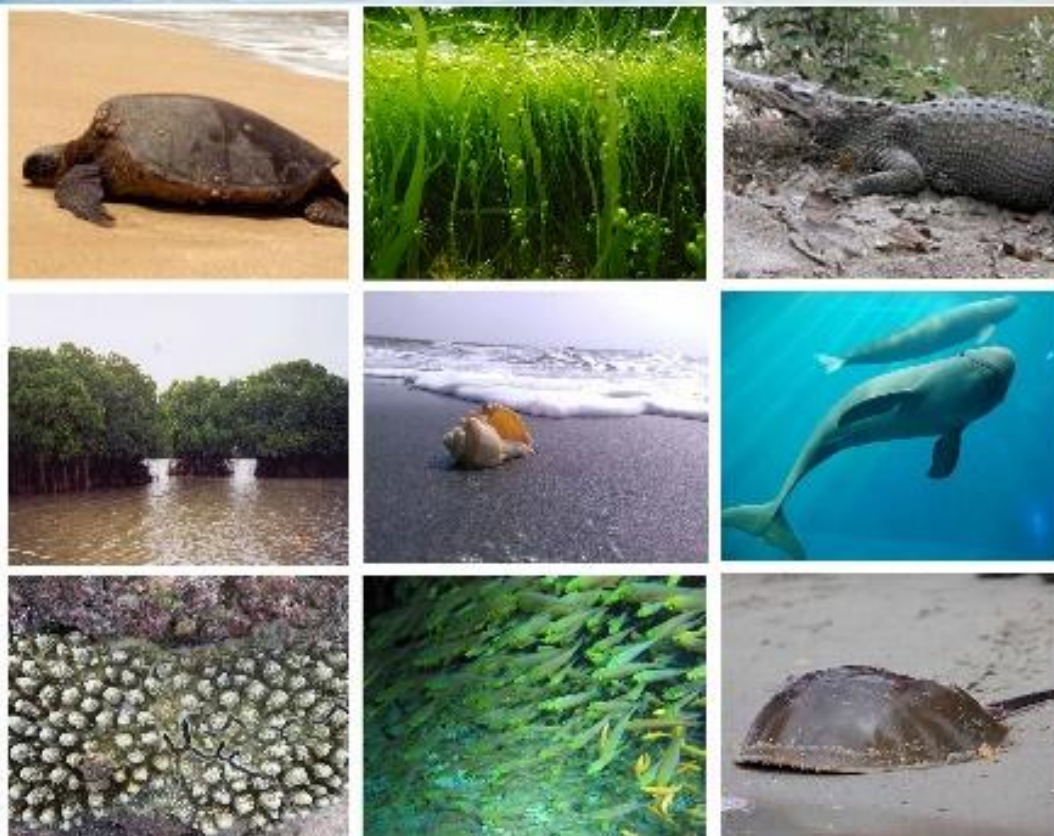


Marine Biodiversity Manual for Sampling/Collection and Analysis



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MANUAL ON MARINE ECOLOGY AND BIODIVERSITY
Procedure for Sample Collection and Analysis

PREFACE

Marine biodiversity refers to the species richness and abundance in the world's oceans, seas and coastal ecosystems. Since the world is covered with approximately 70% water, the amount of life in the marine environment is enormous. In the ocean, over-fishing and other threats to species' populations reduce resources for society, have altered ecosystems, and put many mammals, birds, reptiles, and fish in danger of extinction. It is well known that biodiversity plays a vital role in maintaining the functionality and productivity of ecosystems. It also makes habitats more resilient to environmental change. Current concerns about the marine ecosystems and the loss of biodiversity, drives the need to measure spatial and temporal variation in biodiversity from local to regional and global scales.

Coastal areas in India, as elsewhere in the world, are bestowed with various highly productive ecosystems, such as mangroves, coral reefs, ecologically sensitive habitats such as mudflats, sand dunes, rocky shores, etc., which support rich biodiversity. Health of these specialized ecosystems is very much essential for the sustainable coastal and offshore fisheries and thus the nation's economy and people's livelihood.

Marine biodiversity comprises of a wide variety of flora and fauna like bacteria, plankton, benthic organisms, seagrasses, seaweeds, mangroves, corals, other invertebrates like crustaceans, molluscs, etc., and vertebrates like fishes, turtles, birds, mammals, etc. There exists a number of methods for studying these wide variety of organisms. All methods are selective, at least for body size by excluding smaller and/or larger organisms. Such bias should be explicitly recognised in the design and interpretation of field data. Because of methodological biases a comprehensive sampling of marine biodiversity across habitats, body sizes and trophic levels would need to use a variety of complementary methods.

In India, the scope for development in the coastal sectors is increasing and the need to create baseline data for the purpose of EIA for getting EC to develop the coastal industries. And for this purpose, for the qualitative and quantitative assessment of various marine flora and fauna, a methodology manual has been compiled using the various methods practiced. This manual is intended for field observations and sampling marine biodiversity and provide examples of methods.

Various eminent scientists, academicians and professionals from these disciplines of marine biodiversity have contributed in compilation of this manual. I strongly believe this methodology manual will be very much useful, not only for consultants and also for the project proponents to get good understanding on various aspects of marine ecology and biodiversity.

Date: xx December 2018

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1.1. Introduction

Marine microbiology encompasses study of microbes that live not only in the surficial marine waters but also the abyssal depths and from the coastal to the offshore regions. Marine microbes live in strictly aerobic to strictly anaerobic niches; thrive at extremes of pressure i.e. 1 atm in the surface to several hundreds of atm in deep trenches; survive near 0°C to those multiply above 100°C. Though they can thrive in a broad range of Eh, light, temperature, many microbes have an absolute requirement for sodium, potassium and magnesium ions, chloride and ferric ions and grow very well above pH 7.0 or near pH 8.0. In general, marine microbes thrive well around 35 PSU and pH 8.0. Thus distribution of marine microbes revolves around these very characteristic parameters.

1.2. Ecological Significance

From the time of their origin, single cell organisms - initially anaerobic and later aerobic – have served as essential catalysts for all of the chemical reactions within the biogeochemical cycles that shape planetary change and habitability.

Marine microbes carry out half of the primary production on the planet. Microbial carbon re-mineralization, with or without oxygen, maintains the carbon cycle. Microbes account for >90% of the respiration in the oceans. They control global utilization of nitrogen through N₂ fixation, nitrification, nitrate reduction and denitrification and drive the bulk of sulfur, iron and manganese biogeochemical cycles.

Marine microbes regulate the composition of the atmosphere, influence climate, recycle nutrients and decompose pollutants. Without microbes, multicellular animals on earth would not have evolved or persisted over the past 500 million years.

International Census of Marine Microbes (ICoMM) envisages to catalogue the known bacterial diversity and to explore / discover unknown microbes. (<http://icomm.mbl.edu/>).

As of now cultivation-based studies have described > 10,000 bacterial and archaeal species and an estimated 200,000 protistan species. Cultivation independent studies that rely on molecular methods such as the sequencing of 16S ribosomal RNA (rRNA) genes show microbial diversity to be approximately 100 times greater.

The importance of marine microbes to our biosphere cannot be overstated. Since the microbial census began, several major scientific breakthroughs in microbial diversity and microbial ecology have occurred. Owing to the rapid developments in high-throughput and relatively cost-effective sequencing technologies like massively parallel DNA sequencing, it has become possible to deeply

explore microbial genetic diversity of environmental samples in both qualitative and quantitative ways.

1.3. Culturability concern

As the existence of the microbial life was recognized only relatively recently, about 300 years ago, the knowledge we have gained is still inadequate. Microorganisms still represent the largest reservoir of undescribed biodiversity. The recent techniques such as Ribosomal RNA genes - sequencing have helped us survey the biodiversity sufficiently faster and comprehensively. Even though, an impressive number of bacteria (ca. 30,000 species) are represented in "Gen Bank" our view of the microbial world is far from complete.

The term "the great plate count anomaly" is used to describe the difference in orders of magnitude between the numbers of cells that form colony forming units (CFU) on nutrient media and that countable by microscopic examination. Marine ecosystem is a typical example of this phenomenon as only 0.01 to 0.1% of marine bacteria are culturable (Amann et al., 1995).

One of the biggest challenges for marine microbiologists today is how to increase the percentage of cultivability or in other words how to culture these unculturables. Recently, high-throughput culture (HTC) methods were developed, which allowed large number of microbial isolates to be recovered by dilution to extinction in natural sea-water media.

1.4. Method of collection and Identification (water & sediments)

Many distinct marine ecosystems and their microbial assemblages have been identified and studied ranging from ice-swept polar seas to deep-sea thermal vents.

Microorganisms present in the polluted areas are used as health indicators to identify the polluted sites and also for evaluating the impact of pollution. Following are some of the health indicator bacteria:

- i) *Escherichia coli*
- ii) *Citrobacter freundii*
- iii) *Klebsiella pneumoniae*
- iv) *Salmonella* serotype *typhimurium*
- v) *Pseudomonas aeruginosa*
- vi) *Staphylococcus aureus*
- vii) *Vibrio cholerae*
- viii) *Streptococcus faecalis*

Enumeration of the health indicating bacterial population will be useful in the activities related to prevention and control of pollution.

Sediment is collected using a Grab (van Veen grab for shallow depths and Petersen for deeper areas). Most grabs penetrate to depth of 10 cm or more (less on hard-packed sand) and collect the

sample, which is semicircular in cross section. The upper 5-10 cm of the sediment which is considered to be microbiologically most active is of interest for analyses of microbial pathogens.

Note: Sediment samples are collected using van Veen grab (0.1m² area) from shallow depths. In this grab the long arms attached to grab bucket exert a considerable leverage for closing the jaws.

1.5. Collection of Samples

1.5.1. Water

Materials required for field sampling:

- Niskin Sampler or bucket
- Sterile bottles (plastic/glass)
- Ice box, Plastic bags, Rubber bands, Labels, Marker

Water samples are collected using either a clean bucket (surface water) or Niskin sampler (subsurface and deep water) rinsed with 70% alcohol and sterile distilled water. Water from the Niskin sampler or bucket is transferred into a sterile glass or plastic bottle, immediately following collection.

The bottle is labeled with required details such as Station No., Date, Time, Depth (m), Tide, etc.

Note: In case the sampling area is turbid filter the water sample through 200µm bolting silk /sterile Nuclepore Whatman No. 1 filter paper)

1.5.2. Sediment

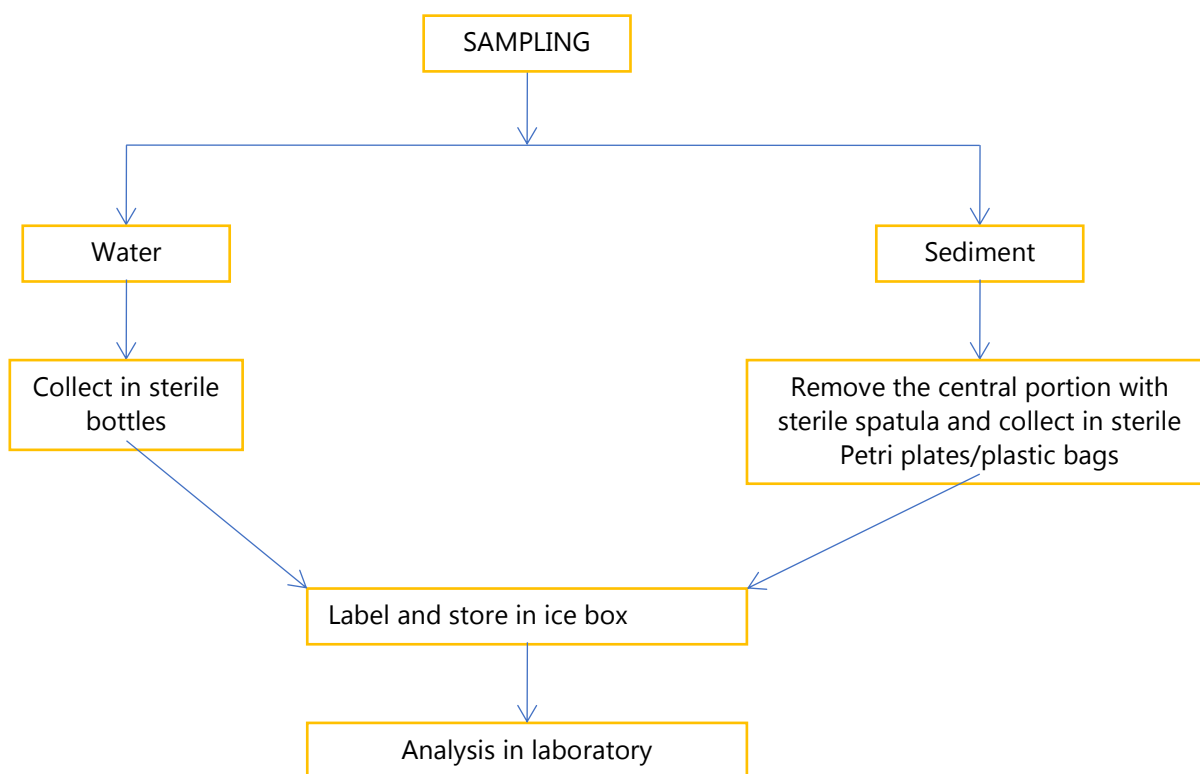
Materials required for field sampling:

- Van Veen grab
- Plastic bags/Petri plates, Spatula, Absorbent Cotton, Alcohol
- Ice Box, Rubber bands, Labels, Marker

Collection: Sediment sample is collected using a van Veen grab (from shallow depths up to 10 cm). Central portion of the sediment upto a depth of 10 cm is removed with a sterile spatula and transferred into a plastic bag or sterile Petri plate; label the samples as mentioned above.

Transportation: The samples are brought to the laboratory under ice-cold condition, as quickly as possible, for further processing.

Flow Chart:



1.6. Enumeration of Marine Bacteria

Counting bacteria in natural environment has been a long standing endeavour for aquatic microbial ecologist. Estimation of culturable bacteria has evolved in several stages. Enumeration of culturable bacteria is based on ability of single bacterium to form colonies on marine agar plates.

A variety of methods have been developed for the enumeration of microbes. These methods measure cell numbers, cell mass, or cell constituents that are proportional to cell number.

There are various approaches used for estimating the sizes of microbial populations through direct and indirect counts of cells or estimation of microbial biomass.

Cells can be counted directly under microscope or by an electronic particle counter, but the disadvantage is that live and dead cells cannot be distinguished.

Cells can also be counted using fluorescent dyes. One of the most widely used fluorescent dye for counting the number of bacterial cells is acridine orange which stains both living and dead cells by interacting with DNA and protein components of cells.

Microorganisms in a sample are diluted or concentrated and grown on a suitable medium; the development of growing microorganisms (for example, colony formation on agar plates) is then used to estimate the numbers of microorganisms in the original sample.

1.6.1. Viable Count

Plate method (Culture method):

The most common procedure for the enumeration of bacteria is the viable plate count. In this method, serial dilutions of a sample containing viable microorganisms are plated onto a suitable growth medium. The suspension is either spread onto the surface of agar plates (spread plate method), or is mixed with molten agar, poured into plates, and allowed to solidify (pour plate method). The plates are then incubated under conditions that permit microbial reproduction so that colonies develop, that can be seen without the aid of a microscope. It is assumed that each bacterial colony arises from an individual cell that has undergone cell division. Therefore, by counting the number of colonies and accounting for the dilution factor, the number of bacteria in the original sample can be determined.

The viable count is an estimate of the number of cells. Because some organisms exist as pairs or groups and because mixing and shaking of the sample does not always separate all the cells, we actually get a count of the "colony forming units".

The bacteria are allowed to grow on seawater nutrient agar medium for a fixed period of time and at room temperature. Each single cell grows into a colony and the numbers of colonies are counted.

Materials required at Laboratory (APHA 23rd Ed 2017):

- i) **Sterilising Autoclave:** Sterilization of media, solutions and equipment before use and contaminated items afterwards; melting solidified agar media for use within range of temperature at 121-124°C and 15 lbs of pressure. The size required depends on the volume of work to be undertaken.
- ii) **Incubator:** capable of maintaining a temperature of 35-37 °C (\pm 0.5 °C).
- iii) **Laminar Flow Chamber** with Ultra violet lamps
- iv) **Laboratory balance:** Locate balance in area, without rapid air movement, even surface to prevent vibration accuracy \pm 0.05 g, with weighing scoop.
- v) **Oven** for use at 170 °C
- vi) **Test tube:** Small volumes (10 ml) of liquid media/agar slopes/sterile solutions for inoculation (held in test tube rack; dry non-absorbent cotton wool plug or plastic cap prevents contamination).
- vii) **Petri dishes:** (Himedia)
 - a. Plastic: pre-sterilised for streak/spread/lawn/ pour plates;
 - b. Glass: only for materials for sterilization by hot air oven, *e.g.* paper discs
- viii) **Spreader (glass/plastic):** Making lawn/spread plates

- ix) **Erlenmeyer flask** (250 ml)
- x) **Pipettes:** 1.0-ml graduated in 0.01-ml divisions and 10-ml graduated in 0.1-ml divisions presterilized, disposable, glass or plastic with cotton plugs, Millipore or equivalent, or sterile, disposable, 1.0-ml (milliliter) hypodermic syringes.
- xi) **Nonabsorbent cotton wool:** Plugs for test tubes, flasks and pipettes.
- xii) **Water bath** or equivalent, capable of maintaining a temperature of $(45 \pm 2) ^\circ\text{C}$.
- xiii) **Bunsen burner:** Sterilization of wire loops and (with alcohol) metal forceps and glass spreaders.

Preparation of culture media:

Before sterilization, ensure ingredients are completely dissolved, using heat if necessary. Avoid wastage by preparing only sufficient for either immediate use (allowing extra for mistakes) or use in the near future. Normally allow 15-20 cm³ medium/ Petri dish. Dispense in volumes appropriate for sterilization in the autoclave/pressure cooker.

- i. **Nutrient agar:** Suspend 28 grams in 1000ml of half strength sea water. Adjust the pH to 7.5 ± 0.2 of the medium. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C). Transfer 15-20 ml/plate under sterile condition.
- ii. **MacConkey Agar:** Suspend 50 grams of the medium in one liter of distilled water. Mix well until a uniform suspension is obtained. Heat with frequent gentle agitation and boil for one minute. Sterilize in autoclave at 121°C (15 lbs. sp) for 15 minutes. Cool to 45°C and pour into Petri dishes. Allow the plates to solidify and place them upside down to avoid excessive moisture in the surface of the medium.
- iii. **XLD agar:** Suspend 55 grams of the medium in one liter of distilled water. Heat with frequent agitation until a temperature of approximately 90°C . Do not boil. Transfer immediately into a water bath at about 50°C . Pour into Petri plates as soon as it has cooled. The medium should have a reddish color and be clear, or almost clear. Excessive heating or a prolonged stay in the water bath produces precipitation. When this occurs, reactions are satisfactory, but colonies may be slightly smaller. This precipitation can be eliminated by paper filtration.
- iv. **TCBS agar:** Suspend 88 grams of the medium in one litre of distilled water. Mix from 10 to 15 minutes. Heat with frequent agitation and boil for 1 minute until completely dissolved. Cool to $45\text{-}50^\circ\text{C}$ and pour in Petri dishes. Do not sterilize in an autoclave.
- v. **Cetrimide Agar:** Suspend 45.3 grams of the medium in one litre of distilled water. Add 10 ml of glycerol. Heat agitating frequently, and boil for one minute. Dispense and sterilize and autoclave at $118\text{ to }121^\circ\text{C}$ (12-15 lbs. sp.) for 15 minutes.

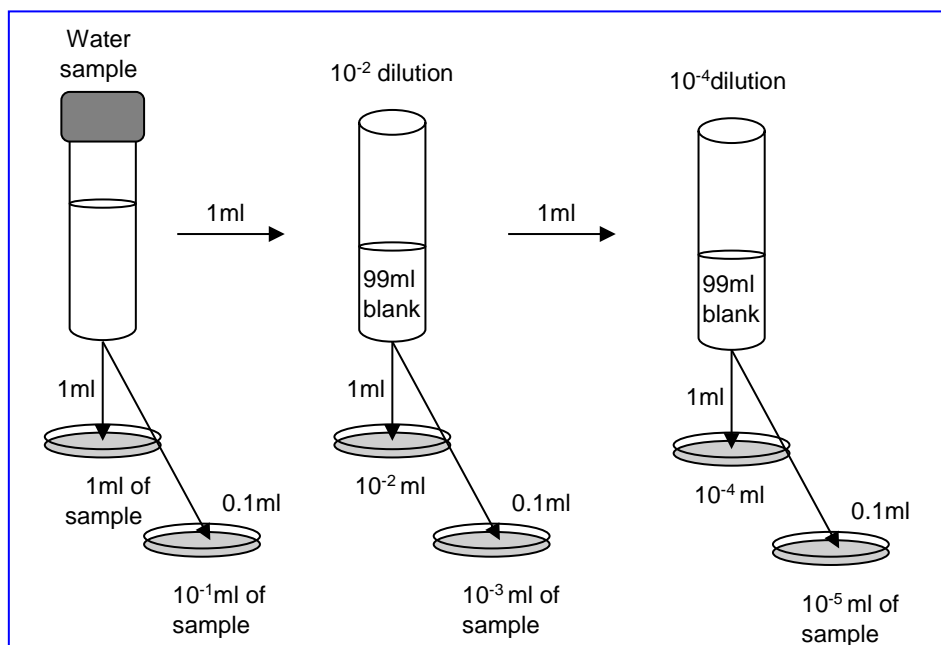
Note: Test the salinity and decide the strength of the sea water depending on the location of sampling. Salinity of the sample is measured with a salinometer (you may use a refractometer also).

Procedure:

a. Serial dilution of water samples: By Prescott et al (2005).

- i) Label four 9.9 ml saline tubes 10^{-2} , 10^{-4} , 10^{-6} , and 10^{-8} , respectively.
- ii) Vortex the unknown sample to ensure an even distribution of bacteria.
- iii) Measure 0.1 mL of the sample dilute in 9.9 mL of saline solution and mix thoroughly. (This means a 1:100 dilution or 10^{-2}).
- iv) Using a sterile pipette, transfer 0.1 mL of the 10^{-2} dilution to another test tube containing 9.9 mL of the saline solution, and repeat the step, in order to have 10^{-4} and 10^{-6} dilutions. Mix thoroughly.
- v) Label each Petri dish and aseptically transfer 1.0 ml from the 10^{-4} dilution tube to the plate labeled 10^{-4} .
- vi) Spread the inoculum on the surface of the agar in each plate using an alcohol-dipped, flamed, metal spreader.
- vii) Dip the spreader into the alcohol jar and quickly take it through the flame and let the alcohol burn off after each spreading.
- viii) Let the agar solidify and incubate (at 37°C for nutrient agar and 30°C for PDA and Sabourad media).
- ix) Allow the surface of the agar to dry before you move or invert the plates. Incubate the plates at 37°C for 24 hrs.
- x) Calculation.

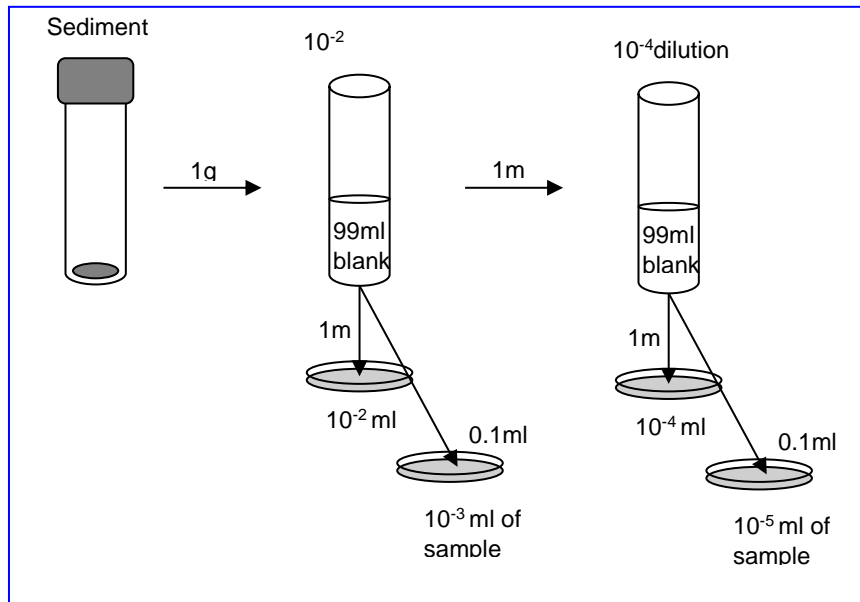
$$\text{Total Viable count / ml} = \frac{\text{No. of colonies}}{0.1 \text{ ml}}$$



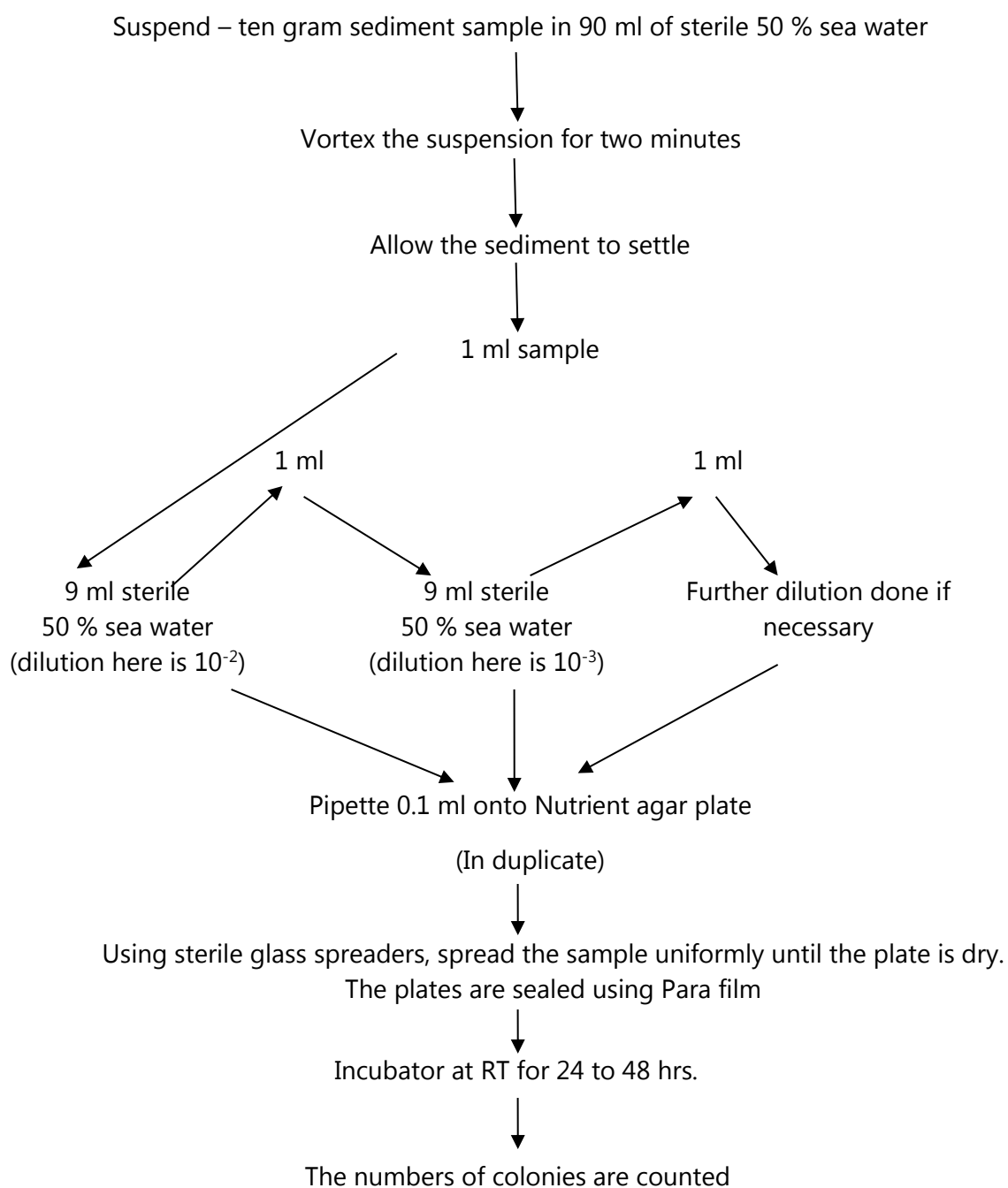
b. Preparation of soil/sediment: Sediment and Soil samples were processed using the method of [Adesemoye et al. \(2006\)](#).

- i) If necessary, place some soil in a sterile mortar and break up the lumps with a pestle.
- ii) Weigh 10 g of the sample soil. Suspend in the 90 mL of sterile 50 % saline solution and mix thoroughly. (This means a 1:100 dilution or 10^{-2}).
- iii) Using a sterile pipette, transfer 0.1 mL of the 10^{-2} dilution to another test tube containing 9.9 mL of the saline solution, and repeat the step, in order to have 10^{-4} and 10^{-6} dilutions. Mix thoroughly.
- iv) Label each Petri dish and add aliquots of the dilutions and media.
- v) Slowly, move each Petri dish so that the samples become mixed with the culture medium.
- vi) Let the agar solidify and incubate.
- vii) Calculation

$$\text{Total Viable count / g dry wt} = \frac{\text{No. of colonies}}{\text{Dilution factor}}$$



Procedure: Flow Diagram



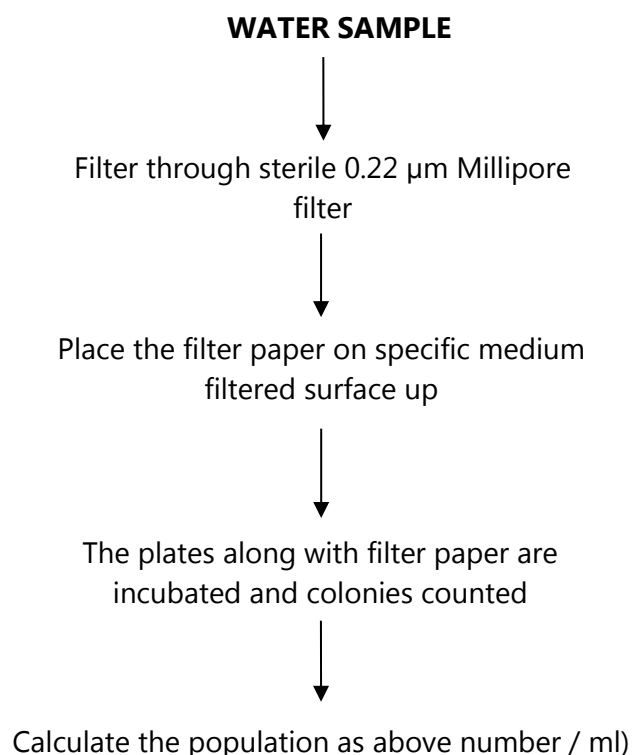
$$\text{Total Viable count / g dry wt} = \frac{\text{No. of colonies}}{\text{Dilution factor}}$$

Note: The water in which sediment is suspended is filtered using a pre – weighted filter paper and it is dried and weighed.

1.6.2. Membrane filter method

Materials Required:

- Filtration Unit
- Millipore filter paper (0.22 μm)



1.7. Enumeration of Health Indicator Bacteria

Health indicator bacteria are enumerated on selective media. Himedia have different types of selective media as shown below.

- HiCrome E. coli Agar (M 1295)
- HiCrome Salmonella Agar (M1296)
- M-Enterococcus Agar

Advantages of HiCrome media:

- i) Abundance of colonies with desired characteristics representing is more compared to other selective media.
- ii) Easy identification based on colour of the colony.
- iii) Single medium can be used to differentiate various groups of bacteria.

1.8. Isolation and Purification of Bacterial Strains

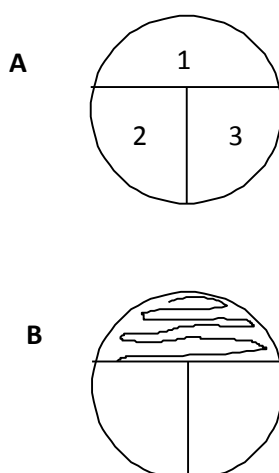
Isolation of bacteria in pure culture is the first and the important step. For that, one should clearly observe the plates and try to distinguish colonies based on their morphological parameters like colour (on special media), shape, size, opacity, pigmentation, etc. A random selection mechanism is also followed when the colonies appear to be similar and too numerous to count (TNTC). Often, plates with 30 to 300 colonies are ideal for counting and for choosing colonies for purification. Once selection is made, then the purity of the isolate is tested by using quadrant streaking method outlined in figures A to D. Purification is to obtain the desired bacteria from the plate using a loop and streaking on to solid medium to get contaminant free pure culture. A pure culture should yield colonies that appear similar to one another and microscopic observation of the culture should reveal cells that are reasonably similar to each other in appearance, particularly in regard to cell diameter and Gram reaction

Materials required:

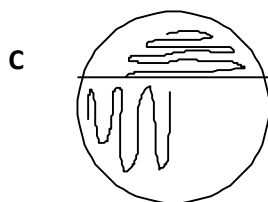
- Nutrient agar plates
- Inoculation needle (loop)
- Light Microscope.

Procedure:

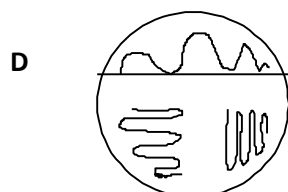
- The desired colonies are marked.
- Using a sterile loop the individual colonies are picked out and transferred on to nutrient agar plate.
- The isolated culture is streaked on the agar plate as shown. Streak a loopful of culture slightly back and forth on the surface of the agar over section 1 as shown in diagram B.



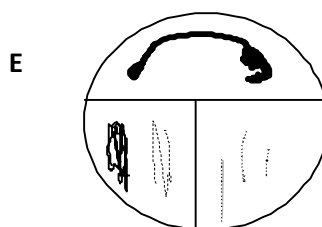
- Flame the needle and allow it to cool. Draw the loop over section and then streak back and forth as mentioned earlier.



v) Repeat the same.



vi) Incubate the dish in an inverted position to prevent drops of condensed water on lid from falling onto the agar surface. Section 1 will develop the thickest amount of growth, while the following sections will usually have well isolated colonies.



1.8.1. Preparation of Culture for Sending

After the identification of the respective culture streak it on a nutrient agar slant.

Preparation of agar slants

- i) Transfer about 1ml of nutrient agar into the storage vial (Laxbro cat no: SV- 2R) for stab or screw cap tubes 5ml for slant, autoclave and keep it in tilted position at an angle of 45° for slants.
- ii) Leave it for two days to check any contamination.
- iii) Pick a single pure colony from nutrient agar plate and transfer it into these vials/slants.
- iv) Streak it on the slant or stab inoculate.
- v) Incubate it for one day.

1.8.2. Colour chart for identification of HiCrome media recommended

Bacteria	M1295	M1296
<i>E.coli</i>	Blue	Pink red
<i>Streptococcus faecalis</i>	M-Enterococcus agar	Red colour

//List of media and bacteria identified and color photographs of bacterial cultures may be included, with special reference to TVC, TC, FC, *E.coli* Like Organisms, *Salmonella* LO, *Shigella* LO, *Pseudomonas aeruginosa* LO, *Proteus klebsiella* LO, *Vibrio* LO, *Vibrio parahaemolyticus* LO, *Vibrio cholerae* LO, etc., being reported in EIA report as well as other important pathogenic bacteria.//

CHAPTER 2

PHYTOPLANKTON

2.1. Introduction

Phytoplankton, microalgae of the sea, are unicellular microscopic plants and range in size from 1/1000 of a millimeter to 2 millimeters. Most of them float in sea surface and are drifted by sea currents including tidal currents. Phytoplankton has different types of organisms. Common ones are diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae) and blue green algae (Cyanophyceae). Based on size, the phytoplankton is classified as Netphytoplankton (>20 micron) Nannoplankton (between 5 and 20 micron) and Picoplankton (5 microns and less).

Nutrients like nitrate, ammonium, phosphate and silicate with micro levels of trace elements like are essential for growth of the phytoplankton. Since they produce organic matter in the sea and also form as food for a wide range of marine organism including whales, shrimp, snails, and jellyfish, they are called as Primary producers. The phytoplankton have high rate of reproductive capabilities with cells doubling time of species is low as 12 -24 hrs.

2.2. Distribution of Phytoplankton

Major groups of phytoplankton are distributed in brackish water zones of estuaries (salinity around 15 PSU), mangroves, coastal waters and open waters. Most of the phytoplankton are present in euphotic zone (upto which light that is required for synthesis of organic matter by phytoplankton).

2.3. Algal bloom

The concentration of essential nutrients like ammonium, nitrate and phosphate greatly influence the growth and reproductive behavior of phytoplankton. The phytoplankton experience abnormal growth when the nutrient levels reach very high and lead to occurrence of blooms. These blooms vary in colour depending on the species that causes bloom. If such blooms are noticed in the sampling site samples could be collected and analyzed for the species and its density.



Red tide



Red tide

2.4. Sample Collection

Estimation of population of phytoplankton in the form of cell nos/litre or biomass g/m^3 is one of the most essential requirements to assess the status of productivity of a water body.

In the marine environment, sampling sites are dependent on water current, depth, water column stability, fresh water in flow, river runoff, off-shore sites prone for bloom etc.

Plankton is not distributed uniformly throughout the water but has a patchy distribution in both space (vertical and horizontal) and time (between day and night, winter and summer). This means, for example, that sampling with a particular size of mesh, or during the night, or during the ebb tide will influence the results and the interpretation.

Phytoplankton can be estimated through quantitative and qualitative sampling. For quantitative analysis subsurface water sample (1 lit) may be collected and preserved. Phytoplankton may be collected in surface, mid depth and bottom. For qualitative analysis net sampling can be carried out.

There are various methods for collecting samples for phytoplankton analysis, depending on whether a quantitative or qualitative analysis is desired. Irrespective of methods, samples should be preserved soon after collection and where possible, live samples should also be examined.

2.4.1. Quantitative Sampling

Surface water samples are collected by dipping a well-rinsed bucket over the side of the boat; the bucket is half filled with water and brought back into the boat. Fill the provided sample bottles. Standard sampling depths for "surface" water samples are 0.1 m and 0.5 m.

Water sampling bottles: The sampling bottles can be used manually to collect water samples at "surface" or by scuba diver to collect water samples at desired depths.



Fig. 2.6 Surface Sample Bottle

(Source: <http://www.marinebio.net/marinescience/01intro/tosamp.htm>)

Water Samplers: Water samplers such as Van Dorn Bottle (Fig. 2.7.), Niskin Bottle (Fig. 2.8.), etc, with closing mechanisms are commonly used for obtaining water samples from the desired depths with help of messenger. This method is used mainly for collecting small forms of plankton. Earlier periods Nansen sampler was also used.



Fig. 2.7. Van Dorn Bottle

(Source: <http://www.marinebio.net/marinescience/01intro/tosamp.htm>)



Fig. 2.8. Niskin Bottle

(Source: <http://www.marinebio.net/marinescience/01intro/tosamp.htm>)

Precautions:

- Water is collected at the sampling site in bottles or water samplers of 5 litres or more.
- Sterile bottles should be preferred.
- While collecting the water samples, there should be minimum disturbance of water to prevent avoidance reaction by plankton.
- The plankton are then concentrated by allowing them to settle, centrifuging or fine filtration.

2.4.2. Qualitative Sampling

Phytoplankton samples from the sub-surface should be collected at the depth of 0.5 to 1 m. Plankton nets are widely used for sampling phytoplankton such as fine meshed (using a 5, 10 or 20 μm) phytoplankton nets.

Several plankton nets are available which can be used for phytoplankton sampling. Phytoplankton collection can be carried out either directly using the bolting silk cloth No. 25 with pore size 64 μ m.

Standard Plankton Net

The standard plankton net is used for qualitative analysis of the phytoplankton. Simple plankton net is conical in shape. The diameter of the mouth is 15 cm and length of the net is 60 cm. The ratio of net length to the net mouth diameter should be maintained between 3:1 and 5:1. It is made up of metal or plastic ring at its wide end. The narrow end of the net is aided with removable PVC container to collect the plankton sample filtered through the net.

The ring at the mouth is bridled down through three nylon ropes tightened finally to the main towing sample in horizontal manner the same net can be towed in horizontal direction at the surface by removing the weight from the distal end. The net used for the purpose is made of monofilament nylon.

The front and tail parts of the net are reinforced with non-porous textile cuffs. The gauzes used in nets are made of different materials such as bolting silk, polyester, nylon etc. Sampling with small (mouth diameter 15 cm; length 110 cm) fine-mesh (5 or 10 μ m) monofilament nylon nets has proved to be very successful in retaining high quality of phytoplankton.



Simple Plankton Net

(Source: http://www.aquaticresearch.com/simple_plankton_nets.htm)

The size of the mesh can be selected according to the target sizes of the phytoplankton groups. The standard size range is considered from 5 to 20 μ m. The speed at which net is towed should be given consideration. The speed of the net towed should be restricted to <2 knots. The net should be washed frequently after use and allowed to air dry. The condition of the net should be inspected for pin size holes and other wear and tear.

Procedure for towing the net

- Ensure rope is securely fastened at the plankton net opening and that the dead end is tied to the boat.

- At the designated site, deploy the net while the boat is underway. Do not lower the net until you are instructed to do so. Lower the collecting vessel end first, paying out the tow line slowly as the net moves away from the boat.
- Net hauls may be made at the surface or at any desired depth. If the sample is to be taken at some depth beneath the surface, a depressor weight may be attached to the towing line in front of the net.
- The plankton net will be towed by slowly moving the boat in circles, or in a horizontal direction at depth 0.5-1m below the surface in order to account for horizontal patchiness of algal species.
- Generally, the towing speed should not exceed 1 ms⁻¹ (2 knots). When nets with fine meshes (less than 20 µm) are used, speed even below 0.3 ms⁻¹ (0.5 knot) is advisable in order to reduce clogging to minimum.
- Date, location (station number), tow sample number, diameter of net, mesh size of net, start time of tow, vessel or current speed and end time of tow should be noted. Write the same information on a sample label for the collection bottle.
- Watch the net at all times and inform the skipper immediately if the net comes close to the boat propellers.
- At the end of the tow, pull the net back into the boat. Keep the mouth of the net up, and the collection vessel end down. Record the end time of tow in notebook.
- Gently wash down the outside of the net with seawater. This action flushes any plankton stuck in the netting into the sample bottle.
- Remove the plankton collection vessel (or open its end) and empty the plankton into a sample bottle. The collection may be preserved for later analysis.
- Wash the net without the collection vessel end 2-3 times by dipping into the water. Also wash the end sample bottle and re-attach it to the end of the net for the next deployment.

Calculation of the volume of water filtered through a net

To quantify the population or density, it is essential that the volume of the water filtered through the net should be known. This can be determined approximately by using the formula:

A plankton net with a circular mouth filters a volume of water approximately equal to a cylinder whose length is the tow distance and whose radius is the radius of the net. To compute the number of cubic meters of seawater that have been sampled by the plankton net, we use the following general formula for the volume of a cylinder:

$$V = \pi r^2 \times L$$

V = volume in cubic meters (m^3) of seawater

π (pi) = 3.1415926536...

r = radius of net opening (in m),

L = Length (distance net was towed, in m)

Clogging of the nets introduces an error in this calculation, however, and since clogging increases with the volume of water filtered the use of nets with flow meters is strongly recommended.

The determination of the volume of water filtered through any plankton net is essential for the estimation of the standing crop and for calculating the abundance of each planktonic organism (especially zooplankton) in a cubic meter (m^3) of seawater.

When nets are towed behind a vessel, "L" (length of tow in meters) is calculated by knowing the **speed** of the vessel in meters per hour, and the **time** (or duration; the decimal fraction of an hour that the net was fishing). Length is calculated by the formula:

Length or distance of tow = Boat speed \times Time.

Since most vessels have speed measured in **knots** (nautical miles per hour), you first need to multiply by 1852 meters per nautical mile \times Speed in knots to find the speed in meters per hour. If the time (duration) was measured in minutes, you must divide minutes by 60 min/hr to convert the time to hours.

Example: For example, a 0.5 m diameter plankton net is towed for 15 minutes at a vessel speed of 2 knots. Calculate the total volume of water filtered. One knot is 1 nautical mile/h; there are 1852 m in a nautical mile.

The speed in meters per hour = $1852 \times 2 = 3704$ meters/hour

Time in hours = $15/60 = 0.25$ h

Length or distance of tow = Boat speed \times Time.

$$= 3704 \text{ m/h} \times 0.25 \text{ h} = 926 \text{ m}$$

$$V = \pi r^2 \times L$$

$$V = 3.1416 \times (0.25 \text{ m})^2 \times (926 \text{ m})$$

$$V = 181.82 \text{ m}^3 \text{ [volume of water filtered]}$$

2.5. Concentration of Sample

When large volume of water is collected through net sampling, in which the number of phytoplankton cells appears less dense, the sample in volume can be reduced using one of the following methods.

- **By settling:** The sample can be kept in a measuring cylinder for settlement after preservation. Later settled portion can be collected by siphoning the top water out by using narrow tube.

- **By using plankton concentrator:** The sample can be filtered immediately after sampling using plankton concentrator. In this, the sample is passed through a stiff tube of PVC or Perspex with filter of nylon monofilament net attached at the bottom.
- **By centrifugation:** Sample is centrifuged for 10 to 15 min at 1500 to 2000 rpm. The supernatant is removed by decanting.

Labeling: Soon after the sample is collected the bottles must be clearly labeled with a water proof pen. 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-colored water proof marker or wax pencil.

Sample Storage and transportation: All sample containers will be stored in an insulated cooler on ice immediately after collection to maintain them at a temperature of approximately 4° C until transport to the laboratory.

Sample Preservation: To analyze the sample in live condition, it should be stored in a refrigerator in a partially filled container or in ice bags in the dark or at ambient temperature. In this case the samples should be examined immediately. If the samples are to be analyzed later in the lab, it should be preserved appropriately with suitable preservatives. Different types of phytoplankton preservatives are:

i) Preservation with Lugol's Iodine Solution

Acidic	Alkaline	Neutral	Modified
20 g Potassium iodide	20 g Potassium iodide	20 g Potassium iodide	10 g Potassium iodide
10 g Iodine (I ₂)	10 g Iodine (I ₂)	10 g Iodine (I ₂)	5 g Iodine (I ₂)
20 g conc. Acetic acid	50 g conc. Acetic acid	200 mL Distilled water	20 mL Distilled water
200 mL Distilled water	200 mL Distilled water		50 mL with 5 g anhydrous sodium acetate dissolved

- Add 0.2 to 0.8 mL of Lugol's solution for 100 mL of sample.
- Preserved samples should always be stored in dark to avoid reaction with sunlight.
- Different types of Lugol's solution can be used according to the requirement.
- Lugol's iodine is suitable for all phytoplankton but acidic Lugol's solution dissolves the coccoliths of Coccolithophores commonly present in marine water and estuaries.

- Modified Lugol's Iodine solution preserves the Coccolithophores but is not suitable for other flagellates.

ii) Preservation with Formaldehyde

Formaldehyde ensures the long-term preservation of phytoplankton. It is necessary to fix the phytoplankton sample immediately to prevent any change due to light, temperature, etc.

- Acidified Formaldehyde
- Buffered Formaldehyde
- Neutralized Formaldehyde

Acidic	Buffered	Neutral
20% formaldehyde solution (HCHO)	1 L 37% formaldehyde	20% formaldehyde solution (HCHO)
50 mL Glacial acetic acid (CH ₃ COOH)	20 g sodium borate, Na ₂ B ₄ O ₇ , Maintain pH=7.5	100 gm Hexamethylene tetramine

- For 100 mL of sample 2 mL of Formaldehyde should be added.
- For net phytoplankton formaldehyde is added to make one third of volume of the sample.

2.6. Sample Analysis

2.6.1. Sedimentation

Sedimentation allows phytoplankton to settle down to the bottom of the sedimentation flask. Sedimentation of the sample can be done by using 1-liter conical measuring cylinder or in Utermohl's tubular chamber.

2.6.2. Counting

Sedgewick-Rafter Counting Slide

The Sedgewick-Rafter counting slide is a device used for plankton counting. This can be manipulated and provides reproducibility of results with calibrated microscope with eyepiece measuring device. This is a quick method for quantifying samples with high cell numbers. The slide is comprised of a transparent base, which has a centrally mounted chamber (50 mm x 20 mm x 1 mm deep) and can hold 1 mL of sample. The base of this chamber has a ruled 1 mm grid, so that the 1 mL sample is subdivided into single micro liters. This chamber is covered over by a cover glass, which protects the sample from drying out and disturbances by air currents. The sample is then counted using a compound microscope.

$$F = (1000/\text{Number of Squares Counted})$$

To obtain a final result expressed as cells L⁻¹ the following equation is used to calculate the multiplication factor (F). F is dependent on the number of squares of the base of the cell counted during the analysis.

Examples of F for the Sedgewick-Rafter slide:

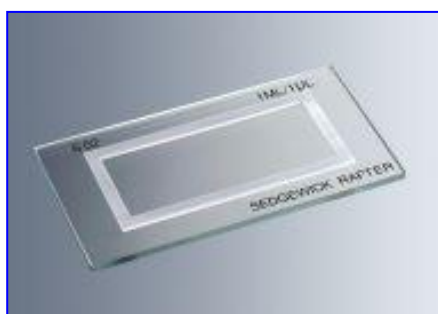
4 rows (200 squares) are counted.

$$\begin{aligned} F &= (1,000/200) * 1,000 \\ &= 5 * 1,000 \\ &= 5,000 \end{aligned}$$

50 rows (1000 squares) or the entire slide is counted.

$$\begin{aligned} F &= (1,000/1,000) * 1,000 \\ &= 1 * 1,000 \\ &= 1,000 \end{aligned}$$

- It allows quick analysis of samples with high density.
- It has been proven for its reproducibility results between 10000 and 100000 cells L-1.
- It is economical in use.
- It is difficult to use this with objectives having high magnification (40X).
- Not suitable for studying nanoplankton.



Sedgewick Rafter counting chamber with lid

<http://www.marienfeld-superior.com>

2.7. Sample Identification

Taxonomists should have hands on experience and high degree of skill and patience for the identification of the species and adequate training should be included in the program.

Phytoplankton may be identified with standard manuals for identification, few of which are listed in the references.

A high quality of microscope is essential for phytoplankton enumeration and identification. The ideal microscope should have phase contrast, oil immersion, and several magnifications (for example - 10X, 40X and 100X). Many species of phytoplankton appear transparent under light microscope. Hence, different techniques should be used to improve the contrast for observation. Differential Interference Contrast (DIC) and Phase contrast are used. The two commonly used microscopes for identification and counting are standard compound microscope and inverted microscope.

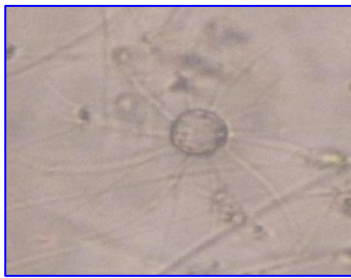
Standard compound microscope: Standard equipment is a set of 10X or 12.5X oculars and 10X, 20X, 40X and 100X objectives. Objectives are used to provide adequate working distance for the counting chamber. Magnification requirements vary with the plankton fraction being investigated, the type of microscope, counting chamber used and optics.

Inverted microscope: The objectives for the inverted microscope are below a movable stage and the illumination comes from above, permitting view of organisms that have settled to the bottom of the chamber. The advantage of the inverted microscope is that by a simple rotation of the nosepiece a specimen can be examined directly in the settling chamber at any desired magnification.

2.8. Conclusion

Data collected on qualitative and quantitative aspects of phytoplankton may be compiled and processed using various statistical tools and presented as per requirement of the project. Phytoplankton is placed at base level in the food chain of sea. It plays an important role in the ecosystem as primary producer and also in climate change by absorbing carbon-di-oxide in the seawater. Phytoplankton are consumed by zooplankton, fish larvae, etc., and these zooplankton are known as secondary producers in the food chain of sea.

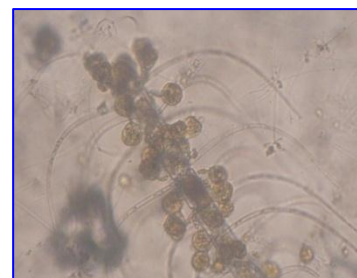
Identified phytoplankton species



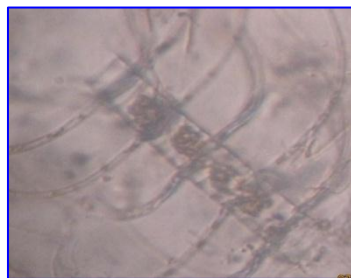
Bacteriastrum sp.



Bellerochea malleus



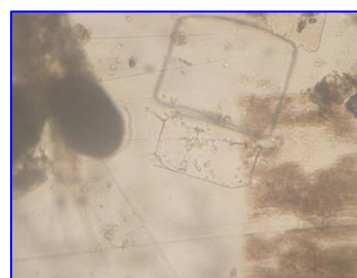
Chaetoceros sp.



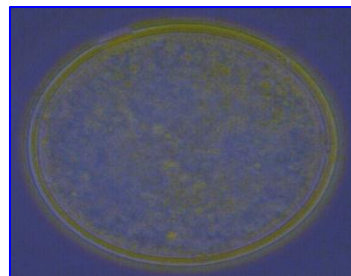
Chaetoceros sp.



Odontella sp.



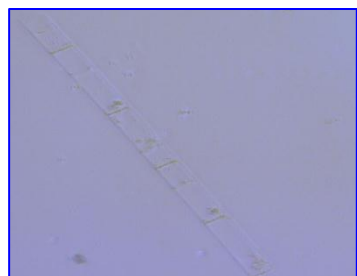
Odontella sp.



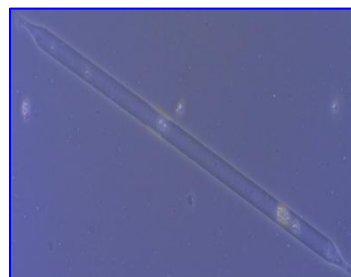
Coscinodiscus sp.



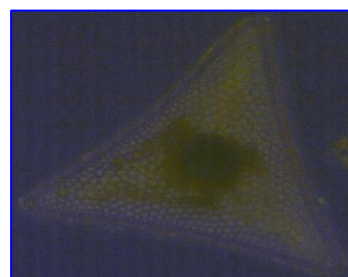
Pleurosigma sp.



Leptocylindrus sp.



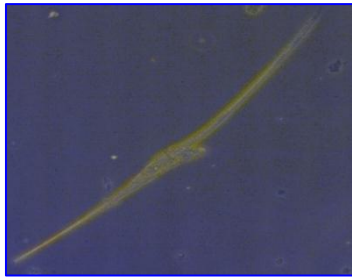
Rhizosolenia sp.



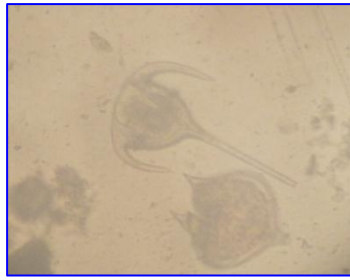
Triceratium sp.



Navicula sp.



Ceratium furca



Ceratium tripos



Ceratium macroceros



Dinophysis caudata



Protoperidinium sp.

3.1. Introduction

Taxonomically, zooplankton range from the most primitive unicellular organisms (protozoans) to vertebrates (fish larvae). Despite their quite limited swimming capacity, they perform day-night rhythmic vertical displacements (migration) of hundreds of meters, most of them to feed at night in surface waters. Their main food source is phytoplankton. However, zooplankton not only include herbivores but also carnivores, omnivores, detritivores and also parasites.

Zooplankton occurrence and distribution influence pelagic fishery potentials. The fishes mostly breed in areas where the planktonic organisms are plenty so that their young ones could get sufficient food for survival and growth.

3.2. Classification of zooplankton

In general, zooplankton are classified in three ways based on different criteria.

- They are divided into holoplankton (whole life as planktonic; eg. copepods, chaetognaths) and meroplankton (planktonic only for a part of life cycle; eg. larvae of benthic molluscs, barnacles).
- Zooplankters are classified into neritic and oceanic. Neritic plankton inhabit inshore waters upto about 200 m depth. Beyond that oceanic plankton prevail.
- Zooplankton were also classified based on size which is given below after incorporating some modifications.

Group	Size range	Examples
Megaplankton	> 20 cm	metazoans; e.g. jellyfish; ctenophores; salps and pyrosomes (pelagic Tunicata); Cephalopoda; Amphipoda
Macroplankton	2→20 cm	metazoans; e.g. Pteropods; Chaetognaths; Euphausiacea (krill) ; Medusae; ctenophores; salps, doliolids and pyrosomes (pelagic Tunicata); Cephalopoda; Janthinidae (one family of gastropods); Amphipoda
Mesoplankton	0.2→20 mm	metazoans; e.g. copepods; Medusae; Cladocera; Ostracoda; Chaetognaths; Pteropods; Tunicata; Heteropoda
Microplankton	20→200 μm	large eukaryotic protists; most phytoplankton; Protozoa, Foraminifera; tintinnids; other ciliates; Rotifera; juvenile metazoans- Crustacea (copepod nauplii)

3.3. Sample collection

Zooplankton collection involves primarily the filtration of sea water by net, collecting the water in bottles/ water samplers or by pumps. The sampling success will largely depend on the selection of a suitable gear; mesh size of netting material, time of collection, water depth and sampling strategy. There are three main methods of zooplankton collection, which are as follows:

3.3.1. Bottles / water samplers:

This method is used mainly for collecting smaller forms or micro-zooplankton. Water is collected in bottles / water samplers of 5 to 20 litre capacity. Surface water can be collected by immersing the bottle into water. While collecting water samples, there should be minimum disturbance of water to prevent avoidance reaction by plankton. Von Dorn bottles or water samplers with closing mechanisms are commonly used for obtaining samples from desired depths. Micro zooplankton are then concentrated by allowing them to settle, centrifuging or fine filtration.

The advantage of this method is that it is easy to operate, and sampling depths are accurately known. The disadvantage is that amount of water filtered is less. Bigger or macro zooplankton and rare forms are usually not collected by this method and so it is unsuitable for qualitative and quantitative estimations.

3.3.2. Pumps

Pumps are normally used on board the vessel/boat. The inlet pipe is lowered into the water and the outlet pipe is connected to a net of suitable mesh size. The zooplankton is filtered through the net. This method is used for quantitative estimation and to study the small scale distribution of plankton.

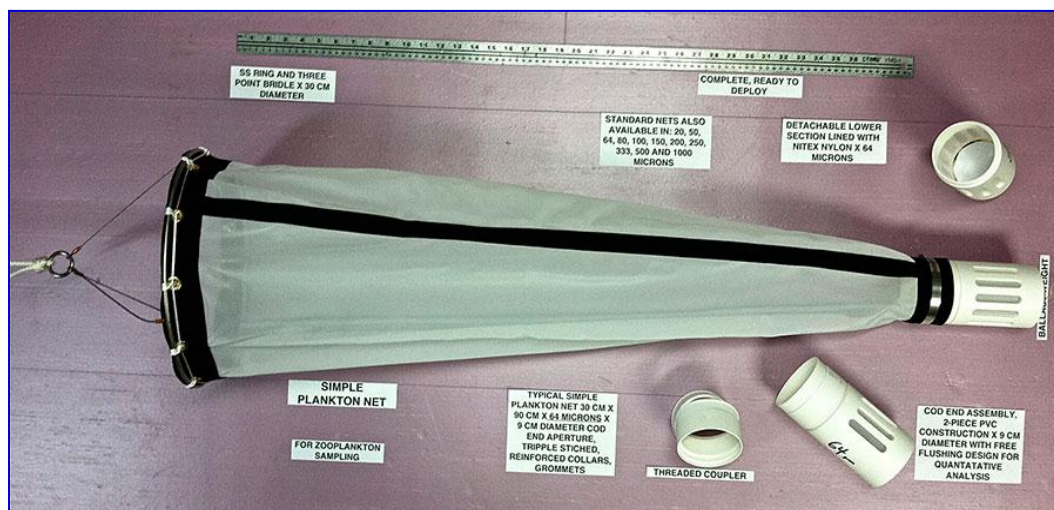
The advantage of this method is that the volume of the water pumped is known and continuous sampling is possible. However, sampling depth is limited to a few meters and it is difficult to obtain samples from deeper layers. Disadvantage is that larger plankton especially the gelatinous forms like the medusae, ctenophores and siphonophores etc can be damaged.

3.3.3. Nets

The most common method of zooplankton collection is by a net. The amount of water filtered is more and the gear is suitable both for qualitative and quantitative studies. The plankton nets used are of various sizes and types. The different nets can broadly be put into two categories, the open type used mainly for horizontal and oblique hauls and the closed nets with messengers for collecting vertical samples from desired depths.

Despite minor variations, plankton net is conical in shape and consists of ring (rigid/ flexible and round/ square), the filtering cone and the collecting bucket for collection of organisms. The collecting bucket should be strong and easy to remove from the net. The netting of the filtering cone is made of bolting silk, nylon or other synthetic material. The material should be durable with

accurate and fixed pore size. The mesh should be square and aperture uniform. The mesh size of the netting material will influence the type of zooplankton collected by a net. The nets with finer mesh will capture smaller organisms, larval stages and eggs of planktonic forms and fish eggs while those with coarse netting material are used for collecting bigger plankton and fish larvae. Sometimes combinations of nets with mesh of different pore sizes are used.



Simple plankton net

(Source: http://www.aquaticresearch.com/simple_plankton_nets.htm)

There is a great variety of mesh available from the finest to the coarse pore sizes. The mesh size of 0.2 mm of monofilament nylon is usually used for collecting zooplankton for taxonomic and productivity studies. In addition to the mesh size, the type, length and mouth area of the net, towing speed, time of collection and type of haul will determine the quality and quantity of zooplankton collected.

The zooplankton collections can be made either by horizontal, oblique or vertical hauls. In the horizontal sampling, the net is towed at a slow speed usually for 5 to 10 minutes. The towing speed of the net should be such that the maximum amount of water enters through the mouth of the net for better filtration and gear used can withstand the strain. The towing speed of the net recommended for horizontal samples is 1.5 to 2.0 knots. When the towing speed is more, a static cone of water develops thus diverting water outside the net and consequently reducing the effective filtration. The net may also be damaged.

Most of the zooplankters migrate vertically in response to light conditions. Their occurrence is poor in upper layers during daytime. For better quantitative and qualitative zooplankton collections, the suitable time for horizontal zooplankton sampling would be before dawn, after dusk or night.

The net should be submerged in water. When the currents are strong the depressors are used to keep the nets in desired position. The horizontal collections are mostly carried out for the surface and subsurface layers.

In oblique hauls, the net is usually towed above the bottom. The disadvantage of this method is that the sampling depth may not be accurately known. The net may be damaged if it touches the substratum.

The vertical haul is made to sample the water column. The net is lowered to the desired depth and hauled slowly upwards. The zooplankton sample collected is from the water column traversed by the net. Closing mechanisms are used to sample a specific body of water. The samples taken with closing nets are analysed to study zooplankton abundance at different depths.

3.3.4 Types of nets

Various types of nets are available to collect zooplankton samples. The choice of net and type of haul should be determined based on the objectives of the study.

- Most commonly used is *Heron -Tranter (HT)* net. It has a square frame and the filtering cone of mesh size of 0.2 mm. The mouth area of the net is 0.25 m². The net is used mainly for collecting horizontal and oblique zooplankton samples.
- For vertical hauls, the nets used are *Nansen Vertical Closing Net*, *Indian Ocean Standard Net (IOSN)* and the *Clark-Bumpus* sampler. These nets are with closing mechanism and are employed to sample at a particular depth.
- To sample whole vertical column in the oceanic waters, a Multiple Opening and Closing Sampler with 5-10 nets is used. This gear is used for zooplankton collections simultaneously at different depths. The nets are closed by means of messengers before retrieval of samplers.
- A high-speed sampler called Hardy's Continuous Plankton Recorder is towed behind the ships or vessels to take continuous samples over long distance.

Precautions: Whatever type of net is used for sampling, it should be thoroughly washed after each tow so that any planktonic material adhering to the mesh of the filtering cone or other part of the plankton net should be pushed into the collecting bucket to prevent contamination of samples with collections from the previous hauls. Washing of nets will also prevent clogging especially when there is a bloom or the finer mesh is used for obtaining the samples. The nets should also be checked for ruptures or holes through which the plankton can escape resulting in the loss of sample. After each haul, the zooplankton sample is transferred into a cleaned and dried glass beaker of half to one litre capacity. The debris or extraneous material should be removed. Replicate hauls are made whenever possible.

3.3.5 Flow meter

For quantitative plankton sampling it is imperative to know the actual amount of water passed through the net. For this purpose, flow meter is used. The flow meter has a multi bladed propeller, which is rotated by the flow of water.



Flow meter



Sampling with flow meter

Flow meter reading and calculations

A flow meter has to be fitted in the middle of the frame of the zooplankton net to estimate the quantity of water filtered through the net, for quantitative estimation of plankton collected. Flow meter is a small device with a propeller at one end and there is a small window on one side where the revolutions of the propeller are indicated in numbers. For the purpose of calibrating the flow meter, the net fitted with flow meter has to be towed for a known distance either vertically or horizontally. The number of revolutions made by the flowmeter during the haul has to be noted from the flowmeter.

The volume of the water column through which the net travelled is then calculated using the formula $\pi r^2 h$, where r is the radius of the mouth ring and h is the known depth or the horizontal distance. By using the volume of water column and the number of revolutions made by the flowmeter, the volume of water which can be filtered in one revolution is found out. This is the calibration factor which can be used to multiply the number of revolutions made at each haul for a particular station to calculate the volume of water filtered by the net.

3.4. Fixation

The necessity of proper fixation and preservation of zooplankton needs no emphasis. The poorly fixed and preserved samples would render their subsequent analysis difficult. The whitish precipitate and ruptured exoskeletons can be seen in the improper fixed samples. The zooplankton deteriorates rapidly in tropics. After the sampling, the fixation of samples should be carried out, as early as possible, at least within 5 minutes after the collection to avoid damage to animal tissue by bacterial action and autolysis. An ideal fixative should be cheap and which kills the animals quickly. Again it should be non-corrosive or toxic in nature. The most common fixing and preserving reagent is (4-5%) formaldehyde (formalin). It is the cheapest fixative and the zooplankton samples can be stored for number of years.

The other fixatives occasionally used are ethanol, picric acid, acetic acid etc. Analytical grade formalin should be used for fixation as the commercial formalin is often contaminated with iron compounds which produce a brown precipitate of iron hydroxide which renders the zooplankton identification difficult. The concentrated formalin should be diluted with fresh water, seawater or preferably with water from the sampling area to avoid undesirable osmotic effects. The dilution is in the ratio of 1 part formalin and 9 parts of fresh water or seawater. The pH of the fixative should be around 8.0.

It is advisable to use buffered formalin. The commonly used buffers are borax (sodium tetraborate) or hexamethylene tetramine. The buffers are added in an amount of 200 g to one litre of concentrated formalin. The fixative usually renders the zooplankton body tissues hard and brittle. The additives viz. propylene phenoxetal and propylene glycerol (2 to 5 %) are added to fixatives for flexibility of specimens, resistance to bacteria and moulds.

3.5. Preservation

After fixation, the zooplankton are transferred and stored in airtight containers with sufficient quantity of preservative. While transferring, due care should be taken so that no part of the zooplankton sample is lost. Various types of preservatives are available. The buffered formalin (4 to 5%) is mostly used both as fixative and as the preservative. The other preservative used is 70% ethanol or 40% isopropanol. The ethanol is used for preserving museum specimens but it is costly and volatile. Glycerine is often added to formalin to prevent shrinkage of specimens, drying of the material and to facilitate retaining colours of zooplankters. For better shelf life of the zooplankton samples, the preservative should be changed within the first 6 months.

It would be better to store the preserved zooplankton samples in well ventilated room at temperature less than 25°C. The samples should be kept in the wide mouth glass/polyethylene jars. A good quality of pre-printed labels, on which the collector's name, fixative and preservative used and other field information written should be put into the jars for ready reference at the time of sample analysis.

3.6. Analysis of the samples

The basic analysis consists of measurements of biomass (standing stock), enumeration of common taxa and species.

3.6.1. Biomass

The term biomass denotes the live weight or the amount of living matter present in the zooplankton sample. The value obtained is used to evaluate the secondary productivity and fishery potentials of the study area. The biomass is estimated by the following methods.

- i. Volumetric (displacement volume and settling volume) method
- ii. Gravimetric (wet weight, dry weight and ash free dry weight) method
- iii. Chemical method

Prior to determination of biomass, larger zooplankters such as medusae, ctenophores, salps, siphonophores and fish larvae should be separated from the zooplankton sample and their biomass taken separately. The total biomass would be the biomass of bigger forms plus the biomass of the rest of the zooplankton. It should be indicated under remark as given on the analysis sheet.

- i) Volumetric method:** The total zooplankton volume is determined by the displacement volume method. In this method the zooplankton sample is filtered through a piece of clean, dried netting material. The filtered zooplankton is transferred with a spatula to a measuring cylinder with a known volume of 4 % buffered formalin. The displacement volume is obtained by recording the volume of fixative in the measuring jar displaced by the zooplankton. The settled volume is obtained by making the sample to a known volume in the measuring jar. The plankton is allowed to settle for at least 24 hours before recording the settled volume.

- ii) Gravimetric method:** The weight measurement should be done preferably in laboratory. It is carried out by filtering the zooplankton. The zooplankton weight is taken on pre-determined or weighed filter paper or aluminium foil. The wet weight is expressed in grams. The dry weight method is dependable as the values indicate the organic content of the plankton. Analysis such as the dry weight is determined by drying an aliquot of the zooplankton sample in an electric oven at a constant temperature of 60°C. The values are expressed in milligram. Ash free dry weight method is also occasionally used.

- iii) Chemical method:** In this method, the live zooplankton samples are dry frozen. Before analysis, the samples are rinsed with distilled water. Measurement of constituent elements such as carbon, nitrogen, phosphorus and biochemical elements viz. protein, lipid and carbohydrates are made. Sometimes the biochemical values of a particular taxon and species are undertaken to evaluate food energy transfer at higher trophic levels. The calorific content of the plankton can be used as an index of zooplankton biomass.

3.6.2. Biomass (standing stock)

After estimation of zooplankton biomass, the standing stock values are converted into per cubic meter and is calculated as follows:

a.) Volume of zooplankton (ml/m ³)	Total volume of zooplankton Volume of water filtered (V)
b.) Wet weight of zooplankton (g/m ³)	Total wet weight of zooplankton Volume of water filtered (V)
c.) Dry weight of zooplankton (mg/m ³)	Total dry weight of zooplankton Volume of water filtered (V)

3.6.3. Faunal enumeration

Information on the faunal composition and the relative abundance of different zooplankton taxa and their species is obtained by counting the plankters present in the samples. For enumeration it is recommended that the subsample or an aliquot is taken for the common taxa. However, for the rare groups, the total counts of the specimens in the samples should be made. For enumeration of zooplankton the subsample or aliquot of 10 to 25% is usually examined. However, the percentage of aliquot can be increased or decreased depending on the abundance of zooplankton in the sample.



Folsom Plankton Splitter

Instruments are available for splitting the sample into fractions such as *Folsom Plankton Splitter*. The zooplankton sample to be sub-sampled is poured into the drum and the drum is rotated slowly back and forth. Internal partitions divide the samples into equal fractions.

The fraction may be poured again into the drum for further splitting. The process is repeated until a suitable subsample is obtained for counting. The splitter is thoroughly rinsed to recover the organisms, which may be sticking onto the wall of the drum.

The sample is usually split into 4 subsamples. One of the subsamples is used for estimation of dry weight, the second for counting the specimens of common taxa, the third for relative abundance of species and the fourth fraction is kept as reference collection.

Plastic or glass pipettes are also used to take the subsample for counting. The Stempel Pipette is used to obtain a certain volume (0.1 to 10 ml). The zooplankton sample in a glass container is diluted to a known volume and is stirred gently. The Stempel Pipette is then used to remove the subsample or aliquot for counting.

a) Counting

After splitting, the next step in the analysis is to sort and count the specimens. There is a primary and secondary sorting. In the former type the sample is separated into 30 to 40 taxonomic groups (Appendix II). Whereas in the secondary stage the important groups of organisms or specimens are further separated or sorted into their respective families and genera.

The zooplankton group diversity is higher in the marine environment. The counting should be done under the microscope and when the specimen of a particular group is seen, a tally mark is made on the sheet. When different groups are to be counted simultaneously, a multiple counter is used. All the specimens present in the subsample are counted with proper records on the data sheet. The total

numbers of organisms are later calculated for the whole sample depending on the percentage of subsamples examined. Image analysis systems are being tried for rapid counting of common taxa and their species. For sorting, the help of illustrations could be taken.

b) Clearing

The fixed specimens must be cleared of any attached material such as detritus or precipitate. This can be done by removing the extraneous substances with fine forceps/needles without damaging the specimens. Examination of external features becomes easier after clearing the specimens. To study the internal structures staining of specimen is required.

c) Staining and dissection

Light staining of the specimens is carried out by adding a few drops of rose Bengal, lignin pink, chlorazol black E and methylene blue added to the lactic acid. Borax carmine is used for staining small zooplankton, larval stages of crustaceans and ichthyoplankton (fish eggs and fish larvae). Rose Bengal is usually added to the zooplankton samples when the preservation is done. The dissection of stained specimens is carried out under stereoscopic dissecting microscope with fine needles on the cavity slides.

3.7. Species identification

Correct species identification is prerequisite for understanding distributional pattern, seasonal variability and community structure of zooplankton in an aquatic ecosystem. It is a specialized work and requires patience, experience and sufficient published literature. The initial identification of common species could be done with the help of illustrated checklists. The taxonomic experts should later confirm the identification. The identified and labelled specimens should be kept properly for further reference. For identification of species a stereoscopic dissecting microscope, good quality glass slides, cover slips, stainless steel fine forceps, dissecting needles, pipettes and chemical reagents are required. It involves various steps such as cleaning of specimens, staining, dissection and slide preparation.

The species under different taxa should be identified and enumerated with the help of experts in different taxon and standard and descriptive manuals for identification. Data on faunal and species occurrence and abundance is useful to evaluate the biodiversity of any ecosystem or area.

3.7.1. Species diversity

Species diversity is defined as the number of species present in an area. The values can be used to assess the health of the environments. The members of species are less in the polluted areas. The species diversity is calculated by the method given by.

Shannon and Weaver (1949)

$$H' = - \sum_{i=1}^n P_i \log_2 P_i$$

Where P_i = proportion of the number of individuals of species i to the total number of individuals ($P_i = n_i / N$)
 n = total number of species.
 N = total number of individuals and
 n_1 & n_2 are the respective number of individuals of each species

Margalef (1968)

$$D = S - 1 / \log_e N$$

Where S = is number of species and
 N = the total number of individuals

Pielou (1966)

Evenness $E = H' / \log_2 S$

Where H' = is the Shannon Weaver's index
 S = the total number of species

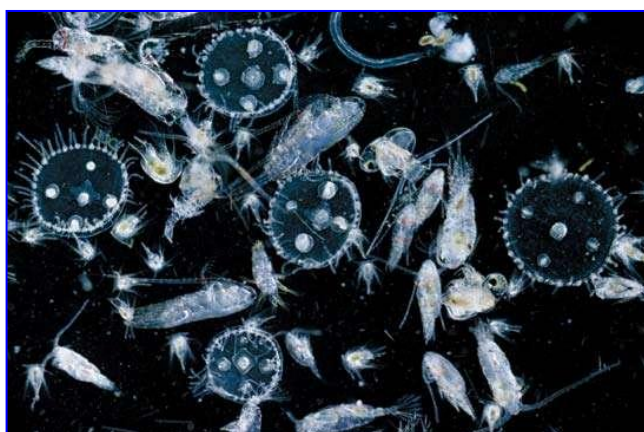
3.7.2. Faunal Composition

- a) Total number of zooplankton specimens/ individuals of all groups
Total counts of the specimens (say x).
 Volume of water filtered (V)

$$\text{No}/\text{m}^3 = x/y \text{ (No. can also be expressed/ } 100 \text{ m}^3 \text{ or } 1000 \text{ m}^3\text{)}$$

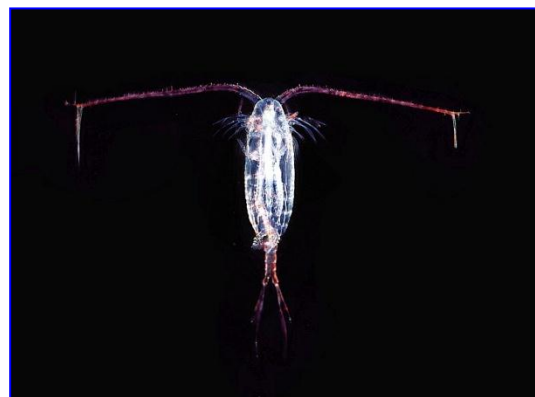
- b) Total number of specimens of a particular zooplankton taxon
Total counts (x)
Volume of water filtered (Y)
 $\text{No}/\text{m}^3 = x / y$

Assorted marine zooplankton species are shown below:





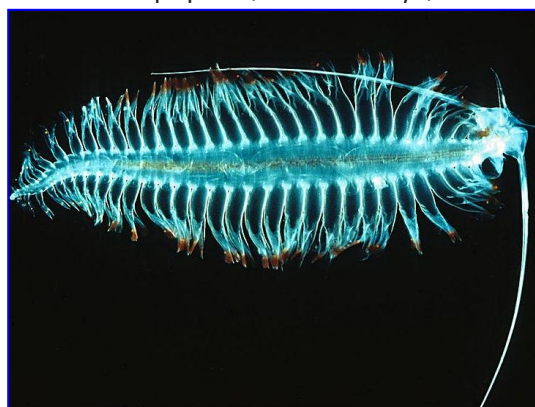
Antarctic krill (Euphasidae)



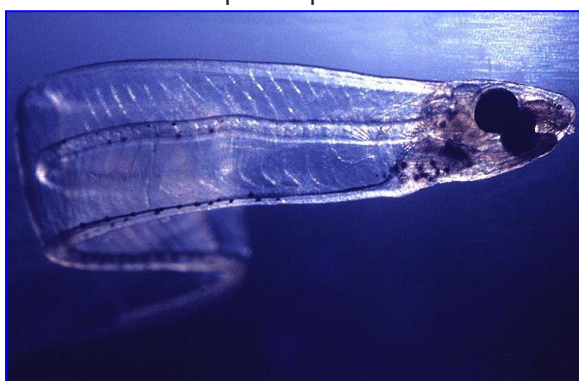
Copepod (*Calanoida* sp.)



Siphonophora



Tomopteris sp. (polychaete)







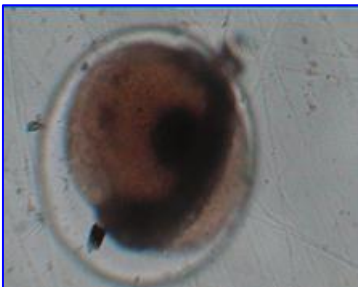

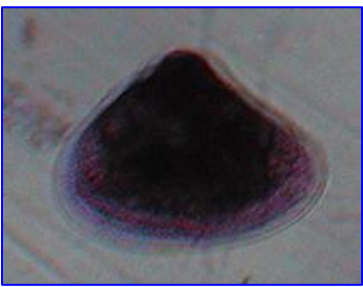










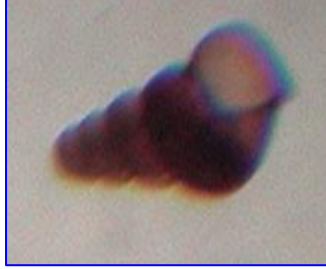
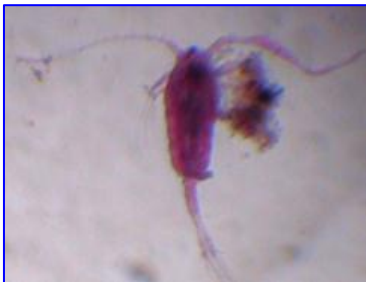
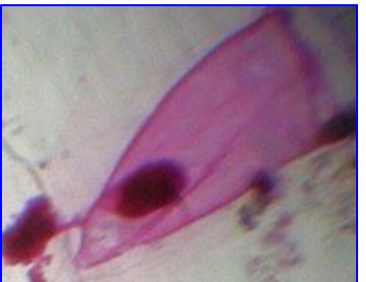




Eel larva






Amphipod (crustacean)

Zooplankton

		
<i>Calanopia minor</i>	<i>Subeucalanus flemingeri</i>	<i>Macrosetella</i> sp.
		
<i>Clausocalanus minor</i>	<i>Eucalanus attenuatus</i>	<i>Corycaeus</i> sp.
		
Fish egg	<i>Oithona brevicornis</i>	Bivalve veliger
		
Crab zoea	<i>Centropages furcatus</i>	Polychaete larvae

		
<i>Sagitta</i> sp.	<i>Oithona spirostris</i>	Fish larva
		
<i>Eucalanus elongatus</i>	<i>Pseudocalanus elongatus</i>	Gastropods Veliger
		
<i>Tortanus barbatus</i>	<i>Diphysis</i> sp.	<i>Copilia vitrea</i>
		
<i>Creseis</i> sp.	<i>Evadne</i> sp.	<i>Paracalanus parvus</i>

		
<i>Microsetella</i> sp.	Mysids	<i>Lucifer</i> sp.

CHAPTER 4

MARINE BENTHOS

4.1. Introduction

Benthic community comprises of a wide range of organisms from bacteria to plants (phytobenthos) and animals (zoobenthos) and from different levels of food web. In this chapter, we shall discuss about zoobenthos (bacteria, seaweeds, seagrasses are dealt with in other chapters). Some of the well-known groups of zoobenthos are worms (eg. polychaetes, oligochaetes), molluscs (eg. bivalves, gastropods), and crustaceans (eg. amphipods, decapods).

Based on size, benthos may be classified into

- macrobenthos (retained on 0.5 mm screen /sieve),
- meiobenthos (that pass through 0.5 mm sieve but retained on 0.062 mm sieve), and,
- microbenthos (which pass through 0.062 mm sieve).

However, the division of the benthos according to size is only arbitrary as the size of organisms may change with age (Wolf, 1983).

Based on the position they occupy on or in bottom sediments, benthos can be classified as:

- *infauna* - animals that live *in* sediments, almost all worms and bivalves belong to this category, and,
- *epifauna* - organisms that live *on* the surface of bottom sediments; many crabs and gastropods are considered epifauna.

Benthic invertebrates play an important role in transitional ecosystems, by filtering phytoplankton and then acting as a food source for larger organisms such as fish, thereby linking primary production with higher trophic levels.

Some of the benthic communities are often used as biological indicators because they can provide information on environmental conditions due to their sedentary nature and ability to accumulate pollutants, etc., on their body tissues.

Polychaetes, molluscs and crustaceans are the major taxa that occur in subtidal and intertidal benthos, among other groups.

Polychaetes: Polychaetes, are usually the most abundant animals living within sand and mud on the seashore. They can be divided into two groups as errant (free-moving) forms and sedentary forms, although the distinction between the two groups is not always sharp. Many polychaetes are strikingly beautiful and are coloured red, pink, or green or possess a combination of colors. Some are iridescent, owing to the presence of crossed layers of collagen fibers in the cuticle.

Crustaceans: Meiobenthic species include ostracods and the cyclopid and harpacticoid copepods. Harpacticoids are an especially abundant group whose members crawl over or burrow through soft sediments. Also included in this size category are the tanaids. Benthic decapod crustaceans include the familiar isopods, amphipods, crabs, lobsters, and shrimp, and the group has both epifaunal and infaunal representatives. Decapods show their greatest diversity in shallower waters, but a few species live at depths of 5000-6000 m.

Molluscs: Phylum Mollusca includes the most conspicuous and familiar invertebrates which include clams, oysters, squids, octopods and snails. Abbott (1954) has estimated the total number of molluscs to be 1,00,000 that include 80,000 gastropods, 10,000 bivalves and 5,000 of other classes. Molluscs are highly adaptive. The geographical distribution of this group is worldwide and molluscs are dominant organisms in any littoral, shallow and sublittoral ecosystems.

4.2. Description of benthic biotopes

Benthic organisms typically occupy intertidal and subtidal areas of the shore and seabed.

4.2.1. Intertidal biotope

The intertidal zone, in marine aquatic environments is the area of the foreshore and seabed that is exposed (to air) at low tide and submerged at high tide, i.e. the area between high and low tidal extremes.

In the intertidal zone, most common organisms are small and most are relatively uncomplicated organisms, because of a variety of reasons; such as intermittent supply of seawater, wave action, high exposure to sun, temperature extremes, high salinity due to evaporation, etc.

Various types of intertidal regions exist, such as, rocky shore, sandy shore, muddy shores, etc.

Rocky shore: A typical rocky intertidal shore can be divided into four vertical zones, based on height and tidal influence. They are splash zone (above the spring high-tide line), high, mid and low intertidal zone. The splash or spray zone is the highest and driest area.

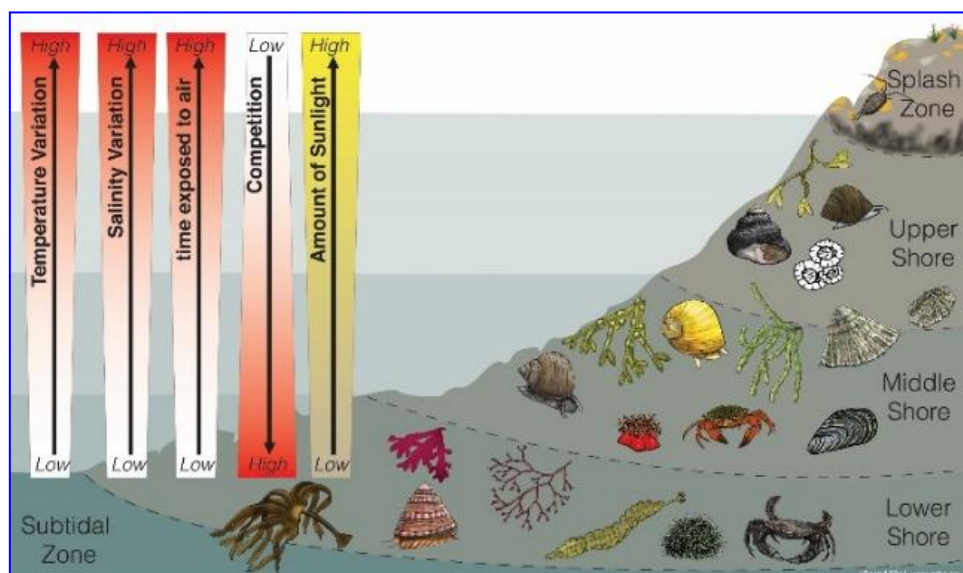
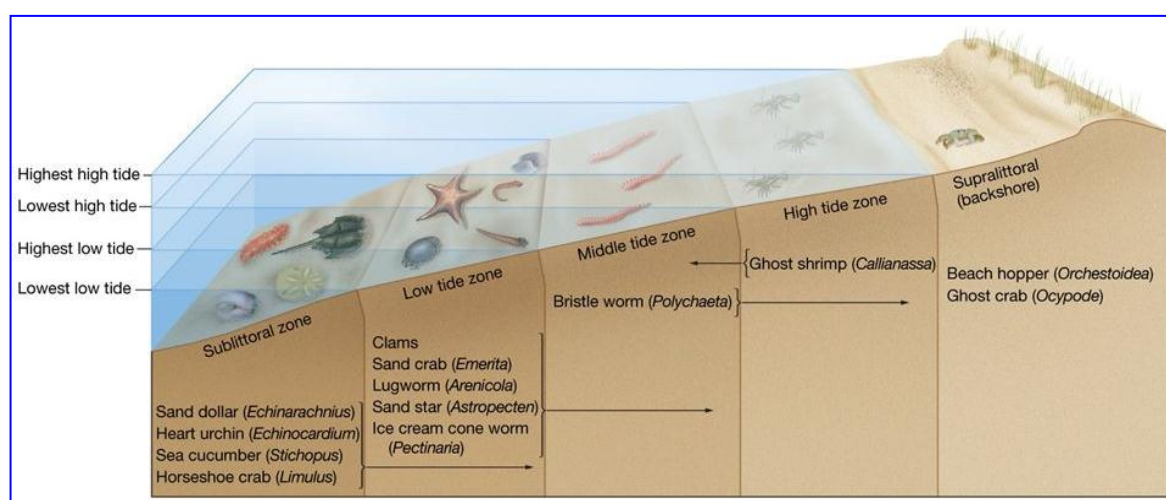


Illustration of rocky shore

Sandy shore: Sandy ecosystems are the most common intertidal habitat around the world. The sediment on coastal beaches is sorted by the physical action of water currents, wind and waves. How much the sand grains are sorted depends greatly on the amount of wave action, geographical location and beach aspect.

Larger invertebrates of the sandy beach include polychaete worms, clams, whelks and crustaceans, which can be scavengers, predators, and filter- or deposit feeders.

The tropical beaches are more species rich, whereas temperate beaches tend to support higher abundance and biomass. Sandy beach surf zones serve as important nursery and foraging areas for fishes. Beaches are important nesting areas for marine turtles and shorebirds.



Sandy shore

Muddy shore: Mud banks are well known for their fishery during the monsoon months. The calm waters of the mud banks act as a temporary fishing harbour. Mud banks support rich variety of fauna.

4.2.2. Subtidal region

The subtidal areas shall extend to various depths and can comprise of rocky, sandy and muddy substrate and support rich faunal resources.

4.3. Types of benthic habitats

Three categories of benthic habitats are envisaged to study the geographic distribution and abundance of benthic organisms. They are estuarine, inshore/nearshore and continental shelf habitats, each characteristic of distinct geomorphic, environmental setting.

Estuarine habitats consist of periodically flooded substrates where tidal seawater is diluted by flowing fresh water. This mix of fresh and ocean waters usually forms a horizontal salinity gradient that varies by area and location with seasonal variations in fresh water inflow and tidal action. The largest number of estuarine organisms are benthic and include hydrozoans, anthozoans, annelid worms, crustaceans, molluscs and fishes. Such organisms attach to the bottom substrate, bury in the mud, or live in crevices (Smith, 1974). Molluscs, including gastropods and bivalves, also occur in this zone. Salinity is the major factor that determines the distribution of benthic organisms.

Inshore or nearshore habitats include seagrass meadows, salt marshes, mangrove forests, coral reefs and tidal flats. "Inshore-dependent" species are dependent upon inshore ecosystems for essential activities such as reproduction (as spawning or nursery areas), migration, or feeding. Moreover, without such critical inshore habitats, these species would not exist in abundance. The living resources of inshore waters are predominantly juveniles - often the young of species hatched offshore and carried by currents to coastal waters or downstream migrants from headwater spawning sites.

Continental shelf is the shallow underwater extension of a continent up to 200m depth. The substratum of this zone is generally of a soft consistency and is largely composed of sand, mud and clay. The ample supply of food and oxygen and the optimum conditions of temperature, light intensity and salinity are responsible for the richness of the fauna in this zone.

4.4. Sample collection

4.4.1. Sampling devices

To efficiently sample the benthic fauna a sampler must be able to penetrate to a depth sufficient to capture the organisms present. Most report that majority of species and individuals to be present in the upper 5 to 10 cm, although large burrowing molluscs and crustaceans may be found deeper.

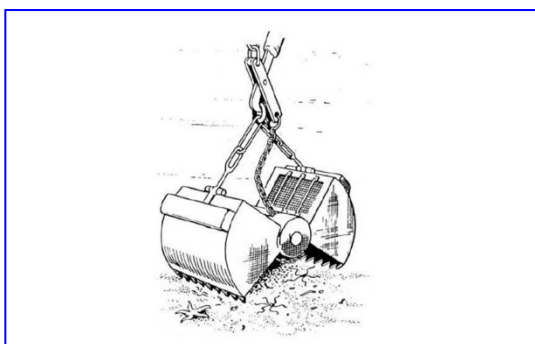
In the intertidal region, 625 cm² wooden quadrat is placed and the substrate is dug out for 10 cm. Sub tidal forms can be quantitatively estimated using Petersen grab covering an area of about 625 cm² penetrating 5 - 10 cm depth and calculated to a square meter.

Devices used to sample soft bottom macrobenthos basically comprise grabs, dredges, box-corers and hand nets. Grabs and corers are suitable for quantitative studies, i.e. when collection of a defined amount of sediment is required. These devices allow good reproducibility and reliable replicates of the samples. A variety of grabs and box corer is available for sampling. The choice of the most suitable device depends on various factors including operational conditions, substrate and physical characteristics of the investigated habitat.

The Petersen grab is found to be effective to sample in mangrove and estuarine environment while long armed Van – Veen grab, Smith–McIntyre grab and box corer are effective in collecting sediments in deep water bodies (inshore, continental shelf and continental slope). Besides sediment grabs, dredges, trawls and traps are also used for collecting benthos for qualitative purposes.

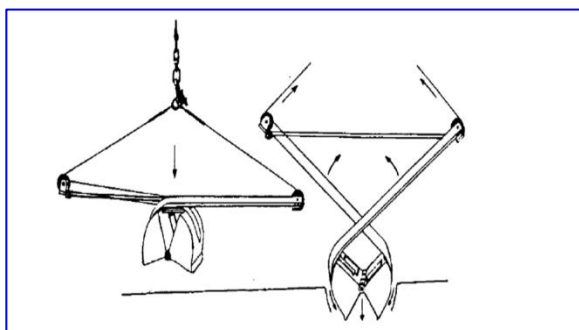
Petersen grab: Petersen grab consists of two buckets hinged together, which are held in an open position during lowering. The top of each bucket has a gauze-covered window to allow water to escape while the grab is closing. However, this offers some resistance to the rapid lowering of the gear, which is desirable when sampling in deeper water. When on the bottom, the lowering rope slackens, allowing a release hook to operate so that on hauling the two buckets close together before the grab leaves the bottom.

Disadvantages of Petersen grab for sampling in other than soft muds and sheltered waters include premature operation of the release during descent due to momentary slackening of the rope as the ship rolls, failure to penetrate sufficiently deeply into the sediment, losses due to the jaws not closing completely and inadequate sampling due to an oblique upward pull when closing due to drift of the ship on station. For these reasons the Petersen grab has not been much in use in recent years, many workers choosing alternative sampling gear.



Petersen grab in sampling position on the seafloor. After the release hook has actuated, an upward pull exerted on the central chain closes the two buckets of the grab (After Hardy, 1959.)

Van Veen grab: The Van Veen grab improves on the Petersen grab in having long arms attached to each bucket, thus giving better leverage for closing. The arms also tend to prevent the grab from being jerked off the bottom, should the ship roll as the grab is closing. On the other hand, the arms may pull the grab to one side if, through drift of the ship the upward pull for closing is oblique. The Van Veen grab is easily operated by a rope and, therefore, it is suitable for relatively deep waters but has the disadvantage that it takes an uneven mouth-shaped bite of the sediment.



Long arm, warp rigged Van Veen grab in sampling operation. (Redrawn from Rumohr 1999.)

The area sampled by a device is, in general, a compromise between ease of handling and the need to get representative samples; a high number of relatively small samples often yield better information than a very small number of large samples. A surface area of about 200–600 cm² is typical for grabs deployed in transitional environments. Replicate samples are usually needed because of the variability in spatial distribution of macrobenthic organisms.

A sampling device must recover relatively undisturbed samples to a depth sufficient to collect the majority of the burrowing organisms dwelling in the deeper layers of the soft bottom. In coastal wetlands a sample depth of about 15–20 cm is usually adequate.

4.5. Sieving

Samples are sieved in order to remove fine sediments and any other extraneous material. Once on board the boat, the grab is opened above a plastic bucket and the sample gently removed. Before sieving, the sample should be described and notes on surface characteristics, individual density, occurrence of organic detritus, etc. should be recorded in a sampling form.

When clay sediments are present, which is common in estuaries, it is advisable to break up the sediment in water inside the bucket by adding filtered seawater and stirring gently. (Filtered water is used to avoid the introduction of unrelated small organisms.)

The sample is then sieved; water is sprinkled directly onto the sample with a low-pressure nozzle in order to prevent any damage to animals. If the boat is too small or time is short, the samples can be kept in watertight plastic bags in a thermally isolated container and, during the warm season, cooled with icepacks.

The delicate process of sieving should be performed very carefully in order to avoid any damage to the fragile organisms and to ensure that all animal present in the sample are collected. In order to separate macrofauna, a sieve of 1 mm or 0.5 mm mesh is used.

A 1 mm mesh is preferable when the sediment contains a large amount of detritus, as often happens in wetlands and estuarine environments, in order to prevent clogging of the sieve. In any case the sieve must have an adequate surface to avoid clogging. Aquatic vegetation present in the sample should be cautiously removed from the surface of the sample, rinsed apart and the resulting water sieved.

All material retained on the sieve, including organisms, shell fragments, vegetal debris and coarse sediment grains, are transferred to appropriate containers.

The material is removed from the sieve using a water jet and conveyed through a funnel into a fine mesh bag (a nylon sock) fixed to the outlet of the funnel. The bag is then put into a suitable plastic container and labelled.

Containers must be labelled both internally and externally; the external label can be written with a permanent marker, the internal label can be made of tracing paper, written in lead pencil or Indian ink. The labels must record: the station code, the sample code, sampling date and split number for any sampling replicates.

4.6. Fixation and preservation

The sieved material is fixed as a whole in the plastic container. The volume of the fixative should be approximately three times the volume of the sample. The presence of considerable quantities of organic matter requires a larger quantity of fixative. The fixation of organisms may be achieved within a couple of days. The most common fixative for benthic organisms is a 10% formalin solution (or 4% formaldehyde). Formalin is a commercial aqueous solution of 40% formaldehyde. Formalin is an acid; therefore it should be buffered in order to avoid the dissolution of calcareous parts of the organisms. Extreme caution should be used in the manipulation of formalin because formaldehyde is toxic and carcinogenic. Less toxic fixatives are available as an alternative; for example, an alcohol such as denaturated ethanol can be used. It does not perform as well as a fixative, but it is much less toxic than formalin.

After a few days, the samples are fixed and can be removed from the fixing solution, rinsed and placed in a preserving solution. The most common preservative is an aqueous solution of ethanol composed of 70% ethanol and 5% glycerin. Some researchers find it useful to stain the sample to accelerate the sorting procedure. One of the most common stains is Rose Bengal, which should be used carefully and sparingly because it is considered carcinogenic.

4.7. Laboratory procedures

4.7.1. Sorting

Sorting consists of picking up from the sieved material all the animals that were alive at the moment of the sampling. Sorting procedures are performed under fume hoods to prevent inhalation of vapours of residual toxic substances. Large samples can be subdivided into sub-samples of roughly equal size that can be sorted more comfortably. The sub-samples should be placed in different jars with preserving solution and labelled.

A small quantity of unsorted material is placed on a tray for an initial general sorting for larger organisms with the help of a magnifying lens. Shell fragments, vegetal debris or coarse detritus in the sample should be rinsed in a separate container and checked for the presence of invertebrates.

Large organisms are placed in appropriate containers making sure that no other smaller animals are attached to their bodies. Fine sorting is performed under a dissection microscope. During this phase a small quantity of the sample is spread onto a Petri dish and carefully examined to identify the organisms.

Organisms are picked up and placed in different containers according to the main taxonomic groups, usually polychaetes, other worms (oligochaetes, nematodes, nemertines, etc.), bivalves, gastropods, amphipods, other crustaceans, insects, cnidarians, sponges and other animals.

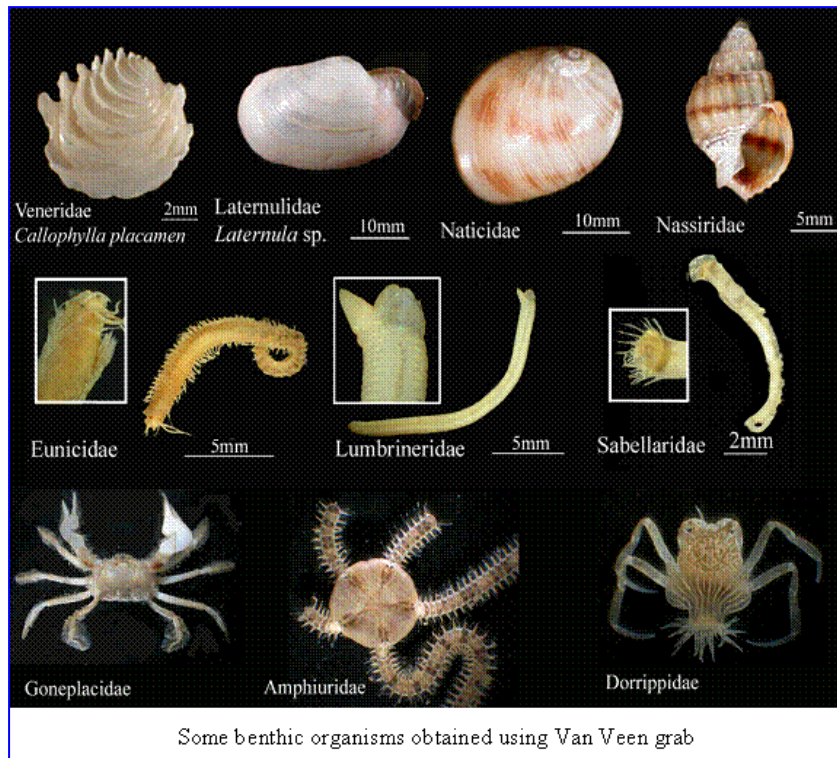
4.7.2. Identification procedures

Once sorted, animals are identified to the taxonomic level required by the investigation. For monitoring purposes, the level of taxonomic identification required is sometimes the family or even higher level, but often the species level is required. The instruments used in identification are a dissecting stereomicroscope coupled with a compound microscope when observation of fine details is needed.

Identification is done with a help of identification keys. There are keys for any major group of organisms, for example, gastropods, bivalves, amphipods etc. For correct identification, accurate analytical keys for the geographic region from which the samples were taken should be used. All taxa can be identified to their species or generic to the extent possible with the help of standard taxonomic references (e.g. Polychaeta: Fauvel (1953), Day (1967); Decapoda: FAO Identification sheets (1983), Alcock (1985); Mollusca: Abott and Dance (1982), Subba Rao (2003); Fishes: Smith and Heemstra (1986), www.marinespecies.org).

To catalogue species correctly it is strongly recommended that the international checklists of species, e.g. the European Register of Marine Species (ERMS) or Integrated Taxonomic Information System (ITIS), or national checklists are consulted. A high level of expertise is often required to achieve complete identification of the benthic fauna of a coastal wetland.

Specieswise abundance data are useful in calculations of various indices and deriving meaningful information about the status of environment.



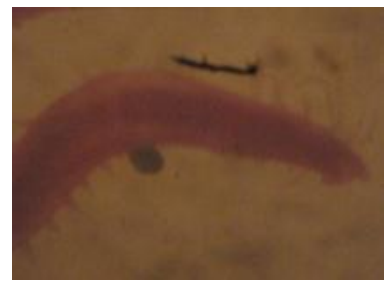
Macrobenthic organisms



Pisone indica



Prionospio sp.



Capitella sp.



Glycera sp.



Nereis sp.



Lacydonia sp.



Glycinde sp.



Gyptis sp.



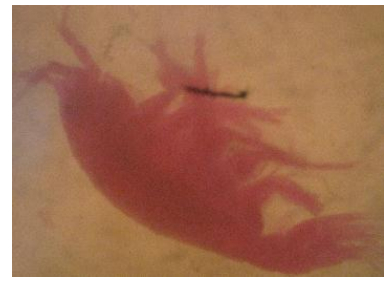
Ancistrosyllis sp.



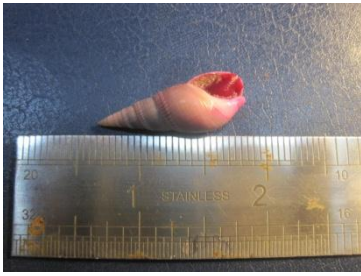
Onuphis sp.



Cirratulus sp.



Amphipod



Bullia vitata



Oliva sp.



Umboonium sp.

5.1. Introduction

The reef building (hard) corals are the building blocks of coral reefs and they cover nearly 0.9% of the world ocean. Coral reefs provide home for a number of marine fauna and flora. Large portions of the world's coral reef are reported to occur within the Indian Ocean (Venkataraman, 2006). Till today, 1574 scleractinian coral species belonging to 25 families are reported from the world, of which 19 families, 92 genera, 592 species are reported from India. **The most diverse region of the world for coral reefs is located in coral triangle viz. Philippines, Indonesia, Malaysia and Papua New Guinea, with between 500 and 600 species of coral species in each of these countries. Unfortunately, these are also some of the most threatened coral reefs of the world.**

5.2. Coral Reefs in India

In India, the reefs are distributed along the east and west coasts. It covers about 5,790 km² and is divided into three major zones: The Andaman and Nicobar Islands; the coral reefs of the mainland; and the Lakshadweep Islands. Reefs from mainland include Gulf of Kachchh, Gulf of Mannar, Ratnagiri, Malvan and Orissa coast.

India has four major coral reefs viz., Gulf of Kachchh, Palk Bay and Gulf of Mannar, Andaman and Nicobar Islands and Lakshadweep.

Gulf of Kachchh: GoK comprises 42 islands. Tidal range in the Gulf is as great as ~12 m. Gulf of Kachchh has fringing reefs, but due to high tidal amplitude, high temperature, salinity changes and high suspended particulate loads, which control coral settlement, coral growth and gamete release, these reefs are restricted with shallow water corals.

Palk Bay and Gulf of Mannar: In Palk bay there is a barrier reef, less than 200 m wide and at the depth of 6.0 m. The lagoon is shallow and can be waded through at lowest tides. Thus, this reef restricts encrusting and boulder corals. Different types of reef forms such as shore platforms, patch, and fringing type are observed in Gulf of Mannar regions. The Gulf of Mannar reefs are developed around a chain of 21 islands that lie along the 140 km stretch between Tuticorin and Rameswaram. The islands are centered about 8 km away from the main land. They are a part of the Mannar barrier reef, which are about 140 km long and 25 km wide between Pamban and Tuticorin. Narrow fringing reefs are located mostly at a distance of 50 to 100 m from the Islands. On the other hand patchy reefs rise from depths of two to nine m and extend to one to two km in length. Reef flat is extensive in almost all the



reefs in the Gulf of Mannar. However, unusual monsoon, coral mining and high sedimentation load affect visibility, and mostly restricted to large corallites possessing corals like Faviidae.

Lakshadweep Islands: Lakshadweep Islands are scattered in the Arabian sea about 255 – 450 km away from Kerala, which consist, chain of coral atolls and reef on a continuous submarine banks like Andaman western invisible coral banks, covering a distance of over 2000 km. Geographically, the Islands lie between 8°N - 12°3' N lat. and 71°E - 74°E long. There are six tiny islands, 12 atolls, three reefs and five submerged banks, covering an area of 32 km² with lagoons occupying about 4200 km². The islands consist of coral formations built up on the Laccadives – Chagos submarine ridge rising steeply from a depth of about 1500 m to 4000 m off the west coast of India. The Islands are flat and scarcely rise more than 2m. They are made up of coral sand and boulders that have been compacted into sandstone. Coral reefs of the Islands are mainly atolls except one platform at Andrott. The reef flat occupies 136.5 km² area, sea grass occupies 10.9 km² and lagoon occupies 309.4 km². The depth of the sea increases outside the coral reef and can reach up to 1500 - 3000 m. *Acropora*, *Pocillopora*, *Psammocora* and some encrusting faviids dominate the Lakshadweep reefs.

Andaman and Nicobar Islands: The coral reefs of this archipelago are of fringing type and a barrier reef to the west has also been reported with a lagoon up to 40 m deep on the western side, but its precise coordinates are yet unknown. Hence, the reefs of the area still largely remain unstudied. A deep oceanic ridge along 10° N separates the Andaman group and Nicobar group of Islands. Coral reefs of Andaman and Nicobar Islands can be grouped into five major zones as follows: North Andaman, Middle Andaman, South Andaman, Little Andaman and Nicobar reefs.

Malvan and Ratnagiri coast: The west coast of India is reported patchy submerged banks with isolated coastal formation. Coral patches have been recorded in the intertidal regions of Ratnagiri and Malvan and at Gaveshani bank, 100 km west to Mangalore. Malvan coast forms part of Western Ghats where Sahyadri ranges gradually meet the Arabian Sea. Most of the reef area is exposed during low tide (especially spring tide). Reef bottom is covered with fine sediment which is brought from leeward hills during monsoon.

5.3. Species diversity of corals

In India, 592 species of corals have been reported so far. Among the four major reef areas of India, Andaman and Nicobar Islands are found to be very rich and Gulf of Kachchh the poorest in species diversity. Lakshadweep Islands have more number of species than the Gulf of Mannar. About 97% of Indian genera recorded from Andaman and Nicobar Islands, whereas other reefs constitute merely 40%. This indicates the high degree of coral diversity in Andaman and Nicobar Islands. Interestingly Andaman and Nicobar Islands have all the families (100%) which are recorded from other major reefs of India.

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Indian coral reefs are mainly dominated by family Acroporidae, Faviidae, Poritidae, Fungiidae and Agariciidae. Acroporidae alone contributes maximum coral diversity with 183 species.

Coral families recorded from different coral reef areas in India

Sl. No.	Families	Species (Genus)				
		India	Andaman Nicobar Islands	Palk bay and Gulf of Mannar	Lakshadweep	Gulf of Kachchh
1.	Acroporidae	183 (4)	168 (4)	53 (3)	47 (3)	9 (2)
2.	Agariciidae	35 (6)	35 (6)	9 (4)	10 (3)	-
3.	Astrocoeniidae	3 (2)	3 (2)	-	-	-
4.	Caryophylliidae	15 (6)	13 (6)	3 (3)	3 (2)	2 (2)
5.	Dendrophyllidae	33 (8)	29 (8)	9 (6)	5 (2)	5 (3)
6.	Euphyllidae	10 (4)	10 (4)	-	2 (2)	-
7.	Faviidae	98 (18)	97 (18)	26 (9)	34 (12)	16 (7)
8.	Flabellidae	2 (2)	1 (1)	-	1 (1)	-
9.	Fungiidae	52 (12)	52 (12)	3 (3)	15 (6)	-
10.	Meandrinidae	1 (1)	1 (1)	-	-	-
11.	Merulinidae	9 (3)	9 (3)	3 (2)	3 (2)	1 (1)
12.	Mussidae	31 (7)	31 (7)	4 (3)	5 (3)	2 (2)
13.	Oculinidae	5 (1)	5 (1)	3 (1)	3 (1)	3 (1)
14.	Pectinidae	15 (5)	15 (5)	4 (4)	3 (3)	3 (3)
15.	Poritidae	59 (3)	55 (3)	13 (2)	15 (3)	7 (2)
16.	Pocilloporidae	22 (3)	22 (3)	3 (1)	6 (2)	1 (1)
17.	Rhizangiidae	2 (2)	1 (1)	2 (2)	-	-
18.	Siderastreidae	16 (4)	16 (4)	4 (4)	4 (1)	5 (4)
19.	Trachyphylliidae	1 (1)	1(1)	-	-	-
	Total	592 (92)	564 (89)	139 (48)	156 (46)	54 (28)

Gulf of Kachchh: Coral diversity in Gulf of Kachchh is very poor when compared to other major coral reefs in India. Families such as Asterocoeniidae, Pocilloporidae, Euphyllidae, Oculinidae, Agariciidae, Fungiidae and Trachyphylliidae are absent. Among the 92 genera recorded in India, only 28 are reported so far from this region. *Montipora venosa*, *Cosinaria monile*, *Hydnophora excesa*, *Turbinaria peltata*, *Goniastrea pectinata*, *Platygyra sinensis*, *Cyphastrea serialia*, *Porites compressa* and *Goniopora stutchburyi* are some of the common species found in all the islands of

Gulf of Kachchh. Species such as *Siderastrea savignayana* and *Acanthastrea hillae* are reported only from Gulf of Kachchh.

Lakshadweep Islands: There are 15 families, 46 genera and 156 species reported from these Islands. Families such as Astrocoeniidae, Pectiniidae and Trachyphylliidae are absent. Among the 156 scleractinian speices *Acropora humilis*, *A. muricata*, *A. intermedia*, *A. hyacinthus*, *Pocillopora verrucosa*, *Euphyllia glabrescens*, *Galaxea fascicularis*, *Psammocora contigua*, *P. haimeana*, *Pavona maldivensis*, *P. clavus*, *Fungia danai*, *Podobacia crustacea*, *Hydnophora microconos*, *Favites abdita*, *Goniastrea retiformis*, *Platygyra daedalea*, *P. sinensis*, *Leptastrea bottae*, *Porites solida*, *P. lichen* and *P. minicoensis* are common in these Islands.

Gulf of Mannar and Palk Bay: Fourteen families, 48 genera and 139 species are reported from this area. Families such as Euphyllidae and Trachyphylliidae are absent. Only 40 genera are reported so far. Species such as *Montipora monasteriata*, *M. informis*, *M. spumosa*, *M. turgescens*, *M. venosa*, *M. verrucosa*, *M. digitata*, *M. millepora*, *M. manauliensis*, *Acropora digitifera*, *A. secale*, *A. intermedia*, *Pocillopora verrucosa*, *Porites mannarensis*, *P. exserta* and *Goniopora stutchburyi* are common in these islands.

Andaman and Nicobar Islands: Information on the occurrence of the various species is based on studies made by several workers. Among India's four major reefs, Andaman and Nicobar Islands are showing maximum diversity. There was no significant compilation on coral diversity after Venkataraman *et al.* (2003). The recent studies in India resulted in 564 zooxanthellate and azooxanthellate coral species occur all over India, which contributes 60% of Global coral diversity.

Comparison of the scleractinian corals in the major reefs of India

Scleractinia	Gulf of Kachchh	Lakshadweep	Palk bay and Gulf of Mannar	Andaman & Nicobar Islands	Total
Families	12	15	14	19	19
Genera	28	46	48	89	92
Species	54	156	139	564	592

5.4. Methods of collection

All species of corals are protected under Wildlife Act, 1972. It should be collected only with permission from the Chief Wildlife Warden of the state

5.4.1. Collection

Collection of corals is important for identification of biodiversity studies and for the museum. Hammer, chisel and a sack are equipments required for coral collection. Colour photographs should be taken before collecting or immediately before making the collection of the specimen. For shallow water collection, same procedure should be adopted. Underwater notes can be made on a slate, with surface scratched with fine flint paper. A soft pencil should be used. The pad may be cleaned with soap water and a brush every time before going for collection. Small pieces of coral collection as well as the dead coral pieces found on the shore are not useful for identification

purposes (e.g. *Acropora*). Collection should be minimized for conservational reasons, and never be performed without proper authorization from the forest authorities since all the scleractinian corals are protected under Wildlife Act. Dive number, locality, depth, colour and description of the immediate surroundings should be written with a waterproof marker on a solid plastic label attached to the specimen with a soft nylon line.

Mylar sheet or a white slate may be used for repeated reuse after transcribing the data and scouring it with cleanser. Some people prefer to use an opaque water proof paper made by Nalgene as a permanent record, eliminating the need for transcription. You can secure the data sheets to the clipboard with rubber bands or elastics, and tie on your pencil. If you want a pencil that won't float away when it becomes untied, you can use Faber- Castell solid graphite pencils.

5.4.2. Cleaning of coral skeleton

Once they are collected from the reef immediately it should be tied with a label. Only the labelled specimens should be rotted in fresh water for a week, and the water changed a few times during the process. Then the specimens should be cleaned with a strong water jet and gelatinous epidermis if any, be removed with a pair of forceps. The rotting procedure may be continued for another week, if necessary. The cleaned specimens should be dried in the sun for a fortnight. Rotting and bleaching with hypochlorite solution or bleaching powder should be avoided, as it makes the corals crisp.

5.4.3. Labelling

The cleaned specimens should be properly labelled. The label should indicate the locality (name of the reef, name of the island, name of the district, station number, latitude and longitude and depth of the reef and collection) date of collection, name of the collector, name of the boat/survey and the cruise number etc. The information on the label may be made with printed/photocopied on an overhead projection sheet (transparent plastic sheet with nylon twain) and information may be written using a glass-marking pen.

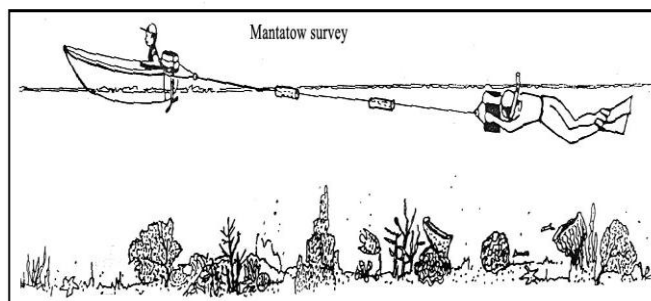
A readymade species list may be prepared on an underwater paper with locality, station number, date, latitude, longitude, dive number, name of the person as shown in the figure below. Along with this species list, information on the different features of the coral colony may be noted on an underwater slate or underwater paper.

The information such as colour of the species, size of the colony, new recruits, other coral associates, fishes, topography drawing, bleaching, crown of thorn fish infestation can be noted before emerging from the water. It is always important to go to the field with a buddy and the name of the buddy should be written in the notes, the different pre-dive preparations, list of precautions taken, etc., may also be included on the notes. The weather condition, season and the environmental conditions and sightings of anchor damage, ghost net, etc., will be valuable information about the reef.

5.4.4. Manta Tow Survey

The Manta tow technique is used to assess broad changes in the benthic communities of coral reefs where the unit of transect is often an entire reef, or large portion thereof. It enables visual assessment of large areas of reef within a short time and is highly recommended for determining the effects of large-scale disturbances such as those caused by cyclonic storms, coral bleaching and outbreaks of *Acanthaster planci* (Crown- of- thorns starfish). The technique is also useful for selecting sites that are representative of large areas of reef.

This technique involves towing an observer, using a rope and manta board, behind a small boat powered by an outboard motor. Tows are carried out at a constant speed around the perimeter of a reef and are broken into units of two-minute duration. During each two-minute tow, observations are made on several variables (e.g. percent cover of live coral, dead coral and soft coral). These are recorded onto data sheets as categories. Additional information may be collected, dependent on the survey objectives, e.g. percent cover of sand and rubble, and numbers of *Acanthaster*, *Diadema* or Tridacnid clams.



Sample Manta Tow Data Sheet

Location:			Date:	
Observer:			Time:	
Tow	Percentage Live Cover			Other Features
	Stony Corals	Octo corals	Algae	

5.4.5. Transect Method: *Line Intercept Transect (LIT)*

Line intercept transects are used to assess the sessile benthic community of coral reefs. The community is characterized using lifeform categories, which provide a morphological description of the reef community. These categories are recorded on data sheets by divers who swim along lines, which are placed roughly parallel to the reef crest at depths of three meters and 10 m at each site. The LIT is used to estimate the cover of an object or group of objects within a specified area by calculating the fraction of the length of the line that is intercepted by the object. LIT has been used for objectives ranging from large-scale spatial problems, to morphological comparisons of coral communities, and studies assessing the impact of natural and anthropogenic disturbances.

Life form categories used for conducting Line Intercept Transect studies for assessing corals for Environmental Impact Assessment as well as Status of live coral cover of a given area.

Categories		Code	Remarks
Hard coral			
Dead coral		DC	Recently dead, white to dirty white
Dead coral with algae		DCA	This coral is stranding, skeletal structure can still be seen
Acropora	Branching	ACB	At least 2° branching,
	Encrusting	ACE	Usually the base-plate of immature <i>Acropora</i> forms,
	Submassive	ACS	Robust with knob or wedge-like for
	Digitate	ACD	No 2° branching, typically like
	Tabular	ACT	Horizontal flattened plates
Non-Acropora	Branching	CB	At least 2° branching
	Encrusting	CE	Major portion attached to substratum as a laminar plate
	Foliose	CF	Coral attached to one or more points, leaf-like, or plate like appearance
	Massive	CM	Solid boulder or mound
	Submassive	CS	Tends to form small columns, knobs, or wedges
	Mushroom	CMR	Solitary, free-living corals of the <i>Fungia</i>
	<i>Heliopora</i>	CHL	Blue coral
	<i>Millepora</i>	CME	Fire coral
	<i>Tubipora</i>	CTU	Organ-pipe coral, <i>Tubipora musica</i>
Other Fauna			
Soft coral		SC	Soft bodied corals
Sponges		SP	
Zoanthids		ZO	Examples are <i>Platythoa</i> ,
Others		OT	Ascidians, anemones, gorgonians, giant clams, etc.,
Algae	Algae assemblage	AA	Consists of more than one species
	Coralline algae	CA	

Categories		Code	Remarks
	<i>Halimeda</i>	HA	
	Macroalgae	MA	Weedy/fleshy browns, reds, etc.,
	Turf algae	TA	Lush filamentous algae often found inside damselfish territories
Abiotic	Sand	S	-
	Rubble	R	Unconsolidated coral fragments
	Silt	SI	-
	Water	WA	Fissures deeper than 50 cm
	Rock	RCK	-
Others		DDD	Missing data

Model data sheets used for collection of Line Intercept Transect to study the status and live cover of coral reefs during Environmental Impact assessment study

LINE INTERCEPT DATA

Location

Reef name Reef zone Reef latitude

Date Time Wind Cloud Longitude

Turbidity Light Top

Depth Sea Tide Temp Bot Salinity Bot

Replicate Site N° Collector

Remarks

Benthos	Transition	Occurrence	Field code	Notes

Analysis

- Summary data showing percent cover and number of occurrences of each lifeform may be calculated using the line intercept data. After calculating the intercept (length) from the transition points recorded along the transect, the percent cover of a lifeform category is calculated.

$$\text{Percent cover} = \frac{\text{Total length of category} \times 100}{\text{Length of transect}}$$

$$\text{Percent cover lifeform 1} = \frac{L1 + L3 + L5 + L7}{Y} \times 100$$

$$\text{Percent cover lifeform 2} = \frac{L2 + L4 + L6}{Y} \times 100$$

- These analyses will provide quantitative information on the community structure of the sample sites. Successive samples can also be compared when the sites have been sampled repeatedly over time.
- If reefs have been selected to represent both disturbed and pristine sites, then comparison of change detected in these sites may allow recognition of change due to disturbance from natural and human-induced pressures. This provides a predictive tool in reef management.
- Where rigorous statistical comparisons of reef community structures within and between sites are needed, greater replication of transects at each sampling site will be required.

5.4.6. Random Swim Technique

The random swim technique provides good information on abundance and species richness, but not on population density. The entire census period is spent searching for unrecorded fish species rather than recording other data of the fish. To obtain reliable data, replicate sample censuses must be conducted.

Variations of this technique appear in Jones and Thompson (1978), and Kimmel (1985). The basic technique for a 50-minute census is presented below.

- Begin the census at a random location in the selected reef area.
- The census period is divided into five 10-minute intervals. Record the name of each species in the interval in which it is first seen.
- To estimate its abundance, each species is given a score based on the interval within which it is first observed. Your data sheet may look something like this:

Model Random Swim Data Sheet for conducting live and dead coral cover

23/01/2018: Upper platform, coral cover: 21%, Depth: 50'				
0-10min	11-20 min	21-30 min	31-40 min	41-50 min
Score:	Score:	Score:	Score:	Score:

Analysis: It's usually important to analyze data on frequency of occurrence, abundance, richness, evenness and diversity of species at individual sites and sites. Data on changes in relative abundance and frequency of occurrence can provide information on population changes for individual species. Changes in average size and structure of size classes of important species can also be evaluated.

5.5. General Surveys and Maps

Most of the biological monitoring methods focus on censusing small sections of a coral reef (several square meters) and detecting changes over time. However, you may need to quickly survey a large area in certain situations. For example, a general survey of most or all of a reef will be essential if you want to assess storm damage or determine the appropriate boundaries for a marine park. A preliminary survey can also be helpful in selecting a study area for a long – term monitoring program. The use of aerial photographs and a manta tow survey are two techniques used in making this kind of general assessment.

Aerial Photography, ground truthing, mapping, GPS, GIS

These are the techniques to assess the extent of corals, recording their status, and to prepare maps/GIS maps for further reference and monitoring. GPS positions with accuracy need to be collected along with data collection.

Aerial photographs provide a good starting point for a general survey of a coral reef. At a scale of 1: 5000, many reef features are visible, and if the photos are taken during calm and clear conditions, water clarity may allow resolution of major features to a depth of 60 feet or more.

Aerial photos with high resolution can sometimes be used to identify major zones and habitats, although changes in the condition or abundance of reef organisms do not generally show up. For "ground truthing" to verify the zonation patterns or habitat types, you may need to self-contained underwater breathing apparatus (SCUBA) dive or snorkel.

Base maps can be created from enlarged aerial photographs (usually at 10" x 10"). It is best to use professionally produced aerial photographs with a known scale. Maps developed from aerial photographs/base maps can be incorporated into Geographic Information System, which enables compilation of various data/info for assessment, long term monitoring etc.

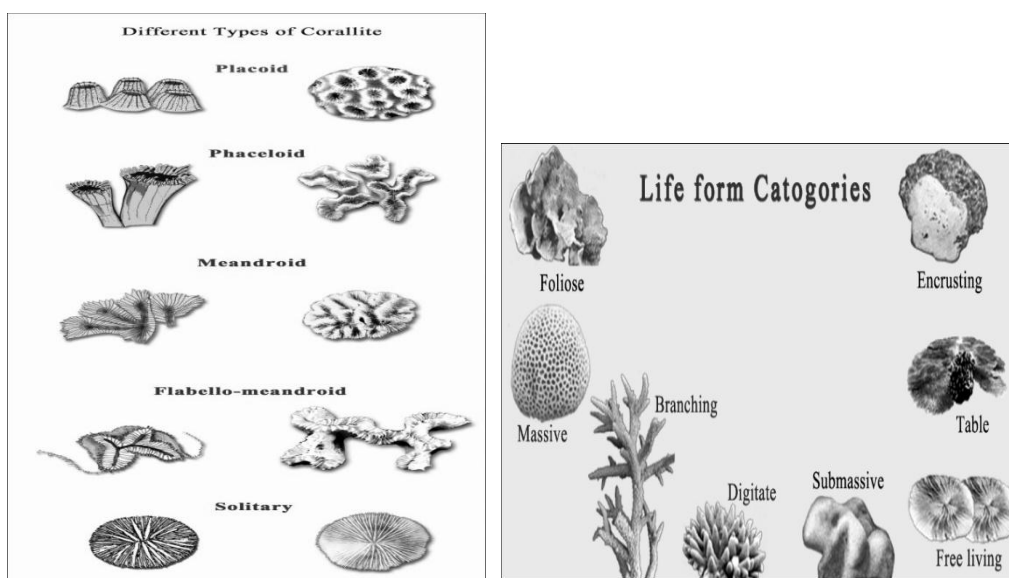
GPS could be used and the field locations, presence of coral type, species, its condition, size, etc, and also underwater photo, videos can be linked to GIS based Information System. Such GIS based

information system will be useful in long term monitoring. Further, overlay of occurrence of corals with corresponding depth contour chart will enable to understand the depthwise distribution of various coral species. GIS will also be helpful in delineating the boundaries, different zones for protection, conservation, etc, by the appropriate authorities.

A GIS is a powerful way to integrate and display large amounts of geographic data. However, geographic information systems are only as good as the data they are based on, and some of the data must be collected through labour-intensive field techniques.

5.6. Identification of species of Hard Corals

Most often the word coral refers to hard corals from the Order Scleractinia. Scleractinian corals are divided into reef-building corals (hermatypic corals), which form the primary structure of coral reefs, and non-reef building corals (ahermatypic corals), which do not contribute significantly to reef formation. Hermatypic corals usually contain millions of tiny algal cells, called zooxanthellae, within their tissues. These algae are a primary energy source for the reef-building activities of hermatypic corals.



The ability to recognize individual coral species is essential to decide on reef management and protection. However, there are a number of characteristics that can make corals particularly elusive and difficult to identify. Corals may be identified by experience mostly based on life form like, foliose, massive, branching, digitate, submassive, table, encrusting, free living etc. Visual observations in intertidal area and photographs made underwater can be used for identification with the help of standard identification manuals and with the help of experts. Different types of corals familywise are given below.

List of Families and Genus

Acroporidae



*Montipora**
Anacropora?
*Acropora**
*Astreopora**

Astrocoeniidae

*Stylocoeniella**
Stephanocoenia
*Palauastrea ramosa**
*Madracis kirbyi**

Pocilloporidae



*Pocillopora**
*Seriatopora**
*Stylophora**

Euphyllidae



*Euphyllia**
Catalaphyllia
Nemenzophyllia
*Plerogyra**
*Physogyra**

Oculinidae



Oculina
Simplastrea
Schizoculina
*Galaxea**

Meandrinidae

Meandrina
Dichocoenia
Dendrogyra
Gyrosmlia
Montigya
Eusmlia
Ctenella

Siderastreidae



*Pseudosiderastrea**
Anomastrea
*Siderastrea**
*Psammocora**
*Coscinaraea**
Horastrea

Agariciidae



*Agaricia**
*Pavona**
*Leptoseris**
*Coeloseres**
*Gardinoseris plannulata**
*Pachyseris**

Fungiidae

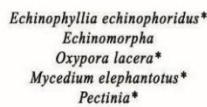


*Cycloseris**
*Diaseres distarta**
Cantharellus
*Heliofungia actiniformis**
*Fungia**
*Ctenactis echinata**
*Herpolitha limax**
*Polyphyllia talpina**
*Sandalolitha robusta**
*Halomitra pileus**
Zoopilus
*Lithophyllon undulatum**
*Podabacia crustacea**

Rhizangiidae

Astrangia

Pectiniidae



*Echinophyllia echinophoridus**
Echinomorpha
*Oxypora lacera**
*Mycedium elephantotus**
*Pectinia**

Merulinidae



*Hydnophora**
Paraclavaria
*Merulina ampliata**
Boninastrea
*Scapophyllia cylindrica**

Dendrophylliidae



*Turbinaria**
Duncanopsammia
Balanophyllia
Heteropsammia

Poritidae



*Porites**
*Stylaraea**
Poritipora
*Goniopora**
Alveopora?

Mussidae



Blastomussa
Micromussa
*Acanthastrea**
Mussismilia
Isophyllia
*Lobophyllia**
*Symphyllia**
Mussa
Scolymia
Mycetophyllia
Australomussa
Indophyllia
*Cynarina lacrymalis**

Faviidae



Cladocora
Caulastrea
Erythraestrea
Manicina
*Favia**
Barbattoia
*Favites**
*Goniastrea**
*Platygyra**
Australogyra
*Oulophyllia crista**
*Leptoria phrygia**
Diploria
Colpophyllia
*Montastrea**
*Plesiaestrea versipora**
Oulastrea
*Diploastrea heliopora**
*Leptastrea**

Caryophylliidae

Heterocyathus



Parasimplastrea
*Cyphastrea**
Solenastrea
*Echinopora**
Moseleya

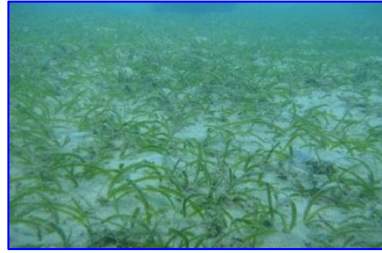
Trachyphylliidae

Trachyphyllia

Coral reef associated organisms



Seaweed



Sea grass



Mangroves



Sponges



Sponges



Gorgonians



Gorgonians



Polyclads



Polyclads



Tetralia glaberrima



Pilodius pugil



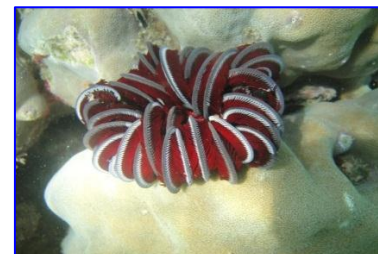
Molluscs Seaslugs



Molluscs Seaslugs



Echinoderm *Linckia laevigata*



Himerometra robustipinna



Heniochus acuminatus



Pomacanthus annularis



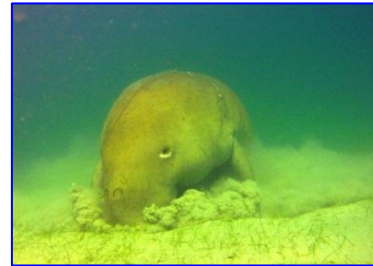
Laticauda columbrina



Laticauda columbrina



Dugong dugon



Dugong dugon

MANGROVES AND COASTAL VEGETATION**6.1. Introduction**

Mangroves occur in tropical and sub-tropical regions of the world. The mangrove forests occupy about 15.2 million hectares in 123 countries, located between 30° South & 30° North (FAO, 2007). The mangroves are among the most productive forests.

Mangroves thrive in a harsh environment that varies with salinity, tidal regime, strong wind velocity, high temperature, and muddy anaerobic soil. The mangroves are remarkably adapted to such adverse conditions where no other trees can survive. The mangroves are structurally and functionally unique to have well-developed aerial roots, viviparous germination, adaptable to high salinity, and highly efficient in nutrient retention.

Mangrove forests are rich in biodiversity by supporting a diverse group of terrestrial and aquatic organisms. The mangrove forests provide diversified habitats, such as water bodies, forests, litter forest floor, and mudflats, for a wide variety of organisms. The calm waters in the forests are ideal nursery and breeding grounds for fish and shellfishes, while the aerial roots, lower trunks of trees and forest floor support a varied fauna of oysters, snails, barnacles, crabs and other invertebrates. The forest also supports terrestrial animals, such as birds, reptiles, insects and mammals. Hence mangroves are biologically diverse and ecologically dynamic (Kathiresan and Bingham, 2001).

6.2. Coastal sand dunes

Coastal sand dunes are mounds or ridges on the shore, and the sand dunes are formed by sand, deposited from the sub-tidal and intertidal regions. Wind is one of the most important factors, helping in formation, movement and distribution of the sand dunes. The sand dune vegetation is a different plant community with remarkable ability to tolerate adverse conditions of drought, nutrient deficiency, high winds, salt sprays and sand blast.

6.2.1. Distribution of coastal sand dunes in India

Coastal sand dunes are distributed all along the sandy coast, which occupies half of the Indian coast. The coastal sand dunes are abundant along the central west coast of India from Goa to Kerala because of the extensive sandy areas.

India has a total of 338 species of coastal sand dune flora. The west coast has more number of coastal sand dune flora (267species) than the east coast (163species). The botanical family-Fabaceae is predominant due its ability to fix nitrogen and to overcome the nutrient-deficiency of the sandy substrate.



View of mangrove forest dominant with *Avicennia* (A) and *Rhizophora* (B)



A view of coastal sand dune vegetation dominant with *Ipomoea pes-caprae* (A) and *Spinifex littoreus*, ("Mustache of Ravana") (B)

6.3. Distribution of mangroves

There are three major types of mangrove forests: (1) river-dominated, (2) tide-dominated and (3) interior mangrove forests. Fringing mangrove forests occur along the borders of protected shorelines and islands, influenced by daily tidal range.

India has a total area of 4,740 km² under mangroves, accounting for 2.8 % of the world's mangrove vegetation and 0.14 % of the country's total geographical area. The mangroves are found along the coastlines of nine States and three Union Territories. The Sundarbans of West Bengal has the largest mangrove cover (44% of total), while Gujarat has the second largest (23 % of total). About 58 % of the total mangrove cover is found along the east coast (Bay of Bengal), 29 % on the west coast (Arabian Sea) and the remaining 13 % on the Andaman and Nicobar Islands.

There are only 39 core mangrove species, in association with 3,972 other species that include mangrove associates, seagrasses, marine algae, microbes, lichens, prawns, lobsters, crabs, insects, mollusks, finfish, amphibians, reptiles, birds, and mammals. Altogether 4,011 species consisting of 920 floral and 3,091 faunal species are present in the mangrove forest systems of India. In other

words, the faunal component occupies 77 %, whereas the floral component is 23 %, and thus the faunal component is about 3.5-fold higher than floral component. No other countries in the world have recorded so many species to be present in the mangrove forest ecosystems.

6.4. Methods of sample collection

Collection and Preservation: A complete twig with buds, flowers and fruits will be collected, washed, except the flowers and pressing immediately between newspapers, within half an hour. Time consuming collections may temporarily be kept in polythene bags. The flowers will be preserved in Kew spirit (prepared by mixing 43 parts of rectified spirit, 44 parts of distilled water, 7 parts of formalin and 6 parts of glycerol). The wood, bark and fruits will be preserved in jars containing the preserving fluid (prepared by 95 parts of water, 4 parts of commercial 40% formalin and 1part of Borax powder).

Pressing: Specimens provisionally pressed in the field should be unpacked and trimmed to fill the mounting sheet. All the specimens except very delicate ones should be immersed in denatured spirit in a rectangular plastic tray (45 x 30 x 15 cm) to kill the tissues to prevent their decay. After immersing, the materials may be kept on a plastic sieve plate 45 x 30 cm to drain off the extra spirit.

Drying: All the materials will be dried by keeping them in hot air oven between the newspapers. Flanked on either side by blotters or very tightly fastened with strong roped between lattice iron presses to prevent shrinkage during drying. Succulent materials may need to be slightly boiled before drying.

Poisoning: After drying, poisoning is to be done by immersing the materials in mercuric solution (prepared by dissolving 25 g of HgCl_2 in 1 litre of denatured spirit) and then arranged back in newspaper folders under pressure for at least 3 hours.

Mounting and Storing: All specimens will be sewed with thin, but strong thread on stiff mount board (42 x 28 cm); knots on the upper surface, but laterally. Delicate materials and broken, brittle fruits are spread inside a paper flap previously mounted on the board. Fumigation may be carried out for overnight with 3:1 mixture of ethylene dioxide and carbon tetrachloride. The herbarium sheets may be arranged by either serial number of family genus-species-order, within which serial order can be followed and can be stored. The sheets may be stored in 5-ply card board boxes with inside measurements of 32.5 x 20 x 38 cm.

Succulent materials like those of Aizoaceae, Cactaceae and Euphorbiaceae will be preserved in liquids such as 5% aqueous solution of formaldehyde or 70% alcohol. The specimens preserved in formaldehyde become flaccid. Due to its offensive odour, many have switched over the use of 70% alcohol as the liquid preservative. To retain green colour of the specimens, preservatives containing 90 ml of 50% alcohol, 5 ml of formaldehyde, 2.5 ml of glycerin, 2.5 ml of glacial acetic acid, 20 gm of cupric chloride, and 2.5 gm of uranium nitrate, is used.

Identification of mangroves and littoral flora: This needs to be done in consultation with the experts and by referring to the standard manuals. For the EIA report preparation, occurrence of true mangrove species, associated species, coastal sand dune vegetation list of species, their distribution, sparse/dense, dominance, etc., may be collected and compiled. There are few more further studies that can be taken up as further studies, which are given below. These studies are more useful for mangrove forest structure, data on stem, no. of plants, their characteristics, such as dbh, so that the mangrove plant biomass, etc., can be estimated.

6.5. Mangrove forest structure

This method is used to Provide baseline data on the diversity and structure of a mangrove community at a particular site, monitor long-term changes and provide a quantitative measure of species composition, stem density, and basal area of trees.

This information can be useful for interpreting other parameters, such as leaf trapping ability and LAI. Changes to basal area, stem density and canopy cover can be indicators of ecosystem health. It is a time-consuming method and should only be used to study long-term changes to mangrove forests.

- i) Establish a transect running at right angles from the sea to the land, with 10 x 10m quadrats in each forest zone along the transect.
- ii) Use the compass to establish the bearing to follow.
- iii) Identify the major forest types or zones along the transect.
- iv) For each forest type, find an area to the left of the transect that is representative (in terms of floristics and structure) of that mangrove community.
- v) Within each quadrat, record the canopy cover, species type, tree height, sapling/seedling number and stem diameter.

Note:

- If two quadrats are to be established, ensure that they are at least 20m apart.
- If monitoring a homogenous forest type or a narrow mangrove fringe along a creek, transects can be established parallel to the shoreline. Quadrats can be placed where the forest is representative of the mangrove community, or at regular intervals.
- If there are a large number of trees or shrubs in the plot and the canopy within the plot is even, only record half the plot (but note which side of the plot it is). For plots with very large numbers of trees/shrubs (e.g. *Aegiceras* communities) it may be necessary to reduce the plot size.

6.5.1. Analysis of structure and composition

Forest structure will be analyzed in the sampling plot of 10 m x 10 m dimension. Data will be recorded on the name of species, number of each species, plant height, diameter at the breast height of 1.3 m (dbh) (Fig. 6.3.), and frequency of occurrence of the species in different plots.

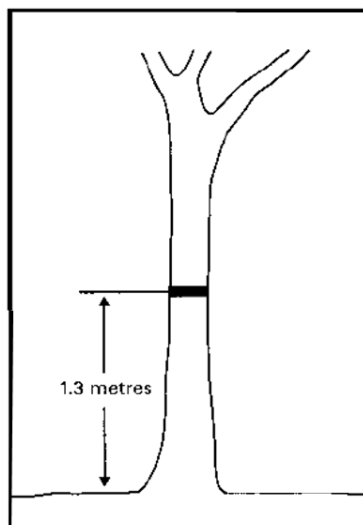


Fig. 6.3. Measurements recorded at breast of height

6.5.2. Estimation of canopy cover

- Walk along the centre line and longest edges of the plot and record where the canopy starts and finishes along these lines. This includes recording breaks in the canopy along these lines.
- Sum the distance that canopy covers along these lines.
- Divide the figure by 3 and multiply by 2 to give a percentage cover for a 50 x10m plot.
- If the length of the plot is more or less than 50m you will need to adjust the figures accordingly.
- If the canopy consists of more than one species of mangrove, estimate the percentage that each species contributes to the total canopy cover.

Note: Dominance is not the same as canopy cover; the total of all species must equal 100 per cent. For example, if there is a 70 per cent canopy cover and only one species, canopy dominance by that species is 100 per cent.

6.5.3. Measurement of stem diameter

- Measure the stem diameter of each tree or shrub at breast height (1.3m above the ground). Measure only those trees or shrubs with a height of 2m or more.
- Record result as diameter at breast height (DBH) (Fig. 6.4.). A regular tape measure measures circumference only. Record this as circumference at breast height and calculate DBH by dividing this result by π (approximately 3.14).

- If carrying out long-term monitoring, hammer a galvanized nail (half of its length) into stems 10cm below where measurements have been taken, to provide a reference point for future measurements. Note this on the datasheet.

6.5.4. How to measure irregularly shaped trees

- Irregularly shaped trees are very common in mangrove forests. If an irregularity occurs at breast height (Figure 8), use the following procedures to measure diameter:
- For multiple stems that fork below breast height; where stem diameter is 2.5cm or greater, measure the diameter of each stem at breast height, and record all results in the same box on the datasheet. Do not count each stem as a separate tree.
- For multiple stems that fork at breast height; take the measurement slightly below the swelling caused by the fork. For buttress roots, take the measurement 30cm above the uppermost prop root or buttress.
- For trunk swellings, take the measurement slightly above or below the swelling.
- Some smaller mangrove forests may be naturally stunted or dwarf-like. In such situations these criteria are not suitable for determining growth status.

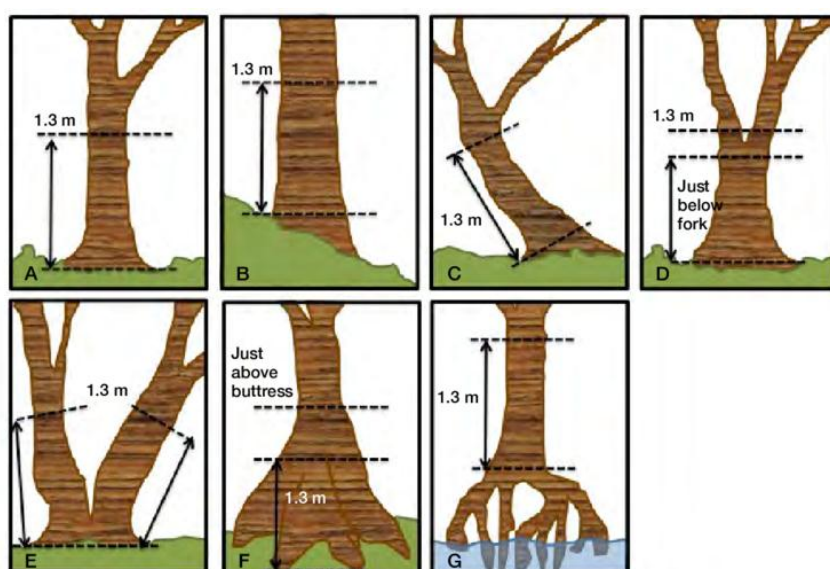


Fig. 6.4. Measuring the stem diameter of irregularly shaped tree

6.5.5. Calculations

Based on the data, the following will be calculated.

- Density of each species (No./ha) = No. x 10,000 m² divided by plot area of 10 m x 10 m
- Total density of all species = Sum of all species densities
- Basal area (m²) of each species = 0.005 x dbh

- Total basal area of all species (m²/ha) = sum of all species basal area divided by plot area of 10 m x 10 m multiplied by 10,000 m²
- Relative density (%) = no. of individuals of a species divided by total no. of individuals of all species multiplied by 100
- Relative dominance (%) = total basal area of a species divided by basal area of all species multiplied by 100
- Relative frequency (%) = frequency of a species divided by total frequency of all species in different plots multiplied by 100
- Importance value of a species = relative density + relative dominance + relative frequency
- Complex index = no. of species + stand density + basal area + height
- Species diversity will be calculated as the Shannon index (H) based on importance value of a species (Ni) and sum of importance value for all the species (N) by using the formula.

$$H = \sum \frac{Ni}{N} \log \frac{N}{Ni}$$

6.6. Biomass and Biomass Carbon

Allometric equations will be followed for estimating the biomass of mangroves (Kaufman and Donato, 2012) as follows.

$$\begin{aligned} \text{Trunk weight, } W_s &= 0.0696 \times \rho \times (D^2H)^{0.931} \\ \text{Leaf weight, } W_L &= 0.135 \times \rho \times D_B^{1.696} \\ \text{Above-ground weight, } W_{\text{top}} &= 0.251 \times \rho \times D^{2.46} \\ &\quad (D_{\text{max}}=49 \text{ cm}) \\ \text{Root weight, } W_R &= 0.199 \times \rho^{0.899} \times D^{2.22} \\ &\quad (D_{\text{max}}=45 \text{ cm}) \\ \text{Total biomass} &= W_{\text{top}} + W_R \end{aligned}$$

Where,

D – Trunk diameter at breast height at 30 cm above ground in Rhizophoraceae members in centimeter and at 130 cm in Avicenniaceae members

D_B – Trunk diameter at the lowest living branch in centimeter

H – Tree height in meter

ρ – Wood density of trunk in ton per m³ (=0.752)

6.7. Calculation of carbon biomass

The biomass values are converted into carbon biomass values in kilograms by multiplying with a factor of 0.42. Carbon biomass per hectare is calculated by multiplying the carbon biomass with tree density per hectare. The Carbon biomass values are converted into carbon-dioxide equivalent values by multiplying with a factor of 3.67.

7.1. Introduction

Seaweeds are group of plants that live either in marine or brackish water environments and dominate the rocky intertidal regions of most oceans. Seaweed zone is one of the conspicuous and wide-spread biotope in the shallow marine environment (Fig. 7.1).

Seaweeds are found in the coastal region between high tide to low tide and in the sub-tidal region up to a depth where 0.01 % photosynthetic light is available. Plant pigments, light, exposure, depth, temperature, tides and the shore characteristic combine to create different environment that determine the distribution and variety among seaweeds.

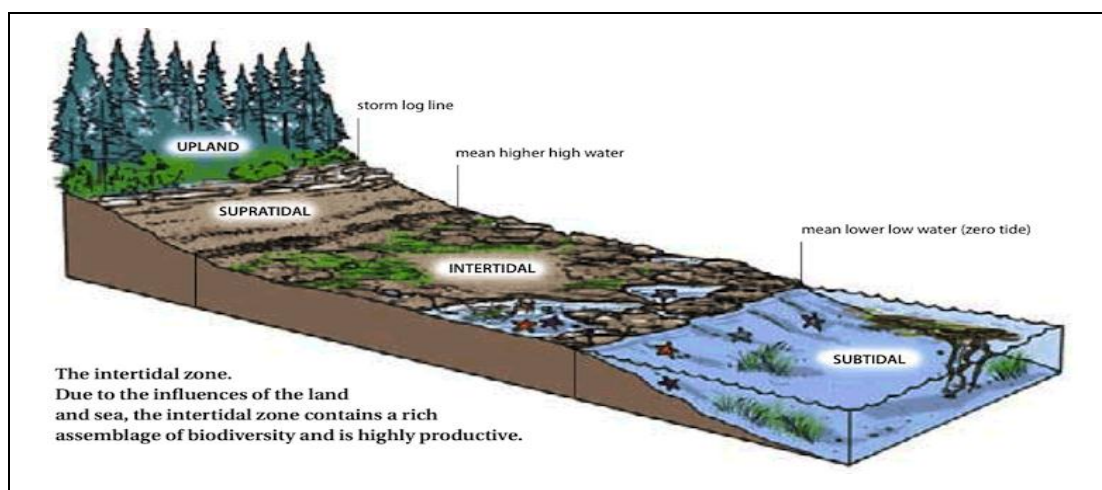


Fig. 7.1. Schematic presentation of beach showing intertidal and subtidal areas

The seaweeds are totally different from higher plants as they neither have true leaves, stems and roots or vascular systems. Whole body of the plant is called thallus that consists of the holdfast, stipe and blade (Fig. 7.2). The holdfast resembles the root of the higher plants but its function is for attachment and not for nutrient absorption. The holdfast may be discoidal, rhizoidal, bulbous or branched depending on the substratum it attaches. The stipe resembles the stem but its main function is for support of the blade for photosynthesis and for absorption of nutrients from surrounding sea water. The blade may resemble leaves of the higher plants and have variable forms (smooth, perforated, segmented, dented, etc.). The important functions of the blade are photosynthesis and absorption of nutrients.

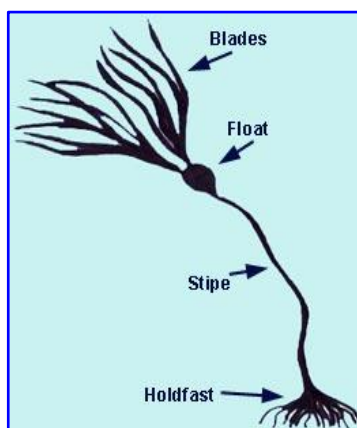


Fig. 7.2. Structure of seaweed

(http://www.mesa.edu.au/marine_algae/default.asp)

Worldwide, 9200 species of seaweeds are reported (www.patentlens.net) with red algae comprising of 6000, brown algae 2000 and green algae 1200 species.

Seaweeds are classified based on their pigments into three major groups. They are 1. Green Algae (Chlorophyceae), 2. Brown Algae (Phaeophyceae) and 3. Red Algae (Rhodophyceae) based on the predominance of pigments chlorophyll a and b, fucoxanthin and phycoerythrin respectively.

7.2. Green Algae (Chlorophyceae):

Green algae are found in the fresh and marine habitats. They range from unicellular to multicellular, microscopic to macroscopic forms. Their thalli vary from free filaments to definitely shaped forms. The photosynthetic portion of the thalli may be moderately to highly calcified appearing in variety of forms as fan shaped segments, feather like or star shaped branches with teeth or pinnules and clavate or globose branchlets. They possess pigments as chlorophyll a & b as well as carotenes, lutein and zeaxanthin. These algae reproduce sexually or asexually by forming spores. The vegetative propagation is also achieved through fragmentation.

- Green algae are mostly found in the intertidal zone and mangrove swamps.
- From the Indian waters, 213 species under 43 genera have been reported (Kaliaperumal *et al*, 2004).
- Important food source for fishes, crustaceans and gastropods. Green algae like *Ulva* sp., *Caulerpa* sp. and *Enteromorpha* sp. are used as salad and in soups. Food reserves are starch and fructosans.
- Bioactive compounds like diterpenes, sesquiterpenes, triterpenes and ceramides have been isolated from a number of genera especially *Caulerpa* sp., *Chaetomorpha* sp. and *Ulva* sp.

- Bioactive compounds from green algae are known to have antifungal, antitumor and antiviral properties.

Example: *Ulva sp.*, *Enteromorpha sp.*, *Chaetomorpha sp.*, *Codium sp.*, *Caulerpa sp.*, etc.,



Ulva lactuca



Chaetomorpha sp



Caulerpa racemosa

7.3. Brown algae (Phaeophyceae)

Brown algae are exclusively marine forms, mostly found in intertidal and subtidal areas. They have different forms from simple, freely branched filaments to highly differentiated forms. They are varying in colouration from olive yellow to deep brown. Colouration is due to accessory carotenoid pigment and fucoxanthin. *Dictyota*, *Ectocarpus*, *Laminaria* etc. are rich in fucoxanthin, while species of *Fucus* are poor in fucoxanthin. Most of the littoral brown algae are rich in xanthophylls and fucoxanthin. Other photosynthetic pigments are chlorophyll a & c, β -carotene and xanthophylls. These algae also reproduce sexually and asexually. Several species also reproduce vegetatively by fragmentation.

Many species have large massive thalli with special air bladder, vesicles or float to make them buoyant.

- Brown algae are mostly marine. They play an important role in marine environment both as food and habitat for marine organisms.
- From the Indian waters, 289 species under 37 genera have been reported (Kaliaperumal et al., 2004).
- Range from small sized filamentous forms of about 1 cm to giant kelps of 20-60 m.
- Algin, a hydrocolloid substance extracted from brown algae is used as a thickening agent in food products like sherbert, ice cream etc. and as a stabilizer in paints and ointments.
- Important algin yielding genera available in India are *Sargassum*, *Turbinaria*, *Spatoglossum*, *Rosenvingea* and *Chnoospora*.
- The brown algae *Laminaria* and *Fucus* are important seafood in China, Japan and Korea. Plants like *Pelvetia* are used as chicken feed and cattle feed in European countries.

Example: *Sargassum*, *Laminaria*, *Turbinaria*, *Dictyota*, etc.



Sargassum wightii



Turbinaria ornata



Hypnea valentiae

7.4. Red Algae (Rhodophyceae)

They are exclusively marine, except for few species. They vary in size and shape. They are either epiphytes, grow as crust on the rocks or shells as a large fleshy, branched or blade like thalli. The thallus is basically filamentous, simple or branched, free or compacted. They inhabit intertidal to subtidal to deeper waters. Colouration of rhodophyta is due to water soluble pigments, the red phycoerithrine and blue phycocyanin. Other pigments present are chlorophyll a & b, carotenoid, etc. These algae seldom reproduce asexually. Sexual reproduction is very complicated, involving several structures after fusion of gametes.

- Red algae are eaten by fish, crustaceans, worms, gastropods and also humans.
- Red algae are used to produce agars, which are gelatinous substances used as a food additive and in science labs as a culture medium. Red algae are rich in calcium and sometimes used in vitamin supplements.
- Coralline algae are red algae. They help build tropical coral reefs. These algae secrete calcium carbonate to build a hard shell around their cell walls. There are both upright forms of coralline algae, which look very similar to coral, and encrusting forms, which grow as a mat over hard structures such as rocks and the shells of organisms like clams and snails.
- Coralline algae are found from intertidal area upto 270 m depth or more deep in the ocean, where light will penetrate water.

Example: *Gelidiella* sp., *Gracilaria* sp., *Eucheuma* sp., *Ceramium* sp., *Acanthophora* sp, etc.,



Gelidiella acerosa



Porphyra sp.



Laurencia sp.

7.5. Seagrasses

Seagrasses are the marine flowering plants. They are the only angiosperms that successfully grow in intertidal and subtidal marine environment. Seagrasses belong to the families, Hydrocharitaceae and Potamogetonaceae and they are in no way related to the terrestrial grasses of Poaceae. As mangrove and coral reef ecosystems are closely associated with the seagrass ecosystem, there is a lot of export of organic matter and nutrients from the latter. Seagrass meadows are highly productive and dynamic ecosystems.

- Seagrasses are placed under the class Monocotyledonae. About 66 species under 14 genera are reported worldwide (Manisseri et al., 2012).
- From the Indian coast, 14 species under seven genera have been reported (Maurice Schwartz, 2005).
- These plants are important for structuring a number of ecosystems, stabilizing coastlines, providing food and shelter for diverse marine organisms and act as a nursery ground for many fishes of commercial importance.
- Seagrasses play an important role in carbon and nutrient cycling in the marine environment. Seagrass meadows are also involved in nitrogen cycling through nitrogen fixation.



Cymodocea rotundata



Halophila stipulacea



Halophila beccarii

7.6. Distribution of seaweeds and seagrass along Indian coast

Seaweeds

Along the coastline of India, the littoral and sub littoral rocky areas support good growth of different seaweeds. There is luxuriant growth of seaweeds along the Southeast coast of Tamil Nadu, from Mandapam to Kanyakumari; Gujarat coast; Lakshadweep Island and the Andaman and Nicobar Islands. Fairly rich seaweed beds are present in the vicinity of Mumbai, Ratnagiri, Goa, Karwar, Varkala, Kovalam, Vizhinjam, Visakhapatnam and few other places such as Chilka and Pulicat lakes. In India, 844 species are reported (Oza and Zaidi, 2001) and their commercial exploitation has been commenced since 1966.

Seagrass

Being an unique component of coastal habitat, 72 named seagrass species is estimated worldwide, represented by 14 genera in six families (Hydrocharitaceae, Cymodoceaceae, Posidoniaceae, Zosteraceae, Ruppiaceae and Zannichelliaceae) in which 14 sea grass species in India are grouped under Cymodoceaceae and Hydrocharitaceae (Jagtap *et al.*, 2003). Most of the species form healthy meadows along the south east coast Gulf of Mannar and Palk bay) of Tamilnadu and Islands of Andaman and Nicobar and Lakshadweep.

7.7. Sample collection for Seaweeds and Seagrasses

The collection of seaweeds in the field is done during low tide. It is necessary to go for collection one or two hours before the time of low tide as per tide tables. This will give more time for seaweed collection and to observe seaweeds in the natural habitat. It is important to make notes on the description of the site location, topography, associated flora and fauna and other related parameters. Although, there are number methods to collect seaweeds, we consider here two methods which are practical and easy to study. a) Line transect or belt transect method and b) Random Quadrant Method.

Note: These two methods are described here for sample collection in intertidal areas. For sampling in subtidal areas, these methods need to be modified as mentioned subsequently.



Fig.3 Seaweed sampling site

Materials necessary for sample collection.

- Polyethylene bags, knife or scalpel
- Labeling materials (pen/pencil, labels, marker pens, rubber bands, etc.)
- Field note book
- Long rope (about 50 m long)
- Quadrant 0.25 m^2 / 1 m^2
- Monopan balance

7.7.1. Line transect or belt transect method

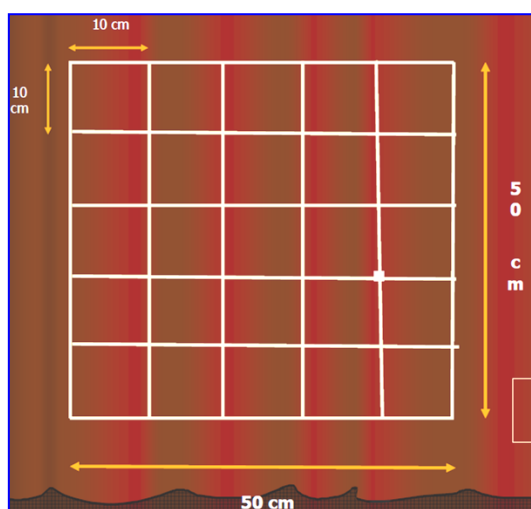
To prepare a quantitative assessment of the marine vegetation in a given area, a line or belt transect is laid perpendicular to the coast from high tide to the low tide with the help of long rope (**Fig. 3**). Sampling points along the rope can be marked depending on the gradient and the expanse of the intertidal area. In case the intertidal area is small, sampling points can be marked at 5 m intervals along the rope and if intertidal area is quite large the sampling point can be marked at 10 or 20 m along the rope.

- A quadrant measuring 0.25 m^2 area is placed at the sampling points in triplicate covering an area of 5 m^2 on either side of the sampling points.
- Seaweed species present within the quadrant are collected (collect complete plant as far as possible along with the hold fast).
- Seaweed specimens can be removed by hand but those specimens which are closely adhering to the substrate such as crustose and mat forming seaweeds can be removed with the help of knife or scalpel. The specimen that grow close to the rocks can be removed with the rocks using geologists pick axe or any other similar tools.

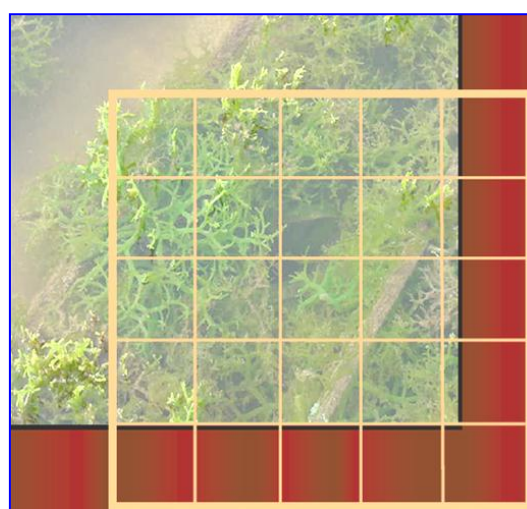
- All the collected specimens should be counted species wise and number of individuals in each species should be noted for quantitative assessment of abundance, density, frequency, species richness, species diversity, percentage cover etc. with statistical consideration.
- All the collected specimens from the quadrant should be weighed to estimate standing crop biomass.
- Collected material should be kept in the polyethylene bags/containers with proper labeling for further preservation and identification at the later stage in the laboratory.

7.7.2. Random Quadrant Method

This method involves collection of random samples. This can be done by selecting sampling points in the area and using quadrant. Sampling points should be selected in such a manner that every species of the study area has good chance being selected. This type of sampling is usually done in the area where the intertidal expanse is very narrow with steep gradient and also in the area where distribution is patchy. It is also employed for qualitative estimation of the seaweed.



A-Quadrant method



B-Quadrant sampling

(Source: Use of Transect Quadrat Method in Transect Seaweed Assessment by E.G. Fortes)

Note: For collection of seaweeds in subtidal area, similar methods as in the case of intertidal area i.e. belt/ line transect and random sampling methods are used. Snorkeling technique is employed to sample shallow depth (0.5 to 3m) and SCUBA diving is employed in case of deeper depth (3.0 to 30m deep). A quadrant of 1.0 m² area is used for collecting seaweed samples at the marked points. Seaweeds present in the quadrant are collected in polyethylene bags and analysed / processed.

Seagrasses

Data collection can be done systematically by using line transect method by placing quadrant 0.25m x 0.25m, which begins with a site survey research to look at the condition of vegetation seagrass along the seagrass beds (English et al., 1997).

During collection, seagrasses should be uprooted with care to keep the underground parts intact and washed in the field itself to remove sediments and epiphytes without making any damage to the plants.



Seagrass estimation using quadrat

Then the specimens should be poisoned with 1% mercury chloride solution and pressed and dried for preservation. The preserved materials could be pasted on mounting boards. If the specimens are slender and fragile, then they should be spread neatly on a mounting board submerged in a tray containing water and the board should be gradually lifted allowing the excess of water to drain. The board with the specimen should then be kept in blotters for drying. After drying, rectified spirit saturated with mercuric chloride can be brushed on the plants and allowed to dry.

7.7.3. Field data sheet

It is important to maintain accurate records of the field sample collection. All information should be recorded in the field data sheet. The field data sheet must include the date, time, locality of sample collection, sampling position, transect/quadrant number, etc. Data on occurrence, abundance of seaweeds species need to be recorded in data sheet for laboratory analysis of density, cover, biomass, diversity etc. Other important/ unusual field observations, ecological parameters, need to be included in field notes. These notes will help great deal during the processing of the sample and interpretation of the data. Good quality data collection and critical observations are essential components of sampling programme.

7.7.4. Labeling

Correct labeling of the sample is important as it ensures sample identification. Label should contain, date, locality, sample code etc. Labelling should be clean, secure, water proof, non-smearing and with necessary information. It should be ensured that label should not get detached during the storage and transportation.

7.8. Preservation

7.8.1. Wet Preservation

- All the adhering materials such as sand particles and other debris as well as epiphytes should be removed from the seaweeds before preservation.
- A solution of 5 -10 % formaldehyde in seawater should be prepared to preserve the seaweed sample.
- Before adding the preservative, water from the polyethylene bags / containers should be drained and sufficient preservative should be added. Fumes of the formaldehyde would help to fix and preserve the seaweed material. Polyethylene bags should be tied with rubber bands properly to prevent leakage during transportation.

7.8.2. Dry preservation (herbarium)

Material required for preparing herbarium is as follows:

- Plastic trays, Forceps
- Specimen mounting paper (herbarium sheets)
- Cheese cloth, Blotting paper
- Herbarium wooden press
- Painting brush, Pencils, knife etc.
- Polyethylene bags.

7.8.3. Procedure for preparing herbarium

- Fresh specimen should be cleaned of sand particles, rocks, shells, mud and other adhering materials and epiphytes.
- A tray containing fresh water (half filled) should be taken and specimen to be mounted be placed in the water.
- A herbarium sheet, size smaller than the tray to be inserted from below the specimen and then spread the specimen on the herbarium sheet with the help of brush in such a way that overlapping of the specimen is minimized.
- After mounting the specimen on the herbarium sheet, sheet is lifted slowly and tilted to one side to allow water to drain gradually without disturbing the mounted specimen.
- Remove the sheet and properly arrange the specimen with the help of forceps or needle if required.
- To blot dry, herbarium sheets are placed on the newspaper sheets or blotting paper to remove the remaining water from the herbarium.
- A cheese cloth is placed on the top of the specimen in such a way that it covers entire specimen.

- Now place another sheet of the blotting paper over the herbarium sheet.
- Once, all the specimen to be mounted are ready, herbaria are piled one above the other and then placed between the two sheets of the wooden press. The press is tied tightly with appropriate pressure by a rope.
- The press is kept at room temperature for 24 hrs. After 24 hours, blotting papers are required to be replaced. The process of replacing blotting papers is repeated till the time specimen is free of moisture.
- On drying of the specimen, the specimen gets attached to the paper due to the phycolloid present in the seaweed.
- The cheese cloth is carefully removed and herbarium sheet is properly labeled containing collection number, name of the specimen, locality, date of collection and other ecological details.
- Sometimes, specimen are thick and do not stick to herbarium sheets. In such cases gum or glue may be used to stick the specimen or specimen may be tied with thread.
- Prepare three to four more sheets of each specimen. One for yourself, one to send away for identification, one to file in museum and also for distribution and for exchange. Sheets can be placed in the polyethylene bags and sealed and stored.

7.9. Identification

Seaweeds can be identified using standard identification manuals and with the help of taxonomical experts.

Though, identification of seaweed species is difficult, time consuming, it is equally an interesting, challenging and humbling experience. Beginners should get familiar, first with herbarium specimen from the museum or reference collection before going for the field collection. Colour and morphological differences between different genera/ species and taxonomic characteristic are required to be carefully studied. Only thorough practice of handling and distinguishing the plants in the natural habitat will help a great deal in learning seaweed identification.

Taxonomic identification key should be followed to identify the seaweed specimen. <http://www.niobioinformatics.in/seaweed/introduction.htm> is an ideal source for taxonomic key, classification, etc. The taxonomic description of the specimen and anatomical characteristic of the specimen to be identified should be referred from the books, monograph, reference herbaria etc. Once the specimen is identified tentatively, it can be ascertained by comparing it with the herbarium from reference center, through internet, or expert in the field.

7.10. Quantitative assessment of abundance

To obtain more realistic picture of the structure and dynamics of seaweeds one should resort to statistical consideration.

7.10.1 Density

It is the count of the number of individual of the species and the total area sampled.

$D = n/A$ where,

D = density,

n = total number of individuals of the species

A = total area sampled.

7.10.2 Frequency

It is the number of samples in which species occur and total number of samples taken.

$F = j/k$ where,

F = frequency

j = number of samples in which species occur

k = total number of samples

7.10.3 Cover

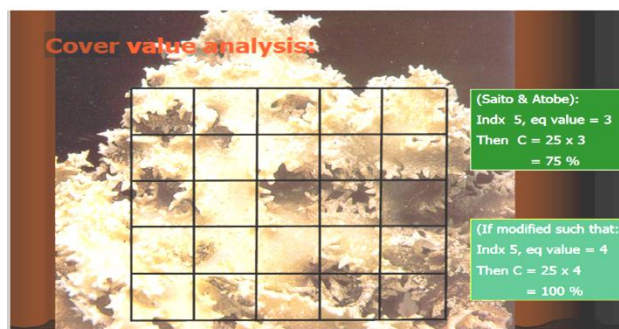
This is the proportion of the ground or the substratum occupied by the individuals of the species.

$C = a/A$ where,

C = cover

a = total area covered by species

A = total area sampled.



Cover value analysis example

(Source: Use of Transect Quadrat Method in Transect Seaweed Assessment by E.G.Fortes)

7.10.4 Standing Crop Biomass

It is the weight of existing species in the given area at any one time.

$B = (D) (TW/n)$ where

B = biomass

D = density

TW = sum of the weights of individual species in a sample.

n = number of individuals in the sample

8.1. Introduction

Fisheries is the largest food production system to derive raw material from nature. According to the Central Marine Fisheries Research Institute, the marine fish landings along the Indian coast was 3.7 million tonnes in 2016 (CMFRI, 2017) out of estimated potential yield of 4.4 million tonnes. Though it indicates that there is a potential to increase the marine fish catch by 0.7 million tonnes, this potential resource is available only in the offshore area and offshore fishing fleet is necessary to harvest these resources.

In the marine sector, the country has 50 fishing harbours and 1281 landing centres. The marine fishing practices are of wide range and are operated by 72,559 mechanised boats (with inboard engine), 71,313 motorised boats (with outboard motor) and 50,618 non-motorised boats (DAHDF & CMFRI, 2012). The major gears are the trawls, gillnets, ring-seines, purse-seines, dolnets, hooks and line and longlines.

While about 1400 species are caught, 200 species of finfish, crustaceans such as penaeid and non-penaeid prawns, crabs, lobsters; and cephalopods such as squids, cuttlefish and octopus are commercially important. Bivalves such as mussels, edible oysters and clams also contribute to the commercial fisheries. Among finfish, oil sardine, Indian mackerel, sciaenids, ribbonfish, Bombay duck, threadfin breams, major perches and carangids contribute substantially to the catches.

Fisheries operate at widely differing organizational levels ranging from self-employed single operators through informal microenterprises to formal sector businesses (as in the case of motorised craft). There are also large differences in the boat types and fishery dynamics between different maritime states. This sector, therefore, is not homogenous within the country and maritime states.

8.2. Method of collection of fish samples

For an EIA study of marine sector, data/information on the status of marine fisheries in the surrounding area, results of experimental trawl catch and major species that contribute to the fishery resources need to be incorporated.

Review and collation of existing information on various aspects such as fish catch statistics during previous years/seasons, fishing gears and crafts used, specieswise landing, fish processing facilities, etc. Adequate and accurate data need to be collected. Experimental trawls need to be carried out as a means of understanding the status of fisheries in the study area.

The primary aim of the field survey is to gather adequate information for subsequent fisheries impact prediction and evaluation, formulation of proposed mitigation measures and monitoring requirements.

The field surveys must be undertaken by suitably trained and competent personnel with adequate knowledge in fisheries and laboratory works. The data obtained shall be quantified and statistical analysis shall be applied wherever appropriate.

Information required for fisheries stock assessment shall include, but not be limited to

- a. the volume and status of fisheries resources and composition of commercially important species in the study areas;
- b. the level and pattern of fishing activity and fisheries production in the study area;
- c. sites of fisheries importance such as nursery and spawning grounds of commercially important species of fish, crustaceans, molluscs and other marine organisms, and seasonal occurrence of juvenile and spawning stocks in the study area;
- d. activities other than fisheries in the area.

8.3. Experimental trawl surveys

In order to assess the fishery potential of the region, exploratory and experimental fishing has to be done. Accordingly, experimental trawl fishing can be conducted using a suitable commercial mechanized stern trawler. The area covered need to be adjacent to the project site and preferably at 5 -10 m depth. Though various gears may be available, two types of bottom trawls, shrimp trawl with 34m head-rope and cod end mesh size of 10mm and fish trawl with 40m head-rope with a cod end mesh size of 20mm are used normally. The duration of each haul can be approximately 1 hr and the towing speed varied between 2 and 3 knots. The catch shall be sorted out into various groups/species and weighed. Fish samples including prawns and crabs collected from the trawl survey should be examined for the maturity stages. Using the experimental trawl survey data, both biomass and density of fish stocks, can be calculated following swept area method (Sparre et.al. 1989). This method assumes that the mean catch in weight per unit area is an index of stock abundance.

The area swept by the trawl is: $\text{Area} = DW \text{ (km}^2\text{)}$,

where

D is the distance covered by the trawl during one haul

W is the width of the path swept by the trawl.

The Biomass (B) for the given area was estimated following the formula:

$$B=S \text{ (mean CPUE/Q)}$$

where

S is the stratum area,

CPUE is the catch per unit effort,

Q is the catch ability coefficient, which is normally taken as 0.5.

Then the density of fish stock is calculated:

Biomass/Area.



Trawling



Trawling



Haul



Assorted Fish catch

For obtaining best results for fish stock assessment, appropriate methodology should be used in the best possible way. To find out the suitability of methodology used in the study to address the stated objectives, the four main aspects of research methodology, namely design, sampling, data collection and data analysis should be evaluated.

The objective of fish collection is to identify all fish species in the habitat using all possible combinations of gear types. There are a number of methods to collect fish specimens. The general approach and methodology may vary from case to case, depending on the nature of the fisheries and the latest development in methods and techniques.

- Information on sedentary animals like bivalves can be collected by hand-picking. Often the best results are obtained by using a variety of gear types to sample, thus ensuring that wide range of fish species and sizes is collected.
- Data can also be collected from fish landing centres or from offices of State Fishery Departments, etc.
- If the assessment is on fishes in the coral reefs, snorkelling and underwater diving will be required and fish abundance can be assessed following line transect surveys and underwater photography. Snorkelling is generally restricted to a pre-selected number of habitat units and involves recording the species, numbers (actual), and in most instances the size classes of fish observed.

Many methods exist for assessing coral reef fisheries, ranging from conventional statistical methods that quantify biomass levels and estimate maximum sustainable yield (MSY), to methods that can be applied where fishery data are limited. MSY is defined as the largest average catch or yield that can continuously be taken from a stock under existing environmental conditions. It is also called as maximum equilibrium catch, maximum sustainable yield, sustainable catch (NOAA, 2006)

A stock assessment provides information to guide fishery management by allowing managers to detect changes in the condition of fish stocks over time. These changes may trigger a management response, aimed at meeting one or more target objectives of the fishery. For example, if managers assess a stock and detect that too many juvenile fish are being harvested, leaving the stock with a low reproduction potential, managers can use this information to set limits on catch or size of the fish being caught.

More detailed stock assessments may be warranted in some situations. Detailed biological information should be collected from captured fish including species, life stage, length and weight, maturity, and age (through analysis of scales, otoliths, or fin rays). When collecting information on the size of fish in a population, the most important measurement is the length. For measuring weight of fish in the field, different types of weigh scales can be used. Commonly used scales include top-loading electronic balances, beam balances, and spring scales. All fish captured should be identified by species and age class, and measured for length (± 1 mm) and weight (± 0.1 g).



Measuring fish length as part of Stock Assessment

Sex determination can be done externally or internally, depending on the species. Recording the maturity of specimens is another important aspect. Accurate determination of maturity is best

accomplished through direct observation of the gonads by dissection in the laboratory. However, classification can also be done based on external observations in some species.

8.4. Preservation

Careful and correct preservation procedures in both the field and laboratory are important for ensuring the quality of the collected specimens or tissues. Fixatives of correct concentration, appropriate containers, clean and sharp dissecting tools, waterproof data form/labels, and complete observations will add to the quality and value of the sampling. Preservation techniques vary depending on how the samples will be used. Formalin is commonly used to preserve collected specimens. It is recommended that a solution of 10% formalin be used for the preservation of fish specimens.

8.5. Identification of fish

- With growing consciousness about the need to conserve biodiversity, the need for taxonomists and species identification has been growing. However, more and more individuals without a taxonomic background, such as fishery inspectors, data collectors, traders and others, have been tasked with the complex and often difficult assignment of identifying aquatic species. These less experienced users are often faced with confusing and inadequate information on the species they encounter and to identify them reliably.
- Publications such as the species catalogues and field guides produced by the FAO Fish Finder Programme, and web resources, such as Fish Base (www.fishbase.org) and the Catalogue of Fishes (<http://researcharchive.calacademy.org/research/ichthyology/catalog/collections.asp>) offer guidance to resolve issues regarding the correct scientific name for a species.
- Nonetheless, greater efforts are needed to ensure correct identification of aquatic resources under management and conservation regimes. For detailed information on different approaches for fish species identification, refer Fischer (2013).

Scientific identification of fishes is based mainly on external characters such as body shape, length, depth, mouth and nature of fish spines, scales, etc. The features of fish that are commonly used for species identification are given in Fig. 8.1.

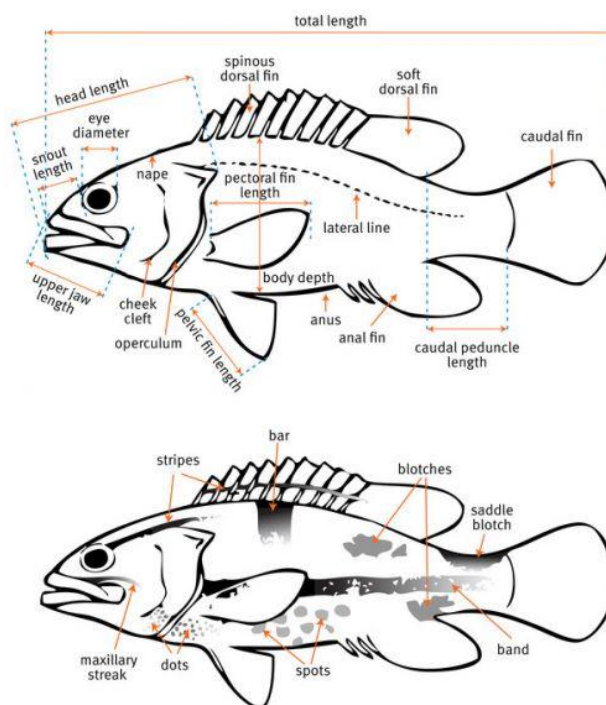


Fig. 8.1. Key features of fish identification (Source: <https://www.daf.qld.gov.au/fisheries/species-identification>)

Fish identification keys depend upon measurement of meristic and morphometric characteristics. A meristic is a countable trait, such as number of gill rakers or number of dorsal fin spines. Morphometrics examines the size and shape using a measurable trait, such as total length or standard length or wet weight, which can be gauged as a length, mass, angle or ratio of other measurements. Meristic traits and morphometrics are often used to classify taxa, sometimes down to the species level or sub-species level.

Prior to modern genetic techniques, meristics and morphometrics were the principal foundation for fish taxonomy and systematics. Even today, meristics and morphometrics are commonly used for species identification and ground-truthing genetic analyses with phenotypic traits.

Among many publications on species identification of Indian fishes, the following are useful guides for field identification (Jones and Kumaran 1980, FSI 2009, Talwar 1995, Talwar and Kacker 1984, and Rajan2003).

Some of the marine fishes, crustaceans, molluscs, etc, collected through trawling are given below.



Sphyraena jello



Saurida sp.



Alepes diedaba



Stolephorus commersonii



Gazza minuta



Siganus canaliculatus



Sepia sp.



Octopus sp.



Loligo sp.



Nibea maculata



Scomberomorus guttatus



Pampus argenteus



Carangoides sp.



Johnius sp.



Narcine sp.



Arius sp.



Decapterus sp.



Trichiurus sp.



Zebrias quagga



Psettodes erumei



Sphyræna sp.



Chirocentrus sp.



Carangoides malabaricus



Thryssa sp.



Pomadasys kaakan



Nemipterus japonicus



Megalaspis cordyla



Leiognathus sp.

Polynemus sextarius

Kathala axillaris

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