Planktonic diversity in Indian waters

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Phytoplankton is microscopic, plant-like organisms that drift in the water. These single-celled photo synthesizers can either swim using their flagella or be carried by the currents. They are an essential part of aquatic food chains and are found in almost all bodies of water. German biologist Victor Hensen first used the term "plankton" in 1887 to describe these microorganisms.

Phytoplankton thrives in the open surface waters of lakes, rivers, and oceans, constituting a diverse group of organisms that include both prokaryotic and eukaryotic species. These unicellular algae play a pivotal role in the primary productivity of aquatic ecosystems through their photosynthetic processes, which form the foundation of the food web, supporting zooplankton, fish, and various other aquatic life forms.

According to Field et al. (1998), marine phytoplankton contribute approximately half of the planet's net primary production. Furthermore, phytoplankton are instrumental in carbon dioxide sequestration and oxygen production. Geider et al. (1997) reported that in marine environments, phytoplankton biomass accounts for nearly 48% of the total carbon fixed on Earth's surface.
The marine environment regulates the phytoplankton population through various elements such as the presence of nutrients, the level of thermal separation, and the impact of zooplankton feeding.

Methods of Phytoplankton Collection:
In order to ensure accurate and comprehensive data collection, it is important to gather samples from multiple locations or stations, rather than restricting the sampling to just one site or station. This will help to avoid an uneven distribution of phytoplankton community structure. By collecting samples from a variety of locations, a sufficient amount of data can be obtained for the analysis of the phytoplankton community structure.
Samples of phytoplankton were gathered using a variety of tools, including bottle samplers, plankton nets, and plankton pumps. Bottle sampling is a straightforward approach for obtaining water samples from a specific depth. It is suggested for the quantitative analysis of phytoplankton structures. To collect surface water samples, a measured volume of water can be collected using a bottle or bucket. However, for subsurface sampling, specialized water samplers such as Meyer's, Niskin, and Friedinger's water samplers can be used.
Phytoplankton sampling is commonly carried out using nets of varying designs, which are known as plankton nets. These nets enable size-selective quantitative sampling by adjusting the towing speed. They can be operated in different ways depending on the sampling requirements. Horizontal towing is effective for collecting surface phytoplankton samples, while vertical towing provides composite samples of the water column. By measuring the diameter of the net mouth opening and the distance traveled through a flow meter, the volume of water sampled can be estimated (Eaton et al. 2003).
Plankton pumps continuously draw water and plankton into the vessel, where it can be filtered through a net for sample collection. Additionally, submersible pumps can be used to collect subsurface phytoplankton samples from a specified depth. Furthermore, plankton pumps enable the collection of integrated phytoplankton samples from the surface to a desired depth.

Phytoplankton fixation and preservation
The samples were preserved using various fixatives such as formalin, Lugol's Iodine, osmic acid, and gluconaldehyde. Among these, formalin is the most commonly used fixative and preservative for phytoplankton.
Formalin is primarily utilized for the preservation and fixation of phytoplankton samples. Commercial formaldehyde is typically diluted to a concentration of 2-5% for this purpose. Marine phytoplankton samples are typically preserved using a 5% formalin solution. An acidified formaldehyde solution made with 20% formaldehyde and 50% acetic acid (in a 1:1 ratio) is also an effective phytoplankton preservative, with the exception of dinoflagellates. Lugol's iodine solution, which consists of 100g of potassium iodide, 50g of crystal iodine, and 100ml of glacial acetic acid in 1 liter of distilled water, is a popular choice for preserving small-sized

phytoplankton. This solution, however, is not suitable for preserving coccolithophorids. Iodine fixes and preserves the color of phytoplankton, while acetic acid helps to preserve flagella and cilia.

For preserving a phytoplankton sample, three to six drops of osmic acid can be utilized. This solution comprises 200 mg of osmium tetroxide dissolved in 10 ml of distilled water and can effectively preserve a 100 ml phytoplankton sample.

Alternatively, a preservation solution consisting of 8 g of glutaraldehyde mixed with 100 ml of distilled water in a 1:1 ratio can also be employed for the same purpose.

Analysis of phytoplankton:

Aquatic ecosystems' primary productivity is typically determined using quantitative analysis of phytoplankton biomass, which also provides an estimate of the organic material available for zooplankton consumption. There are two methods for estimating phytoplankton biomass: total biomass estimation and species or group biomass estimation. Chlorophyll a estimation and direct cell counting are the preferred methods for total biomass estimation, while indirect estimates are based on cellular counts and biovolumes. These methods allow for the assessment of diversity and productivity in the area.
The direct count method involves counting individual phytoplankton cells and recording the number per cubic meter of water using the Sedgwick Rafter. This process involves diluting phytoplankton samples (1 ml) and spreading them uniformly to be counted under a microscope. To determine the total number of phytoplankton cells, multiple replicates of the samples are counted. The formula $N = nv/V$ is then applied, where:
- N represents the total number of phytoplankton cells per liter of water,
- n is the average number of phytoplankton cells in 1 ml of the sample,
- v denotes the volume of phytoplankton concentrate (in ml), and
- V represents the total volume of filtered water.
The chlorophyll *a* estimates were obtained using a spectrophotometric method.

Phytoplankton classification
Fritsch (1935) categorized the algal kingdom into 11 classes, distinguishing them based on morphological characteristics.

1. Chlorophyceae
2. Xanthophyceae
3. Chrysophyceae
4. Bacillariophyceae
5. Cryptophyceae
6. Dinophyceae
7. Chloromonadineae
8. Euglenineae
9. Phaeophyceae
10. Rhodophyceae
11. Myxophyceae

In 1950, Smith grouped algae into seven classifications. Prescott (1984) divided the algal kingdom into seven phyla, including classes and orders. Bold and Wynne (1985) categorized the algal kingdom into nine divisions by introducing the new division Charophyta, which is the most widely recognized classification system among the classical algal taxonomy. Lee (1980) introduced groundbreaking changes in algal classification based on cell structures, such as prokaryotes and eukaryotes, and chloroplast ultrastructure, and this is widely accepted. Lee (1980, 1989, 1999) also proposed a new division, Prochlorophyta, with prokaryotic members containing Chl b.
Lee's modified algal classification from 1989, 1999, and 2008 identified four separate categories based on the development of algal chloroplasts and flagellar structure.

Prokaryotic algae comprise cyanobacteria, while eukaryotic algae can be divided into four groups based on their chloroplast structure. The first group includes Glaucophyta, Rhodophyta, and Chlorophyta, which have chloroplasts with two membranes. The second group consists of Euglenophyta, Dinophyta, and Apicomplexa, which possess one chloroplast ER with a total of three membranes. The third group comprises Cyptophyta, Heterokontophyta, and Prymnesiophyta, which have two chloroplast ERs with a total of four membranes.
Phytoplankton exhibit a wide range of sizes, and Schütt (1892) first classified phytoplankton based on size variations.

Megaplankton	> 20 mm
Macroplankton	2 – 20 mm
Mesoplankton	0.2 – 2 mm
Microplankton	20 – 200 μm
Nannoplankton	2 – 20 μm
Picoplankton	0.2 – 2 μm
Femtoplankton	<0.2 μm

Plankton are broadly classified into two main categories based on their protein content: phytoplankton, which comprise plant-like

organisms, and zooplankton, which consist of animal-like organisms. Phytoplankton encompasses a variety of organisms, including diatoms, dinoflagellates, blue-green algae (cyanophytes), coccolithophores, and green algae.

Bacillariophyceae
Diatoms stand out as the most diverse and abundant single-celled photosynthetic eukaryotes present in virtually all aquatic environments. They represent a significant component of both marine and freshwater floating algae. Diatoms play a crucial role in aquatic biomass within nutrient-rich coastal ecosystems and serve as sensitive indicators of environmental conditions. Consequently, they hold immense ecological significance. Studies by Falkowski et al. (1998), Field et al. (1998), Mann (1999), and Malviya et al. (2016) estimate that diatoms contribute 40-45% of oceanic primary productivity, accounting for roughly 20% of global carbon fixation and oxygen production.

Diatoms are categorized into two main structural types based on their symmetry and organization: centric diatoms and pennate diatoms. Centric diatoms have radial symmetry and are characterized by their valves having a concentric or radiating pattern around a central or lateral point or points. The raphe or pseudoraphe is absent in these diatoms. These cells are usually circular, oval, or elliptical in shape, but may occasionally be polygonal or crescent-shaped. Centrales are more commonly found in open seas and are represented by three suborders: Discoideae, Solenoideae, and Biddulphioideae, which include nine families, 14 subfamilies, and 35 genera. On the other hand, pennate diatoms have bilateral symmetry and are distinguished by their valves having a symmetrical arrangement on both sides of a longitudinal axis. These diatoms have a raphe and are typically elongated and slender in shape. Pennate diatoms are more commonly found in freshwater environments and are classified into several orders based on their shape and other characteristics.

Pennales, also referred to as Pennate Diatoms, are characterized by elongated and symmetrical valves. Their outlines may vary, appearing boat-shaped, oval, cuneate, crescent-shaped, or sigmoid, with markings typically arranged in either pinnate or transverse patterns. These diatoms always feature a true raphe or hyaline median line (pseudoraphe), while processes are absent. In cases where a true raphe is present, the cells are capable of spontaneous movement. Pennales are predominantly found in coastal waters and are classified into three suborders: Araphidineae, Monoraphideae, and Biraphideae, encompassing five families, 10 subfamilies, and 28 genera.

Dinoflagellates (Dinophyceae)
Dinophytes, a type of unicellular, motile algae, are distinguished by their characteristic two-part structure (two halves) and are composed of a multitude of intricately sculptured plates. Some members of this class possess a cellulose envelope and branched filaments. Motile cells of dinophytes have two flagella that they use for locomotion, one longitudinal and one transverse. Dinophytes are also capable of luminescence due to the presence of numerous discoid chromatophores. These organisms often form large, toxic blooms and are more commonly found in seawater than in freshwater.

Chlorophyceae (Green Algae)
Green algae are typically characterized by their unicellular, microscopic, filamentous, or colonial structures. These algae belong to the class Chlorophyta, which is distinguished by the presence of green chromatophores and the same pigment composition (chlorophyll a and b) found in higher plants. Green algae primarily inhabit freshwater environments, although they can also be found in coastal marine waters. They are widely distributed in tropical and subtropical seas and are represented by 15 orders and 22 families.

Cyanophyceae (Blue-green algae)
Members of the Cyanophyceae family, commonly known as blue-green algae, are typically referred to as cyanobacteria. These organisms can be either unicellular or multicellular, and they lack true nuclei or chromatophores. Their cells are smaller in size, blue-green in color, and lack membrane-bound cell organelles. In contrast to bacteria, cyanobacteria possess the ability to perform photosynthesis through chlorophyll a (some also have chlorophyll b or d). There are five orders of cyanobacteria: Chroccocales, Chaemosiphonales, Pleurocapsales, Nostocales, and Stigonematales. Picophytoplanktonic cyanobacteria are more abundant in nutrient-poor offshore waters and are found in almost all seas. Larger cyanobacteria often make up a significant proportion of oceanic phytoplankton.

Prymnesiophyceae (Cocolithophores):The Prymnesiophyceae are a group of single-nucleus flagellates that have a haptonema situated between two smooth flagella. These primarily marine organisms also have freshwater representatives, and their size ranges between 5 and 20 µm. The cells are often covered with scales, with many of these scales being calcified, resulting in the production of coccoliths.

The most prevalent genera of phytoplankton in Indian waters are *Hemidiscus, Coscinodiscus, Triceratium, Biddulphia, Bacteriastrum, Skeletonema, Climacosphenia, Rhizosolenia, Fragilaria, Pleurosigma, Pinnularia, Navicula, Nitzschia, Bacillaria* (bacillariophyceae)*; Ceratium, Noctiluca, Peridinium, Dinophysis* (Dinophyceae)*; Trichodesmium* (Cyanophyceae)*; Globigerina* (Polythalamea) *and Tintinnopsis* (Spirotrichea).

Zooplankton

Zooplankton are aquatic organisms that lack the ability to maintain a stable position against the flow of water. They are composed of a variety of animal taxa, primarily invertebrates, and serve as an essential trophic level in the ecosystem. Zooplankton help to transfer energy from primary producers to secondary consumers, and they play a crucial role in the food chain, making them a critical factor in determining the productivity of marine ecosystems as secondary producers. (Nair, 2001). The use of zooplankton communities is commonly employed to evaluate the productivity and overall health of ecosystems. (Thirunavukkarasu et al., 2013), Some species can serve as potential indicators for various water masses and environmental changes, such as water quality parameters and climate change, although the use of these indicators may vary depending on the specific context. (Russell, 1939). Coastal and marine regions are characterized by significant variability, which arises from fluctuations in water circulation patterns and land-based factors, such as river and sewage flow, resulting in substantial temporal changes.(Walsh, 1988). The production of zooplankton in coastal and inshore waters is primarily determined by seasonal changes caused by monsoons and upwelling. (Nair, 2001). The abundance and composition of zooplankton populations are directly influenced by changes in water quality parameters.(Gaonkar et al., 2010).

Coastal regions are continuously endangered by the discharge of sewage and industrial waste from urban and industrialized areas, which damages their ecosystems. Moreover, shifts in environmental conditions can also impact the abundance and variety of zooplankton. (Calbet et al., 2001).

Methods of zooplankton collection

During the past years, zooplankton have been gathered using various equipment, including water bottles, pumps, and nets. Water bottles are commonly used for collecting smaller plankton forms, such as microzooplankton. To collect water samples, a 5-20 litre capacity sampler is typically employed at the sampling site. To gather surface water, a suitable-sized bottle may be filled by scooping water from the surface. The disturbance of the water should be minimized to prevent plankton from reacting negatively. Von Dorn bottles, named after Dr. William Van Dorn from the Scripps Institute of Oceanography, are commonly utilized for precise depth sampling. They allow for composite sampling from various depths or pooling samples from a single depth, facilitating both horizontal and vertical sampling. Horizontal bottles are frequently employed for sampling at specific levels like the thermocline or above the seabed. They collect whole water samples, ensuring representation across all plankton size classes. Zooplankton obtained in these bottles can be concentrated through settling, centrifugation, or fine filtration. While this method offers accurate sampling depths and ease of use, it filters less water and might miss larger plankton forms and rare species, making it less suitable for qualitative and quantitative assessments.

Pumps, typically used on boats or ships, involve submerging an inlet pipe in the water and connecting an outlet pipe to a net with suitable mesh size to filter zooplankton. This method allows for quantitative estimation and small-scale plankton distribution studies, but it has limitations on sampling depth and may damage larger plankton forms.

Plankton nets, available in various sizes and types, are the most widely used method for collecting zooplankton. They can be open nets for horizontal and oblique hauls or closed nets with messengers for vertical sampling at specific depths. Made of materials like bolting silk or nylon, these nets are conical and consist of a ring, a filtering cone, and a collecting bucket. Plankton nets enable both qualitative and quantitative studies, with mesh size influencing the types of zooplankton collected. Factors such as net type, length, towing speed, and time of collection affect sample quality and quantity.Zooplankton collections can be conducted through horizontal, oblique, or vertical hauls. Horizontal sampling entails

towing the net at a slow speed (1.0 to 2.0 knots) for 10 minutes. Oblique hauls involve towing the net above the seabed, risking inaccurate sampling depth measurement and potential net damage from substrate contact. Vertical hauls are used to sample the water column by lowering the plankton net to the desired depth and slowly retrieving it, capturing zooplankton as it passes through the net. This method is valuable for studying zooplankton abundance at various depths, especially as species often migrate vertically in response to light, though their presence in upper layers is typically low during the day. Effective zooplankton collection often occurs before dawn, after dusk, or at night, with the net submerged in water to start the process. Continuous net samplers and multiple net zooplankton samplers are utilized for continuous or simultaneous sampling. Continuous net samplers, like the Continuous Plankton Recorder by Hardy (1939) and Longhurst Hardy Continuous Plankton Recorder (1966), collect animals on a continuous strip of netting. In contrast, multiple net samplers open and close individual plankton nets in sequence, often employing 5–10 nets to collect zooplankton at various depths simultaneously.Furthermore, there are automated sampling systems and Recording Instrumental Environmental Sampling Systems, exemplified by the one devised by Dunn et al. in 1933a and 1993b, along with the high-speed Gulf-III OCEAN sampler crafted by Nellen and Hempel in 1969. Modern net systems incorporate sensors to measure water qualities and net properties, with flow meters installed to accurately estimate water volume filtered and plankton collected. Calibration involves towing the net with the flow meter attached for a known distance, recording the revolutions made, and calculating the volume of filtered water using the formula $\pi r^2 h$, where r is the mouth ring radius and h is the depth or distance.

Zooplankton-Preservation and Fixation

To uphold the quality of taxonomic studies, it's imperative to promptly fix and preserve specimens following collection. This precaution is essential to prevent degradation, which can arise from factors like bacterial activity, cannibalism, or chemical breakdown. Fixation entails terminating the organism's life while retaining its morphological features, while preservation involves maintaining this fixed state over an extended period. Formaldehyde serves as the most prevalent fixing and preserving agent, typically utilized at a concentration of 4-5%. Buffered formalin storage can effectively preserve zooplankton samples for numerous years. Commonly employed buffers include borax (sodium tetraborate) and hexamethylenetetramine, the latter being added at a concentration of 200 g per liter of concentrated formalin.

Estimation of zooplankton:

i) Quantitative estimation

Zooplankton biomass can be determined using any of the three following methods outlined by Goswami (2004): the volumetric method, which involves measuring displacement and settling volumes; the gravimetric method, which involves calculating wet and dry weights; and the chemical method. To ensure precision in results, it's essential to segregate larger zooplankton specimens like medusae, ctenophores, fish larvae, salps, and siphonophores from the sample and document their biomass individually. The combined biomass of these larger forms, along with the remaining zooplankton, constitutes the total biomass, which should be clearly noted on the analysis sheet.

The volumetric method for determining zooplankton volume employs the displacement volume method, expressed in milliliters per cubic meter. This method involves estimating the displacement volume using a volume determiner. The volume determiner is a transparent cylindrical plastic device with a capacity of 100 milliliters and open ends. One end is equipped with a piece of netting matching the mesh size of the plankton net, and it can be securely sealed over the base, which is made of plastic. The other end features a detachable plastic lid with a side hole and a metallic pointer that aligns with the 50-milliliter mark on the cylinder when fixed over it.

To utilize this method, preserved plankton is poured into the volume determiner, and water is filtered out by repeatedly placing the cylinder over blotting paper until all the water is removed. The cylinder, now containing the plankton, is sealed watertight over its base. Then, an appropriate quantity of 5% formaldehyde solution is slowly dispensed from a 50-milliliter burette without introducing air bubbles until the water level reaches the tip of the needle on the lid. The volume of solution remaining in the burette corresponds to the volume of plankton in the cylinder.

Another method involves using a net with a mesh size equal to or smaller than the one used for collecting plankton. The plankton is filtered through the netting material, and any interstitial water is removed using blotting paper. The plankton is then transferred to a

measuring cylinder containing a known volume of 4% buffered formalin. The difference in solution levels equates to the volume of the plankton.

Alternatively, the volume of plankton can be determined by allowing the sample to settle for at least 24 hours and noting the settled volume. The volume of plankton per cubic meter of water filtered can be estimated by calculating the quantity of water filtered by the net during sampling.

The wet weight and dry weight of the plankton can also be obtained using the Gravimetric method. The wet weight is obtained after filtering and removing interstitial water on a pre-weighed filter paper or aluminum foil, expressed in grams. The dry weight is determined by drying an aliquot of the zooplankton sample in a pre-dried and pre-weighed filter paper in an electric oven at a constant temperature of 60°C for 24 hours, expressed in milligrams. The weight of plankton is then expressed as grams per cubic meter or milligrams per cubic meter.The Chemical method is a technique that involves measuring the levels of elements like carbon, nitrogen, and phosphorus, as well as biochemical components like proteins, lipids, and carbohydrates.

ii) Qualitative estimation

In qualitative assessments, individuals within the sample are identified and counted. Due to the often large number of individuals in a sample, enumerating the entire sample is usually impractical. Instead, a subsample or aliquot of 10%–25% is typically used for enumeration. The percentage of aliquots chosen can vary depending on the zooplankton abundance in the sample.

To obtain the subsample, a Folsom plankton splitter is commonly employed, dividing the sample into two equal halves. This process is repeated until a suitable subsample size is achieved for counting. A Sedgwick Rafter Counting Cell is then utilized for counting, positioned under a stereoscopic microscope. The counts within the subsample are extrapolated to represent the total volume. These numbers are expressed per cubic meter of water, accounting for the volume of water filtered by the net during sampling.

Classification of zooplankton

Zooplankton are typically classified in a number of ways.Initially, they are categorized into two groups: holoplankton and meroplankton. Holoplankton consist of the species that reside exclusively in the pelagic zone throughout their lifetimes, such as copepods and chaetognaths. On the other hand, the meroplankton encompass organisms that only float in the ocean for a portion of their life cycle, such as the larvae of benthic mollusks and barnacles. Additionally, zooplankton are divided into two main types: protozoa and metazoans.Protozoa are a significant group because they multiply rapidly and are often the first organisms to consume algal blooms, such as diatoms. Metazooplankton, on the other hand, have a longer lifespan, ranging from a few days to several years, depending on the species. Thirdly, the zooplankton are classified based on their size. Finally, they are categorized as either neritic or oceanic. Neritic plankton live in inshore waters up to a depth of approximately 200 meters, while oceanic plankton inhabit the open ocean. Within the oceanic regime, they are further divided into epipelagic, mesopelagic, and bathypelagic zones, with the epipelagic and mesopelagic regions being the primary habitats for zooplankton.

Table 1. List of commonly available zooplankton groups/ species

Groups	Species Name	Groups	Species Name
Copepods	*Paracalanus aculeatus*	Sergestids/ Lucifers	*Lucifer hanseni*
	Canthocalanus pauper		*Lucifer penicillifer*
	Paracalanus parvus	Polychaetes	*Pelagobia spp*
	Acartia centrura	Ostracoda	*Cypridina dentata*
	Acartia spinicauda	Appendicularia	*Oikopleura dioica*
	Acartia spp	Salpida	*Thalia democratica*
	Acrocalanus similis	Doliolum	*Dolioletta gegenbauri*
	Acrocalanus spp.	Foraminifera	*Ammonia falsobeccarii*

	Tortanus barbatus		*Ammoniia tepida*
	Tortanus sp.	Amphipods	*Hyperia sp.*
	Centropages furcatus		*Gammarus sp.*
	C.orsinii	Cladocerans	*Penilia avirostris*
	Centropages tenuiremis		*Evadne tergestina*
	Temora turbinata	Tintinnids	*Favella sp.*
	Temora sp.		*Tintinniopsis sp.*
	Eucalanus crassus	Isopods	*Sphaeroma sp.*
	E. subcrassus	Brachyurans	*Crab zoea*
	Euchaeta marina	Cirripedia	*Cypris larvae*
	Euterpina spp	Lobster	*Phyllosoma larvae*
	Microsetella sp.	Molluscans	*Bivalve*
	Oithona sp.		*Gastropod*
	Oncaea sp.	Chordata	*Fish egg*
	Corycaeus sp.		*Fish larvae*
Hydrozoans	*Lensia subtilis*	Decapods	*Penaied larave*
	Lensia subteloides	Crustacea	*Zoea*
	Aequorea conica	Stomatopods	*Alima larave*
Chaetognaths	*Sagitta enflata*		
	Sagitta bedoti		

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