CHAPTER
06

PHYTOPLANKTON TAXONOMY, IDENTIFICATION AND ENUMERATION

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Introduction

Phytoplankton are microscopic, free floating organisms and is the principal primary producers of the oceans. Their size range from 0.2 µm to 2 mm. Phytoplankton contains primary pigments and accessory pigments such as chlorophyll (Chl), carotenoids *etc.* which strongly absorbs the blue and red light of the visible spectra. Phytoplankton also influences the total scattering properties of sea water. Due to their relatively large size, the larger phytoplankton species contributes relatively little to backscattering in the visible spectrum. The principal phytoplankton taxonomic groups include:-

Class Bacillariophyceae	-	Diatoms
Class Pyrrophyceae	-	Dinoflagellates
Class Prymnesiophyceae	-	Coccolithophores
Class Chrysophyceae	-	Silicoflagellates
Class Euglenophyceae	-	Euglenoid flagellates
Class Chlorophyceae	-	Green algae
Class Cyanophyceae	-	Blue-green algae
Class Haptophyceae	-	Brown colored Phytoflagellates (Kennish 2001).

Class Bacillariophyceae (Diatoms)

The class Bacillariphyceae comprises diatoms with fascinating shapes and ornate patterns. The common shapes include rounded (centric) and elongated (pennate). Diatoms are embedded in an outer wall made of silica and known by the frustules. The shape of diatoms are like a lab Petri dish or a soap box, with one valve larger in diameter (the "epivalve") and the other one overlapping the reversed second valve (the "hypovalve"). Some diatoms attach to each other, while some others attach end to end at an angle to form colonies. Diatoms reproduces by asexual or vegetative reproduction. **Sexual** reproduction producing auxospores and resting spores are also reported. The visible accessory pigmentation in diatoms are of golden colour.

Centric diatoms are characterized by their radial symmetry are common to the surface waters. Many centric diatoms have projecting spines, which increases their surface area in relation to the volume of water holding them up, which helps them stay buoyant. Pennate

diatoms have elongated valves and are more likely to be found on shallow bottoms. Pennate diatoms have a groove on their underside, called a "**raphe**," which secretes mucus.

Class Pyrrophyceae (Dinoflagellates)

This name's Greek origin means "spinning tail;" so named because these microscopic organisms have flagella–tiny tails made of protein strands. As the name indicates these are embedded with 2 (dino) flagella. These whip-like flagella helps them to propel in the water and are sometimes used for attachment . The two flagella are dissimilar in size, one wrapped around the body and one extending outward from the body. In dinoflagellates, the accessory pigments are coloured of red to reddish brown. The outer wall of dinoflagellates are made of cellulose. Dinoflagellates are differentiated into two owing to the presence or absence of armoured plates. Some dinoflagellates have thick armoured plates that fit together, like the armour of medieval knights and these plates can slide apart or over each other. Others are unarmored or naked. Bioluminiscence is another peculiarity of dinoflagellates.

Class Prymnesiophyceae (Coccolithophores)

Coccolithophores surround themselves with a microscopic plating made of limestone (calcite). These scales, known as coccoliths, are shaped like hubcaps and are only three one-thousandths of a millimeter in diameter.

Class Chrysophyceae (Silicoflagellates)

Small single-celled flagellates and flagellated colonies. Common in oligotrophic clear waters and humic waters. The algae coming under this family is commonly known as golden algae. The cells are naked or covered by scales, lorica or cell wall. The flagellate cell usually possesses two heterodynamic flagella. This algal family is unicellular or colonial. The pigments are chlorophyll-a, -c and fucoxanthin; this fucoxanthin which give the characteristic colour. It stores energy in the form both as carbohydrate and oil droplets. Presence silica deposition vesicle, flagella apical and unequal in length. Asexual reproduction by binary fission, sporogenesis. Sexual reproduction reported in some members. The lifecycle is haplontic in chrysophyceae.

Class Euglenophycea (Euglenoid flagellates)

The euglenophyceans are basically unicellular flagellate. The cell is usually naked, but some species such as Trachelomonas (Euglenales) possess lorica deposited by iron and magnesium. The most unique feature of the Euglenophyceae is the presence of proteinaceous strips, pelicler strips beneath the cell membrane. The species with many flexible pellicler strips. In the Euglenophyceae, a photosynthetic genus, as well as many colorless phagotrophic or osmotrophic species are present. The green chloroplast is originated via secondary endosymbiosis with a green plant at the common ancestor of the Eutreptiales and Euglenales.

Class Chlorophyceae (Green algae)

Chlorophyceae (chloros, green; phyceae, algal organisation) is commonly known as green algae. Green algae are characterized by the presence of green plastids known as chloroplast. This containing a starch storing region called pyrenoids. The members of Chlorophyceae generally grow in fresh water (about 90%) and the rest in saline water, terrestrial habitat *etc.* The fresh water members such as Volvox, Oedogonium, Spirogyra *etc.* grow in ponds, pools and lakes. Flagella are 1-many, equal in size and inserted either apically or sub-apically. The flagella show typical 9+2 arrangement when viewed under E.M. The cells are eukaryotic in nature. The cell wall is mainly made up of cellulose, which comprised of hydroxyproline glyco-sides or xylans and mannans. The flagellate cells have eye-spot or stigma in the anterior portion, which remain inser-ted at one side of the chloroplast.

Class Cyanophyceae (Blue-green algae)

Members of the class Myxophyceae (Cyanophyceae) are commonly known as blue green algae. The name blue green algae is given because of the presence of a dominant pigment c-phycocyanin, the blue green pigment. In addition, other pigments like chlorophyll a (green), c-phycoerythrin (red), â-carotene and different xanthophylls are also present. The members of this class are the simplest living autotrophic prokaryotes. Nucleus is of prokaryotic nature *i.e.*, devoid of nuclear membrane and nucleolus, Absence of well-organised cell orga-nelles, and Pigments are distributed throughout the chromoplasm (the outer part of proto-plasm). Locomotion is generally absent, but when occurs, it is of gliding or jerky type.

Class Haptophyceae (Brown colored Phytoflagellates)

Unicellular, mostly marine, mostly photosynthetic. Around 80 genera, 500 species (= relatively low diversity). Ecologically important component of phytoplankton communities at all latitudes. Mostly nannoplankton (2-20 micron) size fraction. Mostly motile with 2 flagella. Characteristic feature: presence of a haptonema. Two smooth flagella, equal or unequal in length. Almost all haptophytes are photosynthetic (one species isn't), but most may actually be mixotrophic. All haptophytes contain chlorophylls a and c, as well as ?-carotene, diatoxanthin and diadinoxanthin. In this they resemble heterokonts.

Characters for phytoplankton identification

Phytoplankton are identified based on thier:-

- Cell shape / size
- Mode of life (solitary, colonies, filaments...)
- Organelles : presence / absence

- Plastids: colour, number, shape, ultrastructure
- Cell covering (frustule, scales, theca, cyst...)
- Flagella (number, length, insertion)
- Reserve substances (nature, localisation)
- Other characters (stigma, haptonema, pseudopods, ...)

Sample Collection, Preservation and Enumeration

Sampling Design

Offshore and inshore sampling designs differ according to seasons, locations, depths, substrates, purpose of monitoring and available manpower and equipments. To detect early phases of phytoplankton blooms, frequency of sampling becomes important because blooms can develop over a period of 2 to 3 weeks or be transported into an area. Monitoring of sentinel filter feeding species is also beneficial, e.g., bivalves and tunicates.

Sample Collection

Before sampling begins, the manpower involved has to be thorough in sampling design (transects and adaptive sampling), frequency, collection methods, variables to be measured, data recording and storage, and the best approach to meet the goals of the program. Repetitive sampling at fixed stations is desired for statistical analysis of data (plankton, nutrients, salinity, temperature, *etc.*). Processing enough sample volume is also important. Distance between stations can be critical because of oceanographic conditions. Sampling for planktonic blooms and conditions should involve sampling or profiling with depth because the water column is 3 dimensional. It may also involve monitoring "seed" beds in the benthos to determine and even predict timing of blooms. Sampling for benthic algal blooms requires collecting substrate, e.g., sediments, on a routine basis. Processing requires removal of HA cells for analysis.

Equipments for collection of samples include Bucket, weighted, bottle or Niskin sampler. The bottles used for collecting live samples has to be properly labeled. The label should contain Station Identification No, Latitude/Longitude, Depth at which sample was collected, name of fixatives used and date of sampling. Latitude /Longitude can be identified using GPS unit. A data log sheet also has to be maintained specifying all the details collected for the sample. This may include pH, temperature (using thermometer) *etc.* Lead weighted bottles can be used for collection of phytoplankton samples upto 40m depth. For collecting of sample at depths <200m, Niskin sampler can be used. This uses weights or ropes on wire to trigger the closing of openings in this sampler. The sample collection can be assisted with a portable data profiler which is able to measure pH, Dissolved Oxygen, Conductivity,

TDS, Salinity, ammonium, Nitrate, chloride, Temperature *etc*. When the sampling platform is a Ship, CTD and rosette sampler will serve the purpose.

Fixatives used for sample preservation

Unacidified (Neutral Lugol's Iodine) Solution

1gm potassium Iodide, 0.5 gm Iodine, Dissolve in 3ml of distilled water dilute to 50ml. Use 2%in volume/volume for final concentration.

Gluteraldehyde

25% gluteraldehyde buffered with sodium acetate to a 10% solution. Use in a 1:10 ratio with sample.

Methods for collecting samples

The bottle in which sample is collected has to be rinsed with water sample at the collection point. The samples are collected from discrete depths and transferred to the properly labelled sampling bottles. The bottles are stored in coolers separately for live samples and fixed samples.

Enumeration

1. Using Utermohl Method

The sample bottle is gently inverted nearly 15 times . 10-100ml of sample is measured and transferred to the Utermohlchamber. The chamber is kept idle for 24 hours. The phytoplankton cells are allowed to settle in the chamber. Minimum 30 fields are examined. The phytoplankton are identified upto species level and enumerated. Afterwards, the entire chamber is examined to know the presence of any phytoplankton that is not encountered in the fields. The chamber is examined under inverted microscope. The table below shows the data sheet.

Serial Number	Phytoplankton species	Numerical abundance	Total Number
1	Skeletonema	~~1111~~ ~~1111~~ 11	12
2	Chaetoceros	1111	4
3	Coscinodiscus	~~1111~~	5

Calculation

Cells/Litre=1000*(C*A)/ F*FA*V

Calculation cells/Litre at 100X magnification

Cells/Litre=1000*(C/V)

C = Number of cells counted

A = Area of chamber bottom

F = Number of fields counted

FA = Area of Field view

V = Volume settled

2. Using Nunc Method

The bottle containing samples are inverted nearly 15 times. About 3ml of sample is measured using a pipette. The samples are transferred to Nunc chamber. Cells are allowed to settle for 30 minutes. The cells are identified and enumerated for entire bottom of chamber using inverted microscope. For dense samples, reduced volume (0.3 ml - 0.03 ml) are used for enumeration. Sterile filtered seawater is added to the chamber to level out the sample.

The advantage of Using Nunc chamber is that resolution of cells and morphologyare right upto the edge of the chamber side without distortion, optical transects can be counted easily, and cells can be moved easily for further analysis. The disadvantage includes use of limited volume of sample and higher cost. Limited number of usage of Nunc chambers is also a drawback.

3. Using Sedge-wick Rafter

Sedgewick rafter slides are used for examining 1ml of sample. The slide is designed to occupy 1ml of sample and partitioned into 1000 grids each with an area of $1mm^3$. Identification and enumeration is done in 4x4 grids or on random selection. A minimum of 3 such grids are examined under microscope. The average number is then calculated and scaled for 1ml.

$$N=\frac{(n*v*1000)}{V}$$

Where N = number of cells per litre
n = number of cells per millilitre
V = total volume filtered
v = volume of sub-sample

References

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