4_ANALYSIS OF PHYTOPLANKTON SAMPLES

The identification and counting of phytoplankton cells is something that takes much patience, practice and experience to do correctly. There are a number of taxonomic guides and keys that have been published to assist in the identification of both freshwater and marine algae.

The **unpreserved**, concentrated sample should be examined by microscope for species identification within the first three days, focusing on motile taxa and taxa that degrade under formalin preservative (primarily naked dinoflagellates).

Preserved samples will be examined for taxonomy within 3 months of collection.

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Sample Observation by light Microscope (LM). shake well preserved samples before examination. Phytoplankton specimens are prepared as temporary and permanent slides.

Valve and girdle view of Diatoms

When viewed with the light microscope, diatom shells can present two aspects. When the eipvalve or hypovalve is uppermost, the frustule is said to be '**in valve view'**; if on the other hand the two parts of the girdle (epicingulum and hypocingulum) are uppermost, the frustule is said to be '**in girdle view'**.

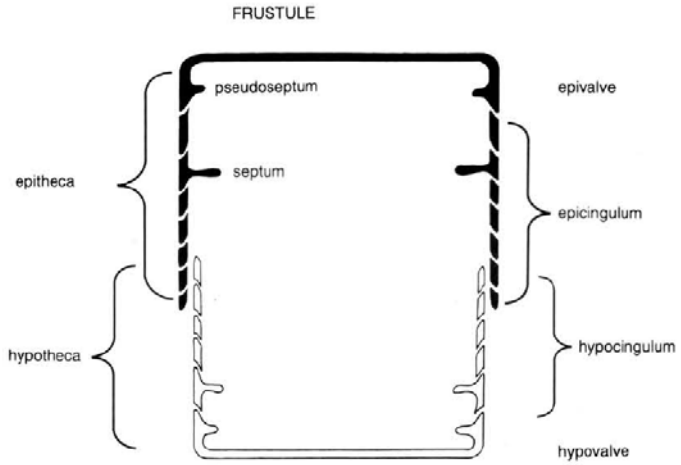


Fig. 1. Gross morphology of the frustule

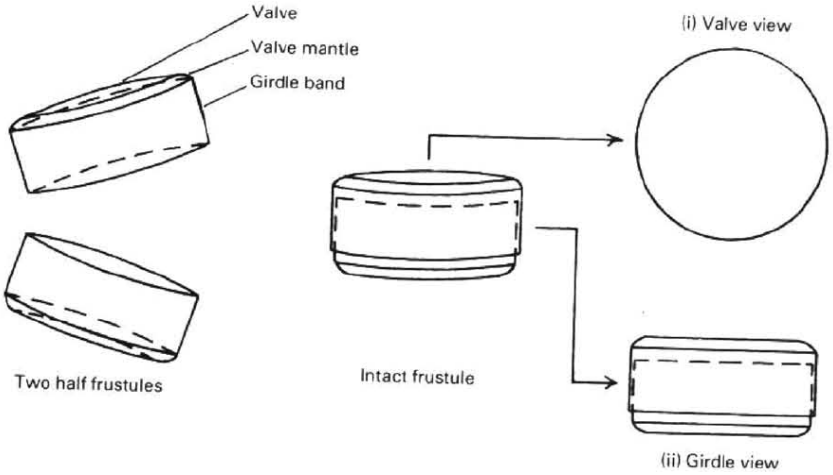


Fig. 2. The basic structure of a diatom cell.

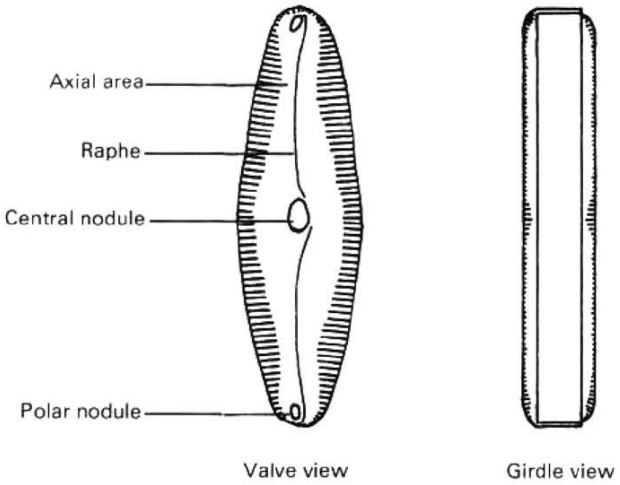


Fig. 3. The structure of a generalized raphe-bearing pennate diatom (after Sykes, 1981).

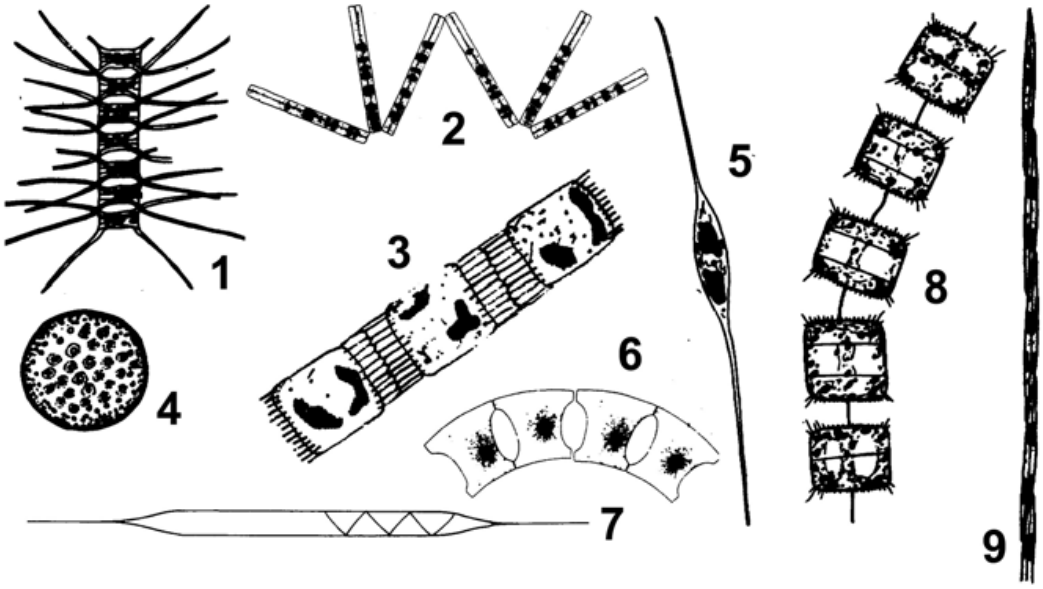


Fig. 1: Some common diatoms that might occur in your field samples. (1) *Chaetoceros* sp.; (2) *Thalassiothrix* sp.; (3) *Skeletonema costatum*; (4) *Coscinodiscus* sp.; (5) *Nitzschia* sp.; (6) *Eucampia* sp.; (7) *Rhizosolenia* sp; (8) *Thalassiosira gravida*; (9) *Nitzschia pungens*, chain.

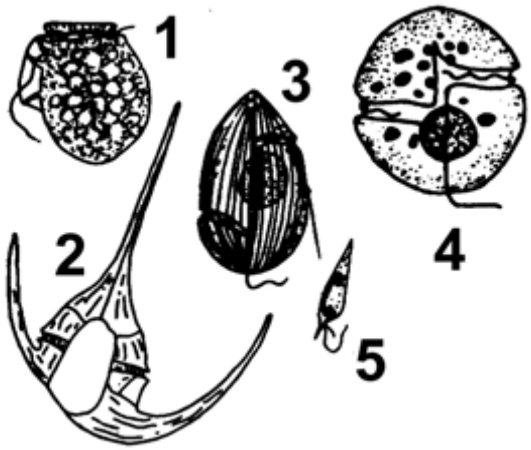
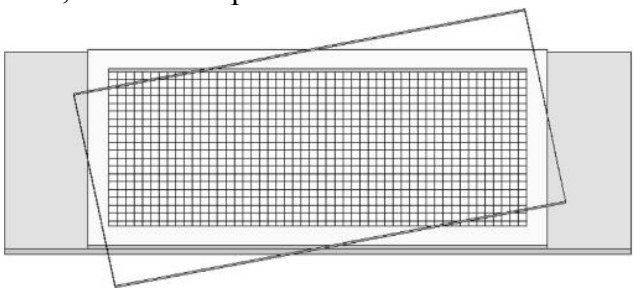


Fig. 2: Some common dinoflagellates that might occur in your field samples. (1) *Dinophysis* sp.; (2) *Ceratium* sp.; (3) *Gyrodinium* sp.; (4) *Gymnodinium* sp.; (5) *Prorocentrum micans*.

Quantitative analysis of phytoplankton
Sedgwick-Rafter cell

There are a number of methods available for counting algal cells in samples. The easiest method is using a Sedgwick-Rafter cell. The Sedgwick-Rafter cell is a four-sided counting chamber that is 50 mm long by 20 mm wide by 1 mm deep, giving a bottom area of 1000 mm^2, and an internal volume of 1 mL. They have a grid engraved on the bottom, with lines 1 mm apart. If correctly calibrated and filled, the volume of sample covering each grid square is 1 mm^3. Both glass and plastic versions are available, with the glass cells being better, but more expensive.



The cells are used on the stage of a normal compound microscope – preferably one with binocular eyepieces. Counting is done at 100× magnification, with higher power being used to identify small sized algal cells.
A very thin microscope cover slip is required to cover the cell.

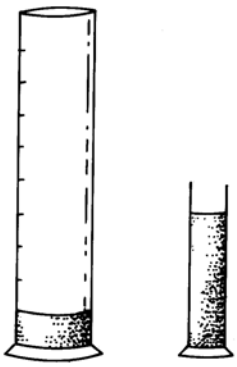
The Sedgewick-Rafter slide is best used when analysing cultures or high biomass blooms. As this method does not require an overnight settling period, it is rapid and can provide a quick assessment of a water sample. It has been proven to provide accurate results between 10,000 and 100,000 cells L^{-1}. The set up cost is low due to the use of a compound microscope.

Procedure

Concentrating the phytoplankton

Samples collected from a dense algal bloom can be analyzed directly, but they usually need to be concentrated prior to analysis.

- Pour a well mixed volume of the sample of water – say 100 ml into measuring cylinder.
- Add Lugol's solution (at the rate of 1 ml per 100 ml). The iodine in Lugols solution not only preserves and stains the phytoplankton but also increase their density by making them become heavy and sink.
- Leave the cylinder to stand for a minimum of 24 hours (or use centrifuge if you have access to one)
- If small nanoplankton are present, a longer sedimentation time may be necessary.
- After the required sedimentation period, most of the phytoplankton cells will have settled to the bottom of the measuring cylinder.

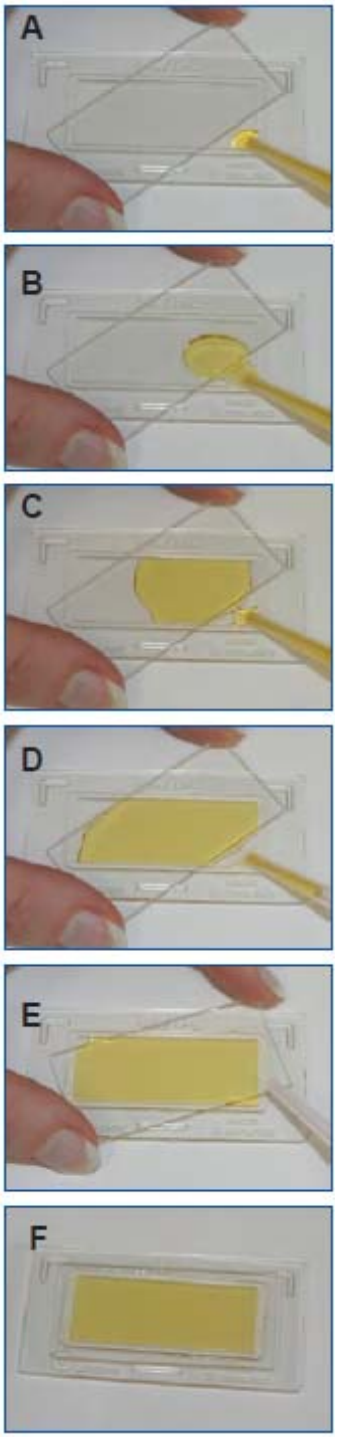


Sedimentation chambers
(100 mL and 10 mL graduated cylinders)

(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

- When the column of water appears clear, the top 90 mL can be gently drawn off using a suction pipette, taking care not to disturb the algal cells at the bottom of the cylinder. This leaves the phytoplankton concentrated in the bottom 10 ml and gives a **10×** concentration. This means that phytoplankton present in 100 ml of the water have now been concentrated into 10 ml, resulting in a concentration factor of 10.
- Sub-samples of this sedimented phytoplankton can be examined under the microscope for **identification**.
- Immediately before commencing a count, the phytoplankton cells in the bottom of the measuring cylinder are resuspended into the remaining 10 mL of sample left in the measuring cylinder by swirling, and a sub-sample of approximately 1 mL of this collected with a large-pore Pasteur pipette.
- This mixed 1-ml sub-sample is then carefully placed in the Sedgwick-Rafter counting cell for counting.
- The cell is full once the cover slip, which should be placed obliquely over the cell prior to filling with one corner open, just begins to float and can be rotated to completely cover the chamber. This avoids introducing air bubbles into the sample. The cell should not be overfilled.
- Once filled, the counting cell should be left to stand on the stage of the microscope for 15 – 20 minutes, to allow the algal cells to settle to the bottom.



Counting method

It is not necessary to count all the cells or 'units' on the bottom of the Sedgwick-Rafter cell. However, a minimum of 30 grid squares (quadrants) should be counted. Or the phytoplankton present in four or five strips of the counting chamber are counted. A horizontal strip corresponds to 50 quadrants. If you observe four horizontal strips for algae counting, you will have observed 4×50 quadrants.

These should be selected randomly, as there is differential sedimentation of algal cells/'units' within the counting cell, with more algae sedimenting closer to the walls than in the centre ('edge effects').

A 'unit' is either an algal cell, filament or colony, depending whether the species being counted is unicellular, filamentous or colonial.

Record the number of grid squares counted as well as the number of algal units counted. If an algal unit lies across the line engraved in the base of the Sedgwick-Rafter cell to delineate a grid square, so that it falls within two squares, the simple rule is that if it lies on the right side of the grid square, include it in the count, but if it lies on the ***left*** side, exclude it. Similarly, if it falls across the top line of the square, include it, but exclude any algal units falling across the ***bottom*** line.

Algal units are often smaller than the width of the lines engraved in the Sedgwick-Rafter cell, so the same applies for any algal units lying within the grid lines delineating a square.

The number of algal units present per mL within the actual water body is calculated as:

$$\text{No. of units/mL} = \frac{(\text{units counted} \times 1000\ \text{mm}^3)}{(\text{no. of grid squares counted} \times \text{concentration factor, which is typically 10})}$$

For **filamentous and colonial** algae, it is then necessary to convert the count in units.mL^{-1} to cells.mL^{-1}. Many green algae have a set number of cells per colony (for example 4, 8, 16, or 32), so, when this is known, it is easy to multiply the units by the cell number per colony to obtain cells.mL^{-1}. However, many other phytoplankton species, especially cyanobacteria, have a variable number of cells per filament or colony. In this instance, it is necessary to count the number of cells in 20 to 30 randomly selected filaments or colonies, and then obtain an average number of cells per colony from these counts.

Further problems arise when samples contain large-sized colonies or aggregations of filaments containing thousands of cells, where it is impossible to count all the cells in each colony or aggregation. In these situations, it is necessary to estimate a portion of the colony or aggregation –say 5% or 10% of the total colony size – and count or estimate the number of cells within that portion. Remember that the colonies or aggregations are three dimensional, with cells overlying cells, and outside of the focal plane at which you are viewing the colony. Once you have an estimate of the number of cells in 5% or 10% of the colony, multiply this by 20 or by 10, respectively, to obtain an estimate of the total cells per colony.
When you do these estimates of average cell numbers per filament or colony to obtain a count in terms of cells.mL^{-1}, the errors can be quite large and are in addition to any statistical counting error. The need to make these estimates arises only during blooms and becomes acceptable because of immediate management needs. Methods to break up large colonies into smaller units to make counting easier (homogenisation, addition of chemicals or sonification) are often inadequate and may destroy a large proportion of the cells present.

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 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 subsample of 1 ml is taken out and placed into Sedgwick-Rafter counting cell (chamber) as described above.

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. Accounting error of 20% means that 20% of the time the samples counted will not be truly representative of the water body.

Bioluminescent Algae

Topic

The ability of a living thing to produce light is known as *bioluminescence*. The light produced is generally bluishgreen, but yellow, red, and other colors have been observed. Most of the organisms that can carry out bioluminescence are marine. In this experiment, you will examine bioluminescence in dinoflagellates.

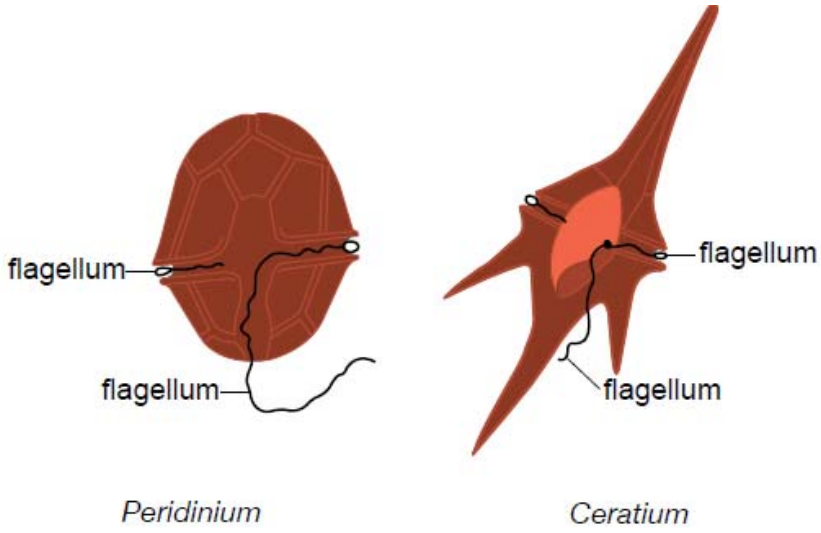


Figure 1

The group of chemicals responsible for light is known as *luciferins*. All biological chemical reactions require enzymes, and the particular enzyme that catalyzes luciferin is luciferase. When luciferin reacts with oxygen, it is *oxidized* to form light energy and oxyluciferin (see Figure). Once a molecule of luciferin is oxidized, it cannot flash any more until is *reduced* to form luciferin again.

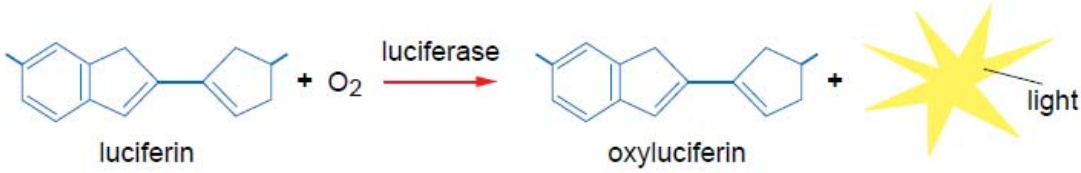


Figure 2

Procedure

1. Prepare a wet-mount slide of dinoflagellates. To do so:
    a. Transfer a drop of dinoflagellates to a slide.
    b. Cover the drop with a cover slip.
2. Place the slide on a compound light microscope, turn on the microscope light, and focus on low power. Observe organisms for a minute or two.
3. Without disturbing the slide, turn off the microscope light and the room lights. Leave both off for about two minutes.
4. While the lights are off, look through the eyepiece of the microscope. Watch the dark slide for a minute or two.
5. While observing through the eyepiece, gently tap the slide to disturb the organisms.