M.Sc. IN FISHERY SCIENCE
LAB MANUAL
2nd Semester

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Fishery Science

MIDNAPORE CITY COLLEGE

PG – II Fisheries Science Lab Manual

FSC-295

PRACTICAL PAPER

(Core course - 4 credits: 50 Marks)

[University Examination - 50]

1. Determination of soil texture, Organic Carbon, Phosphorus, Potassium, Nitrogen, C/N ratio, pH etc.
2. Study of the physico-chemical characteristics of water: pH, Temperature, Dissolved oxygen, Productivity, Free CO2, Hardness, Salinity, Alkalinity, Transparency, Turbidity etc.
3. Identification of different macrophytes collected from freshwater ecosystem.
4. Study of benthic community, aquatic insects from different aquatic ecosystems.
5. Analysis of phytoplankton and zooplankton from different aquatic ecosystems.
6. Dissection & display of reproductive system (male and female) of fish.
7. Dissection and display of pituitary gland and preparation and preservation of PGE.
8. Study of reproductive phase, gonado-somatic index and condition factor of fish.
9. Study of fecundity, ova diameter of different fishes.
10. Study of fish handling and different fish preservation techniques.
11. Calculation of ice requirement for short term freezing.
12. Preparation of fish fillets.
13. Preparation of fish byproducts and value added products.
14. Isolation and identification of fish spoilage causing microbes and their culture.
15. Seminar presentation.

LAB-1: Determination of Soil Texture

Introduction: Soil texture refers to the proportion of sand, silt, and clay particles present in a soil sample. Determining soil texture is essential for understanding soil properties and suitability for different agricultural practices. This lab manual provides a step-by-step guide to determine soil texture using the hydrometer method.

Materials:

1. Soil samples
2. Graduated cylinders (1000 mL and 100 mL)
3. Pipettes (10 mL and 1 mL)
4. Hydrometer
5. Mechanical stirrer
6. Sodium hexametaphosphate (dispersing agent)
7. Distilled water
8. Balance
9. Oven

Procedure:

1. Sample Collection:

    a. Collect representative soil samples from different areas of interest.
    b. Remove any organic material, stones, or debris from the soil samples.
    c. Air-dry the samples thoroughly and break any clumps into fine particles. d. Store the samples in clean, labelled containers.

2. Sample Preparation:

    a. Weigh approximately 50 grams of the soil sample using a balance.
    b. Transfer the soil sample to a clean, dry 1000 mL graduated cylinder.
    c. Add distilled water to the cylinder until the volume reaches the 500 mL mark.
    d. Add 1 g of sodium hexametaphosphate (dispersing agent) to the cylinder.
    e. Mix the contents thoroughly using a mechanical stirrer to disperse soil particles.

3. Soil Dispersion:

    a. Allow the soil suspension to settle for about 40 minutes.
    b. Carefully decant the supernatant liquid, taking care not to disturb the settled particles.
    c. Transfer the suspended soil particles to a clean 100 mL graduated cylinder, avoiding any air bubbles.

4. Hydrometer Reading:

a. Immerse the hydrometer gently into the soil suspension, ensuring it does not touch the sides or bottom of the cylinder.
b. Allow the hydrometer to settle for about 5 minutes.
c. Record the initial hydrometer reading, taking note of the position of the meniscus.

5. Calculating Soil Texture:

a. After the initial reading, record subsequent hydrometer readings at regular intervals (e.g., every 30 seconds) for a total of 10 minutes.
b. Calculate the sedimentation rate by subtracting each reading from the initial reading.
c. Plot the sedimentation rate (in g/L/min) against time (in minutes).
d. Determine the time at which the sedimentation rate reaches 0.2 g/L/min.
e. Use a soil texture triangle to determine the soil texture based on the percentage of sand, silt, and clay present.

6. Clean-up:

a. Dispose of the soil suspension responsibly, following the regulations and guidelines of your institution.
b. Clean and dry all equipment thoroughly for future use.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results.

Conclusion: Determining soil texture is crucial for understanding soil properties and optimizing agricultural practices. The hydrometer method provides a reliable means to determine soil texture based on sedimentation rates. By following this lab manual, you can accurately assess the soil texture and classify it accordingly, allowing for informed decision-making in various agricultural applications.

LAB-2: Determination of Soil Organic Carbon by Walkley and Black Method

Introduction: Soil organic carbon (SOC) is an essential component of soil fertility and plays a vital role in nutrient cycling, soil structure, and overall soil health. The Walkley and Black method is a widely used procedure for determining SOC content in soil samples. This lab manual provides a step-by-step guide to determine soil organic carbon using the Walkley and Black method.

Materials:

1. Soil samples
2. Potassium dichromate solution (K2Cr2O7)
3. Concentrated sulphuric acid (H2SO4)
4. Orthophosphoric acid (H3PO4)
5. Fume hood or well-ventilated area
6. Conical flasks (250 mL)
7. Burette
8. Distilled water
9. Glass pipettes (10 mL and 1 mL)
10. Burette stand
11. Heat-resistant gloves
12. Safety goggles
13. Analytical balance

Procedure:

1. Sample Preparation:
    a. Collect representative soil samples from different areas of interest.
    b. Remove any stones, plant residues, or debris from the soil samples.
    c. Air-dry the samples and grind them to a fine powder using a mortar and pestle.
    d. Store the samples in clean, labelled containers.

2. Digestion and Oxidation:
    a. Weigh approximately 10 grams of the soil sample using an analytical balance.
    b. Transfer the soil sample to a clean, dry 250 mL conical flask.
    c. Add 20 mL of concentrated sulphuric acid (H2SO4) to the flask while stirring gently.
    d. Add 1 mL of orthophosphoric acid (H_3PO_4) to prevent foaming during the oxidation process.
    e. Place the flask on a hot plate in a fume hood or a well-ventilated area.
    f. Heat the flask gradually until the mixture turns dark brown, indicating the completion of digestion.
    g. Allow the flask to cool to room temperature.

3. Dichromate Titration:

a. Prepare a potassium dichromate solution ($K_2Cr_2O_7$) by dissolving 11.36 grams in 1 litre of distilled water.
b. Rinse and fill the burette with the potassium dichromate solution.
c. Pipette 50 mL of distilled water into a separate 250 mL conical flask as a blank.
d. Add the blank solution to the burette until the meniscus is near the zero mark.
e. Pipette 50 mL of the cooled soil extract from step 2 into another 250 mL conical flask.
f. Add a few drops of ferrous sulphate solution (FeSO4) as an indicator.
g. Titrate the soil extract with the potassium dichromate solution until the solution changes from green to a reddish-brown colour.
h. Record the volume of potassium dichromate solution used (in mL).

4. Calculation of Soil Organic Carbon:

a. Calculate the soil organic carbon (SOC) content using the following formula:

$$\text{SOC (\%)} = \frac{Vol.of\ K_2Cr_2O_7\ solution\ used \times Normality\ of\ K_2Cr_2O_7\ solution \times 0.003 \times 100}{Weight\ of\ soil\ sample\ (g)}$$

5. Clean-up:

a. Dispose of the hazardous waste (digestion mixture) following the regulations and guidelines of your institution.
b. Clean the glassware thoroughly with water and detergent.
c. Dry the glassware for future use.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results. Follow all safety precautions while working with hazardous chemicals.

Conclusion: The Walkley and Black method provides a reliable and widely accepted technique for determining soil organic carbon content. By following this lab manual, you can accurately quantify the soil organic carbon in your samples, providing valuable information for soil fertility assessment and management practices.

LAB-3: Determination of Soil Potassium Level

Introduction: Potassium (K) is an essential nutrient for plant growth and plays a crucial role in various physiological processes. Determining the potassium level in soil is important for assessing soil fertility and making informed decisions regarding fertilizer application. This lab manual provides a step-by-step guide to determine soil potassium level using the ammonium acetate extraction method.

Materials:

1. Soil samples
2. Ammonium acetate solution (1 M)
3. Distilled water
4. Conical flasks (250 mL)
5. Burette
6. Flame photometer or atomic absorption spectrophotometer
7. Glass pipettes (10 mL and 1 mL)
8. Burette stand
9. Safety goggles
10. Gloves
11. Analytical balance

Procedure:

1. Sample Collection:
    a. Collect representative soil samples from different areas of interest.
    b. Remove any plant residues, stones, or debris from the soil samples.
    c. Air-dry the samples thoroughly and break any clumps into fine particles.
    d. Store the samples in clean, labelled containers.
2. Sample Preparation:
    a. Weigh approximately 10 grams of the soil sample using an analytical balance.
    b. Transfer the soil sample to a clean, dry 250 mL conical flask.
    c. Add 50 mL of 1 M ammonium acetate solution to the flask.
    d. Seal the flask with a stopper and shake it vigorously for 30 minutes to extract potassium from the soil.
    e. Allow the flask to settle for 10 minutes.
3. Filtration:
    a. Prepare a filter paper and funnel assembly.
    b. Place the filter paper inside the funnel and wet it with distilled water.
    c. Filter the contents of the conical flask through the filter paper into a clean container.
    d. Collect the filtrate, which contains the extracted potassium.

4. Potassium Analysis:

    a. Set up the flame photometer or atomic absorption spectrophotometer according to the manufacturer's instructions.
    b. Calibrate the instrument using standard potassium solutions of known concentrations.
    c. Pipette a suitable volume (as recommended by the instrument) of the filtrate into a clean sample cup.
    d. Measure the potassium concentration in the filtrate using the flame photometer or atomic absorption spectrophotometer.
    e. Record the potassium concentration in units of your instrument's measurement (e.g., mg/L or ppm).

5. Calculation of Soil Potassium Level:

    a. Calculate the soil potassium level by multiplying the potassium concentration by the dilution factor (if any).
    b. Convert the potassium level to the desired units (e.g., mg/kg or ppm) if necessary.

6. Clean-up:

    a. Dispose of the filtrate and waste materials responsibly, following the regulations and guidelines of your institution.
    b. Clean the glassware thoroughly with water and detergent.
    c. Dry the glassware for future use.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results.

Conclusion: Determining the soil potassium level is crucial for understanding soil fertility and making appropriate fertilizer recommendations. The ammonium acetate extraction method provides a reliable means to extract and quantify potassium from soil samples. By following this lab manual, you can accurately determine the soil potassium level and use the information for informed decision-making in agricultural practices.

LAB-4: Determination of Soil Nitrogen Level

Introduction: Nitrogen (N) is a vital nutrient for plant growth and plays a significant role in plant development, protein synthesis, and overall crop productivity. Determining the nitrogen level in soil is essential for assessing soil fertility and making informed decisions regarding fertilizer application. This lab manual provides a step-by-step guide to determine soil nitrogen level using the Kjeldahl method.

Materials:

1. Soil samples
2. Sulphuric acid (H_2SO_4)
3. Digestion catalyst (e.g., potassium sulphate, copper sulphate)
4. Distilled water
5. Sodium hydroxide (NaOH)
6. Boric acid (H_3BO_3)
7. Phenolphthalein indicator
8. Ammonium sulphate standard solution
9. Conical flasks (250 mL)
10. Burette
11. Burette stand
12. Titration apparatus
13. Safety goggles
14. Gloves
15. Analytical balance

Procedure:

1. Sample Collection:

    a. Collect representative soil samples from different areas of interest.
    b. Remove any plant residues, stones, or debris from the soil samples.
    c. Air-dry the samples thoroughly and break any clumps into fine particles. d. Store the samples in clean, labelled containers.

2. Sample Preparation:

    a. Weigh approximately 10 grams of the soil sample using an analytical balance.
    b. Transfer the soil sample to a clean, dry 250 mL conical flask.
    c. Add 10 mL of concentrated sulphuric acid (H_2SO_4) to the flask.
    d. Add a digestion catalyst (e.g., potassium sulphate, copper sulphate) to enhance the digestion process.
    e. Heat the flask gently over a Bunsen burner or a digestion apparatus until the mixture reaches a dark brown colour.
    f. Continue heating until white fumes of sulphuric acid disappear and a clear solution is obtained. g. Allow the flask to cool to room temperature.

3. Distillation:

    a. Add approximately 150 mL of distilled water to the cooled flask and mix well.
    b. Transfer the contents of the flask to a Kjeldahl flask or a distillation apparatus.
    c. Add a few drops of phenolphthalein indicator to the flask.
    d. Add sodium hydroxide (NaOH) solution gradually until the solution turns slightly pink.
    e. Connect the distillation apparatus and collect the distillate in a receiving flask containing a known volume of boric acid (H_3BO_3) solution.
    f. Ensure that the distillation setup is airtight to prevent any loss of nitrogen.

4. Titration:

    a. Prepare a standard solution of ammonium sulphate by dissolving a known mass of ammonium sulphate in a specific volume of distilled water.
    b. Pipette a suitable volume (as recommended by the titration method) of the distillate into a conical flask.
    c. Add a few drops of phenolphthalein indicator to the flask.
    d. Titrate the distillate with the ammonium sulphate standard solution until the pink colour changes to a pale yellow or colourless endpoint.
    e. Record the volume of ammonium sulphate solution used (in mL).

5. Calculation of Soil Nitrogen Level:

    a. Calculate the soil nitrogen level using the following formula:
    Nitrogen (%) =

$$\frac{Volume\ of\ (NH_4)_2SO_4\ solution\ used\ \times\ Nitrogen\ concentration\ of\ (NH_4)_2SO_4\ solution\ \times\ 14\ \times\ 100}{Weight\ of\ soil\ sample\ (g)}$$

6. Clean-up:

    a. Dispose of the waste materials responsibly, following the regulations and guidelines of your institution.
    b. Clean the glassware thoroughly with water and detergent.
    c. Dry the glassware for future use.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results.

Conclusion: Determining the soil nitrogen level is crucial for assessing soil fertility and optimizing fertilizer management practices. The Kjeldahl method provides a reliable means to extract and quantify nitrogen from soil samples. By following this lab manual, you can accurately determine the soil nitrogen level and use the information for informed decision-making in agricultural practices.

LAB-5: Determination of C/N Ratio of Soil

Lab Manual - Determination of C/N Ratio of Soil

Introduction: The carbon-to-nitrogen (C/N) ratio is a fundamental parameter in soil science that provides insights into soil organic matter decomposition, nutrient cycling, and microbial activity. The C/N ratio is calculated by determining the carbon (C) and nitrogen (N) contents of the soil sample. This lab manual provides a step-by-step guide to determine the C/N ratio of soil using the combustion method.

Materials:

1. Soil samples
2. Combustion apparatus (e.g., elemental analyser)
3. Crucibles or combustion boats
4. Desiccator
5. Balance
6. Safety goggles
7. Gloves
8. Laboratory furnace (if needed)
9. Calculators or computers for data analysis

Procedure:

1. Sample Collection:
    a. Collect representative soil samples from different areas of interest.
    b. Remove any plant residues, stones, or debris from the soil samples.
    c. Air-dry the samples thoroughly and break any clumps into fine particles.
    d. Store the samples in clean, labelled containers.
2. Sample Preparation:
    a. Weigh approximately 5 grams of the soil sample using an analytical balance.
    b. Transfer the soil sample to a crucible or combustion boat.
    c. Place the crucible or combustion boat in a desiccator to ensure the sample is completely dry.
    d. Record the weight of the soil sample.
3. Combustion:
    a. Use a combustion apparatus (such as an elemental analyser) following the manufacturer's instructions.
    b. Load the dried soil sample into the combustion apparatus.
    c. Initiate the combustion process according to the instrument's operating procedures.
    d. Allow the instrument to run until complete combustion of the sample occurs, releasing carbon dioxide (CO_2) and nitrogen gas (N_2).

e. The instrument will measure the released gases and provide data on the carbon and nitrogen content.

4. Data Analysis:

   a. Record the carbon and nitrogen values obtained from the combustion apparatus.
   b. Calculate the C/N ratio using the following formula:
      C/N ratio = $\frac{Carbon\ content\ (in\ g/kg)}{Nitrogen\ content\ (in\ g/kg)}$

5. Clean-up:

   a. Dispose of the waste materials responsibly, following the regulations and guidelines of your institution.
   b. Clean the crucibles or combustion boats and other equipment thoroughly.

Note: Ensure that the combustion apparatus is properly calibrated and maintained for accurate results. It is recommended to perform multiple replicates and calculate the average C/N ratio for more reliable data.

Conclusion: Determining the C/N ratio of soil is valuable for assessing soil quality and understanding nutrient cycling processes. The combustion method provides a reliable means to determine the C/N ratio by measuring the carbon and nitrogen contents of the soil sample. By following this lab manual, you can accurately calculate the C/N ratio and gain insights into the organic matter dynamics and nutrient availability in the soil.

LAB-6: Determination of Soil pH

Introduction: Soil pH is a fundamental parameter that influences various soil properties, nutrient availability, and microbial activity. Determining the pH of soil is essential for understanding soil acidity or alkalinity and making informed decisions regarding soil management and crop productivity. This lab manual provides a step-by-step guide to determine soil pH using the potentiometric method.

Materials:

1. Soil samples
2. Distilled water
3. pH meter or pH indicator paper/strips
4. Beakers or plastic cups
5. Stirring rod or glass rod
6. pH buffer solutions (pH 4, 7, and 10)
7. Safety goggles
8. Gloves

Procedure:

1. Sample Collection:

    a. Collect representative soil samples from different areas of interest.
    b. Remove any plant residues, stones, or debris from the soil samples.
    c. Air-dry the samples thoroughly and break any clumps into fine particles.
    d. Store the samples in clean, labelled containers.

2. Sample Preparation:

    a. Measure approximately 10 grams of the soil sample using an analytical balance.
    b. Transfer the soil sample to a clean beaker or plastic cup.
    c. Add distilled water to the beaker or cup in a 1:1 soil-to-water ratio (e.g., 10 grams of soil with 10 mL of water).
    d. Stir the mixture well using a stirring rod or glass rod to ensure proper mixing.
    e. Allow the mixture to sit for about 30 minutes to allow the soil particles to settle.

3. pH Measurement:

    a. Calibrate the pH meter according to the manufacturer's instructions, using pH buffer solutions of known pH values (e.g., pH 4, 7, and 10).
    b. If using pH indicator paper/strips, ensure they are appropriate for the pH range being tested (e.g., 0-14).
    c. Immerse the pH electrode of the pH meter into the soil-water mixture or place a small strip of indicator paper into the mixture.

d. Wait for the pH reading to stabilize on the pH meter or observe the colour change on the indicator paper.
e. Record the pH reading or note the corresponding colour on the indicator paper.

4. Calibration Check:

a. Rinse the pH electrode of the pH meter with distilled water after each measurement.
b. Periodically check the calibration of the pH meter using pH buffer solutions to ensure accuracy throughout the analysis.

5. Clean-up:

a. Dispose of the soil-water mixture responsibly, following the regulations and guidelines of your institution.
b. Clean the beakers, stirring rods, pH meter, or other equipment thoroughly.

Note: It is recommended to perform pH measurements in multiple locations within each soil sample and calculate the average pH value for more accurate results. If using pH indicator paper/strips, refer to the colour chart provided by the manufacturer to determine the corresponding pH value.

Conclusion: Determining the pH of soil is essential for understanding soil acidity or alkalinity, which greatly affects plant growth and nutrient availability. The potentiometric method provides a reliable means to measure soil pH using a pH meter or pH indicator paper/strips. By following this lab manual, you can accurately determine the pH of soil samples and use the information for informed decision-making in soil management practices.

LAB-7: Determination of Water pH

Introduction: pH is a measure of the acidity or alkalinity of a solution and is an important parameter in various fields, including environmental science, chemistry, and biology. Determining the pH of water is essential for assessing its quality, understanding chemical reactions, and ensuring appropriate conditions for aquatic life. This lab manual provides a step-by-step guide to determine the pH of water using a pH meter or pH indicator paper/strips.

Materials:

1. Water samples
2. pH meter or pH indicator paper/strips
3. pH buffer solutions (pH 4, 7, and 10)
4. Beakers or plastic cups
5. Stirring rod or glass rod
6. Distilled water (if needed for calibration or dilution)
7. Safety goggles
8. Gloves

Procedure:

1. Sample Collection:
    a. Collect a representative water sample in a clean, labelled container.
    b. Ensure that the container is free from contaminants and residues that could affect the pH measurement.
    c. If necessary, collect a sufficient quantity of water to perform multiple pH measurements or replicate the analysis.
2. pH Meter Calibration (if using a pH meter):
    a. Calibrate the pH meter according to the manufacturer's instructions, using pH buffer solutions of known pH values (e.g., pH 4, 7, and 10).
    b. Rinse the pH electrode with distilled water before and after each calibration.
3. pH Measurement:
    a. If using a pH meter:
        i) Immerse the pH electrode of the pH meter into the water sample, ensuring that the electrode is fully submerged.
        ii) Wait for the pH reading to stabilize on the pH meter display.
        iii) Record the pH reading.
    b. If using pH indicator paper/strips:
        i) Dip a strip of indicator paper into the water sample for a few seconds, ensuring the entire strip is wet.
        ii) Remove the strip and wait for the color to develop.

iii) Compare the color of the indicator paper with the color chart provided by the manufacturer.
iv) Note the corresponding pH value.

4. Calibration Check:

    a. If using a pH meter, periodically check the calibration using pH buffer solutions to ensure accuracy throughout the analysis.
    b. Rinse the pH electrode with distilled water after each measurement.

5. Clean-up:

    a. Dispose of the water sample responsibly, following the regulations and guidelines of your institution.
    b. Clean the beakers, stirring rods, pH meter, or other equipment thoroughly.

Note: It is recommended to perform pH measurements in duplicate or triplicate to ensure reproducibility and calculate the average pH value for more accurate results. If using pH indicator paper/strips, ensure they are appropriate for the pH range being tested.

Conclusion: Determining the pH of water is crucial for assessing its quality and understanding chemical characteristics. Whether using a pH meter or pH indicator paper/strips, accurate pH measurements provide valuable information for various applications. By following this lab manual, you can effectively determine the pH of water samples and utilize the data for informed decision-making in environmental, chemical, and biological studies.

LAB-8: Determination of Dissolved oxygen

Introduction: Dissolved oxygen (DO) is a critical parameter in water quality analysis as it directly affects aquatic organisms and indicates the level of oxygen available for aquatic life. Determining the DO content in water provides insights into its suitability for aquatic habitats and helps assess the impact of pollutants on water bodies. This lab manual provides a step-by-step guide to determine dissolved oxygen in water using the Winkler titration method.

Materials:

1. Water samples
2. BOD (Biological Oxygen Demand) bottles or airtight glass containers
3. Sodium thiosulfate solution (0.025 N)
4. Manganous sulphate solution
5. Alkaline iodide-azide reagent
6. Sulphuric acid (concentrated)
7. Starch indicator solution
8. Burette
9. Burette stand
10. Pipettes (10 mL and 1 mL)
11. Volumetric flask (100 mL)
12. Magnetic stirrer or stirring rod
13. Safety goggles
14. Gloves
15. Analytical balance

Procedure:

1. Sample Collection:
    a. Collect a representative water sample in a clean, labelled BOD bottle or airtight glass container.
    b. Fill the container to the brim to avoid introducing air bubbles.
    c. Store the sample in a cool, dark place to preserve the dissolved oxygen until the analysis.
2. Sample Preparation:
    a. Measure 10 mL of the water sample using a pipette and transfer it to a clean BOD bottle or glass container.
    b. Add 1 mL of manganous sulphate solution to the BOD bottle.
    c. Add 1 mL of alkaline iodide-azide reagent to the BOD bottle.
    d. Close the bottle tightly and mix the contents thoroughly by inverting the bottle several times.
3. Winkler Titration:

a. Prepare an acidified iodide solution by adding 5 mL of concentrated sulfuric acid to 100 mL of distilled water in a volumetric flask.
b. Transfer the contents of the BOD bottle into a conical flask.
c. Add 10 mL of acidified iodide solution to the conical flask.
d. Titrate the mixture immediately with sodium thiosulfate solution (0.025 N) until the yellow colour of iodine disappears.
e. Add a few drops of starch indicator solution and continue titration until the blue colour disappears, indicating the endpoint of the titration.
f. Record the volume of sodium thiosulfate solution used (in mL).

4. Calculation of Dissolved Oxygen:

    a. Calculate the dissolved oxygen content using the following formula:
    Dissolved Oxygen (mg/L) =
    Volume of sodium thiosulfate solution used (in mL) × 0.008 × 1000 / Volume of water sample (in mL)

5. Clean-up:

    a. Dispose of the chemicals and waste materials responsibly, following the regulations and guidelines of your institution.
    b. Clean the glassware thoroughly with water and detergent.
    c. Dry the glassware for future use.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results. Keep the samples and reagents away from light to minimize potential inaccuracies.

Conclusion: Determining dissolved oxygen in water is crucial for assessing water quality and understanding the oxygen availability for aquatic organisms. The Winkler titration method provides a reliable means to quantify dissolved oxygen in water samples. By following this lab manual, you can accurately determine the dissolved oxygen content and utilize the data for informed decision-making in environmental monitoring and management.

LAB-8: Determination of Primary Productivity in Water

Introduction: Primary productivity is the rate at which autotrophic organisms, such as algae and aquatic plants, convert light energy into organic matter through photosynthesis. Measuring primary productivity in water is essential for understanding ecosystem dynamics and assessing the health and productivity of aquatic environments. This lab manual provides a step-by-step guide to determine primary productivity in water using the light and dark bottle method.

Materials:

1. Water samples
2. Light and dark bottles (BOD bottles or similar)
3. Incubator or temperature-controlled chamber
4. Light source (e.g., fluorescent light)
5. pH meter or pH indicator paper/strips
6. Spectrophotometer or colorimeter (if using chlorophyll-a method)
7. Burette
8. Burette stand
9. Pipettes (10 mL and 1 mL)
10. Sodium bicarbonate ($NaHCO_3$) solution (0.1 M)
11. Sodium thiosulfate solution (0.025 N)
12. Analytical balance
13. Safety goggles
14. Gloves

Procedure:

1. Sample Collection:
    a. Collect a representative water sample from the desired location in a clean, labelled light bottle.
    b. Collect a second water sample in a dark bottle, ensuring it is completely covered to prevent light exposure.
    c. Note the date, time, and location of each sample collection.
2. Sample Preparation:
    a. Measure 100 mL of each water sample using a graduated cylinder and transfer them to separate light and dark bottles.
    b. Add 2 mL of sodium bicarbonate solution (0.1 M) to each bottle to provide a carbon source for photosynthesis.
    c. Mix the contents gently by inverting the bottles a few times.
3. Incubation:

a. Place the light bottle under a light source (e.g., fluorescent light) that approximates natural sunlight conditions.
b. Place the dark bottle in a light-sealed incubator or temperature-controlled chamber.
c. Ensure that both bottles are maintained at the same temperature.

4. Incubation Duration:

a. Incubate the light and dark bottles for a specific period, typically 24 hours, to allow for photosynthesis and respiration processes.
b. Ensure that the light bottle receives continuous light exposure, while the dark bottle remains in complete darkness.

5. DO Measurement:

a. After the incubation period, remove the light and dark bottles from their respective environments.
b. Measure the dissolved oxygen (DO) content in each bottle using a DO meter or the Winkler titration method described in a previous lab manual.
c. Record the DO readings for the light and dark bottles.

6. Calculation of Primary Productivity:

a. Calculate the net primary productivity (NPP) using the following formula:
NPP = DO Light Bottle - DO Dark Bottle

7. Additional Method (Chlorophyll-a):

a. Measure the chlorophyll-content in the water samples using a spectrophotometer or colorimeter according to established protocols.
b. Calculate the primary productivity using the chlorophyll-a concentration and the light and dark bottle readings.

8. Clean-up:

a. Dispose of the water samples responsibly, following the regulations and guidelines of your institution.
b. Clean the bottles, glassware, and equipment thoroughly.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results. Ensure proper calibration and maintenance of measurement instruments. Adjust the incubation duration or other parameters as necessary for specific study requirements.

Conclusion: Measuring primary productivity in water provides valuable insights into the productivity and functioning of aquatic ecosystems.

LAB-9: Determination of Alkalinity by Titrimetric Method

Introduction: Alkalinity is a measure of the buffering capacity of water and is an important parameter in assessing water quality and its ability to neutralize acids. Determining alkalinity helps understand the water's capacity to resist changes in pH and its suitability for various applications. This lab manual provides a step-by-step guide to determine alkalinity in water using the titrimetric method.

Materials:

1. Water samples
2. Burette
3. Burette stand
4. 0.02 N sulphuric acid (H2SO4) solution
5. Methyl orange indicator solution
6. Phenolphthalein indicator solution
7. Graduated cylinder
8. Conical flask
9. Safety goggles
10. Gloves

Procedure:

1. Sample Collection:
    a. Collect a representative water sample in a clean, labelled container.
    b. Ensure that the container is free from contaminants that may affect the alkalinity measurement.
    c. Note the date, time, and location of sample collection.
2. Sample Preparation:
    a. Measure 100 mL of the water sample using a graduated cylinder and transfer it to a clean conical flask.
3. Titration:
    a. Add a few drops of phenolphthalein indicator solution to the water sample in the conical flask.
    b. Set up the burette on the burette stand and fill it with 0.02 N sulfuric acid (H^2SO^4) solutions.
    c. Slowly add the sulphuric acid solution from the burette to the conical flask containing the water sample while stirring continuously.
    d. Continue adding the acid solution until a light pink colour persists for at least 30 seconds. e. Record the volume of sulphuric acid solution used (in mL).
4. Calculation of Alkalinity:

a. Calculate the alkalinity of the water sample using the following formula:
   Alkalinity (mg/L as $CaCO_3$) =
   Volume of sulphuric acid solution used (in mL) × Equivalent factor × 5000 / Volume of water sample (in mL)

5. Additional Titration (optional):

   a. Repeat the titration process using methyl orange indicator solution instead of phenolphthalein to determine the presence of carbonate alkalinity.
   b. Record the volume of sulphuric acid solution used (in mL) and calculate the carbonate alkalinity using the appropriate formula.

6. Clean-up:

   a. Dispose of the water sample and residual chemicals responsibly, following the regulations and guidelines of your institution.
   b. Clean the conical flask, burette, and other equipment thoroughly.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results. Ensure proper calibration and maintenance of measurement instruments. Adjust the concentration of sulfuric acid solution or other parameters as necessary for specific study requirements.

Conclusion: Determining alkalinity in water is essential for understanding its buffering capacity and suitability for various applications. The titrimetric method provides a reliable means to measure alkalinity accurately. By following this lab manual, you can effectively determine the alkalinity of water samples and utilize the data for informed decision-making in water quality assessments and environmental monitoring.

LAB-10: Determination of Hardness

Introduction: Hardness is a measure of the concentration of calcium and magnesium ions in water. High levels of hardness can lead to scale buildup, interfere with soap effectiveness, and cause other operational issues. Determining the hardness of water is crucial for assessing its suitability for various applications. This lab manual provides a step-by-step guide to determine water hardness using the EDTA (Ethylene Diamine Tetraacetic Acid) titration method.

Materials:

1. Water samples
2. Erlenmeyer flasks
3. Burette
4. Burette stand
5. EDTA solution (0.01 M)
6. Eriochrome Black T indicator solution
7. Calcium carbonate (CaCO3) standard solution
8. Buffer solution (pH 10)
9. Magnetic stirrer or stirring rod
10. Safety goggles
11. Gloves

Procedure:

1. Sample Collection:
    a. Collect a representative water sample in a clean, labelled container.
    b. Ensure that the container is free from contaminants that may affect the hardness measurement.
    c. Note the date, time, and location of sample collection.
2. Sample Preparation:
    a. Measure 50 mL of the water sample using a graduated cylinder and transfer it to a clean Erlenmeyer flask.
    b. If the water sample contains visible turbidity, filter it using a filter paper or filtration apparatus to remove any particulate matter.
3. Titration:
    a. Add a few drops of Eriochrome Black T indicator solution to the water sample in the Erlenmeyer flask.
    b. Add 2 mL of buffer solution (pH 10) to the flask and mix well.
    c. Set up the burette on the burette stand and fill it with the EDTA solution (0.01 M).

d. Slowly add the EDTA solution drop by drop to the water sample while stirring continuously.
e. Continue the titration until the colour of the solution changes from wine-red (indicating the presence of metal ions) to blue (indicating the formation of a metal-EDTA complex).
f. Note the volume of EDTA solution used (in mL).

4. Calibration Check:

    a. Perform a blank titration using the same procedure but without adding the water sample. This blank titration corrects for any background colour changes.
    b. Record the volume of EDTA solution used (in mL) for the blank titration.

5. Calculation of Hardness:

    a. Calculate the hardness of the water sample using the following formula:
    Hardness (mg/L as $CaCO_3$) =
    [(Volume of EDTA solution used - Volume of EDTA solution used for blank) × Equivalent factor × 1000] / Volume of water sample (in mL)

6. Clean-up:

    a. Dispose of the water sample and residual chemicals responsibly, following the regulations and guidelines of your institution.
    b. Clean the Erlenmeyer flask, burette, and other equipment thoroughly.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results. Ensure proper calibration and maintenance of measurement instruments. Adjust the concentration of EDTA solution or other parameters as necessary for specific study requirements.

Conclusion: Determining the hardness of water is crucial for understanding its quality and assessing its suitability for various applications. The EDTA titration method provides an accurate means to measure water hardness. By following this lab manual, you can effectively determine the hardness of water samples and utilize the data for informed decision-making in water treatment, industrial processes, and environmental assessments.

LAB-11: Determination of Free Carbon Dioxide (CO_2) in Water

Introduction: The presence of free carbon dioxide (CO_2) in water can affect its chemical composition, pH, and overall quality. Measuring the concentration of free CO_2 is important for understanding the carbon cycle, assessing water quality, and studying the impact of CO_2 on aquatic ecosystems. This lab manual provides a step-by-step guide to determine the free CO_2 content in water using the acid-base titration method.

Materials:

1. Water samples
2. Burette
3. Burette stand
4. Sodium hydroxide (NaOH) solution (approximately 0.1 M)
5. Phenolphthalein indicator solution
6. Graduated cylinder
7. Conical flask
8. Magnetic stirrer or stirring rod
9. Safety goggles
10. Gloves

Procedure:

1. Sample Collection:
    a. Collect a representative water sample in a clean, labeled container.
    b. Ensure that the container is free from contaminants that may affect the free CO_2 measurement.
    c. Note the date, time, and location of sample collection.
2. Sample Preparation:
    a. Measure 100 mL of the water sample using a graduated cylinder and transfer it to a clean conical flask.
3. Titration:
    a. Add a few drops of phenolphthalein indicator solution to the water sample in the conical flask.
    b. Set up the burette on the burette stand and fill it with sodium hydroxide (NaOH) solution.
    c. Start adding the NaOH solution from the burette slowly into the conical flask while stirring continuously.
    d. Continue the titration until a faint pink colour persists for at least 30 seconds.
    e. Note the volume of NaOH solution used (in mL).
4. Calculation of Free CO2:

a. Calculate the free CO_2 content of the water sample using the following formula:
   Free CO_2 (mg/L) =
   [(Volume of NaOH solution used × Equivalent weight of CO_2 × 1000) / Volume of water sample (in mL)]

5. Clean-up:

   a. Dispose of the water sample and residual chemicals responsibly, following the regulations and guidelines of your institution.
   b. Clean the conical flask, burette, and other equipment thoroughly.

Note: It is recommended to perform multiple replicates and calculate the average values for more accurate results. Adjust the concentration of NaOH solution or other parameters as necessary for specific study requirements.

Conclusion: Determining the concentration of free carbon dioxide in water provides valuable information about its chemical composition and potential impacts on aquatic ecosystems. The acid-base titration method outlined in this lab manual offers a reliable means to measure free CO2 accurately. By following this manual, you can effectively determine the free CO2 content in water samples and utilize the data for environmental monitoring, research, and water quality assessments.

LAB-12: Determination of Turbidity in Water

Introduction: Turbidity is a measure of the clarity of water and indicates the presence of suspended particles or solids. High turbidity levels can affect water quality, hinder light penetration, and impact aquatic ecosystems. Determining turbidity is essential for assessing the suitability of water for various purposes. This lab manual provides a step-by-step guide to determine turbidity in water using a turbidimeter or a simple visual comparison method.

Materials:

1. Water samples
2. Turbidimeter (or a visual comparison tube)
3. Turbidity standards (Formazin standard solutions or pre-calibrated turbidity standards)
4. Graduated cylinder
5. Distilled water
6. Safety goggles
7. Gloves

Procedure:

1. Sample Collection:

    a. Collect a representative water sample in a clean, labelled container.
    b. Ensure that the container is free from contaminants that may affect the turbidity measurement.
    c. Note the date, time, and location of sample collection.

2. Turbidity Measurement with a Turbidimeter:

    a. Calibrate the turbidimeter according to the manufacturer's instructions, using distilled water as the blank.
    b. Insert the appropriate turbidity standard into the turbidimeter and record the reading.
    c. Rinse the turbidimeter cell with distilled water between measurements.
    d. Insert the water sample into the turbidimeter cell and record the turbidity reading.
    e. Repeat the measurement for multiple replicates and calculate the average turbidity value.

3. Turbidity Measurement with Visual Comparison:

    a. Fill a graduated cylinder with a known volume of water sample (e.g., 100 mL).
    b. Allow the suspended particles to settle for a few minutes.
    c. Choose a turbidity standard of known turbidity value (e.g., 100 NTU).

d. Hold the graduated cylinder and the turbidity standard side by side and observe them under consistent lighting conditions.
e. Adjust the water sample volume, if necessary, to match the turbidity of the standard. f. Record the turbidity value based on the turbidity standard selected.

4. Clean-up:

a. Dispose of the water sample responsibly, following the regulations and guidelines of your institution.
b. Clean the turbidimeter cell, graduated cylinder, and other equipment thoroughly.

Note: Ensure proper calibration and maintenance of the turbidimeter. Use the appropriate turbidity standard range that matches the expected turbidity levels of the water sample. Adjust the measurement method and volume according to the instrument and standards available.

Conclusion: Determining turbidity in water is essential for evaluating water quality and understanding its suitability for various applications. The turbidimeter or visual comparison method described in this lab manual provides a means to measure turbidity accurately. By following this manual, you can effectively determine the turbidity of water samples and utilize the data for environmental assessments, water treatment, and research purposes.

LAB-13: Determination of Transparency by Secchi Disk Method

Introduction: Transparency, also known as water clarity, is a measure of the penetration of light through water. It provides valuable information about the presence of suspended particles and the overall quality of water bodies. The Secchi disk method is a widely used technique to determine transparency. This lab manual provides a step-by-step guide to measure water transparency using the Secchi disk method.

Materials:

1. Water samples
2. Secchi disk (black and white, with marked increments)
3. Measuring tape or rope (sufficient length to reach the desired depth)
4. Boat or other means of accessing the water body (if necessary)
5. Notebook or data sheet for recording measurements
6. Safety goggles
7. Gloves

Procedure:

1. Sample Collection:
    a. Select the location(s) from which you will measure transparency.
    b. If needed, access the water body by boat or other means.
    c. Note the date, time, and location of each measurement.
2. Secchi Disk Preparation:
    a. Attach a measuring tape or rope to the Secchi disk, ensuring it is securely fastened.
    b. Lower the Secchi disk into the water, allowing it to fully submerge to wet it and remove any air bubbles.
    c. Lift the disk out of the water and ensure it is clean and free from any debris or contaminants.
3. Transparency Measurement:
    a. Stand at a point where you have a clear view of the Secchi disk and the water surface.
    b. Slowly lower the Secchi disk into the water, holding the tape or rope vertically and keeping it taut.
    c. Gradually release the tape or rope, allowing the disk to descend freely until it is no longer visible.
    d. Note the depth (in meters or feet) at which the Secchi disk disappears from view. This is the "Secchi depth."
    e. Slowly raise the disk until it becomes visible again, and note the depth at which it reappears. This is the "reappearing Secchi depth." f. If the water body

has varying transparency, take additional measurements at different locations and depths.

4. Calculation and Data Analysis:

    a. Calculate the average of the Secchi depth and reappearing Secchi depth for each measurement location.
    b. Compute the transparency index (Secchi transparency) by averaging the Secchi depth and reappearing Secchi depth.
    c. Record all measurements and calculated values in your notebook or data sheet.

5. Clean-up:

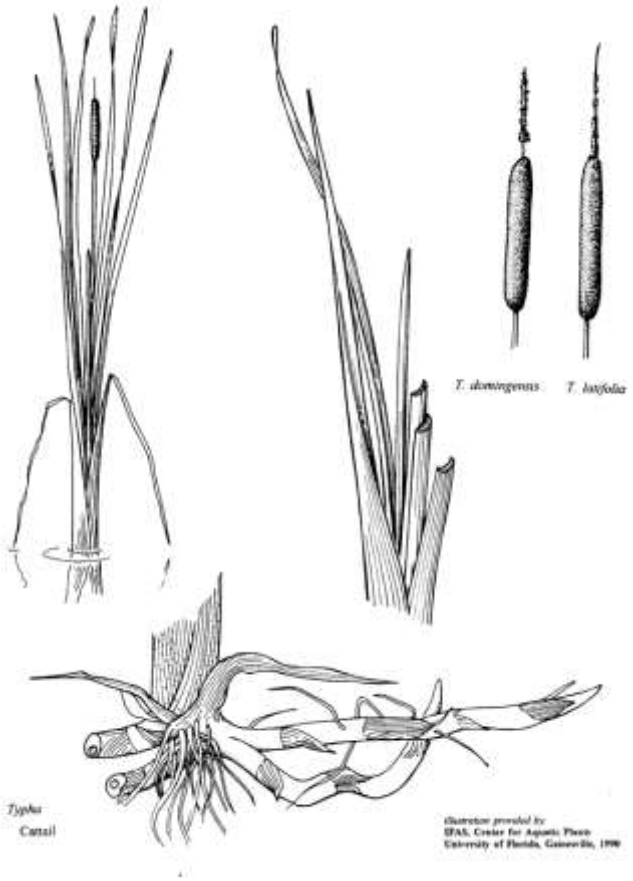
    a. Ensure that the Secchi disk is cleaned and dried properly for future use.
    b. Dispose of any debris or contaminants collected during the measurements responsibly.

Note: It is recommended to perform multiple measurements at different locations and depths to obtain a representative average. Take precautions to ensure safety while accessing the water body and follow any additional guidelines or regulations specific to your study area.

Conclusion: Measuring transparency using the Secchi disk method provides valuable information about water clarity and quality. By following this lab manual, you can effectively determine the transparency of water samples and utilize the data for environmental assessments, monitoring, and research purposes.

LAB-14: Identification of Macrophytes

Typha



Systematic Position

Kingdom – Plantae
Order - Poales
Family - Typhaceae
Genus – Typha

Identifying Character

1. *Typha* leaves are alternate and mostly basal on a simple, joint less stem that bears the flowering spikes.
2. The plants are monoecious, with unisexual flowers that develop in dense racemes.
3. The numerous male flowers form a narrow spike at the top of the vertical stem. Each male (staminate) flower is reduced to a pair of stamens and hairs, and withers once the pollen is shed. Large numbers of tiny female flowers form a dense, sausage-shaped spike on the stem below the male spike.
4. In larger species this can be up to 30 centimeters (12 in) long and 1 to 4 centimeters (0.39 to 1.57 in) thick.

Eleocharis

Systematic Position:

Kingdom - Plantae

Order - Poales

Family - Cyperaceae

Genus – *Eleocharis*

Identifying Character

1. *Eleocharis* species have photosynthetic tube-shaped, leafless green stems but no green leaves (the leaves have been reduced to sheaths surrounding the base of the stems).
2. *Eleocharis* is an erect, rhizomatous, semi aquatic or aquatic, perennial herb.
3. Vegetative reproduction occurs through an extensive rhizome system and is responsible for the maintenance and expansion of existing stands. Sexual reproduction via seed dispersal and seedling establishment is responsible for invasion of new areas.
4. Flower develop singly in axil of glumes, unisexual or bisexual.

Lemna

Systematic Position:

Kingdom: Plantae

Order: Alismatales

Family: Araceae

Subfamily: Lemnoideae

Tribe: Lemneae

Genus: *Lemna*

Specimen: *Lemna minor*

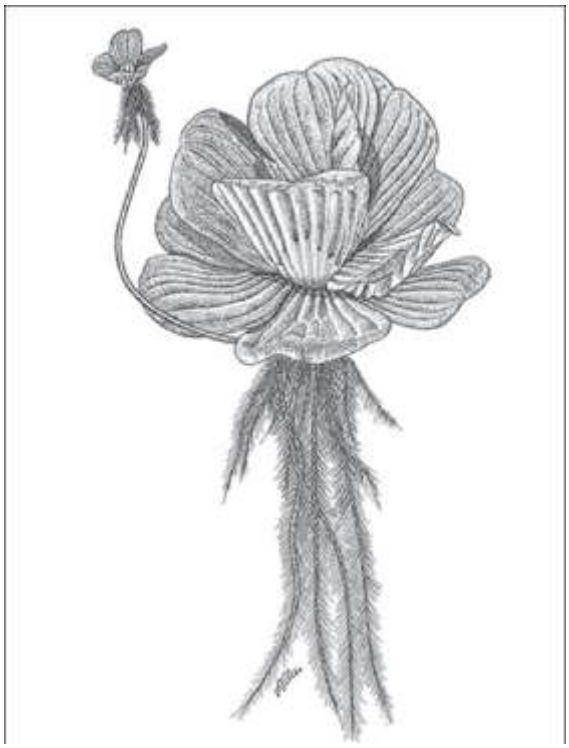
Identifying Character:

1. It is a floating freshwater aquatic plant, with one, two or three leaves each with a single root hanging in the water; as more leaves grow, the plants divide and become separate individuals.

2. The root is 1-2 cm long. The leaves are oval, 1-8 mm long and 0.6-5 mm broad, light green, with three (rarely five) veins, and small air spaces to assist flotation.

3. It propagates mainly by division, and flowers are rarely produced; when produced, they are about 1 mm diameter, with a cup-shaped membranous scale containing a single ovule and two stamens. The seed is 1 mm long, ribbed with 8-15 ribs.

Pistia



Systematic Position:

Kingdom: Plantae

Order: Alismatales

Family: Araceae

Subfamily: Aroideae

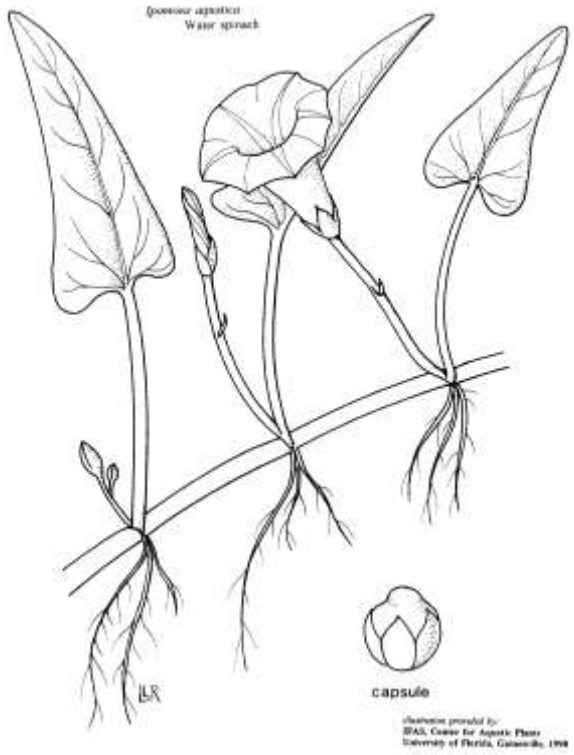
Tribe: Pistieae

Genus: *Pistia*

Identifying Character:

1. It is a perennial monocotyledon with thick, soft leaves that form a rosette.
2. It floats on the surface of the water, its roots hanging submersed beneath floating leaves.
3. The leaves can be up to 14 cm long and have no stem. They are light green, with parallel veins, wavy margins and are covered in short hairs which form basket-like structures which trap air bubbles, increasing the plant's buoyancy.
4. The flowers are dioecious, and are hidden in the middle of the plant amongst the leaves.
5. Small green berries form after successful fertilization. The plant can also undergo asexual reproduction. Mother and daughter plants are connected by a short stolon, forming dense mats.

Ipomoea



Systematic Position:

Kingdom: Plantae

Order: Solanales

Family: Convolvulaceae

Tribe: Ipomoeeae

Genus: *Ipomoea*

Identifying Character:

1. It grows in water or on moist soil. Its stems are 2–3 metres (7–10 ft) or more long, rooting at the nodes, and they are hollow and can float.
2. The leaves vary from typically sagittate (arrow head-shaped) to lanceolate, 5–15 cm (2–6 in) long and 2–8 cm (0.8–3 in) broad.
3. The flowers are trumpet-shaped, 3–5 cm (1–2 in) in diameter, and usually white in colour with a mauve centre.
4. Propagation is either by planting cuttings of the stem shoots that will root along nodes or planting the seeds from flowers that produce seed pods

Eichhornia

Systematic Position:

Kingdom: Plantae

Order: Commelinales

Family: Pontederiaceae

Genus: *Eichhornia*

Species: *E. crassipes*

Identifying Character:

1. Water hyacinth is a free-floating perennial aquatic plant (or hydrophyte) native to tropical and sub-tropical South America.
2. With broad, thick, glossy, ovate leaves, water hyacinth may rise above the surface of the water as much as 1 meter in height. The leaves are 10–20 cm across, and float above the water surface. They have long, spongy and bulbous stalks.
3. The feathery, freely hanging roots are purple-black. An erect stalk supports a single spike of 8-15 conspicuously attractive flowers, mostly lavender to pink in colour with six petals. When not in bloom, water hyacinth may be mistaken for frog's-bit (*Limnobium spongia*).
4. One of the fastest growing plants known, water hyacinth reproduces primarily by way of runners or stolons, which eventually form daughter plants. Each plant can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years.
5. Some water hyacinths were found to grow up to 2 to 5 metres a day in some sites in Southeast Asia

Algal bloom:

An algal bloom is a rapid increase or accumulation in the population of algae (typically microscopic) in a water system. Cyanobacteria blooms are often called blue-green algae. Algal blooms may occur in freshwater as well as marine environments. Typically, only one or a small number of phytoplankton species are involved, and some blooms may be recognized by discoloration of the water resulting from the high density of pigmented cells.

Problems of algal bloom

1. Produce extremely dangerous toxins that can sicken or kill people and animals.
2. Absorbs nutrients from water
3. Sudden death of algal bloom increase the BOD and COD level in water bodies.
4. Create dead zones in the water
5. Raise treatment costs for water
6. Harmful algal blooms negatively impact the food web by decreasing the amount of nutritious, edible phytoplankton that zooplankton and other primary consumers need to survive. These organisms may then starve, leading to decreased food for secondary and higher order consumers.
7. Increased cell concentration can block sunlight from primary producers under the water's surface as well, leading to decreased food and oxygen levels. When the cells in the bloom begin to die it can also lead to decreased dissolved oxygen levels that can be lethal to other aquatic organisms and cause fish kills. Low dissolved oxygen can be made worse by overcast days and warmer temperatures.

LAB-15: Dissection of Pituitary Gland from Fish Head

Introduction: The pituitary gland is a small, pea-sized organ located at the base of the brain. It plays a crucial role in the endocrine system, secreting hormones that regulate various physiological functions in the body. This lab manual provides a step-by-step guide for the dissection of the pituitary gland from a fish head. The pituitary gland can be studied to understand its structure, function, and hormonal regulation in fish.

Materials:

1. Fresh fish head (species of your choice)
2. Dissection tray
3. Dissection scissors
4. Dissection forceps
5. Scalpel or razor blade
6. Magnifying glass or dissecting microscope (optional)
7. Disposable gloves
8. Paper towels or absorbent pads
9. Safety goggles or glasses (recommended)

Procedure:

1. Setup:
    a) Ensure a clean and organized workspace.
    b) Put on disposable gloves and safety goggles/glasses if desired.
2. Preparation of Fish Head:
    a) Rinse the fish head under cold running water to remove any surface impurities.
    b) Pat the fish head dry with paper towels or absorbent pads.
3. External Examination:
    a) Place the fish head on the dissection tray with the ventral side facing up.
    b) Observe the external features of the fish head, including the eyes, mouth, and nostrils.
4. Cranial Incision:
    a) Use the scalpel or razor blade to make a transverse incision across the cranial region, just behind the eyes.
    b) Extend the incision bilaterally towards the gill covers.
5. Skull Cap Removal:
    a) Gently lift and remove the skull cap to expose the brain cavity.

b) Carefully detach any connective tissues or muscles attached to the skull cap.

6. Brain and Pituitary Gland Exposure:

   a) Locate the brain, which is a soft, whitish structure situated within the brain cavity.
   b) Identify the pituitary gland as a small, round or oval-shaped structure at the base of the brain.

7. Pituitary Gland Dissection:

   a) Use the dissection scissors and forceps to carefully isolate and separate the pituitary gland from the surrounding tissues.
   b) Gently lift the pituitary gland with the forceps, taking care not to damage or tear it.

8. Observation and Documentation:

   a) Examine the pituitary gland under a magnifying glass or dissecting microscope, if available, to observe its size, shape, and colour.
   b) Take notes and record any observations or abnormalities of the pituitary gland.

9. Clean-up:

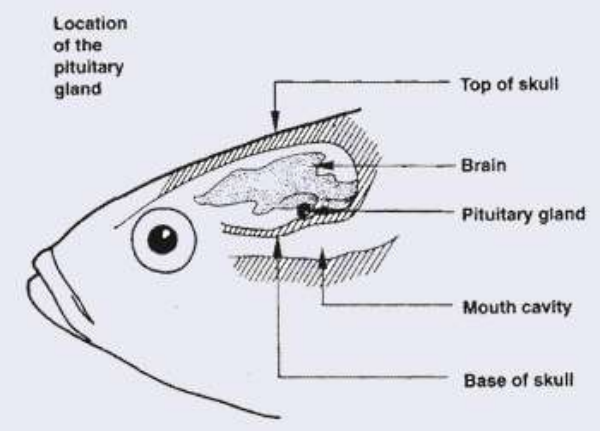
   a) Dispose of the fish head and other biological waste following proper laboratory protocols.
   b) Clean the dissection tools and the workspace with soap and water.

Safety Precautions:

1. Handle sharp instruments, such as scalpels or razor blades, with caution to avoid accidental cuts.

2. Use safety goggles or glasses to protect your eyes from any potential splashes or flying particles.

3. Follow proper disposal procedures for biological waste to maintain hygiene and prevent contamination.



Location of Fish Pituitary Gland

LAB-16: Dissection of Reproductive System in Fish

Introduction: The reproductive system in fish is responsible for the production of gametes and the process of reproduction. Understanding the structure and function of the reproductive organs is essential for studying fish reproductive biology. This lab manual provides a step-by-step guide for the dissection of the reproductive system in fish, focusing on the identification and examination of key reproductive organs.

Materials:

1. Fresh fish (species of your choice)
2. Dissection tray
3. Dissection scissors
4. Dissection forceps
5. Scalpel or razor blade
6. Magnifying glass or dissecting microscope (optional)
7. Disposable gloves
8. Paper towels or absorbent pads
9. Safety goggles or glasses (recommended)

Procedure:

1. Setup:
    a. Ensure a clean and organized workspace.
    b. Put on disposable gloves and safety goggles/glasses if desired.
2. Preparation of Fish:
    a. Rinse the fish under cold running water to remove any surface impurities.
    b. Pat the fish dry with paper towels or absorbent pads.
3. External Examination:
    a. Place the fish on the dissection tray with the ventral side facing up.
    b. Observe the external features of the fish, including the size, shape, and coloration.
4. Ventral Incision:
    a. Use the scalpel or razor blade to make a longitudinal incision along the ventral midline of the fish.
    b. Start the incision from the anus and extend it anteriorly towards the head.
5. Opening the Body Cavity:
    a. Use the dissection scissors to carefully cut through the body wall along the ventral incision.

b. Extend the incision bilaterally towards the pectoral fins.

6. Examination of the Reproductive Organs:

   a. Locate and identify the gonads, which are the primary reproductive organs.
   b. In females, the ovaries appear as whitish or yellowish structures, while in males, the testes are usually elongated and whitish.

7. Dissection of Gonads:

   a. Use the dissection forceps to gently lift and separate the gonads from the surrounding tissues.
   b. Carefully remove the gonads and place them on a clean surface for further examination.

8. Examination and Documentation:

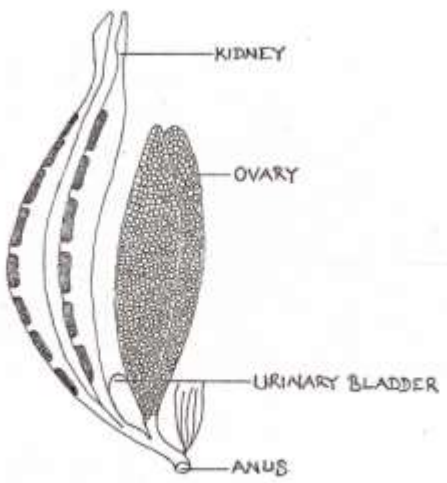
   a. Observe the size, colour, and texture of the gonads.
   b. Take notes and record any observations, including the presence of eggs or sperm.

9. Clean-up:

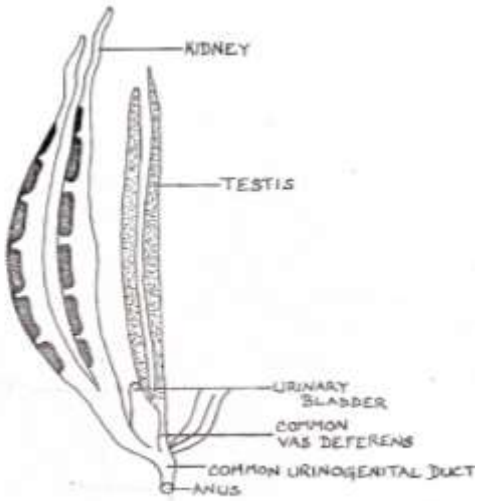
   a. Dispose of the fish and other biological waste following proper laboratory protocols.
   b. Clean the dissection tools and the workspace with soap and water.

Safety Precautions:

1. Handle sharp instruments, such as scalpels or razor blades, with caution to avoid accidental cuts.
2. Use safety goggles or glasses to protect your eyes from any potential splashes or flying particles.
3. Follow proper disposal procedures for biological waste to maintain hygiene and prevent contamination.



Urinogenital system of Tilapia mossambica



Urinogenital system of Tilapia mossambica

LAB-17: Lab Manual for Gonado-Somatic Index (GSI) Calculation in Fish

Introduction: The Gonado-Somatic Index (GSI) is a measurement used to assess the reproductive condition and maturity of fish. It quantifies the ratio of the gonad weight to the total body weight of a fish. This lab manual provides a step-by-step guide for calculating the GSI in fish, which can help in understanding the reproductive status and reproductive potential of fish populations.

Materials:

1. Fresh fish samples (species of your choice)
2. Analytical balance
3. Dissection tools (scissors, forceps, scalpel, etc.)
4. Paper towels or absorbent pads
5. Disposable gloves
6. Calculator or spread sheet software

Procedure:

1. Setup:
    a. Ensure a clean and organized workspace.
    b. Put on disposable gloves for handling the fish samples.
2. Preparation of Fish:
    a. Rinse the fish samples under cold running water to remove any surface impurities.
    b. Pat the fish samples dry with paper towels or absorbent pads.
3. Fish Dissection:
    a. Use the dissection tools to carefully open the abdominal cavity of the fish, exposing the internal organs.
    b. Locate and isolate the gonads (ovaries or testes) from the surrounding tissues.
4. Gonad and Body Weight Measurement:
    a. Weigh the gonads on the analytical balance and record the weight in grams (g).
    b. Weigh the whole body of the fish (without the gonads) on the analytical balance and record the weight in grams (g).
5. GSI Calculation:
    a. Calculate the GSI using the formula: GSI = (Gonad weight / Body weight) x 100.
    b. Multiply the result by 100 to express the GSI as a percentage.

6. Interpretation of GSI:
    a. A higher GSI value indicates a higher proportion of the fish's body weight is attributed to the gonads, suggesting a more advanced reproductive stage.
    b. A lower GSI value indicates a lower proportion of the fish's body weight is attributed to the gonads, suggesting an immature or non-reproductive stage.
7. Documentation and Analysis:
    a. Record the GSI values for each fish sample in a table or spread sheet.
    b. Analyse the GSI data to compare the reproductive conditions among different fish samples or populations.
8. Clean-up:
    a. Dispose of the fish samples and other biological waste following proper laboratory protocols.
    b. Clean the dissection tools and the workspace with soap and water.

Safety Precautions:

1. Handle the fish samples and dissection tools with caution to avoid injury.
2. Use disposable gloves to maintain hygiene and prevent cross-contamination.
3. Follow proper disposal procedures for biological waste.

LAB-18: Lab Manual for Calculating Condition Factor in Fish

Introduction: The condition factor is a measurement used to assess the overall health and well-being of fish. It quantifies the relationship between the weight and length of a fish, providing an indication of its body condition and nutritional status. This lab manual provides a step-by-step guide for calculating the condition factor in fish, which can be used to monitor the health and growth of fish populations.

Materials:

1. Fresh fish samples (species of your choice)
2. Analytical balance
3. Measuring tape or ruler
4. Calculator or spread sheet software
5. Paper towels or absorbent pads
6. Disposable gloves

Procedure:

1. Setup:
    a) Ensure a clean and organized workspace.
    b) Put on disposable gloves for handling the fish samples.
2. Preparation of Fish:
    a) Rinse the fish samples under cold running water to remove any surface impurities.
    b) Pat the fish samples dry with paper towels or absorbent pads.
3. Fish Weight Measurement:
    a) Weigh each fish sample individually on the analytical balance.
    b) Record the weight of each fish sample in grams (g).
4. Fish Length Measurement:
    a) Measure the total length of each fish sample using a measuring tape or ruler.
    b) Measure from the tip of the snout to the end of the tail fin.
    c) Record the length of each fish sample in centimetres (cm).
5. Condition Factor Calculation:
    a. Calculate the condition factor using the formula:
    Condition Factor = $\frac{Fish\ weight}{(Fish\ length)^3} \times 100.$
    b. Raise the fish length to the power of 3 before dividing the weight.
    c. Multiply the result by 100 to express the condition factor.

6. Interpretation of Condition Factor:
    a) A higher condition factor indicates a well-nourished and healthy fish with good body condition.
    b) A lower condition factor suggests a fish that may be experiencing poor nutrition or health issues.
7. Documentation and Analysis:
    a) Record the condition factor for each fish sample in a table or spread sheet.
    b) Analyse the condition factor data to compare the body condition among different fish samples or populations.
8. Clean-up:
    a) Dispose of the fish samples and other biological waste following proper laboratory protocols.
    b) Clean the measuring tools and the workspace with soap and water.

Safety Precautions:

1. Handle the fish samples and weighing equipment with caution to avoid injury.
2. Use disposable gloves to maintain hygiene and prevent cross-contamination.
3. Follow proper disposal procedures for biological waste.

LAB-19: Lab Manual for Estimating Fecundity in Fish

Introduction: Fecundity is an important reproductive parameter that measures the potential reproductive output of fish populations. It refers to the number of eggs produced by a female fish during a spawning event or reproductive cycle. Estimating fecundity provides valuable information for understanding fish reproductive biology and population dynamics. This lab manual provides a step-by-step guide for estimating fecundity in fish.

Materials:

1. Fresh fish (female individuals of the target species)
2. Dissection tray
3. Dissection scissors
4. Dissection forceps
5. Scalpel or razor blade
6. Magnifying glass or dissecting microscope (optional)
7. Disposable gloves
8. Paper towels or absorbent pads
9. Petri dishes or containers for holding eggs
10. Measuring scale or balance
11. Calculator or spread sheet software

Procedure:

1. Setup:
    a) Ensure a clean and organized workspace.
    b) Put on disposable gloves for handling the fish samples.
2. Preparation of Fish:
    a) Rinse the fish under cold running water to remove any surface impurities.
    b) Pat the fish dry with paper towels or absorbent pads.
3. External Examination:
    a) Place the fish on the dissection tray with the ventral side facing up.
    b) Observe the external features of the fish, including the size, shape, and coloration.
4. Ventral Incision:
    a) Use the scalpel or razor blade to make a longitudinal incision along the ventral midline of the fish.
    b) Start the incision from the anus and extend it anteriorly towards the head.

5. Opening the Body Cavity:
    a) Use the dissection scissors to carefully cut through the body wall along the ventral incision.
    b) Extend the incision bilaterally towards the pectoral fins.
6. Dissection of Gonads:
    a) Locate and identify the ovaries in female fish. They are usually two elongated structures on either side of the body cavity.
    b) Carefully remove the ovaries using dissection forceps and place them in a petri dish or container.
7. Egg Counting:
    a) Using a magnifying glass or dissecting microscope, examine the ovaries and count the number of eggs present.
    b) Divide the eggs into groups for more manageable counting.
8. Sampling and Scaling:
    a) Take a subsample of eggs from each group and weigh them using a measuring scale or balance.
    b) Record the weight of each subsample in grams (g).
9. Fecundity Calculation:
    a) Calculate the average weight of the subsamples.
    b) Estimate the total fecundity by extrapolating from the subsample weight to the total weight of all eggs present in the ovaries.
10. Documentation and Analysis:
    a) Record the fecundity estimation for each fish sample in a table or spread sheet.
    b) Analyse the data to compare fecundity among different fish samples or populations.
11. Clean-up:
    a) Dispose of the fish and other biological waste following proper laboratory protocols.
    b) Clean the dissection tools and the workspace with soap and water.

Safety Precautions:

1. Handle sharp instruments, such as scalpels or razor blades, with caution to avoid accidental cuts.
2. Use disposable gloves to maintain hygiene and prevent cross-contamination.
3. Follow proper disposal procedures for biological waste.

LAB-20: Preparation of Fish Fillets

Introduction: The purpose of this lab manual is to provide a step-by-step guide for the preparation of fish fillets. Fish fillets are commonly used in culinary applications and require proper handling and preparation techniques to ensure food safety and quality. This lab will cover the basic process of filleting a fish and preparing fillets for cooking or further processing.

Materials:

1. Fresh whole fish (species of your choice)
2. Cutting board
3. Filleting knife
4. Kitchen shears
5. Tweezers or pliers
6. Clean towels or paper towels
7. Disposable gloves (optional)
8. Plastic bags or containers for storing fillets
9. Ice or a cool environment for temporary storage of fillets
10. Safety goggles or glasses (recommended)

Procedure:

1. Setup:
    a) Ensure a clean and organized workspace.
    b) Put on disposable gloves and safety goggles/glasses if desired.
2. Fish Preparation:
    a) Rinse the whole fish under cold running water to remove any surface impurities.
    b) Pat the fish dry with a clean towel or paper towel.
    c) Place the fish on the cutting board, with the head facing away from you.
3. Removing Scales:
    a) Hold the tail firmly and scrape the fish's scales from tail to head, using the back of the knife or a scaler.
    b) Repeat this process on both sides of the fish until all scales are removed.
    c) Rinse the fish again under cold running water to remove loose scales.
4. Gutting the Fish:
    a) Make a shallow cut along the belly of the fish from the vent (anus) to the base of the head.
    b) Insert the tip of the knife into the belly cavity and carefully cut open the fish.

c) Reach inside the cavity and remove the internal organs, including the guts, liver, and gills.
d) Rinse the cavity thoroughly with cold water to remove any remaining blood or debris.
e) Pat the fish dry with a clean towel or paper towel.

5. Filleting:

a) Lay the fish on its side and make an incision behind the gills, following the natural curve of the fish's body.
b) Starting from the head, insert the knife at a slight angle and cut along the backbone, moving towards the tail.
c) Apply gentle pressure with the knife, following the backbone and separating the fillet from the fish.
d) Repeat the same process on the other side of the fish to obtain the second fillet.
e) Remove any remaining rib bones or pin bones from the fillets using tweezers or pliers.

6. Cleaning and Storing Fillets:

a) Rinse the fillets under cold running water to remove any residual blood or scales.
b) Pat the fillets dry with a clean towel or paper towel.
c) Place the fillets in plastic bags or containers and store them in a cool environment or on ice until further use.

Safety Precautions:

1. Always handle the filleting knife with caution and keep your fingers away from the blade.

2. Use safety goggles or glasses to protect your eyes from any flying scales or bone fragments.

3. Ensure the workspace is clean to prevent cross-contamination and maintain food safety.

4. Dispose of the fish waste properly, following local regulations and guidelines.

Conclusion: This lab manual has provided a detailed procedure for the preparation of fish fillets. Following the steps outlined in this manual will help ensure the proper handling, cleaning, and filleting of fish for culinary purposes. Proper fish fillet preparation is essential for maintaining food safety and quality, and it is crucial to

LAB-21: Lab Manual for Preparation of Fish Oil

Introduction: Fish oil is a valuable product derived from the extraction of oil-rich tissues in fish, such as the liver or fatty parts. It is rich in omega-3 fatty acids, which are beneficial for human health. This lab manual provides a step-by-step guide for preparing fish oil from fish samples.

Materials:

1. Fresh fish samples (preferably oily fish species)
2. Cutting board
3. Sharp knife
4. Blender or food processor
5. Cheesecloth or fine mesh strainer
6. Glass jars or bottles for storage
7. Funnel
8. Paper towels or absorbent pads
9. Disposable gloves

Procedure:

1. Setup:
    a) Ensure a clean and organized workspace.
    b) Put on disposable gloves for handling the fish samples.
2. Preparation of Fish:
    a) Rinse the fish samples under cold running water to remove any surface impurities.
    b) Pat the fish samples dry with paper towels or absorbent pads.
    c) Place the fish on a cutting board.
3. Tissue Selection:
    a) Identify and separate the oil-rich tissues from the rest of the fish, such as the liver or fatty parts.
    b) Remove any scales, bones, or unwanted parts from the selected tissues.
4. Tissue Processing:
    a) Cut the selected tissues into small pieces to facilitate the extraction process.
    b) Place the tissue pieces in a blender or food processor.
5. Blending/Processing:
    a) Blend or process the tissue pieces until a smooth paste or emulsion-like consistency is achieved.

b) Ensure that all tissue particles are well broken down.

6. Straining:

a) Line a funnel with cheesecloth or a fine mesh strainer.
b) Place the lined funnel over a glass jar or bottle.
c) Pour the blended fish mixture into the funnel, allowing the oil to separate and pass through the strainer.
d) Gently press the mixture with a spoon or spatula to extract as much oil as possible.

7. Oil Collection:

a) Allow the oil to pass through the strainer completely, collecting it in the glass jar or bottle.
b) Discard the leftover fish solids trapped in the strainer.

8. Storage:

a) Seal the glass jar or bottle tightly to prevent oxidation and contamination.
b) Store the fish oil in a cool, dark place, such as a refrigerator, to maintain its quality and freshness.

9. Clean-up:

a) Dispose of fish waste and other biological materials following proper laboratory protocols.
b) Clean the blender or food processor, strainer, funnel, and workspace with soap and water.

LAB-22: Lab Manual for Preparation of Fish Fingers

Introduction: Fish fingers are a popular and delicious snack made from boneless fish fillets coated in a crispy breadcrumb coating. This lab manual provides a step-by-step guide for preparing fish fingers, which can be enjoyed as a standalone snack or served as a part of a meal.

Materials:

1. Fresh fish fillets (preferably white fish like cod, haddock, or pollock)
2. Breadcrumbs (plain or seasoned)
3. All-purpose flour
4. Eggs
5. Salt and pepper (optional)
6. Cooking oil (vegetable or canola)
7. Baking sheet or baking dish
8. Plastic wrap or parchment paper
9. Mixing bowls
10. Whisk or fork
11. Paper towels or absorbent pads
12. Disposable gloves

Procedure:

1. Setup:
    a) Ensure a clean and organized workspace.
    b) Preheat the oven to the appropriate temperature (as per fish cooking instructions).
2. Preparation of Fish Fillets:
    a) Rinse the fish fillets under cold running water to remove any surface impurities.
    b) Pat the fish fillets dry with paper towels or absorbent pads.
    c) Cut the fish fillets into rectangular strips of approximately equal size, resembling finger shapes.
3. Coating Station:
    a) Set up a coating station with three separate bowls: one for flour, one for beaten eggs, and one for breadcrumbs.
    b) Season the flour and breadcrumbs with salt and pepper (if desired).
4. Coating Process:

a) Take one fish finger and coat it in flour, shaking off any excess.
b) Dip the floured fish finger into the beaten eggs, ensuring it is fully coated.
c) Transfer the fish finger to the bowl of breadcrumbs and press gently to coat all sides evenly.
d) Place the coated fish finger on a clean plate or baking sheet.
e) Repeat the process for all remaining fish fingers.

5. Frying Method:

a) Heat cooking oil in a deep frying pan or skillet over medium heat.
b) Carefully place the coated fish fingers into the hot oil, ensuring they are not overcrowded.
c) Fry the fish fingers for about 2-3 minutes on each side until golden brown and crispy.
d) Remove the fried fish fingers from the oil and place them on paper towels to absorb excess oil.

6. Baking Method:

a) Preheat the oven to the appropriate temperature (as per fish cooking instructions).
b) Place the coated fish fingers on a greased baking sheet or baking dish.
c) Bake the fish fingers in the preheated oven for approximately 15-20 minutes, or until they are golden brown and cooked through.

7. Serving:

a) Allow the fish fingers to cool slightly before serving.
b) Serve the fish fingers with your choice of dipping sauce, such as tartar sauce, ketchup, or mayonnaise.
c) Garnish with fresh lemon wedges and parsley (optional).

8. Clean-up:

a) Dispose of any waste and clean the cooking utensils and workspace following proper kitchen hygiene practices.

LAB-23: Lab Manual for Preparation of Fish Cutlet

Introduction: Fish cutlets are flavourful and savoury patties made from minced fish and various spices. They are a versatile snack or appetizer that can be enjoyed on their own or used in sandwiches or burgers. This lab manual provides a step-by-step guide for preparing fish cutlets.

Materials:

1. Fresh fish fillets (preferably boneless and skinless)
2. Potatoes (boiled and mashed)
3. Onion (finely chopped)
4. Garlic cloves (minced)
5. Ginger (grated)
6. Green chillies (finely chopped)
7. Fresh coriander leaves (chopped)
8. Bread crumbs
9. All-purpose flour
10. Eggs
11. Salt and pepper
12. Cooking oil (vegetable or canola)
13. Mixing bowls
14. Frying pan or skillet
15. Spatula or slotted spoon
16. Paper towels or absorbent pads
17. Disposable gloves

Procedure:

1. Setup:
    a) Ensure a clean and organized workspace.
    b) Preheat the oven to the appropriate temperature (if baking the cutlets).
2. Preparation of Fish:
    a) Rinse the fish fillets under cold running water to remove any surface impurities.
    b) Pat the fish fillets dry with paper towels or absorbent pads.
    c) Cut the fish fillets into small pieces.

3. Fish and Potato Mixture:

    a) In a mixing bowl, combine the minced fish, mashed potatoes, chopped onion, minced garlic, grated ginger, chopped green chillies, and chopped coriander leaves.
    b) Season the mixture with salt and pepper to taste.
    c) Mix all the ingredients thoroughly until well combined.

4. Shaping the Cutlets:

    a) Take a portion of the fish and potato mixture and shape it into a patty or cutlet of desired size and thickness.
    b) Repeat the process to shape all the cutlets.
    c) Place the shaped cutlets on a plate or tray.

5. Coating the Cutlets:

    a) In a shallow bowl, whisk the eggs until well beaten.
    b) Place the bread crumbs in another shallow bowl.
    c) Dip each cutlet into the beaten eggs, ensuring it is fully coated.
    d) Roll the egg-coated cutlet in the bread crumbs, pressing gently to coat all sides evenly.
    e) Place the coated cutlets on a separate plate or tray.

6. Frying the Cutlets:

    a) Heat cooking oil in a frying pan or skillet over medium heat.
    b) Carefully place the coated cutlets into the hot oil, ensuring they are not overcrowded.
    c) Fry the cutlets for about 2-3 minutes on each side until golden brown and crispy.
    d) Remove the fried cutlets from the oil using a spatula or slotted spoon.
    e) Place them on paper towels to absorb excess oil.

7. Baking the Cutlets (optional):

    a) Preheat the oven to the appropriate temperature.
    b) Place the coated cutlets on a greased baking sheet.
    c) Bake the cutlets in the preheated oven for approximately 15-20 minutes, or until they are golden brown and cooked through.

8. Serving:

    a) Allow the cutlets to cool slightly before serving.
    b) Serve the fish cutlets with your choice of sauce or chutney.
    c) Garnish with fresh coriander leaves (optional).

9. Clean-up:

    a) Dispose of any waste and clean the cooking utensils and workspace following proper kitchen hygiene practices.

LAB-24: Lab Manual for Process of Fish Drying

Objective: To understand and practice the process of fish drying as a method of fish preservation.

Materials:

1. Fresh fish (species of choice)
2. Knife and cutting board
3. Salt or curing mixture
4. Drying racks or screens
5. Weighing scale
6. Timer or clock
7. Data recording sheets

Procedure:

1. Introduction to Fish Drying:
    a) Begin the session with an overview of fish drying as a method of fish preservation.
    b) Discuss the advantages and challenges of fish drying, including increased shelf life, reduced weight and volume, and the need for proper hygiene and environmental conditions.
2. Preparation of Fish for Drying:
    a) Start by selecting fresh fish of appropriate species and size for drying.
    b) Demonstrate the proper technique for cleaning and gutting the fish.
    c) Cut the fish into desired sizes or fillets for drying, considering the thickness and uniformity of the pieces.
3. Curing and salting:
    a) Explain the importance of salting or curing fish before drying to enhance preservation.
    b) Prepare a salt or curing mixture according to a suitable recipe or recommendation.
    c) Apply the salt or curing mixture evenly to all surfaces of the fish pieces.
    d) Allow the fish to rest and absorb the salt or curing mixture for a specified time (e.g., 1-2 hours).
4. Drying Process:
    a) Set up drying racks or screens in a well-ventilated area away from direct sunlight, dust, and pests.
    b) Place the salted fish pieces on the drying racks, ensuring proper spacing between them for air circulation.

c) Monitor and maintain suitable drying conditions, including temperature (around 20-30°C) and humidity (below 60%).
d) Observe and record the drying time at regular intervals, noting any changes in appearance or texture.

5. Monitoring Drying Progress:

a) Check the drying progress by inspecting the fish pieces. They should gradually lose moisture and become firm, leathery, and less sticky.
b) Measure the weight of a few fish pieces at different time intervals using a weighing scale, recording the data on the data recording sheets.
c) Note any observations regarding changes in colour, texture, or aroma during the drying process.

6. Determining Dryness:

a) Determine the dryness of the fish by examining the moisture content and texture.
b) The fish is considered dry when it reaches a moisture content of around 20-30% or when it becomes hard, brittle, and breaks easily.

7. Evaluation and Storage:

a) Evaluate the dried fish for quality, including taste, aroma, and overall appearance.
b) Discuss the importance of proper storage to maintain the quality and prevent rehydration or spoilage.
c) Package the dried fish in suitable containers or packaging materials, ensuring they are airtight and stored in a cool, dry place.

Safety Precautions:

1. Follow standard food safety practices, including hand washing, wearing gloves, and maintaining clean work surfaces.

2. Handle knives and cutting tools with care, ensuring they are sharp and in good condition.

3. Keep the drying area clean, free from pests, and protected from potential contaminants.

Disposal:

1. Properly dispose of any fish waste according to local regulations and waste management guidelines.

2. Clean and sanitize all equipment used during the session to maintain hygiene standards.

LAB-25: Lab Manual for Process of Fish Salting Technique

Objective: To understand and practice the process of fish salting as a method of fish preservation.

Materials:

1. Fresh fish (species of choice)
2. Salt (non-iodized or sea salt)
3. Curing container or basin
4. Weighing scale
5. Timer or clock
6. Data recording sheets

Procedure:

1. Introduction to Fish Salting:
    a) Begin the session with an overview of fish salting as a traditional method of fish preservation.
    b) Discuss the benefits of fish salting, including extended shelf life, enhanced flavour, and reduced microbial growth.
2. Selection of Fresh Fish:
    a) Start by selecting fresh fish of suitable species and size for salting.
    b) Choose fish that are of good quality, without signs of spoilage or damage.
3. Cleaning and Preparation:
    a) Demonstrate the proper technique for cleaning and scaling the fish.
    b) Gut the fish carefully to remove the internal organs.
    c) Rinse the fish thoroughly under cold water to remove any residual blood or debris.
    d) Pat the fish dry with a clean towel or paper towels.
4. Salting Process:
    a) Prepare a suitable container or basin for the salting process.
    b) Spread a layer of salt evenly at the bottom of the container.
    c) Place the fish on top of the salt layer, ensuring there is space between each fish.
    d) Sprinkle a generous amount of salt over each fish, covering them completely.
    e) Continue layering fish and salt until all the fish are salted.
    f) Cover the container with a clean cloth or lid.

5. Salting Time and Monitoring:

    a) Set a timer or clock to monitor the salting time.
    b) The duration of salting depends on the size and thickness of the fish. Generally, smaller fish require less salting time than larger ones.
    c) Monitor the salt penetration by observing changes in colour and texture of the fish. The fish will become firm and less glossy as the salt draws out moisture.

6. Evaluation and Rinsing:

    a) After the designated salting time, carefully remove a fish from the container for evaluation.
    b) Check the fish for desired firmness and salt penetration by pressing it gently with your finger.
    c) If the fish meets the desired texture, rinse off the excess salt under cold running water. If not, return it to the container for additional salting time. d. Repeat the evaluation and rinsing process for each fish.

7. Drying and Storage:

    a) After rinsing, pat the fish dry with a clean towel or paper towels.
    b) Place the salted and rinsed fish on a drying rack or clean surface to allow them to air dry.
    c) Ensure proper ventilation and protection from pests during the drying process.
    d) Monitor the drying time and record any observations regarding changes in texture and appearance.
    e) Once the fish are fully dried, package them in suitable storage containers or bags for long-term storage.

Safety Precautions:

1. Follow standard food safety practices, including handwashing, wearing gloves, and maintaining clean work surfaces.

2. Handle knives and cutting tools with care, ensuring they are sharp and in good condition.

Disposal:

1. Properly dispose of any fish waste according to local regulations and waste management guidelines.

2. Clean and sanitize all equipment used during the session to maintain hygiene standards.

LAB-26: Calculation of Ice Requirement for Short-Term Freezing

Objective: To understand and practice the calculation of ice requirement for short-term freezing of fish or other perishable food items.

Materials:

1. Fish or perishable food items (optional for demonstration purposes)
2. Weighing scale
3. Thermometer
4. Calculator
5. Data recording sheets

Procedure:

1. Introduction to Ice Requirement Calculation:
    a) Begin the session with an overview of the importance of proper ice requirement calculation for short-term freezing.
    b) Explain the significance of maintaining the desired temperature to ensure food safety and quality.
2. Determining the Weight of the Fish or Food Item:
    a) Weigh the fish or food item that needs to be frozen using a weighing scale.
    b) Record the weight on the data recording sheet.
3. Estimating the Heat Load:
    a) Calculate the estimated heat load by multiplying the weight of the fish or food item by its specific heat capacity.
    b) The specific heat capacity of fish is approximately 0.92 Kcal/kg °C. c. Record the calculated heat load on the data recording sheet.
4. Determining the Required Ice Quantity:
    a) Determine the desired storage temperature for the fish or food item. For short-term freezing, it is typically -18°C (0°F).
    b) Calculate the heat of fusion (latent heat) of ice, which is approximately 79.7 Kcal/kg or 144 Btu/lb.
    c) Divide the calculated heat load by the heat of fusion to determine the required ice quantity in kilograms or pounds. Ice Quantity (kg) = Heat Load (Kcal) / Heat of Fusion (Kcal/kg)
    d) Record the calculated ice quantity on the data recording sheet.

5. Monitoring the Freezing Process:

    a) Prepare a suitable container or freezer to hold the fish or food item.
    b) Place a thermometer inside the container to monitor the temperature.
    c) Add the calculated quantity of ice to the container.
    d) Place the fish or food item on top of the ice.
    e) Monitor the temperature regularly using the thermometer and ensure it remains at or below the desired storage temperature (-18°C or 0°F).

6. Evaluation and Documentation:

    a) Observe and record the temperature readings at regular intervals during the freezing process.
    b) Monitor the duration required for the fish or food item to reach the desired storage temperature.
    c) Note any observations regarding the quality, texture, or appearance of the frozen product.

Safety Precautions:

1. Follow standard food safety practices, including handwashing and maintaining clean work surfaces.

2. Handle fish or perishable food items with care to prevent cross-contamination.

3. Use caution when using a weighing scale and handling sharp objects such as knives.

Disposal:

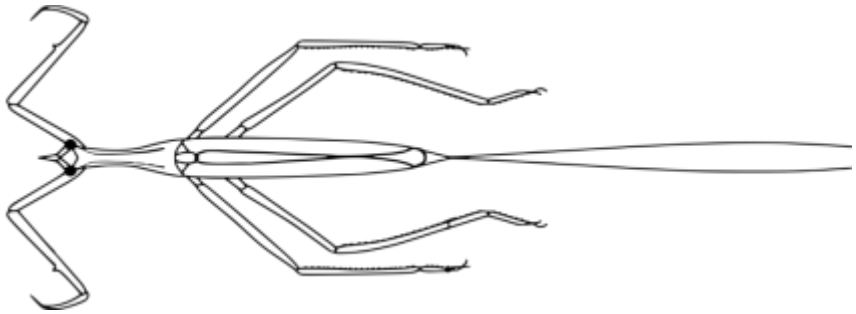
1. Properly dispose of any fish waste or food items according to local regulations and waste management guidelines.

2. Clean and sanitize all equipment used during the session to maintain hygiene standards.

Note: The above procedure is a general guide for calculating ice requirement for short-term freezing. The specific requirements may vary depending on the type of food item and storage conditions.

LAB-27: Identification of Aquatic Insects

Specimen No. 1:



Systematic Position

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Suborder: Heteroptera
Family: Nepidae
Genus: Ranatra

1. Ranatra is a genus of generally slender predatory members of the family Nepidae, known as water scorpions or water stick-insects.
2. Their front legs are strong and are used to grasp prey.
3. They breathe through a pair of long breathing pipes extending from their tails. They eat tadpoles, small fish and other insects, which they pierce with their beak and inject a saliva which both sedates and begins to digest their prey.
4. They are active throughout winter as adults, except in extreme cold.
5. The females lay eggs in vegetation during the springtime. The eggs typically take two to four weeks to hatch and the young take about two months to mature. When fully grown they are 4-5 inches.

Hence, the specimen is *Ranatra*

Specimen No. 2:

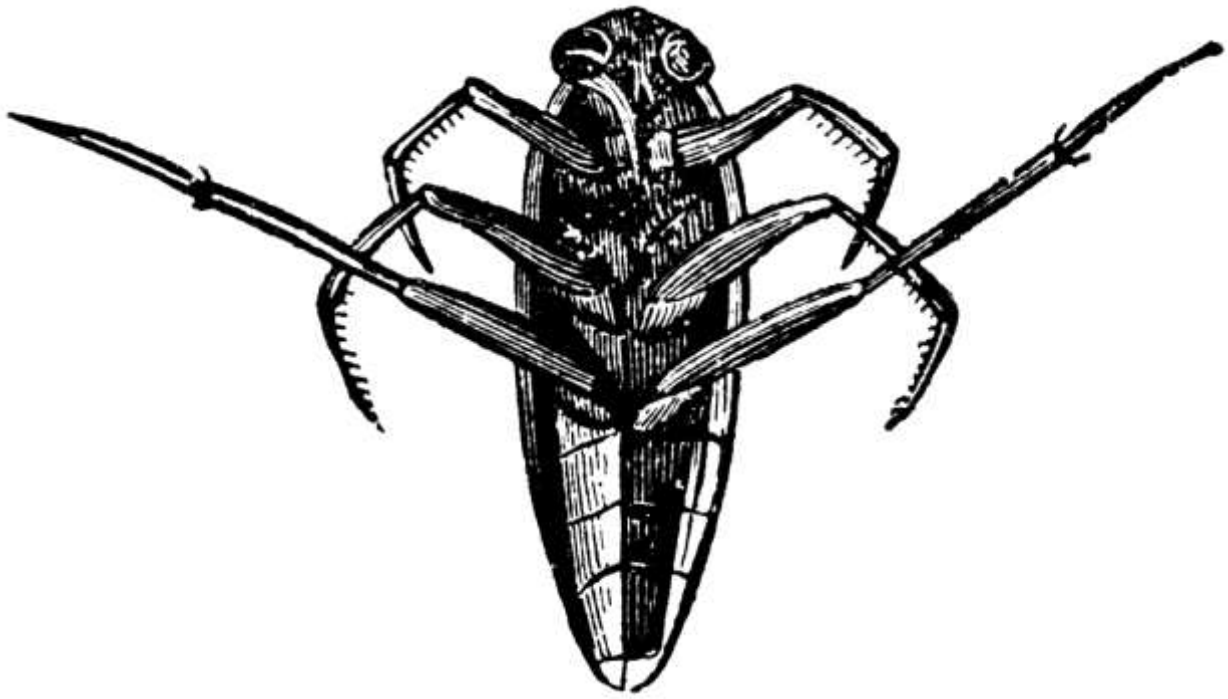
Systematic Position:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Suborder: Heteroptera
Family: Nepidae
Genus: *Nepa*

1. They are oval-bodied, aquatic insects with raptorial front legs.
2. Like other members of the Nepidae, they have a pair of nonretractable cerci-like breathing tubes on the terminal abdominal segment, a characteristic which readily distinguishes them from the Belostomatidae.
3. Their primary staples are other insects and small aquatic vertebrates. They can inflict a painful bite when handled.

Hence, the specimen is *Nepa* sp.

Specimen – 3:



Systematic Position:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Suborder: Heteroptera

Family: Notonectidae

Genus: *Notonecta*

1. Notonecta glauca is a species of backswimmer.
2. *Notonecta* are Hemiptera (true bug) predators, that are approximately 13–16 mm in length.
3. Females have a larger body size compared to males.
4. These water insects swim and rest on their back (hence their common name "Backswimmer" or "Water Boatman") and are found under the water surface.
5. Notonecta supports itself under the water surface by using both pairs of forelegs and the most posterior end of its abdomen and rest them on the water surface. They are able to stay under the water surface by water tension, also known as the air-water interface (Surface tension).

Hence, the specimen is *Nepa* sp.

Specimen – 4:

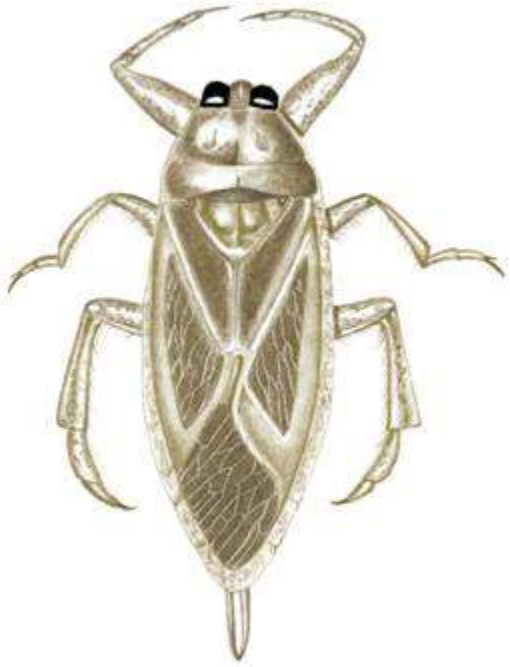
Systematic Position:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Hydrophilidae
Genus: *Hydrophilus*

1. Hydrophilus can reach a length of 33–42 millimetres (1.3–1.7 in).
2. The basic color of the body is dark brown or black.
3. These beetles have streamlined bodies and heads adapted for aquatic life, but they are not powerful swimmers.
4. When they dive they carry a bubble of air under their elytra, while the body is covered by fine hairs trapping a layer of air. Adults are mainly vegetarian, feeding on aquatic plants, but larvae are carnivorous, feeding on tadpoles, snails and small fishes.

Hence, the specimen is *Hydrophilus* sp.

Specimen – 5:



Systematic Position:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Family: Belostomatidae
Genus: *Lethocerus*

1. Lethocerus is a genus of the hemipteran family Belostomatidae, known colloquially as giant water bugs, distributed in tropical, subtropical and temperate areas of the world.
2. the largest true bugs with species capable of reaching a length of over 12 centimetres (4.7 in).
3. Lethocerus sp. have two symmetrical furrows in the inner pad of setae on the fore femur, the external borders of parasternites II and III narrowed and nearly straight, and with the setae of the tarsomeres following the line of the tibial setae.

Hence, the specimen is *Lethocerus* sp.

FSC-296

PRACTICAL PAPER

(Core course - 4 credits: 50 Marks)

[University Examination - 50]

1. Identification of common bacterial, viral, protozoan and fungal diseases of finfishes & shellfishes.
2. Identification of common parasites & parasitic diseases of finfishes and shellfishes.
3. Studies of commonly used aquaculture medicines in West Bengal farms.
4. Hatchery layout and identification of equipment's.
5. Evaluation of quality of fish gametes.
6. Study of maturity status of fish by histology and histochemistry of gonads and related organs.
7. Study of induced breeding technique by natural and synthetic hormones.
8. Cryopreservation of fish gametes.
9. Breeding and larval care of finfish and shellfishes.
10. Preparation of project proposal through chart/ model for different fish productionsystems.
11. Fish market and market survey.
12. Socioeconomic analysis of fishers.
13. Field visit.

FSC-296

LAB-1: Lab manual for Induced Spawning

Preparation of Pituitary Extract – For preparation of gland extract the glands are removed carefully from freshly killed fish called donor fish. For best result the donor fish should be fully ripe and mature. Common carp is the best donor fish, because it breeds through out the year and the individuals are available in all parts of the world. The pituitary glands of such species are relatively large. The gland should be collected prior to spawning.

1. **Removal of Glands:**

The removal of glands can be done by following two processes:

a) Removal through foramen magnum – the foramen magnum was first exposed by removing vertebral parts adhering to skull. Fat is removed first by means of forceps and then cotton piece. A pair of forceps then inserted into foramen magnum dorsally to the brain and anterior part of the brain now detached and remaining is carefully lifted out through the foramen magnum. The gland is then located and removed.

b) Removal of gland by dissecting head – This technique is not used commercially as because the heads are damaged by this process. The first method of removal is less time consuming and economical as the heads are used for human consumptions later. At first the head is dissected using sharp butcher's knife, a portion of scalp is chopped off in a clean cut with one stroke. Fat surrounding the brain is removed with the help of cotton. Olfactory and optic nerves are now severed, and then brain is lifted up and removed. Locate the gland. The gland may come up along with the brain or may remain behind on the floor of brain cavity often covered with a membrane. In any case the gland is carefully removed after separating it from membrane or the brain proper. The gland must not be damaged or torn.

2. **Preservation of Glands:**

The gland after removal needs to be preserved for certain periods or for future use. After 24 hours the glands are kept in fresh absolute alcohol. The glands are taken in absolute alcohol and can be stored in room temperature. In certain countries like Russia the glands are preserved in acetone at 10°C. The glad may be preserved in refrigerator immediately for certain periods. But alcohol preservation of glands is very common and easy methods. It is widely used in India. Glycerine preservation is another technique but less popular in our country.

3. **<u>Preparation of Pituitary Extract:</u>**

- The preserved glands of known quantity are taken out
- Macerated in a homogenizer after evaporation of alcohol with little amount of distilled water. Then the extract is freed of suspended particles by means of centrifugation.

- It is the diluted with required amount of distilled water or 0.3% saline water or a suitable physiological solution.
- The dilution is made at the rate of 0.2 ml/kg body weight of recipient fish.
- The pituitary extract is then centrifuged and only the supernatant solution is used for injection. The extract is now ready for use.

4. Preservation of Extract:

The extract can also be preserved for future use. In this process in place of saline water glycerine is used and extract can be preserved in room temperature or in refrigerator. Other methods of preservation are done by propane and trichloro-acetic acid in place of glycerine.

5. Brood Stock Rearing:

Preparation of Brood fish Pond:-

a. Minimum water area – 1 bigha

b. Depth – 4 to 5 ft.

c. Pond Shape – Rectangular (Advantage in netting operation)

d. Age of Brooders – 2 to 5 years

e. Weight – 2 to 5 kg

Preparation of Pond:-

a) The pond must be prepared 3 – 4 months before giving the injection for induced breeding. Time of breeding is generally from April to July.

b) Application of organic manure (Raw cow dung) @ 1000 kg. per bigha, inorganic manure (Urea, Single Super Phosphate @ 10 kg. per bigha.

c) Application of lime @ 30 kg. per bigha.

d) Racking is necessary to turbulent of the pond bottom.

e) Testing of water.

Stocking of Brood Fish:-

a) 1 bigha pond – 1000 to 1500 kg brooders.

Supplementary Feeding:-

Daily 1% of the total body weight of food should be applied. Mustard oil cake, rice bran, sunflower cake, wheat grains are generally applied as food.

Monthly Care:-

- Organic – RCD @ 1 – 5 kg/bigha
- Inorganic – Urea and SSP 3 kg/bigha (15 days interval)
- Liming – 1 kg/bigha
- Racking

6. Selection of Brooders:

Proper selection of are the key of success in case of induced breeding. The breeders should be healthy, fully ripe and of medium sized. They should preferably come into the age group ranging from 2 – 4yrs and have the weight of 1 – 5kgs. Large sized breeders are avoided for difficulty in handling. For ripe male and female carps, it can be easily identified. The male shows roughness on pectoral fins when belly pressed milt freely oozes out. The ripe female shows relatively smooth pectoral fins and operculum. The eggs are released when the belly is pressed smoothly in female. The belly of ripe female is generally soft and round or budged. The vent is swollen, protruding and pinkish in colour. It is wiser to practice to keep ready adequate stock of potential brooders.

Catla (kg)		Rohu (kg)		Mrigala (kg)	
♂	♀	♂	♀	♂	♀
10 - 15	5 - 8	10 - 11	5 - 6	6 - 10	4 - 5

For Hapa Size = 6 × 3 × 3 cubic ft.

7. Injection to the breeders

The pituitary extract is administered into the body of breeders by means of hypodermic syringe either intra muscular or intra peritoneal. To ensure a higher percentage of fertilisation during induced spawning it is necessary that there is synchronisation between ovulation and milt shading. This is difficult to achieve with a set of breeders having one male and one female. Therefore the common practice is to use a set consisting of one female and two males. For intra muscular injection the fish is laid on its side while held in hand net and the needle is inserted either in the caudal peduncle or in the shoulder. For intra peritoneal the injections are given in the bases of paired pectoral fins. But it is avoided because less expert hand can puncture heard of the fish.

Determination of correct dosage of pituitary extract to be given to the breeders is very important though a difficult matter. Dosage depends upon the size and state of maturity of the recipient (breeders) as well as upon the state of maturity of the donor for the glands. It has been found that the potency of the gland is influenced by the size, the age, the sex, the state of sexual maturity of the donor fish as also the size of the gland itself. Great difficulty is encountered because it is not easy matter to ascertain the state of maturity of fish from external examination. Usually the female is given a preliminary dose of 2-3mg/kg of body wt. The preliminary dose is not given to the male. After an interval of time about 6hrs a second

dose of 5 – 8mg are given per kg of body wt of female. The male was given then the first dose of injection with female @ 2-3mg/kg of body wt. The dose may be depending upon the maturity of fish, age, sex and also the environmental conditions.

8. Spawning:

After injection to the brooders a set of brooders are released into breeding hapa. In hapa breeding the hapa is the fine netting, rectangular in shape and is held by four bamboo poles one at each corner. Closed meshed mosquito netting is preferred for that purpose, as its meshes will allow a good circulation of water and will also not let the laid eggs and milt escape through the meshes. The hapa measures the range of 3m × 1.5m × 1m for breeders weighing to 3 to 5kgs. The height of the hapa should remain about 20cm above to the level of water. The roof can be open or closed. The roof can be opened or closed. The spawning takes place within 3-6hrs following the second dose. It turns out the midnight if the second injection was given in the evening. Successful induced breeding results in the spawn of fertilised eggs. The fertilised eggs are transparent, pearl like where as unfertilised eggs are opaque or whitish.

9. Hatching

The hatching techniques of carps eggs have undergone a lot of changes to get better hatching and survival from the earthen hatchery pits and cement hatchery pits at spawn collection centers. Use of double hatching hapas is being replaced by running water systems, with or without temperature control. The water hardened eggs are measured, their number calculated and a sample is examined to find out percentage of fertilization.

Under field conditions, the eggs are kept in double-walled hapas, outer made of this close-meshed cotton or nylon and inner made of thin round-meshed mosquito net. The inner hapa is smaller, leaving gaps of 15 cm on all sides and also at bottom between the inner and outer hapa. In an outer hapa of the size 2 m × 1 m × 1 m and a suitable size inner hapa a total number of 50,000 to 100,000 eggs can be kept for hatching depending upon fertilization. The eggs are distributed in the inner hatching hapa uniformly. The hatching time is 16–18 hours in the case of Indian major carps and nearly two hours more in silver carp and grass carp at optimum water temperature range of 26-31°C. The hatchlings pass through the inner hapa to outer hapa and inner hapa is removed. Inner hapa with wooden frame is found quite convenient for uniform distribution of developing eggs. Floating hapas are also used for hatching purpose.

In a vertical jar hatchery, hatching jars with a capacity of 6.0 to 6.35 liters, 50,000 eggs can be kept and a rate of flow of water is maintained at 600–800 ml per minute for Indian major carps and 800-1000 ml for silver carp and grass carp. In hatching jars of higher capacity more eggs can be introduced but the flow of water has to be suitably increased.

LAB-2: Lab manual for Hatchery Layout of Eco Hatchery System

Main hatchery unit comprises of

- Spawning/Breeding pool
- Egg incubation pool
- Egg and Spawn collection pool
- Water supply system

A. Overhead Tank:

- ✓ The tank should be at the height of 4 – 6 meters from the ground level.
- ✓ Capacity of the tank ranges from 30,000 – 50,000 liters to meet the requirement of the carp hatchery
- ✓ Shape of the tank should be rectangular and ventilated type
- ✓ Uninterrupted water supply to the hatchery unit during hatchery operation to be maintained/ensured by electric/diesel pump with standby set

B. Eco – spawning/ breeding pool:

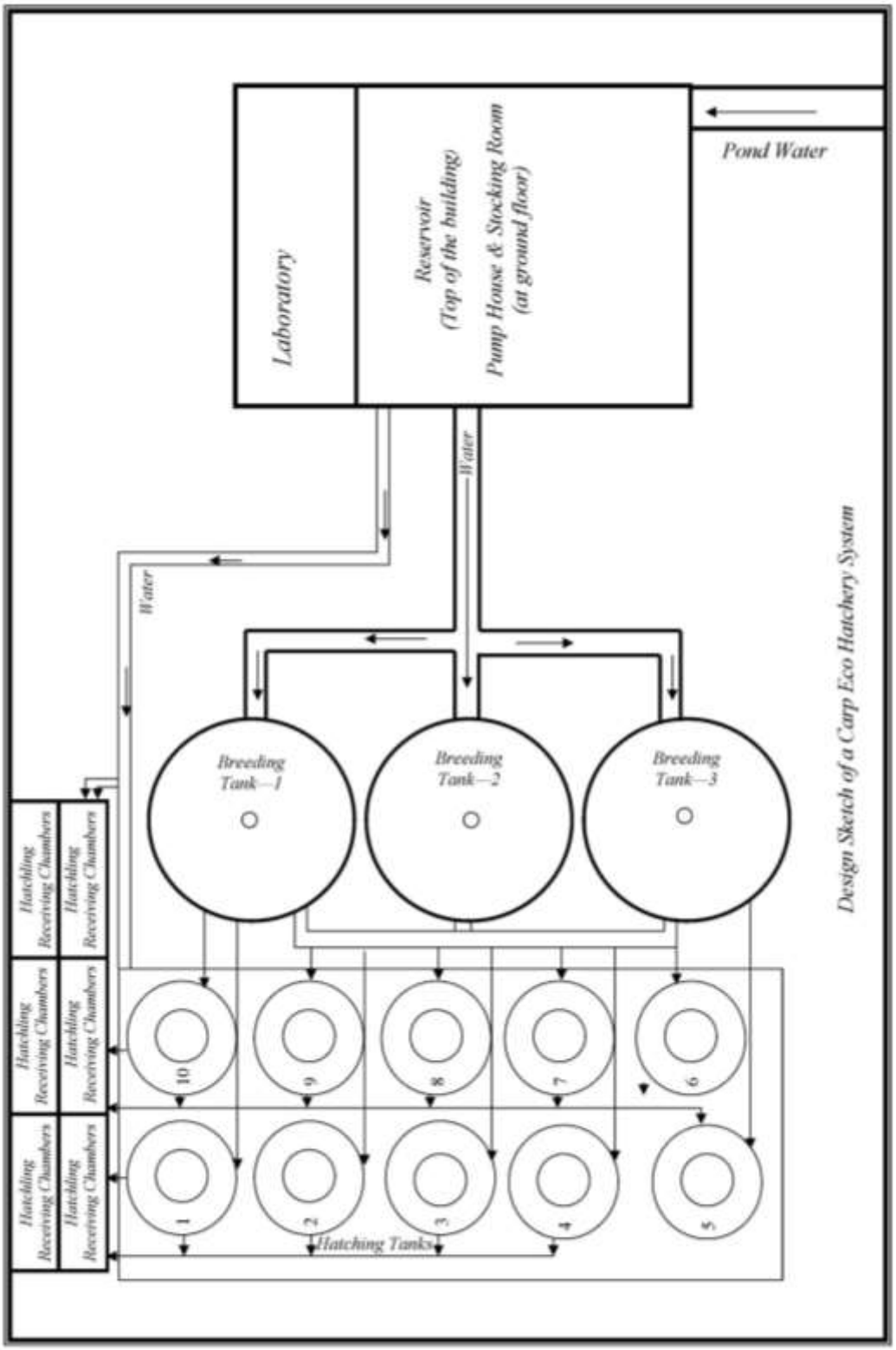
- ✓ It is a circular smooth cistern.
- ✓ It might be made up with brick, RCC or fiber material.
- ✓ The pool should be provided with showers from 1 mt height.
- ✓ Diameter of the pool may vary from 3 to 6 mts. (5 mts for 30 lakhs egg)
- ✓ Depth of the pool would be 1.2 mt for producing 30 lakhs egg.
- ✓ Depth and diameter can be manipulated according to requirement.
- ✓ Single water inlet system (1” – 1.5”dia.) at the base of the pool at 600 to the floor is preferred.
- ✓ Straight egg delivery pipe line (3” to 4”dia) to be provided between the central floor of the spawning pool and the egg incubation unit through which fertilized egg along with water are transferred into incubation tank for hatching.
- ✓ Water in the pool requires D.O. of 6 ppm for conducive spawning of fishes.

C. Hatching pool:

- ✓ Hatching pool is a double chambered (double wall) circular smooth cistern made of Brick, RCC or Fiber material.
- ✓ Water enters into the (outer chamber of the) cistern by a series of duck mouth inlets.
- ✓ Water in the cistern expels out through a screen, encircled over inner chamber.
- ✓ A delivery pipe which is erected on the central outlet hole maintains the water depth of the incubation chamber.
- ✓ A spawn delivery outlet is fixed at the base of the outer wall of the outer chamber and it directs spawn to the spawn collection chamber.
- ✓ The standard size of the outer chamber of the incubation pool ranges from diameter – 2-4 meter depth 1-1.2 meter
- ✓ Mesh size of the screen used in the inner chamber of the incubation pool is 1/80 inch which prevents eggs and spawns escaping out.
- ✓ An egg incubation chamber with good quality of water can hold an average of 7 lakhs eggs per cu. Meter.

D. Seed collection chambers:

- ✓ A chamber adjacent to or in between the spawning and incubation pool can serve both for egg collection and spawn collection purposes.
- ✓ Separate chambers can also be constructed for egg and spawn collection.
- ✓ Chamber should provide a water cushion and collection of eggs and/or spawn is done by gravity.
- ✓ Size of egg collection chamber – 1 x 1.2 x 1 meter.
- ✓ Size of spawn collection chamber – 2 x 1.2 x 1 meter.


Laboratory
Reservoir
(Top of the building)
Pump House & Stocking Room
(at ground floor)
Pond Water
Water
Water
Breeding Tank—1
Breeding Tank—2
Breeding Tank—3
Hatchling Receiving Chambers
Hatchling Receiving Chambers
Hatchling Receiving Chambers
Hatchling Receiving Chambers
Hatchling Receiving Chambers
Hatchling Receiving Chambers
10
9
8
7
6
1
2
3
4
5
Hatching Tanks
Design Sketch of a Carp Eco Hatchery System

LAB-3: Lab manual for commonly used aquaculture medicines in West Bengal farms.

The commonly used aquaculture medicines in West Bengal farms, like in many other regions, can vary depending on the specific diseases or health conditions prevalent in the area. Here are some commonly used aquaculture medicines that may be utilized in West Bengal farms:

1. Antibiotics: Antibiotics are used to treat bacterial infections in fish. Some commonly used antibiotics in aquaculture include oxytetracycline, erythromycin, and florfenicol. However, the use of antibiotics in aquaculture requires caution and should be done under veterinary supervision to minimize the development of antibiotic resistance.

2. Antiparasitics: Antiparasitic medications are used to control and treat parasitic infestations in fish. Examples include formalin, malachite green, and potassium permanganate. These medications are often used to treat parasites like protozoans, monogeneans, and crustaceans.

3. Antifungals: Antifungal medications are used to combat fungal infections in fish, particularly in cases of fungal skin or gill infections. Commonly used antifungal agents include malachite green, potassium permanganate, and copper sulfate.

4. Vaccines: Vaccines are used to prevent certain viral and bacterial diseases in aquaculture. Vaccination is commonly practiced for major diseases like viral infections and bacterial infections. Specific vaccines can be developed and administered based on the prevalent diseases in West Bengal fish farms.

5. Herbal Medicines: Some fish farmers in West Bengal may also use herbal or botanical medicines to maintain fish health and boost immune system function. Herbal extracts, such as neem, garlic, and turmeric, are believed to have antimicrobial and immunostimulant properties.

6. Probiotics: Probiotics are live microorganisms that can improve the intestinal health and overall well-being of fish. They help maintain a healthy gut microbiota and promote disease resistance. Commonly used probiotics in aquaculture include various strains of Bacillus, Lactobacillus, and Saccharomyces.

LAB-3: Lab manual for Evaluation of quality of fish gametes.

Evaluating the quality of fish gametes (eggs and sperm) is essential for successful fish breeding and reproduction. Here is a general outline of a laboratory manual for evaluating the quality of fish gametes:

1. Sample Collection:
    - Collect representative samples of eggs and sperm from the desired fish species.
    - Ensure that the fish used for sampling are healthy and reproductively mature.
2. Visual Examination:
    - Perform a visual examination of the collected eggs and sperm.
    - Assess the external characteristics, such as color, size, shape, and presence of abnormalities or deformities.
3. Egg Evaluation:
    - Measure the size of the eggs using a calibrated microscope or imaging software.
    - Check for egg buoyancy by placing the eggs in water and observing their floating or sinking behavior.
    - Assess egg color, transparency, and overall appearance.
4. Sperm Evaluation:
    - Examine sperm motility by observing a sample of sperm under a microscope.
    - Assess the concentration of sperm using a hemocytometer or other cell counting methods.
    - Check for the presence of motile and immotile sperm, as well as abnormal sperm morphology.
5. Viability Assessment:
    - Perform a viability test to determine the percentage of live eggs or sperm.
    - Use staining techniques like eosin-nigrosin or trypan blue to differentiate between live and dead cells.
    - Count and calculate the percentage of viable gametes based on staining results.
6. Fertilization Trials:

- Conduct fertilization trials to assess the fertilization success rate of the collected gametes.
- Combine a known quantity of eggs and sperm in a controlled environment and monitor the fertilization process.
- Calculate the fertilization rate by determining the percentage of successfully fertilized eggs.

7. Genetic Analysis (Optional):
    - If desired, perform genetic analysis to assess the genetic quality of the gametes.
    - Use techniques like PCR, DNA sequencing, or microsatellite analysis to examine genetic diversity, parentage, or genetic abnormalities.
8. Data Recording and Analysis:
    - Record all observations and measurements in a standardized format.
    - Analyze the data to identify patterns, correlations, or any significant deviations from expected quality parameters.
    - Use appropriate statistical methods to interpret the results and draw conclusions.
9. Quality Classification:
    - Classify the gametes into different quality categories based on the evaluation results.
    - Establish criteria for determining high-quality, medium-quality, or low-quality gametes.
10. Interpretation and Decision-making:

- Evaluate the overall quality of the gametes based on the collected data.
- Make informed decisions regarding the use of the gametes for breeding purposes, such as selecting high-quality gametes for fertilization or considering alternative breeding strategies for low-quality gametes.

LAB-4: Lab manual for Cryopreservation of fish gametes.

Cryopreservation is a technique used to preserve fish gametes (eggs and sperm) at ultra-low temperatures, allowing them to be stored for extended periods. Here is a general outline of a laboratory manual for cryopreservation of fish gametes:

1. Sample Collection:
    - Collect representative samples of eggs and sperm from healthy and reproductively mature fish.
    - Use gentle stripping or massage techniques to obtain the gametes without causing damage.
2. Sample Preparation:
    - Dilute the collected sperm in an appropriate medium, such as a buffered saline solution or extender.
    - Wash the eggs with a suitable solution to remove any debris or mucus.
3. Cryoprotectant Solution Preparation:
    - Prepare a cryoprotectant solution by dissolving an appropriate cryoprotective agent, such as dimethyl sulfoxide (DMSO) or glycerol, in a suitable medium.
    - Determine the optimal concentration of cryoprotectant based on previous research or species-specific protocols.
4. Gamete Cryopreservation:
    - Mix the prepared cryoprotectant solution with the sperm or eggs in a ratio that achieves the desired final cryoprotectant concentration.
    - Transfer the cryoprotected gametes into suitable cryovials or straws for freezing.
5. Cooling:
    - Cool the cryovials or straws slowly using a controlled-rate freezer or liquid nitrogen vapor.
    - Follow a predetermined cooling protocol that gradually decreases the temperature to prevent ice crystal formation and cell damage.
6. Liquid Nitrogen Storage:
    - Transfer the cooled cryovials or straws to liquid nitrogen storage tanks.
    - Ensure that the gametes are stored in containers specifically designed for cryopreservation to maintain their viability.

7. Thawing:
    - Thaw the cryopreserved gametes by quickly immersing the cryovials or straws in a water bath at an appropriate temperature (determined based on species-specific protocols).
    - Minimize the thawing time to prevent damage to the gametes.
8. Post-Thaw Assessment:
    - Evaluate the post-thaw viability of the gametes using appropriate techniques.
    - Assess parameters such as motility, fertilization capacity, hatching success, or genetic integrity.
9. Data Recording and Analysis:
    - Record all observations, measurements, and experimental conditions in a standardized format.
    - Analyse the data to assess the effectiveness of the cryopreservation protocol and identify any factors affecting gamete viability.
10. Optimization and Refinement:

- Based on the results, refine and optimize the cryopreservation protocol to improve gamete survival and functionality.
- Consider adjusting cryoprotectant concentration, cooling rates, or other parameters to enhance cryopreservation success.

Problems:

Cryopreservation of fish gametes presents several challenges and potential problems that can affect the success and viability of the preserved gametes. Some common problems encountered during fish gamete cryopreservation include:

1. Cryoinjury: Cryopreservation involves exposing gametes to low temperatures, which can cause cellular damage and cryoinjury. Ice crystal formation, osmotic stress, and toxicity of cryoprotectants can all contribute to cryoinjury, leading to reduced viability and functionality of the gametes after thawing.
2. Ice Crystal Formation: The formation of ice crystals during freezing and thawing can damage cellular structures and membranes. Large ice crystals can pierce cell membranes and cause irreversible damage. Controlling ice crystal formation is crucial for maintaining the integrity of gametes during cryopreservation.
3. Cryoprotectant Toxicity: Cryo protectants are necessary to protect gametes during freezing and thawing. However, some cryo protectants can be toxic to cells, especially

at high concentrations or when exposure times are prolonged. Finding the optimal cryoprotectant concentration that balances protection and toxicity is a challenge.

4. Variability among Species: Different fish species have varying sensitivity to cryopreservation procedures. Some species have gametes that are more resilient to freezing and thawing, while others are more susceptible to cryoinjury. Optimizing cryopreservation protocols for each species can be time-consuming and require species-specific knowledge.

5. Genetic and Biological Factors: Individual variation among fish within a species can affect the success of cryopreservation. Genetic factors, such as differences in the composition of cell membranes or antioxidant capacity, can influence the tolerance of gametes to freezing and thawing. The age, health, and reproductive condition of the fish can also affect gamete quality and cryopreservation outcomes.

6. Post-thaw Survival and Functionality: Even with successful cryopreservation, post-thaw survival and functionality of gametes may still be compromised. Reduced fertilization rates, impaired embryonic development, or decreased hatching success can occur even if the gametes survive the freezing and thawing process. Ensuring that the cryopreserved gametes retain their full functionality post-thaw is a significant challenge.

7. Research and Development: Cryopreservation techniques for fish gametes are still being developed and optimized for various fish species. Limited knowledge and understanding of specific cryopreservation requirements for certain fish species can hinder progress and lead to lower success rates.

LAB-5: Model Bank Project for Eco-Hatchery

Introduction:

The commonly cultivated six species - Catla ([p) Rohu (*Labeo rohita*), Marigal (*Cirrhinus mrigala*) Silver carp (*Hypothalmichthys molitrix*), Grass carp (*Ctenopharyngodon idella*) and Common carp (*Cyprinus carpio*) are considered to be the best culturable species of fishes in the inland water system. These fishes originally belong to riverine environment and when cultured in standing water bodies such as ponds and tanks, they attain maturity but normally do not breed under confined conditions. Special attempts are therefore made to breed them by artificially creating riverine conditions and stimulating their endocrine system. The technique of breeding fish by other than its natural course is known as INDUCED BREEDING. Induced breeding techniques has been developed for production of quality fish seed of culturable varieties. It is one of the most dependable method of producing pure seed of desired species of fish. Further this technique has helped to produce fish seed in those areas where natural collection of fish seed was not possible.

Method of induced Breeding: Hypophysation - The technique of breeding the fish by administering pituitary gland extract injection is known as induced breeding or hypophysation. The pituitary gland secretes several hormones of which Gonadotropin is the most important for breeding.

The increasing demand of fish pituitaries have now been solved to some extent by the introduction of HCG, now readily available in the market. The HCG is now increasingly becoming popular due to its low cost. A mixture of HCG and pituitary hormone extract in definite proportion are employed successfully for breeding fish.

Collection of pituitary gland:

Glands are collected from ripe fish by removing the upper part of the skull(scalp) by a sharp knife or a bone cutter. After removing the scalp, the brain is exposed which is then cut from the posterior end and lifted up anteriorly. As soon as the brain is lifted the gland can be seen located in the cavity covered by a thin membrane. It is carefully picked up with the help of tweezers and kept immersed in a cavity block or a petri dish in absolute alcohol under cover.

Preservation and storage of pituitary glands:

While exposing and removing the glands great care is taken to avoid any contact with water. It is most important because the hormone of the pituitary gland is soluble in water. There are three methods for preservation of pituitary glands: -

1) preservation in absolute alcohol
2) preservation in acetone
3) preservation by quick freezing

Identification and selection of breeders for spawning:

The success of induced breeding depends on the proper selection of breeders. The identification the sex is made on the basis of the external characters. The mature males are distinguished from the females by the presence of denticulation on the dorsal surface of the pectoral fin which is rough to touch. Further, in males the abdomen is comparatively flat and the vent is not swollen but they ooze milt at slight pressure on their abdomen. The ripe females have soft and bulging abdomen with swollen pinkish genital opening. The presence or absence of pre-anal ridge is also taken into

consideration as a sign of maturity for selection of female breeders. For the production of quality seed and better growth, breeders of standard quality may be selected viz., Catla-3kg and above, rohu, grass and silver carp-2kg and above, mrigal and calbasu-1kg and above.

Maintenance of breeders:

Farm raised breeders give better results. The breeders can also be collected from ponds and rivers. The best time for collection is from November till March. The optimal rate of stocking may be 2000-2500 kg per hectare. Organic manure along with low dose of single super-phosphate (17 to 20 kg. per hectare) may be applied at fortnightly intervals in the pond where catla and silver carp is stocked as major species. Pond/tank stocked with grass carp as the major species need not be manured regularly. It may be fed with submerged aquatic vegetation during winter months and with grass on the advent of the spring (at the rate of 1-2% of the body weight) for acceleration of gonadal development. For other species feed prepared by mixing de-oiled rice bran and oil cake at the rate of 1-2% of the body weight of fish stock is desired in the initial stages. Fish meal containing 30% protein could be a better substitute for oil cake at the later stage (advent of the spring).

The mature male and female breeders are segregated and stocked sex-wise in separate ponds about 1-2 months before their breeding season and their genetic conditions and stage of maturity are checked periodically.

Breeding technique:

After selection of brood fishes from the segregated brooder ponds they are kept in the hapa at fish farms or in fish seed hatcheries for about 6-7 hours for conditioning. After proper conditioning the individual brood fish is weighed in a hand net using a spring balance. The breeders are then ready to receive injection.

Determination of dosage for injection:

Doses of pituitary gland extract are calculated on the basis of milligrams of pituitary gland per kilogram body weight of the recipient fish. Determination of proper dosages of pituitary gland alone or in combination with H.C.G. depends largely on the stage of sexual maturity of the breeders and also to some extent on the environmental (climatic) conditions. Spawning of carp may be obtained by administration of a single dose of 5-10 mg. of pituitary gland per kg. body weight to the female breeders. In males a low dose of 2 mg per kg. body weight is usually given. Better results are however obtained by injecting a preliminary dose of 2-3 mg per kg. body weight to the female fish alone and a second dose of 5-8 mg per kg. body weight after an interval of 6 hours. The males receive only a single dose of 2-3 mg. per kg. body weight at the time of 2nd injection to the female. Both the sexes are then put together in a definite ratio in circular spawning pool inside indoor hatchery.

Preparation of gland extract:

Once proper dosages are determined, the quantity of glands required for injecting the breeders is calculated. The required quantity of glands is then taken out from vials, dried on a filter paper and macerated in a tissue homogenizer with a little distilled water or 0.3% common salt solution. The homogenized glands are then centrifuged and the supernatant liquid is decanted and diluted with the same solvent to a known volume. The following dilutions are recommended.

Weight of brooder Preparatory dose Final dose

1.0 to 2.0 kg 0.50cc/fish 0.75 cc/fish

Above 2 kg 0.75 cc/fish 1.50 cc/fish

Method of injection:

Intra-muscular injection of fish pituitary extract is administered usually in the region of the caudal peduncle a little above or below the lateral line region or near the shoulder region. For injecting the fish, the needle is inserted under a scale, parallel to the body of the fish and then pierced into the muscles at an angle. A 2ml graduated hypodermic syringe is most convenient for injecting most of the fishes. The size of the needle depends upon the size of the breeder to be injected.

Breeding environment:

Success of spawning by hypophysation depends on the hydrological and climatic conditions. Temperature is one of the most important factors. It has been observed that 250-280C is most conducive for breeding. However, spawning could be induced at or below this temperature range. The percentage of fertilisation and hatching under uncontrolled conditions is not very satisfactory. Circulation of fresh water containing 5-9 mg/litre of oxygen promotes better success in spawning, higher fertilisation of eggs and higher recovery of hatchlings from fertilised eggs.

Spawning and hatching:

Eco-hatchery-circular spawning pool:

It is circular cement pool (8 metre in diameter) with 50 cubic metres of water holding capacity. The bottom of the pool slopes to the centre where there is an outlet pipe (10 cm dia) leading to the incubation pond (egg collection chamber). The wall of the spawning pool is provided with diagonally fitted inlet pipes at an angle of 450for circulation of water creating artificial riverine conditions. After circular pool is filled with water, about 80 kg. of females and 80 kg of males are released into the pool. When the breeders start coming up to the surface the valves are opened so that a circular current is created. The speed of water current is maintained at about 30 meters per minute.

The yield of 10 million eggs per breeding operation is usually achieved.

Eco-hatchery-Incubation pool:

As spawning goes on in the spawning pool, the fertilised eggs are led into the incubation pool (3 metre diameter - double walled circular pool, with inner wall of regulated mesh permitting outflow of water) where water at a regulated speed enters through the duck mouth valves fitted on the floor of the outer chamber. The speed of the water is regulated @2.5 litre/sec. in the initial stage and then reduced to 2.0 litre/sec. when movement of embryo inside the eggs starts. After hatching, the speed is again increased to 3.0-3.5 litres/sec. and the hatchlings are allowed to remain there for about 3 days till the yolk sac is absorbed.

Rearing of seed-hatchling to fry/fingerling stage:

Presently two techniques are practiced.

1. Rearing in earthen ponds at the fish farm.

2. Two fazed seed rearing - Phase one inside the hatchery building and phase two rearing in earthen pond at fish farm.

Rearing in earthen ponds at fish farm up to fry stage:

From the hatchling receiving ponds (nursery ponds), the weeds are removed. Unwanted fishes are removed using mahua oil cake containing 4-6% saponin at the rate of 2000-2500kg/ha metre. Lime is applied @ 250-300 kg per ha. for neutralizing acidity and helping mineralisation of organic matter. Manuring of ponds with cow dung @5000kg/ha is done about 15 days before the anticipated date of stocking by broadcasting all over the pond (dose of cow dung is doubled weigh Mauna oil cake is not used). Aquatic insects are controlled suitability of water is tested thereafter ponds are stocked with about 3-4 days old spawn usually in the morning hours. The moderate rate of stocking may be 25-30 lakhs/ha.

Supplementary Feed:

A mixture of finely powdered groundnut/mustard oil cake and rice bran/polish, in equal proportion by weight is supplied to the fry. Cobalt chloride or manganese sulphate (trace elements) @0.01 mg/day/spawn may be added to the feed. Addition of yeast increases survival of fry. Feed may be broadcast all over the pond commencing from the day of stocking. Feeding may be stopped one day earlier to the harvesting. The generally recommended feeding schedule is as under.

Period	**Rate of feeding per day**	**Approximate quantity per one lakh of spawn/day**
1st to 5th day	4 times the initial total weight of spawn stocked	0.56 kg
6th to 12th day	8 times the initial weight of spawn stocked	1.12 kg
13th & 14th day	No feeding	

Feeding not to be suspended in adverse ecological conditions.

Harvesting of fry:

The fry in about 2 weeks' time generally grows to 25-30 mm size. They are harvested with fine meshed (1.5 mm) drag net in the cool morning hours avoiding the cloudy days.

Chinese Fish hatchery - A few tips

- The location of a Chinese Hatchery should ideally be on a sloping high land for economical construction. If sloping land is available the floor level of the spawn collection tank should be adjusted to the ground level for draining out water by gravity.
- The level of eggs transfer outlet located at the centre of spawning tank should be about 10 cm. above the level of central overflow pipe at the top of the hatching tank. This will enable complete transfer of eggs from the spawning tank to use outside the tanks.
- Overflowing water from the hatching tank should not be passed on to the spawn collection tanks but should be put to use outside the tanks.
- Separate fresh-water supply lines should be installed from the overheads water tank to each tank i.e. spawning tank, hatching tanks and spawn collection tanks in order to ensure independent working of each.

- Water spraying arrangements should be provided for aeration and oxygenation in the three tanks.
- Eggs transfer pipe should discharge the water along with eggs into the hatching tank in between the two walls of the hatching tank to avoid damage of eggs.
- The walls of the spawning tanks should be provided with water inlet pipes installed in a diagonal position to create a circular water flow during the spawning period.
- Where water flow is insufficient for circulation, multiple-chambered hatching pools with paddle wheels for circulation may be constructed.

A. Capital cost

ECO-HATCHERY

Item	**Cost (Rs.)**
1. Renovation of tank of 2 ha water spread area involving excavation up to 1-foot depth	30,000
2. Circular breeding pool & hatching pools	
i. Breeding pool of 8 m diameter	30,000
ii. 3 Hatching pools of 3m diameter @ Rs. 15,000/- per pool	45,000
3. Overhead tank of 5000 gallons capacity	50,000
4. Shallow tube well 8"x6"x200'	25,000
5. Pump set (5HP)	20,000
6 Generator set with 10 KVA alternator	50,000
7. Guard shed and office room	25,000
8. Brood stock-5 tonnes	150,000
9. Contingent expenses for nets, equipments hapas etc.	30,000
	455,000

B. Recurring cost

I. Cultural cost for 3 preparatory months

i.	Feeding of brood stock @ 3% body weight for 5 tons of fish with 13,500 per kg artificial feed (150 kgsx30 daysx3 months) @ Rs.3/- per kg.	40,500
ii.	Salary of 2 guards-cum-labour @ Rs.450/pm	37,000
iii.	Cost of netting 2 times a month for 3 months	600
		43,800

II. Cultural cost for five operating months

	Feeding of brood fish @ 1.5% body weight for 5 tons of fish with 11,250 kgs artificial feed (75 kgs.x30 daysx5 months) @ Rs.3/kg	33,750
ii.	Wages of 2 guards-cum-labour @Rs.450/month for 5 months	4,500
iii.	Operation cost of electric pump/generator on equal ratio @ Rs.5.00 per hour for 5 hrs. daily (Rs.5x5hrsx30 daysx5months)	3,750
iv.	Additional labour for 5 months	36,000
v.	Misc. cost for pituitary glands, equipment, electricity etc.	19,250

		97,250

III. Cultural cost for 4 post-operatory months

i.	Manuring, liming and fertilising @Rs.450/- ha/month	3,600
ii.	Wages for 2 guards-cum-labour @Rs.450/- ha/month	3,600
iii.	Cost of periodical netting @ Rs.50/- per.month	400
iv	Other expenses	300
		7,900

Total Recurring cost (I+II+III) = Rs.1,48.900/-

C. Unit cost

i.	Capital cost	Rs.4,55,000
ii.	Recurring cost	Rs.1,48,900
		Rs.6,03,900

PRODUCTION

A. Installed Capacity

	Unit	size	No.	Capacity
i.	Spawning pool	8 m dia	1	150-200 kgs. brood fish
ii.	Hatching pools	3m dia	3	150 litres fertilized egg per pool or 450 litres of fertlised eggs from 3 pools requiring 8-0 kgs. of female brood fish or 160 kgs. of total brood fish.

B. Expected utilisation of installed capacity

i.	Per batch requirement of female brood fish	80 kgs.
ii	Per batch requirement of brood fish for single run (male or female)	160 kgs
iii.	Total number of hatchery runs @5 run/month for 5 months	25 runs
iv.	Total requirement of brood fish in 5 months (160 kgs.x25 runs)	4000 kgs
v	Requirement of breeder for 5 months operation (considering 90% of the brood fish will attain maturity and 90% of the matured breeders would respond to hypophysation)	4940 kgs
vi.	Number of spawn produced per kg body weight of female brood fish (considering @ 1.2 lakh/kg female, 90% fertilisation 80% hatching rate) * say 80,000	80000 nos.
vii.	Spawn produced from female brood fish in a single run	64 lakhs

C. Expected Income

I Gross income/run

I.	Gross income/run	
i)	From sale of 51.2 lakhs of major carp spawn (80% of the produce) @ Rs.200/lakh	Rs. 10,240
ii)	From sale of 12.8 lakhs of exotic carp spawn (20% of the produce) @Rs.500/lakh	Rs.6,4000
		Rs. 16,640

II	Gross income/month (Rs. 16,640/-x5 runs)	Rs. 83,200
III	Gross income in 5 operative months	Rs.4,16,000
iv	Net income	Rs. 2,67,050

* The fecundity may be as high as 2.0 lakh/kg of body weight of female brood fish. However, an average rate of 1.2 lakh/kg body weight is considered for purpose of economics.

Financial Analysis of Eco Hatchery

Particulars	**Years**				
	1	**2**	**3**	**4**	**5**
Capital Cost	4.55	-	-	-	-
Recurring Cost	1.49	1.49	1.49	1.49	1.49
Total	7.04	1.49	1.49	1.49	1.49
Income	2.08	4.16	4.16	4.16	4.16
Net Income	-4.96	2.67	2.67	2.67	2.67
NPV of cost@15%	2.32				
NPV of Benefits@15%	9.82				
NPV@15%	Rs2.32 lakhs				
BCR	1.24:1				
IRR	40%				

LAB-6: Model Bank Project for Shrimp Farm

1. INTRODUCTION

India is endowed with a long coastline and hence offers scope for large exploitation of marine wealth. Till a few years back, fishermen in India were involving themselves in traditional marine fishing. In the seventies fishermen started concentrating on catching prawns more commonly known as `shrimps' due to high profitable return on the same on account of their export value. Brackish water prawn farming started in a big way during 91-94 especially in the coastal districts of Andhra Pradesh and Tamil Nadu. Subsequently due to disease problems, litigation in supreme court and other social and environmental problems the sector suffered a huge set back and most of the corporate farms were closed. However, the small units continued to do farming and adopting extensive prawn farming systems. The shrimp farming has now been regulated with the establishment of Aquaculture Authority of India as per directions of Supreme Court for issuing licenses and overall supervision. It is commonly said that after Green and White Revolution in India, it is time for Blue Revolution to exploit the huge potential in fisheries sector. Shrimps are called the "Pinkish Gold" of the sea because of its universal appeal, unique taste, high unit value and increasing demand in the world market.

2. Scope for brackish water shrimp farming

The over exploitation of shrimp from natural sources and the ever-increasing demand for shrimp and shrimp products in the world market has resulted in the wide gap between the demand and supply shrimp in the International market. This has necessitated the need for exploring new avenues for increasing prawn production. The estimated brackish water area suitable for undertaking shrimp cultivation in India is around 11.91 lakhs ha. spread over 10 states and union territories viz... West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Pondicherry, Kerala, Karnataka, Goa, Maharashtra and Gujarat. Of this only around 1.2 lakhs ha. are under shrimp farming now and hence lot of scope exists for entrepreneurs to venture into this field of activity. The following table gives the state-wise potential and present level of development as on March 1996.

Sr.	State	Estimated brackish water area (ha.)	Area under cultivation (ha.)
1	West Bengal	405,000	34,660
2	Orissa	31,600	11,000
3	Andhra Pradesh	150,000	50,000
4	Tamil Nadu	56,000	2,879
5	Pondicherry	800	37
6	Kerala	65,000	14,657
7	Karnataka	8,000	3,500
8	Goa	18,500	650
9	Maharashtra	80,000	716
10	Gujarat	376,000	884
		1,190,000	118,983

3. Location of the project

The first and foremost requirement for entering into this venture is the acquisition of suitable land. The details of land identified/surveyed in coastal districts are available with the department of fisheries of the concerned State Governments and with the Regional offices of the MPEDA functioning in the coastal states of India. A suitable site is one that can support optimum conditions for the growth of shrimps at targeted production level. Most of the lands available along the coastline are owned by the State Governments. In some cases, the entrepreneur has to get it on long term lease from the revenue authorities of the State Government. If it is a private land, one has to preferably purchase on outright basis. While selecting the site for the project, the entrepreneur should ensure the following :

i) Area should be accessible preferably by a road even during the monsoon season.

ii) Mangrove area with large tree stumps should not be selected.

iii) Site should have good pollution free water supply of both freshwater and brackish water. Water quality parameters required for maximum feed efficiency and maximum growth of Penaeus monodon are given below:

	Water Parameters	**Optimum level**
1	Dissolved Oxygen	3.5-4 ppm
2	Salinity	10-25 ppt
3	Water Temperature	26-32 (C 0)
4	pH	6.8-8.7
5	Total nitrite nitrogen	1.0 ppm
6	Total ammonia (less than)	1.0 ppm
7	Biological Oxygen Demand (BOD)	10 ppm
8	Chemical Oxygen Demand (COD)	70 ppm
9	Transparency	35 cm
10	Carbon dioxide (less than)	10 ppm
11	Sulphide (less than)	0.003 ppm

iv) The areas should be flood free

v) Location with a natural slope, for proper drainage should be selected.

vi) Social problems due to competing use of water resources and drainage of waste water should be properly taken care of.

vii) Availability of necessary infrastructure namely electricity, ice factory, cold storage, communication facilities etc., are necessary for successful management.

4. **Borrowers' profile**

Complete details of the entrepreneurs, partnership firm, registered company should be given. Qualification and experience of the promoters, net worth of the borrowers, other activities undertaken by them, financial ability etc., have to be furnished.

5. **Technical feasibility of the project**

As the project envisaged new technologies, the borrowers may take the help of a competent outside agency to prepare the technical feasibility report on the project. However, to serve as a guidelines to the entrepreneurs in this regard the technical parameters are given in Annexure-I. However, the first and foremost requirement of the project is to get a license from Aquaculture Authority as per the existing norms and as per guidelines issued by Supreme Court.

6. **Physical and financial outlay**

Details of the physical and financial outlays involved for setting up of 5 ha. Brackish water prawn farm is furnished in Annexure No. II. It can be seen therefrom that the total cost including working capital expenses for raising the first crop for a 5 ha. Prawn farm works out to Rs.37.60 lakhs. While submitting the project to the banks for sanction of loan entrepreneurs are expected to submit detailed plan and estimates for all the civil works to be undertaken as also invoices of various items to be purchased from the suppliers.

7. **Margin money and bank loan**

The entrepreneur has to bring in 25% of the project cost out of his own resources and the balance of 75% will be provided by banks as bank loan. However, NABARD could consider providing margin money loan assistance in suitable and eligible cases as per the guidelines contained in circular no. DPD 67/92-93 dated 24.2.1993.

8. **Rate of refinance**

NABARD refinance is available for projects for setting up of shrimp farms provided the same is technically feasible and financially viable. NABARD is agreeable to provide refinance as per existing norms.

9. **Financial viability**

For undertaking shrimp culture within CRZ and outside CRZ the following assumptions have been made

		Improved Traditional (within CRZ)	Extensive (outside CRZ)
i.	Farm Size	5 ha	5 ha
ii.	Culture period	4-4 ½ months	4-4 1/2 months
iii.	Stocking density (PL-20)	50,000/- ha	1,00,000/- ha
iv	Survival	70%	65%
v	Expected production	1.2 tonnes/ha/crop	2.5tonnes/ha/crop
vii	Price of shrimp has been taken as Rs.250/kg		

The financial analysis for extensive system of shrimp farming has been shown in Annexure No. III. Results of the analysis are as under:
i. NPW at 15% DF - Rs.61.314 lakhs.
ii. BCR at 15% DF - 1.515
iii. IRR is more than 50 percent

10. **Marketing**

Because of huge gap between supply and demand of shrimps in local as well as international market, there may not be any problem in marketing the same. Shrimps can either be sold directly by the farmers in the market or sold to exporters for processing before export. Shrimps can be exported in frozen form with head on, head less, battered and breaded, or IQF products or any other form with value addition. The prawn has to be packed as per requirements of importing countries and therefore this should be decided after a detailed market survey. It is always advisable to get in touch with local distributing agents of the customer country. Hygienic packaging, display and appearance of the packet are key factors to attract consumers of importing countries.

11. Rate of interest

As per existing RBI guidelines interest rate to be charged to the ultimate borrower for loan exceeding Rs.2 lakhs will be as decided by the lending Bank. NABARD's interest rate for refinance would be as per existing norms prevailing at the particular time.

12. Repayment period

As can be seen from Annexure No. IV. the borrower will be able to repay the bank loan in 5 years with a moratorium of one year on repayment of principal.

13. Security

Security from the ultimate beneficiaries has to be obtained as per the rules of financing banks which have to be in conformity with the guidelines of RBI.

14. Conclusion

As shrimps have got good export potential, establishment of shrimp farms as per the model scheme indicated above is recommended for consideration by banks for financing.

ANNEXURE I

Technical Parameters for establishing a extensive shrimp farm

A -1. Design and Construction of shrimp farm :

An extensive shrimp farm should be of the size 0.4 - 0.5 ha. and preferably drainable from the management point of view. The ponds generally should have concrete dikes, elevated concrete supply canal with separate drain gates and adequate life supporting devices like generators and aerators.

The design, elevation and orientation of the water canals must be related to the elevation of the area with particular reference to the mean range of tidal fluctuation. The layout of the canals and dikes may be fitted as closely as technically possible to existing land slopes and undulation for minimizing the cost of construction.

2. General Earth Work

It is normally carried out in the following order:

1. Site clearing
2. Top soil stripping

3. Staking of centre lines and templates
4. Preparation of dike foundation
5. Excavation of drainage canals
6. Construction of dikes (peripheral and secondary)
7. Forming and compaction of dikes.
8. Excavation of pits for gates.
9. Levelling of pond bottom.
10. Construction of gates and refilling of pits
11. Construction of dike protection.

The top soil may be set aside and should again be spread later to preserve pond bottom fertility.

3. The essential components of a shrimp farm are: -

1. Ponds
2. Water intake structure
3. Store room for feed and equipment
4. An area for cleaning of the harvest
5. A workshop and pump house
6. Watch and ward room, office and a mini laboratory.

B. **Ponds**

From the management point of view, it is better to go in for ponds of 0.4 ha-0.5 ha size. These ponds should be preferably completely drainable. The ponds are partitioned by secondary dykes. In order to render over all protection to the cultured stock and all related structures a perimeter dyke also can be constructed.

The height of the perimeter dyke will depend upon the following factors, such as :

1. Height of water level in the area.
2. Elevation above mean sea level.
3. Height of free board.
4. The percentage allowance for soil shrinkage.

The partition dykes determine the size and limit of each grow out pond and its height is determined by the following factors namely:

1. The height of water column in the pond
2. Free board
3. Wave action
4. Shrinkage factor

The shrinkage factor is decided by the type of soil like heavy, medium and light soils.

C. **Gates**

They regulate the inflow and outflow of water into the pond and also are responsible for maintaining the desired water column in the pond. The main gates are constructed on the perimeter dyke and are usually located on the partition dykes and they regulate the water column in the individual ponds. It can be made out of concrete or PVC or Asbestos piping.

D. **Drain canals**

They are generally trapezoidal in cross section and its discharge capability is decided by area of cross section and velocity of water flow.

E. **Pond preparation**

Proper pond preparation will ensure higher production. The main objectives of pond preparation are :

1. To eradicate weed fishes and organisms
2. To remove obnoxious gases
3. To improve the natural productivity of the pond eco system
4. To maintain high water quality for proper growth and higher survival percentage.

Eradication of unwanted organisms is usually carried out by draining out the entire water and drying the pond bottom till it cracks. This also helps in removal of obnoxious gases and oxygenation of the pond bottom. It also improves the fertility of the soil.

Liming is done for correcting the pH and to kill pathogenic bacteria and virus. In undrainable ponds mahua oil should be applied @ 200 ppm to eradicate the weed fishes. After around two weeks' time organic and inorganic fertilisers are applied to enrich the soil and water. Once the thick lab-lab is formed the water level is raised and the pond is made ready for stocking.

F. **Selective stocking:**

The most suitable species for culture in India are the Indian white prawn *Penaeus indicus* and tiger prawn P. Monodon. The stocking density varies with the type of system adopted and the species selected for the culture. As per the directives of Supreme Court only traditional and improved traditional shrimp farming can be undertaken within the CRZ with a production range of 1 to 1.5 tonnes/ha/crop with stocking density of 40,000 to 60,000/ha/crop. Outside CRZ extensive shrimp farming with a production range of 2.5 to 3 tonnes/ha/crop with stocking density of 1,00,000/ha/crop may be allowed.

In order to have uniform growth of the cultured animal it is always advisable to go in for hatchery reared seeds.

G. **Food and feeding**

Shrimp diets may be supplementary or complete. In an extensive system the shrimps need a complete diet. Although natural food items have good conversion values but they are difficult to procure in large quantities and maintain a continuous supply.

At present most of the aquaculture farms depend on imported feed with a FCR of 1:1.5 - 1.8. The feeding could be done by using automatic feed dispensers, or by broadcasting all over the pond. If feeding trays are employed in selected pockets in the pond wastage in feed can be reduced.

H. **Harvesting:**

Complete harvesting can be carried out by draining the pond water through a bag net and hand picking. The average culture period required is around 120-150 days during which time the prawns will grow to 20-30 gm size (depending on the species). It is possible to get two crops in a year. Harvested shrimps can be kept between layers of crushed ice before transporting the consignment to

market.

ANNEXURE II

Estimated physical and financial outlay involved for setting up of a shrimp farm

A.	CAPITAL COST	Rs. lakhs
a)	Earth work for construction of ponds, drainages and feeder canals etc. (20000 m 3) Rs.25/m3	'5.0
b)	Lining of feeder canal	0.7
c)	Water inlet structure for ponds (2 Nos.)	0.5
d)	Water outlet structure for ponds (10 Nos.)	1
c)	Main outlet sluices (2 Nos.)	0.3
d)	Pump House, generator shed cum workshop etc	1
g)	Office, laboratory and stores	2
h)	Watchman shed	0.2
i)	Drinking water storage and supply network	0.75
j)	Pumps (3 Nos. Mixed flow pump of 25 HP each)	2.55
k)	Aerators (10 Nos. 1 HP)	2
l)	Electrical installations	2
m)	Generators (7 nos. X 30 KVA)	4
n)	Lab and farm equipment`	1
o)	Miscellaneous expenditure	1
	TOTAL	24
b.	OPERATIONAL COST FOR THE FIRST CROP	
a)	Seed @ Rs.300/1000 Nos. For 2 lakhs	3
b)	Feed @ Rs.40/kg for 15,000 kg	6
c)	Chemicals and manures for pond preparation (@ Rs. 15,000/ha)	0.75
d)	Fuel and electricity	1.5
e)	Repairs and maintenance	0.5
f)	Harvesting	0.25
g)	Labour for pond preparation	0.2
h)	Staff salary	0.92
	1 Farm manager 10,000 x 4	
	1 Mechanic 5,000 x 4	
	Farm hands (2) 4,000 x 4	
	Watchman (2) 4,000 x 4	
i)	Office expenses and Misc. expenses	0.5
		13.62
	Total outlay for 5 Ha	Rs.37.62 lakh Say 37.60
	Total outlay per Ha.	Rs.7.52 lakh appx.

Note: 1) Detailed plan and estimates are to be furnished along with the project.

2) Invoices for purchase of various items to be enclosed.

ANNEXURE III

Statement showing Financial Analysis for Shrimp Culture in 5 ha. Farm (Rs. lakhs)

		I year	II to VIII years	
A	COST			
1	Fixed Cost	24	--	
2	Recurring Cost	13.6	27.2	
	TOTAL COST	37.6	27.2	
B.	BENEFIT	25	50	
	NET INCOME	-12.6	22.6	
	Discount Factor at 15%	0.87	3.17	
	Net Present worth of cost	-32.71	86.22	= 118.936
	Net Present worth of benefits	21.75	158.5	= 180.25
	NPW at 15% discount factor	61.314 BCR = 1.515		
	Discount factor at 50%	0.67	1.25	
	NPW at 50%	-8.44	28.25	= 19.808
		Internal Rate of Return is more than 50%		

ANNEXURE IV

STATEMENT SHOWING REPAYMENT OF PRINCIPAL AND PAYMENT OF INTEREST (ILLUSTRATIVE)

Total Outlay = Rs.37.6 lakhs
Margin (25%) = Rs.9.4 lakhs
Bank Loan = Rs.28.2 lakhs
(Rs. lakhs)

Year	Bank Loan Outstanding at the beginning of the year	Net Income		Repayment		Bank Loan Outstanding at year end	Net Surplus
			Interest	Principal	Total		
1	2	3	4	5	6	7	8
1	28.20	25.00	4.23	--	4.23	28.20	20.77
2	28.20	22.60	4.23	7.07	11.30	21.13	11.30
3	21.13	22.60	3.17	8.13	11.30	13.00	11.30
4	13.00	22.60	1.95	9.35	11.30	3.65	11.30
5	3.65	22.60	0.55	3.65	4.20	--	18.40

LAB-7: Model Bank Project for Composite Farm

Model Bankable Project for Composite Fish Culture

Introduction:

Fish is the cheapest and most easily digestible animal protein and was obtained from natural sources from time immemorial for consumption by human beings. However, due to over exploitation and pollution, the availability of fish in natural waters have declined considerably forcing scientists to adopt various methods to increase its production. Fish farming in controlled or under artificial conditions has become the easier way of increasing the fish production and its availability for consumption. Farmers can easily take up fish culture in village ponds, tanks or any new water body and can improve their financial position substantially. It also creates gainful employment for skilled and unskilled youths. The technology developed for fish culture in which more than one type of compatible fishes are cultured simultaneous is the most advanced and popular in the country. This technology is known as Composite Fish Culture. This technology enables to get maximum fish production from a pond or a tank through utilization of available fish food organisms in all the natural niches, supplemented by artificial feeding. Any perennial fresh water pond/tank retaining water depth of 2 metres can be used for fish culture purpose. However, the minimum level should not fall below one metre. Even seasonal ponds can also be utilised for short duration fish culture.

1.1. Fish species involved in composite fish culture

Depending on the compatibility and type of feeding habits of the fishes, the following types of fishes of Indian as well as Exotic varieties have been identified and recommended for culture in the composite fish culture technology:

Species Feeding Habit Feeding zone

Indian Major Carp

Catla	Zoo plankton feeder	Surface feeder
Rohu	Omnivorous	Column feeder
Mrigal	Detritivores	Bottom feeder

Exotic carps

Silver carp	Phytoplankton feeder	Surface feeder
Grass carp	Herbivorous	Surface, column and marginal areas
Common carp	Detritivores/Omnivorous	Bottom feeder

2. Potential: The area under tanks and ponds available for warm fresh water aquaculture is estimated to be 2.85 million ha. In addition, 0.78 million ha of swamps, beels, etc. and low lying water logged area not good for agriculture as also any agriculture land can be converted for fish farming. Out of the total inland fish production around 60% is contributed by the culture sector. The average productivity from ponds at present is to the tune of 2160 kg/ha/year. This shows the tremendous scope for fish culture in the country. The area of 4.56 lakh ha. brought under scientific fish culture by 1997-98 is only 16% of the potential area of tanks and ponds available for development showing immense possibilities for horizontal expansion of composite fish culture.

3. Technical Parameters: Technical parameters of composite fish culture has been enclosed as annexure - I which includes site selection, items of development, pre-and post stocking operations, stocking density, fertilisation, feeding etc.

4. Margin:

The margin money may be considered @ 5, 10 & 15% for small, medium and large farmer respectively and 25% for companies and partnership firms.

5. Subsidy

Subsidy is available for various items like Pond Development, construction of New Ponds, first year inputs etc. under a centrally sponsored subsidy scheme implemented by majority of the State Governments through FFDA's for different categories of farmers, details of which may be obtained from concerned Fisheries Departments.

6. Eligible Borrowers

The following categories of borrowers are eligible to avail credit.

a) An Individual.

b) A company.

c) A Partnership firms.

d) A co-operative society.

e) A group of fish farmers.

Training in fish farming is being provided by the FFDA's to the eligible borrowers and it is essential that the borrower has prior knowledge of fish farming before ailment of bank loan.

7. Financial Outlay:

The details of Capital Cost and Recurring Cost have been indicated in annexure - II. As per annexure the capital cost for excavation of 1 Ha pond works out to be Rs. 1,75,000/- and the recurring cost as Rs 26,000/-. However, the cost is indicative and actual assessment of the cost parameters have to be done while submitting the project to the bank.

8. Repayment

Repayment of bank loan is possible in 6-8 years in equated annual instalments with moratorium on repayment of principal for the first year.

9. Financial Analysis:

As per financial analysis shown in annexure the scheme is financially viable. The financial parameters are as follows

i). NPW @ 15% Rs: 101106
ii). BCR @ 15% 1.51: 1
iii). IRR 25%

10. Rate of Refinance

NABARD provides refinance assistance for fish culture to commercial banks, cooperative banks and Regional Rural Banks. The rate of refinance is fixed by NABARD from time to time.

11. Rate of interest

Interest rate to be charged to the ultimate borrowers would be as indicated by bank/RBI/NABARD

from time to time depending on quantum of loan amount and the agency providing the loan.

12. Security

Security from the ultimate beneficiaries may be obtained as per the guidelines of RBI issued from time to time.

Annexure - I

Technical Parameters

Technical parameters that needs to be considered for Composite Fish Culture project are as follows:

1. Selection of Pond:

The main criteria to be kept in mind while selecting the pond is that the soil should be water retentive, adequate supply of water is assured and that the pond is not in a flood prone area. Derelict, semi derelict or swampy ponds can be renovated for fish culture by dewatering, desilting**,** repair of the embankments and provision of inlet and outlet. The pond may be owned by the individual or taken on lease in which case the lease period should be more or coterminous with the repayment period. The eligible items of pond development are as follows:

i)	Desilting of existing ponds
ii)	Deepening of shallow ponds.
iii)	Excavation of new ponds.
iv)	Impoundment of marginal areas of water bodies.
v)	Construction / repairs of Embankments.
vi)	Construction of Inlets / Outlets.
vii)	Any other item like civil structures, watchmen huts, water supply arrangements / electricity supply arrangements etc. depending on requirements of the project based on its size etc.

2. Pond Management:

Pond Management plays a very important role in fish farming before and after the stocking of fish seed. Various measures that are required to be undertaken in pre and post stocking practices are tabulated below:

A) Pre-stocking:

In case of new ponds, pre-stocking operations starts with liming and filling of the pond with water. However, the first step for existing pond requiring development deals with clearing the pond of unwanted weeds and fishes either by manual, mechanical or chemical means. Different methods are employed for this.

i) Removal of weeds by Manual/Mechanical or through Chemical means.

ii) Removal of unwanted and predatory fishes and other animals by repeated netting or using mahua oil cake @ 2500 kg/ha metre or by sun drying the pond bed.

iii) **Liming -** The tanks which are acidic in nature are less productive than alkaline ponds. Lime is used to bring the pH to the desired level. In addition, lime also has the following effects -

a) Increases the pH.
b) Acts as buffer and avoids fluctuations of pH.

c) It increases the resistance of soil to parasites.
d) Its toxic effect kills the parasites; and
e) It hastens organic decomposition.

The normal doses of the lime desired ranges from 200 to 250 Kg/ha. However, the actual dose has to be calculated based on pH of the soil and water as follows:

Soil pH	Lime (kg/ha)
4.5-5.0	2,000
5.1-6.5	1,000
6.6-7.5	500
7.6-8.5	200
8.6-9.5	Nil

The pond is required to be filled with rain water or water from other sources after liming in case it is a new pond.

iv) Fertilisation:

Fertilisation of the pond is an important means of intensifying fish culture by increasing the natural productivity of the pond. The fertilisation schedule has to be prepared after studying the quality of the pond soil. A combination of both Organic and Inorganic fertilisers may be used for best results. The fertiliser programme has to be suitably modified depending on the growth of the fish, available food reserve in the pond, physicochemical conditions of the pond and climatic conditions.

a) Organic	:	Organic manure to be applied after a gap of 3 days from the date of liming.
b) Inorganic	:	Cow dung @ 5000 kg/ha or any other organic manure in equivalent manurial value
	:	Inorganic fertilisation to be undertaken after 15 days of organic manuring. Requirement of nitrogenous and phosphate fertilisers would vary as per the nature of the soil fertility indicated below. However, any one of the nitrogen and phosphate fertilisers could be used as per given rate.

Inorganic Fertiliser Application (kg/ha/month)

Soil fertility status	Ammonium sulphate	Urea
1. Nitrogen (mg/100 g soil) i) High (51-75) ii) Medium (26-50) iii) Low (upto 25)	70 90 140	30 40 60
2. Phosphorus (mg/100 gm soil)	**Single super phosphate**	**Triple super Phosphate**
i) High (7-12) ii) Medium (4-6) iii) Low (upto 3)	40 50 70	15 20 30

B) STOCKING:

The pond will be ready for stocking after 15 days of application of fertilisers. Fish fingerlings of 10 cm size (approx.) should be used for stocking @ 5000 nos. per hectare. However, if fingerlings of smaller size are used, suitable allowance may be made accounting for mortality. Depending on availability of seed and market condition, stocking can be of 3, 4 or 6 species combinations in the following ratio.

Species combination (ratio)

Species	3-species	4-species	6-species
Catla	4.0	3.0	1.5
Rohu	3.0	3.0	2.0
Mrigal	3.0	2.0	1.5
Silver Carp	-	-	1.5
Grass Carp	-	-	1.5
Common Carp	-	2.0	2.0

C)POST STOCKING:

a) Supplementary feeding:

Fishes need much more food than what is available naturally in the pond. Fishes can be fed with a mixture of bran and oilcake in equal quantities daily. The feed should be placed on a bamboo tray and lowered to the pond bottom or it can be sprayed at the corners. After some time the fishes will get used to this type of feeding and aggregate at the same place at particular time. The recommended feeding rate is as under:

Culture period	Quantity per day in kgs.
I quarter	1.5 to 3
II quarter	3 to 6
III quarter	6 to 9
IV quarter	9 to 12
Total (for the year)	1,655 to 2,700

b) Manuring:

i) Organic manuring may be done in monthly instalments @ 1000 kg/ha.
ii) Inorganic fertilisation may be done at monthly intervals alternating with organic manuring. However, the monthly rate of fertilisation will depend on pond productivity and the growth of the fishes. It should be ensured that excess fertilisation does not take place which may result in eutrophication.

D) Harvesting:

Harvesting is generally done at the end of 1st year, when the fishes attain average weight of 750 gms. to 1.25 kg. A production of 4 to 5 tons/ha can be obtained in a year. However, for the purpose of working out economics' a production level of 3 tons/ha/year may be considered. Harvesting is done by partial dewatering and repeated netting. In some cases, complete dewatering of ponds is resorted to.

3) Vertical expansion of fish culture:

A number of measures are now being employed by the entrepreneurs to increase the per hectare production of fish. Important measures adopted are stocking of Yearlings by stunning the growth of

fish seed during first year, heavy stocking and multiple harvesting after the fishes attain a size of 500 gms., multiple stocking and multiple harvesting, use of aerators, integrated fish farming with animal husbandry activities like dairy, poultry, piggery or duckery to get daily organic manuring to the pond thus increasing its fertility. It is possible to increase the per hectare production of fish to 7 to 10 tonnes per ha per year by employing different methods as indicated above.

Annexure - II

Indicative Unit Cost and Income for 1 Ha pond requiring 1-meter excavation

Item	New ponds Excavation up to 1 metre depth
A. Capital cost: 1.Excavation 10,000 m3@Rs.15/m3 2.Construction of inlet/outlet (L.S.) 3.Equipments & Gears (L.S.) 4.Total	150000 20000 5000 175000
B. Recurring cost: 1.Lime 500 kg @ Rs. 5/kg 2.Fingerlings 5000 Nos.@ Rs. 400/1000 Nos. 3.Organic manure(cow dung) 15 tonnes @Rs.300/ton 4.Urea 330 kg@Rs.5/kg 5.Triple Super Phosphate165 kg@Rs.5 per kg 6.Mustard oil cake1350 kg@Rs.6/kg 7.Rice Bran: 1350 kg@ Rs.3/kg 8. Insurance cost@4% of Seed and Fertilizers 9. Miscellaneous including Harvesting, Marketing expenses and Watch and Ward etc.	2500 2000 4500 1650 825 8100 4050 960 2415 27000
C. Income: 1. Production (From second year onwards) 2. Sale Price (per Kg) 3. Total Income	3000 Kg Rs 30/- Rs 90,000/-

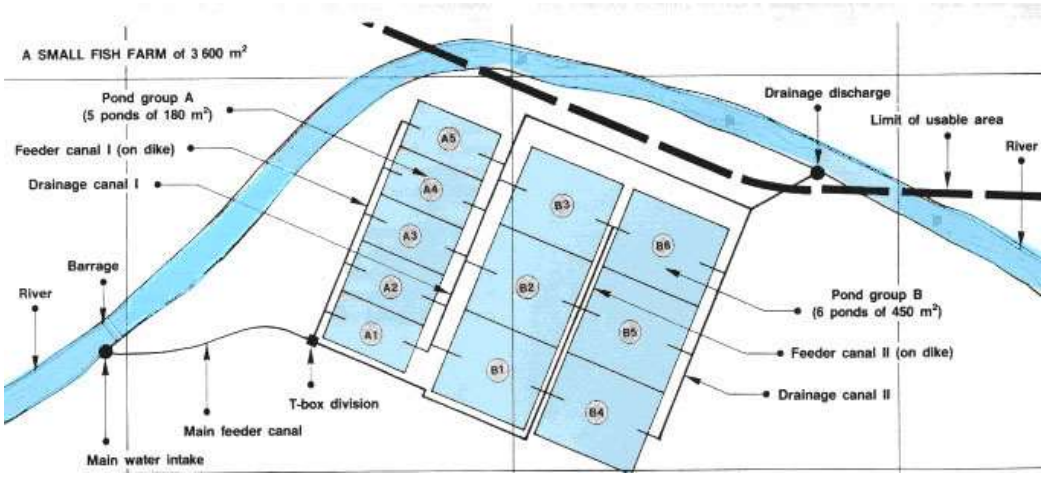
ANNEXURE- III

Statement showing Financial Analysis for Composite Fish culture in New Ponds(Indicative)

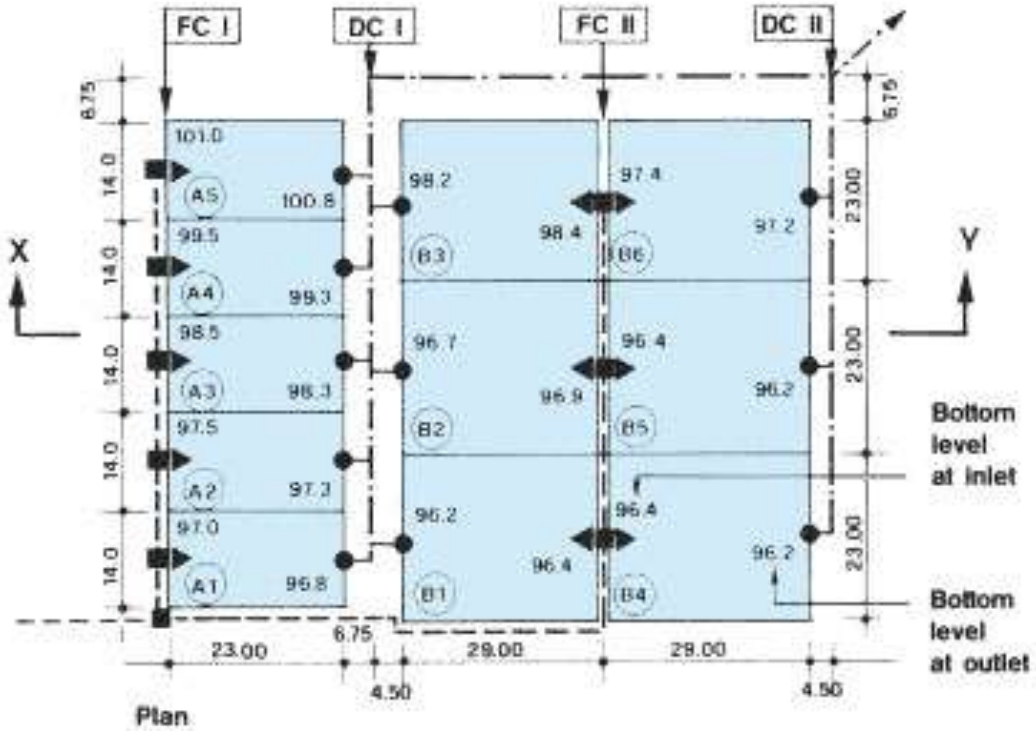
(Amt in Rs.)

A. Cost	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8
1. Fixed Costs	175,000	-						
2. Recurring Costs	27,000	27,000	27,000	27,000	27,000	27,000	27,000	27,000
Total	202,000	27,000	27,000	27,000	27,000	27,000	27,000	27,000
B. Benefits								
1. Income from sale of fish	-	90,000	90,000	90,000	90,000	90,000	90,000	90,000
2. Net Income	-202,000	63,000	63,000	63,000	63,000	63,000	63,000	63,000
3. NPV Costs	273,332							
4. NPV Benefits	374,438							
5. NPV	101,106							
6. BCR	1.51:1							
D. IRR	25 %							

LAB-8: Lab manual for Layout of Aqua farms.



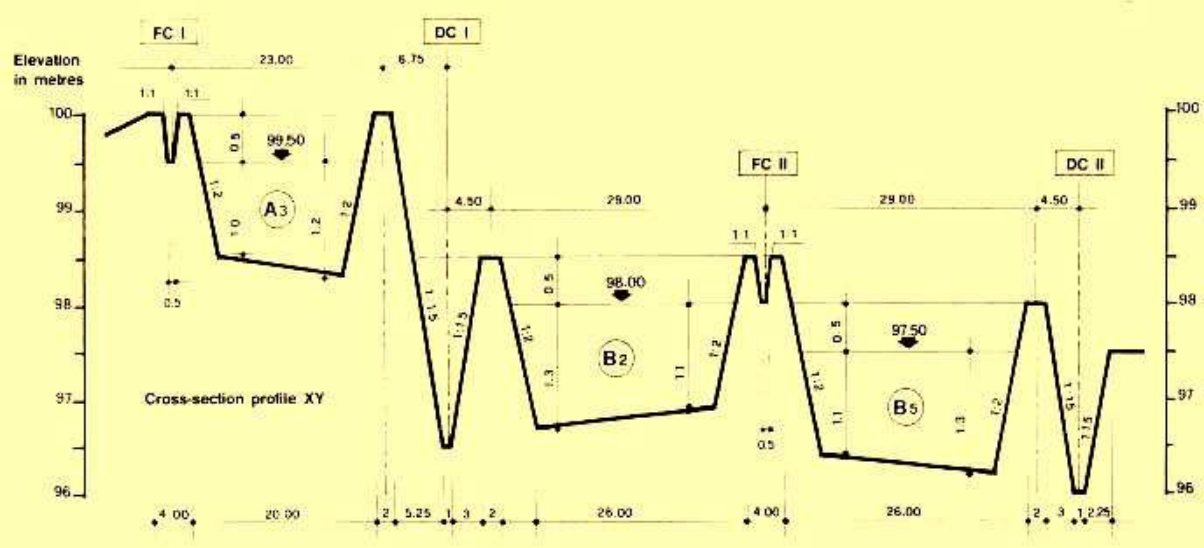
General Layout of the Farm



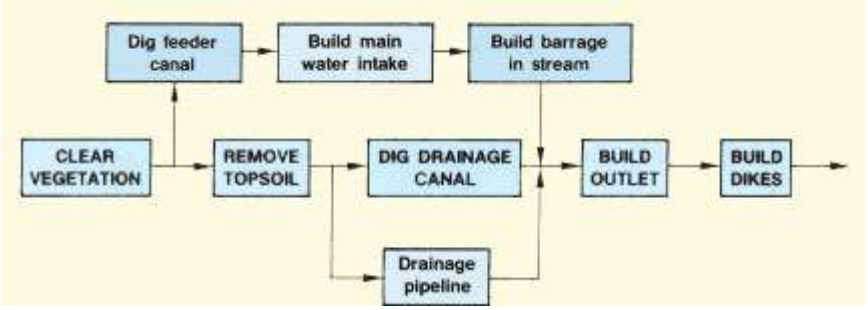
Detailed Layout of the Farm

KEY
- Feeder canal (FC)
- Drainage canal (DC)
- Pond inlet
- Pond outlet
- T-or X-division box
- Elevation measurements

NOTES:
Water depths of pond group A: 1.0 to 1.2 m
Water depths of pond group B: 1.1 to 1.3 m
Freeboard: 0.50 m



Cross section Layout of the Farm



Schedule Activity

The farm having

1) Five small rearing pond of 180 m^3.
2) Six large pond of 450 m^3.
3) Feeder Canal – 200 m.
4) Dikes – 150 m^3

Working hours for the construction of the farm

1) Working hours for manual construction of rural ponds – 130 hrs.
2) Feeder canal – 50 hrs.
3) Excavation/dikes construction – 600 hrs.
4) Inlet/outlet pipes – 10 hrs.
5) Total working time – 785 hrs.

LAB-9: Major Groups of Parasites – (Modified from Hoffman 1999)

Fungi: Usually filamentous, nonseptate, Ichthyophonus odten occurs as spheres.

Protozoa: Commonly referred as single celled animals including ciliates, flagellates, and sporozoans.

Monogenea: Flukes with flattened body; posterior attachment organ (haptor) which bears hooks or clamps; lack true suckers; attach on exterior body of fish host (some exceptions) and exhibit simple life cycles with no intermediate hosts.

Trematoda (Digenetic): Flukes with flattened body; oral and ventral suckers (some exceptions); and exhibit complex life cycles involving multiple hosts.

Cestoidea (Tapeworms): Worms with flattened segmented body (usually); and head (scolex) usually bears suckers, hooks or suctorial grooves, occasionally, no organs of attachment.

Nematoda (Roundworms): Thin, elongated worms with cylindrical body covered with rigid cuticle, one or both ends attenuated; no organs of attachment.

Acanthocephala (Spiny-headed worms): Body cylindrical, sometimes slightly flattened; and spectacular hook bearing eversible proboscis present.

Crustacea (Parasitic): External parasites may be louse like (*Argulus*); worm like (*Lernaea*); or grub-like (*Salmincola, Ergasilus*).

Hirudinea (Leeches): External parasites, some dorsoventrally flattened, others more cylindrical; and body segmented with anterior and posterior sucker.

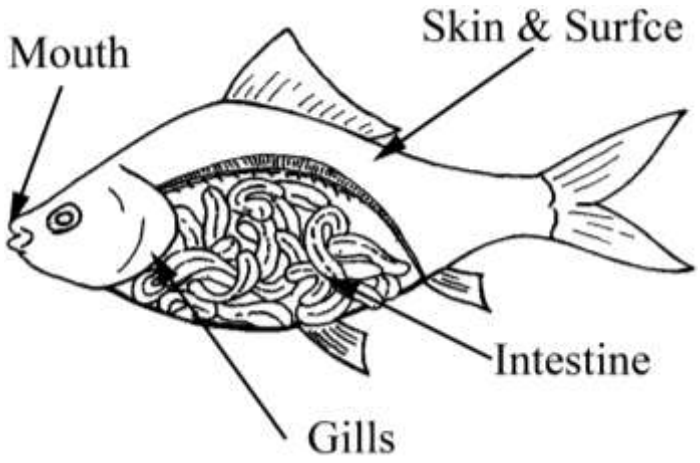
Glochidia: Larval fresh water clams encapsulated in fins and gills; resemble clams with shells; and some armed with hooks.

LAB-10: Key to the Major Taxa of Adult Parasites

1. Individual organisms are microscopic and single celled (except *Ichthyopthirius*), may be multi nuclear .....**Protozoa**
2. Body with segmentation, distinct annual ring present, anterior attachment organ present, which may include hooks and muscular suckers, posterior, attachment organ is not present, gut absent.....**Cestoidean**
3. Anterior attachment organ present which may include hooks, and muscular suckers, posterior attachment organ not present, gut present..... **Hirudinea**
4. Posterior attachment organ present which may include hooks (hamuli) and marginal hooks, external parasites, body with out segmentation, annual rings.....**Monogenea**
5. Posterior attachment organ not present, possess circumoral and ventral suckers except for blood dwelling species; internal parasites..... **Trematoda**
6. Anterior spine proboscis present.....**Acanthocephalan**
7. Anterior spine not proboscis present.....**Nematoda**
8. Body not formed if two hinged valves possess appendages for attachment to host surface; organism not encysted, external parasite..... **Crustacea**

LAB-11: Key to the Major Taxa of Adult Parasites

Different Regions of Fish and Parasites



Skin and Fin Surfaces:

Fungi – External Fungi: *Saprolegnia and relatives, Exophiala pisciphila*
Protozoan – Ecto-parasitic protozoa (*Ambiphyra, Amphiletus, Bodomonas, Chilodonella, Epistylis, Ichthyobodo, Ichthyophthirius, Oodinium, Trichodina, Myxobolus squamalis*
Monogenean – *Gyrodactylus spp.* usually not other monogegenean parasites
Trematoda – Metacercariae of many species, including *Neascus* (Black Spot)
Crustacean – *Argulus, Lernaea, Ergasilus, Salmincola*

Gills:

Fungi – *Dermocystidium*
Protozoan –*Ambiphyra, Amphiletus, Bodomonas, Chilodonella, Epistylis, Ichthyobodo, Ichthyophthirius, Oodinium, Trichodina, Myxobolus, Tricophyra*
Monogenean – *Gyrodactylus, Dactylogyrus, Cleidodiscus, and many other species.*
Trematoda – Metacercariae of many species, *Sanguinicola*
Crustacean – *Argulus, Lernaea, Ergasilus, Salmincola, Achtheres*

Mouth:

Protozoa: *Apiosoma, Myxosporea*
Trematoda*: Leuceruthrus*
Nematoda*: Philometra nodulosa*
Copepoda*: Lernaea, Salmincola*

Blood:

Protozoa*: Trypanosoma, Trypanoplasma, Babesiosoma, Dactylosoma,*
Trematoda*: Sanguinicola*
Nematoda*: Philometra sanguine, P. Obturans*

LAB-12: Examination for Fish Protozoan Parasites

1. Examine skin, fins, and gills for larger parasites that can be seen in naked eye. If the fish is small it can be placed in Petridis with water or normal physiological solution and examined with dissecting microscope. Skin, gills, fins of larger specimen can be removed and examined similarly. These can be stored in 10% formalin solution.

2. Prepare mucous of wet mount by scarping the dorso-ventral surface of the fish with the dull side of scalpel blade. Transfer the mucous to a clean microscope slide, add a drop of saline water on it & cover with cover slip. Examine the smaller parasites in student microscope with 100X to 450X magnification.

3. Remove operculum with scissor. If the fish is small, remove the entire gill arch and transfer to the slide. Add saline and cover with cover slip. For larger specimen, the mucous from gills are collected or gill filaments are taken on slide. Examine the slide with 100X magnification followed by 450X magnification.

4. Open the fish. Examined the body cavity for encysted parasites. Remove small amount of blood from heart. Dilute 1:1 with saline and examine at 100X magnification. Blood smears can also be prepared at this and later stained for blood sporozoa.

5. Viscera of fish were removed. The intestinal fluids were collected on a microscopic slide with required amount and cover slip was added on it. The process was followed to see endoparasites like nematodes etc. Then the intestinal tract were cut open with entire length and examined under dissecting microscope for helminth parasites. The tracts were flush with saline water and the gut contents examined separately from the tract wall.

6. Individual organs – of larger fish were collected and transferred to the saline in Petri dish. Squashes of kidney, liver, spleen and gonads were prepared. Swim bladders were removed by not deflating it. Then examined under dissecting microscope. Gall bladder was also removed with fine forceps examined properly. The wet prepared mount slide to see it properly under microscope. Each eye was removed and examined under dissecting microscope to observe any movement of parasites, flukes.

7. The head was cut open with length wise and brain was removed. Some of brain was squashed on to the slide and saline water with cover slip was added on it. The slide was then examined under microscope.

LAB-13: Identification of Some Parasitic Fish Diseases

A. Ichthyophthiriasis:

Species Affected: Fry and fingerlings of C. catla, L. rohita in nursery and rearing ponds.

Causative agents: *Ichthyophthirius multifiliis*

Diagnosis

- Anorexia (loss of appetite, refusing all food, with consequential wasting)
- Hiding abnormally , Flashing , Rubbing and scratching against objects
- Ich infections are usually visible in the form of characteristic white spots on the side of the fish. The white spots are pockets of fish epithelia containing *Ichthyophthirius* cells called trophozoites or trophonts, which feed on the tissues of the host and may grow to 1mm in diameter. A smear should show ciliates if white spot is present.
- Fins are folded and show white spots about 1mm in diameter.
- Eyes may appear cloudy.
- Gill infection will cause breathing at the surface and fast respiration. Gill examination may show numbers of such white spots.

Treatment

Prophylactic: Maintain the good quality of the water, placed a bamboo pole inside the pond vertically, which help to rub the fish body against it and parasite may dislodged from fish body. Water should be monitored during the treatment course in case there is any loss of filter activity.

Therapeutic: Hourly bath with formalin @ 1:5000 formalin solutions for seven days.

Bath in 2% NaCl for 7 days or more (Gopalakrishnan 1963, 1964) An alternative treatment is prolonged salt immersion at 1-2 ppt (parts per thousand), i.e. 1-2 grams per litre

B. Trichodiniasis:

Species Affected: Indian Major Carps, Oreochromis mossambicus

Causative Agents: Trichodina reticulate, Trichodina nigra. (Trichodiniasis)

External Symptoms:

- Clinically fish usually exhibit flashing and become lethargic.
- There is an increase in mucus production causing a white to bluish haze on the skin. The skin may develop ulcers and the fins may fray.
- If the gills are involved, the fish may have severe respiratory distress.
- The gills turn pale and there is creamish coating on the gill due to excessive secretion of mucous.

Treatment:

Prophylactic: The presence of these ciliates indicates deterioration of water quality thus, the measures to be adopted are: 1) Water quality should be improved, 2) Stocking density should not be high.

Therapeutic: NaCl treatment @ 2 – 3% till the fishes is stressed. $KMnO_4$ treatment 4 mg/l in pond water. Formalin treatment @ 25 mg/l in pond water or @ 100 mg/l for bath treatment with aeration.

C. Whirling Disease:

Species Affected: Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss*), Indian Major Carps.

Causative Agents: Whirling disease is caused by *Myxobolus cerebralis*, a parasitic protozoan

External Symptoms:

- Mass mortalities in fry
- Convulsive movements
- Faster breathing, jerking backwards movements
- Many fish swim with a whirling motion ('tail chasing')
- Erratic, nervous darting movements until exhausted
- Darkening of skin from the anus to the tail ('blacktail')
- Clinical signs of disease in an infected animal
- Spinal curvature
- Skull deformation
- Shortened gill plates

Treatment

Prophylactic: Maintain the good quality of the water, remove excess organic load from water

Therapeutic: Hourly bath with formalin @ 1:5000 formalin solutions for seven days. Bath in 3 - 5% NaCl for 7 days, Bath treatment with potassium permanganate solution.

D. Gyrodactylosis:

Species Affected: Indian major and minor carps, Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*), and lake trout (*Salvelinus namaycush*)

Causative agents: The causative agent for gyrodactylosis is *Gyrodactylus salaris* (a small parasitic flatworm-fluke).

External Symptoms:

- high mortality
- scrubbing (rubbing against objects in response to skin irritation) and flashing (darting and twisting of fish and erratic swimming)
- ulcers on infected fish
- peeling of skin
- fish appear pale
- excess mucus on skin
- frayed fins

Treatment:

Prophylactic: Maintain good quality of water; stop the entry of unwanted fishes in the culture system, regular liming of water and use of potassium permanganate in pond water can check the disease out break

Therapeutic: Use of CaO in pond water according to the need and pH of the water, Bath treatment in 5% NaCl solution. Bath treatment in potassium permanganate solution, Formalin bath treatment @ 100mg/l, application of formalin @ 25ppm.

E. Dactylogyrosis:

Species Affected: Indian major and minor carps, Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*), and lake trout (*Salvelinus namaycush*)

Causative agents: The causative agent for dactylogyrosis is *Dactylogyrus sp* (a small parasitic flatworm-fluke).

External Symptoms:

- High mortality, the respiratory function of fish is affected. There is hyperplasia in gill epithelium. At the site of attachment the gill tissue erosion may occur.
- Ulcers on gill regions of infected fish
- Gills appear pale
- Excess mucus on gills

Treatment:

Prophylactic: Maintain good quality of water; stop the entry of unwanted fishes in the culture system, regular liming of water and use of potassium permanganate in pond water can check the disease out break

Therapeutic: Use of CaO in pond water according to the need and pH of the water, NaCl bath treatment @ 3 – 5% solution for 10 – 15 minutes. Formalin bath treatment @ 100 mg/l of water 25 mg/l of water body. $KMnO_4$ treatment @ 4 mg/l is effective.

F. Argulosis:

Species Affected: Indian major and minor carps,

Causative agents: The causative agent for Argulosis is *Argulus sp* (small crustacean parasites).

External Symptoms:

- Presence of small white transparent, oval shaped parasites on the skin
- Ulcers on skin of infected fish, ulcerated areas may turn black in advanced stages
- Erratic swimming behaviour
- Loss of appetite

Treatment:

Prophylactic: Maintain good quality of water; stop the entry of unwanted fishes in the culture system, regular liming of water and use of potassium permanganate in pond water can check the disease out break; affected pond should be dried in sun for few days.

Therapeutic: Use of CaO in pond water @ 0.1 – 0.2 mg per litter, NaCl bath treatment @ 3 – 5% solution for 10 – 15 minutes. Formalin bath treatment @ 100 mg/l of water 25 mg/l of water body. KMnO4 treatment @ 4 mg/l is effective, use lindone or diptrex SP – 80 in pond water.

G. Lernaeosis:

Species Affected: Indian major and minor carps,

Causative agents: Lernaea bengalensis

External Symptoms:

- In light infestation on the body, the fishes become restless and try to rub their body against hard substratum
- Erratic swimming behaviour
- Loss of appetite
- Sloughing of scales
- Show signs of ulceration
- Heavy mortality in fish pond

Treatment:

Prophylactic: Maintain good quality of water; stop the entry of unwanted fishes in the culture system, regular liming of water and use of potassium permanganate in pond water can check the disease out break; affected pond should be dried in sun for few days, in this stage 100mg/l of lime can be applied in the pond bottom.

Therapeutic: Use of CaO in pond water @ 0.1 – 0.2 mg per litter, NaCl bath treatment @ 3 – 5% solution for 10 – 15 minutes. Formalin bath treatment @ 100 mg/l of water 25 mg/l of water body. $KMnO_4$ treatment @ 5 mg/l is effective, use lindone or diptrex SP – 80 in pond water @ 0.2ppm to 0.5ppm.

LAB-14: Identification of Some Bacterial Fish Diseases

A. Dropsy

Species affected:

Juveniles and adult of *Catla catla Labeo rohita*, and *Cirrhinus mrigala*

Symptoms:

- This disease is characterised by a swollen or hollow abdomen (Ascites).
- A concentration of fluid in the body tissues and cavities causes the fish's abdomen to become swollen and appear bloated.
- Swollen areas may exhibit a 'pine-cone' appearance caused by the fish's scales sticking out. You can best see this by viewing your fish from the top.
- Fish may also stop feeding, appear off-colour, become listless and/or lethargic, have sunken eyes, and hang at the top or stay at the bottom.
- Very often due to the secondary infection the scales fall off ulceration become deep with necrosis.
- The condition affects the fish's internal organs, ceasing proper function.

Causative agents:

Aeromonas hydrophila (The organisms were gram (–) ve, motile, nonsporulating rods, occurring singly).

Treatment:

Prophylactic:

- Dropsy is not very contagious; however, if a fish is diagnosed with dropsy, it is important to remove it from the pond as soon as possible.
- If there are multiple fish, treat the afflicted fish in a specially established "sick tank" (Quarantine).
- Dropsy can spread from the ill fish, possibly causing stress among the other fish in the tank community. This extra stress may make the others vulnerable to dropsy or other forms of disease.
- The water body is treated with KMnO4 @ 1 mg/l. maintaining water quality is always extremely important.
- It should always be checked first, because it is often the cause of disease in aquarium fish. Frequent water changes can work to prevent the spread of disease by "watering

down" the concentration of disease agents, and by reducing stress on the tank occupants.

- All tanks need a 10-25% water change on a weekly basis. The best method of changing water is siphoning the water from the bottom, removing debris and fecal matter from the gravel.

Therapeutic:

- Application of KMnO4 @ 5 mg/l (Gopalakrihnan, 1963).
- Treatment may consist of antibiotics targeting the causative agent.
- They work best in the very early stages of dropsy. A more hands-on approach is to raise the aquarium's temperature a few degrees- slightly higher than usual.
- Adding Epsom salts to the water at (a rate of 20 mg/L) helps to encourage the fish to expel unnecessary damaging fluids.

B. Columnaris (Cotton Wool Disease)

Species affected:

Catla catla Labeo rohita, Cirrhinus mrigala Ctenopharyngodon idella

Symptoms:

- Grayish-white film on skin, damaged fins, ulcers, yellow to gray patches on gills, tissue on head may be eaten away.
- Effect on host
- The causative bacterium penetrates the epidermis and dermis & form red ulceration in musculature. The gill lamellae very often erode and fringes of gill filament are lost due to necrosis.

Causative agents:

Flexibacter columnaris (Kumar et al 1986)

Treatment:

Prophylactic:

- Improve the quality of water.

Therapeutic:

- Must be treated immediately with Over-the-counter antibiotic medications. Very contagious – disinfect tank, rocks, net, etc.
- Dip treatment with KMnO4 @ 500 mg/l. Treat pond with KMnO4 @ 3 to 5 mg/l.

C. Ulcer:

Species Affected:

Catla catla Labeo rohita, Cirrhinus mrigala Ctenopharyngodon idella, Clarias sp and H. molitrix

External symptoms:

- Initially pimple like reddish areas appears on the body, upper surface of the skin.

Causative agents:

Aeromonas and *Pseudomonas* cause such ulcers on fish body stressed by high stocking and bad quality of water.

Treatment:

Prophylactic:

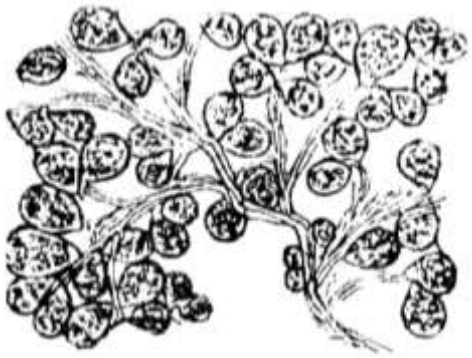
- The water body should be treated with KMnO4 @ 1 mg/l. Water quality has to be improved.

Therapeutic:

- OTC antibiotic treatment,
- Pond treatment - KMnO4 @ 5 mg/l,
- sulphadiazine treatment with food @ 100 mg/kg, terramycine @ 75-80mg/kg of body weight for 10-12days in food,
- Chloramphenicol treatment @ 20 – 30 mg/kg of body weight can be given as injection.

LAB-15: Identification of Parasites

Specimen No. 1



Taxonomy:

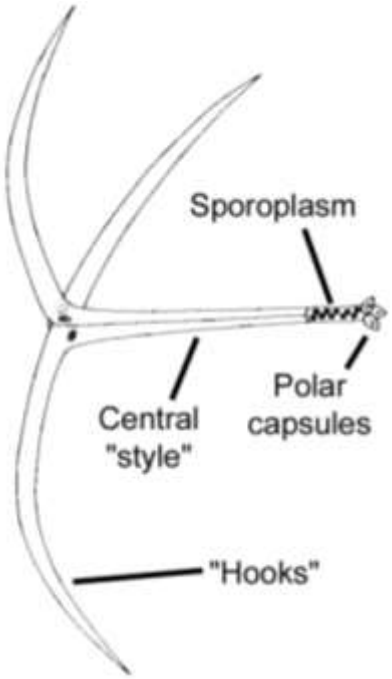
Kingdom: Animalia
Phylum: Ciliophora
Class: Ciliatea
Subclass: Petritrichia
Order: Petritrichida
Family: Sessilina
Genus: *Zoothamnium*

Identifying Character:

- Body highly form-constant, slender and elongated vase-shaped, only slightly constricted below peristomial collar with maximum width at oral border.
- Peristomial disc large and flattened
- Cells not very sensitive to stimuli. When contracted, zooids usually elongated triangle-shaped but does not contact as strongly as in many of its congeners.
- Pellicle smooth when observed at low magnification, fine striations recognizable only under high magnification (x 400 or higher), on which no granules or any other pellicular structure are visible.

Hence the specimen is *Zoothamnium sp*

Specimen No. 2



Taxonomy:

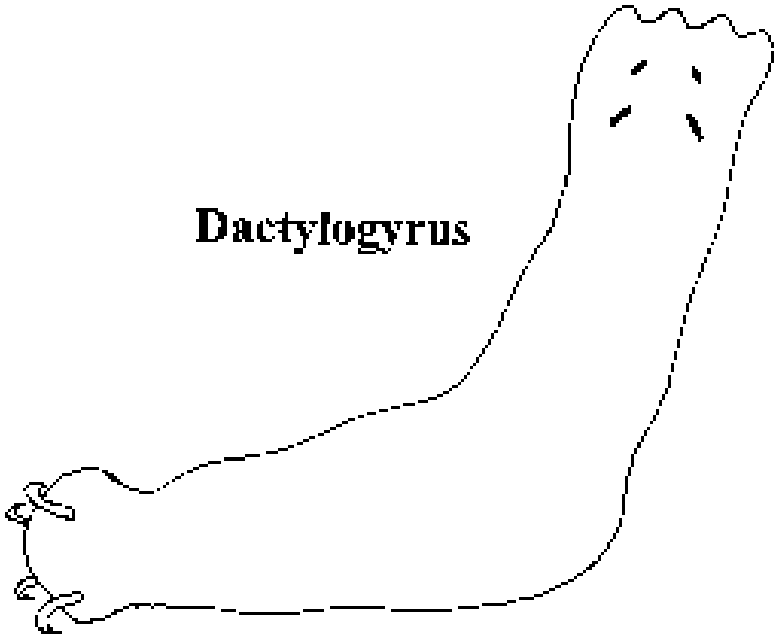
Kingdom: Animalia
Phylum: Cnidaria
Class: Myxosporea
Order: Bivalvulida
Family: Myxobolidae
Genus: *Myxobolus*

Identifying Character:

- The stages that infect fish, called triactinomyxon spores, are made of a single style three processes or "tails" that are each about 200 micrometers long.
- There are also three polar capsules, each of which contains a coiled polar filament.
- Myxospores, which develop from sporogonic cell stages inside fish hosts, are lenticular.

Hence the species is *Myxobolus* sp

Specimen No. 3



Taxonomy:

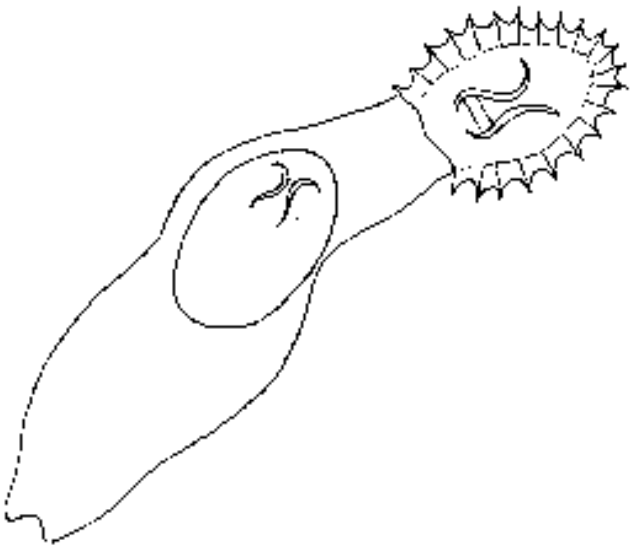
Kingdom: Animalia
Phylum: Platyhelminthes
Class: Monogenea
Order: Dactylogyroidea
Family: Dactylogyridae
Genus: *Dactylogyrus*

Identification of Parasites:

- The parasites are found from the gills of carps
- Body is bilaterally symmetrical & dorsoventrally flattened.
- Adult without cilia and rhabdite
- Attachment organs and 14 marginal hooks are present

Hence the Specimen is *Dactylogyrus* sp.

Specimen No. 4



Taxonomy:

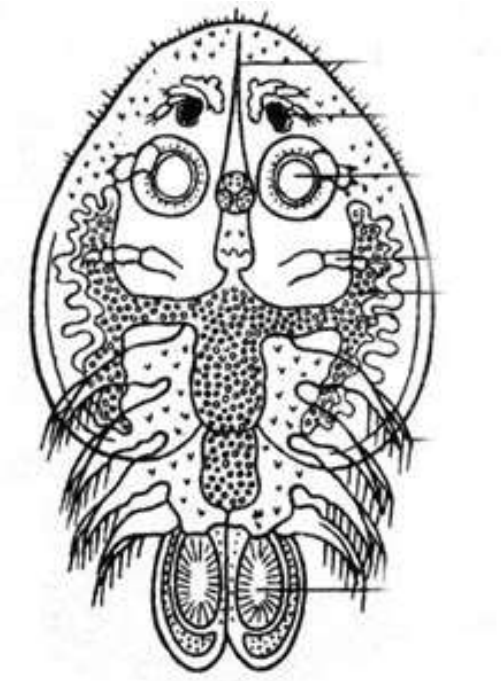
Kingdom: Animalia
Phylum: Platyhelminthes
Class: Monogenea
Order: Monopisthocotylea
Family: Gyrodactylidae
Genus: *Gyrodactylus*

Identifying Character:

- The body is bilaterally symmetrical dorsoventrally flattened (monogeneans).
- The anterior end is generally rounded and is provided with sucker which provides a grip over the host body for feeding and locomotion.
- No eye spot is present
- The posterior end is armed with opisthaptor and acts as an attachment organs
- Present of 16 pairs of marginal hooks and one or more pair of median hooks.
- Squamodisc is present oval & elevated structure (an attachment organ)

Hence the Specimen is *Gyrodactylus* sp

Specimen No. 5



Taxonomy:

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Mandibulata
Class: Crustacea
Order: Arguloidea
Family: Argulidae
Genus: *Argulus*

Identifying Character:

- Body is devisable into cephalothorax, thorax & abdomen. The body is broad & oval.
- The head fused with first thoracic segments
- The cephalothorax is covered with broad carapace.
- The first antenna is modified into attachment organs. The second antenna is uniramous.

Hence the specimen is *Argulus sp.*

Specimen No. 6

Taxonomy:

Kingdom : Animalia
Phylum : Ciliophora
Class : Ciliatea
Subclass : Petritrichia
Order : Sessilida
Family : Vorticellidae
Genus : *Vorticella*

Identification of Parasites:

- Vorticella has a bell-shaped body and attached to the substrate by means of a stalk.
- The stalk is contractile, there is an inner element called the spasmoneme that can contract very rapidly.
- Contractile elements also extend into the body of the cell so the body rounds up when the cell contracts.
- This is a solitary species, in that the cells do not form colonies, although it is not unusual to find many individual *Vorticella* cells living side-by-side and to form a cluster.

Hence the specimen is *Vorticella*

Specimen No. 7

Taxonomy:

Kingdom: Animalia
Phylum: Ciliophora
Class: Ciliatea
Subclass: Petritrichia
Order: Petritrichida
Family: Trichodinidae
Genus: *Trichodina*

Identifying Character:

They always have of flying saucers, hovering and skimming over the surface of the gill or skin.

The shape of the body is hemispherical varying from flat disc to a bell shaped one

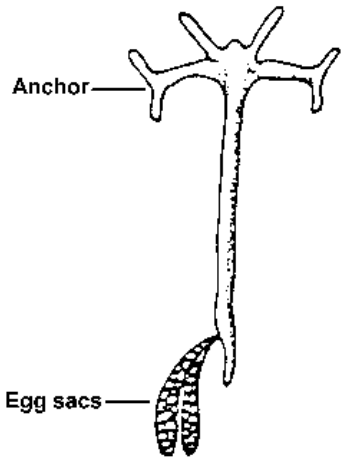
They are top-hat shaped when viewed from the side.

It is concave on its aboral surface

When viewed from the top it is possible to see an outer ring of cilia and the concentric rings and hook-like denticles of the sucking disc.

Hence the specimen is *Trichodina*

Specimen No: 8



Taxonomy:

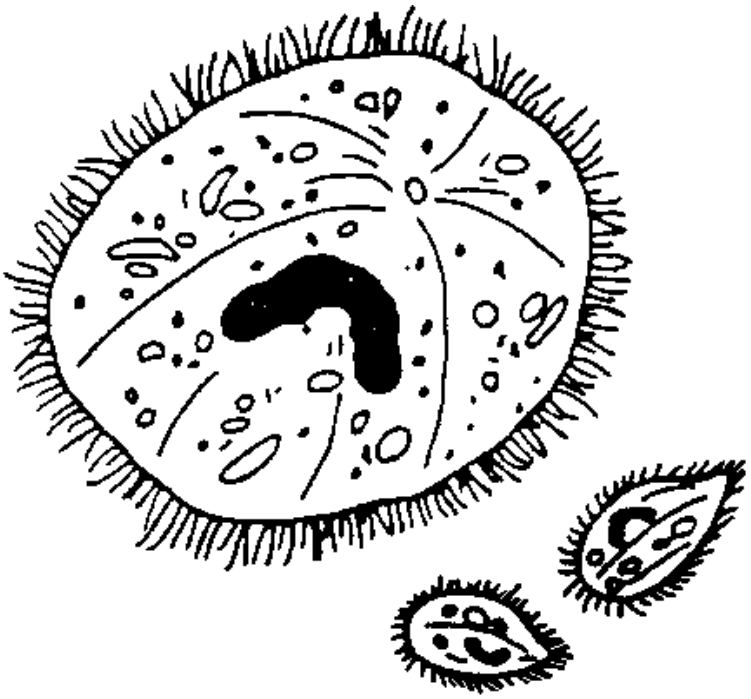
Kingdom: Animalia
Phylum: Arthropoda
Class: Maxillopoda
Order: Cyclopoida
Family: Lernaeidae
Genus: *Lernaea*

Identifying Character:

- The mature female is about 9 mm in length on average. Her cephalothorax has four horns, which vary slightly in length.
- The horns are conical and soft. The anterior ventral pair is simple, while the dorsal pair is T-shaped.
- The head of the copepod is a small bump that projects between the horns.
- The female has a slim, cylindrical neck that gradually enlarges into a larger trunk. Its abdomen is short and rounded at the end and has three segments.
- The mouth-tube is absent in the adult female.
- The structure of the mouthparts themselves is not understood very well.
- The first maxilla is nodular and is tipped with a small chitinous projection. The second maxilla terminates into two stout claws.
- The male differs greatly from the adult female, but shares similar morphology to that of the free-swimming female.
- The antenna of the male is shorter than in the female, it has a larger claw as well, the antennule is segmented into six segments

Hence the provided Specimen is *Lernaea sp*

Specimen No: 9



Taxonomy:

Kingdom: Animalia
Phylum: Ciliophora
Class: Oligohymenophorea
Order: Hymenostomatida
Family: Ichthyophthiriidae
Genus: *Ichthyophthirius*

Identification of Parasites:

- "Ich" is the largest known parasitic protozoan found on fishes.
- Adult organisms are oval to round and measure 0.5 to 1.0 mm in size.
- The adult is uniformly ciliated and contains a horseshoe-shaped nucleus which can be seen in older individuals.

Hence the provided specimen is *Ichthyophthirius sp.*