 ml) of each sample to a scintillation vial. One drop of conc. orthophosphoric acid is added to the vial, and, after mixing, the sample is left unstoppered in a ventilated cupboard for two days. During this time, the dissolved radiocarbon will be exchanged with atmospheric CO_2 . After this, 10 ml of Lumagel scintillation liquid is added, and the radioactivity assimilated by the algae is measured, using liquid scintillation counting. Other scintillation liquids may be used instead of Lumagel, but the ratio of sample to scintillation liquid volumes must be adjusted according to the specifications of the liquid in question.

Sources of error

Care must be taken not to allow any radiocarbon in the stock solution to be exchanged with atmospheric carbon dioxide. The pH of the solution should be kept above 9.5.

Calculation and expression of results

Assimilated carbon at each sample depth is to be calculated according to the following formula:

$$C = 1.05 \times C_1 \times \frac{R_v - R_p}{R_t - R_k}$$

Where:

C = concentration of assimilated inorganic carbon, mg m^{-3}

- 1.05 = rejection coefficient for radiocarbon
- C_1 = concentration of inorganic carbon in the sample, mg m^{-3}
- R_v = radioactivity of the light sample (average of two determinations, dpm or Bq)
- R_p = activity of the dark sample, dpm or Bq
- R_t = radioactivity added to the sample, dpm or Bq (average of two determinations, calculated to the same volume as R_v , R_p and R_k)
- R_k = mean activity of the two dark control samples (surface, deepest sample), dpm or Bq.

The results are expressed as assimilated carbon $\text{mg C m}^{-3} \text{d}^{-1}$ (for each analyzed depth) and as $\text{mg C m}^{-2} \text{d}^{-1}$ (value integrated for the water column).

Net and gross community production

When the concentration of inorganic carbon is low (growing season average $<0.5 \text{ g C m}^{-3}$), the relative change caused by respiration may be so high that the radiocarbon method underestimates primary production. In such cases, the net and gross production of the community should also be measured by the dissolved inorganic carbon or oxygen method. For this determination, two light bottles (without ^{14}C) are incubated at each sampling depth together with respiration samples (see section 4.2). Net production equals the loss of dissolved organic carbon or increase of oxygen in the light bottles. Gross production is net production plus respiration.

3.2 CHLOROPHYLL CONCENTRATION

Introduction

Chlorophyll concentration indicates the trophic level of the lake. It is also a measure of the phytoplankton's production potential. Though all autotrophic algae contain chlorophyll, the chlorophyll concentration need not be directly correlated with biomass; their relationship is affected, for instance, by illumination, nutrient concentrations and the species composition of the phytoplankton assemblage.

Algal pigments are extracted into organic solvent, in which the concentrations are measured spectrophotometrically. Quantitatively, the most important algal pigment is usually chlorophyll *a*, which is determined monochromatically. Other chlorophylls (especially bacteriochlorophylls) and their degradation products may absorb partially on the same wavelengths as chlorophyll *a*. Consequently, the observed concentration may include other pigments in addition to chlorophyll *a*.

Ethanol, methanol and acetone are commonly used in chlorophyll extraction. In the Integrated Monitoring, chlorophyll is determined using ethanol as the extractant. It is less of an occupational risk than methanol or acetone and has proved more effective than acetone for many algal groups (e.g. blue-greens and greens). During extraction, the samples are to be heated, which will inactivate the chlorophyllase enzyme and enhance extraction.

Field work

The samples are taken with a 1 m tube sampler (Fig. 2). When feasible, other types of sampler may be used, but the samples should completely cover the whole water column.

The sampling depths should be chosen according to the type of lake, and five depth intervals should be sampled in each case. The sampling intervals are 0-1, 1-2, 2-3 and 3-4 m, or otherwise spanning the epilimnion, plus a column sample extending down into hypolimnion, to 1 m above the bottom. When the lowest sample is taken, incorporation of bottom sediment must be avoided. Samples are taken at two sites, and samples from the same depths are pooled in darkened plastic containers. To avoid unnecessarily large volumes from the lowermost sample column, known aliquots from sample water can be collected in the container.

Subsamples for chlorophyll are taken from containers in plastic bottles (darkened, for instance, with black adhesive film). The samples should be kept in crushed ice during transport.

Laboratory work

Chlorophyll samples must be filtered on the sampling day. (An absolute limit is the morning of the following day, assuming that the samples have been stored at +4°C.) The samples (nor-

mally 0.1 - 2 litres) are filtered through glass-fibre filters (e.g. Whatman GF/F) with a vacuum <20 kPa. The filters are dried in darkness. If the determinations cannot be continued immediately, the dried filters must be stored in darkness and frozen (at least -20°C). The maximum storage time is 1 month.

Chlorophyll is extracted from the algae by immersing the filters in 94% ethanol. The volume of the extractant (5 to 25 ml) must be known precisely. The extraction is carried out in glass or plastic tubes with air-tight stoppers. The sample tubes are to be placed for 5 minutes in a 75°C water bath so that the sample is completely submerged. When taken out of the

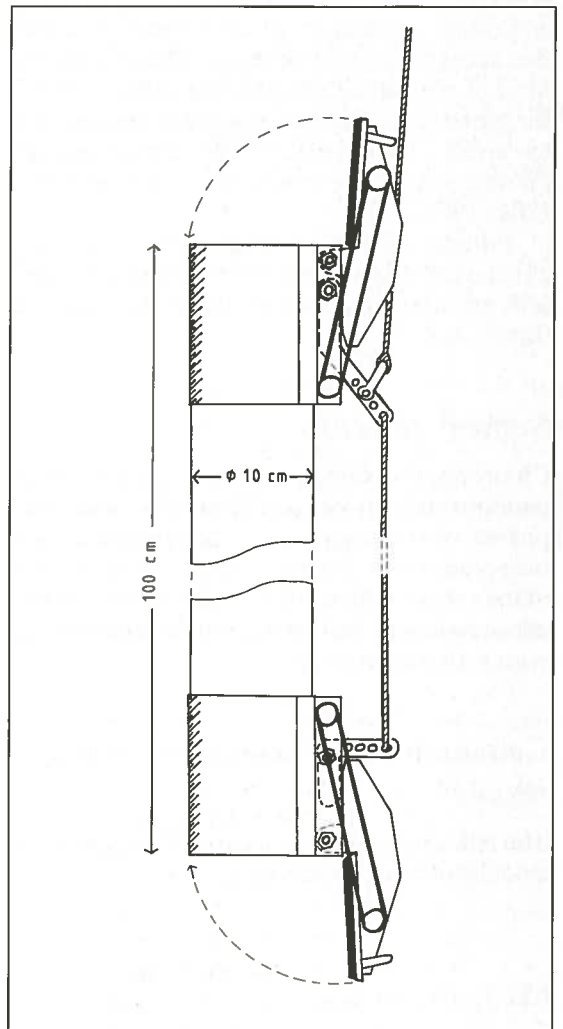


Fig. 2. Tube sampler (model Sormunen). The sampler is closed by pulling the rope.

bath, the tubes are allowed to cool to room temperature. If determination cannot continue immediately, the samples can be kept overnight at +4°C and protected from light. The absorbance of the centrifuged or filtered extract (glass-fibre filters) is measured spectrophotometrically at 665 and 750 nm. The blanks are measured with 94% ethanol.

The bacteriochlorophylls of autotrophic bacteria may absorb at 665 nm, thus causing high chlorophyll values in some anoxic waters (Takahashi & Ichimura 1970), even though chlorophyll *a* may not be present. The possible presence of bacteriochlorophyll in anoxic waters should be checked by plotting the absorption spectrum of the sample.

Chlorophyll results may also be affected by phaeopigments. To detect such interference, additional absorbance measurements of acidified subsamples may be made. The absorbance ratio of non-acidified/acidified samples is 1.7 for pure chlorophyll *a* samples at 665 nm and lower for samples affected by phaeopigments (Wintermans & De Mots 1965, Lorenzen 1967, Edler 1979).

Filtered water and extractant volumes and cuvette length should be selected so as to obtain 665 nm absorbance readings in the range of 0.005 - 0.8.

Sources of error

Chlorophyll is sensitive to both light and temperature, and this should be kept in mind at all phases of the work. Slow disintegration will take place even on frozen filters. The turbidity of the extract will bias the results, so the absorbance reading at 750 nm should not exceed 0.01 with a 10 mm cuvette.

Calculation and expression of the results

The chlorophyll concentration of the sample is calculated with the formula:

$$\text{Chl} = (A_{665} - A_{750}) \times \frac{V_1 \times 10^3}{V_2 \times l \times 83.4}$$

where:

Chl = chlorophyll *a* concentration of the sample, mg m⁻³

V₁ = ethanol volume, ml

A₆₆₅ = sample absorbance at 665 nm (absorption maximum of chlorophyll *a*)

A₇₅₀ = absorbance at 750 nm (turbidity)

83.4 = constant; absorption coefficient of chlorophyll *a* in 94% ethanol

V₂ = sample volume filtered, l

l = cuvette length, cm

The results are expressed as mg m⁻³ (for each depth zone) and mg m⁻² (integrated for the entire water column). The depth of the water column must be given in connection with the integrated value.

3.3 PHYTOPLANKTON FLORA AND BIOMASS

Introduction

The biomasses of phytoplankton species (>2 μm) are determined by counting their numbers and measuring their cell volumes. The algal volume is converted to biomass by assuming the specific density of the cells to be equal to that of water, i.e. 1.0 g cm⁻³.

The species composition of phytoplankton is greatly dependent on the physical and chemical characteristics of the water. Thus, the flora and changes therein are sensitive indicators of both short-term changes and the general state of the environment. Because of the naturally rapid succession of phytoplankton assemblages, short-interval sampling throughout the year is needed to obtain information that can be reliably related to changes in physical and chemical parameters.

Both autotrophic and mixotrophic species are included in phytoplankton, whereas all heterotrophic forms are included in the Protozoan. The distinctive criterion between these two groups is the presence of chloroplasts in the former group. Blue-green algae, or cyanobacteria, are included in phytoplankton (not bacteria) in this context.

Field work

Subsamples for the counting of phytoplankton are taken from the same pooled samples as for chlorophyll samples (see section 3.2). They are

taken in 220-250 ml glass bottles, on which the 200 ml volume level is marked so that any evaporation during storage can be detected. Bottles with self-sealing plastic screw caps are preferred. The sample is fixed with acid Lugol's solution, 1 ml of which is added to the flask immediately after sampling.

Live samples used for qualitative species identification are taken with a 10 μm plankton net by vertical hauls through the water column (starting depth: 1 m above bottom) or, if necessary, as stratified samples by first collecting water in a container and sieving it through the net (for this procedure, see zooplankton sampling, Ch. 5). Live samples are taken to the laboratory in thermos flasks or in an insulated box and stored in a cool place ($\leq 8^\circ\text{C}$). Live samples must be examined within two days of sampling.

Diatom and dry slide samples are taken in the same manner as live samples and stored with Lugol's solution (0.5% of the sample volume). Alternatively, they can be taken without the plankton net as pooled samples (e.g. 1 litre) from the same waters as the phytoplankton biomass samples. In this case, the algae must be concentrated by allowing them to settle for several days and removing excess water.

Lugol's solution

The acid Lugol's solution is prepared according to the following formula:

20 g potassium iodide
200 ml distilled water
10 g iodine
20 ml conc. acetic acid

The potassium iodide and then the iodine are dissolved in the water and acetic acid is added. The ready solution is stored in dark glass bottles in a refrigerator.

Sample storage

Phytoplankton samples must be stored in a dark, cool place ($< +8^\circ\text{C}$). During storage, the samples should be checked regularly so that any losses of sample volume or fixative can be corrected. If the sample colour fades, Lugol's solution must be added. For the detection of colour change, clear glass bottles are better than brown ones. The storage time before microscopy

should not exceed one year.

After the samples have been counted according to the monitoring protocol, the remaining samples from each sampling are pooled, by transferring an equal volume (e.g. 40 ml) of each depth into a bottle. These samples must be stored for at least five years, and they should be available for intercalibration and other such purposes.

Species enumeration and biomass determination

Microscopic enumeration of phytoplankton shall be entrusted only to a person well experienced in this special task. Continuity is also desirable, so the same person should perform the task for as long as possible.

The phytoplankton sample is thoroughly shaken, and some of it is poured into a settling chamber (Utermöhl 1958). After settling, the upper cylinder is removed and the sample in the lower part is examined with an inverted microscope.

If the samples contain algal species that are unsuitably dark coloured by Lugol's solution, these may be cleared by 0.1 N sodium thiosulphate. Thiosulphate solution is added to the sample just before settling and only the minimum amount necessary for colour change in the sample should be used. A more diluted Lugol's solution could adversely affect storage of the samples.

The 50 ml chamber is generally suitable for natural lakes, but the volume can be chosen according to the density of phytoplankton. Preferably only one chamber size should be used for all samples from a given lake, because the sample size may affect the results to some extent. The samples must be stabilized to room temperature before settling, and the temperature should remain constant during the settling time (at least 24 h, in darkness). Before the cylinder is removed, some drops of water should be placed on the cuvette plate in order to prevent air bubbles from entering the chamber. Any underpressure in the chamber may be balanced by carefully opening the upper lid, by sliding it sideways and then closing before the cylinder is removed. The sample should be examined on the same day. If the microscopic examination is delayed, the sample should be stored in a cool,

moist place to prevent the formation of air bubbles.

Some algae may remain floating in the water. In such cases a few drops of detergent (e.g. dish-washing medium) may be added to the water and the sample allowed to settle for two days. Ultrasonication may be performed in an effort to make blue-green algae sink, but the colonies easily break apart in this treatment. Because the detergent and ultrasonic treatments may be harmful to some algae, parallel untreated samples should always be counted for comparison.

Microscopic counting is done with an inverted microscope using phase-contrast optics. A lower magnification (100 to 200x) is used for large but rather scarce species, and the whole sample should be screened. A higher magnification (400 to 600x) is used for abundant species and species that would be difficult to recognize with the low magnification; these are counted along two transects crossing the centre of the counting area at right angles. The width of the transect to be covered is usually equal to the diameter of the field of vision, but a narrower transect may be used with the aid of a suitable ocular grid. All observed species should be identified if possible. Each species is enumerated only once, in one or other of the magnifications.

The average cell dimensions of each phytoplankton species should be measured with the aid of a calibrated ocular scale. The specific volumes should be calculated in accordance with Edler (1979) and Willén & Tikkanen (1992, p. 32).

In addition to phytoplankton, Protozoa are also enumerated in the same transects, but their numbers are treated separately from the phytoplankton counts (cf. section 5.1). If there is any doubt as to whether a particular taxon should be included in phytoplankton or not, the presence or absence of chloroplasts should be checked by epifluorescence microscopy. Autofluorescence can often be detected in recently fixed samples, but live samples are to be preferred.

Biomass calculation

The total biovolume of a single species is calculated with the formula:

$$B = n \times \frac{A_1 \times v}{A_2 \times V \times 10^3}$$

where:

- B = biovolume of the species per unit volume of water, $\text{mm}^3 \text{m}^{-3}$; as converted to fresh weight biomass, mg m^{-3}
- n = observed number of counting units
- A_1 = settling chamber area, mm^2
- A_2 = counted area, mm^2
- v = average counting unit volume of the species, μm^3
- V = sample volume settled in the chamber, ml

The biomass of a single species is expressed as mg m^{-3} and the total phytoplankton biomass as g m^{-3} . The integrated total biomass of the water column is expressed as g m^{-2} , and the water column height must be given together with the integrated value.

Permanent slides

The slide preparations will remain as permanent documentation of the flora. Mounted slides are necessary for proper identification of diatoms and certain scaled species.

Diatom slides

Diatoms may be concentrated by centrifuging sample water in a conical tube. After the water has been decanted off, organic matter is destroyed by boiling in 65% nitric acid. This can be made with a Bunsen-burner (in a ventilated cupboard, avoiding excessive heating!), until the brown exhaust fume turns white. The concentrated acid is diluted by adding distilled water, and the sample is then centrifuged and decanted. This is repeated three times to rinse the acid away. Finally, a suitable amount of the white residue, mainly consisting of diatom frustules, is pipetted onto a coverslip and allowed to dry in a draught-less, dust-free place. The coverslip is then mounted on a slide with a suitable mounting medium, e.g. Hyrax.

Dry slides

A small quantity of concentrated sample is pipetted onto a coverslip, and the moisture is

allowed to evaporate. The coverslip is then heated, with the sample upwards, on an electric plate until the organic matter has oxidized and the residue turned white. The coverslip is then glued, sample downwards, and at the sides only, onto a slide so that it remains slightly elevated; no mounting medium is applied.

Photographic documentation of phytoplankton

Algae may be photographed using a special microscope camera, or an ordinary camera equipped with microscope adapter. Colour slides are preferable, but black-and-white film should also be used because it has a longer life time. Live samples, in particular, may also be filmed on video.

All the species observed during the sampling year should be photographed at the appropriate magnification in the settled samples, diatom or

dry slides or live samples (preferably several exposures of each species). Species that are difficult to identify, have taxonomic interest or significant morphological variability, should be particularly carefully documented. Photography is an indispensable component of electron-microscopic work.

Digital image-processing technology will offer almost unlimited potential for storage and treatment of photographic material. Digitized pictures are readily available for inspection on the computer screen, appropriate filing systems will allow convenient and rapid management of large picture libraries, and other information can be added to the video pictures (sample code, magnification, etc.). Digitized images are easy to distribute via the telephone network. The rapid development of these technologies will enable effective documentation of biological materials in the Integrated Monitoring and will diminish the need for storage of samples.

4 Bacterioplankton

4.1 BACTERIAL DENSITY AND BIOMASS

Introduction

Bacteria are largely responsible for mineralization of organic matter in waters. As their activity is affected by e.g. acidification and eutrophication, they are useful indicators of environmental change. In the Integrated Monitoring bacteria are counted using epifluorescence microscopy after staining with acriflavine (Bergström & *al.* 1986) or DAPI (Porter & Feig 1980).

Field work

No separate bacterial samples are taken in the field; instead bacteria are to be determined from phytoplankton samples. Thus, sampling sites and sample depths are the same as for phytoplankton samples (Table 1 and sections 3.2-3.3).

In case of acriflavine staining Lugol's solution can be used as a preservative. However, with certain other stains, brown colour of Lugol's solution may affect the fluorescence. In such cases, colour can be removed by adding 0.1 N sodium thiosulphate to the sample before making the preparation (Pomroy 1984, cf. section 3.3). Instead of Lugol's solution, the bacteria can be preserved in glutaraldehyde, though this is harmful to health and is thus not recom-

mended. If glutaraldehyde is used, the bacterial samples should be taken in the field separately in small glass bottles (e.g. 25 ml) and preserved in 1% glutaraldehyde (taking into account the concentration in the stock solution).

Laboratory work

The staining solution is prepared by dissolving 260 mg of acriflavine into 100 ml of distilled or deionized water. Water used for preparing the solutions, for rinsing or for dilution should be free of bacteria and is therefore filtered through 0.2 μm cellulose ester or equivalent filter.

Before filtering bacteria sample the funnel should first be rinsed with distilled or deionized water. To ensure equal flow over the whole filtering surface, a glass-fibre filter is used on the support plate of the filtration unit. A black polycarbonate filter ($\text{\O} 25 \text{ mm}$, pore size ca. 0.45 μm) is placed on the glass-fibre filter, and 5 ml of distilled or deionized water is injected into the funnel; this is done to avoid significant relative differences in the depth of water column between the centre and margins of the filters because of the surface tension. Then (typically), 1 ml of sample is added and water is filtered with a low vacuum (<20 kPa). Near the end of the filtering, without interrupting the suction, the funnel is rinsed with 2 ml of water. Immediately after all the water has gone through, about 1 ml of acriflavine is injected onto the filter. This is done through a 0.2 μm disposable

filter to avoid bacterial contamination. The vacuum is then released and the bacteria are stained for about 10 minutes. Next, the vacuum is switched on, and, once the filter is dry, it is carefully removed with forceps. The vacuum is released only after removing the filter, thus minimizing the amount of stain left on the filter.

The dried filter is placed on a microscope slide. One drop of immersion oil is placed in the centre, and the sample is covered with a coverslip. The slides must be stored in darkness and they will remain suitable for microscopy for several days. If, however, the samples have to be stored for long periods, the persistence of the fluorescence must be tested in advance.

Bacteria are enumerated and measured by epifluorescence microscopy at 1000-1200x magnification. An oil-immersion objective with a 90-100x magnification and a numerical aperture of at least 1.25 should be available for the microscopy.

An ocular grid is used for the counting, with 10 to 20 visual fields randomly chosen on the slide. The bacteria are counted in a constant number of grid squares in each of the fields. An optimum count is 10-20 cells per field. The volume of at least 50 randomly chosen bacteria are measured on each slide. An ocular grid containing different-sized circles may be a useful aid for measurement purposes.

It is possible to substitute computerized image analysis for manual enumeration of bacteria. Besides saving time, the automated procedure will eliminate the subjective aspect of the analysis. Among the major prerequisites for the image analysis of bacteria, however, are algorithms which produce cell sizes independent of fluorescence brightness.

Sources of error

External bacteria contamination must be avoided at all stages of the work. This must be checked each time with blank slides, made by repeating all the steps in the filtering procedure, except that no sample water is added.

Calculation and presentation of results

Bacterial density in water is calculated with the equation:

$$T_b = \frac{A_1}{A_2} \times \frac{n}{V}$$

where:

- T_b = bacterial density, cells ml⁻¹
- A_1 = filter surface area, mm²
- A_2 = total area of microscopically enumerated squares, mm²
- n = counted number of bacteria
- V = filtered sample volume, ml

Total volume and biomass of bacteria is calculated with the equation:

$$B_b = T_b \times v \times 10^{-3}$$

where:

- B_b = total volume of bacteria per unit volume of water, mm³ m⁻³; or mg m⁻³ as fresh-weight biomass,
- v = mean cell volume, μm³

The bacterial density of each sample is expressed as cells per ml and their biomass as mg m⁻³. The integrated bacterial biomass of the water column is given as mg m⁻². For this purpose, the height of the water column must also be indicated.

4.2 DECOMPOSITION

Dark fixation of inorganic carbon

As heterotrophic bacteria take part of their carbon in inorganic form, dark fixation of carbon is an indicator of bacterial activity.

Inorganic carbon dark fixation is determined along with the primary production measurement, by *in situ* incubation of dark bottles with radiocarbon as tracer (section 3.1). Light exposure of the dark fixation samples is to be minimized at all stages of the work. ¹⁴C dark fixation values are also needed in the determination of primary production.

Inorganic carbon dark fixation is calculated with the formula:

$$C_p = 1.05 \times C_i \times \frac{R_p - R_k}{R_t - R_k}$$

Where:

C_p = carbon dark fixation, mg C m^{-3}

1.05 = rejection coefficient for radiocarbon

C_i = inorganic carbon concentration of the sample, mg m^{-3}

R_p = radioactivity of the darkened sample, dpm or Bq

R_k = mean activity of the two dark control samples (at surface and lowermost depths; dpm or Bq)

R_t = radioactivity added to the sample, dpm or Bq (average of two determinations, calculated to the same sample volume as R_p and R_k)

The results are expressed as $\text{mg C m}^{-3} \text{d}^{-1}$ (for each depth) and $\text{mg C m}^{-2} \text{d}^{-1}$ (water column); for the integrated value, the height of the column is also to be given.

Respiration

Respiration is the catabolic process counteracting primary production. The rate of respiration is determined in connection with the determination of primary production as either an increase in dissolved inorganic carbon (e.g. Salonen 1981) or consumption of oxygen in darkness (e.g. Väättänen 1979, Kuparinen 1987). The sampling depths and incubation time are the same as in the determination of primary production. At least four replicate samples are taken from each depth; two samples are used to determine initial concentrations, while the other samples are darkened and incubated *in situ*. Hypolimnetic respiration can roughly be estimated from the accumulation of DIC or decrease of O_2 during stagnation periods.

Carbon method

The dissolved inorganic carbon (DIC) method is feasible only if the laboratory is equipped with a precise carbon analyzer (standard deviation of results <1% of the mean). The DIC method is ideal for waters with low concentrations (< 2 g m^{-3}) of DIC, because then the relative difference between the final and initial concentrations of DIC is larger making the respiration estimates more exact. Otherwise, the oxygen method should be used (see below).

DIC samples are to be taken from the sample container before all other samples. Samples for the determination of the initial concentration and for the incubation in lake are taken simultaneously through a splitter into 25 ml glass-stoppered bottles of known volume. When the bottles are filled, at least twice the bottle volume is allowed to flow over the brim, and no airspace should be left after stoppering. The bottle to be incubated must be darkened immediately.

The samples for the determination of initial concentration of DIC are to be packed in crushed ice and protected from light for transport to the laboratory, where the concentration of DIC should be immediately measured. This procedure is repeated the next day for the incubated samples.

To ensure maximum reproducibility in the injection of samples into the carbon analyzer, the bottles are warmed to $+20^\circ\text{C}$ in a water bath immediately before the determination. The bottles should be kept in the bath only the time required to reach the wanted temperature. Five replicates of each sample are injected and their median value is recorded.

Respiration is calculated as the difference between the final and initial concentrations, and is expressed as $\text{mg C m}^{-3} \text{d}^{-1}$ (for each depth) and as $\text{mg C m}^{-2} \text{d}^{-1}$ (for the water column); water column height is to be indicated for the latter value.

Oxygen method

Oxygen concentration is determined by a modified Winkler titration procedure. This method is applicable to waters that contain no excessive amounts of reducing or oxidizing compounds. It should be preferred over the DIC method when the inorganic carbon concentration is more than 2 g m^{-3} .

The reagents are prepared according to standard methods (e.g. American Public Health Organization 1971). The sampling procedure is similar to that described above for the DIC method.

After the bottles have been filled (or after incubation, as appropriate), the reagents - 1 ml of manganese(II) sulphate (or manganese(II) chloride) solution and 1 ml of alkaline iodide solution - are injected into the bottles below the water surface. The temperatures of the samples should not change before the reagent is added.

After the reagents have been added, the sample bottle is stoppered and the contents mixed by turning it upside down a few times. The sample is stored in darkness until the oxygen determination continues in the laboratory.

Before titration, 1 ml of phosphoric acid is added to the bottle, and, after reclosing, it is turned up and down until the precipitate is dissolved. The determination must be finished within 45 minutes of adding the acid.

The titration is done directly in the sample bottle. If necessary, small volume can be taken out from the bottle to make space for thiosulphate titrant. In such case an appropriate multiplication factor (original volume/titration volume) should be applied in the calculation formula. In the titration sodium thiosulphate solution is added to the bottle until the colour turns yellowish brown. Then about five drops of starch solution are added, and the titration is continued until the blue colour disappears for the first time. A precision burette, in which the sodium thiosulphate consumption can be read to an accuracy of at least 0.01 ml, is to be used.

Amperometric, potentiometric, or photometric titration may also be applied. The precision

of the oxygen determination should be about 0.1 percent.

The oxygen concentration of the sample is calculated with the formula:

$$P = 8000 \times a \times v \times (V - 2)^{-1}$$

where:

P = dissolved oxygen in the sample water, g m⁻³,

a = concentration of the sodium thiosulphate solution, mol l⁻¹,

v = volume of the sodium thiosulphate solution consumed, ml,

V = volume of the sample bottle, ml (the 2 ml overflow caused by reagent addition is subtracted from this).

Respiration is calculated as the difference between initial and final concentrations, and is expressed as mg O₂ m⁻³ d⁻¹ (discrete depths), and as mg O₂ m⁻² d⁻¹ (water column; the thickness of the water column investigated is to be given in connection with the integrated value).

5 Zooplankton

Zooplankton is a sensitive indicator of environmental change (e.g. acidification). As zooplankton is grazing on phytoplankton, monitoring is also warranted for proper interpretation of changes in the phytoplankton. For practical reasons, zooplankton is divided into protozoan and other zooplankton. The Integrated Monitoring focuses mainly on the latter group. Proper study of protozoan plankton would involve special expertise and considerable resources. In the Integrated Monitoring, zooplankton (excl. Protozoa) are sampled from different depths and concentrated with a plankton net.

5.1 PROTOZOAN PLANKTON

The term protozoan plankton refers to heterotrophic, microscopic eucaryotic organisms that are usually solitary, but sometimes occur in colonies. The criteria for distinguishing between phytoplankton and protozoan plankton are given in section 3.3.

Protozoan plankton is not monitored in the proper sense; nevertheless, major changes in the species composition and biomass thereof are noted. Protozoans encountered in the phytoplankton counting transects are identified as far as possible (e.g. Kahl 1930-1935, Lee *et al.* 1985), photographed and their volume measured (cf. section 3.3). Some of the Protozoa may be difficult to identify even at the generic level. The unidentified ones are measured, grouped into size classes, and their volumes included in

the total protozoan biomass. The results are expressed as the density of individuals (ind. m^{-3}) and as fresh-weight biomasses (mg m^{-3}). Because large protozoans may appear quite randomly on the counting transects, the results should be considered merely indicative.

5.2 ZOOPLANKTON (EXCLUDING PROTOZOA)

Field work

Zooplankton samples are taken from the pooled samples as described in section 3.2. After the other samples (chlorophyll, phytoplankton, bacterioplankton and protozoan plankton) have been extracted, the remaining water (ca. 11-12 l for each 1-m sequence) is taken as the zooplankton sample. Thus the total sample volume at each sampling is 55-60 l. The water is poured through a conical 50 μm mesh plankton net, provided with an outlet tap at the end. After the retained zooplankton has been transferred to a 250 ml sample bottle, the inside of the net is rinsed with filtered (25 μm) water from a wash bottle, and any remaining animals are also transferred to the bottle. Samples are preserved with formaldehyde (final concentration 4%).

For carbon biomass determination, a separate water column sample is taken with a 50 μm plankton net at one sampling site. The net is lowered to 1 m above bottom and then lifted to the surface at an even pace. It is emptied into a 250 ml plastic bottle. If necessary, additional

hauls may be taken to obtain enough animals. This sample is brought into the laboratory, maintaining the original temperature as closely as possible. The sample is fixed with formaldehyde (4% of water volume) and divided into 3 or more subsamples which are frozen immediately at -20°C (Salonen & Sarvala 1985). Smaller subsamples allow more immediate carbon determination after thawing.

In addition to the above samples, taking live samples from the whole column is recommended for qualitative observation. Live samples are transported to the laboratory in thermos flasks or in an insulated box and stored in a cool place ($\leq 8^{\circ}\text{C}$) until they can be examined (within two days of sampling).

In wintertime, zooplankton densities may be affected by changes in the illumination under the ice due to sampling procedure. Sampling should therefore be performed at an untouched site and as soon as possible after making the sample holes in the ice.

Laboratory work

The counting of each species is done separately for each depth interval sampled. For the purposes of microscopy, each sample is divided into two fractions by sieving it through a $200\ \mu\text{m}$ mesh sieve. The larger fraction, i.e. that retained by the sieve, is washed into a sample jar and stored in 4% formaldehyde. The $<200\ \mu\text{m}$ filtrate is divided into subsamples by pouring equal quantities (e.g. 30 ml at a time) from the sample jar into 3 to 5 subsample jars. The sample should be thoroughly mixed immediately before each pouring. This is repeated, e.g. five times. Each subsample should contain at least 100 individuals. The $<200\ \mu\text{m}$ fraction subsamples are counted with an inverted microscope by screening the entire cuvette area (cf. phytoplankton count, section 3.3). Each species is enumerated only from a number of subsamples permitting a sufficient count, i.e. 50 to 100 specimens. The large-sized species ($>200\ \mu\text{m}$) are counted from the sieving residue with a dissecting microscope. The entire fraction should usually be analyzed; splitting, if warranted, is done in the same manner as for the small frac-

tion. In addition to the adults, eggs (incl. attached) and other juvenile stages are enumerated. Within each species, different size-classes and morphs are counted separately.

Permanent slides are made of the samples as needed for documentation and further investigations by mounting the specimens in a water-soluble mountant. The remaining samples are to be stored for at least five years and kept available for interested parties. Zooplankton is documented by photography and video in the same manner as phytoplankton.

Biomass is determined preferably as carbon content. Alternatively, dry-weight determination may be used. Dry weight is converted to carbon by assuming that the carbon content is 50% of (ash-free) dry weight. Biomass determination should focus primarily on crustacean species.

For carbon determination, the frozen subsamples are thawed, and a definite number of individuals of each species (at least 30 ind. of dominant species) is selected at random. Biomass values for each discrete depth are calculated from species counts and the species-specific mean carbon content.

It is impossible to achieve direct biomass measurements for each species. Thus, earlier regressions for length/carbon or biomass/carbon, obtained for the study lake or for other lakes, may be needed. The carbon contents of crustaceans and large Rotatoria ($>0.5\ \mu\text{g C}$) should be determined at least monthly; those of other organisms are determined as resources permit.

Sources of error

Large species may escape the sampler, thus resulting in unduly low biomass values.

Expression of results

Individual densities are expressed as ind. l^{-1} and biomass as mg C m^{-3} . For the entire water column, the respective values are ind. m^{-2} and mg C m^{-2} ; the water column height should be indicated in connection with the integrated values.

6 Aquatic macrophytes

Submersed and floating-leaved plants, helophytes and – in general – plants found in water and visible to the naked eye are considered aquatic macrophytes. Study of them will provide information on the quality of the water body and its littoral bottoms. Macrophytes react to changes in the environment, but generally more slowly than planktonic algae. Thus, they will indicate long-term, cumulative changes in the aquatic system.

Aquatic macrophyte study consists of aerial photographic mapping complemented with field investigations and monitoring of permanent vegetation transects. Aerial photographs give an overview of the lake's vegetation and also serve the purpose of documentation. Transect monitoring provides more detailed and precise data on the vegetation.

6.1 AERIAL AND FIELD MAPPING OF VEGETATION

Aerial photography, documenting the occurrence and extent of macrophyte stands, should be done every sixth year (preferably the same year on all lakes in nearby areas). The recommended time is between mid-July and the end of August, when the stands are at their densest.

Preferably a customized aeroplane, from which vertical photos can be taken through an aperture in the bottom of the fuselage, should be used, though oblique photos taken through the

aeroplane window are also useful. The photographs should be taken at noon, when long shadows will not hamper their interpretation.

The stands of different species are distinguished by their specific shades of colour. Therefore, colour slide film should be preferred, and black-and-white film used only as complementary material. False-colour images should also be taken, as these may reveal vegetation and site differences that would not otherwise be visible. If possible, a stereo-image camera should be used; stereo pairs will greatly aid interpretation of the vegetation. The flying elevation and the focal length of the camera should be known, as this information will enable image-reduction calculation; the scale of the image on film should be approximately 1:10000.

A field study of the area is necessary for the correct identification of plants. The entire area to be mapped (including submersed vegetation) must be investigated. In the field, the species, the borders of their stands and other information (bottom type, etc.), are to be marked on a sketch map based on the aerial photos taken previously. As soon as possible after the fieldwork, a final version of the vegetation map should be compiled, always using the same symbols for the different species and stands. The vegetation stands are numbered on the map, and an explanatory list describing the characteristic features of each numbered stand is provided.

6.2 PERMANENT VEGETATION TRANSECTS

A few permanent transects, to be studied every third year, should be established at each lake. The number of transects needed depends on the surface area and length of shoreline of the lake, and can be estimated with the following formula (Jensen 1977):

1. lake area $\geq 0.20 \text{ km}^2$:

$$\text{NPA} = C \times \frac{\text{LSA}}{\sqrt{\pi \times \text{LA}}}, \text{ where:}$$

$$C = \frac{P_{mj}}{2} + \frac{\text{LA} - \text{LA}_{mj}}{\text{LA}_{mj}}$$

2. lake area $< 0.20 \text{ km}^2$:

$$\text{NPA} = \frac{\text{LSA}}{\sqrt{\pi \times \text{LA}}}$$

where:

NPA = total number of transects required (rounded to nearest integer)

LSA = length of the shoreline, km

LA = lake area, km^2

P_{mj} = least number of transects required for lakes in the particular size-class (Table 3)

LA_{mj} = lower limit of the size class allotted to the lake (e.g. 0.20 km^2 in the size-class III; Table 3)

The transects should represent the range of bottom and vegetation types encountered in the lake. A transect should start either at the mean water level mark or at the landward margin of a distinct vegetation stand, and it should be extended at right angles to the shoreline as far out as macrophytes occur on the bottom. A transect can be extended to the opposite shore, particu-

Table 3. Size classification of lakes for the determination of macrophyte transect numbers (Jensen 1977). LA = lake area, P_{mj} = minimum number of transects for each size-class.

Size class	LA (km^2)	P_{mj}
I-II	<0.20	2
III	0.20 -0.39	2
IV	0.40 -0.79	4
V	0.80 -1.59	6
VI	1.60 -3.19	8
VII	3.20 -6.39	10
VIII	6.40 -12.79	12
IX	12.80 -25.59	14
X	25.60 -51.19	16
XI	51.20 -102.39	18

larly in shallow lakes where macrophytes are present throughout. A transect crossing from shore to shore equals two transects in the above formula.

Each transect should be defined with fixed points: conspicuous trees, stones, etc. on opposite shores marking the imaginary transect across the lake. The distance between the starting point and the nearby fixed point should be known (the fixed point itself may be the starting point). The actual starting and end points of the transect should also be marked with posts driven into the soil or bottom; furthermore, a compass reading of the precise direction of the transect should be taken. In small lakes where ice movements are negligible, additional posts, e.g. at 10 m intervals, may be used as markers. Each transect must be carefully documented.

When analyzing the transect, a string marked at one-metre intervals should be drawn between the end-posts (the string should be of a quality that does not stretch or shrink in water). Contiguous one-metre squares are examined along the transect (their breadth may be judged visually) and from each square the species present, water depth, bottom quality and other observations (e.g. fertility of the plants) are recorded.

At the deep end, an underwater inspection tube may be used - or divers sent down - as necessary.

Biomass determination is done on evenly spaced squares along the transect, or outside it if necessary to avoid long-term wear on the vegetation. Depending on the vegetation structure, the squares may be taken at every five or ten metres; additional squares may be needed to obtain representation of all vegetation patches crossed by the transect. All above-ground phytomass is removed from a selected area (e.g. 0.5 x 0.5 m). Biomass samples of submersed species are taken either with a long-handled cutter or by diving.

For fresh-weight determination, the plants should not be superficially wet. For dry-weight determination, a subsample of the fresh sample will usually suffice. Dry weight is determined after 24 h drying at 105°C.

In one-species stands, the biomass can be determined by counting the shoot density on 50-100 evenly spaced squares (0.5 x 0.5 m). Altogether 50 to 100 individual shoots are randomly selected and sampled from the stand; their fresh

weight is determined, e.g. in bunches at 20 shoots each; one such bunch will suffice for dry-weight determination. The above-ground biomass in the stand may then be calculated by multiplying the average shoot density by the average shoot biomass.

In connection with the field mapping and transect analyses, herbarium samples of plant specimens are also to be taken. In addition, samples of macroscopic algae should be collected and preserved in formaldehyde (final concentration of formaldehyde 2% of volume).

Presentation of results

For each transect, the frequency of occurrence of each species in the 1 m² squares is given (percentage of the total of those squares in which the species was present). The biomass is expressed as g m⁻² (average dry mass of each species stand). The extent of each stand should be measured and their total biomasses estimated on the basis of the aerial photos and field mapping.

7 Zoobenthos

Introduction

Zoobenthos or bottom-dwelling animals are indicative of conditions prevailing on the bottom and in adjacent water mass. Profundal benthic conditions may be thoroughly altered by eutrophication, which increases the amount of decomposing organic matter and can thus lead to oxygen deficiency. In the littoral zone, vegetation is a key factor determining zoobenthos composition.

In the Integrated Monitoring, both profundal and littoral zoobenthos are followed (ISO 1988, Meriläinen 1984, Meriläinen & Hynynen 1990). The monitoring is restricted to macrofauna, which for practical purposes is defined as animals retained by a 0.5 mm mesh sieve.

Profundal samples are taken quantitatively with an Ekman grab. Littoral benthos is sampled with a hand net, which is not a quantitative method but will give a good representation of the species composition of the fauna.

Sampling frequency

Both profundal and littoral samples are taken after the ice melts in the spring (not later than end of May), and in September-November.

Field work

Profundal

For profundal benthos sampling, a representative bottom site should be chosen; not necessarily the deepest part of the lake if this is limited in area, but rather a relatively plain deep area with a reasonably homogeneous bottom quality. A circle at least 20 m in diameter is established as the benthos sampling site, and its centre is marked with a float. The proper size for a representative area should be judged by the lake area and bottom morphology.

Samples are taken randomly along the perimeter of the defined circle. Compass directions, with the site centre as origin, are drawn randomly for five sampling points on the perimeter. The sampling points are determined in the field with the aid of a rope attached to the float and equal in length to the chosen circle radius. The boat should be anchored while the sampling is being done. In practice, some deviation must be allowed in the radial direction, so the actual sampling area is a circular 2-4 m broad belt.

The bottom at the sampling site should be soft enough to allow quantitative sampling with the Ekman grab. On harder bottoms, the grab may be operated with rods down to a depth of at least 5 m. On excessively soft bottoms, a higher grab than described below, or e.g. a tube sampler, should be used.

The Ekman grab should have a box at least 250 mm in height, and the sampling area should be at least 0.02 m². The grab is lowered steadily until bottom contact is made. After the mechanism is released, the sampler should be lifted steadily from the bottom and it should immediately be placed in a pail or above a sieve. The quality of the sample is checked visually by opening the upper lids, and then the sampler may be emptied into the pail or sieve. If the sampler leaks during lifting, or if the sample is otherwise disturbed, it should be rejected. Observations regarding sediment constitution, colour, smell and other characteristics should be recorded in the field.

The sieve mesh size should be 0.5 mm (this is to be checked on a dissecting microscope with an ocular grid), and it should have sides at least 150 mm in height. Each sample is sieved separately by moving the sieve in the water, with the net just below water surface. The sieving residue, including the animals, is rinsed with water into a sample jar with a volume of e.g. 500 ml. Animals caught in the sieve mesh should be removed with forceps, and the sieve then rinsed clean. The sample is immediately preserved with ethanol (final concentration ca. 70%).

If the deep zoobenthos sampling site appears anoxic, a similar set of samples should be taken from a representative site in the oxic profundal.

Littoral zone

Sampling sites are chosen from the vicinity of macrophyte transects (not on the transects themselves), so that the sites represent the same vegetation and bottom types as the transects.

Littoral zoobenthos is sampled using a triangle-frame hoop net with a long handle (Fig. 3). The handle should be marked in centimeters to facilitate depth recording. The mesh size of the nylon net hoop should be 0.5 mm.

At least one sample should be taken from each vegetation zone, and sampling sites should be chosen from areas of densest vegetation (not at zone margins if only one sample for each vegetation type is taken). In shallow water, the sampling is best done by wading: while moving slowly forward, the person taking the samples stirs the bottom with his feet and collects the suspended material in the net, which should be kept close to the stirred area and tilted above it. For one sample, stirring and netting should

typically continue for about one minute, during which time a strip of about 2 m in length is covered in the direction of the shoreline. In deeper water, down to several meters, the sampling may be done from a boat using a long-handled sampler and stirring the sediment with the frame of the net. Care must be taken not to rip the net.

When the net is lifted, the material is washed down to the bottom of the hoop. The net is then turned inside out and the contents emptied into a pail. Animals caught in the mesh are carefully removed with forceps. The sample is sieved, and the sieving residue transferred to one or several sample jars (volume e.g. 0.5 l). The sample is stored in ethanol (final concentration ca. 70%). The net should be carefully rinsed clean before the next sample is taken.

The Ekman grab may also be used for littoral benthos samples if the bottom is sufficiently soft.

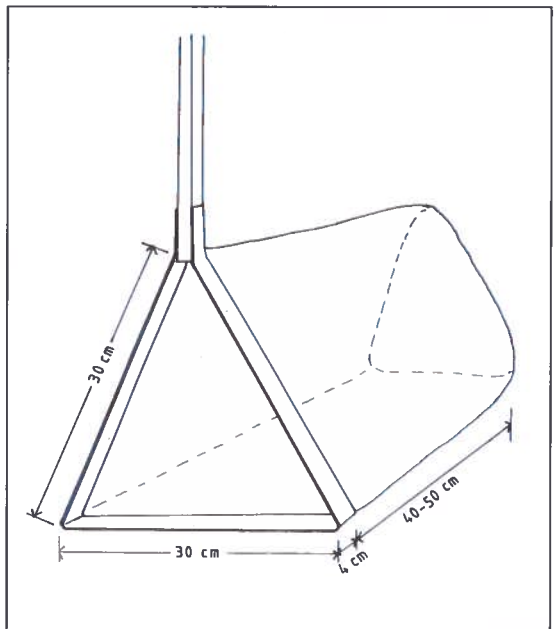


Fig. 3. Triangle-frame hoop net for littoral zoobenthos sampling.

Laboratory work

The samples are stored in a dark place. During storage the biomass of the animals changes. To achieve good comparability between samples, the biomass determination should not be done earlier than 1 month after sampling. The biomass is determined as wet weight, because this allows further storage of the material for taxonomic analyses. For enumeration and weighing, the animals are sorted by species or group into petri dishes containing storage medium or pure water. To make the picking easier, the animals can be stained with Bengal red, a few drops of which should be added to the sample jar at least one day earlier. The enumeration is done in connection with the species identification.

Before weighing, the animals are kept in pure water for 10 min. Grouped according to the desired taxonomic precision, the animals are first placed for a moment on filtering paper, and then shifted onto a piece of preweighed aluminium foil, which is slightly folded closed.

This foil bag with the animals in it is weighed at an accuracy of 0.1 mg, and the fresh-weight biomass is calculated by subtracting the foil weight from the gross weight.

Permanent microscope slides are prepared as necessary for species identification (e.g. of heads of small Oligochaeta).

After all animals are removed, the sieving residue is discarded. The animals are stored in tight-capped glass jars in 70% ethanol into which glycerol is added (50 ml glycerol in 1000 ml of ethanol solution).

Expression of results

Of the quantitative samples, the results for each species are given as ind. m^{-2} and as $g\ m^{-2}$ (fixed wet-weight biomass). The qualitative or semiquantitative results of the littoral samples are indicated as individual numbers and biomass values for each sample and as percentage proportions for each taxon.

8 Fish

Introduction

Fish populations may react sensitively to environmental changes. A major problem in fish studies is that no single method alone will give a complete picture of the fish stock in a body of water (Bagenal 1978). In the long run, comparable results will, however, be achieved by repeating the fishing experiments the same way each time.

In the Integrated Monitoring, the fish community structure, relative abundance proportions and growth rate of the species are monitored by test fishing. In addition, the population structure of perch (*Perca fluviatilis*) is studied by the mark-recapture method and its spawning success is monitored (Rask 1983). Heavy metal concentrations in fish are also monitored (Optimum programme, Table 2).

Test fishing

The lakes are netted every 2 years in August (Table 2). A gill net series with mesh sizes of 12, 15, 20, 25, 30, 35, 45, 60 and 75 mm should be used.

The netting experiment must always be done at the same sites, which are to be marked on maps. A suitable number of netting sites (and netting nights) is one for each ten hectares of water. At each site, all the nets should be kept in the same depth zone, clearly either above or

below the thermocline. Both the epilimnion and the hypolimnion of the pelagial and the littoral should be netted.

The nets are to be set in the afternoon and lifted the next day after 14 to 16 hours. Observations on weather, water quality and any slimy growth on the nets should be recorded. The count of each species and the total weight of the catch are recorded. A random sample of (preferably) 100 specimens of each species present in the catch is taken for body length (mm), weight (g) as well as for age and growth determination. The other fishes are only measured (size interval 10 mm). Age is determined from scales for cyprinids and coregonids, from the operculum and scales for perch, from the cleithrum and metapterygoideum bones for pike (*Esox lucius*) and from the otolith for burbot (*Lota*). The scale samples of the cyprinids are extracted from between the tip of the pectoral fin and the lateral line; those of whitefish and vendace (*Coregonus* spp.) from between the pelvic fins.

Mark-and-recapture

A known number of fish caught in the lake are marked and released back into the lake. In a subsequent catch, the proportion of marked individuals will reveal what proportion of the entire stock was marked in the initial catch. When this procedure is followed over a number

of years, the mortality and age distribution of the fish can also be estimated.

The marking catch is done in the perch spawning season (in spring). Metal net weirs with a mesh size of 1/2 inches are placed evenly along the littoral of the lake. The weirs are examined daily for about two weeks. During the catch, at least 10% of the lake's perch population should be marked by making a cut into a fin. The method of marking should be specific for each year. During marking, the length of the fish are recorded to an accuracy of 10 mm. The time that the fish are exposed to air should be kept to a minimum. After about 2 initial catching days, which can be reserved solely for marking, a record should also be kept of marked (including year of marking) and non-marked individuals. At the end of the catching period, at least 100 specimens are weighed to establish the length/weight relationship and biomass of the population. Weighing at the end of the two-week study period will reveal the population biomass after spawning, i.e. without sexual products. The 1-year age class is excluded from the 1/2 inch mesh weir samples.

Echosounding

Population densities of pelagial fish may be estimated by echosounding. If feasible, this may be used as a complementary monitoring method.

Spawning success

The mortality of perch roe is studied in the same lakes as the mark-and-recapture method is applied (Table 2). A sample of 300 to 500 eggs, representing 10 roe strings, is collected immediately after spawning. The eggs are reared in the lake at a depth of 0.5 m in plastic boxes with shadowing lids and open sides, covered with e.g. 1.0 mm mesh nylon net. Viable and dead eggs are enumerated on three occasions: first at the beginning of the experiment and last slightly before hatching (about 2 weeks from spawning); the spawn must be kept fully immersed even during counting. Another sample of 50 to 100 eggs, for establishing the fertilization percentage of the roe, is brought to the laboratory in lake water and allowed to develop at a temperature of 10-15°C.

In addition to perch, the survival of pike roe may be studied. Samples of 50 to 100 eggs are placed to each of four boxes made of plastic and nylon net. These are incubated at a depth of 0.5 m. Mortality is monitored by a daily count and removal of dead eggs.

Heavy metal analysis

Heavy metal determinations of fish, principally pike and perch, are preferably done in every lake (Table 2).

Five specimens of pike, about 1 kg each (not less than 0.6 kg), should be caught. A smaller number may have to suffice if there is reason to believe that the catch would significantly affect the lake's pike population. In the case of perch, two size-classes (about 20 g and 100 g) and five specimens of each are sampled. The fish are weighed and their length measured; they are then wrapped in aluminium foil and deep-frozen in plastic bags classified as foodstuff packing material. The selection of heavy metals to be analyzed must be considered lake-specifically; for the methods see e.g. Verta *et al.* (1990).

Fish diseases and fish kills

Systematic monitoring of fish health is beyond the scope of the Integrated Monitoring. However, observations of fish diseases should be recorded. Samples of diseased fish should be properly deep-frozen and forwarded to specialists.

It is rather improbable that massive fish kills will occur in the lakes covered by the Integrated Monitoring. If, however, fish kill is observed, fish apparently dying but still alive should be collected for closer studies.

Fish sample repository

The fish sample repository has been set up to provide prolonged storage of samples (for decades) for eventual future needs, and facilitation of long-term quality control of the analyses. The fish are stored deep-frozen. As the samples will require extensive storage space, the material to be stored must be carefully selected. The material should include temporally representative samples of the major fish populations in the monitored lakes; in addition, specific samples of potential future interest should be stored.

9 Sedimentation

Introduction

Sedimented particulate matter in water consists of autochthonous material produced in the lake and of allochthonous material carried into the lake from its drainage area and via atmospheric transport. Sedimented matter is an important energy source for profundal biota. The quantity of organic matter depends on phytoplankton production, for instance. Hypolimnetic anoxia may result from excessive organic sedimentation.

In the Integrated Monitoring, sedimented matter is monitored quantitatively throughout the year with sediment traps suspended in the deepest part of the lake. The trap is a cylindrical vessel, open at the top, in which matter collects. Sediment is removed from the traps at regular intervals. No preserving chemicals are used.

Sampling and laboratory work

Sediment traps are suspended 2 m above bottom. The height/diameter ratio of the collecting cylinder should be at least 5:1, and the diameter ≥ 5 cm (Bloesch & Burns 1980). A vertical row of small holes drilled in the side of the cylinder, closed with stoppers during use, provides easy removal of excess water during sampling.

The sampling assembly consists of the following components, connected with a rope: surface float; submersed float; sampling cylinder

in its frame; anchor (Fig. 4). The submersed float must be big enough to keep the sampler rope vertical and it should be placed at least 2 m above the cylinder to prevent sedimentation of periphytic growth on it from entering the cylinder. The cylinder should be positioned horizontally at a distance of at least 20 cm from the rope. For the sake of balance in the vertical position, two cylinders, placed symmetrically on both sides of the rope, may be feasible. If there are significant currents in the lake, the suspending frame may be equipped with a tail fin to keep the cylinders away from the lee of the rope.

The optional sampling interval is two weeks. The trap is lifted to the surface slowly and evenly, trying to avoid resuspension of the collected sediment. Excess water is allowed out of the cylinder, either by opening the stoppered holes one at a time, or through a siphon tube. Some water must remain in the cylinder to prevent loss of the sample. After the sample and the remaining water has been transferred to a sample jar, the traps (and floats, if necessary) are scrubbed clean and lowered back into the water.

In the laboratory, the sample is thoroughly mixed and split into a number of equal subsamples. These are allowed to settle for a few hours, after which the excess water is decanted off, and the samples are stored in a deep-freeze.

As an alternative method, subsamples can be filtered through preweighed Whatman GF/C filters. Then, before drying and dry-weight determination, large animals (particularly copepods and *Chaoborus* larvae) apparently alive before freezing are picked out with forceps. For this purpose, the subsample volume should not be so high that the sedimented matter covers any animals on the filter. The filtering method should be preferred when a significant number of invertebrates is collected in the sediment trap.

One or several of the subsamples are subsequently analyzed, and at least one should be kept for the sample repository.

After thawing, the subsamples are dried for 24 h at 60°C and weighed. Organic carbon, total nitrogen and total phosphorus are determined from the dried samples. Ignition residue is obtained by incinerating a dry sample overnight at 450°C. Organic matter is calculated by subtracting the weight of the residue from the dry weight.

Expression of results

The quantity of sedimented matter is calculated with the equation (other parameters analogously):

$$W_a = \frac{W_m \times k \times 10^4}{n \times A}$$

where:

W_a = sedimented matter (as dry matter),
mg m⁻² d⁻¹

W_m = weighed dry matter of the subsample,
mg

k = ratio of total sample vol. to subsample vol.

n = sampling interval, d

A = sampling area of the cylinder, cm²

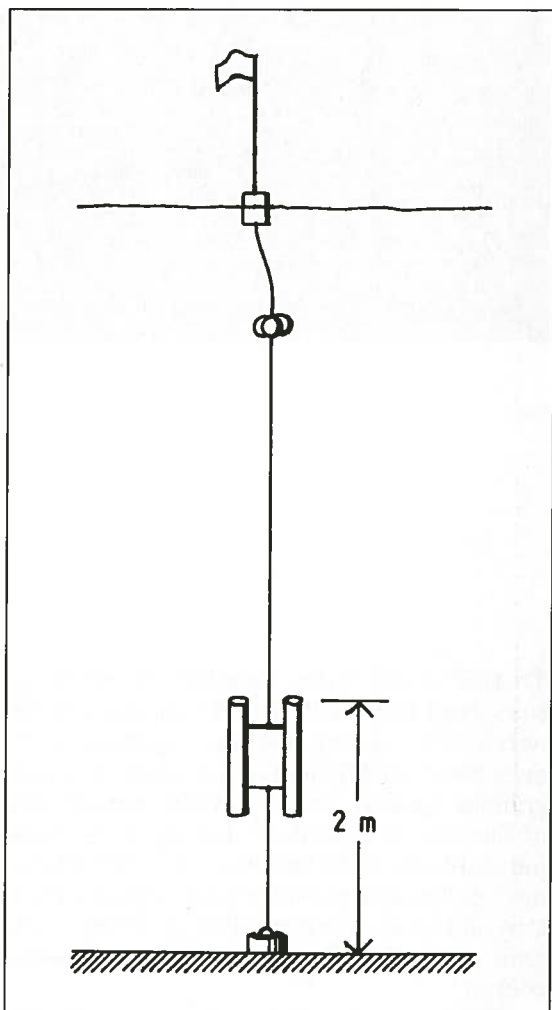


Fig. 4. Sediment sampling assembly with two cylindrical traps.

10 Quality control

To achieve and retain comparability of the results, both temporally in the long run and between different sites, continuous quality control must be an integral part of the monitoring programme. Quality control includes maintenance of the sample repository, testing of methods, intercalibration and internal control of the laboratories. Quality control involves advance planning and it takes time; possibly up to 10% of the total work time should be allotted to quality control.

The sample repository facilitates subsequent checking of chemical determinations or taxonomic issues, for instance. In addition, the repository provides material for further research as necessary in the future. In this capacity, it also serves scientists not directly involved in the Integrated Monitoring. In addition to the original samples, an essential part of the repository consists of pictorial material, which should be stored on computer. Practical procedures for forwarding material to the repository (permanent samples, photos, etc.) are given in each chapter of this manual.

Standardization of all methods is desirable, but it must also be possible to include new methods in the programme. Furthermore, some of the methods may not be applicable to all the monitoring areas. Any differences in results caused by the use of varying methods must always be tested. The goal is to achieve directly comparable results, even when different methods are involved. If this is impossible, conversion factors should be worked out for transforming the data of long time-series into values comparable with the results of the latest method.

Internal quality control at the laboratories includes the use of control samples, the origin of which is unknown to the laboratory personnel. Such samples can be replicates or parallels of samples already analyzed, and originating from a site other than that indicated. Control samples can constitute a few per cent of the total number of samples.

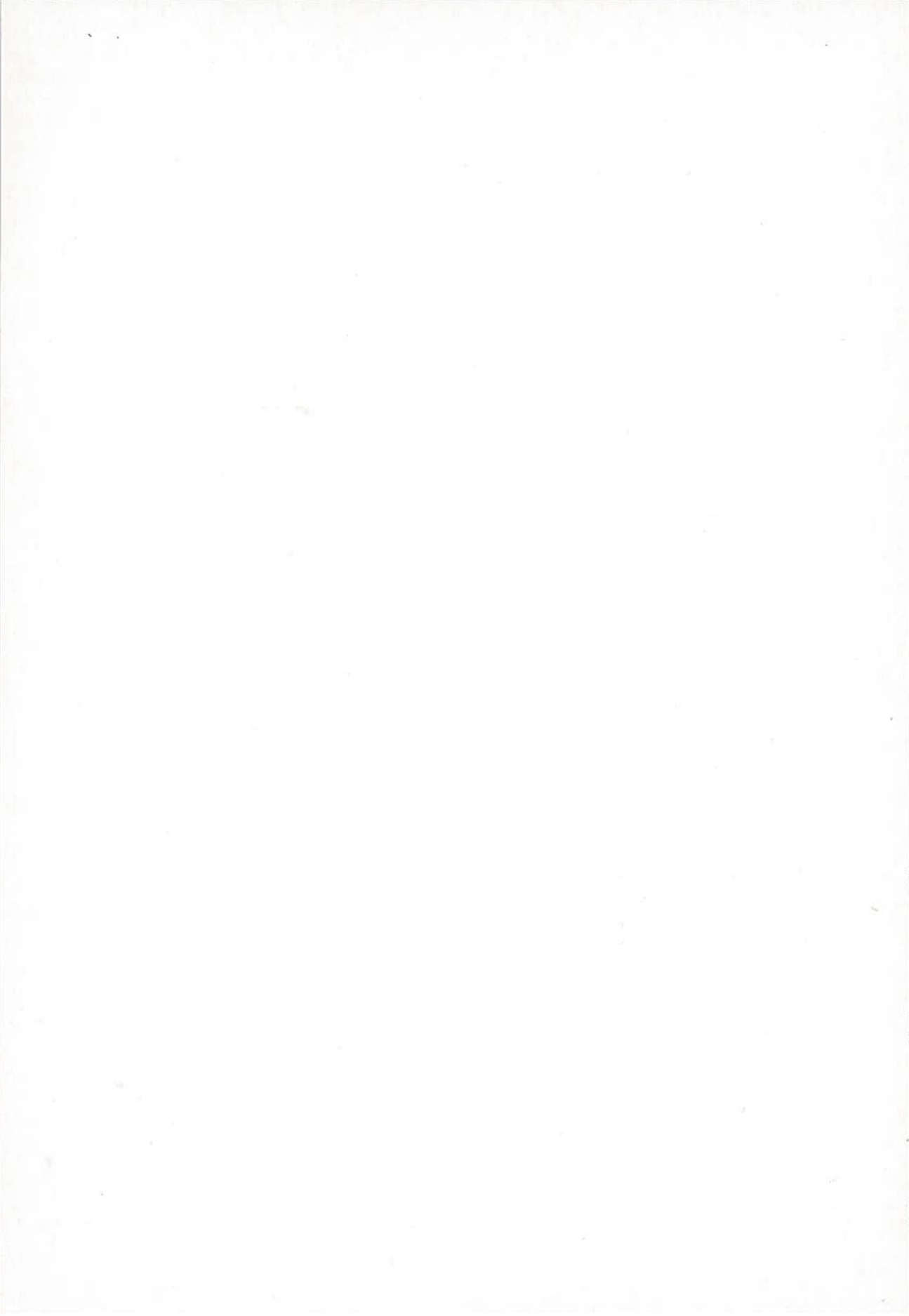
Intercalibration control should be conducted regularly between laboratories involved in the Integrated Monitoring, and also between these laboratories and others outside.

Literature

- American Public Health Organization 1971: Standard methods for the examination of water and waste water. 13th ed.
- Bagenal, T. (ed.) 1978: Methods for assessment of fish production in fresh waters. IBP Handbook 3. 3rd ed. Oxford. 365 pp.
- Bergström, I., Heinänen, A. & Salonen, K. 1986: Comparison of acridine orange, acriflavine and bisbenzimidazole stains for enumeration of bacteria in clear and humic waters. *Applied and Environmental Microbiology* 51: 664–667.
- Bloesch, J. & Burns, N.M. 1980: A critical review of sedimentation trap technique. *Schweiz. Z. Hydrol.* 42: 15–55.
- EDC 1993: Manual for Integrated Monitoring. Programme Phase 1993–1996. UN ECE Convention on Long-Range Transboundary Air Pollution. International Co-operative Programme on Integrated Monitoring on Air Pollution Effects. Environmental Report 5. Environment Data Centre (EDC), Helsinki. 114 pp.
- Edler, L. (ed.) 1979: Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. *Baltic Marine Biologists*. Publication 5. 38 pp.
- Golterman, H.L. & Clymo, R.S. (eds.) 1970: Methods for chemical analysis of fresh waters. IBP Handbook 8. Blackwell Scientific Publications. Oxford and Edinburgh. 2nd printing. 166 pp.
- International Organization for Standardization ISO 1988: Water quality - Design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters. International Standard ISO 8265. 9 pp.
- Jensen, S. 1977: An objective method for sampling the macrophyte vegetation in lakes. *Vegetatio* 33: 107–118.
- Kahl, A. 1930–1935: *Urtiere oder Protozoa. Die Tierwelt Deutschlands* 18: 1–180 (1930), 21: 181–398 (1931), 25: 399–650 (1932), 30: 651–886 (1935).
- Kuparinen, J. 1987: Production and respiration of overall plankton and ultraplankton communities at the entrance to the Gulf of Finland in the Baltic Sea. *Marine Biology* 93:591–607.
- Lee, J.J., Hutner, S.H. & Bovee, E.C. (eds.) 1985: An illustrated guide to the Protozoa. Society of Protozoologists. Lawrence, Kansas. 629 pp.
- Lorenzen, C.J. 1967: Determination of chlorophyll and phycocyanin: spectrophotometric equations. *Limnol. Oceanogr.* 12: 343–346.
- Meriläinen, J.J. 1984: Zonation of the macrozoobenthos in the Kyrönjoki estuary in the Bothnian Bay, Finland. *Ann. Zool. Fennici* 21:89–114.
- Meriläinen, J.J. & Hynynen, J. 1990: Benthic invertebrates in relation to acidity in Finnish forest lakes. In: Kauppi, P., Kenttämies, K. & Anttila, P. (eds.), *Acidification in Finland*, pp. 1029–1049. Springer Verlag, Berlin, Heidelberg.
- Niemi, M., Kuparinen, J., Uusi-Rauva, A. & Korhonen, K. 1983: Preparation of algal samples for liquid scintillation counting. *Hydrobiologia*. 106: 149–156.
- Pomroy, A.J. 1984: Direct counting of bacteria preserved with Lugol iodine solution. *Applied and Environmental Microbiology* 47: 1191–1192.
- Porter, K.G. & Feig, Y.S. 1980: The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* 25:943–948.
- Rask, M. 1983: The effect of low pH on perch, *Perca fluviatilis* L. I. Effects of low pH on the development of eggs of perch. *Ann. Zool. Fennici* 20: 73–76.
- Rebsdorf, A. 1972. The carbon dioxide system in freshwater. A set of tables for easy computation of total carbon dioxide and other components of the carbon dioxide system. Freshwater Biological Laboratory, Hillerød. 66 pp.

- Salonen, K. 1981: Rapid and precise determination of total inorganic carbon and some gases in aqueous solutions. *Water Res.* 15: 403-406.
- Salonen, K. & Sarvala, J. 1985: Combination of freezing and aldehyde fixation. A superior preservation method for biomass determination of aquatic invertebrates. *Arch. Hydrobiol.* 103: 217-230.
- Schindler, D.W. 1987: Detecting ecosystem responses to anthropogenic stress. *Can. J. Fish. Aquat. Sci.* 44 (Suppl. 1): 6-25.
- Steemann Nielsen, E. 1952: The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Perm. Int. Explor. Mer.* 18: 117-140.
- Takahashi, M. & Ichimura, S. 1970: Photosynthetic properties and growth of photosynthetic sulfur bacteria in lakes. *Limnol. Oceanogr.* 15: 929-944.
- Utermöhl, H. 1958: Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Internat. Verein. Limnol.* 9: 1-38.
- Verta, M., Mannio, J., Iivonen, P., Hirvi, J.-P., Järvinen, O. & Piepponen, S. 1990: Trace metals in Finnish headwater lakes - Effects of acidification and airborne load. In: Kauppi, P., Kenttämies, K. & Anttila, P. (eds.), *Acidification in Finland*, pp. 883-908. Springer Verlag, Berlin, Heidelberg.
- Vollenweider, R.A. (ed.) 1974: A manual on methods for measuring primary production in aquatic environments. *IBP Handbook 12*. Blackwell Scientific Publications, Oxford. 2nd ed. 225 pp.
- Väätänen, P. 1979: Microbial activity in brackish water determined as oxygen consumption. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* 12: 32-37.
- Willén, T. & Tikkanen, T. 1992: Växtplanktonflora. (Phytoplankton flora; in Swedish.) *Naturvårdsverket*. Solna. 280 pp.
- Wintermans, J. F. G. M. & De Mots, A. 1965: Spectrophotometric characteristics of chlorophylls a and b and their pheophytins in ethanol. *Biochim. Biophys. Acta* 109: 448-453.

1. Santala, Erkki (toim.): Pienet jäteveden maapuhdistamot. Ohjeita 1 - 10 talouden jätevesien maaperäkäsittelystä. Helsinki 1990.
2. Pajula, Heikki & Pasonen, Aarre: Ojitustoimitus. Helsinki 1990.
3. Heino, Soini; Kinnunen, Ilpo; Nissinen, Raimo K. & Pajula, Heikki: Putkiojien suunnittelu. Helsinki 1990.
4. Vesirakennustyön haittojen vähentäminen. Helsinki 1991.
5. Saviranta, Leena & Vikman, Hannu (toim.): Suomen vesihuollon suuntaviivat. Helsinki 1990.
6. Syrjälä, Kari; Kaarikivi-Laine, Ulla; Pajula, Heikki; Jaakkola, Mauri & Timonen, Risto: Vesirakennustöiden kiinteistökohtaiset sopimukset ja sopimuslomakemallit. Helsinki 1990.
7. Britschgi, Ritva; Hatva, Tuomo & Suomela, Tapani (toim.): Pohjavesialueiden kartoitus- ja luokitusohjeet. Helsinki 1991.
8. Kurttila, Terttu: Maisemanhoito vesistöarakentamisessa. Helsinki 1991.
9. Patoturvallisuusohjeet. Helsinki 1991.
10. Mäkelä, Ari; Antikainen, Sari; Mäkinen, Irma; Kivinen, Jarmo & Leppänen, Tuula: Vesitutkimusten näytteenottomenetelmät. Helsinki 1992.
11. Kyllästämötyöryhmä, Viitasaari, Sauli (toim.): Kyllästämöiden ympäristö- ja työturvallisuus. Helsinki 1991.
12. Velvoitetarkkailutyöryhmä, Vuoristo, Heidi (toim.): Yleisohjeet velvoitetarkkailusta. Helsinki 1992.
13. Hatva, Tuomo & Suomela, Tapani (toim.): Pohjavesiasioiden kehittäminen vesi- ja ympäristöhallinnossa. Helsinki 1992.
14. Salojärvi, Kalervo: Suosituksia sisävesien siikaistutuksista. Helsinki 1992.
15. Hatva, Tuomo; Hyyppä, Juho; Ikäheimo, Jukka; Penttinen, Heikki & Sandborg, Matti: Soranoton vaikutus pohjaveteen. Raportti V: Soranotto ja pohjaveden suojele. Helsinki 1993.



Many processes occur more rapidly in lakes than in terrestrial environments and thus require higher sampling frequencies. This increases the costs and time needed for hydrobiological research and monitoring programmes. However, if the monitoring is implemented with frequencies matching the life cycles of aquatic organisms, it will be better able to discriminate between causes and their effects. This is particularly relevant in view of the variety of different episodic situations occurring in small headwater lakes during relatively short time periods.

A collection of methods for the biological monitoring of lakes is described in this manual. Of course, no method is universal for all situations, but the authors believe that the proposed methods are reliable in most cases. In addition to the simplicity and robustness of the methods, much attention has been paid to integration of different procedures so that minimum time would be consumed in the field and the laboratory.

This manual was prepared for the purposes of the UN ECE International Co-operative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems. In Finland, the intensive national hydrobiological monitoring programme was attached to the International Programme and involved preparation of the instructions for researchers. The authors hope that other parties can also make good use of this manual.