B.Sc. FISHERY SCIENCE
LAB MANUAL
3rd Semester

Prepared By
Biological Science Dept.
Fishery Science

MIDNAPORE CITY COLLEGE

BFSC SEMESTER-III

BFSC-301: Fish Immunology

Collection, separation and identification of fish leucocytes. Separation of blood plasma and serum. Differential counting - RBC and WBC by Haemocytometer. Study of different types of leukocytes and isolation of macrophages. Precipitin reactions - Agglutination test, immunogel diffusion, double immuno diffusion, radial immuno diffusion assay, ELISA. Methods of vaccine preparation and techniques of fish immunization.

BFSC-302: Marine Biology

Study of common instruments used for collection of phytoplankton, zooplankton and benthos. Collection, preservation and analysis of phytoplankton, zooplankton, sea weeds, Collection preservation and analysis of inter tidal organisms.

BFSC-303: Fishery Oceanography

Field visits and operation of oceanographic instruments- Nansen reversing water sampler, Bathythermograph, Grabs, Corers, Current meters, Tidal gauges, Echo-sounder. Measurement of temperature, Transparency, pH. Determination of DO, Salinity, Ammonia, Nitrate, Nitrite, Phosphate and Silicate in sea water.

BFSC-304: Fish Food Organisms

Methods of collection and identification of different live food organisms. Laboratory scale culture of selected live food organisms (green algae, spirulina, chetoceros, rotifer, Moina, copepod). Evaluation of live food organisms. Decapsulation and hatching method of brine shrimp cyst.

BFSC-305: Ornamental Fish Production and Management

Identification of common ornamental fishes and plants. Fabrication of all-glass aquarium. Setting up and maintenance of Aquarium accessories and equipment. Conditioning and packing of ornamental fishes. Preparation of feed. Setting up of breeding tank for live bearers, barbs, goldfish, tetras, chiclids, gouramis, fighters and catfishes. Identification of ornamental fish diseases and prophylactic measures.

BFSC-306: Genetics and Breeding

Problems on Mendelian inheritance (qualitative genetics) - monohybrid and dihybrid ratios and epistasis. Problems on quantitative traits, response to selection and heritability. Estimation of rate of inbreeding and heterosis. Mitotic and meiotic chromosome preparation. Demonstration of protocol of androgenesis, gynogenesis and polyploidy. Problems on gene and genotypic frequency. Gamete cryopreservation protocols and quality evaluation of fish milt.

BFSC-307: Physiology of Finfish and Shell fish

Estimation of oxygen consumption, Osmoregulation, ammonia excretion and carbon-dioxide output. Influence of temperature and salinity on metabolism. Haematology of fin and shellfishes. Histological techniques.

BFSC-308: Inland Fisheries

Analysis of species composition of commercial catches at landing and assembling centers, sampling and familiarization of commercially important groups. Observations and experimental operations of selected fishing crafts and gears in inland / estuarine waters. Maintenance of records on catch data. Visit to Dept. of fisheries, lakes and reservoirs, net making yards.

BFSC-309: Aquaculture Engineering

Evaluation of potential site for aquaculture. Calculation of area of regular and irregular plane surfaces, Trapezoidal and Simpson's rule, volume of regular and irregular shape as applied to stacks and heaps, calculation of volume of pond. Land survey – chain surveying, compass surveying, leveling, plane table surveying and contouring; soil analysis for farm construction. Design and layout plan of fresh water and brackish water farms and hatcheries. Design of farm structure: ponds, dykes and channels. Earth work calculations- excavation, embankment, longitudinal slope and cross slope, calculation of volume of earth work as applied to roads and channels and water requirement calculation. Visit to different types of farms.

BFSC-301: Fish Immunology

Practical – 1:

COLLECTION, SEPARATION AND IDENTIFICATION OF FISH LEUCOCYTES

Leucocytes play an important role in the fight against infection in all animals. Therefore for any immunological study, familiarity with leucocytes of the species would be very important. Leucocytes can be obtained from a number of sites e.g., peripheral blood, haemopoietic/lymphoid organs and induced inflammatory exudates. Though peripheral a good source, collection of sample could be a problem when dealing with small fish. The caudal vein is the preferred site for bleeding large fish. Cardiac puncture can also be performed.

Examination of Blood cells:

Most hematologists use stained smears for the identification of different leucocytes types.

Materials:

1. Glass slides
2. Microscope
3. Stains such as Leishman or Giemsa
4. Phosphate buffer pH 6.8
5. Methanol

Methods:

1. A drop of blood is placed on one edge of a glass slide. Using another slide placed at an angle of 350, the drop of blood is smeared quickly on the slide. The smear is allowed to dry in the air. The smear can be stained by Leishman or Giemsa stain. For Giemsa stain, the smear is fixed for 1-2 minutes in methanol.

2. The smear is stained using Leishman stain for one minute. The stain is diluted with twice the volume of phosphate buffer and left for 2 minutes. The slide is then washed with phosphate buffer and allowed to dry.
3. Giemsa stain is diluted 1+9 in phosphate buffer. This working solution is poured on the slide and allowed to stain for 20-35 minutes. Alternatively undiluted stain is used for 5 minutes. This slide is washed in phosphate buffer and allowed to dry.
4. The slides are examined under oil immersion objective. Fish blood contains predominantly erythrocytes (which are nucleated), lymphocytes thrombocytes, granulocytes (neutrophils, eosinophils and very rarely basophiles) and monocytes. The characters of some of the cells are given below.

Thromobocytes: are spindle to ovoid in shape, about 8 microns in diameter with homogeneous grey-blue cytoplasm and centrally located, elongated, often clefted nucleus. These are common in blood.

Lymohocytes: are round in shape with homogeneous grey-blue_cytoplasm and centrally located spherical or clefted nucleus. These are common in blood. In spleen

and kidney smears, large immature lymphocytes with larger amount of cytoplasm are common.

Granulocytes: are round in shape usually containing granules, which may be eosinophilic, neutrophilic or very occasionally basophilic. The nucleus is central or eccentric, irregular, highly polymorphic with several lobes. These are rare to common in blood. In kidney or spleen smears, neutrophils are more common.

Monocytes-Macrophages: Monocytes are round and macrophages are amorphous in shape. Macrophages are not commonly found in peripheral blood but can be seen in smears from the anterior kidney. The nucleus is generally irregular or kidney shaped and eccentric. The cytoplasm is non granular, grey-blue-purple in colour and may have vacuoles and few inclusions.

Observation and finding results:

Features of leukocytes –

Neutrophil	Not present
Heterophil	Nuclei lobed or un lobed
Eosinophil	Usually pale, spherical to rod shaped granules. Nuclei usually un lobed. Cytoplasm blue
Basophil	Round deep blue to purple granules which often mask outlines of lobed (mammals) or un lobed (non-mammalians) nucleus
Lymphocyte	Small, medium or large with round to irregular nucleus. Sometimes azurophilic granules or vacuoles in the light blue cytoplasm. Immunocytes are activated lymphocytes with ample, dense blue cytoplasm
Monocyte	Large cells with un lobed or lobed nuclei and much grey-blue cytoplasm. Vacuoles and fine azurophilic granules may be present in cytoplasm. Macrophages are transformed monocytes which have digested debris

Red blood cells of A, fish: A1, Erythrocytes; A2, lymphocytes; A3, neutrophilic granulocytes; A4, fine-grained acidophilic granulocyte; A5, coarse-grained acidophilic granulocyte; A6, thrombocytes.

Practical – 2:

LYMPHOCYTE SEPARATION FROM FISH BLOOD

Introduction:

The Lymphocyte Separation Medium (LSM) is an iso-osmotic, low viscosity medium containing polysucrose and sodium diatrizoate adjusted to a density of 1.0770 +/- 0.0010 g/ml. This medium offers a quick and reliable method for the simple isolation of vertebrate mononuclear cells and lymphocytes from de-fibrinated EDTA blood.

Principle:

White blood cells (WBCs) or leukocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist, but they are all produced and derived from lymphoid organs. Leukocytes are found throughout the body, including the blood and lymphatic system. There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function. A major distinguishing feature of some leukocytes is the presence of granules; white blood cells are often characterized as granulocytes or agranulocytes as shown in the following table:

Type		Microscopic appearance	Diagram	Nucleus
Granulocytes	Neutrophil			Multilobed
	Eosinophil			Bi-lobed
	Basophil			Bi-lobed or tri-lobed
Agranulocytes	Lymphocyte			Eccentric
	Monocyte			Kidney bean shaped

Materials required:

Sl No.	Materials	Remarks
1	Lymphocyte Separation Medium (LSM) 1077	All the materials were provided in HiPer® Lymphocyte Separation Kit.
2	Centrifuge Tube (15 ml)	
3	Polypropylene Tube (0.5 ml)	
4	Diluent Buffer	
5	Giemsa's Stain	
6	Cedar wood Oil	
7	Trypan Blue (0.5%)	
8	Methanol	
9	EDTA/Heparin coated collection tube	
10	Cotton	
11	Pipettes	
12	Hemocytometer	
13	Microscopic slides & coverslips	
14	Micropipettes & Tips	

Procedure:

Lymphocyte Layer Separation and viability count:

1. Collect 4 ml of blood in the EDTA coated collection tube, using sterile syringe and needle.
2. Mix immediately by inverting or vigorously shaking the tube for EDTA to be uniformly distributed.
3. Dilute the blood by adding 4 ml of diluent buffer.
4. Take 2.5 ml of LSM 1077 in a new 15 ml centrifuge tube. Overlay the LSM with 7.5 ml of diluted blood.
5. Centrifuge at 2300 rpm for 30 minutes in a fixed angle rotor.
6. Using a clean glass pasteur pipette carefully remove the lymphocyte layer in a new collection tube.
7. Add 5 ml of diluent buffer to the lymphocyte layer. Mix by gentle pipetting and centrifuge at 1900 rpm for 10 minutes. (This step helps to reduce the number of platelets).
8. Discard the supernatant obtained from above step.
9. Resuspend the pellet in 500 µl of diluent buffer.
10. Take 10 µl from above step in new collection tube and add 40 µl of diluent buffer and 50 µl of Trypan Blue.
11. Place a coverslip on the Neubauer chamber of the haemocytometer.
12. Cover one side of the Neubauer chamber of the haemocytometer with the sample.
13. Observe and count the live and dead cells under 45X magnification in a light microscope.

Differential Staining:

1. Spin the tube from step 9 at 2300 rpm for 10 minutes.
2. Discard the entire supernatant leaving around 50 µl in the tube.
3. Resuspend the pellet in the same solution and make a smear on a microscopic slide.
4. Air dry the slide for 5-10 minutes.
5. Add 5-10 ml of methanol to cover the slide. This step helps in fixing the cells onto the slide.
6. Discard the methanol carefully.
7. Add 15 ml of staining solution and incubate for 30 minutes at room temperature.
8. Observe under oil emulsion lens and look for the lymphocytes and monocytes.

Observation and Result:

The viable and dead cells were counted under the microscope (in four WBC chambers of the haemocytometer) after trypan blue staining and the following data were recorded:

Observation and Result:

The viable and dead cells were counted under the microscope (in four WBC chambers of the haemocytometer) after trypan blue staining and the following data were recorded:

Viable cells (cells without dye)	Dead cells (cells with dye)
8	1

Calculate the number of total cells as follows:

Area of one WBC chamber = L X H (1 mm X 1 mm)

= $1\ mm^2$

Depth of Haemocytometer is 0.1 mm

Hence Volume of 1 WBC chamber = Area X Depth

= 1 mm X 0.1 mm

= $0.1\ mm^3$

4 WBC chambers were counted so, volume of 4 WBC chambers is 4 X 0.1= $0.4\ mm^3$

$$\text{Total no. of cells (cells/ml)} = \frac{\text{Total cells}}{\text{Volume of WBC chamber}} \text{ X 1000 X Dilution Factor}$$

$$= \frac{9}{0.4 mm^3} \text{ X 1000 X 5}$$

$$= 1.12 \text{ X } 10^5 \text{ cells / ml}$$

Percentage of isolated lymphocytes, monocytes and total granulocytes were counted under the microscope after performing the differential staining and recorded in the following table:

No. of total cells	No. of viable cells	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)
9	8	65	2	33

Practical – 3:

DIFFERENTIAL COUNTING –

RBC AND WBC BY HAEMOCYTOMETER (NEUBAUER CHAMBER)

Aim:

To study the differential counting of RBC and WBC by Haemocytometer.

Introduction:

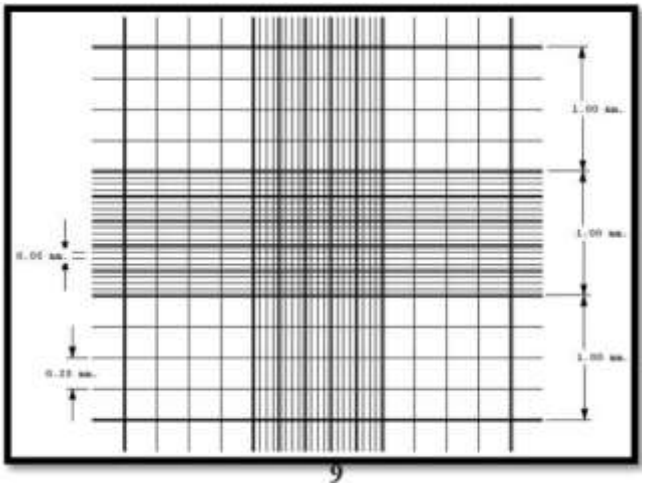
The hemocytometer (or haemocytometer) is a counting-chamber device originally designed and usually used for counting blood cells. The hemocytometer was invented by Louis-Charles Malassez and consists of a thick glass microscope slide with a rectangular indentation that creates a chamber.

General features of the neubauerchamber:

Neubauer's chamber is a thick glass plate with the size of a glass slide (30x70x4mm). The counting region consists of two square shaped ruled areas. There are depressions or the moats on either side or in between the areas on which the squares are marked thus giving an "H" shape.

The ruled area is 3mm2 divided into 9 large squares each with a 1 mm^2 area. The large central square (which can be seen in its entirely with the 10X objective), is divided into 25 medium squares with double or triple lines. Each of these 25 squares are is again divided into 16 small squares with single lines, so that each of the smallest squares has an area of 1/400 mm^2.

The glass cover is a squared glass of width 22 mm. The glass cover is placed on the top of the Neubauer chamber, covering the central area. The ruled area is 0.1 mm lower than the rest of the chamber. So that when a cover slip is kept on the counting region, there is a gap of 0.1 mm (1/10mm) between the cover slip and the ruled area.



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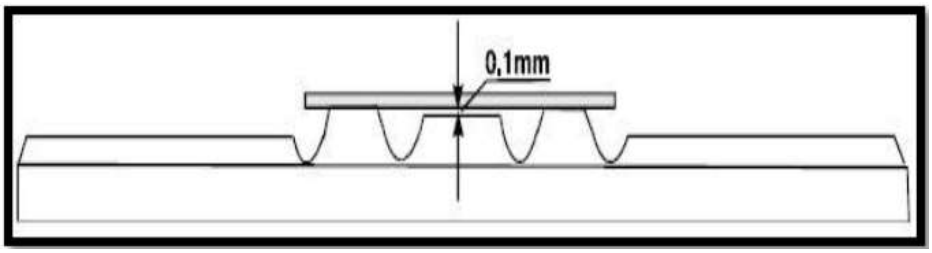
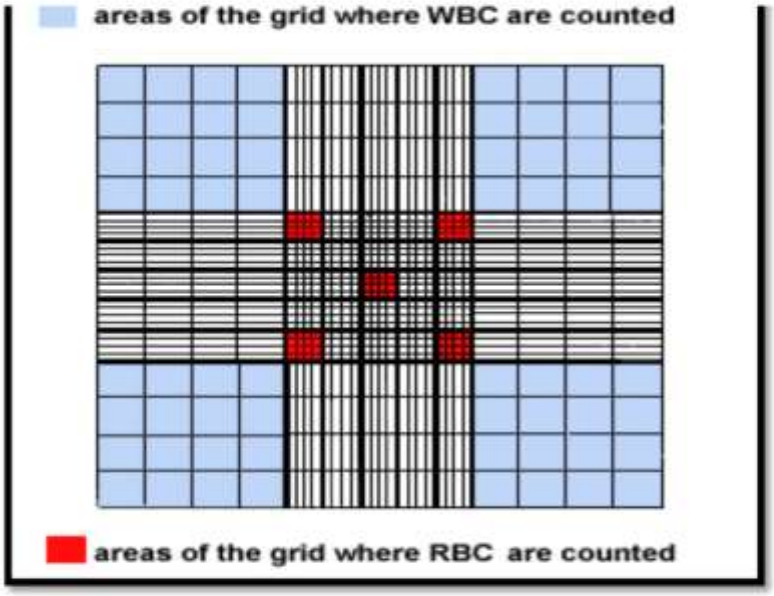


Cell counting areas in neubauer chamber:

The counting can be done either in the central large square or in the corner squares, depending on the size of the cells under study.



WBC Counting Area: The four large sqaures placed at the corners are used for white blood cell count. Since their concentration is lower than red blood cells a larger area is required to perform the cell count.

RBC Counting Area: The large center square is used for RBC counts. As already stated, this area is subdivided into 25 medium sqaures, which in turn are each divided into 16 squares. Of the 25 medium sqaures, only the four corner squares and the center square within the large center square are used to perform RBC counts.

Platelet Counting Area: The large center square is used to count platelets. Platelets in all 25 squares within the large center square are counted.

Practical – 3:

Using the neubauer chamber:

1. Sample Preparation:

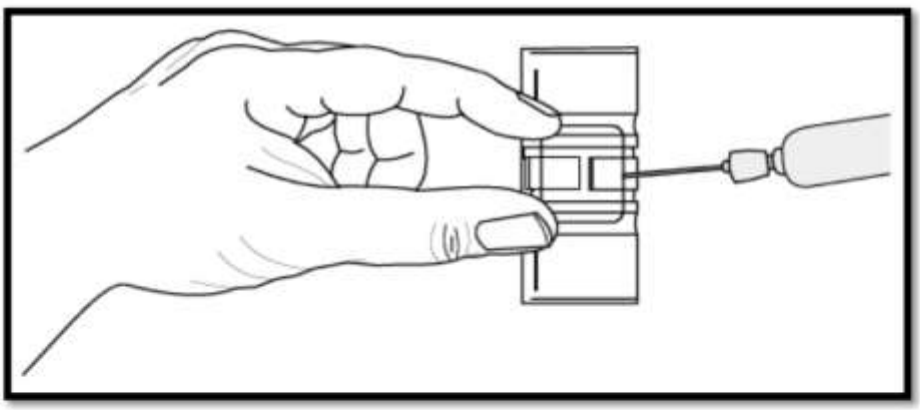
Depending on the type of sample, a preparation of a dilution with a suitable concentration should be prepared for cell counting. Typically, the concentration range for a cell count with Neubauer chamber is between 250,000 cells / ml and 2.5 million cells / ml. An appropriate dilution of the mixture with regard to the number of cells to be counted should be used. If the sample is not diluted enough, the cells will be too crowded and difficult to count. If it is too dilute, the sample size will not be enough to make strong inferences about the concentration in the original mixture.

2. Preparing Neubauer Chamber:

Clean the Neubauer chamber and the cover slip with 70% EtOH. Put the glass cover on the Neubauer chamber central area. Use a flat surface to place the chamber, like a table or a workbench.

3. Introducing the sample into the Neubauer chamber:

With a pipette, carefully draw up around 20 ml of the cell mixture (dilution). Place the pipette tip against the edge of the coverglass and slowly expel the liquid until the counting chamber is full. Capillary action will help to ensure that the counting chamber is full, but care should be taken not to overfill the chamber. A volume of 10 ml is sufficient to fill one counting chamber.

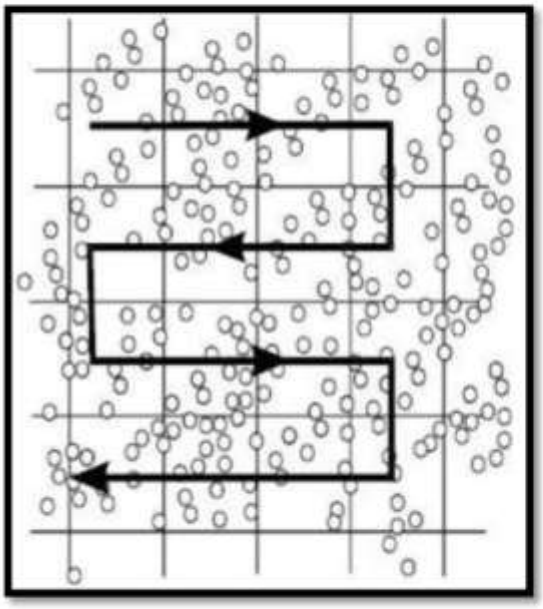


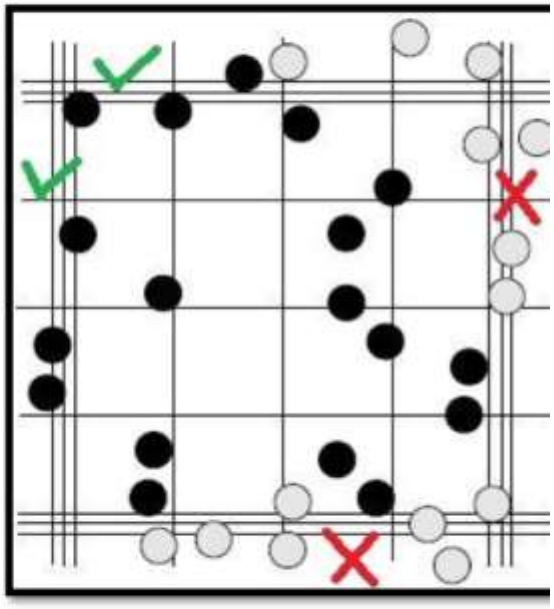
4. Microscope focusing and Cell Counting:

- Place the Neubauer chamber on the microscope stage. Using the 10X objective, focus both onto the grid pattern and the cell particles.
- As 10X is appropriate for WBC counting, count the total number of cells found in 4 large corner squares.

- To count the RBCs and Platelets, the microscope must be switched to 40X objective. Count the cells in the respective areas as stated early.
- Write down the amount of cells counted

If cells are touching the 4 perimeter sides of a corner square, only count cells on 2 sides, either the 2 outer sides or 2 inner sides.



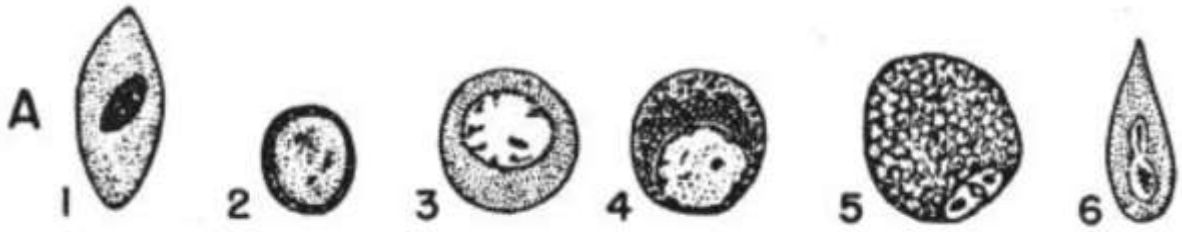


Calculation:

The total number of cells per microliter of sample can be calculated from the number of cell counted and area counted. This is because the ruled areas of the chamber contain an exact volume of diluted sample. Since only a small volume of diluted sample is counted, a general formula must be used to convert the count into the number of cells/microliter.

$$\text{Particles per µl volume} = \frac{\text{Counted particles}}{\text{Counted surface (mm}^2\text{)} \cdot \text{Chamber depth (mm)} \cdot \text{Dilution}}$$

The dilution factor used in the formula is determined by the blood dilution used in the cell count. The depth used in the formula is always 0.1. The area counted will vary for each type of cell count and is calculated using the dimensions of the ruled area.



Red blood cells of A, fish: A1, Erythrocytes; A2, lymphocytes; A3, neutrophilic granulocytes; A4, fine-grained acidophilic granulocyte; A5, coarse-grained acidophilic granulocyte; A6, thrombocytes.

Practical – 8

METHODS OF VACCINE PREPARATION AND TECHNIQUES OF FISH IMMUNIZATION

Aim:

To develop vaccine (formalin killed whole-cell antigen) of pathogenic strain (*Aeromonas hydrophila*) and the techniques of fish immunization.

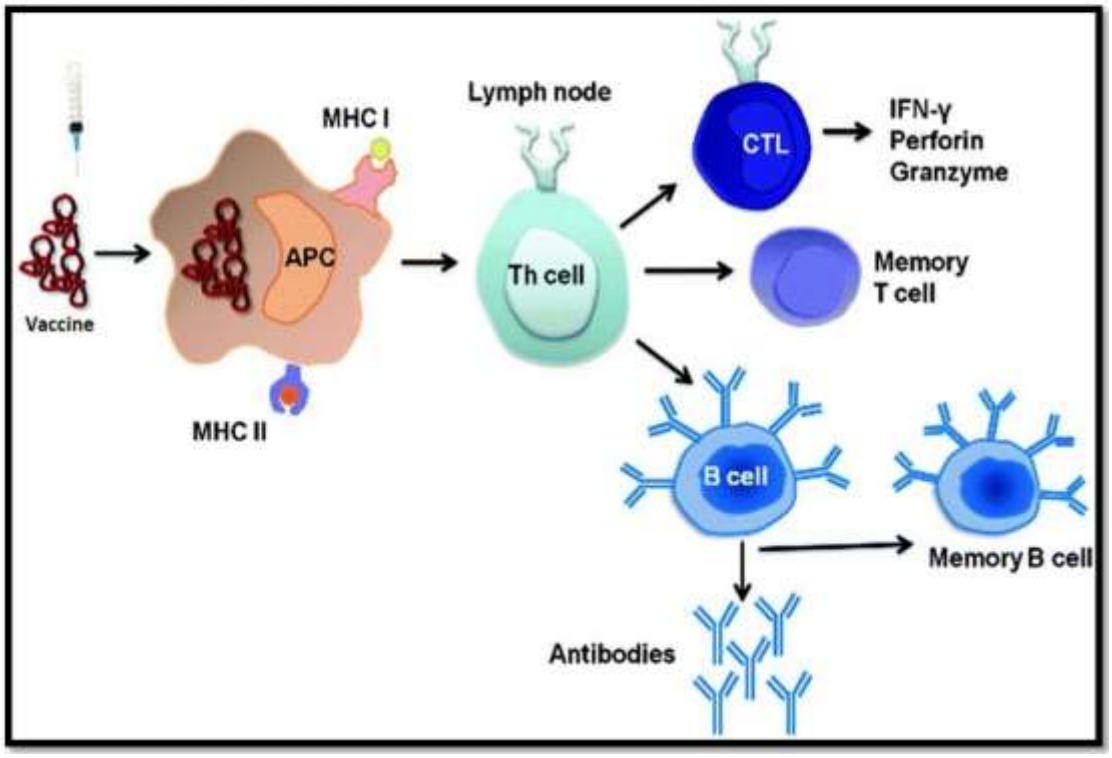
Introduction:

A vaccine is a biological preparation that improves immunity against the particular disease. Vaccines contain an agent that identified a disease causing micro-organisms & is often made from weakened or killed forms of the microbes, its toxins or one of its surface proteins. It stimulates the body's immune system to recognize the agent as foreign, destroy it & remember it, so that the immune system can more easily recognize & destroy any of these micro-organisms that it later encounters.

Characteristic of ideal vaccines:

- Should not be toxic or pathogenic i.e. it should be safe.
- Should have very low level of side effects in normal individuals.
- Should not cause problems in individuals with impaired immune system.
- Should not spread either within the vaccinated individual or to other individuals.
- Should not contaminate the individuals .
- Should be effective in producing long lasting humoral & cellular immunities.
- The techniques of vaccination should be simple.
- It should be cheap.

Mechanism of vaccine action:



Materials required:

1. Live fish
2. Tryptic soy broth (TSB)
3. Tryptic soy agar (TSA)
4. Pathogenic strain (*Aeromonas hydrophila*)
5. Petri plate
6. Test tube
7. Inoculation loop
8. Spreader
9. Incubator
10. Cotton
11. Cooling centrifuge machine
12. Centrifuge tube
13. Laminar flow cabinet
14. Bunsen burner
15. Phosphate buffer saline (PBS)
16. Refrigerator
17. Adjuvant (IFA)
18. Syringe
19. NSS

Procedure:

The cell suspension of *A. hydrophila* strain was prepared, following the method:

i) These strains, maintained on tryptic soy agar (TSA) slants, were streaked on to fresh TSA plates and incubated at 30°C for 24 h to get young discrete colonies.
ii) Two young colonies were aseptically picked, transferred to 10 ml of tryptic soy broth (TSB) and incubated at 30°C for 24 h.
iii) This 24 h old culture was then transferred to 300 ml TSB and re-incubated at 30°C for 24 h.
iv) The cells were harvested by centrifugation at 7500 rpm for 30 min at 25°C in a cooling centrifuge.
v) The cell pellets were washed thrice by centrifugation with sterile phosphate buffer saline (PBS, pH 7.2) and finally re-suspended in 10 ml of sterile PBS and kept at 4 °C.
vi) The number of cells/ml of suspension was determined by spread plating on TSA after incubation at 30°C for 24 h.

Administration/ immunization methods:

Three main vaccines administrating methods

I. Injectable vaccines
II. Immersion vaccines
III. Oral vaccination

Injectable vaccines:

- The vast majority of immunized fish are vaccinated by injection into the peritoneal cavity.
- Vaccine is injected in the abdominal area of each anaesthetised fish (>50g). Now also micro vaccines (0.05ml).
- The needle is inserted into the peritoneal cavity at a 45º angle to a depth of approximately 0.5 cm.
- Vaccines can also delivered by intramuscular injection.
- Intraperitoneal delivery is good for healthy fish with minimum stress & protect the fish throughout the lifetime.
- Modern vaccines will protect against 5 or more different diseases from a single shot.

Immersion vaccines:

Two application methods: dip and bath

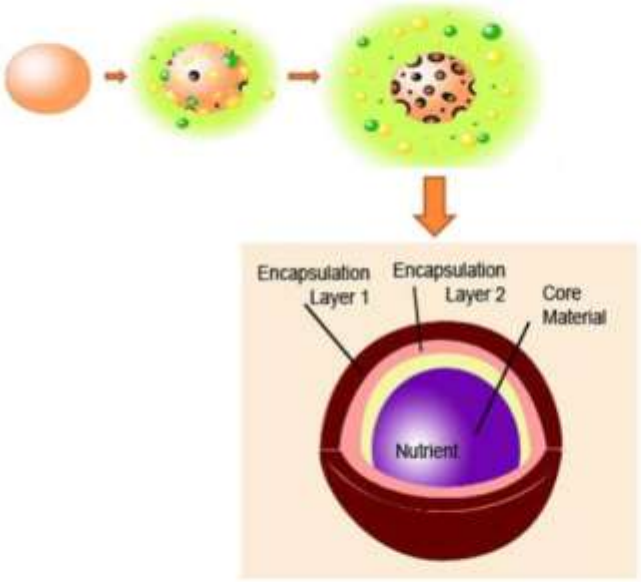
- **Dip vaccination:** More widely used. Rapid vaccination of large numbers of fish (up to 100kg of fish / litre of vaccine solution). Small fish (1-5 gm) immersed for very short duration (30 seconds) in a highly concentrated vaccine solution (1 part vaccine to 9 parts water).
- **Bath vaccination:** Larger fish are exposed for a longer period, usually one to several hours, in a lower concentration of vaccine.

Dip vaccination

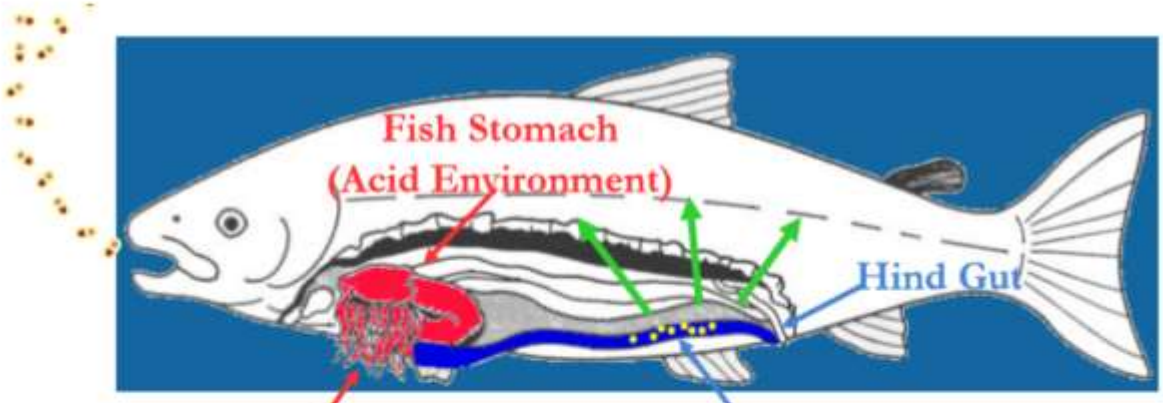
Bath vaccination

Oral vaccination:

- In oral vaccination, the vaccine is either mixed with the feed, coated on top of the feed (top dressed) or bio-encapsulate.
- Bio-encapsulation is used where fish or shrimp fry are to be vaccinated. In this case, live feed such as artemia nauplii, copepods or rotifers are incubated in a vaccine suspension and then fed to the fry.



When vaccines are used as top dressing in feed, a coating agent is usually applied, either to prevent leaching of the antigen from the pellets or to prevent breakdown of the antigen in the acidic environment of the stomach.



1. In the acid environment of the fish stomach, the feed pellets are digested. The Antigens themselves are protected by the APV and pass through intact.
2. Antigens are delivered to the area of the hind gut where they are absorbed and activate an effective immune response in the fish.

Observation:

Groups	Mortality effect at immunized/ vaccinated periods		Mortality effect at *A. hydrophila* challenged periods.	
	Number	**Clinical signs**	**Number**	**Clinical signs**
Injectable vaccinated group				
Immersion vaccinated group				
Oral vaccinated group				
NSS injected group (Positive control)				
Control fish				

BFSC-302: Marine Biology

P-1 . Procedures to be followed for the collection of plankton and benthos:

Time of collection

Unlike some of tideless seas like Mediterranean, the seas that lap Indian shores have tides which may range from a fraction of metre to prodigious 6 ½ m as in Gulf of Cambay. There are two high tides and two ebb tides in 24 hours. Both high and ebb tides occur roughly 50 minutes later each day i.e., tides in our country are predominantly semidiurnal. At new moon and full moon, the sea water rises unusually high and recedes unusually low; these are called spring tides. During the first and last quarters of the moon, we have neap tides, when the sea water neither comes up very high nor recedes very low; obviously it is preferable to visit the seashore at the time of spring low tides, when the maximum portion of the shore is exposed.

On our shores, the monsoon is so vigorous that a good part of our shore life in the intertidal region gets washed away or dies due to the lowering of salinity arising out of dilution by rainwater. It is futile to seek a variety of shoreline during this season. On cessation of the monsoonal rains, marine life starts to colonize the intertidal region and grows to maximum abundance in winter. After winter, the increasing warmth starts to affect shore life, so that it starts to decline and is washed off once again during the rainy season. The best time of year for observing and collecting intertidal life is therefore during winter preferably late winter.

Place of collection:

The animals and plants on the seashore are not distributed haphazardly; each kind occupies a well-defined zone. What we find on seashore will depend not only on the state of the tide but also on the type of shore. Seashores can be roughly classified rocky, shingle, muddy and sandy.

Shingle or pebble beaches are covered by smooth rounded stones. These cannot retain water between them and waves constantly roll them about so that life

cannot settle on them and shingle beaches are therefore barren and not worth any attention.

Sandy beaches are exposed to surf (waves), these waves like the upper layers of sand in a cloud of abrasive particles. This scouring action is harmful to life and only those which can take refuge by burrowing can survive. A sandy beach therefore looks apparently barren until one starts digging. We can then find typical sand dwelling animals like the ghost crab (Ocypode), mole crab (Emerita), cockle (Cariata), razor shell (Solen) and wedge clam (Donax) among others.

Mud consists of very fine particles, which can accumulate only where there is restricted wave action. The fine size of mud particles enables easy burrowing and also prevents drying up of mud even at the surface. Typical mud dwellers are the sea anemones (Para condylacten), tapertry clam (Paphia), lug worm (Arenicola) etc.

Rocky shores may be either in the form of steep cliffs running down into the sea or extensive flat slopes with many fissures and crevices. There we may also include man-made stones or concrete structure as such as marinas and wharf piles for example. In contrast to mud flats and sandy beaches where animals can easily burrowe, rocky shores which are exposed to strong wave action can provide shelter only to animals or plants which can cling to the rocky surface. Typical rock dwellers are the Acorn barnacles (Balanus), Periwinkle (Littornia), Oyster (Ostrea), Mussel (Perna), hydroides (Obelia), bryozoans (Membranipora), rock sea anemones and sea weeds such as Ulva, Entermorpha, Padina and so on.

On the rocky shores, we have the limpet (Cellana), the sea hare (Aplysia), many gastropod snails and most types of sea weeds. A large variety of life forms are unable to tolerate desiccation like fishes, octopi and sea slugs are found in rocky pools, which retain some water even at ebb tide.

Although we have classified the seashores into rocky, sandy and muddy, we may also have mixed shores, having two or all three components. Then a sandy beach open into mud flats at a lower level and also have rounded stones or

boulders on it. Such places provided ideal collecting sites as they have all the three components of the seashore.

At some places in India for example in the Gulf of Kutch (Port Okha, Pirotan island), south eastern coast near Rameswaram, Lakshadweep islands and Andman & Nicobar islands, we have extensive coral reefs...

Marshes occurring in patches in many of our creeks and estuaries and in vast areas like Sunderbans of Bengal, are typically inhabited by mangroves – terrestrial trees which have secondarily invaded the sea, very successfully.

Method of collection

On rocky shores (faces), since, most of the animals and plants live attached to the surface, they can be easily seen and picked up. However, quite a lot of animals hide between the rocks. In rock pools, on sandy beaches and mud flats, what is visible at first glance is but a small fraction of the total population, as much of it lives under stones or burrows into the ground.

Most of the animals in a rock pool can be collected by turning over stones. As this will disturb the animals, the active fishes and prawns will move away to hide in cracks and the crabs will scuttle away, you can see the slowly wriggling brittle stars and worms or a contracting sea anemone. Remember always to turn the stone back to its original position, so that the sessile animals such as sponges, sea anemones, tube dwelling worms, hydroids and bryozoans are not exposed to the sun's heat or to their enemies. Brittle stars, many worms and some sea slugs are very sensitive to handling and may break into pieces. Therefore, gently slide them into a glass bottle or polythene bag containing sea water.

Animals on rock faces will require to be scraped off with some force; an old chisel or scalped is ideal for the purpose. Limpets should be suddenly wrenched off while they are relaxed; once they are disturbed and on their guard it is very difficult to prise them off.

Burrowing animals can be dug out with a shovel or small rake. Conservation of wildlife in sea is as important as on land. Be content to watch the seashore life intently. If you must collect do not overdo it. One specimen of each kind, if required as a museum specimen may be taken away but no more. If you desire to study it alive, remember that most people do not have the expertise to maintain marine animals alive for more than a day or two. It is best to return the animals to the seashore near the place where you collected them. It is worth remembering that batch of students go on the rampage overturning each and every stone they come across in the seashore not bothering to return to its original position thereby collecting vast number of each and every form of life they come across, only to throw them in dustbin at end of the day. Do not be one of them.

A rectangular hand net is suitable for catching fishes or even large crabs if you are worried about their claws hipping your fingers. Animals with sharp spines like sea urchins, spider crabs and lobsters should be handled carefully with blunt-tipped, forceps which are ideal for lifting up delicate and soft animals like worm and sea slugs. Remember that many animals are venomous or can inflict a sting. Some of hydroid colories and jelly fish can give a sting. Spines of fish like sting ray, scorpion fish or catfish and of some sea urchins also contain venom. Any cutor wound should be immediately bathed with clean water and antiseptic applied on them.

The animals can be placed in polythene bags or glass bottles and larger ones in plastic buckets. Small and delicate animals such as sea slugs can best be kept in small vials. It is almost impossible to search for minute forms such as sea spiders, skeleton shrimps, amphipods and some worms directly on the seashore. It is best to bring back a colony of hydroids, seaweeds or sponges and keep them in an enamel or glass tary or jar over night. By next day lack of O2 in the water will force these tiny animals to drop to the bottom from where they can be picked up.

Animals can be preserved in 4% formation prepared by diluting it with sea water. or they may be washed with tap-water and preserved in 70% alcohol or methylated spirit. Storage of animals with limy shells (crustacean, Molluscs) for a long time in formalin dissolves the shell. They are best preserved in alcohol. Remember that formalin is very poisonous and also injurious to our eyes and skin.

Shorts are better than trousers for wading in shallow water. Many animals are most abundant and some animals like sea stars are only found at places which they are always submerged in water, even during spring low tides.. A cap or hat which can be secured against wind is essential in our climate. Shoes should be with laces; sandals and chappals have a disconnecting habit of ending up with broken straps. It is painful to walk barefooted in mud, for there are always shells with sharp edges and near cities even broken glass bottles. On rocks overgrown with barnacles thick soled shoes are a must.

Hunter boots are good for shore collection, as long as the treads on the soles are not worn out. Smooth rubber, soles involve the risk of slipping and hurting oneself. The black rubberized composition on waterproof shoes meant for wear in rainy season is ideal as the shoes grip rock firmly. On very slippery rocks in heavy surf, the three-point contact method of moving is best i.e., always had either both hands and one foot, or one hand and both feet, in contact with rocks. On some shores with very flat slopes or high tidal range, the water at high tide can come rushing faster than you can walk (you cannot run in uneven, slippery rocks or in soft mud). Be careful to come out well in time before the incoming tide overtakes you or while collecting on a high patch of ground surrounded by low shore level, you may suddenly find yourself surrounded by water unless you are vigilant. when collections are done in evening, it is a good idea to have an electric torch for use, as it may become dark while you are still at some distance from dry, even land.

Many timid animals as well as contractile animals like sea anemones and corals are best seen while submerged. When the sea bottom is exposed, they are either hidden or appear as shapeless, blobs. The use of a glass bottom boat in shallow water enables one to see under water without getting wet. You can make a water glass by knocking off the bottom from an old bucket and replacing it with a round piece of glass. Mae it watertight. You can hold bucket so that its bottom is below the water surface and put your head into the bucket. Then you can not only see sea life but can also dive down in shallow waters to collect it.

P-2: Fixation and Preservation of Plankton

Phytoplankton

The collected plankton should be preserved immediately for future analysis. This is especially critical in tropical seas owing to higher temperatures. Plankton collected for years in an area will help to know about its fertility.

Though a number of preservatives are available for planktonic organisms, only a few are commonly used for fixing and preserving phytoplankton. The various methods of fixing and preserving the phytoplankton samples are discussed below.

Formalin

It is a widely used fixative and preservative for a variety of organisms including plankton. The commercial formalin is obtained as a 40% (saturation limit) formaldehyde dissolved in water. It may also have some menthol content which though is a poisonous substance serves as a fixative.

The formalin has to be stored in inert glass or plastic containers and not in metal containers as the formalin reacts with the latter. For ordinary use, formalin may be stored in amber coloured bottles and kept in cool temperature. It is also worthy of mention here that, if, the formalin is stored in light coloured bottle and is exposed to sunlight, a poisonous white precipitate is developed due to the formation of paraformaldehyde. Though for ordinary uses, this may be filtered through a filter paper and used for taxonomic studies, generally paraformaldehyde formed formalin should not be used.

The commercial formalin may also contain dissolved impurities such as iron and formic acid which disintegrate the shells of some planktonic organisms. When impure formalin is used, the iron comes out as a brown flocculant precipitate and the plankton are likely to get entangled in it. Such precipitation of iron can be prevented by adding Rochelle salt (sodium potassium tartrate) at the rate of 10 g of salt to 11 of 40% commercial formalin. The acid content of commercial formalin however, may be neutralized, by the addition of excess of calcium carbonate. Alternately, the commercial formalin may also be neutralized in the following way. One litre of 20% neutralized stock formaldehyde solution is prepared by diluting 200 ml of 40% formaldehyde solution with 800 ml of filtered seawater to which 0.5 g borax (Sodium borate)and 6.5 g disodium hydrogen phosphate are added. Then the solution is allowed to settle for about 24 hours. The supernatant solution is siphoned out and used.

For preserving net phytoplankton sample, 2% neutralized formaldehyde (i.e. 5% formalin) may be used. The volume of the supernatant preservative should atleast equal the settled volume of plankton.

Formaldehyde is poisonous and its fumes are known to irritate the skin, nose and lungs and cause serious cases of dermatitis. Hence, rubber gloves should be worn when preparing formaldehyde solution or at the time of preservation of plankton samples in formaldehyde.

Lugol's solution

It is a good preservative especially for nanophytoplankton of Utermohl's tubular chambers . A 100 ml of Lugol's solution may be prepared as detailed below: 10 g of potassium iodide and 5 g of doubly sublimed iodine are dissolved in 20 ml of distilled water and to this, 50 ml of distilled water and 5g of sodium acetate or 5ml of 10% acetic acid are added. This solution is made up to 100ml with more of distilled water. For about 250 ml of water sample containing nanophytoplankton, about five drops of this preservative is quite sufficient. However, for phytoplanktonm samples containing more of coccolithophorids, this preservative should not be used as their cells may turn black with this preservative.

Osmic acid

This preservative is prepared by adding 200 mg of Osmium tetroxide to 10 ml of distilled water. This preservative is added at the rate of 3-6 drops per 100 ml phytoplankton sample.

Glutaraldehyde

This solution is prepared by mixing 8 g of glutaraldehyde in 100ml of distilled water. This preservative is added to the phytoplankton sample in the ration of 1:1.

The phytoplankton especially diatom plankton should not be stored in glass bottles as their silica shells may otherwise get dissolved in polythene bottles or other metal containers in the course of time.

Zooplankton

Narcotisation, fixation and preservation

Zooplankton samples intended for taxonomic study need to be narcotised, fixed and preserved (stored) , in that order r, immediately after being caught . Otherwise autolysis, bacterialaction, cannibalism or chemical deterioration will set in .

Narcotisation

The narcotisation of zooplankters is a vital aspect especially before their fixation and preservation. Narcotics (analgesics) which are soluble either in water or lipids are mainly used with zooplankton to prevent their contraction and distortion at fixation, thereby ensuring ready identification of the preserved organisms . I t has been found that full narcos occurs in about 24 hrs in most of the zooplankters .

Narcotising Agents and Methods of Narcotisation:

i) **Oxygen starvation**: In this method, a little quantity sea - water i s first boiled for 5 - 10 minutes and then cooled to room temperature . The transfer of planktonic animals to this medium will narcotise them.

ii) **Excess CO2:** The planktonic animals present in seawater may be narcotised by bubbling pure C0 2 from a cylinder of gas.

iii) **CO**: This may be added to the seawater containing zooplankters by bubbling the gas from a cylinder. This operation should be done in a fume chamber .

iv) **Freezing** : The zooplankters in their sea-water medium may be kept in - 1 ° C t or 3 0 – 60 min. After that the seawater is thawed and decanted. The narcotised animals are the fixed.

v) **Ethanol**: Pure ethanol may be added drop by drop to the sea- water containing live zooplankters. However, 70% ethanol has been found to be more effective in planktonic polychaetes such a s tomopte rids and for the larvae of benthic species.

(vi) **Formaldehyde**: Planktonic molluscs can be narcotised using formaldehyde as follows:

a) The animals are first placed in about 50 - 500 times their own volume in seawater and allowed to expand.

b) Three drops of 1 % formaldehyde are added to every 100 ml of sea-water every 15 minutes for 1 h.

c) Six drops of formaldehyde are added every 15 minutes during the second hour.

d) During the third hour, 12 drops are added.

e) The addition of formaldehyde is continued until the animals become completely insensitive.

(vii) Magnesium chloride and magnesium sulphate: Although these chemicals are not exactly narcotics, they are cheap and simple to use and make the planktonic animals insensitive.

a) **MgCI2 ' 6H20**

Planktonic coelenterates, polychaetes (tomopterids and polychaete larvae), chaetognaths and pteropod molluscs may be narcotised using 7% MgCI2 . 6H20 solution. The transfer of these animals to this solution induces superficial narcosis in about 20 min. For the veliger larvae of molluscs, a 2% MgCI2 . 6H20 solution (prepared in freshwater and sea-water in equal volume) has been found to be effective. This solution is added gradually to the sea - water containing actively swimming larvae until they are extended and immobilised.

b) **Mg S04 . 7H20**

Planktonic coelenterates, tomopterids and polychaete larvae may be narcotised using 20% Mg . S04 . 7H20 solution.

(viii) **Acetone-chloroform** mixture and glycerine-alcohol mixture: A 1 % solution of acetone-chloroform mixture is effective in narcotising pteropod and heteropod mollusks in the plankton. Similarly. a mixture of 1 part glycerine, 2 parts 70% alcohol and 2 parts sea-water may also be used for these groups of plankton.

(ix) **Menthol**: This is useful for narcotising planktonic coelenterates and pteropod molluscs. 12 g of methanol and 13 g of chloral hydrate are ground in a mortar and the mixture added to the medium containing live plankters. Narcotisation may occur in 15 - 30 hrs.

P-3 Fixation and Preservation of Zooplankton:

Fixation is the application of a chemical (fixative) to kill an organism but retain its morphological characteristics as far as possible. Preservation is the maintenance of the fixed condition for extended periods of time. The chemical solutions for fixation and for preservation may be the same or a different one may be used for the latter .

Fixatives and preservatives and their preparation

Formaldehyde: Its characteristics and uses:

(i) The commercial formaldehyde available to biologists has the

following composition:

Formaldehyde : 37 - 41%* (* For calculation , 40 % is considered .)

Methanol : 7-13%

Formic acid : 0.025% (by wt)

pH : 2.8 - 5

Ash content : 0.01 % (by wt)

Fe : 2 ppm

Cu : 5 ppm

Water : 46 - 56%

(i i) Formaldehyde is the cheapest among the known preservatives.

(iii) It is known to have preserved plankters in good condition for more than 50 yrs (Anon., 1968).

(iv) 2 to 4% formaldehyde in sea-water is the best reagent both as a fixative and as ' a preservative for most marine zooplankters.

A 4% solution is normally used if the plankton-to-preservative ratio exceeds 1 : 9. and a 2% solution is used when the above ratio does not exceed 1 : 9. For

example, for a 10 ml plankton concentrate , 90 ml of 2% formaldehyde solution may be added or 180 ml of a 4% solution .

(v) The preferable of pH of formaldehyde solution for preserving a mixture of collected zooplankton is 7.

In order to obtain such a favourable pH, sodium glycerophosphate is added at the rate of 5 g per 100 ml of concentrated formaldehyde.

(vi) Dilution of formaldehyde for preserving marine zooplankton mixtures is done with sea-water .

(vii) The additive propylene glycol is added at the rate of 5 ml per 1 00 ml diluted formaldehyde to increase the bactericidal and fungicidal properties of the formaldehyde solution .

Preparation of formaldehyde solutions : The concentrated formaldehyde (i . e., 40%) is usually used for fixing the zooplankters after the catch . However , for preservation, 2 to 4% solution is commonly employed. In the preparation of a 4% solution , 10 ml of 40% formaldehyde is dissolved in 90 ml sea - water or distilled water . Similarly , 100 ml of 2% formaldehyde would contain 5 ml of 40% formaldehyde . The prepared solutions are normally kept in low temperatures , i . e. , below 20°C.

Neutralisation of formaldehyde: As the commercial formaldehyde has considerable acidity, it is always advisable to use the formaldehyde solution whether concentrated or diluted only after neutralising it . To neutralise the acidity of the fixing (40%) and preserving (2 - 4%) formaldehyde solutions , excess CaC03 may be added when the pH of these solutions rises to 7 . When formaldehyde solutions neutralised with CaC03 are stored in steel containers , the dissolved iron occasionally present may produce brown deposits. This can be prevented by using the following solutions:

(i) Neutralisation of 40% formaldehyde fixative : This is prepared by adding 10 g of Rochelle salt (potassium sodium tartrate - KNaC4H406 . 4 H20) and 10 g of CaC03 to 1 litre of 40% commercial formaldehyde.

(ii) Preparation of 2 - 4% neutralised formaldehyde solution : 5 g of Rochelle salt is first dissolved in 1 litre of tap-water . To 875 ml of this solution , 120 ml of 40% commercial formaldehyde and 10 g of CaC03 are added . '

Formaldehyde fixation of general zooplankton: Two methods are commonly employed.

To an empty container (say 500 ml capacity) neutralised 40% formaldehyde is added so as to make a final strength of 4% when the container is completely filled with sea-water and zooplankton . In this case, 50 ml of neutralised 40% formaldehyde is added to the 300 ml of sea - water earlier poured into the container . The living, drained or concentrated zooplankters are then added and the jar topped up with sea-water . The jar is temporarily screened down and gently inverted a few times to mix the contents .

(ii) In the second method , 4% neutralised formaldehyde in sea- water or in distilled water is first added to half the level of an empty jar . The freshly collected and concentrated zooplankters are then added with a minimum of sea - water . Subsequently , more of the 4% formaldehyde solution is added until the jar i s full .

Such fixed zooplankton . are left for a day or two , then filtered once again . The animals are finally preserved (stored) in small jars with plastic screw caps in the neutralised 5% formaldehyde solution or in 75% ethanol . In replacing the fixative with the preservative , the fixative is carefully decanted . The sample jar is then filled to about three-quarters with sea-water or distilled water to which the correct volume of concentrated preservative (2.5 ml of buffered 40% formaldehyde or 7 . 5 ml of preservative with additives per 100 ml container volume) is added. Subsequently, the jar is topped up with sea-water or distilled water and sealed with a secure lid. It is also better if the jar is inverted several times to scatter the animals through the preservative. Such bottles should be checked monthly or quarterly for evaporation or animal condition. The plankton bottles should preferably be stored in a cool dark room.

Plankton storage bottles and labeling: Generally glass containers with wide mouths and screw-on plastic lids or good quality polyethylene jars with polypropylene lids are suitable for plankton fixation and storage. It is also essential that apart from an external label, an internal label written on water - resistant paper be placed in every jar containing a zooplankton sample.

This internal label should contain the following information:

1. Station number

2. Date of sampling

3. Duration of sampling

4.Sampling depth

5.Type of net, mouth size and mesh size

6.Type of haul (horizontal , vertical, etc.)

7. Number of turns recorded by flowmeter

8. Collector's name

Details on external label

1 . Bottle no.

2. Station no .

3. Date of sampling

4. Day/Night

5. Sky

6. Time

7. Depth of sampling site

8. Type of net

9. Mesh aperture

10. Flowmeter reading

11 . Collector's name

Methods of Fixation and Preservation of Individual Groups of Zooplankton

Calcareous plankton

These include planktonic foraminifers and certain gastropod molluscs such as Janthina and Creseis, larvae of pelecypods and gastropods which possess calcareous skeletons, namely tests or shells made of calcium carbonate. These are fixed and preserved in 2% formaldehyde (pH 8 - 8.5) as follows: 5 ml of 40% formaldehyde buffered with borax is added to 90 ml of sea-water containing living calcareous plankton . The buffered formaldehyde is prepared with 1000 ml of 40% formaldehyde to which are added 30 g of sodium tetraborate (NCI2S407 ' H20 - b or ax) , 4 . 5 ml of propylene glycol and 0.1 ml of propylene phenoxetol . It is advisable to have the volume ratio of plankton biomass to preservative fluid at 1 : 9 and storage temperature at 15 ° C.

Coelenterates(Cninadarians)

The fixation of live cnidarians in a large glass jar may be done by gently adding 40% neutralised formaldehyde . Alternatively, the animals may be gently trans f e r r e d to a 4% formaldehyde fixative using a spoon .

These coelenterates are preserved in 2 % formal dehyde solution prepared with additives as follows: 10 ml of stock solu tio n (prepared using 500 ml of 40% formaldehyde to which the additives , viz. , 50 ml of propylene phenoxetol and 45 0 ml of propylene glycolare added) is added to 90 ml of sea - water or distilled water . Hydromedusae may be best preserved in 1 % phenoxetol in distilled water and i n the same fluid neutralised with 1 % hexamine.

Ctenophores(Acnidarians)

The animals are fixed in 1 % trichloroacetic acid (1 g in 99 m l se a- water) for 30 minuts . They are then temporarily preserved in 100 m l of sea- water containing 1 ml of stock solution (50 ml of 4% formaldehyde containing 5 ml of propylene phenoxetol and 45 ml of propylene glycol). After a week, the anima l s are permanently transferred to 1 0 0 ml of sea -w ate r containing 5 ml of the above stock solution .

Chaetognaths

These are fixed with 5 % neutralised for maldehyde which is prepared as follows . A solution containing 1 I of tap-water and 5 g o f Ro c hel l e salt is prepared. Then 125 ml of 40 % commercial for maldehyde and 10 g or CaC03 are added to just 875 m l of the a fore saidsolution . Fixatives containing osmic acid or Souin's fluid can also b used for chaetognaths .

The chaetognaths can be preserve d in 2% form a ldehyde in sea-water at pH 6 - 7. 1 % phenoxetol in distilled water and 5 0% e thylene glycol in distilled water are also added as preservative s .

Polychaetes

The fixation of adult planktonic polychaetes is done by slowly adding neutralised formaldehyde until a concentration of about 2% is reached. They are preserved in either 2% neutralised formaldehyde or 70 - 80% ethanol.

The fixation of polychaete larvae i s done by pipetting Bouin's fluid heated to 60 ° C into s mall volume of seawater containing the swimming larva e. The dead larvae are then transferred to cold Bouin's fluid f or 1 to 3 hours and subsequently was heed and stored in ethanol.

C r u s t ace a ns:

For either fixation or preservation of crustacean zooplankton the following solution is prepared and used. 5 - 10 ml of neutralised for maldehyde (prepared by adding 30 - 40 g of sodium tetraborate to 1000 ml of 4 0 % formal dehyde) is added to 90 - 95 ml of filtered sea-water . The pH of this solution should be between 7 and 8. The ratio of plankton biomass to the preservative fluid should be approximately 1 : 4 by volume .

Important type species may be preserved in a 70% ethanol-distilled water solution. Specimens from a formaldehyde-sea-water solution may be transferred to an ethanol-d i stilled water solution as follows: The specimens , after thorough rinsing in distilled water , are first immersed in 30% ethanol for 10 m i n . Subsequently, the animals are immersed i n 50% ethanol for 1 h and finally stored in 70% ethanol . A few drops of glycerol may be added to this solution , especially for the preservation of copepod and decapod plankton .

Planktonic mollusks These include bivalve and gastropod veligers, shelled pteropods and heteropods and naked pteropods. The shell of these organisms is composed of aragonite, a most suitable form of calcium carbonate. Hence the pH of preservatives suitable to these organisms should drop to 6. Other- wise severe corrosion of the shells will take place. Further, the shells, usually shiny, translucent, coloured and hard, may lose their sculpture and become opaque - white, then chalky-white, soft and brittle. Such shells may break easily when touched and eventually dissolve completely.

Veliger larvae: The veliger larvae of ' molluscs can be fixed with 90 - 95% aqueous ethanol which will prevent the dissolution of calcium carbonate. They may be preserved in a solution prepared as follows: 10 cc of 40% formaldehyde (buffered with sodium bicarbonate to pH 10) is added to 11 of filtered seawater containing 100 g of commercial sugar. The sugar besides reserving the colour of organisms, acts as a clearing agent and as a safety factor against evaporation.

The veliger larvae can also be fixed in a 40% 'formaldehyde solution buffered to pH 8 with sodium glycerophosphate. After washing in fresh- water, they can be stored in a solution of 1 % propylene phenoxetol and 5 - 10% glycerol in freshwater .

Pteropods and heteropods: These animals can be fixed using 70% ethanol or 4% formaldehyde sea - water buffered to pH 8 ' - While the shelled forms are preserved in 70% ethanol, the naked forms are best preserved in 3 - 4% formaldehyde buffered to pH 8.

Thaliaceans : The salps, doliolids and pyrosomas may be fixed in 4% formaldehyde . Subsequently, they are best preserved in 70% aqueous ethanol .

Appendicularians : The appendicularians , for example Oikopleura and Fritillaria , are fixed using 4% formaldehyde in sea-water. While fixing, equal volumes of plankton and 4% formaldehyde are selected . Subsequently, they are preserved in 2% formaldehyde in sea-wate r with a pH o f 6,

P-5 Sea bottom Sampling devices

Several types of technology are used to collect marine sediments from research ships. These devices include surface samplers and sediment corers. Surface samplers collect only the uppermost layers of the ocean floor. These devices include dredges and benthic grabs. Dredges are heavy nets attached to metal frames that are dragged along the bottom behind a ship. The nets themselves can be made of chain. Dredges are useful when collecting samples from hard surfaces such as the rocky bottoms of coastal habitats.

Benthic surface grab samplers look like giant metal jaws. They dig into the bottom and take a bite of the sediment. These samplers are good for collecting softer, sandy or silty sediments that do not contain rocks. A box corer is a cross between a surface sampler and a sediment corer. It is a special device that is used to collect an undisturbed sample of the top surface layers and the sediment underneath.

This coer collects a 1-2 foot thick “box of mud”. A box core usually contains some overlying water, a surface “floc” layer where newly deposited particles are suspended, and frequently the core collects some benthic animals such as worms and starfish. Box corers are used to collect mud and silt but not sand.

The most common types of sediment corer used by marine geologists to collect mud and silt are those that collect a tube of sediment. The tube of sediment collected can range in length from several feet to hundreds of feet. A gravity corer consists of a metal tube called a barrel, a plastic

liner, and a heavy weight. The plastic liner is inserted into the metal tube before it is lowered over the side of the ship. This plastic liner will contain the sediment core when it is retrieved from the ocean floor. The corer is lowered to the bottom where the weight on the top drives the barrel and liner into the sediment. Gravity corers generally sample sediment layers up to 6 feet long (or 2 meters). After collection, the sediment core within the hard plastic liner is slid out of the core barrel, caps are placed on both ends, and the core is stored in a large walk-in refrigerator. Scientists will split the core in to 2 halves length-wise “working half”[Nazeer1] . is used for various analyses. The other core half is saved as the “archive half”.

Piston corers look like gravity corers, but they are much longer, much heavier and collect a longer core sediment. A cylindrical piston system at the top of the coring unit, which can slide down the core barrel with the help of weights, acts to drive the corer deep into the sediment. This gives scientists a very compact, long sediment core with very little water. Due to the force with which the piston core is driven into the mud, the very top is often lost and not sampled. However, this corer is able to penetrate much deeper into the seafloor, thus collecting much older sediments than what a gravity corer or a box corer can obtain. This is because the deeper the sediment is below the surface of the seafloor, the older it is.

Several other types of coring devices are also useful to marine scientists. The multicorer punches 6 or more, 1-2' long tubes into the bottom, all at once. It also relies on gravity and weights and is most useful in sticky, muddy sediments. On a really big scale, the international Deep-Sea Drilling Project (DSDP) uses drilling technology from the oil and gas industry to obtain cores of 10's –100's of meters long. These long cores give scientists an excellent overview of the geologic and oceanographic history over thousands of years. The cores are obtained from a specialized ship, designed to operate like an oil drilling platform used to collect oil from beneath the seafloor. Obtaining cores that are hundreds of meters long from a ship takes many hours. This process can be very challenging and occasionally dangerous! This is especially true when the seas are rough and/or there is a lot of ice in the areas such as in the Antarctic. In general, the success or failure of all types of sediment coring done from oceanographic research ships is very much dependent upon the sea state.

Today various types of underwater vehicles are also being used to collect sediment samples. Submersibles or remotely operated vehicles (ROVs) collect sediments using small push corers less than one foot long. These plastic tubes are pushed into the surface sediment by the submersible vehicle's/ROV's mechanical or hydraulic arm. The recovered sample is placed in a sample bucket or cage on the front of the underwater vehicle and is transported back to the ship. The advantage of this type of sediment sampling is that the scientist, either inside the submersible or controlling the ROV with the use of cameras from the ship, can visually target the sampling site. All other shipboard coring methods described above are done "blindly"—the corer is lowered over the side of the ship to the seafloor without the scientist being able to see exactly what is being sampled.

BFSC-303: Fishery Oceanography

P-1 : NISKIN WATER SAMPLER

Niskin bottles. Open Niskin bottles are attached to a cable and lowered to water depths where seawater samples are to be obtained for chemical analysis. A metal messenger “trips” each bottle on the cable individually, causing it to fill with water and close securely.

A rosette cluster. Water collecting bottles are arranged around a rigid, circular frame in a rosette pattern. Technicians are able to close the bottles individually as the array is lowered or raised through the water column.

Sampling Depths

Chemists must establish the exact sampling depth for each bottle. Otherwise, the analytical work, no matter how accurate, is of limited use in determining the exact chemical structure of the water column. A common technique is to measure the length of the cable between the ocean surface and the depth at which the bottle was triggered by the messenger. However, the cable rarely hangs straight down, because of the drift of the ship relative to the bottles on the cable. Depth corrections are applied by measuring the angle of the cable and by noting the difference between the temperature readings on the pressure-protected and unprotected thermometers mounted on the sampling bottles. (Temperature discrepancies are indicators of water pressure,which is a function of water depth). When near-bottom water samples are collected, it is customary to attach a pinger(a pulsing sound source) to the free end of the cable. Sound signals reflected off the sea floor and transmitted to the ship are used to determine the distance between the pinger and the bottom to within a meter or so.

Analytical Procedures

Analytical procedure reveal temperature and salinity of water. recorded in the reversing thermometers, which are fastened to water-sampling bottles, Better precision (up to 0.0001°C) is obtained by using temperature-sensitive materials, such as quartz crystals, which vibrate at frequencies that depend on temperature. These signals are transmitted electronically to the ship. This allows the temperature of the water to be monitored continuously as the instrument is lowered.

Because the composition of seawater is constant, chemists traditionally have determined water salinity by chemical titration-the process of standardizing silver nitrate against a normal seawater sample of known chemical composition.The electrical conductivity of seawater, which is proportional to the total concentration of dissolved ions, is now used routinely to determine salinity rapidly. The salinometer compares the electrical conductivity of an unknown sample with that of a known, standard sample of seawater, and converts the difference into a salinity value after correcting for temperature effects. An important instrument called the CTD (conductivity, temperature, depth) consists of a salinometer, an

electronic thermometer, and a pressure sensor. As it is lowered through the water column, the CTD transmits electronic signals to the ship, where they are stored in a shipboard computer for analysis later.

P-2 GRABS/CORERS-PRACTICALS:

Various simple but durable devices are available for collecting sediment samples from the ocean floor, even from the deepest, most inaccessible parts of the sea. A long-established technique is scraping the ocean bottom with a dredge-a rigid metal frame to which is attached a sampling bag made of chain or tough netting. Dredges are suitable for obtaining large, bulk samples of either rock or sediment. As they are dragged, however, the bite the bottom indiscriminately and mix samples together in the sampling bag. Also fine sediment such as mud tends to be washed out of the sample. Because of these effects, oceanographers employ dredges almost exclusively to collect hard rock rather than soft sediment. Less disturbed samples mud and sand are collected by grab samplers-spring –loaded metal jaws that take a bite out of the bottom and close tightly around the sediment sample.

Dredges and grab samplers merely sample the surface layer of sediment. Deeper penetration of soft sediment is accomplished by gravity corer. This hollow metal tube, known as a core barrel, is pushed into the sediment by the force of gravity. The corer is lowered to the bottom, where the heavy weight at the top of the device drives the barrel into the sediment. A plastic liner that has been inserted into the core barrel allows oceanographers to extract the sediment core intact from the sampler and also serves as a temporary storage container. Gravity corers are capable of taking cores of between 1 and 2 meters long, depending on the properties of the sediment. Sediment cores longer than 20 meters are routinely obtained by piston corers. This type of corer has a piston that slides up the core barrel as it penetrates the bottom. The action of the piston extrudes water from the core barrel, allowing the sediment core to enter the liner with minimal disturbance and compaction. Once the core is on deck, the plastic liner with its sample of sediment is extruded from the core barrel and is taken to a laboratory for detailed examination. Geologists carefully study the layering and composition of sediment particles to determine the geological history of the earth.

At present, the best technique for sampling the ocean bottom is plat form drilling, which was first developed by petroleum engineers on land and is now adapted to the ocean, even the deep ocean. The procedure is very expensive, but the scientific results are priceless. Marine geologists not only recover cores of sediment more than 1 kilometer in length, but also they can drill into the hard rock of the crust beneath the sedimentary layers. The Glomar Challenger—the 122-meter-long vessel that completed an illustrious international career of drilling, even in the remotest regions of the oceans, collecting hundreds of kilometers of core sample—has been retired.

P-3 : COLLECTION OF SEDIMENT SAMPLES

Two types of sediment samplers (grabs and corers) are used for collecting sediment from coastal and off-shore waters. The following samplers are commonly used in oceanographic surveys.

1. Petersen grab

It is consisting of two hinged pincer like buckets which are sent down to the sediments in open condition. As the drawing line slackens, the release mechanism is activated. In retrieval, the two buckets come together and thus a semi-circular section of sediment is cut and entrapped. The drawing line is then pulled off and the grab which is now in a closed condition is made open in a tray or bucket. As in the case of Van Veen's grab, this grab is also not activated by a metallic messenger.

2. Ekamn-Birge grab

This is the first and commonest grab devised for use in muddy bottom by Ekman (1911) and Birge (1922). The two shovels which are kept open against very strong spring action by means of two chains are closed from above by means of a drop weight. (metallic messenger). Immediately after this operation, one can pull the grab out of the bottom and finally out of the water column. It is very heavy and is made of brass in order to avoid rusting in the water. The upper portion is box shaped and is closed by two movable covers which fall in under the pressure of the water when the grab is sent down. The basal surface of this grab is about 250cm2.

3. Van Veen's grab

It is also a very convenient and reliable grab devised by Van Veen (1936). The working principle of this grab is more or less similar to that of Ekman-Birge. However, it is held open by a small bar and is not operated open by a metallic messenger. During operation, the grab is sent down the bottom when the two shovels spread out so that the bar is released automatically. The draw rope is attached in such a way that with the pull from above, the two shovels of the grab aremade to close tightly.

P-4 : Reversing thermometers

In the earliest, temperature measurements at some depth below the surface where made by bringing a water sample up to the deck of a ship in an insulated bucket and measuring the sample temperature with a mercury thermometer. Although these measurements were not accurate, they gave the first evidence that below the top 1000m the ocean is cold even in the tropics. They also showed that highly accurate measurements are required to resolve the small temperature differences between different ocean regions at those depths.

The first instrument that (through the use of multiple sampling and averaging) achived the required accuracy of 0.001ºC was the reversing thermometer. It consists of a mercury filled

glas pipe with a 360º coil. The pipe is restricted to capillary width in the coil, where it has a capillary appendix. The instrument is lowered to the desired depth. Mercury from a reservoir at the bottom rises in proportion to the outside temperature. When the desired depth is reached the thermometer is turned upside down (reversed), but the flow of mercury is now interrupted at the capillary appendix, and only the mercury that was above the break point is collected in the lower part of the glass pipe. This part carries a clibratd gradation that allows the temperature to be read when the thermometer is returned to the surface.

To eliminate the effect of pressure, which compresss the pipe and causes more mercury to rise above the break point during the lowering of instrument, the thermometer is enclosed in a pressure resistant glass housing. If such a " protected reversing thermometer" is used in conjunction with an "unprotected reversing thermometer" (a thermometer exposed to the effect of pressure), the difference between the two temperature reading can be used to determine the pressure and thus the depth at which the reading were taken. The reversing thermometer is thus also an instrument to measure depth.

Reversing thermometers require a research vessel as platform and are used in conjunction with Nansen or Niskin bottles or on multi-sample devices.

P-5 : Nansen and Niskin bottles

The measurement of salinity and oxygen, nutrients and tracer concentrations requires the collection of water samples from various depths. This essential task is achieved through the use of "water bottles". The first water bottle was developed by Fritj of Nansen and is thus known as the Nansen bottle. It consists of a metal cylinder with two rotating closing mechanisms at both ends. The bottle is attached to a wire. When the bottle is lowered to

desired depth it is open at both ends, so the water flows through it freely. At the depth where the water sample is to be taken the upper end of the bottle disconnects from the wire and the bottle is turned upside down. This closes the end valves and traps the sample, which can then be brought to the surface.

In an "ocean graphic cast" several bottles are attached at intervals on a thin wire and lowered into the sea. When the bottles have reached the desired depth, a metal weight ("messenger") is dropped down the wire to trigger the turning mechanism of the uppermost bottle. The same mechanism releases a new messenger from the bottle; that messenger now travels down the wire to release the second bottle, and so on until the last bottle is reached.

The Nansen bottle has now widely been displace by the Niskin bottle. Based on Nansen's idea, it incorporates two major modifications. Its cylinder is made from plastic, which eliminates chemical reaction between the bottle and the sample that may inter face with the measurement of tracers. Its closing mechanism no longer requires a turning over of the bottle; the top and bottom valves are held open by strings and closed by an elastic band. Because the Niskin bottle is fixed on the wire at two points instead of one(as is the case with the Nansen bottle) it makes it easier to increase its sample volume. Niskin bottles of different sizes are used for sample collection for various tracers.

Nansen and Niskin bottles are used on conjunction with reversing thermometers. On the Nansen bottle the thermometers are mounted in a fixed frame, the reversal being achieved by the turning over of the bottle. On Niskin bottles thermometers are mounted on a rotating frame.

P-6 Conductinity, Temperature and Depth System(CTDs)

Today's standard instrument for measuring temperature , salinity and often also oxygen content is the CTD, which stands for conductivity, temperature, depth. It employs the principle of electrical measurement. A platinum thermometer changes its electrical resistance with temperature. If its incorporated in an electrical oscillator, a change in its resistance produces a change of the oscillator frequency, which can be measured. The conductivity of seawater can be measured in a similar way as a frequency change of a second oscillator, and a pressure change produces a frequency change in a third oscillator. The combined signal is sent up through the single conductor cable on which the CTD is lowered. This produced a continuous reading of temepratrue and conductivity as functions of depth at a rate of up to 30 samples per second.

Bathythermograph

Electrical circuits allow measurements in quick succession but suffer from “instrumental drift”, which means that their calibration changes with time. CTD systems therefore have to be calibrated by comparing their readings regularly against more stable instruments. They are therefore always used in conjunction with reversing thermometers and a multi-sample device

Current meters

Ocean currents can be measured in two ways. An instrument can record the speed and direction of the current, or it can record the east-west and north-south components of the current. Both methods require directional information. All currents meters therefore incorporate a magnetic compass to determine the orientation of the instrument with respect to magnetic north. Four classes of current meters can be distinguished, based on the method used for measuring current magnitude.

Mechanical current meters use a propeller-type device,a Savonius rotor or a paddle-wheel rotor to measure the current speed, and a vane to determine current direction. Propeller sensors often measures speed correctly only if they point into the current and have to be oriented to face the current all the time. Such instruments are therefore fitted with a large vane, which turns the entire instrument and with it the propeller into the current.

Propellers can be designed to have cosine response with the angle of incidence of the flow. Two such propellers arranged at 90º will resolve current vectors and do not require an orienting vane.

The advantage of the Savonius rotor is that its rotation rate is independent of the direction of exposure to the current. A Savonius rotor current meter therefore does not have to face the

current itn any particular way, and its vane can rotate independently and be quite small, just large enough to follow the current direction reliably.

With the exception of the current meter that uses two propellers with cosine response set at 90º to each other, mechanical current meters measure current speed by counting propeller or rotor revolutions per unit time and current direction by determining the vane orientation at fixed intervals. In other words, these current meters combine a time integral or mean speed over a set time interval (the number of revolutions between recordings) with an instantaneous reading of current direction (the vane orientation at the time of recording). This gives only a reliable recording of the ocean current if the current changes slowly in time. Such mechanical current meters are therefore not suitable for current measurement in the oceanic surface layer where most of the oceanic movement is due to waves.

The Savonius rotor is particularly problematic in this regard. Suppose that the current meter is in a situation where the only water movement is from waves. The current then alternates back and forth, but the mean current is zero. A Savonius rotor will pick up the wave current irrespective of its direction, and the rotation count will give the impression of a strong mean current. The paddle-wheel rotor is designed to rectify this; the paddle wheel rotates back and forth with the wave current, so that its count represents the true mean current.

Mechanical current meters are robust, reliable and comparatively low in cost. They are therefore widely used where conditions are suitable, for example at depths out of reach of surface waves.

Electromagnetic current meters exploit the fact that an electrical conductor moving through a magnetic field induces an electrical current. Sea water is a very good conductor, and if it is moved between two electrodes the induced electrical current is proportional to the ocean current velocity between the electrodes. An electromagnetic current meter has a coil to produce a magnetic field and two sets of electrodes, set at right angle to each other, and determines the rate at which the water passes between both sets. By combining the two components the instrument determines speed and direction of the ocean current.

Acoustic current meters are based on the principle that sound is a compression wave that travels with the medium. Assume an arrangement with a sound transmitter, and let receiver B located downstream. If a burst of sound is generated at the transmitter it will arrive at receiver B earlier than at receiver A, having been carried by the ocean current.

A typical acoustic current meter will have two orthogonal sound paths of approximately 100mm length with a receiver/transmitter at each end. A high frequency sond pulse is transmitted simultaneously from each transducer and the difference in arrival time for the sound travelling in opposite directions gives the water velocity along the path.

Electromagnetic and acoustic current meters have no moving parts and can therefore take measurements at a very high sampling rate (up to tens of readings per second). This makes them useful not only for the measurement of ocean currents but also for wave current and turbulence measurements.

Acoustic Doppler current profilers (ADCPs) operate on the same principle as acoustic current meters but have transmitter and receiver in one unit and use reflections of the sound wave from drifting particles for the measurement. Seawater always contains a multitude of small suspended particles and other solid matter that may not all be visible to the naked eye but reflects sound. If sound is transmitted in four inclined beams at right angle to each other, the Doppler frequency shift of the reflected sound gives the reflecting particle velocity along the beam. With at least 3 beams inclined to the vertical the three components of flow velocity can be determined. Different arrival times indicate sound reflected at different distances from the transducers, so an ADCP provides information on current speed and direction not just at one point in the ocean but for a certain depth range; in other words, an ADCP produces a current profile over depth.

Different ADCP designs serve different purposes. Deep ocean ADCPs have a vertical resolution of typically eight meters (they produce one current measurement for every eight meters of depth increase) and a typical range of up to 400m. ADCPs designed for measurements in shallow water have a resolution of typically 0.5m and a range of up to 30m. ADCPs can be placed in moorings, installed in ships for underway measurements, or lowered with a CTD and multi-sample device to give a current profile over a large depth range.

P-8 Remote sensors:

Sea level can also be measured from satellites. An altimeter measures the distance between the satellite and the sea surface. If the satellite position is accurately known this results in a sea level measurement. Modern altimeters have reached an accuracy of better than 5cm. The global coverage provided by satellites allows the verification of global tide models. When the tides are substracted, the measurements give information about the shape of the sea surfaces and, through application of the principle of geostrophy, the large scale oceanic circulation.

P-9 : Determination of Phosphate:

Introduction

Phosphate-P occurs in natural waters and in waste waters almost solely as phosphate. These are classified as orthophosphate, condensed phosphate and organically bound phosphate. It is largely a measure of orthophosphate and it occurs in both dissolved and suspended form.

Principle

Ascorbic acid method

Ammonium molybdate and potassium antimonyl tartarate reacts in acid medium with orthophosphate to form a heteropoly phosphomolybdic acid that is reduced to intensely colored molybdenum blue by ascorbic acid and the extinction is measured at 885 nm.

Reagents

Ammonium molybdate: Dissolve 15g of ammonium molybdate in 500ml of distilled water.

Sulphuric acid: Add 140 ml of conc. H_2SO_4 to 900 ml distilled water.

Ascorbic acid solution: Dissolve 27g of ascorbic acid in 500 ml distilled water.

Potassium - Antimonyl - Tartarate (PAT): Dissolve 0.34 of PAT in 250ml of distilled water.

Mixed reagent: To prepare 500 ml of mixed reagent, mix together 100 ml ammonium molybdate, 250ml H_2SO_4, 100ml ascorbic acid and 50 ml PAT. Prepare this reagent for use and discard any excess.

Procedure:

Take 100ml of sample in conical flask.

Add 10+0.5ml of mixed reagent.

After 5 min and preferably within 2-3 hrs, measure the extinction of the solution at 885 nm.

Calculation:

Mg at – P/lt =Extinction x F

Where, F=Factor value

P-10: Determination of silicates in sea water

Principle

The water sample is allowed to react with molybdate under conditions which result in the formation of the conditions which result in the formation of the silicomolybdate,

phosphomolybdate and arcenomolybdate complexes. A reducing solution, containing metal and oxalic acid is then added which reduces the silicomolybdate complex to give a blue reduction compound and simultaneously decomposes any phosphomolybdate or arcenomolybdate, so that interference from phosphate and arcenate is eliminated. The extinction of the resulting solution is measured at 810 nm.

Reagents

Ammonium Molybdate solution: Dissolve 4.0 gm of analytical reagent quality ammonium para molybdate in about 300 ml distilled water, add 12 ml of concentrated HCl, mix and make up to 500 ml using distilled water.

Metal-sulphite solution: Dissolve 6 gm of anhydrous sodium sulphite in 500 ml distilled water and then add 10 gm of metal.

Oxalic acid solution: Dissolve 50gm of analytical reagent quality oxalic acid dihydrate in 500 ml distilled water.

Sulphuric acid solution 50%: 250 ml concentrated H_2SO_4 dilute to 250 ml of distilled water.

Reducing reagent: Mix 100 ml of metal sulphite solution with 60 ml of oxalic acid solution, add slowly 60 ml of 50% H_2SO_4 and make up to 300 ml with distilled water.

Procedure

Add 10 ml molybdate solution to 50 ml conical flask or measuring cylinder.

Pipette 25 ml of the see water in to the cylinder or conical flask and mix it.

Allow the mixture to stand for 10 min but not more than 30 min.

Add the reducing reagent rapidly so as to make the volume exactly 50 ml and mix it.

Allow the solution to react for 2-3 hrs to complete the reduction of the silicomolybdate complex.

Measure the extinction at 810 nm, alter correction with turbidity values.

Calculation

Concentration of Silicate silica= mg/l

Mg at Sig-Si/l = [E x F]

Where,

F= Factor value

E= Extinction values obtained

P-11: Determination of DO

1. Introduction

Dissolved oxygen content is one of the vital environemental characteristics of seawater. Its determination may be required either in the raw seawater sample or to assess primary production.

2. Principle

When the solutions of manganese and alkaline iodide are added to the seawater sample, the following reactions normally take place.

Manganese ion reacts with the hydroxide of the alkaline iodide to give Mn(OH)2.

Mn(OH)2 reacts with the dissolved oxygen present in the water sample to give Mn(OH)3.

In the presence of acid (above pH 1.5), Mn(OH)2 reacts with iodide to liberate iodine.

The iodine liberated is titrated against Na2S2O3 to convert the iodine to iodide.

The sequence of the above reactions are explained through the following formulae:

$Mn^{2+} + 2OH^- \rightarrow Mn(OH)_2$

$2\ Mn(OH)_2 + \frac{1}{2}\ O_2 + H_2O \rightarrow 2Mn(OH)_3$

$2Mn(OH)_3 + 3\ I^- + 6H^+ \rightarrow 2Mn^{2+} + I_3^- + 6\ H_2O$

2S2O32- + I-3 □ S4O62- + 3I-

Range of detection: up to 10 ml O2/1

3. Reagents

a. Manganese II chloride tetrahydrate

40 g of manganous chloride tetrahydrate is dissolved in 100ml distilled water. (or)

b. Manganese sulphate reagent

48 g of MnSo4. 4H2O or 40 g of MnSo4. 2H2O or 36.5 of MnSo4.H2O is dissolved in water and the volume is made up to 100 ml with distilled water.

c. Alkaline iodide

60 g of Kl and 30g of KOH are dissolved separately in a minimum amount of water. Both these solutions are mixed together and this solution is then made up to 100ml with distilled water.

d. Sulphuric acid

50ml of conc. H2 So4 is carefully added to 50ml of distilled water (This mixture should be cooled during mixing).

e. Sodium thiosulphate

2.482g of Na2S2O3.5 H2O is dissolved and made up to 1000ml with distilled water. The normality of this solution is 0.01N.

f. Starch solution

1 g of soluble starch is dispersed in 100ml of distilled water. The solution is quickly heated to boiling. One drop of phenol or formaldehyde is added to every 50ml of starch solution to check microbial degradation.

4. Procedure

The seawater sample for which the dissolved oxygen content is to be determined is slowly siphoned out into a BOD bottle with the help of a rubber tube. When the water sample is allowed to overflow in this BOD bottle the following reagents are added. One ml of manganous solution is added first to the water sample keeping the tip of the pipette just below the surface of the water. In the same way, one ml of alkaline iodide is added and the bottle is carefully stoppered. The bottle is tilted upside down so that Mn(OH2) reacs with the dissolved oxygen present in the sample to give a slightly brown coloured Mn(OH3). Extra care should be taken not to permit the entry of any air bubble inside the BOD bottle. The estimation of dissolved oxygen may be done immediately or in a day or two. If the analysis is not carried out immediately, the mouth of the BOD bottle should be thoroughly sealed with wax and the bottle is kept in a water bath (at room temperature) so as to avoid the impact of increasing temperatures.

At the time of titration, one ml of sulphuric acid is slowly added to the sample and the bottle is stoppered. The bottle is tilted side wise, so that, all the precipitates are dissolved and the solution turns brownish due to the liberarian of iodine.

The thiosulphate solution of the given normality (0.01N) is then taken in the burette and is kept ready for titration. 50ml of sample from the reagent fixed BOD bottle is taken in a conical flask and is titrated against the thiosulphate till a hay yellow colour appears. At this point, one ml of starch is added to this solution and it turns blush indicating the presence of iodine. The titration is continued till the blue colour disappeard. The titration is repeated to

get concordant values. The oxygen content of the water samples is calculated using the following formula:

$$O_2 \text{ ml/1} = \frac{CF \times N \times E \times 0.698 \times 1000 \times V\,t}{Vs}$$

Where

CF=correction factor value

correction factor value = Total volume of the stoppered bottle

Total volume of stoppered bottle-2

N= Normality of thiosulphate (0.01N)

E=Equivalent weight of O2 (8)

0.698 = To convert mg/l into ml/l.

1000= To convert the value to 1000ml or 1 litre

V t =Titre Value

Vs = Volume of sample taken for titration

If the oxygen content is to be calculated in mg/l, then the conversion factor value (0.698) of the above formula should be avoided. In the case of brackishwater and sea water samples, it is customary to express the value of oxygen content in ml/l and in freshwater samples, mg/l or ppm.

The above formula is derived by treating the dissolved oxygen and thiosulphate as reactants. The reaction and the calculation are as given below.

V1 N1 (Dissolved oxygen) = V2N2 (Thiosulphate)

Since V1 = Vs, V2=V t

N2 = 0.01 and N1 has to be calculated.

Thus, N1 = V t x 0.01

Vs

Normality x Equivalent weight = Weight in g/l

Thus, Wt of the dissolved oxygen g/l = V t x 0.01 x 8

Vs

Weight of dissolved oxygen (mg/l) = V t x 0.01x 8 x 1000

Vs

In order to convert the dissolved oxygen content from mg/l to ml/l, the above formula is multiplied by 0.698.

Thus,

Dissolved Oxygen (ml/l) = V t x 0.01x 8 x 1000 x 0.698

Vs

Correction factor (CF) is included in the above formula to rectify the defect caused by the addition of MnSo4 and alkaline iodide solution by replacing 2ml of the water sample.

The total volume of the stoppered bottle can be measured by filling the bottle with tap water and then stoppering it to remove the excess water. The water remaining in the bottle is measured by pouring the contents in a measuring cylinder.

The oxygen solubility of water sample is usually calculated by using the standard values if the salinity and temperature of the water samples have already been estimated. For calculating the oxygen solubility at different combinations of salinity temperature refer Table 11 (Vide Annexures).

By calculating the difference between the dissolved oxygen content and the oxygen solubility values, Apparent Oxygen Production (AOP) and Aparent Oxygen Utilization (AOU) can be calculated. These two parameters function as reliable indices of oxygen production due to photosynthesis and oxygen utilization due to community respiration.

BFSC-304: Fish Food Organisms

P-1 : CULTURE OF CLADOCERANS

Introduction

Cladocerans are commonly called as "water fleas". Moina and Daphnia are the commonest representatives of this group of crustaceans and are quite popular as live food in aquahatcheries. This is because of their easy availability in nature and easy adaptability to captive conditions. Owing to these traits these cladocerans come in handy as relatively inexpensive live food in aquahatcheries. Moina and Daphnia belong to the Family Daphnidae of the Order Cladocera under Sub-class Branchiopoda of the Class Crustacea and Phylum Arthropoda of the animal kingdom. Moina and Daphnia inhabit fresh and low saline waters and also in some sewage lagoons.

Morphology

Body is laterally compressed and enclosed in a bivalved shell or a large fold of carapace (except head) in both Moina and Daphnia. Daphnia is distinguished from Moina by the presence of prominent caudal spine. Moina has a pair of prominent caudal setae whereas Daphnia has small setae. In both the cases, head is round and bears a pair of large biramous antennae, a pair of small antennules and a compound sessile eye. Large biramous antennae are the chief organs of locomotion. Thorax bears 5 pairs of appendages (Fig.). Moina measures about 0.5 to 1.0 mm in length and 0.2 to 0.6 mm in width whereas Daphnia measures 0.5 to 2.5 mm in length and 0.3 to 1.0 mm in width.

Food and feeding

Food and feeding habits of Moina and Daphnia are same. They feed on algae, bacteria, fungi, protozoans and organic debris.

Reproduction

Generally Moina and Daphnia reproduce parthenogenetically. The eggs are laid in large brood pouch situated between abdomen and posterior part of carapace. The eggs undergo complete development in brood chamber before being released as a firstinstar, which is similar in morphology as that of adult female. A batch of eggs in brood chamber is termed as 'brood'. The young ones are released in small batches known as 'Clutches'. In the total life spawn of a parthenogenetic female, a sexual phase occurs by generating sexual males. These males after mating with parthenogenetic females turn to sexual female, which result in production of resting eggs known as 'ephippia'. These ephippia can be stored for initiating the fresh culture as and when desired. They remain in viable condition for about 2 to 3 months.

P-1.1

Culture Of

MoinaStock

culture

For pure culture of Moina stock culture need to be developed. In order to start stock culture, collection of Moina is done from freshwater ponds and tanks with the help of a scoop net having 250-500 micron mesh. After collection, the content of the net is placedin a plastic bucket and brought to the laboratory. The sample is then diluted by adding fresh clear water and examined under a microscope to pick up Moina with the help of a fine dropper whenever observed. Each Moina so picked up are inoculated in 20 mlglass tube containing 10 ml of filtered water. Feeding of Moina is done with yeast at the rate of 200 ppm or Chlorella at a cell density of $10x10^6$ cells per million. Each gravid Moina produces about 8 to 10 off springs in about 24 hours. Dilution of the test tube cultures is done daily through several 100 ml beakers. The volume is increased to 1 to 2litre beakers or jars. Feeding is continued in similar manner as in the test tube culture. After 4 to 5 days, these jar cultured Moina are used as inoculum in mass culture tanks.

Mass culture

The mass culture technique for Moina is almost similar to that of Brachionus. Different authors have developed various techniques by feeding pure algal cultures i.e. *Chlorella, Chlamydomonas, Scenedesmus* etc. or by using phased manuring techniques with various inorganic fertilizers and organic manures.

First Method

In this method, the culture tanks are treated with groundnut oilcake, single superphosphate and urea at the rate of 75 ppm, 20 ppm and 8 ppm respectively. After fertilization, the tank is inoculated with Chlorella or mixed phytoplankton. Algal blooms develop within 3-4 days. Moina is inoculated at the rate 40-50 individuals per litre depending on the availability of the stock culture. Moina multiplies rapidly, feeding on phytoplankton blooms, bacteria and small particles of groundnut oilcake. It attains apeak density of 20,000-25,000 individuals per litre in 5 to 7 days after inoculation. After attaining peak density it is regularly harvested to feed the larval stages. As a result of Moina multiplication and reduction of nutritional status of water, Chlorella concentration declines. In order to maintain optimum Chlorella concentration, partial water exchange from tank bottom and refertilisation with 75 ppm groundnut oilcake is done at an interval of 4 to 5 days after commencement of first harvesting of Moina.

P-1.2

Culture of Daphnia

Stock culture and mass culture techniques of Daphnia are similar to that of Moina culture.

Maintenance of culture

Regular uninterrupted supply of Moina and Daphnia to the aquahatcheries needs judicious management. This involves risk of bacterial contamination. In order to avoid bacterial contamination, a new approach has been evolved as a prophylactic treatment. In this method, probiotics are introduced in Moina and Daphnia culture systems andalso in the larval rearing tanks. This helps in providing a microbiologically balanced system by suppressing pathogenic bacteria.

P-2 CULTURE OF INFUSORIA

Introduction

Successful production of seed of finfish and shellfish species in aqua hatcheries mainly depends on the supply of abundant quantity of proper live food organisms at appropriate time. Live food organism in this respect serve as "Living Capsules ofNutrition". Providing appropriate live food organisms at appropriate time playa majorrole in achieving optimum growth and survival of the young ones of finfish and shellfish. Selection of suitable live food organisms depends on mouth size, age and size of larvae of finfish and shellfish. Infusoria are most primitive of all organisms in the animalkingdom. Besides being small in size, they are soft bodied and nutritionally rich. Owing to these qualities, they serve ideally as starter feed for early stages of finfish and shellfish in aqua hatcheries. The tiny microscopic single celled animalcules, collectively called as infusoria, belong to the Class Ciliata under the Phylum Protozoa of the animal kingdom. Infusorian microorganisms inhabit ponds and tanks of freshwater, brackish water and marine habitats having decaying weeds, organic matter and foul smelling debris. Infusoria feed upon the microorganisms such as bacteria, algae, and flagellates and also on debris. Cilia present on the body act as chief locomotory and food catching organelles in most of the infusoria. Two types of reproduction occur in infusoria i.e. asexual and sexual. Asexual reproduction occurs by binary fission and sexual reproduction by conjugation.

Culture of infusoria

The most commonly cultured freshwater species are *Paramaecium* and *Stylonychia.* These organisms are cultured using several methods. A few common methods are described below

By using banana pealings

By using hay infusion

Take dry hay (straw) into a pan and pour boiling water over it. Transfer the hay together with water to a jar or aquarium. After this, repeat the process as described under banana pealing method above.

By using lettuce leaves

Aquarium set-up with banana pealings for culture of infusoria. In place of hay, lettuce

leaves can also be used for the culture of infusoria. But in this case, pouring of boiling water will not be required, plain water will do. After this, follow the same process as stated for banana pealings above.

By using milk

Add a teaspoon of skimmed milk or two pinches of milk powder to the culture tank filled with 50 litre of water. Thereafter, follow the same process as described for using banana pealings.

By using apple snail

Keep the snail, *Ampullaria globosa* in an aquarium and feed with lettuce leaves or water plants such as Hydrilla. The droppings (faeces) of snail will contain half digested leaves and will give rise to a large population of infusoria, when added to the water.

CULTURE OF MICROWORMS

Some of the micro-worms form important food items for the post-larvae, juveniles, adults and brood stock of finfish and shellfish. Among the microworms *Tubifex* and *Chironomid* larvae are commonly used as live food for the maintenance of ornamental fishes, and larvae and post-larvae of freshwater prawns.

Tubifex

Tubifex worm belongs to the Family Tubificidae of the Order Oligochaeta of the Class Chaetopoda under the Phylum Annelida. The commonly available species is *Tubifex tubifex*. Tubifex worms are commonly called as sludge worms. Tubifex worms inhibit in sewage drains. When present in plenty, the worms can be seen as reddish wriggling carpet in sewage drains. The anterior end embedded in mud while the posterior end waves about. They jerk into the mud when disturbed. Tubifex worms are long and slender. There is no distinct head. At the extreme anterior end there is a lip like structure known as the prostomium which is not counted as segment. The first segment lies immediately behind prostomium. The mouth present in the first segment is known as peristomium while the anus lies in the last segment and is known as anal segment. The coelom is specious and devised into segment compartments by the inter-segmental septa. Some of the segments in the anterior end become fused together to form a swollen structure known as the clitellum. Tubifex worms feed on decaying organic matter, detritus, vegetable matter which commonly available in sewage drains.

Tubifex worm is a hermoprodite, because it has both male (testes) and female (ovaries) organs in the same animals. These minute reproductive organs attached to the ventral side of the body wall in the celomic cavity. In mature specimens, the reproductive organs are clearly found on the ventral side of the body.

Culture of Tubifex

Tubifex can be easily cultured on mass scale in containers with 50 to 75 mm thick pond mud at the bottom, blended with decaying vegetable matter and masses of bran and bred. Continuous mild water flow is to be maintained in the container, with a suitable drainage system. After the arrangement of the system, the container is inoculated with Tubifex worms, which can be obtained from nearby muddy canals or sewage canals. Within 15 days, clusters of Tubifex worms develop and this can be removed with mud in masses by means of a spade and kept in large wide mouth plastic container. When worms will come to the surface due to lack of oxygen, they are collected and washed under brisk stream of water to remove residual mud attached to the body. Since their guts still may contain mud, which they have eaten. Therefore, they should be kept long enough under stream of water for the mud to be evacuated from the intestine. After proper cleaning only, the worms are fed to the fish.

Chironomid

Chironomid larvae belong to the Family Chironomidae of the Order Diptera under the Class Insecta of the Phylum Arthropoda. Chironomid larvae are commonly called as blood worms. Chironomid flies attract towards foul smell where organic matter decays and lay eggs. The eggs hatch into Chironomid larvae. Larvae initially live in soft tubes made from organic matter. As larvae grow, come out of tubes and swim vertically in water, by showing wriggling movement. Fully grown larvae are dark red in colour. Chironomid larva looks like an annelid worm. Body is segmented and head is free. It is about 1.0 to 1.5 mm in length. Larva has three legs, one in front and the other two at rear end of body. Rear end (tail) has tufts of hairs.

The worm Chironomid larvae are herbivorous in feeding habits and feed on algae, detritus, decaying organic and vegetable matter etc. Chironomid flies lay eggs on organic matter, which is immersed in water. The eggs hatch straight into proboscis larvae, which in turn metamorphose in to adult flies. The larvae initially live in soft tubes and become free swimming in water.

Culture of Chironomid larvae

Flat trays filled with water are added with soil and composted manure or organic matter or decaying vegetable matter to attract chironomid flies to deposit eggs. Some times the chironomid flies even lay eggs in clear stagnant waters also. Each female lay a batch of about 20,000 eggs which hatch out in about 3 days. The larvae are herbivorous in feeding habit and feed on algae, detritus and decaying vegetable matter. Initially the larvae live in soft tubes made up of organic matter, which can be clearly seen at the bottom of the try. After 2 to 3 days, they come out of the tubes and freely swim in water vertically. The larvae are harvested with scoop net and washed thoroughly before feeding. It constitutes one of the staple food items in the ration of nearly all carnivorous young fishes.

QUALITATIVE AND QUANTITATIVE ANALYSIS OF PLANKTON

Collection and Preservation of

PlanktonCollection

Bottle method:-
Bottle method is ideal for small collections. It is mainly used for the collection of water samples from any desired depth of shallow ecosystems from a stationary vessel - near shore waters, estuaries and mangroves. Surface water can be obtained by gently scooping water into a container of a suitable size from the leeward side of the ship.

Net method:-
Plankton of >50 μm size can be collected by ordinary net sampling. This method could preferably used for qualitative plankton collections, as large quantity of water is filtered. A net is towed vertically, horizontally or obliquely.

Fixation

Fixation is the application of a chemical (fixative) to kill an organism but to retain its morphological characteristics as for as possible. Commonly used fixative is formalin (5%). Add formalin in the ratio of 5-10 to 90 parts (v/v). Invert the sample bottle after adding fixative for even dispersing. The pH of both solutions should be maintained around 7.6 — 8.3.

Preservation

Preservation is the maintenance of the fixed condition for extended periods of time. Specimens after one week fixation are used for preservation after thorough washing with distilled water. Formalin (2.5 — 5.0%) is used in the ratio of 1:9 (sample to preservative). pH should be maintained at 7.0. To preserve the natural color of the plankton, fish and crustaceans may preserve in phenolic antioxidant such as 40% emulsifiable concentrate of butylated hydroxytoluene (BHT) or butylated hydroxyamisole (BHA).

The direct Concentration of the collected plankton by sedimentation (and occasionally by centrifugation) is a pre-requisite for accurate qualitative and quantitative analysis.

Qualitative estimation

Preparation of the sample: -

1. Take one litre of water sample in a glass bottle.
2. Add 10ml of Lugol's Iodine and allow it to stand for at least 24 hrs to ensurecomplete sedimentation (centrifuge if necessary).
3. Remove the supernatant liquid with the help of a pipette.
4. Further concentrate the remaining sample upto 10-100ml depending on the number of plankton.

Plankton enumeration

I. Drop Count Method: -

1. Shake the concentrated sample and put quickly one drop on a clean micro slide with the help of a standard dropper holding it vertically.
2. Carefully cover the whole drop with a cover slip of suitable size so that thesample does not run out.
3. Put the slide under microscope and focus one edge of the cover slip.
4. Count the phytoplankton / zooplankton species-wise.
5. Shift the slide to the next field.
6. In this way observe the whole coverglass and work put planktonic estimation at least for 5-10 drops depending on the density of plankton.

BFSC-305: Ornamental Fish Production and Management

Specimen No. 1 - *Carassius auratus auratus* (Linnaeus, 1758) Goldfish

Class - Actinopterygii
Order - Cypriniformes (Carps)
Family - Cyprinidae (Minnows or carps)

Short description

Dorsal spines (total): 3 - 4; Dorsal soft rays (total): 14-20; Anal spines: 2 - 3; Anal soft rays: 4 - 7; Vertebrae: 30. Body stout, thick- set, caudal peduncle thick and short. Head without scales, broadly triangular, interorbital space broad, snout longer than eye diameter, maxillary reaching posterior nostril or not quite to eye, barbels lacking on upper jaw. Lateral line complete. Dorsal and anal fins with serrate bony spines, pelvic fins short, broad and thoracic. Nuptial tubercles of male fine, on opercula, sometimes on back and a few on pectoral fins. Hybridize readily with carp, hybrids intermediatein most characteristics. Caudal fin with 17-19 rays. Last simple anal ray osseous and serrated posteriorly; no barbels. Pigmentation: Wild-caught specimens, olive brown, slate olive, olive green, with a bronze sheen, silvery, grayish yellowish, gray-silver, through gold (often with black blotches) to creamy white; yellowish white or white below. Cultured forms vary through scarlet, red-pink, silver, brown, white, black and combinations of these colors.

Size / Weight / Age

Max length: 32.0 cm SL male/unsexed; common length: 10.0 cm TL male/unsexed; max. Reported age: 41 years.

Environment

Freshwater; pH range: 6.0 - 8.0; dH range: 5 - 19 benthopelagic; potamodromous; depth range 0
- 20 m

Climate / Range

Subtropical; - 41°C; 53°N - 22°N

Distribution

Asia: central Asia and China, and Japan. Introduced throughout the world. Asian form of the goldfish. Several countries report adverse ecological impact after introduction.

Biology

Inhabit rivers, lakes, ponds and ditches with stagnant or slow-flowing water. Occur in eutrophic waters, well vegetated ponds and canals. Live better in cold water. Feed mainly on plankton,

benthic invertebrates, plant material and detritus. Goldfish lay eggs on submerged vegetation. Oviparous, with pelagic larvae. They last long in captivity. Maximum recorded salinity is 17 ppt, but unable to withstand prolonged exposure above 15 ppt. Used as an experimental species. Valued as ornamental fish for ponds and aquaria; edible but rarely eaten. Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 100 cm. Reported individual hooked by an angler in a lake in Poole, Dorset measured 40 cm (16 in), weighing 2.3 kg (Practical Fishkeeping, 2010).

Reproduction

Goldfish may only grow to sexual maturity with enough water and the right nutrition. Most goldfish breed in captivity, particularly in pond settings. Breeding usually happens after a significant temperature change, often in spring. Males chase females, prompting them to release their eggs by bumping and nudging them. A pregnant goldfish is called a "twit".

Goldfish, like all cyprinids, are egg-layers. Their eggs are adhesive and attach to aquatic vegetation, typically dense plants such as Cabomba or Elodea or a spawning mop. The eggs hatch within 48 to 72 hours.

Within a week or so, the fry begins to assume its final shape, although a year may pass before

they develop a mature goldfish color; until then they are a metallic brown like their wild ancestors. In theirfirst weeks of life, the fry grow quickly—an adaptation born of the high risk of getting devoured by the adult goldfish (or other fish and insects) in their environment.

Some highly bred goldfish can no longer breed naturally due to their altered shape. The artificial breeding method called "hand stripping" can assist nature, but can harm the fish if not done correctly. In captivity, adults may also eat young that they encounter.

Specimen No. 2 - *Poecilia reticulata* (Peters, 1859) Guppy

Class - Actinopterygii (Ray-finned fishes)Family - Cyprinodontiformes
Order - Poeciliidae (Poeciliids)

Size / Weight / Age

Max length: 3.5 cm SL male/unsexed; (Ref. 43281); 6 cm SL (female); common length: 2.8 cm TL male/unsexed;

Environment

Freshwater; brackish; benthopelagic; pH range: 7.0 - 8.0; dH range: 9 - 19; non-migratory

Climate / Range

Tropical; 18°C - 28°C (Ref. 1672); 14°N - 2°N, 67°W - 52°W

Distribution

South America: Venezuela, Barbados, Trinidad, northern Brazil and the Guyanas. Africa:
Feral populations reported from the coastal reaches of Natal Rivers from Durban southwards, as well as in the Kuruman Eye and Lake Otjikoto in Namibia.

Short description

Dorsal spines (total): 0; Dorsal soft rays (total): 7-8; Anal spines: 0; Anal soft rays: 8 - 10

Biology

Inhabits warm springs and their effluents, weedy ditches and canals. Found in various habitats, ranging from highly turbid water in ponds, canals and ditches at low elevations to pristine mountain streams at high elevations. Occurs in wide variety of habitats with low predation pressure, usually in very small streams and densely vegetated lakes and springs. Has a wide salinity range but requires fairly warm temperatures (23-24 °C) and quiet vegetated water for survival. Feeds on zooplankton, small insects and detritus. One of the most popular aquarium fishes with many standardized varieties. Used in genetics research. Female reaches 5 cm SL. Males mature at 2 months and females at 3 months of age. Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 60 cm. A very popular and widely available species in the aquarium trade.

Reproduction

Guppies are highly prolific livebearers. The gestation period of a guppy is 21–30 days, with an average of 28 days, varying according to water temperature. Males possess a modified tubular anal fin, the gonopodium, located directly behind the ventral fin, which is flexed forward and used as a delivery mechanism for one or more balls of spermatozoa. The male will approach a female and will flex his gonopodium forward before thrusting it into her and ejecting these balls.

After the female guppy is inseminated, a dark area near the anus, known as the gravid spot, will enlarge and darken. Just before birth, the eyes of fry may be seen through the translucent skin inthis area of the female's body. When birth occurs, individual offspring are dropped in sequence over the course of an hour or so.

Guppies prefer water temperatures of about 26 °C (79 °F) for reproduction. The female guppy has drops of between two and 50 fry at a time, typically ranging between 5 and 30. After giving birth, the female is ready for conception again within only a few hours. Guppies have the ability to store sperm up to a year, so the females can give birth many times without depending on the presence of a male. From the moment of birth, each fry is fully capable of swimming, eating, and avoiding danger. If not kept separate, the older, mature guppies will eat the fry, so the use of a breeder box, net breeder, or a separate 20–40 litres (4–9 imp gal; 5–11 US gal) tank is recommended. Live plants may be used as hiding places for the fry.

Young fry take roughly three or four months to reach maturity. In the aquarium, they are usuallyfed finely ground flake foods, baby brine shrimp or, unless they are put in a separate tank, uneaten food from the adults. In addition, they nibble on algae.

Guppies have been selectively bred to produce a variety of colors and patterns. In the wild, male guppies are dull black or brown in colour, with some coloured spots, while females are fully dull grey. The wild guppies that showed the most colours in each generation were bred to produce the "fancy guppies" seen in pet stores and guppy shows today.

The guppy has been successfully hybridised with various species of molly (*Poecilia latipinna* or *velifera*), e.g. male guppy and female molly. However, the hybrids are always males and appear to be infertile. The guppy has also been hybridized with the Endler's livebearer (*Poecilia wingei*)to produce fertile offspring.

Specimen No 3 - *Poecilia sphenops* (Valenciennes, 1846) Black Molly

Class - Actinopterygii (Ray-finned fishes)
Order - Cyprinodontiformes (Rivulines, killifishes and live bearers)Family - Poeciliidae (Poeciliids)

Size / Weight / Age

Max length: 6.0 cm TL male/unsexed; (Ref. 50894); common length : 4.0 cm TL male/unsexed;

Environment

Freshwater; brackish; pH range: 7.5 - 8.2; dH range: 11 - 30 benthopelagic; non-migratory

Climate / Range

Tropical; 18°C - 28°C; 24°N - 1°S, 101°W - 69°W

Distribution

Central and South America: Mexico to Colombia. Often confused with Poecilia mexicana.

Biology

Feeds on worms, crustaceans, insects, plant matter. The black variety (Black molly) is a very popular aquarium fish and is marketed throughout the world. In the aquarium it feeds on green algae and also readily accepts dried food. Minimum aquarium size 60 cm.

Specimen No. 4 - *Xiphophorus maculatus* (Günther, 1866) Sword Tail

Class - Actinopterygii (Ray-finned fishes)
Order - Cyprinodontiformes (Rivulines, killifishes and live bearers)Family - Poeciliidae (Poeciliids)

Size / Weight / Age

Max length: 4.0 cm TL male/unsexed; 6 cm TL (female); common length: 2.4 cm TL male/unsexed; Length at first maturity - 1 - 4.2 cm

Environment

Freshwater; pH range: 7.0 - 8.0; dH range: 9 - 19 benthopelagic; non-migratory

Climate / Range

Tropical; 18°C - 25°C; 23°N - 17°N, 99°W - 86°W

Distribution

North and Central America: Ciudad Veracruz, Mexico to northern Belize. At least one country reports adverse ecological impact after introduction.

Short description

Dorsal soft rays (total): 8-10. Distinct line of black pigment midventrally on caudal peduncle absent. Greatest body depth about 2 times in SL. Ventral rays of caudal fin of males not elongated into a sword. Male gonopodium falling short of caudal fin base, without a membranous protuberance, third ray with a strong hook.

Biology

Adults occur in warm springs, canals and ditches with typically slow-moving water, silt bottoms and weedy banks. Also inhabit creeks and swamps. Feed on worms, crustaceans, insects and plant matter. Used for genetic research. Several color varieties are popular aquarium fishes, where they attain sexual maturity after 3-4 months and reproduce easily. Aquarium keeping: minimum aquarium size 60 cm.

Breeding:

The Mickey Mouse Platy is sexually mature as early as four months of age, which means young fish should be sexed and separated when they are very young. Female platys who mate will retain sperm packets and can continue to give birth without mating again for a

number of months

Once mating has occurred, and the eggs are fertilized, it takes about 30 days for the fry to emerge. The temperature can slow down or speed up the process (warmer water shortens the gestation period). Typical broods are 40-60 fry, which are born live.

As the fry develop, the belly of the female will become lar ger. Eventually the eyes of the fry can be seen through the stretched belly of the mother. As birthing time draws near, you should be prepared to shelter and protect the fry. Otherwise the parents, and any other fish in the tank, will eat most, if not all of them.

One option is to place female in a breeding trap just before birth. The trap is designed so the fry fall through slits that are too small for the mother to follow. The negatives of this is that the small trap is stressful for the mother, and must be done before she begins giving birth.

Another method is to have a separate birthing/nursery tank that is heavily planted with fine leafed vegetation. As the fry are born, they hide in the plants. Once the mother had given birth toall her fry, she is removed, thus protecting the fry.

The fry are fully formed very tiny fish. Initially they need very fine foods to feed upon. Freshly hatched brine shrimp are ideal, but liquid or powdered fry food will do fine. Feedings are required several times per day, which means debris will build up more quickly in the tank, thus requiring daily water changes.

Specimen No. 5 - *Puntius tetrazona* (Bleeker, 1855) Sumatra barb

Class - Actinopterygii (Ray-finned fishes)Order - Cypriniformes (Carps)
Family - Cyprinidae (Minnows or carps)

Size / Weight / Age

Max length: 7.0 cm TL male/unsexed; (Ref. 7050)

Environment

Freshwater; pH range: 6.0 - 8.0; dH range: 5 - 19 benthopelagic

Climate / Range

Tropical; 20°C - 26°C; 6°N - 3°S, 95°E - 118°E

Distribution

Asia: Sumatra and Borneo. Introduced widely and has been reared in several countries in facilities for breeding aquarium fishes.

Description

Four tiger-like black vertical stripes on an orange-yellow body make it obvious where this member of the barb family got its common name. Red edged fins and nose add even more color to the popular Tiger Barb. In recent years, selective breeding has created several color variations that include green, black, red, and albino.

Biology

Feeds on worms, small crustaceans and plant matter. Aquarium keeping: in groups of 5 or more individuals; not to be kept with long-finned fishes; minimum aquarium size 60 cm.

Reproduction

The tiger barb usually attains sexual maturity at a body length of 2 to 3 centimeters (0.8 to 1.2 inches) in total length, or at approximately six to seven weeks of age. The females are larger with a rounder belly and a mainly black dorsal fin, while the males have a bright, red nose with adistinct red line above the black on their dorsal fins. The egg-layers tend to spawn several hundred eggs in the early morning in clumps of plants. On average, 300 eggs can be expected from each spawn in a mature brood stock population, although the number of eggs released will increase with the maturity and size of the fish. Spawned eggs are adhesive, negatively buoyant in freshwater and average 1.18 ± 0.05 mm in diameter.

Tiger barbs have been documented to spawn as many as 500 eggs per female. With proper conditioning, females can spawn at approximately two week intervals.

Once spawning is finished, they will usually eat any of the eggs they can find. It is usually necessary to separate the fish from the eggs after spawning to prevent them from being eaten.

Specimen No. 6 - *Puntius conchonius* (Hamilton, 1822) Rosy barb

Class - Actinopterygii (Ray-finned fishes)Order - Cypriniformes (Carps)
Family - Cyprinidae (Minnows or carps)

Size / Weight / Age

Max length: 14.0 cm TL male/unsexed; (Ref. 4832)

Environment

Freshwater; pH range: 6.0 - 8.0; dH range: 5 - 19 benthopelagic

Climate / Range

Subtropical; 18°C - 22°C (Ref. 1672); 40°N - 8°N

Distribution

Asia: Afghanistan, Pakistan, India, Nepal, and Bangladesh. Reported from Myanmar. Introduced worldwide and now very popular with aquarists.

Description

This pinkish fish is one of the larger species of Barbs growing up to 6 inches (14 cm) in length. Their color becomes bolder during their mating periods. The male has a brighter pinkish color and the female is slightly plumper. Also note that females do not have any reddish color in their fins while males do. A black dot is present on caudal peduncle. They may weigh up to 12 oz when fully grown but can weigh much less during adolescence. They are mature at 2.5 inches.

Biology

Inhabits lakes and fast flowing hill streams. One of the hardiest of the barbs, undemanding and beautiful; most impressively colored during the mating period, when the normally silvery male takes on a rich claret flush and the slightly larger female becomes more luminous. Can be kept together with other small fishes. Feeds on worms, crustaceans, insects and plant matter. Prolific spawner that tolerates low water temperatures.

Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 80 cm.

Breeding

When the female is ready to spawn, she will appear swollen with eggs. The males will circle and chase the females, repeatedly nudging her head and belly area. Spawning usually occurs in the early morning, and lasts several hours resulting in several hundred eggs. Eggs are usuallydeposited in groups of plants, and the pair will attempt to eat any that they are able to locate.

The young hatch in 24 to 36 hours, depending on water temperature. A day later, the young fish will hang on the plants, and/or the sides of the tank if the breeding takes place in an aquarium. In about six days the young are free-swimming and will seek out food. In captivity, they can be fed newly hatched brine shrimp.

Rosy barb has been successfully hybridized with the Tiger Barb (*Puntius tetrazona*), i.e. crossing female Tiger Barb with male Rosy Barb. The hybrids reach maturity and were all males, however they are sterile.

Specimen No. 7 - *Epalzeorhynchos bicolor* (Smith, 1931) Redtail sharkminnow

Class - Actinopterygii (Ray-finned fishes)Order - Cypriniformes (Carps)
Family - Cyprinidae (Minnows or carps)

Size / Weight / Age

Max length : 12.0 cm TL male/unsexed; (Ref. 7020)

Environment

Freshwater; ; pH range: 6.5 - 7.5; dH range: ? - 15 demersal

Climate / Range

Tropical; 22°C - 26°C (Ref. 13371)

Distribution

Asia: Chao Phraya basin, Thailand. Reported from the Mekong basin.

Description

The Red Tailed Black Shark has a black body with a red tail (hence thename). The Red Tailed Black Shark has a downwards facing mouth with two pairs of barbels. The female isslightly large than themale.

Biology

Inhabits mainstream rivers and floodplains. Omnivorous, feeding on plant matter and small benthic animal. Tens of thousands of specimens are exported annually from Thailand for the ornamental trade, all now captive bred. Aquarium keeping: solitary, adults are territorial and may 'bully' other fish; minimum aquarium size 120. It is currently critically endangered in the wild, but common in aquaria, where it is prized for its deep black body and vivid red or orange tail.

Specimen No. 8 – *Betta splendens* (Regan, 1910) Siamese fighting fish

Class - Actinopterygii
Order - Perciformes
Family - Osphronemidae (Gouramies)

Short description

Dorsal spines (total): 1; Vertebrae: 29 - 34. Reddish bars on opercula. Max length: 6.5 cm TL male/unsexed; max. Reported age:
2 years. Although known for their brilliant colors and large, flowing fins, the natural coloration of *B. splendens* is a dull green and brown, and the fins of wild specimens are relatively short. Brilliantly colored and longer finned varieties (i.e. Veil tail; Delta; Super delta; and Half-moons) have been developed through selective breeding.

Biology

Occur in standing waters of floodplains, canals, rice paddies and medium to large rivers. Feeds on zooplankton, mosquito and other insect larvae. Air breather and bubble nest builder. Used in behavioral studies. Males will fight each other. The many colorful varieties are popular aquarium fish; however, the holding of the males in very small containers should be discouraged. Aquarium keeping: several females for one male; minimum aquarium size 60 cm.

Environment

Freshwater; pH range: 6.0 - 8.0; dH range: 5 - 19 benthopelagic

Climate / Range

Tropical; 24°C - 30°C (Ref. 1672); 22°N - 8°N, 99°E - 107°E

Distribution

Asia: Mekong basin.

Specimen No. 9 – *Pterophyllum scalare* (Schultze, 1823) Freshwater angelfish

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Cichlidae (Cichlids)

Size / Weight / Age

Max length: 7.5 cm SL male/unsexed;

Environment

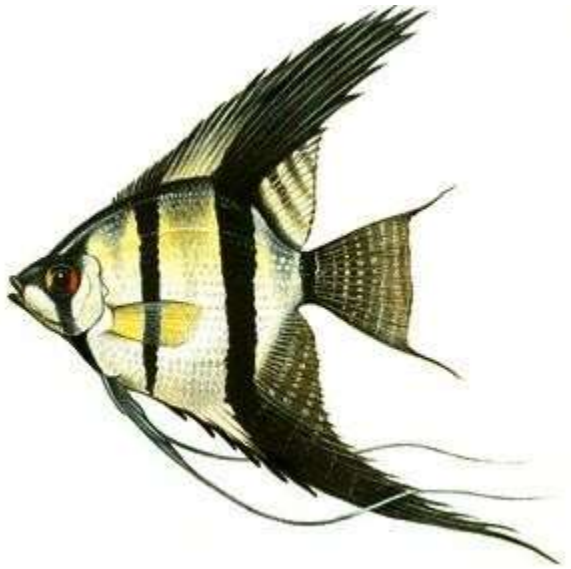
Freshwater; pH range: 6.0 - 8.0; dH range: 5 - 13 benthopelagic

Climate / Range

Tropical; 24°C - 30°C; 6°N - 10°S, 78°W - 51°W

Distribution

South America: Amazon River basin, in Peru, Colombia, and Brazil, along the Ucayali, Solimões and Amazon rivers; rivers of Amapá (Brazil), Rio Oyapock in French Guiana; Essequibo River in Guyana.



Short description

Body compressed and disc-shaped; dorsal and anal spiny rays increasing in length from anterior to posterior part of the fin; first branched rays also very long; body height at anal fin level 1.07 to 1.29 times in SL; body color silvery with dark vertical bars (7 in juveniles, 4 in adults).

Biology

Inhabit swamps or flooded grounds where the aquatic and riverine vegetation are dense and thewater is either clear or silty. Its color is deeper in clear water. One of the most popular of all the tropical aquarium fish. Maximum length 15 cm TL. Aquarium keeping: in groups of 5 or more individuals; keep pairs in small tanks for breeding; minimum aquarium size 100 cm.

Specimen No. 10 - *Astronotus ocellatus* (Agassiz, 1831) Oscar

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Cichlidae (Cichlids)

Size / Weight / Age

Max length: 45.7 cm TL male/unsexed; common length: 24.0 cm TL male/unsexed; max. Published weight: 1,580 g.

Environment

Freshwater; pH range: 6.0 - 8.0; dH range: 5 - 19 benthopelagic.

Climate / Range

Tropical; 22°C - 25°C; 4°N - 15°S, 78°W - 47°W

Distribution

South America: Amazon River basin in Peru, Colombia and Brazil; French Guiana. Reported fromArgentina.

Short description

Dorsal spines (total): 12 - 14; Dorsal soft rays (total): 19-21; Anal spines: 3; Anal soft rays:
15 - 17. Large mouth with thick lips; 7 preopercular pores; first gill arch without lobe; gill rakers short and thick with many denticles;
dorsal and anal fins bases densely scaled; many branched rays; body color dark with bright orange opercle margin and ventral parts of the lateral sides of the body; often a black rounded blotch with orange margin at caudal fin base.

Biology

Preferably inhabits quiet shallow waters in mud-bottomed and sand-bottomed canals and ponds (Ref. 5723). Feeds on small fish, crayfish, worms and insect larvae. Quite popular with aquarists but not for aquaculturists because of its slow growth.

Specimen No. 11 - *Symphysodon discus* (Heckel, 1840) Discus

Class - Actinopterygii
Order - Perciformes
Family – Cichlidae

Size / Weight / Age

Max length: 12.3 cm SL male/unsexed;

Environment

Freshwater; benthopelagic; pH range: 4.2 - 6.2; dH range: 0 - 1

Climate / Range

Tropical; 26°C - 30°C; 1°S - 3°S

Distribution

South America: Amazon River basin in Brazil, near the mouth of the Negro River, in the lower Abacaxis River, and in the lower Trombetas River.

Description

Like cichlids from the genus *Pterophyllum*, all *Symphysodon* species have a laterally compressed body shape. In contrast to Pterophyllum, however, extended finnage is absent giving *Symphysodon* a more rounded shape. It is this body shape from which their common name, "discus", is derived. The sides of the fish are frequently patterned in shades of green, red, brown, and blue.

Reproduction and sexual dimorphism

Another characteristic of *Symphysodon* species is their care for the larvae. As for most cichlids, brood care is highly developed with both the parents caring for the young.
Additionally, adult discus produces a secretion through their skin, which the larvae live off during their first few days. This behavior has also been observed for Uaru species. However when bred in captivity the larvae will tend to live off their parents secretion for up to 2 weeks.

Specimen No. 12 - *Trichogaster leerii* (Bleeker, 1852) Pearl gourami

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Osphronemidae (Gouramies)

Size / Weight / Age

Max length: 12.0 cm TL male/unsexed;

Environment

Freshwater; benthopelagic; pH range: 6.0 - 8.0; dH range: 5 - 19

Climate / Range

Tropical; 24°C -

28°C**Distribution**

Asia: Malay Peninsula, Thailand and Indonesia (Sumatra and Borneo).

Description

These fish reach about 12 centimetres(5 in) TL. The body is a brownish- silver colour, covered in a pearl-like pattern with a distinct black 'lace' running from the fish's head, and gradually thinning towards the caudal fin. The patterning of this fish has given rise to many popular names, such as the Lace Gourami and the Mosaic Gourami. The 'lace' refers to the long black line running through the fish's body, whereas 'Mosaic' refers more towards the pearl, or mosaic-like patterning of the body.

Male specimens of this fish, typical of many gouramis, are generally larger and more colourful than their female counterparts. They exhibit bright orange colouring around the throat region, which at breeding time becomes much brighter and is used to court the female. Males also exhibit somewhat of an orange tinge in their fins, with the exception of the caudal (tail) fin. The male also has longer fins, with a more pointed dorsal fin and extended anal fin rays

Biology

Occurs in swamps and streams, usually among dense vegetation. Found in lowland swamps withacidic water. Aquarium keeping: minimum aquarium size 120 cm.

Specimen No. 13 - *Helostoma temminkii* (Cuvier, 1829) Kissing gourami

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Helostomatidae (Kissing gourami)

Size / Weight / Age

Max length: 30.0 cm TL male/unsexed;

Environment

Freshwater; pH range: 6.0 - 8.0; dH range: 5 - 19 benthopelagic; potamodromous

Climate / Range

Tropical; 22°C - 28°C (Ref. 1672); 16°N - 6°S

Distribution

Asia: Thailand to Indonesia.

Description

Typical of gourami, the body is deep and strongly compressed laterally. The long-based dorsal (16–18 spinous rays, 13–16 soft) and anal fins (13–15 spinous rays, 17–19 soft) mirror each other in length and frame the body. The posterior softest rays of each of these fins are slightly elongated to create a trailing margin. The foremost rays of the jugular pelvic fins are also slightly elongated. The pectoral fins are large, rounded, and low-slung. The caudal fin is rounded to concave. The lateral line is divided in two, with the posterior portion starting below the end of the other; there are a total of 43–48 scales running the line's length.

The most distinctive feature of the kissing gourami is its mouth. Other than being terminal rather than superior (as in other gourami families), the kissing gourami's mouth is highly protrusible as its family name suggests, the lips are lined with horny teeth. However, teeth are absent from the premaxilla, dentaries, palatine, and pharynx. The gill rakers are also well- developed and numerous. The visible scales of the body are ctenoid, whereas the scales of the top of the head are cycloid. Kissing gourami reach a maximum size of 30 centimeters (12 in) TL. There is no outward sexual dimorphism and is difficult to almost impossible to

distinguish the sexes.

There are two colour morphs encountered: greens, which have lengthwise lateral stripes and opaque, dark brown fins; and pink, which have a rose to orangey pink body and silvery scales, with transparent pinkish fins. Green fish originate from Thailand while pink fish origiante from

Java. There is also a "dwarf" or "balloon pink" variety, which is a mutated strain of the pink gourami that are offered to hobbyists. The "balloons" are named so for their smaller and rounder bodies.

Biology

Occurs in lakes and rivers. Prefers slow-moving water with thick vegetation. These fish are omnivorous and need both plant and animal matter in its diet. Feeds on a variety of plants and animals, including green algae and zooplankton as well as aquatic insects near the water surface. Male kissers will occasionally challenge each other; however, the "kissing" itself is never fatal but the constant bullying can stress the other fish to death. They often do in fact kill other fish by sucking their 'fish slime' (mucus) off of their skin as food, which opens the victim fish up to infections. These fish may be useful as algae eaters to control algae growth. To preventdigging and to present enough surface area for algae growth, the substrate should consist of large-diameter gravel and stones.

Aquarium keeping: not recommended for home aquariums; minimum aquarium size 150 cm.

Specimen No. 14 - *Paracheirodon innesi* (Myers, 1936) Neon Tetra

Class - Actinopterygii
Order - Characiformes
Family – Characidae

Size / Weight / Age

Max length: 2.2 cm SLr

Environment

Freshwater; pelagic; pH range: 5.0 - 7.0; dH range: 1 - 2

Climate / Range

Tropical; 20°C - 26°C (Ref. 1672)

Distribution

South America: Black water or Clearwater stream tributaries of the Solimões River.

Description

The neon tetra has a light-blue back over a silver-white abdomen. The fish is characterized by an iridescent blue horizontal stripe along each side of the fish from its nose to the base of the adipose fin, and an iridescent red stripe that begins at the middle of the body and extends posteriorly to the base of the caudal fin. Most, if not all, will develop an olive green sheen lining their backs. The fish is completely transparent (including fins) except for these markings. During the night, the blue and red become silver as the fish rests—it reactivates once it becomes active in the morning. It grows to approximately 3 cm (1.2 in) in overall length. Sexual dimorphism is slight, the female having a slightly larger belly, and a bent iridescent stripe ratherthan the male's straight stripe.

Biology

Feeds on worms, small insects, crustaceans and plant matter. In tank, female lays a relatively small number of eggs, which hatch in about 24 hours. Most popular aquarium fish. Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 60 cm.

Breeding

The male is slender, and the blue line is straighter. The female is rounder, producing a bent blue line. Some say that the females look plumper when viewed from above but this is disputed. However, the 'straightness' of the line and the plumpness of the female might occasionally be due to the eggs she is carrying.

To breed Neon Tetras, place a pair of the species in a breeding tank without any light, and gradually increase the lighting until spawning occurs. Other inducers include mosquito larvae and a hardness of less than 4 degrees. Some also recommend letting the level of

nitrates rise, then do at least 50% water change to simulate the fresh rain the tetras get in their natural habitat, the Amazon. It is recommended that everything you place in the aquarium be sterilized,

as well as the aquarium top. Because the adults will often eat newly-hatched fry, it is best to remove them as soon as the eggs have been laid. The eggs are especially sensitive to light. Eggs will hatch within 24 hours of the laying. Fry can be fed infusoria, especially rotifers and egg yolk for 1 to 4 weeks, followed by nauplii of brine shrimp, shaved cattle liver, and formulated diets. Fry will achieve their adult coloration at approximately one month of age. Adults can spawn every two weeks.

Nutrition

Neon tetras are omnivores and will accept most flake foods, if sufficiently small, but should also have some small foods such as brine shrimp, daphnia, freeze-dried bloodworms, tubifex, which can be stuck to the side of the aquarium, and micro pellet food to supplement their diet. A tropical sinking pellet is ideal as most brands of these include natural color enhancers that bringout the color in neon tetras. Some frozen foods including frozen blood worms add variety to their diet.

Specimen No. 16 - *Paracheirodon axelrodi* (Schultz, 1956) Cardinal Tetra

Class - Actinopterygii
Order - Characiformes
Family - Characidae

Size / Weight / Age

Max length: 2.5 cm SL male/unsexed;

Environment

Freshwater; pH range: 4.0 - 6.0; dH range: 5 - 12 pelagic; non-migratory

Climate / Range

Tropical; 23°C - 27°C

Distribution

South America: Upper Orinoco and Negro River basins.

Description

Growing to about 3 cm (1.25 in) total length, the cardinal tetra has the striking iridescent blue line characteristic of the *Paracheirodon* species laterally bisecting the fish, with the body below this line being vivid red in color, hence the name "cardinal tetra". The cardinal tetra's appearance is similar to that of the closely related neon tetra, with which it is often confused; the neon's red coloration extends only about halfway to the nose, and the neon's blue stripe is a less vibrant blue.

Biology

Occurs mainly in shoals in the middle water layers. Feeds on worms and small crustaceans. Breeding in captivity is possible but difficult and most specimens in the aquarium trade are caught in the tributaries of the Rio Negro and Orinoco. Eggs hatch in 24 to 30 hours and fry are free-swimming after 3 to 4 days in captivity. One of the most popular and beautiful aquarium fishes. Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 60 cm.

Specimen No. 17 - *Paracheirodon axelrodi* (Boulenger, 1895) Black Tetra

Class - Actinopterygii
Order - Characiformes
Family - Characidae

Size / Weight / Age

Max length: 7.5 cm SL male/unsexed;

Environment

Freshwater; pelagic; pH range: 6.0 - 8.0;dH range: 5 - 19

Climate / Range

Subtropical; 20°C - 26°C; 11°S - 30°S, 64°W - 48°W

Distribution

South America: Paraguay and GuaporéRiver basins to Argentina.

Description

The black tetra has a roughly tetragonal body shape and is greyish in colour, fading from light at the nose to near black at the tail. Two prominent black vertical bars appear just posterior of the gills.

Biology

Occurs in the middle and upper water layers. Feeds on worms, small crustaceans and insects.

Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 60 cm.

Breeding

The black widow tetra reaches sexual maturity at about two years of age. They will spawn in water 78 degrees F. A separate spawning tank, heavily planted and at least 15 gallons, is recommended. Like most characins, black tetras spawn by intermittently releasing and fertilizing eggs among the plants. These fish are frequent egg-eaters and must be removed after spawning. The young are easy to rise, subsisting on newly hatched brine shrimp or powdered processed foods.

Specimen No. 18 - *Hyphessobrycon eques* (Steindachner, 1882) Serpae Tetra/ Jewel Tetra

Class - Actinopterygii
Order - Characiformes
Family – Characidae

Size / Weight / Age

Max length: 4.0 cm SL

male/unsexed;Length at first

maturity: 2.1 cm **Environment**

Freshwater; ; pH range: 5.0 - 7.8; dH range: 10 - 25 benthopelagic

Climate / Range

Tropical; 22°C -

26°C**Distribution**

South America: Amazon, Guaporé and Paraguay River basins.

Description

Serpae tetra has distinct red body coloration. The shades vary from bright red to reddish brown. The red body is decorated with a black comma-shaped mark that is found right behind the gill cover. Some Serpae tetra specimens have a very small marking or have no marking at all. As your Serpae tetra grows older, the marking will grow smaller. The anal fin, ventral fins and tail are all red, and the anal fin is fringed with black and white layers. The dorsal fin of the Serpae tetra is tall and black, and has a thin white fringe. This fringe can sometimes have a reddish hue.

Biology

A gregarious species which positions itself near the surface between the stems of emerging plants. It is frequently found in stagnant waters. They are generally peaceful, but when there are too many of them during feeding, they bite each other's fins. Feeds on worms,

crustaceans, insects and plants. Oviparous. This species is part of a complex of `blood' tetras, hybrids of which are commonly offered in the aquarium trade. Aquarium keeping: aggressive; in groups of 5 or more individuals; minimum aquarium size 60 cm.

Specimen No. 19 - *Hyphessobrycon pulchripinnis* (Ahl, 1937) Lemon Tetra

Class - Actinopterygii
Order - Characiformes
Family – Characidae

Size / Weight / Age

Max length: 3.8 cm SL male/unsexed; (Ref. 40342); 3.6 cm SL (female); max. Published weight:
1.4 g; max. Published weight: 1.2 g

Environment

Freshwater; benthopelagic; pH range: 5.5 - 8.0;

Climate / Range

Tropical; 23°C -

28°C**Distribution**

South America: Tapajós River basin.

Description

The lemon tetra is one of the deeper-bodied tetras, contrasting with slender, torpedo-shaped relations such as the cardinal tetra and the rummy nosed tetra, whose approximate body shape when seen from the side is that of a lozenge, (often referred to as a diamond, is a form of rhombus). The basic body color of an adult specimen is a translucent yellow, with a pearlescent

lustre emanating from the scales in particularly fine specimens. The dorsal and anal fin of the fish are marked with black and yellow: specifically, the anal fin is hyaline (glass-like appearance), with a black outer margin, the anterior three or four rays being an intense, acrylic lemon-yellow in hue, while the dorsal fin is principally black with a yellow central patch. The tailfin is mostly hyaline but in fine specimens (particularly alpha males) acquires a gunmetal-blue lustre. The pectoral fins are hyaline whilst the pelvic fins are translucent yellow, becoming more solidly and opaquely yellow with black posterior edges in fine specimens (again, alpha males are particularly notable in this regard). The eye is a notable feature of this fish, the upper half of the iris being an intense red, in some specimens almost

gemstone-ruby in appearance. The colour of this part of the iris is an indicator of the health of the fish: if this red coloration fades, or worse still turns grey, then this is an indicator of serious disease in the fish. In common with many characins, the lemon tetra possesses an adipose fin. This fin may acquire a black border, particularly in males, though this is not a reliable characteristic. Black areas of coloration on adult fishes frequently acquire a glossy sheen, enhancing the beauty of the fish.

Determining the gender of the fish is achieved in adult specimens by examining the black outer border of the anal fin. In female specimens, this consists of a fine black line, appearing almost as if drawn onto the fin with a fine pencil. In male specimens, particularly alpha males, the border is conspicuously wider, and in breeding alpha males, can cover up to a third of the total area of the anal fin. This is the only reliable means of differentiating gender in this species - while males frequently have taller and more pointed dorsal fins, some females also possess tall, pointed dorsal fins, and thus this characteristic is not reliable. Ripe females are fuller bodied, particularly when viewed from directly above, as the body cavity expands to accommodate the eggs forming within the female's reproductive tract.

Juvenile fishes are usually translucent with only a hint of the colour of the adult. Additionally, the gender characteristics in juvenile fishes are not fully formed, so differentiating juveniles intomale and female individuals is extremely difficult.

Biology

Feeds on worms, crustaceans and plant matter

Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 60 cm.

Reproduction

Lemon tetras exhibit an interesting behavior pattern in the aquarium, replicated by several other characin species, in which males will adopt 'landmarks' within the aquarium and use these as places from which to display as maturity approaches. Displays are principally performed between rival males, which position themselves in a slightly head-up posture, unpaired fins held erect to appear as large and as imposing as possible and swim forwards with 'flicking' movements of the body. If two rival males approach closely, they will then begin to make passes at each other, which to the causal observer look like attacks: this is an entirely ritualized behavior, best referred to as 'jousting', where the males make darting movements toward each other but pull away at the last moment. No damage is incurred by either contestantin these events, and evenly matched males that are at a similar level in the social hierarchy will continue such behavior for 30 minutes or more at a time. This behavior serves not only to establish the social rankings of the males, but also serves as an indicator of reproductive fitness to the watching females.

In the wild, the lemon tetra is a communally spawning fish. Tens of thousands of pairs will spawn together, and choose thickets of fine-leaved aquatic plants as the repository for their fertilized eggs. This behavior has several ramifications for captive reproduction, as will be duly noted.

When ripe females are receptive, males will court them, after a chase sequence through aquatic foliage in which several males may pursue an individual female, breaking off to pursue a different female as the opportunity arises, resulting in the aquarium in mad dashes hither and thither. Eventually, close observation will see a male court a female in some secluded area of aquatic foliage. The courting gesture of the male consists of a quivering motion, with a head- down posture, and the 'flicking' of the unpaired fins in such a manner as to generate flashes of yellow coloration in the visual field of the female. These flashes will be readily visible to the observing aquarist. If the female is ready to spawn, then the pair will

migrate to fine-leaved aquatic plants such as Cabomba or Java Moss, whereupon the pair will adopt a side by side position. The release of eggs and sperm is signaled by a quivering motion upon the part of both

fishes, followed by an 'explosive' parting of the couple accompanied by the appearance of a cloud of eggs. The eggs are non-adhesive, and fall through the foliage, coming to rest either in thicker foliage at the base of the plants, or on the substrate.

One of the problems presented to the aquarist by this species is that of egg-eating. in the wild, as has already been noted, tens of thousands of pairs spawn simultaneously, and in order to reducethe competition for their own offspring, a pair will turn around and eat some of the eggs being produced by neighboring spawning pairs. The same instinct, needless to say, manifests itself in the aquarium, but in an aquarium setting, the only eggs present are usually those of the single spawning pair. Consequently some of the pair's own eggs will be devoured. Special measures to reduce losses through this behavior are therefore required if the aquarist is interested in obtaining as large a yield of fry as possible. Special 'egg traps' have been devised for the purpose
- these may consist of evenly spaced glass rods in a frame, a layer of glass marbles on the aquarium bottom, or some other such device. The purpose of all these designs is the same - to allow eggs to fall through small gaps into a space where they will be beyond the reach of the adults, who are unable to pass through the same gaps. However, the speed of movement of the adult fishes is such that even with an 'egg trap' in place, a small percentage of eggs will still be eaten.

A large adult female in prime condition may produce as many as 300 eggs.

A breeding aquarium for the lemon tetra thus requires good quality filtration and aeration, fine leaved plants, and an 'egg trap' present to prevent large-scale losses because of the aforementioned parental instincts. Temperature should be slowly raised over a period of a few days to 28°C, the pair conditioned with copious quantities of live foods if possible, and the aquarium should be sited so as to receive illumination by morning sunshine, as this is a well- documented spawning stimulus for the lemon tetra. Parent fishes should be removed from the breeding aquarium and returned to the main aquarium once spawning is complete. Sometimes, best results are obtained by using two males with one female.

Specimen No. 20 - *Acanthurus leucosternon* (Bennett, 1833) Powder blue surgeonfish

Actinopterygii (Ray-finned fishes)Perciformes (Perch-likes)
Acanthuridae (Surgeonfishes, tangs, unicornfishes)

Size / Weight / Age

Max length: 54.0 cm TL male/unsexed; (Ref. 30573); common length: 19.0 cm TLmale/unsexed;

Environment

Marine; reef-associated; depth range 0 - 25 m (Ref. 9710), usually 0 - 25 m (Ref. 27115)

Climate / Range

Tropical; 23°C - 28°C; 26°N - 30°S, 31°E - 129°E

Distribution

Indian Ocean: eastern Africa to the Andaman Sea, southwest Indonesia and Christmas Island; with range extended to Bali, Indonesia in Western Pacific.

Short description

Dorsal spines (total): 9; Dorsal soft rays (total): 28-30; Anal spines: 3; Anal soft rays: 23 - 26. Blue with a white chest. Head black with a broad white band from pectoral-fin base to throat. Nodistinct white spot or broad white band below eye. Dorsal fin yellow (except white margin and black sub marginal line). Anal and pelvic fins white.

Biology

Inhabits shallow, clear coastal and island coral reefs. Usually found on reef flats and along upperseaward slopes. May occur singly or in large feeding aggregations. Monogamous. Feeds on benthic algae; on small, sparsely scattered algae and small growths in crevices. Caught with nets. Marketed fresh.

Specimen No. 21 - *Chrysiptera cyanea* (Quoy & Gaimard, 1825) Sapphire devil

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Pomacentridae (Damselfishes)

Size / Weight / Age

Max length: 8.5 cm TL male/unsexed;

Environment

Marine; reef-associated; non-migratory; depth range 0 - 10 m.

Climate / Range

Tropical; 30°N - 30°S**Distribution**

Indo-West Pacific: eastern edge of the Indian Ocean and Western Australia to New Guinea, New Britain, Solomon Islands, Marianas and Caroline Islands, Indonesia, Philippines, Taiwan and Ryukyu Islands. Also known from Vanuatu and New Caledonia; Palau and Yap in Micronesia; Samoa.

Short description

Dorsal spines (total): 13; Dorsal soft rays (total): 12-13; Anal spines: 2; Anal soft rays: 13 - 14. Recognized by the brilliant light-blue color. This species exhibits a marked sexual dichromatism: juveniles and females usually have a small black spot at the rear base of the dorsal fin and (at least in Micronesia) lack yellow; males have a bright yellow snout and tail and sometimes, latter all orange in some regions; also lack the black spot.

Biology

Adults are found amongst rubble and coral of clear sheltered lagoons and sub tidal reef flats. They occur in groups of a male and several females or juveniles. Feed on algae, pelagic tunicates and copepods. Oviparous, distinct pairing during breeding. Eggs are demersal and adhere to the substrate. Males guard and aerate the eggs.

Specimen No. 22 - *Gramma loreto* (Poey, 1868) Royal gramma

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Grammatidae (Basslets)

Size / Weight / Age

Max length: 8.0 cm TL male/unsexed;

Environment

Marine; reef-associated; depth range 1 - 60 m (Ref. 9710), usually 1 - 40 m

Climate / Range

Tropical; 22°C -

27°C**Distribution**

Western Central Atlantic: Bermuda, Bahamas, and Central America to northern South America.

Short description

Dorsal spines (total): 12; dorsal soft rays (total): 10. Bicolored: purple (appearing blue underwater) in front, bight orange-yellow behind.

Biology

Often found in caves or under ledges. Swim with belly toward substratum, thus under ledges seen upside down. Feed on ectoparasites of other fishes. Males show various types of nest care behavior. Retreat into recesses when alarmed. Have been reared in captivity.

Specimen No. 23 - *Heniochus acuminatus* (Linnaeus, 1758) Pennant coralfish

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Chaetodontidae (Butterflyfishes)

Size / Weight / Age

Max length: 25.0 cm TL male/unsexed; common length : 15.0 cm TL male/unsexed;

Environment

Marine; brackish; reef-associated; depth range 2 - 75 m, usually 15 - 75 m

Climate / Range

Tropical; 30°N - 35°S**Distribution**

Indo-Pacific: East Africa and Persian Gulf to the Society Islands, north to southern Japan, south to Lord Howe Island. Throughout Micronesia.

Short description

Dorsal spines (total): 11 - 12; Dorsal soft rays (total): 22-27; Anal spines: 3; Anal soft rays: 17 - 19. Distinguished from the very similar H. diphreutes by the longer snout, rounder shape and longer and more angular anal fin.

Biology

Inhabit deep, protected lagoons and channels, and the deeper parts of outer reef slopes. Juveniles are often solitary while adults occur in pairs. A planktivorous species that generally remains within a few meters of the reef. Juveniles may sometimes pick on parasites on the epidermis of other fish. Oviparous. Form pairs during breeding.

Specimen No. 24 - *Pomacanthus imperator* (Bloch, 1787) Emperor angelfish

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Pomacanthidae (Angelfishes)

Size / Weight / Age

Max length: 40.0 cm SL male/unsexed; (Ref. 30573); max. reported age: 14 years (Ref. 72479)

Environment

Marine; reef-associated; non-migratory; depth range 1 - 100 m (Ref. 48391)

Climate / Range

Tropical; 31°N - 28°S, 32°E - 144°W

Distribution

Indo-Pacific: Red Sea and East Africa to the Hawaiian, Line and Tuamoto islands, north to southern Japan and the Ogasawara Islands, south to the Great Barrier Reef, New Caledonia, and the Austral Islands. Not found in Easter Island, Rapa and the Marquesan Islands.

Short description

Dorsal spines (total): 13 - 14; Dorsal soft rays (total): 17-21; Anal spines: 3; Anal soft rays:
18 - 21. Juveniles are bluish black with concentric white circles. Adults blue with close-set horizontal yellow stripes on the sides and the adjoining dorsal and anal fins. Snout white; eye enclosed in a black vertical bar with blue edge; pectoral fin base area also black. Transformation to the adult color pattern occursover the size range 8 - 12 cm.

Biology

Juveniles are encountered under ledges, or in holes of outer lagoon patch reefs or semi-protected areas of exposed channels and outer reef flats. Sub adults move to reef front holes

and surge channels. Large adults inhabit ledges and caves in areas of rich coral growth on clear lagoon, channel, or seaward reefs. Benthopelagic. Feed on sponges and other encrusting organisms; also on tunicates. Form pairs. Young and adults may clean much larger fishes such assunfish. Frequently exported through the aquarium trade. Juveniles are distinguished by a whitedorsal-fin margin.

Specimen No. 25 - *Pterois antennata* (Bloch, 1787) Broadbarred firefish

Class - Actinopterygii (Ray-finned fishes)
Order - Scorpaeniformes (Scorpionfishes and flatheads)
Family - Scorpaenidae (Scorpionfishes or rockfishes)

Size / Weight / Age

Max length: 20.0 cm TL male/unsexed;

Environment

Marine; reef-associated; depth range 2 - 50 m

Climate / Range

Tropical; 42°N - 40°S, 30°E - 137°W

Distribution

Indo-Pacific: East Africa to Marquesan and Mangaréva islands, north to southern Japan, south to Queensland, Australia and Kermadec (Ref. 8879) and Austral islands.

Short description

Dorsal spines (total): 13; Dorsal soft rays (total): 11-12; Anal spines: 3; Anal soft rays: 6. Reddish to tan with many dark bars on body; median fins with scattered dark spots; tentacle above eye long and with dark bands. Adults with bluish black blotches near the base of the pectoral fins.

Biology

Occurs in lagoon and seaward reefs. Hides in crevices under rocks and coral formations during the day and hunts at night. Typically with head towards the safety of their hide-out or narrow passage. Feeds on shrimps and crabs. Venomous and capable of inflicting a painful sting. Solitaryor in groups, under ledges and holes.

Specimen No. 26 - *Synchiropus splendidus* (Herre, 1927) Mandarinfish

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Callionymidae (Dragonets)

Size / Weight / Age

Max length: 6.0 cm TL male/unsexed; (Ref. 2334)

Environment

Marine; reef-associated; depth range 1 - 18 m (Ref. 9710), usually 1 - 18 m (Ref. 27115)

Climate / Range

Tropical; 24°C - 26°C (Ref. 27115)

Distribution

Western Pacific: Ryukyu Islands to Australia.

Short description

Dorsal spines (total): 4; Dorsal soft rays (total): 8; Anal spines: 0; Anal soft rays: 6 - 8. Rare individuals have a bright red ground color. Preopercular spine.

Biology

Inhabits shallow protected lagoons and inshore reefs. Found on silty bottoms with coral and rubble. Usually in small groups spread over small area. Has been reared in captivity. Well-known aquarium fish.

Specimen No. 27 - *Arothron nigropunctatus* (Bloch & Schneider, 1801) Blackspotted puffer

Class - Actinopterygii (Ray-finned fishes)
Order - Tetraodontiformes (Puffers and filefishes)Family - Tetraodontidae (Puffers)

Size / Weight / Age

Max length: 33.0 cm TL male/unsexed; (Ref. 9710)

Environment

Marine; reef-associated; depth range 3 - 25 m (Ref. 30874)

Climate / Range

Tropical; 30°N -

32°S**Distribution**

Indo-Pacific: East Africa to Micronesia and Samoa, north to southern Japan, south to New South Wales. Replaced by Arothron diadematus in the Red Sea.

Short description

Dorsal spines (total): 0; Dorsal soft rays (total):
10-11; Anal spines: 0; Anal soft rays: 10 - 12. Body covered with prickles and with large black spots.

Biology

Inhabit coastal to outer reef crest and slopes with rich invertebrate growth. Adults often inpairs. Feed on corals (usually Acropora tips), crustaceans, mollusks, sponges, tunicates and algae.

Specimen No. 28 - *Pterapogon kauderni* (Koumans, 1933) Banggai cardinal fish

Actinopterygii (Ray-finned fishes)Perciformes (Perch-likes)
Apogonidae (Cardinalfishes)

Size / Weight / Age

Max length: 8.0 cm TL male/unsexed; (Ref. 35396)

Environment

Marine; demersal, usually - 2 m

Climate /

RangeTropical

Distribution

Western Central Pacific: Apparently restricted to Banggai Islands, Indonesia. Threatened by extinction due to collection for the aquarium trade (Ref. 27438, 35396). Proposal to be listed in CITES Appendix II was submitted (Ref. 87100).

Short description

Dorsal spines (total): 8; Dorsal soft rays (total): 14; Anal spines: 2; Anal soft rays: 13. Easily recognized by its tasseled first dorsal fin, elongate anal and second dorsal fin rays, deeply forkedcaudal fin and striking color pattern.

Biology

Common around the jetty, in silty sand bottoms with seagrass (*Enhalus acoroides*). Associated with *Diadema setosum*, the long-spined sea urchins. Individuals of 2 to 60 hover directly above the urchins, with the younger ones about 2-3 cm SL staying closer to the urchins. The fishretreat among the spines when threatened. Probably feed on small benthic and planktonic crustaceans at night. Male incubates the eggs until hatching; the large egg about 2.5 mm in diameter, with the young remaining within the mouth cavity for undetermined period after hatching (Ref. 9936, 48635). Newly hatched larvae have no planktonic period. Juveniles also useanemones for protection. Has been reared in captivity.

Specimen No. 29 - *Nemateleotris decora* (Randall & Allen, 1973) Elegant fire fish

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Ptereleotridae (Dartfishes)

Size / Weight / Age

Max length: 9.0 cm TL male/unsexed; (Ref. 9710)

Environment

Marine; reef-associated; depth range 25 - 70 m (Ref. 1602), usually 25 - 70 m (Ref. 27115)

Climate / Range

Tropical; 23°C - 27°C; 30°N - 23°S

Distribution

Indo-Pacific: Mauritius to Samoa, north to Ryukyu Islands, south to New Caledonia.

Short description

Dorsal spines (total): 7; Dorsal soft rays (total): 27-32; Anal spines: 1;
Anal soft rays: 28 - 31

Biology

Found over hard, open bottoms at the bases of reefs and over sand and rubble patches; also observed in deep coastal to outer reef drops-offs subject to strong currents. Often in pairs. Monogamous. Feeds on zooplankton, especially copepods and crustacean larvae. Darts into a hole when alarmed.

Specimen No. 30 - *Amphiprion clarkia* Yellowtail clownfish

Class - Actinopterygii (Ray-finned fishes)Order - Perciformes (Perch-likes)
Family - Pomacentridae (Damselfishes)

Size / Weight / Age

Max length: 15.0 cm SL male/unsexed; (Ref. 6113); max. reported age: 11 years (Ref. 11318)

Environment

Marine; reef-associated; non-migratory; depth range 1 - 60 m (Ref. 58652)

Climate / Range

Tropical; 30°N - 30°S, 47°E - 172°W

Distribution

Indo-West Pacific: Persian Gulf to Western Australia, throughout the Indo-Australian Archipelago and in the western Pacific at the islands of Melanesia and Micronesia, north to Taiwan, southern Japan and the Ryukyu Islands.

Short description

Dorsal spines (total): 10; Dorsal soft rays (total): 15-16; Anal spines: 2; Anal soft rays: 13 - 14. Highly variable in color and several geographical and localized forms. Two white bands, one behind the eye and one above the anus. Caudal fin white, sometimes yellowish, but always lighter than rest of the body.

Biology

Adults inhabit lagoons and outer reef slopes. Omnivorous. Oviparous, with elliptical eggs. Monogamous. Oviparous, distinct pairing during breeding. Eggs are demersal and adhere to the substrate. Males guard and aerate the eggs.

Specimen No. 31 - *Danio rerio* (F. Hamilton, 1822) Zebra Fish

Class - Actinopterygii
Order - Cypriniformes
Family – Cyprinidae

Size / Weight / Age

Max length: 3.8 cm SL male/unsexed; (Ref. 41236)

Environment

Freshwater; benthopelagic; pH range: 6.0 - 8.0; dH range: 5 - 19

Climate / Range

Tropical; 18°C - 24°C; 33°N - 8°N, 66°E - 98°E

Distribution

Asia: Pakistan, India, Bangladesh, Nepal and Myanmar. Reported from Bhutan. Appearance in Colombian waters presumably by escape from an aquarium fish rearing facility.

Short description

Vertebrae: 31 - 32. Five uniformly, pigmented, horizontal stripes on the side of the body, all extending onto the end of caudal fin rays. Anal fin distinctively striped. Lateral line absent. Rostral barbels extend to anterior margin of orbit; maxillary barbels end at about middle of opercle. Branched anal fin rays 10-12. Vertebrae 31-32.

Biology

Adults inhabit streams, canals, ditches, ponds and beels. Occur in slow-moving to stagnant standing water bodies, particularly rice-fields; and lower reaches of streams. Common in rivulets at foot hills. Feed on worms and small crustaceans; also on insect larvae. Breed all year round. Appears to be primarily an annual species in the wild, the spawning season starting just before the onset of the monsoon. Domesticated zebrafish live on average 3.5 years, with oldest individuals surviving up to 5.5 years. Spawning is induced by temperature and commences at the onset of the monsoon season. Food availability also acts as cue for breeding. Growth rate is a vital guiding environmental factor for sexual

differentiation for this species as observed in a study. In this same study, frequency and amount of food prior to and throughout gonadal differentiation period resulted in more individuals differentiating to become females and is more pronounced in hybrid than pure bred groups. Often used for mosquito control. Popular for aquarium purposes. Used as a model system (=organism) for developmental biology. Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size 60 cm.

Specimen No. 32 - *Balantiocheilos melanopterus* (Bleeker, 1851) Tricolor shark minnow

Class - Actinopterygii (Ray-finned fishes)Order - Cypriniformes (Carps)
Family - Cyprinidae (Minnows or carps)

Size / Weight / Age

Max length: 35.0 cm SL male/unsexed;

Environment

Freshwater; benthopelagic; pH range: 6.0 - 8.0; dH range: 5 - 12

Climate / Range

Tropical; 22°C - 28°C (Ref. 1672); 20°N - 6°S

Distribution

Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra and Borneo. Becoming rare orextinct in many river basins.

Short description

Silver body with black margins on dorsal, caudal, anal and pelvic; lower lip with a posterior groove forming a pocket opening backwards.

Biology

Found in mid-water depths in large and medium-sized rivers and lakes. Feeds on phytoplankton, but mostly on small crustaceans, rotifers as well as insects and their larvae. Aquarium keeping: in groups of 5 or more individuals; minimum aquarium size >150 cm.

Specimen No: 01

Taxonomic Classification:

Kingdom: Plantae
Phylum: Angiosperms
Class: Monocots
Order: Alismatales
Family: Alismataceae
Genus: *Echinodorus*

Identifying Character:

- An aquatic plant with submergent leaves 40 – 60 cm long attached by petioles flatly triangular.
- Leaf blades are lanceolate or narrowly oval.

Comments:

Echinodorus grisebachii is commonly known as Amazon sword plant, although other plants are also known under this common name. The aquatic plant is cultivated for and used in ponds and artificial aquatic habitats. It is native to Cuba, Central America, and South America as far south as Brazil and Bolivia.

Specimen No: 02

Taxonomic Classification:

Kingdom: Plantae
Phylum: Angiosperms
Order: Nymphaeales
Family: Cabombaceae
Genus: *Cabomba*

Identifying Character:

- Fanwort is a submersed, sometimes floating, but often rooted, freshwater perennial plant with short, fragile rhizomes.
- The erect shoots are upturned extensions of the horizontal rhizomes.
- The shoots are grass green to olive green or sometimes reddish brown. The leaves are of two types: submersed and floating.
- The submersed leaves are finely divided and arranged in pairs on the stem. The floating leaves, when present, are linear and inconspicuous, with an alternate arrangement.
- They are less than 1/2 inch (13 mm) long and narrow (less than 1/4 inch or 6 mm). The leaf blade attaches to the centre, where there is a slight constriction.
- The flowers are white and small (less than 1/2 inch (13 mm) in diameter), and are on stalks which arise from the tips of the stems.

Comments:

Cabomba caroliniana, commonly known as Green Cabomba, is an aquatic perennial herbaceous plant native to North America. Cabomba species to grow in the aquarium but needs a strong bright light. Plant in groups. Can grow fast in good conditions. If the water circulation is too strong or with some fish (which like to nibble at it), because of its brittle stems, filters may become clogged.

Specimen No: 03

Taxonomic Classification:

Kingdom: Plantae

Phylum: Angiosperms

Class: Monocots

Order: Alismatales

Family: Hydrocharitaceae

Genus: *Vallisneria*

Identifying Character:

- Vallisneria is a submersed plant that spreads by runners and sometimes forms tall underwater meadows.
- Leaves arise in clusters from their roots. The leaves have rounded tips, and definite raised veins.
- Single white female flowers grow to the water surface on very long stalks. Male flowers grow on short stalks, become detached, and float to the surface.
- The fruit is a banana-like capsule having many tiny seeds. Sometimes it is confused with the superficially similar Sagittaria when grown submerged.

Comments:

Various strains of Vallisneria are commonly kept in tropical and subtropical aquaria. They oxiginate the aquarium and give food to the fish.

AQUASCAPING

A. Introduction:

Aquascaping is the craft of arranging aquatic plants, as well as rocks, stones, cave work, or driftwood, in an aesthetically pleasing manner within an aquarium in effect, gardening under water. Aquascape designs include a number of distinct styles, including the garden-like Dutch style and the Japanese-inspired nature style. Typically, an Aquascape houses fish as well asplants, although it is possible to create an aquascape with plants only, or with rockwork or other hardscape (rocks and wood) and no plants.

Although the primary aim of aquascaping is to create an artful underwater landscape, the technical aspects of aquatic plant maintenance must also be taken into consideration. Many factors must be balanced in the closed system of an aquarium tank to ensure the success of an aquascape such as filtration, fertilization, lighting, and algae control etc.

B. Principles

1. Plant all groups in odd numbers.
2. Fine leaved plants look best in the mid to back center of a tank, with heavier leavedplants toward the edges.
3. Don't use red in the middle as they have a heavy, dark, feel.
4. Dark leaves (red or dark green) look best toward back edges, with light colored leavestoward the center.
5. Arrange plants and hardscape to provide good contrast of light and dark areas
6. Light colored sand provides good contrast to plants.
7. When rocks are used, use multiple sizes, mixing large and small rocks, as in nature.
8. Rock edges should generally be rounded.
9. Aquascapes with unplanted sand in front is a good alternative to the traditional"Nature Aquarium" style of all foreground covered with foreground plants.
10. An attractive layout alternative is a slope up from near the middle up to the two backcorners.

C. Design and styles

1. Dutch Style:

The Dutch style aquascaping utilises multiple plants with different leaf colours,

sizes, and textures. This style was developed in 1930s in Netherlands, as freshwater aquarium equipment became commercially available. More than 80% of the aquarium floor is coveredwith plants, and little or no substrate is left visible.

2.Japanese Style:

A contrasting approach is the nature aquarium or Japanese style, introduced in 1990s by Takashi Amano. This style draws particularly from the Japanese aesthetic concepts of Wabi-sabi, which focuses on transience and minimalism as sources of beauty. Plants with small leaves like, small aquatic ferns, and java moss are often used to emulate grass or moss. Colours are more limited than in the Dutch style, and the hardscape is not completely covered. Fish or freshwater shrimp are usually selected to complement the plants and controlalgae.

3.Iwagumi style:

The Iwagumi term in Japanese means "rock formation" and refers to a layout where stones play a leading role. Aquarium with gray stones arranged to form a tall pointed structure at the right, and a similar but smaller structure at the left.

The stone peaks and the foreground are largely but not entirely covered by a short layer of fine textured green plants.

4.Jungle Style:

Some hobbyists also refer to a jungle (or wild jungle) style, separate from either the Dutch or nature styles, or incorporating some of the features of them both. The plants are left to assume a natural, untrimmed look. Unlike nature style, the jungle style does not follow clean lines, or employ fine textures. A jungle canopy effect can be obtained using combinations of darker substrates, tall plants growing up to the surface, and floating plants that block light, offering a dappled lighting effect.

5.Biotopes Style:

The styles above often combine plant and animal species based on the desired visual impact, without regard to geographic origin. Biotope aquascapes are designed instead to replicate exactly a particular aquatic habitat at a particular geographic location, and not necessarily to provide a gardenlike display. Plants

and fish need not be present at all, but if they are, they must match what would be found in nature in the habitat being represented, as must any gravel and hardscape, and even the chemical composition of the water. By including only those organisms that naturally exist together, biotopes can be used to study

ecological interactions in a relatively natural setting.

6.Paludarium Style:

Paludarium is an aquarium that combines water and land inside the same environment. Thesedesigns can represent habitats including tropical rainforests, jungles, riverbanks, bogs, or even the beach. In a paludarium, part of the aquarium is underwater, and part is above water. Substrate is built up so that some land regions are raised above the waterline, and the tank is only partially filled with water. Unlike other aquarium setups, paludariums are particularly well-suited to keep amphibians.

IV) Techniques of Aquascape:

- In addition to design, freshwater aquascaping also requires specific methods to maintain healthy plants underwater. Plants are often trimmed to obtain the desired shape, and they can be positioned by tying

them in place inconspicuously with thread.

- Use aquarium-safe fertilizers, commonly in liquid or tablet form, to help the plants fill out more rapidly.
- It is also necessary to support photosynthesis, by providing light and carbon
- dioxide. Use of animals that consume algae, such as some fish, shrimp, or snails, to clean thealgae that collect on the leaves and tank.

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VALUE ADDITION IN ORNAMENTAL FISH CULTURE

Aquariculture, the culture of living jewels in the confined auatic system has emerged as the second largest hobby and one of the most favourite commercial business in the world. The ever increasing demand for the ornamental fishes has led to the global trade of them where India is trying to make a significant contribution through value addition and popularization ofindigeneous varieties. The aquarium fishes get high value because of its coloration routine, graceful behaviour, peculiar body morphology, and endemicity. But one of the greatest challenge faced by the industry is to replicate the natural colour in the captive environment. So in this regard, the colour enhancement of fish by using eco-friendly feed as well as fabrication of ornamental fish tanks, cultivation of ornamental plants, toys and other decorative for tanks are equally important as the development of breeding techniques to make this in the industry a success.

1. Value addition by color Enhancement

The aesthetic value decides the demand of the fish and therefore the market value of them. Skin coloration is an important factor in this regard. Colour enhancement in fish helps to increase the quality, cost and thus the market value of ornamental fishes. This is possible byadministration of pigment enriched eco-friendly feed. The constant intake and adequate level of carotenoids in the feed are essential to optimize the coloration as the biosynthesis of carotenoids is not happens in the fish. Carotenoid pigments give red, orange and yellow coloration. Both synthetic and natural carotenoids can be used for this purpose. Much cheaper sources of carotenoids are plant-based sources which include Alfa-alfa (*Medicago sativa*), Carrot (*Daucus carota*), Marigold flower (*Tagetes erecta*), China rose (*Hibiscus rosa sinensis*), etc. In animal-based sources, astaxanthin is the predominant carotenoid rich in crustacean discards. Dried shrimp meal, red crab meal, krill meal are commercially available as fish feed. However high ash content, chitin digestibility issue limit its rate of inclusion inthe feed. Some of the microalgal sources are also available commercially like *Hematococcus fluvialis*, *Dunaliella salina*, *Arthospira maxima,* etc. Examples of commercially available carotenoids are Lucanthin Pink (contain 10% astaxanthin) and Carophyll Red (contain 10% Anthaxanthin). Along with coloration, carotenoids also have functions like it act like a 1) Vitamin A precursor 2) Antioxidant 3) Growth enhancer, etc. Synthetic carotenoids have some disadvantages like residue

problem, deteriorating effect on the environment and they are costly. Further research is needed to make improved feed for better coloration.

Source: Role of feed additives in pigmentation of ornamental fishes. International Journal of Fisheries and Aquatic Studies 2017; 5(2): 684-686

2. Value addition by transgenesis

The possibility of easier genetic manipulation in fish has led to the success of devolvement of genetically modified organism by transgenesis. It helps to bring out new color variants of ornamental fish to increase the aesthetic value and demand by the market. Even though it has some disadvantages, further improvements can definitely bring novel color variants. Such value-added aquarium. This brand fish is patented and trademarked, available in themarket in bright red, green, orange-yellow, blue, and purple fluorescent colors. Recently other variants also
developed with six attractive fluorescent color combinations, including Starfire red, cosmic blue, electric green, galactic purple, sunburst orange, and moonrise pink. The other goals oftransenesis include the intensification of growth and food conservation, increase tolerance to environmental variables like temperature and salinity and development of disease resistant forms.

3. Value addition by Painting or Dyeing or tattooing

Painted fishes are artificially colored fishes to increase the appeal to the customers. The artificial coloring or juicing is possible by
injecting the fish with bight fluorescent color dye, dipping the fish into a dye solution, or by feeding the fish with food
containing desired dye. This is done to develop exotic colors in fish which is not possible byline breeding. The coloring is not permanent; usually, last for six to nine months. Blueberryor strawberry Oscar which is available in the market is an example of dyed fish. Tattooed

fishes with different patterns with different colors are also available in the market. Tattooingis done with a low-intensity laser with a dye. Administration of some of the hormone also showed to increase the coloration in fish.

BFSC-306: Genetics and Breeding

P-1: Monohybrid Cross

Problems

1. A dominant allele **G** produces grey guppies. Its recessive allele **g** produces gold guppies. A group of homozygous grey guppies and gold guppies were crossed and their F_1 progeny were then test crossed. Determine the expected genotypic and phenotypic ratios among the test cross progeny.

2. In the case of goldfish, the cross of normal eye X telescopic eye yields normal and telescopic eyed progeny in equal proportions, but telescopic eyed X telescopic eyed always gives rise to only the telescopic eyed progeny. What does this tell about the genotype of normal and telescopic eyes in goldfish ? Which phenotype is dominant?

3. The colour of the parent and offsprings of grass carp is given below. Explain the results for the crosses and mention the genotypes of all the individuals.

Sl. No.	**Parents**	**Progeny colouration**
1.	**Wild x Wild**	**Wild**
2.	**Wild x Albino**	**Wild**
3.	**Albino x Wild**	**Wild**
4.	**Albino x Albino**	**Albino**
5.	**Albino x Wild**	**530 Wild, 480 Albino**

4. Normal eyes of gold fish is a dominant trait. Telescopic eyes is the alternative recessive trait. When a pure normal eye gold fish is crossed to a telescopic one,what fraction of F_2 is expected to be heterozygous ?

5. In carp, blue colour is recessive to non blue colour. What coloured carp will you choose to breed a given non blue colour fish in order to find its genotype ? Write the type of cross ?

6. Koi carps with known phenotype but with unknown genotype produced the listed progeny.

Sl. No.	**Parents**	**Offsprings**	
		Pattern	**No pattern**
1.	**Pattern x No pattern**	820	780
2.	Pattern x Pattern	1180	390
3.	No pattern x No pattern	0	1500
4.	Pattern x No pattern	3500	0
5.	Pattern x Pattern	2800	0

Using **D** for the light yellow pattern gene and **d** for no pattern give the most probable genotype of each parent.

7. In carps blue colour is recessive to non-blue colour. A non blue colour carp male has blue colour female parent.
8. What is the genotype of the fish and female parent ?

What are the possible genotypes of male parent ?

If the fish is crossed with a blue coloured female, What are the possible genotypes of their offsprings ?

8. Two light yellow pattern Japanese carps on breeding produce 1678 light yellow pattern and 554 no pattern offsprings. Give the genotypes of the parents and justify your answers with reasons. (Given : Light yellow pattern is dominant to no pattern). If a pure light yellow pattern carp is crossed with a hybrid light yellow pattern, what will be the genotypic ratio of the offsprings ?
9. When a red Medaka was crossed with wild type, the offspring were all wild type (green), but when these offspring were crossed among themselves the result was approximately three greenish offspring to one red. Explain dominant and recessive colour in Medaka. What is the type of inheritance in Medaka?.
10. A dominant gene '**+**' is responsible for the normal pigmentation of body colour in catfish; its recessive allele '**a**' produces albino. A testcross of a normal type female gave 5200 normal and 5500 albino in the F_1. If the normal F_1 females are crossed to their albino F_1 brothers, What genotypic and phenotypic ratios would be expected in the F_2? Diagramme the results using the appropriate genetic symbols.

P-2: Incompletely Dominant Genes

Problems

1. In red tilapia, the allele for red body colour has an effect that is incompletely dominant over grey or black colour allele. If a cross between two tilapias produced 32 red, 68 pink and 28 black tilapias, what are the phenotypes of the parents ?

2. Calculate the ratios of body colour in red tilapia among the offsprings produced for the following crosses ?

a) red x red

b) red x pink

c) black x pink

d) pink x pink

3. When a transparent scaled (T') goldfish is crossed to a normal scaled goldfish, all the progeny are calico type (T T'). From a cross of two such F_1s repeatedly made, the F_2 showed 6000 transparent scaled, 15000 calico and 5000 normal scaled goldfishes. How this trait is inherited? Give the genotypes of the parents, F_1 and F_2 progeny.

4. Give the genotypic and phenotypic ratio for the offsprings produced for the following crosses in the siamese fighting fish.

a) steel blue x steel blue

b) steel blue x blue

c) steel blue x green

d) blue x blue

e) blue x green

f) green x green

5. Three alleles determine the ABO blood type in brown trout, *Salmo trutta fario*. Alleles $I^a I^a$ produces the A phenotype, $I^b I^b$ produces the B phenotype, and $I^o I^o$ produces O phenotype. The effects of I^a and I^b are dominant to O but are co dominant with each other. What are the genotypes of the following parents ?

Sl. No.	**Phenotypes of parents**	Phenotypes of offspring (proportions			
		A	**B**	**AB**	**O**

1.	**B x B**	-	¾	-	¼
2.	B x AB	-	½	½	-
3.	B x A	-	½	½	-
4.	B x A	¼	¼	¼	¼
5.	B x AB	¼	½	¼	-
6.	B x O	-	1	-	-

6. In Tilapia, gold individuals were shown to be homozygous for the recessive allele (GG), while normal (wild) coloured individuals were homozygous for the normal (WW) allele (black colour) expression. Hetrozygotes (WG) displayed a "bronze" skin colour. Give the genotypic and phenotypic ratios for the offsprings produced among the following cross.

a) GG x WW

b) WG x WG

c) WG x WW

d) WG x GG

P-3: Dihybrid Cross

Problems

1. In goldfish, orange red colour (B) is dominant over blue (b) and normal eyes (D) over telescopic eyes (d). If the alleles segregate independently, write the most probable genotype for the parents for each of the following cross.

Sl. No.	Phenotypes of parents	Phenotypes of the offspring			
		ORN	ORT	BN	BT
1.	**ORN x ORN**	890	310	290	110
2.	ORN x ORT	180	190	0	0
3.	ORT x BN	200	0	210	0
4.	BN x BN	0	0	280	90
5.	ORT x ORT	0	320	0	100
6.	ORN x ORT	460	160	0	0
7.	ORN x ORT	290	310	90	110

ORN– orange red normal,

BN– blue normal,

ORT– orange red telescopic,

BT–blue Telescopic

2. Normal spine in guppy is governed by a dominant gene (Sn) and curved spine by its recessive allele (Sc). Grey colour results from the action of the dominant genotype (G -) and gold from the recessive genotype (gg). Determine the expected genotypic and phenotypic ratios in the progeny for the following crosses.

a) SnSc Gg x SnSc Gg

b) SnSc GG x ScSc Gg

c) SnSc gg x SnSn gg

d) ScSc gg x ScSc gg

3. In the case of minnows Grey-green **(G**) colour is dominant to pink colour. Normal fin (N) is dominant to long fin (n). If a homozygous Gray-green with normal fin male minnow is crossed with a homozygous pink and long fin female What will be the genotypes and phenotypes of the F_1 and F_2 offsprings.

4. In the case of Nile tilapia, two loci **A** and **B** are involved in producing normal body colouration. The recessive alleles **a** and **b** in combination, and only in combination, would result in pearl colouration. Either recessive allele, when present alone, would not alter the normal colouration of the fish. Determine the expected genotypic and phenotypic ratios in the progeny from the following mating.

a) AA BB x aa bb

b) Aa Bb x Aa Bb

c) Aa BB x AA Bb

d) aa Bb x aa Bb

P-4: Lethal genes and Epistasis

Problems

1. The **S** gene in *Tilapia aurea* is an example of a dominant lethal gene. The gene **S+** produces saddleback and the gene **++** produces normal fin. Find out the genotypic and phenotypic ratios for the following crosses.

a) Saddleback x Saddleback

b) Saddleback x Normal

c) Normal x Normal

2. In common carp, a dominant gene (**L**) is lethal in homozygous condition. Produces light coloured pigmentation in heterozygous condition (**L**l) and normal pigmentation in recessive condition **(ll**). What will be the phenotype for the F_1 produced by a crossing of light coloured and normal pigmented fish ?

3. The genotypes and phenotypes of different varieties of common carp are mentioned below :

SSnn, Ssnn - scaled or wild carp

ssnn - "scattered" mirror

SSNn, SsNn - linear mirror

ssNn - "nude" or "leather"

SSNN, SsNN and ssNN are non viable (lethal genes)

Calculate the phenotypic and genotypic ratio of the offsprings produced from the following crosses of common carp. Also mention the phenotype of the parents.

a) ssNn x ssnn

b) ssNn x ssNn

c) Snn x ssNn

d) SsNn x ssNn

e) SsNn x SsNn

f) ssnn x ssnn

4.In gold fish, the gene **M** is epistatic with respect to the gene **S**. Genes **m** and **s** produce albino. The following combinations of genes are possible.

MS and **Ms**-dark fishes,

mS-light fishes,

ms-albino fishes.

By using the punnet square find out the phenotypic and genotypic ratio for the mating of the gold fish with following genotypes.

a) Two heterozygous MmSs dark gold fish

b) Mm Ss x mm ss

c) MM Ss x mm ss

d) MM Ss x Mm Ss

e) Mm Ss x MM ss

P-5: Sex determination and sex linked genes

Problems

1. The presence of three types of sex chromosomes allows different sex ratios to be obtained in various crosses of the platy. Find out the sex ratios for the following crosses.
a) XX × XY
b) WY × YY
c) WX × YY
d) WX × XY
e) WY × XY
f) XX × YY
2. Find out the sex ratio of F_1 hybrid progeny from a cross of a female *Oreochromis mossambicus* and a male *O. hornorum*?
3. In the case of medaka, *Oryzias latipes* the mechanism of sex determination is XY system. Find out the sex ratio among the progeny produced from the following crosses.
a) XX × XX (T)
b) XY (T) × XY
c) XY (T) × YY (from the second cross)
d) YY (T) × YY (from the second cross)
e) XX × YY (from the second cross)
4. Find out the genotypic and phenotypic ratio for the mating of a female grey and maculatus male guppy?
5. Find out the sex ratio for the progeny of the following crosses produced in guppy ?
a) caudalis ♀ × caudalis ♂
b) transparent tailed ♀ × caudalis ♂
c) caudalis ♀ × transparent tailed ♂
6. In the *Lebistes reticulates* there is a black spot on the dorsal fin. In crosses of black spot males with normal females half the progeny have the black spot and other half do not. Black spot occurs only in males. Moreover, black spot does not occur in males unless the father expressed the trait. Result a diagrammatic explanation of these results showing which is the homogametic sex. Show what would be expected if the gene controlling black spot were located on the alternative sex chromosome to that you have postulated.
7. In platy, a cross between a `Nigra' female (**N**) and a lightly pigmented male (**W**) produced 'Nigra' sons and 'white' daughters which, in an F_2, produced approximately equal frequencies of 'Nigra' and 'white' individuals of each sex. Interpret the results of the above cross?

P-6: Counting of fish sperm and checking the motility

Counting of fish sperm and checking the motility

Sperm count and motility are the most commonly used parameters to evaluate sperm quality, since sperm count must be more and motile to achieve fertilization. Unlike mammal, the teleost spermatozoa are found immotile in the testis as well as in seminal fluid. Healthy fish spermatozoa on activation shows a vigorous movement. The motility of such activated spermatozoa is evaluated by six point (++++++) scale after adding 100 times of water to a drop of milt.

Motility percentage Score Condition

0 --10 + All dead

11--30 ++ Slightly active

31--50 +++ Oscillating

51--70 ++++ Moving

71--90 +++++ Active

91--100 ++++++ Excellent

Procedure

1. Collect spermatozoa directly from the testis (for catfish and murrels). Otherwise strip the milt by pressing the abdomen gently (for carps).

2. Take 10 µl of sperm sample and add 490 µl of diluent (HBSS) in a microcentrifuge.

3. Draw 10 µl of the above diluted sample and add 90 µl of diluent (1:50 and 1: 10 dilution).

4. Transfer a drop of the milt content to a clean haemocytometer. Each square is divided into16 smaller cells (4x4). Each of these smaller cells has a volume of 1/4000 mm^3

5. Count the sperm heads in each small cells from the 5 squares marked “x”. It gives a total of 80 cells (4x4x5). This is the number of sperm per 1/4000 mm^3

6. Calculate the concentration of sperm as given below:

Con. (sperm cells /ml) = Average mean x 4000 x 1000 x dilution factor

7. Check the motility under a microscope after adding a drop of tap water

P-7: Cryopreservation of fish sperm

Cryopreservation of fish sperm

Short-term preservation or storage of gametes ranging from a few hours or a few weeks, usually at low temperatures, can also be used in hatcheries to overcome temporary shortage of gametes, asynchrony in artificial and natural spawning, transportation of gametes and selective breeding. First successful cryopreservation of fish sperm was reported in the 1950s. The success of the cryopreservation of sperm depends on the seasonality, varying sperm quality, collection techniques, diluents used, precise freezing and thawing regimes, storage conditions, and post-thaw fertilization techniques.

The development of successful protocols for long-term freezing will allow the storage of disease-free gametes of desirable strains and species for future use and also facilitate the comparison and evaluation of new strains of fish with original parental stock at minimum cost. In addition, frozen gene banks may be used in conjunction with live gene banks. A major advantage of this approach is that the effective population size can be dramatically increased at relatively low cost by storing spermatozoa from a large number of individual males. Unlike sperm of higher vertebrates fish spermatozoa remain quiescent and become activated the moment they come in contact with water. In most of the fishes spawning in freshwater, spermatozoa remain motile for 2-3 min, and in carps energetic movement of sperm is only for a short duration of 30-60 secs during which they are capable to fertilize the eggs.

Cryopreservation methodology includes proper collection of fish milt; addition of extenders to prevent depletion of sperm energy reserve and to maintain sperm in quiescent condition but alive; using of cryoprotectants reduce thermal shock;, and freezing and thawing techniques to minimize sperm damage.

Materials

Hank's Balanced Salt solution

Cryoprotectants - Methanol

- Glycerol

- DMSO (10%)

Straws, Cryocan and its accessories

Procedure

1. Collect the milt directly by pressing the abdomen of the matured male fish (in carps) or cut the testes and homogenize (in the case of catfish) in 10 ml HBSS (refrigerated at 4°C)
2. Dissolve the cryoprotectants to HBSS or 0.9%NaCl.
3. Dilute the sperm suspension at 1:4 in the various cryoprotectant solution.
4. Draw the suspension into 0.5 ml french straws.
5. Equilibrate for 15 min at 10°C prior to freezing.
6. Place the straw in a strainer tray suspended above liquid nitrogen in a circular insulated tank.

BFSC-307: Physiology of Finfish and Shell fish

P-1 : NISKIN WATER SAMPLER

Niskin bottles. Open Niskin bottles are attached to a cable and lowered to water depths where seawater samples are to be obtained for chemical analysis. A metal messenger “trips” each bottle on the cable individually, causing it to fill with water and close securely.

A rosette cluster. Water collecting bottles are arranged around a rigid, circular frame in a rosette pattern. Technicians are able to close the bottles individually as the array is lowered or raised through the water column.

Sampling Depths

Chemists must establish the exact sampling depth for each bottle. Otherwise, the analytical work, no matter how accurate, is of limited use in determining the exact chemical structure of the water column. A common technique is to measure the length of the cable between the ocean surface and the depth at which the bottle was triggered by the messenger. However, the cable rarely hangs straight down, because of the drift of the ship relative to the bottles on the cable. Depth corrections are applied by measuring the angle of the cable and by noting the difference between the temperature readings on the pressure-protected and unprotected thermometers mounted on the sampling bottles. (Temperature discrepancies are indicators of water pressure,which is a function of water depth). When near-bottom water samples are collected, it is customary to attach a pinger(a pulsing sound source) to the free end of the cable. Sound signals reflected off the sea floor and transmitted to the ship are used to determine the distance between the pinger and the bottom to within a meter or so.

Analytical Procedures

Analytical procedure reveal temperature and salinity of water. recorded in the reversing thermometers, which are fastened to water-sampling bottles, Better precision (up to 0.0001°C) is obtained by using temperature-sensitive materials, such as quartz crystals, which vibrate at frequencies that depend on temperature. These signals are transmitted electronically to the ship. This allows the temperature of the water to be monitored continuously as the instrument is lowered.

Because the composition of seawater is constant, chemists traditionally have determined water salinity by chemical titration-the process of standardizing silver nitrate against a normal seawater sample of known chemical composition.The electrical conductivity of seawater, which is proportional to the total concentration of dissolved ions, is now used routinely to determine salinity rapidly. The salinometer compares the electrical conductivity of an unknown sample with that of a known, standard sample of seawater, and converts the difference into a salinity value after correcting for temperature effects. An important instrument called the CTD (conductivity, temperature, depth) consists of a salinometer, an electronic thermometer, and a pressure sensor. As it is lowered through the water column, the CTD transmits electronic signals to the ship, where they are stored in a shipboard computer for analysis later.

P-2 GRABS/CORERS-PRACTICALS:

Various simple but durable devices are available for collecting sediment samples from the ocean floor, even from the deepest, most inaccessible parts of the sea. A long-established technique is scraping the ocean bottom with a dredge-a rigid metal frame to which is attached a sampling bag made of chain or tough netting. Dredges are suitable for obtaining large, bulk samples of either rock or sediment. As they are dragged, however, the bite the bottom indiscriminately and mix samples together in the sampling bag. Also fine sediment such as mud tends to be washed out of the sample. Because of these effects, oceanographers employ dredges almost exclusively to collect hard rock rather than soft sediment. Less disturbed samples mud and sand are collected by grab samplers-spring –loaded metal jaws that take a bite out of the bottom and close tightly around the sediment sample.

Dredges and grab samplers merely sample the surface layer of sediment. Deeper penetration of soft sediment is accomplished by gravity corer. This hollow metal tube, known as a core barrel, is pushed into the sediment by the force of gravity. The corer is lowered to the bottom, where the heavy weight at the top of the device drives the barrel into the sediment. A plastic liner that has been inserted into the core barrel allows oceanographers to extract the sediment core intact from the sampler and also serves as a temporary storage container. Gravity corers are capable of taking cores of between 1 and 2 meters long, depending on the properties of the sediment. Sediment cores longer than 20 meters are routinely obtained by piston corers. This type of corer has a piston that slides up the core barrel as it penetrates the bottom. The action of the piston extrudes water from the core barrel, allowing the sediment core to enter the liner with minimal disturbance and compaction. Once the core is on deck, the plastic liner with its sample of sediment is extruded from the core barrel and is taken to a laboratory for detailed examination. Geologists carefully study the layering and composition of sediment particles to determine the geological history of the earth.

At present, the best technique for sampling the ocean bottom is plat form drilling, which was first developed by petroleum engineers on land and is now adapted to the ocean, even the deep ocean. The procedure is very expensive, but the scientific results are priceless. Marine geologists not only recover cores of sediment more than 1 kilometer in length, but also they can drill into the hard rock of the crust beneath the sedimentary layers. The Glomar Challenger—the 122-meter-long vessel that completed an illustrious international career of drilling, even in the remotest regions of the oceans, collecting hundreds of kilometers of core sample—has been retired.

P-3 : COLLECTION OF SEDIMENT SAMPLES

Two types of sediment samplers (grabs and corers) are used for collecting sediment from coastal and off-shore waters. The following samplers are commonly used in oceanographic surveys.

1. Petersen grab

It is consisting of two hinged pincer like buckets which are sent down to the sediments in open condition. As the drawing line slackens, the release mechanism is activated. In retrieval, the two buckets come together and thus a semi-circular section of sediment is cut and entrapped. The drawing line is then pulled off and the grab which is now in a closed condition is made open in a tray or bucket. As in the case of Van Veen's grab, this grab is also not activated by a metallic messenger.

2. Ekamn-Birge grab

This is the first and commonest grab devised for use in muddy bottom by Ekman (1911) and Birge (1922). The two shovels which are kept open against very strong spring action by means of two chains are closed from above by means of a drop weight. (metallic messenger). Immediately after this operation, one can pull the grab out of the bottom and finally out of the water column. It is very heavy and is made of brass in order to avoid rusting in the water. The upper portion is box shaped and is closed by two movable covers which fall in under the pressure of the water when the grab is sent down. The basal surface of this grab is about 250cm2.

3. Van Veen's grab

It is also a very convenient and reliable grab devised by Van Veen (1936). The working principle of this grab is more or less similar to that of Ekman-Birge. However, it is held open by a small bar and is not operated open by a metallic messenger. During operation, the grab is sent down the bottom when the two shovels spread out so that the bar is released automatically. The draw rope is attached in such a way that with the pull from above, the two shovels of the grab aremade to close tightly.

P-4 : Reversing thermometers

In the earliest, temperature measurements at some depth below the surface where made by bringing a water sample up to the deck of a ship in an insulated bucket and measuring the sample temperature with a mercury thermometer. Although these measurements were not accurate, they gave the first evidence that below the top 1000m the ocean is cold even in the tropics. They also showed that highly accurate measurements are required to resolve the small temperature differences between different ocean regions at those depths.

The first instrument that (through the use of multiple sampling and averaging) achived the required accuracy of 0.001ºC was the reversing thermometer. It consists of a mercury filled glas pipe with a 360º coil. The pipe is restricted to capillary width in the coil, where it has a capillary appendix. The instrument is lowered to the desired depth. Mercury from a reservoir at the bottom rises in proportion to the outside temperature. When the desired depth is reached the thermometer is turned upside down (reversed), but the flow of mercury is now interrupted at the capillary appendix, and only the mercury that was above the break point is collected in the lower part of the glass pipe. This part carries a clibratd gradation that allows the temperature to be read when the thermometer is returned to the surface.

To eliminate the effect of pressure, which compresss the pipe and causes more mercury to rise above the break point during the lowering of instrument, the thermometer is enclosed in a pressure resistant glass housing. If such a " protected reversing thermometer" is used in conjunction with an "unprotected reversing thermometer" (a thermometer exposed to the effect of pressure), the difference between the two temperature reading can be used to

determine the pressure and thus the depth at which the reading were taken. The reversing thermometer is thus also an instrument to measure depth.

Reversing thermometers require a research vessel as platform and are used in conjunction with Nansen or Niskin bottles or on multi-sample devices.

P-5 : Nansen and Niskin bottles

The measurement of salinity and oxygen, nutrients and tracer concentrations requires the collection of water samples from various depths. This essential task is achieved through the use of "water bottles". The first water bottle was developed by Fritj of Nansen and is thus known as the Nansen bottle. It consists of a metal cylinder with two rotating closing mechanisms at both ends. The bottle is attached to a wire. When the bottle is lowered to desired depth it is open at both ends, so the water flows through it freely. At the depth where the water sample is to be taken the upper end of the bottle disconnects from the wire and the bottle is turned upside down. This closes the end valves and traps the sample, which can then be brought to the surface.

In an "ocean graphic cast" several bottles are attached at intervals on a thin wire and lowered into the sea. When the bottles have reached the desired depth, a metal weight ("messenger") is dropped down the wire to trigger the turning mechanism of the uppermost bottle. The same

mechanism releases a new messenger from the bottle; that messenger now travels down the wire to release the second bottle, and so on until the last bottle is reached.

The Nansen bottle has now widely been displace by the Niskin bottle. Based on Nansen's idea, it incorporates two major modifications. Its cylinder is made from plastic, which eliminates chemical reaction between the bottle and the sample that may inter face with the measurement of tracers. Its closing mechanism no longer requires a turning over of the bottle; the top and bottom valves are held open by strings and closed by an elastic band. Because the Niskin bottle is fixed on the wire at two points instead of one(as is the case with the Nansen bottle) it makes it easier to increase its sample volume. Niskin bottles of different sizes are used for sample collection for various tracers.

Nansen and Niskin bottles are used on conjunction with reversing thermometers. On the Nansen bottle the thermometers are mounted in a fixed frame, the reversal being achieved by the turning over of the bottle. On Niskin bottles thermometers are mounted on a rotating frame.

P-6 Conductinity, Temperature and Depth System(CTDs)

Today's standard instrument for measuring temperature , salinity and often also oxygen content is the CTD, which stands for conductivity, temperature, depth. It employs the principle of electrical measurement. A platinum thermometer changes its electrical resistance with temperature. If its incorporated in an electrical oscillator, a change in its resistance produces a change of the oscillator frequency, which can be measured. The conductivity of seawater can be measured in a similar way as a frequency change of a second oscillator, and a pressure change produces a frequency change in a third oscillator. The combined signal is sent up through the single conductor cable on which the CTD is lowered. This produced a continuous reading of temepratrue and conductivity as functions of depth at a rate of up to 30 samples per second.

Electrical circuits allow measurements in quick succession but suffer from "instrumental drift", which means that their calibration changes with time. CTD systems therefore have to be calibrated by comparing their readings regularly against more stable instruments. They are therefore always used in conjunction with reversing thermometers and a multi-sample device.

Bathythermograph

Current meters

Ocean currents can be measured in two ways. An instrument can record the speed and direction of the current, or it can record the east-west and north-south components of the current. Both methods require directional information. All currents meters therefore incorporate a magnetic compass to determine the orientation of the instrument with respect to magnetic north. Four classes of current meters can be distinguished, based on the method used for measuring current magnitude.

Mechanical current meters use a propeller-type device,a Savonius rotor or a paddle-wheel rotor to measure the current speed, and a vane to determine current direction. Propeller sensors often measures speed correctly only if they point into the current and have to be oriented to face the current all the time. Such instruments are therefore fitted with a large vane, which turns the entire instrument and with it the propeller into the current.

Propellers can be designed to have cosine response with the angle of incidence of the flow. Two such propellers arranged at 90º will resolve current vectors and do not require an orienting vane.

The advantage of the Savonius rotor is that its rotation rate is independent of the direction of exposure to the current. A Savonius rotor current meter therefore does not have to face the current itn any particular way, and its vane can rotate independently and be quite small, just large enough to follow the current direction reliably.

With the exception of the current meter that uses two propellers with cosine response set at 90º to each other, mechanical current meters measure current speed by counting propeller or rotor revolutions per unit time and current direction by determining the vane orientation at fixed intervals. In other words, these current meters combine a time integral or mean speed over a set time interval (the number of revolutions between recordings) with an instantaneous reading of current direction (the vane orientation at the time of recording). This gives only a reliable recording of the ocean current if the current changes slowly in time. Such mechanical current meters are therefore not suitable for current measurement in the oceanic surface layer where most of the oceanic movement is due to waves.

The Savonius rotor is particularly problematic in this regard. Suppose that the current meter is in a situation where the only water movement is from waves. The current then alternates back and forth, but the mean current is zero. A Savonius rotor will pick up the wave current irrespective of its direction, and the rotation count will give the impression of a strong mean current. The paddle-wheel rotor is designed to rectify this; the paddle wheel rotates back and forth with the wave current, so that its count represents the true mean current.

Mechanical current meters are robust, reliable and comparatively low in cost. They are therefore widely used where conditions are suitable, for example at depths out of reach of surface waves.

Electromagnetic current meters exploit the fact that an electrical conductor moving through a magnetic field induces an electrical current. Sea water is a very good conductor, and if it is moved between two electrodes the induced electrical current is proportional to the ocean current velocity between the electrodes. An electromagnetic current meter has a coil to produce a magnetic field and two sets of electrodes, set at right angle to each other, and determines the rate at which the water passes between both sets. By combining the two components the instrument determines speed and direction of the ocean current.

Acoustic current meters are based on the principle that sound is a compression wave that travels with the medium. Assume an arrangement with a sound transmitter, and let receiver B

located downstream. If a burst of sound is generated at the transmitter it will arrive at receiver B earlier than at receiver A, having been carried by the ocean current.

A typical acoustic current meter will have two orthogonal sound paths of approximately 100mm length with a receiver/transmitter at each end. A high frequency sond pulse is transmitted simultaneously from each transducer and the difference in arrival time for the sound travelling in opposite directions gives the water velocity along the path.

Electromagnetic and acoustic current meters have no moving parts and can therefore take measurements at a very high sampling rate (up to tens of readings per second). This makes them useful not only for the measurement of ocean currents but also for wave current and turbulence measurements.

Acoustic Doppler current profilers (ADCPs) operate on the same principle as acoustic current meters but have transmitter and receiver in one unit and use reflections of the sound wave from drifting particles for the measurement. Seawater always contains a multitude of small suspended particles and other solid matter that may not all be visible to the naked eye but reflects sound. If sound is transmitted in four inclined beams at right angle to each other, the Doppler frequency shift of the reflected sound gives the reflecting particle velocity along the beam. With at least 3 beams inclined to the vertical the three components of flow velocity can be determined. Different arrival times indicate sound reflected at different distances from the transducers, so an ADCP provides information on current speed and direction not just at one point in the ocean but for a certain depth range; in other words, an ADCP produces a current profile over depth.

Different ADCP designs serve different purposes. Deep ocean ADCPs have a vertical resolution of typically eight meters (they produce one current measurement for every eight meters of depth increase) and a typical range of up to 400m. ADCPs designed for measurements in shallow water have a resolution of typically 0.5m and a range of up to 30m. ADCPs can be placed in moorings, installed in ships for underway measurements, or lowered with a CTD and multi-sample device to give a current profile over a large depth range.

P-8 Remote sensors:

Sea level can also be measured from satellites. An altimeter measures the distance between the satellite and the sea surface. If the satellite position is accurately known this results in a sea level measurement. Modern altimeters have reached an accuracy of better than 5cm. The global coverage provided by satellites allows the verification of global tide models. When the

tides are substracted, the measurements give information about the shape of the sea surfaces and, through application of the principle of geostrophy, the large scale oceanic circulation.

P-9 : Determination of Phosphate:

Introduction

Phosphate-P occurs in natural waters and in waste waters almost solely as phosphate. These are classified as orthophosphate, condensed phosphate and organically bound phosphate. It is largely a measure of orthophosphate and it occurs in both dissolved and suspended form.

Principle

Ascorbic acid method

Ammonium molybdate and potassium antimonyl tartarate reacts in acid medium with orthophosphate to form a heteropoly phosphomolybdic acid that is reduced to intensely colored molybdenum blue by ascorbic acid and the extinction is measured at 885 nm.

Reagents

Ammonium molybdate: Dissolve 15g of ammonium molybdate in 500ml of distilled water.

Sulphuric acid: Add 140 ml of conc. H_2SO_4 to 900 ml distilled water.

Ascorbic acid solution: Dissolve 27g of ascorbic acid in 500 ml distilled water.

Potassium - Antimonyl - Tartarate (PAT): Dissolve 0.34 of PAT in 250ml of distilled water.

Mixed reagent: To prepare 500 ml of mixed reagent, mix together 100 ml ammonium molybdate, 250ml H_2SO_4, 100ml ascorbic acid and 50 ml PAT. Prepare this reagent for use and discard any excess.

Procedure:

Take 100ml of sample in conical flask.

Add 10+0.5ml of mixed reagent.

After 5 min and preferably within 2-3 hrs, measure the extinction of the solution at 885 nm.

Calculation:

Mg at – P/lt =Extinction x F

Where, F=Factor value

P-10: Determination of silicates in sea water

Principle

The water sample is allowed to react with molybdate under conditions which result in the formation of the conditions which result in the formation of the silicomolybdate, phosphomolybdate and arcenomolybdate complexes. A reducing solution, containing metal and oxalic acid is then added which reduces the silicomolybdate complex to give a blue reduction compound and simultaneously decomposes any phosphomolybdate or arcenomolybdate, so that interference from phosphate and arcenate is eliminated. The extinction of the resulting solution is measured at 810 nm.

Reagents

Ammonium Molybdate solution: Dissolve 4.0 gm of analytical reagent quality ammonium para molybdate in about 300 ml distilled water, add 12 ml of concentrated HCl, mix and make up to 500 ml using distilled water.

Metal-sulphite solution: Dissolve 6 gm of anhydrous sodium sulphite in 500 ml distilled water and then add 10 gm of metal.

Oxalic acid solution: Dissolve 50gm of analytical reagent quality oxalic acid dihydrate in 500 ml distilled water.

Sulphuric acid solution 50%: 250 ml concentrated H_2SO_4 dilute to 250 ml of distilled water.

Reducing reagent: Mix 100 ml of metal sulphite solution with 60 ml of oxalic acid solution, add slowly 60 ml of 50% H_2SO_4 and make up to 300 ml with distilled water.

Procedure

Add 10 ml molybdate solution to 50 ml conical flask or measuring cylinder.

Pipette 25 ml of the see water in to the cylinder or conical flask and mix it.

Allow the mixture to stand for 10 min but not more than 30 min.

Add the reducing reagent rapidly so as to make the volume exactly 50 ml and mix it.

Allow the solution to react for 2-3 hrs to complete the reduction of the silicomolybdate complex.

Measure the extinction at 810 nm, alter correction with turbidity values.

Calculation

Concentration of Silicate silica= mg/l

Mg at Sig-Si/l = [E x F]

Where,

F= Factor value

E= Extinction values obtained

P-11: Determination of DO

1. Introduction

Dissolved oxygen content is one of the vital environemental characteristics of seawater. Its determination may be required either in the raw seawater sample or to assess primary production.

2. Principle

When the solutions of manganese and alkaline iodide are added to the seawater sample, the following reactions normally take place.

Manganese ion reacts with the hydroxide of the alkaline iodide to give Mn(OH)2.

Mn(OH)2 reacts with the dissolved oxygen present in the water sample to give Mn(OH)3.

In the presence of acid (above pH 1.5), Mn(OH)2 reacts with iodide to liberate iodine.

The iodine liberated is titrated against Na2S2O3 to convert the iodine to iodide.

The sequence of the above reactions are explained through the following formulae:

$Mn^{2+} + 2OH^- \rightarrow Mn(OH)_2$

$2\ Mn(OH)_2 + ½\ O_2 + H_2O \rightarrow 2Mn(OH)_3$

$2Mn(OH)_3 + 3\ I^- + 6H^+ \rightarrow 2Mn^{2+} + I_3^- + 6\ H_2O$

$2S_2O_3^{2-} + I_3^- \rightarrow S_4O_6^{2-} + 3I^-$

Range of detection: up to 10 ml O_2/1

3. Reagents

a. Manganese II chloride tetrahydrate

40 g of manganous chloride tetrahydrate is dissolved in 100ml distilled water. (or)

b. Manganese sulphate reagent

48 g of $MnSo_4.\ 4H_2O$ or 40 g of $MnSo_4.\ 2H_2O$ or 36.5 of $MnSo_4.H_2O$ is dissolved in water and the volume is made up to 100 ml with distilled water.

c. Alkaline iodide

60 g of Kl and 30g of KOH are dissolved separately in a minimum amount of water. Both these solutions are mixed together and this solution is then made up to 100ml with distilled water.

d. Sulphuric acid

50ml of conc. H2 So4 is carefully added to 50ml of distilled water (This mixture should be cooled during mixing).

e. Sodium thiosulphate

2.482g of Na2S2O3.5 H2O is dissolved and made up to 1000ml with distilled water. The normality of this solution is 0.01N.

f. Starch solution

1 g of soluble starch is dispersed in 100ml of distilled water. The solution is quickly heated to boiling. One drop of phenol or formaldehyde is added to every 50ml of starch solution to check microbial degradation.

4. Procedure

The seawater sample for which the dissolved oxygen content is to be determined is slowly siphoned out into a BOD bottle with the help of a rubber tube. When the water sample is allowed to overflow in this BOD bottle the following reagents are added. One ml of manganous solution is added first to the water sample keeping the tip of the pipette just below the surface of the water. In the same way, one ml of alkaline iodide is added and the bottle is carefully stoppered. The bottle is tilted upside down so that Mn(OH2) reacs with the dissolved oxygen present in the sample to give a slightly brown coloured Mn(OH3). Extra care should be taken not to permit the entry of any air bubble inside the BOD bottle. The estimation of dissolved oxygen may be done immediately or in a day or two. If the analysis is not carried out immediately, the mouth of the BOD bottle should be thoroughly sealed with wax and the bottle is kept in a water bath (at room temperature) so as to avoid the impact of increasing temperatures.

At the time of titration, one ml of sulphuric acid is slowly added to the sample and the bottle is stoppered. The bottle is tilted side wise, so that, all the precipitates are dissolved and the solution turns brownish due to the liberarian of iodine.

The thiosulphate solution of the given normality (0.01N) is then taken in the burette and is kept ready for titration. 50ml of sample from the reagent fixed BOD bottle is taken in a conical flask and is titrated against the thiosulphate till a hay yellow colour appears. At this point, one ml of starch is added to this solution and it turns blush indicating the presence of iodine. The titration is continued till the blue colour disappeard. The titration is repeated to get concordant values. The oxygen content of the water samples is calculated using the following formula:

O2 ml/1 = CF x N x E x 0.698 x 1000 x V t

Vs

Where

CF=correction factor value

correction factor value = Total volume of the stoppered bottle

Total volume of stoppered bottle-2

N= Normality of thiosulphate (0.01N)

E=Equivalent weight of O2 (8)

0.698 = To convert mg/l into ml/l.

1000= To convert the value to 1000ml or 1 litre

V t =Titre Value

Vs = Volume of sample taken for titration

If the oxygen content is to be calculated in mg/l, then the conversion factor value (0.698) of the above formula should be avoided. In the case of brackishwater and sea water samples, it is customary to express the value of oxygen content in ml/l and in freshwater samples, mg/l or ppm.

The above formula is derived by treating the dissolved oxygen and thiosulphate as reactants. The reaction and the calculation are as given below.

V1 N1 (Dissolved oxygen) = V2N2 (Thiosulphate)

Since V1 = Vs, V2=V t

N2 = 0.01 and N1 has to be calculated.

Thus, N1 = V t x 0.01

Vs

Normality x Equivalent weight = Weight in g/l

Thus, Wt of the dissolved oxygen g/l = V t x 0.01 x 8

Vs

Weight of dissolved oxygen (mg/l) = V t x 0.01x 8 x 1000

Vs

In order to convert the dissolved oxygen content from mg/l to ml/l, the above formula is multiplied by 0.698.

Thus,

Dissolved Oxygen (ml/l) = V t x 0.01x 8 x 1000 x 0.698

Vs

Correction factor (CF) is included in the above formula to rectify the defect caused by the addition of MnSo4 and alkaline iodide solution by replacing 2ml of the water sample.

The total volume of the stoppered bottle can be measured by filling the bottle with tap water and then stoppering it to remove the excess water. The water remaining in the bottle is measured by pouring the contents in a measuring cylinder.

The oxygen solubility of water sample is usually calculated by using the standard values if the salinity and temperature of the water samples have already been estimated. For calculating the oxygen solubility at different combinations of salinity temperature refer Table 11 (Vide Annexures).

By calculating the difference between the dissolved oxygen content and the oxygen solubility values, Apparent Oxygen Production (AOP) and Aparent Oxygen Utilization (AOU) can be calculated. These two parameters function as reliable indices of oxygen production due to photosynthesis and oxygen utilization due to community respiration.

P1. Fry's modified respirometer

Materials required: Fry's respirometer.

Description

A respirometer is a device used to measure the rate of respiration or respiratory quotient (RQ) of a living organism by observing its movement or activity in the medium. Many gadgets or devices can be attached to it measure different parameters like amount of dissolved O_2, CO_2, NH_3 etc. Hence, this instrument can be helpful in study of effect of age, activity, disease, light, chemicals and other factors on the respiration of fish.

'Fry's modified respirometer' is a modified from of 'Fry's modified respirometer' originally developed by fry. The modifications were brought by kutty *et al*. (1971). In contrast to fry's respirometer which was bulky and had moving sensors, this modified respirometer is not so bulky, but with enough space for movement of fish and has fixed sensors. These sensors count the activity or movement of fish inside the respirometer chamber.

The 'Fry's modified respirometer' has two circular chambers made up of transparent material (glass or plastic). The inner-shorter chamber has light arrangements, which is sensed by the sensors and also used to view the movement of organism. The larger outer circular chamber is meant for the observation of movement of fish or organism. It can be filled with water and fish can be kept for observation. The light falling from the inner chamber falls on the sensors attached outside the outer chamber. When the light is obstructed due to movement of fish, it is registered on the activity counter, attached to the sensors. The transparent chambers may be encased with a black box like cover with 4 small windows at the 4 lateral sides for observation. The display (LCD) shows the activity sensed by the sensors. A thermometer can also be in-built to record the temperature.

Number of sensors = 8
Height of each sensor = 18cm
Distance between adjacent sensors = 25cm
Radius of inner chamber = 17cm (r_1)
Radius of outer chamber alongwith inner chamber = 30cm (r_2)
Height of chambers (depth) = 19cm (h)
From above measurements we can calculate the volume or capacity of the outer chamber as

$V = \pi (r_2^2 - r_1^2) \times h$

Hence volume of outer chamber = $\pi (30^2 - 17^2) \times 19$ cm^3

$= 22/7 \times (30+17)(30-17) \times 19$ cm^3

$= 22/7 \times 47 \times 13 \times 9$ cm^3

$= 36485.429$ cm^3

$= 36.485$ l

P2. Estimation of oxygen consumption by the fish

Materials Required

- Fish
- Jar with water
- Glasswares used for DO estimation as burette
- Pipettes
- DO bottle
- Beakers
- Conical flask
- Weighing balance.

Principle

Dissolved oxygen can be estimated at regular time interval preferrebly after 30 minutes for a sample of water with fish and the decrease in the concentration of oxygen can be taken as that consumed by the fish.

Oxygen dissolved in water can be estimated using the winkler's titration method.

Following is the principle involved in DO estimation. Manganous sulfate reacts with NaOH or KOH to give white manganous hydroxide.

$Mn^{+2}SO_4 + 2KOH \rightarrow Mn^{+2}(OH)_2 + K_2SO_4$

$2Mn^{+2}(OH)_2 + O2 \rightarrow 2Mn^{+4}O(OH)_2$

Addition of H_2SO_4 dissolves the brown manganese oxide yielding manganic sulfate, which reacts instantly with iodide to yield iodine.

$Mn^{+4}O(OH)_2 + 2H_2SO_4 \rightarrow Mn^{+4}(SO_4)_2 + 3H_2O$

$Mn^{+4}(SO_4)_2 + 2KI \rightarrow Mn^{+2}SO_4 + K_2SO_4 + I_2$

In the effect oxygen oxideses Mn^{+2} to Mn^{+4} and Mn^{+4} oxidises I^- to I_2. Iodine is then determined titremetrically to the starch as an end point indicator.

Reagents

1. **Managanous sulfate ($MnSO_4$) solution:** 480g $MnSO_4.4H_2O$ or 400g $MnSO_4.2H_2O$ or 364 g of $MnSO_4$ H_2O is dissolved in distilled water and the volume is made upto 1l.
2. **Alkaline iodide:** 500g of NaOH or 700g of KOH and 135g BaI or 150g KI is dissolved with 1l distilled water.
3. Concentrated H_2SO_4
4. Starch indicator (1%) – This should be prepared at the time of need by adding 1g starch in 1l distilled water.

5. Sodium thiosulphate solution (0.01N) is dissolved in previously boiled distilled water and g of $Na_2S_2O_3$ the volume is made upto 1l. The solution is stored in a coloured bottle and may be preserved with 2 or 3 pellets of NaOH.

6. Procedure

For the determination of dissolved oxygen, 50ml of water sample can be added with 1ml each $MnSO_4$ solution and alkaline iodide solution one after another. The precipitate is allowed to form.

At the time of titration the bottle with sample is unstoppered and 1ml H_2SO_4 is added to dissolve the ppt. a clear yellow solution appears.

This solution can be taken in conical flask and titrated against 0.01N $Na_2S_2O_3$ solution until pale hay-yet low colour appears. At this stage 3-4drops of 1% starch is added and the titration is continued till the disappearance of blue colour.

Experimental fish can be introduced into a 1.5l jar containing water. Before introducing the fish, its weight was taken and the amount of dissolved oxygen was also estimated using winkler's titration method.After 30 minutes estimate the amount of dissolved oxygen in the water. The difference in oxygen levels between the samples gives the level of oxygen consumed by the fish in 30 minutes. After 30 minutes second sample was taken and the dissolved oxygen was estimated. The difference in the oxygen level between the samples give the quantum of oxygen consumed by the fish at fixed time interval. From the estimated values, the oxygen consumed by the fish is calculated as ml/l/kg/hr of fish.

Results

Dissolved oxygen can be calculated as = O_2 (ml/l) = N x E x 0.698 x 1000 x V_t/ V_s

Where, N =Normality of standard solution (0.01N)

E = Equivalent wt of O_2 (8)

V_t =Volume of titrant

V_s =Volume of sample

0.698 =conversion factor (mg → ml)

P3. Effect of salinity on oxygen consumption by the fish

Materials Required

- Salt (Nacl)
- Fish
- Jar with water
- Weighing balance
- Various glasswares used for DO estimation.

Principle

Dissolved oxygen in water can be estimated by winkler's titration method. It (DO) can be estimated at time interval of 30 minutes and the difference in oxygen levels between the samples give the level of oxygen consumed by fish in 30 minutes.

Procedure

Prepare the solutions containing different salt concentration. Introduce the fish into the containers having different salt concentration. Take the weight of the fish before starting the experiment. Before introducing the fish estimate the dissolved oxygen in water sample by winkler's titration method.

After 30 minutes, again estimate the amount of DO in the water. The difference in the oxygen level between the samples taken after 30 minutes, gives the quantum of oxygen consumed by the fish at fixed time interval. From the estimated values, the oxygen consumed by the fish is calculated as ml/lit/kg/hr of fish.

Result

Dissolved oxygen in a water sample can becalculated as
O_2 (ml/l) = N x E x 0.698 x 1000 x V_t/ V_s
N = Normality of standard solution (0.01N)
E = Equivalent wt. of O_2 (8)
V_t and V_s = volume of titre and sample in ml

Observation

D.O. before introduction of fish:
O_2 consumed by fish in 30 minutes:
Wt. of fish:
O_2 consumed by fish:

P4. Estimation of amount of CO_2 released by fish

Materials Required

- Fish
- Jar with water
- Burette
- Pipette
- Beakers
- Conical flask
- Weighing box
- Reagents.

Principle

Dissolved CO_2 can be estimated at regular time interval for a sample of water with fish and the increase in the concentration of CO_2 can be taken as that released by fish.

CO_2 dissolved in water can be calculated by titrimetry. Following is the principle involved in CO_2 estimation.

CO_2 reacts with NaOH or Na_2CO_3as follows

$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$

$Na_2CO_3 + H_2O + CO_2 \rightarrow 2NaHCO_3$

These two reactions indicate the two possible methods for determining CO_2 in water. Titration with either standard NaOH (N/44)or standard Na_2CO_3 (N/22) to the phenolphthalein endpoint.

Reagents

1. Phenolphthalein indicator
2. N/44 NaOH solution or N/22 Na_2CO_3 solution.

Preparation of reagents: Phenolphthalein indicator is prepared by dissolving 0.5g of phenolphthalein in 50ml of 95% $C_2H_5OH$and 50ml CO_2 free distilled water. Standard NaOH solution (N/44) is prepared by 0.91g of anhydrous NaOH dried at 145^oC and cooled in desiccators is dissolved and diluted to 1l with CO_2 free water. The standard solution should be made fresh before every use.

Procedure

A known volume of water is taken in a jar with fish. 100ml water sample is taken before introduction of fish. Add 10drops of phenolphthalein indicator in the flask and the sample solution is titrated with N/44 NaOH solution till the end point (light pink) appears. Same procedure is repeated with the water sample taken from the jar with the interval of 30 minutes.

Calculation

Amount of dissolved CO_2 = 10 x Volume of titrant (mg/l)

Observation

Volume of water in jar:

Weight of fish:

Initial amount of dissolved CO_2:

Amount of dissolved CO_2 (30 minutes after introduction of fish):

Total amount ofCO_2 released by fish in 30 min:

Amount of CO_2released by fish in 1 hour:

Amount of CO_2released by fish = 32.66 x 1000 / 45 = 725.78 mg CO_2/kg/hr

Result

Amount of CO_2 released by fish is 725.78 mg CO_2/kg/hr.

P5. Estimation of amount of ammonia excreted by fish

Materials Required

- Fish
- Jar with water
- Pipette
- Conical flask
- Measuring cylinder
- Beakers etc.
- Spectrophotometer
- Aluminium foil
- Balance for weighing fish.

• Principle

In a weakly alkaline solution, NH_3 reacts with hypochlorite to form monochloramine,which in the presence of phenol, catalytic amount of nitropruside ions and an excess of hypochlorite yields in dophenols. The reaction leading to this coloured complex is not yet fully understood. Probably aquinone chlorimide maybe formed in the intermediate step.

Reagents

1. Ammonia fesh water – for preparation of reagents.
2. Phenol – nitroprusside solution – 27g phenol and 0.3g sodium nitroprusside di-hydrate diluted to 1l with ammonia free distilled water.
3. 0.84 M sodium hydroxide (NaOH) solution.
4. 6.25% sodium hypochlorite (NaOCl) or bleaching powder solution.
5. 1.19 M trisodium citrate ($C_6H_5Na_3O_7.2H_2O$) solution.

6. Procedure

25ml of water sample is taken in a conical flask and 1mlof citrate solution is added. Now 1ml of phenol-nitroprusside and 1ml of NaOCl or 3ml of bleaching powder solution is added. The contents are mixed well and the flask is covered with aluminium foil. The flask is then kept in dark for about 2 hours. If the colour develops within 5 minutes, the analysis is repeated with fresh sample. After 2 hours the sample is taken to the spectrophotometer for concentration determination by measuring optical density(OD).

Again after 30 minutes of introduction of fish, the water sample can be taken to spectrophotometer after adding reagents for the estimation of NH_3-N.

Preparation of standard solution

0.0382g of NH_4Cl is dissolved in ammonia free water and is made upto 100ml. This solution contains 100 m g NH_3N/ml.1ml of chloroform may be added as preservation. Working standards of various concentrations of 100 to 500 m g NH_3-N/litre are prepared by diluting the solution.

Water samples and different standard solutions are taken to the automatic spectrophotometer. There the concentration of ammonia (NH_3-N)is found out using visible monochromatic light of 630nm. The standards of known centrations are used to compare and calculate the concentration of sample by using optical density.

Observation

Weight of fish:

Initial concentration of ammonia prior to introduction of fish:

Concentration of ammonia(30min after introduction of fish):

Ammonia excreted by fish per hour:

Result

Ammonia (NH_3-N) excreted by fish:

BFSC-308: Inland Fisheries

P1. Appraisal of various morphometric and meristic characters used for the identification of freshwater fishes

Materials required

- Fish specimens, dissection instruments, divider or a dial reading calliper, stainless steel ruler with measurements to mm, balance, marker, tray, dissection board etc.,

Procedure

- Collect freshwater and estuarine fish specimens belonging to wide range of orders either from the fish landing centre or from the institute museum
- Wash them in running water
- Keep all the specimens in individual trays
- Observe the following morphometric and meristic features of the fishes collected
- Draw illustrations wherever needed

Observation

List out all morphometric and meristic features

MORPHOMETRIC AND MERISTIC CHARACTERS OF FRESHWATER FISHES USED FOR CLASSIFICATION AND IDENTIFICATION

The term, morphometry denotes the measurement of the structures and parts of organisms. The term, meristics denotes the counting of quantitative features of a fish, such as its number of fins or of scales.

I. MORPHOMETRIC MEASUREMENTS

Sharp pointed needle-like dividers or dial-reading calipers are used for taking body measurements. A Stainless steel ruler with measurements to millimeter is recommended for accurate readings.

The various body measurements of a fish are:

1. **TOTAL LENGTH**

The greatest distance between the most anterior projecting part of the head (even if it is prolonged beak as in *Hemiramphus*) to the posterior most tip of the caudal fin, including filamentous prolongations, if any. The measurement is a straight line and is not taken over the curves of the body. Where the caudal fin has either of the lobes longer than the other, the maximum length is taken. Where the jaws are unequal, the mouth is closed and measured from the tip of the jaw that is longer.

2. **STANDARD LENGTH**

The straight distance from the anterior most part of the head to the end of the vertebral column. In actual practice, the flexure line of the body over the caudal peduncle is taken as the posterior most point. Some authors however take this as mid of the caudal fin base.

3. **BODY DEPTH**

The vertical measurement from a point in the body of the fish on its back where its height is the greatest to a straight line to the ventral surface or profile. The fleshy or scaly structures pertaining to fin bases are excluded. It need not necessarily be in middle of the fish.

4. **HEAD LENGTH**

A Straight measurement of the distance from the tip of the snout to the most distant point on the opercular membrane on the upper angle of gill opening. Any fleshy membrane of the gill cover is also included.

5. **HEAD DEPTH OR HEIGHT OF HEAD AT OCCIPUT**

The perpendicular distance measured from the midline at the occiput vertically downwards to the ventral contour of the head or the breast. In actual practice, one arm of the divider is placed at the occipital crest and the other arm below the head vertically opposite to the upper arm of the divider, to form a vertical lie. Unlike body depth this measurement is not taken up to ventral profile line.

6. **HEAD WIDTH**

Straight distance across the head in a ventral position: if the opercles are dilated they are forced into a reasonably normal position.

7. **EYE DIAMETER**

The distance between margins of the cartilaginous eyeball across the cornea.

8. **SNOUT LENGTH**

The distance from the most anterior midpoint on the snout or upper lip to the front hard margin of the orbit.

9. **INTERORBITAL WIDTH**

The least distance between the bony rims between inner margin of the eyes.

10. **PRE-DORSAL LENGTH**

A straight measurement from the midpoint or tip of the snout or upper lip or the anterior most part of the head to the structural base of the anterior most dorsal fin ray. In fishes with a dorsal spine, the basal bone of the dorsal fin, also called the nuchal shield, is taken as the structural base.

11. **POST-DORSAL LENGTH**

A straight line measurement from the structural base of the dorsal fin to the flexure line of the body over the caudal peduncle or the end of the vertebral column.

12. **PRE-PELVIC DISTANCE**

A straight distance from the midpoint of the base of the pelvic fin to the anterior most point of head.

13. **HEIGHT OF DORSAL OR LENGTH OF DORSAL FIN SPINE**

It is measured from the anterior point of junction with the body to anterior tip of the fin or spine even if the other rays do not reach this point.

14. **LENGTH OF BASE OF SPINOUS OR SOFT DORSAL ADIPOSE DORSAN ANAL FIN**

The greatest distance measured in a straight line between the anterior most and posterior most

points of junction with the body.

15. **LENGTH OF PECTORAL HEIGHT AND PELVIC FINS, DORSAL FIN OR SPINES**

Measured between its origin or place of insertion into the body to its extreme tip.

16. **DEPTH OR LEAST HEIGHT OF CAUDAL PEDUNCLE**

The least vertical distance from the dorsal to ventral profile at the narrowest part of the caudal peduncle. It is a straight measurement.

17. **LENGTH OF CAUDAL PEDUNCLE**

An oblique measurement from the last point of contact of anal fin posteriorly to the end of the vertebral column or the flexure line of the body over the caudal peduncle.

18. **LENGTH OF LONGEST FIN RAY**

Measured from the structural base of the longest fin ray to its tip.

19.LENGTH OF OCCIPITAL PROCESS

The distance from the point of origin or base of the occipital process on the supra occipital bone to its posterior most tip where it meets the basal bone of the dorsal fin, the latter is excluded. Where it meets the inter-neural shield, the shield is excluded.

20. **POST-ORBITAL LENGTH**

The greatest distance from the posterior edge of orbit to the posterior tip of the fleshy operculum.

21. **SUB-ORBITAL WIDTH**

The least distance from the lowermost margin of the orbit to sub-orbital or pre-orbital margin.

22. **WIDTH OF GAPE OF MOUTH**

The greatest transverse distance across the opening of the mouth, without stretching the mouth opening.

23. **WIDTH OF PREMAXILLARY TEETH BAND**

The maximum width of the band

24. **WIDTH OF BASE OF OCCIPITAL PROCESS**

The maximum width at base of occipital process where it originates from the supra-occipital bone.

II. **MERISTIC COUNTS**

The accurate enumeration of meristic data or counts of fin rays is of diagnostic importance. Great care should be taken to count accurately the simple and branched rays and present the data in charts of frequency distribution wherever possible.

1. **FN RAYS**

All paired and median fins in bony fishes have long, mobile filament like prolongations called rays. The movements of the fins are due to the action of the muscles, these movements being possible of the articulations and often flexibility of these rays. The term “ray” also

applies to spines whether they are included within the membrane of a fin or not.

Three chief types of fin rays are encountered:

(i) SOFT RAY – Are often very thin, flexible and most often branched.

(ii) HARD RAYS – Are a number of soft rays united solidly to compose hard rays which are rigid and sometimes sharply pointed. These are formed of cartilage, and when freed from the membranous sheath holding them, clearly show the juxtaposed soft rays. Some of perches and sisorids have such hard rays. The outermost caudal fin ray called the principal ray is also a hard ray are often preceded by hard rays.

(iii) SPINOUS RAYS – Are made of bone tissues, and are harder and stronger than rays. These are usually not covered by the membrane of the fin and often are with serrations or teeth. They are common in catfishes. These are cited in the fin formulae by capital roman numerals (I, II, III etc). Hard and soft rays can be distinguished easily. Soft rays are so constructed that they can be readily split into right and left in the median. Hard rays on the other hand do not have such cleavage flexure.

2. **SIMPLE RAY**

Is either soft or hard, flexible but without any branching at its tip or elsewhere; in the anal fine often these rays remain within the membranous covering and should be teased out. These are indicated in the fin formulae by lower-case roman numerals (i, ii, iii etc). The rays of a fin are not at all uniform. Hard rays and branched soft rays or un-branched and branched soft rays may be found together in two fins one following the other as in perches.

3. **BRANCHED RAY**

Branches either from the base or middle or tip of the ray. These are stated in the fin formula by Arabic numerals.

4. **RUDIMENTARY RAY**

Is an undeveloped ray grading into different stages of a developed one. If included in the count, it should be specifically mentioned. Generally it is excluded.

5. **CAUDALFIN RAYS**

Are counted from the outermost to the inner ones including the smallest one at the inner end of the fin base. A good magnification is often needed for this count.

6. **COUNTING FIN RAYS**

In the case of the rayed dorsal fin, the posterior most ray is taken as the first ray and the count is done towards the anterior direction. In the case of anal fin the direction is from anterior to posterior.

7. **LATERAL LINE SCALE COUNTS**

The number of scales with perforation along the lateral line up to the structural caudal base or end of hypural plate or at the flexure line of the body over the caudal peduncle. The scales wholly on the caudal fin base or beyond this point without such perforations are excluded. If the lateral line is divided as in loaches, cichlids and anabantids, the lateral line count includes all the pored scales in the upper and lower line: only the count on the lower line starts from the scale following that directly below the last one on the upper line. Indicated as L.L. or L.I.

in fin formulae. If the lateral line is incomplete or absent, or if all the scales do not have pores or perforations, the scales themselves may be counted along the normal course of line. Near the had the first scale to b counted is one which touches the pectoral girdle and which is followed by a scale which does not touch it. Transverse scale counts are taken as scale rows crossing an imaginary vertical line. Scales from the anterior base of fins are generally taken. The number and nature of the striae and pores on the scales are important.

8. **SCALES FROM DORSAL FIN TO LATERAL LINE**

Generally the count is taken from the insertion of the first dorsal fin including the small scales, counting downward and backward following the natural scale row to but not including the lateral line itself. The small scales at base of the fin are however included.

9. **SCALES BELOW LATERAL LINE OR FROM LATERAL LINE TO PELVIC BASE**

Are counted as above but from below upwards and forward from base of pelvic to lateral line. Small scales are included. The scale nearest the pelvic fin in counted as half when, it is so. When counting upward and forward, if the scale rows are found wavy backward and forwards, the backward rows are taken.

10. **PREDORSAL SCALES**

The number of scales from the origin of the dorsal fin to the occiput. All scales which wholly or partly intercept the straight midline running from the origin of the dorsal fin to occiput is taken, Indicated as P.D.S in fin formulae.

11. **PREANAL SCALES**

Counted from the anterior most base of the anal fin to the vertical base of the occiput or end of the scale rows on the head.

12. **SCALES IN LATERAL OR LINEAR SERIES**

Where the lateral line is absent, the number of transverse rows of scales between the occiput and the structural base of the caudal fin is counted. Indicated as L.r. in fin formulae.

13. **CIRCUMPEDUNCULAR SCALES**

Scales around the least depth of caudal peduncle. It is always better and useful to indicate the number of circumpeduncular scales above and below the lateral line.

14. **BRANCHIOSTEGAL RAYS**

Numerous tiny tin bones arranged fanwise from the lower edge of the opercle to the ventral surface of the head and covered by the branchiostegal membrane. Care should be taken to include the most anterior branchiostegals which are likely to be very short, slender and hidden.

15. **GILL RAKERS**

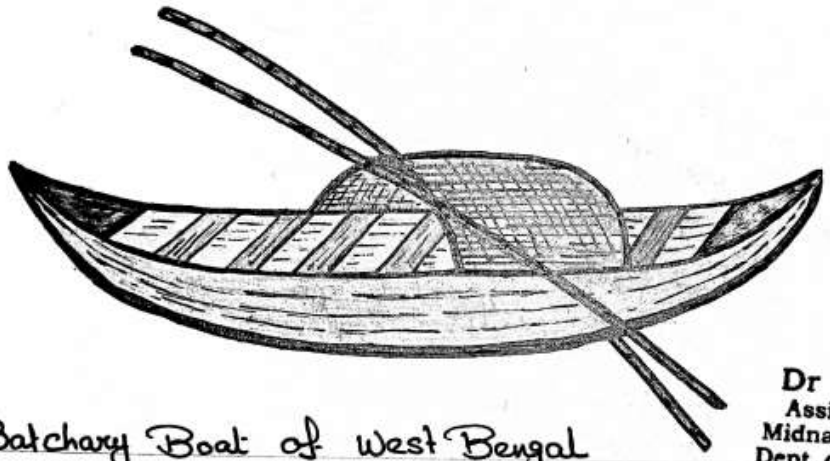
Thin needle like filamentous prolongations on the gill arches. For count purposes, the rakes on the first arch is taken. If the counts of both the upper and lower limbs of the gill arch are taken they are separated by a plus (+) sign; generally the lower limb alone is considered. If rudiments are included this fact should also be mentioned.

16. **PHARYNGEAL TEETH**

“Throat teeth” borne on two bones which are modified fifth gill arches, each with one to three

rows of teeth. The in each row are counted and are indicated in formula in order from left to right. For example 2,5 – 4,2 would indicate that the pharyngeal bone of the left side has two teeth in the outer row, five in the inner whereas the right bone has only four teeth in the inner row and two in the outer. The formula 3-3 would show that no teeth are developed in the outer row. The number and series of pharyngeal teeth are of classificatory value.

P-2: FISHING CRAFTS AND GEARS IDENTIFICATION



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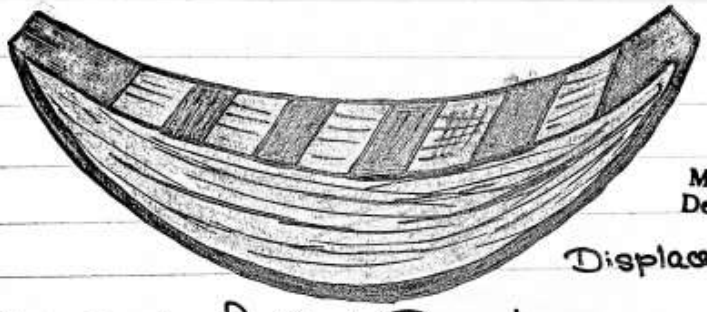
Batchary Boat of West Bengal

1. Size : 8.4-20.4m OAL
2. Most common dimensions are : 13.05 x 1.3 x 10.48 m.
3. The stern is as high as bow
4. Carvel built boat with planks butted together by staples.
5. Used for operation of drift net, bag net & dip nets.



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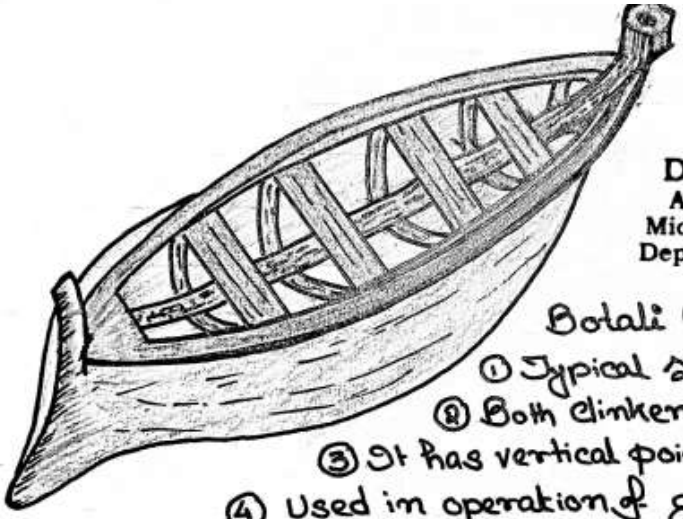
Displacement : 3tons

Chot Boat of West Bengal

① Short over hangs & high free board.
② Typical boat measures : 10.2m x 2.55m x 1.1m -
③ Used for operation of seine net & bag net.



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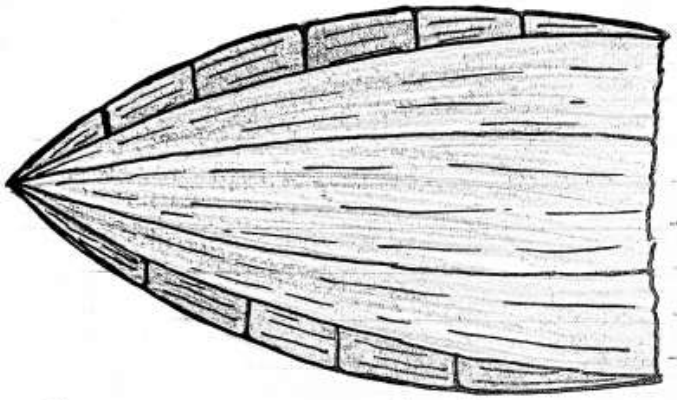
Botali (Dingi) of Orissa

① Typical size : 3.5x0.45x0.45m.
② Both clinker & carvel boats are present
③ It has vertical pointed stem & stern.
④ Used in operation of gill nets to catch hilsa & Pomfrets.

Clinker built boat is known as Pattiya.



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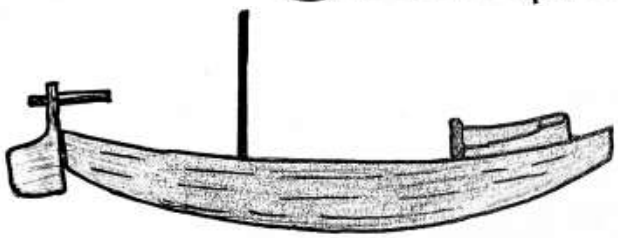
Catamaran of Orissa.
Also known as Ganjam type catamaran with distinctive features.

① The boat is made of 5 logs, which are pegged.
② Logs are narrows to a point in the forward where 2 pieces fitted to give sharp beak point.
③ At the aft, the craft abruptly truncated.
④ 3 medium logs are full length pieces while sides are made of old logs and pegged to give require shape.
⑤ used to operate gill nets from beaches.



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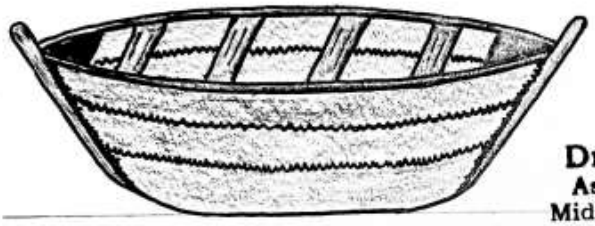
Nava of Orissa & Andhra Pradesh

① Narrowed round bottom boat without any keel.
② Heavily framed, Carvel/Planked built with short fore deck & longer outer aft deck.
③ Size: 10.5 x 1.95 x 0.68 m, displacement 4 tonnes.
④ Carries one mast & latten sail.
⑤ Mainly used for gill nets, Shore Seines, boat Seines & long lines.



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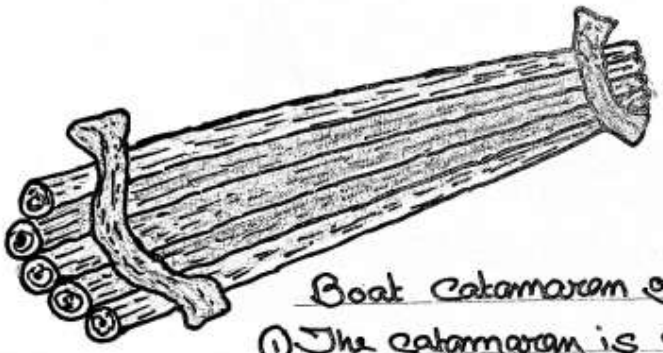
Masula Boat / Bare Boat of Orissa & Andhra Coast

① Non rigid, clumsy open boat without ribs.
② Average size of the boat: 8.4m x 2.4m x 1.2 m.
③ Planks made of mango wood & stiched with palmyrah leave fibres, keeping continuous rope in between the ~~Planks~~ seams.
④ Triangular sails used from bamboo mast.
⑤ Used in operation of gill nets, lines & boat seines.



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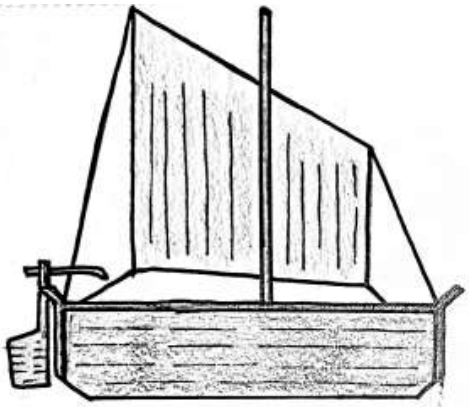
Boat Catamaran of Tamil Nadu Coast

① The catamaran is made of 3-5 logs.
② The size of the catamaran varies from 6.15m to 6.9m.
③ A small triangular sail is used to carry the craft to the fishing ground.
④ They are normally worked in a pair to operate Madivalai, a boat seine.



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Tuticorin Boat of Tamil Nadu Coast

1. Long relatively narrow, nearly vertical stems & sterns.
2. Sheer line is almost straight.
3. One mast with lug sail & sail.
4. Typical boat measures 8.7 x 1.8 x 0.6-0.33 m.
5. The boat is indegenous but mechanised with few modifications to operate gill & seine nets.

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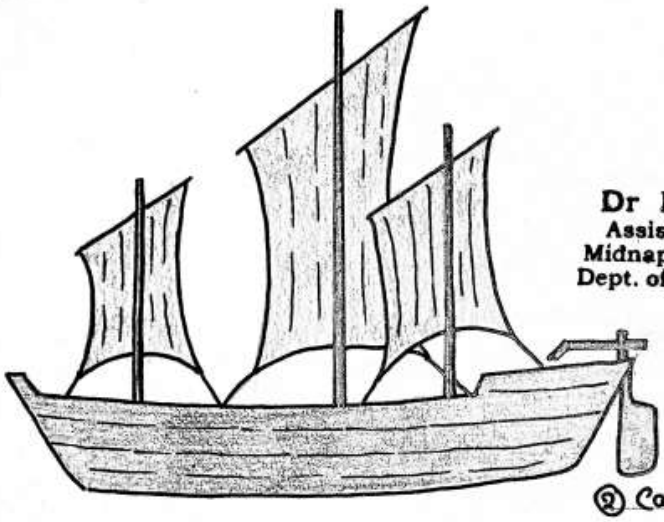
Adiramapatnam Fishing Canoe of Tamil Nadu Coast.

1. They are shorter & majority of them are dugouts with dash strakes.
2. Usually employ one mast, occasionally 3 masts are used.
3. The balance board locally known as Kadisu is exceptionally long & permanently fixed.
4. The boat is very common & used to operate gill nets, seine nets, bagnets, hooks & lines.



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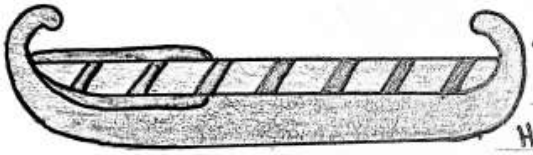
Kala Dhoni of Tamil Nadu.

① Heavy transform stern.

② Carries largest & heaviest balance board.

③ Rigged with 3 masts & lug sails.

④ The fastest traditional fishing boat.



Dugout Canoe

Kerala is the home of dugout, though the boat is found along the Gujarat coast, Maharashtra, Karnataka & some extent in Tamil Nadu.

① The boat is made by scooping out material from single large trunks of trees like Mango, aini.

② Keel portion is thicker than the sides.

③ Propulsion by sails or paddles.

④ 3 different dugouts differ in size and use.

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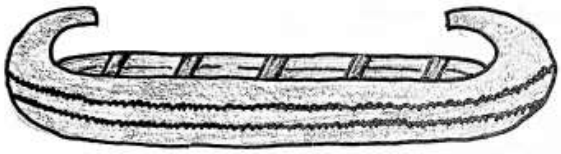
a) Larger – (9.6–10.5 X 0.9 X 0.75 m) commonly known as odoms. displacement 3 tonnes used in pair to operate boat seine.

b) Medium (Thonies) (7.2 X 0.9 X 0.6 m) displacement - 2 tonnes. used to operate beach seines, gill nets & lines.

c) Small (~~Bethapani~~ Bepuhoni) 6.9 - 7.8 m used to operate gill nets & lines.



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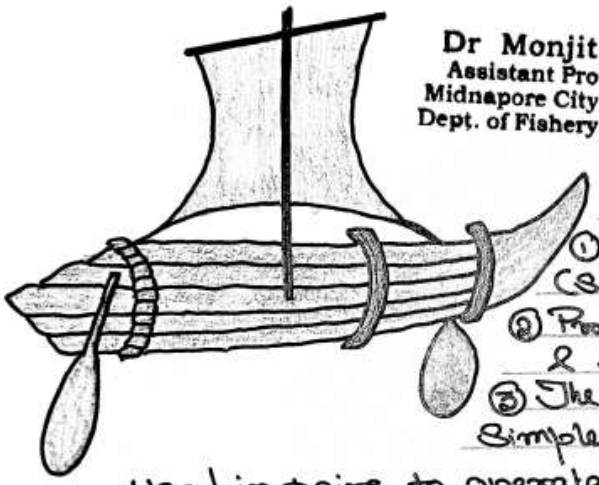
Plank Built Canoes of Kerala Coast.

① Built with planks in the shape of dugout. Locally known as Chemboke or Tonna Vallam.
② Wooden planks are sewen with coir ropes. A layer of black pitch is applied inside & outside to make watertight.
③ Use paddle for propulsion, occasionally sails.
④ Size: Larger one 11-12m x 1-1.5m x 0.8m (displacement: 5t)
Smaller one 6-7m x 0.9m x 0.6m (displacement: 3-4t)
Used for Shore Seines & Gill nets.



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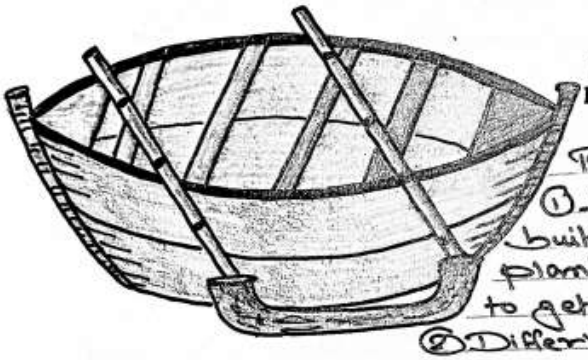
Raft Catamaran of- Kerala
Locally known as Chalakkadi.
① The raft is made of 3-5 logs (Soft Wood) & tied with coir ropes.
② Propulsion with split bamboo oars & sails.
③ The raft is very traditional & simple, used over 10,000 years.
Used in pairs to operate boat seines & individually to operate long lines in deeper waters.



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Rampani Boat of Maharashtra

① Long deep, Widely spread built up canoes. In some cases planks are built over the dugouts to get wider beam.

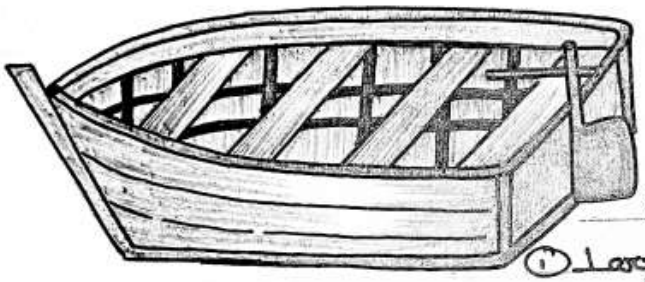
② Differ from normal built up canoe without riggers to give stability.

③ Narrow keel, stem & stern look alike.

④ No mast is carried.

⑤ Typical boat measures 15.0 x 1.80 x 2.70 m

The boat is used to operate a seine net (Beach Seine) locally known as 'Rampani'.



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Machwa Type Wahan of Gujarat Coasts.

① Large deep sea vessel.

② Average Size: 9.6 m - 10.2 m x 2.36 m (Beam)

③ Raked Stem, Square Stern.

④ Decked fore & aft.

⑤ Rudder is large & heavy.

⑥ Single mast with tateen sail.

Used for operation of Doll nets & Gill nets. 4-45 shots of gill nets are operated from each boat with 7-8 crew.

Machwa of Kutch (Gujarat Coast)

① The boat is commonly known as Karanja Boat

② The size of the boat 14.1m x 3.3m x 0.9m

③ Long over hanging bow with raked stem.

④ The keel is short in relation to length.

⑤ 2 mast (Raked forward) with lateen sails.

The boat is used to operate gill nets, dol nets & seine nets.

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Salpati of Maharashtra

① One of the best fishing boat of India with excellent sea going qualities.

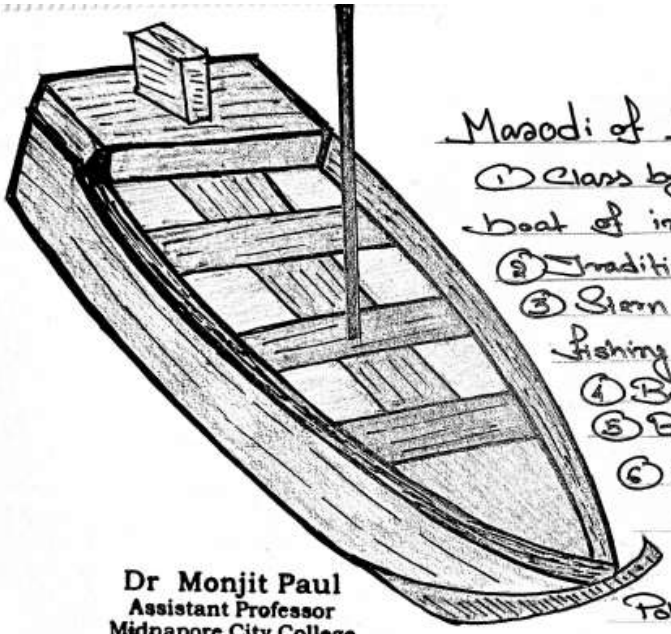
② Mechanised easily without great changes.

③ OAL - 9 - 13.5m

④ Planking is laid carvel fashion but seams are rabbated.

⑤ Medium gunwale & transform stem.

Popularly known as GalBoat, used along the coast of Maharashtra to operate gill nets.



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Masodi of Lakshadweep Islands.

① Class by itself, supirior than any boat of inland.
② Traditional sailing cum rowing boat
③ Stern is broader provided better fishing platform.
④ Boat carries two lateen sails.
⑤ Boat Measures : 12.5 x 3.0 m.
⑥ Suitable for mechanisation.

The boat is used in Minicoy island for Tuna Pole & Line Fishing.

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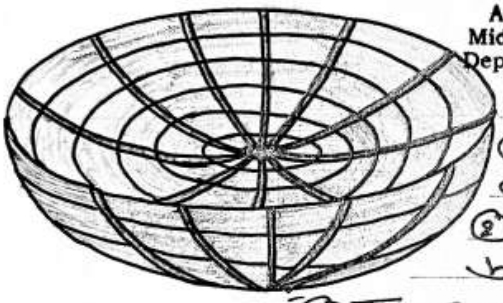

Coracles

① The coracles are dominant craft of South Indian Reservoirs.
② Large Wide mouth flat bottom baskets made of split bamboo.
③ The common size is 3.6 m dia. at mouth
④ Manned by one-two fishermen.

All types of fishing gears including gill nets, seine nets, tong lines, cast nets are operated from each coracle.

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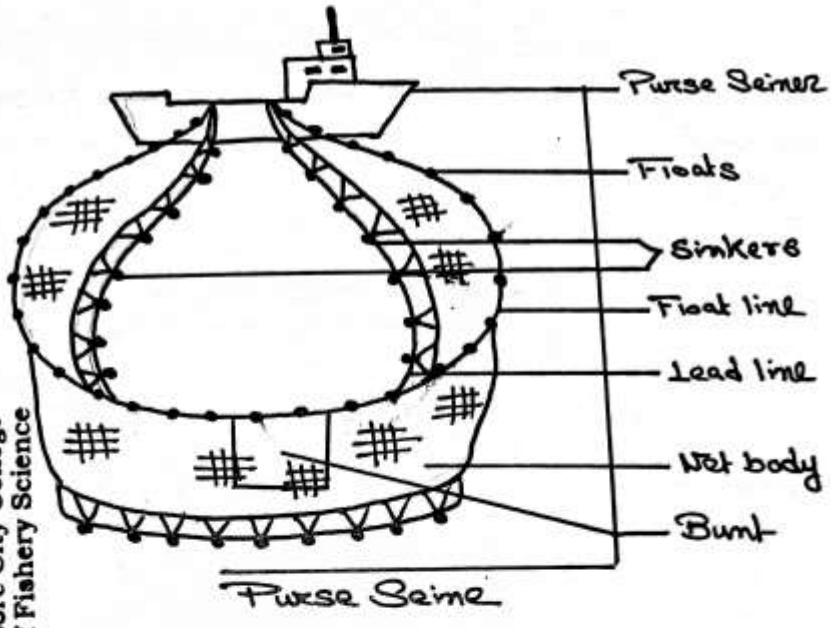


Structure of Purse Seine

Design, Shape, Size of the purse seine vary greatly with operation, target species, dept etc. A typical purse seine could consist following parts :-

① Bunt : This is the section of net where catch is concentrated prior to its removal. The netting of bunt should be stronger & made of thicker twines.

② Main Body : Largest part of the net extending from bunt to facilitate surrounding the shoal of the fish.

③ Selvedges - Consists of a few rows of meshes & thicker twines to protect the net from damages.

④ Float & Lead line - The upper selvedges are attached with float line & lower selvedge to the lead line.

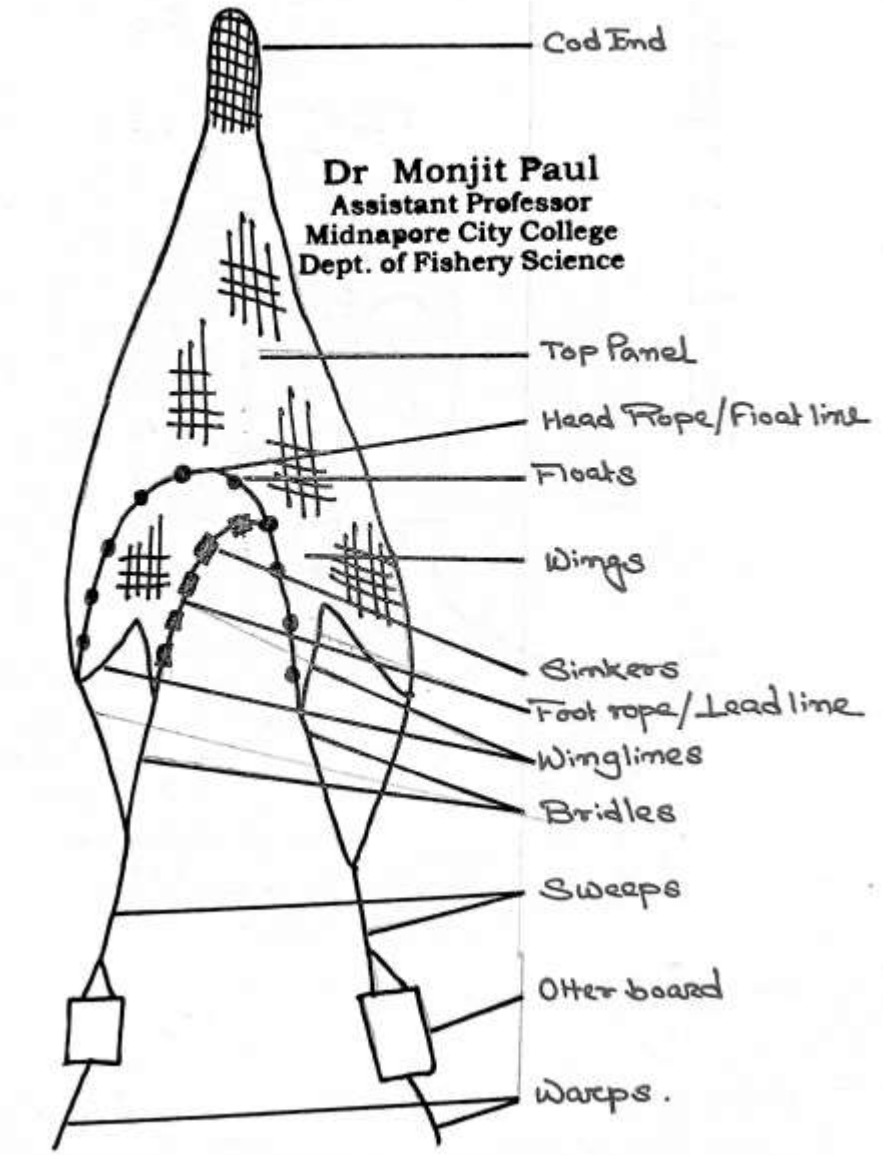
Bridles & tow line: Bridles are ropes attached to the float line & lead line on either end are connected a tow line of sufficient length to facilitate setting & hauling operations.

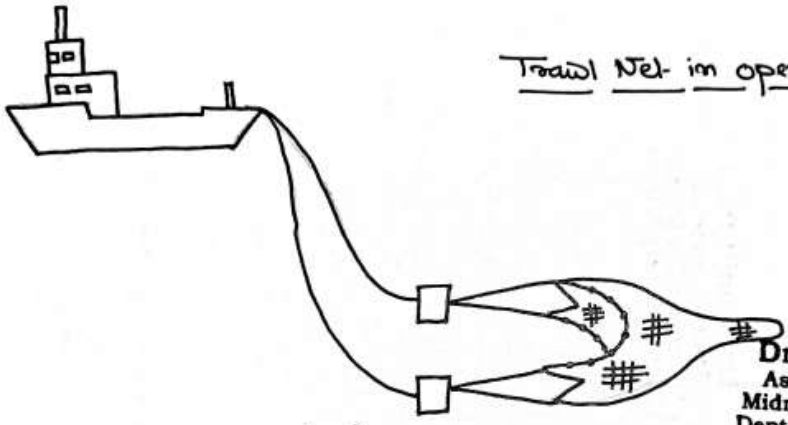
Floats & Sinkers: Sinkers are attached to the lead line to attain 1-3kg/m to 8kg/m weight. Floats are used to maintain the total buoyancy 1.5 to 3.5 times of total underwater weight. Higher buoyancy is maintained in the bunt area of the net.

Operation of Seine Net:

Seine fishing is the method of fishing that employs the surrounding of net, called a seine, that hangs vertically in water with its bottom edge held down by weights & its top edge buoyed by floats. Seine nets can be operated from the shore (Shore/Beach Seine) or from the boat (Boat Seine).

A typical diagram of Trawl Net





Trawl Net in operation

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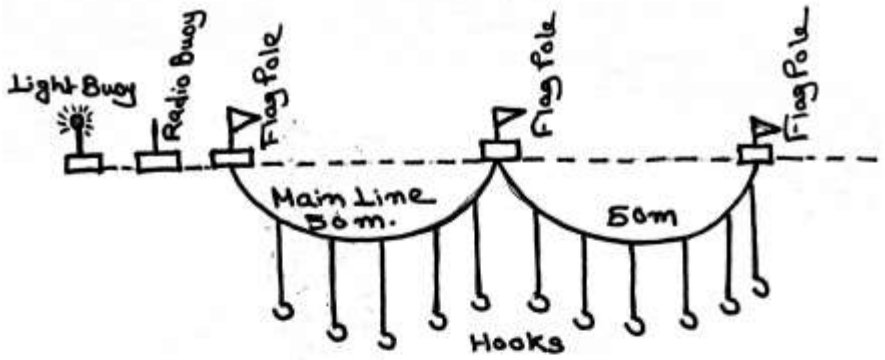
Trawling is a method of fishing that involves pulling a fishing net through the water behind one or more boats. The net is used for trawling is called trawl. Trawling today is heavily regulated in some nations. Capture of undesirable species - turtle, sea lions is the main concern.



A typical long Lines.

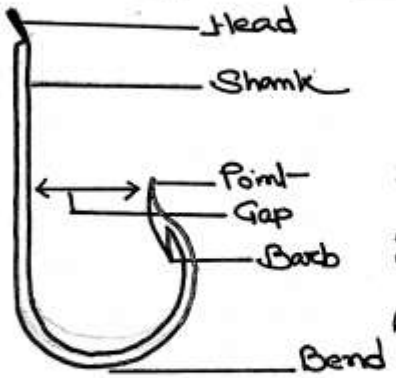


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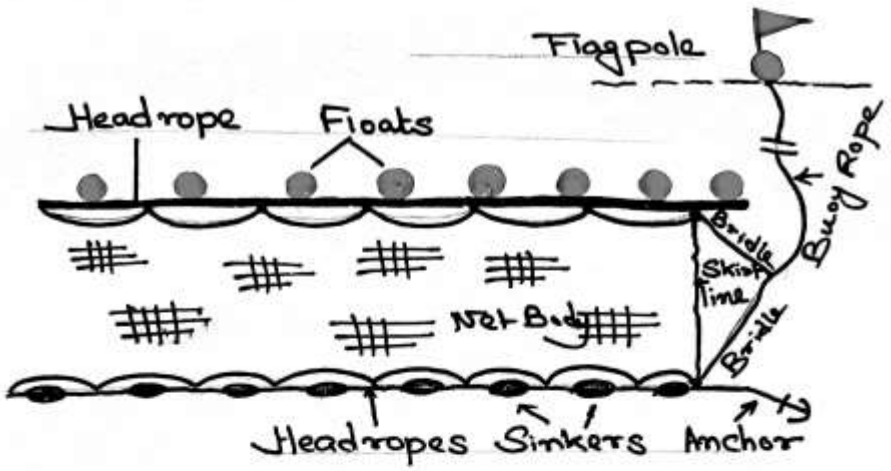
Diagram of a typical Hook

Hooks & Lines are passive gear where fish is attracted by a natural or artificial bait (lures), placed on hook.
Hooks are metalic points, used to catch the fish by ripping them.
The fishing gear is generally operated in a very wide range of depths to catch large fishes like Tuna, Swordfish etc.



A typical diagram of Gill Net

Gillnetting is a fishing method (passive) uses gill nets – a vertical panel of netting that hang from the line with regularly placed floaters.

Fishes are caught by gill nets in three ways –

① Wedged – held by the mesh around the body
② Gilled – held by the mesh slipping behind the opercula.
③ Tangled – held by teeth, spines, maxilleries or other protrusions of the body

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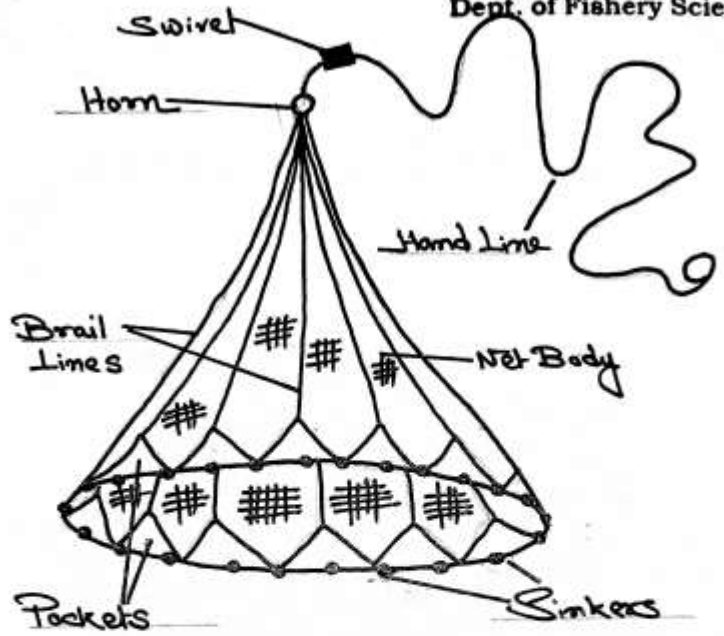

Cast Net

A cast net is also known as throw net. It is a circular net with small weights distributed around its edges. The net is cast or thrown by hand in such manner that spreads out while it is in air, before it sinks into the water. The net is effective to catch fishes from lakes, reservoirs.

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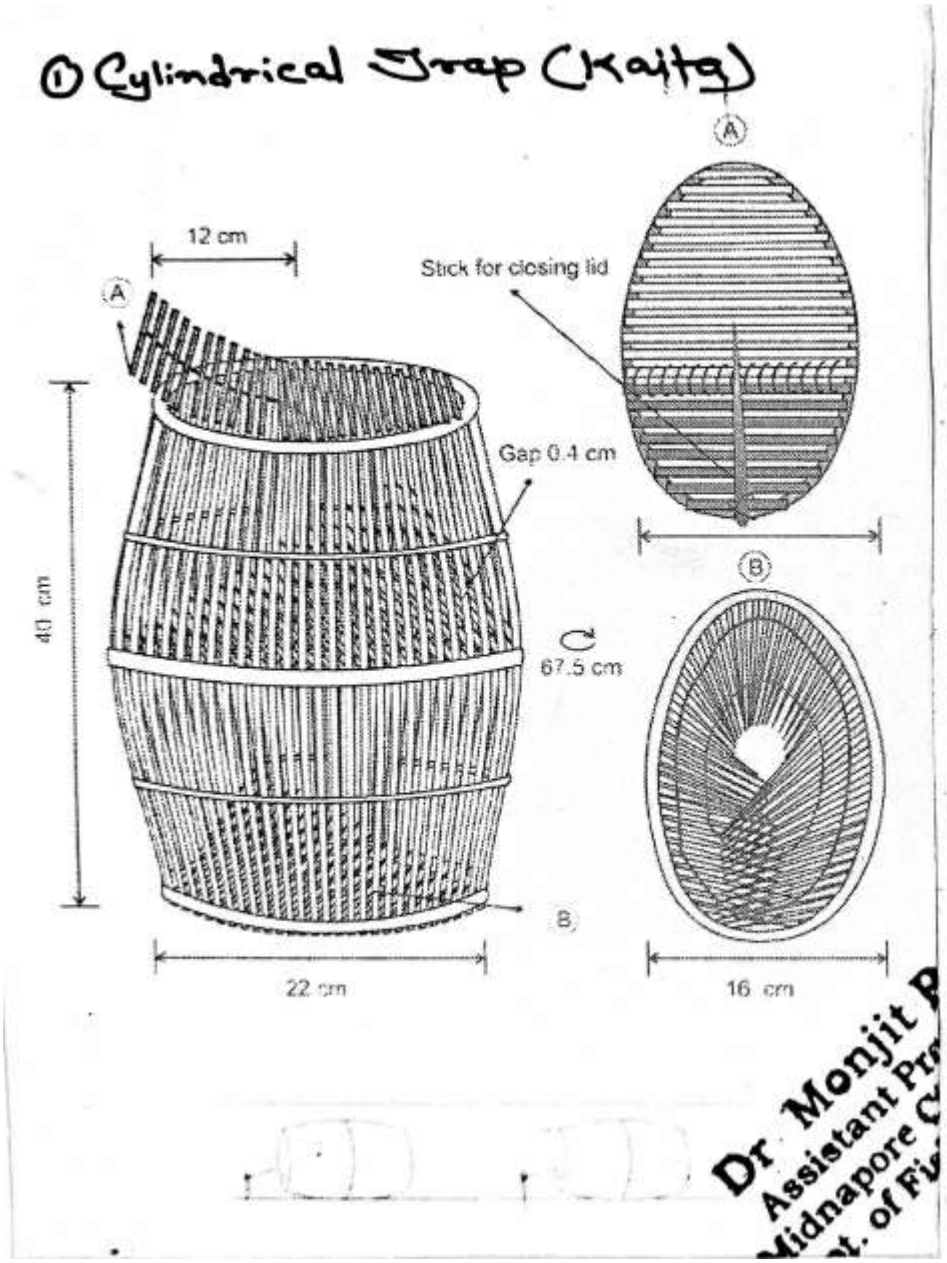
① Cylindrical Trap (Kaitg)
A
12 cm
Stick for closing lid
Gap 0.4 cm
40 cm
67.5 cm
B
22 cm
16 cm

① Cylindrical Trap (Kaita)

Size: 32 x 20 c.m (Bamboo Made)

Area of operation: Shallow Water bodies

Target Species: Small prawns, Mastacembelus spp., Puntius spp., Channa spp., Anabas spp.

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② Cylindrical Trap (Dingora/Bandh Dingra)

Size: 65 x 23 cm (Made of Bamboo)

Area of operation: Paddy fields

Target Species: Mystus spp., Wallago spp. Heteropneustes spp., Clarias sp., Puntius spp Channa spp., Anabas spp.,

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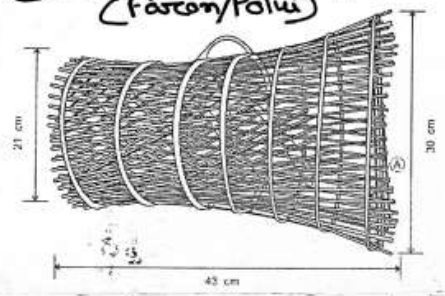
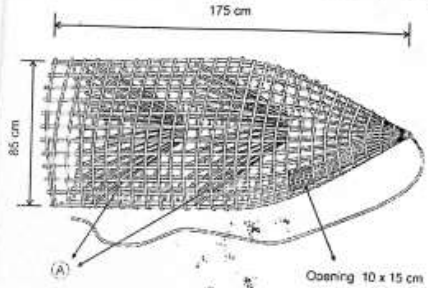
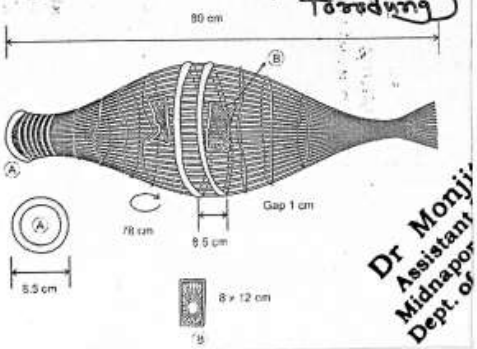
③ Cylindrical Trap (Faron/Polui)

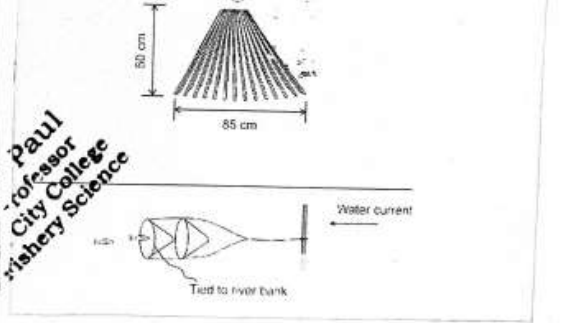


④ Cylindrical Trap (Dui Mukhi Paori)



⑤ Spindle Trap (Seppa/ Toradung)





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(3) Cylindrical Trap (Faram/Polui)
Size: 43X21 cm
Made of Chopped bamboo.
Area of operation: Paddy Fields
Target Species: Small prawns, Mystus spp., Puntius spp., Betia spp., Channa spp., Anabas spp.

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(4) Cylindrical Trap (Dui Mukhi Paori)
Size: 210X88 cm/175X85 cm
Made of Chopped bamboo.
Area of operation: River & Beels.
Target Species: Notopterus spp., Channa spp.

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(5) Spindle Trap (Sepa/Tordung)
Size: 80X8.5X86 cm
Made of Chopped Bamboo.
Area of operation: River & Beels.
Target Species: Prawn & miseellaneous fishes.

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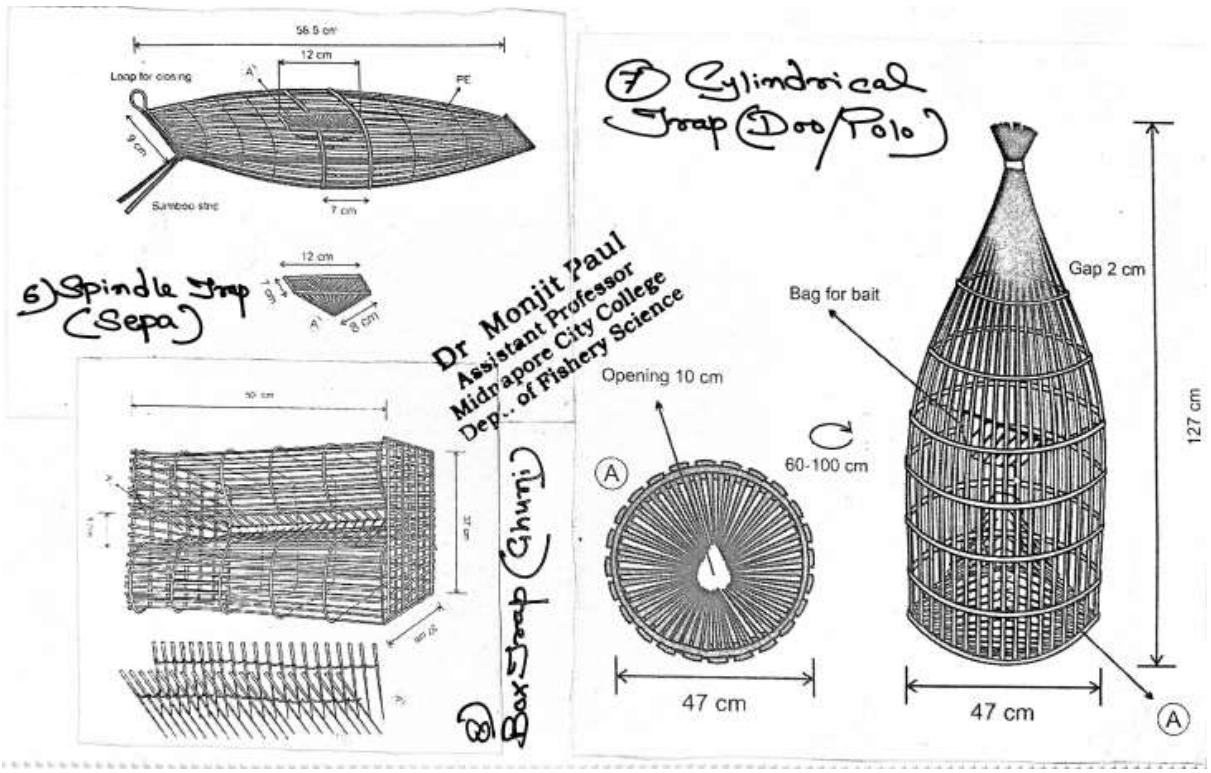
56.5 cm
12 cm
Loop for closing
A
PE
9 cm
Bamboo strip
7 cm
5) Spindle Trap (Sepa)
12 cm
7 cm
A
8 cm
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50 cm
37 cm
6) Box Trap (Ghuni)
7) Cylindrical Trap (Doo/Polo)
Opening 10 cm
A
60-100 cm
47 cm
Bag for bait
Gap 2 cm
127 cm
47 cm
A

⑥ Spindle Trap (Sepa)
Size : 58.5 x 30 x 15 cm
Made of Chopped bamboo.
Area of operation : Rivers & Beels
Target Species : Prawns & Small Fishes.

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⑦ Cylindrical Trap (Doo/Polo)
Size : 60-100 cm Dia
Made of Chopped bamboo.
Area of Operation : Paddy Fields & other Shallow Water bodies
Target Species : Miscellaneous fishes

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8) Box Trap (Ghoni/Ghuni)
Size : 50 x 37 x 37 cm
Made of Chopped bamboo.
Area of operation : River, Beels, Canals
Target Species : Catfishes, Murrels, Puntius

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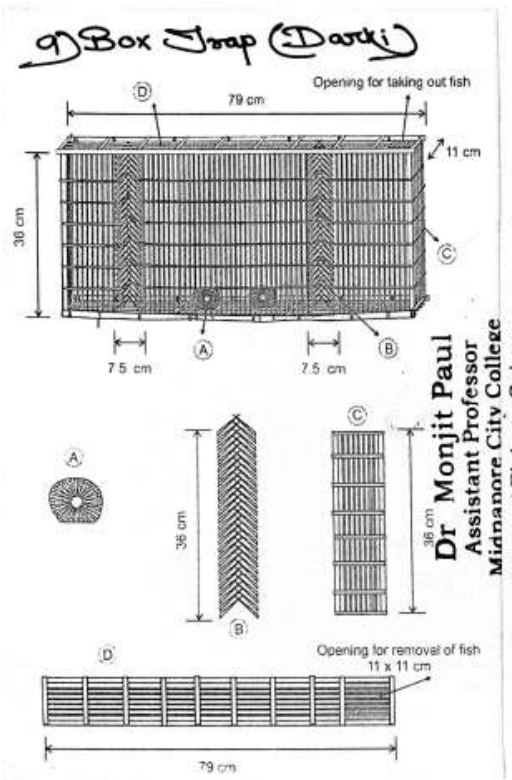

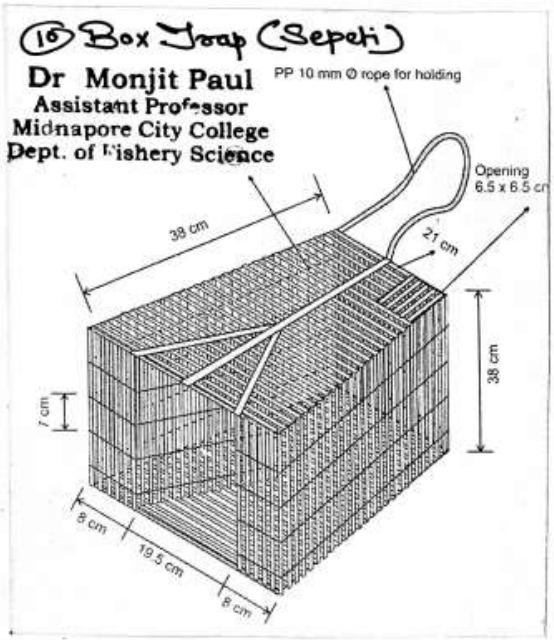

9) Box Trap (Dorki)

Size: 79 x 36 cm

Made of Chopped bamboo.

Area of operation: River, Cannals, Beels.

Target Species: Prawns, Eel & other fishes

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10) Box Trap (Sepeti)

Size: 98 x 35 cm

Made of Chopped bamboo.

Area of operation: Cannals, Rivers & Beels

Target Species: Catfishes & Miscellaneous

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BFSC-309: Aquaculture Engineering

Practical - 1

Experiment No 1 - Folding and unfolding of chain

Folding and unfolding of chain

Two chain men are required for measuring the length of a line, which is greater than a chain length. The more experienced chainman remains at the zero ends or the rear end of the chain who is called as the follower. The other chainman holding the forward handle is known as the leader.

Unfolding the Chain

To unfold a chain, the chainman keeps both the handles in the left hand and throws the rest of the chain in forward direction with his right hand. The other chainman assists in removing the knots, if any for making it straight.

Folding a Chain

Bring the two halves of the chain so as to make them lie along each other by pulling the chain in the middle. Then, starting from the middle portion take two pairs of links at a time with the right hand and place them obliquely across, with the left hand.

Experiment 2 - Line marking

Line marking

The follower holds the zero end of the chain at the terminal point while the leader proceeds forward with the other handle in one hand and a set of 10 arrows and a ranging rod in the other hand. When he is approximately one chain length away, the follower directs him to fix his pole in the line with the previous pole. When the point is ranged, the leader makes a mark on the ground, holds the handle with both the hands and pulls the chain so that it becomes straight between the terminal point and the point fixed. The leader then puts an arrow at the end of the chain, swings the chain slightly out of the line and proceeds further with the handle in one hand and the rest of the arrows and the ranging rod in the other.

The follower also takes the handle in one hand and the ranging rod in the other and follows the leader until the leader has approximately traveled one chain length. The follower puts the zero end of the chain at the first arrow fixed by the leader and ranges the leader who in turn stretches the chain straight in the line and fixes the second arrow in the ground and proceeds further.

Experiment 3 - Ranging

Ranging

While measuring the length of a „Survey Line" or „Chain line", the chain or tape must be stretched straight along the line joining its two terminal stations. If the length of the survey line is less than the length of the chain, there will be no difficulty in doing so. If the length of the survey line exceeds the length of the chain, intermediate points have to be established in line with the two terminal points before chaining is started. "The process of fixing or establishing such intermediate points is known as Ranging".

Ranging Code Signals

Direct ranging by eye

Direct ranging is done when the two ends of the survey lines are intervisible. In such cases ranging can either be done by eye or through some optical instruments such as line ranger or Theodolite.

Let A and B be the two points at the ends of a survey line. One ranging rod is

erected at the point „B" while the surveyor stands with another ranging rod at point

„A" holding the rod at about half meter length. The assistant then goes with another ranging rod and establishes the rod at a point approximately in the line with AB, at a distance not greater than one chain length from „A". The surveyor at

„A" then signals the assistant to move transverse to the chain line, till he is in line

with A and B. Similarly other intermediate points can be established.

Indirect ranging or Reciprocal ranging

Indirect ranging is resorted to when both the ends of the survey line are not intervisible either due to high intervening ground or due to long distance between them. In such a case, ranging is done indirectly by selecting two intermediate points M1 and N1 very near to the chain line in such a way that from M1 both N1 and B are visible, while from N1 both M1 and A are visible.

Two surveyors station themselves at M1 and N1 with ranging rods. The person at M1 then directs the person at N1 to move to a new position N2 in the line with M1B. The person at N2 then directs the person at M1 to move to new position M2 in line with N2A. Thus the two persons are now at M2 and N2 which are near to the chain line than the positions M1 and N1. The process is repeated till the points M and N are located in such a way that the person at M finds the person at N in

line with MB and the person at N finds the person at M in line with NA. After having established M and N other points can be fixed by direct ranging.

Experiment 4 - To erect a perpendicular to chain line

To erect perpendicular to a chain line from a point on it

AB is the chain line. It is required to erect a perpendicular to the chain line at point „C‟ on it. Establish a point „E‟ at a distance of 3m from „C‟. Take 10m tape and put the zero (0) end of the tape at „E‟ and the 10m end at „C‟. The 5th and 6th meter marks of the tape are brought together to form a loop of 1m. The tape is now stretched tight by fastening the ends E and C. The point „D‟ is thus established.

Angle DCE will be 90º.

To drop a perpendicular to chain line from a point outside it

AB is the chain line. It is required to drop a perpendicular to a chain line AB from point „D‟ out side it. Select any point „E‟ on the chain line. With „D‟ as center, and DE as radius, draw an arc to cut the chain line at F. Bisect EF at „C‟ and then CD is perpendicular to chain line AB.

Experiment 5 - To run a parallel to chain line through a given point To run a parallel to chain line through a given point

AB is the chain line; C is the given point through which parallel line is to be drawn. Through „C‟ drop a perpendicular CE to the chain line. Measure CE. Select any other point F on the line and erect a perpendicular FD. Make FD=EC. Join CD.

Practical – 2

Experiment 1- Obstacle to chaining and ranging

In chain surveying, sometimes the chainman is unable to measure the distance between two points directly, due to obstacles. Hence it has to be found out by indirect measurements. Basically there are three types of obstacles

Experiment 1: Obstacle to ranging but not chaining

In this type of obstacle the ends are not intervisible and it is quite common, except in a flat country.

AB is the line in which A and B are not visible from intermediate point on it. Through A, draw a random line AB1 in any convenient direction but as nearly towards B as possible. The point B1 should be chosen in such a way that B1 is visible from B and BB1 is perpendicular

to the random line. Measure BB1. Select C1 and D1 on the random line and erect perpendicular C1C and D1D on it. Join CD and prolong.

Experiment No 2 - Obstacle to chaining but not ranging

There are two types of obstacle in this case

When it is possible to chain round the obstacle

PQ is the chain line. Select two points A and B on either side of the obstacle. Setout equal perpendiculars AC and BD. Join CD and measure it. AB = CD.

When it is not possible to chain round the obstacle

AB is the chain line. Select a point „C‟ on the line and draw perpendicular CD to it. Bisect it at E. Erect a perpendicular to the line CD at „D‟. Range „F‟ in line with GE and measure DF. Then GC = DF.

Experiment 3 - Obstacle to both chaining and ranging Obstacle to both chaining and ranging

A building is the typical example for this type of obstacle. Select two points A and B on one side of the obstacle. Erect perpendiculars AC and BD of equal length.

Join CD and prolong it past the obstacle. Choose two points E and F on the line CD and erect perpendiculars EG and FH equal to that of AC or BD. Join GH and prolong it. Measure DE. BG = DE

Practical - 3

Experiment No 1 - Determination of elevation of different points

To find out the elevation (reduced levels (R.L.)) of different points shown on the ground with respect to a given arbitrary bench mark (B.M.) Apparatus: Dumpy level with accessories, Levelling staff etc.

Procedure

Practical - 4

Experiment No 1 - Determination of elevation between water source and pond site

Aim

To find out the difference of elevation between water source and pond site and to draw the profile of the ground by levelling.

Apparatus

Dumpy level with accessories, Levelling staff, Tape, Ranging rods, Arrows, Chain etc.

Procedure

- Stretch the chain along the centre line of the plot from the water source to pond site.
- Mark the stations along the central line at equal intervals. (Say 5, 10, 20 mt depending upon the slope of the ground.)
- Set up the instrument near the water source in the proceeding direction of work. So that the Bench Mark (B.M.) should be visible clearly. Carry out temporary adjustments to the instrument to bring it properly leveled.
- Take the back sight (B.S) by holding the leveling staff on the B.M. Find out the height of instrument (H.I.) for that set up of the instrument. Take the intermediate sight (I.S.) at different points; calculate the reduced level (R.L.) of the same. Take fore sight (F.S.) on the first turning point for same set up of the instrument and find out the R.L.
- Now, shift the instrument to a new position in the direction of pond site, so that the staff held at turning point 1 is clearly visible.
- Set up the instrument and level it properly by carrying out temporary adjustments. Note that the staff should be kept at the turning point 1 itself.
- Now take the B.S. on the turning point 1 who‟s R.L. is previously determined with respect to first position of the instrument. B.S. + R.L. of turning point 1 will give new H.I.
- Continue the same procedure until the pond site is reached. However, staff readings on that point will be F.S. only. Enter the above readings in the level book and calculate the R.L. of water source, turning point, height of instrument and R.L. of the site. Draw the profile of the ground to suitable scale

Practical - 5

Experiment No 1 - Plotting of the position of objects by radiation method Aim:

To plot the position of given ground points by radiation method.

Apparatus:

Plane table with accessories, Ranging rods, Tape, Arrows, etc.

In this method a ray is drawn from the instrument station towards the point, the distance is measured between the instrument station and the point, and the point is located by plotting to suitable scale the distance so measured. This method is more suitable when the distances are small and single instrument can control the points to be detailed.

Procedure:

Experiment No 2 - Plotting of the position of objects by intersection method Aim

To determine the distance between two inaccessible points (Inter section method)

Apparatus

Plane table with accessories, Tape, Ranging rods, Arrows etc.

Procedure

Practical - 6

Experiment No 1 - Drawing of contour lines by direct method Aim

To draw the contour lines for given elevations (Direct method)

Apparatus

Dumpy level, Levelling staff, Tape, Plane table with accessories, Arrows etc.

Procedure

- Set up the level at a convenient place, so that all the points and bench mark are visible properly.
- Do the “temporary adjustments” properly and make the line of sight exactly

horizontal.

- Take the back sight by focusing the telescope towards the staff held on the bench mark.
- Calculate the elevation of line of sight as height of instrument.
- The staff reading is calculated so that the bottom of the staff is at an elevation equal to the value of contour. Eg: when the height of instrument is 101.80mt, the staff reading to get a point on the contour of 100.00 m will be

1.8 m.

- Taking one contour at a time, the staff man is directed to keep the staff on the ground so that reading of 1.8 m is obtained every time (say A, B, C, D etc.)
- Set the plane table on the ground near to the levelling instrument at instrument station say 'X“. Level it. Transfer the ground point on to the sheet by means of plumbing fork, thus getting „x“ representing „X“.

- With the help of trough compass mark the north direction on the sheet Clamp the table.
- Pivoting the alidade about „x", sight it to the first point say „A" on the ground.

Draw a ray along the fiducial edge of the alidade.

- Measure the distance between ground station „X" and the point A. Plot this distance to suitable scale along the ray marked.
- Similarly sight the other points on the ground. Measure their distance and plot it on the sheet for same scale (say B, C, D etc.)
- Join these points to get the contour line for an elevation of 100 m.
- By using same procedure draw the other contour lines.

Practical - 7

Experiment No 1 - Drawing of contour lines by indirect method Aim

To draw the contour lines for given elevation (Indirect method)

Apparatus

Dumpy level, Levelling staff, Tape, Arrows etc.

Procedure

Practical - 8

Experiment No 1 - Earth work calculation for dyke preparation Longitudinal section

A given section is said to be in cutting or excavation, when the formation line at that section is lower than the existing ground. On the other hand a given section is said to be in filling or embankment, when the formation line at that section is higher than the existing ground.

The slope of the ground along a given line is known as the longitudinal slope. On the other hand slope of the ground perpendicular to the given line is known as cross slope or transverse slope. Cross section of earthen channel / dykes either in banking or in cutting is usually in the form of a trapezium and the quantity of earthwork may be calculated by the following method.

Volume of earth work = Cross sectional area x Length

The Cutting or filling areas of trapezoidal sections are calculated by the given equation,

$A = BY + KY2$

where,

A = Cross sectional area of the section B= Width of the section

Y= Depth of cut or Height of bank K= Slope of the section

Usually slope of the section is considered only horizontal to vertical.

Problems

1) An embankment is to be built to a length of 150 m, with top width 1 m and to a height of 3 m. The side slope recommended is 1:1. Calculate the quantity of soil required for it.

Given B=1mt. L=150 m. Y=3mt K=1

$A = BY + KY2 = 1x3 + 1x32 = 12\ m2$

Volume of soil required = A x L = 12 x 150 = 1800 m3

2) An embankment is to be built with 4 m. wide at the top with side slope 1 to 1 and. Height 3 m. Assuming the ground to be level, calculate the volume of earth required to construct an embankment if of 200 m. length.

Computation of volume of earth work in the ponds

1) Excavation is to be done to make a pond of 80 m. long, 50 m. width at the bottom and 3 m. deep. The side slopes of excavation is 1.5: 1 (H: V). Assuming the surface of the ground to be level, calculate the volume of earth work.

2) Excavation is to be made to dug a pond 30 m long, 20 m wide at the top and having side slope of 2:1 (H:V). Calculate the volume of excavation if the depth is 4 m. The ground surface is level.

Practical - 9

Experiment No 1 - Earth work calculation for channel making Aim:

Computation of volume of earth work in the channels.

Problems:

1. Compute the depth of cut and height of bank at the various sections along the

2. Calculate the quantity of earth work for 200 m. length channel in uniform ground with height of banks at the two ends being 1.0 m and 1.6 m. The formation width is 5 m, side slope is 2:1 (H: V). Assume that there is no transverse slope.

Use (i) Mid-sectional area method (ii) Mean sectional area method.

3. Compute the volume of earth work along the given line from the following data

Assume the following Formation width = 10 m. Side slope = 2:1

Use

4. Compute the volume of earth work from the following data

Formation width = 10 m Side slope

Practical - 10

Experiment No 1 - Distribution of grain size in coarse grained soil Aim

To find out the grain size distribution of coarse grained soil by sieving.

Apparatus

IS sieves, Pans, Vibrating machine, Weighing machine, Weights etc.

Procedure

- Collect some quantity of soil sample received from the field and dry it in the oven.
- Take 5 kg oven dried soil sample, keep it in a tray and soak it with water.
- Puddle the sample thoroughly in water and transfer the slurry to a 4.75 mm sieve which separates the sample into gravel and sand fractions. Collect the materials in separate containers.
- Sieve the dried sample retained in 4.75 mm sieve through 63 mm 20mm, 10mm and 4.75 mm by hand sieving. The mass of the material retained on each sieve should be recorded.
- Wash the material passed through 4.75 mm sieve through a 75 micron sieve to separate the silt and clay particles from the sand fraction. Collect the material retained on 75 micron sieve in container and keep it in the oven.

- The dried material retained on 75 micron sieve is carefully placed on top of the sieve pan of 2 mm size followed by 1mm, 600, 425, 300, 212, 150 and 75 micron size respectively.
- After pouring the soil into the top most sieve it is closed tightly by a lid and the whole set of sieve is kept in a vibrating machine. It is allowed to vibrate for 10-15 minutes.
- The weight of soil retained in each pan is found out by weighing.
- Cummulative weight of soil retained, its percentage weight and the percentage of finer is calculated.
- The material passing through 75 micron sieve may be used for sedimentation analysis.
- A graph is plotted on a semi-logarithms graph sheet taking sieve size or particle size as abscissa percentage finers as the ordinate.