Phytoplankton Identification Manual

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#### FOREWORD

Since its inception in 1966 the National Institute of Oceanography is involved in taxonomic classification of marine phytoplankton, zooplankton, benthos and other flora and fauna under the Project "Measurement and Mapping of Marine Resources". Although the mandate of the project has been diversified with changing times, the taxonomic identification continues to remain the thrust area for all biological projects, especially those dealing with baseline studies on ecobiology and environmental pollution. Visiting post-graduate and postdoctorate students constantly look for information on taxanomic identification which is spread over several books and journals.

The project "Survey and Inventerisation of Coastal Biodiversity (West coast) funded by Ministry of Environment and Forests (MoEF), New Delhi, provided an opportunity to bring together taxonomic experts from various disciplines. Their efforts have resulted in preparation of this manual. This manual provides details of taxonomic classification and description of the concerned organisms / species. All the figures are well illustrated and detailed identification key is provided. This should surely guide even a beginner to understand the identification procedure.

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#### PREFACE

Marine phytoplankton which constitutes diatoms, dinoflagellates, blue-green algae, silicoflagellates, cocolithophors etc. contributes about 95% of primary production in the oceans. On this depends the secondary production (zooplankton) and tertiary production (fish, shellfish, mammals, etc.). Since phytoplankton serve as a basic food source for animals in the sea their presence in large numbers may indicate the abundance of commercially important fish and shellfish populations.

Coastal waters around India contain diverse groups of phytoplankton. The biodiversity of these organisms is threatened due to large scale release of domestic and industrial wastes. Hence there is a need to study these organisms in more details.

The organization of this manual is made with a broad outline to accommodate the specific need of our Indian coastal waters. The manual is divided in chapters which include - method of collection, preservation and identification procedures. Species chosen for description were selected to provide good representation of the commonly important species to give full spectrum of *Chaetoceros* to be found in phytoplankton. The details should help the students as well as researches to be thorough in the method of collection and identification of the different phytoplanktonic species. We take the responsibility of any inadvertent errors in this manual.

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## 1. Introduction

Phytoplankton ('phyto' = plant; 'planktos' = made to wander) are single celled marine algae, some of which are capable of movement through the use of flagella while others drift with currents. These microscopic plants range in size from 1/ 1000 of a millimeter to 2 millimeters and float or swim in the upper 100 m of the ocean, where they are dependent on sunlight for photosynthesis. In addition to light and oxygen ( $O_2$ ), they require basic simple inorganic chemical nutrients, such as phosphate (PO<sub>4</sub>) and nitrate (NO<sub>3</sub>). They also require carbon in the form of carbon dioxide (CO<sub>2</sub>). Some phytoplankton, the diatoms, also require a form of silicon (silicate, SiO<sub>4</sub>) because they have a "glass-like" shell.

The marine phytoplankton come in a myriad of shapes, sizes, and forms, some of them quite beautiful. Some drift on currents while others have an ability to move around with the aid of flagella (*Gymnodinium sanguineum*). Some live as single cells while others form chains or colonies. Marine algae are extremely important to life on earth—probably the most important living organisms on the planet. They impact us in at least three ways. First, they appear to be a significant factor in controlling atmospheric carbon dioxide ( $CO_2$ ), a green house gas, which in turn can influence heat retention in the Earth's atmosphere. Secondly, the phytoplankton and bacteria are the basis of the marine food web. At this level, inorganic nutrients like phosphate, nitrate, and carbon dioxide are converted to larger more complex organic molecules necessary for life. In turn, these microscopic organisms provide the food for the higher trophic levels in the food web or larger organisms higher in the food web, such as zooplankton, fishes and mammals. For example, bivalve shellfish (oysters, mussels, scallops, clams) almost exclusively consume phytoplankton for their food.

And lastly, marine algae are important because they can produce a variety of highly toxic compounds—marine biotoxins. These compounds, some of which can be released to the surrounding water while others are retained in the phytoplankton, can enter the food web and accumulate in fish and shellfish. In most cases, fish and shellfish do not appear to be affected by these potent compounds, but organisms higher in the food web, such as marine mammals and humans, can be made ill or even die. It is this very lack of affect on the fish and shellfish that we consume that makes marine biotoxins so dangerous, since there is no outward sign that can forewarn the consumer. In virtually all cases, the marine biotoxins produced by these phytoplankton, can only be detected

through laboratory analysis.

If conditions are right, phytoplankton can sometimes grow and reproduce at such a high rate that they create dense, highly colored patches in the water. When this happens, because the growth rate is so high, they deplete necessary nutrients from the water, particularly dissolved oxygen ( $O_2$ ). When this happens fish can suffocate. This sudden depletion in a small contained area can be a serious problem in aquaculture since the fish are constrained in pens and cannot escape into more oxygenated waters.

Algal Blooms: Most of the time, marine waters are characteristically blue or green and reasonably clear. In the temperate waters of the northern latitudes, water is seldom as clear as seen in tropical areas, where visibility can exceed 50-75 feet. In temperate waters, the limits of visibility or murkiness is usually the result of algae in the water. However, in some unusual cases, a single microalgal species can increase in abundance until they dominate the microscopic plant community and reach such high concentrations that they discolor the water with their pigments, these "blooms" of algae are often referred to as a "Red tide". Although referred to as "Red tides", blooms are not only red, but can be brown, yellow, green, or milky in color. These blooms can be caused by high concentrations of toxic algal species and referred to as a "Harmful Algal Bloom" (abbreviated as HAB), however non-toxic species can also bloom and harmlessly discolor the water. Adverse effects can likewise occur when algal cell concentrations are low and these cells are filtered from the water by shellfish such as clams, mussels, oysters, scallops, or small fish. Many animals at higher levels of the marine food chain are impacted by harmful algal blooms. Toxins can be transferred through successive levels of the food chain, sometimes having lethal effects.

## 2. Methods of samplings

There are three methods of sampling of phytoplankton. 1)Bottle samples 2) Plankton pumps 3) Plankton nets

#### 2.1 Bottle Samplers:

Sampling by water sampler is the recommended method (Sournia 1978 p. 33) to obtain a correct picture of the quantitative composition of the phytoplankton. A water bottles sample contains all but the rarest organisms in the water mass

sampled and includes the whole size spectrum from the largest entities, like diatom colonies to the smallest single cells (Tomas, 1997). These are ideal for quantitative phytoplankton collections as required quantities of water can be collected from the desired depth. Water samples are generally used from vessels, ships or fish trawlers. Bottle sample method is a simplest method as generally used for the collection of water samples from any desired depth of shallow systems like the near shore water, estuaries and mangroves.

2.1.1 Meyers water sampler (Fig 1): It consists of an ordinary glass or perhaps bottles of about 1-2 liter capacity and is enclosed with a metal band. It is weighted below with a lead weight and there are two strong nylon graduated ropes. One tied to the neck of the bottle and the other to the cork. While operation, the corked up (closed bottle) is let down to the desired depth where the stopper is jerked open by a strong pull of the cork rope. Water flows into the bottles and then the cork rope is released to keep the cork closed. Afterwards, using the neck rope, the bottle containing the water sample is taken out of the water columns. Up to a depth of only 20m, this type of water samples could be used.

2.1.2 Friedinger's Water Sampler (Fig 2): It is made of Plexiglas or Perspex with two hinged covers. While operation, the sampler is sent down in an open state to the desired depth and can be closed by a drop weight messenger, which falls down inside on sliding rail and closes the covers and makes the bottle water tight. By this way, the water together with the planktonic organisms of the specified column is trapped inside.

2.1.3 Niskin water sampler (Fig 3a, 3b): It is employed for taking water samples for phytoplankton enumeration from subsurface levels to various depths. These bottles are non-metalic, free-flushing sampler recommended for general purpose water sampling. These samplers can be individually or serially attached on a hydrocable and activated by messenger, or placed in any kind of Multisampling System (like G.O., Sea Bird, Falmouth Scientific and Small Multisampler System), and activated by remote or preprogrammed command. The Standard PVC Niskin type Sampler is made of gray PVC (RAL 7011), spring closure made of latex tubing with optional stainless steel spring closure, clamp bolts for attachments on a cable and mounting blocks for Multisampling System attachment. Delivery is made with lanyards for loading on both, cable and Multi Sampling Systems. All metal parts are made out of special V4A-stainless steel. Specially made V-LIP seal rings avoid leaking of the sampler. When the sampler is lowered, the clamp at the lower end and the plug valves are in open condition, so that, water can pass through the sampler. The sampler is held in this position by the wire rope. When the messenger is dropped down the rope, it strikes the release, shutting the valves closed by a locking device. The water sample of the desired depth so trapped in the bottle can then be pulled up onto the vessel in a closed condition. For collection water samples from different depths simultaneously, a series of water samplers are suspended one above the other from a wire rope and are lowered into the depths in the open state. In this case, the messenger releases another messenger that was attached to the wire clamp before lowering. The second messenger closes the next lower sampler releasing a third messenger and so on.

## 2.2 Plankton pumps

Plankton pumps are integrating samplers that pump a continuous stream of water to the surface and the phytoplankton can then be rapidly concentrated by continuous filtration. Because the pumps can collect continuously as the tube is lowered through the water column the samples are integrated from surface to desired depth. This method has its disadvantages, however e.g., breaking up colonies, breaking of large *Chaetoceros* setae, and breaking into pieces long pinnate cells like *Thalassiothrix* spp.

#### 2.3 Plankton nets:

Nets permit quantitative studies, since the mesh size will select the type of phytoplankton collected. Sampling by nets is highly selective, depending on the mesh size of the gauze, net towing speed, and the species present in the water. *Chaetoceros* setae, for instance, may form a fine network inside the gauze and very small single cells, which in other cases pass through the meshes, are retained. On the other hand, net with very fine meshes (5 or 10  $\mu$ m) often filter too little water to provide an adequate diatom sample. The most useful mesh size for collecting diatoms is 25  $\mu$ m. Net hauls have the advantage of a simultaneous collection and concentration of the plankton providing sufficient for species identification.

A typical plankton net usable in the surface layers is conical in shape and has the following constituents (Fig 4). A net ring made up of stainless steel and wrapped and sealed with polythene tubing is present anteriorly. To this, a nonfiltering portion made of a coarse khaki cloth is attached using button and hole system. The filtering portion is made of monofilament nylon material as described earlier and is followed by again a non-filtering portion of khaki cloth. To the latter, a metal net bucket provided with a stop cock is tied with a strong twine.

The determination of the volume of water filtered through any plankton net is essential for the estimation of the standing crop. The volume of water traversed by the net is determined as an approximate value by the formula  $v = r^2 d\Pi$ . Where, V, volume of the water filtered by the net; r, radius at the mouth of the net; d, distance through which the net is towed.

The water collected through the different water samplers is either centrifuged or passed through fine mesh nylon or filter papers to separate the plankton present in it. The smaller the sub sample the fewer number of rare species will be ob-

tained. On the other hand, there is no point in concentrating large quantities of a sample rich in one or a few species. Concentration by settling, centrifugation and filtration are the most used methods.

Plankton concentration is generally used to over come the damages caused, to certain groups of phytoplankton especially the setoid diatoms and dinoflagellates, by vacuum filtration, centrifugation. The simple plankton concentrator, which is quite gentle in its action, consists of a stiff tube (1.2 cm dia: 10cm height) of Perspex or PVC to the bottom of which a filter is attached. A filter paper (Whatman No. 42) or membrane filters supported by monofilament nylon netting which serves as the filter is glued at the bottom of the tube with the aid of ethylene dichloride. While using the tubes is dipped slowly into a beaker containing the phytoplankton sample. Through the filter water flows slowly upward into the tube and is removed with a large pipette. By forcing the tube downward, the rate of flow through the filter can be increased.

## Centrifugation:

With the help of an electrical centrifuge 5-20ml of water sample is centrifuged for above 10-20 mins at 1500-2000 rpm. The supernatant water is removed by decanting. The plankton is precipitated by adding a few drops of 1% Potassium aluminium sulphate or fixed weak neutralized formalin or Lugol's solution.

## 3. Fixation and Preservation

If collected samples kept alive, they should be stored in an ice chest or refrigerator and then only for a few hours. For long term analysis the collected plankton should be preserved in fixatives and preservative. A very widely used fixative and preservative for a variety of organisms including plankton is formalin. The commercial formalin is obtained as a 40% (Saturation limit) formaldehyde dissolved in water. The formalin has to be stored in inert glass or plastic containers and not in metal containers as the formalin reacts with the latter. The commercial formalin may also contain dissolved impurities such as iron and formic acid which disintegrate the shells of some planktonic organisms. The acid content of commercial formalin however, may be neutralized, by the addition of excess of calcium carbonates. For preserving net and other phytoplankton sample, 2% neutralized formaldehyde (i.e formalin) may be used.

3.1 Lugol's Solution: It is a good preservative especially for flagellated and ciliated phytoplankton to retain the flagella and cilia. It consists of 10g iodine and 20g potassium iodine dissolved in 200ml of distilled water and 20g of glacial acetic acid. The solution can be made up a few days ahead and stored in a dark bottle for convenience. Lugol's solution is added in a ratio of 1 part to 100 parts of the seawater sample. For about 250ml of water sample containing nanophytoplankton about five drops of this preservative is quite sufficient.

### 3.2 Bottling and Labeling.

*Bottling:* Storage of phytoplankton especially diatoms in bottles made of soft glass is preferred. Because surface water is usually under saturated with silicate, storage in bottles of high quality glass like Pyrex which does not release much silicate or plastic bottles may result in slow solution of delicate frustules or spines of diatoms. This may happen in plastic bottles in one to a few years. Further use of glass of very low quality for storage of phytoplankton may result in precipitates. The bottles are closed by a leak proof cork. After the analysis of the plankton, contents of the bottles and for permanent storage of plankton, or wax coating is given around the cork of the bottle after the latter's closure. This would help avoiding the loss of formalin by evaporation in the long run.

Labeling: Proper labeling of the collected and bottled plankton samples is essential. All types of information regarding plankton collection should be written on the labels so that, the plankton samples can be identified accurately. The label should contain enough information about the sample collected in order to assure proper identification of the sample. The label is written with a light coloured water proof marker or wax pencil.

## 4. Preparation for light microscopy

Common diatoms can be identified by examination of raw (without acid cleaned) material in a water mount. Common diatoms such as *Chaetoceros spp* and *Rhizosolenia* spp are identified by their gross morphology and special structures like *Chaetoceros* setae and the shape of the *Rhizosolenia* its and process (Tomas, 1997). However, this method is not effective for identifying the essential morphological structures of other genera; for example, the areolation and processes of *Coscinodiscus* and *Thalassiosira* and the striation and raphe structure of *Navicula* and *Pesudo-nitzschia* (Tomos, 1997). Organic part and cell contents, which obscure the image, have to be removed. Acid cleaning is one of the methods used to separate diatoms frustules into single valves on which structures of diatoms are best seen .

4.1 Acid cleaning: Before acid cleaning salt particles associated with the diatoms in a test tube should be washed by rinsing and centrifuging in distilled water. Then test tube with the sample is allowed to dry by removing water. Adding some hydrochloric acid to the test tube dissolves the calcareous matter and also loosens any diatoms that may be attached to the debris. After allowing the test tube with the sample for one or two days, the test tube is well shaken and the solid matter including the diatoms is allowed to settle at the bottom. The acid is then decanted off and the sediment is washed by adding water and pouring off again after allowing time for the solids to settle. Finally most of the water is poured off and concentrated sulphuric acid is added slowly and carefully. Until red fumes are no longer evolved, small crystals of potassium dichromate are then added at intervals. The sulphuric-chromic acid mixture is then poured off and water is added. Acid and dichromate treatment must be repeated until cleaning is complete if the diatoms are not yet properly cleaned with water.

4.2 Specimen mounting : For, mounting, diatoms are put in a drop of distilled water on a cover slip that has been smeared with a little Mayer's egg albumen which is prepared by mixing 50ml of white of egg with 50ml of glycerin and 1g of sodium salicylate. After allowing the water to evaporate, the diatoms on the coverslip are thoroughly dried by heating and then using any mounting media like Canada balsam, Styrax, Hyrax or DPX mount can be done. After cooling the specimen-mounted slide excess resin is trimmed off by a knife and the preparation is finally sealed with nail polish or wax.

Glycerin mounting and polyvinyl lactophenol mounting are other methods of mounting diatoms. These are more convenient to mount the diatoms in slides directly by embedding them in polyvinyl lactophenol.

Canada balsam is ideal for permanent mounts. For longer preservation, diatoms can also be cleaned and stained with methylene blue and Bengal pink. Subsequently they are embedded in Canada balsam in microscopic slides and covered with cover glasses.

## 5. Identification of species

Identification of diatoms in water samples is usually best done by using phase contrast optics, which reveal especially well lightly silicified structures, like delicate *Chaetoceros* setae, and also the organic chitan threads in *Thalassiosiraceae*. (Tomas, 1997).

It is essential to know which side of the diatoms cell is viewed. Intact single cells with a short pervalvar axis tend to lie up under the coverslip (*Coscinodiscus radiatus* and *Pleurosigma sp*). Diatoms like *Corethron* and *Rhizosolenia* with a pervalvar axis longer than the cell diameter or the apical axis turn girdle side upwards. Colony types like *Chaetoceros, Fragilariopsis and Thalassiosira*) are normally seen in girdle view in a water mount. Diatoms like *Thalassionema, Asterionellopsis* and *Pseudo-nitzschia* show either valve or girdle side. Cylindrical and discoid diatoms are readily recognized by the general circular outlines in valves view. When the cells are viewed properly the next step is to look for special features like setae in Chaetoceraceae, shape of linking processes in *Skelotonema* and in unpreserved material, organic threads from the valve in Thallassiosiraceae.

Frustular elements cleaned of organic material may also be oriented in various

ways in a permanent mount (Tomas, 1997). Flattened valves with a low mantle will usually be seen in valve view (Some *Coscinodiscus* spp., most *Navicula* spp.), while valves with a high mantle and protuberances may appear in girdle view (*Eucampia* and *Rhizosolenia*). Lightly silicified bands shaped as those in *Rhizosolenia* and *Stephanopyxis* often lie with girdle side up.

## 6. Bacillariophyceae (Diatoms)

Diatoms are extremely widespread and occur as the dominant organisms of many diverse habitants. They are particularly conspicuous in both marine and freshwater phytoplankton. They are characterized by four main features : 1). The cell walls are silicified and show characteristic secondary structures. 2). The photosynthetic pigments include chlorophylls a and c, together with the xanthophylls, fucoxanthin. 3). Food storage products include fats and chrysolaminarin. 4). The motile states possess a single pantonematic flagellum.

Although it has been possible to identify a number of structural developments in the Xanthophyta and Chrysophyta paralleling those in the Chlorophyta, the range of form has been much more restricted. The reduction in the range of vegetative types is even more marked in the diatoms, in which only unicellular and colonial forms can be recognized.

### 6.1 Structure of the diatom cell:

The diatom cell wall (frustule) consists of two parts, the one fitting over the main part of the box (Fig. 5A). The outer part (the 'lid') is the epitheca and the inner part is the hypotheca. Each half consists of the main surface, the valve, and the overlapping connecting bands; the two bands constitute the girdle. The diatom cell can therefore be viewed from two directions, the girdle view and the valve view (Fig. 5 B,C). The axis between the middle of the two valves is the long axis (most diatoms are broader than long) and is called the pervalvar axis, and the one at right angles to this is the valvar plane.

All diatom frustules have silicified walls, although in *Phaeodactylum tricornutum* only one valve is silicified. The walls contain hydrated silica and, since no other element can replace silicon, growth of diatoms show an absolute requirement for silicon, and if other elements are present in adequate amounts, growth is proportional to the silicon concentration. The silicified wall also contains an organic component, which has been called 'pectin' although there is no definitive chemical evidence for this statement.

The diatom cell wall is not of uniform thickness, and periodic arrangements of thicker and thinner areas produce a complex series of 'markings' on the cell surface. The general arrangements and symmetry of these 'markings' are important criteria for the division of the Bacillariophyceae into the two orders, the Centrales and the Pennales. In the former they are arranged with reference to a central point (Fig. 5E)(this basic picture may be altered when the cell is angular). In the later, however, the main structural element on the cell surface is a spine, and the finer secondary structures are arranged as lateral branches (Fig. 5B). Thus, the value of pinnate forms has a narrow axial area thickened at each end (polar module), and a dilated central area usually having a median thickening (central nodule). The axial region sometimes has a slit (raphe)which runs from one polar nodule to the other; whereas in other species a lighter region of the axial area gives the superficial appearance of a raphe, and this is called a *pseudoraphe*.

The secondary structures of the diatom cell wall represent the fine sculpturing which occur over much of the value surface. These structures are extremely variable; their nomenclature is sometimes equally variable and much confusion has resulted. Examination in the electron microscope has confirmed four basic kinds of secondary structures : 1. punctae are small perforations of the valve surface and are frequently arranged in regular lines, or striae, 2. aerolae are larger, depressed box-like structures , 3.Canaliculi are narrow tubular channels through the valve wall and 4. costae are ribs formed by the heavy deposition of silica.

From the voluminous literature on the detailed arrangements and elaborations of these four basic kinds of structures, two main wall types are recognized: first, the laminar walls consisting of a single silicified layer with a great variety of patterns on it (Fig. 5 F), and second , the locular walls consisting of two parallel layers with a complex reticulum of cross walls between them (Fig.5 G). Examples of species with lamina walls include *Synedra fulgens*, *Fragilaria construens* and *Didymosphenia geminata*. Species in which the wall is of the locular type include *Triceratium favus*, *Coscinodiscus asteromphalus* (in both of these species the locular nature of the wall can be seen with the light microscope) and *Stephanopyxis palmeriana*.

### 6.2 Gross vegetative structure:

The general shape and superficial appearance of the diatom cell is variable and many of these gross variations are useful for a preliminary identification of a given alga. There are six main types of morphological elaborations which appear to be correlated with the planktonic habit: 1). the flat discoid shape of many centric diatoms (e.g. *Cyclotella comta*, Fig.5 D, E); 2). the needle shape of species such

as *Rhizosolenia* and *Synedra*; 3). the long, sometimes coiled filaments of some species of *Melosira*; 4). the development of elongated bristles (*Stephanodiscus*) or horns (*Chaetoceros*) from the edge of the valves; 5). the stellate colonies of *Asterionella* and *Tabellaria*; and 6). the frequent production of extensive mucilaginous envelopes (e.g. *Cyclotella planctonica*).

Although, most diatoms are unicellular, a number of colonial species is also known. There appears to be three basic kinds of colony formation. Firstly, cells are sometimes joined together by a localized production of mucilage to form stellate colonies of *Asterionella* of the filamentous forms of *Melosira*. Secondly, some colonies consists of many cells embedded in a common mucilaginous envelope, and in some genera the envelope has a tubular structure (e.g. some species of *Navicula, Cymbella and Nitzschia*) The third type of colony consists of cells joined by special outgrowth such as the spines of *Chaetoceros*.

### 6.3 Cell Division:

Diatoms usually divide at night and the plane of division is always at right angles to the longitudinal axis, that is, parallel to the valve surfaces. The first indication that cell division is imminent is that the cell increases in size and the two halves separate slightly. Mitotic division of the nucleus is followed by fission of the protoplast in a plane parallel to the valve faces. New siliceous valves are then deposited on the two fresh protoplasmic surfaces. As the connecting bands develop, the valves of the parent cell separate and the new silica valve becomes the hypotheca of each daughter cell.

The daughter cell having the original hypotheca of the parent (it is now the epitheca of the daughter cell) is smaller than the parent cell. Thus, in a population of diatoms there is normally a progressive decrease in the average cell size (those species which do not show a progressive decrease in size are usually weakly silicified and the constancy of size is probably related to the plasticity of the cell wall).

## 6.4 Classification of Diatoms:

As pointed out in the beginning the diatoms can be divided into two orders, the Centrales and the Pennales; this classification being based largely on the symmetry and orientation of the secondary structures on the valve surface. 1. Centric diatoms are non-motile, whereas many species of the pinnate forms exhibit a gliding movement which is, in some way, dependent on the presence of a raphe.

2. Sexual reproduction of the Centrales is oogamous whereas that of the pinnate species is generally isogamous. 3. although species of the two groups sometimes inhabit the same regions, Centrales are more commonly planktonic and marine, whereas occurrence of Pennales are less in marine water.

The more detailed classification of diatoms depends almost entirely on the structure of the siliceous skeleton. The Centrales are divided into three major groups on the basis of cell shape and are the presence or absence of particular processes. Genera such as *Coscinodiscus, Cyclotella* and *Melosira* are disc-shaped with no processes, whereas the valve surfaces of genera such as *Biddulphia* and *Chaetoceros* have various horns. A third group containing genera such as *Rhizosolenia* and *Corethron* also have a complex girdle structure.

The classification of the pinnate diatoms is based largely on extent of development of the raphe. *Tabellaria* and *Asterionella* are examples of the group of diatoms which only posses a pseudoraphe. Amongst the other forms it is possible to identify an increasing tendency for the development of a raphe; the valves of *Eunotia*, for example, show the beginnings of raphe development. *Achnanthes* and *Cocconeis* have a raphe on one value only, but most genera (e.g. *Navicula*, *Bacillaria* and *Nitzschia*) have a raphe on each valve.

### Order: Centrales:

#### Family: Coscinodisceae

Skeletonema costatum (Greville) Cleve (Fig. 6): Valves small, lens shaped with rounded ends and form long and slender chains with the help of marginal spines; space between cells larger than cell; dia.,  $12-15 \,\mu$ m.

*Cyclotella meneghiniana* Kutzing (Fig. 7): Cell disc shaped with a number of regularly arranged striations which do not reach center; dia.,  $17-24 \mu m$ .

*Cyclotella striata* (Kutzing) Grunow (Fig. 8): Cells resemble *C. meneghiniana*; with evenly striated border; central area of the cell coarsely punctuate; dia., 15- $30 \mu m$ .

*Coscinodiscus eccentricus* Ehrenberg (Fig. 9): Cell disc shaped; hexagonal markings seen; areolae of same size (6 in  $10\mu m$ ) and arranged in tangential series; margin striated and 18-20 striae in  $10\mu m$ ; dia., 340-104  $\mu m$ .

#### Family: Actinodisceae

Asterompalus flabellatus (Brebisson) Greville (Fig. 11): Cell slightly convex; valves slightly ovate; middle sector lines unbranched; 7-8 slightly curved hyaline rays, of which one is narrower; length 37-63  $\mu$ m and breadth 32-55  $\mu$ m.

*A. wyvillei* Castracane (Fig. 12): Valves rounded with 15 straight hyaline rays, of which, one is narrower; sector lines branched; dia., 71-74  $\mu$ m.flattened at ends; numerous disc-shaped chromatophores; dia., 3-16  $\mu$ m and length 10-91  $\mu$ m.

#### Family: Soleniae

*Lauderia annulata* Cleve (Fig 13a, b): Cells from straight chain; cells cylindrical with convex valves; valves with a depression in middle and raised at margin; adjacent cells touch raised portions; valves with numerous spines and varying length; dia., 53-83  $\mu$ m.

Schroederella delicatula (Peragallo) Pavillard (Fig. 14): Cells cylindrical and form chains; valves with depressions in middle; valve ends with a crown of spines; a spine like pore canal present at center of each valve; dia., 14-41µm.

*Leptocylindrus danicus* Cleve (Fig 15): Cells cylindrical and form chains; valves flattened at ends; numerous disc-shaped chromatophores; dia., 21-24µm

*Rhizosolenia cylindrus* Cleve (Fig. 16): Cells cylindrical and valves with fairly truncated ends; presence of large and bent spines; cell wall hyaline; dia., 21-24  $\mu$ m.

*R. crassispina* Schroeder (Fig 17): Cell cylindrical and valves possess truncated ends; apical processes broadened at base and hair-like afterwards; numerous disc-shaped chromatophores; dia.,  $41-54 \mu$ m; length  $145-278 \mu$ m.

#### Family: Chaetocereae

*Chaetoceros lorenzianus* Grunow (Fig. 18): Cells from straight chains; apertures of varying sizes; terminal setae thicker, somewhat shorter than other setae and run parallel to chain axis; inner setae longer and interlocking; setae four sided;

length of cell, 18-61 µm.

*C. didymus* Ehrenberg (Fig 19): Cells from straight chains; a charasteristic semicircular knob like structure present in middle of each valve; distict interlocking of setae; two plate-like chromatophores present; length of cell, 22-40  $\mu$ m.

*C. diversus* Cleve (Fig. 20): Cells form compact and short chains; apertures very small; setae of some cells thicker, tubular and spinous; other inner setae and terminal ones hair-like; length of cell,  $5-9\mu m$ .

### Family: Biddulphieae

*Eucampia zoodiacus* Ehrenberg (Fig 21): Cells flat united to form spirally twisted chains with characteristic blunt processes; valves concave in middle so that a wide aperture between two cells; intercalary bands faint; length of cell, 42-61µm.

*Ditylum brightwellii* (West) Grunow (Fig 22a, b): Cells prism shaped with three cornered valver plane; valve margin wavy; a circlet of short spines on valves ends and a long hollow spine at center of the valve; side of valve measures  $42-144 \mu m$ .

*Biddulphia sinensis* Greville (Fig 23): Cells forming short chains, cylindrical and square to rectangular in girdle view; however, ovate to lanceolate in valvar plane; presence of two thin blunt horns at corners of valve and two long and thin spines nearer to horns characterize this species; length of cell 82-215  $\mu$ m.

*B. mobiliensis* Bailey (Fig 24): Cell resembles *B. sinensis* to some extent; cells moderately squarish with slender horns at corners of valves; length of cell 24-81  $\mu$ m.

## Order: Pinnales

## Family: Fragilariodeae

*Fragilaria oceanica Cleve* (Fig 25): Cells rectangular in girdle view and form compact ribbon-like chain; valves broadly lanceolate with rounded ends; pseudoraphe narrow linear; transapiacl striae 14 in 10  $\mu$ m; length of cell, 11-32  $\mu$ m; breadth 6  $\mu$ m.

*Thalassionema nitzschioides* Grunow (Fig. 26): Cells form zig-zag chains and linear-rectangular in girdle view; cells rest at protoplasmic cushions found at junctions; linear-lanceolate in valve view; marginal striae 12 in 10  $\mu$ m; length 20-66  $\mu$ m; breadth 3m.

Asterionella japonica Cleve (Fig. 27): Cells form spiral colonies; frustules linear, narrow with parallel sides and knob-like at base; striae not clearly seen; length 43-106  $\mu$ m; breadth 7-11  $\mu$ m.

### Family: Naviculoideae

*Gyrosigma balticum* (Ehrenberg) Rabenhorst (Fig 28): Valves linear with curved and truncated ends; raphe excentric and central area small, oblique; transverse and longitudinal striae equidistant, 11-12 in 10  $\mu$ m; length of cell 290-338  $\mu$ m; breadth 28-30  $\mu$ m.

*Pleurosigma galapagense* Cleve (Fig. 29): Valves very slightly sigmoid; ends blunt; raphe somewhat sigmoid; transverse striae 18 in 10  $\mu$ m and oblique striae 15 in 10  $\mu$ m.

*Diploneis weissflogii* (A. Schidt) Cleve (Fig. 30): Valves broad and strongly constricted at center; ends fairly rounded; central nodule with horns; transeverse costae 9 in 10  $\mu$ m; length 28-58  $\mu$ m; breadth 10-25  $\mu$ m (away from constriction) and 6-15  $\mu$ m (at constriction).

*Navicula longa* (Gregory) Ralfs (Fig. 31): Valves long rhombic with fairly pointed ends; axial area narrow; central area small; striae 9-11 in 10  $\mu$ m; length 52-56  $\mu$ m; breadth 10 $\mu$ m.

*N. sigma* (Kutzing) W. Smith (Fig. 32): Valve linear; somewhat sigmoid in girdle view and straight in valve view; bulge at center and gradually diminishing in size towards end; keel punctae 5-6 in  $10\mu$ m; length, 280-310  $\mu$ m; 10-11  $\mu$ m.

*N. closterium* (Ehrenberg) W. Smith (Fig. 33): Valve spindle shaped in middle; extremities beak like and slightly curved in opposite directions; striae not visible;

length 30-160  $\mu$ m; breadth 3-7  $\mu$ m.

## 7. Pyrrophyceae (Dinoflagellates)

The motile unicellular forms of the dinoflagellates are sometimes important constituents of phytoplankton populations and are only next to diatoms as far as the phytoplankton biomass is concerned. Although, motile unicells form the bulk of the class a number of non-motile and multi-cellular types also occur. The dinoflagellates are unicellular, single or pseudocolonial and show wide variations in morphology. The size of these organisms ranges from 0.001 to 2 mm; however, most of the species have a size below 0.2 mm.

The presence of 2 flagella, one encircling the body is located in the transverse furrow otherwise known as 'girdle' or 'cingulum' and the other located in the longitudinal furrow or 'sulcus' trailing behind is the characteristic feature of most of the planktonic dinoflagellates (Fig 34). The body of the cell is covered by an envelope (cell wall) made of cellulose, pellicle, valves or plates. The plates when present form the theca and are usually arranged in specific series of taxonomic importance. The arrangements of plates and plate formulas of certain genera of dinoflagellates are shown in Figs 34-37. Based on certain characteristics, the theca may be of five types a) no distinct plates in theca (e.g. *Amphidinium, Noctiluca, Oxyrrhis*); b) theca of thin polygonal plates (e.g. *Gymnodinium, Gyrodinium*); c) theca of thik plates each with distingshable shape (e.g. *Gonyaulax, Glenodinium*); d) theca of two valves (e.g. *Prorocentrum*). The highly ornamental forms are provided with striations, ridges, horns, spines, tentacles (lists).

The cell has normally a large and fingerprint-like nucleus and two vacuoles. Among the latter, the larger one is known as 'pusule' which is said to help in phagocytosis. The other vacuole is small and its function is not known. The chromatophores may or may not be present; if present, they are few, small and conspicuously coloured (green , yellow, brown or orange). Pigments such as cholorophyll a. c,  $\beta$  carotene, fucoxanthin, dinoxanthin, peridinin and diadinoxanthin. The other components of the cell are oil globules, ocelli, eye spots (stigmata), nematocytes, trichocysts and internal siliceous star-shaped or net work structure.

Majority of dinoflagellates are autotrophic and a few are holozoic, saprophytic or phagotrophic. In the autotrophic dinoflagellates, the products of the photosynthesis are starch and lipids. The class Pyrrophyceae comprises two groups, viz. Desmophyceae and Dinophyceae

Group: *Desmophyceae*: It is much smaller group and has only two genera, viz. *Prorocentrum* and *Exuviaella*. Both the flagella in these organisms arise from the

anterior end of the cell and hence the cingulum and sulcus are absent. The cell wall is not composed of separate plates unlike in dinophyceans but has only a longitudinal suture which divides the cell into two valves. The reproduction is by longitudinal division while the cell is motile. During division, the suture dividing the two valves separates, so that after fission, each daughter cell retains one valve from parent.

## Order: Prorocentrales

Genus: *Prorocentrum*: Cells of species generally elongated, oval and armoured; composed of two opposing longitudinal valves connected by suture and intercalary bands; chromatophores small and yellowish-brown.

*P. micans* Ehrenberg (Fig 38): Cells variously shaped from oval to almost circular and compressed laterally; apical teeth and protrusions may or may not be present; however, apical platelet present; valves with poroids (pits) pores, reticulations, spines or other surface markings; length 34-52 µm, breadth 15-18 µm.

*P. rostratum* Stein, (Fig 39): Body compressed laterally with a blunt apex; a finger or rostratum like process present; valves narrow and pointed posteriorly with an apical tooth on each valve; length, 98  $\mu$ m; bredth, 18 $\mu$ m.

Genus: *Exuviaella* Cienkowski: Cells of species oval or subspherical; absence of anterior projection; two lateral large and brown chromatophores; nucleus posterior.

*E. compress* Barley and Ostenfeld (Fig 40): Cell oval and not compressed ; each valve with a smooth tooth anteriorly; two plate-like yellow chromatophores; length  $20-25 \,\mu$ m; breadth 18-24  $\mu$ m.

Group: *Dinophyceae*: This group differs from Desmophyceae in having a cingulum which divides the cell into an anterior epicone and a posterior hypocone. The girdle houses a hand-like transverse flagellum which arises through a pore and causes the cell to spin to some extent on its axis. The sulcus is also present in this group and it runs from the posterior end of cell part way forwards. The longitudinal flagellum which arises from a pore in the sulcus, runs back and usually beyond the cell trailing behind in the water.

### Order: Dinophysiales

Genus: *Dinophysis* Ehreneberg: Cells of this species compressed laterally; epitheca small or rudimentary with oblique set girdle tentacles (lists); upper list funnel shaped projecting beyond epitheca and strengthened by radial ribs; left sulcal list not well developed.

*D. caudata* var. *pedunculata* Schmidt (Fig 41): Hypotheca with distinctive protuberances but without posterior sail; length 65-115 µm.

Genus: *Phalacroma* Stein: Body not much compressed; epitheca is elevated above transverse lists which are uniformly developed and not conspicuous; chromatophores absent.

*P. argus* Stein (Fig. 42): Body laterally ovate and wider behind girdle; epitheca and hypotheca rounded; girdle lists ribbed; right sulcal list concave, length 72  $\mu$ m.

*P. cuneus* Schutt (Fig 43): Body cuneate laterally; epitheca low and broadly rounded; hypotheca posteriorly narrowly rounded to subacute; margin of left sulcal list slightly sigmoid. 71  $\mu$ m.

### Order: Peridiniales

*Noctiluca* Suriray: Body kidney or sphere shaped; no girdle and hence epicone and hypocone not distinct; deep sulcus; short longitudinal flagellum and transverse flagellum represented by a mobile membrane or tooth; well developed tentacle at the posterior end of sulcus.

*N. miliaris* Suriray (Fig 44): Only one species known under the genus *Noctiluca*; characters similar to that of genus; dia., 200-2000  $\mu$ m.

Genus: *Protoperidinium* Ehrenberg: Top shaped; presence or absence of antapical horns; ventral plate (1<sup>st</sup> apical) of epitheca may be 4, 5 or 6 sided; 2<sup>nd</sup> intercalary plate 4, 5 or 6 sided; apical horn affixed or tapering; plate formula 4', 3a, 7", 5" and 2"".

*P. ovatum* (Pouchet) Schutt (Fig. 45): Cell slightly compressed; epitheca low, dome-shaped and tapering sharply into small apical horn; hypotheca also low, dome-shaped and with two small antapical spines; sulcus subantapical or reaching antapex; five-sided 1<sup>st</sup> apical and four-sided or five-sided 2<sup>nd</sup> intercalary; 68-70  $\mu$ m by 45-60  $\mu$ m.

*P. crassipes* Kofoid (Fig 46): Body low and stout; slightly compressed dorsoventrally; ventral side rather concave but dorsal convex; apical horn conical abtuse; antapicals short, stout and close together; antapicals end in a blunt, semi-truncated projection with 2-3 points; right antapical longer than left; 70-85 by 60-72  $\mu$ m.

Genus: *Gonyaulax* Diesing: Girdle equatorial and left handed; sulcus indented and occupies whole venteral area; plate formula 3' 6", 6"', 1p and 1""; chromatophores yellow to dark brown.

*G. polygramma* Stein (Fig 47): Body elongated, spindle shaped and swollen midbody; both ends projecting into two long horns; length, 135 µm; breadth, 40 µm.

Genus: *Ceratium* Schrank: Cell dorso-ventrally flattened; girdle left-handed with lists; epitheca with long hollow apical horn and hypotheca with two hollow antapicals; chain formation in some species and heteromorphic; chromatophores numerous yellow; plate formula 4',5", 5" and 2"".

*C. tripos* var. *atlanticum* Ostenfeld (Fig 48): Some what large species; body as broad as long; epitheca's left contour slightly convex and right contour strongly convex; horns strong; apical broader below, larger than the others; antapicals diverging from one another; antapicals of equal size, bent; 115-130  $\mu$ m by 20-25  $\mu$ m.

*C. pulchellum* B. Schroder (Fig. 49): Robust species; eiptheca with steep left and very convex right side; apical horn long and strong, slightly wider in middle; base of hypotheca strongly convex; antapicals short; less stronger than apical; left curved slightly diverging or parallel to apical; right equal in length or shorter than left, 130-140 by 60-65  $\mu$ m.

*C. breve* (Ostenfeld and Schmidt) Schroder (Fig. 50): Body with short horns; epitheca's right contour strongly convex and left contour steep; hypotheca with evenly convex base; anatapicals very strong; slightly parallel with apical horn; 75-80 by  $60 \mu m$ .

*C. karstenii* Pavillard (Fig. 51): Strong body; right contour of the epitheca convex; apical horn slightly bent at base; antapical slender; right antapical longer than left antapical and bent distally towards apical horn; left anatapical at times curved; 90-95 by 25-32  $\mu$ m.

*C. contortum* (Gourret) Cleve (Fig. 52): Cell resembles *C. karstenii* to some extent; epitheca oblique on right, right contour strongly convex; apical horn twisted, S shaped; horns slender; antapicals unequal, right longer than left and twisted towards apical horn; 90-94 by 25-30  $\mu$ m

## 8. Micrometry

By micrometry, while viewing through a microscope, the length, breadth and other details of an organism are measured. The size determination of the phytoplankton forms an important aspect, especially, in preparing the report on the occurrence of new species or taxonomic studies for publication.

In micrometry a occular micrometer (graticule) plays an important role. The ocular micrometer is a circular glass piece which contains a scale of lines which are engraved or photographically reproduced (Fig. 53). This scale is of 10 mm in length divided into ten equal divisions. Thus on the scale of the ocular micrometer one hundred divisions of 100  $\mu$ m each.

*Calibration:* Ocular micrometer is mounted on the diaphragm inside the eyepiece of the chosen microscope at the focal of the eyelens. On the diaphragm inside the eyepiece, at which point, the image from the object is also focused, so that, the two can be viewed simultaneously. Now, not only the object in focus, but superimposed on the object, the series of lines of the graticule is equally visible. For the calibration of the graticule, a stage micrometer which is a microscopic slide of 7.5 x 2.5 cm, on which, has been engraved a scale of 1mm long, divided into 100 divisions of 10 $\mu$ m (0.01mm) each (Fig 54). While calibrating, the stage micrometer is first placed on the stage of the microscope. Then it is focused and aligned with the ocular micrometer scale. The stage micrometer is then moved carefully until its zero line is in exact coincidence with that of the ocular meter, in order to find out how many divisions on the stage micrometer scale. From this, the value (in  $\mu$ m) of one division of the ocular micrometer under the chosen microscope with fixed objective and eyepiece powers is calculated.

If 30 divisions of the ocular micrometer correspond with 10 divisions of the stage micrometer scale, then these 30 divisions are equivalent to 100  $\mu$ m. In other words, these 30 divisions occupy 100  $\mu$ m space of the stage micrometer (as one division occupies 10  $\mu$ m of the space in the stage micrometer and the total length of the scale is 1000  $\mu$ m –1 mm). Thus one ocular micrometer division is equal to 100/30 = 3.3  $\mu$ m. This calibrated value of the ocular micrometer is of a particular objective and eyepiece of a microscope.

If size determination of a object is to be done in different objective or eye lens, the ocular micrometer scale is calibrated for all the combinations of the different objectives and eyepieces, all value may be tabulated and can be used whenever it is required.

The size of an individual phytoplankton cell of a species may be determined using the calibrated ocular micrometer and micrometer as follows. For size determination, on the stage of the microscope, the specimen for which the size is to be determined, is now placed instead of the stage micrometer. If the diameter of *Cyclotella* cell is to be determined the zero of the ocular micrometer is focused against the edge of the cell and the number of division of the ocular micrometer occupy the diameter of the cell is found out. Number of calibrated ocular micrometer divisions multiplied by the corresponding calibrated value would

give a diameter of the said cell. For examples, if the graticule divisions are 20 then the diameter of the cell is  $20 \times 3.3 \mu$ m=  $66\mu$ m.

## 9. Measurement of Biomass

Assessment of standing crop of phytoplankton in different periods is essential for any environment as the level of biomass indicates directly or indirectly its fertility and fishery resources. The biomass may be estimated in various ways.

#### 9.1 Chlorophyll measurements:

This method is chiefly employed to estimate phytoplankton biomass. The most useful chemical method for determining the total quantity of phytoplankton in seawater is to estimate the amount of chlorophyll usually as chlorophyll a (Parson *et al.*, 1984). This is a rapid method for determining phytoplankton density in a sample involves the extraction and measurement of chlorophyll concentrations. The amount of pigments as chlorophyll a, b, c and phaeophytin is considered as a measure of phytoplankton biomass.

After the collection of the sample, it is filtered through a Millipore (Pore size 0.45  $\mu$ m) or glass fiber (1  $\mu$ m mesh) filter, and is pumped to dryness (Fig. 55). All steps should be carried out in the dark to avoid pigment breakdown. The filter containing the sample is placed in 90% acetone in a plastic vials covered by aluminium foil and shaken vigorously and gently ground with a homogeniser to ensure dissolving of the filter (Millipore) before storage in the refrigerator for 20-24 hr. Some recommend, addition of 1 ml of a 1% Magnesium carbonate suspension on to the filter paper to form a thin bed, which will serve as a precaution against the development of any acidity and subsequent degradation of pigment in the extract.

After 20-24 hrs of extraction in the cold and dark, the plastic vial containing filter paper is brought to room temperature and the volume brought up to the original level by addition of 90% acetone in a graduated centrifuge tube. The solution is centrifuged for about 20 minutes at 5000 rpm and the supernatant solution is considered for the determination of optical density, or transmission percentage which is mainly with the aid of a flourometer (Parson *et al.*, 1984).

#### 9.2 Cell counts:

The direct estimate of phytoplankton cell density as measures of standing crop are usually made by this method. The enumeration of nano and net phytoplankton is done by various counting chambers, however, the most commonly used counting chamber is Sedgwick Rafter cell. The counting cell is filled with the plankton sample and placed on the mechanical stage of the microscope. Then the counting cell is left for about half-an-hour for proper sedimentation. The organisms are then counted from one corner of the counting cell to the other. The Sedgwick Rafter is moved horizontally along the first row of squares and the organisms in each square of the row are thus counted. The rafter is moved to the second row and organisms in each square here are counted. (Few transects may also be counted instead of all the squares. The total number of cells is then computed by multiplying the number of individuals counted in transects with the ratio of the whole chamber area to the area of the counted transects.) Replication of counts of one ml samples is recommended for the statistical treatments. After counting, the sample is to be returned to the jar containing the whole sample. The average values are taken into account for calculation. The total number of phytoplankton present in a liter of water

sample can be calculated using the formula:

Where, N: total number of phytoplankton cells per liter of water filtered;

n: average number of phytoplankton cells in 1 ml of plankton sample.

v: volume of plankton concentrate (ml)

V: volume of total water filtered ( I ).

## 9.3 Cell counts by drop count method:

The common glass slide mounted with a drop of concentrated phytoplankton sample in glycerol and covered with cover slip is placed under the microscope provided with a mechanical stage. The plankton are then counted from the microscopic field of the left top corner of the slide. Then slide is moved horizontally along the right side and plankton in each microscopic field are thus counted. When first microscopic field row is finished the next consecutive row is adjusted using the mechanical device of the stage. In this way all the plankton present in entire microscopic field are counted. If it is difficult to count all the microscopic fields, then few microscopic field may be counted.

The total number of cells then calculated by summing the plankton numbers of all the microscopic fields. If this total number is of one drop of the concentrated phytoplankton, then total number is in 1 ml of the phytoplankton concentration has to be calculated. Before calculating this, number of drops which form 1 ml has to be counted by adding the drops of water into the graduated centrifuge tube. If one drop of concentrated phytoplankton contains some known number then cells present in 1 ml can be calculated.

For example if 16 drops forms 1ml, and suppose 50 planktons are counted in one drop. Then the plankton in 1ml are calculated as follows.

Plankton in 1 ml concentrate = 16 x 5

Plankton per litre = 800 x 1000 ml

= 800000 cells.

### 10. Measurement of Productivity

The basic concept of primary productivity can be summed up in the equation for carbon fixation by autotropic aerobic algae.

 $6CO_2 + 6H2O$   $^{Light} \rightarrow C_6H_{12}O_6 + 6O_2$ 

From this equation it is apparent that a number of methods can be employed to measure the rate of photosynthesis i.e carbon dioxide uptake, oxygen production or the formation of carbon compounds.

According to Litter (1973) and Hoffman and Dawes (1980), of the different methods: such as labeled carbon ( $C^{14}$ ) uptake, oxygen release (oxygen probe), pH ( $CO_2$  uptake), light and dark bottle method (Winkler titration method); the mosteffective method for measuring productivity was  $C^{14}$  uptake.

#### C<sup>14</sup> Method:

Labeled carbon is probably the most extensively used procedure for oceanic studies of productivity. This method essentially advantageous because it is relatively safe, weak  $\beta$ -emission (0.15 Mev) as well as its long half life (4700yr), so that storage offers no major problems.

Procedure: The activity per ml of the working solution needed for the different productivity experiments depends on the production rates expected, duration of incubation, bottle size, etc. Invariably, 0.2-1 ml of the working solution is used per bottle containing water sample.

Water samples for which production rates are to be determined are first collected from the specified depths and are transferred to the light and dark bottles kept in a dark box. Then, a known dose of the working solution is injected rapidly into the bottles with the help of a graduated hypodermic syringe having a needle not shorter than 5 cm. The bottles are then incubated for a known period by suspending them at the respective depths from where the water samples were taken for experimentation. After the incubation is over, the experimental bottles are removed from the depths and are stored in a light-free case until the filtration of water samples is begun. Filtration may be done either on board the ship or in the laboratory. Aliquots of water samples for filtration are rapidly transferred into a suitable vacuum filtration apparatus on to a No. 2 membrane filter or Millipore filter of about 0.5  $\mu$  porocity. The vacuum should be applied at about 0.5 atm which will help avoiding damaging of fragile phytoplankton cells. The filtration

should be done in a semidarkend area.

The filters, after their removal from the filtration apparatus are placed onto planchets which are then kept in a desiccator containing silica gel. Filters obtained from light and dark bottles are then subjected to counting in a Geiger-Muller counter. Under constant light source, the rate of production is obtained in mgC/m<sup>3</sup>/hr by the following formula:

Where, cpm, counts per minute; the total  $CO_2$  is assumed to be constant in oceanic waters and the value is 90 mg  $CO_2$ /l; 1.06, a correction factor for the isotope discrimination effect and to be used as the <sup>14</sup>C incorporation will be slow compared to <sup>12</sup>C; 1000 to convert the value for m<sup>3</sup>; 12/4 to get the value of C from  $CO_2$  as the molecular weight of  $CO_2$ , 44 and the atomic weight of C, 12.

Data of production -	Net activity (cpm of light bottle-cpm of dark bottle)	) X	Total CO <sub>2</sub>	Х	1.06 X 1000 X 12/44
(photosynthesis)	cpm added	H	s of incubation	۱	
(mgC/m <sup>3</sup> /hr)					

## 11. Bibliography:

Hoffman, W. E. and C. J. Dawes., 1980. Photosynthetic rates and primary production by two Florida benthic red algal species from a salt marsh and a mangrove community. *Bull. Mar. Sci,.* 30: 358-364

Littler, M. M. 1973. The productivity of Hawaiin fringing-reef crustose corallinaceae and an experimental evaluation of production methodology. Limnol. Oceanogr. 18: 946-952.

Parsons, T. R., Y. Maita and C. M. Lalli., 1984. *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, New York.

Sournia, A.(ed). 1978. Phytoplankton manual. In *"Monographs on Oceanographic Methodology* 6," pp 337. UNESCO, Paris.

Tomas C. R., 1997. *Identifying marine phytoplankton*. Academic press, Harcourt Brace and Company, Toronto. Pp. 858







Fig. 3







A, B, C, *Pinnularia viridis*; A, transverse section, B, valve-view, C, girdle view, D, E, *Cyclotella comta*; D, girdle view; E, valve view, F, laminar wall, G, locular wall c.n. central nodule, e, epitheca; g, girdle; h, hypotheca; p.a, pervalvar axis, p.n, polar nodule; r, raphe, v, valve; v.p. valvar plane.



A, B, C, *Pinnularia viridis*; A, transverse section, B, valve-view, C, girdle view, D, E, *Cyclotella comta*; D, girdle view; E, Valve view, F, laminar wall, G, locular wall c.n. central nodule, e, epitheca; g, girdle, h, hypotheca; p.a, pervalvar axis, p.n. polar nodule; r, raphe, v, valve; v.p. valvar plane.























Fig. 49



Fig. 51



Fig. 48



Fig. 50



Fig. 52

