#### PLANKTON TAXONOMY

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Plankton includes all those organisms that live suspended in the water column of aquatic ecosystems. Unlike nektons, which swim actively against water currents, plankton are characterized by feeble power of locomotion and drift in the water column at the mercy of currents, winds, and tides. The term "plankton" was coined by Victor Hensen from the Greek word "plankton" meaning drifter or wanderer.

Plankton comprises diverse organisms ranging in size from microscopic viruses to macroscopic forms such as large colonial jellyfishes. Plankton are fundamental in maintaining the intricate balance of marine ecosystems, as both primary producers and as a crucial food source, channeling energy produced through photosynthesis to higher orders in the trophic levels that are exploitable by man. Besides their contribution to global primary production, they are also responsible for 50% of global oxygen production synthesised as by-product of photosynthesis. Their photosynthetic activity not only sustains marine life but also plays a critical role in the ocean's biological pump and carbon cycle, sequestering carbon from the surface to the deeper ocean in turn aiding in regulating earth's climate. They also play a vital role in the nutrient cycling and biogeochemical cycling of elements through their process of respiration, moulting, defecation, death and decomposition. Furthermore, plankton are often considered as sensitive indicators of perturbations associated with climate change and anthropogenic interventions in the aquatic ecosystems. The characteristics of plankton such as short life cycle, ectothermic nature, high sensitivity of their physiological and metabolic processes to subtle changes in habitat makes them an ideal indicator while evaluating ecosystem changes. Thus understanding and conserving these tiny wanderers are essential for maintaining the intricate health and sustainable productivity of our oceans. The training notes below will provide detailed insight on the significance of both phytoplankton and zooplankton community in aquatic ecosystems, their sampling and enumeration methodologies, and taxonomic identification.

### **Phytoplankton**

Phytoplankton are microscopic autotrophs capable of synthesizing organic carbon from dissolved inorganic nutrients in the ocean using the sun as the source of energy. Through the process of primary production, they are responsible for generating 50% of global productivity and forms the base of ocean food web. Besides their role in organic carbon synthesis, they are also vital in generating  $2/3^{rd}$  of the atmospheric oxygen levels. They also have a crucial role in mitigating impacts of global warming occurring in conjunction with excessive CO<sub>2</sub> release to the atmosphere, through their process of primary production and subsequent carbon sequestration to deeper ocean.

Phytoplankton encompasses a diverse community consisting of both eukaryotic and prokaryotic organisms spanning wide size ranges and taxonomic diversity. The size of phytoplankton usually range between  $\leq 2\mu m$  to 200 µm. The predominant phytoplankton representatives of aquatic ecosystems are diatoms, dinoflagellates, cyanobacteria, green algae, chrysophytes and coccolithophorids.

### Classification of phytoplankton based on size

As the size of the phytoplankton community has an upper hand in determining the pathways of carbon cycling, type of food web active in the ecosystem, nutrient uptake and biological pump, they are often classified as,

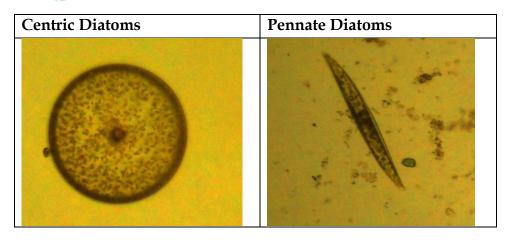
- **Picoplankton** Phytoplankton having size ≤2µm. Mostly include autotrophic bacterioplankton and smaller phytoplankton
- **Nanoplankton** Phytoplankton having size in between 2 20µm. Include the majority of the autotrophic diatoms, dinoflagellates, and coccolithophores.
- **Microphytoplankton** Phytoplankton having size between 20-200 µm. Include larger chain forming diatoms and flagellates.

### Major phytoplankton groups

Phytoplankton comprises a diverse community of photosynthetic organisms classified according to their morphological, physiological and genetic characteristics. The predominant members of phytoplankton are representatives of classes, Chlorophyceae, Bacillariophyceae, Dinophyceae, Cyanophyceae, and Prymnesiophyceae. Members from Raphidophyceae and Cryptomonads also contribute significantly to the phytoplankton community of freshwater and brackish water ecosystems.

### 1. Diatoms (Bacillariophyceae)

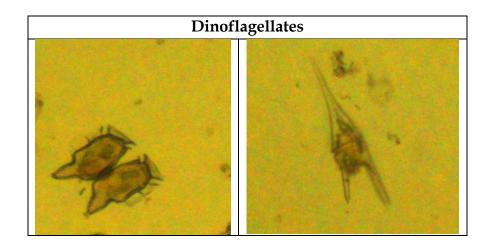
Diatoms, often referred as the "jewels of the sea," come under a diverse phytoplankton class bacillariophyceae ranging from unicellular, multicellular to colonial forms. They are distinguished by their intricate siliceous outer wall (frustule), which gives them a distinctive appearance under the microscope. These microorganisms are remarkable for their stunning diversity in shape and structure. They possess pigments such as chlorophyll *a*, chlorophyll *c*, and fucoxanthin, which enable them to carry out photosynthesis efficiently. Diatoms comprise two major forms distinguished by their shape i.e., *Centric diatoms* the discoidal forms and dominant members of the pelagic phytoplankton community, and the *Pennate diatoms*, the elongate/ bilaterally symmetrical forms and the dominant members of the benthic phytoplankton community. They are commonly found in cold, nutrient-rich waters worldwide, and have a crucial role in sustaining oceanic production and in regulation of global biogeochemical cycles. Some common centric diatoms are *Coscinodiscus*, *Thalassiosira*, *Cyclotella* while the pennate forms include *Navicula*, *Fragillaria*, *Diploneis* etc.



### 2. Dinoflagellates (Dinophyceae)

Dinoflagellates are unicellular flagellated protists that possess an organic cell wall and contain photosynthetic pigments such as chlorophyll *a*, chlorophyll *c*, and peridinin. They are characterised by two flagella, the *longitudinal flagella* lying in surface groove called *sulcus* and the *transverse flagella* lying in surface groove called *cingulum*. Dinoflagellates are often called as fire plants as they are bioluminescent and emit light upon disturbance. Some members of dinoflagellates are known for forming toxic algal blooms and discoloration of waters (Red tides). Dinoflagellates are often identified based on the shape of their cell (round, oval or elongated), flagella arrangement, ornamentation of their cellulose armor called thecal plates and chloroplasts, cell extensions (horns, spines) etc.

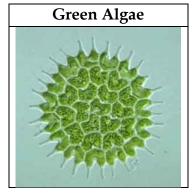
Common dinoflagellate representatives are *Gymnodinium*, *Prorocentrum*, *Dinophysis*, *Ceratium*, *Gonyaulaux* etc.



### 3. Green Algae (Chlorophyceae)

Green algae, encompass a wide range of unicellular, colonial, and multicellular algae. They are distinguished by their green pigmentation, due to presence of photosynthetic pigments such as chlorophyll *a* and *b*, as well as various accessory pigments carotenoids and xanthophylls. Their cell walls are primarily composed of cellulose. Green algae display diverse forms, from simple unicellular species to complex multicellular filamentous or parenchymatous forms. They typically inhabit

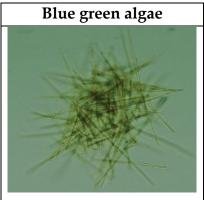
freshwater environments, although some species are found in marine habitats. Cell wall structure, color and shape of cell and chloroplasts aid in the identification of green algae. Common green algae found in our waters are *Spirogyra, Cladophora, Agmenellum, Pediastrum* etc



### 4. Cyanobacteria (Cyanophyceae or Blue-green algae)

Cyanobacteria, often referred to as blue-green algae, are a group of prokaryotic microorganisms commonly found in diverse habitats, including freshwater, marine, and terrestrial environments. Despite their name, they are not true algae but rather belong to the domain Bacteria. Cyanobacteria are characterized by their blue-green pigmentation, primarily due to the pigment phycocyanin and chlorophyll *a*. They possess cell walls made of peptidoglycan, similar to other bacteria. Cyanobacteria can be unicellular, colonial, or filamentous in structure, and they often form blooms in nutrient-rich waters. Some cyanobacteria are capable of fixing atmospheric nitrogen, and thus have crucial role in nitrogen cycling. Certain species are known to produce toxins posing risks to aquatic ecosystems and human health.

Eg. Anabaena, Microcystis, Nostoc, Trichodesmium, Lyngbya etc

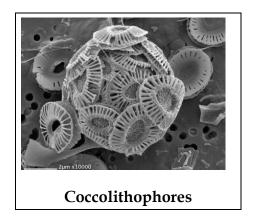


### 5. Coccolithophores (Prymnesiophyceae)

Coccolithophores are a unique group of marine phytoplankton belonging to the division Haptophyta. They are characterized by their distinctive calcium carbonate plates called coccoliths, surrounding their cells. These tiny, unicellular organisms are typically spherical in shape and range in size from few to several micrometers in diameter. Coccolithophores are photosynthetic, containing chlorophyll a and c2 as well as other pigments like fucoxanthin and diadinoxanthin. They play a significant

role in the global carbon cycle, as their calcification process removes carbon dioxide from the ocean and contributes to the formation of carbonate sediments on the seafloor. Coccolithophores are abundant in nutrient-rich surface waters, particularly in temperate and subtropical regions, where they form blooms visible from space. Despite their small size, coccolithophores have a profound impact on marine ecosystems and biogeochemical processes, influencing the climate system through their role in carbon sequestration and albedo feedback mechanisms. They are often identified based on the intricate morphology of coccoliths as wells as their cell structure.

Eg: Emiliania, Coccolithus, Calcidiscus etc.



### **Phytoplankton sampling**

Phytoplankton sampling methodology is a crucial aspect of marine research, and various methods are employed to ensure accurate quantification and analysis of phytoplankton abundance and diversity. The choice of sampling method depends on factors such as the depth of water being sampled, the target size range of phytoplankton, and the specific objectives of the study. For surface water sampling in coastal waters, estuaries, or lagoons, the techniques commonly used include,

### 1. Water Samplers

Water samplers like Niskin bottles, Nansen bottles, and Van Dorn samplers are frequently utilized to collect phytoplankton samples. These samplers allow the collection of subsurface samples at various depths. They are typically made of flexible or rigid plastic tubes. They are immersed in water in open conditions and are closed at the top at specific depths via a messenger. The capacity of these samplers usually ranges from 5 to 20 liters. In oceanic regions, large-sized Niskin bottles are often used in conjunction with CTD (Conductivity, Temperature, and Depth) probes. The CTD probe provides data on the physical properties of seawater, such as temperature and salinity, while the Niskin bottles collect water samples at discrete depths simultaneously.

### 2. Towing Plankton Nets

Surface phytoplankton samples can also be collected by towing nets with mesh sizes ranging from 5 to 20  $\mu$ m. Towing nets offer the advantage of filtering

larger volumes of water at a time, allowing the concentration of phytoplankton, including larger and rarer cells. Once the water samples are collected, phytoplankton are typically concentrated from the samples through settling or sieving using mesh sizes ranging from 20 to 200  $\mu$ m, depending on the target size range of the organisms being studied.

### 3. Plankton pumps

Plankton pumps are an efficient method for collecting phytoplankton samples in shallow estuaries and coastal waters. These pumps facilitate continuous pumping and filtration of water, allowing for the integration of plankton samples from the surface down to a specified depth. The basic design of a plankton pump involves an inlet pipe that is lowered to the desired depth and an outlet pipe covered with a mesh of appropriate size, which is submerged in a tank of known volume. As water is pumped through the system, phytoplankton and other particles are captured by the mesh, allowing for the collection of a representative sample. A meter integrated into the pump records the volume of water filtered during the sampling process. While plankton pumps enable the filtration of large volumes of water, there are considerations regarding potential damage to phytoplankton cells due to frictional resistance within the pump mechanism. Care must be taken to minimize any adverse effects on the integrity of the collected samples.

### Niskin sampler

### **Plankton pump**

### **Plankton nets**





Image courtesy: serc.si.edu



### **Fixing and Preservation**

Phytoplankton samples collected are immediately preserved in acidified Lugol's iodine solution added in the ratio of 1: 100 volumes of water. Lugol's iodine solution helps in the discharging gas enclosed in vacuoles of cells and also kills, stains and weighs the algae. 4-5% formaldehyde solution is also added as a phytoplankton

preservative. Phytoplankton samples should be stored in iodine-proof glass bottles appropriately labeled with the date, time and area of collection. Samples should be stored in dark and cool conditions and cells should be counted as soon as possible as long-term storage leads to distortion and damage of cells. After fixation, phytoplankton are concentrated for enumeration by the settling and sedimentation process. In this method, the fixed phytoplankton samples are settled in Utermöhl sedimentation chambers of 5-100 ml capacity for 24 hours after which the cells concentrated at the settling plate of the Utermöhl chamber is separated and examined under an inverted microscope.

### **Enumeration and Estimation**

Identification and enumeration of the phytoplankton samples are carried out either using a standard compound microscope or an inverted microscope of suitable magnification. The preferred magnification for observing picoplankton is 1000x; for nanoplankton is 200 – 630x and for microplankton is 100-200x. After concentrating the phytoplankton cells through the settling and sedimentation procedure, the cells are enumerated under microscope following the Sedgwick rafter cell counting method. In this method, 1 ml of concentrated phytoplankton sample is poured into the Sedgewick rafter counting chamber having 1 ml sample holding capacity using a micropipette. Later a cover glass is placed over the chamber and is kept idle for 5 minutes to allow the phytoplankton cells to get settled. Once the cells get settled at the base of the counting chamber it is enumerated under the microscope with suitable magnification.

Phytoplankton species in 1 litre of the sample is estimated by,

where, N = Number of planktonic algae per litre of water filtered, n = Average number of planktonic algae in 1ml, v = Volume of plankton concentrate in ml, and V = Total volume of water filtered in litre

### Phytoplankton biomass estimation

Phytoplankton biomass estimation usually involves the measurement of chlorophyll *a*, the universal photosynthetic pigment in autotrophic organisms. As estimation of photosynthetic pigments provides a quantitative measure of taxonomy, biomass and physiological state of the phytoplankton it is often taken as the proxy of phytoplankton biomass in the aquatic ecosystems Chlorophyll *a* estimation involves the following steps,

- Filtration of water samples For chlorophyll *a* estimation, about 500ml 2litre of water samples (depending on the area sampled) are filtered using a 47 mm Whatman GF/F filter paper of pore size 0.7μm using a suction pump.
- 2. Extraction of pigments After filtration, the filter papers are immersed in 10 ml of 90% acetone and kept overnight at 4°C under dark for the pigment extraction.

- 3. **Concentration of pigments** –The extracted pigment is concentrated by centrifugation at 3000 rpm for 10 minutes.
- 4. **Estimation** The extracted pigment is estimated either using spectrophotometer, fluorometer or HPLC.

In Spectrophotometer, the concentration of chlorophyll corresponds to the absorbance of light by the sample. In spectrophotometric estimation of chlorophyll-*a*, after the centrifugation process, the clear supernatant obtained is transferred to a 10 mm path length cuvette and extinction at 750, 665, 645 and 630 nm is measured against a blank solution of 90% acetone. The amount of pigment in the sample is calculated following Strickland and Parsons (1972) as,

C (Chlorophyll *a*) = 11.6  $E_{665}$  -1.31 $E_{645}$  -0.14 $E_{630}$ C (Chlorophyll *b*) = 20.7  $E_{645}$  - 4.34 $E_{665}$  - 4.42 $E_{630}$ C (Chlorophyll *c*) = 55  $E_{630}$  - 4.64 $E_{665}$  - 16.3  $E_{645}$ 

Where, **E** = Absorbance at different wave length obtained and corrected by the 750nm reading

The Concentration of pigments in the sample,

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mg/pigment/m^3 = C * 10/V
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Where, **C** is the value obtained from the Strickland and Parsons equation and **V** is the volume of water filtered.

In fluorometer, concentration of chlorophyll corresponds to the emission of light by the sample. Chlorophyll *a* absorbs the blue light and fluoresce in the red region of the electromagnetic spectrum. In HPLC phytoplankton biomass estimated through the quantification of specific marker pigments of each taxa.

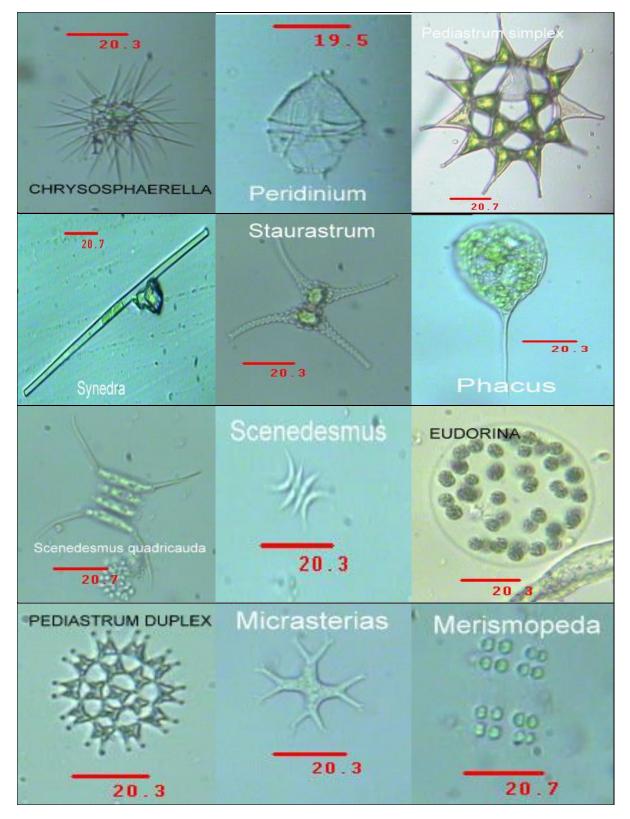
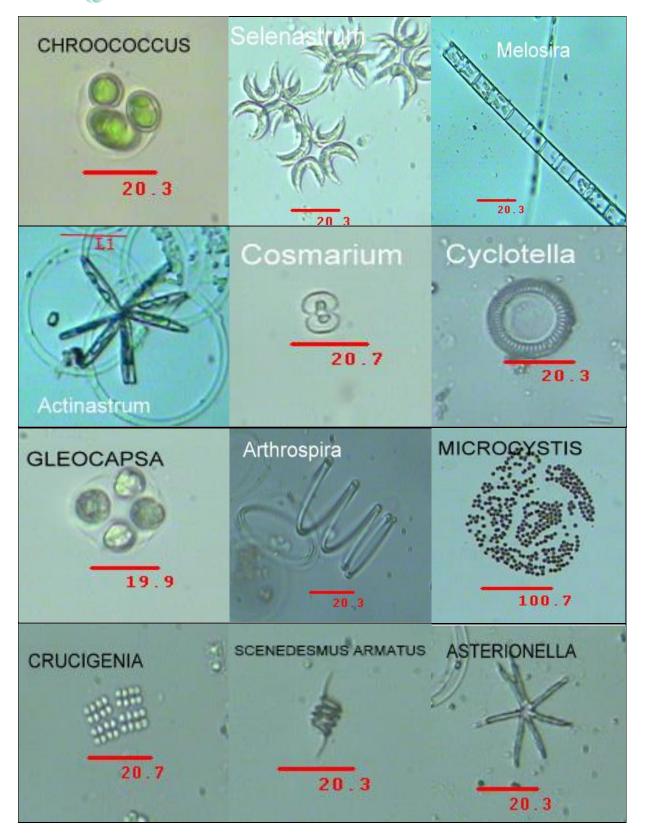
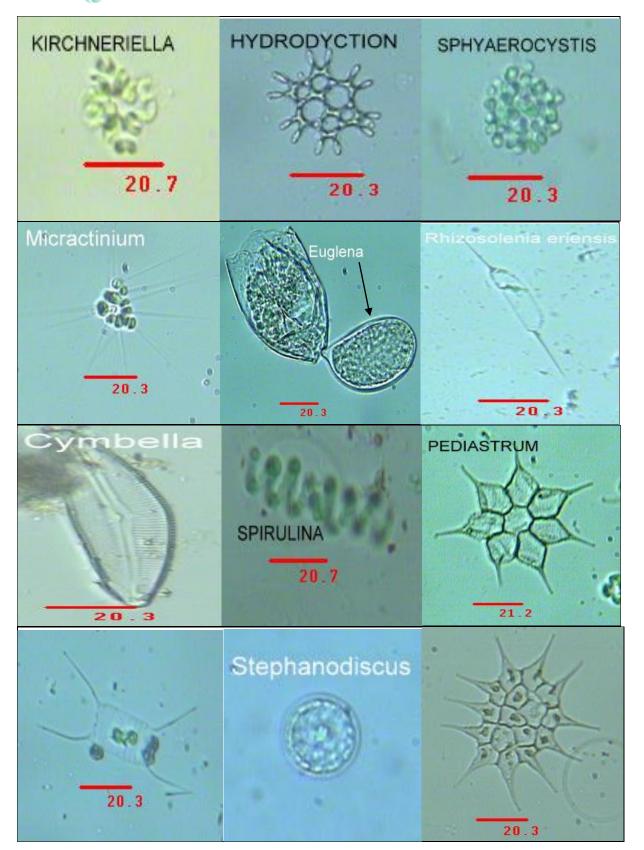
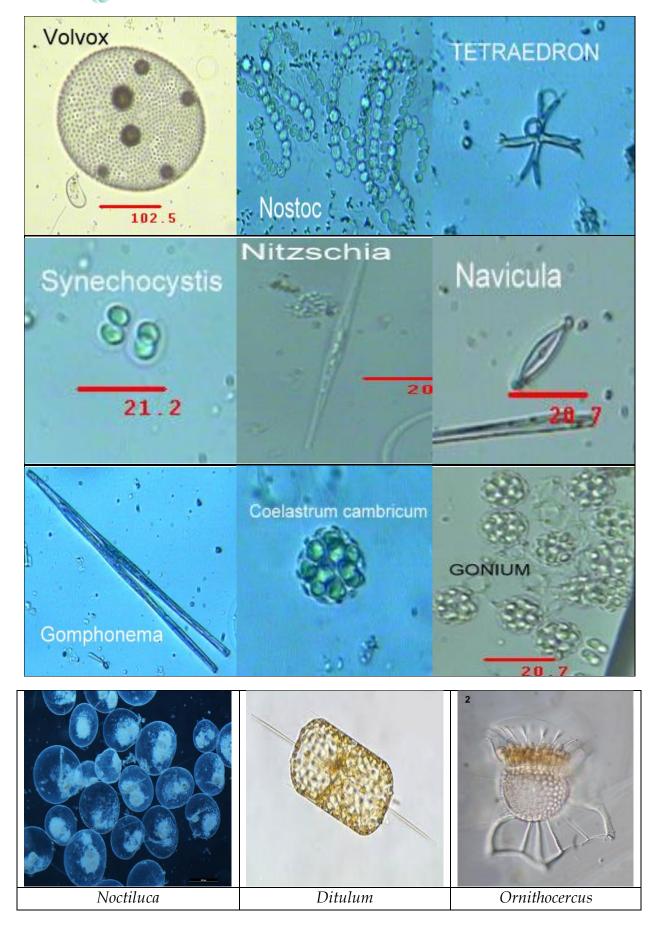


Fig. Common phytoplankters seen in aquatic systems (including fresh water and marine)











*Ceratium* sp.

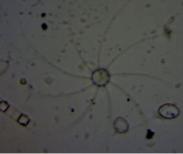


Planktoniella sol



Skeletonema costatum





*Bacteriastrum* sp.



*Navicula* sp.

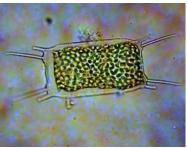




Protoperidinium sp.



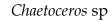
Pleurosigma sp.



Biddulphia sp.



R Rhizosolenia sp.



Coscinodiscus sp.



### Mesozooplankton

Mesozooplankton comprises of a taxonomically diverse array of heterotrophic organisms spanning size range of 0.2 -20 mm. They form a vital intermediary link in the pelagic food web transferring carbon produced by primary producers (Phytoplankton) to higher trophic levels exploitable by man. Their wide distribution patterns, diversity and their crucial role in the trophodynamics of the aquatic ecosystem make them the chief index of the utilization of aquatic biotopes at the secondary level. Moreover, their significance in the nutrient regeneration and biogeochemical cycling of aquatic ecosystems further makes planktonology an inevitable part of ecological monitoring programmes.

### Classification of mesozooplankton based on duration of planktic stage

**1.** *Holoplankton* are those organisms that spend their entire life drifting in the water column. They include representatives of nearly every phylum of the animal kingdom.

Eg: Copepoda, Ostracoda, Appendicularia

**2.** *Meroplankton* - Almost all aquatic organisms, be they are nekton or benthos are known to have a planktic stage in their life cycle and based on the duration they spend as plankton in the pelagic realm before getting transformed either as nekton or benthos are categorized as meroplankton.

Eg: Trochophore and veliger larvae of benthic worms and molluscs; nauplii and zoea of bottom and surface living crustaceans and also eggs and larvae of fin fishes.

### Classification of mesozooplankton based on body composition

Zooplankton, based on their external body composition are categorised as

- 1. *Crustacean zooplankton,* are mainly the arthropodan representatives armed with chitinous exoskeleton. Predominant crustacean representatives include taxa like Copepoda, Ostracoda, Mysidacea, Amphipoda and Isopoda.
- 2. *Gelatinous zooplankton*, are mesozooplankton having more than 90% of water content in their body. Common gelatinous zooplankton representatives are Hydrozoa, Ctenophora and the appendicularian representatives like Salps and Doliolids.
- 3. Gastropod zooplankton

Pteropod and Heteropod molluscs often constitute the major *gastropod* members among the mesozooplankton community.

### **Collection and Preservation of mesozooplankton**

In shallow estuaries and coastal waters, mesozooplankton are collected by towing a Working Party 2 (WP-2) net, of mesh size ranging from 70 to 200  $\mu$ m and mouth area 0.28 m<sup>2</sup>. The net is towed horizontally just below the surface (speed ~1 knot) for 10

minutes. A digital flow meter (Hydro Bios, Model- 438110) attached across the mouth of the net estimates the volume of water filtered through the net. For obtaining vertically stratified plankton samples from oceanic regions, a Multiple Plankton Net comprising of an assemblage of five nets are often used. Each net is used to collect samples of a particular depth zone and are controlled by ship board electronic sensors. The zooplankton samples collected are preserved immediately in 4% formaldehyde solution.



Working party net

### Biomass (ml. m<sup>-3</sup>) and abundance (ind. m<sup>-3</sup>) estimation

Biomass of zooplankton is generally measured following the displacement volume method, after removing the large detrital particles and gelatinous organisms and is expressed as m1. m<sup>-3</sup>. Later, the mixed zooplankton samples are manually sorted in the laboratory into their respective taxa. The abundance of each taxa can be estimated by counting all the individuals present in the sample or in aliquots depending on the biovolume. Samples having a displacement volume greater than 5 ml biovolume can be subdivided into aliquots of adequate measure, either using a Folsom splitter or a Stempel pipette.



Fig: Folsom Splitter



Bogorov's counting chamber

Image courtesy: Hydrobios

### Enumeration of mesozooplankton

The segregated zooplankton taxa from the sample are enumerated using a Bogorov's counting chamber under a stereo zoom microscope.

The abundance of each taxonomic group can be calculated as,

Abundance(ind.  $m^{-3}$ ) =  $\frac{\text{Number of organisms}}{\text{Volume of water filtered}(m^3)}$ 

The relative contribution of particular taxon towards the total zooplankton population can be calculated as,

# $Relative abundance = \frac{Number of individuals of a particular taxon}{Total number of organisms} \times 100$

### Taxonomic Identification of mesozooplankton

Zooplankton, collected by employing various methods such as vertical and horizontal towing of plankton nets of different mesh sizes, light traps to attract migrating organisms, plankton pumps for continuous water filtration, are preserved onboard using 4% formalin. Once collected, mesozooplankton samples undergo meticulous identification procedures. After biomass estimation, samples are sorted under a dissecting microscope to separate them into various taxonomic groups based on their morphology and size. Subsequently, detailed morphological analysis is conducted using compound microscopes, often aided by identification keys and atlases. Molecular techniques such as DNA barcoding and sequencing provide additional accuracy, particularly for cryptic or morphologically similar species. Staining methods highlight specific structures, while digital imaging systems and software assist in rapid identification and enumeration. Through these integrated approaches, researchers can accurately assess zooplankton species composition, abundance, and distribution, elucidating their vital role in aquatic ecosystems.

### Major mesozooplankton groups

### Phylum Cnidaria

- Class Scyphozoa
- Gelatinous organism
  - Umbrella shaped body



### Phylum Ctenophora

- ✓ "Comb Jellies"
- ✓ Eight comb plates



### Phylum: Annelida

- Class: Polychaeta
- Order: Tomopteridae
- Holoplanktonic



### Phylum Arthropoda

- Order: Cladocera
- "Water fleas"
- 0.2-0.6mm
- Mostly freshwater or neritic

### Phylum Cnidaria

- Class: Hydrozoa
- Order: Siphonophorae
- Bell shaped body



### Phylum: Chaetognatha

- "Arrow worms"
  - Carnivorous organism
  - Torpedo shaped body



### Phylum Arthropoda

- Sub phylum: Crustacea
- Subclass: Copepoda
- "Insects of the sea"
- Dominant zooplankton
- Largest biomass on earth



### Phylum Arthropoda

- Order: Mysida
- Brood pouch in females
- Statocyst in uropods
- Pleopods absent or reduced



### Phylum Arthropoda

- Order: Euphausiacea
- "Krill"
- Exposed gills
- Large brown eyes



### Phylum Arthropoda

- Order: Amphipoda
- "Different footed
- Scavengers & detritivores
- Hyperiidae planktonic



### Phylum Arthropoda

- Class: Ostracoda
- 0.2 -30 mm in size
- Bioluminescent





### Phylum Arthropoda

- Family: Luciferidae
- "Light bearer"
- Long rostrum
- Estuarine or Neritic



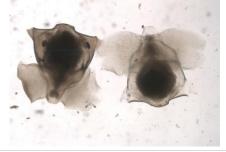
### Phylum Arthropoda

- Order: Isopoda
- "Similar footed"
- Dorsoventrally flattened



### Phylum Mollusca

- Pteropoda
- Thecosomata –Sea butterflies
- Holoplanktonic gastropods
- Body enclosed in calcified shell



### Phylum Mollusca

- Super family: Pterotracheoidea
- Heteropods / Sea elephants
- Transparent body without shell



### Phylum Chordata

- Class Appendicularia
- Order: Copelata
- Gelatinous holoplankton
- Solitary tunicates
- Lives inside house/test of protein
- Shape resembles tad pole larvae



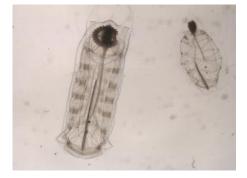
### Phylum Chordata

- Subphylum: Tunicata
- Class: Thaliacea
- Order: Doliolidae
- Holoplanktonic
- Gelationous
- Barrel shaped



### Phylum Chordata

- Subphylum: Tunicata
- Class: Thaliacea
- Order: Salpidae



### Meroplanktonic Crustaceans

Zoea

Megalopa

Mysis





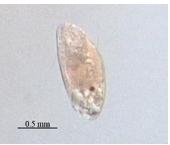


## **Alima** Stomatopoda larvae



**Phyllosoma** Lobster larvae **Cypris** Barnacle larvae





Pic courtesy: wikipedia

Meroplanktonic molluscans

**Bivalve larvae** 

## Gastropoda larvae

Cephalopod larvae





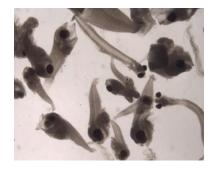
Meroplanktonic chordates



Fish eggs

Fish larvae





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