BIODIVERSITY OF CHILIKA THROUGH THE LENS OF FOLDSCOPE

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Abstract

Chilika is the largest brackish water lagoon located on the east coast of India in Odisha. The lagoon is a biodiversity hotspot and is one of the Ramsar sites of India. Many new species of flora and fauna have been documented from this lagoon. The lagoon also supports a rich diversity of seagrasses and so far six species of seagrasses have been reported from this lagoon. In this study, we aim to test the utility of foldscope as an on-site portable microscope to analyze the biodiversity of Chilka. We specifically targeted the seagrass meadows for the sampling of microalgae, zooplankton, polychaetes, and other benthic organisms. Our continuous long-term monitoring in combination with foldscopy showed that on board microscopic observation reduces the observational time lag between sample collection and observation. Samples were mounted on board without preservation and many live biological processes were captured and recorded using foldscope. Foldscope was proved to be an efficient cheap portable microscope for carrying out on-site assessment of biodiversity in Chilika Lagoon.

Keywords

Chilika, Foldscope, Seagrass, Epiphytic Community, Planktons, Benthos.

1. Introduction

Chilika, the largest brackish water coastal lagoon of Asia, lies on the east coast of India and lifeline of the state of Odisha, is a designated Wetland of International Importance (Ramsar Site under the Convention on Wetlands) since 1981 (Behera et al., 2018a) (Fig.1). Chilika is a welcoming wintering ground for millions of migratory avifauna from different countries. The highly productive lagoonal ecosystem supports India's most productive fisheries. The rich fishing grounds sustain the livelihood of more than 2,00,000 fisherfolk who live in and around Chilika. It is the hot spot of biodiversity and many rare, vulnerable, and endangered species listed in the IUCN Red List of threatened animals inhabit the lagoon for at least part of their life cycle. The presence of unique salinity (Behera et al., 2017a) gradient enables the lagoon to host a wide range of biodiversity which includes 317 species of fish, 224 species of water birds (including 97 inter-continental migrants) and 729 species of angiosperms with several of economic value. It is also one of the few lagoons in the world that support Irrawaddy Dolphin (*Orcaella brevirostris*). Its fringe areas and the islands are a habitat for 399 phytoplankton sp., 14 algae, 729 plants, 37 zooplanktons sp., 61 protozoa, 6 porifera, 7 coelenterata, 29 platyhelminthes, 36 nematoda, 31 annelids, 136 mollusca, 62 crustacea, 5 echinoderms, 1 protochordata, 314 fish, 7 amphibia, 30 reptilia, 225 birds, and 19 mammals.

Fig.1: Chilika Lagoon a designated Ramsar site located in the Odisha State.

Chilika lagoon also supports the luxuriant growth of seagrasses due to the shallow depth and brackish salinity regime. Seagrasses are flowering plants that grow in brackish and marine water. Seagrasses (Behera et.al, 2017b) require optimal temperature, sunlight, and saline condition to grow and expand. As per the latest ground survey carried out by Chilika Development Authority, Bhubaneswar in the year 2018, about 152.3 sq km area of Chilika lagoon is covered by seagrass meadows. Seagrasses in Chilika are mostly confined to shorelines and islands where water is shallow and sunlight penetration is high. In Chilika, seagrasses play a key role in maintaining the biological diversity and productivity. These submerged plants act as an effective ecological filter for the nutrient and pollutants and store carbon in their biomass and sediments. Seagrass meadows acts as foraging and feeding ground for many commercially important prawn and fish species and supports dense growth of epiphytic organisms on their leaves. The presence of benthic microscopic organisms in the seagrass meadows facilitates nutrient recycling and keeps the ecological health of lagoon in good condition. The epiphytic algae that grow over the seagrasses are grazed by a variety of invertebrate species. The seagrass meadows provide shelter, food, protection, spawning, and nursery grounds to finfish and shellfish and thus contribute significantly to the lagoon fishery. Thus, their protection and restoration are essential for sustainable management of biodiversity and ecosystem functions.

A Foldscope is an optical microscope that can be assembled from simple components, including a sheet of paper and a lens with the cost less than US$1(Fig.2). More details on the foldscope can be found at https://www.foldscope.com/. It is a part of the "frugal science" movement which aims to make cheap and easy tools available for scientific use in the developing world where resources are limited. It is an optical microscope that can be assembled from a punched sheet of cardstock, a spherical glass lens, a light emitting diode, and a diffuser panel, along with a watch battery that powers the LED. Once assembled, the Foldscope is about the size of a bookmark. The Foldscope weighs 8 grams and comes in a kit with multiple lenses that provide magnification from 140 X to 2,000 X. The kit also includes a magnetic coupler that can be attached to the Foldscope to allow coupling of smartphone with it for recording of live images. The main purpose of Foldscope is to make science easier and available to everyone and diagnosis of diseases quicker to the people from resources constrained settings.

Fig.2: Foldscope. A paper Microscope. Picture Credit: https://www.foldscope.com/

In October 2015, Department of Biotechnology, Government of India announced a program to make Foldscopes available across India in different colleges and programs keeping in mind that it may be used as a teaching tool for students in biology, chemistry, and physics. Wetland Research and Training Centre, a nodal laboratory of Chilika Development Authority which is located in Balugaon was one of the selected institutions which got a chance to explore and utilise the foldscope as a tool to understand the biodiversity of Chilika, seagrasses, and epiphytic microbial communities associated with seagrass meadows. The two main objectives of the research projects were (i) to capture microscopic organisms and their biological processes in the field using foldscopy and (ii) to sensitize the fishermen and the local community about the seagrass biodiversity of Chilika.

2. Materials and Methods

2.1 Study area and sampling

The water and sediment sampling was carried for microalgae, plankton and benthos from the seagrass meadows covering the central sector and southern sector of the Chilika lagoon (Fig. 3) which supports luxurious growth of seagrasses. The sampling was done for the 9 months period in between the June 2018- February 2019.

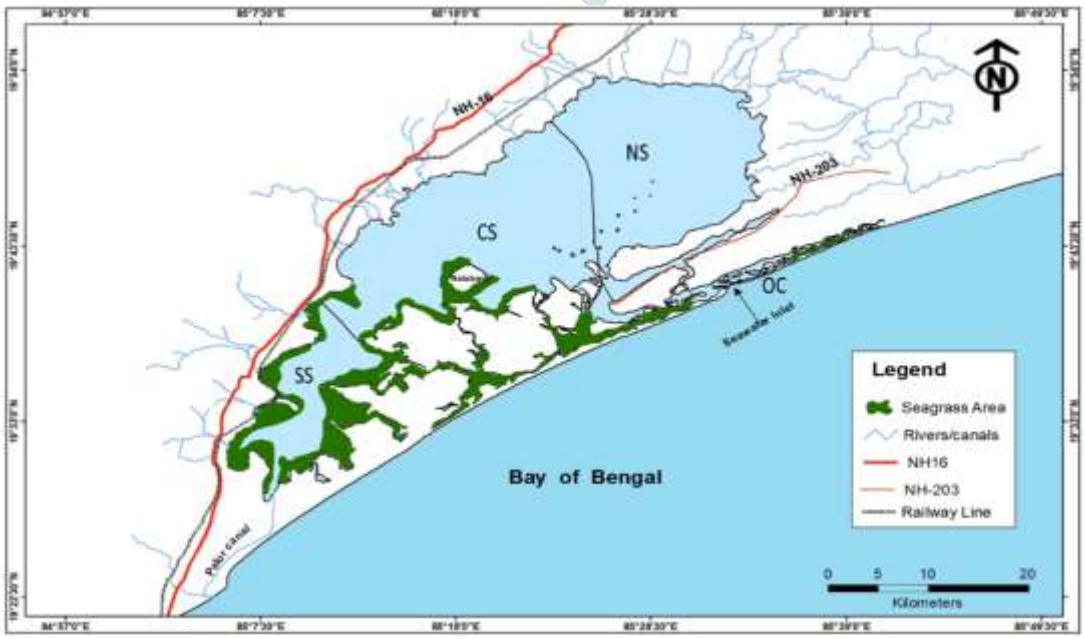


Fig.3: Map of Chilika showing the four different sectors primarily on the basis of salinity regime : Northern sector (NS), Central sector (CS), Southern sector (SS) and Outer Channel (OC). The green area represents seagrass distribution in Chilika.

2.2 Phytoplankton sampling

The phytoplankton sampling (Srichandan et.al., 2015) was done from the overlying water columns above the seagrass meadows (Fig. 4a). Onboard for collecting the phytoplankton, plankton net (mesh size 20 µm, mouth diameter 0.25 m, model KC Denmark) was used to filter ~100 L of lagoon water. Then, the phytoplankton samples were immediately mounted on foldscope using glass slides and cover slip.

2.3 Zooplankton sampling

Zooplankton samples were collected from seagrass beds through horizontal hauling using plankton net of 120 µm (model KC Denmark). The collected zooplankton samples were mounted on a glass slide to be observed under Foldscope (Fig.4b).

2.4 Benthic sampling

Seagrass meadow sediment samples were collected using a Van Veen grab sampler and sediments were sieved through 0.5 mm mesh size to collect the macro-benthic organisms. The macro-benthic organisms were identified up to their best possible taxonomic level with the help of foldscope (Fig.4c).

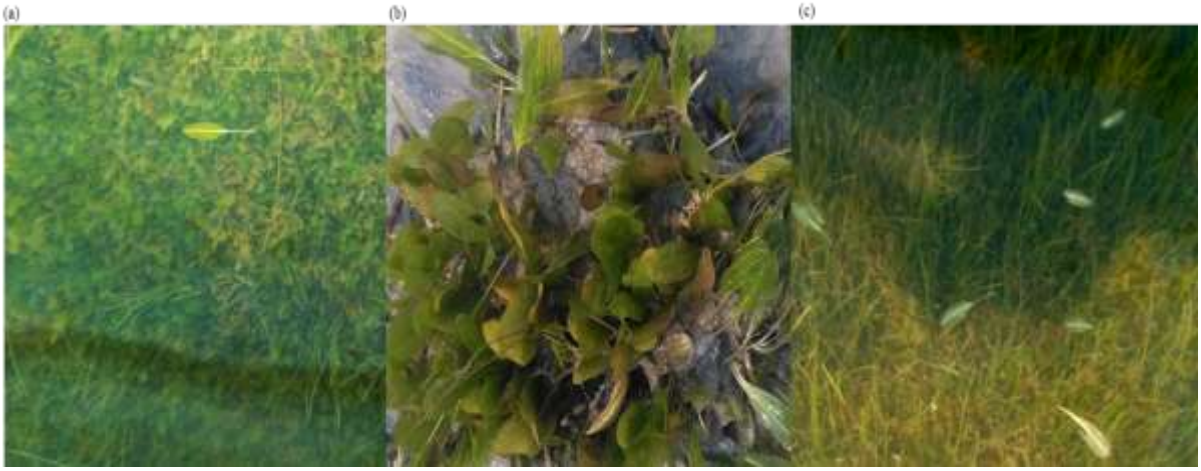
2.5 Macroalgae sampling

The epiphytic algae present over the seagrass leaves were dislodged and mounted on foldscope. The chloroplast arrangement and the colouration of algal filaments were studied under foldscope to identify the species (Fig.4d).

Fig.4: Sampling in Chilika. (a)Phytoplankton (b) Zooplankton (c) Benthos, and (d) Macroalgae.

3. Results and Discussion

Our ground survey on seagrass biodiversity documented five species; *Halophila ovalis*, *Halophila ovata*, *Halophila beccari*, *Halodule pinifolia* and *Halodule uninervis* from the Chilika Lagoon. The *H. ovalis* and *H. pinifolia* were the two most commonly occurring seagrasses in Chilika. (Fig.5). All pictures and video generated in this study are available at https://microcosmos.foldscope.com/?author=3049. Fig. 5: Seagrasses of Chilika. Two major genera of seagrasses found in Chilika. (a) *Halophila* sp. (b) *Halophila* sp. outside water and (c) *Halodule* sp.



3.1 Planktonic Larvae

Many benthic organisms are free swimming organism and their larval stages are planktonic in nature. While sampling for plankton, many planktonic larvae were also collected and observed under the foldscope. These planktonic larvae are discussed in the following sections:

3.1.1 Fish juvenile

During the planktonic survey in Nalabana Bird Sanctuary, we caught fish juveniles and were able to video record the pumping of internal organs. Melanin pigments dots were identified and feeding action of larvae was recorded and studied (Fig.6a).

3.1.2 Polychaete larvae

The shore of Chilika has an abundant amount of dead and decomposing plant material. These act as a refuge to many larvae and juveniles. Water samples from shore of the lagoon were collected and observed under the foldscope. The polychaete larvae caught during the shore sampling was mounted under foldscope to be observed under the lens. The larvae's feeding mechanism was captured. It was seen that the larvae also have filter feeding mechanism and it filter feeds the organic matter (Fig.6b). Polychaete, also known as bristle worm belongs to phylum Annelida and generally their outer surface is made up of chitin. They are the organic matter indicator; the presence of these organisms specifies the abundant presence of organic matter in the water body.

3.1.3 Veliger larvae

A veliger is the planktonic larva of many kinds of sea snails and freshwater snails, as well as most bivalve molluscs (clams) and tusk shells. These larvae happen to be free living and are often carried from one place to another through water currents. They complete their larval stage in planktonic form before settling on the benthos. We encountered a live veliger larva doing active filter feeding in the water samples collected from the Chilika Lagoon. The foldscopy helped us to capture the filter feeding mechanism of a gastropod veliger larva from the surrounding water. The fleshy part of the veliger is called velum through which water current was seen passing in and out. The velum is also used as the swimming organ as well as for the respiration. The velum contains ciliary bands which produces the currents (Fig.6c)

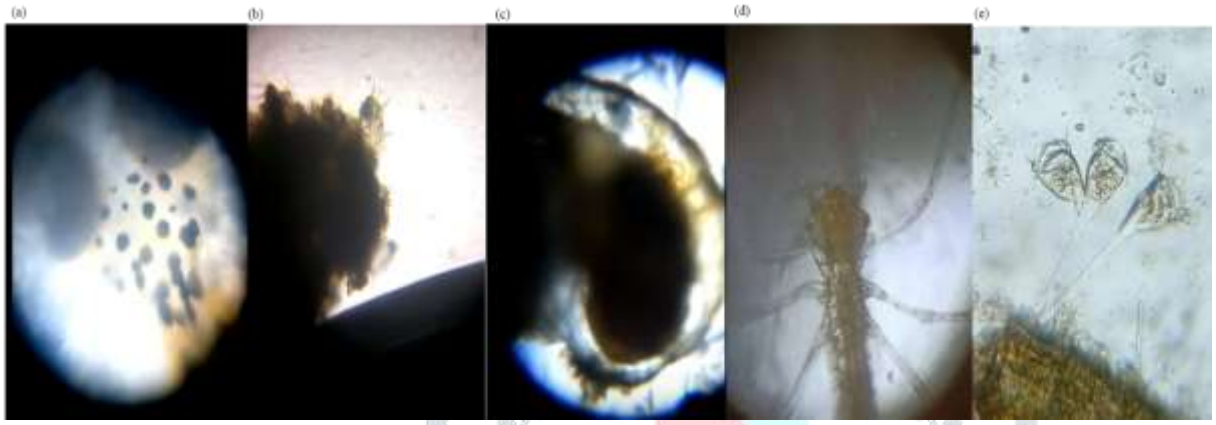
3.1.4 Damsel nymph

Chilika Lagoon supports dense growth of submerged and emergent macrophytes. These macrophytes are also sheltering niches to a variety of flies and insects that feeds and rests on these plants. During sampling from northern sector of the lagoon which supports luxurious growth of freshwater aquatic weeds such as *Eichhornia*, *Potamogeton* and *Vallisneria*, water samples were collected. The sampling captured a young damselfly nymph, zygopteran insect of the order odonata which includes the dragonflies. Live specimen of nymph was analysed through foldscope. We noted the coloured patterns on its slender body with the two eyes distinctly separated from each other. This aquatic insect develops through 10 – 12 immature stages (instars) before emerging out in the water as an adult winged insect. The nymph mostly resides in the calm waters of ponds or lakes and in vegetation where it feeds on water fleas (cladocerans), water mites, small insect larvae, etc (Fig.6d).

3.1.5 *Vorticella*

Vorticella is a protist (protozoan) belonging to the Phylum Ciliophora and are found in freshwater ponds, lakes, rivers and streams. It has a peculiar inverted bell shaped structure often attached by a long highly contractile stalk to some submerged objects like weeds, animals, rocks, and algal filaments etc. Due to the bell-shaped body, they are often called 'bell-animalcule'. The bell-shaped body of *Vorticella* is attached to substratum by a long, thin, un-branched and highly contractile stalk. Adults also swim freely if their stalks are cut, or if they have to detach themselves from the substrate due to unfavourable conditions. *Vorticella* exhibits high degree of contractility and irritability to any mechanical stimulus and also responds to external stimuli. When irritated, it's all activities cease at once; the stalk is retracted and becomes coiled into closed spiral to reduce its size, then the disc is withdrawn and covered over by a peristomial lip. There are oral cilia specialized for making water currents that beat to bring food closer to its mouth (Fig.6e).

Fig.6. Planktonic larvae found during Phytoplankton sampling. (a) Fish juvenile, (b) Polychaete larvae, (c)Veliger larvae, (d) Damsel Nymph, (e) *Vorticella*.



3.2 Zooplankton

Zooplankton are the primary consumer linking phytoplankton with the secondary consumer in a food chain or food web. They play a vital role in supporting the fishery resources of Chilika by being the major food source for them. Zooplankton are heterotrophic plankton that ranges from microscopic organisms to large organism such as jellyfish. Zooplankton samples observed under foldscope are discussed as below:

3.2.1 Female copepod

A matured female copepod captured from water samples of outer channel of Chilika lagoon was observed under foldscope with egg sacs dangling from the sides of the genital segment of the urosome. The swirling movement of water in the region below the antenna was also seen and recorded. The movement process is actually the feeding process which is created by the millipedes and antenna which forces the water current to pass through the mouth. Some copepods are known to carry fertilised eggs until they hatch out as nauplius. The nauplius passes through 6 naupliar stages and 5 adult like instars before finally molting into adults (Fig.7a).

3.2.2 Rotifer

Rotifer was observed under foldscope in water samples collected from shoreline of Chilika where active decomposition of macrophytes and macroalgae was occurring (Fig.7b). Rotifer, are multi-cellular animals often referred as "wheel-bearer" due to presence of the crown of cilia around their mouth. They primarily feed on dead or decomposing organic materials, as well as unicellular algae and other phytoplankton and are considered as primary consumers. Rotifers are in turn prey to carnivorous secondary consumers, including shrimp and crabs.

3.2.3 *Mysis*

Foldscopy captured the beating heart of a Mysis (Fig.7c). *Mysis* is a marine zooplankton belonging to the order Mysida of the malacostracan superorder of Peracarida. *Mysis* are also known as opossum shrimps due to the presence of marsupium or brood pouch in the female which carry the larvae until maturity. They can be distinguished from their stalked eyes and thorax, to which the appendages are limited. They are the preferred food for many marine organisms such as cephalopods, young seahorses, cultured shrimps, etc. due to their high protein and fat content.

Fig. 7: Zooplankton samples from Chilika observed under Foldscope. (a) Female Copepod, (b) Rotifer and (c) *Mysis*.

3.3 Benthic Organism

Benthos include worms, clams, crabs, lobsters, sponges, and other tiny organisms that live in the bottom sediments. Benthic animals are much more abundant in the shallow waters where seagrasses are abundant. Seagrass meadow support a diverse benthic communities and offer several ecosystems services such as nutrient cycling, foraging and breeding ground for many fishes and crabs. Following benthic organisms were analysed under foldscope:

3.3.1 Oligochaete

Oligochaetes are well-segmented worms and most have a spacious body cavity (coelom) used as a hydro-skeleton. They are found abundantly in the seagrass meadow of Chilika lagoon. Foldscopy helped to understand the fluid movement inside an oligochaete specimen (Fig.8a).

3.3.2 Amphipod

Amphipods were recovered after sieving the sediments collected from seagrass beds. The movement, pigmentation and feeding mechanism was captured under foldscope (Fig.8b). Amphipoda is an order of malacostracan crustaceans with no carapace and generally with laterally compressed bodies and are mostly detritivores or scavengers. They are mostly marine animals, but are found in almost all aquatic environments.

3.3.3 Isopod

Isopod samples were collected from seagrass bed sediments of Chilika Lagoon to be observed under Foldscope (Fig.8c). Isopods are one of the most diverse crustaceans in terms of shape, size and habitats. They dwell in a wide variety of habitats ranging from pelagic (free-swimming) to interstitial spaces of sand grains and are often abundant in seagrass meadows. Some of them also live as parasites on other animals either temporarily or permanently. Isopods are mainly detritivores, carnivores, parasites and sometimes filter feeders.

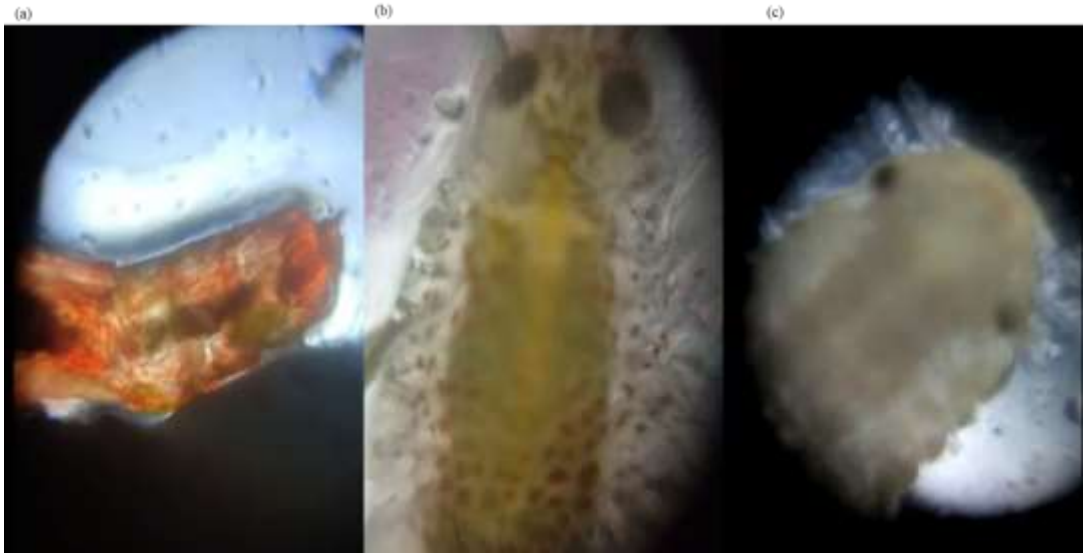


Fig. 8 Benthic samples collected from Chilika and observed under Foldscope. (a) Oligochaete, (b) Amphipod and (c) Isopod.

3.4 Macroalgae

Macroalgae can be subdivided into the blue algae (Cyanophyta), green algae (Chlorophyta), brown algae (Phaeophyta) and the red algae (Rhodophyta) (Ross et al. 2008). Chilika lagoon supports the growth of marine algae because of its varying salinity and nutrient regime at different locations during different seasons. Chlorophyta and Rhodophyta were the two main groups occurring in different sectors of Chilika. Using foldscope, we observed *Spirogyra* (Fig.9a), a genus of filamentous charophyte green algae of the order zygnematales that has a characteristic helical or spiral arrangement of the chloroplasts. In Chilika lagoon, the spirogyra filaments formed dense epiphytic growth on seagrasses meadows. Other than *Spirogyra*, algae namely *Oscillatoria* (Fig. 9b), *Polysiphonia* (Fig.9c) and *Ceramium* (Fig.9d) species were found in the southern sector of Chilika either attached to the seagrasses or to the rocks of shorelines. *Polysiphonia* is a red alga with filaments reaching a length of about 30 cm. These algae were found attached to the rocks of the small islands of Chilika lagoon.

3.4.1 Diatom *Biddulphia* sp.

Diatoms are a dominant group of algae found in Chilika lagoon. These are single-celled photosynthetic algae quite abundant in aquatic environment. When algae attached to a rock samples were analysed under foldscope, they were identified to be *Biddulphia* sp. under the group diatom. Their cells are robust, rectangular in girdle view, elliptical in valve view, with prominent elevations at the poles. Often occurs in zig-zag chains generally found near shore or attached to substrates such as seaweeds and rock (Fig.9e)

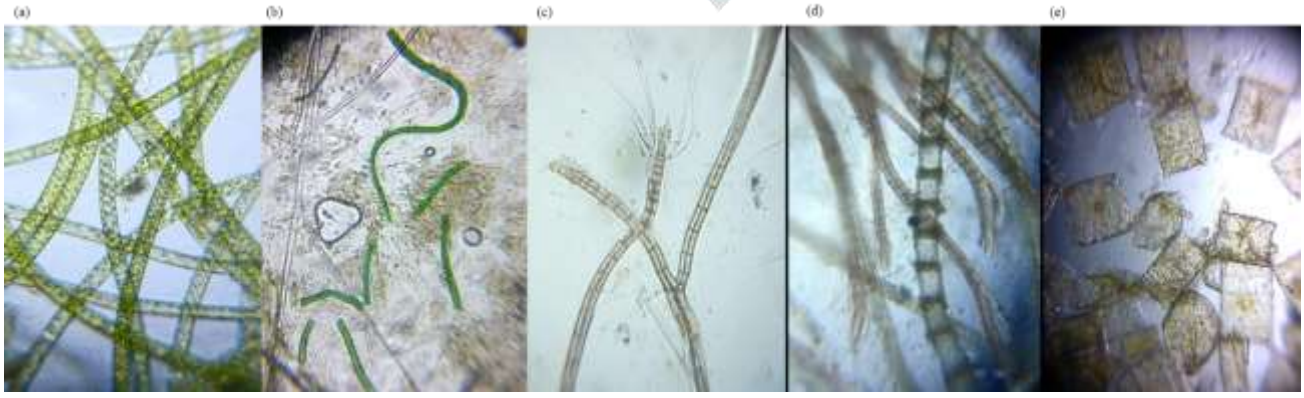


Fig. 9: Macroalgae of Chilika observed under Foldscope. (a) *Spirogyra*, (b) *Oscillatoria*, (c) *Polysiphonia*, (d) *Ceramium* and (e) *Biddulphia*.

4. Conclusion

Foldscope, a paper microscope proved to be an excellent portable microscope that allowed the observation of biological samples within few minutes after their collection. This elimination of observational lag period allowed us to capture many biological processes such as filter feeding, cilia motion, etc in real-time. The portable nature of the foldscope in combination with easy mounting of samples allows anyone to do microscopy without much training. Due to low price, foldscope was also appreciated by many school and colleges which were located in close proximity to Chilika. In such resource constrained organizations, foldscope will allow easy access of science to everyone and students will get a chance to do the science by themselves and magnify their curiosity.

Acknowledgement

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Legends to Figures

Figure 1. Chilika Lagoon a designated Ramsar site located in the Odisha State.

Figure 2. Foldscope. a paper microscope. Picture credit: https://www.foldscope.com/

Figure 3. Map of Chilika showing the four different sectors primarily on the basis of salinity regime Northern sector (NS), Central sector (CS), Southern sector (SS), and Outer Channel (OC). The green area represents seagrass distribution in Chilika.

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