

## Algae

In addition to macro-invertebrates, algae are an informative group to monitor as indicators for environmental degradation. For our monitoring purposes we will concentrate on microscopic algae (called phytoplankton) that are suspended freely in the water. All phytoplankton species are sensitive to environmental changes, but some are more sensitive than others. Changes in the diversity of phytoplankton algae species and their relative numbers can be used to gauge water quality changes. The greater the diversity of phytoplankton, in general, the better is the health of the water body. If your monitoring program records a drop in phytoplankton diversity, this may indicate water quality is declining (although natural seasonal changes may also cause fluctuations in the numbers and diversity of algae).

Algae are extremely important in an ecological sense in any aquatic environment for two reasons. Firstly, they are the primary producers of all aquatic food chains, and therefore all aquatic animals and decomposers are dependent on them. Secondly, in the process of food production through photosynthesis, they use up the dissolved CO<sub>2</sub> in the water and release oxygen. The photosynthetic process is important for maintaining sufficient oxygen levels in the water to meet the respiration needs of aquatic animals.

Knowledge about the types and numbers of algae can provide valuable insights into water quality and aquatic food webs. Like most plants, algae produce oxygen and take up nutrients while they are growing, but when they die and decay they can release nutrients. They therefore have a major influence on the nutrient status of a water body. Furthermore, the decay process requires oxygen, so if large numbers of algae are decaying they use up oxygen in the water and can cause fish and macro-invertebrates to die.

The many kinds of phytoplankton are classified into many different classes and families. For our purposes we will group them into four groups, the **blue-green algae**, the **green algae**, the **diatoms** and the **flagellates**.

Blue-green algae in particular are important to monitor because of their potential impact on human and animal health and because high numbers often indicate high nutrient levels in the water. These algae (also called cyanobacteria) reduce water quality when they are present in large numbers. Some produce toxins, odours or thick scum on the water surface. The toxins can cause death and illness in stock, and gastroenteritis, liver damage and skin and eye irritations in humans. The blue-green algae to particularly watch for (because of their public health implications) are *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria* and *Aphanizomenon* species.

Even though the identification and counting of phytoplankton requires a microscope and a certain level of biological expertise and training, participants in water quality monitoring programs are encouraged to develop skills in correctly recognising and counting the above blue-green algae. In addition, it would be useful to develop skills in identifying some green algae, diatoms and flagellates. Some illustrations of each of these groups are included (see pages 32 - 38) to assist with identification. Check with your local co-ordinator regarding possible access to colour photographs of algae.

### Changes in algal populations

A number of environmental factors influence the growth of phytoplankton in an aquatic environment. Some of the more important ones are: nutrient levels (perhaps the most significant), light, water temperature, pH,



salinity and turbidity. Of the nutrients, phosphorus (in its dissolved form as the phosphate ion) and nitrogen (largely in the nitrate or nitrite form, but also as the ammonium ion) greatly influence algal growth. Many studies have reported a high correlation between algal growth and nutrient availability in the water.

While algal blooms may occur under natural conditions, in recent years they have increased in frequency and intensity. The reasons appear to include pollution of waterways with nutrients (especially through sewage and industrial effluents), reduction in water flow (through irrigation, industrial and domestic uses) and degradation of river and lake ecosystems.

## Monitoring program for algae

The first step in monitoring algae is to design an appropriate monitoring strategy. Then you are able to choose appropriate site(s), collect the samples, concentrate the water

sample you collected, and finally count the algal cells using a microscope and a special counting slide called a Sedgwick-Rafter Chamber.

## Designing a phytoplankton - monitoring program

A phytoplankton-monitoring program can be set up for a number of reasons and the specific goals determine when and where to sample. It is important therefore to clearly define your goals and design your monitoring program before beginning to collect samples. Assess your resources and available time, and seek advice from the water authorities in your State regarding selection of methods, sampling sites, frequency of sampling, counting techniques etc. to meet your specific goals.

## Choosing your sampling sites

Your choice of sampling site(s) will depend on your monitoring goals.

### Goal for the monitoring program

To assess the quality of the water reaching the consumer or entering the treatment plant

To determine the effectiveness of water-treatment systems

To give sufficient warning of deteriorating water quality in large river systems

To study the long-term changes in a river as reflected by the algae in the water

To gather baseline data and monitor a reservoir or lake for algae

### Sampling sites

Sampling around offtake points will be most important.

Samples of both treated and untreated water should be taken.

Data-sharing with a monitoring team taking samples from a monitoring point 2-3 days, flow time, upstream will be essential.

Several strategically located sites must be carefully selected.

Sampling should be undertaken at several sites and depths because the water is usually poorly mixed. Sites should include one immediately upstream and one immediately downstream of weirs where appropriate, you should also take one sample from the worst-affected area, one from the water off-take area and one from immediately after treatment.

Refer also to the notes on selecting your monitoring site in the Getting Started section of this manual.

## Collecting representative samples

A **representative sample** is one that is typical of the bulk of water being assessed.

In **small to medium-sized flowing streams** the water is generally well mixed, and when monitoring for algae you can collect a representative sample from midstream at a depth of between 10-20 cm below water surface. In **larger streams and rivers**, sampling 1-2 metres away from the bank using a bottle attached to a sampling pole or rod would suffice.

During summer, the water in **lakes and reservoirs** may not be well mixed unless there is turbulence and high winds. If suitable sampling equipment like depth samplers is available then samples taken at varying depths could be examined. For simple identification purposes, a sub-surface dip sample taken from 10-20 cm below the water surface would be sufficient.

**Accumulated floating scums** (concentrated masses of algae) are difficult to count without dilution and should not be included in the water sample for counting purposes.

However, you should note the presence of scum on your Algae Record Sheet and give an estimate of the area it covers. (N.B. Blue-green algae scums can be highly toxic if ingested and can cause skin irritations, so care should be taken in collecting a water sample under these conditions). If collecting samples, avoid body contact, use implements to collect the sample so you do not use your bare hands and place the samples in separate bottles for identification.

## When to sample

It is best to sample at a particular time each day. **Between 8.30 and 10.00 a.m. is the preferred time.** This is because the algae move up in the water to the surface, towards the sunlight, in the morning. In the afternoon the blue-green algae tend to sink to lower

regions. By sampling at roughly the same time on each sampling occasion you can directly compare the algal results from different days.

## Equipment needed

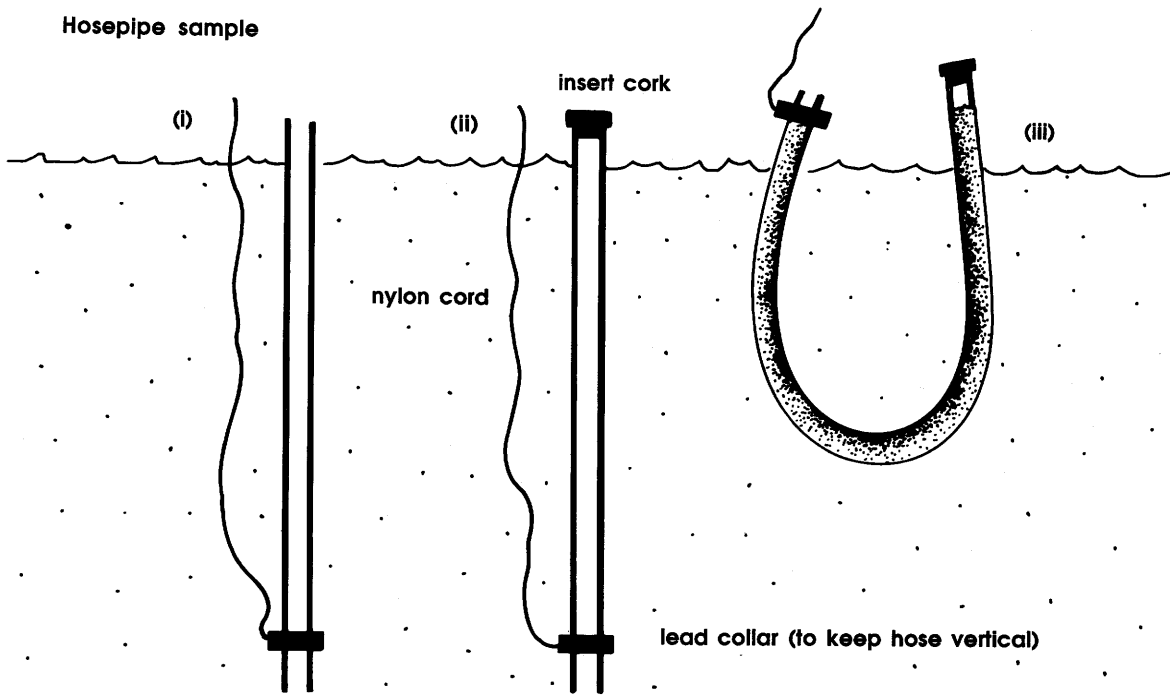
- ◆ A hose-pipe sampler
- ◆ A depth sampler
- ◆ A microscope
- ◆ A Pasteur pipette
- ◆ Graduated glass pipettes (1-mL)
- ◆ A plastic Sedgwick-Rafter Cell (for counting algal cell numbers)
- ◆ Cover slips
- ◆ Sedimentation chambers (100-mL and 10-mL graduated cylinders)
- ◆ Lugols solution

## Hose-pipe sampler

A hose pipe sampler is a weighted tube (such as a length of hose) that can be used to obtain a water 'column' sample. The algae in this sample can then be identified and counted to gain a picture of the algae present through this layer of water.

Hose-pipes are easy to make yourself. They are generally about 20 mm in diameter and 4 - 5 m long but can be varied in length to suit the size of the water body being sampled. Weight the hose at one end and attach a string to that end. Drop vertically into the water, with both ends open. Then cork the top end when the tube is full of water and use the string to pull the lower end up and out of the water. Pull the corked hose-pipe completely out of the water, and empty its contents into a bucket. This water sample - taken over 1 or 2 or 5 metres (according to what is appropriate at your sampling site) - is mixed well in the bucket and then a sub-sample of 500 mL or 1 litre taken as an integrated-water-column sample.

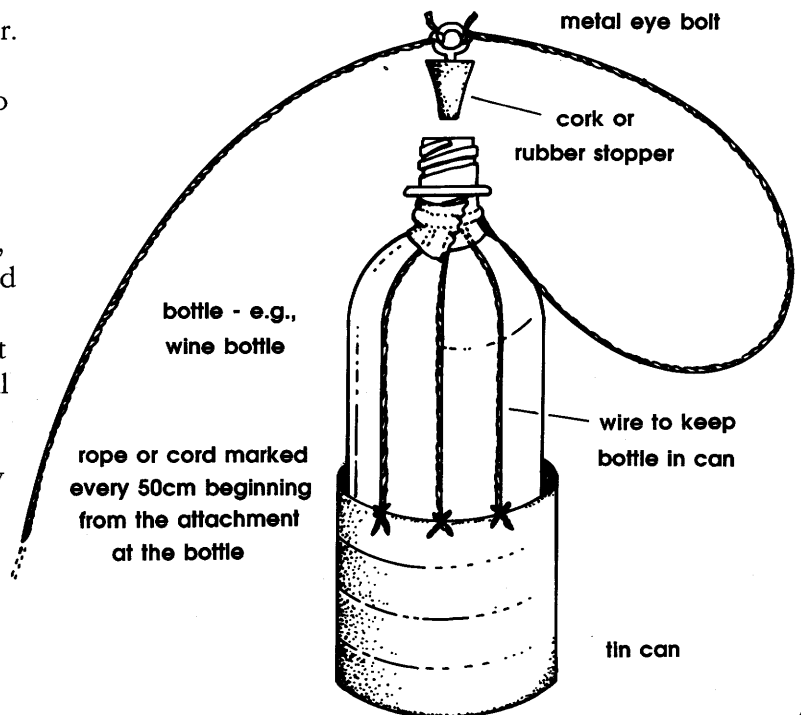
### Hosepipe sample



### Depth sampler

Another sampling device is the depth sampler. A home-made version can be made by weighting a bottle. The bottle can be used to sample for nutrients or plankton, or to measure physical conditions with depth. Smaller bottles may be used for monitoring shallow streams. Stopper the weighted bottle, then lower it into the water. When the desired sampling depth is reached, tug the cord on the cork to pull it out of the bottle. Note that this may be hard to do, as water pressure will be acting on the cork.

Using the water sample you can then identify and count the algae present at the chosen sampling depths.



## Preparing your sample for algal identification and counting

Soon after the sample is collected the bottles must be clearly labelled with a waterproof pen. It is best to examine samples as early as possible after collection, while the algae remain alive because many features helpful in identifying algae are more clearly seen in live specimens. If you have to wait a few hours after collection to examine a sample it would be desirable to keep the sample cold on ice or in a portable refrigerator. However, in some instances, particularly for counting purposes, it is necessary to stain them and preserve the algae. If possible, therefore, identify the phytoplankton in your sample first, before staining them for counting.

Lugols solution is the preferred preservative and staining reagent. Samples can be preserved and kept (in dark bottles away from light) for as long as 1 year in 0.3% Lugols solution - that is, 0.3 mL of Lugols solution per litre of sample. Lugols solution may be available from chemical suppliers or chemists.

### To make up Lugols solution:

Dissolve 20 g potassium iodide and 10 g iodine crystals in 180 mL of distilled water and add 20 mL glacial acetic acid (take care when handling, as this acid is corrosive). The Lugols solution can be added to water samples drop by drop, using a Pasteur pipette. Note that Lugols solution has a shelf life of about 6 months.

### Concentrating the phytoplankton

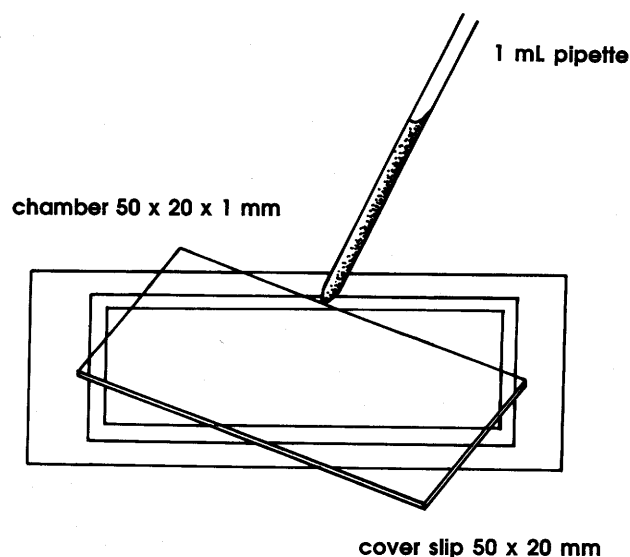
Pour a well-mixed volume of the sample of water - say 100 mL into a measuring cylinder. Add Lugols solution (at the rate of 1 mL per 100 mL) and allow the water to stand overnight (or use a centrifuge if you have access to one) so that the phytoplankton cells sink. (The iodine in Lugols solution not only preserves and stains the phytoplankton but also increases their density by making them become heavy and sink to the bottom.)

When the column of water appears clear, gently siphon off the top 90 mL without disturbing the sediment at the base of the cylinder. This leaves the phytoplankton concentrated in the bottom 10 mL. A well-mixed 1-mL subsample is then taken and placed in the Sedgwick-Rafter counting cell for counting.

This means the phytoplankton present in 100 mL of the water have now been concentrated into 10 mL, resulting in a concentration factor of 10. (Subsamples of this sedimented phytoplankton can also be examined under the microscope for identification.)

### Counting the phytoplankton in your sample

If the water sample contains sufficient number of algae and concentration is not required, a direct count can be undertaken. The sample is thoroughly mixed, treated with a few drops of Lugols solution, mixed again and allowed to stand for 30 to 60 minutes. The iodine is used in this instance as a stain. The sample is mixed again and a subsample of 1 mL taken out using a graduated pipette, and the subsample placed into the Sedgwick-Rafter counting cell (chamber) under gravity.



*Sedgwick-Rafter counting cell*



### Sedimentation chambers

(100 mL and 10 mL graduated cylinders)

- (i) 100 mL sample (or less) sedimented with 1 mL Lugols Iodine for 24 hours, 90 mL siphoned to waste
- (ii) 10 mL remaining is resuspended and 1 mL taken for counting

### Counting procedure

The Sedgwick-Rafter counting cell limits the volume and area of the sample to enable easier counting and calculation of phytoplankton numbers. The counting cell is marked with grids to assist with counting.

Before filling the cell, place the cover slip diagonally across the cell (see the diagram). Placing the cover slip in this way will help prevent formation of air bubbles in the cell corners. The cover slip will rotate slowly and cover the inner portion of the cell during filling. Do not overfill the chamber, as this will make the depth greater than 1mm and so invalidate the calculations. A large-bore pipette should be used to transfer the sample into the chamber.

The phytoplankton sample placed into the Sedgwick-Rafter counting chamber is allowed to stand on a flat surface for 20 minutes to enable the phytoplankton to settle.

It is then transferred to the stage of an upright light microscope and securely positioned, ready for counting.

### Counting strips in the Sedgwick-Rafter chamber

The number of strips to be counted is a function of the precision desired and the number of units (cells, colonies or filaments of algae) per strip. You can calculate the number of phytoplankton in the cell using the following formula.

$$\text{No. per mL} = \frac{C \times 1000}{L \times D \times W \times S}$$

- where: C = number of organisms counted  
 L = length of each strip (cell length) (mm)  
 D = depth of a strip (cell depth) (mm)  
 W = width of a strip (grid image width) (mm)  
 S = number of strips counted.

### Counting fields in the Sedgwick-Rafter chamber

In samples containing many phytoplankton (10 or more per field), make field counts rather than strip counts. Count the phytoplankton in random fields each consisting of one grid. The number of fields counted will depend on the density of the phytoplankton and the statistical accuracy desired.

Calculate the number of phytoplankton per mL as follows:

$$\text{No. per mL} = \frac{C \times 1000}{A \times D \times F}$$

- where: C = number of organisms counted  
 A = area of field (grid image area) (mm)  
 D = depth of field cell depth (mm)  
 F = number of fields counted

Multiply or divide the number of cells per millilitre by a correction factor to adjust for sample dilution of concentration.

### Example of strip counting using the Sedgwick-Rafter cell

The phytoplankton present in four or five strips of the counting chamber are counted.

The Sedgwick-Rafter chamber holds 1 mL. It has the dimensions 50 mm long by 20 mm

wide. The depth of the chamber stage is 1 mm. Since 1mL takes up the full capacity of the chamber, it should occupy 1000 cu. mm.

There are 50 quadrants in the length and 20 quadrants in the width of the chamber (comprising a total 1000 quadrants). A horizontal strip corresponds to 50 quadrants. If you observe four horizontal strips for algae counting, you will have observed 4 x 50 quadrants.

Those 200 quadrants out of 1000 quadrants equate to one-fifth of the quadrants. If you counted x number of algal cells those four in strips, then (5 x) algal cells occur in 1000 quadrants or 1 mL.

$$\text{Algal cells per mL} = \frac{\text{Number of algal cells counted} \times 1000}{\text{Number of quadrants observed}}$$

Due to the fact that algae are seldom evenly distributed in a water body, an agreed 20% level of error has been generally acceptable when counting phytoplankton. A counting error of 20% means that 20% of the time the samples counted will not be truly representative of the water body.

### Recording and interpreting your results

The following Algae Record Sheet should be used to record the diversity and numbers of phytoplankton found in your samples. The presence or absence of blue-green algae is a major indicator of water quality, so particularly note any changes over time in the numbers of *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria* and *Aphanizomenon* species.

Similarly, decreases over time in the diversity of green algae, diatoms and flagellates in your water body indicate a decline in water quality.

### Reporting blue-green algal blooms

It's important to report blue-green algal blooms detected by your monitoring, because of the wide range of water-users who could be affected. Rapid reporting to the

responsible water authority in your area will allow it to sample the site so that its aquatic biologists can verify the identification and numbers of organisms. The authority can then ensure that control procedures are implemented and that water-users are made aware of any water use problems.

As a guide, three alert levels based on algal cell counts have been determined:

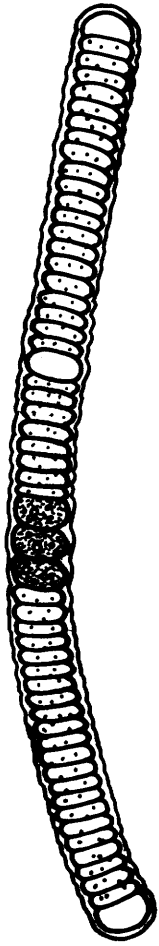
- ◆ **alert level I** (500 - 2000 potentially toxic blue-green algal cells per mL)
- ◆ **alert level II** (2000 - 15000 potentially toxic blue-green algal cells per mL); any cell count over 2000 cells per mL is regarded as a level of concern for drinking-water supplies
- ◆ **alert level III** (greater than 15000 potentially toxic blue-green algal cells per mL)

The illustrations provided in this manual are those commonly found in fresh water. The drawings aim to highlight the main structural features relating to each type of alga. These features can usually be observed through a microscope and are the basis for identification.

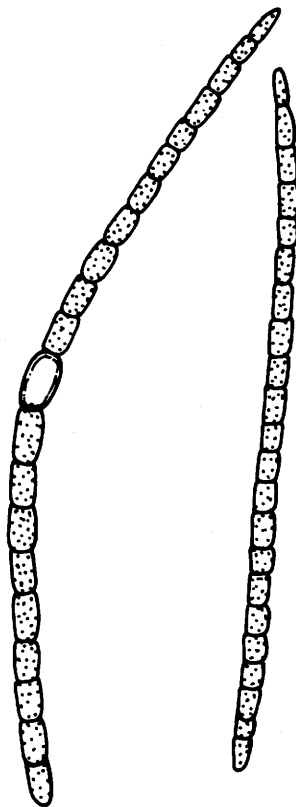
As the species level of identification is regarded as a specialist job, it has been largely omitted with the exception of a few distinct and frequently seen algae. The degree of magnification of the various algal drawings has not been shown. Those who want to know more are encouraged to refer to algal taxonomic books.

# Blue-green algae

(greatly enlarged - varying magnifications)



*Nodularia*



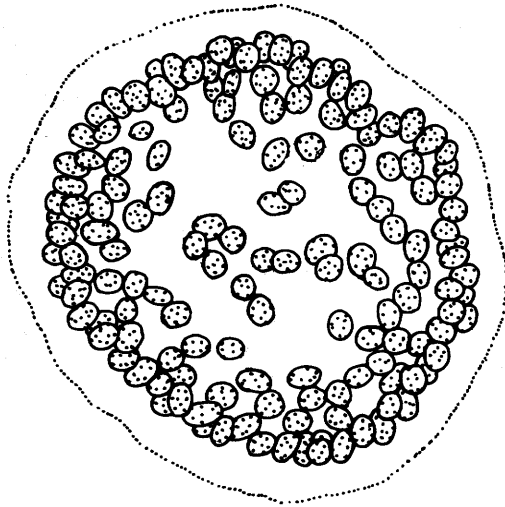
*Aphanizomenon*  
species



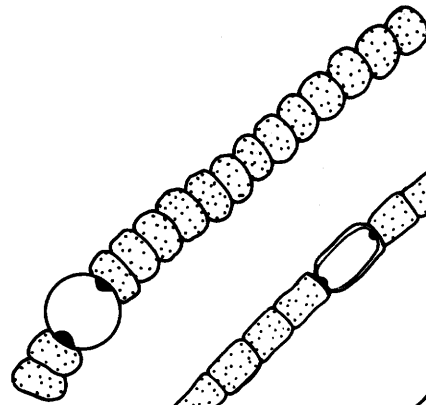
*Microcystis*

Blue-green algae

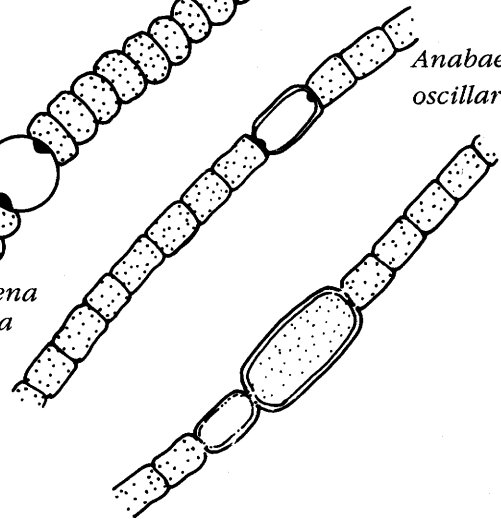
(greatly enlarged - varying magnifications)



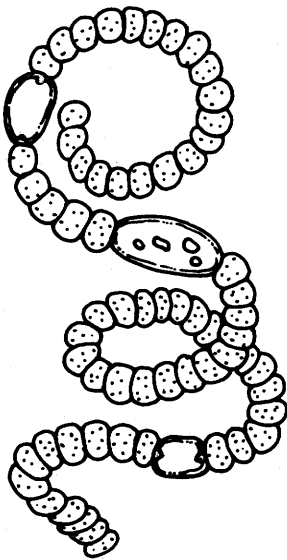
*Coelosphaerium*



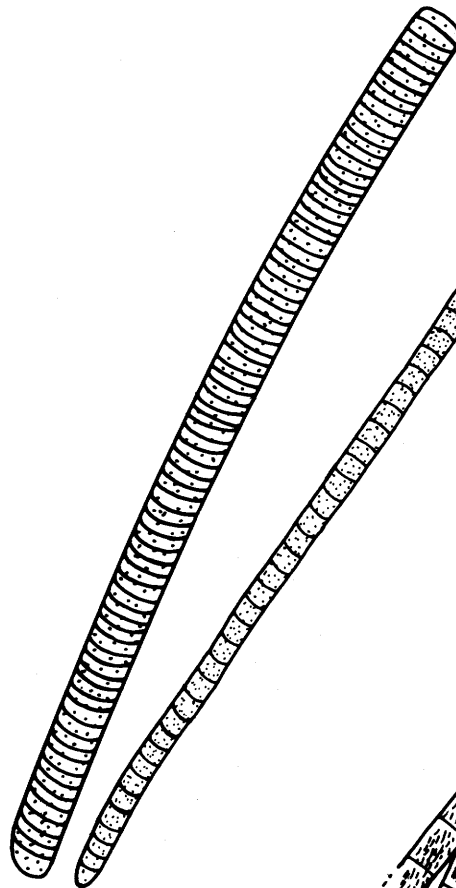
*Anabaena solitaria*



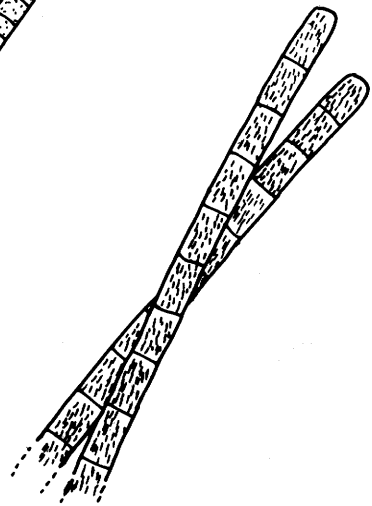
*Anabaena oscillarioides*



*Anabaena circinalis*



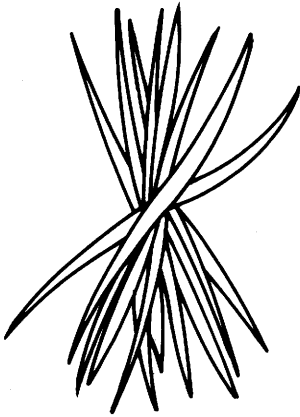
*Planktothrix mougeotii*



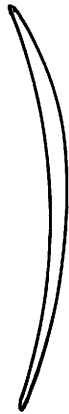
*Planktothrix species*

**Green algae**

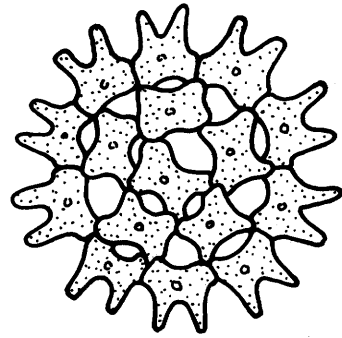
*(greatly enlarged - varying magnifications)*



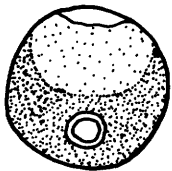
*Ankistrodesmus falcatus*



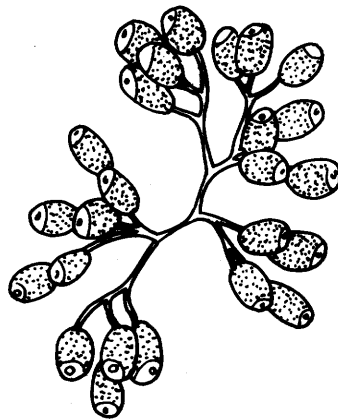
*Ankistrodesmus*



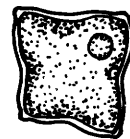
*Pediastrum*



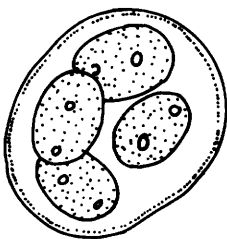
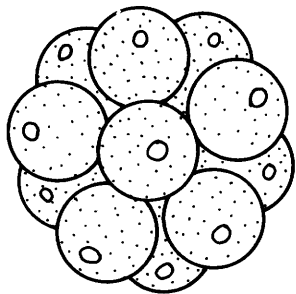
*Chlorella vulgaris*



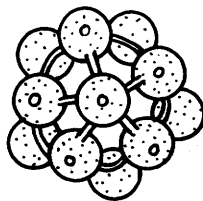
*Dictyosphaerium*



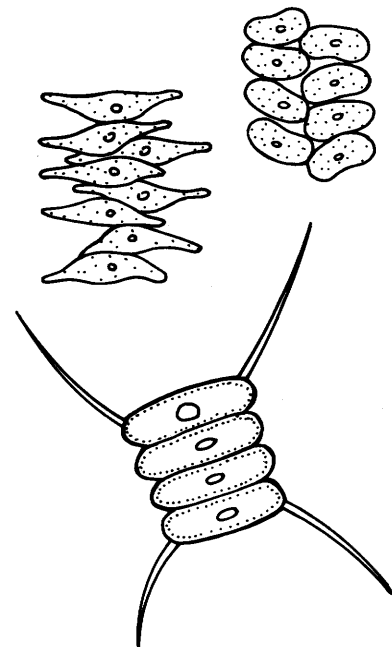
*Tetraedron*



*Oocystis*



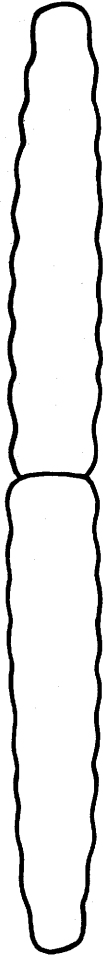
*Coelastrum  
species*



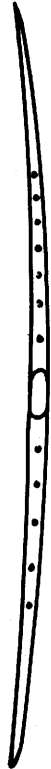
*Scenedesmus  
species*

Green algae

(greatly enlarged - varying magnifications)



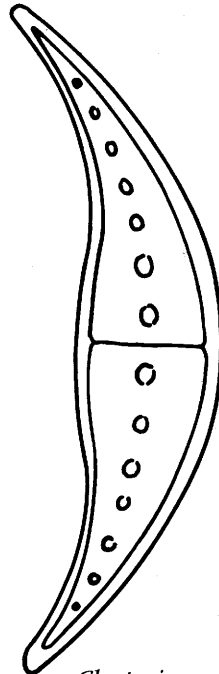
*Pleurotaenium*



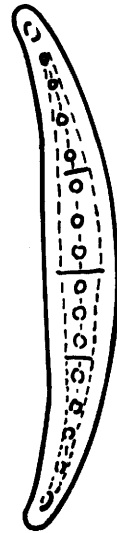
*Closterium aciculare*



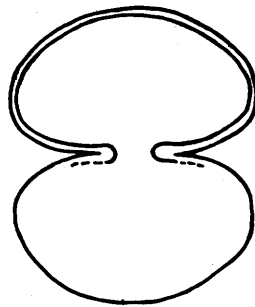
*Closterium setaceum*



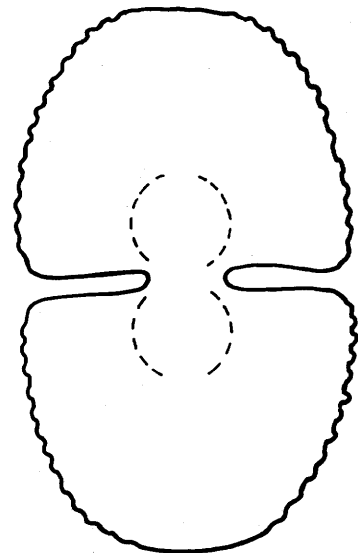
*Closterium ebrenbergi*



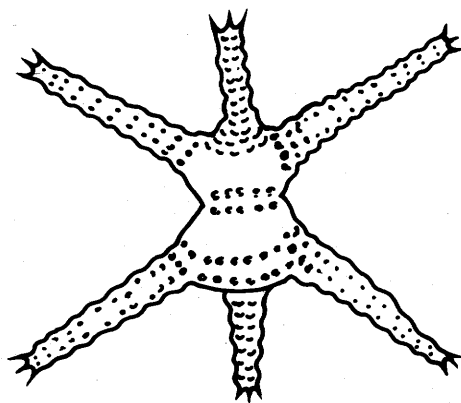
*Closterium species*



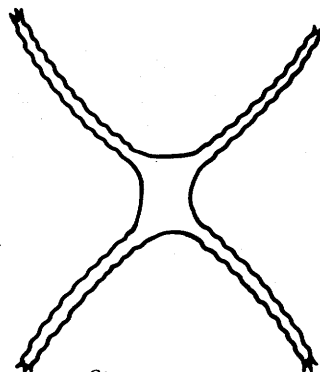
*Cosmarium*



*Cosmarium magnifici*



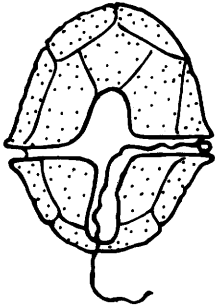
*Staurastrum pringue*



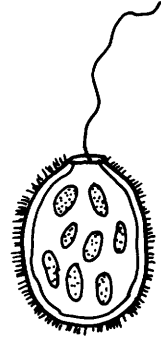
*Staurastrum nodulosum*

# Flagellate algae

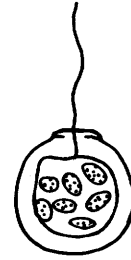
(greatly enlarged - varying magnifications)



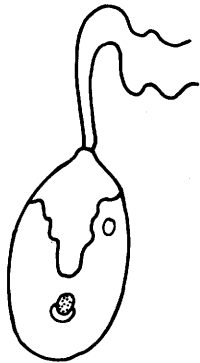
*Peridinium*



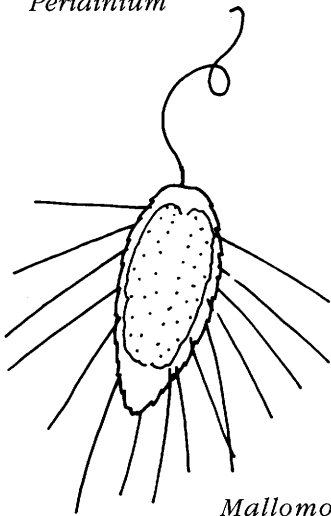
*Trachelomonas*



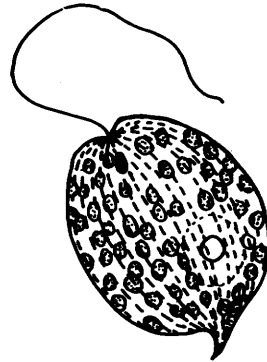
*Trachelomonas volvocina*



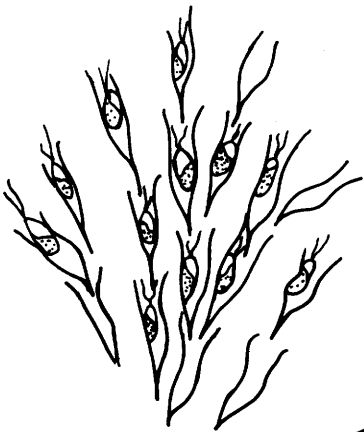
*Chlamydomonas*



*Mallomonas*



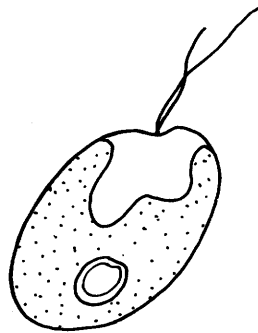
*Phacus pleuronectus*



*Dinobryon*



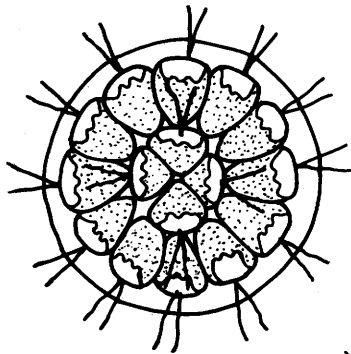
*Cell detail*



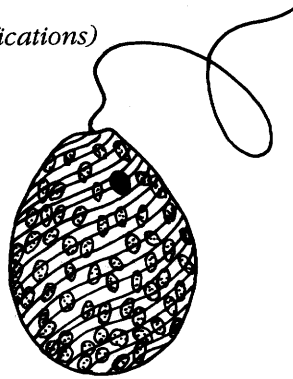
*Cryptomonas*

Flagellate algae

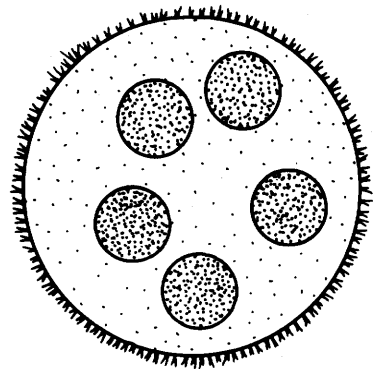
(greatly enlarged - varying magnifications)



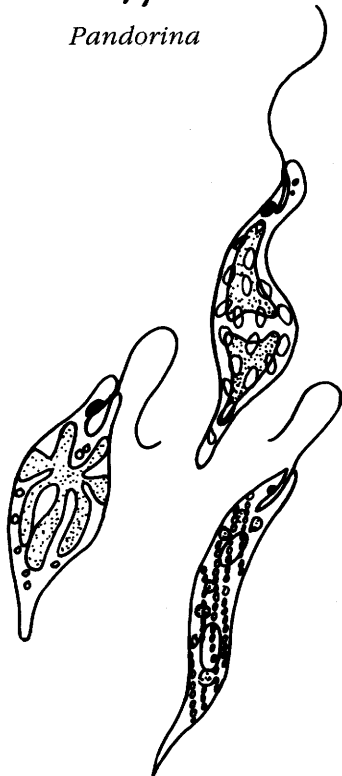
*Pandorina*



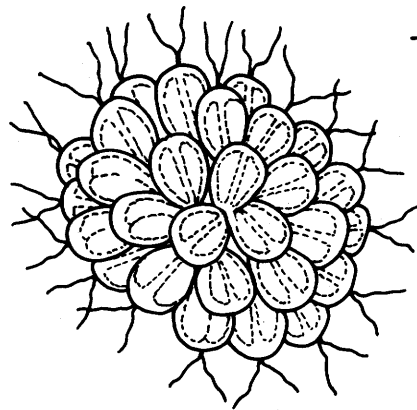
*Lepocinilis*



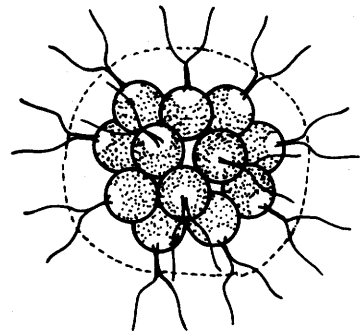
*Volvox*



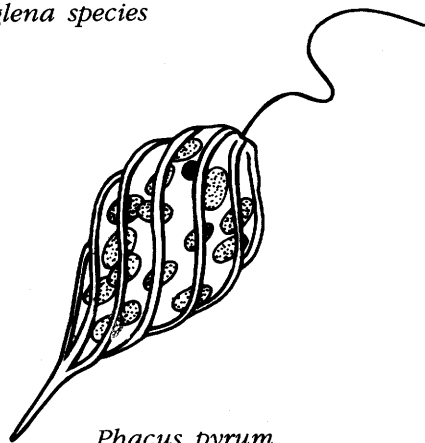
*Euglena species*



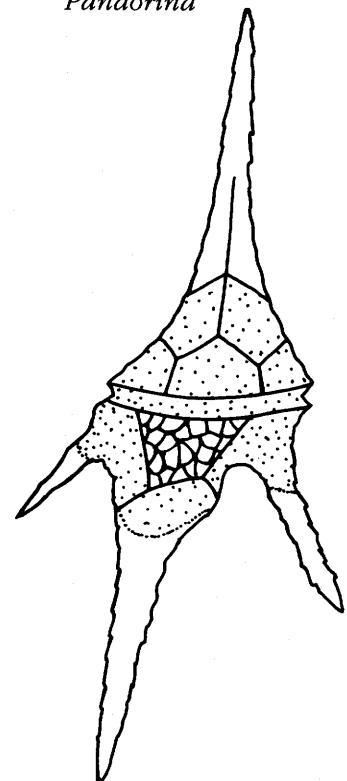
*Synura*



*Pandorina*



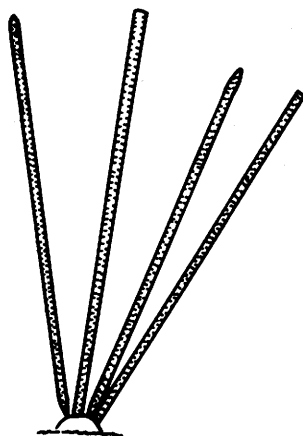
*Phacus pyrum*



*Ceratium*

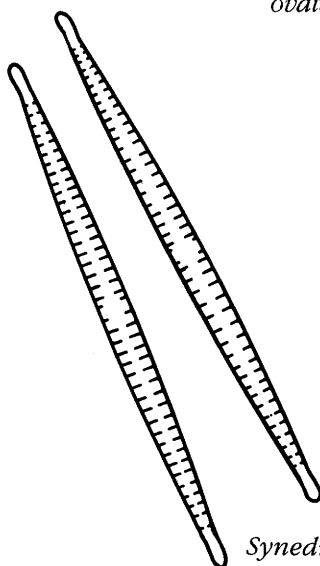
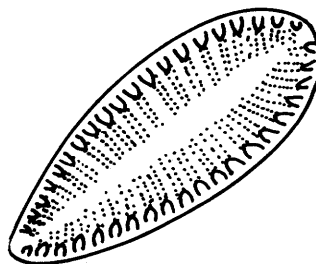
# Diatoms

(greatly enlarged - varying magnifications)

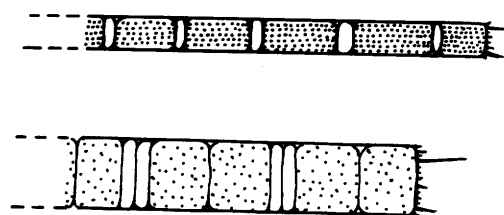


*Synedra ulna*

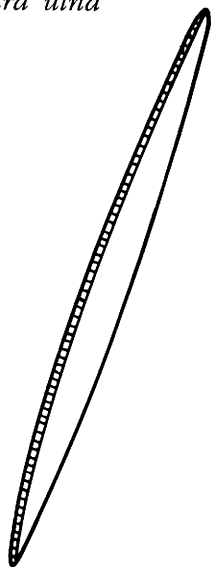
*Surirella ovata*



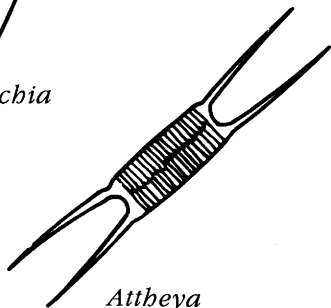
*Synedra acus*



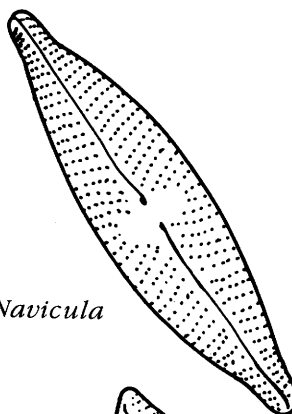
*Melosira granulata*



*Nitzschia*



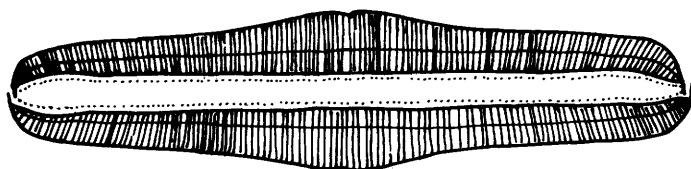
*Attheya*



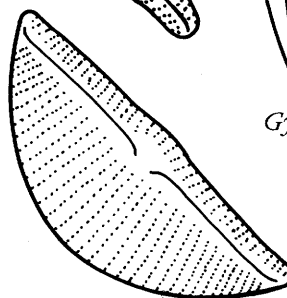
*Navicula*



*Gyrosigma*



*Rhopalodia*



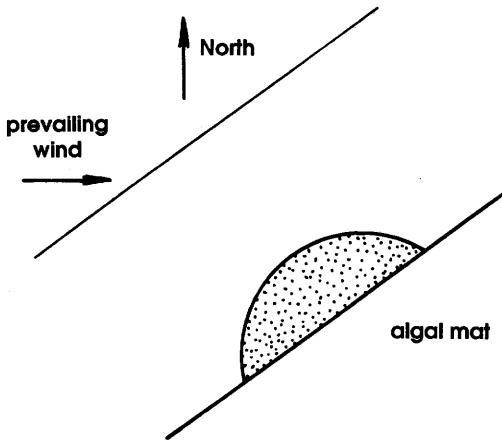
*Cymbella*



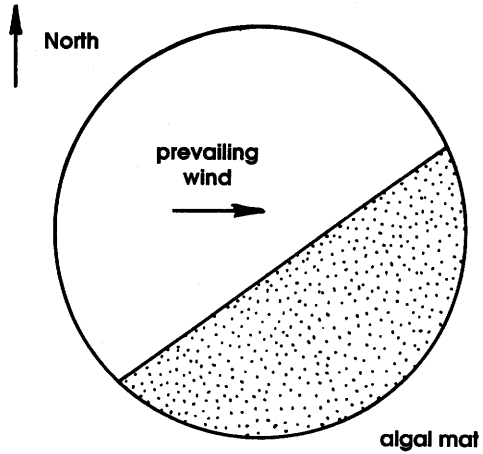
# Algae Record - Sheet 2

1. Mark the position of any algal blooms on a sketch

River example



Wetland example



2. Field Observations

	No	If Yes describe:
Coloured water:	<input type="checkbox"/>	<input type="checkbox"/> (colour) _____
Smelly water:	<input type="checkbox"/>	<input type="checkbox"/> (smells like) _____
Death of: fish	<input type="checkbox"/>	<input type="checkbox"/> (numbers) _____
birds	<input type="checkbox"/>	<input type="checkbox"/>
sheep	<input type="checkbox"/>	<input type="checkbox"/>
cattle	<input type="checkbox"/>	<input type="checkbox"/>
other animals	<input type="checkbox"/>	<input type="checkbox"/>

3. Note the colour of the bloom

Clear

0

1

Light green

2

3

Dark green

4