

**ANIMAL PHYSIOLOGY,  
PARASITOLOGY AND IMMUNOLOGY  
(DZ00L21)  
(MSC - ZOOGOLY)**



**ACHARYA NAGARJUNA UNIVERSITY**

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**DM PRACTICAL – III**  
**ANIMAL PHYSIOLOGY, PARASITOLOGY AND**  
**IMMUNOLOGY**

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**Dr.N.GOPALA RAO**

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**Dr.K.VEERAAIAH**

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**Dr.K.VEERAAIAH**

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- Expt. No.3 :- Test for uric acid

3.5 Quantitative estimation of ammonia

Quantitative estimation of urea

**Dr.K.VEERAAIAH**

**DM EXPERIMENT - 3.1****Experiment No. 1: Estimation of Haemoglobin content in the given blood sample.**

**Aim:** To determine the haemoglobin (Hb) content in the given blood sample

**Method:** Sahil's method of Hb estimation

**Apparatus:** Haemometre, needles, cotton, Haemoglobin pipette, Hellige's haemometre tube

**Reagents:** N/10 Hcl, blood sample, distilled water.

**Principle:** On treatment with dil Hcl acid, the Haemoglobin will be broken down into Haem and globin and forms peculiar colour. This colour will be matched with the standard.

**Procedure:** The standard diluted Hcl (of 1:10 dilution) will be taken into the Hellige's haemometre tube upto the mark of 20. The distilled water and stop clock must be kept ready for diluting the reaction and to record the reaction time.

A small pad of cotton dipped in 90% alcohol be used to wipe the finger tip. The skin of the finger be punctured with needle until the blood flow freely later the oozed blood was sucked through the haemoglobin pipette upto the mark of 0.02 ml. Care was taken that air bubbles should not enter into the pipette.

The blood collected into the pipette be transferred into the Hellige's haemometre tube which is already containing N/10 dil Hcl. The contents were mixed well with the help of stirring rod, and left for 10 minutes when acid haematin is formed. Then the distilled water was added drop by drop constantly until the colour of the contents matches with the colour of the standard.

**Result:** The result was expressed in grams/or % of Hb of blood.

**Significance of Haemoglobin (Hb):** Haemoglobin is the most important pigment of the blood imparting red colour to it. It forms the integral part of the erythrocytes. It contains globin and iron components of haem. The amount of haemoglobin varies in mammals and also in adult males, females and children.

**Normal Haemoglobin concentration:**

Male	:	14-18 gms
Female	:	12-18 gms
At Birth	:	23 gms
Increased	:	Macrocytic anaemia
Decreased	:	Microcytic anaemia

## EXPERIMENT - 3.2

### QUALITATIVE IDENTIFICATION OF PROTEINS

#### Aim

To identify the presence of proteins in the given sample.

#### Apparatus

Test tubes, Pipettes etc.

#### Reagents

1. Millon's reagent
2. Millon-Nasse reagent
3. Biuret reagent
4. Ninhydrin reagent
5. Concentrated nitric acid
6. 1N sodium hydroxide
7. 10% Trichloroacetic acid

#### Preparation of reagents

##### 1. Millon's reagent

Dissolve 100 gms of mercuric sulphate in 250ml of concentrated nitric acid slowly by stirring and adding small amount at a time. Brown fumes will evolve and continue the stirring till the fumes disappear. Dilute the solution, adding 2 volumes of distilled water.

##### 2. Millon - Nasse reagent

Take 10 gms of powdered magnesium in a flat bottomed conical flask and add enough distilled water to cover the magnesium powder and shake well. Now slowly add 250ml of cold saturated solution of oxalic acid under running water bath to reduce the heat. Filter the contents and to the filtrate add 2 drops of glacial acetic acid and make up the solution to 1 litre with distilled water.

**3. Biuret reagent**

Dissolve 3 gms of copper sulphate and 9 gms of sodium potassium tartarate in 500ml of 0.2N sodium hydroxide solution. To this add 5 gms of potassium iodide and make up the volume to 1000ml with distilled water.

**4. Ninhydrin reagent**

Dissolve 0.1 gms of Ninhydrin powder in 100ml of distilled water or acetone.

**5. Concentrated Nitric acid****6. 1N sodium hydroxide**

Dissolve 4gms of sodium hydroxide in 100ml of distilled water.

**Procedure**

Before starting the experiment, the solubility of the proteins should be checked. Almost all the forms of proteins are soluble in water under normal conditions. Hence, homogenate the tissues in distilled water for conducting the various reactions.

**Experiment No-1 (Millon's test)****Principle**

Millon's reaction is due to the presence of hydroxy phenol group in proteins which on titration gives red precipitate of coloured mercury compounds with tyrosine.

**Procedure**

Take 5ml of the sample into a test tube and add 1ml of Millon-Nasse reagent and shake thoroughly.

**Result**

A deep red colour solution indicates the presence of proteins.

**Experiment No-2 (Ninhydrin test)****Principle**

The amino acids present in the proteins undergo oxidative deamination in presence of Ninhydrin resulting in the formation of hydrindantin and ammonia which gives a violet blue colour.

**Procedure**

Take 5ml of the sample solution into a test tube and add 0.5 ml of 0.1% Ninhydrin reagent. Boil the contents for 1 or 2 minutes and leave it to cool.

**Result**

Appearance of blue colour indicates the presence of proteins.

**Experiment No-3 (Biuret test)****Principle**

The Biuret reaction occurs because of the peptide linkage in the proteins. It is based on the fact that the  $-CONH$  groups of proteins form a purple coloured complex in an alkaline medium.

**Procedure**

Take 5ml of the sample into a test tube and add 4ml biuret reagent and keep the mixture at  $37^{\circ}C$  for 10 minutes.

**Result**

Development of purple blue colour indicates the presence of proteins with peptide bonds.

**Experiment No-4 (Xanthoprotein test)****Principle**

This reaction is due the presence of phenolic group in the proteins. On reaction with nitric acid it gives an yellow precipitate of polynitro compounds; which turns to organge colour by the addition of dilute sodium hydroxide.

**Procedure**

To 5ml of the sample add a few drops of concentrated nitric acid and boil for 2 minutes. After cooling, add few drops of dilute sodium hydroxide.

**Result**

Appearance of yellow colour and changing to the orange colour indicates the presences proteins.

**Experiment No-5 (Trichloroacetic acid Test)****Principle**

Trichloroacetic acid precipitates the proteins by the formation of an insoluble white precipitate. In acid medium, proteins behave as cations and react with negative charges of the acid to form insoluble salt.

**Procedure**

Take 5ml of the sample into a test tube and add 5ml of 10% trichloroacetic acid solution.

**Result**

Formation of a white precipitate indicates the presence of proteins.

## QUANTITATIVE ESTIMATION OF PROTEINS

### Aim

To estimate the amount of proteins quantitatively present in the given sample.

### Method-I

Biuret method

### Apparatus

Test tubes, pipette, conical flasks, burette measuring jar, water bath, centrifuge etc

### Reagents required

#### 1. Biuret reagent

Dissolve 3gms of copper sulphate and 0.9 gms of sodium potassium tartarate in 500ml of 0.2 N sodium hydroxide solution.

To this solution is adds 5gms of potassium iodide and make up the volume to 1000ml with 0.2N sodium hydroxide

#### 2. Standard protein solution

Dissolve 500mg of Bovine serum albumin in 100ml of water. The concentration obtained is 5mg/ml.

3. 10% Trichloro acetic acid –dissolve 10 gm of TCA in 100 ml of distilled water.

4. 1N NaOH.

### Principle

This is the most commonly used method and is based on the fact that the –CO-NH- groups of protein form a purple complex with copper ions in alkaline medium. Since all proteins contain the peptide bond, the method is fairly specific and there is little interference from other compounds. Some substances like urea and biuret interfere because they possess – Co-NH-groups. Other interfering materials are reducing sugars like glucose which interact with cupric ions in the reagent.



## Procedure

Pipette out into a series of tubes 0.1, 0.2, 0.3 -----1.0 ml of the protein solution and make up the total volume to 4ml with the addition of water. The blank tube will have only 4ml of water. Add 6ml of biuret reagent to each tube and mix well. Keep the tubes at 37°C for 10 minutes during which a purple colour will develop. The optical density of each tube is measured at 520nm (green filter) using the reagent blank. Draw the standard graph with concentrations on x-axis and optical density values on y-axis.

Now take 50 mg of tissue sample and homogenate the tissue with 5ml of 5% Tri chloro acetic acid. Take the sample in the test tube and centrifuge at 2000 rpm for 15 minutes. Discard the supernatant from the test tube and adds 1ml of 1 N NaOH to the denatured protein that settle's down. From this solution take 0.2 ml into a test tube and add. 6ml of biuret reagent to it. Mix well and keep the test tube at 37°C for 10 minutes. The optical density of the solution is measured at 520nm and with the standard graph drawn earlier.

## Result

The amount of protein content present in the sample will be obtained from standard graph in mg/ml.

## Method – II

### Modified Biuret Method

The above method works well in most cases but sometimes one comes across coloured extracts or, the solution may contain interfering material like glucose, In such cases the following modified procedure is to be followed.

## Reagents required

### 1.10% Trichloroacetic acid (TCA)

10 gm of trichloroacetic acid (TCA) is dissolved in 100ml of distilled water.

### 2. Biuret reagent

Dissolve 3 gms of copper sulphate and 0.9 gms of sodium potassium tartarate in 500ml of 0.2 N sodium hydroxide solution. To this solution is added 5gms of potassium iodide and make up the volume up to 1000ml with 0.2N sodium hydroxide.

3. Ethyl ether

4. 1 N NaOH

### Principle

The  $-\text{CO}-\text{NH}$ -groups of protein form a purple complex with copper ions in an alkaline medium. When TCA is added, this denatures the protein which precipitates out. The precipitated protein will give the colour when it reacts with biuret reagent.

### Procedure

Aliquots of the extract are distributed into different tubes as before and volume made to 1ml with water. To each tube add 1ml of 10% trichloroacetic acid solution (TCA). This denatures the proteins which precipitate out. The tubes are now centrifuged at 3000 rpm for 10 minutes when the denatured proteins settle down, and the supernatant is decanted out. To the precipitate 2ml of ethyl ether is added, mixed and recentrifuged. This helps in the removal of any residual TCA. The final dry precipitate is suspended in 4ml water and mixed well. (Do not expect this to dissolve). Add 6ml of biuret reagent and the alkali in the reagent will dissolve the precipitate. Developed the colour as before and estimate the protein comparing with the standard graph.

Now take 50mg of tissue sample and homogenate the tissue with 5ml of 5% Trichloroacetic acid. Take the sample in the test tube and centrifuge at 3000 rpm for 10 minutes. The supernatant is decanted out. To the precipitate 2ml of ether is added mixed and recentrifuged. To the final precipitate add 1ml of 1 N NaOH. Take 0.2 ml from it and add 6ml of biuret reagent with which the colour will develop. Take the optical density value and plot it on the standard graph drawn earlier.

### Result

The amount of protein present in the sample will be estimated from the standard graph in mg/ml.

### Method-III – Lowry's method

#### Reagents required

- a. 2% sodium carbonate in 0.1N NaOH.
- b. 0.5% copper sulphate solution in 1% sodium potassium tartarate solution (to be prepared fresh).

- c. Mix 50 ml of reagent A with 1 ml of reagent B, just prior to use.
- d. Folin-Ciocalteu reagent: This is commercially available and has to be diluted with equal volume of water just before use. The reagent can also be prepared in the laboratory. Into a 2 litre flask, measure out 100 gms sodium tungstate, 25gms of sodium molybdate, 500ml distilled water, 50ml 85% phosphoric acid and 100ml conc HCl. The mixture is refluxed gently for about 10 hours with a condenser. After cooling 150 gms of lithium sulphate, 50ml of distilled water and a few drops of bromine are added and boiling continued for another 10 minutes without the condenser. This helps to remove excess bromine. After cooling, the volume is made up to 1000ml and filtered if necessary. The filtrate should not have any greenish tint. If it has, it is boiled with bromine once more. This is the stock reagent and is diluted with equal volume of water just before use.
- e. The standard protein solution of 5mg/ml concentration (Bovine serum albumin) and is diluted to 20 times.

### Principle

This method is about 10 times more sensitive than the biuret method. The reagent, called Folin-Ciocalteu reagent, is quite complex and contains phosphomolybdic acid and tungstate. The aromatic amino acids, tryptosine and tryptophan, present in proteins react with these and produce a dark blue colour.

### Procedure

Aliquots of protein solution are pipetted out as in previous experiments and the total volume made to 4ml with distilled water. To each tube 5.5 ml of the alkaline mix (reagent c) is pipetted out, mixed well and allow to stand at room temperature for 10 to 15 minutes, 0.5 ml of the reagent is pipetted out into each tube mixing rapidly after each addition. The tubes are left as such for 30 minutes and the blue colour formed is measured at 650 nm (or red filter). A proper blank without the protein is used. The standard graph is drawn with concentration on x-axis and optical density values on y-axis. (as mentioned in method I)

Now take 50 mg of tissue sample and homogenate the tissue with 5ml of 5% Trichloroacetic acid. Take the sample in the test tube and centrifuge at 2000 rpm for 15 minutes. Discard the supernatant from the test tube and add 1ml of 1 N NaOH to the denatured protein that settle's down. From this solution take 0.2ml into a test tube and add 5.5ml of alkaline mix (reagent c). Allow the solution to stand at room temperature for 10 to 15 minutes. 0.5ml of the reagent is pipetted out into the test tube mix rapidly after each addition. The test tube is left as such for 30 minutes and

the blue colour formed is measured at 650 nm. The optical density value of the sample is taken and on the standard graph drawn earlier.

### **Result**

The amount of protein content present in the sample will be obtained from standard graph in mg/ml.

## EXPERIMENT - 3.3

### QUALITATIVE IDENTIFICATION OF CARBOHYDRATES

#### Aim

To identify the presence of carbohydrates in the given sample.

#### Apparatus

Test tubes, Pipettes

#### Reagents

1. Molisch's reagent
2. Fehling Benedict's solution
3. Concentrated hydrochloric acid
4. Iodine solution (0.005 N)
5. Concentrated sulphuric acid solution
6. Saturated Picric acid solution
7. 40% sodium hydroxide

#### Preparation of the reagents

##### 1. Molisch's reagent

Dissolve 5 gms of O-naphthol in 100ml of ethanol.

##### 2. Fehling Benedict's solution

###### Solution A

Dissolve 1.73 gms of copper sulphate in 20ml of distilled water.

###### Solution B

Dissolve 17.3 gms of Sodium Citrate and 10 gms of Sodium Carbonate in 75ml of distilled water and filter if necessary.

Add solution A slowly to the alkaline citrate solution and make up the volume to 100 ml with distilled water.

**3. Concentrated hydrochloric acid****4. Iodine solution (0.005N)**

Dissolve 3gms of potassium iodide and 1gm of iodine in 100ml of distilled water.

**5. Concentrated Sulphuric Acid solution****6. Saturated Picric acid solution****7. 40% Sodium Hydroxide**

Dissolve 40 gms of sodium hydroxide in 100ml of distilled water.

**Experiment No-1 (Molisch test)****Principle**

The test is based on the dehydration of carbohydrates by an acid. The monosaccharide (carbohydrate) on reaction with concentrated sulphuric acid will form hydroxy methyl furfural. This furfural then condenses with O-naphthol in presence of sulphuric acid to form a violet coloured product.

**Procedure**

Homogenize the tissues / samples in 5% trichloroacetic acid to eliminate the carbohydrates. To 2ml of the filtrate / supernatant add few drops of Molisch's reagent and then to this mixture add 1ml of concentrated sulphuric acid slowly and carefully by the sides of the test tube without mixing.

**Result**

A violet coloured ring formed, indicates the presence of carbohydrates.

**Experiment No-2 (Fehling – Benedict's test)****Principle**

The aldehydes and ketones of Mono-Saccharides reduce the Copper Sulphate to Cuprous Oxide. Cuprous Oxide being red in colour indicates the presence of monosaccharides.

**Procedure**

Homogenize the tissues / sample in 5% Trichloroacetic Acid and to 2ml of filtrate, add 2ml of Benedict's reagent and boil for 2 minutes.

**Result**

Formation of green coloured precipitate turning to brick red indicates the presence of carbohydrates.

**Experiment No-3 (Iodine test)****Principle**

Iodine is known to form coloured complexes with Polysaccharides (starch, glycogen) giving characteristic blue colour of Mischler-iodine complex.

**Procedure**

Homogenize the tissues / sample in 5% Trichloroacetic Acid and filter/centrifuse. To 2ml of the filtrate/ supernatant, add 1ml of Hydrochloric Acid and 1ml of iodine solution.

**Result**

A blue colour solution indicates the presence of polysaccharides.

**Experiment No-4 (Picric acid test)****Principle**

The monosaccharides will reduce the picric acid to form a red coloured Picramic acid.

**Procedure**

Homogenize the tissues / sample in 5% Trichloroacetic Acid and to 3ml of filtrate / supernatant, add 1ml of 40% Sodium Hydroxide.

**Result**

A red coloured precipitate indicates the presence of carbohydrates.

## QUANTITATIVE ESTIMATION OF CARBOHYDRATES

### Aim

To estimate the amount of carbohydrates quantitatively present in the given sample.

### Apparatus

Test tubes, pipettes, burette, measuring jar, water bath, centrifuge.

### Method-I – Anthrone method

#### Reagents required

1. Anthrone reagent (0.2% in conc.  $H_2SO_4$ )
2. Standard glucose solution (10mg/ 100ml)
3. 5% Trichloroacetic acid
4. 1 N NaOH

### Principle

Carbohydrates are dehydrated by conc  $H_2SO_4$  to form furfural. Furfural condenses with anthrone to form a blue coloured complex which is measured colorimetrically.

### Procedure

Pipette out into a series of test tubes, different volumes of glucose solution (range 0-100 mg) and make up to volume of 1ml with water. To each test tube add 4ml of the anthrone reagent and mix well. Cover the tubes with marbles on top and keep them in a boiling water bath for 10 minutes. Cool to room temperature and measure the optical density at 620 nm (or red filter) using a blank (a tube containing 1ml of water and 4ml of reagent). Draw the standard graph with concentrations on x-axis and optical density on y-axis.

Now take 50 mg of tissue sample and homogenate the tissue with 5ml of 5% trichloroacetic acid. Take the sample in the test tube and centrifuge at 2000 rpm for 15 minutes. Take the supernatant from the test tube and discard the precipitate. From the supernatant take 0.2 ml and add 4ml of anthrone reagent and mix well. Keep the test tube in water bath for 10 minutes with lid closed, cool it to temperature and measure the optical density at 620 nm (or red filter). Plot the O.D value on the standard graph drawn earlier and the concentration of carbohydrates is estimated from the standard graph.



The test can be made with various sugars such as maltose and methyl  $\alpha$ -glucoside also.

### Result

The amount of carbohydrates present in the sample is estimated from the graph in mg/ml.

### Method-II – Benedict's Quantitative method

#### Reagents required

1. Benedict's quantitative solution : Dissolve 200 gms of sodium citrate, 75 gms of anhydrous sodium carbonate and 125 gms of potassium thiocyanate in about 600 ml of water with gentle heating. Filter, cool and add 18 gms of copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) dissolve in about 100ml of water pour this slowly, stirring continuously Add 5ml of 5% potassium ferrocyanide solution and make up to 1.0 litre with distilled water. If the solution is not clear, filter.
2. Sodium carbonate (anhydrous)

#### Principle

The reagent will react with the standard sugar solution molecules and forms a bulky white precipitate first which is cuprous thiocyanate. When sugar solution is added, the last trace of the colour will disappear when carefully done, 25ml of Benedict's reagent is equivalent to 50 mg of glucose, 53 g of fructose, 68.8 mg lactose and 74mg of maltose.

#### Procedure

Pipette out 25ml of the reagent into a conical flask (about 100ml capacity) and add 3gms of sodium carbonate. Heat the mixture to boiling meanwhile take the standard sugar solution (0.5g/100 ml) in a burette and slowly run this solution into the boiling reagent. A bulky white precipitate is formed first, which is cuprous thiocyanate. At this stage add the sugar solution slowly till the last trace of colour has disappeared note the volume of sugar solution required. In this procedure, there is no standard curve. When carefully done, 25ml Benedict's reagent is equivalent to 50mg of glucose, 53mg of fructose, 68.8 mg of lactose and 74mg of maltose.

#### Result

The volume of sugar solution required to dissolve the bulky white precipitate is noted and compared with the standard values given in the procedure.

## PRACTICAL – 3.4

# QUALITATIVE IDENTIFICATION OF NITROGENOUS WASTES

### Aim

To identify the Nitrogenous wastes present in the given sample.

### Apparatus

Test tubes, Pipettes etc.

### Reagents

1. Nessler's reagent
2. Urease (enzyme)
3. Saturated sodium carbonate solution
4. Follin's uric acid reagent

### Experiment No.1 Test for Ammonia

Method : Nesslerization method as described by Bergmeyer (1965)

### Principle

The ammonia that is present in the sample reacts with Nessler's reagent and forms a brown coloured product called dimercuric ammonium iodide indicating the presence of ammonia in the sample.

### Procedure

Take 5ml of the sample into a test tube and add 0.5 ml of Nessler's reagent.

### Result

Appearance of brown coloured precipitate indicates the presence of ammonia in the given sample.

**Experiment No.2 Test for UREA**

Method : Nesslerization method as described by Bergmeyer (1965)

**Principle**

Urea that is present in the sample will be hydrolysed with urease and liberates ammonia. The ammonia so liberated will react with Nessler's reagent and forms a brown colored product called dimercuric ammonium iodide indicating the presence of urea in the given sample.

**Procedure**

Take 5ml of the sample into a test tube. To it add 0.5ml of urease solution and after few minutes add 0.5ml of Nessler's reagent.

**Result**

Appearance of brown coloured precipitate indicates the presence of urea in the given sample.

**Experiment No.3 Test for uric acid**

Method: Natelson method (1954)

**Principle**

Uric acid present in the sample will be extracted into the sodium carbonate solution. This solution specifically reacts with follin uric acid reagent and develops blue colour indicating the presence of uric acid in the given sample.

**Procedure**

Take 5ml of the sample into test tube. Add 1ml of saturated sodium carbonate solution to it and mix thoroughly, immediately after few minutes, add 0.5ml of Follin's uric acid reagent.

**Result**

Appearance of blue coloured precipitate indicates the presence of uric acid in the given sample.

## PRACTICAL – 3.5

# QUANTITATIVE ESTIMATION OF AMMONIA

### Aim

To estimate the amount of ammonia present in the given sample.

### Method

Nesslerisation method by Berg Mayer (1965)

### Apparatus

Test tube, Pipette etc

### Reagents

Nessler's reagent

### Principle

The ammonia that is present in the sample will react with Nessler's reagent and forms a brown coloured product called dimercuric ammonium iodide. The intensity of the colour is directly proportional to the amount of ammonia that is present in the sample.

### Procedure

Take 5ml of sample in a clean dry test tube and add 0.5ml of Nessler's reagent slowly. The contents in the test tube are mixed thoroughly and the intensity of the colour is read at 495nm in the spectrophotometer against a reagent blank which is prepared with 5ml of distilled water and 0.5ml of Nessler's reagent.

### Calculation

$$\frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times \frac{\text{Amount present in standard}}{\text{Volume of sample taken}} \times 1000$$

### Result

The amount of ammonia present in the sample solution is expressed in ng/litre

## QUANTITATIVE ESTIMATION OF UREA

### Aim

To estimate the amount of urea present in the given sample.

### Method

Nesslerisation method by Berg Mayer (1968)

### Apparatus

Test tubes, pipette etc

### Reagents

Urea present in the sample will be hydrolysed with urease enzyme and the ammonia so liberated is estimated by using Nessler's reagent.

### Procedure

Take 5ml of the sample into a clean dry test tube and add 0.5ml of urease solution. After few minutes add 0.5ml of Nessler's reagent to the sample. The colour intensity is read at 495 nm in spectrophotometer against a reagent blank which is prepared with 5ml of distilled water, 0.5ml of urease solution and 0.5ml of Nessler's reagent.

### Calculation

$$\frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times \frac{\text{Amount present in standard}}{\text{Volume of sample taken}} \times 1000$$

### Result

The amount of urea present in the given sample solution is expressed in mg/litre.

## M.Sc. Zoology (Final) DM. PRACTICAL-III

### ANIMAL PHYSIOLOGY, PARASITOLOGY AND IMMUNOLOGY

1. Estimation of hemoglobin content in the given blood sample.
2. Qualitative identification and quantitative estimation of proteins in the given sample.
3. Qualitative identification and quantitative estimation of carbohydrates in the given samples.
4. Qualitative identification of nitrogenous wastes, viz., ammonia, urea and uric acid.
5. Quantitative estimation of ammonia and urea in the given sample.

### SPOTTERS

Study of prepared slides and museum specimens of selected parasites of representative groups of protozoans, helminthes and immunology.

1. *Entamoeba histolytica*
2. *Leishmania donovani*
3. *Trypanosoma gambiense*
4. *Trichomonas*
5. *Fasciola hepatica* (Liver fluke)
6. Miracidium
7. Redia with Cercaria
8. Liver fluke – *Cercaria*
9. *Fasciola* – metacercaria
10. *Schistosoma haematobium*
11. *Taenia solium* (Tapeworm)
12. *Taenia solium* – mature proglottid
13. *Taenia solium* – gravid proglottid
14. *Echinococcus granulosus*
15. Transverse section of female *Ascaris lumbricoides*
16. Transverse section of male *Ascaris lumbricoides*
17. *Ancylostoma duodenale*
18. *Trichinella spiralis* cyst in muscles
19. *Wuchereria bancrofti* (*Filaria bancrofti*)
20. *Dracunculus medinensis*
21. Bone marrow
22. Thymus (T.S.)
23. Lymphnode (T.S.)
24. Liver abscess
25. Lung abscess

### 1. *Entamoeba histolytica*

# 1. *Entamoeba histolytica*

Phylum: Protozoa

Class: Sarcodina

Order: Amoebida

## Characters:

1. It is commonly known as dysentery amoeba.
2. It occurs in the colon of man and feeds on the mucous membrane destroying the tissues by an enzyme which it secretes.
3. The size of *E. histolytica* varies from 0.06 mm to 0.05 mm in diameter.
4. It is dimorphic occurring in two forms:
  - (i) trophic or trophozoite. (ii) precystic or minuta
5. Both these forms occur in man. Trophozoite is pathogenic, while minuta is non-pathogenic.
6. Cytoplasm is differentiated into ectoplasm and endoplasm.
7. Endoplasm contains a single spherical nucleus and food vacuoles.
8. Contractile vacuole is absent.
9. One or two pseudopodia are present.
10. Reproduction by binary fission and spore formation.
11. It completes its life cycle in the primary host (Direct life cycle); no intermediate host is utilized to complete the life cycle.
12. Mode of infection is oral. Man is infected by taking contaminated food or water with cysts.
13. The feeding habits of the parasite cause amoebic dysentery; it involves the destruction of the tissues of the host and discharge of large quantities of mucus and blood.

**Primary host:** Man

**Intermediate host:** No involvement

**Name of disease caused:** Amoebic dysentery or Amoebiasis.

**Infective stage:** Tetranucleate cyst

## Clinical symptoms of the disease:

- (i) The trophozoites penetrate the mucosa and submucosa of intestine and cause its necrosis and cause small wounds or abscesses.
- (ii) Persons suffering from amoebic dysentery has repeated blood-mixed, slimy and foul smelling motions.
- (iii) Formation of abscesses in lung and brain usually prove fatal; such abscesses are common in liver.

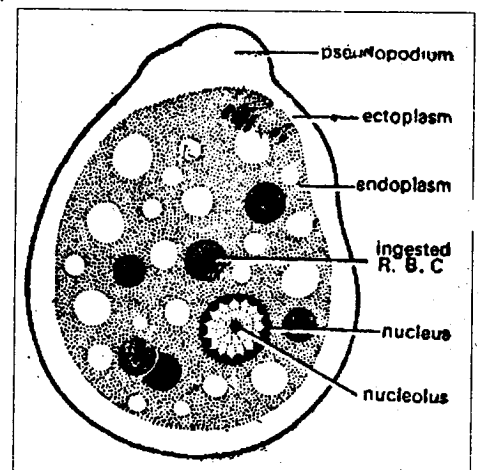


Fig. 1. *Entamoeba histolytica*

## Prophylaxis:

Amoebiasis can be prevented by taking uncontaminated food and water; using boiled water.

## 2. *Leishmania donovani*

Phylum: Protozoa  
Class : Mastigophora  
Order : Kinetoplastida

### Characters:

1. *Leishmania donovani* lives as an intracellular parasite in leucocytes or cells of liver, spleen, bone marrow, lymphatic glands etc.
2. It occurs as amastigote form in man, measuring 2-4  $\mu$  in diameter with a limiting membrane, the periplast or pellicle.
3. The cytoplasm contains an oval nucleus, rod shaped or dot like kinetoplast and a parabasal body.
4. In the secondary host i.e., the sand fly, it appears as leptomonad form. Here the body is elongated and measures 14-20  $\mu$  in length and 1.5 – 3.5  $\mu$  in width.
5. A round nucleus is present in the centre of the body.
6. An oval kinetoplast lies transversely near the anterior end of body, a head of which is a small basal granule from which a flagellum is given out.
7. Reproduction by binary fission.
8. It is digenetic parasite and requires two hosts for completion of its life cycle.
9. Transmission by sand fly.

**Primary host:** Man

**Intermediate host:** Sand fly (*Phlebotomus argentipes*)

**Name of disease caused:** Kala-azar or Black fever or Dumdum fever.

**Infective stage:** Leptomonad stage (flagellated stage) to man, leishmania stage to sand fly.

### Clinical symptoms of the disease:

- (i) Early symptoms of Kala-azar include swelling, high fever and enlargement of spleen and liver. Kala-azar is also characterized by anaemia and emaciation.
- (ii) It is followed by general weakness, anaemia due to reduction in number of blood cells and a peculiar darkening of skin.
- (iii) The defense mechanism of body becomes so weak that the patient is unable to resist pathogens.

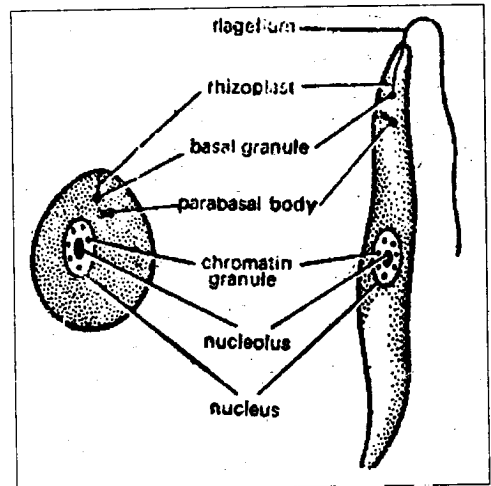


Fig. 2. *Leishmania*

### Prophylaxis:

Destruction of the habitat of the sand flies by the use of insecticides.

Protection from sand fly bites.



### 3. *Trypanosoma gambiense*

Phylum: Protozoa  
Class: Mastigophora  
Order: Kinetoplastida

#### Characters:

1. *Trypanosoma* is polymorphic.
2. It has a colourless, slender elongated, spindle-shaped body tapering at both ends, ranging in length from 15 to 16  $\mu$ .
3. Body is covered by the periplast or pellicle somewhat compressed laterally and twisted spirally.
4. The anterior end is more pointed than the posterior end and it bears the flagellum.
5. The body is convex on one edge and concave on the other, the convex edge of the body is thrown into irregular folds and is called undulating membrane.
6. Nucleus is rounded and central in position.
7. Contractile vacuole are absent.
8. Reproduction takes place by longitudinal binary fission.

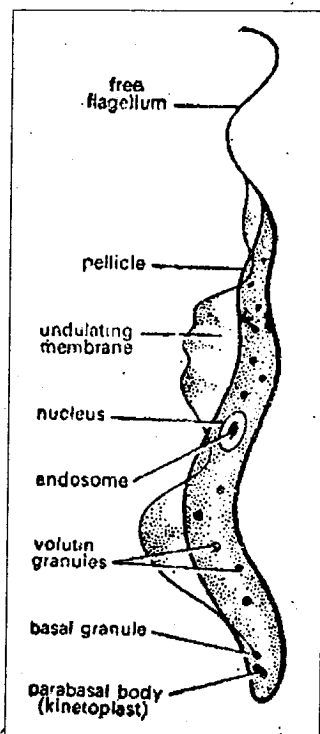


Fig. 5. *Trypanosoma gambiense*

9. Trypanosomes are found as parasites in the blood of vertebrates and are transmitted to them by the blood sucking invertebrates. The parasites first live in the plasma of the blood of an infected man, but later, find their way into the cerebrospinal fluid. When the parasites are in blood, the infected man gets a kind of fever termed Gambia fever. But when they reach central nervous system, the patient goes to a lethargic condition, which has given the name sleeping sickness of the disease.
10. Infected Tse-tse fly bites a man and injects metacyclic forms of the parasite into the blood of man through inoculation.

**Primary host:** Man

**Intermediate host:** Tse tse fly (*Glossina palpalis*)

**Name of disease caused:** *Gambian trypanosomiasis* or West African sleeping sickness.

**Infective stage:** Metacyclic stage in man, short and stumpy forms in tse-tse fly.

**Clinical symptoms of the disease:**

- (i) The disease sleeping sickness is caused when the parasites invade cerebrospinal fluid of central nervous system.
- (ii) An irregular recurrent fever, weakness, loss of weight, anaemia, increase in pulse rate and severe head ache.
- (iii) In due course, the patient falls asleep, first at regular intervals and ultimately enters in coma. Death is always the ultimate fate.

**Prophylaxis:**

Elimination of vector hosts by the use of insecticides.

Avoiding the bites of insects by using mosquito nets.

#### ***4. Trichomonas***

## 4. *Trichomonas*

Phylum: Protozoa  
Class: Mastigophora  
Order: Trichomonodina

### Characters:

1. *Trichomonas* is a common multiflagellate parasite, living in the intestine of many vertebrates. Three species are found in man: *T. hominis* in the colon, *T. buccalis* in the mouth and *T. vaginalis* in the human vagina. The last has also been found in male urethra. These parasites feed on bacteria and debris contained in their habitats.
2. They exist only in the trophozoite phase and have no cystic phase.
3. The body is pear shaped tapering posteriorly, provided with four flagella of which one is directed backwards united to the body by an undulating membrane.
4. Body measures 5-20  $\mu$  in length.
5. Cytostome is present anteriorly, used for ingestion of food.
6. Blepharoplast is minute situated at the anterior end. blepharoplast is club shaped parabasal body.
7. The body is supported by a cylinder of microtubules, the axostyle, which extend backwards and projects from posterior end as spike. The spike helps to anchor the animal while feeding.
8. Reproduction takes place exclusively by longitudinal binary fission.

**Primary host:** Vertebrates (Man, cattle) and Invertebrates (Leeches, termites)

**Intermediate host:** No int. host

**Name of disease caused:** *Trichomoniasis*

**Infective stage:** Trophozoite phase

### Clinical symptoms of the disease:

The three parasites have little pathogenic importance; they feed on bacteria and debris contained in their habitats. *T. hominis* is associated with a persistent type of diarrhoea and *T. buccalis* with inflammatory pyorrhoea. *T. vaginalis* causes inflammation in the vaginal and urethral mucosa.

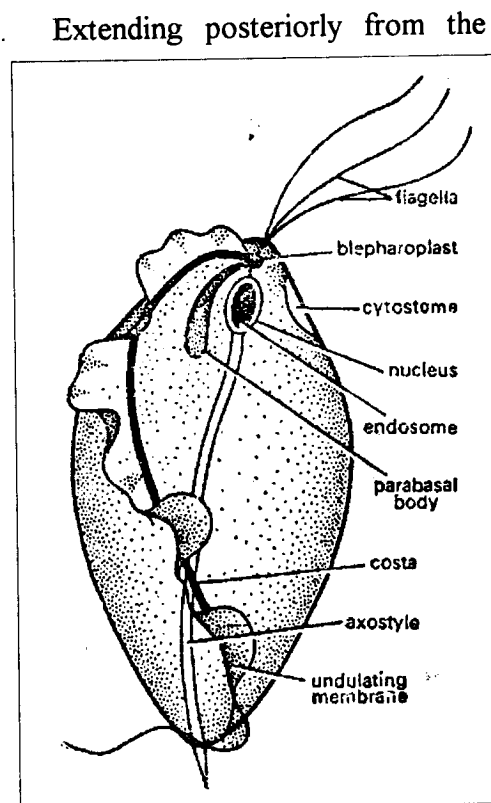


Fig. 4. *Trichomonas*

### Prophylaxis:

Trichomoniasis can be prevented by maintaining personal hygienic conditions.

## 5. *Fasciola hepatica* (Liver fluke)

Phylum: Platyhelminthes

Class: Trematoda

Order: Digenea

### Characters:

1. *Fasciola hepatica* is found as an endoparasite in the bile ducts of liver of sheep all over the world.
2. Body is conical in shape and leaf like. It is dorso-ventrally flattened measuring about 25-30 mm in length and 4-5 mm in breadth.
3. The colour of body is generally pinkish. The margins are blackish or brownish due to the presence of vitelline glands.
4. Anterior end is somewhat broad and rounded, while posterior end is bluntly pointed.
5. An oral sucker is situated apically and a larger highly muscular ventral sucker (acetabulum) is located a little posterior to the oral sucker. The posterior sucker serves for attachment.
6. Mouth is situated at the anterior end and is surrounded by the oral sucker.
7. Digestive system is simple, pharynx is muscular, oesophagus short and branched and diverticulated intestine.

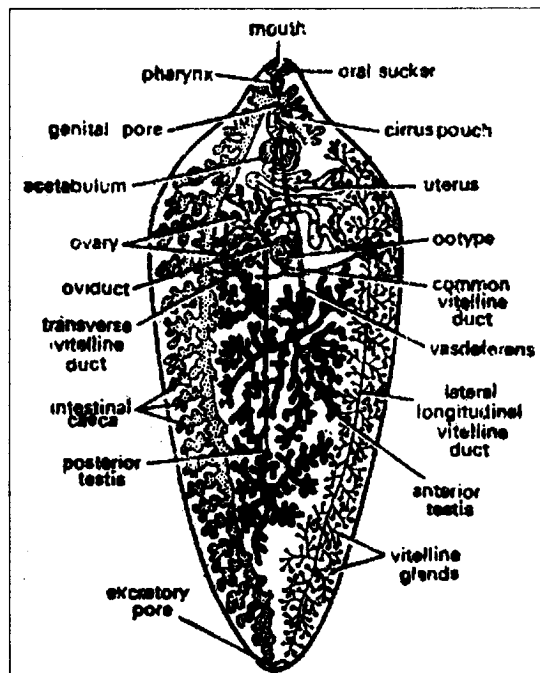


Fig. 5. *Fasciola hepatica*

8. Between the oral and ventral sucker is a median common genital pore through which pass eggs to the exterior.
9. Excretory pore lies at the extreme posterior end of the body.
10. Hermaphroditic. Male system consists of testes, vasa deferentia, seminal vesicle, ejaculatory duct and penis, while female system comprises ovary, uterus and vitelline glands.

**Primary host:** Sheep

**Intermediate host:** Snail (*Lymnaea*, *Planorbis* etc.)

**Name of disease caused:** Liver rot or Fascioliasis

**Infective stage:** Miracidium to snail, Metacercaria to sheep

**Clinical symptoms of the disease:**

- (i) Acute fascioliasis or liver rot occurs during the preadult migration of the flukes in the parenchyma of the liver, and sometimes in other organs, for about 8 weeks.
- (ii) The liver becomes swollen and in some instances, the swollen liver capsule may rupture due to host-parasitic interactions.
- (iii) The by-products produced by worms are very toxic and cause anaemia, diarrhoea, eosinophilia etc.
- (iv) Chronic fascioliasis occurs beyond 12 weeks, when the flukes have reached the bile ducts and are maturing sexually.
- (v) The adult fluke causes anaemia, haemorrhages in liver and sometimes in other internal organs. Production of new red blood corpuscles may be suppressed due to fascioliasis..

**Prophylaxis:**

Liver flukes prove a real agony to sheep.

The complete eradication of the parasite is done by destroying, the intermediate host, the snail.

## 6. Miracidium

### Characters:

1. It is the first larval stage involved in the life cycle of *Fasciola hepatica* and other parasitic digenetic trematodes.
2. Miracidium larva comes out from the fertilized egg and leads a free life.
3. It is microscopic, dorso ventrally flattened, conical in shape, free swimming larval stage.
4. Body is uniformly covered with cilia.
5. It has an outer layer of hexagonal cells, arranged in five rows, beneath this layer is a thin layer of muscles.

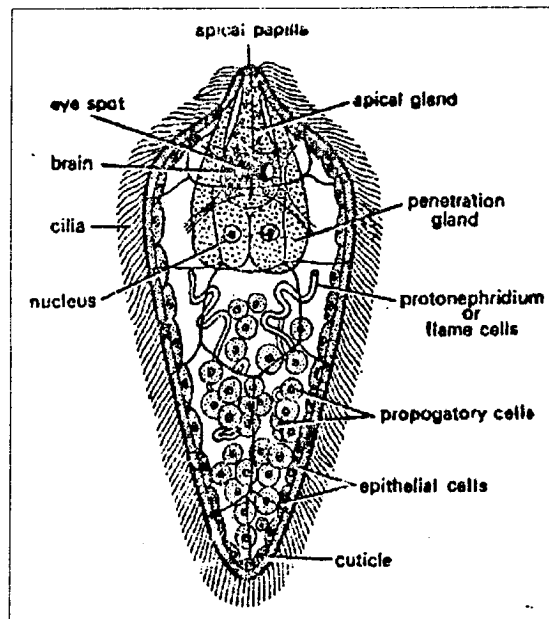


Fig. 6. Miracidium larva of *Fasciola hepatica*

6. Anterior end is produced into a conical lobe, the apical papilla.
7. Internal structures, apical gland, cephalic or penetration glands, brain, two eye spots, two flame cells, rudimentary gut and germ cells are seen.
8. Miracidium larva swims in search of an intermediate host which is *Limnaea truncatula* for about 4-30 hours.
9. If it does not come in contact with a suitable host, it dies.
10. After getting the suitable host, it pierces into tissues of snail by apical papilla and reaches the roof of its respiratory chamber.
11. Then changes take place in its organization – its cilia are cast off, the eyes, nerve ganglion and excretory organs degenerate. The larva develops a cavity inside and becomes an enlarged sac. In this stage it is known as a sporocyst.

## 7. Redia with Cercaria

### Characters:

1. Redia larva develops from the germ cells of the sporocyst. When fully developed, the rediae come out of the sporocyst by the rupture of the body wall.
2. Each redia is an elongated and cylindrical form, measuring 1.3 – 1.6 mm in length.
3. Anterior end bears the mouth leading into muscular pharynx, which finally leads into sac like intestine.
4. Just behind the pharynx is a muscular ring like swelling known as collar, which helps in locomotion.
5. Posterior region is also provided with two stumpy processes known as lappets helpful in locomotion.

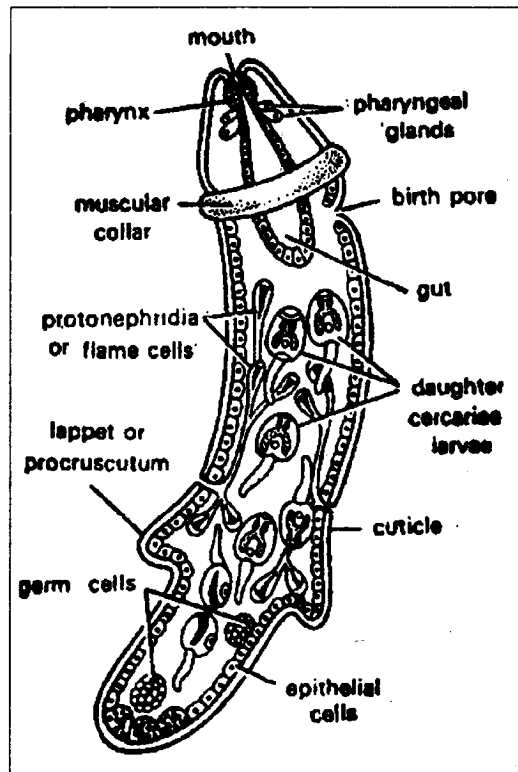


Fig. 7. Redia larva of *Fasciola hepatica*

6. Just posterior to collar a permanent aperture, the birth pore is seen.
7. The space between the body wall and intestine contains few germ cells.
8. Germ cells often gives rise to second generation the daughter rediae.
9. Redia gives rise to a new type of larva known as cercaria larva from the germ cells.
10. Cercaria larva comes out from redia through birth pore.

## 8. Liver fluke - *Cercaria*

### Characters:

1. Each redia produces 14 to 20 cercaria larvae. Cercaria is the infective stage to primary host.
2. They leave the body of redia through its birth pore and enters the snail's digestive gland.
3. Morphologically, cercaria bears a close resemblance with the adult fluke.
4. Cercaria is about 0.25 – 0.35 mm long and is an oval larva with a tail. It has no eyes.
5. The body wall consists of cuticle, musculature and subepithelium.
6. On the other side, there is a mouth with a sucker, a suctorial pharynx, long oesophagus and a bifid intestine.
7. A ventral sucker or acetabulum is present on the ventral side.
8. A large number of dark, brown cells called cystogenous glands are found beneath the cuticle.
9. There is a pair of excretory ducts, each with several flame cells.

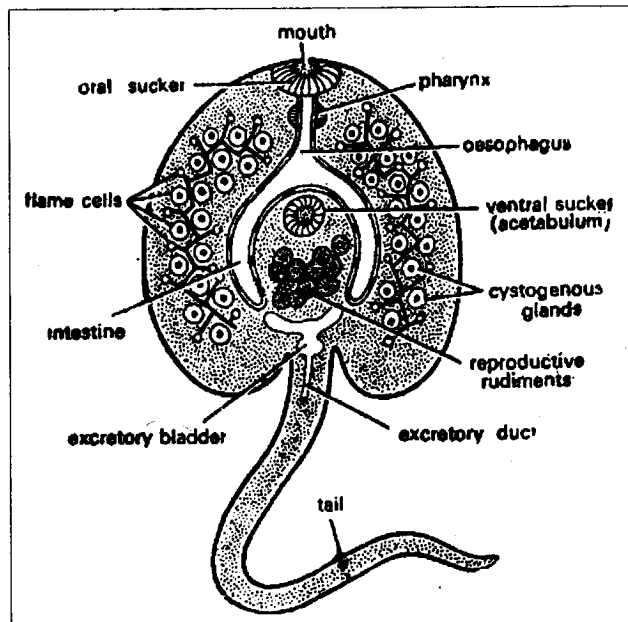


Fig. 8. Cercaria larva of *Fasciola hepatica*

10. Rudiments of reproductive organs develop from the germ cells near the hind-end.
11. From the digestive glands of the snail, the cercariae pass into the pulmonary sac and then escape into the surrounding water.
12. Cercaria swims about 5 minutes to an hour. Afterwards, it settles down on green leaves of water plants.
13. When cercaria enters the primary host or undergoes encystation, tail casts off and the body becomes rounded and forms around itself a thick brownish cyst wall from the cystogenous glands.

The encysted cercaria is known as metacercaria which is swallowed by the final host, sheep.



## 9. *Fasciola* - metacercaria

### Characters:

1. Cercaria which comes out of the body of the snail swims with its tail. Within 2 or 3 hours, the cercaria settles on a blade of grass or the leaf of a plant. Its tail is cast off, and its body is covered with a gelatinous cyst formed by the gland cells. The cercaria is now infective and this encysted larva or metacercaria may be swallowed by the final host, the sheep. As many as a thousand metacercariae may be found attached to a single grass blade.
2. It is a juvenile fluke having a rounded form with a diameter of about 0.2 mm.
3. It resembles cercaria larva but differs from later in the absence of tail, cystogenous glands and presence of thick cyst wall.
4. Excretory bladder opens directly through a single pore.
5. Germ cells or the genital rudiments are present.
6. Cyst provides protection against short periods of desiccation.
7. The vertebrate host (sheep, goat etc.) gets the infection by grazing on grass, leaves and other vegetation to which metacercarial cysts are attached.
8. The cyst wall is dissolved in the intestine of the host and the young fluke is set free to migrate to the bile duct. The cycle is then repeated.

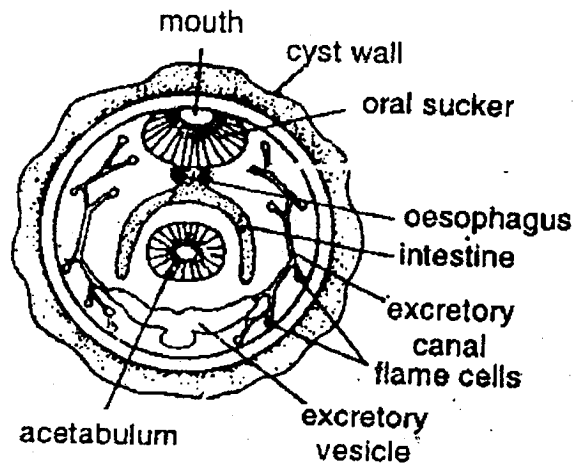


Fig. 9. *Fasciola* - metacercaria

## 10. *Schistosoma haematobium*

Phylum: Platyhelminthes

Class: Trematoda

Order: Digenea

### Characters:

1. *Schistosoma haematobium* is commonly called the blood fluke of man.
2. Body is long, slender in form and greyish or pinkish in colour.
3. Sexes are separate.
4. Male is usually 8 to 16 mm in length, has a cylindrical stout and flattened body.
5. Female is longer than male, usually 15 to 20 mm in length, has a more slender delicate cylindrical body.
6. Males carry females permanently in their gynecophoric canals formed by the infolding of the ventral body wall.
7. Both male and female worms are provided with oral and ventral suckers but the ventral sucker is larger and powerful in male.
8. Digestive system is simple and consists of oesophagus and forked intestine. Pharynx absent.
9. Male reproductive system consists of 4-5 testes, vasa deferens and seminal vesicle.
10. Female system has an elongated ovary, oviduct, vitellaria and an uterus.
11. *Schistosoma* lives in the hepatic portal system and mesenteric vessels of man.

**Primary host:** Man

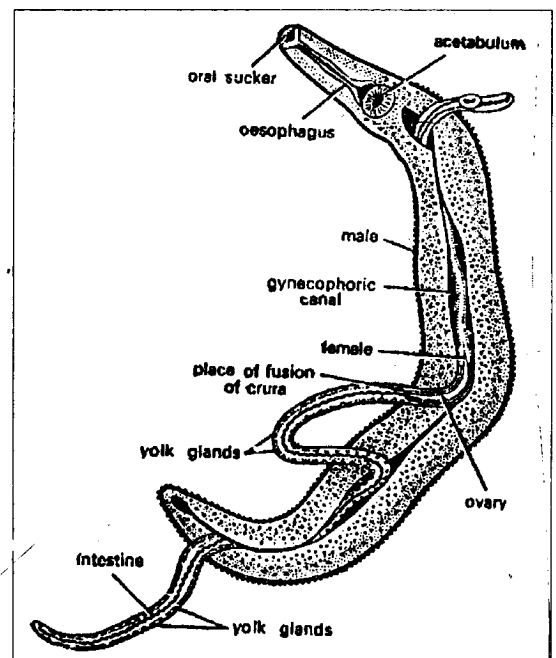
**Intermediate host:** Snail (*Bulinus truncatus*)

**Name of disease caused:** Schistosomiasis or Bilharziasis

**Infective stage:** Cercaria larva to man, miracidium larva to snail.

### Clinical symptoms of the disease:

- (i) During migration of the eggs through the wall of the urinary bladder or colon, haemorrhage generally occurs.
- (ii) Swelling of liver and spleen takes place.
- (iii) In case of heavy infections, mature worms may migrate to the brain, lungs, uterus, oviduct and gonads. Their presence at these sites provoke pathological changes.



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Fig.10. *Schistosoma haematobium* (male and female)

### Prophylaxis:

Prevention of pollution of water with human excreta; destruction of the snail vector in endemic areas; avoidance of swimming, bathing, wading or washing in infected water.

## 11. *Taenia solium* (Tapeworm)

Phylum: Platyhelminthes  
 Class: Cestoda  
 Order: Taenioidea  
 (or)  
 Cyclophyllidea

### Characters:

1. *Taenia solium* is commonly called as “tapeworm of man”. The body is elongated and flattened dorsoventrally like a ribbon or tape.
2. Body consists of scolex or head, neck and strobila or body segments.
3. Scolex is smaller than the head measuring about 1 mm in diameter. It is the organ of attachment, bears four suckers and a rostellum which has a double circlet of hooks about 28 to 32 in number.
4. Behind the scolex is a thin unsegmented neck.
5. Strobila or body consists of large number of segments about 800 or more in number. Each segment is termed a proglottid.
6. Each mature proglottid contains a set of male and female reproductive organs, a part of excretory and nervous system and a lateral genital opening.
7. Pig is infected by bladder worm or cysticercus after eating contaminated human faeces.
8. Man in turn gets infection by consuming measly pork.
9. *Taenia solium* is commonly found in the intestine of man in places where pork is eaten as food.

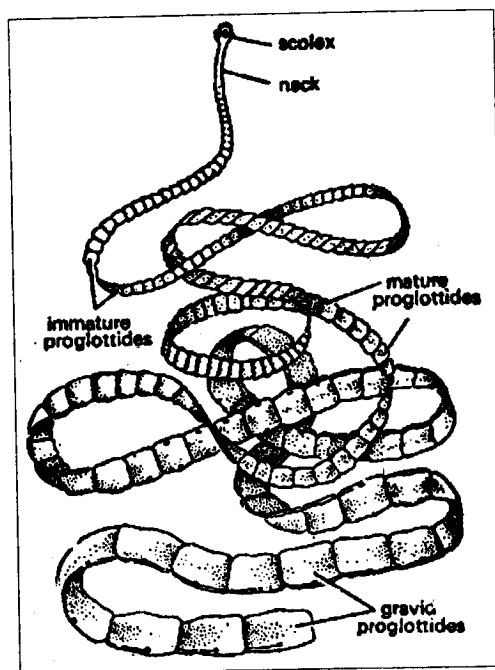


Fig. 11. *Taenia solium*

**Primary host:** Man

**Intermediate host:** Pig

**Name of disease caused:** *Taeniasis*

**Infective stage:** Cysticercus larva to man, onchosphere to pig.

**Clinical symptoms of the disease:**

- (i) The scolex of adult worm remains embedded in the mucous lining of alimentary canal for the attachment. There may be abdominal discomfort, diarrhoea, nervousness, nausea and epilepsy etc.
- (ii) Sometimes, man may accidentally ingest eggs or proglottids with contaminated food. Serious effects may result from cysticercosis. Cysticerci are commonly located in striated muscles, liver, eye and the central nervous system and damage may occur in these organs.

**Prophylaxis:**

1. Avoidance of eating raw or undercooked meat (pork).
2. Adequate meat inspection in the slaughter house.
3. Proper sanitary control of sewage disposal.
4. Effective treatment of infected individual with antihelmintics.
5. Defecation in open places should be avoided.
6. Selling of measly pork should be prevented.

## 12. *Taenia solium* - mature proglottid

### Characters:

1. Each mature segment is longer than broad.
2. Both the lateral sides of each proglottid or segment contain lateral longitudinal nerve cords and lateral excretory canals.
3. Each mature proglottid has a complete set of male and female reproductive organs.
4. Male reproductive organs consist of testes, vasa efferentia, vas deferens and cirrus.
5. Testes are numerous spherical bodies distributed through out the proglottid.
6. Female reproductive organs comprise bilobed ovary, oviduct, ootype, vagina, uterus and vitellaria.
7. Oviduct divides into two ducts, one leading to vagina opening into genital atrium by female genital opening and the other into uterus.

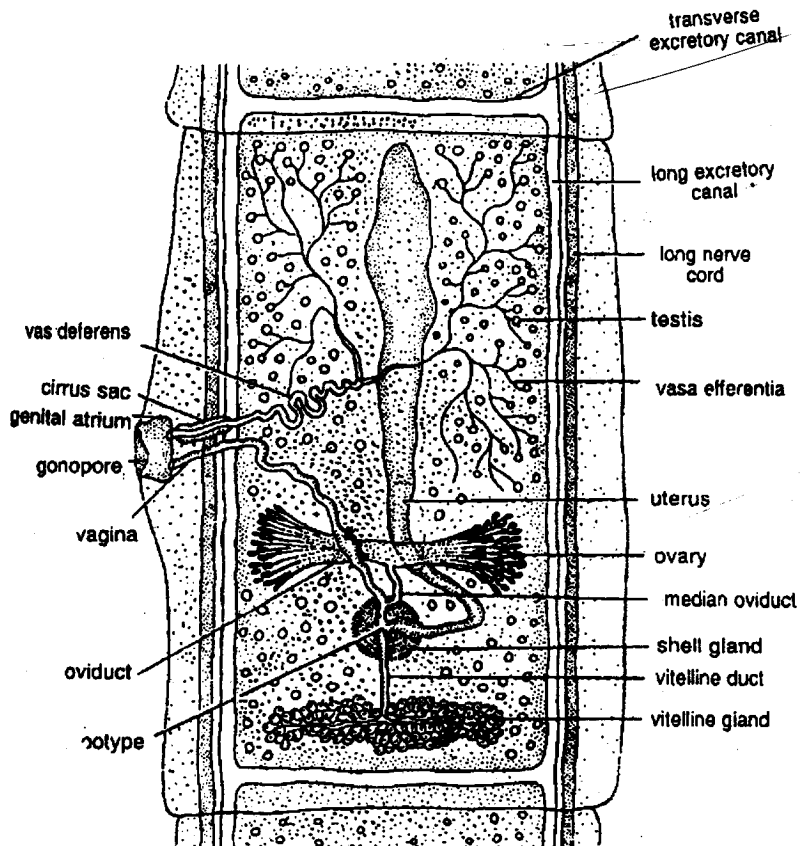


Fig. 12. *Taenia solium* - mature proglottid

### 13. *Taenia solium* – gravid proglottid

#### Characters:

1. In tapeworm, the gravid or ripe proglottids are situated posteriorly.
2. Each gravid proglottid is longer than broad and has a highly branched uterus filled with fertilized eggs.
3. Other structures have disappeared in ripe proglottid.
4. Ripe proglottids are detached from the strobila and pass out from the intestine of host with the faeces.
5. The phenomena of detachment of ripe proglottids from strobila is known as apolysis.

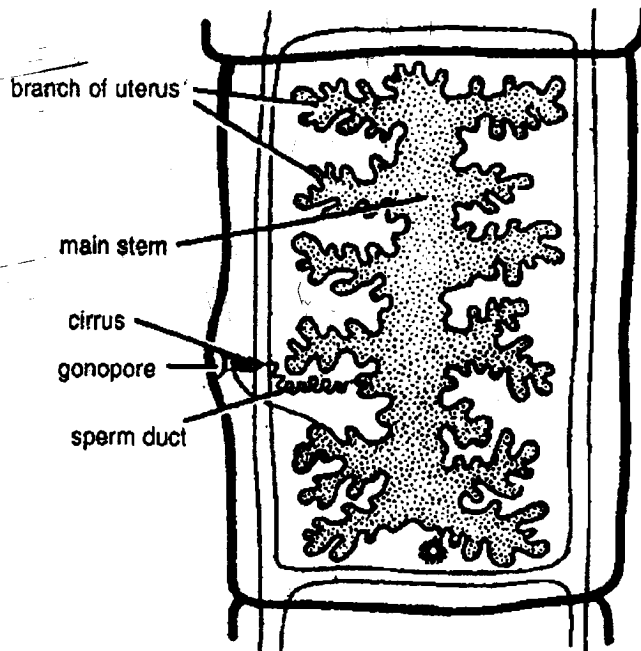


Fig. 13. *Taenia solium* - gravid proglottid

## 14. *Echinococcus granulosus*

Phylum: Platyhelminthes  
 Class: Cestoda  
 Order: Taenoidea  
 (or)  
 Cyclophyllidea

### Characters:

1. *Echinococcus* is commonly known as hydatid worm. In its adult stage, it is found attached to the mucous membrane of the small intestine of the dog.
2. It measures from 2-8 mm in length.
3. The body consists of a scolex and three segments.
4. The scolex or head bears four suckers and a protrusible rostellum provided with double rows of 30 to 36 hooks.

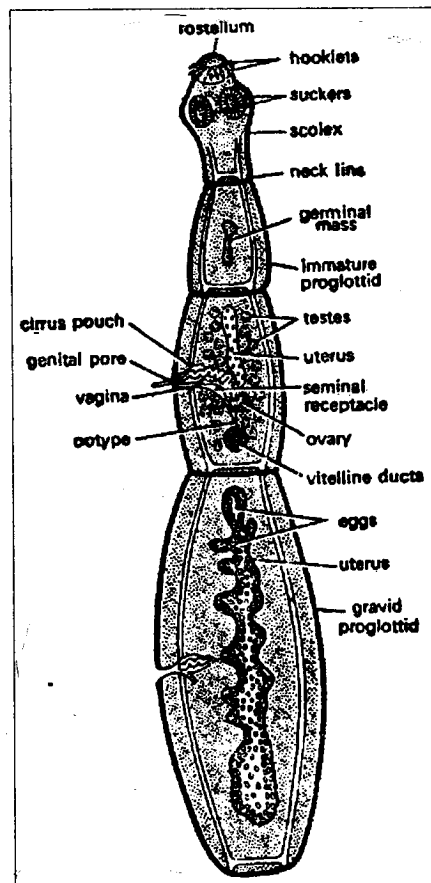


Fig. 14. *Echinococcus granulosus*

5. The narrow part of the scolex, behind the suckers, forms the neck. It is the area of strobilization.
6. The strobila consists of usually three proglottids. First proglottid is generally immature, second proglottid is mature, while the third is large and gravid.
7. Hermaphroditic and the mature segment contains a single set of genital organs.
8. Male reproductive system consists of spherical testes, vas deferens and cirrus.
9. Female reproductive system comprises ovaries, oviduct, vitellaria, ootype, uterus and vagina.
10. Gravid segment is elongated containing branched uterus with ocospheres; it is discharged with faeces.
11. Hydatid cyst or echinococcus cyst or larval stage occurs in man and other domestic animals, e.g. monkey and cattle (usually found in liver).
12. *Echinococcus* is cosmopolitan in distribution, especially in sheep and cattle-grazing areas.
13. Herbivorous animals like sheep and oxen are also infected, when they swallow the grass contaminated with eggs.
14. Man may become infected if he eats the eggs through polluted water or green vegetables.

**Primary host:** Dog and other carnivores

**Intermediate host:** Man

**Name of disease caused:** Hydatid cyst disease or echinococcosis

**Infective stage:** Ochosphere to man, Brood capsules to dog

(Broad capsules are internal buds having scoleces of parasites)

**Clinical symptoms of the disease:**

- (i) Bladders or cysts are harmful when they develop rapidly.
- (ii) They cause inflammatory reactions in the surrounding tissue of the host resulting in the formation of fibrous tissue.
- (iii) The escaping hydatid fluid not only cause diarrhoea, abdominal pain etc., but also death of the host sometimes.

**Prophylaxis:**

1. Prevention of infection of dogs.
2. Deworming of dogs in endemic areas.
3. Maintenance of personal prophylaxis (cleaning of hands before eating).



## 15. Transverse section of female *Ascaris lumbricoides*

### Characters:

- Body wall consists of cuticle, epidermis and muscle layer.
  - Cuticle is delicate, transparent, elastic membrane covering the outer surface of the body.
  - Epidermis is syncytial and lies below the cuticle.
  - Below the epidermis is a muscular layer consisting of a single layer of spindle-shaped cells arranged longitudinally.
- Four longitudinal lines, one dorsal, one ventral, and two laterals are seen at their respective places.
- Lateral excretory canals are embedded within the lateral longitudinal lines.
- Dorsal and ventral nerves are seen within the dorsal and ventral longitudinal line. There is a spacious body cavity between the alimentary canal and the body wall which is a pseudocoel.
- Pseudocoel contains the transverse section of alimentary canal, uteri, oviducts and ovaries. The female reproductive system consists of a pair of ovaries which are tubular like the testes.
  - Ovaries have central rachis with the young eggs clustered around it.
  - Oviducts contain eggs in its lumen.
  - Uteri contain a large number of unfertilized eggs.

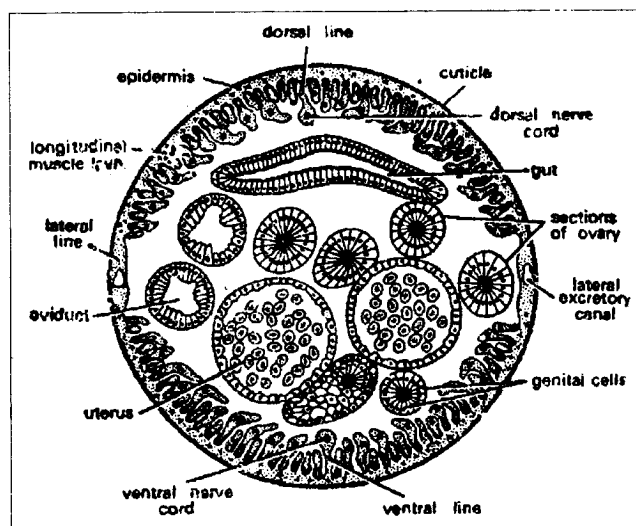


FIG 15 T.S. of female *Ascaris lumbricoides*

## 16. Transverse section of male *Ascaris lumbricoides*

### Characters:

1. Body wall is composed of cuticle, epidermis and muscle layer.
  - (i) Cuticle is delicate, transparent, elastic membrane covering the outer surface of the body.
  - (ii) Epidermis is syncytial (without distinct cell boundaries and with scattered nuclei) and lies below the cuticle.
  - (iii) Below the epidermis is a muscular layer consisting of a single-shaped cells arranged longitudinally.
2. Epidermis is thickened into ridges in four regions which project into the body cavity as the four longitudinal lines, i.e., two lateral, one dorsal and one ventral.
3. Due to the presence of four longitudinal lines, four bands of muscles two dorso-lateral and two ventro-lateral are easily differentiable.
4. Two lateral excretory canals are seen within the two lateral lines.
5. Dorsal and ventral nerves run in the dorsal and ventral lines respectively.
6. The space between the alimentary canal and body wall is known as pseudocoel.
7. Transverse section of alimentary canal, testes, vas deferens and seminal vesicles are seen within the pseudocoel.

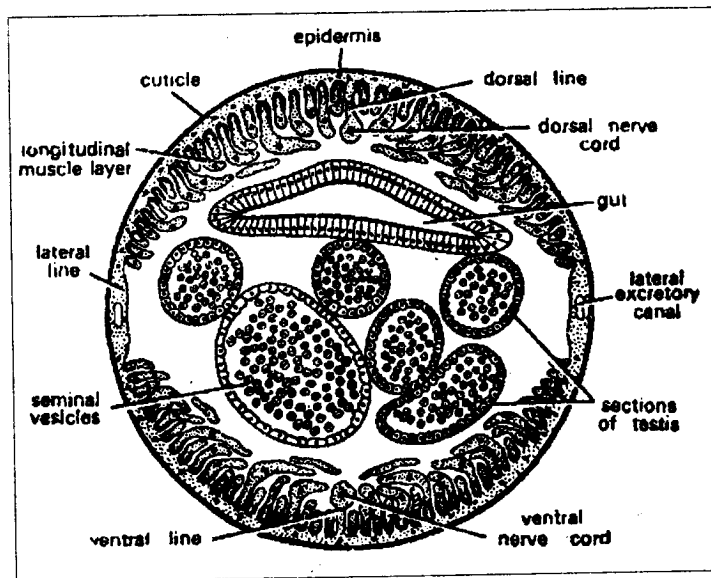


Fig. 16. T.S. of male *Ascaris lumbricoides*

## 17. *Ancylostoma duodenale*

Phylum: Aschelminthe

Class: Nematod

Order: Stongyloide.

### Characters:

1. *Ancylostoma duodenale* is found as an endoparasite in the intestine particularly in the jejunum of man. It is usually found in tropical and subtropical regions.
2. *Ancylostoma duodenale* is commonly known as old world hook-worm.
3. Mature worm is cylindrical in shape, narrow anteriorly and white or ivory grey in colour.
4. Buccal cavity is oval and the buccal capsule is made of articulated grooved portion.

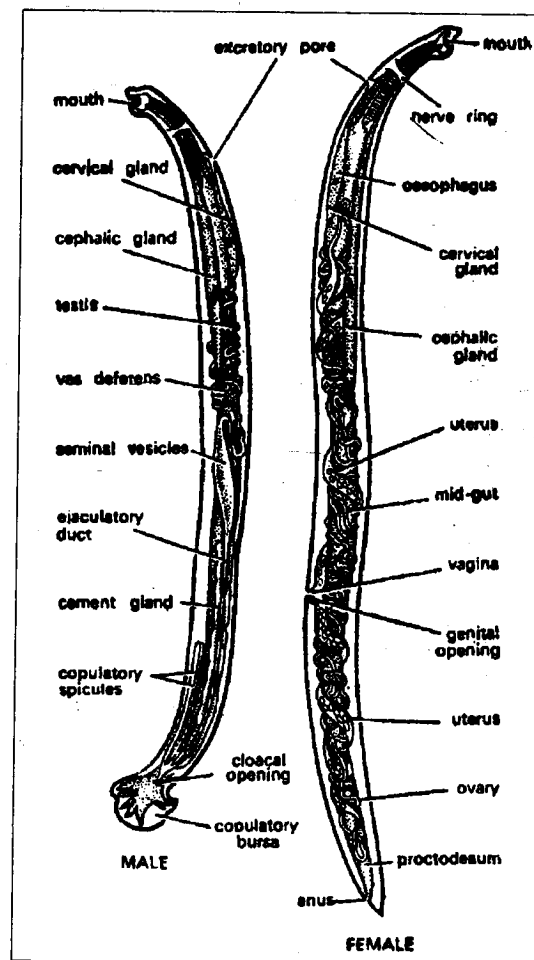


Fig. 17. *Ancylostoma duodenale*

5. Males measure 8-11 mm in length and females measure 10-13 mm in length.
6. Excretory pore mid-ventral in position just behind the nerve ring.
7. Male is provided with a copulatory bursa which is broader than long and supported by fleshy rays.
8. Vulva of the female is behind the middle of the body and the tail is tipped by a minute spine.
9. Fertilization is internal and occurs in the intestine of host.
10. Fertilized eggs pass out with the faeces and after sometime develop into infective stage. Nonfeeding filariform larva is the infective stage infection occurs by larval penetration.

**Primary host:** Man

**Intermediate host:** ---

**Name of disease caused:** Ancylostomiasis

**Infective stage:** Filariform larva

**Clinical symptoms of the disease:**

- (i) Internal bleeding, severe anaemia, weakness, loss of resistance and pneumonia.
- (ii) Mental and physical growth is retarded in children and growing young people.
- (iii) Unchecked infection may lead to fatty degeneration of heart, liver and kidneys, ending in death.
- (iv) Inflammation of the skin and lungs during larval migration.

**Prophylaxis:**

**Attack of adult parasite:** Treatment of carriers and diseased persons simultaneously with anthelmintics proper hygienic habits should be maintained in the community.

**Attack on larvae:** Prevention of soil-pollution by proper control of sewage disposal. Disinfection of feces or soil.

**Personal protection:** Walking with bare foot should be avoided. Wearing of shoes and gloves may prevent larval penetration.

## 18. *Trichinella spiralis* cyst in muscles

Phylum: Aschelminthes

Class: Nematoda

Order: Trichuroidea

### Characters:

1. *Trichinella spiralis* is an endoparasite in the intestine of man, pig, rat and other vertebrates and cosmopolitan in distribution.
2. It is commonly known as trichina worm. It is rare in our country.
3. It is the smallest human nematode parasite. Males measuring 1.4 to 1.6 mm and females 3.0 to 4.0 mm in length.
4. Male has two large papillae.
5. The females are viviparous and penetrate more deeply into the intestinal wall of the host and deposit the larvae.
6. The large numbers of larvae in the intestinal wall are distributed through out the body by the lymphatic and blood streams. The larvae may be carried to all parts of the body but they are incapable of development in parts other than voluntary muscles. The larvae which do not reach the voluntary muscles perish.
7. The juvenile worms penetrate deeply into the ends of skeletal muscles such as diaphragm and intercostals etc., and form lemon shaped cysts. Under encystment the larvae may live for several years.

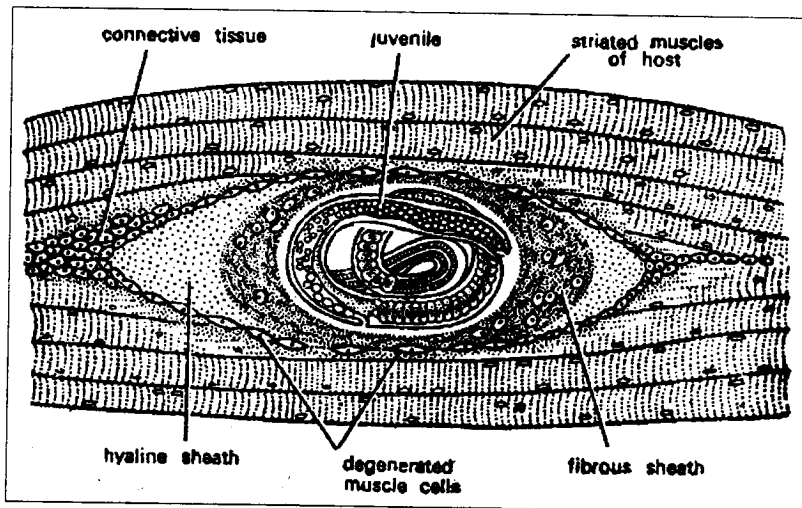


Fig. 18. Encysted larva of *Trichinella spiralis*

**Primary host:** Man, pig, rat and other vertebrates.

**Intermediate host:** ---

**Name of disease caused:** Trichinosis.

**Infective stage:** Non-feeding, third stage, active Filariform larva.

**Clinical symptoms of the disease:**

1. Fever, gastro-intestinal symptoms including diarrhoea, muscle pain, nausea, eosinophilia, weakness etc.
2. Death may occur as a result of heart failure, respiratory complications and kidney malfunction.
3. Symptoms like the typhoid may be seen when adult females burrows in the intestine to deposit larvae.

**Prophylaxis:**

Prevention of pollution of drinking water by infected individual. Chemical treatment of water-supply for eradication of cyclops. Drinking water should be either strained, filtered or boiled.

## 19. *Wuchereria bancrofti* (*Filaria bancrofti*)

Phylum: Aschelminthes

Class: Nematoda

Order: Filarioidea

### Characters:

1. It is commonly known as filarial worm.
2. It is the most important human parasite in the lymph glands or ducts.
3. Adult worms are creamy white in colour, filiform and cylindrical in shape with both ends tapering and terminating bluntly. Sexes are separate.
4. Head slightly swollen and provided with two rows of small sessile papillae.
5. Mouth is unarmed and devoid of buccal capsule.
6. Male is about 40 mm in length with the caudal end curved.
7. Female is about 80 to 100 mm in length bearing the vulval opening just behind the anterior extremity of the body; viviparous.
8. Female worms give birth to microfilariae which are surrounded by delicate membrane or sheath.
9. Microfilaria is long cylindrical provided with a striated cuticle.
10. Microfilariae may either live in lymph or migrate into the blood capillaries.

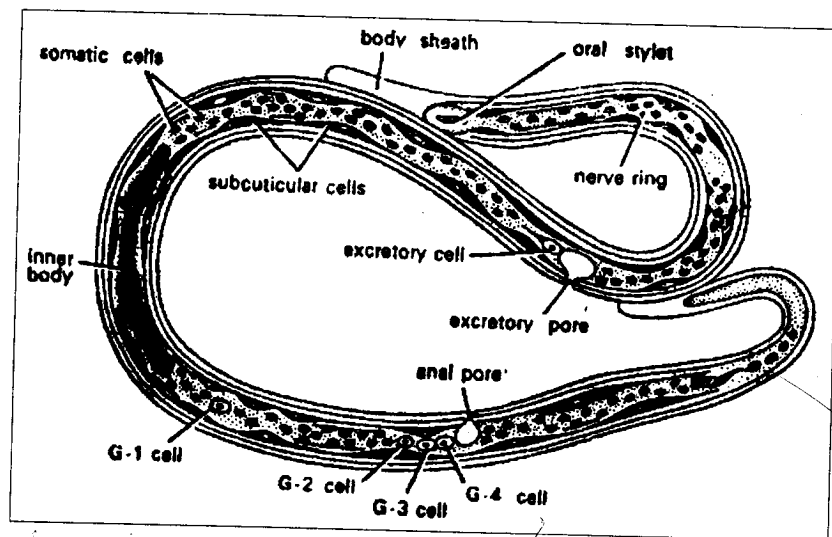


Fig.19. Microfilaria of *Filaria bancrofti*

11. Culex mosquito sucks the blood along with microfilariae from the infected host.
12. The further development of microfilariae takes place in the stomach and thoracic muscles of the mosquito.
13. When the infected mosquito next bites a man, the larvae pass to the point of bite and penetrate the superficial skin to find their way in to the lymphatic vessels. Here they settle down and attain sexual maturity.

**Primary host:** Man

**Intermediate host:** *Culex* mosquito

**Name of disease caused:** Filariasis or Elephantiasis or wuchereriasis

**Infective stage:** Microfilaria larva

**Clinical symptoms of the disease:**

- (i) Mild infection produces no serious symptoms. It causes filarial fever, mental depression, head ache etc.
- (ii) In heavy infection, accumulation of living or dead worms eventually blocks the lymphatic vessels and glands.
- (iii) Generally lower limbs, legs, scrotum in male are affected.

**Prophylaxis:**

Filariasis can be controlled in two ways:

1. Protection against adult mosquitoes.

Spraying DDT in the houses

Fumigation in human dwellings

Using mosquito nets and repellents.

2. Eradication of mosquito larvae.

Spraying insecticides in the breeding places.

Spraying of pyrethrum oil on the stagnant water places like ditches, ponds etc.

Rearing larvicidal fish like Gambusia in water tanks and ponds.



## 20. *Dracunculus medinensis*

Phylum: Aschelminthes  
Class : Nematoda  
Order: Dracunculoidea

### Characters:

1. It is commonly called as Guinea worm. Sexes are separate.
2. It lives in the deeper subcutaneous tissues of man.
3. The anterior end is blunt while the posterior end is pointed.
4. The adult male measures 4 cm in length. Its hind end is curved and bears 10 pairs of genital papillae and a pair of pineal spicules.
5. Adult female measures 70-120 cm in length. Its hind end is straight and bears a spine-like process.
6. The oesophagus is distinguished into an anterior narrow muscular region and a posterior broader glandular region.
7. The female reproductive system is didelphic with opposite uteri.
8. Females are viviparous. The alimentary canal and vulva are atrophied in mature females.
9. Mature female migrates to the superficial layers of the skin in those parts of the body which are likely to come in contact with cold water.
10. On coming in contact with water, the worm ruptures, releasing larvae in water. The larvae swim about but do not develop further unless swallowed by female cyclops. Here the female larvae moult twice and become infective.
11. Infection occurs when cyclops is swallowed by a new host along with drinking water.

**Primary host:** Man

**Intermediate host:** Cyclops

**Name of disease caused:** Dracunculosis or Guinea worm disease

**Infective stage:** Larvae to cyclops, infected cyclops with larvae to man.

### Clinical symptoms of the disease:

- (i) Guinea worm disease is characterised by asthma, nausea, vomiting, diarrhoea etc.
- (ii) Mature female migrates to the superficial layers of the skin. Toxic substances are produced under the skin which results in the formation of a blister, leading to an ulcer.

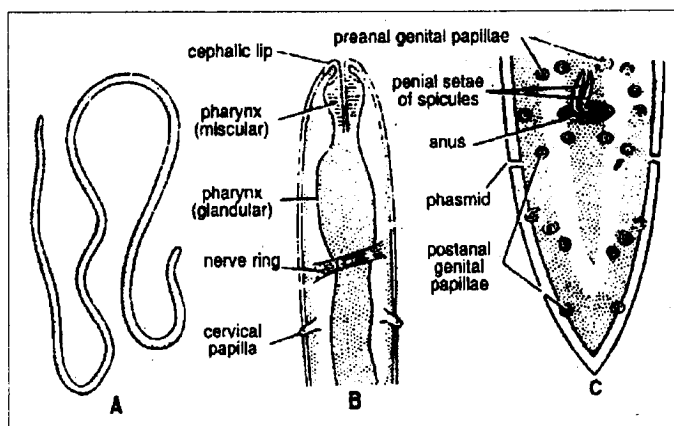


Fig.20. *Dracunculus medinensis*. A-Entire, B-Anterior end of male. C-Posterior end of male (ventral view).

**Prophylaxis:** By drinking water free from cyclops (drinking boiled water).

## 21. Bone marrow

The system of the body which is responsible for all types of immune responses is called the immune system. The immune system is constituted by the lymphoid organs and cells. Based upon the functional development of the lymphocytic cells, lymphoid organs can be classified into three groups:

1. Lymphoid organs involved in genesis of lymphocytes, e.g. yolk sac of embryo, fetal liver and bone marrow.
2. Primary lymphoid organs, e.g. thymus and avian Bursa Fabricus or Bursa equivalent tissue.
3. Peripheral lymphoid organs e.g. lymph nodes, spleen, Payer's patches, tonsils, appendix etc.

The lymphoid organs involved in the genesis of lymphocytes contain a pool of undifferentiated stem cells which migrate from these organs to the primary lymphoid organs through circulation. All lymphocytes are derived from these stem cells.

Bone marrow remains as the chief source of stem cells in adult life. Lymphopoiesis along with genesis of other haemopoietic cells occur in red bone marrow. The red bone marrow is present in most of the bones in fetal and early post natal life. In adults, the red bone marrow is found in flat bones. The remaining marrow is replaced by fat cells and is known as yellow marrow. Bone marrow is also an important peripheral lymphoid organ containing mature T and B lymphocytes.

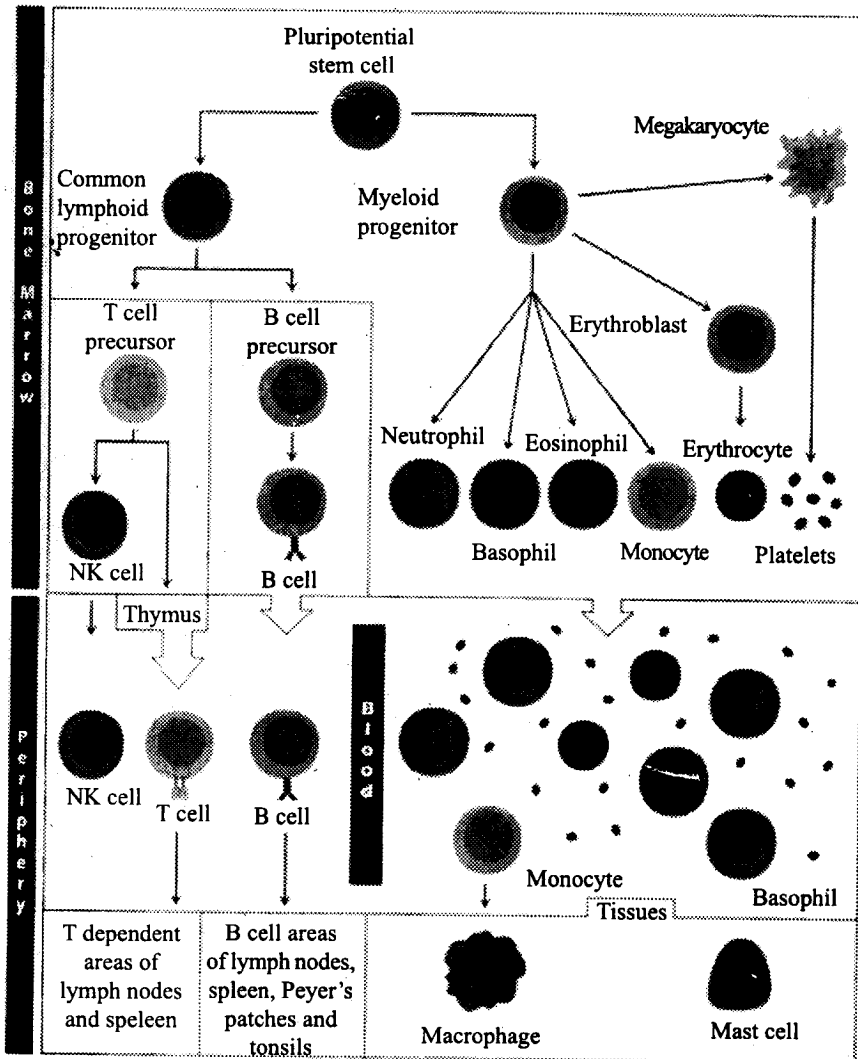


Fig. 21. The developmental pathway of various cell types from pluripotent bone marrow stem cells.

## 22. Thymus (T.S.)

Thymus is a primary lymphoid organ involved with immunological differentiation of lymphocytes. It is the primary lymphoid organ. The thymus is located behind the upper part of the sternum. It has two lobes surrounded by a fibrous capsule. The whole organ is divided into lobules by connective tissue septa. The lobules are differentiated into an outer cortex and an inner medulla. The peripheral part of each lobule is densely populated with lymphocytes around concentrically arranged epithelial cells forming the structures known as Hassall's corpuscles. The cortex is crowded with populations of proliferations with small lymphocytes. The medulla consists of mainly of epithelial cells and Hassall's corpuscles.

The primary function of the Thymus is the production of thymic lymphocytes. It is the major site of proliferation of lymphocytes. Lymphocytes produced in the thymus are called "Thymus (T dependent) lymphocytes" or "T cells" of cell mediate immune system. Then cells leave the thymus, circulate in peripheral blood and lymph and colonise in the thymus-dependent areas of peripheral lymphoid organs. But, of the lymphocytes produced, only about 1% leave the Thymus. The rest are destroyed locally. The thymus confers immunological competence on the lymphocytes during their stay in the Thymus.

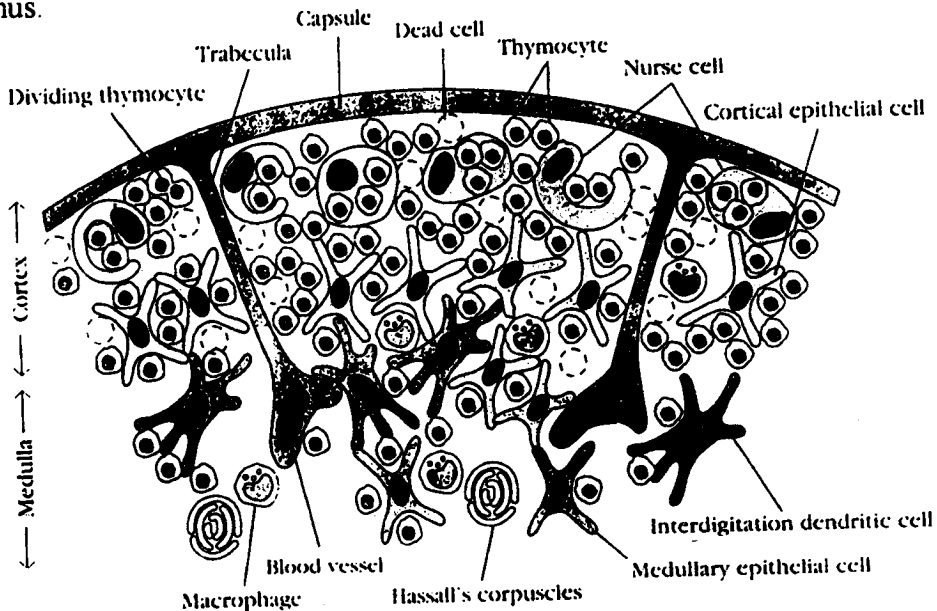


Fig. 22 . Diagrammatic cross-section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae).

## 23. Lymphnode (T.S.)

Basing on the functional development of the lymphocytic cells lymph nodes are classified as peripheral lymphoid organs. In the lymphnodes functionally mature lymphocytes colonize and respond to antigenic stimuli. These are also the organs in which antibodies are formed and the cells for cell mediated immune responses are born. Lymphnodes act as filters in lymphatic vascular tree.

The lymphnodes are small round or ovoid bodies; present along the course of lymphatic vessels. Lymphnodes are surrounded by a fibrous capsule. The lymph node can be differentiated into an outer cortex and an inner medulla. In the cortical region are accumulations of lymphocytes - these are called primary lymphoid follicles (PLF). Upon antigenic stimulation germinal centers (secondary lymphoid follicles) develop in PLF region. Antigen processing dendritic macrophages are also present in follicles. In the medulla, the lymphocytes are arranged as elongated branching rods (medullary rods). The follicles and medullary cords contain B lymphocytes and constitute Bursa dependent areas. Between the follicles and medullary cords, there is an intermediate zone of paracortical area; this area contains T lymphocytes and constitutes thymus dependent area.

Lymph nodes phagocytose foreign materials and microorganisms.

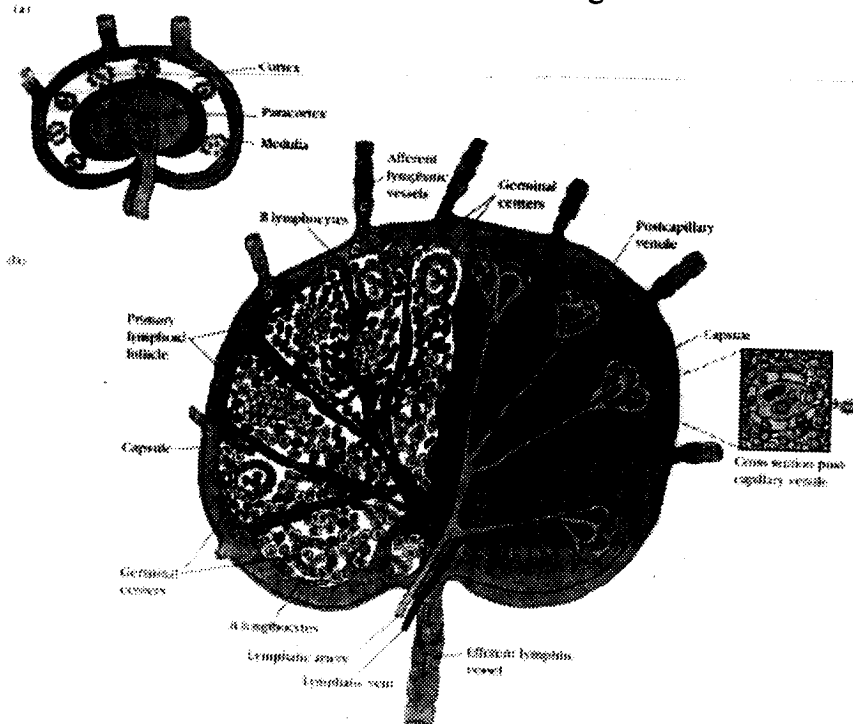


Fig. 23 Structure of a lymph node. (a) The three layers of a lymph node support distinct microenvironments. (b) The left side depicts the arrangement of reticulum and lymphocytes within the various regions of a lymph node. Macrophages and dendritic cells, which trap antigen, are present in the cortex and paracortex. The right side of (b) depicts the lymphatic artery and vein and the postcapillary venules

## 24. Liver abscess

The human digestive system consists of the alimentary canal and its associated glands. The various organs of the digestive systems are in the following sequence: mouth, oesophagus, stomach, duodenum, jejunum and ileum (duodenum, jejunum and ileum together are called small intestine), colon and rectum (colon and rectum together are called large intestine). Salivary glands, liver and pancreas are associated with the digestive system. Liver is the target organ to be affected by the toxins. Liver secretes bile which contains bile pigments and bile salts. The secretions of the liver and pancreas pass into the duodenum. The bile secreted by the liver is normally stored in gall bladder.

Lesions of wounds in the liver cause ulcers in this organ. When liver is invaded by pathogenic microbes, protozoan parasites or metzoan parasites pathological reactions occur in the hepatocytes. In chronic or heavy infections the lesions would lead to abscess. The pathological reactions may result in the destruction of hepatocytes, hemorrhages and damage to liver trabuculae. Ultimately, the function of the liver may be stopped and the affected patients may become weak. Amoebic liver abscess varies greatly in size. The area of abscess appears reddish brown in colour and shows necrotic liver tissues. Timely diagnosis and prophylactic measures may improve the condition.



Fig. 24 Macroscopic pathology of amoebic liver absces

## 25. Lung abscess

In human beings, the organs of respiratory systems are nose, nasal passage, trachea, bronchi, lungs and diaphragm. The lungs lie in the chest cavity or thoracic cavity. Lungs are separated from the abdominal cavity by a muscular partition called diaphragm. Two thin membranes, pleura cover the lungs. The trachea or wind pipe leads to bronchi. Each bronchus divides in the lungs to form a large number of smallest bronchioles. Each bronchiole has small air sacs at its rear end called alveolus. The thin, alveolur wall is surrounded by blood capillaries. It is in the alveoli gas exchange takes place.

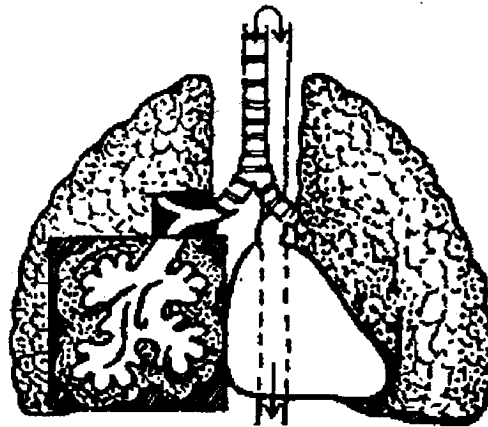


Fig. 25. Larvae breaking out of pulmonary capillaries into lung alveoli

Lung abscess may be caused by inhaling irritant fumes, pathogenic microbes and by lodging pathogenic larvae or parasites. When lungs are diseased, breathing difficulties occur and gas exchange is also reduced. To keep good health, it is important to breath unpolluted air and to stay away from unhygienic conditions of the environment.

## DISTANCE MODE PRACTICAL MANUAL

### PRACTICAL - IV

Determination of dissolved oxygen, free CO<sub>2</sub>, alkalinity, total hardness, chlorides, COD/BOD.

- 4.1.1 Determination of Dissolved Oxygen
- 4.1.2 Estimation of Total Free CO<sub>2</sub>
- 4.1.3 Estimation of Total Alkalinity
- 4.1.4 Estimation of Total Hardness
- 4.1.5 Estimation of Chlorides
- 4.1.6 Estimation of Chemical Oxygen Demand (COD)

***Dr.K.VEERAAIAH***

### PRACTICAL – II

Spectrophotometer/Colorimeter Principles & Instrument Working Estimation of Phosphates and Nitrites :

- 4.2.1 Spectrophotometer/Colorimeter Principles & Instrument Working
- 4.2.2 Estimation of Orthophosphates
- 4.2.3 Estimation of Nitrites

***Dr.K.VEERAAIAH***

Identification and mounting of common zooplanktonic organisms in the ponds –

- 4.3.1 Rotifers,
- 4.3.2 cladocerans
- 4.3.3 copepods

***Dr. P. PADMAVATHI***



**IV. Identification of benthos in ponds –**

4.4.1 Insects,

4.4.2 insect larvae,

4.4.3 molluscs

4.4.4 aquatic vegetation

**Dr. P. PADMAVATHI**

V Dissection and mounting of the Pituitary gland of the fish – Preparation, dosage and injection of pituitary extract for induced breeding of fish

**Dr. N. GOPALA RAO**

VI Spotters – Fishes of aquacultural importance

**Dr. N. GOPALA RAO**

## PRACTICAL - 4.1.1

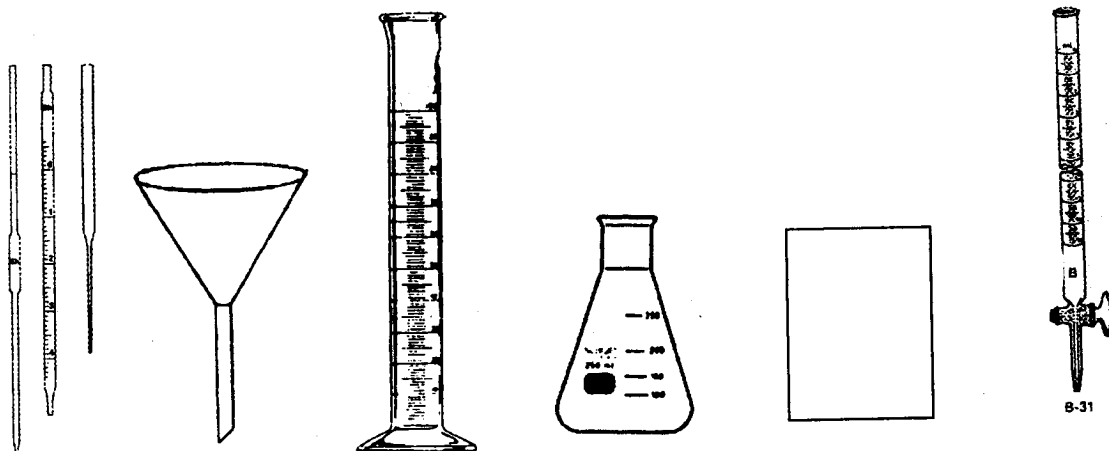
### DETERMINATION OF DISSOLVED OXYGEN

#### Aim

To estimate the amount of dissolved oxygen in given water sample by using Winkler's method.

#### Apparatus

Burette, Burette stand, pipette, volumetric flask, sampling bottle, conical flask measuring jar.



Pipette Funnel Measuring jar Conical flask Porcelain tile Burette

#### Reagents

- A. Winkler's A
- B. Winkler's B
- C. Winkler's C (Conc. Sulphuric acid)
- D. Hypo solution
- E. Starch solution

## Preparation of Reagents

### A. Winkler's A

$MnSO_4 \cdot 5H_2O$  (50%) solution. Dissolve 500gm of  $MnSO_4 \cdot 5H_2O$  in 1 litre of distilled water. No iodine should be liberated when 1ml of reagent is added to 50ml of acidified KI solution.

### B. Winkler's B

Alkaline Iodide Solution; This is an alkaline iodide oxide solution. Dissolve 400 gm of KOH or NaOH in 560ml of distilled water and add 900gm of NaI or KI and heat the solution until KI gets dissolved.

### C. Winkler's C

$H_2SO_4$  Add 500ml of  $H_2SO_4$  slowly to 500ml of distilled water.

Note: Never add water to the acid.

### D. Hypo solution (0.025N)

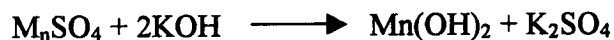
Dissolve 6.2gm of  $Na_2 S_2 O_3 \cdot 5H_2O$  and 1 gm of  $Na_2CO_3$  to 1 litre of distilled water.

### E. Starch solution(1%)

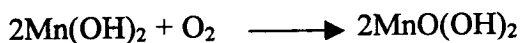
Dissolve 1 gm of starch in 100 ml of distilled water and warm it to  $80^{\circ}C$  to  $90^{\circ}C$  stir it well and allow it to cool.

## Principle

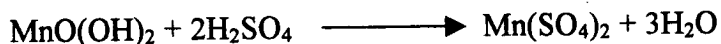
This method depends upon the oxidation of manganous hydroxide (bivalent –manganese) by the oxygen dissolved in the water resulting in the formation of a tetravalent compound. When the water containing the tetravalent compound is acidified, free iodine is liberated from the oxidation of potassium iodide. The free iodine is chemically equivalent to the amount of dissolved oxygen present in the sample; and is determined by titration with a standard solution of sodium thiosulfate (0.005N). The reactions involved by the addition of reagents (KI, KOH,  $MnSO_4$  and  $H_2SO_4$ ) to the water are as follows.



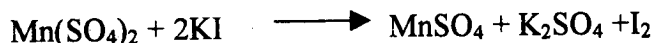
If the white precipitate is obtained, there is no dissolved oxygen in the sample. A brown precipitate indicates that oxygen was present and reacted with the manganous hydroxide forming manganic basic oxide.



After the addition of sulphuric acid this precipitate is dissolved forming manganic sulphate



There is an immediate reaction between this compound and the potassium iodide previously added liberating iodine and resulting in the typical iodine coloration (brown) to the water.



The number of moles of iodine liberated by the reaction is equivalent to the number of moles of oxygen present in the sample. The quantity of iodine is determined by titrating a portion of the solution with a standard solution of sodium thiosulfate.



### Procedure

Take 250 or 300 ml BOD bottle and dip in the water in standing position so that the water enters into the bottle slowly. Care should be taken to avoid the entry of the air bubbles. After filling the bottle with the sample water keep the stopper to the bottle while it is still in the water. To the sample immediately add 2ml of winkler's A and 2ml of Winkler's B. Carefully stopper the bottle without introducing air bubbles and mix vigorously by inversion. Allow the precipitate to settle. Shake vigorously again and allow the precipitate to settle to at least the bottom third of the bottle volume in the dark chamber. After taking out of the dark chamber add 2ml of Winkler's C and shake the bottle until all the precipitate is dissolved. Take 50ml of the sample in a conical flask and titrate against 0.05N Hypo solution which is taken in the burette. Titrate until the solution turns straw yellow colour and then add 2 drops of starch solution. The content in the flask turns to blue colour. Titrate until the blue colour is disappeared and note down the burette reading of rundown Hypo. The amount of oxygen present in the sample is calculated by using the formula.

Amount of  $\text{O}_2$  in the sample =

$$\frac{\text{Volume of hypo rundown} \times \text{Normality of Hypo} \times 8 \times 1000}{\text{Volume of sample water} \times \text{correction factor}}$$

### Result

The amount of  $\text{O}_2$  in the sample water is obtained in mg/lit.

## PRACTICAL – 4.1.2

ESTIMATION OF TOTAL FREE CO<sub>2</sub>**Aim**

To estimate the amount of total free CO<sub>2</sub> present in the given sample.

**Apparatus**

Burette, Burette stand, Pipette, Conical flask, Measuring jar, Porcelain tile etc.

**Reagents**

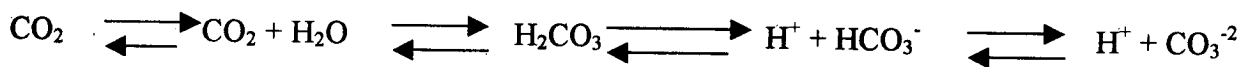
1. H<sub>2</sub>SO<sub>4</sub> (0.02N)
2. Phenolphthaline indicator
3. Methyl orange indicator

**Preparation of reagents****1. H<sub>2</sub>SO<sub>4</sub> (0.02N)**

0.1 N of H<sub>2</sub>SO<sub>4</sub> is prepared by diluting 3ml of conc. H<sub>2</sub>SO<sub>4</sub> in 100ml of distilled water. This is standardized against sodium carbonate of 0.1 N appropriate volume of H<sub>2</sub>SO<sub>4</sub> and diluted to obtain a standard, 0.02 N H<sub>2</sub>SO<sub>4</sub>.

**Principle**

Free CO<sub>2</sub> in the air is often in equilibrium with dissolved CO<sub>2</sub> in the water. This equilibrium together with the equilibria taking place in water is represented by taking an equation.



The concentration of CO<sub>2</sub> in the atmosphere tells about the respiration by aquatic organism and can substantially the quantity of CO<sub>2</sub> in water at any given time and place. The amount of CO<sub>2</sub> may be determined by the titration method. This method is based on the principle that the difference is volume of the titrant between the phenolphthalene alkalinity end point. Total alkalinity and point is equivalent to the total CO<sub>2</sub>. The pH of the sample is first brought to 8.2 by careful addition of CO<sub>2</sub> free strong acid base. Then the solution is titrated with strong acid to the total alkalinity end point as in the normal alkalinity determination.

**Procedure**

Take 50ml of the sample in the conical flask and add 2 or 3 drops of phenolphthaleine indicator. It develops a pink colour which tells that the pH is above 8.3. Here,  $\text{CO}_3^{2-}$  is abundant in water. The  $\text{CO}_3^{2-}$  is converted to  $\text{HCO}_3^-$  and then to carbonic acid  $\text{H}_2\text{CO}_3$  by titrating it against  $\text{H}_2\text{SO}_4(0.02\text{N})$  and the acid rundown from the addition of methyl orange to change of its colour and considered for total  $\text{CO}_2$ . The acid rundown from the Burette decolourise after adding phenolphthaleine. Then 1 or 2 drops of methyl orange is added and titration is continued until all bicarbonate is converted into carbonic and when phenolphthaleine is added to the sample water, if it doesn't develop any colour, it is understood the pH value is less than 7.5. 2 or 3 drops of alkali is added to increase the pH to that of  $\text{CO}_3^{2-}$  and total  $\text{CO}_2$  can be estimated. The total acid rundown is noted and is used for calculation of the total amount of  $\text{CO}_2$  is calculated by this formula.

$$\frac{\text{Volume of H}_2\text{SO}_4 \text{ run down} \times \text{Normality of HCl} \times 1000}{\text{Volume of the sample taken}}$$

$$\text{Total free CO}_2 =$$

**Result**

Total free  $\text{CO}_2$  present in the sample is obtained is milliequivalents (meq)/lit.

## PRACTICAL – 4.1.3

## ESTIMATION OF TOTAL ALKALINITY

**Aim**

To estimate the total alkalinity of the given sample.

**Apparatus**

Burette, Burette stand, Pipette, Measuring jar, Conical flask, Porcelain tile etc.

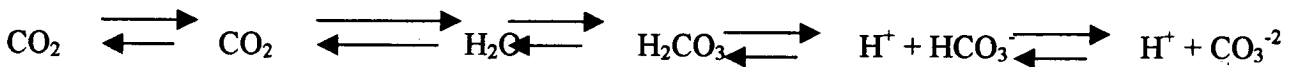
**Reagents**

1. HCl or H<sub>2</sub>SO<sub>4</sub>
2. Methyl orange indicator
3. NaOH solution

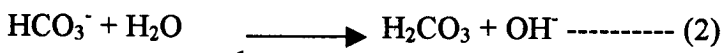
**Principle**

Alkalinity of the water refers to the quantity and kinds of dissolved substances present in it. The property of alkalinity in water is caused primarily by the presence of bicarbonates, carbonates and hydroxy ions and less frequently by borates, silicates and phosphates. The interrelationship of CO<sub>2</sub> and other major components of alkalinity may be described as follows.

Free CO<sub>2</sub> in the air is often in equilibrium with dissolved CO<sub>2</sub> if the water and may be reported as

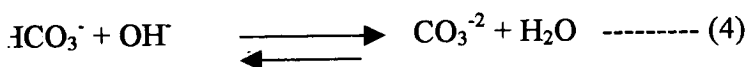


After equilibrium is established in the water the resulting conditions and exemplified by the following reactions



The hydroxyl ion formed in equation number (1) and (2) show why waters with high carbonate content are alkaline. Acid H<sup>+</sup> ions of neutrality i.e., where equal quantities of H<sup>+</sup> ions and OH<sup>-</sup> ions are present.

The equilibrium shown by the above equation explains the buffering capacity of the alkaline water i.e the water tends to resist change in pH as long as this equilibrium is in existence. The addition of H<sup>+</sup> ions removes the OH<sup>-</sup> ions from the sample but more OH<sup>-</sup> ions, are immediately formed by the reaction of the carbonate with water as long as excess carbonates present. Therefore pH remains practically the same as before the addition of until all available supply of carbonates and bicarbonates ions are exhausted similarly when OH<sup>-</sup> ions are added they react with bicarbonate ions.



Alkalinity is thus the equivalent titrable base in equilibrium with carbonates or bicarbonates and is determined by titration with a standard solution of a strong acid (Eg = 0.02N H<sub>2</sub>SO<sub>4</sub>). To certain equivalence point as given by indicator solution the indicator phenolphthalein commonly used for measurement of that position of alkalinity contributed by hydroxyl and carbonate ion while indicator methyl orange or mixed indicator responding in the pH range below 5 is used to measure alkalinity contributed by carbonate without complete analysis of the sample one cannot know exactly whether the alkalinity exists primarily as hydroxyl bicarbonate or carbonate to facilitate calculation of all alkalinities grouped together and calculated in terms of CaCO<sub>3</sub> and is termed as total alkalinity in terms of CaCO<sub>3</sub>.

### Procedure

In the next step, take 50ml of the sample water in a clear conical flask and add 2 drops of Phenolphthalein indicator, a pink colour develops. Since Phenolphthalein gives colour above pH 8.2, it indicates that the sample contains OH<sup>-</sup> ion or CO<sub>3</sub><sup>2-</sup> ion or both. Then the sample is to be titrated against HCl of known normality. On addition of acid, the colour of the sample will disappear making CO<sub>3</sub><sup>2-</sup> ions to HCO<sub>3</sub><sup>-</sup> ions.

Then to this solution add 2 drops of methyl orange mixed indicator, the sample gives pale yellow colour indicating the presence of bicarbonates, then, it is titrated against HCl until the pale yellow colour changes to light orange. This indicates the conversion of HCO<sub>3</sub><sup>-</sup> ions to carbonic acid H<sub>2</sub>CO<sub>3</sub>. The volume of the acid rundown is noted and the total alkalinity of the sample is calculated as follows.

Phenolphthalein alkalinity in terms of CaCO<sub>3</sub>

$$\frac{\text{Volume of acid rundown} \times \text{Normality of acid} \times 1000 \times 50}{\text{Volume of sample taken}}$$

Methyl orange alkalinity in terms of CaCO<sub>3</sub>



Volume of acid rundown x Normality of acid x 1000 x 50

—————→  
Volume of sample taken

Total alkalinity of the sample in terms of  $\text{CaCO}_3$   
Phenolphthalein alkalinity + Methyl orange alkalinity

### Result

The total alkalinity of the sample is obtained in mg/litre.

**PRACTICAL - 4.1.4****ESTIMATION OF TOTAL HARDNESS****Aim**

To estimate the total hardness of the given water sample.

**Apparatus**

Burette, Burette stand, Conical flask, Porcelain tile, Measuring jar etc.

**Reagents**

1. 0.01N EDTA
2. Ammonia buffer
3. Eriochrome black T indicator

**Preparation of Reagents****1. 0.01N EDTA**

Dissolve 18.6 gms of ethylene diamine tetra acetic acid in 1000ml of distilled water.

**2. Ammonia buffer**

( $\text{NH}_4\text{OH} + \text{NH}_4\text{Cl}$  mixture) To 142 ml of concentrated ammonia 17.5 gms of ammonium chloride is added and make it upto 250ml with distilled water.

**3. Eriochrome black T indicator**

Dissolve 100mg of Eriochrome black T indicator in 100 ml of methanol or ethanol.

**Principle**

The hardness of water is a measure of the capacity of the water to precipitate soap. Dissolved ions in the water combines with soap to form insoluble precipitates and delay the formation of suds until this combination is complete. Precipitated materials from such hard waters also form scale and other incrustations in vessels in which they are heated.

The substances that cause this effect are primarily the salts of calcium and magnesium polyvalent metals, such as aluminum, iron, strontium, manganese, and zinc, also contribute to hardness, but their concentrations are usually so low in natural waters that their contribution are

insignificant in comparison to those of calcium and magnesium. Usually the bicarbonate ions of calcium and magnesium predominate and, on evaporation, precipitate as carbonates.

The salts that cause water hardness are usually expressed together in terms of equivalent calcium carbonate concentrations. Hardness is sometimes classified as temporary, or carbonates hardness (calcium and magnesium carbonates which are largely decomposed when heat drives off  $\text{CO}_2$ ) and permanent or non-carbonate hardness. Carbonate hardness can be removed by boiling, which causes precipitation of carbonates. The function of calcium and magnesium remaining a solution as Sulfates, Chlorides and Nitrates after boiling constitutes the residual non carbonate hardness.

The total hardness is measured by titration against  $\text{Na}_2\text{EDTA}$  which has the capacity to bind with Ca and Mg ions. This binding is very specific with reference to pH. A buffer is added to bring the pH to the required strength (pH = 8-10). The erichrome black T indicator forms a soluble wine red complexes with  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions. These complexes are less stable than the complexes of some metals with EDTA.

When the indicator is added to the water to be analysed, it forms complexes with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions and the colour of the solution becomes wine red. When the water is titrated with EDTA these complexes are decomposed as the result of removal of  $\text{Ca}^{2++}$  and  $\text{Mg}^{2++}$  ions, which form more stable complexes with EDTA and the indicator anions goes into solution. At the equivalence point the wine red colour of the solution changes to blue owing to accumulation of indicator anions.

### Procedure

Take 20ml of the sample water in a conical flask and add 1ml of ammonium buffer solution in order to raise the pH to 10. Add 3-4 drops of erichrome black T indicator to the above solution. The solution turns to wine red in colour. Then titrate the solution against 0.01N EDTA solution until the colour changes from wine red to blue in colour. The change in colour indicates the end point of the titration. The volume of EDTA used is to be noted. Repeat the experiment until concurrent values were obtained. The total hardness is usually expressed in parts per million (ppm) or mg/litre. The total hardness of water is calculated using the formula.

$$\text{Total Hardness} = \frac{\text{Volume of EDTA used} \times \text{Conc. of EDTA} \times 1000}{\text{Volume of the sample}}$$

### Result

The total Hardness of the sample water is obtained in mg/lit or ppm.

## PRACTICAL – 4.1.5

# ESTIMATION OF CHLORIDES

### Aim

To estimate the amount of chlorides present in the given sample of water.

### Apparatus

Burette, Burette stand, Pipette, Conical flask, Measuring jar, Porcelain tile etc.

### Reagents

1.  $\text{AgNO}_3$  silver nitrate
2.  $\text{K}_2\text{CrO}_4$  potassium chromate

### Preparation of Reagents

#### 1. $\text{AgNO}_3$ (0.0141 N)

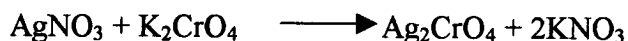
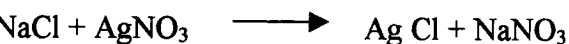
Dissolve 2.72 gms of  $\text{AgNO}_3$  in 1000 ml of distilled water.

#### 2. $\text{K}_2\text{CrO}_4$ (50%)

Dissolve 50 gms of potassium chromate in 100ml of distilled water.

### Principle

Chlorides are present in the water as  $\text{NaCl}$ ,  $\text{MgCl}$ ,  $\text{KCl}$  and other metallic chlorides when the silver nitrate is added to the sample the replacement reaction takes place by forming  $\text{AgCl}$ .  $\text{K}_2\text{CrO}_4$  forms a yellow colour with  $\text{AgNO}_3$  and forms reddish brown colour with silver chloride. This reddish brown colour indicates the end point and  $\text{K}_2\text{CrO}_4$  acts as indicator.



### Procedure

Take 50ml of sample water in a clear conical flask and add few drops of potassium chromate which is an indicator, a pale yellow colour is formed in the sample. Now titrate the solution against  $\text{AgNO}_3$  solution. As long as the free chlorides are present no precipitate is seen in the sample. After the complete precipitation of the chlorides, a reddish brown precipitate is seen in the sample, this is to be noted as end point and repeat the experiment twice or thrice to obtain concurrent values and calculation are made as shown below;

$$\text{Chloride salinity} = \frac{\text{AgNO}_3 \text{ rundown} \times \text{Normality of AgNO}_3 \times 1000}{\text{Volume of sample taken}}$$

### Result

Salinity of sample water is obtained in mg/lit.

# PRACTICAL -4.1.6

## ESTIMATION OF CHEMICAL OXYGEN DEMAND

### Aim

To estimate the chemical oxygen demand in the given sample of water.

### Apparatus

Burette, Burette stand, Pipette, Conical flask, Beakers, measuring jar and porcelain tile etc

### Reagents

1.  $\text{H}_2\text{SO}_4$  (1+1)
2. Starch solution 1%
3. Potassium iodide solution 10%
4. Hypo (0.005 N)
5. Potassium permanganate solution

### Preparation of reagents

1.  $\text{H}_2\text{SO}_4$  (1+1) = 100 ml of distilled water is added to 100ml of  $\text{H}_2\text{SO}_4$ .

2. Starch solution 1%

Dissolve 4 gms of starch in 100ml of distilled water and boil it upto  $80^\circ\text{C}$ .

3. Potassium iodide solution (KI) 10%

Dissolve 100 gms of KI in 100ml of distilled water.

4. Hypo (0.005 N)

Dissolve 6.2 gms of hypo in 100ml distilled water.

5. Potassium permanganate solution  $\text{KMnO}_4$

Dissolve 1.975 gm of  $\text{KmnO}_4$  is 100ml of distilled water.

## Principle

Estimation of  $\text{CO}_2$  only in the sample cannot speak about the actual productivity of water. In the process of chemical oxygen demand the inorganic materials such as  $\text{Fe}$ ,  $\text{Mn}$ ,  $\text{NO}_3^-$  will get oxidized by utilizing dissolved oxygen in the sample water which takes about 4 hours or more. When  $\text{KI}$  is added to the sample Iodine is liberated in equal amount of  $\text{O}_2$ . In nutrient rich water when  $\text{KI}$  is added the colour appears pale when compared with black.

## Procedure

Take 100ml of sample water in the conical flask and add 10ml of  $\text{N}/80 \text{ KmnO}_4$  solution and 10ml of (1+1)  $\text{H}_2\text{SO}_4$ . In the same way 100ml of distilled water is taken and the reagents are added as above which acts as a blank. Both the sample and the blank are kept in dark chamber for about four hours. Then 50ml from sample and blank are taken separately and titrated against hypo till pink colour disappears and then add 1 or 2 drops of starch and titrate till blue colour disappears.

COD is calculated using the formula

$$\frac{(a-b) \times \text{Normality of Hypo} \times 8 \times 1000}{\text{Volume of the sample}}$$

Where  $a$  = Volume of hypo rundown for distilled water

$B$  = Volume of hypo rundown for sample water

## Result

C.O.D for sample water is obtained in  $\text{mg/hr}$

## DISTANCE MODE PRACTICAL MONUAL

### PRACTICAL – 4.2

4.2.1 Spectrophotometer/Colorimeter: Principles & Instrument Working

4.2.2 Estimation of Phosphates

4.2.3 Estimation of Nitrites

*Dr.K.VEERAAIAH*

### PRACTICAL – 4.2.1

## SPECTROPHOTOMETER / COLORIMETER

### Principles and Instrument Working

Colorimetry is based upon the matching of a colored solution representing an unknown concentration of the substance undergoing analysis with a standard solution of specific color representing the substance in known concentration.

Determinations involving quantitative estimations of color are known as colorimetric analyses. The proportion of the various wavelengths of light absorbed is directly related to the concentration of light absorbing material. The intensity of the remaining transmitted colour is also a measure of the concentration of the solution. Analytical procedures based upon the direct measurement of light absorption at specific wavelengths or regions of the spectrum are known as photometric, procedures, and the instruments used are photometers or spectrophotometers. A colorimetric procedure is used in which a colored solution representing the substance in unknown concentration is brought to an exact match with a standard color representing the substance in known concentration. While a photometric procedure is one based upon the direct measurement of color intensity in terms of the light absorbing power of the solution at a specific wavelength of the spectrum. Unlike colorimetric procedures, which are limited to the visible portion of the spectrum, the general principles of photometric procedures are as applicable to the absorption of radiant energy in the ultraviolet and infrared portion of the spectrum as they are to the absorption of light in the visible region. For uniform absorbing medium, the proportion of the radiation passing through it is called the transmittance. The extent of radiation absorption is referred to as absorption (A). This is originally called optical density (O.D). Transmittance is usually expressed on a range of 0 to 100% and absorption has no units and varies from 0 to infinity.

### Beer-Lambert's law

This law states that the extinction is proportional to concentration of the absorbing substance and to the thickness of the layer.

$$E = \Sigma \lambda cd$$

Where  $\Sigma \lambda$  = Molar extinction coefficient for the absorbing material at wavelength.

$\lambda$  (is units of  $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ )

$c$  = concentration of the absorbing solution (molar)

$d$  = light note is the absorbing material (cm)

Since the molar extinction coefficient  $\Sigma \lambda$  of a compound may be very large, usually the extinction given by 1 cm thick of a 1% solution of the compound is taken  $-E_1$  %. However, under certain circumstances, the Beer Lambert's Law may not be applicable. For example, the substance may ionize or polymerize at higher concentration, or it may give a turbid solution, which might increase or decrease the apparent extinction.

### Working

The spectrophotometer or colorimeter is used to measure absorbance. The instrument produces light of selected wavelength, directs it through a sample and measures the intensity of light transmitted by the sample. The instrument consists of a light source, a monochromator, a sample chamber, detector and meter or recorder.

### Light source

For absorption measurements in the UV region, a high pressure hydrogen or deuterium lamp is used. These lamps produce radiation in the 200 to 320 nm range. In the visible region a tungsten lamp is used with a wavelength range of 320 to 800 nm.

### Monochromator

Both lamps mentioned above produce a continuous commissions of all wavelengths within their range. Thus a spectrophotometer must have an optical system to select monochromatic light. This is done using a prism or a grating. Prisms made of glass are used for visible region and quartz for UV region. The light emerging from the monochromator does not consist of a single wavelength but a group of wavelengths called spectral slit width, band width or wave band. Diffracting grating monochromator consist of series of ruled lines on a transparent base. Diffraction of white light gives rise to a series of overlapping spectra. Usually in a monochromator a fore prism preselects a portion of the spectrum of a light source which is then diffracted to obtain monochromatic light. The major advantage of diffraction grating monochromator is that their resolving power is directly proportional to the closeness of the lines and hence they are superior to prisms. Before the monochromatic light



impinges on the sample, it passes through a series of slits, lenses, filter and mirrors. This optical system concentrates the light increases the spectral quality and focuses it on the sample. The light passes through a slit before reaching the sample this slit width can be adjusted. It determines both the intensity of light reaching the sample and the spectral purity of the light decreasing the slit width increases spectral purity.

#### **Fig 4.2.1.2**

#### **Sample chamber**

The sample is placed in a tube of cuvet of glass, quartz and other transparent material. Glass tubes are for visible spectrum and quartz cuvetts are for U.V light.

#### **Detector**

The intensity of light that passes through the sample depends upon the amount of light absorbed by the sample. There is a photo multiplier tube which detects the small amount of light energy and amplifies this by a cascade of electrons accelerated by dynodes and converts it into an electrical signal which is then read by a measuring instrument.

#### **Applications**

##### **Qualitative analysis**

Visible and UV spectra may be used to identify class of compounds in both pure state and biological preparations e.g., proteins, nucleic acids, cytochromes, chlorophylls.

##### **Quantitative analysis**

Many biological compounds can be measured using UV and visible spectrophotometers eg. Proteins at 280nm and nucleic acids at 260 nm.

## PRACTICAL – 4.2.2

# ESTIMATION OF ORTHOPHOSPHATES

### Aim

To estimate the amount of phosphates ( $P_{04}$ ) present in given water sample by calorimetric method or spectrophotometric method.

### Apparatus

Burette, Funnel Burette stand, Pipette, Conical flask, Porcelain tile measuring jar etc.

### Reagents

- A.  $KH_2PO_4$  standard solution,  $40\mu\text{g/ml}$  of  $PO_4\text{-P}$
- B.  $H_2SO_4$ , 4N
- C. Molybdate- antimony solution
- D. Ascorbic acid (about 0.1M)

### Preparation of Reagents

#### A. $KH_2PO_4$ standard solution

Dissolve 0.1757 gm of  $KH_2PO_4$  potassium dihydrogen ortho phosphate is dissolved in 1 litre of distilled water. This solution contains  $40\mu\text{g p}$  per ml. 25ml this solution is diluted with 975ml of distilled water to make the concentration to  $1\mu\text{g/ml}$ .

#### B. $H_2SO_4$ 4N

55ml of  $H_2SO_4$  is dissolved in 445 ml of distilled water.

#### C. Molybdate – antimony solution

Dissolve 4.8 gm of ammonium molybdate and 0.1g sodium antimony tartrate in 400ml of 4N  $H_2SO_4$ .

#### D. Ascorbic acid

Dissolve 2.0 gm of ascorbic acid in 100 ml of distilled water.

### Principle

In strongly acid solutions ortho-phosphate will form a yellow complex with molybdate ions, which can then be reduced to a highly coloured blue complex. If ascorbic acid is used as a reducing agent, the formation of the blue colour is stimulated by antimony which is directly proportional to the concentration of phosphate present in the sample.

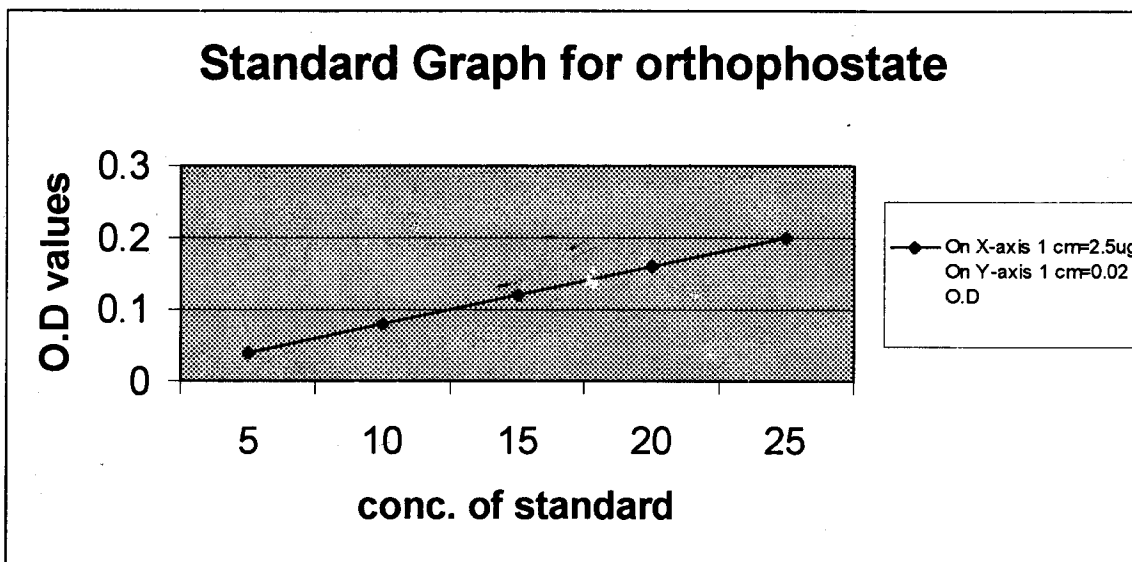
### Procedure

Take six beakers of 100ml capacity each and the standard solution is taken in increasing concentrations such as 2.5ml, 5ml, 7.5ml, 10ml, 12.5ml is the five of the above beakers. Make the solution in each of the beakers upto 25ml by adding distilled water. In the sixth beaker take 25ml of distilled water which acts as a blank. To the above six beakers add 5ml of molybdate antimony solution and 2ml of ascorbic acid. Now the solution in all the beakers are made upto 40ml by adding 8ml of distilled water. The solution turns to blue colour in 10 minutes. The intensity of the colour is measured by a spectrophotometer and optical density values are noted at wavelength of 680 nm. A calibration graph is to be plotted taking O.D values on 'y'-axis and concentrations of solutions on the 'x'-axis. A straight line will be obtained on the graph. This graph is called standard graph for the orthophosphates. (Figure 4.2.2.1)

Now take 50ml of the sample water in the beaker. Add 5ml of molybdate antimony solution and 2ml of ascorbic acid to the above sample. Wait for 10 minutes till the solution in the beaker turns to blue colour. Then the optical density (O.D) values of sample water is observed in the spectrophotometer. Mark the O.D values on the standard graph already prepared above and observe the concentration of the phosphates present in the sample by marking on the standard graph.

### Result

The concentration of phosphates present in the sample water is obtained in  $\mu\text{g}/50\text{ml}$ . Fig. 4.2.2.1



**PRACTICAL – 4.2.3****ESTIMATION OF NITRITES****Aim**

To estimate the amount of nitrites ( $\text{NO}_2$ ) present in the given sample.

**Apparatus**

Burette, Burette stand, Pipette, Conical flask, Porcelain tile, Measuring jar etc.

**Reagents**

1. Potassium Nitrite  $\text{KNO}_2$  (0.1 N)
2. 6N HCl
3. Sulphonamide
4. Ammonium sulphomate
5. N-1 Napthalene ethylene diamine dil HCl.
- 6.

**Preparation of reagents****1.  $\text{KNO}_2$  standard (0.1 N)**

Dissolve 1.064 gms of  $\text{KNO}_2$  in little distilled water and add 1ml of 5 N NaOH and make it upto 250ml with distilled water this solution contain 700 mg  $\text{NO}_2/\text{l}$ .

**2. Working standard**

Dilute the standard 200 times by taking 5 ml of standard and dilute it to 1000ml of distilled water. This solution contains  $3.50\mu\text{g NO}_2/\text{ml}$ .

**3. 6N HCl (50%)**

50 ml of distilled water is added to 50ml of HCl.

**4. Sulphonilamide (0.2%)**

Dissolve 2gms of sulphanlamide in 1000ml of distilled water.

**5. N-1 Napthalane ethylene diamine di HCl (0.1%)**

Dissolve 0.17 gm of N-1 napthalane ethylene diamine di HCl in 100ml of distilled water.

**6. Ammonium sulphamate**

Dissolve 5gms of  $\text{NH}_4\text{NH}_2\text{SO}_3$  in 100ml of distilled water and store it at room temperature.

**Principle**

In a strongly acid medium  $\text{KNO}_2$  reacts with sulphanilamide to form a diazonium compound. The diazonium compound reacts with N-1 naphthyl ethylene diamine di HCl to form a strongly coloured azo-compound. If any  $\text{NO}_2^-$  is left at this stage it will destroy the azo-compound, so that almost colour will develop and sample will appear to contain almost no  $\text{NO}_2^-$ . Possible excess of  $\text{NO}_2^-$  is therefore destroyed by adding ammonium sulphamate first before azo-compound is formed.

**Procedure**

Take six clean beakers of 100ml capacity each and take the working standard in the increasing concentrations such as 3ml, 6ml, 9ml, 12ml and 15ml in the first five beakers. In the last sixth beaker take 25ml of distilled water which is taken as blank. Make up the different concentration in the five beakers to 25ml with distilled water.

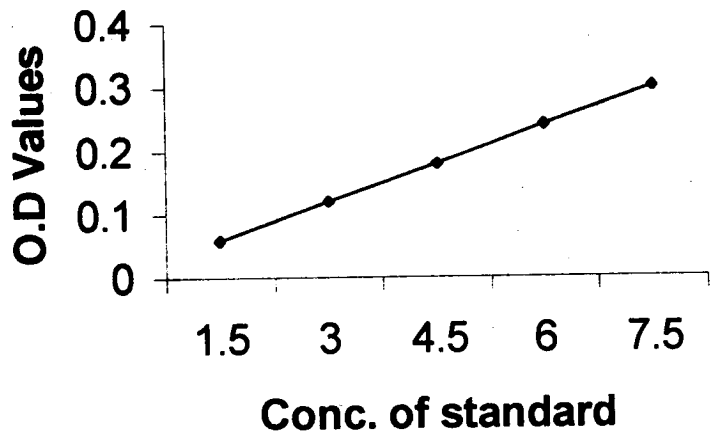
To each beaker of the above add 25ml of sulphanilamide and 1ml of 6N HCl. After 3 minutes add 0.5ml of N-1 naphthyl ethylene diamine di HCl and make up the solution in each beaker to 50ml with distilled water. After 15-30 minutes the solutions in the beaker will develop pink colour. The intensity of the colour is measured in spectrophotometer at 540nm and optical density of the each solution in the each beaker is plotted on the graph taking concentrations on x axis and optical density O.D. values on Y-axis, the graph thus attained is known as standard graph. (Figure 4.2.3.1)

Now take 50ml of the sample water in the beaker and add 2.5 ml of sulphanilamide and 1ml of 6N HCl. After 3 minutes add 0.5 ml of N-1 naphthyl ethylene diamine di HCl, again after 3 minutes add 0.5 ml of ammonium sulphamate. After 15-30 minutes the solution in the beaker will develop a colour. The optical density values of the sample water is noted and compared with standard graph drawn already with the known concentrations, thus the concentration of nitrites present in the sample will be obtained depending on the optical density values.

**Result**

The amount of nitrites present in the given sample is obtained in  $\mu\text{g}/50\text{ml}$ .

### Standard graph for Nitrites



—●— on X axis 1 cm =  
1.5ug, on y axis 1  
cm = 0.03 O.D  
value

### 4.3. Identification and mounting of common zooplanktonic organisms in the ponds –Rotifers, copepods and cladocerans

Dr. P. PADMAVATHI

#### 4.3.1 ROTIFERS

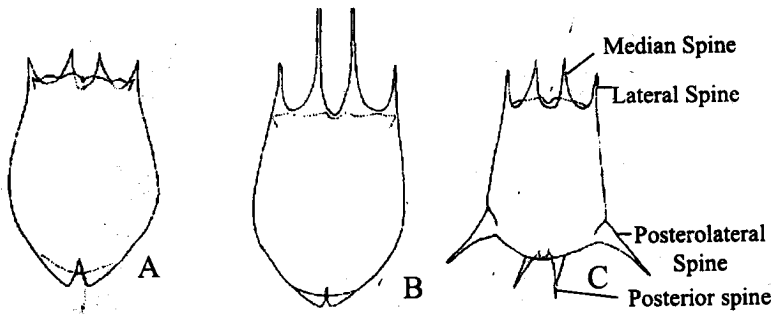
1. *Brachionus calyciflorus*
2. *Brachionus falcatus*
3. *Brachionus forficula*
4. *Brachionus caudatus*
5. *Brachionus angularis*
6. *Keratella tropica*
7. *Filinia longiseta*
8. *Asplanchna* sp.
9. *Hexarthra* sp.
10. *Polyarthra* sp.

#### 4.3.2. CLADOCERANS

1. *Daphnia* sp.
2. *Ceriodaphnia* sp.
3. *Moina micrura*
4. *Diaphanosoma* sp.

#### 4.3.3. COPEPODS

1. *Heliodiaptomus viduus*
2. *Paradiaptomus greeni*
3. *Allodiaptomus raoi*
4. *Mesocyclops* sp.
5. *Thermocyclops* sp.

***Brachionus calyciflorus***

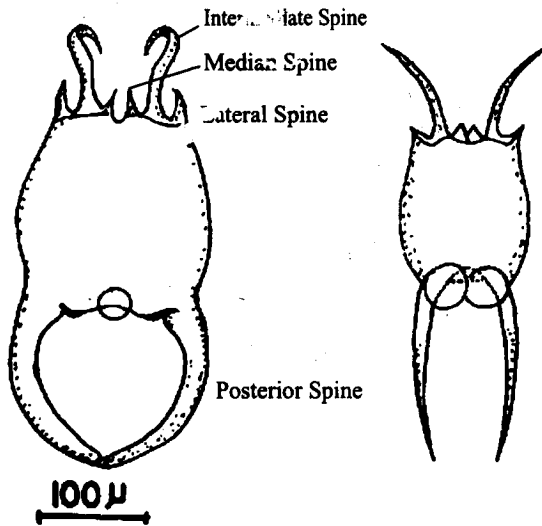
Phylum : Rotifera  
 Class : Monogononta  
 Order : Ploima  
 Family : Brachionidae  
 Genus : *Brachionus*  
 Species : *calyciflorus*

Morphologic variation A) Ventral View B,C) Dorsal view

1. Lorica is very slightly compressed dorsoventrally and is not separated into dorsal and ventral plates.
2. It is somewhat oval in shape.
3. Anterior dorsal margin with four broad based spines of variable length.
4. The medians are longer than laterals.
5. The ventral margin is somewhat elevated with a shallow 'V' or 'U' shaped notch.
6. Posterior spines may be present or absent. Lateral posterior spines are commonly absent. Foot opening is flanked with a pair of spines but the degree of development of these spines may vary.
7. This is an exceedingly variable species in size, length of dorsal spines and in the presence and length of posterior spines.
8. These are the secondary producers in ecological food pyramid and form the natural food for plankton feeding fishes.

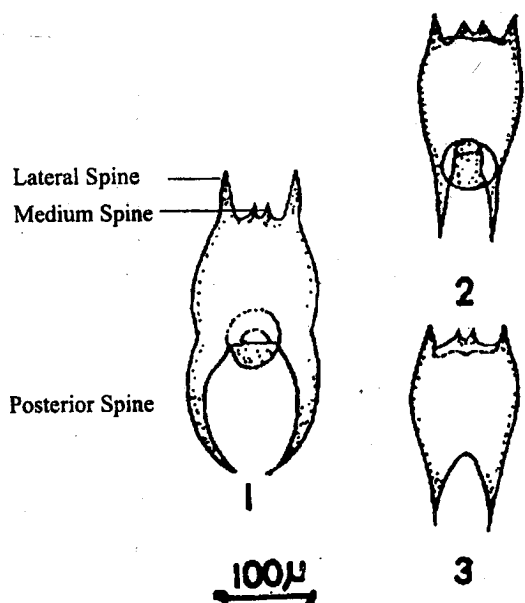


### *Brachionus falcatus*



Phylum : Rotifera  
Class : Monogononta  
Order : Ploima  
Family : Brachionidae  
Genus : *Brachionus*  
Species : *falcatus*

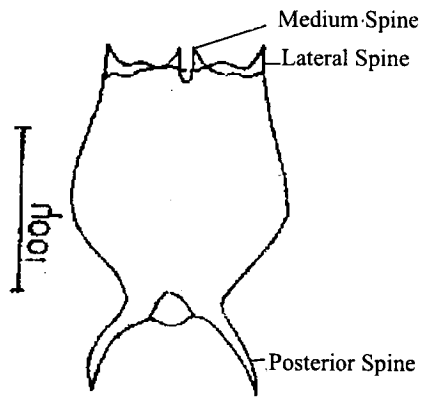
1. Lorica moderately compressed dorsoventrally.
2. Anterior dorsal margin with 6 spines. Intermediate spines are larger which curve ventrally. The lateral and median spines have lateral sinusoids with a slight median sinus.
3. Posterior spines are two, long and widely separated at their bases. They are usually bent inwards converging towards their free ends.
4. These are the secondary producers in the ecological food pyramid and form natural food for plankton feeding fishes.

*Brachionus forficula*

Phylum : Rotifera  
 Class : Monogononta  
 Order : Ploima  
 Family : Brachionidae  
 Genus : *Brachionus*  
 Species : *forficula*

1. Dorsal view, 2. Ventral view; 3. Ventral view, Female without eggs.

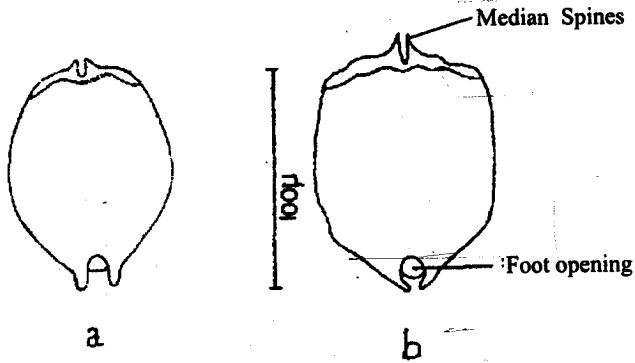
1. Lorica moderately compressed dorsoventrally.
2. Dorsal anterior margin with four spines. The laterals are longer than the medians. Intermediates absent.
3. The spines may be pointed or rounded at the tips.
4. The ventral margin elevated and undulate with a shallow unflanked median sinus.
5. Posterior spines are two, stout, usually long, widely separated at their bases and taper into blunt points. On the inner aspect of these posterior spines, near their bases are knee-like swellings.
6. These are the secondary producers in the ecological food pyramid and form the natural food for plankton feeding fishes.

***Brachionus caudatus***

Phylum : Rotifera  
Class : Monogononta  
Order : Ploima  
Family : Brachionidae  
Genus : *Brachionus*  
Species : *caudatus*

1. Lorica moderately compressed dorsoventrally.
2. The two median spines in the anterior dorsal margin separated by a 'U' – shaped sinus.
3. Lateral spines developed.
4. The ventral margin is slightly elevated or undulate with a shallow median sinus.
5. The posterior margin has two spines separated at the bases about half of the width of lorica. These spines are usually divergent and strongly bent ventrally.
6. This species is very variable in the degree of development of anterior spines and also in the mode of origin of posterior spines.
7. These are the secondary producers in the ecological food pyramid and form the natural food for plankton feeding fishes.

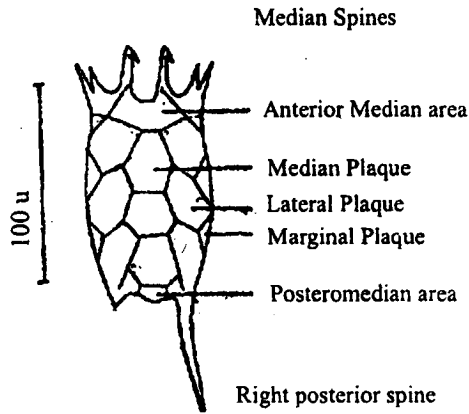
### *Brachionus angularis*



Phylum : Rotifera  
 Class : Monogononta  
 Order : Ploima  
 Family : Brachionidae  
 Genus : *Brachionus*  
 Species : *angularis*

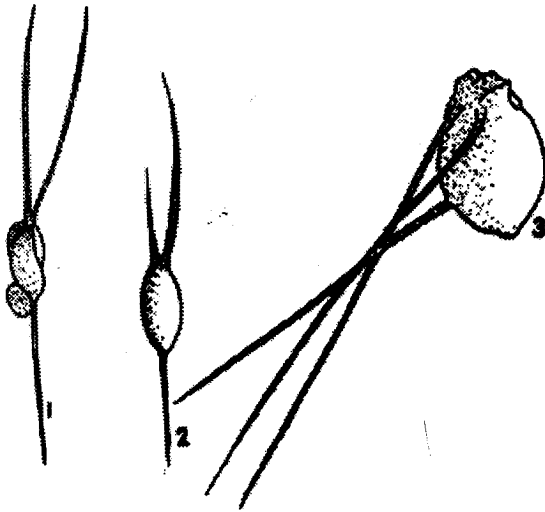
a) *Brachionus angularis angularis*. b) *B. angularis bidens*

1. Lorica moderately compressed dorsoventrally, anterior dorsal margin with two median spines divided by a 'U' shaped sinus.
2. The lateral and intermediary spines are usually absent or sometimes weakly developed.
3. Intermediates are more commonly developed than laterals.
4. The ventral margin is somewhat elevated with a shallow median sinus.
5. The foot opening is flanked laterally by cuticular protruberances.
6. Posterior spines are absent.
7. The dorsal plate has a pattern of cuticular ridges.
8. These are the secondary producers in the ecological food pyramid and form natural food for plankton feeding fishes.

*Keratella tropica*

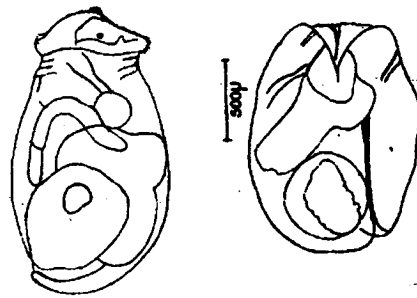
Phylum : Rotifera  
 Class : Monogononta  
 Order : Ploima  
 Family : Brachionidae  
 Genus : *Keratella*  
 Species : *tropica*

1. Body compressed dorsoventrally.
2. Lorica narrows markedly at the base of the posterior spines so that the width at the base of anterior spines is more than at the posterior margin.
3. Anterior dorsal margin with 6 spines. Medians are stout, long and curved ventral ward. Laterals usually little longer than intermediates.
4. Lorica with 2 unequal posterior spines. Right one is always longer than the left one, sometimes the left spine is totally absent.
5. The foundation pattern of dorsum consists of three median hexagonal plaques. Small four sided plaque is present between last hexagonal plaque and posterior border of lorica. There are three pairs of lateral polygons and 3 pairs of marginals.
6. Lorica pustulated
7. These are secondary producers in the ecological food pyramid and form natural food for plankton feeding fishes.

***Filinia longiseta***

Phylum : Rotifera  
Class : Monogononta  
Order : Ploima  
Family : Testudinellidae  
Genus : *Filinia*  
Species : *longiseta*

1. Lorica somewhat flexible.
2. Body long, fairly cylindrical with two movable anterior lateral bristles and one posterior bristle arising from ventral surface.
3. Lateral bristles are twice as long as body and posterior bristle.
4. Two eyes are present on the head.
5. Foot is absent.
6. These are the secondary producers in the ecological food pyramid and form natural food for plankton feeding fishes.

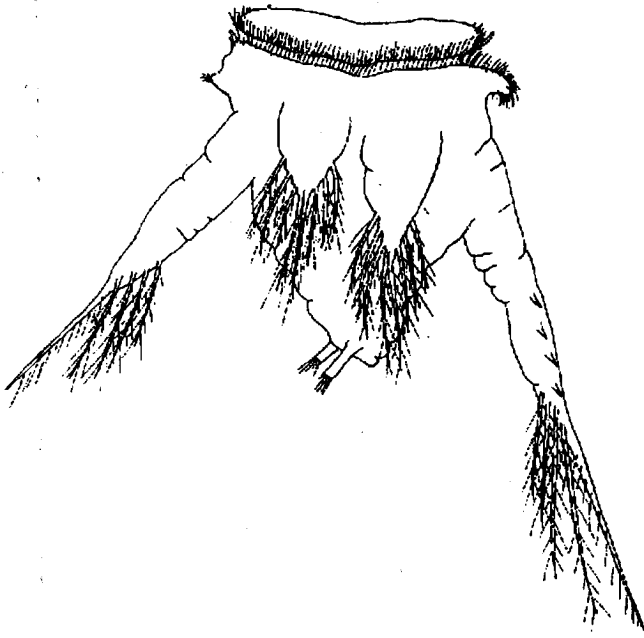
***Asplanchna* sp.**

Expanded form

Contracted form

Phylum : Rotifera  
Class : Monogononta  
Order : Ploima  
Family : Asplanchnidae  
Genus : *Asplanchna*

1. Body transparent, sac-shaped, greatly contractile and without distinct lorica.
2. Spines or appendages are absent
3. Corna reduced to a thin course of cilia around the head.
4. Body cavity large with small stomach and no intestine.
5. Foot is absent
6. Ovary horse-shoe shaped. Often viviparous, with one or several embryos.
7. It is highly predaceous on other small planktonic organisms
8. It forms natural food for plankton feeding fish.

***Hexarthra* sp.**

Phylum : Rotifera

Class : Monogononta

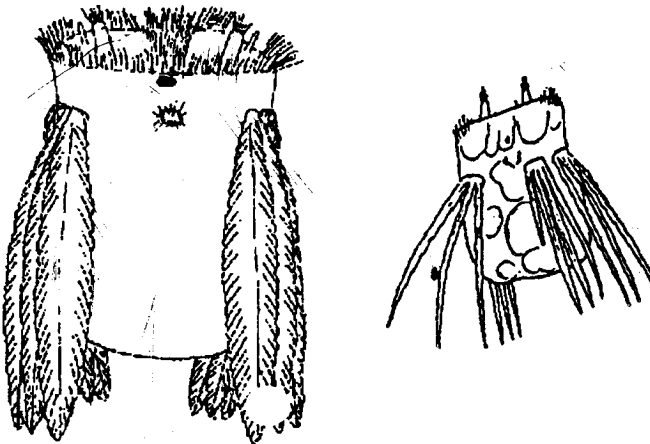
Order : Ploima

Family : Synchaetidae

Genus : *Hexarthra*

1. Body oval, slightly compressed dorsoventrally
2. Body bears six-arm-like appendages. Appendages are hollow out growths of body wall, containing striated muscles, and bearing setae.
3. Foot or attachment discs are absent.
4. It forms natural food for plankton feeding fish.

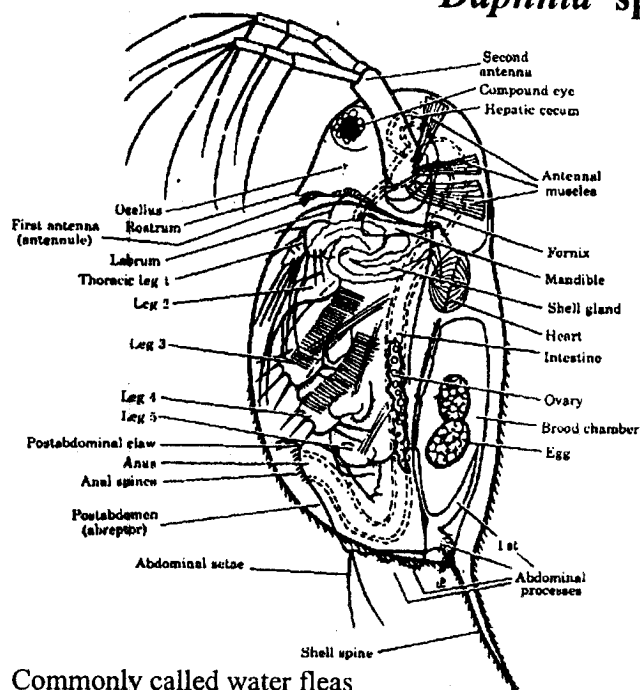


*Polyarthra* sp.

Phylum : Rotifera  
Class : Monogononta  
Order : Ploima  
Family : Synchaetidae  
Genus : *Polyarthra*

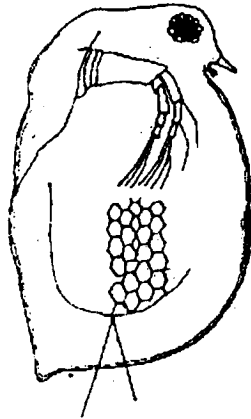
1. Body flattened dorso-ventrally.
2. Body bears 4 groups of 3 sword shaped (also called paddles), equal sized, serrated blades. 2 groups are dorsolateral and 2 groups are ventrolateral in position, arising from large lateral muscles near anterior end.
3. Foot or attachment discs are absent.
4. It forms natural food for the plankton feeding fish.

## *Daphnia* sp.



Phylum : Arthropoda  
 Class : Crustacea  
 Sub-class : Branchiopoda  
 Order : Cladocera  
 Family : Daphnidae  
 Genus : *Daphnia*

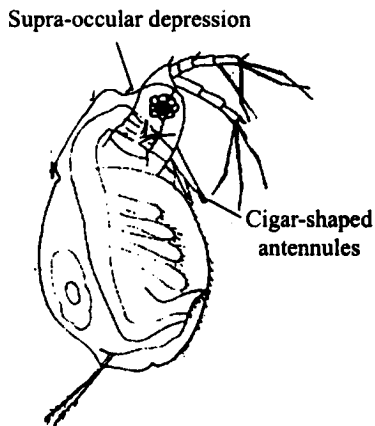
1. Commonly called water fleas
2. Large cladocerans, the females are larger (upto 2-3mm) than males (1.0mm).
3. There is a distinct head and body, covered by a bivalved carapace/shell which is composed of a single piece with no hinges dorsally.
4. The head bears 2 pairs of sensory appendages.
  - A) Antennules, which carry sense hairs, the olfactory setae.
  - B) Antennae, which are the main organs of locomotion and are large. Each consists of a stout basal segment, a segmented dorsal ramus and a segmented ventral ramus. The two ramii bear a variable number of plumose setae and the setation formula has taxonomic value in identifying genera and species. The setal formula for *Daphnia* is 0-0-1-3/1-1-3.
5. In females, rostrum is well developed and pointed. Males do not possess a rostrum and the antennules are large.
6. The fornix is well developed which terminates in a sharp spine posteriorly.
7. The body is oval with both dorsal and ventral edges gently curved and meeting at the posterior margin to form the *shell spine*. The spines along the ventral margin arise from about the middle and extend as far as the cervical region along the dorsal edge.
8. Shell surface with squarish or rhomboidal markings.
9. The body is divided into thorax and abdomen. Thorax bears five pairs of lobed, leaf like thoracic legs bearing numerous hairs and setae. In *males*, the first leg is modified into hook and bears a long flagellum.
10. Post-abdomen bears two long abdominal setae, two terminal claws, anal spines (9-10) and 3 abdominal processes of which the anterior most is the largest and serves to hold the eggs in position. It has taxonomic importance in identification.
11. *Daphnia* are likely to be found in temporary pools of clear and weedy water, in small ponds and in lakes. It forms natural food for the plankton feeding aquatic organisms.

***Ceriodaphnia* sp.**

Phylum : Arthropoda  
Class : Crustacea  
Sub-class : Branchiopoda  
Order : Cladocera  
Family : Daphnidae  
Genus : *Ceriodaphnia*

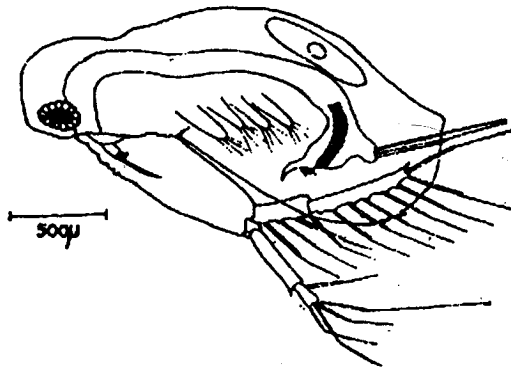
1. Very small forms, measuring 0.5 to 1.0 mm.
2. The head is small, with a conical pointed process.
3. The antennules are short in females.
4. Second antennae small, biramous, cylindrical. Setal formula – 0,0,1,3/1,1,3.
5. The valves are rounded or oval in shape with distinct hexagonal markings on the surface.
6. The valves end posteriorly in a sharp angle or as a short spine.
7. The ephippium, if present, is somewhat triangular in shape.
8. Post-abdomen is large with 5-8 anal spines. Abdominal claws moderately long, simple or slightly curved.
9. Common planktonic form in freshwater bodies and form the natural food for plankton feeding fishes.

## *Moina micrura*



Phylum : Arthropoda  
Class : Crustacea  
Sub-class : Branchiopoda  
Order : Cladocera  
Family : Moinidae  
Genus : *Moina*  
Species : *micrura*

1. Adult females measure about 1.0 mm (Males are rare and much smaller with long antennules).
2. Head and body rounded and transparent.
3. The head is without a rostrum, large and bent downwards.
4. The antennules are long, cigar-shaped and movable, arising from the ventral surface of the head.
5. Setal formula of antennae is 0,0,1,3/1,1,3.
6. There is a depression above the eye known as the supra-ocular depression.
7. The postabdomen invariably has a bident tooth and a number of ciliated or feathered spines.
8. The abdominal setae are long.
9. The ephippium has one or two sexual eggs.
10. Most common planktonic form in all freshwater habitats and forms the preferential food for plankton feeding fishes, especially the cultured Indian major carps.

***Diaphanosoma* sp.**

Phylum : Arthropoda

Class : Crustacea

Sub-class : Branchiopoda

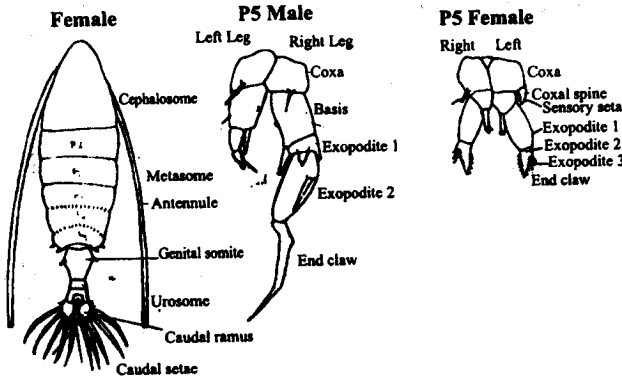
Order : Cladocera

Family : Sididae

Genus : *Diaphanosoma*

1. Medium sized, elongated forms, some what rectangular in shape.
2. The head is large with no rostrum.
3. The antennae are broad and flattened with two segments in the dorsal and three segments in the ventral ramus. Setal formula is  $\frac{4, 8-9}{0, 1, 4}$
4. The post abdomen has no anal spines but instead each claw has 3 spines.
5. Most common planktonic form in all freshwater habitats and forms the preferential food for plankton feeding fishes, especially the cultured Indian major carps.

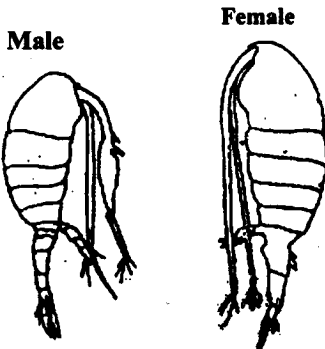
## *Heliodiaptomus viduus*



Phylum : Arthropoda  
 Class : Crustacea  
 Sub-class : Copepoda  
 Order : Calanoida  
 Family : Diaptomidae  
 Genus : *Heliodiaptomus*  
 Species : *viduus*

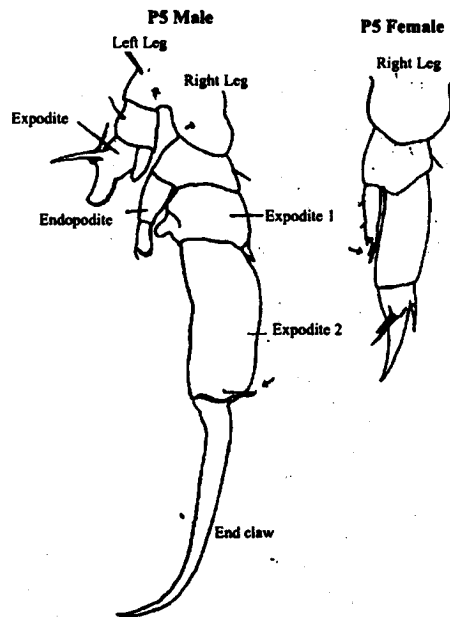
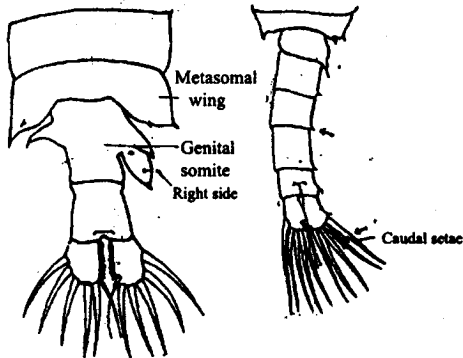
1. One of the most common diaptomids. (Average length ♀ 1.95 mm; ♂ 1.82 mm)
2. Body is elongated and cylindrical in shape.
3. a. The body is divided into anterior, wide, 6 segmented metasome (6<sup>th</sup> segment merged with 5<sup>th</sup> segment) and a posterior, narrow 5 segmented (in ♂) or 2 to 4 segmented (in ♀) urosome, separated by a distinct major articulation. This articulation is between the somite of fifth legs and the genital somite (first segment of urosome). The first body segment is referred to as the cephalic segment or cephalosome.
  - b. The last urosomal segment called anal somite, is slightly bifid posteriorly and carries a pair of plate like caudal rami. Each ramus carries posteriorly five long tapering caudal setae of equal length and a short dorsal seta with a distinct basal joint.
  - c. The metasome bears 6 oral appendages and legs 1-5 (P<sub>1-5</sub>)
  - d. Legs 1-4 are natatory (used for swimming) and the legs 5 are modified in both sexes for mating and has taxonomic value. Fifth leg is almost symmetrical in females and asymmetrical in males, the right leg is elongated.
  - e. Antennules (A<sub>1</sub>) are long, uniramous and 25 segmented. In females both the antennules are similar and in male, the right one is geniculated, also called grasping antennule.
4. Antennules (A<sub>1</sub>) extend beyond the metasome
5. In females, metasomal wings are almost symmetrical. In males, right metasomal wing with 2 hyaline spines.
6. In females, posterior border of third and fourth pedigerous segments bear a transverse row of minute spinules on the dorsal side.  
 In males, only 4<sup>th</sup> pedigerous segment has transverse row of dorsal spinules forming a "spinular girdle"
7. P<sub>5</sub> ♂: Coxal process in the form of long, curved and conspicuous spines RP<sub>5</sub>♂. Exopodite 1 bears a spinous process at distal outer corner and a roughly trapezium – like or triangular chitinous process on distal posterior aspect. Exopodite 2 bears a stout proximal lateral spine. These three spines form a triangle. The long tapering end claw has an angular base. Endopod long, narrow and cylindrical.  
 P<sub>5</sub> ♀: Left coxal spine longer than the right. Claws asymmetrical, left claw denticulated on both margins and the right on inner margin only; endopodites long and cylindrical with 2 curved spinules and hairs on tip.

## *Paradiaptomus greeni*



Phylum : Arthropoda  
 Class : Crustacea  
 Sub-class : Copepoda  
 Order : Calanoida  
 Family : Daptomidae  
 Genus : *Paradiptomus*  
 Species : *greeni*

**Female urosomee      Male urosomee**



1. One of the largest diaptomids. (Average length ♀ 2.3 mm, ♂ 2.2 mm)
2. The body is elongated and cylindrical in shape. Extremities of both ♀ and ♂ body are pinkish or violet pinkish in colour.
3.
  - a. The body is divided into anterior, wide, 6 segmented metasome (6<sup>th</sup> segment merged with 5<sup>th</sup> segment) and a posterior, narrow 5 segmented (in ♂) or 2 to 4 segmented (in ♀) urosome, separated by a distinct major articulation. This articulation is between the somite of fifth legs and the genital somite (first segment of urosome). The first body segment is referred to as the cephalic segment or cephalosome.
  - b. The last urosomal segment called anal somite, is slightly bifid posteriorly and carries a pair of plate like caudal rami. Each ramus carries posteriorly five long tapering caudal setae of equal length and a short dorsal seta with a distinct basal joint.
  - c. The metasome bears 6 oral appendages and legs 1-5 (P<sub>1-5</sub>)

d. Legs 1-4 are natatory (used for swimming) and the leg 5 are modified in both sexes for mating and has taxonomic value. Fifth leg is almost symmetrical in females and asymmetrical in males, the right leg is elongated.

e. Antennules ( $A_1$ ) are long, uniramous and 25 segmented. In females both the antennules are similar and in male, the right one is geniculated, also called grasping antennule.

4. Antennules ( $A_1$ ) from 16<sup>th</sup> – 25<sup>th</sup> segments pinkish or violet pink in colour in both sexes.  $A_1$  short extending slightly beyond the metasome.

5. In female, metasomal wings are large and lamellate; genital segment with a bilobed projection on right margin and a digit's form process on left margin.

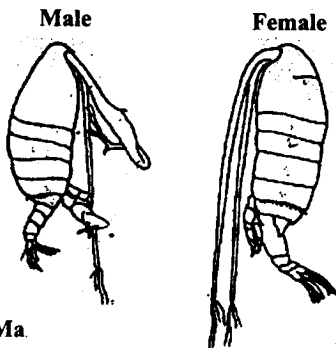
6. Male urosome 5 – segmented, each segment bears a bulbous, sensory lobe tipped with a fine hair; outer seta on the right caudal ramus bears a small tooth on its inner margin.

7.  $RP_5$  ♂: Exopodite 1 has a knob-like projection internally and a stout conical process externally. Endopodite long, 2 – segmented and clavate in shape with a spinule and hairs on its rounded tip.

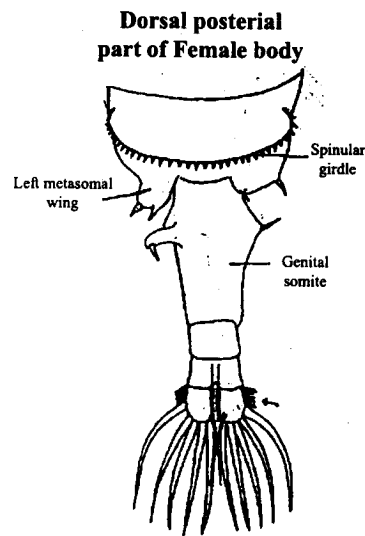
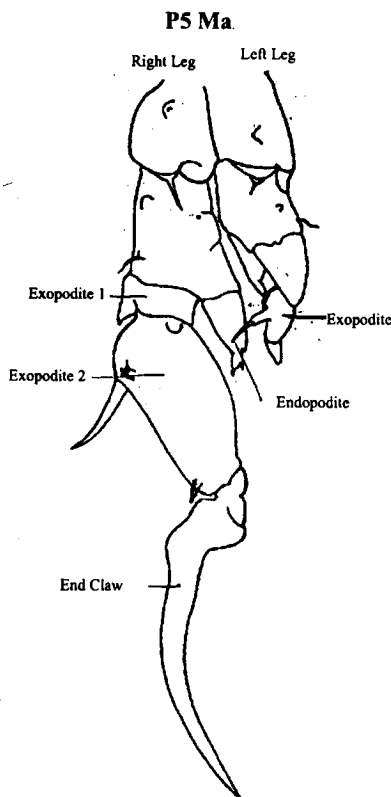
$LP_5$  ♂: Exopodite broad, fringed with fine hairs along the inner margin and the external margin bears 2 long, stout processes which cross each other.

8.  $P_5$  ♀: Endclaw (Exp.2) moderately strong and blunt at the apex, carrying along the internal margin a row of variable number of minute denticles. Exp. 3. is very small and bears two unequal spines, the outer one being relatively longer and stronger than the inner one. Endopodite 1 segmented somewhat shorter than exopodite 1 and bears at the apex two unequal spines and a short spinule. The inner margin of endopodite carries two sensory bristles, one in the proximal half and the other in the distal half.



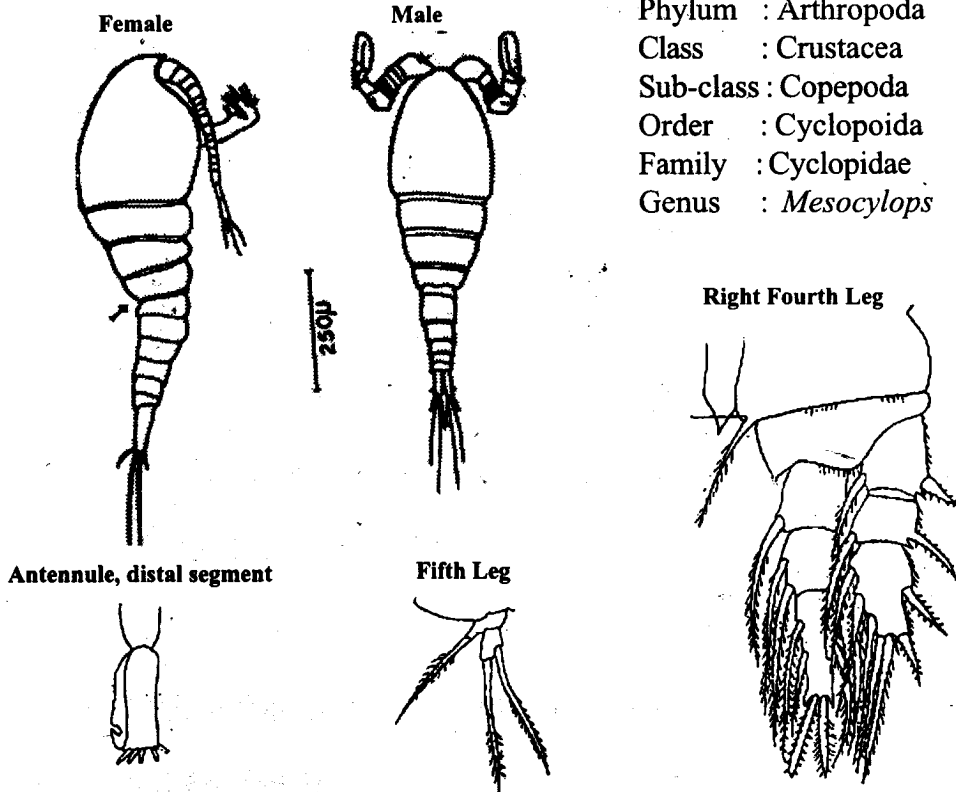
*Allodiaptomus raoi*

Phylum : Arthropoda  
 Class : Crustacea  
 Sub-class : Copepoda  
 Order : Calanoida  
 Family : Diaptomidae  
 Genus : *Allodiaptomus*  
 Species : *raoi*



1. One of the most common diaptomids. These are small and slender forms. (Average length: ♀ 1.45 mm, ♂ 1.30 mm)
2. Body elongated and cylindrical in shape.
3. a. The body is divided into anterior, wide, 6 segmented metasome (6<sup>th</sup> segment merged with 5<sup>th</sup> segment) and a posterior, narrow 5 segmented (in ♂) or 2 to 4 segmented (in ♀) urosome, separated by a distinct major articulation. This articulation is between the somite of fifth legs and the genital somite (first segment of urosome). The first body segment is referred to as the cephalic segment or cephalosome.
  - b. The last urosomal segment called anal somite, is slightly bifid posteriorly and carries a pair of plate like caudal rami, each ramus carries posteriorly five long tapering caudal setae of equal length and a short dorsal seta with a distinct basal joint.

- c. The metasome bears 6 oral appendages and legs 1-5 ( $P_{1-5}$ )
  - d. Legs 1-4 are natatory (used for swimming) and the leg 5 are modified in both sexes for mating and has taxonomic value. Fifth leg is almost symmetrical in females and asymmetrical in males, the right leg is elongated.
  - e. Antennules ( $A_1$ ) are long, uniramous and 25 segmented. In females both the antennules are similar and in male, the right one is geniculated, also called grasping antennule.
4. Antennules ( $A_1$ ) extend beyond the caudal rami.
  5. In female, metasomal wings are asymmetrical; Left metasomal wing oblong with rounded apex and distinctly longer than right wing. Left margin of the ♀ genital somite bears a digitiform process tipped with a long spine at right angles to the process, right margin with a conical projection carrying a long spine.
  6. Female and male carry a 'spinular girdle' on the posterior margin of the penultimate metasomal segment (fourth segment).
  7.  $RP_5♂$ : Second exopodite segment has one long and stout proximal and a small distal spine near the base of end claw. End claw slightly angular at the base and blunt apically. Endopodite large, conical with narrow terminal parts carrying two spinules.  
 $LP_5♂$ : Exopodite with a broad, thumb-like terminal process formed as a cap and long curved inner process with a distinct basal joint; inner process at right angles to the exopodite.
  8.  $P_5♀$ : Right coxal spine much smaller than left, Endopodite unsegmented, long (shorter than first exopodite segment) cylindrical with prominent spinules on the sloping tip. Claw bears a stout, short, conical spine and a long, slender spine on the indistinct exopodite 3.

*Mesocyclops sp.*

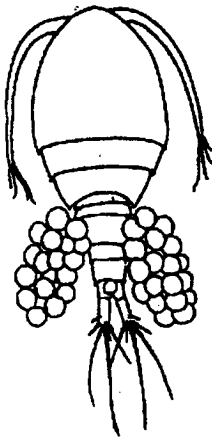
Phylum : Arthropoda  
 Class : Crustacea  
 Sub-class : Copepoda  
 Order : Cyclopoida  
 Family : Cyclopidae  
 Genus : *Mesocyclops*

1. Most common and larger cyclopoids. Length excluding caudal setae:  
 ♀ 0.9 – 1.3 mm, ♂ 0.75 mm - 0.9 mm.
2. a. Body is pear shaped with a distinctly broad metasome with 5 segments and narrow urosome, with 4 segments in ♀ and 5 segments in ♂, with the major articulation between the somites of P<sub>4</sub> and P<sub>5</sub> (i.e. thoracic segments 5 and 6). The first body segment is referred to as cephalic segment or cephalosome.
- b. The caudal rami carry distinctly unequal caudal setae.
- c. The antennules (A<sub>1</sub>) 17 segmented and never much longer than the metasome. Both antennules of the male are geniculate and modified for grasping the female.
- d. Fifth legs (P<sub>5</sub>) in both sexes are much reduced, uniramous without an enlarged basal segment and are similar.
- e. Males often have rudimentary sixth legs.
- f. The ovigerous female carries eggs in two ovisacs attached to the sides of the genital somite. Spermatophore is kidney – shaped and two may be attached to the female genital somite.

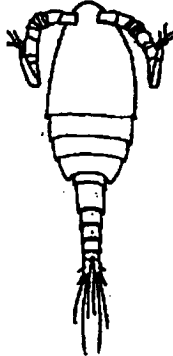
- 3 Last 2 segments of Antennules together longer than the previous three. Last 2 segments have hyaline membrane and on the last segment it has several indistinct notches or serrations or with one deep notch.
4. Leg 4: Both spinous projections on connecting lamella small, terminal endopodite is long and has two terminal seta, of which the inner one is slighter shorter than outer.
5. P<sub>5</sub> of both sexes consisting of two distinct segments, distal segment is small and has two spines or setae. Inner spine is at the middle of distal segment and shorter than the terminal seta.

## *Thermocyclops*

Female



Male



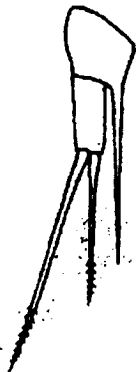
Phylum : Arthropoda  
 Class : Crustacea  
 Sub-class : Copepoda  
 Order : Cyclopoida  
 Family : Cyclopidae  
 Genus : *Thermocyclops*

Antennule, last segment

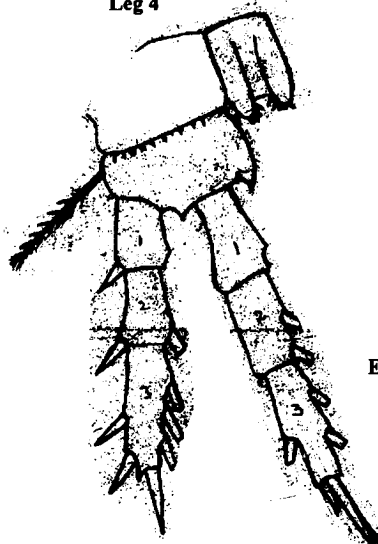


Hyaline membrane

Leg 5 (P5)



Leg 4



Endopodite

1. Small cyclopoids, less than 1 mm long.
2. a. Body is pear shaped with a distinctly broad metasome with 5 segments and narrow urosome, with 4 segments in ♀ and 5 segments in ♂, with the major articulation between the somites of P<sub>4</sub> and P<sub>5</sub> (i.e. thoracic segments 5 and 6). The first body segment is referred to as cephalic segment or cephalosome.
  - b. The caudal rami carry distinctly unequal caudal setae.
  - c. The antennules (A<sub>1</sub>) 17 segmented and never much longer than the metasome. Both antennules of the male are geniculate and modified for grasping the female.

- d. Fifth legs ( $P_5$ ) in both sexes are much reduced, uniramous without an enlarged basal segment and are similar.
- e. Males often have rudimentary sixth legs.
- f. The ovigerous female carries eggs in two ovisacs attached to the sides of the genital somite. Spermatophore is kidney – shaped and two may be attached to the female genital somite.
3. The last two segments of the antennules with smooth, narrow, hyaline membrane.
  4. Last thoracic segment small, only little broader than genital somite.
  5. Leg 4: Inner terminal seta of last endopodite segment is distinctly longer than outer seta and shorter than the last endopodite segment.
  6. Leg 5: 2 Segmented and the distal segment with 2 apical setae, of which the inner seta is somewhat spiniform and longer than outer normal seta.

#### 4.4 Identification of benthos in ponds – Aquatic insects, insect larvae, molluscs and aquatic vegetation

Dr. P. PADMAVATHI

##### 4.4.1. AQUATIC INSECTS

1. *Lethocerus*
2. *Laccotrephes*
3. *Anisops*
4. *Ranatra*
5. *Gerris*
6. *Eretes*
7. *Cybister*

##### 4.4.2. INSECT LARVAE

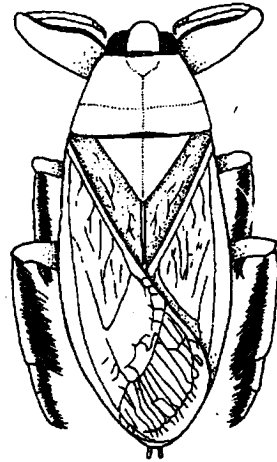
1. *Chironomus*
2. *Chaoborus*
3. Mayfly nymph
4. *Eristalis*
5. *Tabanus*

##### 4.4.3. MOLLUSCS

1. *Pila virens*
2. *Bellamyia bengalensis*
3. *Lymnaea acuminata*
4. *Thiara (Melanoides) tuberculata*
5. *Thiara scabra*
6. *Gyraulus*
7. *Lamellidens marginalis*

##### 4.4.4. AQUATIC VEGETATION

1. *Eichhornia*
2. *Pistia*
3. *Azolla*
4. *Lemna*
5. *Wolffia*
6. *Salvinia*
7. *Nymphaea*
8. *Typha*
9. *Ipomoea*
10. *Marselia*
11. *Hydrilla*

*Lethocerus*

Phylum : Arthropoda

Class : Insecta

Order : Hemiptera

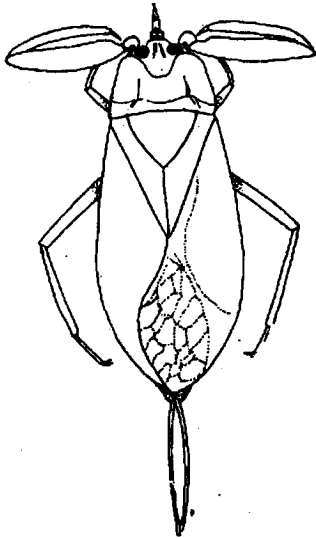
Family : Belostomidae

Genus : *Lethocerus*

1. Commonly called **Giant Water Bug**.
2. It is highly predacious and feeds on fish fry and fingerlings. It usually comes out during night time towards light.
3. Body broad, oval and flat. Measures more than 1.5 inches.
4. Antennae shorter than head.
5. Membrane of hemelytra (front wings) reticulately veined. Furrow of the wing membrane shallowly 'S' shaped.
6. Apical appendages of the abdomen short and flat, retractile.
7. First segment of the beak shorter than second.
8. Forelegs are raptorial and other two pairs are flat, long and hairy to help in swimming. Anterior femora grooved for the reception of the tibiae.



## *Laccotrephes*



Phylum : Arthropoda

Class : Insecta

Order : Hemiptera

Family : Nepidae

Genus : *Laccotrephes*

1. Commonly called **water scorpions** (due to superficial resemblance of forelegs to the pedipalps of scorpion).
2. Usually found in the ponds under the aquatic plants and mud, and also in fish ponds. It feeds voraciously on tender fish hatchlings (spawn).
3. Body elongate and flat
4. Legs slender and not extremely long. Hind tarsi with distinct claws. Anterior femora but little longer than tibia.
5. Antennae short, shorter than head and slender.
6. Prothorax is much broader than the head.
7. Membranes of hemielytra (front wings) reticulately veined.
8. It can be recognized by the long respiratory tubes extending from the tip of the abdomen. It is formed by the two 'cerci' and permits the bug to feed or rest on the bottom of shallow ponds and obtain the air from the surface.
9. Front legs modified for grasping the prey to paralyse while the piercing beak inflicts a saliva-like poison.

## *Anisops*



Phylum : Arthropoda

Class : Insecta

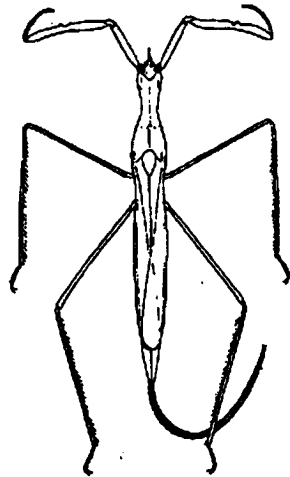
Order : Hemiptera

Family : Notonectidae

Genus : *Anisops*

1. It is very common in nursery ponds of fish farms. They usually occur in huge numbers and feed voraciously on the carp spawn and hence it is very harmful.
2. Antennae shorter than head. Antennae four segmented and the last segment is much shorter than the penultimate.
3. Head inserted into prothorax region.
4. Legs dissimilar, hind legs fringed and flattened for swimming. Front tarsi normal, hind tarsi with indistinct setiform claws.
5. Abdomen stores air on the ventral side of the body and swims on its back hence the common name, "*back swimmer*".

## *Ranatra*



Phylum : Arthropoda

Class : Insecta

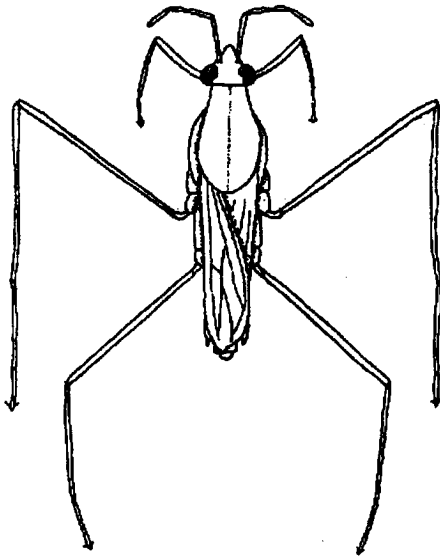
Order : Hemiptera

Family : Nepidae

Genus : *Ranatra*

1. Commonly called **water stick insect**.
2. It is generally found among the aquatic vegetation in stagnant waters.
3. Body very elongated, legs long and slender.
4. Antennae short and slender, shorter than head.
5. Prothorax is narrower than the head.
6. These are predacious insects. The first pair of legs used for catching the prey.
7. Anterior femora considerably longer than tibia.
8. Hind tarsi with distinct claws.
9. Membranes of hemielytra (front wings) reticulately veined.
10. It has a pair of breathing tubes at the hind end of the abdomen.
11. It is harmful to tender spawn and fry in fish nursery ponds.

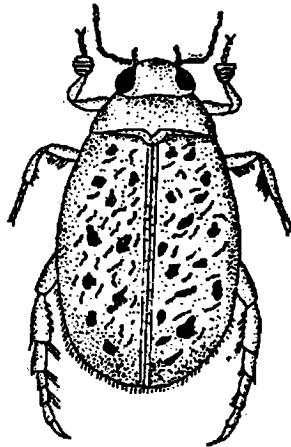
## *Gerris*



Phylum : Arthropoda  
Class : Insecta  
Order : Hemiptera  
Family : Gerridae  
Genus : *Gerris*

10/1

1. Commonly called **water strider or pond skater**.
2. Commonly found in large numbers gliding along or jumping on the surface of the ponds.
3. Length of the body is about 2 cm.
4. It has a thin dark brown flat body with relatively long legs.
5. Antennae long and first segment of antennae longer than the second and third.
6. It has short non-functional wings.
7. It is harmful to tender fish spawn in nursery ponds.

*Eretes sticticus*

Phylum : Arthropoda

Class : Insecta

Order : Coleoptera

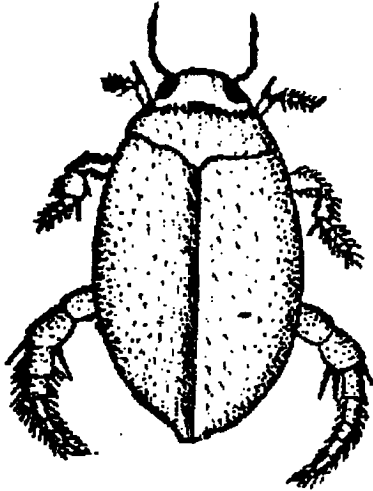
Family : Dytiscidae

Genus : *Eretes*

Species : *sticticus*

1. Form obovate, narrow anteriorly, dilated posteriorly, depressed on the dorsal side, moderately shiny.
2. This species is extremely variable in size, form and colour.
3. Elytra testaceous to fawn colour covered anteriorly with black irritations, with yellow line of attachment along the suture and three similarly placed longitudinal lines.
4. Lateral borders of elytra serrated in the posterior half.
5. Head with black spot on the vertex.
6. Scutellum visible.
7. Pronotum with black band.
8. Hind margins of the fore basal metatarsal segment on both anterior and posterior faces fringed with golden yellow cilia, over-lapping the basis of next segment.
9. Females are larger than males.
10. Occurs in muddy water and rarely in ponds with vegetation.
11. They occur in large number in turbid waters, cement nurseries constructed for rearing carp fry, ponds without aquatic vegetation and when vegetation is present, they are collected away from the vegetation.
12. They occur in many parts of the world.
13. It is harmful to the tender fish spawn and fry in nursery ponds.

## *Cybister*



Phylum : Arthropoda

Class : Insecta

Order : Coleoptera

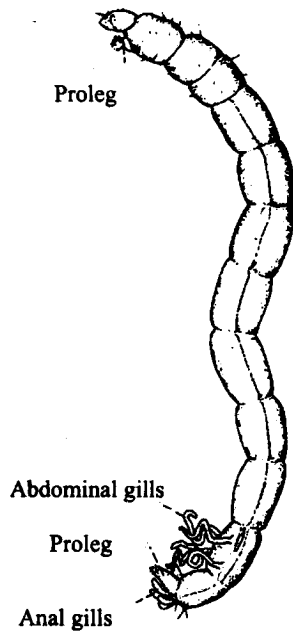
Family : Dytiscidae

Sub-family: Dytiscinae

Genus : *Cybister*

1. Commonly called predacious diving beetle.
2. Form elongate, ovate, moderately broad behind, (narrower in the posterior side). The middle line with greenish metallic iridescence pronotum and elytra have a yellow lateral borders extending upto the apex.
3. Body covered with hard elytrae.
4. Antennae are long and filiform.
5. Hind legs are flattened which is an adaptation for swimming.
6. Male & Female differ significantly in size. 23- 28 mm & ? 25-39 mm.
7. It usually occurs in ponds associated with aquatic vegetation like *Ceratophyllum* in debris and along the walls of cement nurseries and in open stagnant water.

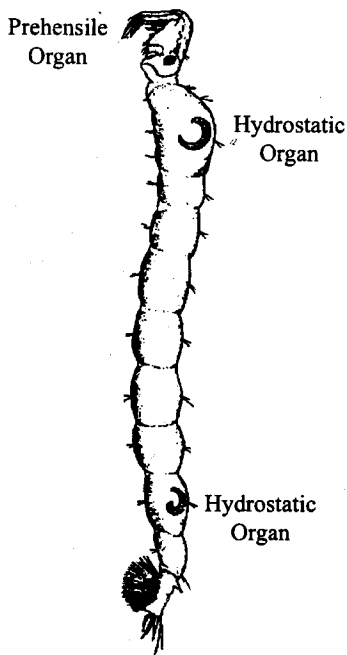
## *Chironomus*



Phylum : Arthropoda  
Class : Insecta  
Order : Diptera  
Sub-order: Nematocera  
Family : Tendipedidae  
(=Chironomidae)  
Sub-family: Tendipedinae  
(=Chironominae)  
Genus : *Chironomus*  
(=*Tendipes*)

1. Commonly known as **blood-worms**.
2. Inhabits bottom of the freshwater bodies.
3. The larvae is slender, construct tubes and feed on algal matter.
4. Head capsule complete and not retractile.
5. A pair of prolegs are present both the first and last abdominal segments.
6. 8<sup>th</sup> abdominal segment with fingerlike ventral gills called anal gills which helps in respiration.

## *Chaoborus*

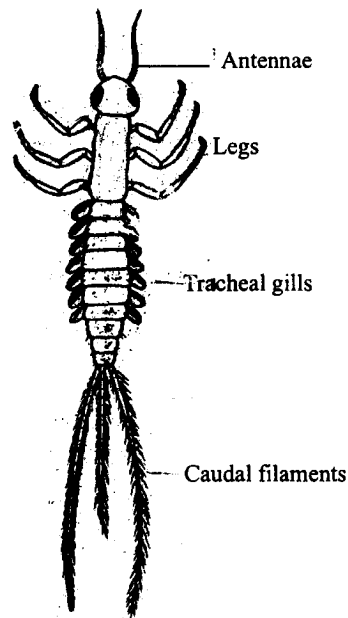


Phylum : Arthropoda  
Class : Insecta  
Order : Diptera  
Sub-order: Nematocera  
Family : Culicidae  
Sub-family: Chaoborinae  
Genus : *Chaoborus*

1. Commonly called **phantom midges**.
2. Body transparent, and leads planktonic life.
3. It is notoriously predacious, lives in benthic and mid waters.
4. Body either not flattened with serially arranged ventral suckers or if flattened, the suckers are 8 in number.
5. Head capsule complete and not retractile
6. Pseudopods lacking.
7. Thoracic segments fused into an enlarged complex which is distinctly broader than the abdomen.
8. Antenna developed into a prehensile organ with long, strong apical spines.
9. Eighth abdominal segment without an elongated respiratory siphon.
10. Hydrostatic organs present in thorax and seventh abdominal segment, useful for swimming.

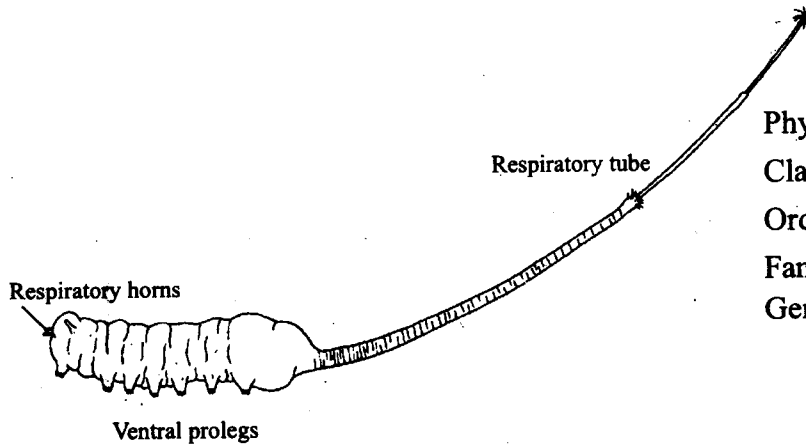


## Mayfly Nymph



Phylum : Arthropoda  
Class : Insecta  
Order : Ephemeroptera  
Family : Baetidae

1. Mature nymphs are characterized by an elongated body, large head, well-developed mandibulate mouth-parts, stout legs, long filiform antennae, large compound eyes, and three rather prominent ocelli.
2. Large, paired tracheal gills on the lateral or dorsal surface of most of the abdominal segments (generally 1-7) constitute the most characteristic feature and serve to distinguish mayfly nymphs from all other aquatic insects.
3. At the tip of the abdomen are usually three long, segmented fringed caudal filaments.
4. Mayfly nymphs occur in all types of freshwaters, wherever there is an abundance of oxygen. Some occur only in vegetation; some only on mud, debris, gravel or rock bottoms; many burrow in mud or debris.
5. Mayfly nymphs are almost entirely herbivorous, although some feed on exuviate, small invertebrates and dead nymphs.

***Eristalis (=Tubifera)***

Phylum : Arthropoda

Class : Insecta

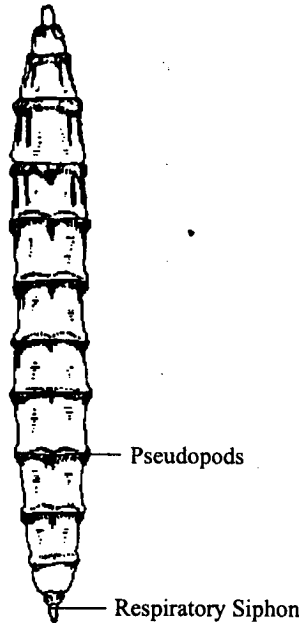
Order : Diptera

Family : Syrphidae

Genus : *Eristalis*

1. Commonly called "**Rat-tailed maggots**" or "**flower flies**".
2. Species of the genus *Eristalis* are the common aquatic larvae and occur in sewage, polluted streams, rivulets and decaying vegetation at the edges of the ponds.
3. The body is covered with fine spinules, and the thoracic and abdominal segments are wrinkled and indistinct.
4. The head is small and retracted into the body.
5. There are 7 pairs of *ventral prolegs*, a pair of short, dorsal, *anterior respiratory horns* that terminate in a series of minute openings, and an elongated *caudal respiratory tube*.
6. The caudal respiratory tube is three segmented, telescopic from two to four times the length of the body proper when extended, and has two spiracular openings at its tip.
7. *Eristalis* is inactive and feeds on decaying organic material in shallow water bodies (to permit the tip of the extended caudal respiratory tube to be projected just above the surface)

## *Tabanus*



Phylum : Arthropoda

Class : Insecta

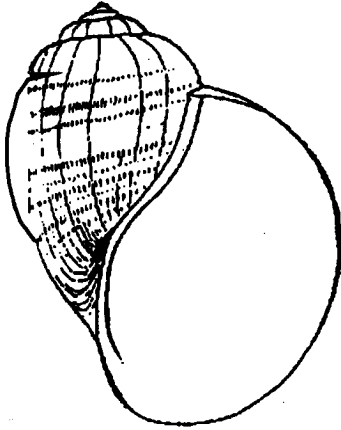
Order : Diptera

Sub-order: Brachycera

Family : Tabanidae

Genus : *Tabanus*

1. Commonly known as horse-flies
2. Larva elongated, cylindrical, tapered at both the ends and coloured.
3. Body is divided into a minute head, 3 thoracic segments, 8 abdominal segments and a short posterior siphon.
4. Head capsule well developed, usually sclerotized dorsally. Free part of head retractile.
5. Each abdominal segment with a girdle of pseudopods which may bear hooks or which may be reduced to fleshy swellings.
6. The 8<sup>th</sup> abdominal segment ends with a respiratory siphon which is more or less elongated when exerted.

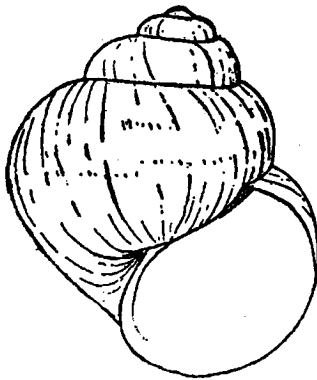
***Pila virens***

Class : Gastropoda  
Sub-Class: Prosobranchiata  
Order : Mesogastropoda  
Family : Pilidae  
Genus : *Pila*  
Species : *virens*

1. Commonly called **apple snail**.
2. Shell ovoid or semiglobose. Sutures deeply impressed. Spire elevated. Sutures acuminate with a distinct carination of the whorls on the outside. Spire prominent and conical.
3. It is variable in its colour and shape of the spire and difficult to distinguish it from *Pila globosa*.
4. It is amphibious in habit, performs both aerial and aquatic respiration.
5. It's meat is used as food for ducks, and shrimps.
6. Breeding habit: Viviparous

Distribution : Common in South India and Maharashtra.

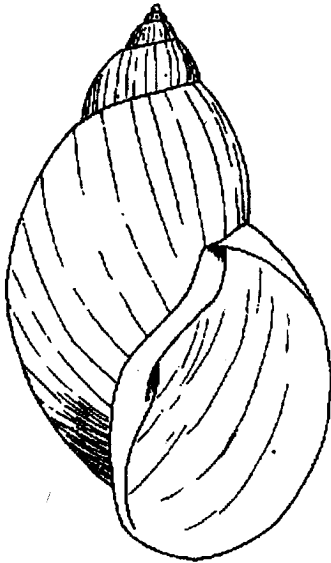
## *Bellamyia bengalensis*



Phylum : Mollusca  
Class : Gastropoda  
Sub-class: Prosobranchiata  
Order : Mesogastropoda  
Family : Viviparidae  
Genus : *Bellamyia*  
Species : *bengalensis*

1. Shell ovate, smooth, without traces of distinct ridges or sculptures.
2. Adult shell medium sized with less distinct three or more chocolate brown spiral bands.
3. Surface has fine transpiral and spiral striations.
4. Spire conical, acuminate with Four or Five narrow flattened whorls.
5. Sutures deep
6. Body whorl broad, aperture pyriform
7. Outer lip thin and sharp
8. Fertilization internal, *palial* oviduct enlarged and acts as the uterus. It contains embryos of various stages of development, young ones are shed into the water.
9. It is considered as pest of *Azolla pinnata*, a plant used as a bio-fertilizer in paddy fields.
10. Distributed throughout India.

### *Lymnaea acuminata*



Phylum : Mollusca  
Class : Gastropoda  
Sub-class: Pulmonata  
Order : Basommatophora  
Family : Lymnaeidae  
Genus : *Lymnaea*  
Species : *acuminata*

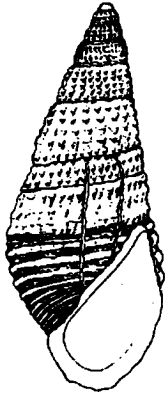
1. Shell generally thin and usually more than 10 mm height. Shell dirty white to pale brown and regularly ovate in outline. Sculpture consists only fine curved vertical striations.
2. Sutures well impressed and oblique in position.
3. Body whorl large and inflated.
4. Spire almost always exerted, never scalariform or canalized at the base.
5. Spire short, usually with not more than 4 whorls, tapering into acuminated tip.
6. Columella spirally twisted.
7. It occurs in permanent water bodies with abundant vegetation.
8. Acts as secondary host for helminth parasites.

***Thiara (Melanoides) tuberculata***

Class : Gastropoda  
Sub-Class: Prosobranchiata  
Order : Mesogastropoda  
Family : Thiaridae  
Genus : *Thiara*  
Species : *tuberculata*

1. Shell with a high spire and moderately large body valve, generally not more than 12 whorls.
2. Whorls evenly rounded, dark red brown spots and flames either irregularly distributed or longitudinally arranged on the shell surface.
3. Sculptured with vertical ribs and spiral striae, (giving a tubercle appearance) distinct and raised on the upper whorls but flattened on the lower one.
4. Ovoviviparous and parthenogenetic
5. Distribution: Throughout India except Kashmir. Commonly occur in streams, rivers, irrigation canals and stagnant water pools, often extending into brackish waters.

## *Thiara scabra*



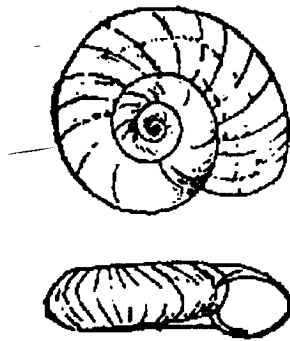
Class : Gastropoda  
Sub-class: Prosobranchiata  
Order : Mesogastropoda  
Family : Thiaridae  
Genus : *Thiara*  
Species : *scabra*

1. Commonly known as **melanids**
2. Height of body whorl considerably more and spire generally as high as body whorl. Sculptured with conspicuous nodules, granules or axial ridges.
3. Shell generally spinous or sharply nodulose.
4. Whorls angular between suture and the periphery.
5. Whorls descending in steps, (pagoda like), with a crown of spines below the suture.
6. Shell with spiral ridges.
7. It prefers slow or fast moving waters but may also occur in ponds. Ovoviviparous.

Distribution: India except Kashmir.

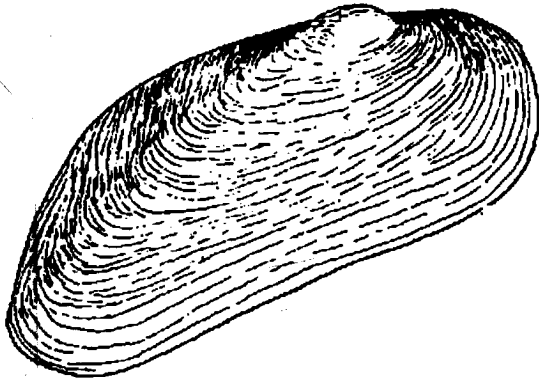


## *Gyraulus*



Phylum : Mollusca  
Class : Gastropoda  
Sub-class: Prosobranchiata  
Order : Mesogastropoda  
Family : Planorbidae  
Genus : *Gyraulus*

1. Shell small and discoidal, with the whorls rounded.
2. Aperture oblique and somewhat deflected.
3. In many species the surface of the shell is covered with hair like processes of periostracum and in addition is spirally striate.
4. It occurs in association with aquatic vegetation.

*Lamellidens marginalis*

Phylum : Mollusca  
Class : Bivalvia  
Order : Unionida  
Family : Unionidae  
Genus : *Lamellidens*  
Species : *marginalis*

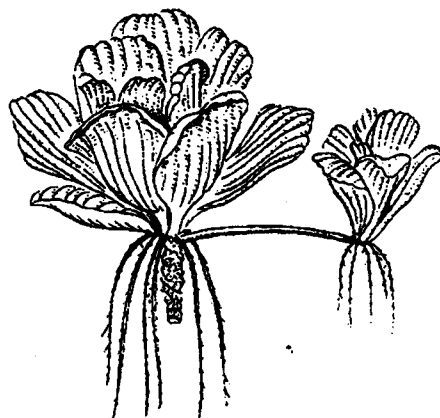
1. Shell oblong, ovate, thin, very smooth.
2. Periostracum blackish brown and shiny, light brown border along the ventral margin.
3. Posterior side broad, roundedly angular.
4. Anterior side short and narrow,
5. Dorsal margin little curved, ventral margin slightly contracted in the middle.
6. Hinge with two cardinals in the right valve, left valve with one feebly developed cardinal, two curved lateral teeth.
7. This species is capable of producing pearls and hence has great importance in freshwater pearl culture.
8. It occurs both in standing and flowing waters.
9. Distributed in India, Bangladesh, Myanmar, Sri Lanka, etc.,

## *Eichhornia*



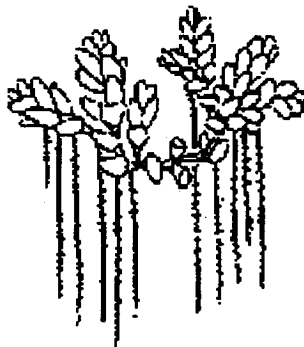
1. Commonly called **water Hyacinth**.
2. It is a perennial and a free floating hydrophyte
3. Plants large with rosettes of leaves either floating or rising above the water. The petiole is swollen near the middle portion with bladder like structure consisting internally large air chambers. Leaf blades are broadly rounded or rarely ovate circular in shape.
4. Flowers in terminal clustures.
5. Roots are long, hanging in water in clustures and with prominent root pockets. Roots fibrous, folded and pinkish violet in colour.
6. These restrict the light penetration in the water and inturn hamper the plankton production, limits the living space for fish, upsets the equilibrium of physio-chemical parameters of water, particularly dissolved oxygen and nutrients, and provide shelter to predatory and weed fishes. All of which finally result in poor fish production.
7. It is processed in biogas plants to produce slurry to be used as manure for fish ponds.

### *Pistia*



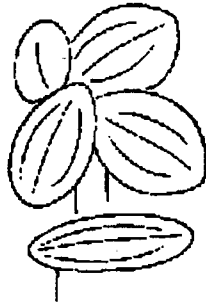
1. It is a perennial and a free floating weed.
2. Stem tubular with soft internode; some internodes bear a cup of leaf in a dense – rosette.
3. Leaves are sessile, highly segmented arranged in circular manner.
4. Roots simply white, fibrous, folded with fibrilles.
5. These restrict the light penetration in the water and inturn hamper the plankton production, limits the living space for fish, upsets the equilibrium of physio-chemical parameters of water, particularly dissolved oxygen and nutrients, and provide shelter to predatory and weed fishes. All of which finally result in poor fish production.

## *Azolla*



1. commonly called **water fern**. Free-floating plants with roots.
2. Plants small with several to many leaves inserted on an axis.
3. Axis horizontal, leaves overlapping, not more than 5mm long, not in a rosette.
4. *Azolla* is inhabitant of tropical as well as temperate rice growing regions.
5. *Azolla* is well established as a nitrogenous biofertilizer in agriculture as well as in fish ponds.
6. It is also used as a fodder for livestock, swine and poultry. Recently it is used as a supplementary feed for fishes in the form of pellets.
7. It fixes atmospheric nitrogen (N) efficiently due to the presence of symbiotic heterocystous blue-green alga, *Anabaena azollae* present in the cavities on dorsal lobes of the leaf (cavities of sporophytes).
8. The average daily N fixing rate is reported to range from 1.0 to 2.6 kg N/ha. Hence it is often referred to as a miniature nitrogen factory. It was observed that about 10-12 t of *Azolla* can be applied to a ha of water body per annum, contributing 25-30 kg N.
9. Its suitability over other biofertilizers like blue-green algae is due to high N fixing capacity, rapid multiplication fast decomposition, rapid availability of N to the ambient system and non-interference with other biota.
10. One kg of phosphorus application to *Azolla* in field cultivation results in fixation of 5-10 kg N.
11. Existence of thick *Azolla* mat considerably suppresses the growth of submerged weeds.

### *Lemna minor*



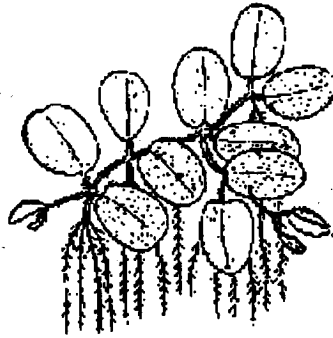
1. Commonly called **duck weed**. Small or minute, gregarious aquatic plants. These are cosmopolitan, floating in still water.
2. Plants reduced to a few small flat floating fronds.
3. Lacks distinct stems and leaves.
4. Fronds are light green in colour, 2-5 mm long, round or ovate. They are nearly flat on both sides and are usually 3 nerved.
5. Fronds bear a single root.
6. Root sheath not appendaged, root cap obtuse
7. Reproduction through budding and seeds.
8. These restrict the light penetration in the water and in turn hamper the plankton production, limits the living space for fish, upsets the equilibrium of physio-chemical parameters of water, particularly dissolved oxygen and nutrients, and provide shelter to predatory and weed fishes. All of which finally result in poor fish production.
9. Severe infestations of this plant occasionally impede boat travel.
10. Extremely valuable as a food source for water fowl, especially puddle ducks.

## *Wolffia*



1. Commonly called **water meal**.
2. These are diminutive, floating rootless plants. Individual plants scarcely visible to the naked eye, often forming large green masses on the water surface.
3. Leaves or fronds solitary or paired, globular to ellipsoidal, 0.5-1.5 mm long, 0.3-1.0 mm wide, green on all surfaces.
4. Grows in close association with other species of the duckweed family. However, plants are capable of survival in damp vegetation along marginal areas of sloughs and ponds. Usually occurs in marshes, ponds and lakes.
5. Mainly found in tropics and subtropics. It usually presents no navigational or recreational impairment, except in very high concentrations.
6. Water meal serves as an extremely important food source to ducks and other water fowl. Recently, attempts are being made to prepare pellets to feed fish and other aquatic organisms.

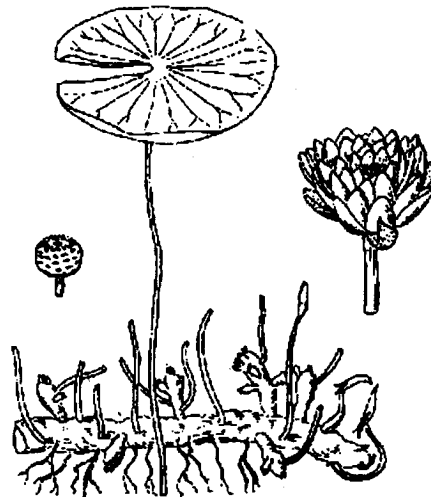
### *Salvinia*



1. It is a floating weed. Common throughout India in lakes, pools, tanks, etc. with high organic matter.
2. Strand floating stem giving off short petiole or sessile fronds on the upper side.
3. Leaves small, dimorphic, arranged in 3 whorls. Two leaves at a node are normal, floating, and *spongy* and a third modified into filiform submerged root-like segments.
4. These restrict the light penetration in the water and inturn hamper the plankton production, limits the living space for fish, upsets the equilibrium of physio-chemical parameters of water, particularly dissolved oxygen and nutrients, and provide shelter to predatory and weed fishes. All of which finally result in poor fish production.



## *Nymphaea*



1. These aquatic plants rooted in the bottom but having their leaves and flowers above the water surface, hence considered as emergent weeds.
2. These found scattered everywhere in lakes and ponds.
3. The stem is modified as creeping jointed rhizome embedded in the pond mud.
4. Leaves are short and characterized by waxy coating and hence do not hold any moisture on the upper surface. The leaf when young pink in colour.
5. Rhizome is present at the bottom of the ponds by numerous adventitious roots.
6. These restrict the light penetration in the water and in turn hamper the plankton production, limits the living space for fish, upsets the equilibrium of physio-chemical parameters of water, particularly dissolved oxygen and nutrients, and provide shelter to predatory and weed fishes. All of which finally result in poor fish production.

## *Typha*

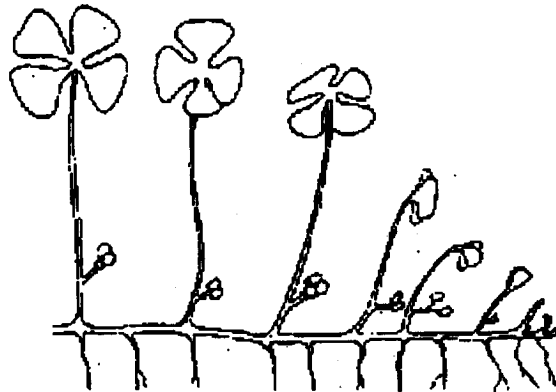


1. Commonly called cattails.
2. Commonly found in stagnant waters, brackish water or slow running rivers and streams, which do not dry up in the hot season.
3. It is a tall perennial aquatic marginal plant.
4. The main portion of the stem is modified into a thick rhizome, creeping underneath the muddy soil.
5. Leaves are elongated, linear, erect, spongy, very smooth all over, arising in cluster from the node.
6. Numerous small flowers are arranged cylindrically in a dense spike.
7. Roots are adventitious. Superficially fixed in the pond mud at the node of rhizome.
8. Cattails spread by sending out rhizomes, in addition to proliferation by seeds.
9. These propagate on the margins and shallow areas of water bodies. In fish culture ponds, these absorb nutrients from the water vigorously leading to poor production of plankton and fish. These also provide shelter to predatory and weed fishes and obstruct net operations.



1. These are rooted aquatic plants and infest the shallow littoral areas of the water body and hence considered as marginal weeds.
2. The stem is profusely branched, hollow and jointed, modified for creeping plants. The main portion of the plant is embedded in the margin of the pond.
3. Leaves are single and alternate with long petiole, the margin of the leaf is entire and apex is pointed.
4. Roots are adventitious and fibrous, found at the node. Some are freely hanging in the water; some are rooted in the mud.
5. Common throughout India in all water bodies.
6. These plants propagate on the margins and shallow areas of water bodies. In fish culture ponds, these absorb nutrients from the soil and water vigorously, leading to poor production of plankton and fish. Also provide shelter to predatory and weed fishes.
7. These also harbour many forms of littoral copepods and cladocerans as well as many other invertebrates.

### *Marselia*



1. It is a marginal weed, abundant in warm regions of ponds, pools, puddles, etc.
2. Rhizome is slender, branched, creeping just below the soil. Adventitious roots are present at nodes.
3. Long-stalked quadrifoliate compound leaves arise from the nodal region of the rhizome. The petiole is long, slender and weak.
4. Peduncles with 2-6 sporocarps, adnate to the base of the petiole.
5. These plants propagate on the margins of water bodies. In fish culture ponds, these absorb nutrients from the soil and water vigorously leading to poor production of plankton and fish.

## *Hydrilla*



1. It is a submerged rooted aquatic herb of shallow stagnant freshwaters occurring throughout India.
2. Stem is branched, slender with a long or short internodes.
3. Leaves reduced in size and thickened. They are green in colour and mostly whorled.
4. Roots fibrous, slender and attached at the nodes.
5. In ponds these absorb nutrients from the soil and water, leading to poor production of plankton and fish.
6. These obstruct the netting operations in the fish ponds.

## DMP-IV : Aquaculture

### 4.5.1 Dissection and mounting of the Pituitary gland of the fish – Preparation, dosage and injection of pituitary extract for induced breeding of fish

#### Dissection and Mounting of the pituitary gland of the fish

Pituitary gland in a fish lies on the ventral side of brain immediately behind the optic chiasma in a concavity on the floor of the brain box known as sella turcica and enclosed by a thin membrane called Durameter (Fig. 1).

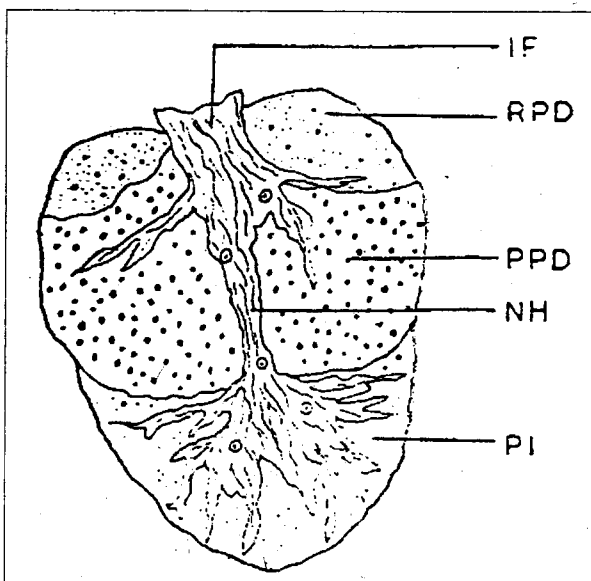


Fig. 1 Showing different parts of the pituitary gland of *Labeo rohita* in a mid-sagittal section. IF, Infundibular stalk; NH, Neurohypophysis; RPD, Rostral pars distalis; PPD, Proximal pars distalis; Pi. Pars intermedia.

For the preparation of the extract the pituitary gland is removed from some donor fish. For best results, the donor fish should be a fully ripe fish in advanced stages of its maturity just prior to spawning period. The donor fish may be freshly caught or preserved in ice until use. In general, *Cyprinus carpio* should be chosen widely as donor fish as it is available throughout the year. This fish gives larger gland. The gland does not show species specificity in its action of induced fish breeding in the recipient fish.

#### Methods of Gland collection

The pituitary gland may be collected by either of the following two methods.

1. Through foramen magnum.
2. By dissecting and removing a portion of the scalp.

**1. Removal of gland through foramen magnum:** The foramen magnum is first exposed in a cutfish head by removing vertebral parts adhering to the skull. Fat is removed from above the brain parts by means of forceps first then by means of a cotton piece. A pair of forceps are now inserted into the foramen magnum dorsally to brain. The anterior part of the brain is now detached and the remaining brain is carefully lifted out through the foramen magnum. The pituitary gland localised within it is carefully removed.

**2. Removal of gland by dissection of fish head:** Using a sharp butcher's knife that is a portion of the scalp (brain case) is chopped off in a clean cut with one stroke. Fat surrounding the brain is now removed with the help of cotton. Olfactory and optic nerves are now severed and the brain is lifted up and removed to trace out the pituitary gland. Then the gland lying behind the optic chiasma is lifted up and removed. The gland is to come up alone, with the brain or may remain behind the floor of the brain cavity often covered with a membrane. The gland is carefully removed after separating it from the membrane of the brain proper. The gland should not be damaged or torn. The pituitary gland after removal is mounted in a watch glass with little water for display.

**Note:** The first method is easier and less time consuming. It is more economical because the fish-cut heads may be still sold out after removal of the gland.

The second method will not be preferable because it renders fish head useless for sale as the operation brings about complete mutilation.

### **Preparation of pituitary extract**

The pituitary glands, immediately after collection, are kept in absolute alcohol for dehydration. After 24 hours, the alcohol is changed for further dehydration and defatting. The glands are then weighed and preserved in fresh alcohol contained in dark colored phials and stored either at room temperature or in a refrigerator. The weight of glands may vary from 7.0 to 18.8 mg in rohu weighing 1.0 to 3.6 kg, and 3.0 to 22.8 mg in mrigal (0.3 to 3.5 kg in weight).

The required quantity of glands are taken out of the phials and the alcohol allowed to evaporate. The glands are then macerated with a tissue homogenizer either in distilled water or 0.3% saline. Further, dilution is usually made by adding the same fluid to render the total solute at the rate of 0.2 ml per kg weight of breeders. Usually the volume of the dose does not exceed 1 ml. The gland suspension is then centrifuged and the supernatant fluid drawn into a hypodermic syringe for injection.

Ibrahim and Chaudhuri (1966) have devised a method for preserving pituitary extract in glycerine. In this method, a known quantity of pituitary glands, after homogenizing in distilled water, is diluted with  $\frac{1}{3}$  of the total volume of extract calculated at the desired concentration and the suspension is kept under refrigeration for 24 hours. Pure glycerine is then added to make up the total volume of the extract (i.e. 3 ml of extract will have 1 ml of water and 2 ml glycerine). The extract, with glycerine added is again kept in refrigerator for another 24 hours, after which it is centrifuged and the supernatant fluid transferred into ampoules for future use. The advantage of this method is that it permits hormone extraction from a large number of glands at a time, ensuring uniform hormone potency per unit volume of the extract and saving time in the preparation of injection dose. Glycerine extract is found to retain its potency from 9 to 61 days at room or under lower temperatures.

### **Injection of pituitary extract**

Intramuscular injection of pituitary extract is administered with a hypodermic syringe (with B.D. needle No.22 for fishes weighing 1.0 to 3.0 kg, needle No.19 for larger ones and needle No.24 for smaller spawners) at the caudal peduncle or shoulder region near the base of the dorsal fin. Both the injected males and females are kept together in a breeding hapa for spawning.

### **Dosage**

In usual practice, the female alone is injected with a stimulating dose of 2 to 3 mg/kg weight followed by a second dose of 5 to 8 mg/kg weight after a lapse of 6 hours, if required. One set that is two males per female by weight are given a single dose, each of 2.3 mg/kg weight at the time of second injection to the female if spawning is not effected. Both the injected males and the females are kept together in a breeding "hapa" for spawning.

**Dr. N. GOPALA RAO**

*M.Sc., Ph.D.*

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## DMP-IV : Aquaculture

### 4.6.1 Spotters – Fishes of aquacultural importance:

## *CYPRINUS CARPIO*

**Classification:**

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniforms

Family: Cyprinidae

**Characters:**

1. It is commonly known as “Bangaru Thega”.
2. It is found in fresh water ponds, lakes, reservoirs, tanks and other capturable areas.
3. It is a detritus omnivorous bottom feeder.
4. Head is large with wide mouth.
5. Body is elongated and covered with scales.
6. Dorsal fin is quite large reaching upto the tail.
7. Ventral fins are abdominal.
8. Cyprinus is not a native of India but is introduced into the lakes of Nilgiri mountain ranges from China and South-East Asia.
9. There are 3 varieties of *Cyprinus* sp. viz.,  
*C. carpio* var. *specularis* (Mirror carp)  
*C. carpio* var. *communis* (scale carp)  
*C. carpio* var. *nudus* (Leather carp)

Out of the above, var. *specularis* is much more familiar in India and was introduced in Nilgiris from Ceylon.

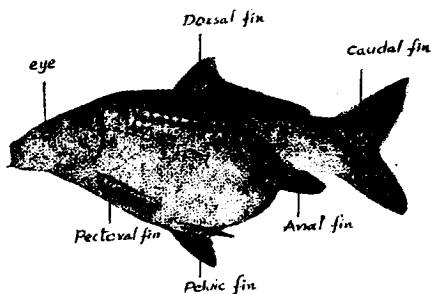


Fig. *Cyprinus carpio*

10. This fish is used in induced fish breeding Technique in order to produce pure fish seed variety.
11. This fish breeds in riverine condition but in confined waters this is forced to breed under artificial conditions

## CATLA CATLA

### Classification:

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

### Characters:

1. It is commonly known as "Bocche" in Telugu and Katla in Hindi.
2. Body is short with rounded abdomen
3. It is a silvery fish with a pinkish tinge.
4. It has wide mouth with prominent lower jaw.
5. Eyes are large and situated in the anterior half of the head.
6. Barbels are absent.
7. Upper lip is absent and the lower lip is very thick.
8. Dorsal fin is inserted above tip of pectoral fin with 17-19 rays.
9. Anal fin has 8 rays out of which five are branched.

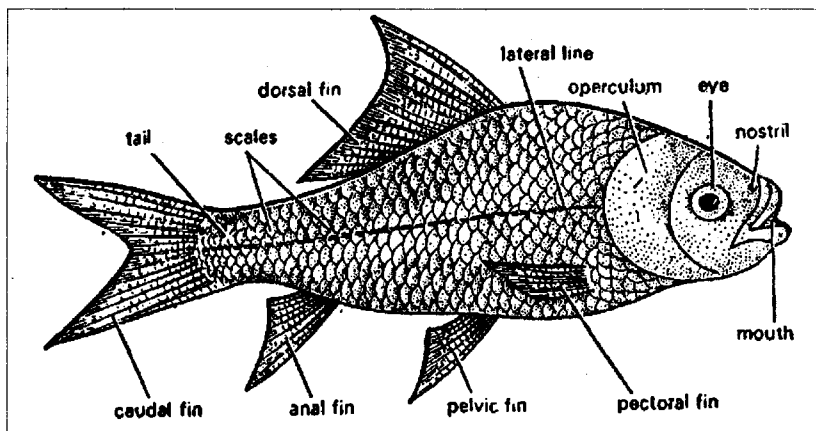


Fig. *Catla catla*

10. Caudal fin is forked.
11. Lateralline is complete with 40-43 scales.
12. This is the fastest growing Indian major carp food fish growing upto 180 cm in size and 1-2 kg/year.
13. It is a surface zooplankton feeder found in fresh waters.
14. It is used in induced fish breeding technique to obtain pure fish seed variety for fish farmers.
15. It is found in India, Pakistan, Bangladesh, Nepal and Thailand.

## ***LABEO ROHITA***

**Classification:**

Phylum: Chordata

Class: Pisces

Sub-class: Teleostemi

Order: Cypriniformes

Family: Cyprinidae

**Characters:**

1. It is the most famous carp and commonly known as *Calcutta gande*.
2. The colour of the fish is bluish black along the back reddish black along the sides and silvery in the abdominal area.
3. Body is elongated with moderately rounded abdomen measuring upto 1 metre in length.
4. Scales are large and orange to reddish in colour in the centre.
5. Head is prominent with blunt snout.
6. Mouth is transverse and semi oval.
7. Barbels are absent.
8. Lips thick, fleshy, fringed covering both the jaws, continuous at an angle of mouth forming a labial fold.
9. It is relished very much as food.
10. This fish is a column micro vegetation feeder.

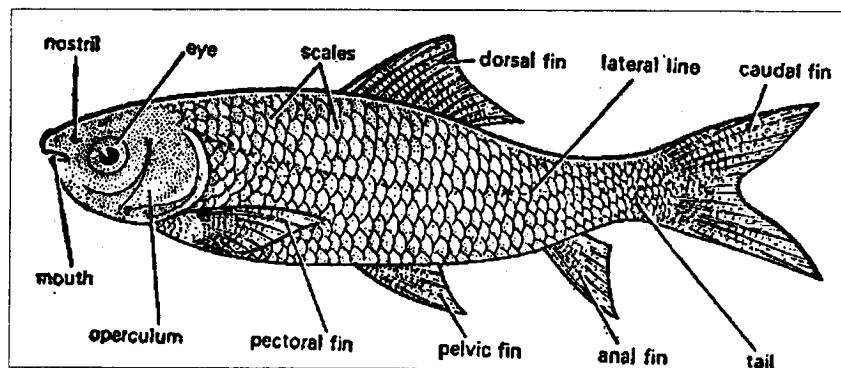


Fig. *Labeo rohita*

11. *Labeo rohita* can be identified by the presence of scales i.e. 6 to 6½ between lateral line and the base of pelvic fin.
12. It is economically important food fish used in induced fish breeding to obtain pure fish seed for farmers.
13. It is found in freshwater of Northern, Central and Southern India.

## *CIRRHINUS MRIGALA*

**Classification:**

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

**Characters:**

1. It is commonly called mrigal or yerra monu found in freshwater bodies.
2. The body is covered by large cycloid scales. The scales are absent on the head.
3. The colour of the body is silvery, dark grey along the back sometime with copper tinge.
4. Mouth is wider with thinner lips.
5. Barbels small in fold of lip.
6. Pectoral, pelvic and anal fins are orange in colour tipped with black.
7. Caudal fin is sharply forked.
8. It gives best sport on rod and line and is used as food.
9. It is a bottom detritus-omnivorous feeder.
10. It is important food fish of Indians.

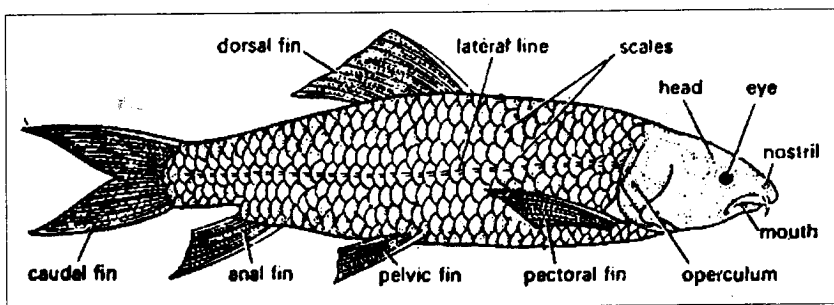


Fig. *Cirrhinus mrigala*

11. This is used in induced fish breeding technique.
12. They spawn once in a year during monsoon.
13. Fecundity is 1 - 2.1 lakhs/kg body weight.
14. This fish is popularly used in composite fish culture and in integrated fish farming.
15. It is found through out India, Nepal and Bangladesh.

## ***CTENOPHARYNGODON IDELLA***

**Classification:**

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

**Characters:**

1. This fish is commonly known as grass carp or white amur.
2. This fish is known as exotic fish introduced in India in 1959 into the Cuttack substation from Japan.
3. Head is depressed and flattened.
4. Mouth is depressed and flattened.
5. The upper jaw is slightly longer than the lower jaw.
6. Eyes are large and lateral in position.
7. Barbels are absent.
8. Dorsal fin is inserted slightly ahead of pelvic fin consists of 10 rays (Branched & Simple).
9. Anal fin is short with 8 branched and 2 simple rays.
10. Caudal fin is forked.

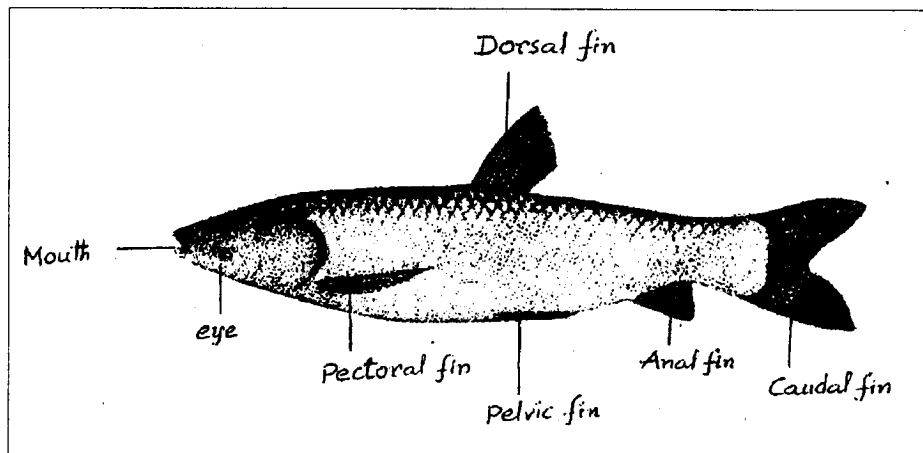


Fig. *Ctenopharyngodon idella*

11. Scales are ctenoid.
12. Lateral line is continuous with 40-42 scales.
13. It is a column feeder, feeds on macro vegetation.
14. It is used to control Aquatic weeds in the pond.
15. It was found in Siberia, Manchuria, China, USSR but now it has been introduced into India.

***HYPOPHTHALMICHTHYS MOLITRIX*****Classification:**

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

**Characters:**

1. It is commonly known as Vendi Chepa or silver carp.
2. It is a phytoplanktonic surface feeder.
3. Snout is bluntly rounded.
4. The body is laterally compressed with a pointed head.
5. Dorsal fin small with less than 9 rays and medially placed.
6. Body is covered with scales. The scales are very small and 110 to 124 in number on lateral line.
7. It has a keeled abdomen.
8. The gills of fish are specially modified as sieving apparatus for sieving small planktonic food.
9. It attains a maximum size of 1.5 – 2 kg/yr and sexual maturity at about 2 years of age.

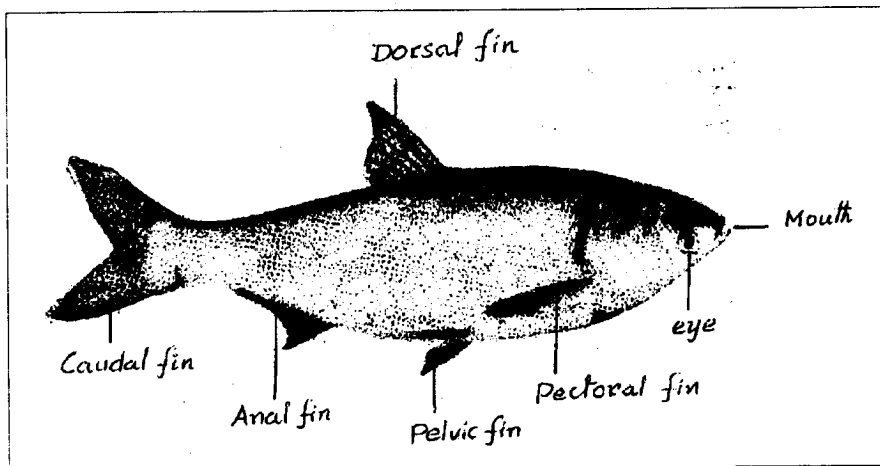


Fig. *Hypophthalmichthys molitrix*

10. It is an exotic fish brought to India from Hongkong in 1959 and was introduced into the ponds of CIFRI, Cuttack.
11. At the age of 3 years, the individual fish can attain a weight upto 7 kg.
12. It is found in fresh waters of Western of India, Nepal, Burma and Philippines.

## *LABEO FIMBRIATA*

**Classification:**

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

Genus: *Labeo*Species: *Fimbriata***Characters:**

1. This is a minor carp found in fresh water areas.
2. Body is moderately elongated, covered with scales.
3. Lips are thick fleshy, fringed covering both the jaws continuous at an angle of mouth forming a labial fold.
4. Dorsal fin rays 19-22. Scales between lateral line and pelvic fin base 6 or 7.
5. It is found in India (W. Bengal, S. India. Not found in W. ghats) & in Nepal, Burma, Pakistan.

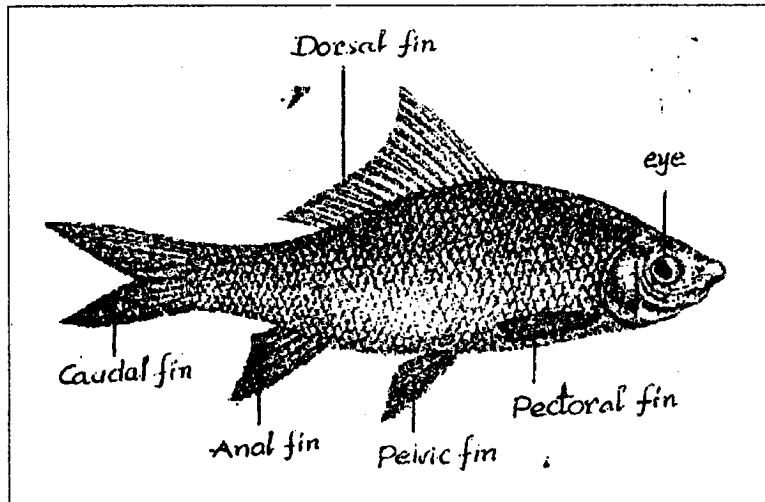


Fig. *Labeo fimbriata*

## *PUNTIUS SARANA*

**Classification:**  
Phylum: Chordata  
Class: Pisces  
Sub-class: Teleostomi  
Order: Cypriniformes  
Family: Cyprinidae

### Characters:

1. It is commonly known as paraka or Gudaparaka.
2. The body is moderately elongated deep and compressed.
3. Head is short.
4. Mouth is arched and it may be anterior or inferior.
5. Eyes are moderate to large and dorso-lateral in position.
6. Two pairs of barbels.
7. Dorsal fin is short and is inserted opposite to pelvic fins.
8. Scales are small, moderate or large.
9. Lateral line consists of 30-34 scales and the scales between midline and lateral line are  $4\frac{1}{2}$  to  $6\frac{1}{2}$  in numbers.

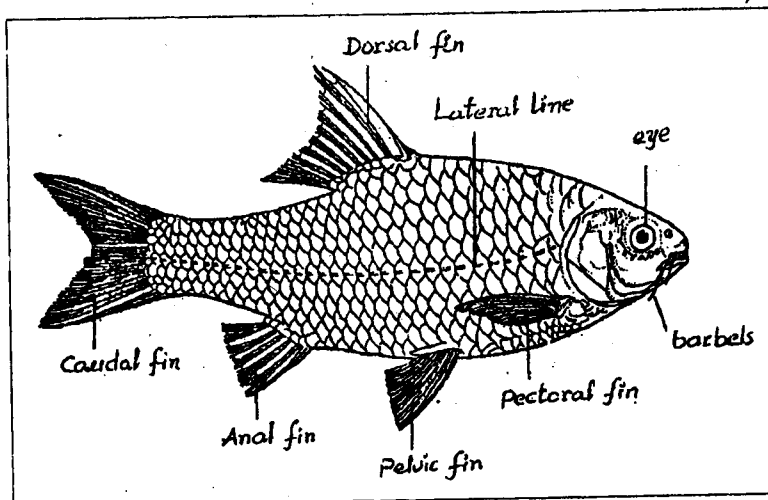


Fig. *Puntius sarana*

0. It is an omnivorous feeder and feeds on detritus filamentous algae, micro vegetation.
1. It breeds during monsoon months.
2. The species have been reported from different parts of India, Pakistan, Bangladesh, Nepal, Srilanka and Burma.



## ***PUNTIUS SOPHORE***

### **Classification:**

Phylum: Chordata

Class: Pisces

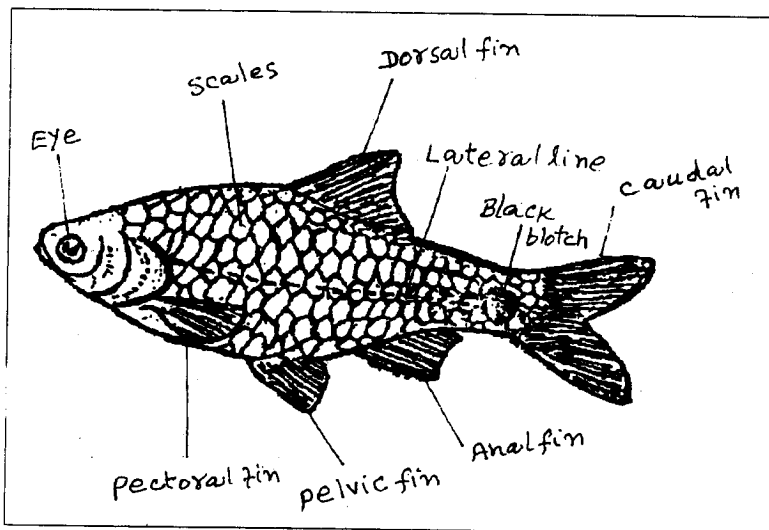
Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

### **Characters:**

1. This is a minor carp found in fresh water.
2. Body is not very deep.
3. Head is short and abdomen rounded.
4. Mouth is arched and it may be anterior or inferior.
5. Eyes are moderate to large and dorso-lateral in position.
6. Barbels are absent.
7. Dorsal fin short inserted nearly opposite to pelvic fins with 11 rays.
8. Anal fin short with 7 to 9 rays.
9. Scales small moderate or large.



**Fig. *Puntius sophore***

10. Lateral line complete with 25-26 scales.
11. A round black blotch is present at the roof of the caudal fin.
12. This species does not attain a table size fish hence it can be removed from the pond farm because it consumes all the available food useful for major carp food fish.

## CHANNA PUNCTATUS

### Classification:

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Channiformes

Family: Channidae

### Characters:

1. It is commonly called live fish and popularly known as snake-heads.
2. Body is elongated anteriorly cylindrical and posteriorly compressed and covered with cycloid scales.
3. Head depressed and covered with large shield like cycloid scales.
4. Dorsal and anal fins are single, long and without spines.
5. Gill opening is wide and lateral in position.
6. Accessory respiratory organs are present in the form of folded linings, richly supplied with blood vessels for taking in air in the paired cavities on the roof of the pharynx.
7. The accessory respiratory organs enable these fishes to survive out of water for a few hours or migrate from one pool to another.
8. It is carnivorous.

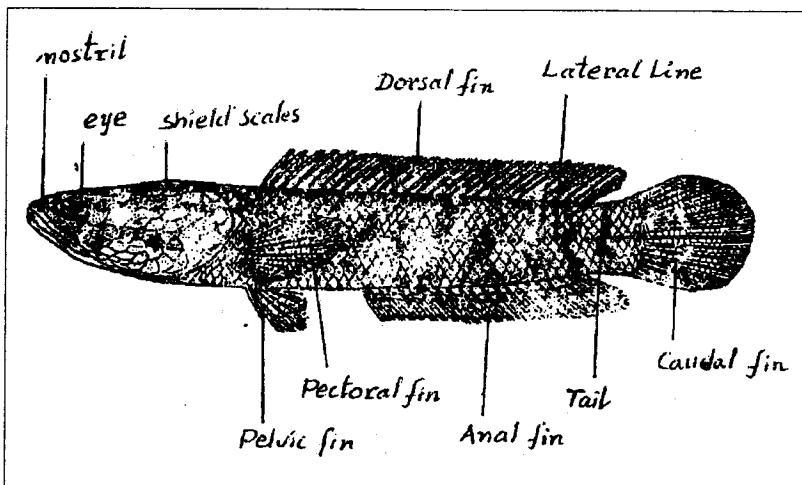


Fig. *Channa punctatus*

9. Air bladder is present and much elongated.
10. It is found in the freshwaters of India, Burma, Ceylon, Malaya, Thailand, China and Islands of East Archipelago.
11. Pelvic fin has more than half the length of pectoral fin.
12. A dark variety with black spots are present. Presence of 15-16 predorsal scales.
13. Found in muddy streams are greenish brown above and yellowish below.
14. In brackish water they may exhibit purple colour. Caudal fin is separate and round in shape.

## *CLARIAS BATRACHUS*

**Classification:**  
 Phylum: Chordata  
 Class: Pisces  
 Sub-class: Teleostomi  
 Order: Siluriformes

### Characters:

1. *Clarias batrachus* is called as mangri in Hindi.
2. The general colour of the body is uniform brown or greyish black.
3. Head depressed with top and sides covered with osseous plates.
4. Sensory barbels are four pairs. Out of these, two pairs of mandibular one pair of maxillary and one nasal.
5. Dorsal fin is long and without spines extending from the neck to the caudal fin.
6. Anal fin also long. No adipose fin.
7. Pectoral fins are provided with spines.
8. Caudal fin is more or less rounded.
9. Accessory respiratory organs are branched tree like especially designed to take oxygen from the air.

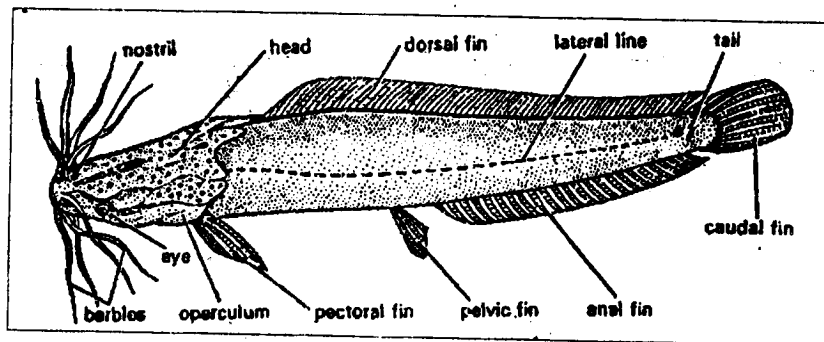


Fig. *Clarias batrachus*

0. The air bladder is connected with internal ear by Weberian ossicles.
1. It is highly nourishing and esteemed as food.
2. It is also used in laboratories for experimental purpose.
3. The size of the eyes are smaller considered to be an adaptation to cope with the muddy habitat.
4. Caudal fin is round and free.
5. *Clarias* is found in Africa and South-West Asia. It occurs in fresh or brackish waters throughout India

## HETEROPNEUSTES FOSSILIS

### Classification:

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Heteropneustidae

### Characters:

1. It is commonly known as "Mapujella".
2. Body is elongated and laterally compressed.
3. Skin without scales.
4. Mouth is narrow and terminal and snout is nearly flat.
5. Head is flattened.
6. Eyes with free circular margins.
7. Barbels are long and four pairs.
8. Dorsal fin is short and has no spine.
9. Ventral fin is situated at the level of dorsal fin.
10. Pectoral fins are strong with poisonous spine.

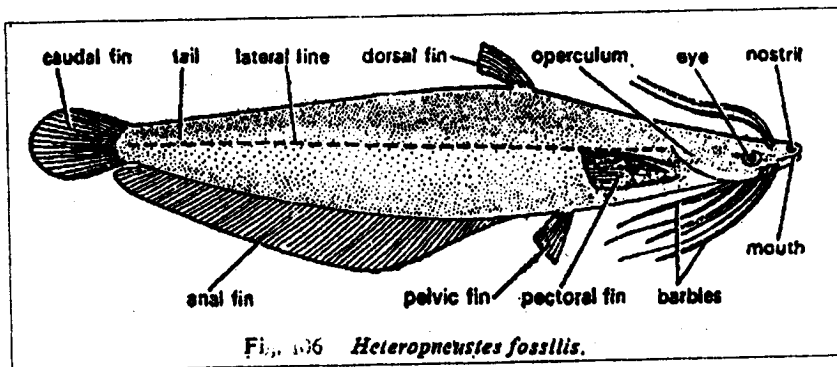


Fig. *Heteropneustes fossilis*

11. Anal fin is elongated reaches upto the caudal fin separated from it by a notch.
12. Gill membranes are not united with the isthmus separated from it by a deep notch.
13. Accessory respiratory organs are present in the form of air sacs which extend into the caudal region.
14. This fish is dark brown in colour but the young one is reddish.
15. This is nourishing food fish and is tasty.
16. It is an important predatory fish.
17. It is found in fresh waters of India, Burma, Pakistan, Srilanka, Nepal, Thailand and Banglades

## OSPHRONEMUS GORAMY

### Classification:

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Perciformes

Family: Osphronemidae

Genus: Osphronemus

Species: Goramy

### Characters:

1. It is commonly known as Gouramy.
2. The colour of the body is greenish brown becoming lighter below.
3. Dorsal fin is shorter than Anal fin.
4. Presence of small conical teeth on the jaws.
5. It is mainly Herbivorous/omnivorous, feeding on fry insect larvae, zooplankton, crustaceans and soft parts of Aquatic vegetation.
6. Male and female take part in nest building.
7. It attains maturity in the 2<sup>nd</sup> year.

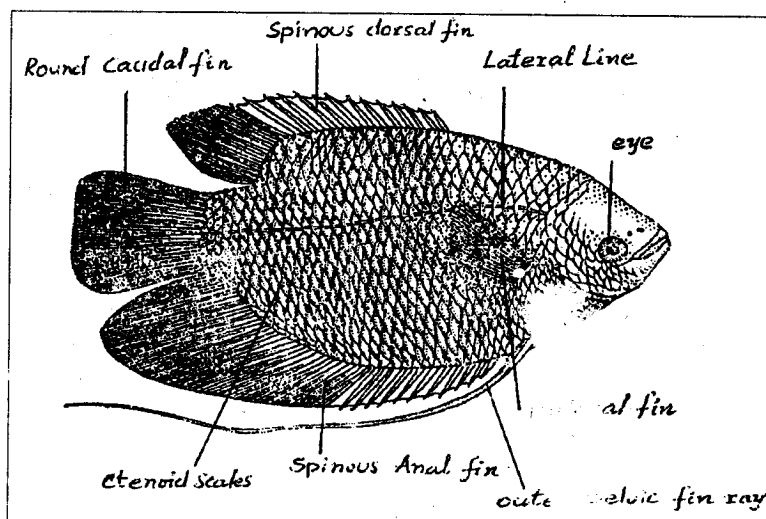


Fig. *Osphronemus goramy*

8. It breeds twice in a year May-June and November-February. The peak breeding season is January.
9. Parental care is important for this fish.
10. This is an exotic species introduced in India into Nilgiri hills of Tamilnadu from the states Java and Mauritius in early 1865.

## *ANABAS TESTUDINEUS*

### Classification:

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

### Characters:

1. *Anabas* is commonly known as climbing perch.
2. It is predator feeds on shrimps, gastropod shells and young fishes.
3. The sensory respiratory organs which are the modification of suprabranchial organs very well developed.
4. The body is laterally compressed and covered with ctenoid scales.
5. Dorsal and Anal fins are long and spinous.
6. Pectoral and pelvic fins are smaller and the caudal fin is well developed.
7. Operculum bears backwardly directed spines.

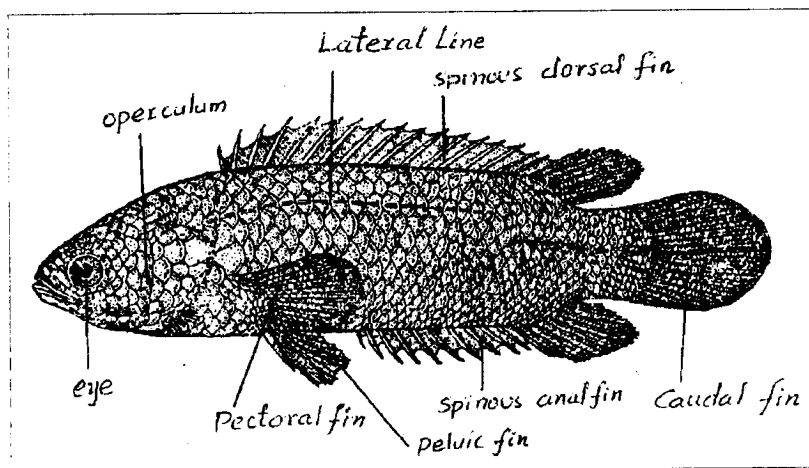


Fig. *Anabas testudineus*

8. This fish can walk on land with the help of fin spines and opercular spines.
9. This fish can live a long time out of water when it remains out of water, it respire by the accessory respiratory organs.
10. It is highly esteemed food fishes at several places.
11. It is found in estuaries and freshwater of India, Ceylon, Burma, Malaya, Peninsula and Archipelago.

## *ETROPLUS SURATENSIS*

**Classification:**

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Perciformes

Family: Cichlidae

Genus: *Etroplus*Species: *Suratensis***Characters:**

1. It is commonly known as pearl spot.
2. Body is oblong, elevated and compressed.
3. Lips thin, jaws equal,
4. Dorsal fin is single with its spinous portion much larger than that of soft portion with 18-19 spines and 14-15 soft rays.
5. Anal spines are 12-13 in number.
6. Body with coloured spots.

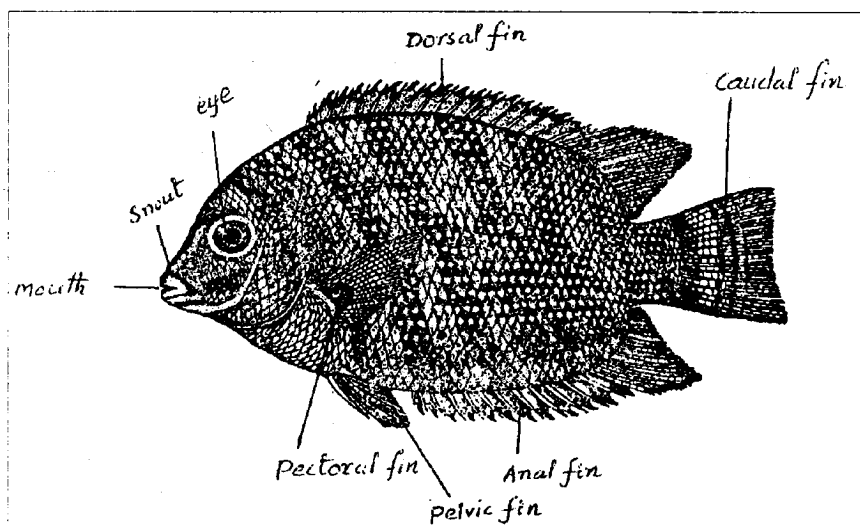


Fig. *Etroplus suratensis*

7. Most of the scales above the lateral line are with a central spot.
8. Snout is spout like.
9. Omnivorous in habit. It feeds preferably on luxuriant growth of aquatic vegetation.
10. It is found in brackish or sea water and also confined to tanks and ponds for breeding.
11. It is found in India especially in Kerala, Tamilnadu, Andhra and Orissa states as well as in Srilanka.

## MUGIL CECPHALUS

**Classification:**  
Phylum: Chordata  
Class: Pisces  
Sub-class: Teleostomi  
Order: Perciformis  
Family: Mugilidae

### Characters:

1. It is commonly known as Grey striped mullet.
2. The colour of the body is greyish and silvery below often with grey stripes lengthwise.
3. Body is elongated and slightly compressed from side to side.
4. Head is short and flattened with broad terminal.
5. Upper lip is thin and smooth.
6. Eye is covered with thick adipose lid.
7. Two dorsal fins, the first one is short with four slender spines.
8. Anal fin originates opposite to the second dorsal fin.
9. Caudal fin with pointed lobes.

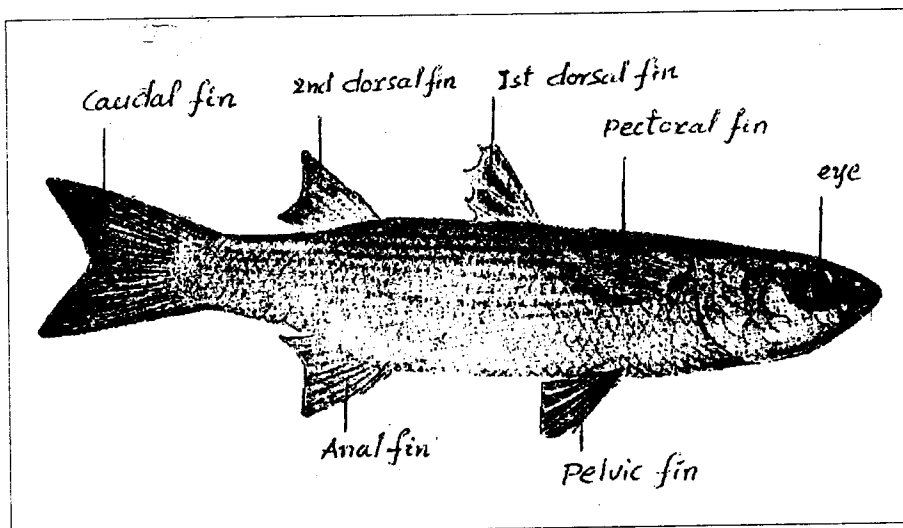


Fig. *Mugil cecphalus*

10. Presence of a dark line along each row of scales in the upper part of the body.
11. It is a filter feeder living on organisms low in food chain.
12. However, different scientists described the mullet as omnivorous, planktonphagous and feeds upon small Crustaceans.



## *CHANOS CHANOS* (Milk fish)

### Classification:

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Cypriniformes

Family: Cyprinidae

### Characters:

1. It is commonly called Milk Fish or White millet.
2. Body is elongated and abdomen is rounded and smooth.
3. The surface of the Head is flat.
4. Single dorsal fin with 13-16 rays arises midway between the front edge of the eye and the base of the caudal fin.
5. Caudal fin is forked with sub-equal lobes.
6. Scales are smaller.
7. Ventrals are inserted under the middle of the dorsal fin.
8. Mouth is small, terminal and transverse.
9. The colour of the body is bright, silvery, greenish along the back and metallic greenish blue on the top of the head.

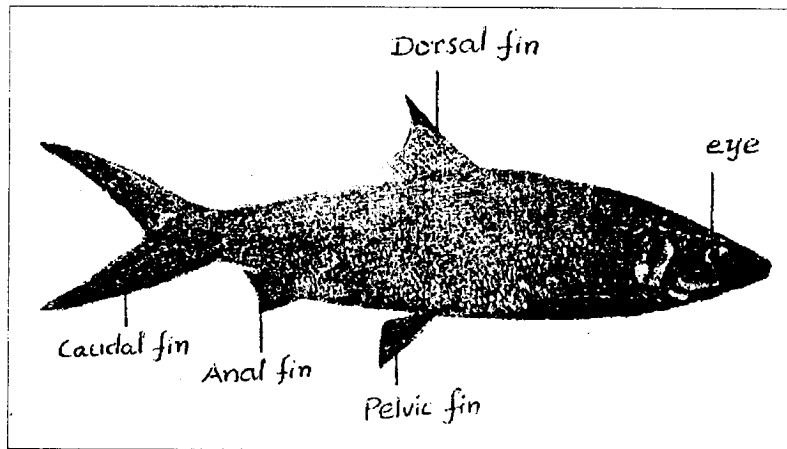


Fig. *Chanos chanos*

10. It is primarily a phytoplankton feeder.
11. It is found in brackish and estuarine areas suitable for cultivation.
12. The fry of *Chanos chanos* occur in great numbers along sandy shallow coasts, tidal creeks and estuarine areas.
13. The post larvae of milk fish 12-14 mm are transparent with one prominent black spot over the air bladder.

## LATES CALCARIFER

### Classification:

Phylum: Chordata

Class: Pisces

Sub-class: Teleostomi

Order: Perciformes

Family: Centropomidae

### Characters:

1. It is commonly known as Sea bass.
2. Scales are ctenoid.
3. Head is of moderate and compressed.
4. Snout is obtusely rounded.
5. Mouth is wide and gape extends to anterior border of eyes.
6. Eyes are moderate and supra-lateral in position.
7. Lower jaw is longer than the upper one.
8. Lips are thin.
9. Presence of two dorsal fins which are united at the base. The first dorsal fin has 7-8 spines.
10. Anal fin has 3 spines and 8-9 rays.

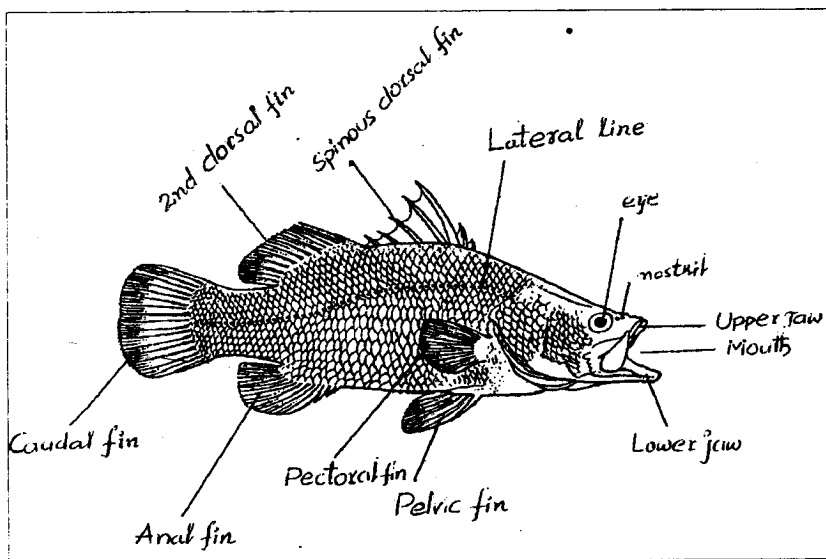


Fig. *Lates calcarifer*

11. Caudal fin is truncate and rounded.
12. Lateral line has 52-60 scales and it is slightly curved.
13. It is a game fish and has better food value.
14. It is an estuarine or brackish water fish.
15. It is a phedatory fish.
16. It is found in different parts of India, Pakistan, Burma, Bangladesh, Thailand and Malaya.

## *PENAEUS MONODON*

**Classification**  
Phylum: Arthropoda  
Class: Crustacea  
Order: Decapoda

### Characters:

1. This is commonly known as Tiger prawn.
2. This is usually found both in brackish water and in seas.
3. Body is divided into thorax and abdomen besides telson.
4. Head consists of a rostrum which is sigmoidal.
5. The body has 3 pairs of walking legs and the abdomen contain 5 pairs of swimming legs.
6. This is grown in ponds usually for 120 days so that to attain a marketable crop.
7. It is a tasty, delicious palatable and has a exportable value.
8. It is benthic in its behaviour and feeds upon animal and plant materials. It also feeds on diatoms and algae. It feeds on lab lab which is a biological association of phyto-zooplankton.
9. The main food item of adults is crustaceans mostly composed of cope pods. It is a detritivore.
10. Body is covered with black and white striped bands, transversely.
11. Head with a pair of compound eyes and a pair of antennules.
12. It is extremely euryhaline in nature and capable of with standing a wide range of salinity and temperature of brackish waters.
13. It has a exportable value of great demand to Japan and USA.

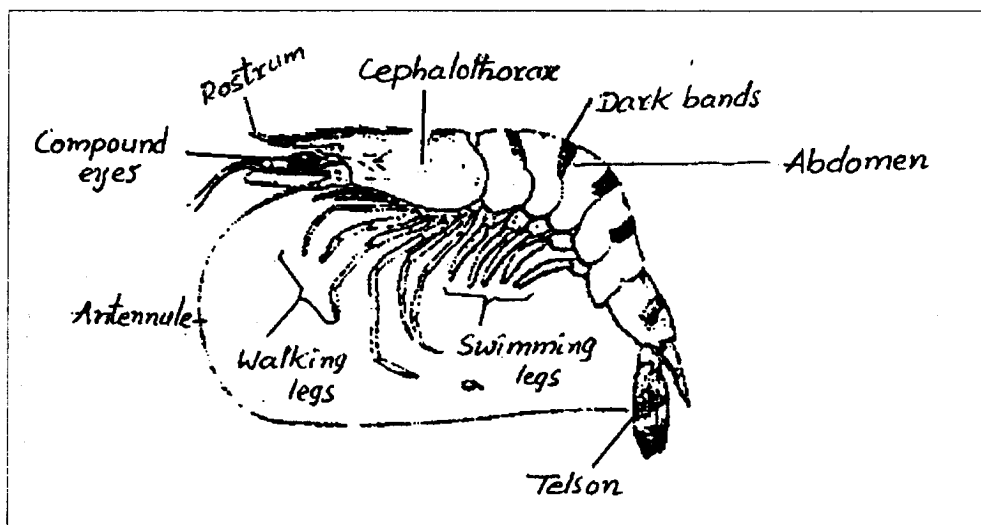


Fig. *Penaeus monodon*

## MACROBRACHIUM ROSENBERGII

### Classification:

Phylum: Arthropoda

Class: Crustacea

Order: Decapoda

### Characters:

1. This is commonly known as giant fresh water prawn.
2. This species is found in freshwaters.
3. This species is suitable for culture in confined waters.
4. The rostrum is long, sword shaped and bears 13-14 rostral spines dorsally and 11 rostral spines ventrally.
5. The second pair of walking legs of males develop abnormally with well developed chela and shows the sexual dimorphism is exhibited.
6. It occurs in rivers, estuaries and coastal areas.
7. It migrates to estuaries during the breeding season.
8. It is the largest among the freshwater prawns attains a maximum length of 32 cms (males) and 25 cm (females) and 450 gr. weight.
9. The breeding season in east-coast is from December to July with a peak in March-May whereas on west-coast it is from August to December.
10. The fecundity is 7000-500,000.

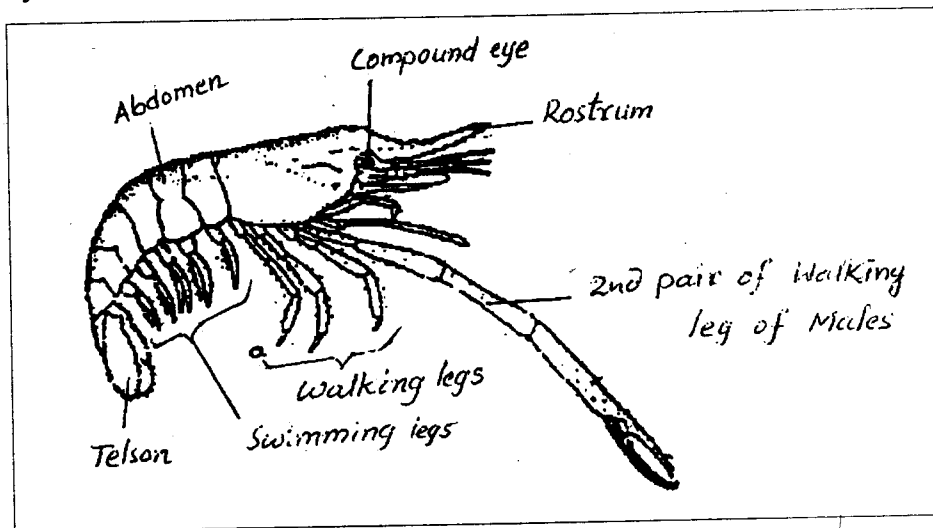


Fig. *Macrobrachium rosenbergii*

11. This is an omnivorous bottom feeder, feeding on molluscs, worms, insects, small crustaceans, vegetable matter, Filamentous algae and tender leaves and stems of aquatic weeds.
12. These are nocturnal animals and they come out at night for food.

13. They under go moultings which depend upon environmental, nutrition, age and season and complete its life cycle in five stages egg larvae, post larve juveniles and adult.
14. Males are bigger than females.
15. These in ditches grow ever upto 750 grams in wild collection.
16. Genital pores of males lying between the base of V walking legs. In females, the genital pores are at the base of III walking leg

17. In females, the eggs can be seen as gray or light orange mass in the dorsal part of cephalothorax.
18. These are cannibalistic grow by moulting. Once it is done the animal goes to shelter to hide and shady corners for protection. Heap of stones, tiles, bricks are used for hide outs. Discarded PVC pipes stone wear pipes, earthen pipes of 2 to 4" diameter are used for shelter.

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- Dr. N. GOPALA RAO  
*M.Sc.,Ph.D.*

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**అధ్యాపకులు, విద్యార్థుల సలహాలు, సూచనలు :**

అధ్యాపకులు, విద్యార్థులు ఈ స్టడీ మెటీరియల్ కు సంబంధించిన సలహాలు, సూచనలు, ముద్రణ దోషాలు తెలియపరచినచో, పునర్ముద్రణలో తగు చర్యలు తీసుకొనగలము. తెలియపరచవలసిన చిరునామా : డిప్యూటీ డైరెక్టర్, దూరవిద్యా కేంద్రం, ఆచార్య నాగార్జున విశ్వవిద్యాలయం, నాగార్జున నగర్ - 522 510.

Course	Year	Title
M.Sc. Zoology	IIInd year	Practical Manual - III, IV



