04 Phytoplankton - collection, estimation, classification and diversity

Gireesh R., Molly Varghese and V.J. Thomas

Central Marine Fisheries Research Institute, Kochi-682 018

Introduction

Phytoplankters are microscopic, unicellular and photosynthetic organisms which freely float in water bodies. They are composed of both eukaryotic and prokaryotic species which colonizes upper euphotic part of the water column ranging from freshwater to ocean conditions. They exhibit remarkable adaptation to remain in floating condition. Phytoplankton cells can range in size from about 1 μm to 1 mm. Like terrestrial plants, these tiny primary producers require sunlight, nutrients and carbon dioxide for their growth and multiplication. The cells of these organisms contain chlorophyll pigments to harvest solar radiations. Phytoplankters photosynthesize in the presence of sunlight using sufficient nutrients, fixing carbon dioxide and releasing oxygen. Hence they play significant role in maintaining carbon budget of atmosphere as well as in seawater and help mitigate global warming. Physical process such as wind and current play significant role in their distribution especially in estuarine and marine conditions. Phytoplankton act as primary link in energy pathway to higher trophic level through various food chains. It supports half of global primary production which directly or indirectly supports almost all marine life. Phytoplankters are a major food source for variety of organisms such as zooplankters, larvae and juveniles of fishes and invertebrates. Today world over the fishery depends on Potential Fishery Zone which is an attribute of pigment characteristic of phytoplankton measured through remote sensing technique.

Phytoplankton usually undergoes a fairly predictable annual cycle, but some species may develop exponentially and form so called blooms. Sometimes, this may cause adverse effect, especially on coastal environment. Not all blooms are toxic. Blooming can cause oxygen depletion and hence it acts as a threat to other marine life especially sedentary organisms like shellfishes. Blooms of certain harmful species produce toxin. If such species are consumed by shellfish or other species, toxin may accumulate and affect organisms of higher trophic levels which is a concern for human health.

The distribution of some commercially important fish and shellfish species and their larvae depend on certain phytoplankton species which act as indicators. The diatom species *Fragilaria oceanica* and *Hemidiscus hardmannianus* have been considered as indicators of oil sardine, *Sardinella longiceps* in the west coast of India. The abundance of coccolithophores is another indicator for herring fishery in European waters while *Fragilaria antarctica* indicates abundance of krill in Antarctic waters. Some dinoflagellates, due to their luminescence help to locate and identify fish shoals during night.

Collection

In coastal waters, estuaries or lagoons, surface samples are collected usually with a clean bucket of measured volume. The subsurface samples from different depths are collected with water samplers such as Van Dorn samplers, Niskin bottles, Meyer's water sampler or Friedinger water sampler. The samples can be obtained using a weighed flexible or rigid plastic tube. The sampler is sent down vertically up to a measured depth and then closed at the top to trap a column of water. In oceanic waters, large size Niskin bottles (5, 8 or 20 litres) are used along with CTD probe to collect samples. The samples are collected in a container.

Fixing and preservation

For enumeration of phytoplankton, the cells must be preserved at the earliest. Formalin is the widely used fixative and preservative of phytoplankton cells. Formalin stored in amber coloured bottles can be kept in cool temperature. For 100 ml water sample, 2 ml of formalin is sufficient. If kept in light coloured bottles, a white precipitate will develop due to exposure of sunlight and form toxic paraformaldehyde.

Lugol's iodine solution is another good preservative especially for diatoms and nanoplanktons and except coccolithophorids. To prepare Lugol's solution 10 g of potassium iodide and 5 g iodine are dissolved in 20 ml of distilled water and to this

50 ml of distilled water and 5 g of sodium acetate or 5ml of 19% acetic acid is added. About five drops are sufficient for 250 ml sample.

Osmic acid is also added as preservative at the rate of 5-6 drops per 100 ml sample. This is prepared by adding 200 mg osmium tetroxide in 10 ml of distilled water.

Gluteraldehyde solution is prepared by mixing 8 gm of gluteraldehyde in 100 ml distilled water and is applied to sample in the ratio 1:1.

Concentration of phytoplankton cells

The collected samples are concentrated by the use of plankton concentrator, centrifuge or settling method. The concentrated phytoplankton samples are stored, especially diatoms, in polythene bottles.

Plankton concentrator

The samples can be filtered immediately using plankton concentrator. In this method, sample is passed through a PVC tube or Perspex tube fitted with nylon net attached at one end.

Centrifuge

Centrifuge is a simple device by which phytoplankton can be concentrated from samples without causing damage to cells. In this method, 10-20 ml of aliquot is centrifuged in an electrical centrifuge at 1500-2000 rpm for 15 to 30 minutes. The supernatant water is decanted until the volume reduced to 1/10 to 1/30 of initial sample. Later, it is suspended in remaining water and add few drops of 1% potassium aluminium sulphate to ensure the precipitation of phytoplankton. Finally the samples are preserved using neutralized formalin or lugol's iodine for further examination under microscope.

Settling method

The water sample can be kept in measuring cylinder for settlement after preservation. Later, settled portion can be separated by siphoning out water from the top.

Staining

The process of staining phytoplankton is species specific. Neutral red and Evans blue are commonly used stains for whole plankton. Flurochromes are used to enhance fluorescence quantum yield, particularly in cyanobacteria. Fluorescein developed by Bentley-Mowat, enhances the green fluorescence and is widely used in marine plankton. The stock solution is prepared by mixing fluorescein hydrate in 0.5% acetone and is used as 0.01% solution in freshly filtered seawater. Equal volume of solution and samples are mixed and used under fluorescent microscope.

Identification

A high quality microscope is essential for enumeration and identification of phytoplankton cells. The ideal microscope should have magnifications of 10x, 40x and 100x and also with oil immersions and phase contrast. Many species are transparent under light microscope. So different techniques are used to improve the contrast of cell identification. Commonly used microscopes for identification and enumeration are Standard Compound Microscope (10x or 12x Ocular and 10x, 20x, 40x or 100x Objectives) and Inverted Microscope. The advantage of inverted microscope is that it allows viewing the organisms settled at the bottom of the chamber.

Enumeration and estimation of phyto-plankton

Biomass can be calculated either by direct count method (no. of cells/ m^3) or chlorophyll estimation by spectrophotometric method. In the direct count method, the cells have to be identified and counted and then expressed as numbers per m^3 of water. For this, Sedgwick Rafter can be used. The sample is spread uniformly as a thin layer and cells are counted. Diluting the stock plankton sample is ideal to avoid clumping or clustering of organisms. The cells are counted individually form one corner of the counter. Replicate cell counts are necessary for accuracy and to avoid any statistical error. The total number of phytoplankton cells present in a litre of water is calculated using the formula,

$N = nv/V$

Where N is the total number of phytoplankton cells per lite of water filtered, n is the average number of phytoplankton cells in 1 ml of sample and v is the volume of plankton concentrate (ml) and V is the volume of water filtered. The unit is expressed as N of cells/litre or $N \times 10^3 / m^3$.

In the spectrophotometric method, the common and most abundant pigment in all photosynthetic organisms, Chlorophyll a is generally used for estimating phytoplankton biomass. A known volume of water collected from surface or subsurface is filtered immediately through a synthetic fiber or glass fiber filter (Millipore, Whatman GF/F filter paper with 0.45 μm pore size) or sample can be brought to laboratory by keeping it in an ice box and filter it later. While filtering two to three drops of magnesium carbonate should be added. After the filtration, filter should be removed for further extraction or should be folded and kept in desiccators at -20ºC until the analysis can be done. The filter is placed in a 15 ml glass or centrifuge tubes and 15 ml of 90% acetone is added and then should be shaken vigorously. These tubes should be closed and kept overnight for 24 hours in a refrigerator in dark. The tubes are removed from the refrigerator and allowed to warm up in the dark nearly to room temperature. The samples are centrifuged at 5000 to 6000 rpm for 10 minutes. The supernatant is decanted into a glass spectrophotometer cuvette (10 cm length) without delay. The spectrophotometic reading of samples are taken at wave lengths 630, 664, 647

and 750 nm. Correction factor for each extinction can be taken by using a blank solution of 90% acetone. The quantity of chlorophyll pigments in the water can be measured by using following formulas.

Chlorophyll a $= 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630}$

Chlorophyll b $= 21.03\ E_{647} - 5.43\ E_{664} - 2.66\ E_{630}$

Chlorophyll c $= 24.52\ E_{664} - 1.67\ E_{664} - 7.60\ E_{647}$

Where E is the absorbance at different wavelengths (turbidity corrected by 750 nm) and the unit of Chlorophyll is expressed as μg/mL.

Chlorophyll mg/m^3 $= C \times v/V \times 10$

Where, v is the volume of acetone in cuvette, V is the volume of water filtered in litres and C is the chlorophyll pigment.

Apart from the spectrophotometric method, high performance liquid chromatography is also used to analyze the pigment concentration present in water. But in this case large volume of water is necessary to be filtered. Now a days, application of remote sensing has an important role in predicting phytoplankton population structure. The spectral property of water is used as tool for determining the pigment concentration. The colour of water is determined by volume scattering in a water body (transmittance). However, in taxonomic point of view, the application of fluorescence mcicroscopy or remote sensing is limited to determination of phytoplankton functional groups only.

Classification

Phytoplankters are classified as microplankton (200-20 μm), nanoplankton (20-2 μm) and picoplankton (2-0.2 μm) according to their size. The first two size classes can be identified by optical microscopy while the third is determined by fluorescence microscopy.

Classification of algae has always been changing. W.H. Harvey (1836) was the first who classified algae into three groups based on colour: (a) Chlorospermae (green algae and fresh water forms) (b) Melanospermae (brown algae) and (c) Rhodospermae (red algae). Thereafter, a lot of workers have described algal classification based on different criteria. The classification proposed by F.E. Fritsch (1933) is still widely recognized and accepted by algal taxonomists. Based on pigment and morphological characters, Fritsch (1935, 1945) classified algae into 11 classes viz. Chlorophyceae, Cryptophyceae, Phaeophyceae, Rhodophyceae, Xantho-phyceae, Dinophyceae, Bacillariophyceae, Chloromonadinae, Eugleniae, Chrysophyceae and Myxophyceae.

The phytoplankton composed of mainly diatoms, dinoflagellates, cocolithoides (prymenophyceae), cyanophytes and green algae.

Diatoms (Bacillariophyceae)

Diatoms are ubiquitous algae (jewels of the plant world) and have very important role in aquatic vegetation of world forming part of the plankton. They are the best known group of phytoplankton and most important in terms of their contribution (approximately 40%) to oceanic primary productivity. They are unicellular, filamentous or some forms colonies and have chlorophyll a, b, βcarotene and fucoxanthin as main light harvesting pigments. The markings of cell wall, structure and position of raphae and nodules are the characteristic features for identification of species. The diatom cell is known as frustules and characteristic feature is possession of silica cell wall. This structure is highly ornamental, which is species specific and often used as means of identification. It is composed of two overlapping halves like pill box or a pair of petri dish. The outer layer is called epitheca while inner is hypotheca. Edges of these two halves together forming girdle. If one half of cell is seen, it is valve view, while only the girdle, then it is girdle view. Diatoms are divided into order centrales and pennales based on symmetry. Centrales are radialy symmetrical about a central point while pennales are bilaterally symmetrical with respect to long axis of the cell.

Centrales: Valves are circular, polygonal or irregular in outline and with ornamentation on the wall; ornamentation is radial or concentric about a central point. Valve have raphae or pseudoraphae. Protoplast with many chromatophores. Centrales are more often seen in open sea.

Centrales are divided into three suborders, 9 families, 14 subfamilies and 35 genera.

Sub order Discoidae: Cells shortly cylindrical, valves circular, hyaline, aerogated or with radiating striations. Eg. *Cyclotella, Melosira, Stephanodiscus*

Sub order Solenoidae: Cells elongate, cylindrical or subcylindrical, complex girdle with numerous bands. Eg. *Rhizosolenia*

Sub order Biddulphiodeae: Cells box shaped, valves with two or more poles provided with horns or bosses. Eg. *Biddulphia, Triceratium*

Pennales: Valves are bilaterally symmetrical or asymmetrical in surface view. The cell wall ornamentation is also bilateral with respect to a long line, along the long axis of cell. Valve always with a raphae or pseudoraphae. Protoplasts with one or two chromatophores. Pennales are more common in coastal waters.

Pennales are divided into three suborders based on presence or absence of raphae. These are further classed into 5 families, 10 subfamilies and 28 genera.

Suborder Araphidae: only pseudoraphae present

Family Fragilarioidaea: Valves mostly straight. Eg. *Asterionella, Fragilaria, Synedra*, etc

Suborder Monoraphidiodeae: Shows the beginning of raphe, no central nodule

Family Eunotioideae: Raphae on one or both valves. Eg. *Cocconies, Acnanthes*, etc

Suborder Biraphidiodeae: Shows raphae on both valves, central nodule is present. Eg. *Pleurosigma, Navicula*, etc

Dinoflagellates (Dinophyceae)

Dinoflagellates are unicellular, flagellates, naked or covered with cellulosic plates (theca). They possess two flagella, one longitudinal while other in furrow and form significant blooms (known as red tides), which are often toxic. They have chlorophyll a, c, phycobilins or fucoxanthin as main light harvesting pigments. Several of them are luminescent and produce light. The perforation in the thecal plates are the characteristic features of dinoflagellates and help in the identification.

Coccolithophores (Prymnesiophyceae)

Mostly occur in marine waters. Size ranges between 5 to 20 μm. Some have flagella while others are devoid of them. They are characterized by possessing two flagella and a fine whip-like structure called haptonema. The cells are covered with scales. One of the important group is coccolithophores. They are two flagellated and filamentous forms with calcified cells.

Green algae (Chlorophyceae)

Green algae are microscopic, uniccelluar, some filamentous or colonial, flagellates or nonflagellates and have chlorophyll a, b and - carotene as light harvesting pigments. Mostly fresh water and saline forms restricted to coastal waters. They are widely distributed in tropical waters and few species are found in Arctic and Antarctic oceans. Picoplankton cannot identify easily due to lack of distinct morphological characters. Green algae are subdivided into two sub-classes Chorophyceae and Charophyceae on the basis of difference in structure, and reproductive organs and methods of reproduction. Plankton forms are mainly comes under the chlorophyceae and majority is fresh water forms. This subclass further divided into 14 orders and 22 families.

Cyanobacteria (earlier Blue-green algae)

The large part of seas consists of prokaryotic unicellular or filamentous organism known as cyanobacteria, as it appear bluish or blue green, formerly called as blue green algae, however, the name is less used today. Unlike the common bacteria, they carry out photosynthesis and referred as part of phytoplankton. Unicellular or multicellular organisms, filamentous or nonfilamentous with or without heterocysts and cell contain phycocyanin pigment. Cyanobacteria is divided into 5 orders (Chroccocales, Chaemosiphonales, Pleurocapsales, Nostocales and Stigonematales)

Diversity

Due to small size, rapid growth rate and spatio-temporal variation of species in relation to environmental conditions, phytoplankters are very sensitive to stress imparted by them. The degree of stress imposed on phytoplankton reflects the change at community level (group or functional). The classwise, orderwise, familywise, genuswise and specieswise numbers of algae listed out from the published information with respect to India is given below:

Region	No. of species		Reference
East coast of India	249	131 Dinoflagellates (7 orders, 19 families, 30 genera), 111 Diatoms (2 orders, 17 families, 43 genera), 7 Cyanophytes (1 order, 2 families, 4 genera)	Geetha Madhav and Kondal Rao, 2004
Andaman Sea	227	58 Dinoflagellates, 164 Diatoms (2 orders, 8 failies), 2 Cyanophytes, 2 Silicoflagellates	Kartik *et al.*, 2012
South east coast of India	185	16 Dinoflagellates, 166 Diatoms (Centrals 94; Pennales 72), 2 Cyanophytes, 1 Silicoflagellates	Sahu *et al.*, 2012
Mangalore coast (west coast of India)	73	22 Dinoflagellates, 46 Diatoms	Karolina *et al.*, 2009
South East Arabian Sea	105	25 Dinoflagellates, 75 Diatoms (Centrals 55; Pennales 20), 1 Cyanophytes, 2 Silicoflagellates, 2 Green algae	Robin *et al.*, 2013
South West Coast of India	67	17 Dinoflagellates (7 genera), 49 Diatoms (Centrals 40; Pennales 9), 28 Genera 1, Cyanophytes (1 genus)	Robin *et al.*, 2010
Nethravathi-Gurupura estuary	80	54 Diatoms (20 orders, 26 families, 33 genera), 5 Dinoflagellates (4 orders, 4 families, 4 genera), 15 Cyanobacteria (6 orders, 6 families, 9 genera), 6 Green algae (5 orders, 5 families, 5 genera)	Shruthi and Rajasekhar, 2014
Tumkur Lake	171	Chlorophyceae, 46 species; Bacillariophyceae, 52 species; Desmidiaceae, 22 species; Euglenophyceae, 27 species; Cyanophyceae, 24 species	Ravishankar *et al.*, 2009

Suggested reading

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