M.Sc. ZOOLOGY LAB MANUAL

4th Semester

Prepared By

Biological Science Dept.

Zoology

MIDNAPORE CITY COLLEGE

PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for PG Zoology fourth Semester. Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers , and non teaching staffs to make this effort little bit complete. Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

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Environmental Management, Biostatistics, Developmental Biology & Neuro-endocrinology

(50 Marks, 4 credit)

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Paper: ZOO 494

1. Biodiversity and Environmental stress

i. Qualitative and quantitative estimation of soil and aquatic biodiversity.

AQUATIC BIODIVERSITY
PLANKTON ANALYSIS

The physical and chemical characteristics of water affect the abundance, species composition, stability and productivity of the indigenous populations of aquatic organisms. The biological methods used for assessing water quality include collection, counting and identification of aquatic organisms; and processing and interpretation of biological data. The work involving plankton analysis would help in:

- Explaining the cause of colour and turbidity and the presence of objectionable odour, tastes and visible particles in waters.
- The interpretation of chemical analyses.
- Identifying the nature, extent and biological effects of pollution.
- Providing data on the status of an aquatic system on a regular basis.

Plankton: A microscopic community of plants (phytoplankton) and animals (zooplankton), found usually free floating, swimming with little or no resistance to water currents, suspended in water, non-motile or insufficiently motile to overcome transport by currents, are called "Plankton". Phytoplankton (microscopic algae) usually occur as unicellular, colonial or filamentous forms and is mostly photosynthetic and is grazed upon by the zooplankton and other organisms occurring in the same environment. Zooplankton principally comprise of microscopic protozoans, rotifers, cladocerans and copepods. The species assemblage of zooplankton also may be useful in assessing water quality.

Plankton, particularly phytoplankton, has long been used as indicators of water quality. Because of their short life spans, planktons respond quickly to environmental changes. They flourish both in highly eutrophic waters while a few others are very sensitive to organic and/or chemical wastes. Some species have also been associated with noxious blooms causing toxic conditions apart from the tastes and odour problems.

Plankton net: The plankton net is a field-equipment used to trap plankton. It has a polyethylene filter of a defined mesh size and a graduated measuring jar attached to the other end. A handle holds the net. The mesh size of the net determines the size range of the plankton trapped

Sampling Procedure: Plankton net number 25 of mesh size 60 µm was used for collecting samples. 100 litres of water was measured in a graduated bucket and filtered through the net and concentrated in a 100 ml bottle. Samples were collected as close to the water surface as possible in the morning hours and preserved for further analysis.

Labelling: The samples are labelled with the date, time of sampling, study area-lake name, sampling site name and the volume measured and pasted on the containers.

Preservation of the sample: Between the time that a sample is collected in the field and until its analysis in the laboratory, physical, chemical and biochemical changes may take place altering the intrinsic quality of the sample. It is therefore necessary to preserve the samples before shipping, to prevent or minimise changes. This is done by various procedures such as keeping the samples in the dark, adding chemical preservatives, lowering the temperature to retard reactions by freezing or by a combination of these methods. For a phytoplankton sample to be analysed for an extended period, commonly two preservatives are used: Lugol's iodine using acetic acid which will stain cells brownish yellow and will maintain cell morphology and of 4% formaldehyde. The samples collected for this study were preserved by adding suitable amounts of 1 ml chloroform to act as the narcotising agent and 2ml of 4% formalin for preservation and analyses.

Concentration technique: The plankton nets were used to collect samples for the qualitative and quantitative estimation of the plankton, by filtering a known volume of water (100 litres) through the net. The sample was allowed to settle for 24-48 hours and was further concentrated to approximately 30 ml by decanting. The concentration factor is used during the calculations.

Qualitative and quantitative evaluation of plankton: Detailed analyses of phytoplanktonic populations are done by estimating the numbers in each species. The phytoplankton consisting of individual cells, filaments and colonies are counted as individual cells. When colonies of species are counted, the average number of cells per colony is counted, and in filamentous algae, the average length of the filament has to be determined.

Mounting the slides: Preserved samples in bottles are mixed uniformly by gentle inversion and then one drop of the sample is pipetted out from a calibrated pipette onto the glass slide for analysis. A cover slip is carefully placed ensuring no air bubbles remain and the cover slip is ringed with a transparent nail enamel to prevent evaporation during the counting process.

Microscope: A binocular compound microscope is used in the counting of plankton with different eyepieces such as 10X and 40X. The microscope is calibrated using an ocular micrometer.

Counting method: Drop count method – In this method one drop of the sample is pipetted out from a calibrated pipette onto a glass slide and the planktonic organisms are counted in strips. The total area under the cover slip represents the number of organisms present per given volume of the sample. This volume expanded to an appropriate factor yields the organisms per litre of water for the lake.

Phytoplankton Counting Units: Some plankton are unicellular while others are multicellular (colonial), posing a problem for enumeration. For analysis, a colony of plankton is accounted as a single count.

Formula for calculating organisms per litre:

Total plankton count per litre = A * (1/L) * (n/v)

Where, A = number of organisms per drop

L = volume of original sample (l)

n = total volume of concentrated sample (ml)

v = volume of one drop (ml)

ESTIMATION OF SOIL BIODIVERSITY

Introduction : Soil biodiversity is an important resource that provides ecosystem processes essential to the functioning of natural and global systems. Our understanding of the species, their interactions, and effect on processes occurring in the soil food web in natural systems are an important contribution to management of land, particularly agriculture. The link between aboveground and belowground diversity is strong, although occurring at different temporal scales for organisms, and changes affecting aboveground diversity and function are reflected in belowground ecosystems. An immediate effect is a decrease in the biological capacity of soils and a change in the regulation of interactions and processes. Knowledge on whether all or a few key taxa are important in this regulation of ecosystems processes is a high priority for planning for future sustainability.

Key Functional Groups : The taxonomic diversity of the soil biota is so high that inevitably some selection must be made. The taxonomic groups described below were selected on the basis of their diverse functional significance to soil fertility (hence the term "target taxa"); and their relative ease of sampling.

1) Earthworms, which influence both soil porosity and nutrient relations through channeling, and ingestion of mineral and/or organic matter.

2) Termites and ants, which influence a) soil porosity and texture through tunnelling, soil ingestion and transport, and gallery construction; b) nutrient cycles through transport, shredding and digestion or organic matter.

3) Other macrofauna such as woodlice, millipedes and some types of insect larvae which act as litter transformers, with an important shredding action on dead plant tissue, and their predators (centipedes, larger arachnids, some other types of insect)

4) Nematodes, which a) influence turnover in their roles as root grazers, fungivores, bacterivores, omnivores and predators b) occupy existing small pore spaces in which they are dependent on water films and c) usually have very high generic and species richness.

5) Mycorrhizas, which associate with plant roots, improving nutrient availability and reducing attacks by plant pathogens.

6) Rhizobia and, when relevant, other N-fixing microsymbionts which transform N2 into forms available for plant growth.

7) Microbial biomass, which is an indirect measure of the total decomposition and nutrient recycling community of a soil. Microbial biomass is contributed by three very diverse taxa: fungi, protists and bacteria (including archaea and actinomycetes), but it is not usually practical to separate these during measurements. Microbial biomass estimation usually depends on relatively crude chemical methods (lysis of cells, followed by determinations of total N (and P), conversion of these values to a C equivalent, and comparisons with unlysed control samples). It may thus have relatively low resolution, but assesses the decomposer community as a whole.

Sampling design:

Overall strategy: Macrofauna, microbiota and soil (for physical and chemical analyses) are sampled in transects, for which the optimum size is 40 x 4m. However, for the quantitative sampling of termites and for a number of above-ground studies (particularly plant functional attributes and C sequestration) quadrats of 40 x 5m have been deployed, and it seems advisable to standardize both above-ground and below-ground work at 40 x 5m (Figure 2). In further amendments to the procedures, pitfall trapping of surface-active invertebrates and a 100m qualitative transect for termites have been added to the sampling. These can take place along one flank of the transect (pitfalls) or in parallel at about 5-10m distance (termite transect). These modifications are intended, in part, to contribute elements of true biodiversity to the dataset by achieving resolution at the species level, but also to mitigate the variability of data from short transects on groups with typically patchy distributions. Replication of transects in each site is also desirable, as it facilitates statistical analysis of the data obtained, though this may not always be practical where time and funding are limited.

Macrofauna : Procedures follow Anderson and Ingram (1993), making use of pitfall traps together with the digging of soil monoliths of dimensions 25x25x30 (depth) cm. An additional 100x2m sampling transect is used for termites.

Sampling from the 40 x 5 m transect : a. 5-10 sampling points (for monoliths) are located and marked. These should be equally spaced along the transect. The larger the number of monoliths, the more comfortable the subsequent statistical analysis of the data obtained (see below). It is suggested that 8 should be the target, although 5 will suffice as a minimum number.

b. 10 pitfall traps are installed at roughly 4m intervals along one flank of the transect. The traps are put in during the afternoon or early evening and emptied 24 hours later. Each trap contains a little water, with a few drops of detergent added to immobilize specimens by drowning. Glass jars of about 10-15cm mouth diameter make suitable traps. Depth of the traps is not critical, but the mouth must be exactly flush with the surface of the ground. A sloped cover (for example an inverted petri dish, or a piece of plywood or plastic), supported on twigs over the jar, is useful to keep rain out.

c. At each sampling point, litter is removed from within a 25cm quadrat and hand-sorted at the site. Following this the exact position of the monolith of is marked with a wooden or metal quadrat of 25x25cm outside dimensions.

d. Isolate the monolith by cutting down with a spade a few centimetres outside the quadrat and then digging a 20cm wide and 30cm deep trench around it. NB. In a variant of the method, all invertebrates longer than 10 cm excavated from the trench are collected; these will be mainly large millipedes and earthworms with very low population densities but representing an important biomass. Their abundance and biomass can be calulated on the basis of 0.42m2 samples, i.e. the width of the block plus two trench widths, squared.

e. Divide the delimited monolith block into three layers, 0-10cm, 10-20cm and 20- 30cm. This can be done conveniently using a machette or parang held horizontally and grasped at both ends. Hand-sort each layer separately. If time is short or the light poor (sorting in closed canopy forest is usually difficult after about 3.30 pm), bag the soil and remove to a laboratory. Ants can be extracted by gently brushing small (handful) quantities of soil through a course (5mm) sieve into a tray: the sieve retains the ants. Bagged soil should be kept out of direct sunlight and sorted within 24 hours (but preferably sooner).

f. Record the number and fresh weight of all animals and identify to at least the taxonomic and functional levels indicated in Table 2 (but preferably further). The presence and weight of termite fungus combs (if any) should also be noted. If a balance is not available in the

field, fresh weight can be approximated for preserved specimens by weighing them after light blotting.

g. Make a list of species, if possible grouped into subfamilies or families. Within each of these, use generic names to generate alphabetical orders. Combine the results from pitfall traps and monoliths to compile this list.

ii. Basic principles for the estimation of heavy metals.

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. The earliest known metals—common metals such as iron, copper, and tin, and precious metals such as silver, gold, and platinum—are heavy metals. From 1809 onward, light metals, such as magnesium, aluminium, and titanium, were discovered, as well as less well-known heavy metals including gallium, thallium, and hafnium. Heavy metals are relatively scarce in the Earth's crust but are present in many aspects of modern life. They are used in, for example, golf clubs, cars, antiseptics, self-cleaning ovens, plastics, solar panels, mobile phones, and particle accelerators.

PRINCIPLE

Heavy metals are here defined as those metallic impurities that are colored by hydrogen sulfide under the conditions of this test. Concentration is determined by visual comparison with lead standards treated similarly. If there is color interference the organic matter in the sample is first destroyed by ignition in the presence of a small amount of sulfuric acid and the residue is dissolved in dilute acid.

SCOPE

The method applies to the determination of heavy metals in corn syrups, corn sugars, refined sugars and syrup solids, including USP dextrose .

SAFETY NOTE

This procedure requires the use of several extremely hazardous chemicals. Thorough knowledge of the dangers and safety procedures is required. Proper protective equipment is also required.

SPECIAL APPARATUS

1. **Muffle furnace:** equipped with pyrometer and capable of operating at controlled temperatures up to 550 °C.
2. **Platinum or silica dishes:** 100 to 200 mL capacity.
3. **Nessler Tubes:** Matching 50 mL tall form tubes and viewing stand.

REAGENTS

1. **Sulfuric Acid solution,** (1:3): Cautiously pour 500 mL of concentrated sulfuric acid (96% H_2SO_4, sp g 1.84, lead free) into 1500 mL of purified water and mix.
2. **Hydrochloric Acid Solution,** (1:3): Add 500 mL of concentrated hydrochloric acid, (37% HCl, sp g 1.19) to 1500 mL of purified water and mix.
3. **Acetic Acid, (CH_3COOH) 6%:** Dilute 60 mL glacial acetic acid, sp g 1.06 to 1.0 L with purified water and mix.
4. **Ammonium Hydroxide Solution, (NH_4OH)** (1:1 v/v): Add 500 mL of concentrated NH_4OH, (28% NH_4OH, sp g 0.90; 14.8 N), to 500 mL of purified water and mix.
5. **Hydrogen Sulfide Solution,** Saturated: Hydrogen sulfide gas is toxic . Use special precautions to contain in hood. Vent only through a scrubber (Notes 3, 4). Bubble hydrogen sulfide (H_2S) gas from a cylinder through cold water until saturated, (about 10 mins.). The saturated solution should produce, at once, a copious precipitate of sulfur when added to an equal volume of 10% ferric chloride solution. Prepare fresh solution just prior to use.
6. **Standard Lead (Pb) Solution, 10 µg/mL:**

Stock Solution:

Dry lead nitrate [$Pb(NO_3)_2$] overnight in a desiccator. Dissolve 0.1600 g of reagent grade lead nitrate in water, add 1.0 mL of concentrated nitric acid (HNO_3) and dilute with purified water to 1.0 L. Store in glass container which is free from soluble lead salts.

Standard Solution:

Pipet 10.0 mL of the stock lead solution and dilute with purified water to 100 mL and mix thoroughly. Prepare fresh standard solutions daily.

PROCEDURE

i. Weigh accurately about 5 g of sample and transfer with a minimum quantity of purified water to a 50 mL Nessler tube .

ii. Pipet 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mL of standard lead solution into 6 separate 50 mL Nessler tubes.

iii. From this point treat all tubes in the same manner. Add 2 mL of 6% acetic acid, dilute to 25 mL with purified water and mix.

iv. Add 10 mL of saturated hydrogen sulfide solution, mix and allow to stand 10 minutes.

v. Compare color in sample tube with that in standard tubes by viewing downward over a white surface.

vi. Determine the volume of standard lead solution (to nearest 0.5 mL) required to obtain a color match. If there is a color interference, follow the ash procedure below:

vii. Weigh about 10.0 g of sample (dry basis) into a platinum or silica dish. Add 5 mL of 1:3 sulfuric acid, distributing the acid uniformly in the sample.

viii. Place the dish on a steam bath or low temperature hot plate to evaporate the water; increase heat to carbonize the sample and expel most of the sulfuric acid.

ix. Place the dish in a muffle furnace at 525-550 °C until the ash is free from carbon (refer to CRA ash procedure).

x. Cool to room temperature. Carefully wash down sides of dish with 3-5 mL of purified water; add 5 mL of 1:3 hydrochloric acid and evaporate to dryness on a steam bath.

xi. Pipet 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mL of standard lead solution into separate beakers.

xii. From this point treat samples and standard in the same manner. Add 5 mL of 1:3 hydrochloric acid and 10 mL of purified water.

xiii. Bring to boil on a hot plate and boil gently for 10 mins. Cool and carefully add 1:1 ammonium hydroxide solution until the solution is neutral to litmus paper. Add 2 mL of 6% acetic acid solution.

xiv. Filter quantitatively if solution is hazy. Transfer solution to Nessler tube, dilute to 30 mL volume with purified water and mix.

xv. Add 10 mL of saturated hydrogen sulfide solution, mix and allow to stand for 10 mins.

xvi. Compare sample color with that of the standards. Determine volume of standard lead solution (to nearest 0.5 mL) required to obtain a color match.

CALCULATION

Heavy metal ppm (as expressed as lead)

= (ml Std. Pb solution)(0.00001)(1000000) / Sample ,Wt., gm

ESTIMATION OF COD

PRINCIPLE:

The chemical oxygen demand (COD) determines the amount of oxygen required for chemical oxidation of organic matter using a strong chemical oxidant, such as, potassium dichromate under reflux conditions. This test is widely used to determine: a) Degree of pollution in water bodies and their self-purification capacity, b) Efficiency of treatment plants, c) Pollution loads, and d) Provides rough idea of Biochemical oxygen demand (BOD) which can be used to determine sample volume for BOD estimation. The limitation of the test lies in its inability to differentiate between the biologically oxidizable and biologically inert material and to find out the system rate constant of aerobic biological stabilization. Most of the organic matters are-destroyed when boiled with a mixture of potassium dichromate and sulphuric acid

producing carbon dioxide and water. A sample is refluxed with a known amount of potassium dichromate in sulphuric acid medium and the excess of dichromate is titrated against ferrous ammonium sulphate. The amount of dichromate consumed is proportional to the oxygen required to oxidize the oxidizable organic matter.

REAGENTS:

Standard Potassium dichromate ($K_2Cr_2O_7$) digestion solution, 0.01667M: Add to about 500 mL

distilled water 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150°C for 2 h, 167 mL conc. H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 mL.

Sulfuric acid reagent: Add H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4 or 10.12 g silver sulphate/L H_2SO_4. Let stand 1 to 2 d to dissolve and mix. This accelerates the oxidation of straightchain aliphatic and aromatic compounds. (1 Kg = 543.47826 mL of H_2 SO_4 and take 20.24 g of Ag_2SO_4 to 2 L of H_2 SO_4 or 22.264 g of Ag_2SO_4 to 2.2 L of H_2 SO_4)

Ferroin Indicator solution: This indicator is used to indicate change in oxidation-reduction potential of the solution and indicates the condition when all dichromate has been reduced by ferrous ion. It gives a very sharp brown color change which can be seen in spite of blue color generated by the Cr^{3+} ions formed on reduction of the dichromate.

Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10M: Dissolve 39.2 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 mL conc. H_2SO_4, cool, and dilute to 1000 mL. Standardize solution daily against standard $K_2Cr_2O_7$ digestion solution as follows: Pipet 5.00 mL digestion solution into a small beaker. Add 10 mL reagent water to substitute for sample. Cool to room temperature. Add 1 to 2 drops diluted Ferroin indicator and titrate with FAS titrant. Molarity of FAS solution = [$VK_2Cr_2O_7$ ×0.1] / (VFAS) Where: $VK_2Cr_2O_7$ = volume of $K_2Cr_2O_7$ (mL); VFAS = volume of FAS (mL)

PROCEDURE:

1. Wash culture tubes and caps with 20% H_2SO_4 before using to prevent contamination.

2. Place sample (2.5 mL) in culture tube and Add $K_2Cr_2O_7$ digestion solution (1.5 mL).

3. Carefully run sulphuric acid reagent (3.5 mL) down inside of vessel so an acid layer is formed under the sample-digestion solution layer and tightly cap tubes or seal ampules, and invert each several times to mix completely.

4. Place tubes in block digester preheated to 150°C and reflux for 2 h behind a protective shield.

5. Cool to room temperature and place vessels in test tube rack. Some mercuric sulfate may precipitate out but this will not affect the analysis.

6. Add 1 to 2 drops of Ferroin indicator and stir rapidly on magnetic stirrer while titrating with standardized 0.10 M FAS.

7. The end point is a sharp color change from blue-green to reddish brown, although the blue green may reappear within minutes.

8. In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.

CALCULATION:

COD is given by COD (mg O_2 /L) = [(A-B) × M ×8000) / (V sample)

Where: A = volume of FAS used for blank (mL) B = volume of FAS used for sample (mL) M = molarity of FAS 8000 = milli equivalent weight of oxygen (8) ×1000 mL/L.

ESTIMATION OF BOD

Biological Oxygen Demand (BOD): The amount of oxygen, taken up by the microorganisms that decompose the organic waste matter in wastewater is known as biological oxygen demand or biochemical oxygen demand. Therefore it is used to measure the amount of certain type of organic water pollution BOD is calculated by keeping a sample of water containing a known amount of oxygen for five days at 20 °C.

The oxygen content is measured again and BOD is calculated. A high BOD indicates the presence of a large number of microorganisms which indicates a high level of pollution in wastewater.

1. Neutralization of Sample: The accuracy of BOD test totally depends upon the proper bacterial growth, present in the water sample. pH of the diluted sample should be adjusted 7.00 ±0.2 before the incubation for five days for proper results. The sample should be neutralized in the following manner.

I) Take 50 ml of sample in a 100 ml beaker.

ii) Measure the pH of the solution by using the calibrated pH meter.

iii) Add the 1N sulfuric acid to adjust the pH if it is higher than 7.00 and 1N sodium hydroxide if pH is lower than 7.00.

iv) Note down the volume of sulfuric acid or sodium hydroxide used to adjust the pH of 50 ml sample to 7.00 ±0.2.

v) Calculate the volume of sulfuric acid or sodium hydroxide required to neutralize the 1000 ml sample.

vi) Add the calculated volume of sulfuric acid or sodium hydroxide to the sample to neutralize. For example, if 2.1 ml of 1N sulfuric acid or sodium hydroxide are used to neutralize 50 ml of sample to pH 7.00 ±0.2. Calculate the volume of 1N sulfuric acid or sodium hydroxide to be added to neutralize the 1000 ml sample as follows: 1N sulfuric acid or sodium hydroxide required = (2.1 ml x 1000 ml)/50 ml = 2100/50 = 42 ml. Note: Hydrochloric acid or other acid containing chlorine should not be used to neutralize the sample because chlorine interferes the results of wastewater BOD.

2. Removal of Chlorine Content: Chlorine is a strong oxidizing agent and it can inhibit the microbial growth during wastewater BOD analysis, so it should be removed from sample before start the analysis. Chlorine can be removed by adding the sodium sulfite to the sample in following manner.

I) Take 50 ml of water sample to be tested in a conical flask.

ii) Add 2.5 ml of acetic acid diluted to 50% with water.

iii) Add 2.5 ml of 10% w/v solution of potassium iodide.

iv) Add 1 ml of starch indicator and titrate with 0.025N sodium sulfite solution.

v) Note down the volume and calculate to add in 1000 ml of the sample as described above in Neutralization of Sample section.

vi) Add the calculated volume of sodium sulfite solution to the sample and mix well to neutralize the chlorine.

3. Preparation of Phosphate Buffer Solution: Dissolve accurate weighed 8.5 gm of potassium dihydrogen phosphate (KH2P04), 21.75 gm of Dipotassium hydrogen phosphate (K2HP04), 33.4 gm of Disodium hydrogen phosphate (Na2HP04.7H20) and 1.7 gm of ammonium chloride (NH4Cl) in 500 ml distilled water. Dilute the solution up to 1000 ml.

4. Preparation of Alkali-Iodide-Azide Reagent: Dissolve 500 gm of sodium hydroxide (NaOH) and 135 gm of sodium iodide (NaI) in distilled water. Make up the solution to 1000 ml of distilled water. Now dissolve 10 gm of sodium azide in this solution.

5. Preparation of Dilution Water: The dilution water for wastewater BOD analysis must be free from organic content. Dilution water can be prepared by the following method.

i. i)Take five liters of double distilled water in a glass container
ii. ii) Aerate the water with the clean compressed air for not less than 12 hours.
iii. iii) Allow to stable for at least 6 hours at 20 °C.
iv. iv) Add 5 ml of 27.5% w/v solution of calcium carbonate.
v. v) Add 5 ml of 22.5 % w/v solution of magnesium sulfate.
vi. vi) Add 5 ml of 0.15% w/v solution of ferric chloride.
vii. vii) Add 5 ml of phosphate buffer solution.
viii. viii) Mix well and allow to stand for 2 hours.

6. Procedure to Determine the Biological Oxygen Demand of Water:

i. Take four 300 ml BOD bottles and add 10 ml of samples to two bottles and fill the remaining volume with dilution water.
ii. Fill remaining two BOD bottle only with dilution water for blank.
iii. Immediately close the bottles when filled and there should not be any air bubble in the bottle.
iv. Mark the bottles as blank and sample.
v. Incubate one sample and one blank bottle at 20 °C for 5 days.
vi. Analyze immediately remaining one blank and one sample bottle of dissolved oxygen (DO).
vii. Analyze incubated bottles for DO after 5 days.

Recommended sample volume for BOD determination:

Bod range (mg/lit)	Sample volume (ml)	Dilution water(ml)
0-10	300	0
11-30	100	200
31-70	100	250
71-150	25	275
151-310	10	290
311-630	5	295
631-1270	2	298
1270-2500	1	299

7. Test for Dissolved Oxygen (DO):

i) Add 2 ml of 36.4% of manganous sulfate ($MnSO_4.H_2O$) solution inserting the tip of pipette tip into the sample because the drops of solution can allow inserting the oxygen into the solution.

ii) Add 2 ml of the alkali-iodide-azide reagent by above method.

iii) Allow reacting the solutions with the oxygen present in the sample.

iv) When precipitates are settled down at the bottom add 2 ml of concentrated sulfuric acid by placing the pipette tip very near to sample surface.

v) Mix well to dissolve the precipitates.

vi) Take 203 ml of sample from BOD bottle into an Erlenmeyer flask.

vii) Titrate immediately with 0.025N sodium thiosulfate solution using starch indicator until blue color disappears and note down the burette reading.

viii) Determine the burette reading for blank in the same manner.

Calculation: Blank correction = B.R. for blank at D0 – B.R. for blank at D5

BOD mg/l = [(B.R. for sample at D0 –D5)– blank correction] x dilution factor

Dilution factor = Bottle volume (300 ml) / Sample volume

[Where: B.R. = burette reading, D0 = Initial, D5 = Day five after incubation]

2. Biostatistics

i. Chi square test for goodness of fit with a Mendelian frequency distribution.

Chi-Square Goodness of Fit Test

When an analyst attempts to fit a statistical model to observed data, he or she may wonder how well the model actually reflects the data. How "close" are the observed values to those which would be expected under the fitted model? One statistical test that addresses this issue is the chi-square goodness of fit test. This test is commonly used to test association of variables in two-way tables , where the assumed model of independence is evaluated against the observed data. In general, the ***chi-square test statistic*** is of the form.

The **Chi-square test** is intended to **test** how likely it is that an observed distribution is due to chance. It is also called a "goodness of fit" statistic, because it measures how well the observed distribution of data fits with the distribution that is expected if the variables are independent.

$$X^2 = \sum \frac{(\text{observed - expected})^2}{\text{expected}}$$

If the computed test statistic is large, then the observed and expected values are not close and the model is a poor fit to the data.

The **goodness of fit** test is used to test if sample data fits a distribution from a certain population (i.e. a population with a normal distribution or one with a Weibull distribution). In other words, it **tells you** if your sample data represents the data **you would** expect to find in the actual population

Example

A new casino game involves rolling 3 dice. The winnings are directly proportional to the total number of sixes rolled. Suppose a gambler plays the game 100 times, with the following observed counts:

Number of Sixes	Number of Rolls
0	48
1	35
2	15
3	3

The casino becomes suspicious of the gambler and wishes to determine whether the dice are fair. What do they conclude?

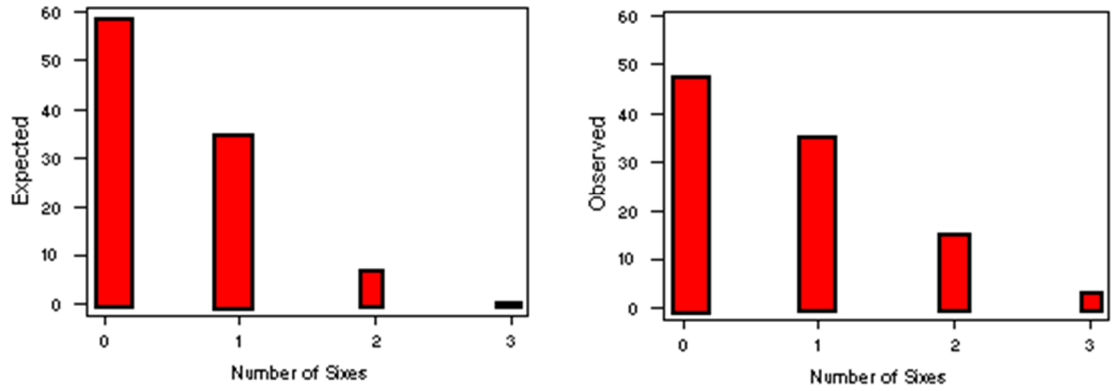
If a die is fair, we would expect the probability of rolling a 6 on any given toss to be 1/6. Assuming the 3 dice are independent (the roll of one die should not affect the roll of the others), we might assume that the number of sixes in three rolls is distributed Binomial (3,1/6). To determine whether the gambler's dice are fair, we may compare his results with the results expected under this distribution. The expected values for 0, 1, 2, and 3 sixes under the Binomial (3,1/6) distribution are the following:

Null Hypothesis:
p_1 = P(roll 0 sixes) = P(X=0) = 0.58
p_2 = P(roll 1 six) = P(X=1) = 0.345
p_3 = P(roll 2 sixes) = P(X=2) = 0.07
p_4 = P(roll 3 sixes) = P(X=3) = 0.005.

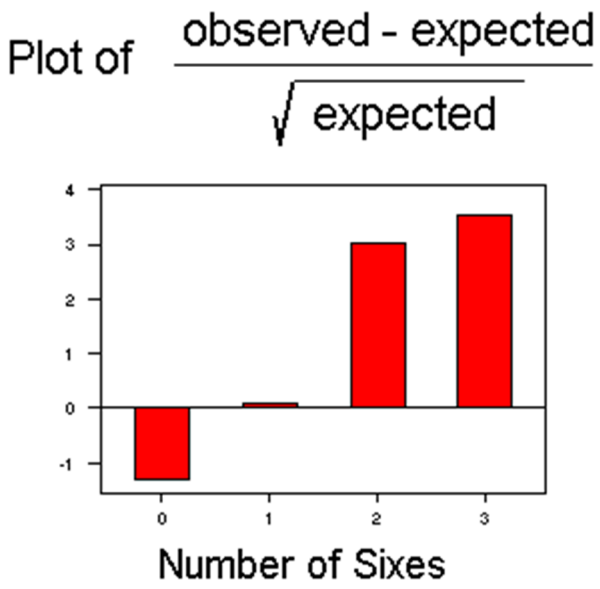
Since the gambler plays 100 times, the expected counts are the following:

Number of Sixes	Expected Counts	Observed Counts
0	58	48
1	34.5	35
2	7	15
3	0.5	3

The two plots shown below provide a visual comparison of the expected and observed values:



From these graphs, it is difficult to distinguish differences between the observed and expected counts. A visual representation of the differences is the *chi-gram*, which plots the observed - expected counts divided by the square root of the expected counts, as shown below:



The chi-square statistic is the sum of the squares of the plotted values,
$(48-58)^2/58 + (35-34.5)^2/58 + (15-7)^2/7 + (3-0.5)^2/0.5$
$= 1.72 + 0.007 + 9.14 + 12.5 = 23.367.$

Given this statistic, are the observed values likely under the assumed model?

A random variable χ^2 is said to have a chi-square distribution with *m* degrees of freedom if it is the sum of the squares of *m* independent standard normal random variables (the square of a single standard normal random variable has a chi-square distribution with one degree of freedom). This distribution is denoted χ^2(*m*), with associated probability values available in Table G in Moore and McCabe and in MINITAB.

The standardized counts (observed - expected)/sqrt(expected) for *k* possibilities are approximately normal, but they are not independent because one of the counts is entirely determined by the sum of the others (since the total of the observed and expected counts must sum to *n*). This results in a loss of one degree of freedom, so it turns out the the distribution of the chi-square test statistic based on *k* counts is approximately the chi-square distribution with $m = k-1$ degrees of freedom, denoted χ^2(*k-1*).

Hypothesis Testing

We use the chi-square test to test the validity of a distribution assumed for a random phenomenon. The test evaluates the null hypotheses H_0 (that the data are governed by the assumed distribution) against the alternative (that the data are not drawn from the assumed distribution).

Let $p_1, p_2, ..., p_k$ denote the probabilities hypothesized for *k* possible outcomes. In *n* independent trials, we let $Y_1, Y_2, ..., Y_k$ denote the observed counts of each outcome which are to be compared to the expected counts $np_1, np_2, ..., np_k$. The chi-square test statistic is q_{k-1} =

$$X^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$= (Y_1 - np_1)^2 + (Y_2 - np_2)^2 + \ldots + (Y_k - np_k)^2$

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np_1 np_2 np_k

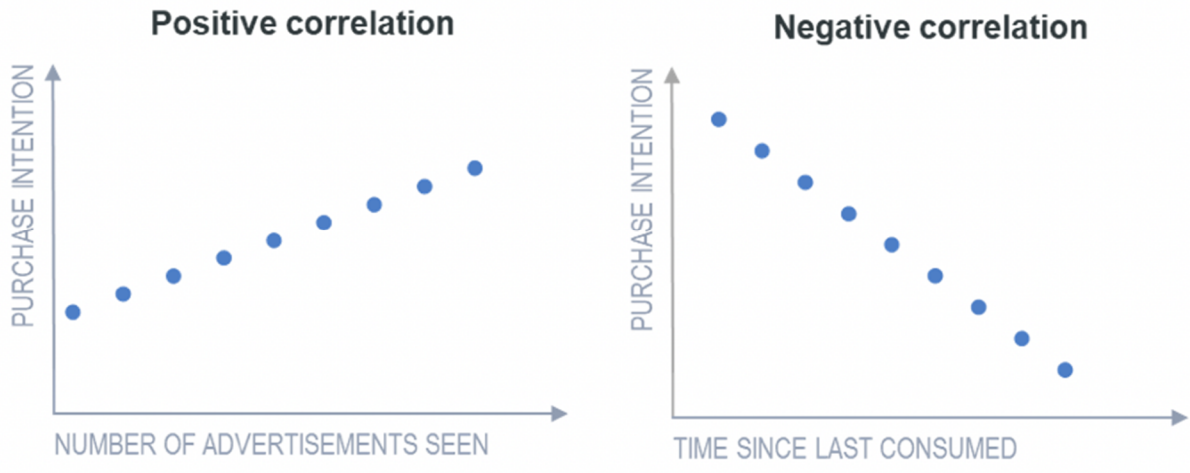
Reject H_0 if this value exceeds the upper α critical value of the $\chi^2(k\text{-}1)$ distribution, where α is the desired level of significance.

Computation and significance test of product – moment r between two continuous measurement variables.

Correlation is usually defined as a measure of the *linear* relationship between two quantitative variables (e.g., height and weight). correlation simply means that there is some type of relationship between two variables. This post will define positive and negative correlation.

When the values of one variable increase as the values of the other increase, this is known as ***positive correlation .***

When the values of one variable decrease as the values of another increase to form an inverse relationship, this is known as ***negative correlation.***



Where it is possible to predict, with a reasonably high level of accuracy, the values of one variable based on the values of the other, the relationship between the two variables is described as a *strong correlation.* A *weak correlation* is one where on average the values of one variable are related to the other.

Pearson's Product-Moment Correlation

The most common measure of correlation is *Pearson's product-moment correlation*, which is commonly referred to simply as the *correlation*, the correlation coefficient, or just the letter *r*.

- A correlation of 1 indicates a perfect positive correlation.
- A correlation of -1 indicates a *perfect negative correlation*.
- A correlation of 0 indicates that there is no relationship between the different variables.
- Values between -1 and 1 denote the strength of the correlation.

FORMULA:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[\, n\Sigma x^2 - (\Sigma x)^2 \,][\, n\Sigma y^2 - (\Sigma y)^2 \,]}}$$

Significance test

To test whether the association is merely apparent, and might have arisen by chance use the t test in the following calculation:

$$t = r\sqrt{\frac{n-2}{1-r^2}}$$

Calculation of the correlation coefficient

❖ A paediatric registrar has measured the pulmonary anatomical dead space (in ml) and height (in cm) of 15 children. Calculate the correlation coefficient.

Child number	Height (cm)	Dead space (ml), y
1	110	44
2	116	31
3	124	43
4	129	45
5	131	56
6	138	79
7	142	57
8	150	56
9	153	58
10	155	92
11	156	78
12	159	64
13	164	88
14	168	112
15	174	101
Total	2169	1004
Mean	144.6	66.933

The calculation of the correlation coefficient is as follows, with x representing the values of the independent variable (in this case height) and y representing the values of the dependent variable (in this case anatomical dead space). The formula to be used is:

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{[\Sigma(x - \bar{x})^2 (y - \bar{y})^2]}}$$

r = 5426.6/6412.0609 = 0.846.

The correlation coefficient of 0.846 indicates a strong positive correlation between size of pulmonary anatomical dead space and height of child. But in interpreting correlation it is important to remember that correlation is not causation. There may or may not be a causative connection between the two correlated variables. Moreover, if there is a connection it may be indirect.

The assumptions governing this test are:

1. That both variables are plausibly Normally distributed.
2. That there is a linear relationship between them.
3. The null hypothesis is that there is no association between them.

The number of pairs of observations was 15. Applying test for significance-

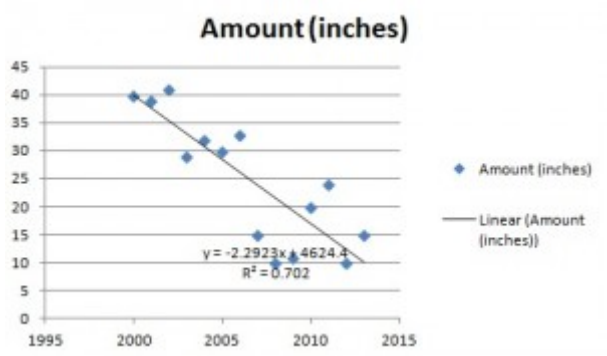
$$t = 0{\cdot}846\sqrt{\frac{15-2}{1-0{\cdot}846^2}} = 5{\cdot}72.$$

Thus the correlation coefficient may be regarded as highly significant. Thus there is a very strong correlation between dead space and height which is most unlikely to have arisen by chance.

iii. Computation of simple linear regression.

Regression analysis can result in linear or nonlinear graphs. A linear regression is where the relationships between variables can be described with a straight line. Non-linear regressions produce curved lines.

The X variable is sometimes called the independent variable and the Y variable is called the dependent variable. Simple linear regression plots one independent variable X against one dependent variable Y. Technically, in regression analysis, the independent variable is usually called the predictor variable and the dependent variable is called the criterion variable. However, many people just call them the independent and dependent variables. More advanced regression techniques (like multiple regression) use multiple independent variables.



Simple linear regression for the amount of rainfall per year.

Regression analysis is used to find equations that fit data. One type of regression analysis is linear analysis. When a correlation coefficient shows that data is likely to be able to predict future outcomes and a scatter plot of the data appears to form a straight line, simple linear regressionis used to find a predictive function. Calculate linear regression, and find the equation y' = a + bx.

EXAMPLE:

subject	Age	Glucose level	xy	X2	Y2
1	43	99	4257	1849	9801
2	21	65	1365	441	4225
3	25	79	1975	625	6241

4	42	75	3150	1764	5625
5	57	87	4959	3249	7569
6	59	81	4779	3481	6561

From the above table, Σx = 247, Σy = 486, Σxy = 20485, Σx2 = 11409, Σy2 = 40022. n is the sample size = 6,

Using the following equations to find a and b.

$$a = \frac{(\Sigma y)(\Sigma x^2) - (\Sigma x)(\Sigma xy)}{n(\Sigma x^2) - (\Sigma x)^2}$$

$$b = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{n(\Sigma x^2) - (\Sigma x)^2}$$

a = 65.1416

b = .385225

Find a:

((486 × 11,409) – ((247 × 20,485)) / 6 (11,409) – 2472)

484979 / 7445

=65.14

Find b:

(6(20,485) – (247 × 486)) / (6 (11409) – 2472)

(122,910 – 120,042) / 68,454 – 2472

2,868 / 7,445

= .385225

Inserting the values into the equation.

y’ = a + bx

y’ = 65.14 + .385225x

iv. Computation of variance ratio (F) and multiple comparison of Scheffe’s F test for one-way anova and their interpretation

What are F-statistics and the F-test?

F-tests are named after its test statistic, F, which was named in honor of Sir Ronald Fisher. The F-statistic is simply a ratio of two variances. Variances are a measure of dispersion, or how far the data are scattered from the mean. Larger values represent greater dispersion.

Variance is the square of the standard deviation. For us humans, standard deviations are easier to understand than variances because they’re in the same units as the data rather than squared units. However, many analyses actually use variances in the calculations.

F-statistics are based on the ratio of mean squares. The term “mean squares” may sound confusing but it is simply an estimate of population variance that accounts for the degrees of freedom (DF) used to calculate that estimate.

Despite being a ratio of variances, you can use F-tests in a wide variety of situations. Unsurprisingly, the F-test can assess the equality of variances. However, by changing the variances that are included in the ratio, the F-test becomes a very flexible test. For example, you can use F-statistics and F-tests to test the overall significance for a regression model, to compare the fits of different models, to test specific regression terms, and to test the equality of means.

To calculate the FF ratio, two estimates of the variance are made.

1. **Variance between samples:** An estimate of σ2σ2 that is the variance of the sample means multiplied by n (when there is equal n). If the samples are different sizes, the variance between samples is weighted to account for the different sample sizes. The variance is also called **variation due to treatment or explained variation.**
2. **Variance within samples:** An estimate of σ2σ2 that is the average of the sample variances (also known as a pooled variance). When the sample sizes are different, the variance within samples is weighted. The variance is also called the **variation due to error or unexplained variation.**

- SSbetween=SSbetween= the sum of squares that represents the variation among the different samples.
- SSwithin=SSwithin= the sum of squares that represents the variation within samples that is due to chance.

To find a "sum of squares" means to add together squared quantities which, in some cases, may be weighted. We used sum of squares to calculate the sample variance and the sample standard deviation in **Descriptive Statistics**.

MSMS means "mean square." MSbetweenMSbetween is the variance between groups and MSwithinMSwithin is the variance within groups.

- MSbetween=SSbetweendfbetween=SSbetweenk−1MSbetween=SSbetweendfbetween=SSbetweenk−1
- MSwithin=SSwithindfwithin=SSwithinn−kMSwithin=SSwithindfwithin=SSwithinn−k

The One-Way ANOVA test depends on the fact that MSbetweenMSbetween can be influenced by population differences among means of the several groups. Since MSwithinMSwithin compares values of each group to its own group mean, the fact that group means might be different does not affect MSwithin.

A One-Way Analysis of Variance is a way to test the equality of three or more means at one time by using variances.

Assumptions

- The populations from which the samples were obtained must be normally or approximately normally distributed.
- The samples must be independent.
- The variances of the populations must be equal.

Hypotheses

The null hypothesis will be that all population means are equal, the alternative hypothesis is that at least one mean is different.

In the following, lower case letters apply to the individual samples and capital letters apply to the entire set collectively. That is, n is one of many sample sizes, but N is the total sample size.

Grand Mean

The grand mean of a set of samples is the total of all the data values divided by the total sample size. This requires that you have all of the sample data available to you, which is usually the case, but not always. It turns out that all that is necessary to find perform a one-way analysis of variance are the number of samples, the sample means, the sample variances, and the sample sizes.

$$\bar{X}_{GM} = \frac{\sum x}{N}$$

Another way to find the grand mean is to find the weighted average of the sample means. The weight applied is the sample size.

$$\bar{X}_{GM} = \frac{\sum n\bar{x}}{\sum n}$$

Total Variation

The total variation (not variance) is comprised the sum of the squares of the differences of each mean with the grand mean.

$$SS(T) = \sum (x - \bar{X}_{GM})^2$$

There is the between group variation and the within group variation. The whole idea behind the analysis of variance is to compare the ratio of between group variance to within group variance. If the variance caused by the interaction between the samples is much larger when compared to the variance that appears within each group, then it is because the means aren't the same.

Between Group Variation

The variation due to the interaction between the samples is denoted SS(B) for Sum of Squares Between groups. If the sample means are close to each other (and therefore the Grand Mean) this will be small. There are k samples involved with one data value for each sample (the sample mean), so there are k-1 degrees of freedom.

$$SS(B) = \sum n(\bar{x} - \bar{X}_{GM})^2$$

The variance due to the interaction between the samples is denoted MS(B) for Mean Square Between groups. This is the between group variation divided by its degrees of freedom. It is also denoted by s_b^2.

Within Group Variation

The variation due to differences within individual samples, denoted SS(W) for Sum of Squares Within groups. Each sample is considered independently, no interaction between samples is involved. The degrees of freedom is equal to the sum of the individual degrees of freedom for each sample. Since each sample has degrees of freedom equal to one less than their sample sizes, and there are k samples, the total degrees of freedom is k less than the total sample size: df = N - k. The variance due to the differences within individual samples is denoted MS(W) for Mean Square Within groups. This is the within group variation divided by its degrees of freedom. It is also denoted by s_w^2. It is the weighted average of the variances (weighted with the degrees of freedom).

$$SS(W) = \sum df \cdot s^2$$

F test statistic

Recall that a F variable is the ratio of two independent chi-square variables divided by their respective degrees of freedom. Also recall that the F test statistic is the ratio of two sample variances, well, it turns out that's exactly what we have here. The F test statistic is found by dividing the between group variance by the within group variance. The degrees of freedom for the numerator are the degrees of freedom for the between group (k-1) and the degrees of freedom for the denominator are the degrees of freedom for the within group (N-k).

$$F = \frac{s_b^2}{s_w^2}$$

Summary Table

All of this sounds like a lot to remember, and it is. However, there is a table which makes things really nice.

	SS	**df**	**MS**	**F**
Between	SS(B)	k-1	SS(B) / k-1	MS(B) / MS(W)
Within	SS(W)	N-k	SS(W) / N-k	.
Total	SS(W) + SS(B)	N-1	.	.

Notice that each Mean Square is just the Sum of Squares divided by its degrees of freedom, and the F value is the ratio of the mean squares. Do not put the largest variance in the numerator, always divide the between variance by the within variance. If the between variance is smaller than the within variance, then the means are really close to each other and you will fail to reject the claim that they are all equal. The degrees of freedom of the F-test are in the same order they appear in the table (nifty, eh?).

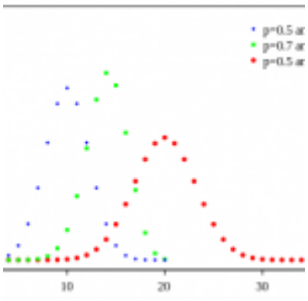
Decision Rule

The decision will be to reject the null hypothesis if the test statistic from the table is greater than the F critical value with k-1 numerator and N-k denominator degrees of freedom.

If the decision is to reject the null, then at least one of the means is different.

V. Significance of observed sex ratios using binomial distribution.

A **binomial distribution** can be thought of as simply the probability of a SUCCESS or FAILURE outcome in an experiment or survey that is repeated multiple times. The binomial is a type of distribution that has two possible outcomes (the prefix "bi" means two, or twice). For example, a coin toss has only two possible outcomes: heads or tails and taking a test could have two possible outcomes: pass or fail.



A Binomial Distribution shows either (S)uccess or (F)ailure.

The first variable in the binomial formula, n, stands for the number of times the experiment runs. The second variable, p, represents the probability of one specific outcome. For example, let's suppose you wanted to know the probability of getting a 1 on a die roll. if you were to roll a die 20 times, the probability of rolling a one on any throw is 1/6. Roll twenty times and you have a binomial distribution of (n=20, p=1/6). SUCCESS would be "roll a one" and FAILURE would be "roll anything else." If the outcome in question was the probability of the die landing on an even number, the binomial distribution would then become (n=20, p=1/2). That's because your probability of throwing an even number is one half.

Criteria

Binomial distributions must also meet the following three criteria:

1. **The number of observations or trials is fixed.** In other words, you can only figure out the probability of something happening if you do it a certain number of times. This is common sense—if you toss a coin once, your probability of getting a tails is 50%. If you toss a coin a 20 times, your probability of getting a tails is very, very close to 100%.
2. **Each observation or trial is** independent. In other words, none of your trials have an effect on the probability of the next trial.
3. The probability of success (tails,heads,fail or pass) is exactly the same from one trial to another.

Binomial Distribution – Formula

First formula

$b(x,n,p)= nCx*P^{x*}(1-P)^{n-x}$ for x=0,1,2,…..n.

where : –
b is the binomial probability.
x is the total number of successes.
p is chances of a success on an individual experiment.
n is the number of trials

4. n>0 ∴ p,q≥0
5. ∑b(x,n,p) = b(1) + b(2) + ….. + b(n) = 1
6. Value of 'n' and 'p' must be known for applying the above formula. Thus existence of binomial distribution highly depends on the knowledge of these two parameters. This is why it is also called **bi-parametric distribution**.

Example: 80% of people those who purchase pet insurance are women. If the owners of 9 pet insurance are randomly selected, then find the probability that exactly 6 out of them are women.

Solution: Find out the 'n' from the problem. Here n = 9

1. Identify 'X'. X = the number you are asked to search the probability for is 6.
2. (Divide the formula then it become easy to get the solution) solve the first part of the formula: – n! / (n-X)! X!

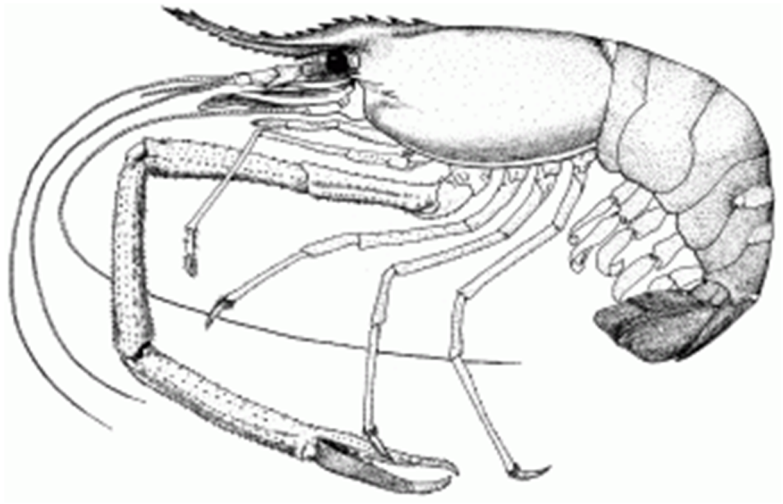
   Now add the variables = 9! (9-6)!*6! = 84. And keep it aside for further uses.
4. Now find out the P and Q. P= the probable chances of success and Q= the possibility of failure. As mentioned in the above question p = 80% or 0.8 so, the probability of failure = 1-0.8 = 0.2 (20%)
5. Now let's do the second part of the formula. $P^x = 0.8^6 = 0.262144$
6. $Q^{(n-x)} = 0.2^{(9-6)} = 0.2^3 = 0.008$ (third part of the formula)
7. Multiply the answer you get from step 3, 5, 6 together

   8×0.262144×0.008 = 0.176

SPECIAL PAPER PRACTICAL

Course No: ZOO 495A: Fishery Practical-II (50 Marks, 4 credit)

Specimen No. – 1



Systematic Position

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Infraorder: Caridea

Family: Palaemonidae

Genus: *Macrobrachium*

Species: *M. rosenbergii*

Identification

1. Having an exoskeleton (external skeleton), a segmented body, and jointed appendages.
2. Presence cuticle made of chitin, often mineralized with calcium carbonate.
3. The rigid cuticle inhibits growth, so it replaces periodically by molting.

Hence, Phylum – Arthropoda

1. The body is composed of body segments, which are grouped into three regions: the cephalon or head, the thorax, and the pleon or abdomen.

2. The head and thorax may be fused together to form a cephalothorax, which may be covered by a single large carapace.
3. The shell around each somite can be divided into a dorsal tergum, ventral sternum and a lateral pleuron.

Hence, Subphylum – Crustacea

1. Its members are characterized by the presence of three tagmata (specialized groupings of multiple segments) – a five-segmented head, an eight-segmented thorax and an abdomen with six segments and a telson, except in the Leptostraca, which retain the ancestral condition of seven abdominal segments.
2. These have abdominal appendages, a fact that differentiates them from all other major crustacean taxa except Remipedia.

Hence, Class – Malacostraca

1. All have ten legs, in the form of five pairs of thoracic appendages on the last five thoracic segments.
2. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainder are pereiopods.
3. In many decapods, however, one pair of legs has enlarged pincers; the claws are called chelae, so those legs may be called chelipeds.

Hence, Order – Decapoda

1. Telson elongate, with 2 pairs of dorsal spines and 2 or 3 pairs of posterior spines.
2. First and second pairs of pereopods with pincers. First pair of pereopods shorter and more slender than second; pincers well developed, normal.
3. Second pair of pereopods more robust than first, often very long and strong in adult males; pincers normal, carpus and merus not subdivided
4. Last 3 pereopods simple without pincers.
5. Exopods on none of the legs.
6. Males without petasma, females without thelycum.
7. Males with an appendix masculine and an appendix interna on the endopods of second pleopods.

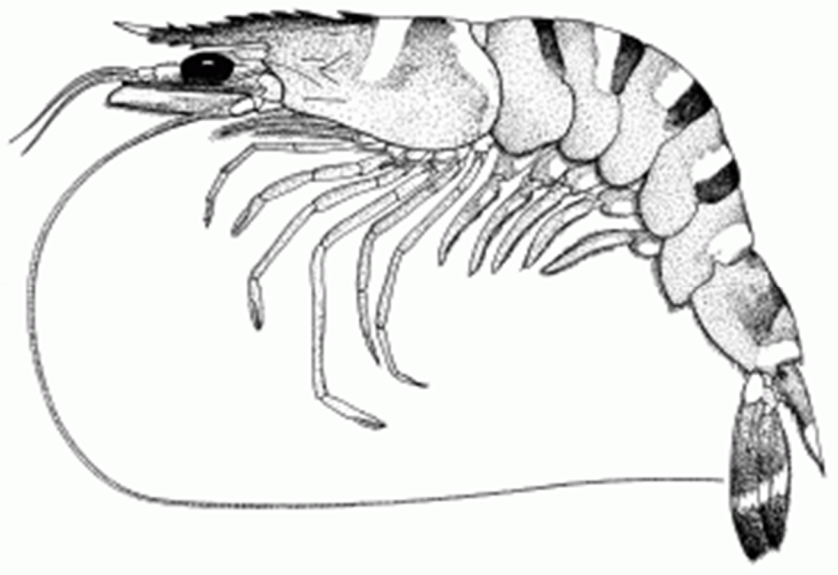
Hence, Family – Palaemonidae

1. Presence of chelae (movable claws) on the first two pairs of walking legs, and the third thoracic segment overlapping the second.
2. The second pair of walking legs greatly lengthened, often equaling or exceeding body length, with very prominent chelae.

3. The rostrum is long in young males (1.2-1.4 X carapace length), but proportionately shorter in older specimens (0.8-1.0 X carapace length). It is curved somewhat upwards, bearing 11-14 dorsal teeth, and 8-10 ventral teeth.
4. Males reach 320 mm, and females can reach 250 mm.

Hence, the Specimen is *Macrobrachium rosenbergii*

Specimen No. – 2



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Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Family: Penaeidae

Genus: *Penaeus*

Species: *Penaeus monodon*

Identification

1. Having an exoskeleton (external skeleton), a segmented body, and jointed appendages.
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3. The rigid cuticle inhibits growth, so it replaces periodically by molting.

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1. The body is composed of body segments, which are grouped into three regions: the cephalon or head, the thorax, and the pleon or abdomen.

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2. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainder are pereiopods.
3. In many decapods, however, one pair of legs has enlarged pincers; the claws are called chelae, so those legs may be called chelipeds.

Hence, Order – Decapoda

1. The pleurae on either side of the second abdominal segment overlap only the third segment.
2. The first three pairs of peraepods are chelate in penaeids.
3. For transferring sperms the male penaeids has petasma and for storing sperms the female has thelycum.

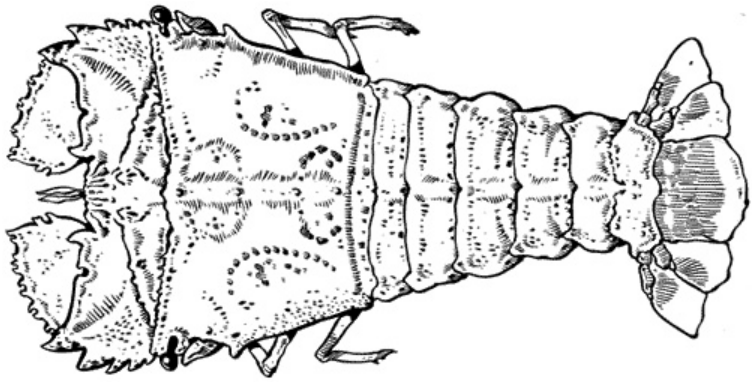
Hence, Family – Penaeidae

1. Females can reach about 33 cm (13 in) long, but are typically 25–30 cm (10–12 in) long and weight 200–320 g (7–11 oz); males are slightly smaller at 20–25 cm (8–10 in) long and weighing 100–170 g (3.5–6.0 oz).
2. The rostrum, extending beyond the tip of the antennular peduncle, has usually seven dorsal and three ventral teeth, and is sigmoid in shape.
3. The adrostral carina reaches almost to the epigastric spine. The carina reaches to the posterior edge of the carapace.
4. The fifth pereiopods have no exopod.
5. The abdomen is carinated dorsally from the anterior one-third of the fourth to sixth somite.

6. Carapace and abdomen are transversely banded with red and white. The antennae are greyish brown. Pereiopods are brown and fringing setae red. When kept in ponds, the colour changes to dark brown, and often to a blackish hue.

Hence, the Specimen - *Penaeus monodon* (giant tiger prawn)

Specimen No. – 3



Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Family: Scyllaridae

Genus: *Thenus*

Species: *Thenus orientalis*

Identification

1. Having an exoskeleton (external skeleton), a segmented body, and jointed appendages.
2. Presence cuticle made of chitin, often mineralized with calcium carbonate.
3. The rigid cuticle inhibits growth, so it replaces periodically by molting.

Hence, Phylum – Arthropoda

1. The body is composed of body segments, which are grouped into three regions: the cephalon or head, the thorax, and the pleon or abdomen.
2. The head and thorax may be fused together to form a cephalothorax, which may be covered by a single large carapace.
3. The shell around each somite can be divided into a dorsal tergum, ventral sternum and a lateral pleuron.

Hence, Subphylum – Crustacea

1. Its members are characterized by the presence of three tagmata (specialized groupings of multiple segments) – a five-segmented head, an eight-segmented thorax and an abdomen with six segments and a telson, except in the Leptostraca, which retain the ancestral condition of seven abdominal segments.
2. These have abdominal appendages, a fact that differentiates them from all other major crustacean taxa except Remipedia.

Hence, Class – Malacostraca

1. All have ten legs, in the form of five pairs of thoracic appendages on the last five thoracic segments.
2. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainder are pereiopods.
3. In many decapods, however, one pair of legs has enlarged pincers; the claws are called chelae, so those legs may be called chelipeds.

Hence, Order – Decapoda

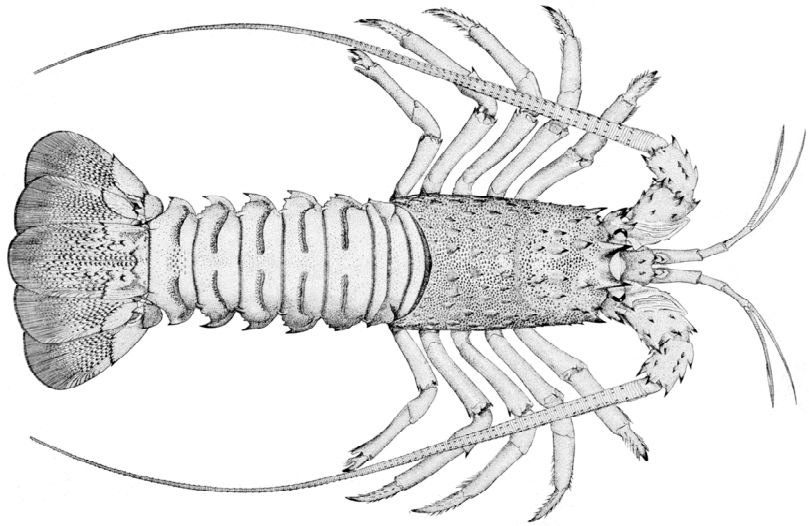
1. It is immovably connected with the antennular somite and the carapace, thereby forming an integral part of the orbit.
2. The fourth element is large, broad and flat; it usually bears teeth on its margins.
3. Carapace is often depressed always with distinct lateral margin.
4. Rostrum is small, and enclosed by antennular somite.

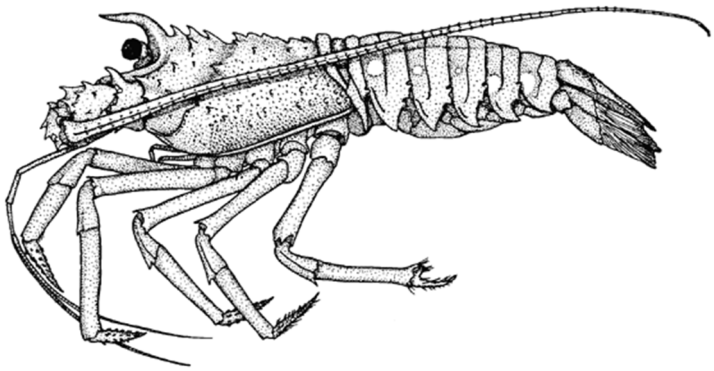
Hence, Family – Scyllaridae

1. Body strongly depressed.
2. Lateral margin of the carapace with only the cervical incision.
3. No teeth on the lateral margin of the carapace, apart from the anterolateral and postcervical.
4. Orbits on the anterolateral angle of the carapace.
5. Exopod of third and first maxilliped without a flagellum; the flagellum of the second maxilliped transformed into a single laminate segment.
6. Fifth leg of female without a chela.
7. Maximum total body length about 25 cm; maximum carapace length about 8 cm.

Hence, the Specimen - *Thenus orientalis* (Flathead lobster).

Specimen No. – 4





Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Family: Palinuridae

Genus: *Panulirus*

Species: *Panulirus argus*

Identification

1. Having an exoskeleton (external skeleton), a segmented body, and jointed appendages.
2. Presence cuticle made of chitin, often mineralized with calcium carbonate.
3. The rigid cuticle inhibits growth, so it replaces periodically by molting.

Hence, Phylum – Arthropoda

1. The body is composed of body segments, which are grouped into three regions: the cephalon or head, the thorax, and the pleon or abdomen.
2. The head and thorax may be fused together to form a cephalothorax, which may be covered by a single large carapace.
3. The shell around each somite can be divided into a dorsal tergum, ventral sternum and a lateral pleuron.

Hence, Subphylum – Crustacea

1. Its members are characterized by the presence of three tagmata (specialized groupings of multiple segments) – a five-segmented head, an eight-segmented thorax and an abdomen with six segments and a telson, except in the Leptostraca, which retain the ancestral condition of seven abdominal segments.
2. These have abdominal appendages, a fact that differentiates them from all other major crustacean taxa except Remipedia.

Hence, Class – Malacostraca

1. All have ten legs, in the form of five pairs of thoracic appendages on the last five thoracic segments.
2. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainder are pereiopods.
3. In many decapods, however, one pair of legs has enlarged pincers; the claws are called chelae, so those legs may be called chelipeds.

Hence, Order – Decapoda

1. Characterized by the lack of chelae on their first pair of pereiopods and the presence in their development of a phyllosoma larval stage.
2. Have typically a slightly compressed carapace, lacking any lateral ridges.
3. Their antennae lack a scaphocerite, the flattened exopod of the antenna.
4. This is fused to the epistome (a plate between the labrum and the basis of the antenna).
5. The flagellum, at the top of the antenna, is stout, tapering and very long. The ambulatory legs (pereopods) end in claws (chelae).

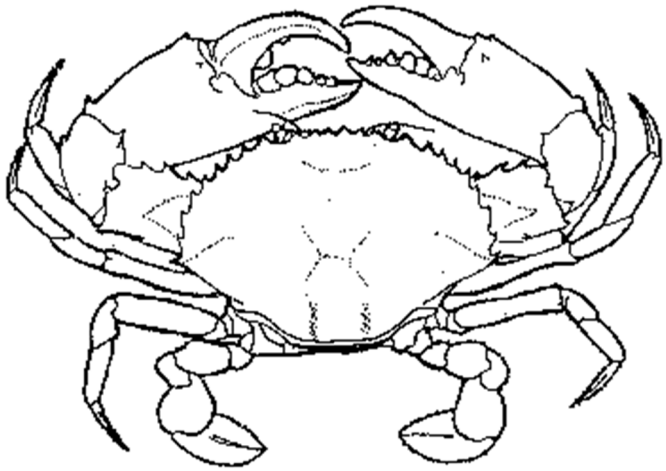
Hence, Family – Palinuridae

1. Long, cylindrical bodies covered with spines.
2. Two large spines form forward-pointing "horns" above the eyestalks.
3. They are generally olive greenish or brown, but can be tan to mahogany. There is a scattering of yellowish to cream-colored spots on the carapace and larger (usually four to six) yellow to cream-colored spots on the abdomen.

4. They have no claws (pincers).
5. The first pair of antennae are slender, black or dark brown and biramous. The second pair of antennae are longer than the body, and covered with forward pointing spines.
6. The legs are usually striped longitudinally with blue and yellow and terminate in a single spine-like point.
7. May reach up to 60 cm (24 in) long, but typically around 20 cm (7.9 in), and is fished throughout its range. Sexual maturity in females is reached at a carapace length of 54–80 mm (2.1–3.1 in).

Hence, the Specimen - *Panulirus argus* (Spiny lobster).

Specimen No. – 5



Systematic Position

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Family: Portunidae

Genus: *Scylla*

Species: *Scylla serrata*

Identification

1. Having an exoskeleton (external skeleton), a segmented body, and jointed appendages.
2. Presence cuticle made of chitin, often mineralized with calcium carbonate.
3. The rigid cuticle inhibits growth, so it replaces periodically by molting.

Hence, Phylum – Arthropoda

1. The body is composed of body segments, which are grouped into three regions: the cephalon or head, the thorax, and the pleon or abdomen.
2. The head and thorax may be fused together to form a cephalothorax, which may be covered by a single large carapace.
3. The shell around each somite can be divided into a dorsal tergum, ventral sternum and a lateral pleuron.

Hence, Subphylum – Crustacea

1. Its members are characterized by the presence of three tagmata (specialized groupings of multiple segments) – a five-segmented head, an eight-segmented thorax and an abdomen with six segments and a telson, except in the Leptostraca, which retain the ancestral condition of seven abdominal segments.
2. These have abdominal appendages, a fact that differentiates them from all other major crustacean taxa except Remipedia.

Hence, Class – Malacostraca

1. All have ten legs, in the form of five pairs of thoracic appendages on the last five thoracic segments.
2. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainder are pereiopods.
3. In many decapods, however, one pair of legs has enlarged pincers; the claws are called chelae, so those legs may be called chelipeds.

Hence, Order – Decapoda

1. Portunid crabs are characterized by the flattening of the fifth pair of legs into broad paddles, which are used for swimming.
2. This ability, together with their strong, sharp claws, allows many species to be fast and aggressive predators.

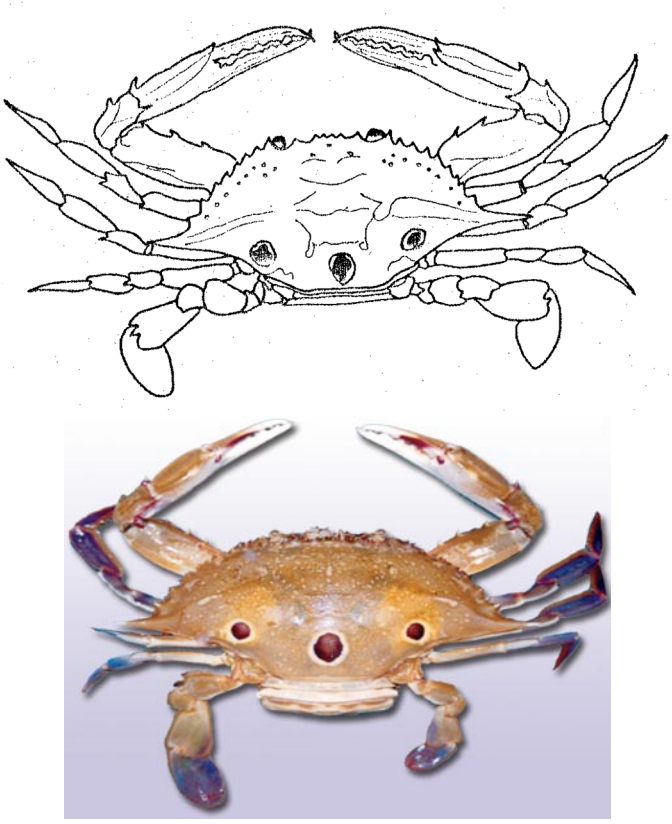
Hence, Family – Portunidae

1. Carapace smooth and glabrous with exception of granular lines on the gastric regions and an epibranchial line starting from the tip of the last antero-lateral tooth and reaching to the branchial regions;
2. Front with 4 subequal and equally spaced teeth with acute to rounded tips; antero-lateral borders with 9 very acute and subequal teeth, last one the smallest. Basal antennal joint short and broad, with a lobule at its antero-external angle.
3. Chelipeds heterochelous; merus with 3 spines on anterior border and 2 spines on posterior; carpus with a strong spine on inner corner and another on outer face; propodus with 2 acute spines at distal end of upper face and a strong knob on inner face at base of fixed finger.

4. Swimming leg without spines on posterior border of either of the joints.

Hence, the Species - *Scylla serrata*

Specimen No. – 6



Systematic Position

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Family: Portunidae

Genus: *Portunus*

Species: *Portunus sanguinolentus*

Identification

1. Having an exoskeleton (external skeleton), a segmented body, and jointed appendages.
2. Presence cuticle made of chitin, often mineralized with calcium carbonate.
3. The rigid cuticle inhibits growth, so it replaces periodically by molting.

Hence, Phylum – Arthropoda

1. The body is composed of body segments, which are grouped into three regions: the cephalon or head, the thorax, and the pleon or abdomen.
2. The head and thorax may be fused together to form a cephalothorax, which may be covered by a single large carapace.
3. The shell around each somite can be divided into a dorsal tergum, ventral sternum and a lateral pleuron.

Hence, Subphylum – Crustacea

1. Its members are characterized by the presence of three tagmata (specialized groupings of multiple segments) – a five-segmented head, an eight-segmented thorax and an abdomen with six segments and a telson, except in the Leptostraca, which retain the ancestral condition of seven abdominal segments.
2. These have abdominal appendages, a fact that differentiates them from all other major crustacean taxa except Remipedia.

Hence, Class – Malacostraca

1. All have ten legs, in the form of five pairs of thoracic appendages on the last five thoracic segments.
2. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainder are pereiopods.
3. In many decapods, however, one pair of legs has enlarged pincers; the claws are called chelae, so those legs may be called chelipeds.

Hence, Order – Decapoda

1. Portunid crabs are characterized by the flattening of the fifth pair of legs into broad paddles, which are used for swimming.
2. This ability, together with their strong, sharp claws, allows many species to be fast and aggressive predators.

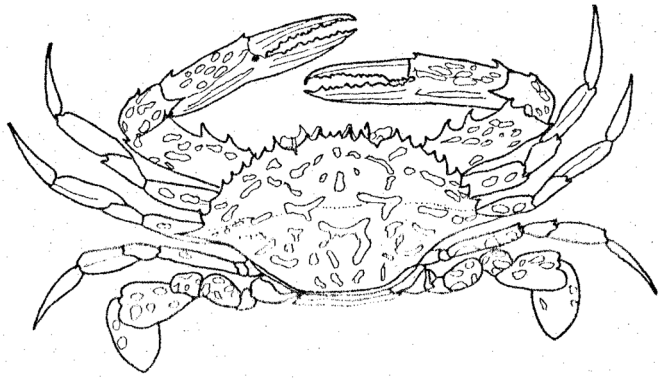
Hence, Family – Portunidae

1. Carapace very broad (breadth 2.0-2.5 times length), with 3 red spots in posterior half, persisting quite long in preserved specimens; surface finely granulated anteriorly, smooth posteriorly; with recognizable mesogastric, epibranchial, and metagastric ridges;
2. Front with 4 triangular teeth, outer pair broader and very slightly more prominent than inner ones;
3. Antero-lateral borders with 9 teeth, first clearly longer and much more pointed than following 7, last one very large and projecting straight out laterally;
4. Postero-lateral junction rounded.

5. Cheliped merus with postero-distal border smooth, anterior border with 3-4 sharp spines; carpus with inner and outer spines;
6. Lower surface of palm smooth. Posterior border of swimming leg without spines or spinules.

Hence, the Species - *Portunus sanguinolentus*

Specimen No. – 7



Systematic Position

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Family: Portunidae

Genus: *Portunus*

Species: *Portunus pelagicus*

Identification

1. Having an exoskeleton (external skeleton), a segmented body, and jointed appendages.
2. Presence cuticle made of chitin, often mineralized with calcium carbonate.

3. The rigid cuticle inhibits growth, so it replaces periodically by molting.

Hence, Phylum – Arthropoda

1. The body is composed of body segments, which are grouped into three regions: the cephalon or head, the thorax, and the pleon or abdomen.
2. The head and thorax may be fused together to form a cephalothorax, which may be covered by a single large carapace.
3. The shell around each somite can be divided into a dorsal tergum, ventral sternum and a lateral pleuron.

Hence, Subphylum – Crustacea

1. Its members are characterized by the presence of three tagmata (specialized groupings of multiple segments) – a five-segmented head, an eight-segmented thorax and an abdomen with six segments and a telson, except in the Leptostraca, which retain the ancestral condition of seven abdominal segments.
2. These have abdominal appendages, a fact that differentiates them from all other major crustacean taxa except Remipedia.

Hence, Class – Malacostraca

1. All have ten legs, in the form of five pairs of thoracic appendages on the last five thoracic segments.
2. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainder are pereiopods.
3. In many decapods, however, one pair of legs has enlarged pincers; the claws are called chelae, so those legs may be called chelipeds.

Hence, Order – Decapoda

1. Portunid crabs are characterized by the flattening of the fifth pair of legs into broad paddles, which are used for swimming.
2. This ability, together with their strong, sharp claws, allows many species to be fast and aggressive predators.

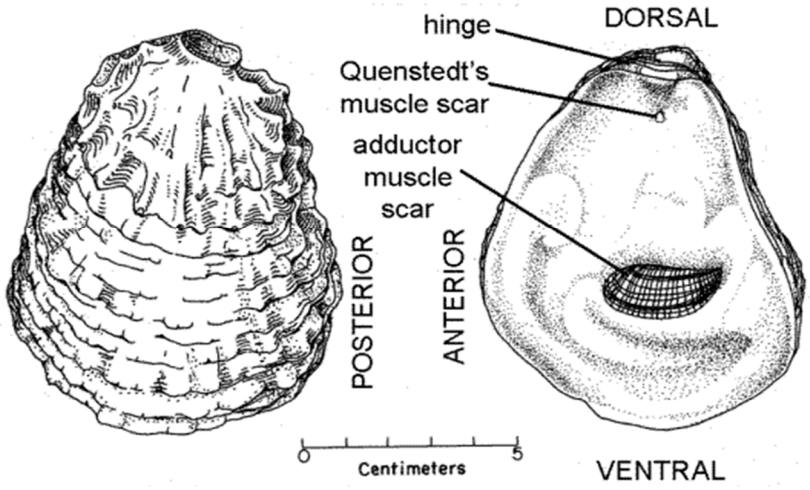
Hence, Family – Portunidae

1. Carapace very broad (breadth just over 2-21/3 times length); surface coarsely granulated, frequently with a short but dense pubescence between the granules;
2. Usually with recognizable mesogastric, epibranchial, and indistinct metagastric ridges, cardiac and mesobranchial ridges with low granular eminences; front with 4 acute teeth, outer pair larger and more prominent than inner ones;
3. Antero-lateral borders with 9 teeth, last one very large and projecting straight out laterally; postero-lateral junction rounded.

4. Cheliped merus with postero-distal border spinous, anterior border with 3-4 (usually 4) sharp spines;
5. Carpus with inner and outer spines; lower surface of palm smooth. Posterior border of swimming leg without spines or spinules.

Hence, the Species – *Portunus pelagicus*

Specimen – 8:



Systematic Position

Kingdom: Animalia

Phylum: Mollusca

Class: Bivalvia

Order: Ostreoida

Family: Ostreidae

Genus: *Crassostrea*

Specimen – *Crassostrea madrasensis*

Identification

1. Presence of a mantle with a significant cavity used for breathing and excretion,
2. Radula resent.
3. This has a single, "limpet-like" shell on top, which is made of proteins and chitin reinforced with calcium carbonate, and is secreted by a mantle covering the whole upper surface.
4. The underside of the animal consists of a single muscular "foot".

Hence, Phylum – Mollusca

1. The shell of a bivalve is composed of calcium carbonate, and consists of two, usually similar, parts called valves covers the laterally compressed bodies. These are joined

together along one edge (the hinge line) by a flexible ligament that, usually in conjunction with interlocking "teeth" on each of the valves, forms the hinge.

2. The shell of a bivalve is composed of calcium carbonate, and consists of two, usually similar, parts called valves. These are joined together along one edge (the hinge line) by a flexible ligament that, usually in conjunction with interlocking "teeth" on each of the valves, forms the hinge.
3. They have no head.

Hence, Class – Bivalvia

1. The shell is characterized by two unequal valves, the interior either porcelain-like or mother-of-pearl.
2. The hinge is dysodont (lacking teeth).

Hence, Order – Ostreoida

1. Shell thick, rugose; inequivalve,
2. Left (lower) valve convex, often forming a deep bowl and frequently overlapping the right valve, usually cemented to the substratum when small, right valve flat or slightly concave.
3. Inequilateral, umbones anterior to midline.
4. Juveniles with small taxodont teeth on each side of the umbones, absent in adults.
5. Ligament internal, thick; inner surface nacreous, with a single adductor scar, elliptical, distinct, and often recessed.

Hence, Family – Ostreidae

1. Abductor muscle elliptical or oblong.
2. Pigmentation if present, is mainly on middle and inner lobs. It doesn’t or rarely extends on the surface of inner lobe.
3. Mantle with or without pigmentation.
4. Anal portion of the rectum ½ to 1 mm in length, slightly directed out of the body.
5. Anal tip with variously folded collar.
6. Anal opening situated at the middle of the ventral margin of abductor muscle.

Hence, the Specimen – *Crassostrea madrasensis*

Specimen – 9:

Systematic Position

Kingdom: Animalia

Phylum: Mollusca

Class: Bivalvia

Order: Ostreoida

Family: Ostreidae

Genus: *Crassostrea*

Specimen – *Crassostrea gryphoides*

Identification

1. Presence of a mantle with a significant cavity used for breathing and excretion,
2. Radula resent.
3. This has a single, "limpet-like" shell on top, which is made of proteins and chitin reinforced with calcium carbonate, and is secreted by a mantle covering the whole upper surface.
4. The underside of the animal consists of a single muscular "foot".

Hence, Phylum – Mollusca

1. The shell of a bivalve is composed of calcium carbonate, and consists of two, usually similar, parts called valves covers the laterally compressed bodies. These are joined together along one edge (the hinge line) by a flexible ligament that, usually in conjunction with interlocking "teeth" on each of the valves, forms the hinge.
2. The shell of a bivalve is composed of calcium carbonate, and consists of two, usually similar, parts called valves. These are joined together along one edge (the hinge line) by a flexible ligament that, usually in conjunction with interlocking "teeth" on each of the valves, forms the hinge.

3. They have no head.

Hence, Class – Bivalvia

1. The shell is characterised by two unequal valves, the interior either porcelain-llike or mother-of-pearl.
2. The hinge is dysodont (lacking teeth).

Hence, Order – Ostreoida

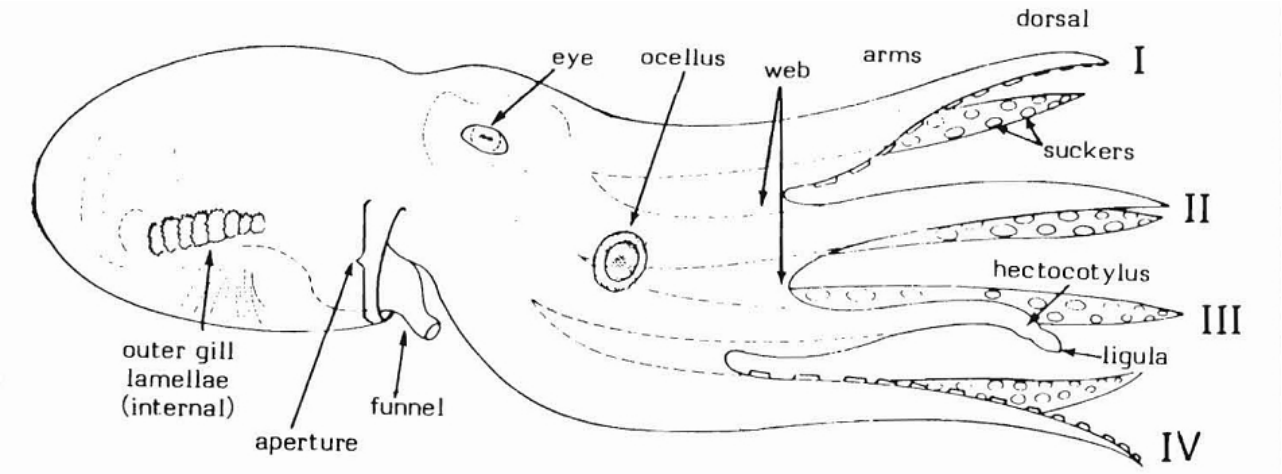
1. Shell thick, rugose; inequivalve,
2. Left (lower) valve convex, often forming a deep bowl and frequently overlapping the right valve, usually cemented to the substratum when small, right valve flat or slightly concave.
3. Inequilateral, umboes anterior to midline.
4. Juveniles with small taxodont teeth on each side of the umbones, absent in adults.
5. Ligament internal, thick; inner surface nacreous, with a single adductor scar, elliptical, distinct, and often recessed.

Hence, Family – Ostreidae

1. Abductor muscle round, bean-shaped.
2. Pigmentation of the mantle pronounced on the edge of the outer and inner lobes.
3. Mantle pigmented black.
4. Anal portion of the rectum 1 to 2 mm in length, markedly directed outwards.
5. Anal tip is simple, slightly funnel shaped.
6. Anal opening situated at the corner of the posterior and ventral margins of abductor muscle.

Hence, the Specimen – *Crassostrea gryphoides*

Specimen – 10



Systematic Position:

Kingdom: Animalia

Phylum: Mollusca

Class: Cephalopoda

Superorder: Octopodiformes

Order: Octopoda

Family: Octopodidae

Genus: *Octopus*

Specimen: *Octopus* sp.

Identification:

1. Presence of a mantle with a significant cavity used for breathing and excretion,
2. Radula resent.
3. This has a single, "limpet-like" shell on top, which is made of proteins and chitin reinforced with calcium carbonate, and is secreted by a mantle covering the whole upper surface.
4. The underside of the animal consists of a single muscular "foot".

Hence, Phylum – Mollusca

1. Exclusively marine animals are characterized by bilateral body symmetry,
2. Presence of a prominent head, and a set of arms or tentacles (muscular hydrostats) modified from the primitive molluscan foot.

Hence, Class – Cephalopoda

1. Octopods have rather short, compact bodies and only eight arms;
2. No trace of the missing second arm pair remains even during embryonic development.

3. Many species are benthic (bottom-living) and crawl over the ocean floor with the mouth facing the substratum.

Hence, Order – Octopoda

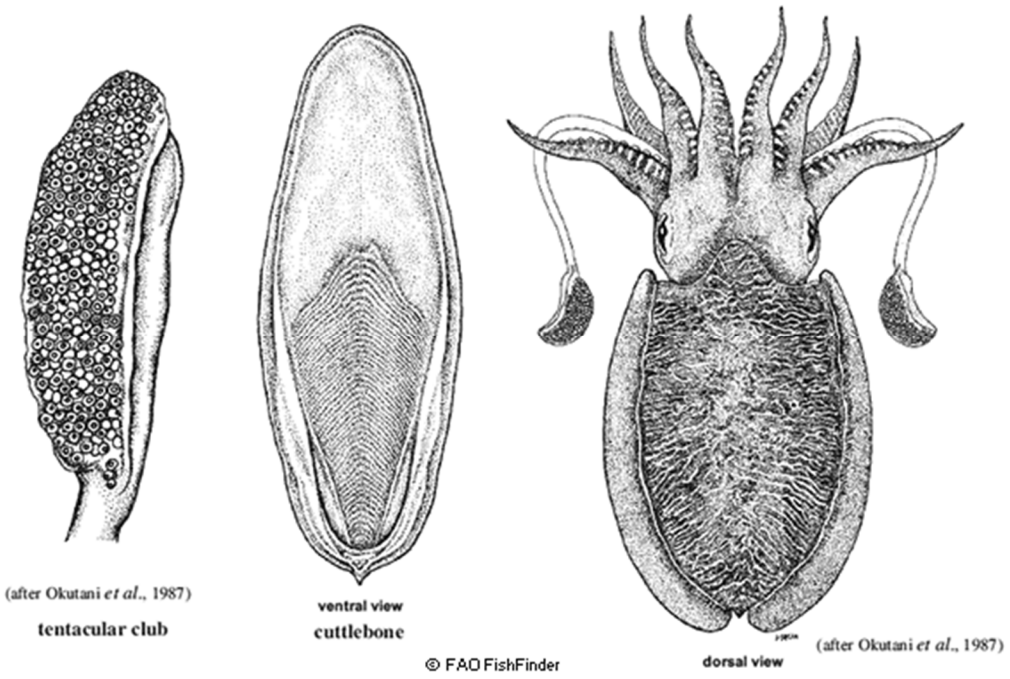
1. Presence of eight circumoral arms.
2. Absence of tentacles.
3. Arm sucker arranged in two rows, without horny rings and stalks.
4. Third arm tip of male spoon shaped.

Hence, Family – Octopodidae.

1. Octopuses are characterized by their eight arms, usually bearing suction cups.
2. They have neither a protective outer shell like the nautilus, nor any vestige of an internal shell or bones, like cuttlefish or squid.
3. The beak, similar in shape to a parrot's beak, and made of chitin, is the only hard part of their bodies.
4. The octopuses in the less-familiar Cirrina suborder have two fins and an internal shell, generally reducing their ability to squeeze into small spaces.
5. Octopuses have three hearts. Two branchial hearts pump blood through each of the two gills, while the third is a systemic heart that pumps blood through the body.

Hence, the Specimen – *Octopus* sp.

Specimen – 11



Systematic Position:

Phylum - Mollusca

Class - Cephalopoda

Order - Sepioidea

Family - Sepiidae

Genus - *Sepia*

Species - *Sepia officinalis*

Identification:

1. Presence of a mantle with a significant cavity used for breathing and excretion,
2. Radula resent.
3. This has a single, "limpet-like" shell on top, which is made of proteins and chitin reinforced with calcium carbonate, and is secreted by a mantle covering the whole upper surface.
4. The underside of the animal consists of a single muscular "foot".

Hence, Phylum – Mollusca

1. Exclusively marine animals are characterized by bilateral body symmetry,
2. Presence of a prominent head, and a set of arms or tentacles (muscular hydrostats) modified from the primitive molluscan foot.

Hence, Class – Cephalopoda

1. This order of species have eggs that attach to a substrate separately or in unorganized groups.
2. They also have two eyelids, and the suckers on their arms are encircled with muscle

Hence, Order – Sepioidea

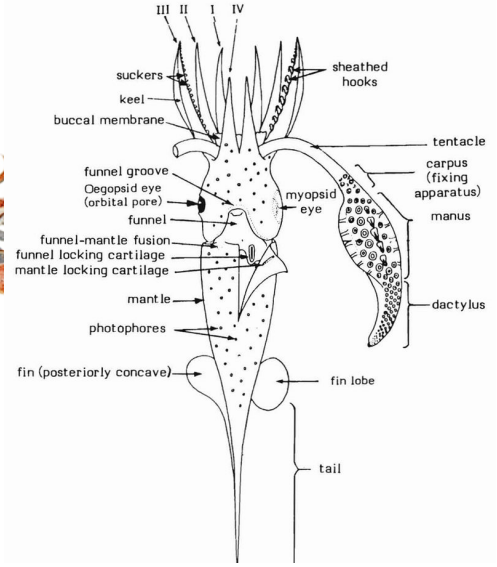
1. These animals have flattened arms, tentacles that can retract into a pocket of the body,
2. An eye pore inside of a ventral eyelid, narrow fins, and the shell of a cuttlebone.

Hence, Family – Sepiidae

1. These cephalopods do not have a pore at the posterior end of their mantle and their cuttlebone is about the same length as their mantle.
2. This species has an oval body and can only grow to a maximum length of 40 cm.
3. In English, the word Sepia refers to a "rich brown pigment prepared from the ink of cuttlefishes". The word officinalis is a Latin word that means "used in medicine".

Hence, the Specimen is *Sepia officinalis*

Specimen – 12



Systematic Position:

Phylum - Mollusca

Class - Cephalopoda

Order - Teuthida

Family - Loliginidae

Genus - *Loligo*

Species – *Loligo vulgarais*

Identification:

1. Presence of a mantle with a significant cavity used for breathing and excretion,
2. Radula resent.
3. This has a single, "limpet-like" shell on top, which is made of proteins and chitin reinforced with calcium carbonate, and is secreted by a mantle covering the whole upper surface.
4. The underside of the animal consists of a single muscular "foot".

Hence, Phylum – Mollusca

1. Exclusively marine animals are characterized by bilateral body symmetry,
2. Presence of a prominent head, and a set of arms or tentacles (muscular hydrostats) modified from the primitive molluscan foot.

Hence, Class – Cephalopoda

1. The main body mass is enclosed in the mantle, which has a swimming fin along each side. These fins, unlike in other marine organisms, are not the main source of locomotion in most species.

2. The skin is covered in chromatophores, which enable the squid to change color to suit its surroundings, making it practically invisible. The underside is also almost always lighter than the topside, to provide camouflage from both prey and predator.
3. Under the body are openings to the mantle cavity, which contains the gills (ctenidia) and openings to the excretory and reproductive systems. At the front of the mantle cavity lies the siphon, which the squid uses for locomotion via precise jet propulsion.

Hence, Order – Teuthida

1. Very muscular body with 3 – 100 mm in size.
2. All member of the family have a cornea that covers the lens of each eye.
3. Presence of a gladius that extends the full length of the mantle and a gill that has a branchial canal.

Hence, Family – Loliginidae

1. Long, moderately slender and cylindrical body. Rhomboid fins comprise two-thirds of the mantle length, though locomotion is via jet propulsion.
2. The posterior border is slightly concave.
3. The head is relatively small and has large eyes which are covered with a transparent membrane.
4. Like almost all squid, this species has ten limbs surrounding the mouth and beak: eight are relatively short arms, and two, which form the tentacles, are long, as they are used to catch prey.
5. The fourth left arm of males is a hectocotylus. The European squid can grow up to 30–40 cm in the mantle length, but more usually they are 15–25 cm long.
6. The males are generally bigger than the females and exhibit more rapid rates of growth.

Hence, the species - *Loligo vulgaris*

Macrophytes

1. ***Eichhornia crassipes*** (Common water hyacinth)

Systematic Position

Kingdom- Plantae

Order-Commelinales

Family-Pontederiaceae

Type-. ***Eichhornia crassipes***

Distinctive features

1. Floating waterweed up to 65 cm tall and the leaves are dark green rounded leaves up to 5–10 cm in diameter.

2. Leaf stalks of young plants are swollen into spongy, bulbous structures; mature plants have elongated leaf stalks.

3. An erect stalk supports a single spike of 8 to 15 flowers. Flowers are light purple with a darker blue-purple with yellow centres.

4. The root system is extensive feathery, fibrous, black to purple.

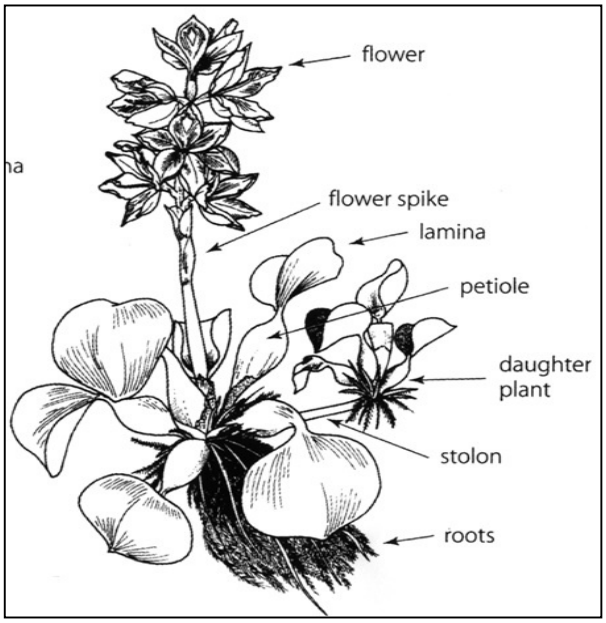
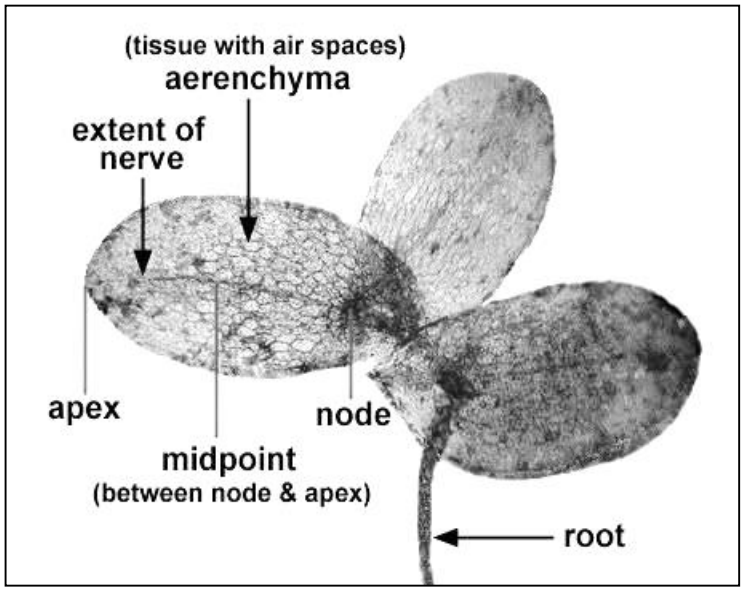


Fig 10. Common water hyacinth



2. ***Lemna minor***

(Duck weed)

Systematic Position

Kingdom- Plantae

Order-Alismatales

Family-Araceae

Type-*Lemna minor*

Fig 11. Duckweed

Distinctive features

1. Perennial aquatic plant usually growing in dense colonies forming a mat on water surfaces.

2. Each plant is a smooth, round, flat disc 0.5 to 1.0 cm wide with mostly green or red upper surface while lower surface is dark red.

3. Produces several minute roots and a pouch containing male and female flowers.

Aquatic insects :

1. ***Ephemera* sp.** (May fly larvae)

Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Insecta

Subclass- Pterygota

Order-Ephemeroptera

Type-*Ephemera*

Distinctive features

1. Sides of the abdomen with plate like, feather like or leaf like gills, usually with three terminal filaments.

2. Tarsal claw single.

3. Older larvae show developing wing pads.

4. Gills feathery, consists of two branchiaes each thickly fringed with filaments.

5. Gills held over back during life.

6. Mandibles project beyond front of the head.

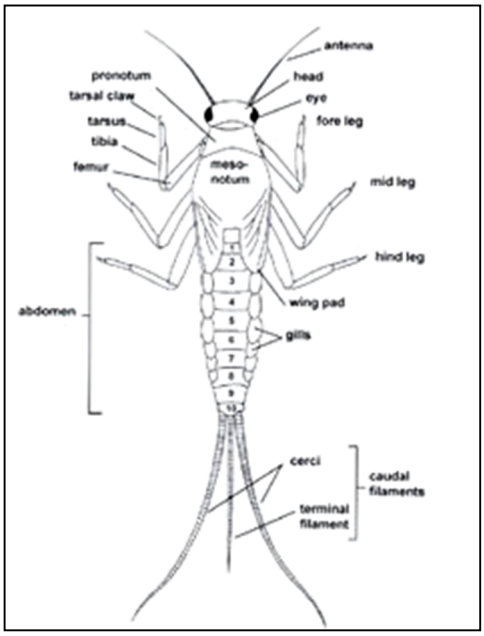
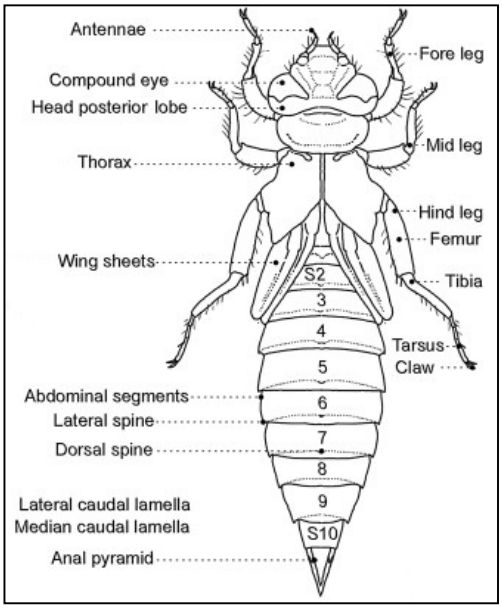


Fig 7.*Ephemera* sp. (May flylarvae)

2.**Dragon fly nymph**



Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Insecta

Subclass- Pterygota

Order-Odonata

Type-Dragon fly nymph

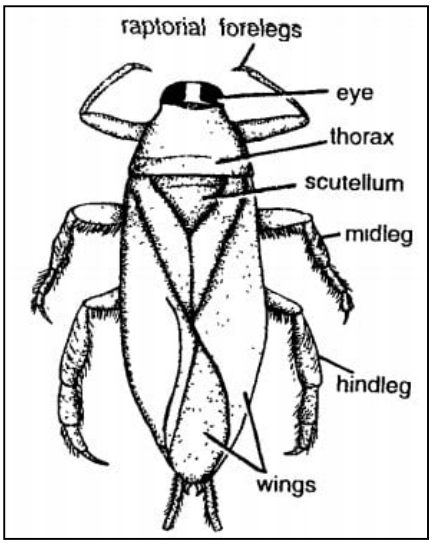
Distinctive features

1. Labium prominent, extensible forming a food capturing structure larger than head.

2. Abdomen terminating in three to five stiff pointed valves, longest less than one third of abdomen.

Fig 8. Dragon fly nymph

3. ***Belostoma* sp.** (Giant water bug)



Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Insecta

Subclass- Pterygota

Order-Hemiptera

Type-*Belostoma*

Distinctive features

1. Large insect with elongated body covered by leathery brownish exoskeleton.

2. Mouth parts piercing and sucking type.

Fig 9.*Belostoma* sp.

3. Legs flat, forelegs shorts and raptorial while posterior legs adopted for swimming and crawling.

4. Thorax broad and somewhat triangular.

Physicochemical Characteristics of water

Determination of Salinity of Water:

- **Chloride:**

Chloride, in the form of the Cl– ion, is one of the major inorganic anions,or negative ions, in saltwater and freshwater. It originates from the dissociation of salts, such as sodium chloride or calcium chloride, in water.

$$NaCl(s) \longrightarrow Na^{+}(aq) + Cl^{-}(aq)$$

$$CaCl_2(s) \longrightarrow Ca^{2+}(aq) + 2\,Cl^{-}(aq)$$

Sources of Chloride Ions:

- River streambeds with salt-containing minerals
- Runoff from salted roads
- Irrigation water returned to streams
- Mixing of seawater with freshwater
- Chlorinated drinking water

Expected Levels:

- Seawater has a chloride ion concentration of about 19,400 mg/L (a salinityof 35.0 ppt).
- Brackish water in tidal estuaries may have chloride levels between 500 and5,000 mg/L (salinity of 1 to 10 ppt).
- Even freshwater streams and lakes have a significant chloride level that canrange from 1 to 250 mg/L (salinity of 0.001 to 0.5 ppt).
- **Requisites:**

Glass wares, Burette, Pipette, Volumetric flasks, Porcelin Basin etc.

- **Reagents:**
- □ Potassium Chromate Indicator K_2CrO_4.
- □ Standard Silver Nitrate $AgNO_3$.
- □ Standard Sodium Chloride NaCl.

- **Procedure:**
- **Step 1:**
- Take 100 ml Sample water in a conical flask.
- **Step 2:**
- Add few drops $K_2Cr_2O_7$ Solution.
- **Step 3:**
- Yellow Colour Appearance.
- **Step 4:**
- Take initial Burette Reading (ml)
- **Step 5:**
- Titrate with $AgNO_3$.
- **Step 6:**
- Brick-Red Colour Appearance.
- **Step 7:**
- End Point of the Titration and Take the Final Burette Reading.

Calculation Concentration of Cl- (ppm) = X * F * 1000 / VWhen,

X= Titre Value of Ag NO_3 (ml) F= Factor (Normality of Ag. NO_3)

V= Vol. of water sample (ml) Used

Salinity (ppt) = Chlorinity * 1.805 + 0. 03

Determination of Organic Carbon.

Introduction:

The role of organic carbon in maintaining the productivity of fishponds is well recognized. Apart from influencing various physic-chemical properties of bottom soils and releasing different nutrient elements to more available forms in the pond environment, it controls an important property of the pond eco-system viz. oxidation-reduction reaction.

Principle:

The rapid titrimetric method of Walkley and Black (1934) using heat of dilution is widely used for determination of OC in soil. This method has an advantage that it excludes less active elementary carbon soil and includes only that part of OC which is involved in maintenance of pond productivity. Under this method organic matter in soilis oxidized with excess standard potassium dichromate ($K_2Cr_2O_7$) using the heat of dilution of added concentrated H_2SO_4. The unutilized $K_2Cr_2O_7$ is then titrated with standard ferrous ammonium sulphate and the amount of OC is determined from the amount of standard K2Cr2O7 used for oxidation.

Requisites:

1. **Glassware:** 500 ml conical flasks, pipette, burette, measuring cylinder,1lit Volumetric flasks.
2. **Reagents:**
   a) 1 N K2Cr2O7
   b) 1 N Fe (NH4)2(SO4) 2
   c) Diphenyl amine Indicator
   d) Concentrated H_2SO_4
   e) 85% H_3PO_4

Procedure:

Step 1: Take 1 g of soil sample in a 500 ml conical flask and moisten itwith a few drops of DW, Keep for about 10 minutes.

Step 2: Add exactly 10 ml of 1 N K2Cr2O7 and then 20 ml of conc. H2SO4.

Step 3: Mix the contents thoroughly and keep in a dark place for 30 min.

Step 4: Then add 200 ml DW and 10 ml of H_3PO_4.Put about 1 ml ofdiphenyl amine indicator to develop a deep blue color.

Step 5: Titrate the solution with standard 1 N Fe (NH4)2(SO4) 2

Step 6: At the end point the blue color suddenly flushes to bright green.

Step 7: Carry out a blank with all the reagents but without Soil.

Calculation:

Organic Carbon (Percent) = (B-A) *0.3

Where,

B= Titration Value (ml) of Fe (NH4)2(SO4) 2 in Blank Set.

A= Titration Value (ml) of Fe (NH4)2(SO4) 2 with Soil.

OC Level in Pond Soils:

Banerjea (1967) categorized fishpond soil of India Under different productivity levels with respect to OC status of the Soil. In his opinion, pond soils having less than 0.5 % OC may be too poor while those in the range of 1.5-2.5 % may be taken as optimum for fish production.

Comments: Chloride ions, when present in considerable concentrations in soil, mayinterfere with OC determination under this procedure. Such interference can be controlled by application of silver sulphate AgSO4. Add 5 g of AgSO4 in 100 ml conc.H2SO4. Use 20 ml of this AgSO4 mixed H2SO4 after addition of K2Cr2O7 for digestion in stead of ordinary H2SO4. Such modification is essential for estimating OCcontent of Brackishwater pond soil.

pH DETERMINATION OF WATER

Principle

Hydrogen ion concentration of water is very important chemical constituent of water. It both affects the biological as well as chemical of water.

pH meter is regulated using a freshly made buffer solutions (pH 4, 7 and 9) and the slope of electrode adjusted against the respective strengths of solutions. Temperature compensation is adjusted manually according to the ambient sample temperature. The electrode is thoroughly rinsed with distilled water before each measurement.

Materials required

a. pH meter

b. Distilled water

c. Sample water

d. Buffer tablets (pH 4, 7 and 9) and corresponding buffer solutions.

Procedure

1. Buffer solution of pH 4.0, 7.0 and 9.0 are prepared by using standard buffer tablets.

2. Electrodes are connected to pH meter.

3. Electrodes are dipped in a buffer solution of pH 7.0.

4. Temperature control of the pH meter is set as per the temperature of the sample water.

5. pH is recorded. The meter gives a reading near 7.0.

6. The electrodes are then removed from buffer solutions and washed with distilled water and dried. The electrodes are dipped to buffer of pH 4.0 and 9.0 consecutively. The meter should correspondingly show a reading of 4.0 and 9.0 respectively.

7. Next the electrodes are dipped in the water sample (test sample). The sample is gently swirled twice and pH is seen in the digital display or in the meter of the instrument. Three such replicates are taken.

8. Mean pH value is taken.

Observation

No. of Observations	pH value	Mean pH value

Inference

Depending upon the pH of the water one can state whether the sample water is acidic, neutral or alkaline.

The pH of sample water is _____. Thus it has _____ pH.

CALCULATION OF LENGTH- WEIGHT RELATIONSHIP OF FISH.

- The length-weight relationship of a fish is basically a measure of its growth pattern and its age.
- It also helps to evaluate the condition, reproductive history, life cycle and the general health of the fish.
- Negative changes in growth rates may result in increased risk of predation and mortality.
- Studies on the length–weight relationship provides the basis for estimation of the average weight of fish of a given `group.
- It also provides the understanding of morphorlogical comparisms between populations.

- Total length (TL; tip of snout to caudal fin end)
- Standard length (SL; tip of snout to caudal peduncle)
- Fork length (FL; tip of snout to mid of forked caudal fin)
- Other length measurements (e.g. width in rays)
- Length as proxy for size overestimates weight in eels, underestimates in puffers and boxfishes

Relationship Between Weight and Length

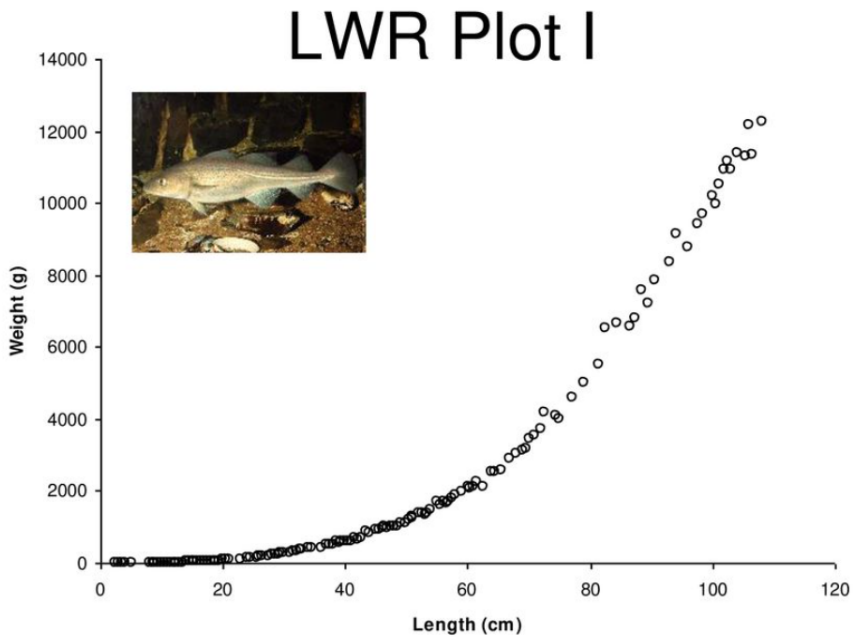
$$W = a * L^b$$

with weight W in grams and length L in cm, to standardize parameters

For parameter estimation use linear regression of data transformed to base 10 logarithms

$$\log_{10} W = \log_{10} a + b * \log_{10} L$$

Plot data to detect and exclude outliers, and to check for growth stanzas



Weight-length data for Cod taken in 1903 by steam trawlers from Moray Firth and Aberdeen Bay. Data were lumped by 0.5 cm length class and thus one point may represent here 1-12 specimens.

Gastrosomatic Index: (GoSI)

GoSI = weight of the stomach / weight of the fish * 100

The gastrosomatic index sometimes indirectly indicates the spawning season in certain species of fin fishes. This index is very low during the peak spawning season because of the more number of empty stomachs. The rise and fall of gastrosomatic index always show an inverse relationship with the Gonadosomatic Index. This is mainly because in fully matured fishes, the Kn value and Gonadosomatic Index are high.

Gonadosomatic Index

Principle: The relative ovary weight or the gonadosomatic index will explain the stages of maturity and spawning periodicity in fishes. The gonads undergo a regular change during the year and when this is accompanied by large changes in females and their seasonal analysis will be the indicative of peak spawning activity.

Equipments/ Reagents: Fresh fish, dissection tools, Weighing balance

Procedure: Take samples, which represent wide range of length groups of fishes. Measure the weight of samples, cut open and remove the gonads. Take weight of the ovary.

$$\text{Gonadosomatic index} = \frac{\text{Wt. of Ovary}}{\text{Wt of fish}} \times 100$$

Observations:

Table:

Graph

Estimation of muscle protein from Indian Major Carp

Principle: The Bradford dye assay is based on the equilibrium between three forms of Coomassie Blue G dye. Under strongly acid conditions, the dye is most stable as a doubly-protonated red form. Upon binding to protein, however, it is most stable asan unprotonated, blue form.

Protein

Red	<=>	Green	<=>	Blue	<=>	Blue-Protein
(470 nm)		(650 nm)		(590 nm)		(590 nm)

H^+ H^+

The Bradford assay is faster, involves fewer mixing steps, does not require heating, and gives a more stable colorimetric response, its response is prone to

influence from non protein sources, particularly detergents, and becomes progressively more nonlinear at the high end of its useful protein concentration range. The response is also protein dependent, and varies with the composition of the protein.

a) Materials Required:

(i) **Equipments:** Spectrophotometer.

Glass or polystyrene cuvettes

(ii) Chemicals/reagents:

- Bradford reagent
- Bovine serum albumin (BSA)

(iii) Glass wares and others:

- Test-tubes
- Pipettes
- Micro centrifuge tubes

b) Reagent Preparation:

- **Protein extraction buffer:**

Tris HCl pH 8.1------ 10mM

EDTA pH 8.0 ---------10mM

β-Mercaptoethanol --- 5mM

PMSF ---------- --0.1 mg/ml

BSA stock solution: 2.0mg/ml in extraction buffer

Procedures:

Protein Extraction

1. Weigh 1-1.5g muscle from specimen. Add 1ml of protein extraction buffer per gram of muscle in a cold mortar and pestle.

2. Grind the tissue in the presence of fine sand or liquid nitrogen until a thick paste is produced. Collect the paste and place them in a 1.5ml micro centrifuge tube and centrifuge for 20 minutes at 12000 rpm at 4^{o}C.
3. Transfer the supernatant to another 1.5ml micro centrifuge tube.

4. Place about 100µl of the supernatant in a tube for quantification of the extracted protein by Bradford method.

Protein Estimation by Bradford method

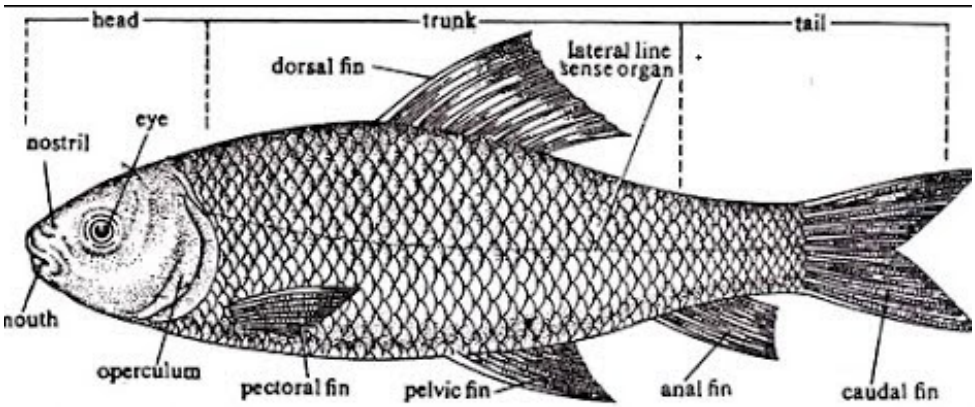
1. Take 100µl of Protein extract containing approximately 10-100µg. As you do not know the protein content of the extract, you will be obliged to run a preliminary assay. Dilute two different concentrations of the extract i.e 20µl and 5µl make up the volume to 100µl with extraction buffer. Add 5ml of dye reagent and mix well. At the same time, prepare a set of standards containing 5, 10, 20, 30, 40, 50, and 100µl of Bovine Serum Albumin (BSA 2.0mg/ml stock in extraction buffer) in separate tubes. Add extraction buffer to each tube to bring the volume to 100 µl. To these tubes also add 5ml of dye reagent and mix well by vortexing. After 5 minutes and before one hour, read the absorbance at 595nm (OD_{595}) against a reagent blank (100 µl of extraction buffer with 1 ml of dye reagent)
2. Calculate the protein concentration in the extract by comparison with the standard curve for BSA. If the OD_{595} for the diluted extract is too high or too low, prepare a more suitable dilution.
3. Different proteins show considerable variation in their dye-binding capacities and so give different responses in the assay. In particular, bovine serum albumin gives a high OD_{595} value and so it is not totally representative of proteins. It is used here for convenience with total leaf extract. If you wish to measure the concentration of a specific protein, it is advised to use a purified form of the same protein as standard.

Table1:

Sl No	PROTEIN		Extraction buffer (µl)	Dye Reagent(ml)	Incubate at at Room Temperature for 30mins to 1Hr	A595 before one hour
	(µl)	(µg)				
1	-	-	100	1.0		
2.	5	10	95	5.0		
3.	10	20	90	5.0		
4	20	40	80	5.0		
5.	30	60	70	5.0		
6.	40	80	60	5.0		
7.	50	100	50	5.0		
8.	100	200	-	5.0		
9.	Unknown Sample (A)	-	-	0.9		
10.	Unknown Sample (B)	-	-	0.9		
11.	Unknown Sample (C)	-	-	0.9		
12.	Unknown Sample (d)					

Calculation: Prepare a standard curve of absorbance versus micrograms of protein and determine the slope y/x from the standard curve, which gives the A_{595} per unit of protein (□g).Hence determine the amount of protein in the unknown sample.

Identification of Freshwater Fishes.



1. Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.

2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**

2. Tail is **homocercal.**

3. Presence of bony endoskeleton.

4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.

2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.

2. Gills are fully filamentous.

3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

1. No scale on the head, head is covered by plates.

2. No teeth in the jaws.

3. Single large dorsal fin.

4. Fins supported by soft fin rays.

5. Lateral line distinct.

Hence, the specimen belongs to the Order Cypriniformes.

1. Body covered with large cycloid scales, no scale on the head.

2. Head prominent with blunt snout projecting beyond the narrow mouth.

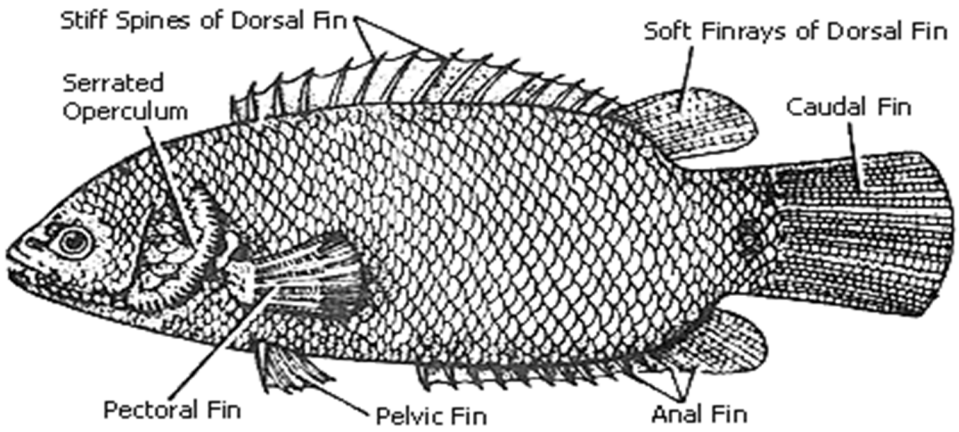
3. Lips thick and horny covering the jaw, having inferior transverse fold.

4. Barbels are very short or absent.

5. Body colour is bluish-brown on the dorsal aspect, and silvery-white with reddish-orange tinge on the ventral aspect.

Hence, the specimen seems to be *Labeo sp.*

Anabus sp.(Koi fish)



1. Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.

2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**

2. Tail is **homocercal.**

3. Presence of bony endoskeleton.

4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.

2. Fins are supported by fin rays.**Hence, the specimen belongs to the Subclass Actinopterygii.**

1. Scales are covered by **epidermis** or absent.

2. Gills are fully filamentous.

3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

1. Dorsal and anal fins are both with spiny and soft rays.

2. Dorsal fins are two in number.

3. Body is covered by **ctenoid** scales.

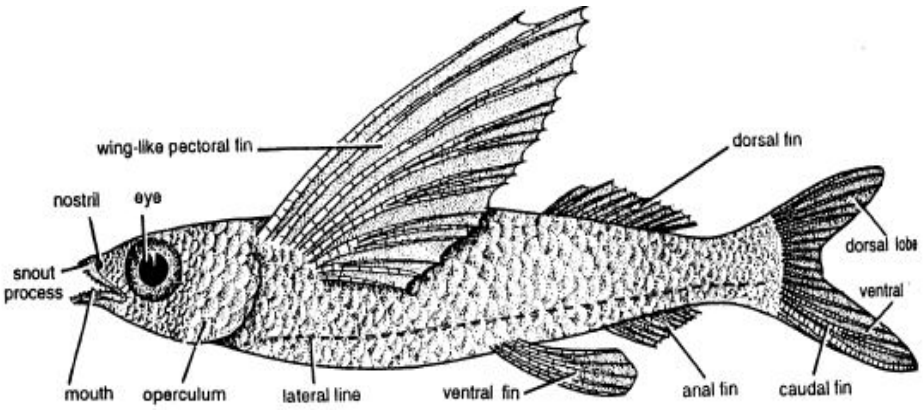
Hence, the specimen belongs to the Order Perciformes.

1. Dorsal and anal fins are divided and supported by anterior spiny and posterior soft rays.

2. Small spiny projections are present along the posterior edge of operculum.

Hence, the specimen seems to be *Anabus sp.*

Exocoetus sp. (Flying fish)



1.
Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.

2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**

2. Tail is **homocercal.**

3. Presence of bony endoskeleton.

4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.

2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.

2. Gills are fully filamentous.

3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

1. Actually spindle like long body.

2. Pectoral fins are very big and modified into flying structure.

3. Pelvic fins are at the abdominal region.

Hence, the specimen belongs to the Order Beloniformes.

1. Pectoral fins are enormously elongated to form wing-like structures. They serve as parachute to sustain the fish in its gliding leap.

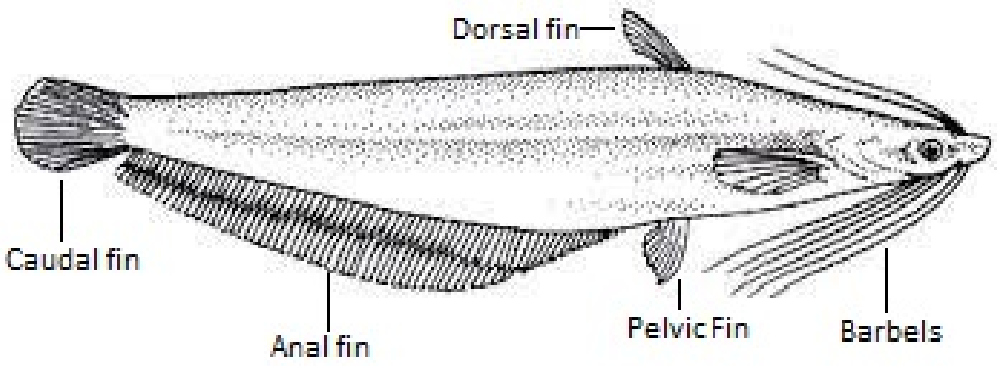
2. Lateral line is located low on the body.

3. Pectoral fin with black spots.

4. Tail is hypobatic and ventral lobe of the tail fin is much elongated and help in skipping over the water.

Hence, the specimen seems to be *Exocoetus sp.*

Heteropneustes sp. (Singhi)



1. Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.

2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**

2. Tail is **homocercal.**

3. Presence of bony endoskeleton.

4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.

2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.

2. Gills are fully filamentous.

3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

1. Pectoral fin with a spine.

2. Barbels present.

3. Scales absent.

Hence, the specimen belongs to the Order Siluriformes.

1. Skin is scaleless.

2. Head is flat with four pairs of **barbells.**

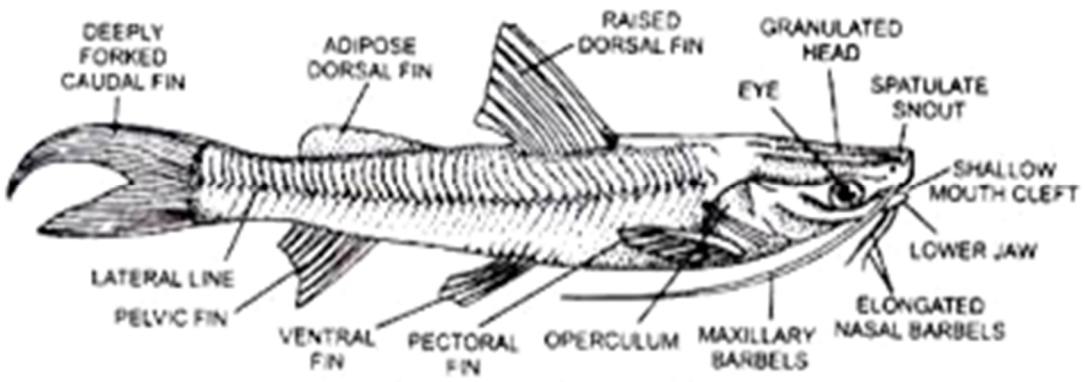
3. First ray of pelvic fin is modified into a serrated spine.

4. Dorsal fin is very small.

5. Caudal fin is separated by a notch from extended anal fin.

Hence, the specimen seems to be *Heteropneustes sp.*

Mystus sp. (Tangra)



1. Presence of hollow, dorsal, tubular nerve cord.

2. Presence of **notochord**, pharyngeal gill slits and post anal tail during some stages in the **ontogeny**.

Hence, the specimen belongs to the Phylum Chordata.

1. Presence of **cranium** and **vertebral column**.

Hence, the specimen belongs to the Subphylum Vertebrata.

1. Mouth is covered by functional jaws.

2. Presence of paired appendages.

Hence, the specimen belongs to the Superclass Gnathostomata.

1. Gills are covered by an **operculum.**

2. Tail is **homocercal.**

3. Presence of bony endoskeleton.

4. Mouth is terminal.

Hence, the specimen belongs to the Class Osteichthyes.

1. Fins have basal support projecting outside the body wall.

2. Fins are supported by fin rays.

Hence, the specimen belongs to the Subclass Actinopterygii.

1. Scales are covered by **epidermis** or absent.

2. Gills are fully filamentous.

3. Presence of swim bladder.

Hence, the specimen belongs to the Super Order Teleostei.

1. Pectoral fin with a spine.

2. Barbels present.

3. Scales absent.

Hence, the specimen belongs to the Order Siluriformes.

1. Presence of **adipose** dorsal fin.

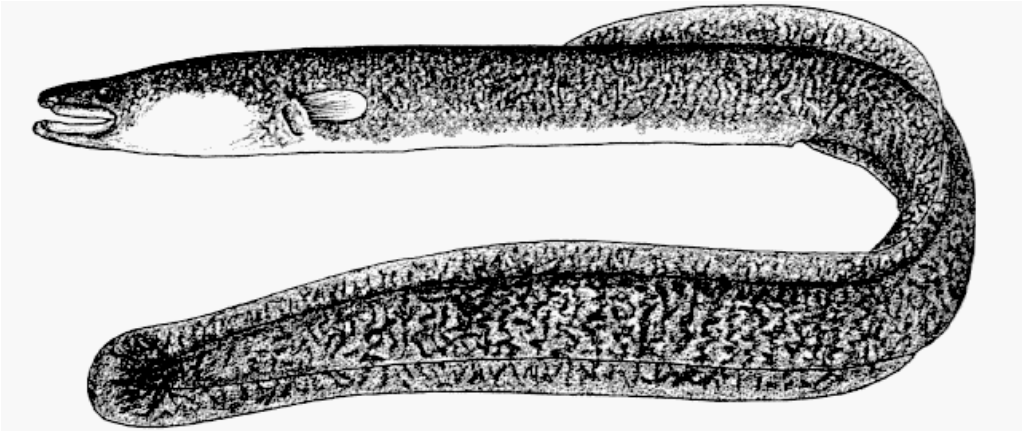
2. Body is blackish above and slivery on the sides.

3. Snout is distinctly long with four pairs of barbells.

4. Presence of light and dark coloured longitudinal bands on bodywall.

Hence, the specimen seems to be *Mystus*

Anguilla sp



Phylum – Chordata

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws

- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Gular plate present in non eel like representatives.
- Branchiostegals usually numerous.

Hence, Super order - Elopomorpha

- Body ill like, smooth, with minute or rudimentary scales, imbedded in the skin or absent
- Gill opening in the pharynx as narrow or wide slits
- Pelvic fins are absent

Hence, Order – Anguilliformes

- Gill openings situated in the pharynx in the form of moderate slits near the base of pectoralfins.
- Nostrils lateral or superior

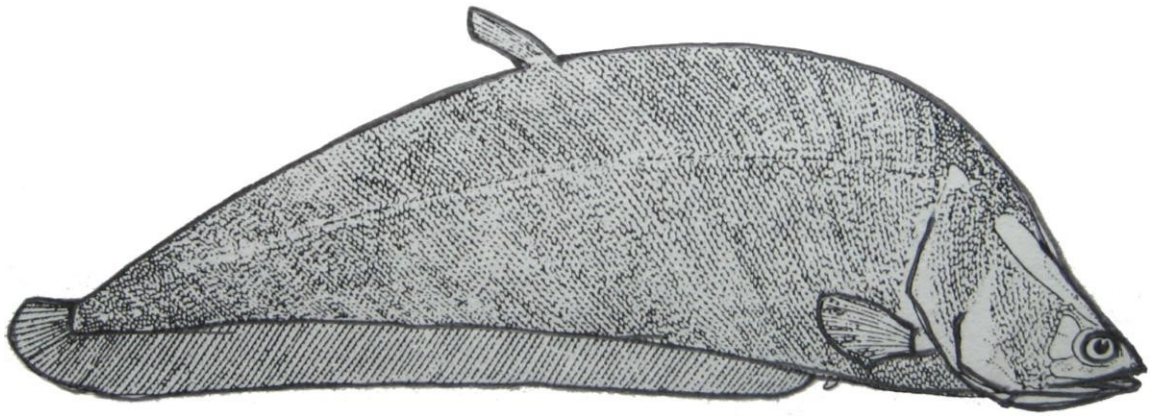
Hence, Family – Anguillidae

- Body elongated, cylindrical, band-shaped, Abdomen rounded, Head long and compressed,Snout pointed.
- Mouth terminal, cleft of mouth wide, extending to the posterior margin of the orbit
- Eyes are very small, superior, in middle of the head, not visible from the bellow ventralsurface
- Lips are thick and well developed
- Jaws are equal
- Caudal fin continued round the end of the tail

Hence, Genus – *Anguilla*

Hence, the provided specimen is *Anguilla* sp

Notopterus sp



Identifying Character:

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- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
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Hence, Subphylum – Vertebrata

- Mouth bounded with jaws

- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Premaxilla firmly bound to the ethmo-vomerine region
- Branchiostegals three to five

Hence, Super Order – Osteoglossomorpha

- Maxillaries well toothed and forming the greater part of the upper jaw
- Maxillaries and premaxillaries firmly bound together and have restricted mobility
- Supra maxillae absent

Hence, Order – Osteoglossiformes

- Abdomen serrated before pelvic fins
- Dorsal profile not compressed as ventral profile
- No barbells
- Dorsal fin single belonging to the caudal portion of vertebral column

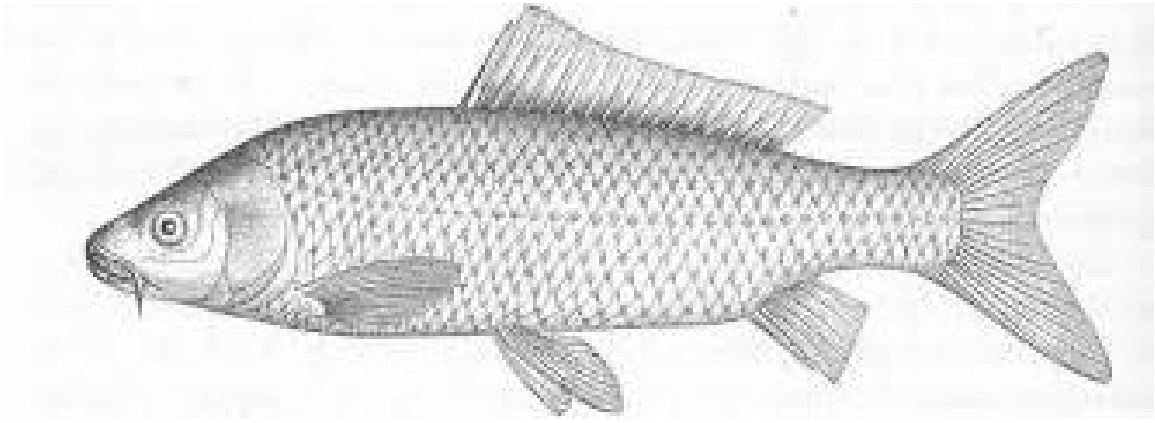
Hence, Family Notopteridae

- Body oblong, laterally compressed
- Abdomen with about 28 pre-pelvic double serration
- Mouth wide, cleft of the mouth extending up to or beyond the posterior border of the eye
- Eyes moderate, superior, in part of the head, not visible bellow the ventral surface
- Lips thin and jaws equal
- Gill membranes partly united

Hence, Genus – *Notopterus*

Hence, the provided specimen is *Notopterus* sp

Cyprinus



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also

- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless
- Barbells often present around the mouth
- No adipose dorsal fin

Hence, Order – Cypriniformes

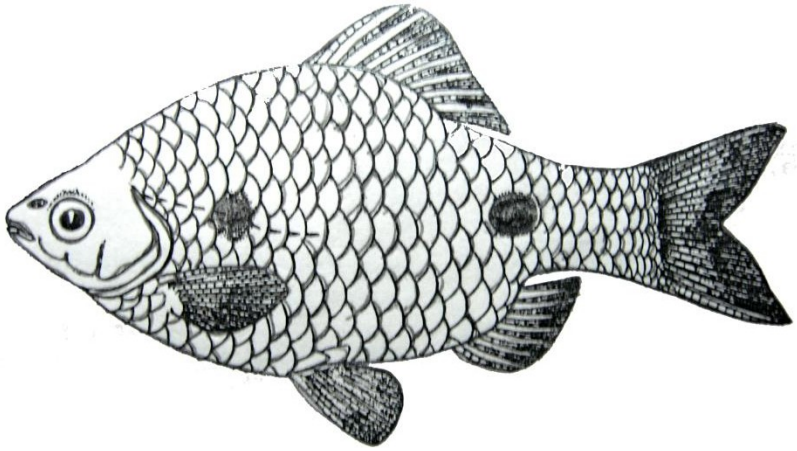
- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or with out sucker
- Developed lips are present

Hence, Family Cyprinidae

- Body robust anteriorly, more or less compressed, with round abdomen
- Head moderate, snout obtusely blunt
- Mouth terminal, oblique cleft not extending to anterior margin of eyes
- Eyes moderate and super lateral, in anterior part of the head visible from below the ventralsurface
- Lips fleshy
- Barbells two pairs, one pair each of rostral and maxillary

Hence, Genus – *Cyprinus*

Hence, the provided specimen is *Cyprinus* sp



***Puntius* sp**

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless
- Barbells often present around the mouth
- No adipose dorsal fin

Hence, Order – Cypriniformes

- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or with out sucker
- Developed lips are present

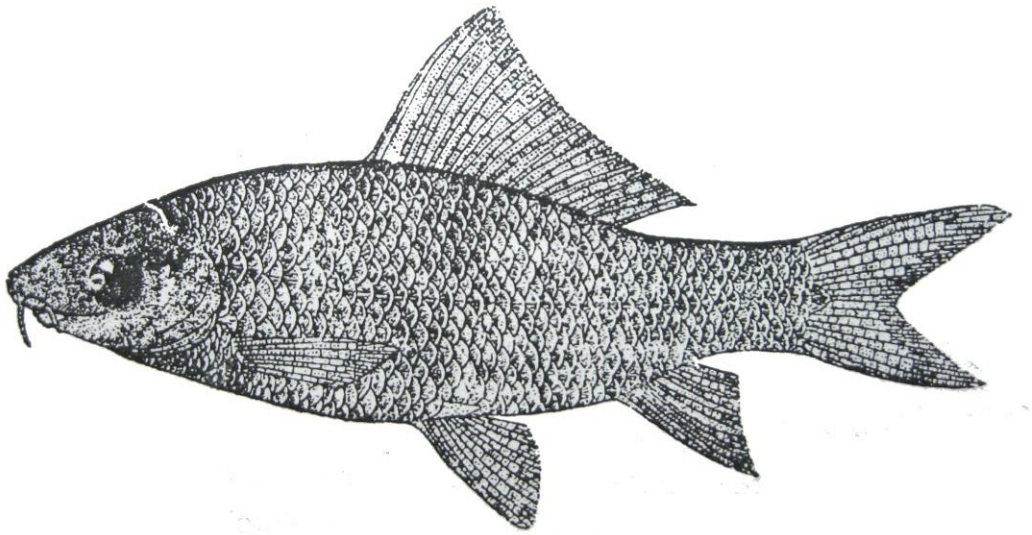
Hence, Family Cyprinidae

- Body short, to moderately elongated, deeply compressed, abdomen rounded
- Head short, snout obtuse conical or pointed
- Mouth arched anterior or inferior not protrusiable
- Eyes moderate to large, dorso lateral, not visible from bellow ventral surface
- Lips thin but covering the jaws, many have lathery lobes, without nay horny covering, jawssimple covered by lips

Hence, Genus – *Puntius*

Hence, the provided specimen is *Puntius* sp

Labeo calbasu



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless
- Barbells often present around the mouth

Hence, Order – Cypriniformes

- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or with out sucker
- Developed lips are present

Hence, Family Cyprinidae

- Body moderately elongated, with round abdomen
- Head fairly large, snout truncated or rounded
- Mouth narrow or moderate, somewhat inferior
- Eyes moderately large, generally situated in the commencement of the posterior half of thehead, not visible from below the ventral surface
- Lips thick and fleshy, fringed, covering both jaws, continuous at angle of mouth forminglabial fold.

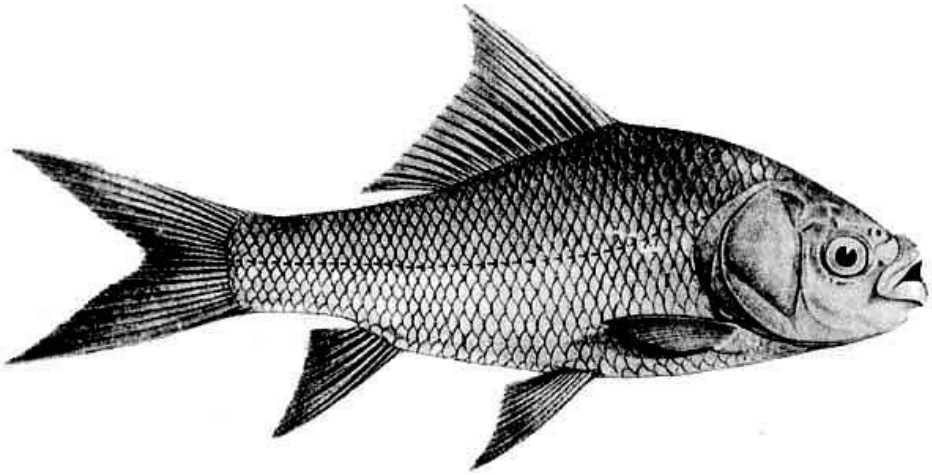
Hence, Genus – *Labeo*

- Dorsal fin inserted above pelvic fin, and 16 - 18 rays,
- Anal fin short with 5 rays (two or three simple)
- Pelvic fin with 8 rays
- Lateral line straight with 40 - 44 scales
- One pair each rostral and maxillary barbells are present
- Colour of the body black, including fins

Hence, species – *calbasu*

Hence, the provided specimen is *Labeo calbasu*

Catla catla



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless
- Barbells often present around the mouth

Hence, Order – Cypriniformes

- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or with out sucker
- Developed lips are present
- Body short and deep

Hence, Family Cyprinidae

- Head broad & large, snout bluntly rounded, may be with pores and thin skin
- Mouth wide, anterior, arched
- Eyes large, in anterior half of the head, visible from below the ventral surface
- Upper lip absent, lower lip moderately thick, continuous and with free posterior margin
- Lower jaw with movable articulation, without prominent knob

Hence, Genus – Catla

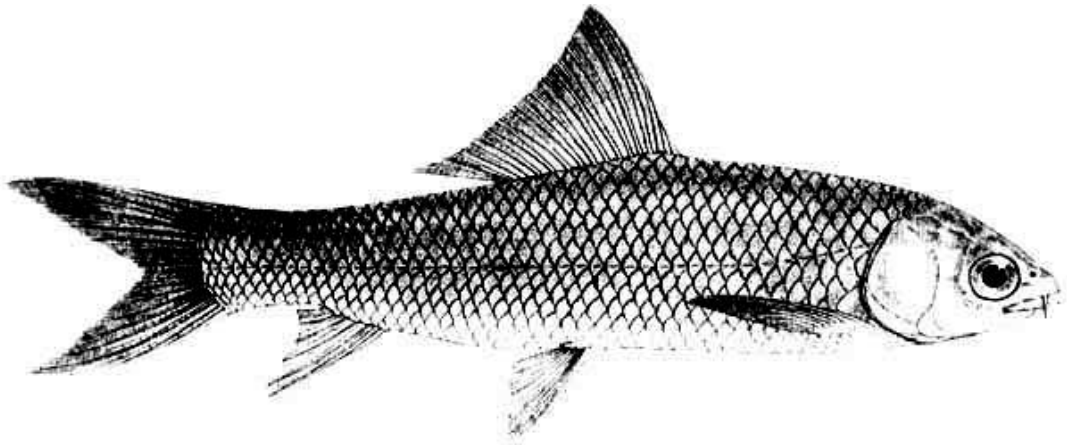
- Body attains a length of about 120 cm in three years
- Dorsal fin long, inserted above the tip of the pectoral fin with 17 to 19 rays (3/4 simple)
- Anal fin short with 8 rays (5 branched)

- Lateral line covered with 40 – 43 scales
- Caudal fin deeply forked

Hence, species – *catla*

Hence, the provided specimen is *Catla catla*

Cirrhinus mrigala



Phylum – Chordata

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless
- Barbells often present around the mouth

Hence, Order – Cypriniformes

- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or with out sucker
- Developed lips are present

Hence, Family Cyprinidae

- Body moderate, elongated, compressed and rounded with round abdomen
- Head short, snout obtusely rounded, may be with pores and thin skin covering
- Mouth broad, transverse
- Eyes moderately large, in anterior half or middle of the head, not visible from below theventral surface
- Upper lip fringed or entire, not continuous with lower
- Lower jaw sharp with a small tubercle at the symphysis, without any cartilaginous coveringinside the jaw

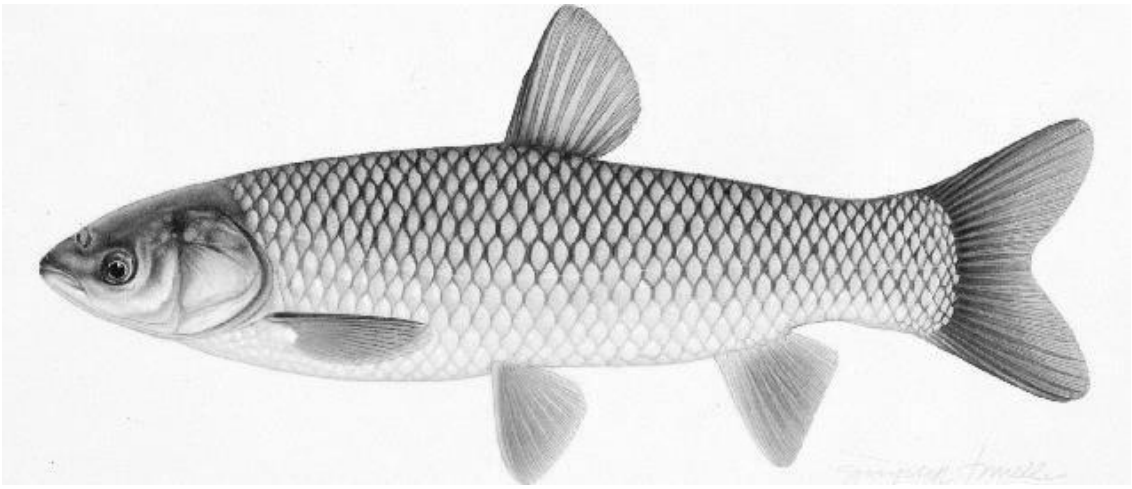
Hence, Genus – *Cirrhinus*

- Body attains a length of about 99 cm in three years
- Dorsal fin long, inserted ahead of the pelvic fin with 15 to 16
- Anal fin short with 7 or 8 rays (2/3 simple)
- Lateral line covered with 40 – 45 scales
- Caudal fin deeply forked or lunate

Hence, species – *mrigala*

Hence, the provided specimen is *Cirrhinus mrigala*

Ctenopharyngodon idella



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless
- Barbells often present around the mouth

Hence, Order – Cypriniformes

- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or with out sucker
- Developed lips are present

Hence, Family Cyprinidae

- Body moderate, elongated, compressed posterior and sub cylindrical in anterior, with roundabdomen
- Head depressed and flattened, snout obtusely rounded,
- Mouth terminal, cleft not extending to the anterior margin of eyes
- Eyes large, lateral in anterior part of the head, may or may not visible just from below theventral surface
- Lips thin without any lobes
- Upper jaw slightly longer than lower and protractile

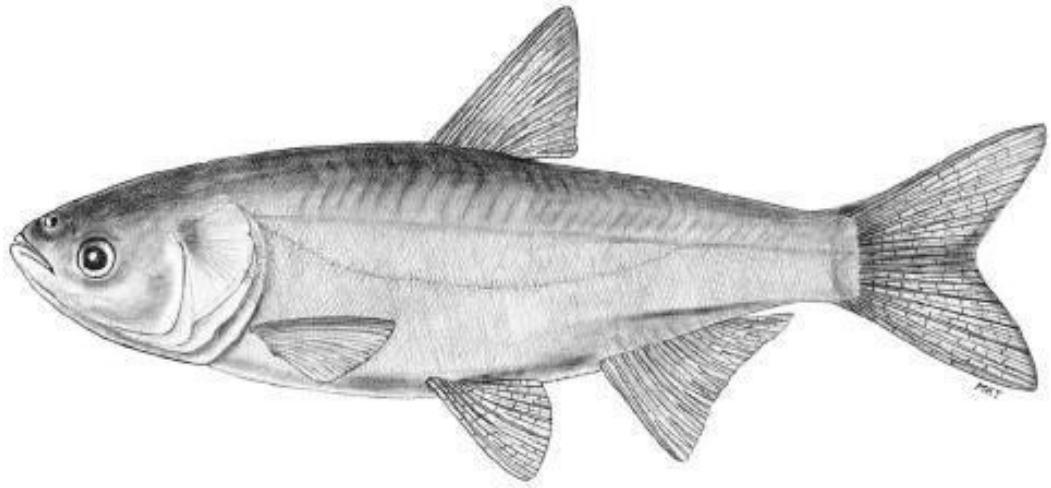
Hence, Genus – *Ctenopharyngodon*

- Body attains a length of about 86 cm in three years
- Dorsal fin inserted slightly ahead of the pelvic fin with 10 rays
- Anal fin short with 10 rays (8 branched)
- Lateral line continuous slightly curved covered with 40 – 42 scales
- Caudal fin deeply forked

Hence, species – *idella*

Hence, the provided specimen is *Ctenopharyngodon idella*

Hypophthalmichthys molitrix



Identifying Character:

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- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species, it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless

Hence, Order – Cypriniformes

- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or with out sucker
- Developed lips are present

Hence, Family Cyprinidae

- Body stout and compressed abdomen strongly compressed with a sharp keel from abdomento vent
- Head moderate, snout bluntly rounded,
- Mouth anterior, large, wide, cleft not extending to the anterior margin of eyes
- Eyes rather small, anterior sub inferior anterior part of the head, sub inferior visible frombelow the ventral surface
- Lips thin
- Upper jaw slightly protruded upward a little longer than lower

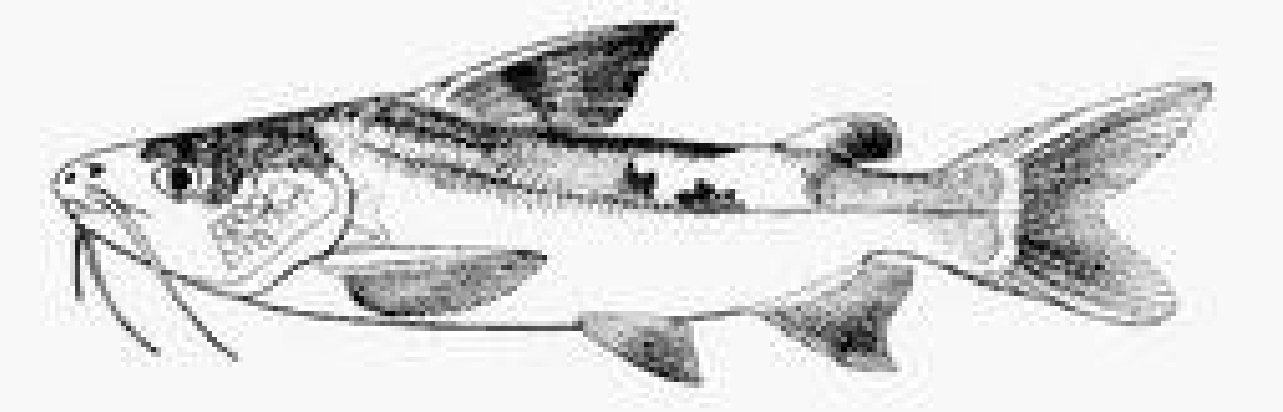
Hence, Genus – *Hypophthalmichthys*

- Body attains a length of about 82 cm in three years
- Dorsal fin inserted behind the pelvic fin or above the tip of the pectoral fin with 10 rays
- Anal fin short with 14 – 17 rays (12 – 14 branched)
- Lateral line continuous slightly curved covered with 110 – 115 scales
- Caudal fin deeply forked

Hence, species – *molitrix*

Hence, the provided specimen is – *Hypophthalmichthys molitrix*

Ompok pabda



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Skin naked with or without bony scutes or plates, never with true scales
- Nearly always one to four pair of barbells
- Adipose fin generally present

Hence, Order – Siluriformes

- Generally large sized elongated fish with compressed body
- Nostrils separated by each other with short distance
- Barbell six or eight, generally well developed

Hence, Family – Siluridae

- Body elongated, compressed with round abdomen
- Head small, broad and depressed
- Mouth superior, moderately wide, its cleft oblique, not extending to the front borders ofeyes, snout bluntly rounded, depressed
- Eyes small, ventral border on the level with corner of mouth, visible from under side of thehead
- Lips thin, Jaws sub equal, lower jaw prominent
- Barbells two pairs, one pair each of maxillary and mandibular, latter occasionally small orrudimentary
- Body attains 17 cm

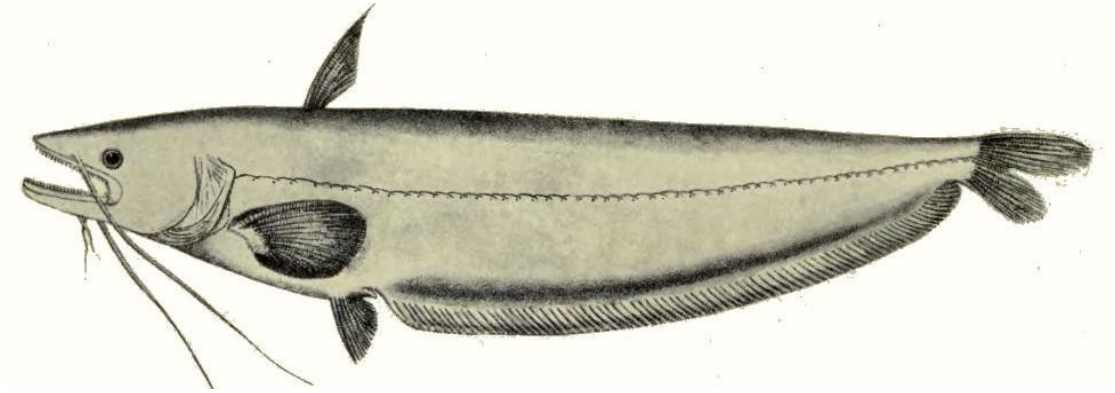
Hence, Genus – *Ompok*

- Rayed dorsal fin inserted above the last half of the pectoral fin with 4-5 rays and withoutany spine
- Adipose dorsal fin absent
- Pectoral fin with 11-14 rays
- Anal fin with 22-56 rays, very long, close to caudal fin, free from it
- Caudal fin forked

Hence, Species – *pabda*

Hence, the provided specimen is *Ompok pabda*

Wallago attu



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Skin naked with or without bony scutes or plates, never with true scales
- Nearly always one to four pair of barbells
- Adipose fin generally present

Hence, Order – Siluriformes

- Generally large sized elongated fish with compressed body
- Nostrils separated by each other with short distance
- Barbell six or eight, generally well developed

Hence, Family – Siluridae

- Body elongated, compressed with round abdomen
- Head large and depressed, snout spatulate, protruded
- Mouth sub terminal, gape wide, reaching to or beyond anterior border of the eyes.
- Eyes small, above the level with corner of mouth, not visible from bellow ventral surface
- Lips thin, Jaws sub equal, lower jaw longer and prominent
- Barbells two pairs, one pair each of maxillary and mandibular

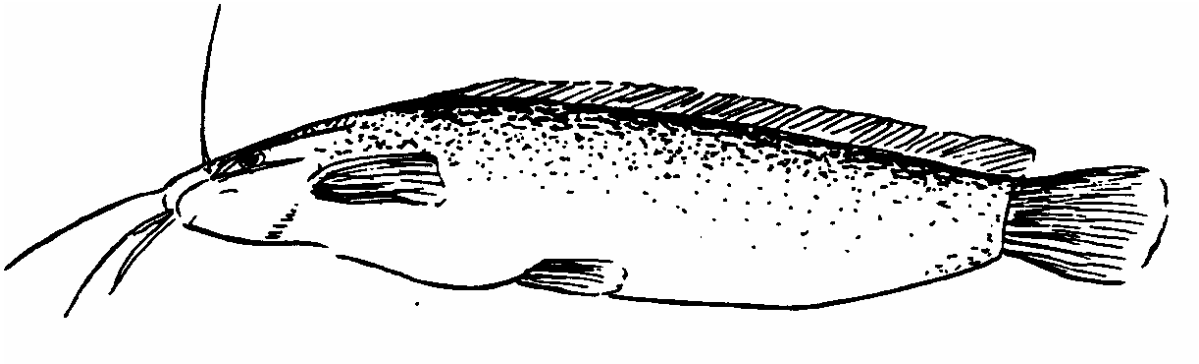
Hence, Genus – *Wallago*

- Rayed dorsal fin inserted above half of the pectoral fin with 5 rays and without any spine
- Adipose dorsal fin absent
- Pectoral fin with 13-15 rays, and feeble smooth spine
- Anal fin long with 86-89 rays, free from caudal fin
- Caudal fin forked with rounded lobes
- Lateral line complete, well marked and simple

Hence, Species – *attu*

Hence, the provided specimen is *Wallago attu*

Clarias batrachus



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws
- Endoskeleton is bony

Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Major trends towards the reduction of number of jaw teeth, may be absent also
- Branchiostegals generally few in numbers, but in some species it may be 15
- Otophysic connections involving the intercalation of bony elements in all

Hence, Super Order – Ostariophysi

- Skin naked with or without bony scutes or plates, never with true scales
- Nearly always one to four pair of barbells
- Adipose fin generally present

Hence, Order – Siluriformes

- Generally large sized elongated fish with compressed body
- Nostrils widely separated, anterior tubular, situated near the tip of the snout
- Barbells 8, moderately developed

Hence, Family – Clariidae

- Body elongated, compressed with round abdomen
- Head moderate, generally depressed, covered with osseous plate dorsally and latterlyforming a cask covering a diverticulum of gill cavity of
- Snout broadly rounded and pointed
- Mouth terminal, fairly wide, transverse
- Eyes small, dorso lateral with free orbital margin, not visible from ventral surface
- Lips fleshy and papillated, jaws sub equal, upper jaw longer
- Barbells four pairs, one pair each of maxillary and mandibular

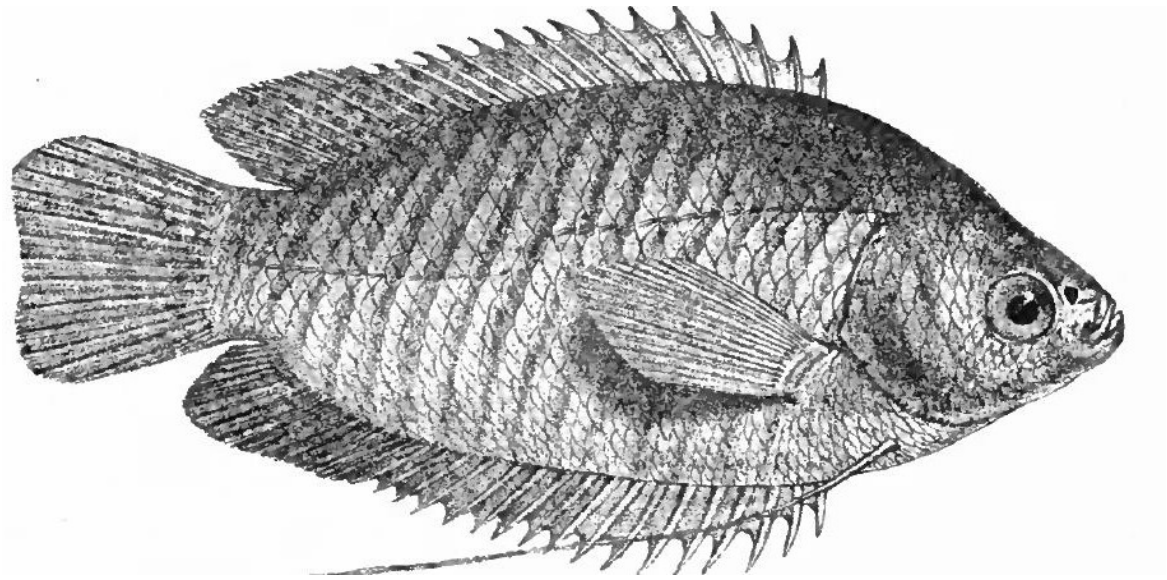
Hence, Genus – *Clarias*

- Rayed dorsal fin long with 62-77 rays without any spine
- Adipose dorsal fin absent,
- Pectoral fin with 7-11 rays, and a strongly serrated spine, enveloped in a skin
- Anal fin long with 45-63 rays
- Caudal fin almost rounded
- Lateral line complete, and simple

Hence, Species – *batrachus*

Hence, the provided specimen is *Clarias batrachus*

Colisa fasciata



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Hence, Super class - Gnathostomata

- Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony
- Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Upper and lower pharyngeal well developed and toothed

- Branchiostegals generally blade like or hair like
- Bones of head with numerous pungent spine
- Otophysic connection rare

Hence, Super Order – Acanthopterygii

- Skin scales, commonly ctenoid,
- Fins are with strong spines

Hence, Order – Perciformes

- Body is short and compressed, anteriorly depressed in slight extent
- Head and body covered with ctenoid scales
- Body elevated, compressed

Hence, Family – Belontidae

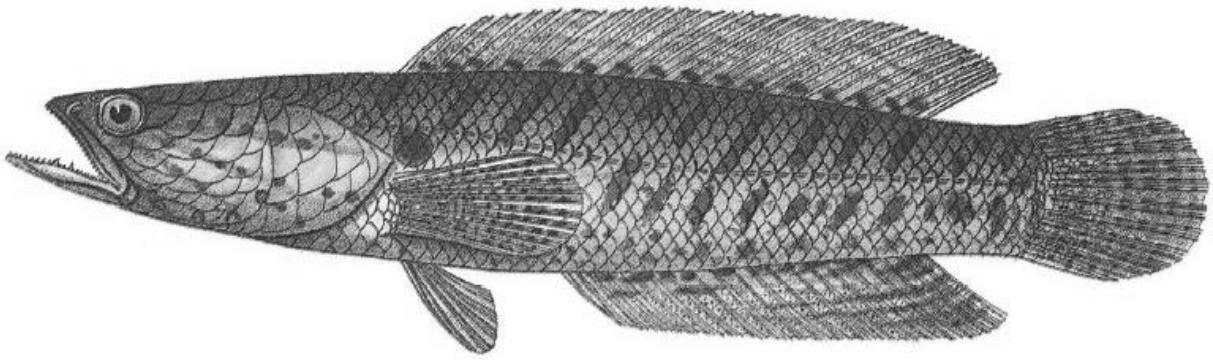
- Head moderate, compressed, Snout blunt
- Mouth upturned, terminal, cleft small.
- Eyes large, lateral in middle of the head, not visible bellow the ventral surface
- Lips thin, jaws sub equal, little protractile

Hence, Genus – *Colisa*

- Single dorsal fin commencing above from near pectoral base, with 15-18 spine & 7-13 rays,number of spine variable
- Anal fin with 15-20 spines and 11-19 rays, number of spines in anal and dorsal fin isvariable
- Pelvic fin form, single elongated, filliform ray
- Caudal fin slightly emarginated or truncate
- Lateral interrupted, with 27-31 scales
- Bands on body, 14 or more

Hence, Species – *fasciata*

Hence, the provided specimen is *Colisa fasciata*



Channa punctatus

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws

Hence, Super class - Gnathostomata

- Endoskeleton is bony, Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony, Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Body elongated with scales, head with plate like scales
- Suprabranchial accessory respiratory organ well developed
- Branchiostegals five

Hence, Order – Channiformes

- Body elongated and sub-cylindrical anteriorly
- Cephalic pits present
- Gills four.

Hence, Family – Channidae

- Body elongated, sub cylindrical anteriorly
- Head depressed with plate like scales
- Mouth opening moderate to wide extending bellow the orbit
- Eyes lateral and moderate
- Lips are moderate
- Teeth present in jaws

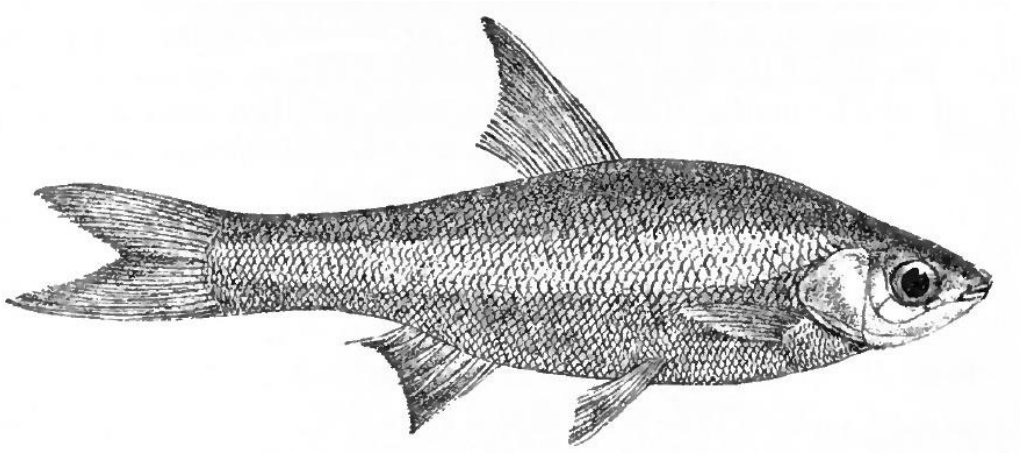
Hence, Genus – *Channa*

- Pelvic fin is more than half of the pectoral fin
- Dorsal fin and anal fin free from caudal fin

Hence, species – *punctatus*

Hence, the provided specimen is *Channa punctatus*

Amblypharyngodon mola



Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
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- Notochord is replaced by vertebral column
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Hence, Subphylum – Vertebrata

- Mouth bounded with jaws

Hence, Super class - Gnathostomata

- Endoskeleton is bony, Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony, Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Body oblong, compressed covered with large cycloid scales, head without scales
- Bony plates never developed
- Jaws, palatine and pterygoid bones are toothless
- Barbells often present around the mouth
- No adipose dorsal fin

Hence, Order – Cypriniformes

- Eyes never covered with skin
- Mouth transverse, superior inferior or terminal, with or without sucker
- Developed lips are present

Hence, Family Cyprinidae

- Body moderately elongated and sub cylindrical, with round abdomen
- Head conical and compressed, snout obtusely rounded
- Mouth wide antero lateral, not protractile
- Upper lip absent
- No barbells present

Hence, Genus – *Amblypharyngodon*

- Lateral line scales – 65 to 75
- Body depth 4 to 4.25 of total body length
- A silvery lateral band with dark markings present on dorsal anal and caudal fin

Hence, species – *mola*

Hence, the provided specimen is *Amblypharyngodon mola*

***Mastacembelus* sp**

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axialskeleton of the body
- Pharyngeal gill slits are present in all members

Hence, Phylum – Chordata

- Notochord is replaced by vertebral column
- Brain is well developed with brain box or cranium
- Gills are present as primary respiratory organ

Hence, Subphylum – Vertebrata

- Mouth bounded with jaws

Hence, Super class - Gnathostomata

- Endoskeleton is bony, Skin is provided with mucous gland
- Scales are ganoids, ctenoid, cycloid, types, some are scale less
- Paired fins are provided with fin rays, which may be cartilaginous or bony
- Gill arches are bony, Gill Chambers are covered by bony operculum

Hence, Class – Osteichthyes

- Body eel like, compressed and elongated
- Mouth non-protractile, elongated and supported by an elongated rod.
- Dorsal and anal fin long.

Hence, Order – Mastacembeliformes

- Body eel like, compressed and elongated, covered with minute sclaes
- Palatine flap like, fused to the ethno-vomer, vomer toothless
- Pyrolic appendages two

Hence, Family – Mastacembelidae

- Snout long, conical, without any transversely striated bones/ bony plates on under surface.

- Preopercle generally spiny at its angle, a pre orbital spine may be absent.
- Dorsal fin inserted above middle of the pectoral with 24-39 detached, depressible spines and50-90 rays.
- Anal fins with 3 spines and 31-98 rays.
- Caudal fin round

Hence, Genus – *Mastacembelus*

Hence, the specimen is *Mastacembelus* sp

Fish food organisms

1. ***Daphnia* sp.**

Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Crustacea

Subclass-Branchiopoda

Order-Cladocera

Type-*Daphnia*

Distinctive features

1. Bilaterally compressed body, enclosed in a transparent bivalve carapace, ending into sharp caudal spine and anteriorly into pointed rostrum.

2. Rounded head bears large **biramous antennae**.

3. Large, sessile, single, huge eye is very distinct.

4. Five pairs of leaf like thoracic appendages and abdomen devoid of appendages.

5. In females, there is a brood pouch near the back.

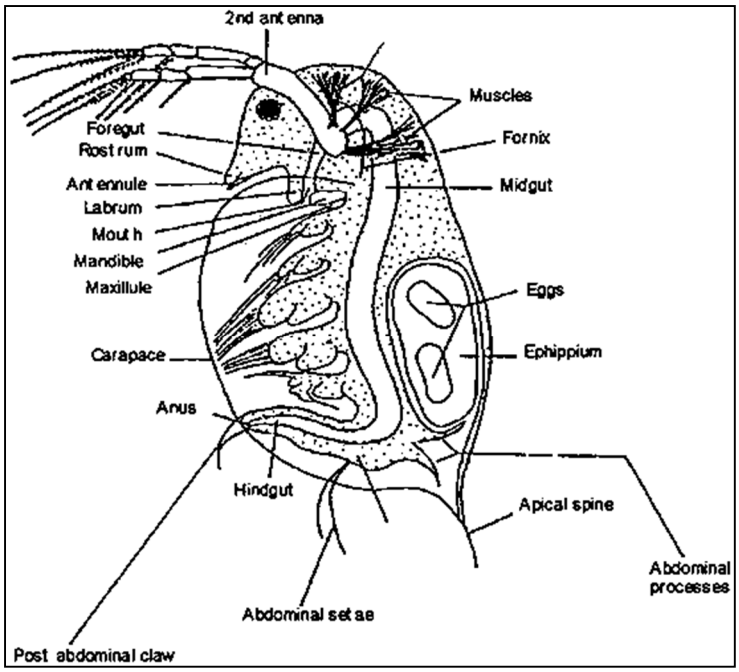


Fig 1.*Daphnia* sp.

2. ***Moina* sp.**

Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Crustacea

Subclass-Branchiopoda

Order-Anomopoda

Type-*Moina*

Distinctive features

1. Body oval shaped.
2. Antennae are branched.
3. Rostrum is in horizontal arrangement.
4. Body terminates into a pair of caudal styles.

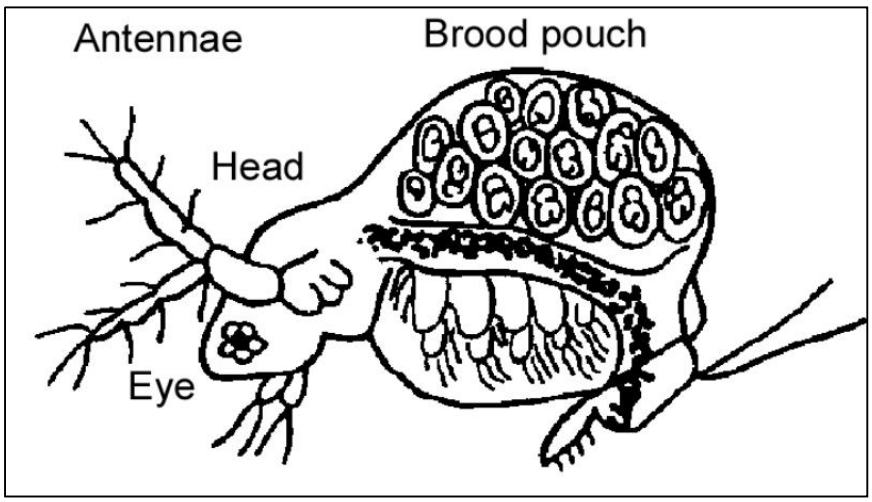


Fig 2.*Moina* sp.

3. ***Cyclops* sp.**

Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Crustacea

Subclass-Copepoda

Order-Cyclopoida

Type-*Cyclops*

Distinctive features

1. Pear shaped body with broad anterior and narrow posterior end.

2. The head and first thoracic segment fuse to form cephalothorax.

3. A median eye is present near the anterior end.

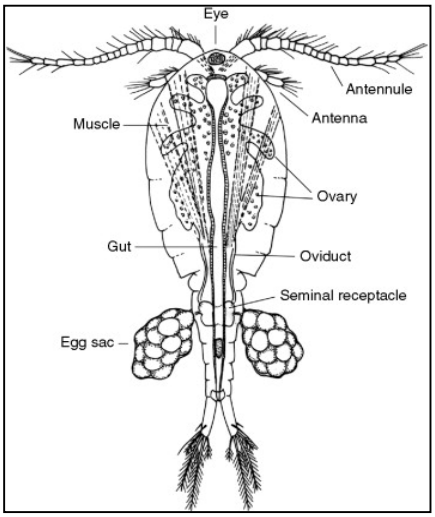


Fig 3.*Cyclops* sp.

4. ***Cypris* sp.**

Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Crustacea

Subclass-Ostracoda

Type-*Cypris*

Distinctive features

1. The body is entirely enclosed within a bivalve carapace.

2. Well developed antennae used in swimming.

3. Gills enclosed within the valves.

4. Head bears four pairs of appendages.

5. Presence of three pairs of thoracic appendages.

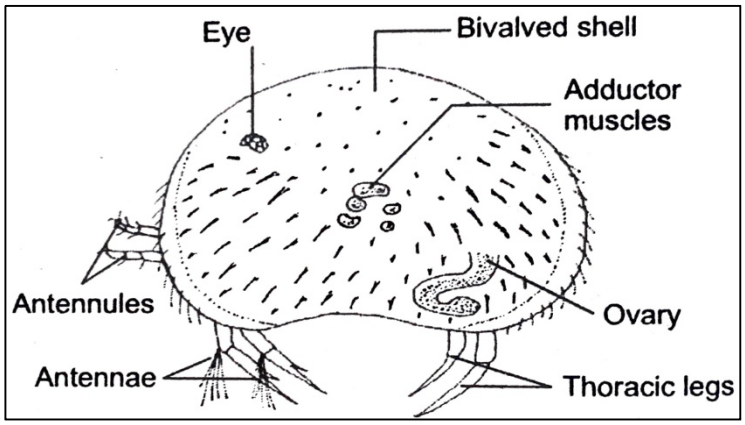


Fig 4.*Cypris* sp.

5. ***Nauplius* larvae**

Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Crustacea

Type-*Nauplius*

Distinctive features

1. Minute, conical and microscopic with broad anterior and narrow posterior end.

2. Body is oval and divided into indistinct head, trunk and anal region.

3. It contains single median eye and frontal and lateral horns.

4. Three pairs of appendages namely uniramous antennules, biramous antennae and biramous mandibles.

5. Larvae contain mouth and gut.

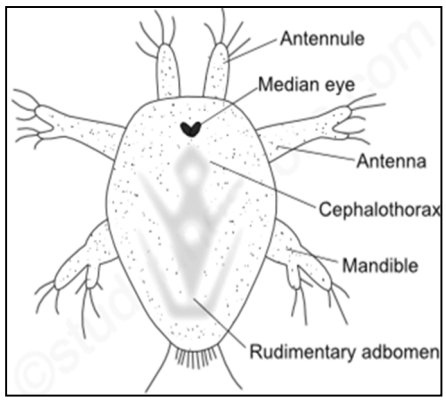


Fig 5. Nauplius larva

6. ***Volvox* sp. (Phytoplankton/Periphyton)**

Systematic Position

Division- Chlorophyta

Class-Chlorophyceae

Distinctive features

1. Free floating fresh water green algae.

2. Each mature *Volvox* colony is composed of up to thousands of cells from two differentiated cell types.

3. Adult somatic cells comprise a single layer with the flagella facing outward.

4. The cells have anterior eyespots that enable the colony to swim toward light.

5. *Volvox* is facultatively sexual and can reproduce both sexually and asexually.

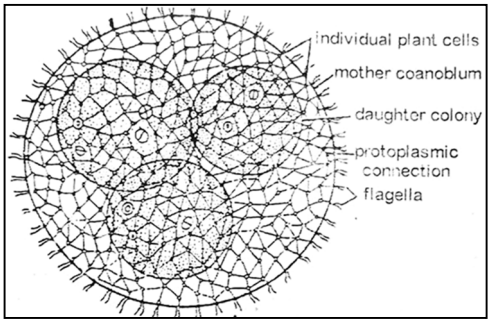


Fig 6.*Volvox* sp.

***Ephemera* sp.** (May flylarvae)

Systematic Position

Phylum- Arthropoda

Subphylum-Mandibulata

Class-Insecta

Subclass- Pterygota

Order-Ephemeroptera

Type-*Ephemera*

Distinctive features

1. Sides of the abdomen with plate like, feather like or leaf like gills, usually with three terminal filaments.

2. Tarsal claw single.

3. Older larvae show developing wing pads.

4. Gills feathery, consists of two branchiaes each thickly fringed with filaments.

5. Gills held over back during life.

6. Mandibles project beyond front of the head.

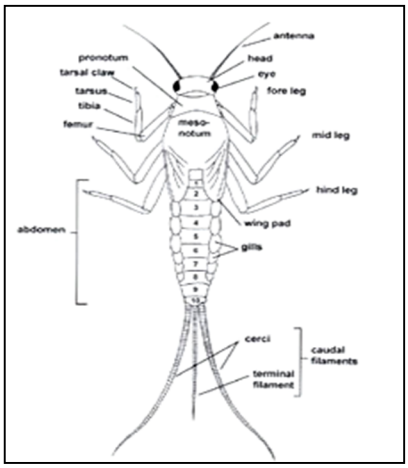


Fig 7.*Ephemera* sp. (May flylarvae)

ARO in Fishes

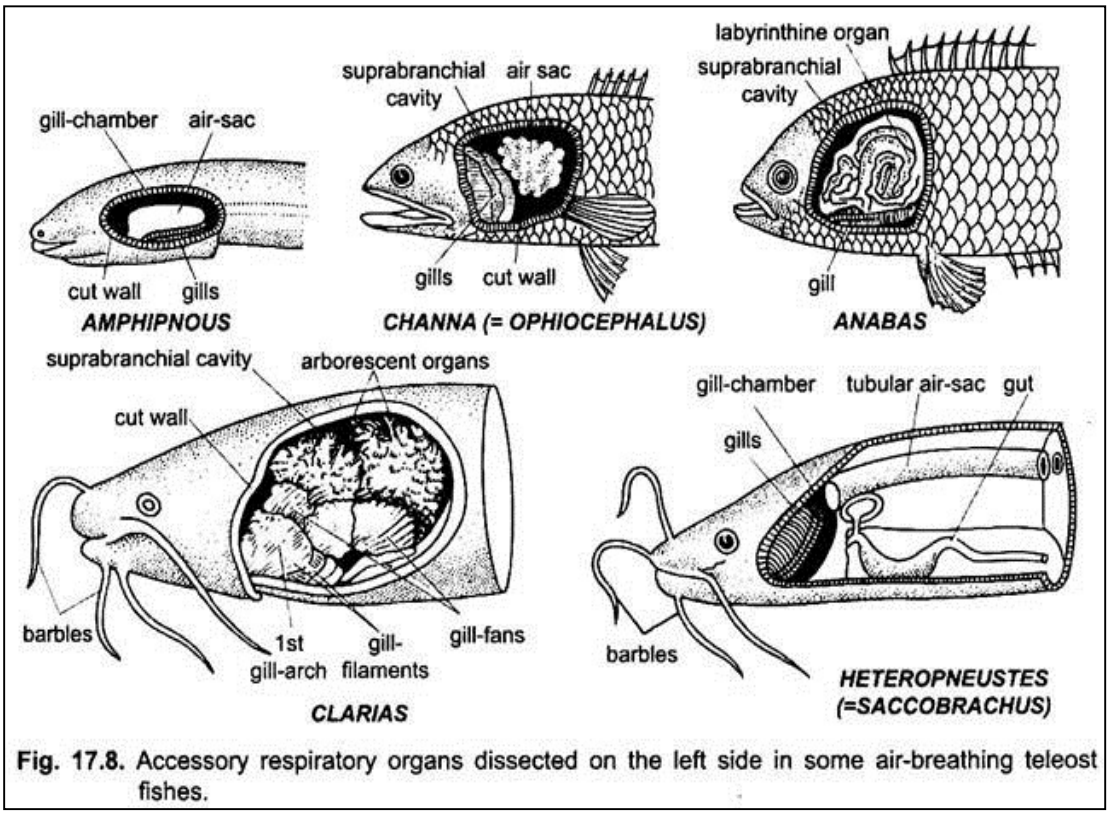


Fig. 17.8. Accessory respiratory organs dissected on the left side in some air-breathing teleost fishes.

BIOCHEMICAL PARAMETER DEMONSTRATION.

ESTIMATION OF CHEMICAL OXYGEN DEMAND FROM WATER SAMPLE

Chemical Oxygen Demand (COD) is the measure of oxygen consumed during the oxidation of oxidisable organic matter by a strong oxidising agent. It is often measured as a swift indicator of organic pollutant in water in both municipal and industrial wastewater treatment plant using both influent and effluent water.

Principle

Potassium dichromate in presence of sulphuric acid generally used as oxidising agent in the determination of COD. The sample is refluxed with potassium dichromate and sulphuric acid in presence of mercuric sulphate to neutralize the effect of chlorides and silver sulphate (catalyst). The excess of potassium dichromate is titrated against ammonium sulphate using Ferroin indicator. The amount of potassium dichromate used is proportional to oxidisable organic matter present in the sample.

Materials required

Reagents

1. **0.25N Potassium dichromate solution**: 12.259g of $K_2Cr_2O_7$ is dissolved in water to make 1L of solution

2. **0.025N Potassium dichromate solution**: 0.25N $K_2Cr_2O_7$ is diluted 10 times.

3. **0.1N Ferrous ammonium sulphate:** 39.2g of $Fe(NH_4)_2(SO_4).6H_2O$ is dissolved in water, adding 20ml of concentrated H_2SO_4 to make it 1L of solution.

4. Ferroin indicator

5. Concentrated H_2SO_4

6. $HgSO_4$

7. $AgSO_4$

Glassware

1. COD flask

2. Conical flask

3. Beaker

4. Glass pipette and glass rod

5. Glass burette with stand

Procedure

1. 20 ml of sample is taken in a 250-500ml COD flask.

2. If the sample is expected to have COD more than 50mg/L, 10ml of 0.025N Potassium dichromate solution is added to the sample.

3. A pinch of $HgSO_4$ and $AgSO_4$ is added.

4. 30ml of sulphuric acid is added to it.

5. The total solution is refluxed for 2 hours on a water bath or hot plate and the refluxed water is made to a final volume of 140ml.

6. 2-3 drops of Ferroin indicator is added and mixed thoroughly and titrated with 0.1N Ferrous ammonium sulphate.

7. A blank is run with distilled water using same quantity of the chemicals.

Calculation

$$\text{COD mg/ml} = \frac{[(b-a) \times \text{strength of } K_2Cr_2O_7 \times 1000 \times 8]}{\text{ml of sample}}$$

a= ml of titrant with sample

b= ml of titrant with blank

ESTIMATION OF DISSOLVED OXYGEN IN WATER

Dissolved oxygen should be measured as quickly and carefully as possible. Ideally, samples should be measured in the field immediately after collection.

Reagent List:

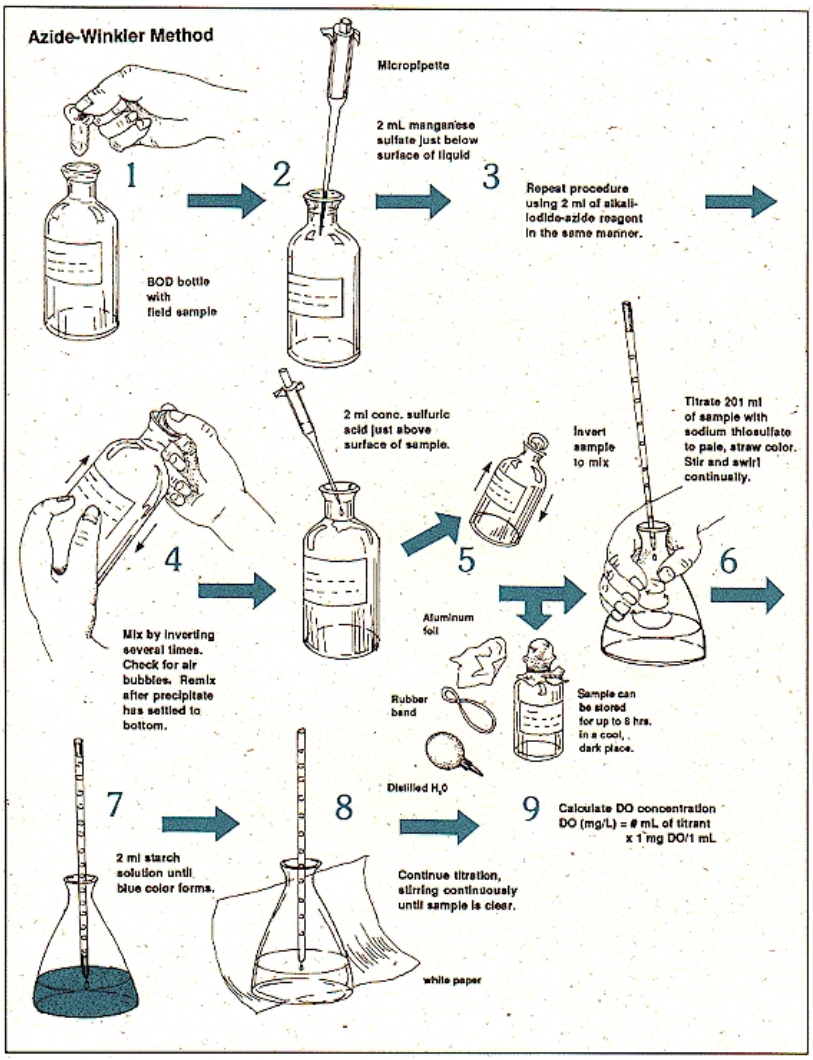
- 2ml Manganese sulfate
- 2ml alkali-iodide-azide
- 2ml concentrated sulfuric acid
- 2ml starch solution
- Sodium thiosulfate

These reagents are available in dissolved oxygen field kits, such as those made by the Hach Company. Please use caution when using these reagents, as they can be hazardous to one's health.

Procedure:

1. Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) stoppered bottle brim-full with sample water.
2. Immediately add 2mL of manganese sulfate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
3. Add 2 mL of alkali-iodide-azide reagent in the same manner.
4. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or floc will appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.
5. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with aluminum foil and a rubber band during the storage period.
6. In a glass flask, titrate 201 mL of the sample with sodium thiosulfate to a pale straw color. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continually stirring or swirling the sample water.
7. Add 2 mL of starch solution so a blue color forms.

8. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue color.
9. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 6 and 8 equals 1 mg/L dissolved oxygen.



CALCULATION:

Dissolved oxygen (mg/lit)= V_1X NX8X1000/V_4(V_2-V_3/V_2)

V_1=volume of titrant

N = Normality of titrant

V_2= vol. of sample bottle

V_3= vol.of reagent added

V_4= vol.of sample titrated

ESTIMATION OF TURBIDITY OF WATER

Turbidity can be measured using several methods. The easiest and least expensive method is through the employment of a Secchi disk. A Secchi disk is an 8-inch diameter disk with alternating black and white quadrants that is lowered into the water column until it can no longer be seen from the surface. The point at which the disk disappears is a function of the lake turbidity. A turbidity tube, or T-tube, can be used as an alternative to lowering a Secchi disk through the water column. The T-tube is a plastic tube with a small-scale Secchi disk pattern at its base. Water samples can be poured into the tube and the clarity of the bottom disk can be used to reveal turbidity.

Secchi Disk Protocol:

1. Slowly lower the Secchi disk into the water on the shady side of the boat until it is no longer visible. Record this depth.
2. Slowly raise the disk until it just becomes visible once again. Record this depth.
3. Average the depths from steps 1 and 2 to get the Secchi depth.
4. This may be repeated for a measurement of precision.

Results

In general, lower turbidity is associated with cleaner, healthier water. Turbidity measurements can vary across different types of environments, so they are especially useful when comparing similar environments or the same water body through time.

Applications

Turbidity measurements can be used for water quality analysis in lakes and streams. Generally the more turbid a lake is, the less biota it will be able to support. Turbid waters inhibit light from penetrating deeply into water column and therefore negatively affect primary productivity and dissolved oxygen available to support other organisms.

Estimation of BOD

Introduction

- BOD stands for Biochemical Oxygen Demand, is the amount of oxygen required for bacteria to decompose biodegradable organic matter at certain temperature for a specific period of time.
- It is used mainly in determination of pollution strength of water in terms of requirement of oxygen, when this wastewater is discharged into water bodies.

- It is also useful in designing of wastewater plants and even this measures the efficiency of some of the treatment processes.
- The organic matter present in wastewater is grouped into two categories. They are –

  a) Carbonaceous Matter –

  This is the amount of oxygen required in sample for microorganisms to decompose the biodegradable carbonaceous matter. This is called 1st stage of decomposition.

  b) Nitrogenous Matter –

  This is called 2nd stage where nitrogenous matter get oxidised by autotrophic bacteria.

  Principle-

  The sample is filled in an airtight bottle and incubated at specific temperature for 5 days. The dissolved oxygen content of sample is determined before and after five days of incubation at 20°C and then the BOD is calculated from the difference of initial and final DO. The initial DO is noted shortly after the dilution is made. After this measurement, whatever the amount of oxygen uptake take place is included in the BOD measurement.

  Apparatus Required –

- BOD incubator – It's specificity is that it maintains the accurate temperature needed throughout the period of experiment.
- Burette and its stand.
- 300ml glass stoppers BOD bottles.
- DO Meter.
- Wash bottle.
- Conical flask.
- Measuring cylinder.
- Pipette with elongated tip.
- Gloves and seal starch.

  PROCEDURE –

- Take two bottles of sample from any river or pond or lake.
- Pour it into the brown bottles or BOD bottles.
- The blank solution is already prepared , so label the three bottles.
- Now, measuring the DO of each bottle and marking it as an initial reading.
- Closing the BOD bottles and seal the mouth of BOD bottles by seal starch, this is to avoid movement of air in and out of bottles.
- After sealing perfectly place all the samples in the incubator for 5 days at 20°C temperature.
- Making sure to wear gloves while performing the whole experiment.

- After 5 days, take out the samples and start to measure the final readings of DO in all samples.
- Also, taking care to clean the DO probe each time while taking readings of samples.
    - **Calculations –**
    - The amount of oxygen required to decompose the organic matter in the given sample is calculated by the difference of the two DO levels.
    - BOD (mg/l) = (D^1 – D^2 / V)
    - Where,
    - D^1 = initial DO reading.
    - D^2 = final DO reading.
    - V = Volume of sample.

Course No: ZOO 495B: Ecology Practical-II (50 Marks, 4 credit)

Estimation of the degree of faunal similarity and association between species

Faunal Similarity Index (Jaccard Index):

The Jaccard Index also known as Jaccard similarity co-efficient is a statistical method for measuring similarity of diversity of sample sets developed by Paul Jaccard. The index measures similarities between finite sample sets and is defined as the size of the intersection divided by the size of the union of the sample sets. Therefore, for a particular species, S, Jaccard Index is

$$S_J = \frac{a}{a + b + c}$$

Where, S_J = Jaccard similarity co-efficient

a = Number of species common to (shared by) quadrates

b = Number of species unique to first quadrate

c = Number of species unique to second quadrate

S_J index can be multiplied by 100 and may be represented in terms of dissimilarity, D_J.

$$D_J = 1 - S_J$$

Sørensen Co-efficient

It is the statistical procedure for measuring the similarity of two samples developed by Thorvald Sørensen. This index equals twice the number of elements common to both sets divided by the sum of the number of elements in each set.

$$S_S = \frac{2a}{a + b + c}$$

Where, S_S = Sørensen similarity co-efficient

a = Number of species common to (shared by) quadrates

b = Number of species unique to first quadrate

c = Number of species unique to second quadrate

S_S index can be multiplied by 100 and may be represented in terms of dissimilarity, D_S.

$$D_S = 1 - S_S$$

Problem:

In two adjacent forest areas, *Bandhavgarh National Park* and *Kanha National Park*, there are six different deer species with overlapping habitat range.

Area	Species I	Species II	Species III	Species IV	Species V	Species VI
Bandhavgarh National Park	30	40	20	10	0	0
Kanha National Park	30	30	0	50	20	10

Estimate the degree of faunal similarity between two areas using Jaccard similarity Index and Søurensen similarity co-efficient.

Ecological association between species

In many ecologic studies there is need to express in a quantitative manner the degree to which two different species are associated in nature. Of the measures of association between species previously proposed, the "coefficient of association" of Forbes is useful. The coefficient of association developed by Forbes considers the number of associated occurrences of two given species in a series of random samples com- pared to the number of such joint occurrences expected by chance.

In order to calculate this coefficient we must therefore count the number of samples in which each of the two species occurs and also the number of samples in which both species occur together.

Let us indicate,

By ***a*** the number of samples in which species A occurs either alone or together with species B,

By ***b*** the number of samples in which species B occurs,

By ***h*** the number of samples in which both species occur, and

By ***n*** the total number of samples examined.

Therefore,

The chance of species A occurring in any particular sample in the series is therefore ***a/n***.

The chance of species B occurring in any sample is ***b/n***.

The chance that species A and B will both occur in any given sample is ***a/n*** X ***b/n***.

The number of samples in the series in which species A and B would be expected to occur together is therefore ***a/n*** X ***b/n*** X ***n*** or ***ab/n***.

To obtain the coefficient we now divide the number of samples (***h***) in which both species are observed to occur by the number of samples (***ab/n***) in which they would be expected to occur by chance:

Coefficient of association = ***h/ab/n*** = ***hn/ab***

A coefficient of association of **1.0** show that the two species under consideration occur together in exactly the number of sample units expected by chance while coefficient smaller than **1.0** show that the species occurs together in fewer samples than would be expected by chance.A coefficient of **0.5** shows that the species are found together only half as frequently as expected by chance while a coefficient larger than **1.0** indicates that the species being compared are associated more frequently than would be expected by chance alone.

The coefficient of association therefore gives a readily understandable indication of the degree to which the association between any two species conforms to expectation on the basis of chance.The value of the coefficient of association between any two given species varies with the abundance of the forms in the area studied.

Estimation of alpha, beta and gamma diversity

Alpha diversity-

Alpha diversity is the mean species in the site at a local scale. Ecologists have used several different definitions of alpha diversity. The term was first introduced by R. H. Whittaker who himself used the term for the species diversity in a single subunit. Definitions of alpha diversity can also differ in what they assume species diversity to be. Often researchers use the values given by one or more diversity indices like Shannon index or Simpson index.

Beta diversity-

It is the ratio between regional and local species diversity and the term was first introduced by R. H. Whittaker. Beta diversity is the measure of change in diversity of species from one environment to another i.e. it calculates the number of species that are not the same in two different environments. There are also indices that measure beta diversity on a normalized scale, usually from 0 to 1. High beta diversity index indicates low level of similarity and vice versa.

Gamma diversity-

It is the total species diversity in a landscape which is determined by two things- (i) the main species diversity in sites at the more local scale and (ii) the differentiation among those sites.

Gamma diversity can be measured for an existing data set at any scale of interest. The smaller the available sample in relation to the area of interest, the more species that actually exist in the area not found in the sample.

Calculations-

- *Alpha diversity-*

$$\mathbf{H} = \Sigma [(p_i) \times \ln (p_i)]$$

Where, $\mathbf{p_i}$ = proportion of total sample represented by species

i = number of individuals of species divided by total number of samples

Problem-

Two environments have a total 12 species a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z and the number for each species are 60, 66, 62, 75, 55 ,50, 70, 47, 44, 53, 35 and 41 respectively. Calculate the alpha diversity by using suitable biodiversity index.

- *Beta diversity-*

Let, $\mathbf{S_1}$ be the total number of species in the first environment.

Let, $\mathbf{S_2}$ be the total number of species in the second environment.

Let, **C** is the number of species that the two environments have in common.

Let, ***x*** is the beta diversity.

Then, $\boldsymbol{x} = (S_1\text{-}C) + (S_2\text{-}C)$

Problem-

Two environments have a total 12 species a, b, c, d, e, f, g, h, i, j, k, l. In environment 1, there are 10 species: a to j and in environment 2, there are 7 species: f to l. Both the environments have 5 species in common. Calculate the beta diversity of the two environments.

- *Gamma diversity-*

Alpha diversity and beta diversity constitute independent components of gamma diversity i.e.

$$\gamma = \alpha + \beta$$

Analysis of the structure of biotic community: Abundance, Relative abundance, Frequency, Species diversity and Dominance indices; Shannon-Weiner diversity index and Importance Value Index

I. Determination of minimum number of quadrats

Requirements:

Metre scale, string, four nails (or quadrat), note book, graph paper, herbarium sheet, cello tape.

Method:

i. Lay down 20-50 quadrats of definite size at random in the grassland to be studied, make a list of different plant species (e.g., A-J) present in each quadrat and note down their botanical names or hypothetic numbers (e.g., A, B, C,…, J) as shown in Table 42. u

ii. With the help of the data available in Table, find out the accumulating total of the number of species for each quadrat.

Name of Species	Quadrat Number												
	1	2	3	4	5	6	7	8	9	10	11	12	...
A	+	+	−	−	+	+	−						
B	+	+	+	+	+	+	−						
C	+	−	−	−	+	+	+						
D		−	+	+	−	+	+						
E					−	−	−						
F						−	+						
G							+						
H						+	−						
I							−						
J						+	−						
Accumulating total number of species	3	4	4	4	5	8	10						

iii. Now take a graph paper sheet and plot the number of quadrats on X-axis and the accumulating total number of species on Y-axis of the graph paper.

Observations and results:

A curve would be obtained. Note carefully that this curve also starts flattening. The point at which this curve starts flattening up would give us the minimum number of quadrats required to be laid down in the grassland.

II. Determination of the minimum size of the quadrat by species area-curve method.

Requirements:

(i) Nails, (ii) cord or string, (iii) metre scale, (iv) hammer, (v) pencil and (vi) notebook.

Method:

i. Prepare an L-shaped structure of 1 × 1 metre size in the given area by using 3 nails and tying them with a cord or string.

ii. Measure 10 cm on one side of the arm L and the same on the other side of L, and prepare 10 x 10 sq. cm area using another set of nails and string. Note the number of species in this area of 10 x 10 sq. cm.

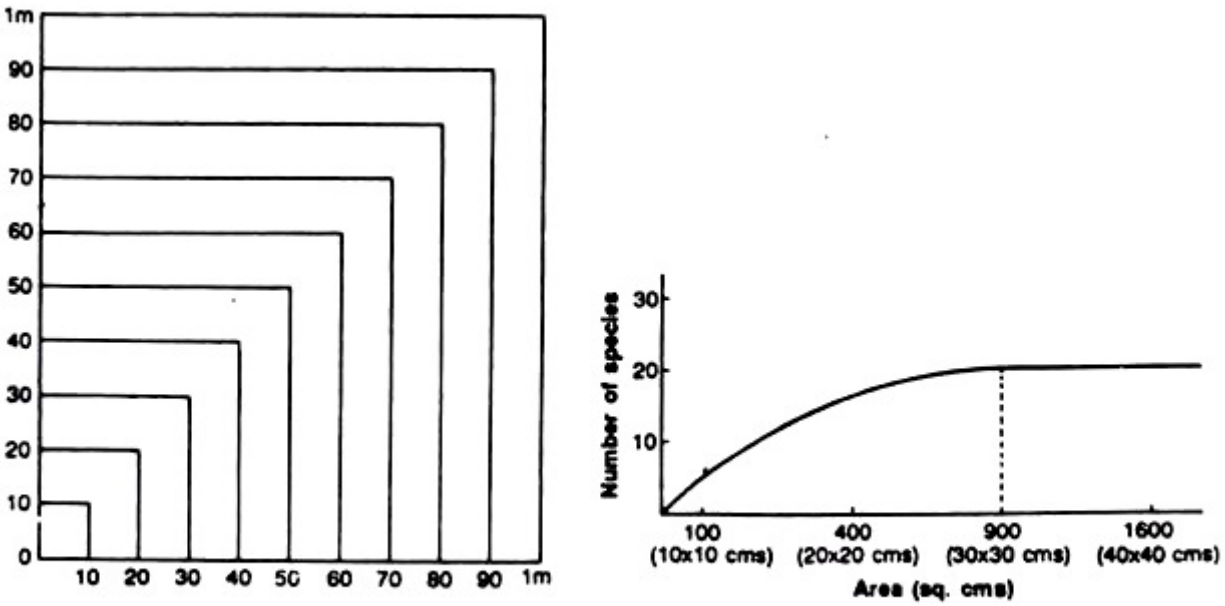


Fig. 67. A, Procedure for determining minimum required size of the quadrat; B, Species area-curve to determine the size of the quadrat.

iii. Increase this area to 20 × 20 sq. cm and note the additional species growing in this area.

iv. Repeat the same procedure for 30 × 30 sq. cm, 40 × 40 sq. cm and so on till 1 × 1 sq. metre area is covered (Fig. 67) and note the number of additional species every time.

Record the data in the following table:

No.	Area	Total no. of species
1.	10 × 10 sq. cm	
2.	20 × 20 sq. cm	
3.	30 × 30 sq. cm	
4.	40 × 40 sq. cm	
:	:	
10.	100 × 100 sq. cm	

v. Prepare a graph using the data recorded in the above table. Size of the quadrats is plotted on X- axis and the number of species on Y-axis (Fig. 67 B).

Observations:

The curve starts flattening or shows only a steady increase (Fig. 67 B) at one point in the graph.

Results:

The point of the graph, at which the curve starts flattening or shows only a steady or gradual increase, indicates the minimum size or minimum area of the quadrat suitable for study.

Study of density, diversity, frequency and abundance

Requirements:

i.Metre scale

ii. String

iii. Four nails or quadrat

iv. Notebook.

A. Frequency

Frequency is the number of sampling units or quadrats in which a given species occurs.

Percentage frequency (%F) can be estimated by the following formula:

$$\%\ \text{frequency (F)} = \frac{\text{Number of quadrats in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

B. Density

Density is the number of individuals per unit area and can be calculated by the following formula:

$$\text{Density (D)} = \frac{\text{Total number of individuals}}{\text{Total number of quadrats studied}}$$

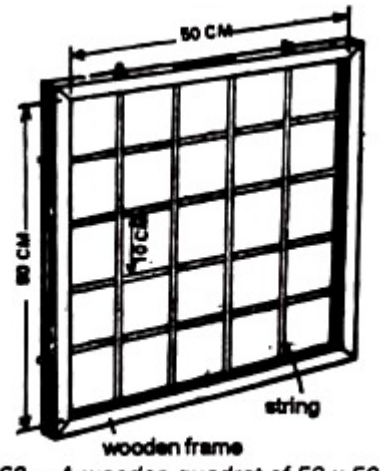


Fig. 68. A wooden quadrat of 50 x 50 cm.

C. Abundance:

Abundance is described as the number of individuals per quadrat of occurrence.

Abundance for each species can be calculated by the following formula:

$$\text{Abundance (A)} = \frac{\text{Total number of individuals}}{\text{Number of quadrats of occurrence}}$$

Method:

Lay a quadrat (Fig. 68) in the field or specific area to be studied. Note carefully the plants occurring there. Write the names and number of individuals of plant species in the note-book, which are present in the limits of your quadrat. Lay at random at least 10 quadrats (Fig. 69) in the same way and record your data in the form of Table 4.1.

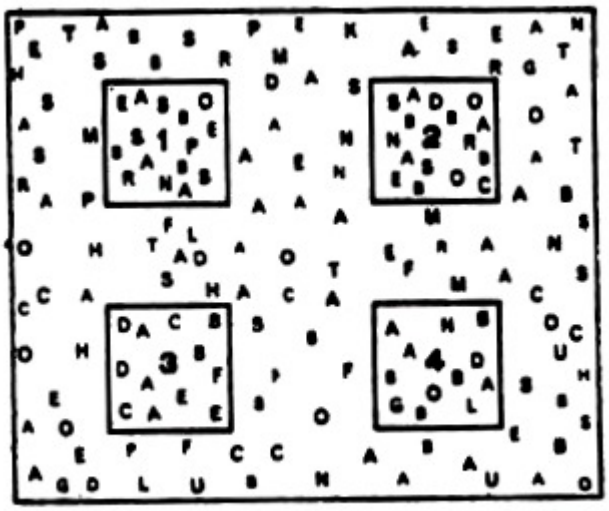


Fig. 69. Sketch of an artificial field showing four quadrats (1–4).

In Table 4.1, % frequency, density and abundance of Cyperus have been determined. Readings of the other six plants, occurred in the quadrats studied, are also filled in the table. Calculate the frequency, density and abundance of these six plants for practice. (For the practical class take your own readings. The readings in Table 4.1 are only to give an explanation of the matter).

Results:

Calculate the frequency, density and abundance of all the plant species with the help of the formulae given earlier and note the following results:

(i) In terms of % Frequency (F), the field is being dominated by…

(ii) In terms of Density (D), the field is being dominated by…

(iii) In terms of Abundance (A), the field is being dominated by…

Observations:

Table: Size of quadrat: 50cm × 50cm = 2500 cm^2

S. No.	Name of plant species	Number of individuals in quadrat number										Total number of quadrats of occurrence	Total number of quadrats studied	Total number of individuals	% Frequency (F)	Density (D)	Abundance (A)
		1	2	3	4	5	6	7	8	9	10						
1.	*Cyperus*	10	9	7	0	0	3	8	15	0	7	7	10	60	70%	6	8.57
2.	*Cassia*	0	0	2	0	3	0	5	0	6	10						
3.	*Cynodon*	50	0	7	41	6	0	0	8	0	5						
4.	*Eclipta*	0	0	4	0	3	0	0	1	0	2						
5.	A	0	0	0	0	2	0	0	1	3	0						
6.	B	5	10	1	0	0	0	3	1	0	2						
7.	C	3	5	0	0	2	1	8	0	2	0						

Problem-

1. The number of 5 species of trees- *Mangiferaindica, Artecartusheterophylus, Ficusreligiosa, Terminaliaarjuna* and *Shorearobusta* were estimated in 10 forest areas.

Sl. No	Plant Species	Number of individuals in forest area									
		1	2	3	4	5	6	7	8	9	10
1.	*Mangiferaindica*	10	9	7	0	0	3	8	15	0	7
2.	*Artecartusheterophylus*	0	0	2	0	3	0	5	0	6	10
3.	*Ficusreligiosa*	50	0	7	41	6	0	0	8	0	5
4.	*Terminaliaarjuna*	0	0	4	0	3	0	0	1	0	2
5.	*Shorearobusta*	15	5	0	0	8	7	0	1	3	0

Calculate the density, frequency and abundance of the tree species.

2. Two species of trees A and B are seen in 5 different areas.

Sl. No	Plant Species	Number of individuals in forest area									
		1	2	3	4	5					
1.	A	35	15	30	3	8					
2.	B	45	20	38	12	24					

Calculate the density, frequency and abundance of the tree species.

Shannon Wiener Diversity Index

In the literature, the terms species richness and species diversity are sometimes used interchangeably. It is suggested that at the very least, authors should define what they mean by either term of the many species diversity indices used in the literature; the Shannon index is perhaps most commonly used. On some occasions, it is called Shannon-Weiner's index.

Shannon-Weiner index is defined and given by the following function---

$$\boldsymbol{H} = \Sigma\ [(p_i)\ x\ \ln\ (p_i)]$$

Where, $\mathbf{p_i}$ = proportion of total sample represented by species

i = number of individuals of species divided by total number of samples

s = total number of species

$$\boldsymbol{H_{max}} = \ln(s) = \text{maximum diversity possible}$$

$$\boldsymbol{E} = \text{Evenness} = H/H_{max}$$

Problem

1. The number of 5 species of trees *Mangifera indica*, *Artocarpus heterophyllus*, *Ficus religiosa*, *Terminalia arjuna*, *Shorea robusta* are 75, 60, 20, 15, and 5 in a forest area. Calculate the species richness using Shannon-Weiner diversity index and Evenness for these sample values.

2. In a savannah grassland ecosystem there are 5 species of antelopes and 5 species of deer residing in the same area competing for food. Their numbers (*n*) are 66, 62, 58, 55, 50, 47, 44, 41, 53, and 35. Calculate the richness and even distribution of species using biodiversity index.

Logarithm Table

Table of base 10, base 2 and base e (ln) logarithms:

x	$\log_{10} x$	$\log_2 x$	$\log_e x$
1	0.000000	0.000000	0.000000
2	0.301030	1.000000	0.693147
3	0.477121	1.584963	1.098612
4	0.602060	2.000000	1.386294
5	0.698970	2.321928	1.609438
6	0.778151	2.584963	1.791759
7	0.845098	2.807355	1.945910
8	0.903090	3.000000	2.079442
9	0.954243	3.169925	2.197225
10	1.000000	3.321928	2.302585
20	1.301030	4.321928	2.995732
30	1.477121	4.906891	3.401197
40	1.602060	5.321928	3.688879
50	1.698970	5.643856	3.912023
60	1.778151	5.906991	4.094345
70	1.845098	6.129283	4.248495
80	1.903090	6.321928	4.382027
90	1.954243	6.491853	4.499810
100	2.000000	6.643856	4.605170
200	2.301030	7.643856	5.298317
300	2.477121	8.228819	5.703782
400	2.602060	8.643856	5.991465
500	2.698970	8.965784	6.214608
600	2.778151	9.228819	6.396930
700	2.845098	9.451211	6.551080
800	2.903090	9.643856	6.684612
900	2.954243	9.813781	6.802395
1000	3.000000	9.965784	6.907755

The natural logarithm table (Equal to or less than 1.0)

n	$\log_e n$	n	$\log_e n$	n	$\log_e n$	n	$\log_e n$
0.01	-4.60517	0.26	-1.34707	0.51	-0.67334	0.76	-0.27443
0.02	-3.91202	0.27	-1.30933	0.52	-0.65392	0.77	-0.26136
0.03	-3.50655	0.28	-1.27296	0.53	-0.63488	0.78	-0.24846
0.04	-3.21887	0.29	-1.23788	0.54	-0.61618	0.79	-0.23572
0.05	-2.99573	0.30	-1.20397	0.55	-0.59783	0.80	-0.22314
0.06	-2.81341	0.31	-1.17118	0.56	-0.57982	0.81	-0.21072
0.07	-2.65926	0.32	-1.13943	0.57	-0.56212	0.82	-0.19845
0.08	-2.52573	0.33	-1.10866	0.58	-0.54472	0.83	-0.18633
0.09	-2.40794	0.34	-1.07881	0.59	-0.52763	0.84	-0.17435
0.10	-2.30258	0.35	-1.04982	0.60	-0.51082	0.85	-0.16252
0.11	-2.20727	0.36	-1.02165	0.61	-0.49430	0.86	-0.15082
0.12	-2.12026	0.37	-0.99425	0.62	-0.47803	0.87	-0.13926
0.13	-2.04022	0.38	-0.96758	0.63	-0.46203	0.88	-0.12783
0.14	-1.96611	0.39	-0.94161	0.64	-0.44629	0.89	-0.11653
0.15	-1.89712	0.40	-0.91629	0.65	-0.43078	0.90	-0.10536
0.16	-1.83258	0.41	-0.89160	0.66	-0.41551	0.91	-0.09431
0.17	-1.77196	0.42	-0.86750	0.67	-0.40047	0.92	-0.08338
0.18	-1.71480	0.43	-0.81419	0.68	-0.38566	0.93	-0.07257
0.19	-1.66073	0.44	-0.82098	0.69	-0.37106	0.94	-0.06187
0.20	-1.60944	0.45	-0.79851	0.70	-0.35667	0.95	-0.05129
0.21	-1.56065	0.46	-0.77653	0.71	-0.34249	0.96	-0.04082
0.22	-1.51412	0.47	-0.75502	0.72	-0.32850	0.97	-0.03046
0.23	-1.46968	0.48	-0.73397	0.73	-0.31471	0.98	-0.02020
0.24	-1.42711	0.49	-0.71335	0.74	-0.30110	0.99	-0.01005
0.25	-1.38629	0.50	-0.69214	0.75	-0.28768	1.00	-0.00000

Determination of soil moisture

Soil moisture was measured using torsion balance moisture meter (Adair Dutt, Kolkata).

Principle:

The moisture content of the wet soil was directly read from the apparatus and then converted into dry weight percentage (md) using the procedure mentioned below.

Requirements:

i. Torsion balance moisture meter.

ii. Forceps

iii. Poly bag

iv. Spatula

v. Digging instrument

vi. Weight pan

Procedure:

1. The weight pan is placed in the moisture meter and the right hand knob is rotated and 0 of the movable scale is brought into the same line.

2. Then little amount of soil sample is placed on the weight pan until the pointer comes in the same line with o marking at the movable scale.

3. The infrared bulb is turned on and by rotating the knob the temperature was fixed at 80oC.

4. After sometime the pointer shifts and the movable scale is rotated anticlockwise until it comes back to the straight line and reading from the scale is recorded.

5. This is repeated until a constant reading is observed.

Calculation:

$M_d = \left(\frac{m}{100 - m}\right) \times 100$ m= moisture content reading; 100-m= weight of dry soil;
m_d =dry weight percentage.

Study of forest/vegetation health- Estimation of tree height, DBH, stand density, canopy density and tree biomass

Estimation of tree height

Introduction

Tree height is the vertical distance between the base of the tree and the tip of the highest branch of the tree. It is one of the parameters commonly measured as part of various tree programs and documentation efforts. Tree height can be measured in a number of ways with varying degree of accuracy.

Materials and method:

Materials required-

i) A long fully grown tree

ii) Measuring tape

iii) Measuring scale/yard scale

Procedure-

Stick method:

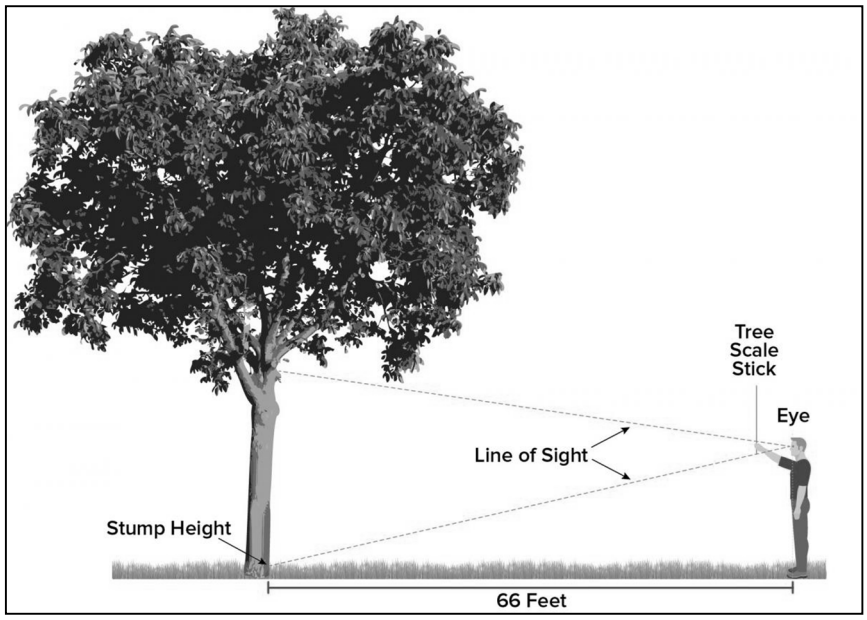
1. At first, the stick is held at arm's length with the free end pointed straight up.

2. For the alignment of the tree with the stick adjustment of a perfect distance is done by moving backward and forward from the tree so that the top of the tree visually aligns with the top of the stick and the base of the tree aligns with the top of the hand at the base of the stick.

3. After alignment, the distance between the top of the hand grasping the base of the stick and the eye is measured.

4. The distance between the top of the hand and the top of the stick is measured.

5. The distance between the eye and the base of the tree is measured.



Calculation-

$$\textbf{\textit{Tree Height}} = \frac{\text{Length of the stick x distance to tree}}{\text{Distance to eye}}$$

$$= \frac{d \times c}{a}$$

Diameter at breast height (DBH)

Introduction

Diameter at breast height or DBH is the standard method for expressing the diameter of the trunk of a standing tree. It is measured t the height of an adult's breast and defined differently in different countries and situations. Generally, DBH is measured at approximately 1.3m (4.3 ft) above ground.

Materials and method:

Materials required-

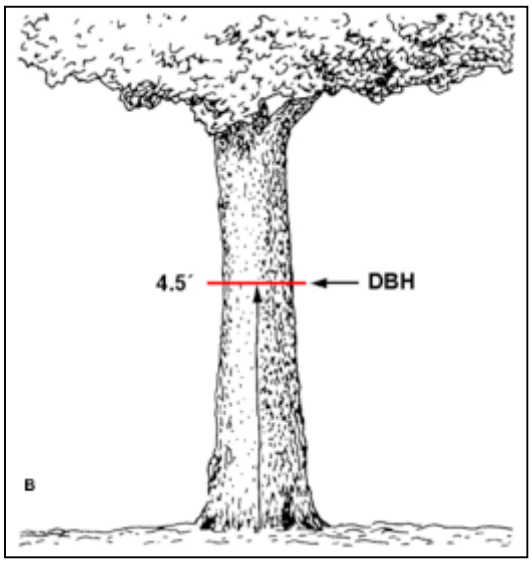
i) A long fully grown tree

ii) Measuring tape

Procedure-

1. A measuring tape actually measures the girth (circumference of the tree).

2. The measure assumes the trunk has a circular cross section and and gives a directly converted reading of the diameter.



Calculation-

$$\boldsymbol{BH} = \boldsymbol{x} \text{ inches}$$

$$\boldsymbol{r} = \frac{\boldsymbol{x} \text{ inches}}{\boldsymbol{2\pi}}$$

$$\textbf{diameter} = 2 \times \boldsymbol{r}$$

Stand density Index (SDI)

Introduction

Stand density index is a measure of the stocking of a stand of trees based on the number of trees per unit area and diameter at breast height (DBH) of the tree of average basal area, also known as the quadratic mean diameter. It may also be defined as the degree of crowding within stocked areas, using various growing space ratios based on crown length or diameter, tree height or diameter, and spacing.

Materials and method:

Materials required-

i) A forest area with well crowding of trees

ii) Measuring tape

Procedure-

Plotting the logarithm of the number of trees per acre against the logarithm of the quadratic mean diameter (or the dbh of the tree of average basal area) of maximally stocked stands generally results in a straight-line relationship. In most cases the line is used to define the limit of maximum stocking.

Calculation-

The maximum density line is expressed by the equation: $\log_{10}N = -1.605(\log_{10}D) + k$

Where,

N = number of trees per acre

D = DBH of the tree of average basal area

k = a constant varying with the species

When the quadratic mean diameter equals 10 inches (250 mm), the log of N equals the log of the stand density index.

In equation form: $\log_{10}SDI = -1.605(1) + k$

Which means that: $k = \log_{10}SDI + 1.605$

Substituting the value of k above into the reference-curve formula gives the equation:

$\log_{10}N = \log_{10}SDI + 1.605 - 1.605 \log_{10}D$

This equation can be rewritten as:

$\log_{10}SDI = \log_{10}N + 1.605 \log_{10}D - 1.605$

The above equation is an expression for computing the stand density index from the number of trees per acre and the diameter of the tree of average basal area.

Assume that a stand with basal area of 150 square feet (14 m^2) and 400 trees per acre is measured. The dbh of the tree of average basal area D is:

$$\sqrt{\frac{150}{400 \times .005454}} = 8.29$$

Substituting this value into the stand density equation gives:

$\log_{10}SDI = \log_{10}(400) + 1.605\log_{10}(8.29) - 1.605 = 2.47$

$SDI = 10^{2.47}$

SDI = 295

The computed value of SDI is often compared to the species maximum to determine the relative "stand density" or stocking of the stand.

Canopy density (Tree crown measurement)

Introduction

A tree crown measurement consists of the mass of foliage and branches growing outward from the trunk of the tree. The average crown spread is the average horizontal width taken from drip-line to drip-line moving around the crown.

Materials and method:

Materials required-

i) A long fully grown tree

ii) Measuring tape

iii) Above head shade of the tree

Procedure-

1. The point on the ground immediately below the branch tip on one end of the measurement is located and marked.

2. Then the point under the branch tip at the opposite side of the crown is located and marked.

Calculation-

$$\textbf{\textit{Average crown head}} = \frac{\text{longest spread + longest cross spread}}{2}$$

Tree biomass

Introduction

The measurement is based on the use of existing measured volume estimates (VOB per ha) converted to biomass density (t/ha). The method presented here is based on existing volume per ha data and is best used for secondary to mature closed forests only, growing in moist to dry climates. The primary data needed for this approach is VOB/ha, that is inventoried volume over bark of free bole, i.e. from stump or buttress to Crown Point or first main branch. Inventoried volume must include all trees, whether presently commercial or not, with a minimum diameter of 10 cm at breast height.

Calculation-

Biomass density can be calculated from VOB/ha by first estimating the biomass of the inventoried volume and then "expanding" this value to take into account the biomass of the other aboveground components as follows

Aboveground biomass density (t/ha) = VOB * WD * BEF

Where,

WD = volume-weighted average wood density (1 of oven-dry biomass per m^3 green volume)

BEF = biomass expansion factor (ratio of aboveground oven-dry biomass of trees to oven-dry biomass of inventoried volume)

Vermitechnology and related matters: Analysis of biota from urban waste materials and identification of suitable specimen for vermicomposting.

Introduction

Earthworms are terrestrial invertebrates belonging to the Order Oligochaeta, Class Chaetopoda, Phylum Annelida, which have originated about 600 million years ago, during the pre-Cambrian era. Earthworms occur in diverse habitat, exhibiting effective activity, by bringing about physical and chemical changes in the soil leading to improvement in soil fertility. An approach towards good soil management, with an emphasis on the role of soil dwellers like earthworms, in soil fertility, is very important in maintaining balance in an ecosystem. The main activity of earthworms involves the ingestion of soil, mixing of different soil components and production of surface and sub-surface castings thereby converting organic matter into soil humus. Earthworms play an important role in the decomposition of organic matter and soil metabolism through feeding, fragmentation, aeration, turnover and dispersion. Earthworms were referred by Aristotle as “the intestines of earth and the restoring agents of soil fertility”. They are biological indicators of soil quality as a good population of earthworms indicates the presence of a large population of bacteria, viruses, fungi, insects, spiders and other organisms and thus a healthy soil.

Ecological strategies of earthworms

Three main ecological groups of earthworms, based on the soil horizons in which the earthworms are commonly found i.e., litter, topsoil and sub soil. Also, three major groups based on ecological strategies: the epigeics, anecics and endogeics.

Epigeic earthworms live on the soil surface and are litter feeders. Anecic earthworms are topsoil species, which predominantly form vertical burrows in the soil, feeding on the leaf litter mixed with the soil. Endogeic earthworms preferably make horizontal burrows and consume more soil than epigeic or anecic species, deriving their nourishment from humus.

Distribution of earthworms

Earthworms occur all over the world, but are rare in areas under constant snow and ice, mountain ranges and areas almost entirely lacking in soil and vegetation. Some species are widely distributed, which are called peregrine, whereas others, that are not able to spread successfully to other areas, are termed as endemic.

Factors affecting earthworm distribution

The distribution of earthworms in soil is affected by physical and chemical characters of the soil, such as temperature, pH, moisture, organic matter and soil texture.

Temperature

The activity, metabolism, growth, respiration and reproduction of earthworms are all influenced greatly by temperature.

pH

pH is a vital factor that determines the distribution of earthworms as they are sensitive to the hydrogen ion concentration. pH and factors related to pH influence the distribution and abundance of earthworms in soil. Most species of earthworms prefer soils with a neutral pH.

Moisture

Prevention of water loss is a major factor in earthworm survival as water constitutes 75-90% of the body weight of earthworms. They have considerable ability to survive adverse moisture conditions, either by moving to a region with more moisture or by means of aestivation. Availability of soil moisture determines earthworm activity as earthworm species have different moisture requirements in different regions of the world. Soil moisture also influences the number and biomass of earthworms.

Organic matter

The distribution of earthworms is greatly influenced by the distribution of organic matter. Soils that are poor in organic matter do not usually support large numbers of earthworms.

Soil texture

Soil texture influences earthworm populations due to its effect on other properties, such as soil moisture relationships, nutrient status and cation exchange capacity, all of which have important influences on earthworm populations.

Importance of earthworms

Effect of earthworms on soil quality

Earthworms, which improve soil productivity and fertility, have a critical influence on soil structure. Earthworms bring about physical, chemical and biological changes in the soil through their activities and thus are recognized as soil managers.

Effects on physical properties of soil

Soil structure is greatly influenced by two major activities of earthworms: Ingestion of soil, partial breakdown of organic matter, intimate mixing of these fractions and ejection of this material as surface or subsurface casts. Burrowing through the soil and bringing subsoil to the surface. During these processes, earthworms contribute to the formation of soil aggregates, improvement in soil aeration and porosity.

Earthworms contribute to soil aggregation mainly through the production of casts, although earthworm burrows can also contribute to aggregate stability since they are often lined with oriented clays and humic materials. Earthworm casts contains more water-stable aggregates than the surrounding soil and by their activity influence both the drainage of water from soil and the moisture holding capacity of soil, both of which are important factors for plant productivity.

Effect on chemical properties of soil

Earthworms bring about mineralisation of organic matter and thereby release the nutrients in available forms that can be taken up by the plants. Organic matter that passes through the earthworm gut is egested in their casts, which are broken down into much finer particles, so that a greater surface area of the organic matter is exposed to ***microbial decomposition***.

Earthworms contribute nutrients in the form of nitrogenous wastes. Their casts have higher base-exchangeable bases, phosphorus, exchangeable potassium and manganese and total exchangeable calcium. Earthworms favour nitrification since they increase bacterial population and soil aeration. The most important effect of earthworms may be the stimulation of microbial activity in casts that enhances the transformation of soluble nitrogen into microbial protein thereby preventing their loss through leaching to the lower horizons of the soil.

Earthworms and organic solid waste management

1. Burning destroys the soil organic matter content, kills the microbial population and affects the physical properties of the soil.

2. It has been demonstrated that earthworms can process household garbage, city refuse, sewage sludge and waste from paper, wood and food industries.

3. In tropical and subtropical conditions ***Eudriluseugeniae*** and ***Perionyxexcavatus*** are the best vermicomposting earthworms for organic solid waste management.

4. The use of earthworms in composting process decreases the time of stabilisation of the waste and produces an efficient bio-product, i.e., vermicompost.

5. Vermicompost and vermiculture associated with other biological inputs have been actually used to grow vegetables and other crops successfully and have been found to be economical and productive.

6. Compost is becoming an important aspect in the quest to increase productivity of food in an environmentally friendly way.

7. Vermicomposting offers a solution to tonnes of organic agro-wastes that are being burned by farmers and to recycle and reuse these refuse to promote our agricultural development in more efficient, economical and environmentally friendly manner.

Vermitechnology

1. Vermitechnology is the use of surface and subsurface local varieties of earthworm in composting and management of soil.

2. It has been recognized that the work of earthworms is of tremendous agricultural importance. Earthworms along with other animals have played an important role in regulating soil processes, maintaining soil fertility and in bringing about nutrient cycling.

3. Earthworms have a critical influence on soil structure, forming aggregates and improving the physical conditions for plant growth and nutrient uptake.

4. They also improve soil fertility by accelerating decomposition of plant litter and organic matter and, consequently, releasing nutrients in the form that are available for uptake by plants.

Vermicomposting

1. Vermicomposting is the biological degradation and stabilization of organic waste by earthworms and microorganisms to form vermicompost.

2. The earthworms fragment the organic waste substrates, stimulate microbial activity greatly and increase rates of mineralization. These rapidly convert the waste into humus-like

substances with finer structure than thermophilic composts but possessing a greater and more diverse microbial activity.

3. Vermicompost being a stable fine granular organic matter, when added to clay soil loosens the soil and improves the passage for the entry of air. The mucus associated with the cast (earthworm cast) being hydroscopic absorbs water and prevents water logging and improves waterholding capacity.

4. The role of earthworms in organic solid waste management has been well established since first highlighted by Darwin and the technology has been improvised to process the waste to produce an efficient bio-product vermicompost.

5. Epigeic earthworms like *Perionyxexcavatus, Eiseniafetida, Lumbricusrubellus*and *Eudriluseugeniae*are usedfor vermicomposting but the local species like *Perionyxexcavatus*has provedefficient composting earthworms in tropical or sub-tropical conditions.

6. The method of vermicomposting involving a combination of local epigeic and anecic species of earthworms (*Perionyxexcavatus*and *Lampitomauritii*) is called Vermitech.

7. The compost prepared through the application of earthworms is called vermicompost and the technology of using local species of earthworms for culture or composting has been called Vermitech.

8. Many agricultural industries use compost, cattle dung and other animal excreta to grow plants. In today's society, we are faced with the dilemma of getting rid of waste from our industries, household etc. In order for us to practice effective waste management we can utilize the technology of vermicomposting to effectively manage our waste.

Conclusion

Environmental Hazards are compounded by accumulation of organic waste from different sources like domestic, agricultural and industrial wastes that can be recycled by improvised and simple technologies. Vermicompost could be effectively used for the cultivation of many crops and vegetables, which could be a step towards sustainable organic farming. Such technologies in organic waste management would lead to zero waste techno farms without the organic waste being wasted and burned rather then would result in recycling and reutilization of precious organic waste bringing about bioconservation and biovitalization of natural resources.

Air pollution monitoring: demonstration of the operating principles High Volume Sampler

Introduction

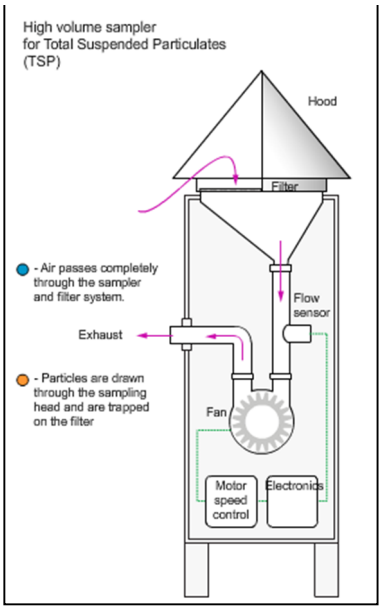
High volume air samplers are instruments used to collect samples of air particles. The difference between high and low volume air samplers is the amount of air sampled. High volume air samplers typically sample more than 1500 cubic metres (m^3) of air over a 24-hour period, while low volume air samplers draw through only 24m^3 of air, or less.

Total suspended particulate matter (TSP)

Total suspended particulate matter (TSP) monitoring measures the total amount of particles suspended in the atmosphere. TSP samples may also be used to determine the levels of chemical elements and compounds in the particles which may pose a risk to human health. An instrument called a high volume air sampler is used to collect TSP samples. The high volume air sampler draws a large known volume of air through a pre-weighed filter for 24 hours.

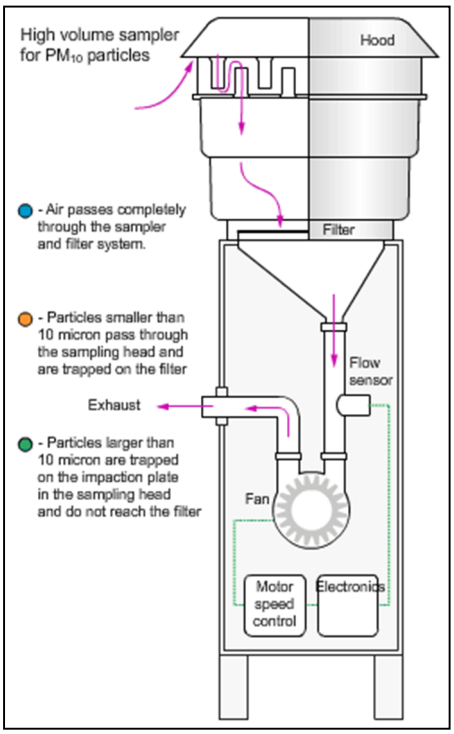
After sampling, the filter is re-weighed and the difference in filter weight is the collected particulate matter mass. Dividing the mass by the volume of air sampled gives the concentration of TSP.

If required, the particulate matter retained on the filter is analysed to determine the concentration of pollutants, such as lead or other metals. Metals can also be monitored continuously using an XACT instrument. The design of the air inlet means that a TSP high volume air sampler is unlikely to collect airborne particles with diameters greater than 100 micrometres (μm) in diameter. This type of sampling usually takes place at 6-day intervals due to the need to manually change the filters.



Particles less than 10 micrometres in diameter (PM_{10})

Particles smaller than 10µm are especially concerning as these particles can enter the human respiratory system and penetrate deeply into the lungs, causing adverse health effects. Motor vehicles and other combustion processes that burn fossil fuels such as power stations, industrial processes and domestic heaters, generate PM_{10}. Dust storms and smoke particles from bushfires can also be another source of PM_{10} missions. Instruments used to measure PM_{10} are either a high or low volume air sampler or a tapered element oscillating microbalance (TEOM). The PM_{10} high or low volume air sampler is similar to that described above for TSP, except that the air sample passes through a size-selective inlet. The inlet removes particles larger than 10µm by using their greater inertia to trap them on a greased plate, while smaller particles pass through the instrument onto the pre-weighed filter.

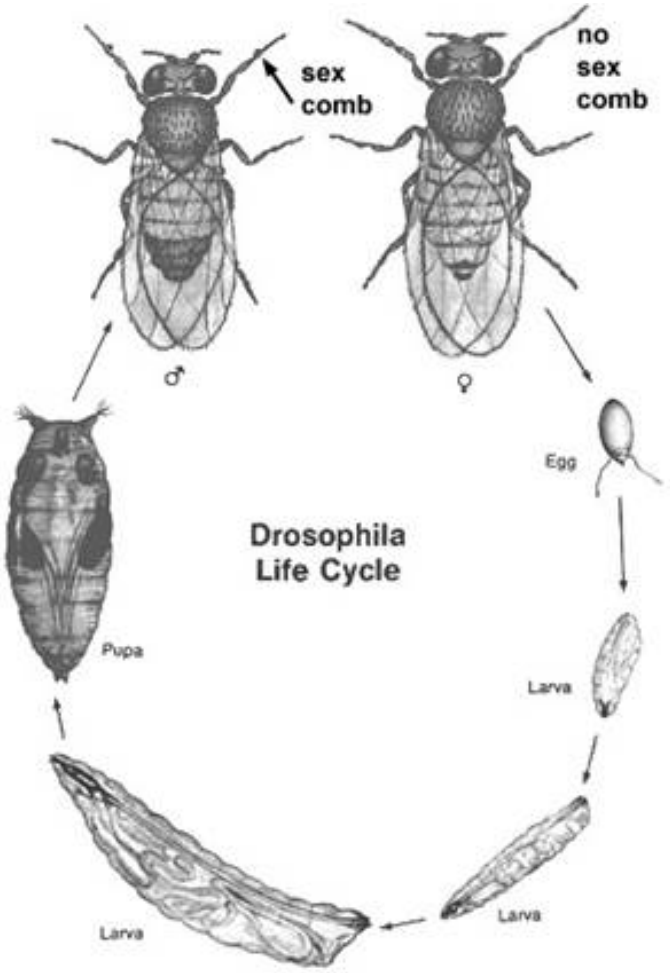


PM_{10} sampler

Measuring the volume of air sampled and weighing the filters before and after sampling determines the concentration of PM_{10} particles in the air.

ZOO 495C: GENETICS & MOLECULAR BIOLOGY (SPECIAL PAPER)

Study of Life cycle of Drosophila



1. Fruit flies are holometabolous insects; that is, they undergo complete metamorphosis during their life cycle. The life cycle consists of four distinct stages: egg, larva, pupa, and adult. The rate of development is dependent on temperature, being more rapid at higher temperatures. For instance, at 20ºC, the life cycle is completed in 14 or 15 days, but at 25ºC, the cycle lasts about 10 days.

2. **Mating and Eggs:** Mated females store sperm to fertilize eggs that are subsequently laid. Therefore, to ensure that the desired cross is achieved, it is necessary to place females that are virgins with their intended male mates. Female flies are unable to mate for several hours after they have eclosed as adults from their pupal cases. Therefore, virgin females can be obtained by clearing all of the flies from a

vial and collecting all newly-eclosed females several hours later. These virgin females can be kept separated from males for several days until needed for crosses.

3. **Oviposition** by the female starts as early as the second day after its emergence from its pupal case. It increases for about a week until a female adult may be laying 50-75 eggs per day for a total of approximately 400-500 eggs in 10 days. The egg is ovoid, covered outside with a thin but strong envelope (chorion) from which project anteriorly two thin stalks whose terminal portions are each flattened into a spoon-like float. The latter serve as "water-wings" to prevent the egg from sinking and drowning in a semiliquid medium. At the anterior end of the egg is a minute pore (micropile) through which the spermatozoa enter the egg as it passes down the oviduct into the uterus. Although many sperm may enter the egg as it passes down the oviduct, only one fertilizes the female pronucleus and the others are soon absorbed in the developing embryonic tissue.

4. **Larva:** The larva is a white, segmented, worm-shaped burrower with black mouth parts (jaw hooks) in the narrower head region. For tracheal breathing it has a pair of spiracles (air intakes) at both the anterior and posterior ends. Since insect skin will not stretch, the young small larvae must periodically shed their skins (cuticle) in order to reach adult size. There are two such molts in *Drosophila* larval development that are accompanied by shedding of the mouth parts as well as the skins. During each period between molts, the larva is called an instar, i.e. the first instar is between hatching and the first molt. Both the size of the larva and the number of teeth on the dark colored jaw hooks are an indication of which instar the larva has reached. After the second molt, the larva (now third instar) feed until ready to pupate. At this stage, the larva crawls out of the food medium onto a relatively dry place, ceases moving, and everts its anterior breathing spiracles.

5. **Pupa:** Soon after everting its anterior spiracles, the larval body shortens and the cuticle becomes hardened and pigmented. A headless and wingless prepupa forms. This stage is followed by the formation of the pupa with everted head, wing pads, and legs. The puparium (outer case of the pupa) thus utilizes the cuticle of the third larval instar. The adult structures that seem to appear first during the pupal period have actually been present as small areas of dormant tissues as far back as the embryonic stage. These localized preadult tissues are called **anlagen** (or **imaginal discs**) and because of the ease in which they can be isolated have often been used in studies of developmental genetics. The main function of the pupa is to permit development of the anlagen to adult proportions. The breakdown of larval tissues to furnish material and energy for this development is therefore a prime feature of pupal metabolism.

6. **Adult:** Adults exhibit a typical insect anatomy, including compound eyes, three-part bodies (head, thorax, and abdomen), wings, and six jointed legs. The various types of bristles and hairs found on the body are characters that we will use to identify different phenotypes of flies.

DNA ISOLATION FROM TISSUE

PRINCIPLE:

The genomic DNA isolation depends on what the application of the DNA after isolation. Generally all methods include the disruption and lysis of cell. This followed sometimes by the removal of RNA and other materials.

In general the separation of DNA from cell and cellular components can be divided into four steps-

I. Cell disruption
II. Cell lysis
III. Removal of proteins
IV. Receving of DNA

REQUIREMENTS:

Micro centrifuged tube, chicken liver, tips, and micro pipette reagents.

REAGENTS AND IT'S PREPARATION:

1. Lysis buffer:- 50ml Tris, 20ml EDTA, 100ml NaCl, 1% SDS proteinase all are mixed well and volume made upto 100ml.
2. TE buffer:- 50μl Tris solution+ 5μl of EDTA solution + DH_2O volume made upto 5ml.
3. PCI (phenol: Chloroform: Isoamyl alcohol):- 25:24:1
4. TAE buffer 1X :- 0.484gm Tris + 0.115ml acetic acid + EDTA 0.25 gm are mixed properly and volume made upto 100ml.
5. DNA loading dye:- glycerol, Bromophenol blue.
6. Ethidium Bromide (EtBr):- 10mg/ml dissolved in 1X TAE buffer.

PROCEDURE:

1. 1 of tissue was added to 50μl of lysis buffer.
2. The tissue was then homogenised by tissue homogenizer which is able to break sufficient cell membrane and nuclear membrane.
3. The homogenised mixture was then stored at 4°C temperature for 15min.
4. The homogenised mixture was then centrifuged at 12000 rpm for 10 min under 4°C temperature.

5. After centrifugugation, supernatant was collected and equal volume of P:C:I was added to microcentrifuged tube.
6. Then this mixture again centrifuged at 12000 rpm for 5min under 4°c.
7. The upper aquas solution was collected and added equal volume of chloroform.
8. After that mixture was centrifuged again at 12000 rpm for 5min at 4°C.
9. Thus upper aquas layer was collected and equal volume of sodium acetate (CH_2COONa^-) and double volume of ethanol (CH_3CH_2OH) were added.
10. Then the mixture was taken properly and stored at -20°C for 1 hr or overnight (as required).
11. After that mixture was centrifuged at 8000 rpm for 8-10 min.
12. Next pellet was treated with 70% ethanol and centrifuged at 8000 rpm for 8min.
13. Then pellets were dried properly by evaporation at alcohol and dissolved in of TE buffer.
14. Load in well of DNA agarose (1%) gel and run the gel.
15. The result was studied by using transluminator.

OBSERVATION:

After sometime it was seens that DNA migrated from –Ve to +Ve in agarose gel. The bands were found just below the well.

RESULT AND INTERPRETATION:

From the above observation we may interpreted that the genome which is absorbed in the well indicates that the animal tissue under investigation contain genomic DNA.

PURIFICATION:

1. RNAse treatment is for 30-35 min at 37°C.
2. A stock solution of protease is prepared and from that stock solution 1/20 th volume is added to the isolation DNA. The preparation is a incubated at 37°C for 1hr.
3. To the preparation of $CHCl_3$ is added for 15 min and centrifuged for 10000 rpm for 10 min.
4. Sample kept on ice ban 10min.
5. Aqueous phase is pipette out in a tube and 2 volume of chilled ethanol is added.

DNA Extraction from Blood Sample

Introduction

Human DNA can be extracted from all the nucleated cells such as hair, tissue, blood etc. certain sources contain high levels of proteins & many types of secondary metabolites that effects DNA purification, highly purified DNA is essential for molecular studies. Here we followed salting out method to extract large quantities of human DNA from whole blood.

Preparation of Reagents

The reagents were prepared as described below:

a. TKM 1 Buffer / Low salt buffer (500 ml): 0.605 g of TrisHCl (10mM) pH 7.6, 0.372 g of KCl (10 mM), 1.016 g of MgCl2

b. Triton-X (10ml): Added 0.1 ml of 100 % Triton-X to 9.9ml of distilled water. (10 mM), 0.372g of EDTA (2mM) was dissolved in 500ml of distilled water

c. TKM 2 Buffer / High salt buffer (100 ml): 0.121 g of TrisHCl (10mM) pH 7.6, 0.074 g of KCl (10 mM), 1.203 g of MgCl2

d. Cell lysis buffer: 10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 10% SDS, pH 7.5

e. SDS: One gram of sodium dodecyl sulphate was dissolved in 10ml distilled water. (10 mM), 0.074 g EDTA (2mM), 0.467 g of NaCl (0.4 M) was dissolved in 100ml of distilled water

f. 6M NaCl : 8.765 g of NaCl was dissolved in 25 ml of distilled water.

g. TE Buffer: 0.030 g of TrisHCl (10mM) pH 8.0, 0.009 g of EDTA (1mM) was dissolved in 100ml of distilled water

DNA extraction Protocol

1. 900 µl of TKM 1 and 50 µl of 1x Triton-X were added to 300 µl of heparinised blood in an autoclaved 1.5 ml eppendorf.

2. Incubated at 370

3. Cells were centrifuged at 8000 rpm for 3 minutes and the supernatant was discarded. C for 5 minutes to lyse the RBCs.

4. This step was repeated 2-3 times with decreasing amount of 1x Triton-X till

RBC lysis was complete and a white pellet of WBCs was obtained.

5. The pellet was resuspended in 500 µl PBS, followed by addition of 400 µl cell lysis buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 10% SDS, pH 7.5) and 10 µl proteinase K (10 mg/ml stock).

6. The sample was vortexed to dissolve the pellet completely and incubated for 2 h at 56°C in a water bath for lysis. An equal volume of phenol (equilibrated with Tris, pH 8) was subsequently added to the tube and mixed well by inverting for 1 min.

7. The tube was centrifuged at 10,000 g (at 4°C) for 10 min, and the aqueous upper layer was transferred to a fresh tube containing equal volumes (1:1) of phenol and chloroform: isoamyl alcohol (24:1).

8. The tube was mixed by inverting for 1 min and centrifuged for 10 min at 10,000 g (at 4°C). The supernatant was then transferred to a fresh tube, and 10 µl of 10 mg/ml RNase A was added.

9. The sample was incubated at 37°C for 30 min before an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inverting the tube for 1 min and centrifuging at 10,000 g (at 4°C) for 10 min.

10. The supernatant was transferred to a fresh tube, and twice the volume of absolute alcohol was added and inverted gently a few times and chilled at 20°C, followed by centrifugation at 10,000 g at (4°C) for 20 min.

11. The supernatant was discarded, 250 µl 70% ethanol was added, and the pellet was tapped gently, followed by centrifugation at 10,000 rpm for 10 min and decanting the supernatant gently.

12. The pellet was air-dried in a laminar air flow, and the dried pellet was resuspended in 50 µl nuclease-free water or 1X TE buffer and frozen at -20°C or -80°C for storage.

Concentration and Purity Determination

i. A quantitative spectrophotometric assay of DNA was performed using a UV-visible spectrophotometer.

ii. Absorbance was measured at wavelengths of 260 and 280 (A260 and A280, respectively) nm.

iii. The absorbance quotient (OD260/OD280) provides an estimate of DNA purity.

iv. An absorbance quotient value of 1.8 < ratio (R) < 2.0 was considered to be good, purified DNA.

v. A ratio of <1.8 is indicative of protein contamination, where as a ratio of > 2.0 indicates RNA contamination.

PRINCIPLE AND METOD OF AGAROSE GEL ELECTROPHORESIS

PRINCIPLE :

Agarose gel electrophoresis used to analyse and quantite nuclic acid. The agarose for agarose gel electrophoresis is purified from agarose. Agarose is a linear polymer made up of repeating units of 1.3-linked β D galactopyranase and 1.4-linked 3.6 anhydro a 1 galactopyranose. Agarose has an average MW of 12000 and contains about 35-40 agarobise units. Agarose in solution exist as left handed double helices. About 7-11 such helices from bundles which extend as long as rods and appear to intertwine with one another, further strengthening the frame work of the gel. The cross links are held together by hydrogen and hydrophobic bonds. By changing the gel conc. the pre size can be altered. Higher the concentration of agarose smaller the pre size and vise versa. Because of large pre size even at low concentration. Agarose gel are widely used for separation of DNA and RNA.

MATERIALS REQUIRED:

1. Agarose solution
2. Ethidium bromide.
3. Electrophoresis buffer

EFFECTS OF AGAROSE CONCENTRATION ON SEPARATION RANGE:

The following table describe the relationship between agarose concentration and separation range of nuclic acid.

AGAROSE CONCENTRATION (%)	SEPARATION RANGE(kb)
0.3	5 to 60
0.6	1 to 20
0.8	0.8 to 10
1.0	0.4 to 8
1.2	0.3 to 6
1.5	0.2 to 4

FACTORS WHICH AFFECT THE RATE OF MIGRATION OF NUCLEIC ACIDS IN AGAROSE GEL:

Rate of migration of nucleic acids in agarose gel depends mainly on five important parameters.

1. AGAROSE CONCENTRATION:

Higher conc. of gel are used for the separation of lower weight DNA & RNA fragments and vice-versa.

2. MOLECULAR WEIGHT:

A duplex DNA fragment migrates at rates inversely proportional to the log molecular weight. A plot of log MW mobility gives a straight line.

3. CONFIRMATION:

Supercoild DNA moves faster followed by linear forms & relaxed open circular forms.

4. APPLIED VALTAGES:

At low voltages (<5V/cm) the rate of migration is directly proportional to the applied voltage.

5. BASE COMPOSITION & TEMPERATURE:

Base composition & running the gel between 4& 30 C don't change the mobilites.

PREPARATION OF STOCK SOLUTION FOR DNA GEL:

Two different buffer systems are used for separation of nucleic acid by agarose gel electrophoresis. There compositions are given below:

i. TBE buffer

ii. TAE buffer:

PREPARATION OF AGAROSE SOLUTION FOR GEL CASTING:

Dissolved the agarose by placing the flashes in boiling water bath cool to Luke warm. Cover the sides of a tray using cello tape & place the comb about 1cm from the top of the tray. Pour the agarose without making any bubbles, cool it for 20mints and take off the combs and uncovered the tapes.

PREPARATION OF SAMPLE LOADING DYE GLYCEROLLS BROMOPHENOL BLUE

30ml glycerol(30%), 250mg bromophenol blue, dH_2O 100ml.

PROCEDURE:

1. The DNA sample is mixed with the loading dye and loaded in the well carefully using pipetman & capillary tube.
2. One the sample is loaded in to the well the cathode is connected towards the top end of the gel and anode(Red positive) terminal is connected towerds the bottom end of the gel. The maximum volume that can be loaded onto a well formed from a 105mm thickness tooth of the comb is 30μl. The electrophoresis is started by switching on the D.C. power pack.
3. The gel is run at 5V/cm. As the bromophenol blue has moved 1cm above the bottom end. The current is switched off the power supply is disconnected and the gel along

with the platform is stain is plastic tray containing 0.5μg/ml ethidium bromide in the sterile distilled water.

4. After about 30-40 min the platform & gel is rinsed with distilled water & by keeping the platform in a standing position, the gel is gently pushed onto the UV transluminator . UV light is switched on and the DNA bands are seen and photographed at 5.6 for 10 seconds with an orange filter.

RESULT: After electrophoresis DNA bands can be visualized under VU light and they appear as orange fluorescence.

FAMILY PEDIGREE ANALYSIS FOR AUTOSOMAL /SEX LINKED, DOMINANT /RECESSIVE TRAIT.

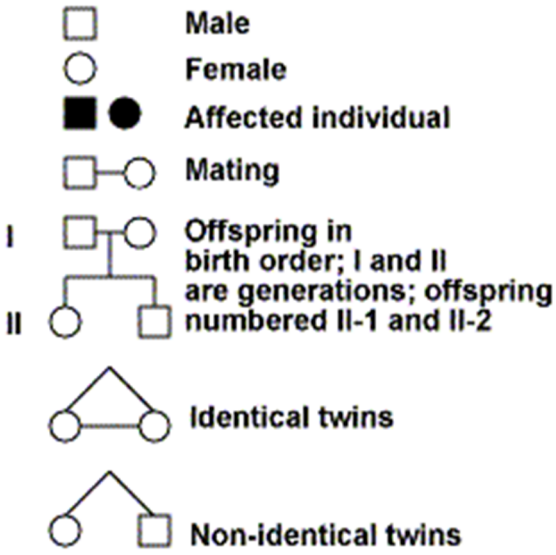
Pedigrees are used to **analyze** the pattern of **inheritance** of a particular trait throughout a family. **Pedigrees** show the presence or absence of a trait as it relates to the relationship among parents, offspring, and siblings. It is usually undertaken if families are referred to a genetic counsellor following the birth of an affected child. The **pedigree analysis** chart is **used** to show the relationship within an extended family. Males are indicated by the square shape and females are represented by circles. It is very important tool for studying human inherited diseases. These diagrams make it easier to visualize relationships with in families, particularly large extended families. Pedigrees are often used to determine the mode of inheritance (dominant, recessive, etc.) of genetic diseases. **Pedigree** analysis is therefore an **important** tool in both basic research and genetic counseling. Each **pedigree** chart represents all of the available information about the inheritance of a single trait (most often a disease) within a family

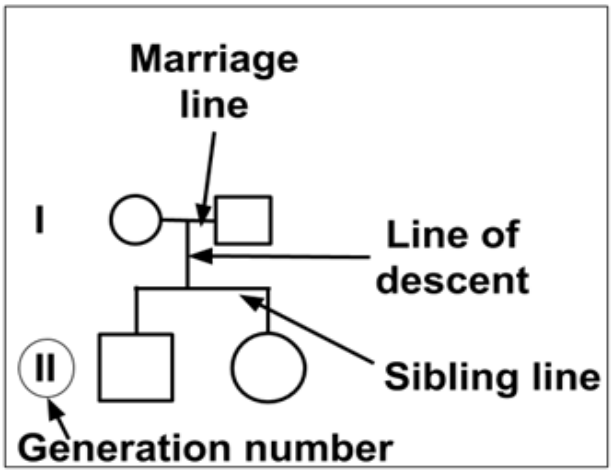
Key terms

Term	Meaning
Pedigree:	Chart that shows the presence or absence of a trait within a family across generations
Genotype:	The genetic makeup of an organism (ex: TT)
Phenotype:	The physical characteristics of an organism (ex: tall)
Dominant allele:	Allele that is phenotypically expressed over another allele
Recessive allele:	Allele that is only expressed in absence of a dominant allele
Autosomal trait:	Trait that is located on an autosome (non-sex chromosome)
Sex-linked	Trait that is located on one of the two sex chromosomes

Term	Meaning
trait:	
Homozygous:	Having two identical alleles for a particular gene
Heterozygous:	Having two different alleles for a particular gene

Reading a pedigree





Pedigrees represent family members and relationships using standardized symbols.

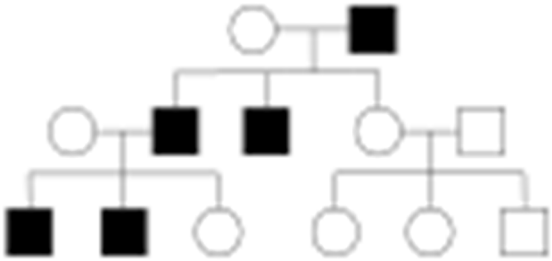
By analyzing a pedigree, we can determine **genotypes**, identify **phenotypes**, and predict how a trait will be passed on in the future. The information from a pedigree makes it possible to determine how certain alleles are inherited: whether they are **dominant**, **recessive**, **autosomal**, or **sex-linked**.

To start reading a pedigree:

1. **Determine whether the trait is dominant or recessive.** If the trait is dominant, one of the parents *must* have the trait. Dominant traits will not skip a generation. If the

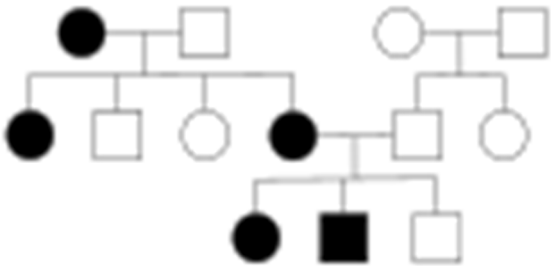
trait is recessive, neither parent is required to have the trait since they can be heterozygous.

2. **Determine if the chart shows an autosomal or sex-linked (usually X-linked) trait.** For example, in X-linked recessive traits, males are much more commonly affected than females. In autosomal traits, both males and females are equally likely to be affected (usually in equal proportions).

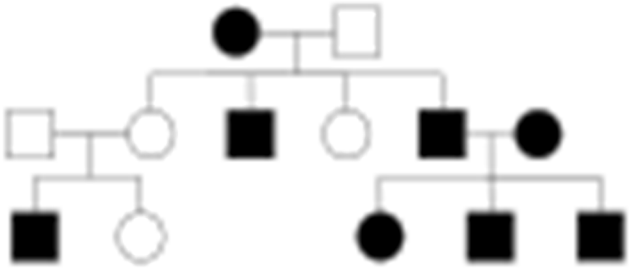


In a Y-linked disorder, only males can be affected. If the father is affected all sons will be affected. It also does not skip a generation.

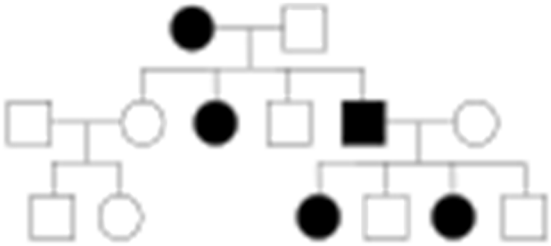
In an Autosomal Recessive Disorder, both parents can not express the trait, however, if both are carriers, their offspring can express the trait. Autosomal recessive disorders typically skip a generation, so affected offspring typically have unaffected parents. With an autosomal recessive disorder, both males and females are equally likely to be affected.



Autosomal Dominant disorders don't skip a generation, so affected offspring have affected parents. One parent must have the disorder for its offspring to be affected. Both males and females are equally likely to be affected, so it is an autosomal disorder.

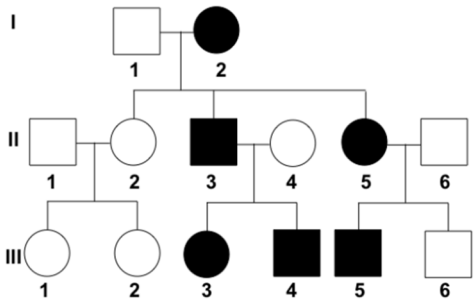


In a X-linked Recessive Disorder, males are more likely to be affected than females. Affected sons typically have unaffected mothers. The father also must be affected for daughter to be affected and the mother must be affected or a carrier for the daughter to be affected. The disorder is also never passed from father to son. Only females can be carriers for the disorders. X-linked recessive disorders also typically skip a generation.



In a X-Linked Dominant disorder, if the father is affected all daughters will be affected and no sons will be affected. It doesn't skip a generation and if the mother is affected she has a 50% chance of passing it onto her offspring

Example: Autosomal dominant trait



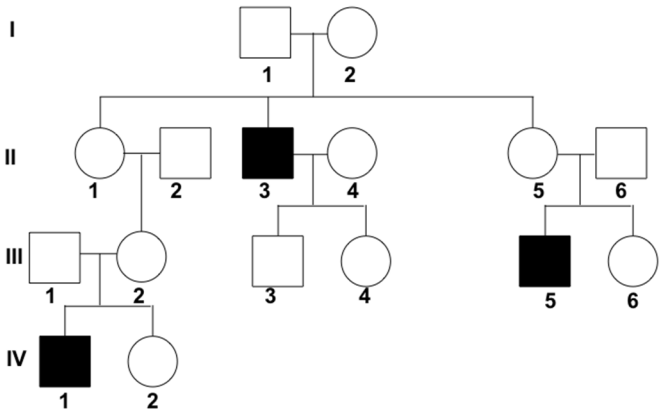
Freckles are small brown spots on your skin, often in areas that get sun exposure. In most cases, **freckles** are harmless. They form as a result of overproduction of melanin, which is responsible for skin and hair color (pigmentation). Overall, **freckles** come from ultraviolet (UV) radiation stimulation. Since freckles are dominant to no freckles, an affected individual such as I-2 must at least have one **F** allele.

The diagram shows the inheritance of freckles in a family. The allele for freckles (**F**) is dominant to the allele for no freckles (**f**). At the top of the pedigree is a grandmother (individual I-2) who has freckles. Two of her three children have the trait (individuals II-3 and II-5) and three of her grandchildren have the trait (individuals III-3, III-4, and III-5).

What is the genotype of 1-2?

The trait shows up in all generations and affects both males and females equally. This suggests that it is an autosomal dominant trait. Unaffected individuals must have two recessive alleles (**ff**) in order to not have freckles. If we notice, I-2 has some children who do *not* have freckles. In order to produce children with a genotype of **ff**, I-2 must be able to donate a **f** allele. We can therefore conclude that her genotype is **Ff**.

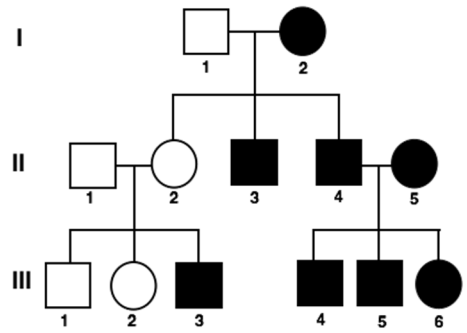
Example: X-linked recessive trait



The diagram shows the inheritance of colorblindness in a family. Colorblindness is a recessive and X-linked trait (X^b). The allele for normal vision is dominant and is represented by X^B. In generation I, neither parent has the trait, but one of their children (II-3) is colorblind. Because there are unaffected parents that have affected offspring, it can be assumed that the trait is recessive. In addition, the trait appears to affect males more than females (in this case, exclusively males are affected), suggesting that the trait may be X-linked

What is the genotype of Generation III - 2?

We can determine the genotype of III-2 by looking at her children. Since she is an unaffected female, she must have at least one normal vision allele X^B. Her two genotype options are then X^BX^B or $X^BX^{b.}$ However, her son (IV-1) is colorblind, meaning that he has a genotype of X^bY. Because males always get their X chromosome from their mothers and Y from their fathers), his colorblind allele must come from III-2. We can then determine that III-2's genotype is X^BX^b, so she can pass the X^b her son.



6. ISOLATION & PURIFICATION OF PROTEIN & CHARACTERIZATION THROUGH SDS-PAGE

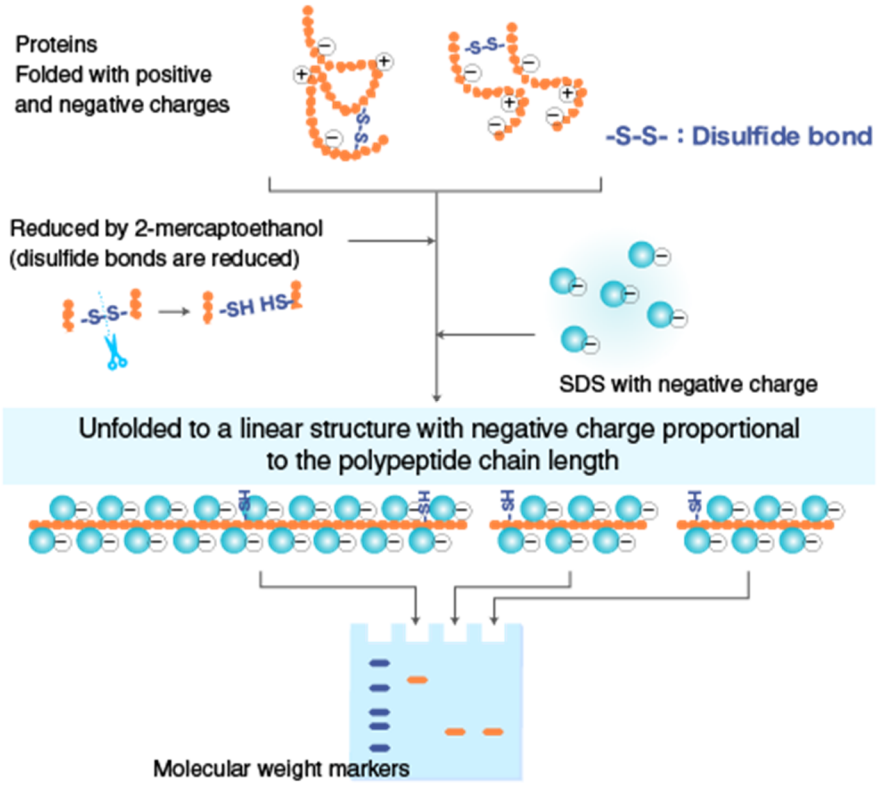
SDS-PAGE (sodium dodecyl sulfate – polyacrylamide gel electrophoresis) is a technique used to separate the proteins according to their masses. Separation of macromolecules under the influence of the charge is called **electrophoresis**. The gel used in **SDA-PAGE** is polyacrylamide and agent which is used to linearize the proteins is SDS.

Principle of SDS-PAGE

Protein samples and ladder are loaded into wells in the gel and electric voltage is applied. A reducing agent such as mercaptoethanol or dithiothreitol (DTT) (in the presence of a detergent i.e. SDS) breaks down the disulfide bridges that are responsible for protein folding; and a detergent such as SDS imparts negative charge to the proteins thereby linearizing them into polypeptides. Polyacrylamide provides a matrix for the polypeptides to run. Polypeptides run towards the positive electrode (anode) through the gel when electric field is applied. Electrophoretic mobility of the proteins depends upon 3 factors:

- **Shape** – All the proteins are in the primary structure after the treatment with a reducing agent. So, shape doesn't affect the protein separation.
- **Charge** – All the proteins are negatively charge proportional to their molecular weight after treatment with SDS. So charge doesn't affect the separation.
- **Size**– proteins get separated solely on the basis of their molecular weight.

Smaller polypeptides move faster because they have to face less hindrance, larger ones move slower because of greater hindrance. Hence proteins get separated only on the basis of their mass.



Materials Required For SDS PAGE

Acrylamide solutions (for resolving & stacking gels).

Isopropanol / distilled water .

Gel loading buffer.

Running buffer.

Staining, destaining solutions.

Protein samples .

Molecular weight markers.

The equipment and supplies necessary for conducting SDS-PAGE includes:

An electrophoresis chamber and power supply.

Glass plates(a short and a top plate).

Casting frame .

Casting stand.

Combs .

Reagents

1. 30% Polyacrylamide solution(29g acrylamide+1g bisacrylamide in 50 mL of water, dissolve completely using a magnetic stirrer, make the volume upto 100mL). Keep the solution away from sunlight.
2. 1.5 M Tris, pH 8.8
3. 1 M Tris, pH 6.8
4. 10% SDS (10 g SDS in 100mL distilled water).
5. 10% ammonium persulfate (0.1 g in 1 ml water). It should be freshly prepared.
6. 10x SDS running buffer(pH ~8.3) - Take 60.6 g Tris base, 288g Glycine and 20g SDS in separate beakers and dissolve them using distilled water. When properly dissolved ,mix three of them and make upto 2L.(working standard is 1X buffer).

Resolving gel (10%)

dH_20	4.0 ml
30% acrylamide mix	3.3 ml
1.5M Tris pH8.8	2.5 ml
10% SDS	0.1 ml

Stacking gel (5%)

dH_20	5.65 ml
30% acrylamide mix	1.65 ml
1.0M Tris pH 6.8	2.5 ml
10% SDS	0.1 ml

10% ammonium persulfate	0.1 ml
TEMED	0.004ml

10% ammonium persulfate	0.1 ml
TEMED	0.004ml

Gel loading buffer:

To make 10 mL of 4X stock:

2.0 ml 1M Tris-HCl pH 6.8.
2. 0.8 g SDS.
3. 4.0 ml 100% glycerol.
0.4 ml 14.7 M β-mercaptoethanol.
1.0 ml 0.5 M EDTA.
8 mg Bromophenol Blue.

Staining solution:

Weigh 0.25g of Coomassie Brilliant Blue R250 in a beaker. Add 90 ml methanol:water (1:1 v/v) and 10ml of Glacial acetic acid ,mix properly using a magnetic stirrer. (when properly mixed, filter the solution through a Whatman No. 1 filter to remove any particulate matter and store in appropriate bottles)

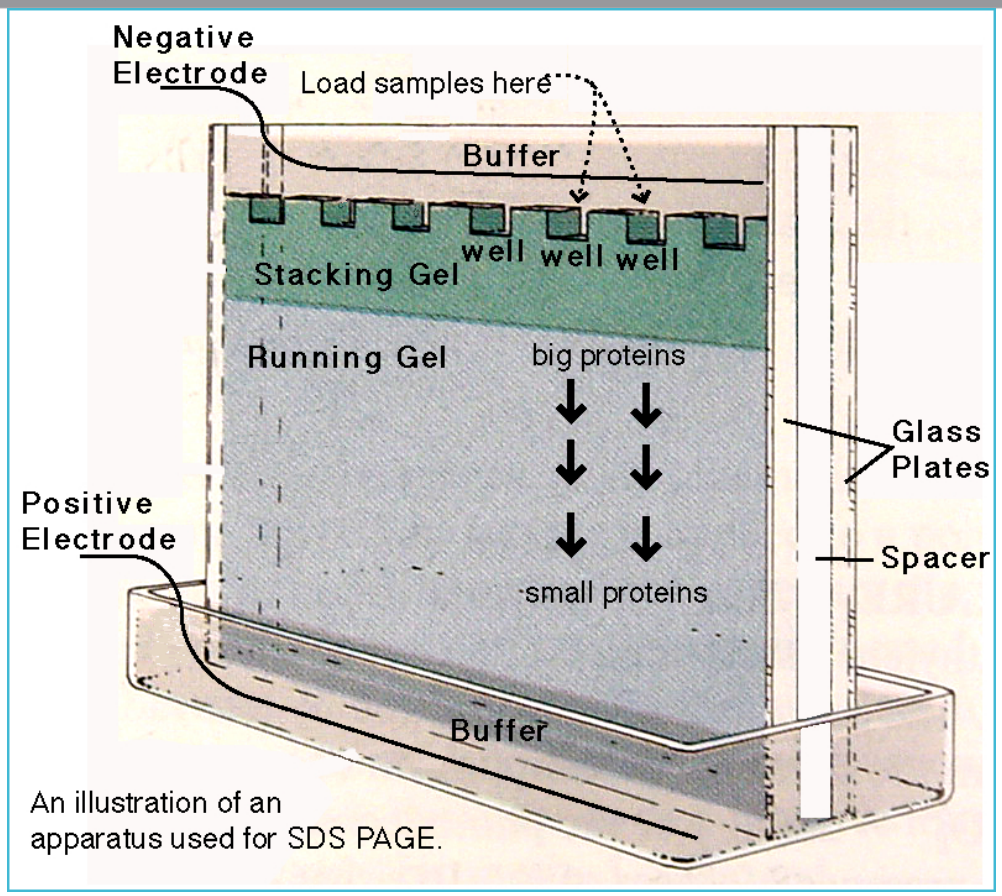
Destaining solution:

Mix 90 ml methanol:water (1:1 v/v) and 10ml of Glacial acetic acid using a magnetic stirrer and store in appropriate bottles.

Major steps of SDS-PAGE

Pouring of the resolving gel:

Resolving gel is poured between two glass plates (one is called short plate and the other one is tall plate), clipped together on a **casting frame** (Fig. 05) Bubbles are removed by adding a layer of isopropanol on the top of the gel. (The level of the gel is predetermined by placing the comb on the glass-plates and leaving approximates 1cm space below the comb. Use a pen to mark the level. Now pour the gel up to this mark.) The gel is then allowed to solidify. When the gel is solidified, remove the isopropanol by using a filter paper.

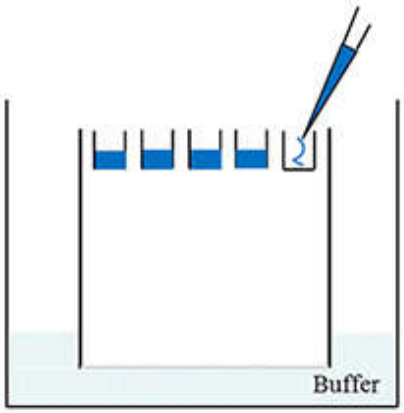


Pouring of the stacking gel:

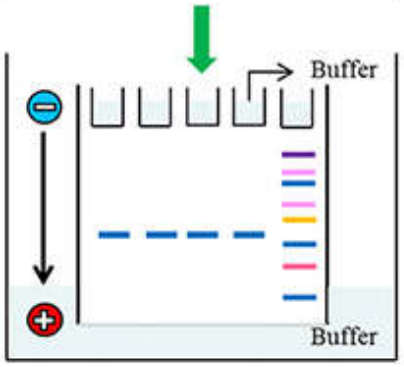
When the resolving gel is solidified, stacking gel is loaded all the way to the top of the glass plates. Comb is placed just after loading. The gel is, then, allowed to polymerize (solidify). When stacking gel is solidified, comb is removed very carefully not damaging the well's shape.

Loading the ladder in wells

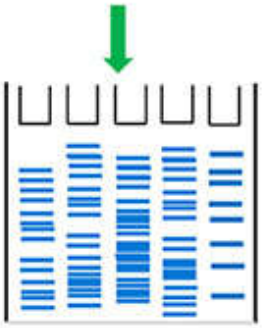
Add the ladder very carefully into the well which is on the extreme right using a micropipette. The samples are loaded into the other wells. Ladder is mostly pre-stained with the known molecular weight proteins.



Protein samples and marker loaded in vertical SDS-PAGE system



Direction of migration of samples in vertical SDS-PAGE system



SDS-PAGE gel after Coomassie blue staining

Loading the ladder in wells

Loading the samples in wells

Samples are loaded in each well with equal amount of the proteins mixture using micropipette. Be careful while loading the samples. Make sure not to damage the size of the wells or not to pour the sample out of the well instead of pouring inside it. At this stage, sample of the proteins appears to be blue because of a dye (bromophenol) used while preparing the sample.

Running the gel by applying voltage

A voltage is applied after dipping the "sandwich of gel and glass plates" in running buffer. Turn of the voltage when the tracking dye has reached or crossed the gel. The gel is further proceeded for the subsequent analysis.

Subsequent analysis – Coomassie Blue Staining

The gel is rinsed with deionized water 3-5 times to remove SDS and buffer. It may create hindrance with the binding of the dye (0.1% Coomassie Blue) to the proteins. The gel is then dipped in Coomassie Blue stain (staining buffer) on a shaking incubator at room temperature. The invisible bands of the proteins beginning to appear within minutes but it takes approximately 1h for complete staining .

3. Western Blotting Hybridization :

Principle:

The western blot (sometimes called the protein immunoblot), or western blotting, is a widely used technique in molecular biology and immunogenetics to detect specific proteins in a sample of tissue homogenate or extract. The western blot method is composed of a gel electrophoresis to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide, followed by an electrophoretic transfer onto a membrane (mostly PVDF or Nitrocellulose) and an immunostaining procedure to visualize a certain protein on the blot membrane.

{The sample undergoes protein denaturation, followed by gel electrophoresis. A synthetic or animal-derived antibody (known as the primary antibody) is created that recognises and binds to a specific target protein. The electrophoresis membrane is washed in a solution containing the primary antibody. Excess antibody is washed off. A secondary antibody is added which recognises and binds to the primary antibody. The secondary antibody is visualised through various methods such as staining, immunofluorescence, and radioactivity, allowing indirect detection of the specific target protein.}

Procedure :

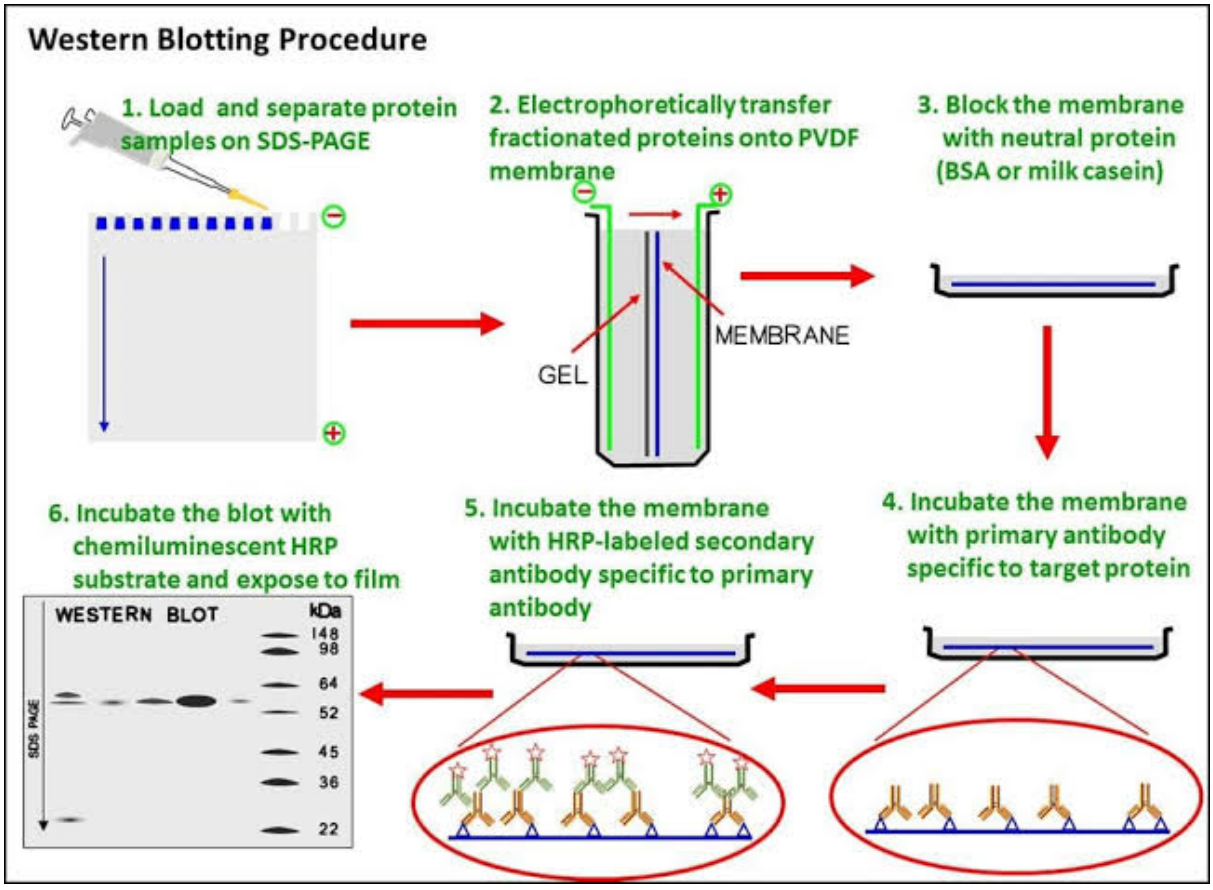
1. Protein samples are often boiled to denature the proteins present. This ensures that proteins are separated based on size and prevents proteases (enzymes that break down proteins) from degrading samples.

2. Protein samples are then electrophoresed to separate native proteins by 3-D structure or denatured proteins by the length of the polypeptide. SDS-PAGE is generally used for the denaturing electrophoretic separation of proteins.

{SDS is generally used as a buffer (as well as in the gel) in order to give all proteins present a uniform negative charge, since proteins can be positively, negatively, or neutrally charged.

3. Following electrophoretic separation, the proteins are transferred to a membrane (typically nitrocellulose or PVDF), where they are blocked with milk (or other blocking agents) to prevent non-specific antibody binding.

4. After blocking, a solution of primary antibody (generally between 0.5 and 5 micrograms/mL) diluted in either PBS or TBST wash buffer is incubated with the membrane for typically an hour at room temperature, or overnight at 4°C. The antibody solution is incubated with the membrane for anywhere from 30 minutes to overnight.



5. After rinsing the membrane to remove unbound primary antibody, the membrane is exposed to another antibody known as the secondary antibody.

{To allow detection of the target protein, the secondary antibody is commonly linked to biotin or a reporter enzyme such as alkaline phosphatase or horseradish peroxidase. Horseradish peroxidase (HRP) is commonly linked to secondary antibodies to allow the detection of the target protein by chemiluminescence.}

6. After the unbound probes are washed away, the western blot is ready for detection of the probes that are labeled and bound to the protein of interest.

{The colorimetric detection method depends on incubation of the western blot with a substrate that reacts with the reporter enzyme (such as peroxidase) that is bound to the secondary antibody. This converts the soluble dye into an insoluble form of a different color that precipitates next to the enzyme and thereby stains the membrane.

Chemiluminescent detection methods depend on incubation of the western blot with a substrate that will luminesce when exposed to the reporter on the secondary antibody. The light is then detected by CCD cameras which capture a digital image of the western blot or photographic film.}

Application :

1. The western blot is extensively used in biochemistry for the qualitative detection of single proteins and protein-modifications (such as post-translational modifications).

2. It is used as a general method to identify the presence of a specific single protein within a complex mixture of proteins.

3. A semi-quantitative estimation of a protein can be derived from the size and color intensity of a protein band on the blot membrane.

4. The western blot is routinely used for verification of protein production after cloning.

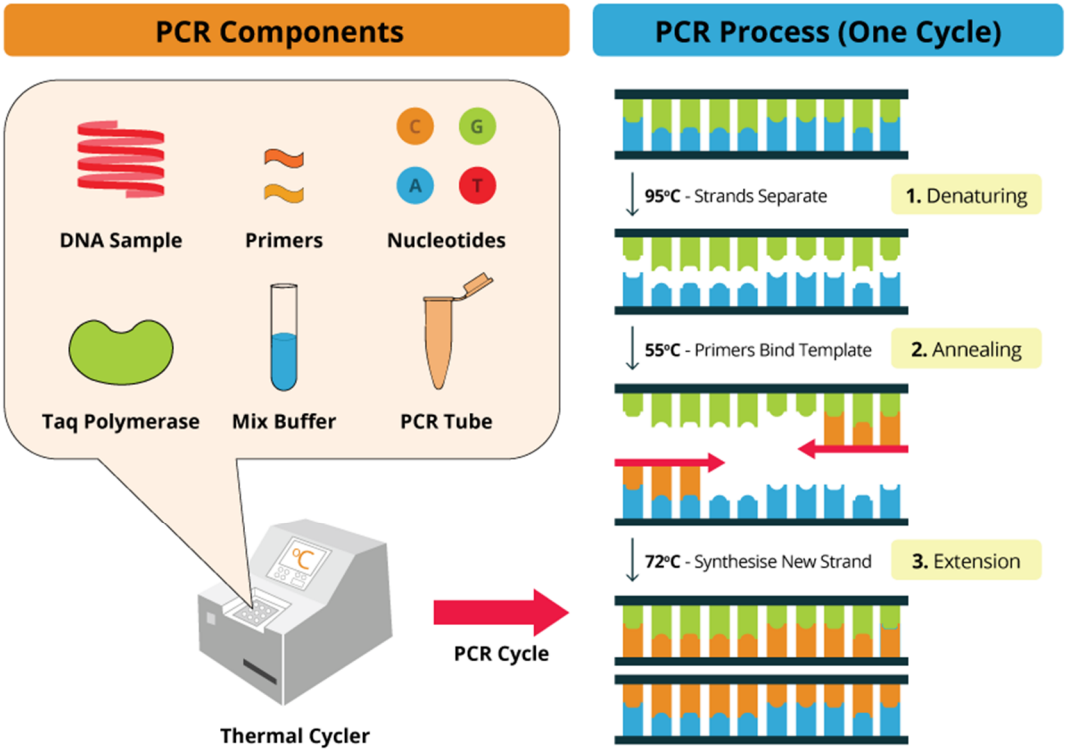
5. It is also used in medical diagnostics, e.g., in the HIV test or BSE-Test.

PCR

Polymerase Chain Reaction (PCR) is a rapid procedure for in vitro enzymatic amplification of specific DNA sequences using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. Repetitive cycles involving template denaturation, primer annealing and the extension of the annealed primers by DNA polymerase, result in the exponential accumulation of a specific fragment whose termini are defined by 5’ end of the primers. The primer extension products synthesized in one cycle can serve as a template in the next. Hence the number of target DNA copies approximately doubles at every cycle. Since its inception, PCR has had an enormous impact in both basic and diagnostic aspects of molecular biology. Like the PCR itself, the number of applications has been accumulating exponentially. It is therefore recommended that relevant scientists and laboratories in developing countries like Nigeria should acquire this simple and relatively inexpensive, but rather robust technology

Principles of PCR

Its principle is based on the use of DNA polymerase which is an in vitro replication of specific DNA sequences. This method can generate tens of billions of copies of a particular DNA fragment (the sequence of interest, DNA of interest, or target DNA) from a DNA extract (DNA template). As the name implies, it is a chain reaction, a small fragment of the DNA section of interest needs to be identified which serves as the template for producing the primers that initiate the reaction. One DNA molecule is used to produce two copies, then four, then eight and so forth.



Procedure

The protocol describes how to amplify a segment of double-stranded DNA in a chain reaction catalyzed by a thermostable DNA polymerase. It is the foundation for all subsequent variations of the polymerase chain reaction.

I. Materials

Buffers and Solutions

10x Amplification buffer
Chloroform
dNTP solution (20 mM) containing all four dNTPs (pH 8.0)

Enzymes and Buffers

Thermostable DNA polymerase
Nucleic Acids and Oligonucleotides
Forward primer (20 µM) in H_2O
Reverse primer (20 µM) in H_2O
Template DNA.

Dissolve template DNA in 10 mM Tris-Cl (pH 7.6) containing a low concentration of EDTA (<0.1 mM) at the following concentrations: mammalian genomic DNA, 100 µg/ml; yeast genomic DNA, 1 µg/ml; bacterial genomic DNA, 0.1 µg/ml; and plasmid DNA, 1-5 ng/ml.

Method

In a sterile 0.5-ml microfuge tube, mix in the following order:

REAGENTS	**AMOUNT(µl)**
Deionized water	37.5 µl
Taq assay buffer(10x)	5 µl
Template DNA	1µl
dNTPs mix	2 µl
Forward primer	2 µl
Reverse primer	2 µl
Taq DNA polymerase	5 µl

The table provides standard reaction conditions for PCR. Mg2+ (1.5 mM) ;KCl(50 mM) ;dNTPs (200 µM) ;Primers(1 µM);DNA polymerase (1-5 units); Template DNA(1 pg to 1 µg).

The amount of template DNA required varies according to the complexity of its sequence. In the case of mammalian DNA, up to 1.0 µg is used per reaction. Typical amounts of yeast, bacterial, and plasmid DNAs used per reaction are 10 ng, 1 ng, and 10 pg, respectively.

1. If the thermal cycler is not fitted with a heated lid, overlay the reaction mixtures with 1 drop (approx. 50 µl) of light mineral oil. Alternatively, place a bead of wax into the tube if using a hot start protocol. Place the tubes or the micro titer plate in the thermal cycler.

2. Amplify the nucleic acids using the denaturation, annealing, and polymerization times and temperatures listed below.

3. Withdraw a sample (5-10 µl) from the test reaction mixture and the four control reactions, analyze them by electrophoresis through an agarose gel, and stain the gel with ethidium bromide or SYBR Gold to visualize the DNA.

4. A successful amplification reaction should yield a readily visible DNA fragment of the expected size. The identity of the band can be confirmed by DNA sequencing, Southern hybridization and/or restriction mapping. If all is well, lanes of the gel containing samples of the two positive controls (Tubes 1& 2) and the template DNA under test should contain a prominent band of DNA of the appropriate molecular weight. This band should be absent from the lanes containing samples of the negative controls (Tubes 3 & 4).

5. If mineral oil was used to overlay the reaction (Step 2), remove the oil from the sample by extraction with 150 µl of chloroform. The aqueous phase, which contains the amplified DNA, will form a micelle near the meniscus. The micelle can be transferred to a fresh tube with an automatic micropipette.