

**PHYTOPLANKTON**

**Aim** *To monitor changes in the chlorophyll concentration of standing and running waters and in counts of phytoplankton in standing waters.*

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**Rationale**

Many water quality problems in inland waters are related to the growth and accumulation of phytoplankton, the microscopic algae which dominate the pelagic environment. Phytoplankton provides an important source of food for many aquatic organisms and plays a key role in nutrient cycling. The simplest method of estimating the biological productivity of a lake is to measure the concentration of phytoplankton chlorophyll present during the growing season. The conventional method of measuring phytoplankton biomass is to filter a sample of water and then extract the photosynthetic pigment chlorophyll *a*, using organic solvents such as acetone, ethanol or methanol. A detailed discussion of the assumptions and problems of chlorophyll determination is given by Vollenweider (1974) and in papers by Marker (1977) and Marker and Collett (1991). Some water quality problems are, however, caused by qualitative rather than quantitative changes in the phytoplankton so it is also useful to know what species are present at different times of the year. The most important taxonomic groups of phytoplankton are the green algae (Chlorophyceae), blue-green algae (Cyanophyceae) and diatoms (Bacillariophyceae – see Protocol FDT, page 81). The identification of phytoplankton to species level is a specialised task but simple guides have been produced by Belcher and Swale (1976) and Pentecost (1984). Quantitative information of this kind is particularly useful in lake studies. The phytoplankton populations in rivers are more difficult to quantify and are strongly influenced by changes in the flow regime.

**Method****PHYTOPLANKTON CHLOROPHYLL**  
(Standing and running waters)

Most investigators in the UK use the hot aqueous methanol method (Talling & Driver 1963) for extracting phytoplankton chlorophyll; cold acetone and cold ethanol methods (Marker 1992; Jespersen & Christoffersen 1987) are used in some laboratories but there is some evidence that incomplete extraction occurs in cold solvent. The hot methanol method appears to be more efficient and is therefore recommended as the standard for ECN at this time. ECN sites must take the necessary steps to ensure that procedures meet statutory health and safety standards (COSHH).

**Equipment**

Water for both chlorophyll analysis and phytoplankton counts in standing waters should be collected using a suitably designed integrating sampler such as the weighted plastic tube (Lund tube) described by Lund and Talling (1957). A variety of polyethylene containers can be used for transporting and storing of the phytoplankton sample; they should be easy to clean and large enough to contain 2–3 litres of water. Containers should be washed in recommended detergent (eg Decon®90), rinsed with tap water in the laboratory and subsequently, at each site, with the standing or running water to be sampled.

**Location**

- **Standing waters**

Water samples should be taken from a central location near the deepest point and the grid reference should be recorded as accurately as possible, usually to within 30–40 m, together with the water depth at the time of sampling. In circumstances where a boat is not available for sampling it is permissible to sample phytoplankton at the lake outflow or from a jetty or dam which projects over deep water.

- **Running waters**

The grid reference of locations from which samples are taken at running water sites will be recorded, usually to within 10 m.

### Sampling

- **Standing waters**

Water samples should be collected preferably fortnightly and not less than quarterly. For deep lakes the Lund tube is lowered into the water to the appropriate depth and the weighted end raised by rope to discharge the contained water into the collecting vessel. If the tube is made of relatively stiff material no closing device is required but the sampler may have to be lowered repeatedly to collect a sufficiently large volume of water. For shallow lakes, of depth 1 m or less, and for sites which cannot be sampled by boat, 'bucket samples' are more appropriate. Water samples are collected in a washed plastic bucket that is either carried into the shallows or lowered from a suitable jetty or wall and plunged below the surface to collect a near-surface water sample. Sub-samples of the collected water can be removed by any convenient, objective, volumetric method as long as care is taken to avoid spillage.

- **Running waters**

Water samples should be collected preferably weekly and not less than monthly. Sampling procedures should follow the guidelines set out in the ECN FSP Protocol (page 49) 'Recommended sampling procedures for water chemistry'. Sub-samples of the collected water can be removed by any convenient, objective, volumetric method as long as care is taken to avoid spillage.

### Pigment extraction

The samples of water collected for chlorophyll analysis should be returned to the laboratory as quickly as possible and stored in the dark at 4°C until they can be processed. In the methanol extraction procedure, a known volume of water is filtered through a glass-fibre (eg Whatman®GF-C) filter and the moist filter paper removed and placed in a test tube. A known volume of 100% methanol is added and the contents of the tube brought to the boil for a few seconds in a water bath or an electrically heated block. The samples are then allowed to stand for 10 minutes at room temperature in the dark before removing the filter papers and centrifuging the extract to remove any particles in suspension. The absorbance of the extract is determined at 665 nm and 750 nm in a 1 cm or 4 cm cuvette as appropriate for the optical density. The absorbance at 750 nm is subtracted from that at 665 nm to correct for general turbidity and background absorption. The chlorophyll *a* concentration in the sample is given by the formula:

$$\text{Chl } a \text{ } \mu\text{g L}^{-1} = v/V \cdot f/l \cdot A$$

where *v* is the total volume of solvent extract in ml, *V* is the volume of the sample filtered in litres, *l* is the light path in cm, *A* is the absorbance at 665 nm corrected for that at 750 nm and *f* is a factor equivalent to the reciprocal of the specific absorption coefficient multiplied by 10 (estimated as 13.9 by Talling and Driver (1963)).

## Method

### PHYTOPLANKTON COUNTS

(Standing waters only)

Preparation for phytoplankton counting may be done in a variety of ways, including centrifugation and the use of sedimentation chambers. In the recommended method, acidified Lugol's iodine (see Appendix I, page 65) is added to a known volume of water in a graduated cylinder immediately after

collection and the sample allowed to stand for 24 hours before siphoning off the supernatant liquid.

### **Equipment**

Water samples should be collected using a suitably designed integrating sampler such as the weighted plastic tube (Lund tube) described by Lund and Talling (1957). A variety of polyethylene containers can be used for removal of the phytoplankton sample. The containers used should be easy to clean and the volume of water used in the graduated cylinders should reflect the presumed productivity of the site, (ie 100 ml for a productive lake and 1000 ml for an unproductive lake). Containers should be washed in recommended detergent (eg Decon®90) and rinsed with tap water in the laboratory and subsequently in lake water at each site.

### **Location**

Water samples for phytoplankton counts will normally be taken at the same location and at the same time as those for phytoplankton chlorophyll determination (see above) (ie they should be collected from a central location near the deepest point). The grid reference of standing water sampling sites should be recorded as accurately as possible, usually to within 30–40 m, together with the water depth at the time of sampling.

### **Sampling**

Water samples for phytoplankton counting should be collected preferably fortnightly and not less than quarterly, using the same methods as are described above for phytoplankton chlorophyll analysis. A note should be made of the presence of significant accumulations of blue-green algae on the water surface at the sampling location.

### **Counting procedures**

A number of cells and chambers have been designed for the microscopic examination of freshwater phytoplankton. One of the most satisfactory methods is that developed by Lund (1959), commonly referred to as the Lund chamber. This simple device is constructed from a rectangular coverslip mounted on thin strips of glass that are then cemented to a glass slide to form a chamber with open ends. A full description of the method of construction is given in Jones (1979), and at least one version is available commercially from the Water Research Centre. The distribution of phytoplankton in a Lund chamber is known to conform to a Poisson distribution (Lund, Kipling & Le Cren 1958) so counting errors are small when compared to the field sampling error. Counts of phytoplankton are made by taxa and related to the volume of the sample; individuals should be identified only to the level of the identifier's expertise. Where sub-sampling is used, its nature must be documented and reported to the CCU. Counts are reported as number of cells per ml by taxon, but where colonial taxa are present they are reported as number of colonies per ml by taxon. Filamentous taxa are reported as length of filament per ml.

### **Archiving samples**

Phytoplankton samples should be archived by preserving a sub-sample of 10–20 ml with a few drops of acidified Lugol's iodine solution in a well-stoppered container. Note that if plastic containers or stoppers are used the iodine will eventually volatilise. However by that time the iodine should have successfully sterilised the sample and should not therefore cause a problem. Archived samples should be checked periodically for evaporative loss.

**Author**

**FPP Protocol**

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## References

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## Appendix I

Lugol's iodine is prepared as follows (Wetzel & Likens 1991).

### Acidified

Dissolve 20 g potassium iodide and 10 g iodine crystals (caution: toxic) in 200 ml distilled water containing 20 ml concentrated glacial acetic acid.

### Non-acidified

Dissolve 20 g potassium iodide and 10 g iodine crystals in 200 ml distilled water.

To preserve samples with Lugol's iodine add 0.3 ml of the solution to 100 ml of sample and store in the dark. For long-term storage add 0.7 ml of the solution per 100 ml of sample and buffered formaldehyde to a minimum of 2.5% final concentration after 1 hour.

## Specification of results and recording conventions

The measurement variables listed below are those required for each FPP sampling location at an ECN Site. Sites submitting data to the ECNCCU should refer to the accompanying Data Transfer documentation for the specification of ECN dataset formats, available on the restricted access Site Managers' extranet. Contact [ecncuu@ceh.ac.uk](mailto:ecncuu@ceh.ac.uk) if you need access to this documentation.

The first 4 key parameters uniquely identify a sample or recording occasion in space and time, and must be included within all datasets:

- [Site Identification Code](#) (e.g. R10) Unique code for each ECN Site
- [Core Measurement Code](#) (e.g. FWC) Unique code for each ECN 'core measurement'
- Location Code (e.g. 01) Each ECN Site allocates its own code to replicate sampling locations for each core measurement (e.g. FWC 01, FWC 02 for different surface water collection points)
- Sampling Date (/time) Date on which sample was collected or data recorded. This will include a time element where sampling is more frequent than daily

ECNCCU 2001

### Core measurement: Phytoplankton (FPP protocol)

The following variables are recorded from samples taken at a recommended frequency of weekly for rivers (chlorophyll only) and fortnightly for lakes (chlorophyll and species concentrations):

Variable	Units	Precision of recording
Site identification code		
Core measurement code		
Location code		
Sampling date		
Sampling time	GMT 24-h clock	1 min
<i>Phytoplankton chlorophyll analysis:</i>		
Chlorophyll <i>a</i>	$\mu\text{g l}^{-1}$	3 sig. figs.
Analysis date		
<i>Species concentration (Lakes only) for each species present:</i>		
Species code	8-digit code <sup>(1)</sup>	(eg 13080100)
Species name	genus species	eg <i>Asterionella formosa</i>
Species type	2-character code <sup>(2)</sup>	
Concentration	indivs $\text{ml}^{-1}$	1
	or $\text{mm ml}^{-1}$ if filamentous	1

#### Notes

<sup>(1)</sup> ECN uses the Whitton *et al.* (1998) coded list of freshwater algae. A machine-readable version of this list is available from the NERC Land-Ocean Interaction Study (LOIS) Web site: [http://www.nwl.ac.uk/~loisys/algal\\_coded\\_list.htm](http://www.nwl.ac.uk/~loisys/algal_coded_list.htm), or via the CCU.

<sup>(2)</sup> Phytoplankton species are categorised as:

CE – Cellular

CO – Colonial

FI – Filamentous

This code determines which units of measurement are used for reporting concentration.