B.Sc. FISHERY SCIENCE
LAB MANUAL
1st Semester

Prepared By
Biological Science Dept.
Fishery Science

MIDNAPORE CITY COLLEGE

BFSC SEMESTER-I

BFSC-101 Principles of Aquaculture

Aquaculture production statistics- world and India. Aquaculture resources of world and India. Components of Aquaculture farms. Estimation of carrying capacity. Practices on prestocking and post stocking management. Growth studies in aquaculture system. Study on waste accumulation in aquaculture system (NH3, Organic matter, CO_2). Analysis of manure.

BFSC-102 Meteorology, Climatology and Geography

Graphic representation of structure of atmosphere; physical layering and compositional layering. Temperature instruments: simple thermometers; Six's Max-Min Thermometer; thermograph. Humidity measurement: hygrometer; psychrometer; relative humidity; dew point. Condensation: observation and identification of various types of clouds. Precipitation: measurement of rainfall using rain gauge. Atmospheric pressure measurement: fortin's mercurial barometer; Aneroid barometer. Isobars: Wind observation and measurement: wind vane; cup anemometer. Geography: The Earth: diagrammatic representation of shape, size, structure, zones, latitudes, longitudes and great circles. Geographical terms used in landscape.

BFSC-103 Soil and Water Chemistry

Principles of Titrimetry, Gravimetry, Potentiometry, Conductometry, Refractometry, Colourimetry, Turbidimetry, Spectrophotometry (UV, Visible, Flame, AAS), Computerized instrument system. Demonstration: demonstration of laboratory glass wares and equipment used in water and soil analysis. Water analysis: measurement of temperature, turbidity. Determination of pH and EC. Determination of salinity, chlorinity, total solids, Redox potential, DO, Free CO2. Determination of total alkalinity, total hardness. Determination of inorganic nitrogen and phosphorus. Soil analysis: Determination of soil texture, soil pH, conductivity, soil available nitrogen, available phosphorus, and organic carbon.

BFSC-104 Fundamentals of Microbiology

Handling of microscopes, Wet mount, smear and hanging drop preparations Micrometry- Determination of size of micro organisms (ocular, stage micrometers). Tools and techniques in sterilization methods: Filtration, dry heat, moist heat, chemical agents Cultivation technique: Media preparation, Isolation - pure culture, subculture. Observation of fungi, bluegreen algae, and protozoans. Staining techniques for bacteria– simple, differential, structural and Biochemical tests: Indole, methyl red, Voges Proskauer, citrate test, oxidase test, catalase ztests. Collection of water and sediment samples for microbiological analysis, Winogradsky cylinder, Isolation, identification and enumeration of various groups of microorganisms from different water bodies including aquaculture systems. Study of bacteria involved in nutrient cycles. Biofilms, water testing for potability, enumeration of coliforms. Antibiotic sensitivity of bacteria - antibiotic sensitivity test – disc diffusion method.

BFSC-105 Fundamentals of Biochemistry

Preparation of normal solution of acid and base, buffers and reagents. Qualitative determination of carbohydrates, proteins and lipids. Estimation of total nitrogen and crude protein of fish tissue. Estimation of carbohydrates in foods. Determination of specific gravity of oil. Extraction and estimation of total lipids in fish tissue. Determination of saponification value, iodine value and free fatty acid value.

BFSC-106 Taxonomy of finfish

Collection and identification of commercially important inland and marine fishes. Study of their external morphology and diagnostic features. Modern taxonomic tools - Protein analysis and electrophoretic studies; Karyo taxonomy - chromosome preparation and identification. DNA barcoding, DNA polymorphism; Visit to fish landing centres to study commercially important fishes and catch composition.

BFSC-107 Information and Communication Technology

Exercises on binary number system, algorithm and flow chart; MS Word; MS Excel; MS Power Point; Internet applications: Web Browsing, Creation and operation of Email account; Analysis of fisheries data using MS Excel. Handling of audio visual equipments. Planning, preparation, presentation of posters, charts, overhead transparencies and slides. Organization of an audio visual programme.

BFSC-108 Taxonomy of Shellfish

Study of external morphology. Collection, preservation and identification of commercially important prawns, shrimps, crabs, lobsters, bivalves, gastropods, cephalopods from natural habitats. Field visits for collection and study of commercially important shellfishes.

BFSC-101 Principles of Aquaculture

Pond preparation

Principle

Pond preparation is an essential practice to the success of fish culture. High organic matter content in neutral soil often promotes higher primary productivity and hence higher fish yield. Good pond preparation can serve a number of purposes such as sterilize and improve soil quality of ponds, induce production of natural foods; It is rich in protein, vitamins, minerals and other essential growth elements that simple supplementary feed cannot complete, Increase fish survival, Maintain fish health, Ensure good growth and yield. Fish yield in pond can also be affected by the presence of predators, deteriorating water quality and improper pond management. Hence, pond preparation is a first step towards ensuring a better pond production.

Procedures

1. Drain water from ponds completely and let ponds dry for 1-2 weeks until the bottom mud cracks.
2. Upon drying, air and sunlight can enhance oxidation of reduced chemical compounds in pond bottom, and eradicate unwanted organisms, predators and micro-organisms.
3. Liming is usually applied during or after the pond drying stage.
4. Liming is an important procedure to increase alkalinity and pH in pond soil, maintain pH (7-8) in pond water, provide sufficient CO2 for phytoplankton growth, and enhance good response to fertilization.

5. The amount of lime applied to ponds depends on the nature of the soil and history of ponds.
6. In general, newly dug ponds require greater amount of initial lime input than aged ponds; loamy soil needs less lime than clayey and acid soil.
7. To enhance the lime reaction with pond soil, the pond bottom should be saturated with water prior to lime application. Lime should be spread evenly on the pond bottom and dike slopes.
8. Liming can be done in three different ways:
9. by broadcast over dried pond which includes the dike walls.
10. by mixing with water and spraying over the pond, and
11. by liming the water flowing into the pond.
12. One to two weeks after liming, ponds are ready to be filled with water. Inlet and overflow pipes should be covered with screen nets to prevent wild fish from being introduced.
13. Some rocks, a piece of bamboo mat or a piece of iron wire can be put under the inlet pipe to protect the soil from being eroded there and the water from becoming too muddy during filling.
14. Fertilization using chemical fertilizers, manure or their combinations starts on the next day after filling and should continue on a weekly basis until water becomes green.
15. When Secchi disk visibility reaches about 30 cm, fish can be stocked. If the Secchi disk is not available, you may roughly test how fertile the pond water is by putting your arm in the water until it reaches your elbow, and then look at your hand. If you can see your palm through the water, you need to add more fertilizers and test again before stocking fish.

2. Analysis of soil pH and liming

Principle

Liming is the application of calcium and magnesium compounds to the soil for the purpose of reducing soil acidity.

Materials required

1. pH meter
2. Glass beaker
3. Distilled water
4. Soil sample
5. Weighing balance
6. p-nitrophenol buffer: To prepare a p-nitrophenol buffer of pH 8.0. Dilute 20 g para-nitrophenol, 15 g boric acid, 75 g potassium hydroxide in one liter of distilled water. Check the pH using a standardized pH meter and adjust to the right pH by adding

7.5% potassium hydroxide (7.5 g/100 ml distilled water) or 1.5% boric acid (1.5 g/100 ml distilled water).

Procedure

1. Weight 20 g of the sieved soil sample in 100 ml glass beaker and add 20 ml of distilled water. Stir intermittently for one hour.
2. Measure the pH of the above solution with a pH meter previously standardized and record the reading.
3. Add 20 ml of p-nitrophenol buffer to the above solution and stir intermittently for 20 minutes. Prior to reading its pH, set the pH meter to pH 8.0 with 1:1 mixture of the p-nitrophenol buffer and distilled water.
4. Read the pH of the soil sample (buffer/distilled water mixture) while stirring vigorously.
5. Use the values of the soil sample pH in distilled water and soil sample in buffered solution to obtain the liming rate from Table 1.
6. If the pH of the soil in the buffered solution is below 7, repeat the analysis with 10 g of soil sample and double the liming rate from Table 1.
7. Table 1. Lime requirement in kg/ha of calcium carbonate (neutralizing value of 100) to increase total hardness and total alkalinity of pond water above 20 mg/L

Mud pH in water	Mud pH in buffered solution									
	7.9	7.8	7.7	7.6	7.5	7.4	7.3	7.2	7.1	7.0
	(kg/ha of calcium carbonate required)									
5.7	121	242	363	484	605	726	847	968	1 089	1 210
5.6	168	336	504	672	840	1 008	1 176	1 344	1 512	1 680
5.5	269	538	806	1 075	1 344	1 613	1 881	2 150	2 419	2 688
5.4	386	773	1	1	1	2	2	3	3	3

			159	546	932	318	705	091	478	864
5.3	454	907	1 361	1 814	2 268	2 722	3 175	3 629	4 082	4 536
5.2	521	1 042	1 562	2 083	2 064	3 125	3 646	4 166	4 687	5 208
5.1	588	1 176	1 764	2 353	2 940	3 528	4 116	4 704	5 292	5 880
5.0	672	1 344	2 016	2 688	3 360	4 032	4 704	5 376	6 048	6 720
4.9	874	1 747	2 621	3 494	4 368	5 242	6 115	6 989	7 974	8 736
4.8	896	1 792	2 688	3 584	4 480	5 376	6 272	7 186	8 064	8 960
4.7	941	1 882	2 822	3 763	4 704	5 645	6 586	7 526	8 467	9 408

Source: Boyd 1979

Generally, the liming material used in ponds is calcium oxide. It is therefore necessary to convert the liming rate to calcium oxide value by multiplying the above rate with 0.56.

P – 3: Eradication of aquatic weeds

Principle

Aquatic weeds can be defined as unwanted and undesirable vegetation that grow profusely in waters. Based on their habitat, the aquatic weeds can be classified into floating, submerged, emergent, marginal, filamentous and algal blooms. The disadvantages of aquatic weeds in ponds include

a. Interference in culture activities
b. Depletion of dissolved oxygen
c. Restrict space for movement of fish
d. Utilize nutrients
e. Hinder netting operation
f. Restrict light penetration
g. Release toxic gases

Procedures

Physical methods:

controlling aquatic weeds by manually (hand picking) or mechanically using various tools such as sickle, scythes, blades, nets, wooden rakes, cullers, haulers, cranes etc., are used to remove aquatic weeds.

Chemical methods

Different types chemicals with different dosages applied to control aquatic weeds depend on time season and type of infestation. Chemicals have to be sprayed over floating leaves or applied to pond water or soil.

Biological methods

Biological method of weed control is the most efficient and useful method without much expensive. The waste vegetative matter is recycled into proteinacious fish flesh. Various biological agents used are fishes, insects, snails and mammals.

P - 4. Eradication of Predatory fishes:

Principle:

Predatory fish prey upon the spawn, fry and fingerlings of carps and the weed fish compete with carp for food, space and oxygen. Therefore predatory and weed fish should be completely eradicated from nursery, rearing and stocking ponds before these ponds are stocked.

Procedure

1. Initially predatory fishes present in the ponds are harvested by netting.
2. If possible, drain the water completely and disinfect the pond bottom with suitable disinfectant.
3. However, where it is not possible, the pond should be treated with fish poison at the rate given in the table.

Calculation of dose

The required quantity of poison can be calculated using the following formulae.

For rectangular ponds:

= Required amount of poison in kg.

For circular ponds:

1. = Required amount of poison in kg

Table 1. Recommended doses of fish poison	
Poison	**Dose (kg/ha/m)**
Bleaching powder	350 – 500
Mohua oil cake	2500
Anhydrous ammonia	20 – 30
Powdered seed of Croton tiqlium	30 – 50
Root powder of Milletia pachycarpa	40 – 50
Seed powder of Milletia piecidia	40 – 50
Seed powder of Barrinqtonia acutanqula	150
Seed meal of tamarind (Tamarindus indica)	1750 –2000
Tea seed cake (Camellia sinensis)	750

P – 5. Application of fertilizers

Principle

Fertilization schedule involving both organic and inorganic fertilizers starts 10–15 days prior to stocking and is prepared on the basis of nutrient status and chemical environment of the pond soil and water.

Procedure

Fertilizer Application

Applying fertilizer in ponds to supply the nutrients needed for plant growth is a fundamental part of fishpond management. Fish production per unit area can be increased as much as five-fold by proper application of fertilizer. Fertilizers are classified into two groups:

Techniques in Fertilizer Application

1. Apply animal manure in heaps of 20-50 kg at several locations in the pond to prevent excessive absorption of dissolved oxygen during decomposition.
2. Apply inorganic fertilizers in fishponds using the platform method. The platform is a table-like structure about 75 sq m positioned with its surface horizontal beneath the water surface. Bamboos are excellent for constructing platforms.
3. Position the platform 15-20 cm below the water surface. Do not place this in corner, or in areas shielded from the wind. The platform saves approximately 20-40% of the required amount of fertilizer.

4. Put the inorganic fertilizers by placing them in gunny sacks suspended in the water to enable this fertilizer to dissolve gradually thus, providing a continuous supply of nutrients for the plankton.

P - 6. Estimate the total potassium permanganate needed to disinfect a pond (pond size: 25 ft×15 ft./ depth 6 ft.) @ 5 ppm rate.

Ans:

The total volume of the pond = 25×15×6 = 2250 cu. Ft.

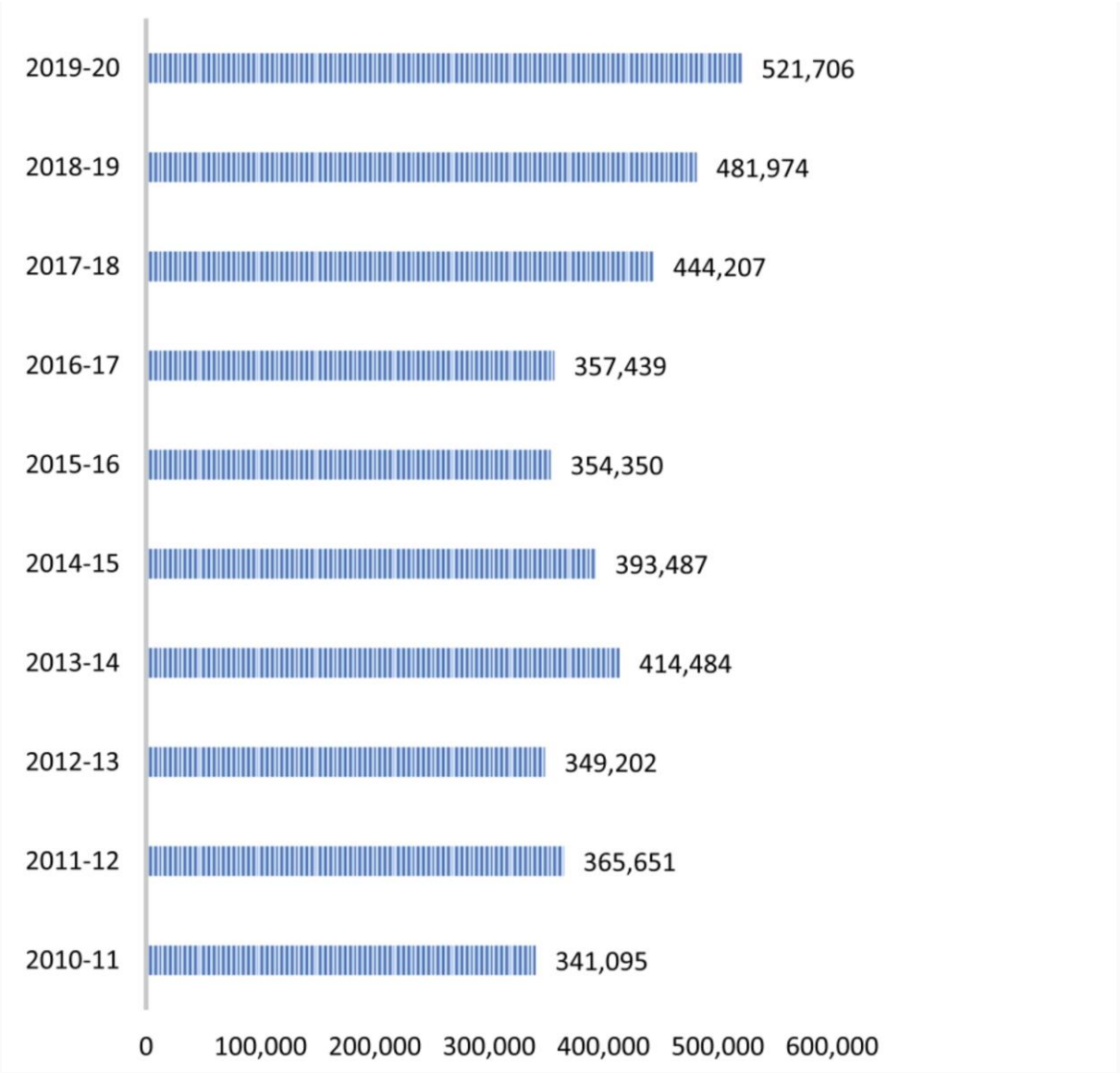
The water volume for 1 cu. Ft. = 28.32 litre

Total water volume of the pond = 2250×28.32 = 63720 litre

Total potassium permanganate needed to disinfect a pond = 63720×5 mg = 318600 mg = 318 gram.

Total potassium permanganate needed to disinfect a pond is 318 gram.

P-7: Plot a bar diagram on Total Fish Seed Production, India (Lakhs Fry)

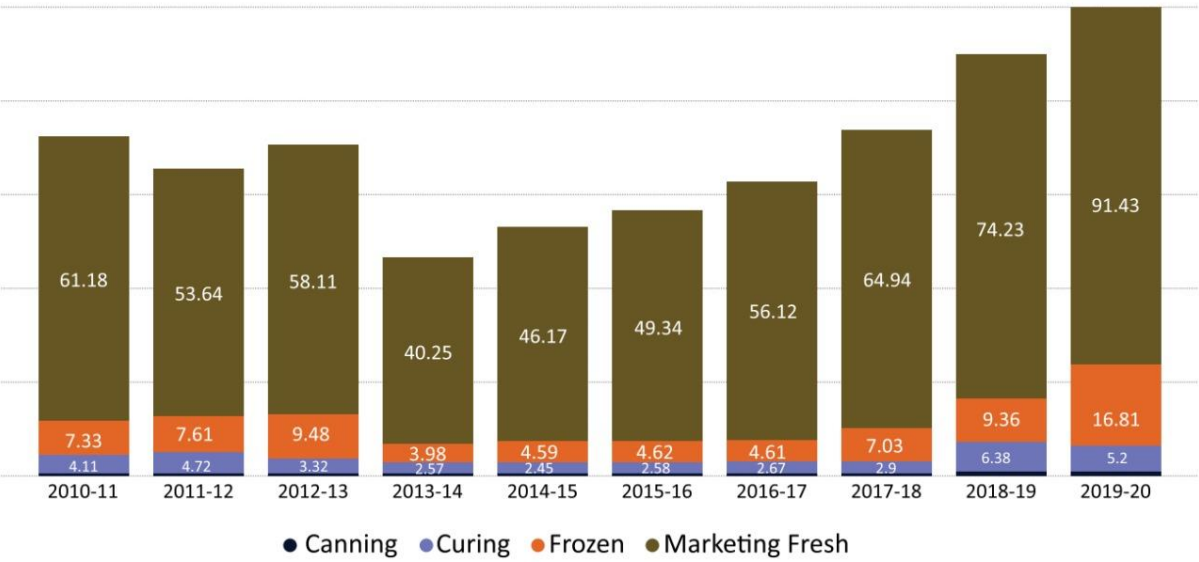


P – 8. Make a bar diagram on following data of disposition of fish catch

Table 2.1: Year-wise Disposition of Fish Catch (In Lakh Tonnes)

Year	Marketing Fresh	Fro-zen	Curing	Can-ning	Reduc-tion	Miscella-neous	Offal for Reduction	Unspeci-fied	Others	Total
2014-15	46.17	4.59	2.45	0.43	3.33	0	0.9	0.21	2.18	60.26
2015-16	49.34	4.62	2.58	0.52	3.21	0	1.06	0.22	0.2	61.75
2016-17	56.12	4.61	2.67	0.5	3.25	0	1.2	0.29	2.4	71.04
2017-18	64.94	7.03	2.9	0.36	3.33	0	1.89	0.38	2.39	83.22
2018-19	74.23	9.36	6.38	1.02	3.28	2.28	0	0.05	1.43	98.03
2019-20	91.43	16.81	5.2	0.36	3.38	3.04	0.08	0.05	1.44	121.79

Source: Department of Fisheries, States Government / UTs Administration



Draw a line diagram of

a) Trend of Export of Fish and Fish Products in Quantity (Lakh Tonnes)
b) Trend of Export of Fish and Fish Products in Value (Rs. Crore) by using following data.

Table 5.1: Trend of Export of Fish and Fish Products:2019-20

Year	Quantity (Tonnes)	Value (₹ Crore)	US Dollar ($: Million)	Unit value (Rs. /Tonnes)	Unit value Index	Annual growth rate (%)	
						Quantity	Value
2010-11	8,13,090.85	1,29,01.47	2,856.92	1,58,671.89	6,367.92	19.85	28.39
2011-12	8,62,021.41	1,65,97.23	3,508.45	1,92,538.46	7,727.08	6.02	28.65
2012-13	9,28,214.67	18,856.26	3,511.67	2,03,145.42	8,152.76	7.68	13.61
2013-14	9,83,755.56	30,213.26	5,007.70	3,07,121.60	12,325.60	5.98	60.23
2014-15	10,51,243.49	33,441.61	5,511.12	3,18,114.75	12,766.79	6.86	10.69
2015-16	9,45,891.90	30,420.83	4,687.94	3,21,609.99	12,907.06	-10.02	-9.03
2016-17	11,34,948.09	37,870.90	5,777.61	3,33,679.62	13,391.45	19.99	24.49
2017-18	13,77,243.70	45,106.89	7,081.55	3,27,515.69	13,144.07	21.35	19.11
2018-19	13,92,558.89	46,589.37	6,728.50	3,34,559.46	13,426.76	1.11	3.29
2019-20	12,89,650.90	46,662.85	6,678.69	3,61,825.42	14,521.01	-7.39	0.16

Source: Marine Products Export Development Authority, Kochi

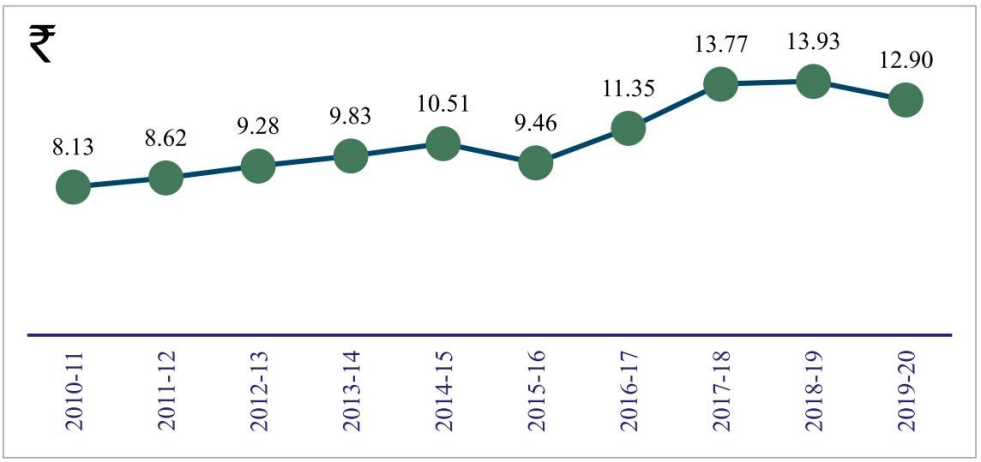
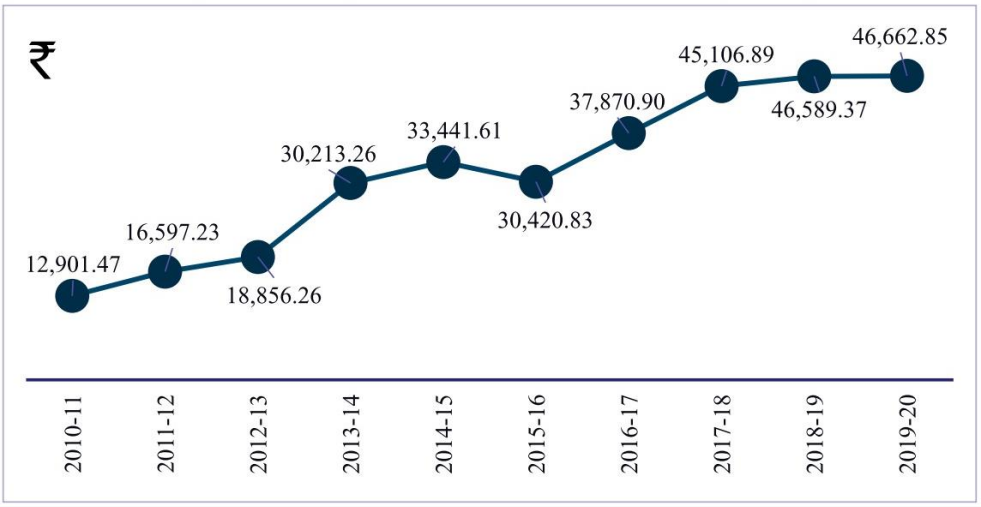


Fig 27: Trend of Export of Fish and Fish Products in Value (Rs. Crore)



Draw a pie diagram on:

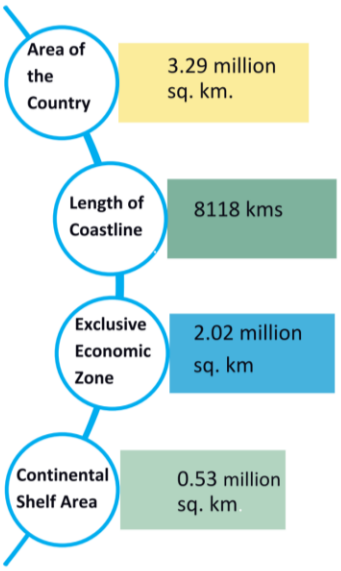
a) Percentage share of Item-wise Export of Fisheries Products (Value)
b) Percentage share of Item-wise Export of Fisheries Products (Quantity)

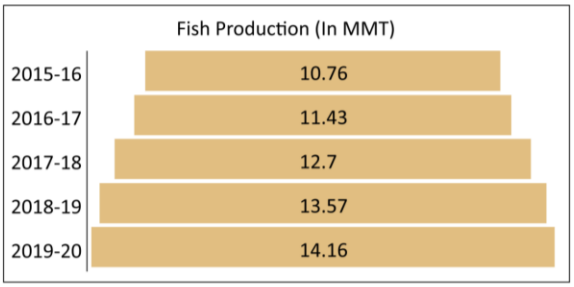
Table 5.2: Item-wise Export of Fisheries Products:2020

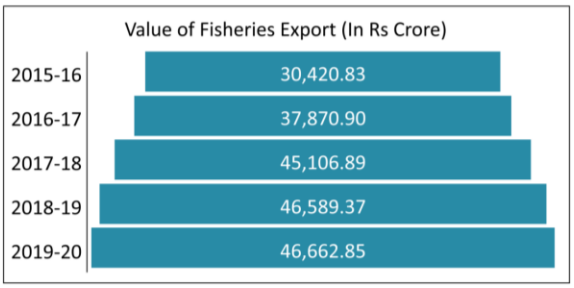
Item		2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	2018-19	2019-20
Frozen Shrimp	Q:	1,51,465	1,89,125	2,28,620	3,01,435	3,57,505	3,73,866	4,34,486	5,65,980	6,14,145	6,52,253
	V:	5,718.13	8,175.26	9,706.36	19,368.30	22,468.12	20,045.50	24,711.32	30,868.17	31,800.51	34,152.03
	$:	1,261.81	1,741.20	1,803.26	3,210.94	3,709.76	3,096.68	3,726.38	4,848.19	4,610.59	4,889.12
Frozen Fish	Q:	3,12,358	3,47,118	3,43,876	3,24,359	3,09,434	2,28,749	2,96,762	3,53,192	3,38,933	2,23,318
	V:	2,623.89	3,284.15	3,296.86	4,294.81	3,778.50	3,462.25	4,460.90	4,674.03	4,916.82	3,610.01
	$:	583.48	683.50	617.59	708.63	619.66	529.85	672.47	733.17	699.09	513.6
Fr Cuttle fish	Q:	59,159	54,671	63,296	68,577	82,353	65,596	63,320	69,183	60,210	70,906
	V:	1,104.57	1,346.72	1,354.28	1,386.98	1,833.21	1,636.11	1,944.50	2,356.46	1,975.97	2,009.79
	$:	244.62	282.72	251.54	228.13	300.69	250.31	292.73	369.88	282.29	286.4
Fr Squid	Q:	87,579	77,373	75,387	87,437	69,569	81,769	99,348	1,00,845	1,01,101	87,631
	V:	1,010.57	1,228.19	1,378.08	1,731.97	1,275.25	1,615.21	2,575.29	2,451.87	2,506.99	2,196.59
	$:	223.67	262.72	256.90	284.60	209.84	247.53	388.64	385.01	359.71	314.23
Dried Item	Q:	79,059	53,721	72,953	67,901	70,544	43,320	61,071	88,997	95,296	84,417
	V:	954.94	562.65	819.90	998.00	1010.16	725.58	871.74	1042.37	1323.45	981.5
	$:	212.22	117.66	152.81	167.89	165.52	111.57	199.77	163.53	189.58	140.81
Live Items	Q:	5,208	4,199	4,373	5,080	5,488	5,493	6,703	7,034	10,179	7,287
	V:	142.15	154.61	197.89	281.85	301.51	308.81	403.75	286.11	388.88	324.26
	$:	31.46	32.46	36.82	46.70	49.62	47.77	61.05	45.41	55.89	46.43
Chilled Items	Q:	21,118	21,278	26,868	19,755	31,404	33,150	31,815	19,501	17,207	21,202
	V:	257.54	357.42	537.11	527.84	635.93	809.50	769.81	647.41	616.22	631.84
	$:	56.93	74.03	99.87	88.48	104.71	124.51	116.02	101.78	89.20	90.34
Others	Q:	97,145	1,14,538	1,12,841	1,09,212	1,24,947	1,13,949	1,41,442	1,72,512	1,55,487	1,42,638
	V:	1,089.67	1,488.24	1,565.78	1,623.50	2,138.94	1,817.87	2,133.59	2,780.48	3,060.53	2,756.84
	$:	242.72	314.16	292.86	272.34	351.31	279.71	320.54	434.58	442.16	397.77
Total	Q:	8,13,091	8,62,021	9,28,215	9,83,756	10,51,243	9,45,892	11,34,948	13,77,244	13,92,559	12,89,652
	V:	12,901.47	16,597.23	18,856.26	30,213.26	33,441.61	30,420.83	37,870.90	45,106.89	46,589.37	46,662.86
	$:	2,856.92	3,508.45	3,511.67	5,007.70	5,511.12	4,687.94	5,777.61	7,081.55	6,728.50	6,678.7

Q: Quantity in M T
V: Value in Rs. Crore
$: US Dollar Million
Source: Marine Products Export Development Authority, Kochi

Draw Aquaculture production statistics Data of India.





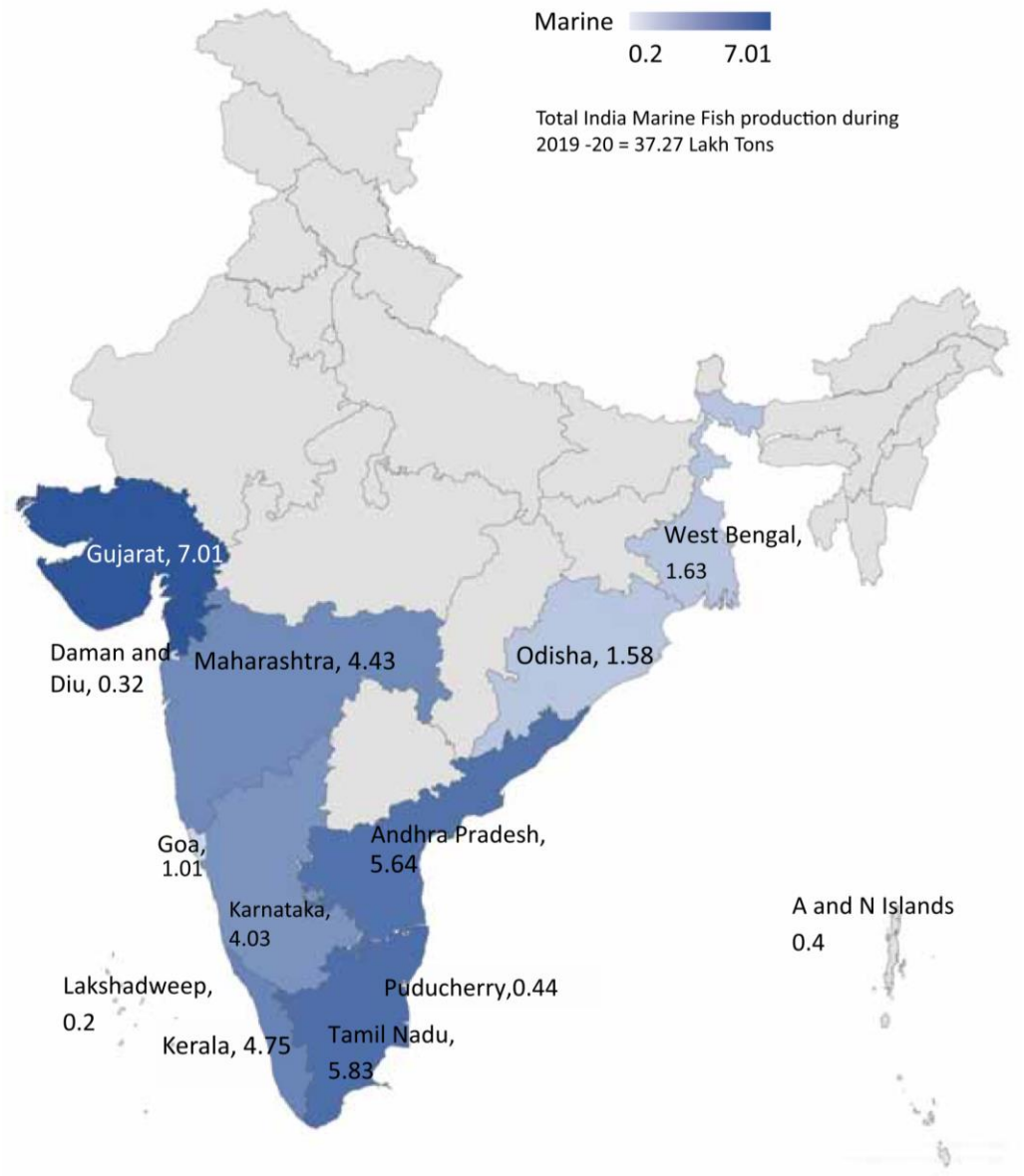


Marine Fish Production:2019-20 3.72 MMT.	Inland Fish Production: 2019-20 10.43 MMT.	Total Expenditure for development of Fisheries Sector: 2019-20 ₹ 64025.86 Lakh
Contribution of Fisheries sector in Indian Economy (GVA): 2018-19 ₹ 2,12,915 Crore	Percentage Contribution of Fisheries sector in India Economy: 2018-19 1.24%	Percentage Contribution of Economy Fisheries sector in India Agriculture Sector: 2018-19 7.28%
Total value of Fisheries Export:2019-20 ₹ 46,662.85 Crore	Total Quantity of Fisheries Export:2019-20 12,89,651 Tons	Total Quantity of world Fish Production 178.5 MMT
Consumption of Fish: 2019-20 (Top Five States) Tripura Kerala Manipur Odisha Assam	Post-Harvest Infrastructure :2019-20 Total Fish landing centres - 1548 Major Fishing Harbours commissioned-7 Minor Fishing Harbours commissioned- 62	Fisheries Welfare: 2019-20 Number of houses sanctioned to Fishermen – 4504 Number of Fishermen Insured - 3,586,721 Relief provided to Fishermen under SCR – 765,194

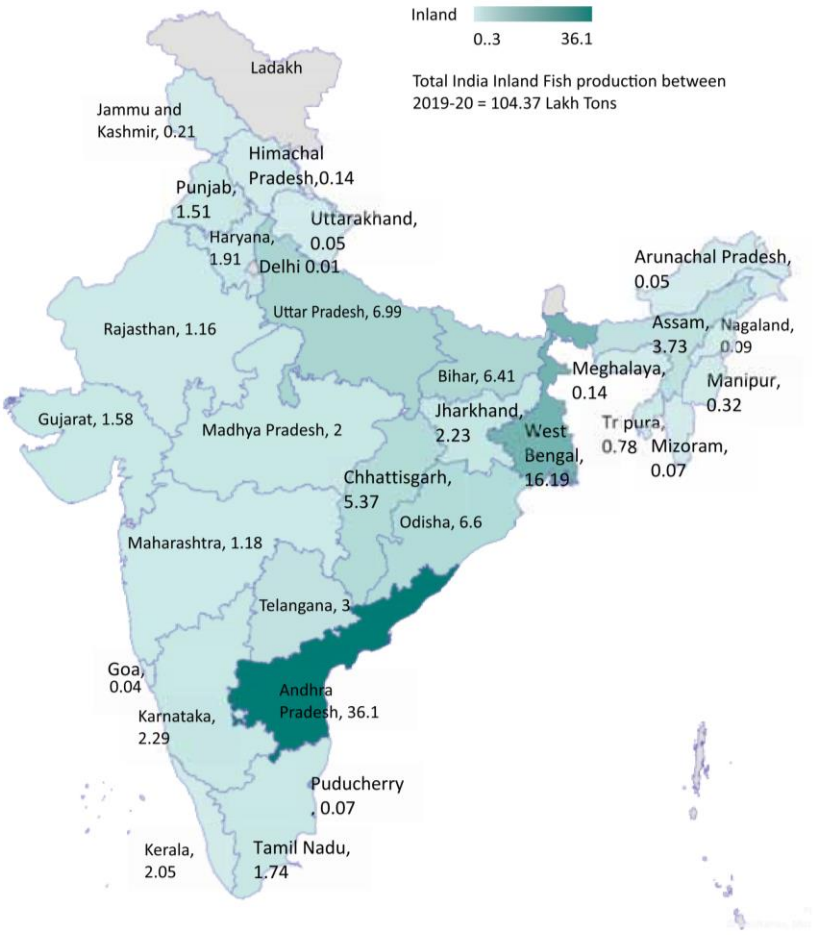
Make a Chart of the Following Data showing the Fish Production in India for the period 1980-1981 to 2019-2020

Table 1.1: Fish Production in India for the period 1980-81 to 2019-20						
Year	Fish production (In Lakh Tonnes)			Annual Average Growth Rate (Percent)		
	Marine	Inland	Total	Marine	Inland	All India
1980-81	15.55	8.87	24.42	4.22	4.6	4.36
1981-82	14.45	9.99	24.44	-7.07	12.63	0.08
1982-83	14.27	9.4	23.67	-1.25	-5.91	-3.15
1983-84	15.19	9.87	25.06	6.45	5	5.87
1984-85	16.98	11.03	28.01	11.78	11.75	11.77
1985-86	17.16	11.6	28.76	1.06	5.17	2.68
1986-87	17.13	12.29	29.42	-0.17	5.95	2.29
1987-88	16.58	13.01	29.59	-3.21	5.86	0.58
1988-89	18.17	13.35	31.52	9.59	2.61	6.52
1989-90	22.75	14.02	36.77	25.21	5.02	16.66
1990-91	23	15.36	38.36	1.1	9.56	4.32
1991-92	24.47	17.1	41.57	6.39	11.33	8.37
1992-93	25.76	17.89	43.65	5.27	4.62	5
1993-94	26.49	19.95	46.44	2.83	11.51	6.39
1994-95	26.92	20.97	47.89	1.62	5.11	3.12
1995-96	27.07	22.42	49.49	0.56	6.91	3.34
1996-97	29.67	23.81	53.48	9.6	6.2	8.06
1997-98	29.5	24.38	53.88	-0.57	2.39	0.75
1998-99	26.96	26.02	52.98	-8.61	6.73	-1.67
1999-00	28.52	28.23	56.75	5.79	8.49	7.12
2000-01	28.11	28.45	56.56	-1.44	0.78	-0.33
2001-02	28.3	31.26	59.56	0.68	9.88	5.3
2002-03	29.9	32.1	62	5.65	2.69	4.1
2003-04	29.41	34.58	63.99	-1.64	7.73	3.21
2004-05	27.79	35.26	63.05	-5.51	1.97	-1.47
2005-06	28.16	37.56	65.72	1.33	6.52	4.23
2006-07	30.24	38.45	68.69	7.39	2.37	4.52
2007-08	29.2	42.07	71.27	-3.44	9.41	3.76
2008-09	29.78	46.38	76.16	1.99	10.24	6.86
2009-10	31.04	48.94	79.98	4.23	5.52	5.02
2010-11	32.5	49.81	82.31	4.7	1.78	2.91
2011-12	33.72	52.94	86.66	3.75	6.28	5.28
2012-13	33.21	57.19	90.4	-1.51	8.03	4.32
2013-14	34.43	61.36	95.79	3.67	7.29	5.96
2014-15	35.69	66.91	102.6	3.66	9.04	7.11
2015-16	36	71.62	107.62	0.87	7.04	4.89
2016-17	36.25	78.06	114.31	1.14	8.63	6.12
2017-18	37.56	89.48	127.04	3.61	14.62	11.13
2018-19	38.53	97.2	135.73	2.58	8.62	6.84
2019-20	37.27	104.37	141.64	-3.2	7.37	4.35
Source: Department of Fisheries, States Government / UTs Administration						

Show Total Marine Fish Production Data of India (2019-2020) in Indian Map



Show Total Inland Fish Production Data of India (2019-2020) in Indian Map



Make a Chart of the Following Data showing the State-wise Fish Production in India for the period 2015-2016 to 2019-2020

Table 1.2: State-wise Fish Production, India (In Lakh Tonnes)										
	2015-16		2016-17		2017-18		2018-19		2019-20	
State/UT's	Inland	Marine	Inland	Marine	Inland	Marine	Inland	Marine	Inland	Marine
Andhra Pradesh	18.32	5.2	21.86	5.8	28.45	6.05	33.91	6	36.1	5.64
Arunachal Pradesh	0.04	0	0.04	0	0.04	0	0.05	0	0.05	0
Assam	2.94	0	3.07	0	3.27	0	3.31	0	3.73	0
Bihar	5.07	0	5.09	0	5.88	0	6.02	0	6.41	0
Chhattisgarh	3.42	0	3.77	0	4.57	0	4.89	0	5.72	0
Goa	0.05	1.07	0.04	1.14	0.06	1.18	0.05	1.15	0.04	1.01
Gujarat	1.12	6.97	1.17	6.99	1.38	7.01	1.42	6.99	1.58	7.01
Haryana	1.21	0	1.44	0	1.9	0	1.8	0	1.91	0
Himachal Pradesh	0.12	0	0.13	0	0.13	0	0.13	0	0.14	0
Jharkhand	1.16	0	1.45	0	1.9	0	2.08	0	2.23	0
Karnataka	1.69	4.12	1.59	3.99	1.88	4.14	1.98	3.9	2.29	4.03
Kerala	2.11	5.17	1.61	4.31	1.89	4.84	1.92	6.09	2.05	4.75
Madhya Pradesh	1.15	0	1.39	0	1.43	0	1.73	0	2	0
Maharashtra	1.46	4.34	2	4.63	1.31	4.75	1	4.68	1.18	4.43
Manipur	0.32	0	0.32	0	0.33	0	0.32	0	0.32	0
Meghalaya	0.11	0	0.12	0	0.12	0	0.13	0	0.14	0
Mizoram	0.07	0	0.08	0	0.08	0	0.07	0	0.07	0
Nagaland	0.08	0	0.09	0	0.09	0	0.09	0	0.09	0
Odisha	3.77	1.45	4.55	1.53	5.34	1.51	6	1.59	6.6	1.58
Punjab	1.2	0	1.33	0	1.37	0	1.35	0	1.51	0
Rajasthan	0.42	0	0.5	0	0.54	0	0.55	0	1.16	0
Sikkim	0	0	0	0	0	0	0	0	0	0
Tamil Nadu	2.43	4.67	1.97	4.72	1.85	4.97	1.7	5.2	1.74	5.83
Telangana	2.37	0	1.99	0	2.7	0	2.84	0	3	0
Tripura	0.69	0	0.72	0	0.77	0	0.7	0	0.78	0
Uttar Pradesh	5.05	0	6.18	0	6.29	0	6.62	0	6.99	0
Uttarakhand	0.04	0	0.04	0	0.05	0	0.05	0	0.05	0
West Bengal	14.93	1.78	15.25	1.77	15.57	1.85	16.19	1.63	16.19	1.63
A and N Islands	0	0.37	0	0.39	0	0.39	0	0.4	0	0.4
Chandigarh	0	0	0	0	0	0	0.01	0	0.01	0
D & Nagar Haveli and Daman and Diu	0	0.23	0.01	0.23	0	0.24	0	0.28	0	0.32
Delhi	0.01	0	0.01	0	0.01	0	0	0	0.01	0
Jammu & Kashmir	0.2	0	0.2	0	0.21	0	0.21	0	0.21	0
Ladakh	-	-	-	-	-	-	-	-	0	0
Lakshadweep	0	0.16	0	0.3	0	0.21	0	0.22	0	0.2
Puducherry	0.07	0.47	0.04	0.46	0.07	0.42	0.07	0.4	0.07	0.44
India	71.62	36	78.06	36.25	89.48	37.56	97.2	38.53	104.37	37.27
Source: Department of Fisheries, States Government / UTs Administration										

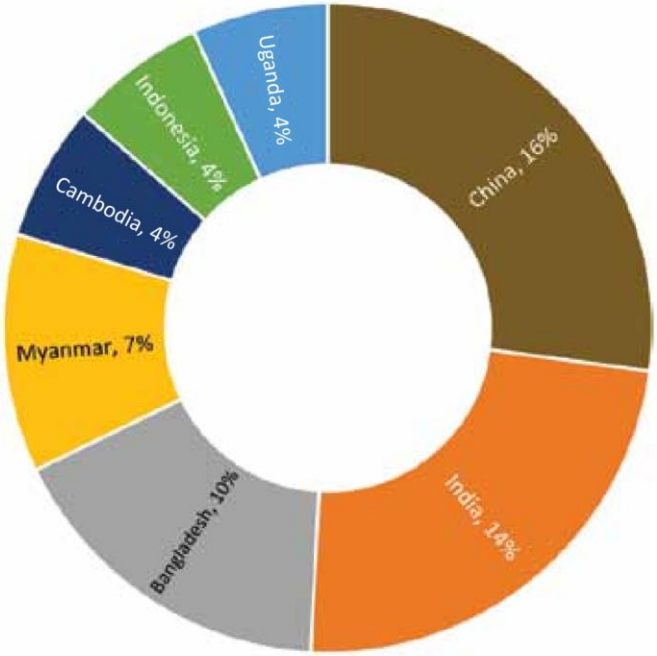
Make a Chart of the Following Data showing the State-wise Fish Consumption Data in India

Table 3.1 State-wise Fish Consumption Data (Per Capita/Year/Kg): 2019-20		
S. N.	States/UT's	Yearly Fish Consumption (Per Capita/Kg.)2019-20
1	Andhra Pradesh	8.07
2	Arunachal Pradesh	3.52
3	Assam	11.72
4	Bihar	8.82
5	Chhattisgarh	4.66
6	Goa	NA
7	Gujarat	9.55
8	Haryana*	NA
9	Himachal Pradesh	2.16
10	Jharkhand	10.32
11	Karnataka	7.56
12	Kerala	19.41
13	Madhya Pradesh	2.76
14	Maharashtra	3.02
15	Manipur	14.1
16	Meghalaya	10.98
17	Mizoram	5.54
18	Nagaland	6.68
19	Odisha	13.79
20	Punjab	0.4
21	Rajasthan	0.01
22	Sikkim	1.16
23	Tamil Nadu	9.6
24	Telangana	8.87
25	Tripura	29.29
26	Uttarakhand	0.49
27	Uttar Pradesh	10.89
28	West Bengal	NA
29	A and N Islands	59.47
30	Chandigarh	NA
31	Daman and Diu, D & Nagar Haveli	NA
32	Delhi	NA
33	Jammu & Kashmir	3
34	Ladakh	NA
35	Lakshadweep	NA
36	Puducherry	30
Source: Department of Fisheries, States Government / UTs Administration		

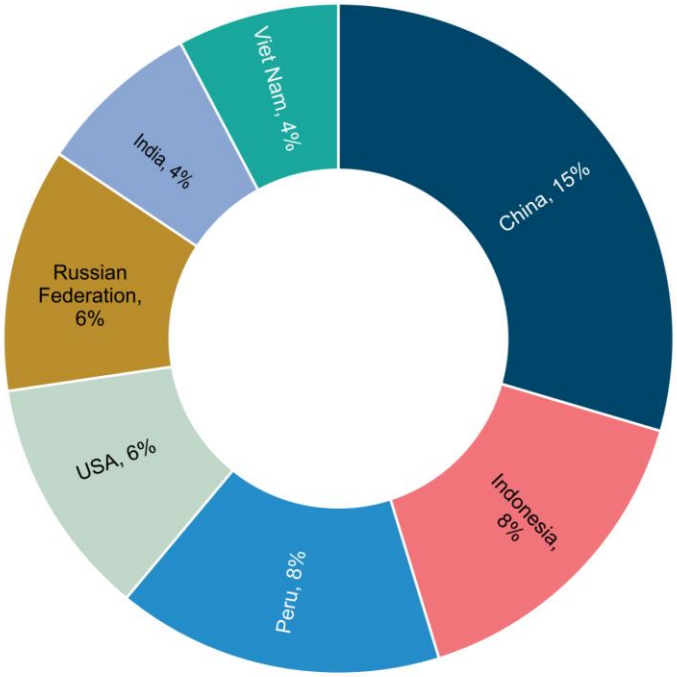
Make a Chart of the Following Data showing Inland Water Capture Production: Major Producing Countries

Table 12.5: Inland Waters Capture Production: Major Producing Countries					
Country	Production				Percentage of total 2018
	2015	2016	2017	2018	
	(million tonnes, live weight)				
Bangladesh	0.102	1.05	1.16	1.22	10
Brazil	0.23	0.22	0.22	0.22	1
Cambodia	0.49	0.51	0.53	0.54	4
Chad	0.1	0.11	0.11	0.11	1
China	1.99	2	2.18	1.96	16
Democratic Republic of the Congo	0.23	0.23	0.23	0.23	2
Egypt	0.24	0.23	0.26	0.27	2
Ghana	0.09	0.09	0.09	0.09	1
India	1.35	1.46	1.59	1.7	14
Indonesia	0.47	0.43	0.43	0.51	4
Iran (Islamic Republic of)	0.09	0.09	0.1	0.11	1
Kenya	0.16	0.13	0.1	0.1	1
Malawi	0.14	0.15	0.2	0.22	1
Mali	0.09	0.1	0.11	0.09	1
Mexico	0.15	0.2	0.17	0.22	1
Mozambique	0.09	0.1	0.1	0.1	1
Myanmar	0.86	0.89	0.89	0.89	7
Nigeria	0.34	0.38	0.42	0.39	3
Pakistan	0.13	0.14	0.14	0.14	1
Philippines	0.2	0.16	0.16	0.16	1
Russian federation	0.29	0.29	0.27	0.27	2
Thailand	0.18	0.19	0.19	0.2	1
Uganda	0.4	0.39	0.39	0.44	4
United Republic of Tanzania	0.31	0.31	0.33	0.31	3
Viet Nam	0.15	0.15	0.16	0.16	1
Total 25 major producers	9.79	10.01	10.53	10.64	89
Total all other producers	1.39	1.36	1.37	1.38	11
All producers	11.15	11.37	11.91	12.02	100
INLAND WATER CAPTURES, BY REGION					
Asia	7.3	7.44	7.9	7.95	66
Africa	2.84	2.87	3	3	25
Americans	0.57	0.6	0.58	0.63	5
Europe	0.43	0.44	0.41	0.14	3
Oceania	0.02	0.02	0.02	0.02	0
Others					0
World total	11.15	11.37	11.91	12.02	100
Source: SOFIA 2020. – State of Fisheries and Aquaculture in the world					

Make a Pie Chart of the Following Data showing Inland Fish Production of Major Countries



Make a Pie Chart of the Following Data showing Marine Fish Production of Major Countries



Make a Chart of the Following Data showing Marine Capture Production: Major Producing Countries

Table 12.6: Marine Capture Production: Major Producing Countries and Territories (million tonnes)					
Country or Territory	2015	2016	2017	2018	Percentage of Total 2018
Argentina	0.8	0.74	0.81	0.82	1
Canada	0.82	0.84	0.81	0.78	1
Chile	1.79	1.5	1.92	2.12	3
China	14.39	13.78	13.19	12.68	15
Denmark	0.87	0.67	0.9	0.79	1
Iceland	1.32	1.07	1.18	1.26	1
India	3.5	3.71	3.94	3.62	4
Indonesia	6.22	6.11	6.31	6.71	8
Iran (Islamic Republic of)	0.54	0.59	0.69	0.72	1
Japan	3.37	3.17	3.18	3.1	4
Malaysia	1.49	1.57	1.47	1.45	2
Mauritania	0.39	0.59	0.78	0.95	1
Mexico	1.32	1.31	1.46	1.47	2
Morocco	1.35	1.43	1.36	1.36	2
Myanmar	1.11	1.19	1.27	1.14	1
Norway	2.29	2.03	2.38	2.49	3
Peru	4.79	3.77	4.13	7.15	8
Philippines	1.95	1.87	1.72	1.89	2
Republic of Korea	1.64	1.35	1.35	1.33	2
Russian Federation	4.17	4.47	4.59	4.84	6
Spain	0.97	0.91	0.94	0.92	1
Taiwan Province of China	0.99	0.75	0.75	0.81	1
Thailand	1.32	1.34	1.31	1.51	2
United States of America	5.02	4.88	5.02	4.72	6
Vietnam	2.71	2.93	3.15	3.19	4
Total 25 major producers	65.11	62.58	64.6	67.83	80
Total all other producers	15.39	15.69	16.61	16.58	20
World total	80.51	78.27	81.21	84.41	100
Source: SOFIA 2020. – State of Fisheries and Aquaculture in the world					

Course: BFSC-102: Meteorology, Climatology and Geography

Graphic representation of structure of atmosphere

Physical layering

Compositional layering:

The atmosphere layer closest to the earth is referred to as the troposphere. Beyond the troposphere are the stratosphere, the ozone layer, the mesosphere, and the thermosphere. The atmosphere is made up of 78% nitrogen, 21% oxygen, and smaller amounts of argon, carbon dioxide, helium, and neon.

There are five layers in the structure of the atmosphere depending upon temperature. These layers are:

- Troposphere
- Stratosphere
- Mesosphere
- Thermosphere
- Exosphere

Troposphere

- It is considered as the lowest layer of Earth's atmosphere.
- The troposphere starts at the earth's surface and goes up from a height of 8 kms (poles) to 18 kms (equator). The main reason for higher height at the equator is the presence of hot convection currents that push the gases upward.
- All kinds of weather changes occurs within this layer.
- This layer has water vapor and mature particles.
- Temperature decreases with increasing height of atmosphere at the rate of 1 degree Celsius for every 165 m of height. This is called Normal lapse rate.
- Tropopause, the transitional zone, separates Troposphere and Stratosphere.

Stratosphere

- It is the second layer of the atmosphere found above the troposphere.
- It extends up to a height of 50 km from the earth's surface.
- This layer is very dry as it contains little water vapour.
- This layer provides some advantages for flight because it is above stormy weather and has steady, strong, horizontal winds.
- The ozone layer is found in this layer.

- The ozone layer absorbs UV rays and safeguards earth from harmful radiation.
- Stratopause separates Stratosphere and Mesosphere.

Mesosphere

- The Mesosphere is found above the stratosphere.
- It is the coldest of the atmospheric layers.
- The mesosphere starts at 50 km above the surface of the Earth and goes up to 80 km.
- The temperature drops with altitude in this layer.
- By 80 km it reaches -100 degrees Celsius.
- Meteors burn up in this layer.
- The upper limit is called Mesopause which separates Mesosphere and Thermosphere.

Thermosphere

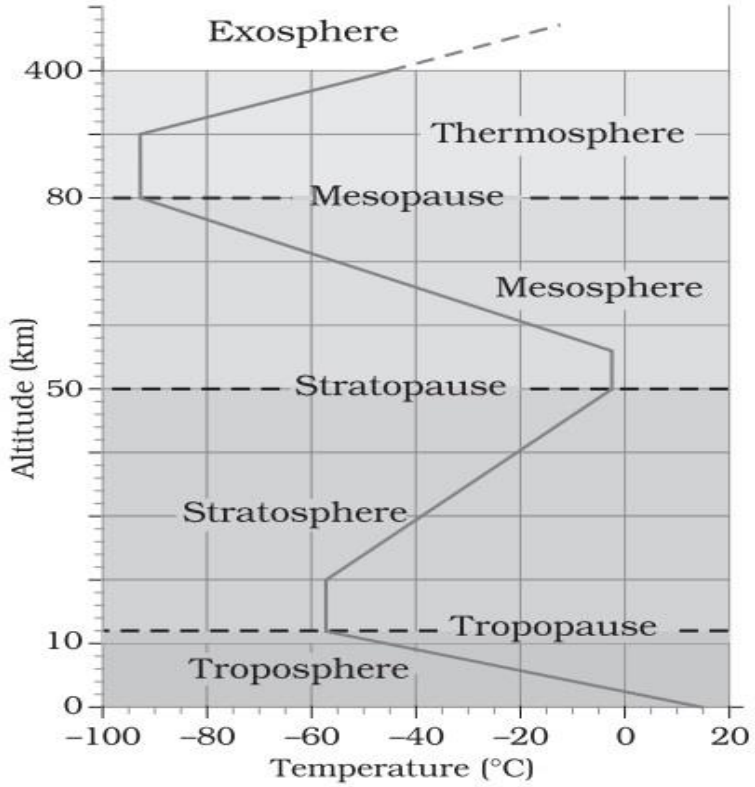
- This layer is found above Mesopause from 80 to 400 km.
- Radio waves that are transmitted from the earth are reflected by this layer.
- The temperature starts increasing again with increasing height in this layer.
- Aurora and satellites occur in this layer.

Ionosphere

- The lower Thermosphere is called the Ionosphere.
- The ionosphere consists of electrically charged particles known as ions.
- This layer is defined as the layer of the atmosphere of Earth that is ionized by cosmic and solar radiation.
- It is positioned between 80 and 400 km above the Mesopause.

Exosphere

- It is the outermost layer of the atmosphere.
- The zone where molecules and atoms escape into space is mentioned as the exosphere.
- It extends from the top of the thermosphere up to 10,000 km.



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Temperature instrument

A thermometer (thermos: hot; matron: measure) is the universal instrument used to measure temperature.

What is simple thermometer?

A thermometer is an instrument for measuring or showing temperature (how hot or cold something is).

Types of Thermometer

Following are the different types of thermometers that we use on a daily basis:

- Clinical thermometer /medical thermometer
- Laboratory thermometer
- Digital thermometer
- Infrared ear thermometer
- Mercury thermometer
- Alcohol in glass thermometer
- Constant pressure gas thermometer

- Constant volume thermometer
- Platinum resistance thermometer
- Thermocouple thermometer
- Pyrometer thermometer

Simple thermometer

A thermometer is a tool that measures temperature — how hot or cold something is. the main uses of the thermometer is *to measure the temperature of an object, environment and atmosphere*.

Use of a simple thermometer:

Use a simple thermometer to get an approximate temperature. Simple thermometers use a glass tube filled with liquid to measure the temperature. As the air around the thermometer gets warmer, the liquid moves up the tube and can be used to measure the temperature closely.

Six's maximum and minimum thermometer:

Six's maximum and the minimum thermometer is used for measuring the day's maximum and minimum temperature. It was invented by Englishman James Six in 1782.

Construction:

It consists of a U-shaped capillary tube with bulbs at both ends. The bend of the U-tube is filled with mercury. One of the bulbs is completely filled with alcohol and the other bulb is partly filled with alcohol. The space in the bulb partly filled with alcohol is initially a vacuum. The thermometric liquid is alcohol, whose expansion and contraction allow the change in temperature to be recorded. Just above the mercury are two dumbbell-shaped steel indices, which are kept in place by tiny steel springs. The two dumbbell-shaped steel indices are reset to their initial positions just above the mercury by means of a horse-shoe magnet.

Working

Maximum Temperature:

Due to a rise in the temperature during the day, the alcohol in the completely-filled bulb expands and exerts a pressure on the mercury. The mercury moves toward the partly-filled bulb and pushes up the index in the capillary closer to the partly-filled bulb. Thus, the index closer to the partly-filled bulb records the maximum temperature.

Minimum Temperature:

Due to a fall in the temperature during the night, the alcohol in the completely-filled bulb contracts. The mercury moves toward the completely-filled bulb and pushes up the index in the capillary closer to the completely-filled bulb. Thus, the index closer to the completely-filled bulb records the minimum temperature.

What is humidity?

Easily stated humidity is simply the amount of water vapour held in the air. Water vapour is the gaseous state of water. As the temperature of the air increases more water vapour can be held since the movement of molecules at higher temperatures prevents condensation from occurring.

There are three main measurements of humidity: relative, absolute and specific.

Absolute humidity (units are grams of water vapour per cubic meter volume of air) is a measure of the actual amount of water vapour in the air, regardless of the air's temperature. The higher the amount of water vapor, the higher the absolute humidity. For example, a

maximum of about 30 grams of water vapor can exist in a cubic meter volume of air with a temperature in the middle 80s.

Relative humidity, expressed as a percent, is a measure of the amount of water vapor that air is holding compared the amount it can hold at a specific temperature. Warm air can possess more water vapor (moisture) than cold air, so with the same amount of absolute/specific humidity, air will have a higher relative humidity. A relative humidity of 50% means the air holds on that day (specific temperature) holds 50% of water needed for the air to be saturated. Saturated air has a relative humidity of 100%.

The relative humidity of an air-water mixture is also defined as the ratio of the partial pressure of water vapor in the mixture to the saturated vapor pressure of water at a given temperature (See what is vapor pressure). *Thus the relative humidity of air is a function of both water content and temperature.*

Specific humidity refers to the weight of water vapor contained in a unit weight (amount) of air (expressed as grams of water vapor per kilogram of air). Absolute and specific humidity are quite similar in concept.

What is dew point?

Dew Point is the temperature at which air is saturated with water and condensation begins. The higher the dew point rises, the greater the amount of moisture in the air.

What is the relationship between Dew Point and Relative Humidity?

Compared to relative humidity, dew point is frequently cited as a more accurate way of measuring the humidity and comfort of the air, since it is an absolute measurement (unlike relative humidity).

The relative humidity is 100 percent when the dew point and the temperature are the same. If the temperature drops any further, condensation will result, and liquid water will begin to form.

If the relative humidity is 100 percent (i.e., dew point temperature and actual air temperature are the same), this does not necessarily mean that precipitation will occur. It simply means that the maximum amount of moisture is in the air at the particular temperature the air is at.

Saturation may result in fog on the ground and clouds aloft (which consist of tiny water droplets suspended in the air).

While dew point gives one a quick idea of moisture content in the air, relative humidity does not since the humidity is relative to the air temperature. In other words, relative humidity cannot be determined from knowing the dew point alone, the actual air temperature must also be known.

Relative humidity is also approximately the ratio of the actual to the saturation vapor pressure.

RH = (Actual Vapor Pressure) / (Saturation Vapor Pressure) X 100%

Where actual vapor pressure is a measurement of the amount of water vapor in a volume of air and increases as the amount of water vapor increases.

Saturated vapor pressure is the maximum VP that can exist at any given temperature.

Air which is at 100% relative humidity (RH) contains water vapor whose VP is its SVP at the given temperature. This corresponds to air which is in equilibrium with liquid water. RH is the ratio VP/SVP expressed as a percentage. 'Dry' air will contain water vapor with a VP which is less than the SVP at the given temperature.

How is humidity measured?

A device to measure relative humidity is called a hygrometer. The simplest hygrometer - a sling psychrometer - consists of two thermometers mounted together with a handle attached on a chain. One thermometer is ordinary. The other has a cloth wick over its bulb and is called a wet-bulb thermometer.

How does a psychomotor measure relative humidity?

A psychrometer also called a sling psychrometer has two thermometers attached. One is dry (often called the dry bulb thermometer) and measures the actual air temperature. The other called the wet bulb thermometer has a wet cloth at the tip. As water molecules evaporate from the surface of the wet bulb they will take heat with them lowering the reading on the thermometer. The rate of evaporation depends on the vapor pressure or amount of water vapor in the air. At 100% relative humidity no water will evaporate from the wet bulb and the

readings on both thermometers will be the same. Comparing the two temperatures in a chart will give the relative humidity.

Sper Scientific Sling Psychrometer to Measure Humidity -- Top is wet bulb -- Bottom is dry bulb

For a Table of Relative Humidity, %---Difference between Readings of Wet & Dry Bulbs

Condensation

Condensation is the process where water vapor becomes liquid. It is the reverse of evaporation, where liquid water becomes a vapor.

Condensation happens one of two ways: Either the air is cooled to its dew point or it becomes so saturated with water vapor that it cannot hold any more water.

Condensation occurs when water vapour (gaseous form) in the air changes into liquid water when it comes in contact with a cooler surface. When the water in the air comes in contact with a cold surface, it condenses to form water droplets.

Basic process of condensation

Condensation of water happens when water changes its phase from gaseous state to liquid or crystal shape. At high pressure and low temperature, any gas can condense. Technically, the process of condensation can happen at any temperature as long as the pressure of the liquid state of the gas is less than the pressure of the condensing gas. The molecules in the matter

slow down during the process of condensation because the heat energy is taken away, which causes a change within the three states of matter, that is it changes the matter into the solid-state.

Condensation – water cycle

- Condensation is important to the water cycle as it is responsible for the formation of clouds.
- Water vapour present in the air is responsible for the formation of clouds which ultimately comes down in the form of rain.
- This phase change of water between solid, liquid and gas is because of the movement of water molecules.
- In vapour form, water molecules are arranged randomly as compared to the liquid state.
- As condensation happens, water molecules become more organized and as a result, heat is released into the atmosphere leading to a change of phase from the vapour state to the liquid state.
- This generally occurs in the atmosphere when warm air rises up and cools down.

For condensation to take place, it is very important that the atmosphere is fully saturated (to reach maximum vapour pressure). Usually, condensation takes place around dust particles or smoke or microscopic bacteria. It plays a very significant role in the water cycle and thus helps in maintaining the water balance in the environment. It is also used in various industrial processes by the scientists and engineers for separating mixtures and manufacturing pure substances.

Clouds

Clouds are classified according to their height above and appearance (texture) from the ground.

The following cloud roots and translations summarize the components of this classification system:

1) Cirro-: curl of hair, high.
2) Alto-: mid.
3) Strato-: layer.
4) Nimbo-: rain, precipitation.
5) Cumulo-: heap.

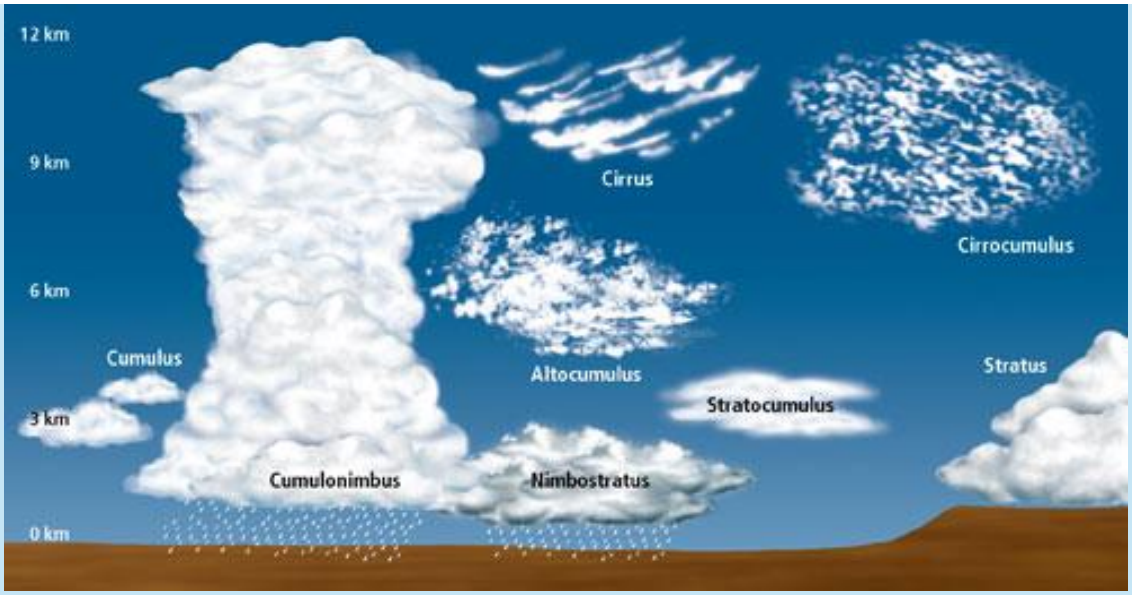


Figure from: www.jason.org/digital_library/201.aspx (defunct)

{Refer to the chart below for examples of the various types of clouds.}

High-level clouds:

High-level clouds occur above about 20,000 feet and are given the prefix "cirro-". Due to cold tropospheric temperatures at these levels, the clouds primarily are composed of ice crystals, and often appear thin, streaky, and white (although a low sun angle, e.g., near sunset, can create an array of color on the clouds).

The three main types of high clouds are **cirrus, cirrostratus, and cirrocumulus.**

Cirrus clouds are wispy, feathery, and composed entirely of ice crystals. They often are the first sign of an approaching warm front or upper-level jet streak.

Unlike cirrus, **cirrostratus clouds** form more of a widespread, veil-like layer (similar to what stratus clouds do in low levels). When sunlight or moonlight passes through the hexagonal-shaped ice crystals of cirrostratus clouds, the light is dispersed or refracted (similar to light passing through a prism) in such a way that a familiar ring or halo may form. As a warm front approaches, cirrus clouds tend to thicken into cirrostratus, which may, in turn, thicken and lower into altostratus, stratus, and even nimbostratus.

Finally, **cirrocumulus clouds** are layered clouds permeated with small cumuliform lumpiness. They also may line up in streets or rows of clouds across the sky denoting localized areas of ascent (cloud axes) and descent (cloud-free channels).

Mid-level clouds:

The bases of clouds in the middle level of the troposphere, given the prefix "alto-", appear between 6,500 and 20,000 feet. Depending on the altitude, time of year, and vertical temperature structure of the troposphere, these clouds may be composed of liquid water droplets, ice crystals, or a combination of the two, including super cooled droplets (i.e., liquid droplets whose temperatures are below freezing).

The two main types of mid-level clouds are altostratus and altocumulus.

Altostratus clouds are "**strato"** type clouds (see below) that possess a flat and uniform type texture in the mid-levels. They frequently indicate the approach of a warm front and may thicken and lower into stratus, then nimbostratus resulting in rain or snow. However, altostratus clouds themselves do not produce significant precipitation at the surface, although sprinkles or occasionally light showers may occur from a thick alto-stratus deck.

Altocumulus clouds exhibit "**cumulo"** type characteristics (see below) in mid-levels, i.e., heap-like clouds with convective elements. Like cirrocumulus, altocumulus may align in rows or streets of clouds, with cloud axes indicating localized areas of ascending, moist air, and clear zones between rows suggesting locally descending, drier air. Altocumulus clouds with some vertical extent may denote the presence of elevated instability, especially in the morning, which could become boundary-layer based and be released into deep convection during the afternoon or evening.

Low-level clouds:

Low-level clouds are not given a prefix, although their names are derived from "strato-" or "cumulo-", depending on their characteristics. Low clouds occur below 6500 feet, and normally consist of liquid water droplets or even super cooled droplets, except during cold winter storms when ice crystals (and snow) comprise much of the clouds.

The two main types of low clouds include stratus, which develop horizontally, and cumulus, which develop vertically.

Stratus clouds are uniform and flat, producing a gray layer of cloud cover which may be precipitation-free or may cause periods of light precipitation or drizzle. Low stratus decks are common in winter in the Ohio Valley, especially behind a storm system when cold, dismal, gray weather can linger for several hours or even a day or two.

Stratocumulus

NImbostratus

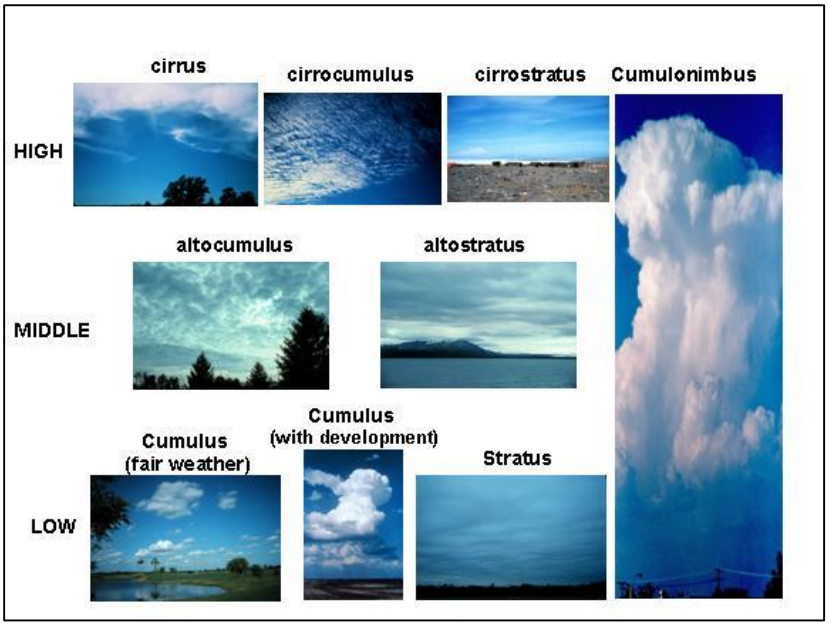
Stratocumulus clouds are hybrids of layered stratus and cellular cumulus, i.e., individual cloud elements, characteristic of cumulo type clouds, clumped together in a continuous distribution, characteristic of strato type clouds. Stratocumulus also can be thought of as a layer of cloud clumps with thick and thin areas. These clouds appear frequently in the atmosphere, either ahead of or behind a frontal system.

Nimbostratus clouds are generally thick, dense stratus or stratocumulus clouds producing steady rain or snow.

In contrast to layered, horizontal stratus, cumulus clouds are more cellular (individual) in nature, have flat bottoms and rounded tops, and grow vertically. In fact, their name depends on the degree of vertical development. For instance, scattered cumulus clouds showing little vertical growth on an otherwise sunny day used to be termed "cumulus humilis" or "fair weather cumulus," although normally they simply are referred to just as cumulus or flat cumulus

A cumulus cloud that exhibits significant vertical development (but is not yet a thunderstorm) is called cumulus congestus or towering cumulus. If enough atmospheric instability, moisture, and lift are present, then strong updrafts can develop in the cumulus cloud leading to a mature, deep cumulonimbus cloud, i.e., a thunderstorm producing heavy rain. In addition, cloud electrification occurs within cumulonimbus clouds due to many

collisions between charged water droplet, graupel (ice-water mix), and ice crystal particles, resulting in lightning and thunder.



Precipitation

Precipitation is any liquid or frozen water that forms in the atmosphere and falls back to the Earth. It comes in many forms, like rain, sleet, and snow. Along with evaporation and condensation, precipitation is one of the three major parts of the global water cycle.

Classification of precipitation

Precipitation of water vapour can be classified into different things and have different methods of formation and few of them are

1. Raindrop

When water droplets combine each other to form bigger water droplets and when water droplets freeze onto a crystal of ice, this process is known as **coalescence**. The rate of fall of small droplets is considered to be negligible, that is the reason behind the clouds not falling from the sky.

Precipitation is only possible when those will form into larger drops by coalescence with the help of turbulence in which water droplets collide, producing even larger droplets. Eventually, the droplets descend and become heavy with coalescence and resistance and finally fall as rain.

2. Snowflakes

Snow crystals form when the temperature freezes the tiny cloud droplets and because water droplets are more in number than ice crystals, the crystals can grow in size at the expense of water droplets as the water vapour causes the droplets to evaporate. These droplets fall from the atmosphere due to their mass as snowflakes.

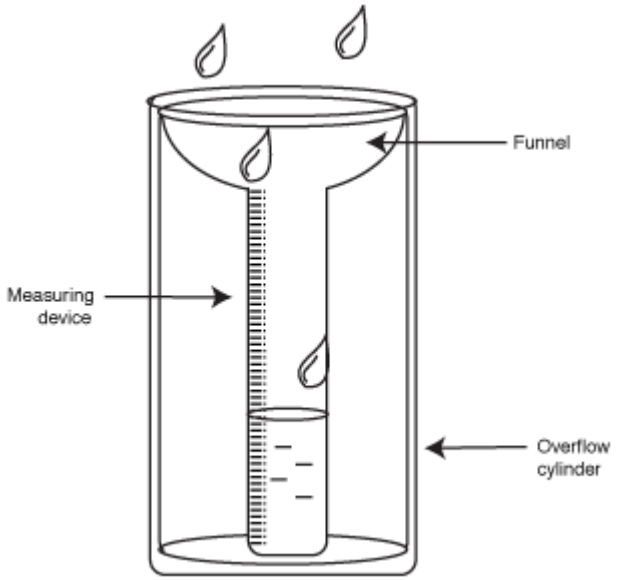
3. Hail

Like other precipitation techniques, hail forms in the storm clouds when supercooled droplets come in contact with dust and dirt. The storm's updraft blows the hailstones up and lifted again after the updraft dissipates.

In meteorology, any result of atmospheric water vapour condensation that comes under cloud gravity is precipitation. **The main types of precipitation include drizzle, rain, sleet, snow, ice pellets, grapple, and hail.** Precipitation happens when water vapour (reaching 100 percent relative humidity) saturates a portion of the atmosphere so that the water condenses and 'precipitates' or falls. Fog and mist are thus not precipitation, but colloids, since the vapour of water does not condense enough to precipitate. Two processes may contribute to air being saturated, likely working together: cooling the air or adding water vapour to the air. As smaller droplets coalesce through collision with other rain drops or ice crystals within a cloud, precipitation forms. Quick, heavy bursts of rain are called "showers" in scattered areas.

Rain gauges are thought to be the most ancient weather instruments, and they're believed to have been used in India more than 2,000 years ago. A rain gauge is really just a cylinder that catches rain. If an inch collects in the cylinder, it means an inch of rain has fallen. It's that simple. Most standard rain gauges have a wide funnel leading into the cylinder and are calibrated so that one-tenth of an inch of rain measures one inch when it collects inside. The funnel is 10 times the cross-sectional area of the tube. Rainfall as low as .01 inches can be measured with this instrument. Anything under .01 inches is considered a trace.

This standard rain gauge is shown in the following figure.



Rain gauge—rainfall measurements.

A rain gauge is an instrument that measures the amount of rainfall at a given time interval.

In the more modern era, a common rain gauge is called the tipping bucket type. A bucket doesn't really tip—a pair of small receiving funnels alternate in the collection of the rain. When one fills up with water, it tips and spills out, and the other comes into place to do the collecting. These little funnels tip each time rainfall amounts to .01 inches. The tip triggers a signal that is transmitted and recorded.

Of course, these rain gauges have a problem when the temperature drops below freezing, so the standard versions are heated for the occasion.

What about snowfall? When snow falls on these heated rain gauges, it melts, and a water equivalent is determined. The recorded precipitation is always expressed in terms of rainfall or melted snow. The snow depth doesn't count—unless, of course, you have to shovel it! Sometimes a foot of snow amounts to just a half-inch of water, other times it amounts to three inches of water. It really depends on the water equivalent of the snow, which varies widely.

On the average, 10 inches of snow is equivalent to one inch of rain, but that's only an average. If a rain gauge measures one inch of water during a snowstorm, an observer can't automatically assume that 10 inches of snow has fallen. The snow depth can only be determined the old-fashioned way—by measuring it.

That depth is determined by taking an average of three or more representative spots. A ruler is stuck into the snow, and its depth is recorded. Because of blowing and drifting, the determination of three or more representative locations is not always easy. You would think that there would be a better way, but there really isn't.

The atmospheric pressure

Definition

The atmospheric pressure is the force exerted by the weight of the Earth's atmosphere, expressed per unit area in a given horizontal cross-section. Thus, the atmospheric pressure is equal to the weight of a vertical column of air above the Earth's surface, extending to the outer limits of the atmosphere.

Units

In meteorology, atmospheric pressure is reported in hectopascals (hPa). 1 hPa is equal to 100 Pa, the pascal being the basic SI (System of International Unit) .

1 Pa is equal to 1 Newton per square meter (N/m2). And 1 hPa is equal to 1mb that was used formerly. The scales of all barometers used for meteorological purposes should be graduated in hPa. Some barometers are graduated in the unit inHg or mmHg. Under standard conditions, the pressure exerted by a pure mercury column which is 760 mm high is 1013.250 hPa, so the conversion factors are represented as follows:

1 hPa = 0.750062 mmHg; 1 mmHg = 1.333224 hPa. And because of the relation between inch and mm (1 inch = 25.4 mm), the following conversion coefficients are provided: 1 hPa = 0.029530 inHg; 1 inHg = 33.8639 hPa; 1 mmHg = 0.03937008 inHg.

Pressure data measured with the barometer should preferably be expressed in hectopascals (hPa).

Principle of Atmospheric Pressure Measurement

Mercury Barometer

(1) Principle of mercury barometer

When a one-meter long, open ended glass tube is filled with mercury and is then turned upside down into a container filled with mercury, part of the mercury flows out of the glass

tube into the container. "Torricellian vacuum" is then produced at the top of the glass tube and the mercury level stabilizes at approximately 76 cm from the mercury level in the container (See Figure 5.1). Torricelli's experiment revealed that such a height indicates the ambient atmospheric pressure. The principle of mercury barometer is to measure atmospheric pressure from precise measurement of this height.

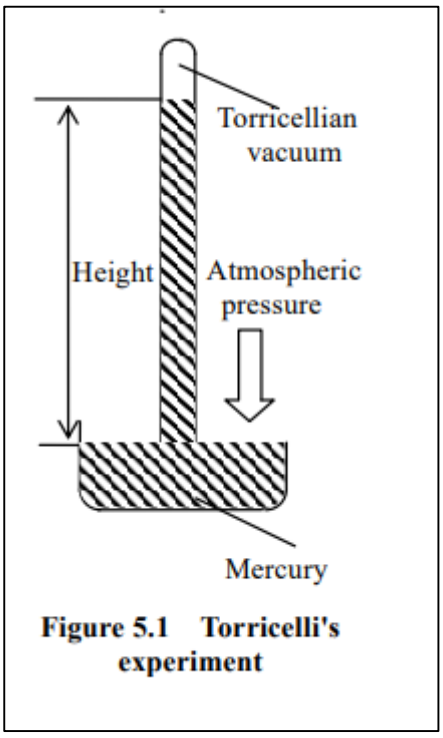


Figure 5.1 Torricelli's experiment

Figure 5.1 Torricelli's experiment Mercury Atmospheric pressure Height Torricellian vacuum 2 (2) Structure of the Fortin barometer As shown in Figure 5.2, a mercury barometer consists of three main parts: the mercury cistern (right), the glass barometer tube (center) and the scale (left). The bottom of the mercury cistern is made of a wash-leather bag (sheepskin). The mercury level can be changed by rotating an adjusting screw. The barometer tube is secured with the wash-leather bag in the upper part of the mercury cistern in order to lead atmospheric pressure from the point at the bounded leather. An ivory pointer is put on the top of the mercury cistern, whose tip indicates

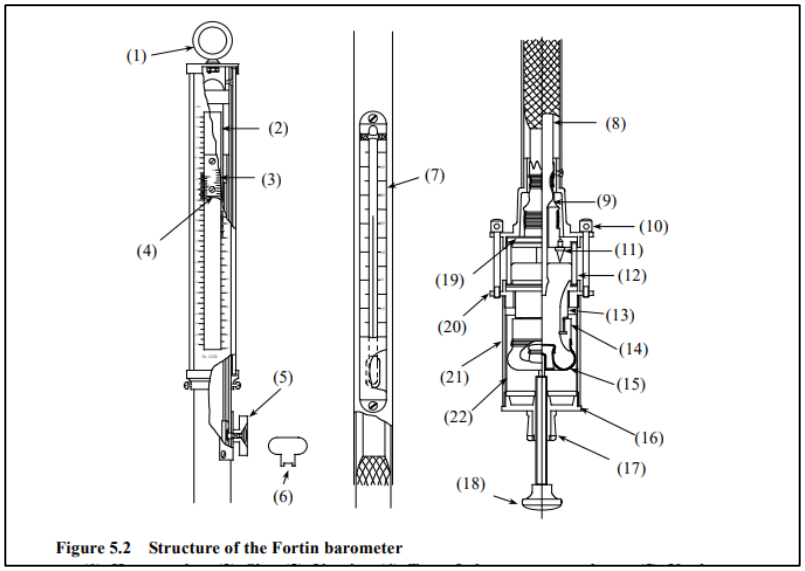


Figure 5.2 Structure of the Fortin barometer

The above Figure shows the structure of the Fortin barometer (1) Hanger ring (2) Slot (3) Vernier (4) Top of the mercury column (5) Knob (6) Pin face wrench (7) Attached thermometer (8) Barometer tube (9) Vent wash- leather (10) Three screws (11) Ivory pointer (12) Glass cylinder (13) External thread wooden frame (14) Internal thread wooden frame (15) Wash-leather bag (16) Under cover (17) Screw bridge (18) Adjusting screw (19) Wooden base for leather washer (20) Metal frame (21) Brass cover (22) Mica plate.

The zero of the scale. When the level of the mercury touches the tip, the atmospheric pressure is read at the top of the mercury column. The precise height of the mercury column is measured with the vernier. The main body has a hanger hook at its top and is used to hang the barometer from a latch on a hanger plate. The bottom is secured to the screw bridge through a vertical axis pivoting link with three screws. Both the hanger hook and the screw bridge can be rotated while the barometer is set on the hanger plate. This allows verticality checks at any time. A mica plate is wound inside the brass cylinder to prevent the direct contact between brass and the wash-leather bag. The plate serves as a heat insulator as well as prevents contamination, discoloration, and wear.

(3) Handling precautions for mercury High-purity distilled and refined mercury is used in mercury barometers. When the mercury surface oxidizes, the interface between the surface and the ivory pointer becomes unclear. Heavily contaminated mercury surface requires cleaning. Since mercury is a toxic substance, it is necessary to pay attention to the following when handling mercury. 1) A container of mercury must be sealed tightly to prevent leakage and breakage. Do not put mercury into any metal containers as mercury reacts and

amalgamates almost all metals except for iron. 2) The floor of the room where mercury is stored or used in large amounts should be shielded and laid with an impervious covering. It must not be stored together with other chemicals, especially with ammonia or acetylene. 3) Mercury has a relatively low boiling point of 357 °C, and produces dangerous poisonous gas if on fire. It must not be stored close to a heat source. 4) Check the mercury handling room and personnel periodically to make sure that the amount of mercury does not exceed the dangerous limit. (The environmental regulation on water contamination affecting personal health limits the total amount of mercury to 0.0005 mg/l.)

(4) Correction of barometer readings The mercury barometer's reading should be corrected to the one and the standard condition. Standard condition is defined as a temperature of 0 °C, where the density of mercury is 13.5951 g/cm3 and a gravity acceleration of 980.665 cm/s2 . During actual observation, the reading should be corrected for the index error, temperature correction, and gravity acceleration as follows:

(a) Corrections on index error Individual mercury barometers include index errors (difference between the value indicated by an individual instrument and that of the standard). The index error is found by comparison with the standard, and the value is stated on a "comparison certificate".

(b) Corrections for temperature The temperature correction means to correct a barometric reading, obtained at a certain temperature, to a value when mercury and graduation temperatures are 0 °C. The temperature of the attached thermometer is used for this purpose. The height of the mercury column varies with temperature, even the atmospheric pressure is unchanged. The graduation of the barometer is engraved so that the correct pressure is indicated when temperature is 0 °C. In a case that when temperature is above 0 °C, the graduation expands and the measured value will be smaller than the true value. This effect of temperature must b be corrected from these two aspects collectively. 4 Correction for the expansion and contraction of mercury is much larger than that for the expansion and contraction of the graduation. The correction value for temperature Ct is expressed as follows: where: H hPa is the barometric reading after the correction for index error. t °C is the temperature indicated by the attached thermometer. μ is the volume expansion coefficient of mercury. λ is the linear expansion coefficient of the tube. There is a small difference in absolute values for correction between temperatures below and above 0 °C. The values for correction at temperatures above 0 °C are negative and those below 0 °C are positive.

(c) Corrections for gravity Gravity affects the height of the mercury column. After the corrections for index error and temperature, the reading under the local acceleration of gravity has to be reduced to the one under the standard gravity acceleration. This is called corrections for gravity. The gravity value for correction Cg is derived by: where: g0 is the standard gravity acceleration. g is the gravity acceleration at an observing point. H is the barometric reading after the index error and temperature corrections H0 is the value already corrected for gravitation. The gravity acceleration used in corrections for gravity value is calculated to the fifth decimal place, in m/s2 . When the gravity acceleration at the observing point is larger than the standard gravity acceleration, the gravity value for correction is positive. Otherwise, the value for correction is negative. To use a barometer for regular observations at a particular location, a synthesis correction table that summarizes values for correction for index error, temperature and gravity should be used.

Aneroid barometer

The aneroid barometer does not use any liquid. It uses changes in shape of an evacuated metal box to measure variations in atmospheric pressure. It has many advantages. It is light and portable, does not have any liquid, can be fixed in any plane, and is calibrated to read pressure directly.

Aneroid barometer mechanism:

A metal cell which contains a small amount of air or a series of these cells combined together operate the aneroid barometer. When the air pressure increases, the sides of these cells come together quickly. The base of the instrument secures one side.

After that, the other side joins the structure of pulleys and levers to a rotating pointer, which moves over a scale on the face of the instrument. This pointer is generally found in black colour.

Moreover, the aneroid barometer comprises of a small capsule which has quite flexible sides. The air has been pumped out of this capsule. Therefore, whenever there is a change in the pressure, the thickness of this capsule changes.

Similarly, whenever there is an increase in the pressure of the atmosphere, the sides of the capsule become compressed. This capsule attaches to a lever which keeps moving the needle as and when the air pressure keeps squeezing the capsule.

This results in registering a higher reading on the scale. These changes are thus magnified by levers and are causes the pointer to move on a dial which is behind the needle. Finally, you will have your weather forecast on a digital read-out device.

Uses of aneroid barometer

When we compare the aneroid barometer to a mercury one, we see it has many benefits over it. In other words, it is definitely superior to it.

The first one has to be quick and easy handling. As you know, mercury is quite poisonous, so there is always a risk with it. Thus, it needs special and cautious handling. However, an aneroid barometer does not have mercury, so one can handle it easily.

Moreover, we can carry an aneroid barometer from one place to another easily without any worry of harm to the device.

That is not the case with a mercury barometer. Similarly, an aneroid barometer is smaller and lighter in comparison to a mercury one, so one can transport it in a car or ship or whatever.

Furthermore, we can easily fit it into an aircraft which will help in sustaining the variations and rolls of the aircraft. Whereas, a mercury barometer won't be able to fit in like this.

Isobar

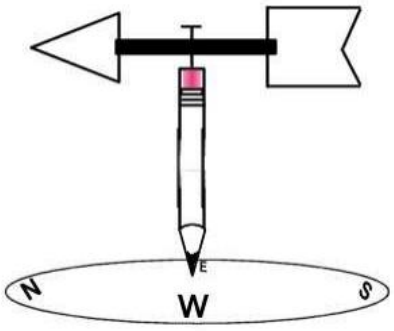
Isobar is an imaginary line on a weather map of constant barometric pressure drawn on a given reference surface. The isobaric pattern on a constant-height surface is extremely useful in weather forecasting because of the close association between pressure and weather. The spacing of isobars indicates the pressure gradient. The close spacing of isobars express the high pressure gradient and wide spacing of isobar express pressure gradient.

Wind observation and measurement:

Wind vane- Cup anemometer Meteorological requirements Wind observations or measurements are required for weather monitoring and forecasting, for wind-load climatology, for the probability of wind damage and estimation of wind energy, and as part of the estimation of surface fluxes, for example, evaporation for air pollution dispersion and agricultural applications.

Wind instruments

Wind Vane: A very old, yet reliable, weather instrument for determining wind direction is the wind vane. Most wind vanes consist of a long arrow with a tail, which is allowed to move freely about a vertical post. The arrow always points into the wind and, hence, always gives the wind direction. Wind vanes can be made of almost any material. At airports, a cone-shaped bag opened at both ends so that it extends horizontally as the wind blows through it sits near the runway. This form of wind vane, called a wind sock, enables pilots to tell the surface wind direction when landing.

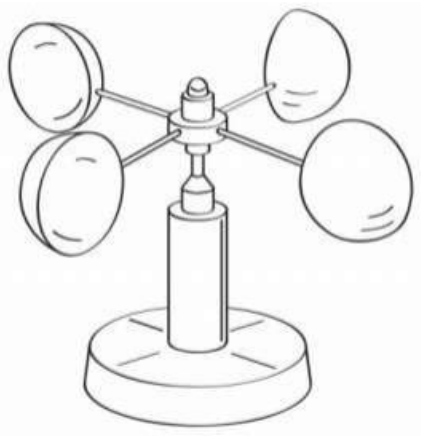


WIND VANE

Anemometer: The instrument that measures wind speed is the anemometer. Most anemometers consist of three (or more) hemispherical cups (cup anemometer) mounted on a vertical shaft.

The difference in wind pressure from one side of a cup to the other causes the cups to spin about the shaft. The rate at which they rotate is directly proportional to the speed of the wind. The spinning of the cups is usually translated into wind speed through a system of gears, and may be read from a dial or transmitted to a recorder.

Anemometer



The wind-measuring instruments described thus far are “ground-based” and only give wind speed or direction at a particular fixed location. But the wind is influenced by local conditions, such as buildings, trees, and so on. Also, wind speed normally increases rapidly with height above the ground. Thus, wind instruments should be exposed to freely flowing air well above the roofs of buildings. In practice, unfortunately, anemometers are placed at various levels; the result, then, is often erratic wind observations.

Earth

Earth is not a perfect sphere. It is an **oblate spheroid. It’s** flattened at the poles and bulges at the equator. The Earth is 12,756km at the equator and 12,714km from pole to pole. We round this up to 13,000km.

Earth, with an average distance of 92,955,820 miles (149,597,890 km) from the sun, is the third planet and one of the most unique planets in the solar system. It formed around 4.5 to 4.6 billion years ago and is the only planet known to sustain life. This is because of factors like its atmospheric composition and physical properties such as the presence of water over 70.8% of the planet allow life to thrive.

Earth is also unique however because it is the largest of the terrestrial planets (one that have a thin layer of rocks on the surface as opposed to those that are mostly made up of gases like

Jupiter or Saturn) based on its mass, density, and diameter. Earth is also the fifth largest planet in the entire solar system.

Earth's Size

As the largest of the terrestrial planets, Earth has an estimated mass of 5.9736×10^{24} kg. Its volume is also the largest of these planets at $108.321 \times 10^{10} km^3$.

In addition, Earth is the densest of the terrestrial planets as it is made up of a crust, mantle, and core. The Earth's crust is the thinnest of these layers while the mantle comprises 84% of Earth's volume and extends 1,800 miles (2,900 km) below the surface. What makes Earth the densest of these planets, however, is its core. It is the only terrestrial planet with a liquid outer core that surrounds a solid, dense inner core. Earth's average density is 5515×10 kg/m^3. Mars, the smallest of the terrestrial planets by density, is only around 70% as dense as Earth.

Earth is classified as the largest of the terrestrial planets based on its circumference and diameter as well. At the equator, Earth's circumference is 24,901.55 miles (40,075.16 km). It is slightly smaller between the North and South poles at 24,859.82 miles (40,008 km). Earth's diameter at the poles is 7,899.80 miles (12,713.5 km) while it is 7,926.28 miles (12,756.1 km) at the equator. For comparison, the largest planet in Earth's solar system, Jupiter, has a diameter of 88,846 miles (142,984 km).

Earth's Shape

Earth's circumference and diameter differ because its shape is classified as an oblate spheroid or ellipsoid, instead of a true sphere. This means that instead of being of equal circumference in all areas, the poles are squished, resulting in a bulge at the equator, and thus a larger circumference and diameter there.

The equatorial bulge at Earth's equator is measured at 26.5 miles (42.72 km) and is caused by the planet's rotation and gravity. Gravity itself causes planets and other celestial bodies to contract and form a sphere. This is because it pulls all the mass of an object as close to the center of gravity (the Earth's core in this case) as possible.

Because Earth rotates, this sphere is distorted by the centrifugal force. This is the force that causes objects to move outward away from the center of gravity. Therefore, as the Earth

rotates, centrifugal force is greatest at the equator so it causes a slight outward bulge there, giving that region a larger circumference and diameter.

Local topography also plays a role in the Earth's shape, but on a global scale, its role is very small. The largest differences in local topography across the globe are Mount Everest, the highest point above sea level at 29,035 ft (8,850 m), and the Mariana Trench, the lowest point below sea level at 35,840 ft (10,924 m). This difference is only a matter of about 12 miles (19 km), which is quite minor overall. If the equatorial bulge is considered, the world's highest point and the place that is farthest from the Earth's center is the peak of the volcano Chimborazo in Ecuador as it is the highest peak that is nearest the equator. Its elevation is 20,561 ft (6,267 m).

Geodesy

To ensure that the Earth's size and shape are studied accurately, geodesy, a branch of science responsible for measuring the Earth's size and shape with surveys and mathematical calculations is used.

Throughout history, geodesy was a significant branch of science as early scientists and philosophers attempted to determine the Earth's shape. Aristotle is the first person credited with trying to calculate Earth's size and was, therefore, an early geodesist. The Greek philosopher Eratosthenes followed and was able to estimate the Earth's circumference at 25,000 miles, only slightly higher than today's accepted measurement.

What is Latitude & Longitude?

The geographic coordinate system is a spherical or ellipsoidal coordinate system for measuring and communicating positions directly on the Earth as latitude and longitude.

The first person to calculate the size of the earth was **Eratosthenes.** He realized that Earth can be divided into a basic grid of lines called Longitudes and Latitudes which would help in pinpointing a location.

Latitude is a geographical coordinate that specifies a point on the Earth's surface, it tells whether a point is either north or south.

Latitude:

Latitude is the angular distance of a point measured in degrees from the center of the earth on the surface of the earth.

As the earth at the poles is slightly flattened, the linear distance at the pole of a degree of latitude is slightly longer than at the equator.

For example, it is 68,704 miles at the equator (0°), 69,054 miles at 45 ° and 69,407 miles at the poles. The average of 69 miles (111 km) is taken.

Note: 1 mile = 1.607 km

Some Important Parallels of Latitudes:

There are four important parallels of latitudes besides the equator (0°), the north pole (90° N) and the south pole (90° S).

- Tropic of Cancer (23½° N) in the northern hemisphere
- Tropic of Capricorn (23½° S) in the southern hemisphere
- The arctic circle at 66½° north of the equator
- The Antarctic circle at 66½° south of the equator

Longitude:

On all latitudes between the Tropic of Cancer and the Tropic of Capricorn, the midday sun is exactly overhead at least once a year. Consequently, this area receives the maximum heat and is called the **Torrid Zone**.

On no latitude beyond the Tropic of Cancer and the Capricorn Tropic, the midday sun never shines overhead.

The angle of the rays of the sun continues to decline towards the poles. As such, the areas bounded by the Tropic of Cancer and the Arctic circle in the northern hemisphere, and the Tropic of Capricorn and the Antarctic circle in the southern hemisphere, have moderate temperatures. These are called **temperate zones**.

Areas in the northern hemisphere between the Arctic circle and the north pole and the Antarctic circle and the south pole in the southern hemisphere are very cold. It's because the sun isn't raising much above the horizon here. These are called **frigid zones**.

Longitude is an angular distance, measured in degrees along the Prime (or First) Meridian east or west equator.

Longitude on the globe is shown as a series of semi-circles running through the equator from pole to pole.

This is the Prime Meridian (0°) from which all other meridians move up from 180 ° east to west.

They have one very important function, determining local time in relation to G.M.T. or Greenwich Mean Time, sometimes called World Time.

Great circle

The circle on the surface of a sphere which lies in a plane passing through the sphere's centre. As it represents the shortest distance between any two points on a sphere, a great circle of the earth is the preferred route taken by a ship or aircraft.

Cylindrical equal area projection

The cylindrical equal area projection presents the world as a rectangle while maintaining relative areas on a map.

The projection was first described by the Swiss mathematician Johann H. Lambert in 1772. Since then, many variations appeared over the years. The projection is appropriate for large-scale mapping of the areas near the equator and generally not recommended for small-scale (world) maps.

Graticule

Cylindrical equal area is a cylindric projection. The meridians are vertical lines, parallel to each other, and equally spaced. The lines of latitude are horizontal straight lines, perpendicular to meridians, and the same length as the equator, but their spacing decreases toward the poles. Both lines form a rectangular grid where each cell covers the same area size

on a spheroid. In this projection, the poles are represented as straight lines across the top and bottom of the grid, the same length as the equator. The graticule is symmetric across the equator and the central meridian.

Distortion

Cylindrical equal area is an equal-area (equivalent) projection. The scale is correct along the standard parallels. Shape, scale, direction, angle, and distance distortion increase with the distance from the standard parallels. Shapes are distorted north-south between the standard parallels (if the equator is not used as the standard parallel) and east-west above the standard parallels. The distortion values are severe near the poles and symmetric across the equator and the central meridian.

PROPERTIES:

i. It is a non-perspective projection.
ii. The pole is represented by an arc of circle.
iii. The tangential scale is true only along the standard parallel.
iv. The tangential scale increase gradually away from the standard parallel toward the pole.
v. The radial scale decreases gradually away from the standard parallel toward the pole.
vi. Inter parallel distance gradually decrease toward pole.
vii. It is an equal area projection.
viii. It is suitable for showing the distribution of any element in the mid-latitude countries or region.

Q1. Draw the graticules of cylindrical equal area projection for the extension of 20oW-60oW & 40oN-40oS at an interval of 10o on a scale of 1:75,000,000

Solve: Calculation Part

STEP-1: Radius of the reduced earth:

R= 640,000,000/75,000,000cm

=8.53cm

STEP-2: Division along the equator for spacing the meridians:

d= (2πR × interval) ÷ 360º

=1.49cm

STEP-3: Distance of the parallel from the equator=R sinΘ

Θ (N/S)	R(cm)	R sinΘ(in cm)
10	8.53	1.48
20	8.53	2.92
30	8.53	4.27
40	8.53	5.48

LANDSCAPE

A landscape is part of the Earth's surface that can be viewed at one time from one place. It consists of the geographic features that mark, or are characteristic of, a particular area. The term comes from the Dutch word landschap, the name given to paintings of the countryside.

Terminology used in landscape

Basic terminology used in landscaping designing which is given below

1) Avenue

A wide road or pathway lined with trees on either side.

2) Buffer

The use of landscape elements to reduce or curtail view, sound or dust with plants or earth berms, wall etc.

3) Canopy/Tree canopy

The average horizontal spread of the tree, taken from dripline to dripline.

4) Climber
A woody or herbaceous plant which either clings to a wall, trellis or other structures or can be supported or trained as it grows.
5) Columnar
A slender, upright plant form.
6) Contour
The form of the land, existing or proposed; a part of the topography
7) Contour Interval
The contour line is imaginary and indicates continuous elevation above mean sea level or an assumed datum line. The difference in elevation or the vertical distance measured between consecutive contour lines.
8) Drainage
Drainage is the natural or artificial removal of surface and subsurface water from an area through use of vegetated/open channel, pipes, drain boards, chambers, etc.
9) Egress
10) Elevation
A contour line or notation of relative altitude with respect to a benchmark, useful in plotting existing or proposed feature
11) Erosion
Wearing a way of soils, rocks, sediments, etc, by gradual action of natural processes (such as water, wind and glacier).
12) Exotic- A plant that is not native to the area in which it is planted.
13) Fence - A barrier of plant or construction material used to define the boundary of an area and to restrict visual and/or physical access.
14) Foliage- The collective leaves of a plant or plants.
15) Geogrid- A deformed or non-deformed netlike polymeric material used with foundation, soil, rock, earth, or any other geotechnical engineering related material as an integral part of the human made project structure or system.
16) Geotextiles - are *permeable fabrics which, when* used in association with soil, have the ability to separate, filter, reinforce, protect, or drain.
16) Geo-Textile- Any permeable textile or fabric (natural or synthetic) used to retain or protect soil and filter and drain water as an integral part of project, structure or system such as terrace garden, etc.
17) Girth of Tree- Girth is a measurement of the circumference of the trunk of a tree, measured perpendicular to the axis of the trunk. It is measured at breast height (1.4 m above ground level).
18) Grade- The slope or lay of the land as indicated by a related series of elevations.

19) Natural Grade- Grade consisting of contours of unmodified natural landform.
20) Finished Grade- Grade accomplished after landscape features are installed and completed as shown on plan as proposed contours or spot levels.
21) Gradient- The degree of slope of a pipe invert or road or land surface.
22) Grading- The gradient is a measure of the slope height as related to the length.
23) Grasses- The slope is expressed in terms of percentage or ratio. The cutting and/or filling of earth to establish finished contours. Plants that characteristically have joint stems, sheaths and narrow blades (leaves).
24) Grass Paver- Grass paver is a permeable structural grid cellular system (concrete, HDPE or any other polymer) for containing and stabilizing gravel or turf.

25) Green Roof- A roof surface of a building that is partially or completely covered with a growing medium and vegetation. Green roofs can be deep (intensive) or shallow (extensive).
26) Green Walls- A supporting structure completely or partially covered with vegetation which is grown with soil or growing medium. It can be either free standing or part of a structure. They include climbing plants such as vines that grow directly on the wall, or walls that comprise of modular panels, containers and an integrated irrigation system.

27) Ground Cover- The planting material that forms a carpet of low height.

28) Hard Landscape- Civil work component of landscape development such as pavements, walkways, roads, retaining walls, sculptures, street amenities, fountains and other elements of the built environment.

29) Hardy Plant- Plants that can withstand harsh temperature variations, harsh wind, pollution, dust, extreme soil conditions, and can tolerate either drought or flooding

30) Heat Island Effect- A phenomenon in which air and surface temperature of an area are higher than nearby areas due to the replacement of natural land cover with pavement, building, and other infrastructure.

31) Hedge- Shrubs or trees (usually of the same species) planted closely together in a linear configuration. A hedge may be pruned to shape or allowed to grow to assume its natural shape.

32) Herb- A plant with a non-woody or fleshy structure. Certain herbs are highly useful for cooking or of high medicinal value.

33) Ingress- A way in, or entrance.

34) Invert- The lowest inside point of a pipe, culvert, or channel.

35) Irrigation- A concrete or stone or similar hard edging along a pathway, or along a road, often constructed with a channel to guide the flow of stormwater.

36) Kerb- A local atmospheric zone where the climate (temperature, humidity, wind, etc) differs from the surrounding areas.

37) Microclimate- The term may refer to areas as small as a few square metres or as large as many square kilometres.

38) Mound- A small hill or bank of earth, developed as a characteristic feature in landscape.The artificial application of water, to assist in growing and maintenance of plants.

39) Mulching- A practice of using a protective covering, usually of organic matter such as leaves, straw, placed around plants to retain moisture, improve soil conditions and prevent the growth of weeds.

40) Permeable Paving-

Paving surfaces that reduce runoff by allowing rainwater to soak through the surface into the underlying sub-base where the water is stored temporarily before allowing it to seep into the ground or flow to the drains.

41) Plants- The living beings consisting of trees, shrubs, herbs, grasses, ferns, mosses, etc, typically growing in a permanent site, absorbing water and inorganic substances through their roots, and synthesizing nutrients in their leaves through the process of photosynthesis.

42) Endemic Plant- The plant which is found only in one geographic location on earth. The species of plant which is not native to a specific location (an introduced species) and has a tendency to spread to a degree believed to cause damage to the environment.

43) Invasive Plant-

44) Native Plant- A plant indigenous to a particular locale.

45) Naturalised Plant- A plant that is established as a part of the flora of a locale other than its place of origin.

46) Screen- A vegetative or constructed hedge or fence used to block wind, undesirable views, noise, glare and the like, as part of in landscape design; also known as "screen planting" and "buffer plantation".

47) Sediment- The product of erosion processes; the solid material, both mineral and organic, that is in suspension, is being transported or has been moved from its site of origin by air, water, gravity or ice.

48) Shelterbelt- Shelterbelt is usually made up of one or more rows of trees or shrubs planted in such a manner so as to provide shelter from wind, and protect soil.

49) Shrub- A woody plant of low to medium height, deciduous or evergreen, generally having many stems.

50) Soft Landscape- The natural elements in landscape design, such as plant materials and the soil itself.

ANALYTICAL INSTRUMENTS

Analytical instruments are devices which are used to measure the physical or chemical properties of assayed substance.

Basic concepts in Instrumental analysis

Colorimetry

In colorimetry, colour reactions using appropriate reagents are made use of to measure concentration of a substance. Higher the concentration of the substance being determined, greater is the intensity of the colour. The intensity of colour is measured using an instrument called photoelectric colorimeter.

Colorimeter deals with the determination of concentration of the substance in solution by measurement of the relative absorption transmittance of light with respect to a known concentration of the substance.

About the instrument

The photoelectric colorimeter is an instrument which helps to determine the extent of absorption of light of particular wavelength through a coloured solution. It consists of

1) A light source
2) Collimating lens
3) Filter to isolate the required band of wavelength
4) Sample holder/cuvette
5) Photoelectric cell – It convert light energy into electric energy
6) Colorimeter – measures the current output

Operations

1) See that a proper filter is in position
2) Check whether the galvanometer needle is on central line of galvanometer scale. If not bring it to null position by adjusting the mechanical zero adjustment knob located on top of the instrument (without switching on the instrument)
3) Switch on the instrument (main lamp) and allow it to warm up for 30 minutes (after filling with blank or distilled water) in the holder. Clear the outside with tissue paper and place it in a sample holder.
4) Place the potentiometer needle at zero reading of the scale & see that galvanometer arresting switch (side switch) is in ON position
5) Adjust the reference knob (which adjust the slit across the reference beam) till the deflected needle is brought to null position
6) Switch off the galvanometer side switch remove the cuvette with reagent blank, clean it with distilled water & fill it (appr. 5 ml) with standard solution, wipe the outer surface & place it in the instrument
7) The galvanometer side switch is ON & needle of galvanometer deflects from central line to right
8) Rotate the potentiometer knob (located in front of the instrument) anticlockwise until the deflected needle is brought back to central line / null position of the

galvanometer. Note down the reading Switch OFF the galvanometer side switch & potentiometer knob is brought back to zero position.

9) Take further readings of all standards using above steps
10) When all the readings are over, clean the sample folder & fill it with distilled water. Before it is inserted, switch OFF the main lamp
11) Plot the readings in a graph paper & connect the points by a straight line (standard curve)
12) Find out concentration of sample from the standard curve

Flame Photometry

Flame Photometry makes use of the characteristic radiation given out by different elements when their atoms are excited in a flame. When a fine atomized spray of a solution of a compound containing the element is introduced into the flame atoms of elements absorbs thermal energy from the flame & get excited. As a result extra nuclear electrons are raised to higher energy level, as they fall back to original lower energy level energy initially absorbed given off in the form of radiation of discrete wavelength. The amount of radiation given out in this manner will be proportional to the conc. of atoms in the flame. When all other things will be equal the concentration of atoms in the flame will depend upon the conc. of elements in the solution.

In flame Photometry the characteristic radiation is isolated using a filter & intensity of isolated radiation is measured by a suitable mechanism such as photoelectric cell & galvanometer.

Essential parts of a Flame Photometer

1) Pressure regulator for the fuel, gas & air
2) Atomizer:- Aspirates the solution & atomize it in the form of mist
3) Burner:- Produce desired flame in which atoms are excited
4) The optical system:- Collects light energy, renders it monochromatic by a filter or monochromator
5) Photosensitive detectors:- usually a barrier layer cell converts light energy into electrical energy
6) Galvanometer:- Measure electric output

7) The fuel gas ordinary LPG gas – air combination can excite only elements like Li, Na, and K while Ca, Mg requires higher flame temperature (3000^{o}C) for excitation and hence acetylene air combination is required.

Operating Flame photometer

1) Switch ON the instrument, warm up to 30 minutes
2) Insert proper filter
3) Start air compressor & adjust flow rate (0.6 kg.cm^{2})
4) Place beaker containing distilled water for aspiration
5) Open gas cylinder & adjust gas / flow regulator, light the burner
6) Adjust the regulator to obtain a non luminous flame with maximum blue cone
7) Aspirate the blank or distilled water
8) Adjust set zero knob of galvanometer to read zero
9) The most conc. standard is aspired first & reading is adjusted to 100 using sensitivity knob
10) Several standards of lower concentration are introduced & reading noted
11) Aspire the blank or distilled water, aspire the unknown solution and note down the reading.

The conc. of unknown solution is calculated from the standard curve prepared with reading on x – axis & conc. on y – axis. Critically the curve should be straight line. At the end of determination the following steps are taken to switch OFF flame potentiometer.

1. After aspiring all the samples turn OFF gas supply
2. Switch OFF galvanometer
3. Run distilled water & allow to flush out the atomizer & burner for sometimes
4. Remove the distilled water & allow only compressed air to flow
5. Switch OFF the compressor after 5 min to cool the instrument

pH meter

The pH meter is actually a voltmeter which measures the potential difference b/w 2 electrode system which share a common electrolyte. The pH meter has 2 electrodes. One is

the reference electrode with a constant reproducible potential and a measuring electrode which develops a potential depending upon H+ ions conc. of solution or suspension.

Parts

1) Temperature compensator knob:- Set it to temperature of solution / suspension
2) Selection switch:- It has a pH knob with 0 – 14 milli volt range with values on stand by or zero position
3) Standardised knob or set buffer knob:- Used to caliberate pH meter with the buffer
4) Meter is groduated in pH unit & milli volt & needle moves over a mirrored centre
5) Electrode assembly:- Separate reference & glass electrode or a combined form of electrode assembly

Operating a pH meter

1. Switch ON the instrument to warm up for about 30 min in the stand by position
2. Lift electrode from distilled water, wash it with distilled water & wipe it with dry tissue paper
3. Set temperature compensation knob about to the temp. of solution
4. Take approximately 30 ml buffer (pH4) into a 50 ml beaker & introduce electrode
5. Turn the function knob to pH mode to read the pH if necessary. Adjust the needles using the set buffer (srandardise) knob to exact pH.
6. Being back the selector switch to stand by mode, lift electrode and wash it with distilled water, wipe it dry.
7. Take 30 ml buffer pH 9.2 in the 50 ml beaker, introduce the electrode into it & turn the function knob to pH mode. The meter should read a pH of 9.2 (double buffer mode). If not the instrument / electrode / buffer is faulty
8. Put selector switch to stand by position, lift electrode, wash, & wipe dry it
9. Take unknown solution / suspension. Introduce electrode into it turn the function knob to pH mode & note the reading
10. Turn the function knob to stand by position, lift the electrode wash with distilled water dry & insert it back in distilled water. Switch OFF the instrument

Conductivity Bridge

The electrical conductivity bridge consists of AC source Wheastone bridge, or conductivity cell and a null indicating device.

Operation

1) ON / OFF switch
2) Resistance / conductance bridge
3) Bridge source selector
4) Range switch
5) Temperature compensator knob
6) Potentiometer deal knob
7) Null indicator / magic eye

Operating Conductivity Bridge

1. Switch ON the instrument to warm up for about 10 minutes
2. keep the sensitivity control at or near the minimum
3. Adjust the temperature compensator knob to the temperature of the solution
4. Clean the conductivity cell with distilled water
5. take the sample solution in a beaker, introduce cell into it
6. Balance the bridge by rotating potentiometer deal knob balance point is indicated by widest shadow of magic eye.
7. Note down the reading & multiply with cell constant specific for each conductivity cell to get specific conductance.

COLLECTION OF SOIL SAMPLES FROM THE FIELD & PREPARATION FOR ANALYSIS

The analysis can be no better than the sample. Soil being heterogeneous in nature, it is important to device a satisfactory method of sampling to get a uniform sample. There is a considerable opportunity for sampling error and hence it is essential that the field be sampled

correctly. For efficient evaluation of soil fertility, a representative soil sample of the area is the most important aspect.

For routine soil fertility testing, first go around the field to be sampled. Variation in slope, colour, texture, management and cropping pattern should be recorded. The demarcate the field according to uniform areas.

Use proper sampling tools. Satisfactory samples can be taken with a soil tube, auger, spade, trowel, pick-axe etc. Locate different spots of sampling in a zig-zag manner in the field. In each spot scrap away surface litter, then take a core sample from the surface to plough depth. Likewise collect from the 10 – 20 spots in the field. Depending upon the area, where crop have been planted in lines or rows sampling should be done in b/w the lines. Do not sample unusual areas. Avoid areas recently fertilized, old bunds mainly marshy spot, near trees, compost heap pr other non-representative locations. Take a uniform slice sample from each spot. If a spade is used, dig a 'V' shaped hole then cut a uniformly thick (2cm) soil from bottom to top of the exposed soil face. Collect the sample on the blade of the spade or in the hand and place it in a clean bucket. Collect all the spot sample from field in to the same bucket. Pour the spot sample from the bucket into a piece of clean paper or cloth in shade & mix thoroughly. Collect the representative composite sample by quartering method. To quarter the sample mix well, divide into 4 equal parts & reject the 2 opposite quarter samples. Mix the remaining 2 portions and repeat the procedure as many times as necessary to arrive at the desired size of sample. If the sample is wet or moist, dry it in shade or in place without direct sunlight. Put into a clean sample container. Fill up the sample information or case history sheet of the sample completely and put inside the sample bag and label neatly. Pencil or ball point pen may be used for labeling. Avoid all the possible contaminations of the samples during sampling. Mixing and packing till the sample reaches to the lab for analysis.

For sampling from soil profile

Sampling from soil profile which is a vertical section of the soil from the surface down to the parent rock is usually done for physico-chemical and mineralogical analysis which is of importance in soil. Genesis, morphology and classification, studies on soil fertility, samples are drawn on horizontal basis. This can be distinguished by colour, texture & structure. In the absence of such horizon differentiation, samples are to be collected at uniform depth. Dig the profile pit in such a way that there is maximum sunshine on one end at the time of sampling. Before sampling, clear away all the litter and plant materials from the surface, but don't

remove grass roots or organic matter embedded in the horizon. Place each horizon wise samples together with a slip of paper for identifying the sample in the numbered bag for transport to the laboratory. Designate different soil horizons as A0, A1, A2, B, B1, B2, B3 etc with other features as well. Detailed field observations may be recorded in soil profile study proforma. For soil survey works, a soil type sample is only required ie a sample taken from a particular spot is typical of the surrounding area. This area may be large or small and its boundaries are determined by putting down test boxes at intervals. These boundaries are then drawn on a map. For this kind of soil survey work, about 2 kg sample is required for detailed analysis.

Depending upon the purpose for which sampling is done, the depth of sampling, time of sampling, frequency of sampling and intensity of sampling are varying as follows.

Depth of sampling

For field crops samples are to be taken to the depth of tillage which can vary from 10 – 30 cm. To study the fertility status of soil for pasture crops, a 10 cm depth is normally sufficient. For deep rooted crops like sugarcane, horticulture crops or under dry farming conditions, samples at different depths are to be taken based on situations. For immobile nutrients like P, K, Ca and Mg, samples to tillage depth will give satisfactory results. For mobile nutrients like nitrates and sulphates, samples should be taken upto a depth of 60 cm. In saline and alkali soil salt crusts on soil surface should be sampled and depth of sampling to be recorded. The samples of lower depth are more important than those of surface sample. Similarly for the site of garden, the subsoil samples are more important than surface soil. For reclamation purpose dig a pit of 3 feet depth in problematic area. Make one side of the pit vertical and mark on it at 15 cm, 30 cm and 60 cm from the surface. Collect 1 kg each of the soil from these layers separately. For garden plantation, sampling should be done from different layers of pit of about 2 m depth. Generally for giving discriminatory fertilizer recommendations samples are collected at 0 – 30 cm depth for annuals and 30 – 60 cm for biennial and perennials. In some cases even from 3 depths are collected especially for the tree crops.

Time of sampling

Under intensive cultivation, sampling should be done every year at same time. If one crop per year is raised sampling once in 3 years is sufficient. However, when the crops shows and deficiency symptoms, it is better to collect samples more frequently and analyze the required

element. In coarse textured soil. For preparation of soil, frequent testing is necessary fertility index / map of village or block level, sampling once in 5 years is more efficient.

Intensity of Sampling

If a field from which sampling is done appreciably uniform in all respects only that field can be treated on a single sampling unit. But normally such soils are more heterogeneous in nature and hence intensity of sampling is decided principally by the objective of sampling, nature of soil, time available and other conditions for plantation crops like rubber, coffee, cardamom etc, intensity of sampling is recommended at one composite sample for every 20 ha. For annual crops, take a representative sample for every 2.5 ha. based on the conditions. For the purpose of preparing soil fertility index map, the intensity of sampling is done at one representative sample for every 10 ha.

Preparation of soil sample for analysis

The soil sample received at the laboratory is registered in lab registers with all case history in field. It is air dried in shade again and spread on a sheet of paper after breaking large lumps if present with a wooden mallet in such a way that the aggregate particles are broken down to ultimate soil particles. Soil thus prepared is sieved through sieve with round holes, the material on the sieve is again ground and sieved till all the aggregates are fine enough to pass through and stones and organic residues/remain on the sieve. Mix well the fine soil got by sieving and store in suitable containers with label on outside and one inside.

Subsampling for analysis

The soil in the container is emptied in a clean thick sheet of paper and evenly spread with a sampling knife. It is heaped into a cone by raising four ends of the paper. This is again mixed well and evenly spread on paper as before. This process is repeated 3 or 4 times to ensure uniformity of the sample and finally spread evenly on paper again. Now it is divided into 4 equal quarters and small quantity of soil is taken from various points in each quarter to get representative sample for analysis.

(4) DETERMINATION OF PH IN THE GIVEN SOIL SAMPLE

I. Soil pH

Principle

The pH of 1:2.5 soil water suspension is estimated using a pH meter. It is a measure of hydrogen ion activity of soil water system and indicate whether the soil is acidic, neutral or alkaline in reaction.

Reagents

Standard buffer solution:- prepare buffer solution of pH 4, 7, 9.2 using commercially available tablets ie (buffer tablets). Dissolve the respective tablets in freshly prepared distilled water and make up the volume to 100 ml. It is necessary to prepare fresh buffer solution after every few days as these solution don't keep for long.

Procedure

Calibrate the pH index using buffer solutions. The pH of the soil determined in 1:2.5 soil water suspension. Take 10g soil sample in a 50 ml beaker. Add 25 ml of distilled water, stir intermittently for 30 min. Stir well and again and take the reading using pH meter.

DETERMINATION OF ELECTRICLA CONDUCTIVITY IN THE GIVEN SOIL SAMPLE

Electrical Conductivity

Principle

Electrical conductivity in soil water system is a measure of concentration of soluble salts and extent of salinity in the soil and is measured by using a conductivity meter.

Reagents

0.01 N KCl solution:- Dry a small quantity of AR grade KCl at 60^0C for 2 hrs in a hot air oven, Weigh 0.7456 g of it and dissolve in freshly prepared distilled water and make it to 1L. This solution gives an electrical conductivity of 1.41 dSm^{-1} at 25^oC.

Procedure

The clear supernatant of 1:2.5 soil – water suspension prepared for pH measurement can be used for estimation of electrical conductivity. Calibrate the conductivity meter using 0.01N KCl solution prepared & determine the cell constant. Determine conductivity of the supernatant liquid.

DETERMINATION OF AVAILABLE NITROGEN IN SOIL (Subbia and Asija, 1956)

Alkaline Permanganate method

The available nutrients are termed as the portion of the plant nutrients accessible for the plant for its growth & function which are shown by the plants from the soil reserve through various mechanisms. The calibration of soil test data with crop responses obtained from the field experiments is used for the establishment of specific procedure for specific nutrient.

Principle

The amount of soil nitrogen released by alkaline permanganate solution is estimated by distillation with sodium hydroxide. The distillate is collected in boric acid and containing mixed indicator and titrate against standard acid – H_2SO_4. The nitrogen so estimated is designated as available nitrogen which is correlated with crop response to nitrogen fertilization.

Procedure

Weigh 20g of the soil and transfer to a distillation flask. Add 20 ml of distilled water and 1 ml of liquid paraffin or 1 g of paraffin wax to control frothing. Put a few glass beads to prevent bumping and then add 100 ml of 0.32% potassium permanganate solution and 100 ml of 2.5% NaOH solution. Distill the contents in a steady rate collecting the librated ammonia in 100 ml conical flask containing 20 ml of 2% boric acid with mixed indicator (0.5 g of bromocresol green, 0.1 g of methyl red in 100 ml of ethyl alcohol). Continue distillation for about half an hour or until 100 ml of distillate is collected in the beaker. Titrate the ammonia collected against N / 50 sulphuric acid. From the titre value, calculate the available nitrogen content in the soil.

Calculation

1 ml of N/50 H2SO4 = 0.0028 g of Nitrogen

$$N\ Kg\ ha^{-1} = \frac{TV \times 0.0028 \times 1000 \times 2.24 \times 10^{6}}{1000 \times 20}$$

Rating of soil

Upto 280 Kg ha^{-1}	-	Low status
280 to 560 Kg ha^{-1}	-	Medium
> 560 Kg ha^{-1}	-	High

DETERMINATION OF AVAILABLE PHOSPHORUS IN SOIL

Determination of plant available P in soil has 2 distinct phases. First the extraction of plant available pool of P present in soil and second, the quantitative determination of the P in the extract. The choice of the colorimetric method for determining P depends on the P conc. in the solution, the conc. of interfering substance in the solution to be analyzed and the particular acid system involved in the analytical procedure. The Molybdenum blue method is the most sensitive & widely used one for soil extracts containing small amount of P.

Principle

In an acid molybdate solution, the orthophosphate ions get precipitated as phosphomolybdate complex forms that can be reduced by ascorbic acid, stannous chloride and other reducing agents. This reduced phosphomolybdate has blue colour. The intensity of the blue colour varies with P concentration but is affected also by other factors such as acidity, arsenates, silicates and substances that influence oxidation – reduction conditions of the system.

As the available pool of P varies depending on the pH of the soil, reagents used for extraction of this pool also are different.

Available P in acidic soils

Available P is commonly extracted using Bray No. 1 (Bray and Kurtz, 1945) which consists of 0.03 NH_4F and 0.025 HCl. The combination of HCL and NH_4F is designed to remove easy acid soluble P forms and largely Calcium Phosphate and a portion of the Fe and Al phosphates. The NH_4F dissolves Fe and Al phosphates by its complex ion formation with these metal ions in acid solution.

Reagents

1) Ammonium Fluoride (NH_4F), 1N:- Dissolve 37g of NH_4F in dissolved water and dilute the solution to 1L. Store the solution in polyethylene bottle.
2) Hydrochloric acid, 0.5N:- Dilute 20.2ml of conc. HCl to a volume of 500 ml of distilled water.
3) Bray No.1:- Add 15ml of 1N NH_4F and 25ml of 0.5N HCl to 460ml of distilled water. Keep in glass for more than 1 year.
4) Ammonium paramolybdate:- Dissolve 12g of ammonium paramolybdate in 250ml of distilled water. Dissolve 0.2908g of potassium antimony tartarate in 100 ml of distilled water. Add these dissolved reagents to 1L of 5N sulphuric acid H_2SO_4 (141ml of conc. H_2SO_4 diluted to 1L). Mix thoroughly and dilute with distilled water to 2L. Store in a pyrex glass bottle in a dark & cool compartment (Reagent A).
5) Ascorbic acid:- Dissolve 1.056g of ascorbic acid in 200ml of Reagent A & mix. This ascorbic acid (Reagent B) should be prepared as required because it does not keep for more than 24 hour.
6) Standard Phosphate solution:- Dissolve 0.4393g of ovendry AR grade KH_2PO_4 (Potassium dihydrous Phosphate) in distilled water and dilute the solution to 1L. 1ml of this solution contains 100 µg of P. From this solution, prepare a 2^{nd} standard of 2 µg 1ml by pipetting out 2ml and diluting to 100ml with distilled water.

Procedure

Extraction

Weigh out 5g of soil to a 100ml conical flask & add 50ml of Bray No 1 reagent and shake for exactly 5 mins. Filter through What man No. 42 filter paper. To avoid interference of fluoride, 7.5ml of 0.8M boric acid (50g of H_3BO_3 per liter) can be added to 5ml of the extract if necessary. Estimate 'P' in the extract by ascorbic acid method. (Watenabae and Olsen 1965).

Estimation by reduced molybdate blue colour method

Pipette out 5ml of the extract into a 25ml volumetric flask and dilute it to approximately 20 ml. Add 4ml of reagent B. Makeup the volume with distilled water and shake the contents well. Read the intensity of colour after 10 min at 660 nm. The colour is

stable for 24 hours and maximum intensity develops within 10 min. The conc. of P in the samples is computed from the standard curve.

Preparation of standard curve

Prepare different concentrations of P taking 1, 2, 3, 4, 5 & 10ml of 2µg/ml P solution in 25ml volumetric flask. Add 25ml of the extracting reagent (Bray No.1) and develop colour as described above by adding reagents B & plot the concentration Vs absorbance curve on a graph paper.

Calculation

Weight of soil taken = W g

Volume of extractant added = 50ml

Volume of extract taken for color development=5ml

Volume made up = 25ml

Available P (mg/kg soil) = Microgram P per ml^1 of the aliquot x $\frac{50}{5}$ x $\frac{25}{5}$

Available P (mg/kg soil) = $\frac{\text{Absorbance for sample} \times 50}{\text{Slope of std. curve}}$

Available P (kg/ha soil) = Available P (mg/kg soil) x 2.24

ESTIMATION OF AVAILABLE POTASSIUM IN SOIL

A relatively small portion of total K in soil is exchangeable (approx. 1%). Exchangeable K generally ranges from < 100 to 200 µg ml^{-1} or more when compared with total K values which is in the order of 1.2%. Water soluble K seldom exceeds a few parts per million except in case of certain saline soils.

Exchangeable plus water soluble K contributes to the plant available pool of K in the soil. Hence most soil test procedures to estimate plant available K involves extractants that replace a significant portion of exchangeable K. In highly weathered soil or soils where parent materials contain little K bearing minerals, the exchangeable K can be depleted by K removal and is replenished by fertilizer application or return by K from plant residue.

Principle

By definition, exchangeable K is that which is free to exchange with cations of salt solution added to soils. But the quantity exchanged from the soil depends on the nature of replacing solution. Hence with the reference to fertility evaluation exchangeable K is defined more specifically as that which is extracted with neutral 1N ammonium acetate minus the water soluble K. In normal soils as the water soluble K is so small there is no appreciable error even it is included and the water soluble K plus exchangeable K represents the available pool. The removal of water soluble K before extraction with ammonium acetate is not recommended because as the salt content of soil solution is decreased, the adsorption of divalent cations in solution increases.

Reagents

1. Neutral 1Normal ammonium acetate solution (CH_3COONH_4):- Dissolve 77.08g of ammonium acetate in distilled water & make up to 1L. Adjust the pH if necessary to 7 with acetic acid or NH_4OH.
2. Standard K solution:- Dissolve 1.908g of dried KCl AR grade in distilled water & make up to 1L so as to get 1000µg/ml K solution

Procedure

Extraction

Shake 5g of soil with 25ml of neutral normal ammonium acetate for 5 minutes and filter immediately through a dry What man No. 42 filter paper. First few ml of the filtrate my be discarded. K concentration in the extract is determined using flame photometer after necessary settings and calibration of the instrument.

Standard curve for potassium

Dilute measured aliquots from the standard solution using ammonium acetate solution to given conc. of 5 – 20 µg/ml of K. After attaching appropriate filter and adjusting the gas and air pressure set reading in the flame photometer as zero for the blank (NH_4COOCH_3-Ammonium acetate) and at 100 for 20µg/ml K. Fluctuation in gas and air pressure doesn't allow steady reading in the meter & must be taken care of.

Calculation

Available K(mg/kg soil) = x/5 x 25/5

Where x = flame photometer reading

Available K ($kgha^{-1}$ soil) = Available K (mg/kg soil) x 2.24

DETERMINATION OF AVAILABLE SULPHUR IN SOIL BY CALCIUM CHLORIDE EXTRACTION

Principle

Different reagents have been prepared for extracting plant available sulphur from soil. This includes water, salt solution such as 0.15% $Cacl_2$. 500 ppm 'P' as $CaH_2(PO_4)$or KH2PO4 and acidic solutions such as 0.5N ammonium acetate + 0.25 N aceticacid and Bray No. 1. Generally PO_4^{2-}solutions extract more sulphate- sulphur from soil than that can be extracted with H_2O or salt solutions because phosphate ions displaces the absorbed SO_4^{2-}which is known to be readily available in plant.

Reagents

1. 0.15% $CaCl_2$ solution:- Dissolve 1.5g of $CaCl_2$ in about 500ml distilled water & make up the volume to 1 litre.
2. Gum acacia solution:- Dissolve 0.25g of chemically pure gum acacia in hot water and filter the solution through Whatman No. 42 filter paper.
3. Then cool the filtrate and dilute to 100ml.
4. Barium Chloride ($BaCl_2$):- Grind analytical grade BaCl2 to pass through 1mm sieve.
5. Concentrated standard sulphate solution (100mg S/l):-Dissolve 0.5434 g oven dried AR K_2SO_4 in distilled water & make up to 1 liter.

Procedure

Extraction by TATABAI, 1982

Shake 10g of air dried processed soil with 50 ml 0.15% $CaCl_2$ solution in 250 ml conical flask for 30 minutes. Filter the extract through Whatman No. 42 filter paper and estimate the SO_4^{2-} content by turbidimetric procedure.

Preparation of standard curve

1. Pipette out 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.50, 2.5ml of standard sulphate solution in separate 25 ml volumetric flask and add 10 ml of extracting solution (0.15% $CaCl_2$) Prepare fresh standard each time when a batch of sample is analyzed.
2. Add 1g of BaCl2 crystal to each flask & swirl to dissolve
3. Add 1ml of 0.25% gum acacia solution & then make up to 25ml with distilled water and shake well
4. Within 5 – 30 minutes of development of turbidity, read the absorbance in spectrophotometer at 440 nm
5. Draw the standard curve with absorbance in Y axis & concentration on X axis

Turbidimetric estimation of sulphur by Massoumi and Cornfield, 1963

1. Pipette out 10 ml of soil extract into 25ml volumetric flask
2. Add 1g of $BaCl_2$ crystal and swirl to dissolve
3. Add 1ml of 0.25gum acacia solution, make up the volume with distilled water and shake well
4. Within 5 – 30 minutes of development of turbidity read absorbance at 440nm on a spectrophotometer.

Calculation

Weight of soil taken = 10 g
Volume of extractant = 50ml
Volume of soil extract taken = 10ml
Volume made up = 25ml
Amount of sulphur (mg/kg of soil) = Concentration from the instrument x $\frac{50}{10} \times \frac{25}{10}$

$$= \frac{\text{Absorbance from the sample} \times 12.5}{\text{Slope of std. curve}}$$

$$= \frac{\text{Reading} \times 12.5}{0.025}$$

ESTIMATION OF EXCHANGEABLE CALCIUM AND MAGNESIUM By EDTA or VERSANATE METHOD

Exchangeable Ca & Mg are extracted with neutral normal ammonium acetate (CH_3COONH_4) and determined by either using AAS(Atomic Absorption Spectrophotometer) or titrimetry..

Extraction

Ammonium acetate extract used for the estimation of K or CEC can be used for the determination of Ca & Mg 10g soil is taken and 50 ml ammonium acetate solution is added and kept for overnight , filtered and used for estimation.

Titrimerty

EDTA (Ethylene diamine tetra aceticacid) strongly complexes the cations Ca^{2+}, Mg^{2+}

Procedure

Titrate first the Ca^{2+} in solution & then Ca^{2+} + Mg and obtain the Mg by difference.

Reagents

- EDTA – 0.01N
- NaOH – 10%
- NH_4Cl, NH_4OH buffer – Dissolve 67.5g NH_4CL in 400ml H_2O.To this add 5 – 10ml of NH_4OH solution. Dilute it
- Hydroxylamine hydrochloride – 5%
- Potassium hexacyano ferrate – 4%
- Tri ethanol amine
- KCN solution – 1%
- Calcon & Erichrome – black – T – indicator:- Calcon 0.2g / 50ml methanol
- Erichrome black – T -0 0.2g / 50ml ”

Procedure

Determination of ‘Ca’ in solution

Pipette 5ml of sample into a beaker. Dilute & add 10drops of KCN, hydroxyl amine hydrochloride and triethanol amine solution. Add 2.5ml of NaOH and 1ml of calcon solution. Titrate with EDTA & calculate the mg of Ca in solution. Colour changes from wine red to blue.

Role of KCN in titration

The KCN is a masking agent which masks heavy metals like Cd, Zn, Ni, Si, etc. triethanol amine mask Ti, Fe3+ & Al

Determination of Ca+Mg

Pipette 5ml of sample into a beaker & dilute to 10ml. Add 15ml buffer solution (NH_4Cl - NH_4OH) 10 drop each of KCN, hydroxyl amine hydrochloride, potassium hexa cyano ferrate & triethanol amine while warming the solution. Continue warming for 3 minute,s. cool & add 10 drops of Erichrome black – T solution. Titrate with EDTA solution to blue end point.

Determining Mg

Mg is calculated from the difference between Ca + Mg & Ca determination

Calculation

1 ml of INEDTA = 20 mg of Ca
1 ml of INEDTA = 0.02 g of Ca
Volume of extract taken = 5 ml
Weight of soil taken = 10 g
Volume of ammonium acetate added = 250 ml
Normality of EDTA = N
Volume of EDTA used for titration of Ca + Mg = V1
Volume of EDTA used for titration of Ca alone = V2

Volume of EDTA used for titration of Mg alone = V1 – V2=Y

$$\% \text{ of Ca} = \frac{V2 \times N \times 0.02 \times 250 \times 100}{\text{weight of soil} \times 5}$$

1 ml of INEDTA = 0.012 g of Mg

$$\% \text{ of Mg} = \frac{Y \times N \times 0.012 \times 250 \times 100}{10 \times 5}$$

ESTIMATION OF CATION EXCHANGE CAPACITY OF SOIL

The cations are displaced in a known quantity of soil sample by ammonium ions by leaching the soil with neutral normal ammonium acetate. The excess of ammonium acetate is removed with alcohol. Adsorbed ammonium ions are then determined by steam distillation.

Reagents

- Neutral normal ammonium acetate: Add 57ml of glacial acetic acid & 68ml of con. Ammonium hydroxide to 800ml water. Dilute to 1litre & adjust the pH to 7.
- Alcohol 60% - Add 500ml of H_2O to 1 litre of absolute alcohol
- 0.1Nsulphuricacid
- 0.1N NaOH for titrating
- Methyl red indicator
- Magnesium oxide

Procedure

- Transfer 10g of the soil to 500ml conical flask, add 250ml Netral Normal ammonium acetate solution & shake the content occasionally for an hour & keep it overnight
- Filter the content through Whatman No 42 filter paper & collect the filtrate in flask
- Wash the soil on the filter paper with 60% alcohol by 2 – 3 washing to eliminate excess of CH_3COONH_4 .This may be determined by adding a small quantity of ammonium chloride to the first lot of alcohol used for washing & then with alcohol until the filtrate gives no test of chloride.When this is attained remove the soil with the filter paper(The leachate can be used to the determination of the individual cations & residue left on the filter paper is used for the determination of soil EC)
- Transfer the residue on Kjeldhat5s distillation flask. Add 200ml water followed by paraffin wax, a pick of NaCl, glass beeds. 2 – 3 mg MgO as 20 ml 1N NaOH
- Carry out distillation, collect the distillate in known volume of 20 ml of 40% boric acid to a conical flask. Few drops of mixed indicator added with absorption of NH_3, Red colour of boric acid change to green and titrate back with 0.1N HCl until the wine red colour.

Calculation

Titre value = 0.1 N acid taken – 0.1 N alkali used for back titration

1ml of 0.1 N acid = 0.1 milli eq. of any cation

SO, CEC = Titre value x 0.1x100 (Cmol per kg of soil)

Wt of soil taken

LIME REQUIREMENT OF SOIL

Buffer Method – Shoemaker *et.al*

Principle

Soil is treated with a buffer solution with a known pH and the pH is taken after a definite period of equilibrium. The depression in pH is proportional to the lime requirement of the soil.

Reagents

Buffer solution
P – nitrophenol – 1.8g
Triethanol amine – 2.5ml
Potassium chromate – 3g
Calcium acetate-2g
Calcium chloride($CaCl_2 . 2H_2O$)_ – 40g

Dissolve this in 800 ml of distilled water & adjust to pH 7.5 with dilute HCl or NaOH. Dilute to 1L.

Procedure

Mix 10g (20g if very sandy) of soil with 20mlof the buffer solution & shake for 10 minutes. Read the pH of the suspension & estimate the lime requirement. If 20g soil is taken, the result should be divided by 2.

pH of soil buffer solution 7.5	Lime required to bring the soil to indicate pH (in tons/acre of pure $CaCO_3$)

	pH6.0	pH 6.4	pH 6. 8
6.7	1	1.2	1.4
6.6	1.4	1.7	1.9
6.5	1.8	2.2	2.5
6.4	2.3	2.7	3.1
6.3	3.1	3.2	3.7
6.2	3.9	3.7	4.2
6.1	4.4	4.2	4.8
6.0	4.8	4.7	5.4
5.9	4.8	5.2	6
5.8	5.2	5.7	6.3
5.7	5.6	6.2	7.1
5.6	6	6.7	7.7
5.5	6.5	7.2	8.3
5.4	6.9	7.7	8.9
5.3	7.4	8.2	9.4
5.2	7.8	8.6	10
5.1	8.2	9.1	11.2
5.0	8.6	10.1	11.8
4.9	9.1	10.6	12.4

GYPSUM REQUIREMENT OF SOIL

Principle

The amount of gypsum required to be added to a sodic soil to lower the ESP to a desired value is found out by titrating a weighed quantity of soil with saturated gypsum solution and finding out the concentration of Calcium and Magnesium in the filtrate.From this the Gypsum requirement can be calculated.

Procedure

5g soil is shaken with 100ml of a saturated gypsum solution of known Calcium concentration for 30 minutes. The solution is filtered and the filtrate is analysed for Ca+Mg

Calculation

Concentration of Ca in the gypsum solution = C1 cmol per kg

Concentration of Ca+Mg in the filtrate = C2 cmol per kg

Gypsum Requirement = $\frac{(C1-C2)x100x100}{1000x5}$ cmol per kg

However for effective use,the Gypsum applied in practice is 1.2to1.5 times the Gypsum requirement calculated

Determination of Dissolved oxygen in water (Winkler's Method)

Introduction

Principle:

$MnSO_4$ reacts with the alkali to form white precipitate of Manganese hydroxide which in the precipitate of O_2 get oxidized to a brown color higher hydroxide, which on acidification liberate iodine equivalent to that of O_2 fixed. The iodine is titrated against thiosulphate using starch as indicator.

Reagents

- Manganese sulphate solution: Dissolve 480 g of $MnSO_4.4H_20$ or 400 g of $MnSO_4.2H_20$ or 363 g of $MnSO_4.H_20$ in 1000 ml distilled water.

- Alkaline iodide solution: Dissolve 500 g NaOH [or 700 g KOH] and 135 g NaI [or 150 g KI] in 1000 ml distilled water (or dissolve 100 g KOH and 50 g KI in 200 ml distilled water).
- Concentrated sulphuric acid.
- Starch solution: Dissolved 2 g laboratory grade soluble starch and 0.2 g salicylic acid in 100 ml hot distilled water.
- Sodium thiosulphate solution (0.025 N): Dissolve 6.203 g of $Na_2S_2O_2.5H_2O$ in 1000 ml distilled water.

Procedure:

- Water sample is collected from surface in 125 ml Dissolved Oxygen bottle avoiding formation of bubbles.
- Add 1 ml alkaline iodide and 1 ml $MnSO_4$ to that Dissolved Oxygen bottle.
- Mix well and then precipitate formed is allowed to settle for few minutes.
- Add 1 ml of conc. H_2SO_4 and shake well to dissolve the precipitate.
- Then from DO bottle take 50 ml solution in conical flask.
- Titrate against std. $Na_2S_2O_3$ using starch as indicator.
- The end point is blue to colorless and titre value is measured.
-

Calculation:

$$DO\ (mg/l) = \frac{A \times N \times V \times 1000 \times 22.4}{B\ (A-L) \times 0.4 \times 0.698}$$

Where,
A= Volume of the DO bottle
N= Normality of $Na_2S_2O_3$
V= Titrate value
B= Volume of sample taken
L= Volume of reagent used

(15) Estimation of free carbon dioxide

Introduction: Dissolved CO_2 has marked effects on the properties of water, if forms a weak carbonic acid solution that changes the pH increases, alkalinity and hardness of water by dissolving minerals.

Principle:

Free CO_2 reacts with NaOH or Na_2CO_3 to form $Na(HCO_3)2$, the completion of the reaction is indicated by the appearance of pink color in the presence with phenolphthalein indicator as pH of 8.3.

Reagents:

- **Std. NaOH solution (0.02 N):** Dissolve 200 ml of stock solution to 1000 ml with distilled water.
- **Stock NaOH solution:** Dissolved 4 g NaOH in 1000 ml distilled water.
- **Phenolphthalein indicator:** Dissolve 0.5 g of phenolphthalein powder in 50ml of 95% C_2H_2OH and add 50ml distilled water.

Procedure:

- Take 50 ml of sample in a conical flask.
- Add 4 to 5 drops of phenolphthalein indicator:
  If the sample is pink color does not appear titrate the sample against standard 0.02 N NaOH and until the pale pink color develop and remain for 30 sec.
- Note down the burette reading.
-

Calculation:

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{V \times N \times 44 \times 1000}{\text{Volume of sample}}$$

V = Volume of 0.02N NaOH
N= Normality of NaOH

Determination of pH by electrometric method

Introduction: Measurement of pH is one of the most important and frequently used test in water chemistry; practically every phase of water supply and waste water treatment. pH is used in alkalinity and CO_2 measurement and many other acid-base equilibrium.

Principle:

The basic principle of the electrometric pH measurement is determination of the activity of the hydrogen ion by potentiometric measurement using a standard hydrogen electrode and a reference electrode.

Apparatus:

pH meter consisting of potentiometer, a glass electrode, a reference electrode and a temperature-compensating device.
Glass electrode: The sensor electrode is bulb of special glass containing a fixed concentration of HCl and a buffered chloride solution in contact with an internal reference electrode.

Procedure:

Before use, remove electrode from storage solution, rinse, and blot, dry with a soft tissue paper.
Calibrate the instrument with standard buffer solution. [Ex: KCl solution of pH 7.0]

Once the instrument is calibrated remove the electrode from standard solution; rinse, blot and dry.
Dip the electrode in the sample whose pH has to be measured.
Stir the sample to ensure homogeneity and to minimize CO_2 entrainment.
Note down the reading (pH) from the pH meter.

(17) Determination of Chloride and Salinity from the Fish-pond 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
- Water softener regeneration

✪ **Expected Levels:**

✡ Seawater has a chloride ion concentration of about 19,400 mg/L (a salinity of 35.0 ppt).

✡ Brackish water in tidal estuaries may have chloride levels between 500 and 5,000 mg/L (salinity of 1 to 10 ppt).

- ✡ Even freshwater streams and lakes have a significant chloride level that can range 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 water sample.

Step 2:

Take 10 ml Std. NaCl Sol. + Add DW to make the Vol. around 100 ml.

Step 3:

Add 0.5 ml of K_2 CrO_4 to the Sol. & Titrate with $AgNO_3$ till appearance of a permanent red precipitation.

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

When,

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

Another 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.

(18) Determination of Organic Carbon from Fishpond Soil

- 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 soil is oxidized with excess standard potassium dichromate (K2Cr2O7) using the heat of dilution of added concentrated H2SO4. The unutilized K2Cr2O7 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 H2SO4

   e) 85% H_3PO_4

✪ **Procedure:**

Step 1: Take 1 g of soil sample in a 500 ml conical flask and moisten it with 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 of diphenyl 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.

BFSC-104 Fundamentals of Microbiology

Microscopy

Introduction: Microscope is a powerful and crucial basic tool in the field of microbiology. The existence of microbial life to the world was introduced first by Antony Van Leeuwenhoek in 1673, with the help of simple, crude, self-made, single-lens microscope having a magnification of about 300. (In the late 16th century several Dutch lens makers designed devices that magnified objects, but in 1609 Galileo Galilei perfected the first device known as a microscope. Dutch spectacle makers Zaccharias Janssen and Hans Lipperhey are noted as the first men to develop the concept of the compound microscope.) Over the years, microscopes have evolved to increase the magnification several thousand-fold. Modern day microscopes are either light microscopes or electron microscopes. Light microscopes use either visible light to illuminate specimens. A compound light microscope is the most common microscope used in microbiology. It consists of two lens systems (combination of lenses) to magnify the image. Each lens has a different magnifying power. A compound light microscope with a single eye-piece is called monocular; one with two eye-pieces is said to be binocular. These are generally used to look at intact cells. Ideally a microscope should be parfocal; that is, the image should remain in focus when objective lenses are changed.

Microscopes that use a beam of electrons (instead of a beam of light) and electromagnets (instead of glass lenses) for focusing are called electron microscopes. These microscopes provide a higher magnification and are used for observing extremely small microorganisms such as viruses. Besides, these are generally used to look at internal structures or details of cell surface.

Common Types of Light Microscopy

Brightfield microscopy: This is the commonly used type of microscope. In brightfield microscopy the field of view is brightly lit so that organisms and other structures are visible against it because of their different densities. It is mainly used with stained preparations.

Differential staining may be used depending on the properties of different structures and organisms.

Darkfield microscopy: In darkfield microscopy the field of view is dark and the organisms are illuminated. A special condenser is used which causes light to reflect from the specimen at an angle. It is used for observing bacteria such as treponemes (which cause syphilis) and leptospires (which cause leptospirosis).

Principle: When the light passes from one medium to another, refraction occurs, i.e., the ray is bent at the interface. The direction and the magnitude of bending are determined by the refractive indices of the two media forming the interface. The **refractive index** is a measure of how greatly a substance slows the velocity of light when light passes from air to glass or vice versa.

When the light rays strike the lens, a convex lens will focus these rays at specific point called **focal point**. The distance between the center of the lens and the focal point is called the **focal length**. Convex lens act as a magnifier. It provides a clear magnifying image at a much closer range. Lens strength is related to focal length. A lens with a short focal length has a more magnification power than a lens having a longer focal length.

Magnification means enlargement. In compound microscope, it is carried out by two-lens system – objective lens and ocular lens. **Objective lens** produces the real image of the specimen, which is projected up into focal plane and then magnified by the ocular lens to produce the final image, as illustrated through the light pathway of compound bright field microscope. Though magnification is important, it has limits. Unlimited enlargement by increasing magnifying power of the lenses is not possible because of the limitations of resolving power.

Microscope should not only produce the enlarged image but also the clear image. **Resolution** or **resolving power** of the lens is its ability to separate two close points as separate entities.

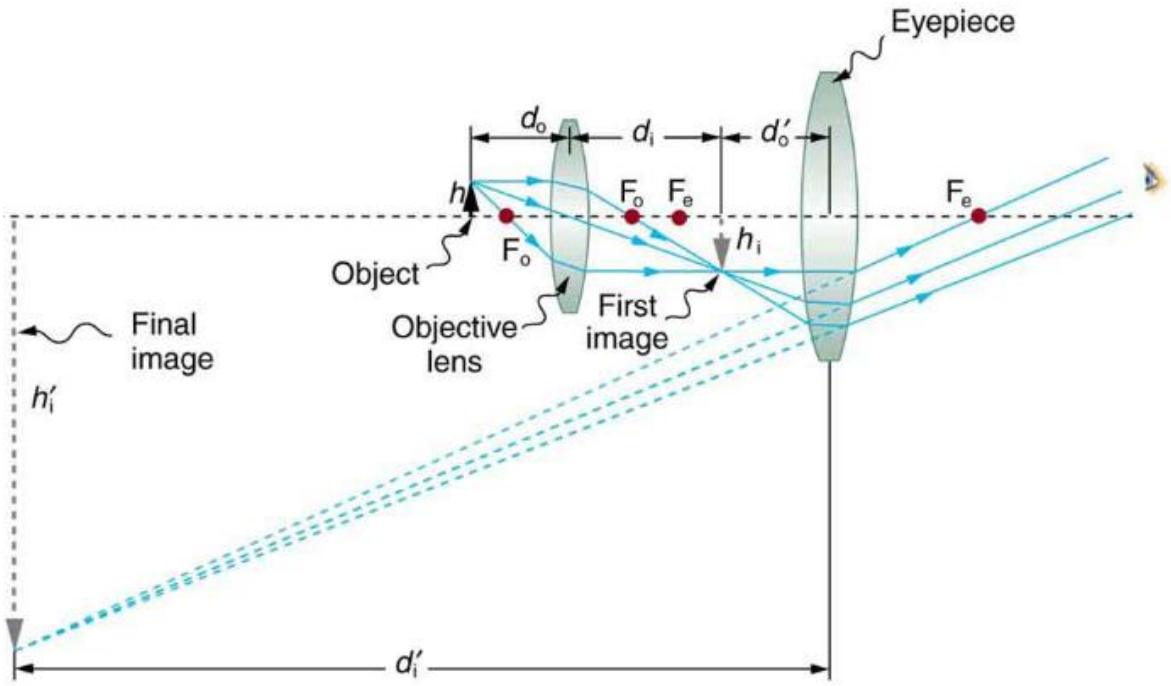
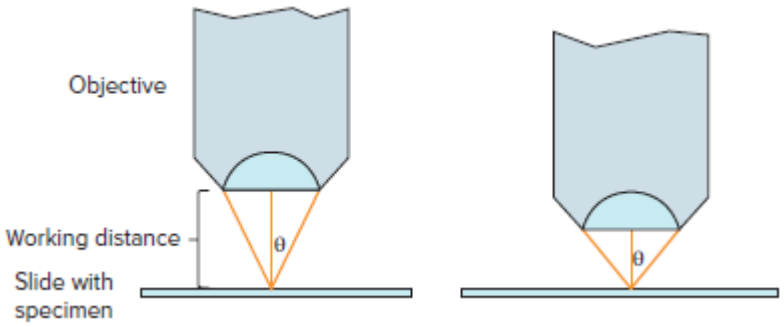


Figure: A compound microscope composed of two lenses, an objective and an eyepiece. The objective forms a case 1 image that is larger than the object. This first image is the object for the eyepiece. The eyepiece forms a case 2 final image that is further magnified.

The minimum distance (d) between two objects that reveals them as separate entities or resolving power of a lens can be calculated by Abbe's equation as:

$$d = \frac{0.5\,\lambda}{\eta sin\phi}$$

Where λ is the wavelength of the light used and η*sin* φ is the numerical aperture (NA).

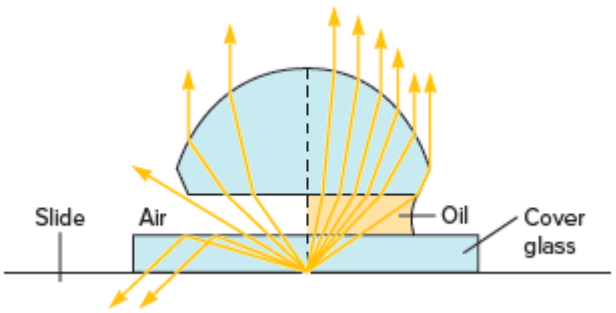


Numerical Aperture in Microscopy: The numerical aperture of a lens is related to a value called the angular aperture (symbolized by θ), which is 1/2 the angle of the cone of light that enters a lens from a specimen. The equation for numerical aperture is n sin θ. In the right-hand illustration, the lens has larger angular and numerical apertures; its resolution is greater and its working distance smaller.

Numerical aperture is a characteristic of each lens and is printed on the lens. It can be defined as a function of the diameter of the objective lens in relation to its focal length. It depends on the refractive index (η) of the medium in which the lens works and also upon the objective itself. Theta (φ) is defined as half of the angle of the cone of light entering an objective. Sin φ cannot be more than 1. Therefore, the lens working in air with refractive index 1 can have N.A.1. η will have to be increased for increasing the resolution. This can be done by using the mineral oil. Wavelength is also an important factor in resolution. With shorter wavelength, the resolving power increases. Just like magnification, resolution also has a limit. The smaller d is, the better the resolution, and finer detail can be discerned in a specimen; d becomes smaller as the wavelength of light used decreases and as the numerical aperture increases. Thus, the greatest resolution is obtained using a lens with the largest possible numerical aperture and light of the shortest wavelength, at the blue end of the visible spectrum (in the range of 450 to 500 nm). By decreasing wavelength, resolving power of the lens cannot be increased indefinitely because –

(a) the visible portion of the electromagnetic spectrum is very narrow and borders of very short wavelengths are found in ultraviolet range of spectrum.

(b) This relationship of Resolving Power with λ is valid only when light rays are parallel. As such 'Resolving Power' is also dependent on another factor, i.e., refractive index. When the light passes from air to glass slide, and from glass slide to air, there is a loss of light due to bending of rays. This reduces the numerical aperture and thus the resolving power of the lens. This loss in light can be compensated by using mineral oil in between glass slide and objective lens. Mineral oil is a colourless liquid having same refractive index as glass. This does decrease the bending of ray, as shown in Figure 1.5, so that more light rays enter the objective lens thus increasing the resolving power. Proper specimen illumination is also important in determining resolution.



Resolving Power of a microscope depends upon the numerical aperture of both condenser and objective, i.e.,

$$d_{\text{microscope}} = \frac{\lambda}{NA_{\text{Objective}} + NA_{\text{Condenser}}}$$

It was found that limit of resolving power of a microscope at best be about 0.2 μm and limit of magnification is about 1000 times the numerical aperture of the objective lens.

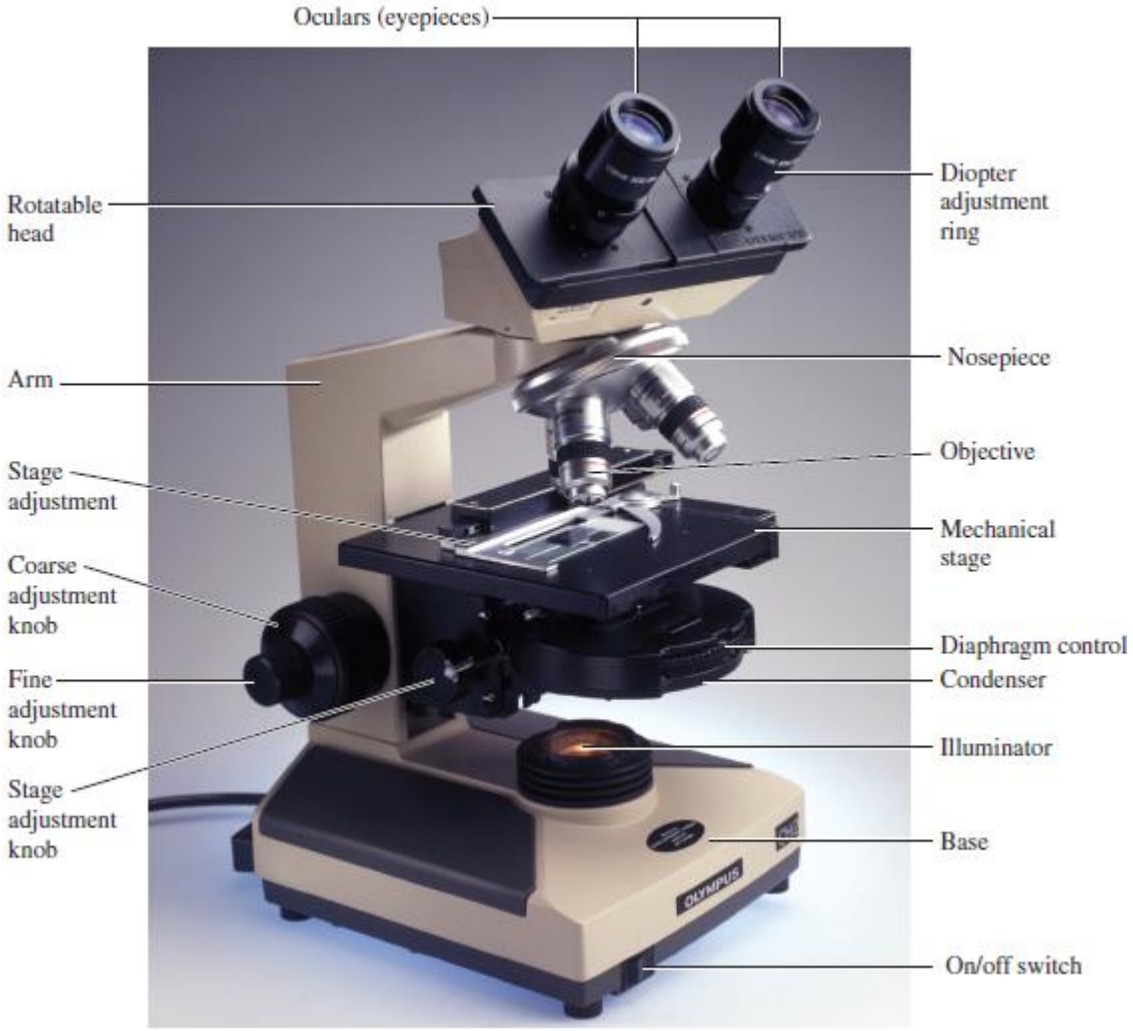


Figure: Compound Microscope

Parts of the Microscope: The components of the microscope are:

1. OCULAR LENS or EYEPIECE — On a binocular scope there are two ocular lenses, one for each eye. These lenses magnify the image at 10× power. The power of the ocular lens multiplied by the objective lens gives the total magnification of the microscope.

2. ARM — A support for the upper portion of the scope. It also serves as a convenient carrying handle.

3. MECHANICAL STAGE CONTROLS — A geared device to move the slide (placed in the slide clamp) precisely.

4. COARSE ADJUSTMENT KNOB — A rapid control which allows for quick focusing by moving the objective lens or stage up and down. It is used for initial focusing.

5. FINE ADJUSTMENT KNOB — A slow but precise control used to fine focus the image when viewing at the higher magnifications.

6. BASE — The part of your microscope that sits on a level, stable support.

7. OCULAR ADJUSTMENT — An adjustment for differences in the focusing abilities of your eyes.

8. DIOPTIC ADJUSTMENT — A horizontal adjustment of the oculars. Adjust for your eyes so you see only one field of view with both eyes open.

9. NOSEPIECE — A circular plate with 4 objective lenses that can be rotated into position for different magnifications.

10. OBJECTIVE LENS — Four separate lenses that magnify the image (10×, 40×, and 100×) depending on the objective in use. The lens is positioned just above the object being viewed.

11. SLIDE CLAMP — A clamp to hold the slide on the stage.

12. STAGE — A platform for placement of the microscope slide.

13. CONDENSER — A lens that concentrates or directs the light onto the slide.

14. IRIS DIAPHRAGM CONTROL — A lever (or rotating disk) that adjusts the amount of light illuminating the slide. Use just enough light to illuminate the object on the slide and give good contrast.

15. FILTER HOLDER — A blue filter rests in this holder below the substage condenser.

16. CONDENSER HEIGHT CONTROL — A knob that controls the height of the condenser.

17. LAMP — The light source.

18. LAMP SWITCH — Turns the lamp “on” and “off”.

Lens Systems of a Microscope: All compound microscopes have three lens systems: the oculars, the objectives, and the condenser. The **ocular**, or **eyepiece**, is a complex piece, located at the top of the instrument, that consists of two or more internal lenses and usually has a magnification of 10×. Most modern microscopes have two ocular (binocular) lenses. Note that they are attached to a rotatable nosepiece, which makes it possible to move them into position over a slide. **Objectives** on most laboratory microscopes have magnifications of 10×, 40×, and 100×, designated as low-power, high-dry, and oil immersion, respectively. The total magnification of a compound microscope is determined by multiplying the power of the ocular lens times the power of the objective lens used. Thus, the magnification of a microscope in which the oil immersion lens is being used is: 10×100=1000. The object is now magnified 1000 times its actual size. The third lens system is the condenser, which is located under the stage. It collects and directs the light from the lamp to the slide being studied. Unlike the ocular and objective lenses, the condenser lens does not affect the magnifying power of the compound microscope. The condenser can be moved up and down by a knob under the stage. A diaphragm within the condenser regulates the amount of light that reaches the slide. Microscopes that lack a voltage control on the light source rely entirely

on the diaphragm for controlling light intensity. In order to maximize the resolving power from a lens system, the following should be considered:

(i) A blue filter should be placed over the light source because the shorter wavelength of the resulting light will provide maximum resolution.

(ii) The condenser should be kept at the highest position that allows the maximum amount of light to enter the objective lens and therefore limit the amount of light lost due to refraction.

(iii) The diaphragm should not be stopped down too much. While closing the diaphragm improves the contrast, it also reduces the numerical aperture.

(iv) Immersion oil should be used between the slide and the 100× objective lens. This is a special oil that has the same refractive index as glass. When placed between the specimen and objective lens, the oil forms a continuous lens system that limits the loss of light due to refraction. Thus, a greater magnification cannot be achieved simply by adding a stronger ocular lens.

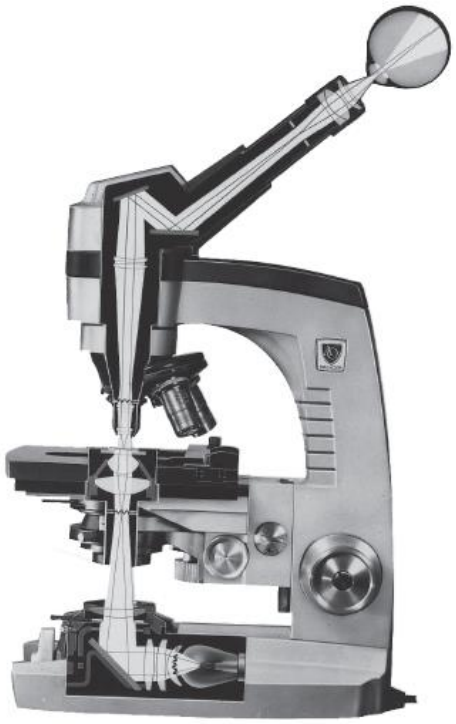


Figure: The light pathway of a microscope.

Use and Care of Microscope:

Proper care and maintenance of microscope is needed. Following points should be kept in mind while handling the microscope:

(i) Instrument should be kept in special cabinets while not in use.

(ii) Microscope should be held firmly by holding the arm with right hand and base with left arm.

(iii) All the lens systems should be cleaned with lens tissue to remove dust, oil, etc., which may decrease the efficiency of the microscope. Blotting paper, cloth or towel should not be used for cleaning.

(iv) If the lens is sticky or oily, the lens can be cleaned with xylol followed by 95% alcohol. The lens is wiped dry with lens paper. This should be performed only if necessary as consistent use of xylol may loosen the lens.

Method of Sterilization

Sterilization describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods.

The various methods of sterilization are:

1. Physical Method

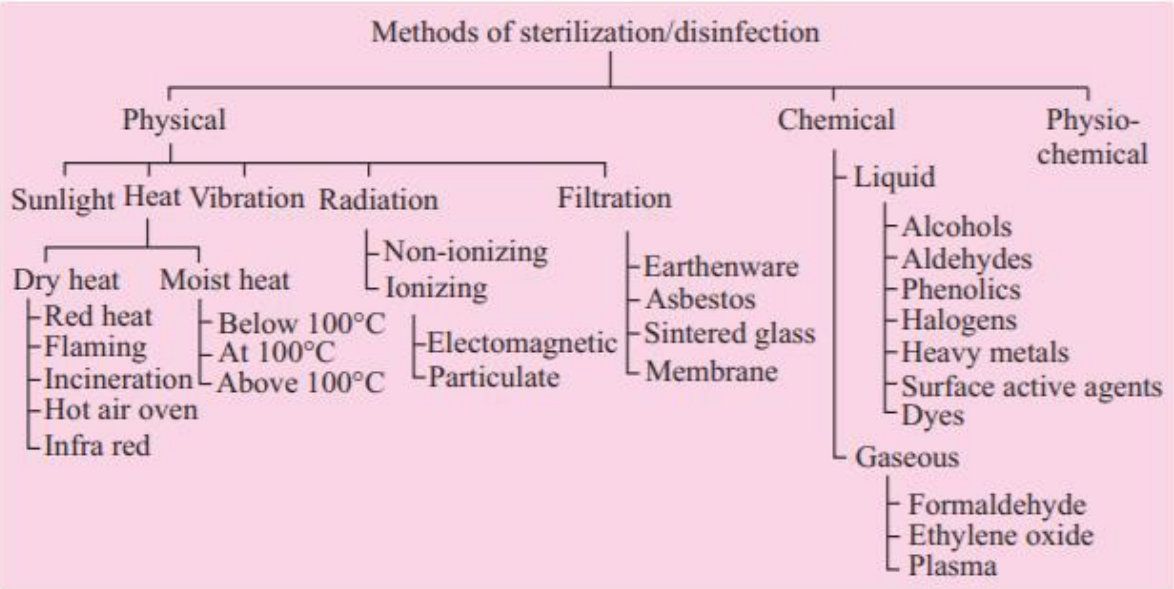
(a) Thermal (Heat) methods

(b) Radiation method

(c) Filtration method

2. Chemical Method

3. Gaseous method



Heat Sterilization: Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required.

This method of sterilization can be applied only to the thermostable products, but it can be used for moisture-sensitive materials for which dry heat (160-180°C) sterilization, and for moisture-resistant materials for which moist heat(121-134°C) sterilization is used.

The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water. The action of heat will be due to induction of lethal chemical events mediated through the action of water and oxygen. In the presence of water much lower temperature time exposures are required to kill microbe than in the absence of water. In these processes, both dry and moist heat are used for sterilization.

Dry Heat Sterilization: Examples of Dry heat sterilization are:

1. Incineration

2. Red heat

3. Flaming

4. Hot air oven

It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hours, depending upon the temperature employed. The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glass-wares and metal surgical instruments.

It is also used for sterilizing non-aqueous thermo-stable liquids and thermostable powders. Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.

Hot-Air Oven:

Dry heat sterilization is usually carried out in a hot air oven, which consists of the following:

(i) An insulated chamber surrounded by an outer case containing electric heaters.

(ii) A fan

(iii) Shelves

(iv) Thermocouples

(v) Temperature sensor

(vi) Door locking controls.

Operation:

(i) Articles to be sterilized are first wrapped or enclosed in containers of cardboard, paper or aluminium.

(ii) Then, the materials are arranged to ensure uninterrupted air flow.

(iii) Oven may be pre-heated for materials with poor heat conductivity.

(iv) The temperature is allowed to fall to 40°C, prior to removal of sterilized material.

Autoclave: The autoclave is a steam-pressure sterilizer. Steam is the vapour given off by water when it boils at 100°C. If steam is trapped and compressed, its temperature rises as the pressure on it increases. As pressure is exerted on a vapour or gas to keep it enclosed within a certain area, the energy of the gaseous molecules is concentrated and exerts equal pressure against the opposing force. The energy of pressurized gas generates heat as well as force. Thus, the temperature of steam produced at 100°C rises sharply above this level if the steam is trapped within a chamber that permits it to accumulate but not to escape. A kitchen pressure cooker illustrates this principle because it is, indeed, an ―autoclave. When a pressure cooker containing a little water is placed over a hot burner, the water soon comes to a boil. If the lid of the cooker is then clamped down tightly while heating continues, steam continues to be generated but, having nowhere to go, creates pressure as its temperature climbs steeply. This device may be used in the kitchen to speed cooking of food, because pressurized steam and its high temperature (120 to 125°C) penetrates raw meats and vegetables much more quickly than does boiling water or its dissipating steam. In the process, any microorganisms that may also be present are similarly penetrated by the hot pressurized steam and destroyed.

Essentially, an autoclave is a large, heavy-walled chamber with a steam inlet and an air outlet. It can be sealed to force steam accumulation. Steam (being lighter but hotter than air) is admitted through an inlet pipe in the upper part of the rear wall. As it rushes in, it pushes the cool air in the chamber forward and down through an air discharge line in the floor of the chamber at its front. When all the cool air has been pushed down the line, it is followed by hot steam, the temperature of which triggers a thermostatic valve placed in the discharge pipe. The valve closes off the line and then, as steam continues to enter the sealed chamber, pressure and temperature begin to build up quickly. The barometric pressure of normal atmosphere is about 15 lb to the square inch. Within an autoclave, steam pressure can build to 15 to 30 lb per square inch above atmospheric pressure, bringing the temperature up with it to 121 to 123°C. Steam is wet and penetrative to begin with, even at 100°C (the boiling point of water). When raised to a high temperature and driven by pressure, it penetrates thick substances that would be only superficially bathed by steam at atmospheric pressure. Under autoclave conditions, pressurized steam kills bacterial endospores, vegetative bacilli, and other microbial forms quickly and effectively at temperatures much lower and less destructive to materials than are required in a dry-heat oven (160 to 170°C).

Temperature and time are the two essential factors in heat sterilization. In the autoclave (steam-pressure sterilizer), it is the intensity of steam temperature that sterilizes (pressure only provides the means of creating this intensity), when it is given time measured according to the nature of the load in the chamber. In the dry-heat oven, the temperature of the hot air (which is not very penetrative) also sterilizes, but only after enough time has been allowed to

heat the oven load and oxidize vital components of microorganisms without damaging materials.

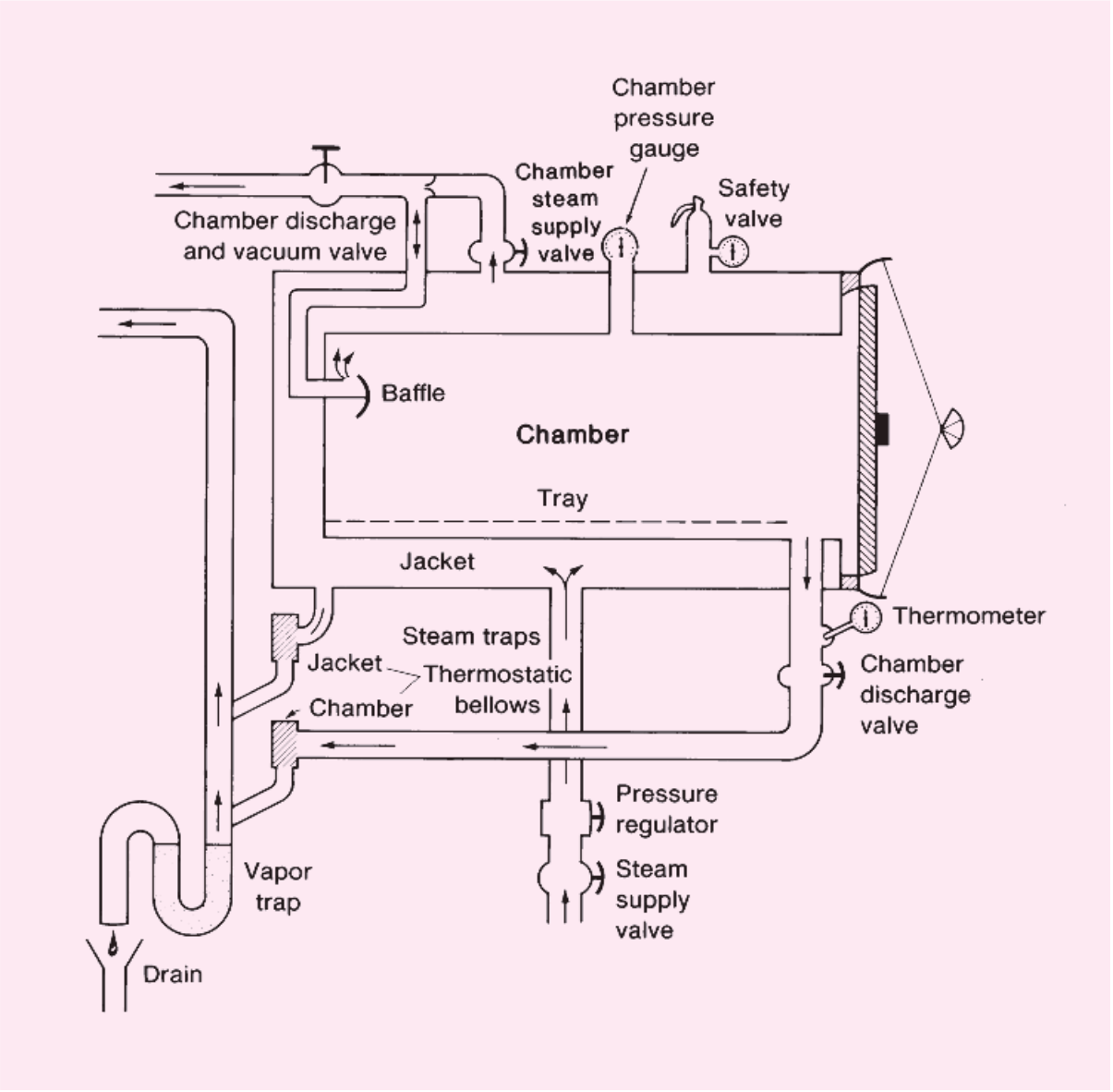


Fig. Autoclave

Table. Pressure-Temperature-Time Relationships in Steam-Pressure Sterilization.

Temperature^{0}C	Steam pressure(lb/sq.inch)	Holding time (minutes)
115-118	10	30
121-124	15	15
126-129	20	10
135-138	30	3

Filtration Sterilization: Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non-viable particles. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas. They are also used in industry as part of the venting systems on fermentors, centrifuges, autoclaves and freeze driers.

Membrane filters are used for sterility testing.

Application of filtration for sterilization of gases: HEPA (High efficiency particulate air) filters can remove up to 99.97% of particles >0.3 micrometer in diameter. Air is first passed through prefilters to remove larger particles and then passed through HEPA filters. The performance of HEPA filter is monitored by pressure differential and airflow rate measurements.

There are two types of filters used in filtration sterilization

(a) **Depth Filters:** Consist of fibrous or granular materials so packed as to form twisted channels of minute dimensions. They are made of diatomaceous earth, unglazed porcelain filter, sintered glass or asbestos.

(b) **Membrane Filters:** These are porous membrane about 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, and polyvinylidene fluoride, or some other synthetic material. The membranes are supported on a frame and held in special holders. Fluids are made to transverse membranes by positive or negative pressure or by centrifugation.

Application of filtration for sterilization of liquids: Membrane filters of 0.22 micrometer nominal pore diameter are generally used, but sintered filters are used for corrosive liquids, viscous fluids and organic solvents. The factors which affects the performance of filter is the titre reduction value, which is the ratio of the number of organisms challenging the filter under defined conditions to the number of organisms penetrating it. The other factors are the depth of the membrane, its charge and the tortuosity of the channels.

Laminar Air Flow: A Laminar flow hood/cabinet is an enclosed workstation that is used to create a contamination-free work environment through filters to capture all the particles entering the cabinet. These cabinets are designed to protect the work from the environment and are most useful for the aseptic distribution of specific media and plate pouring. Laminar flow cabinets are similar to biosafety cabinets with the only difference being that in laminar flow cabinets the effluent air is drawn into the face of the user. In a biosafety cabinet, both the sample and user are protected while in the laminar flow cabinet, only the sample is protected and not the user.

Components/ Parts of Laminar Flow Hood: A laminar flow cabinet consists of the following parts:

1. **Cabinet:** The cabinet is made up of stainless steel with less or no gaps or joints preventing the collection of spores. The cabinet provides insulation to the inner environment created inside the laminar flow and protects it from the outside environment. The front of the cabinet is provided with a glass shield which in some laminar cabinets opens entirely or in some has two openings for the users' hands to enter the cabinet.

2. **Working station:** A flat working station is present inside the cabinet for all the processes to be taken place. Culture plates, burner and loops are all placed on the working station where the operation takes place. The worktop is also made up of stainless steel to prevent rusting.

3. **Filter pad/ Pre-filter:** A filter pad is present on the top of the cabinet through which the air passes into the cabinet. The filter pad traps dust particles and some microbes from entering the working environment within the cabinet.

4. **Fan/ Blower:** A fan is present below the filter pad that sucks in the air and moves it around

in the cabinet. The fan also allows the movement of air towards the HEPA filter sp that the remaining microbes become trapped while passing through the filter.

5. **UV lamp:** Some laminar flow hoods might have a UV germicidal lamp that sterilizes the interior of the cabinet and contents before the operation. The UV lamp is to be turned on 15 minutes before the operation to prevent the exposure of UV to the body surface of the user.

6. **Fluorescent lamp:** Florescent light is placed inside the cabinet to provide proper light during the operation.

7. **HEPA filter:** The High-efficiency particulate air filter is present within the cabinet that makes the environment more sterile for the operation. The pre-filtered air passes through the filter which traps fungi, bacteria and other dust particles. The filter ensures a sterile condition inside the cabinet, thus reducing the chances of contamination.

Principle of Laminar flow hood: The principle of laminar flow cabinet is based on the laminar flow of air through the cabinet. The device works by the use of inwards flow of air through one or more HEPA filters to create a particulate-free environment. The air is taken through a filtration system and then exhausted across the work surface as a part of the laminar flow of the air. The air first passes through the filter pad or pre-filter that allows a streamline flow of air into the cabinet. Next, the blower or fan directs the air towards the HEPA filters. The HEPA filters then trap the bacteria, fungi and other particulate materials so that the air moving out of it is particulate-free air. Some of the effluent air then passes through perforation present at the bottom rear end of the cabinet, but most of it passes over the working bench while coming out of the cabinet towards the face of the operator. The laminar flow hood is enclosed on the sides, and constant positive air pressure is maintained to prevent the intrusion of contaminated external air into the cabinet.

Procedure for running the laminar flow cabinet:

I. The procedure to be followed while operating a laminar flow cabinet is given below:

II. Before running the laminar flow cabinet, the cabinet should be checked to ensure that nothing susceptible to UV rays is present inside the cabinet.
III. The glass shield of the hood is then closed, and the UV light is switched on. The UV light should be kept on for about 15 minutes to ensure the surface sterilization of the working bench.
IV. The UV light is then switched off, and a time period of around 10 minutes is spared before the airflow is switched on.
V. About 5 minutes before the operation begins, the airflow is switched on.
VI. The glass shield is then opened, and the fluorescent light is also switched on during the operation.
VII. To ensure more protection, the working bench of the cabinet can be sterilized with other disinfectants like 70% alcohol.
VIII. Once the work is completed, the airflow and florescent lamp both are closed and the glass
IX. shield is also closed.

Types of Laminar Flow Cabinet: Depending on the direction of movement of air, laminar flow cabinets are divided into two types:

1. **Vertical Laminar Flow Cabinet:** In the vertical flow cabinets, the air moves from the top of the cabinet directly towards the bottom of the cabinet. A vertical airflow working bench does not require as much depth and floor space as a horizontal airflow hood which makes it more manageable and decreases the chances of airflow obstruction or movement of contaminated air downstream. The vertical laminar flow cabinet is also considered safer as it doesn‘t blow the air directly towards the person carrying out the experiments.

2. **Horizontal Laminar Flow Cabinet:** In the horizontal laminar flow cabinets, the surrounding air comes from behind the working bench, which is then projected by the blower towards the HEPA filters. The filtered air is then exhausted in a horizontal direction to the workplace environment. One advantage of this cabinet is that airflow parallel to the workplace cleanses the environment with a constant velocity. The eluent air directly hits the operator, which might reduce the security level of this type of laminar flow cabinets.

Uses of Laminar Flow Hood: The following are some common uses of a laminar flow cabinet in the laboratory:

I. Laminar flow cabinets are used in laboratories for contamination sensitive processes like plant tissue culture.
II. Other laboratories processes like media plate preparation and culture of organisms can be performed inside the cabinet.
III. Operations of particle sensitive electronic devices are performed inside the cabinet.
IV. In the pharmaceutical industries, drug preparation techniques are also performed inside the cabinet to ensure a particulate-free environment during the operations.
V. Laminar flow cabinets can be made tailor-made for some specialized works and can also be used for general lab techniques in the microbiological as well as the industrial sectors.

Precautions: While operating the laminar airflow, the following things should be considered-

I. The laminar flow cabinet should be sterilized with the UV light before and after the operation.
II. The UV light and airflow should not be used at the same time.
III. No operations should be carried out when the UV light is switched on.
IV. The operator should be dressed in lab coats and long gloves.
V. The working bench, glass shield, and other components present inside the cabinet should be sterilized before and after the completion of work.

Biosafety Cabinets: Biosafety Cabinets (BSCs) are enclosed workspaces with a ventilated hood that is designed to contain pathogenic microorganisms during microbiological processes.

The primary purpose of biosafety cabinets is to protect the laboratory personnel and the environment from the pathogenic microorganism as aerosols might be formed during the processing of such microorganisms.

Biosafety cabinets are only used for certain risk group organisms and for processes that might result in aerosol formation.

These cabinets are provided with HEPA-filters that decontaminate the air moving out of the cabinet.

Biosafety cabinets might be confused with the laminar hood as both of these pieces of equipment work as enclosed workspaces. But, laminar hood only provides protection to the sample and not to the personnel and the environment, whereas biosafety cabinets protect all three.

The use of biosafety cabinets or other such physical containment is not required in the biosafety level 1, but depending on the risk assessment, some processes might require such containment.

BSCs are an essential part of biosafety as they minimize the formation of aerosol, protecting the environment, the pathogen, and the laboratory personnel.

Besides, most BSCs also function to sterilize biological materials that are kept inside the cabinets.

Nutritional media and their Preparation

Principle: Different types of media have been formulated for growing bacteria. Media generally contain a carbon source, nitrogen source and some essential minerals and salts. Some media may contain additional nutritional supplements. In addition, solid media contain agar as a solidifying agent. Meat extract and peptone are the commonest sources of carbohydrates and amino acids.

Media are of different types. These are:

1. **Basal Media:** These contain nutrients that support the growth of non-fastidious bacteria. They do not confer any selective advantage, e.g. nutrient agar.

2. **Enriched Medium:** These are solid selective media. These media, in addition to basal nutrients also contain nutritional supplements like blood, serum, etc., which favour the growth of fastidious bacteria. e.g. blood agar, chocolate agar, Löwenstein- Jensen medium, etc.

3. **Enrichment Media:** These are liquid selective media. They favor the growth of some bacteria by extending the lag phase of others e.g. Selenite F broth.

4. **Selective Media:** These media contain ingredients that selectively enable the growth of some species, while inhibiting others e.g. Deoxycholate citrate agar (DCA) medium. This medium is a selective medium for growth of *Salmonella* spp. present in stool which contains a mixed bacteria flora. This medium inhibits *Escherichia coli* and other Gram-negative bacteria.

5. **Differential Media:** These media differentiate between species of bacteria depending on a specific property.

Example: MacConkey agar is a differential medium. This medium is used to demonstrate lactose fermenting properties, and differentiate between lactose and non-lactose fermenting bacteria.

Requirements:

I. Equipment: Bacteriological incubator.

II. Reagents and media: Different kinds of media such as nutrient agar, blood agar, MacConkey agar and Selenite F broth.

III. Specimen: 24-hour broth cultures of *Staphylococcus aureus, E. coli, Proteus mirabilis* and *Salmonella* spp.

Procedure:

1. Inoculate a loopful of the test organism, using a sterile inoculating loop, into appropriately labeled plates and tubes.

2. Incubate the plates and tubes for 18 hours at 37°C.

3. Examine the plate and tubes for growth and record observations.

Quality Control: 1. One un-inoculated set of media as sterility control

2. Nutrient agar: Colonies of non-fastidious bacteria such as *S. aureus*.

3. Blood agar: Haemolytic strain of *S. aureus* streaked on the plate surrounded by a zone of hemolysis.

4. MacConkey agar: Pink, lactose fermenting colonies of *E. coli* and colorless colonies of Proteus spp.

5. Selenite F broth: Growth positive *Salmonella* spp, and growth negative *Proteus* spp.

6. Potato-Dextrose agar: Supporting the growth of fungi.

7. Czapek-Dox agar: Supporting the growth of fungi.

8. Sabouraud agar: Supporting the growth of fungi.

9. Starch casein agar: Supporting the growth of actinomyces.

10. Glycerol asparagine medium: Supporting the growth of actinomyces.

Observations: All the inoculated bacteria (e.g. *S. aureus, E. coli, P. mirabilis* and *Salmonella* spp) produce colonies on the nutrient agar (basal medium) and blood agar (enriched medium). In addition, *S. aureus* may or may not produce haemolysis on the blood agar.

Enumeration of Microbes

Principle: Studies involving the analysis of materials, including food, water, milk, and in some cases—air, require quantitative enumeration of microorganisms in the substances. Many methods have been devised to accomplish this, including direct microscopic counts, use of an electronic cell counter such as the Coulter Counter, chemical methods for estimating cell mass or cellular constituents, turbidimetric measurements for increases in cell mass, and the serial dilution–agar plate method.

Serial Dilution–Agar Plate Analysis: While all these methods (Coulter Counter, chemical methods, turbidimetric measurements) may be used to enumerate the number of cells in a bacterial culture, the major disadvantage common to all is that the total count includes dead as well as living cells. Sanitary and medical microbiology, at times, require determination of viable cells. To accomplish this, the serial dilution–agar plate technique is used. Briefly, this method involves serial dilution of a bacterial suspension in sterile water blanks, which serve as a diluent of known volume. Once diluted, the suspensions are placed on suitable nutrient media. The pour-plate technique is usually employed. Molten agar, cooled to 45°C, is poured into a Petri dish containing a specified amount of the diluted sample. Following addition of the molten-then-cooled agar, the cover is replaced, and the plate is gently rotated in a circular motion to achieve uniform distribution of microorganisms. This procedure is repeated for all dilutions to be plated. Dilutions should be plated in duplicate for greater accuracy, incubated overnight, and counted on a Quebec colony counter either by hand or by an electronically modified version of this instrument. Plates suitable for counting must contain neither fewer than 30 nor more than 300 colonies. The total count of the suspension is obtained by multiplying the number of cells per plate by the dilution factor, which is the reciprocal of the dilution. Advantages of the serial dilution–agar plate technique are- (i) Only viable cells are counted; and (ii) It allows isolation of discrete colonies that can be sub-cultured into pure cultures, which may then be easily studied and identified.

Materials: 24- to 48-hour nutrient broth culture of *Escherichia coli*, test tubes, nutrient broth, agar, conical flask, autoclave, vortex mixture, non-adsorbent cotton, Petri dish, incubator, pipette, micropipette, colony counter.

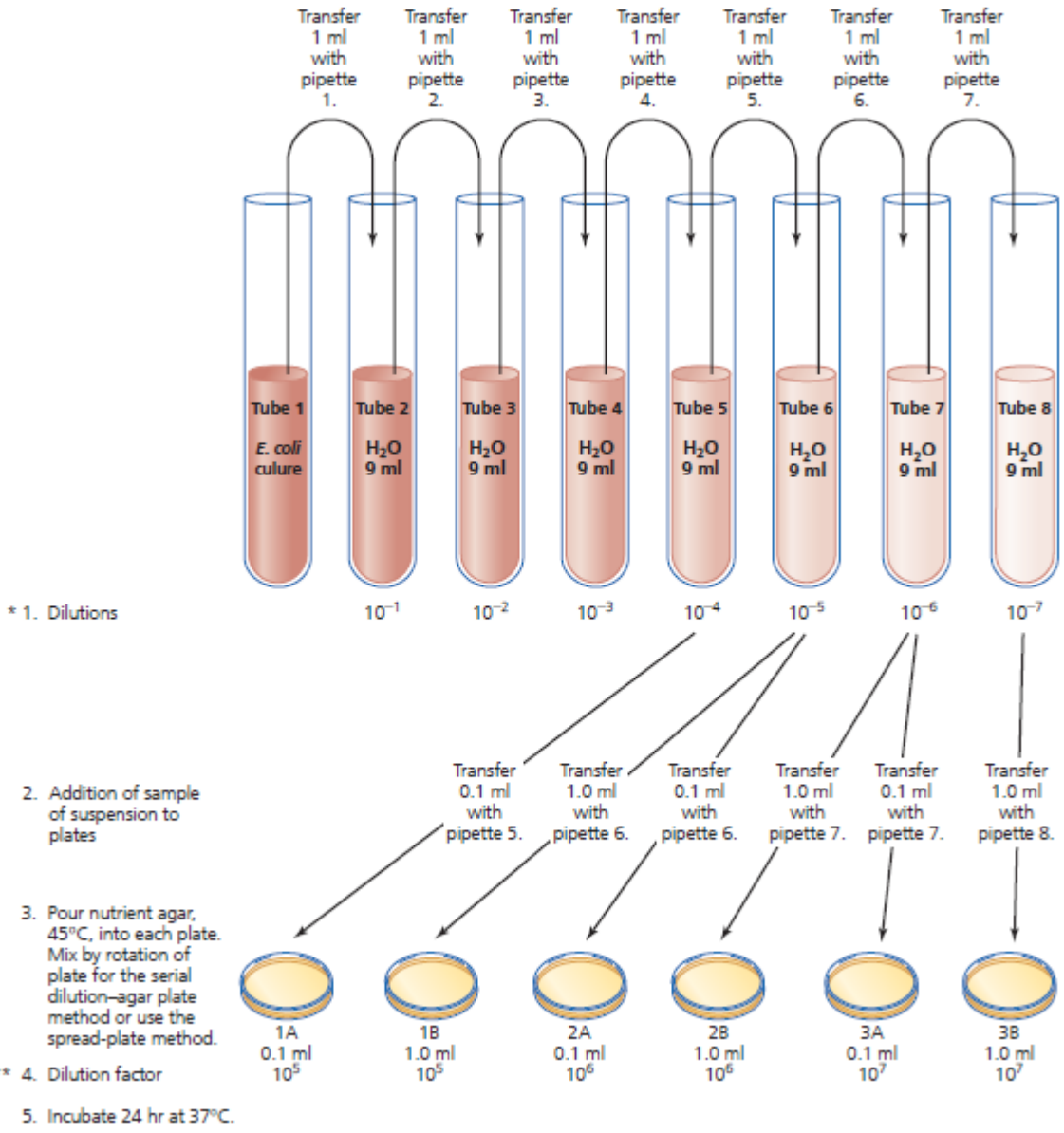
Procedure:

1. Liquefy six agar deep tubes in an autoclave. Cool the molten agar tubes and maintain in a water bath at 45°C.

2. Label the *E. coli* culture tube with the number 1 and the seven 9-ml water blanks as numbers 2 through 8. Place the labelled tubes in a test tube rack. Label the Petri dishes 1A, 1B, 2A, 2B, 3A, and 3B.

3. Mix the *E. coli* culture (Tube 1) by rolling the tube between the palms of hands to ensure even dispersal of cells in the culture.

4. With a sterile pipette, aseptically transfer 1 ml from the bacterial suspension, Tube 1, to water blank Tube 2. The culture had been diluted 10 times to 10^{-1} and follow this procedure for other such tubes.



5. Check the temperature of the molten agar medium to be sure the temperature was 45°C. Remove a tube from the water bath and wipe the outside surface dry with a paper towel. Using the pour-plate technique, pour the agar (100 µl) into Plate 1A and rotate the plate gently to ensure uniform distribution of the cells in the medium.

6. Repeat step 5 for the addition of molten nutrient agar to Plates 1B, 2A, 2B, 3A, and 3B. Once the agar had solidified, incubate the plates in an inverted position for 24 hours at 37°C.

7. After 24 h of incubation, Petri dish were counted for number of colonies grown. Using a Quebec colony counter and a mechanical hand counter, observe all colonies on plates. Statistically valid plate counts are only obtained from bacterial cell dilutions that yield between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated as too numerous to count—**TNTC**; plates with fewer than 30 colonies are designated as too few to count—**TFTC**. Count only plates containing between 30 and 300 colonies. Remember to count all subsurface as well as surface colonies.

8. The number of organisms per ml of original culture is calculated by multiplying the number of colonies counted by the dilution factor:

number of cells per ml = $\frac{number\ of\ colonies \times dilution\ factor}{volume\ (0.1\ ml)}$

Result:

No. of Colonies	Dilution Factor	CFU/ml
40	10^4	4×10^6
76	10^5	7.6×10^7

Interpretation: At 10^{-4} dilution of a soil sample, 4×10^6 CFU/ml of bacteria was obtained.

Hanging Drop Preparation or Wet Mount

Principle: Bacteria, because of their small size and a refractive index that closely approximates that of water, do not lend themselves readily to microscopic examination in a living, unstained state. It is essential to differentiate between actual motility and Brownian movement, a vibratory movement of the cells due to their bombardment by water molecules in the suspension. Hanging-drop preparations and wet mounts make the movement of microorganisms easier to see because they slow down the movement of water molecules. Examination of living microorganisms is useful, however, to do the following:

1. Observe cell activities such as motility and binary fission.

2. Observe the natural sizes and shapes of the cells, considering that heat fixation (the rapid passage of the smear over the Bunsen burner flame) and exposure to chemicals during staining cause some degree of distortion.

Requirements: Compound light microscope, Cavity slide, Cover slip, Petroleum jelly, Normal Saline, Cultures.

Procedure:

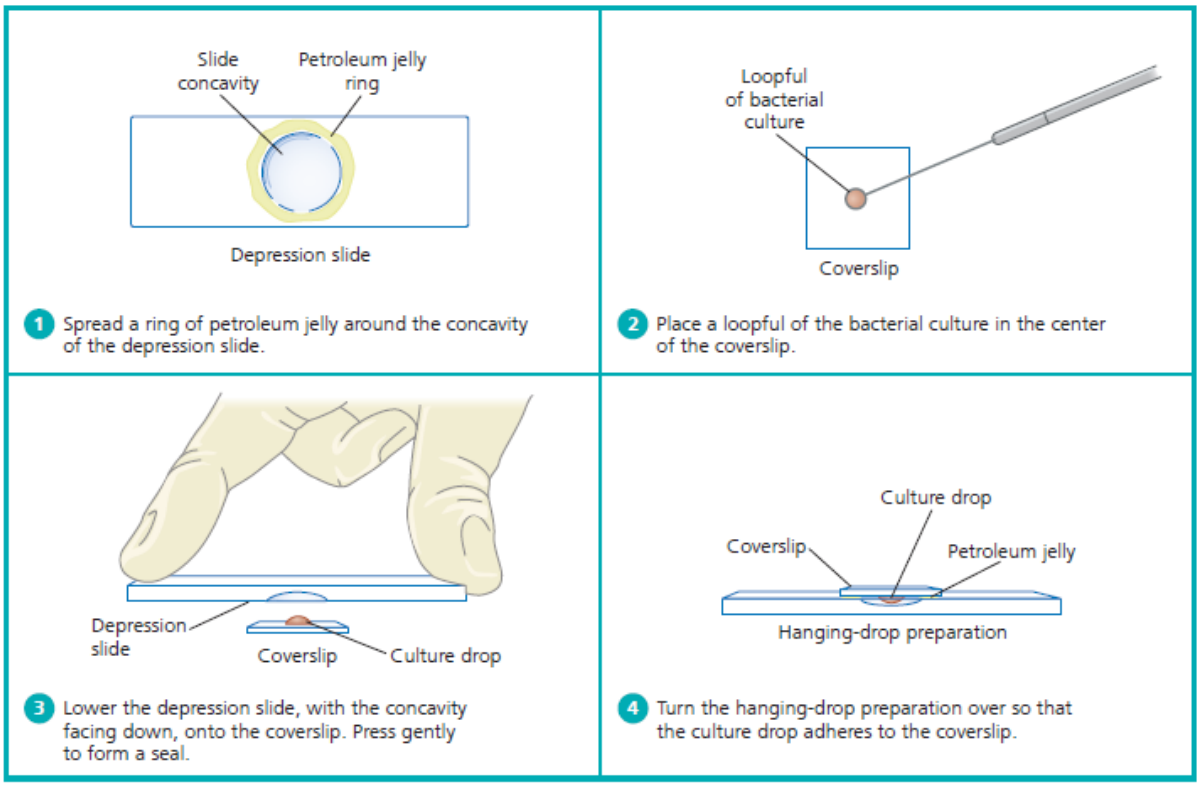
1. With a cotton swab, apply a ring of petroleum jelly around the concavity of the depression slide.

2. Using aseptic technique, place a loopful of the culture in the center of a clean coverslip.

3. Place the depression slide, with the concave surface facing down, over the coverslip so that the depression covers the drop of culture. Press the slide gently to form a seal between the slide and the coverslip.

4. Quickly turn the slide right side up so that the drop continues to adhere to the inner surface of the coverslip.

5. For microscopic examination, first focus on the drop culture under the low-power objective (10×) and reduce the light source by adjusting the Abbé condenser. Repeat using the high-power objective (40×).



Observations: The bacteria showing motility are demonstrated in the hanging drop preparation.

Note: It is important to differentiate active motility from brownian movement. Brownian movement is not true motility, instead it is exibited due to movement of organism as a result of their collision with water molecules. This movement is usually seen around the axis of bacteria.

Isolation of Pure Culture

Introduction: In nature, microbial populations do not segregate themselves by species, but exist with a mixture of many other cell types. In the laboratory, to separate these populations into pure cultures is performed. These cultures contain only one type of organism and allow us to study their cultural, morphological, and biochemical properties.

Principle: The techniques commonly used for isolation of discrete colonies initially require that the number of organisms in the inoculum be reduced. The resulting diminution of the population size ensures that, following inoculation, individual cells will be sufficiently far apart on the surface of the agar medium to separate the different species.

Materials: Nutrient agar, conical flask, inoculation loop or spreader, Petri dish, natural sample.

Procedure:

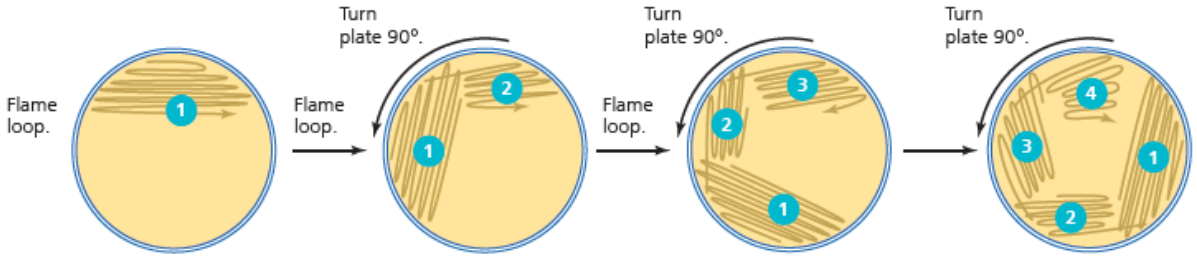
1. The **streak-plate method** is a rapid qualitative isolation method. It is a dilution technique that spreads a loopful of culture over the surface of an agar plate as a means to separate and dilute the microbes and ensure individual colony growth. There are many different procedures for preparing a streak plate-

a. Place a loopful of culture on the agar surface in Area 1. Flame the loop, cool it by touching it to an unused part of the agar surface close to the periphery of the plate, and then drag it rapidly several times across the surface of Area 1.

b. Reflame and cool the loop, and turn the Petri dish 90°. Then touch the loop to a corner of the culture in Area 1 and drag it several times across the agar in Area 2. The loop should never enter Area 1 again.

c. Reflame and cool the loop and again; turn the dish 90°. Streak Area 3 in the same manner as Area 2.

d. Without reflaming the loop, again turn the dish 90° and then drag the culture from a corner of Area 3 across Area 4, using a wider streak. Don't let the loop touch any of the previously streaked areas. The purpose of flaming of the loop at the points indicated is to dilute the culture so that fewer organisms are streaked in each area, resulting in the final desired separation.



Four-way streak-plate technique

2. The **spread-plate technique** requires also the diluted mixture of microorganisms. During inoculation, the cells were spread over the surface of a solid agar medium with a sterile, L-shaped bent glass rod while the Petri dish is spun on a turntable. The step-by-step procedure for this technique was as follows:

a. Place the bent glass rod into a beaker and add a sufficient amount of 95% ethyl alcohol to cover the lower, bent portion.

b. Place an appropriately labeled nutrient agar plate on the turntable. With a sterile pipette, place one drop of sterile water on the center of the plate, followed by a sterile loopful of Micrococcus luteus. Mix gently with the loop and replace the cover.

c. Remove the glass rod from the beaker, and pass it through the Bunsen burner flame with the bent portion of the rod pointing downward to prevent the burning alcohol from running down your arm. Allow the alcohol to burn off the rod completely. Cool the rod for 10 to 15 seconds.

d. Remove the Petri dish cover and spin the turntable. e. While the turntable is spinning, lightly touch the sterile bent rod to the surface of the agar and move it back and forth. This was spread the culture over the agar surface.

f. When the turntable comes to a stop, replace the cover. Immerse the rod in alcohol and reflame.

g. In the absence of a turntable, turn the Petri dish manually and spread the culture with the sterile bent glass rod.

3. The **pour-plate technique** requires a serial dilution of the mixed culture by means of a loop or pipette. The diluted inoculum is then added to a molten agar medium in a Petri dish, mixed, and allowed to solidify.

4. Incubate all plates in an inverted position for 48 to 72 hours at 25°C.

5. The colonies of microorganisms showed characteristics pattern on the Petri dish, which were documented. Particularly for bacteria, the well-isolated colonies were evaluated as follows-

1. **Size:** pinpoint, small, moderate, or large

2. **Pigmentation:** color of colony

3. **Form:** The shape of the colony is described as follows:

a. **Circular:** unbroken, peripheral edge

b. **Irregular:** indented, peripheral edge

c. **Rhizoid:** rootlike, spreading growth

4. **Margin:** The appearance of the outer edge of the colony is described as follows:

a. **Entire:** sharply defined, even

b. **Lobate:** marked indentations

c. **Undulate:** wavy indentations

d. **Serrate:** toothlike appearance

e. **Filamentous:** threadlike, spreading edge

5. **Elevation:** The degree to which colony growth is raised on the agar surface is described as:

a. **Flat:** elevation not discernible

b. **Raised:** slightly elevated

c. **Convex:** dome-shaped elevation

d. **Umbonate:** raised, with elevated convex central region

Result: Three types of colonies were clearly identified- (i) small size, yellow color, circular form with serrate elevation; (ii) small size, milky appearance, entire margin with convex elevation; and (iii) large size, off-white color, lobate margin with undulate margin.

IMViC Test

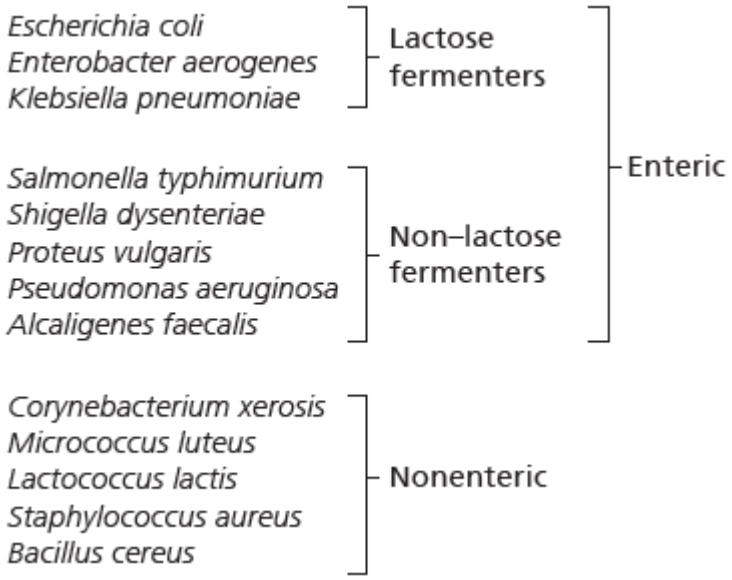
Introduction: The groups of bacteria that will be isolated from the intestinal tract of humans and lower mammals are classified as members of the family Enterobacteriaceae. They are short, gram-negative, non–spore-forming bacilli. Included in this family are

1. Pathogens, such as members of the genera *Salmonella* and *Shigella*.

2. Occasional pathogens, such as members of the genera *Proteus* and *Klebsiella.*

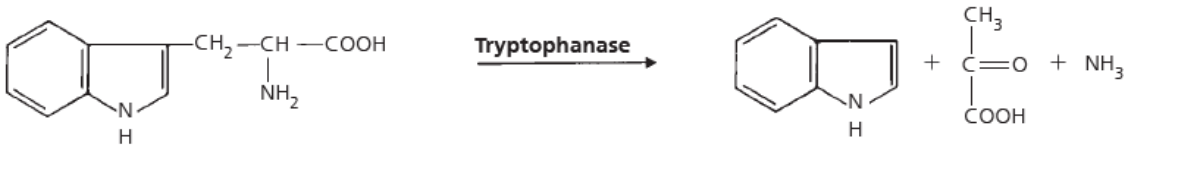
3. Normal intestinal flora, such as members of the genera *Escherichia* and *Enterobacter*, which are saprophytic inhabitants of the intestinal tract.

Microbiologists use the IMViC test to identify members of the Enterobacteriaceae, some of which are powerful pathogens such as members of the genera *Shigella* and *Salmonella*, which cause intestinal infections. Identification of the causative agent may lead to the source of the infection, such as raw food (*Salmonella*) or fecal contamination of food (*Shigella*). This aid healthcare workers in determining the possible number of individuals who have been exposed and who may require medical attention. This test uses the organisms' biochemical properties and enzymatic reactions on specific substrates as a means of identification.

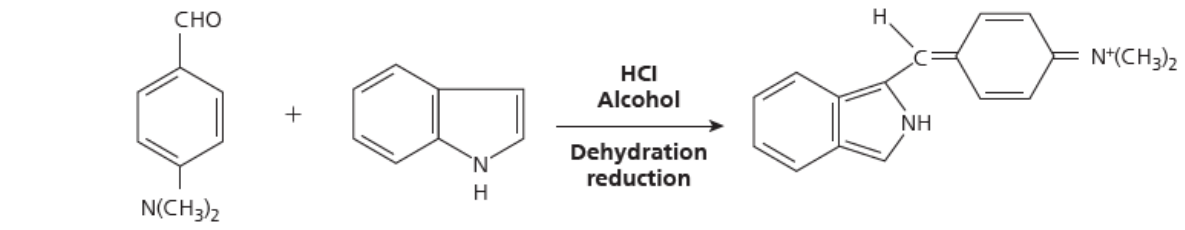


Indole Test

Principle: Tryptophan is an essential amino acid that can undergo oxidation by way of the enzymatic activities of some bacteria. The enzyme tryptophanase mediates the conversion of tryptophan into metabolic products. The below depicted figure showing the chemistry of this reaction. This ability to hydrolyze tryptophan with the production of indole is not a characteristic of all microorganisms and therefore serves as a biochemical marker. In this experiment, tryptone broth, which contains the substrate tryptophan, is used. The presence of indole is detectable by adding Kovac's reagent, which produces a cherry red reagent layer. The reagent produces this color, which is composed of p-dimethylaminobenzaldehyde, butanol, and hydrochloric acid. Indole is extracted from the medium into the reagent layer by the acidified butyl alcohol component and forms a complex with the p-dimethylaminobenzaldehyde, yielding the cherry red color.



Enzymatic degradation of tryptophan



Indole reaction with Kovac's reagent

Materials: Test tubes, beaker, tryptone broth, Kovac's reagent.

Procedure:

1. Using aseptic technique, inoculate each experimental organism into its appropriately labelled deep tube by means of a stab inoculation. The last tube will serve as a control.

2. Incubate tubes for 24 to 48 hours at 37°C.

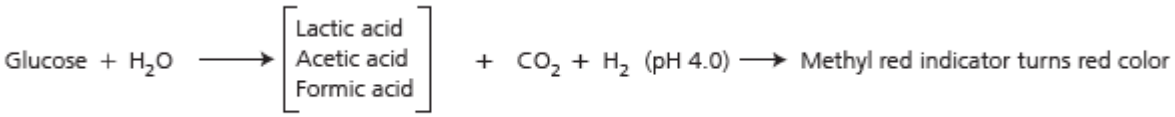
3. Add 10 drops of Kovac's reagent to all deep tube cultures and agitate the cultures gently.

4. Examine the color of the reagent layer in each culture.

Interpretation: Cultures producing a red reagent layer following addition of Kovac's reagent are indole-positive; an example of this is *E. coli*. The absence of red coloration demonstrates that the substrate tryptophan was not hydrolyzed and indicates an indole negative reaction.

Methyl Red Test Voges-Proskauer Test (MR-VP)

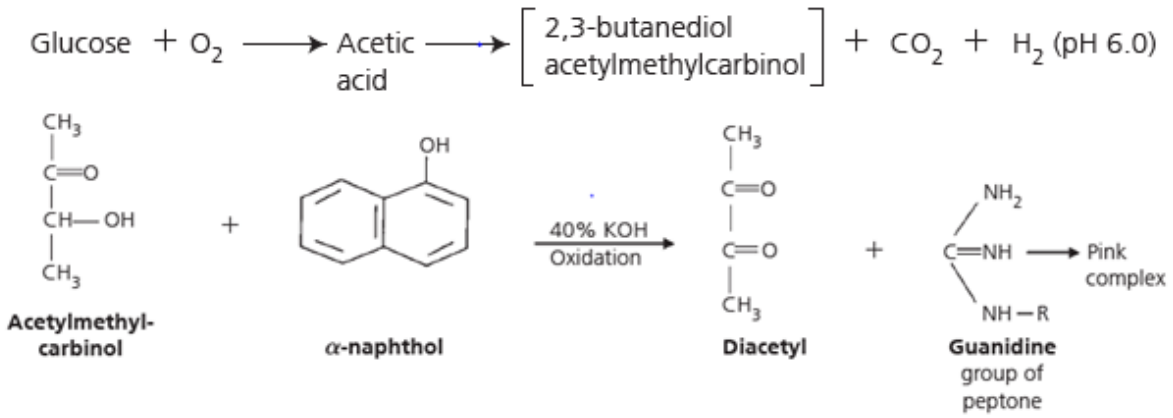
Principle: The hexose monosaccharide glucose is the major substrate utilized by all enteric organisms for energy production. The end products of this process will vary depending on the specific enzymatic pathways present in the bacteria. In this test, the pH indicator methyl red detects the presence of large concentrations of acid end products. Although most enteric microorganisms ferment glucose with the production of organic acids, this test is of value in the separation of *E. coli* and *E. aerogenes*. Both of these organisms initially produce organic acid end products during the early incubation period. *E. coli* stabilizes and maintains the low acidic pH (4) at the end of incubation. During the later incubation period, *E. aerogenes* enzymatically converts these acids to nonacidic end products, such as 2,3-butanediol and acetoin (acetylmethylcarbinol), resulting in an elevated pH of approximately 6. The below figure illustrates the glucose fermentation reaction generated by *E. coli*. At a pH of 4.4 or lower, the methyl red indicator in the pH range of 4 will turn red, which is indicative of a positive test. At a pH of 6.2 or higher, still indicating the presence of acid but with a lower hydrogen ion concentration, the indicator turns yellow and is a negative test.



Glucose fermentation reaction with methyl red pH reagent

The Voges-Proskauer test determines the capability of some organisms to produce nonacidic or neutral end products, such as acetylmethylcarbinol, from the organic acids that result from glucose metabolism. The below figure illustrates this glucose fermentation, which is characteristic of *E. aerogenes*. The reagent used in this test, Barritt's reagent, consists of a mixture of alcoholic α-naphthol and 40% potassium hydroxide solution. Detection of acetylmethylcarbinol requires this end product to be oxidized to a diacetyl compound. This reaction will occur in the presence of the α-naphthol catalyst and a guanidine group that is present in the peptone of the MR-VP medium. As a result, a pink complex is formed,

imparting a rose color to the medium. The below figure illustrates the chemistry of this reaction.



Acetylmethylcarbinol reaction with Barritt's reagent

Materials: Test tubes, beaker, MR-VP broth, methyl red, Barritt's reagent.

Procedure:

1. Transfer approximately one-third of each culture into an empty test tube and label these tubes for the Voges-Proskauer test.

2. Add five drops of the methyl red indicator to the remaining aliquot of each culture (MR tube).

3. Examine the color of all cultures. 4. Based on your observations, determine and record whether each organism was capable of fermenting glucose with the production and maintenance of a high concentration of acid.

5. To the aliquots of each broth culture separated from step 1, add 10 drops of Barritt's reagent A and shake the cultures. Immediately add 10 drops of Barritt's reagent B and shake. Reshake the cultures every 3 to 4 minutes.

6. Examine the color of the cultures 15 minutes after the addition of Barritt's reagent.

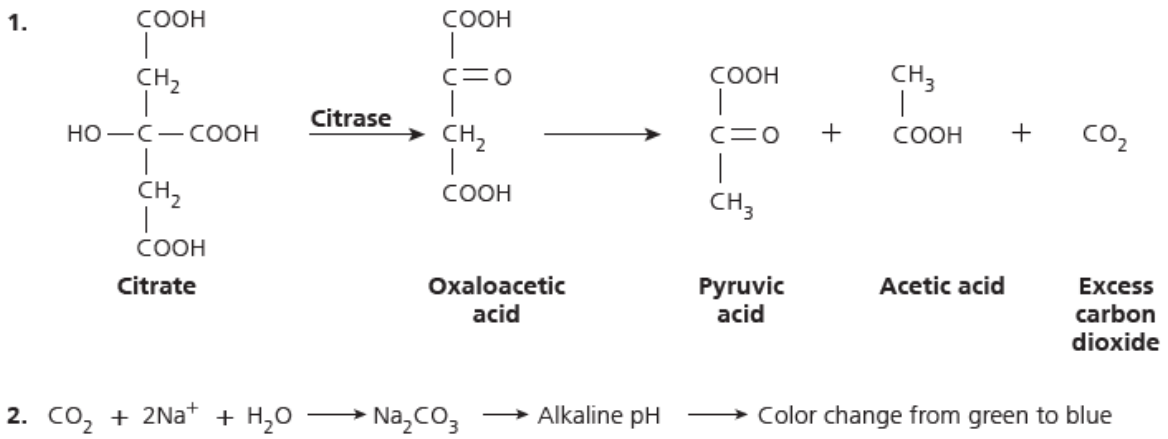
7. Based on your observations, determine and record whether each organism was capable of fermenting glucose with ultimate production of acetylmethylcarbinol.

Interpretation: Test tube showing red color is an indication of MR-positive result, test tube showing pink color is an indication of VP-positive result.

Citrate Utilization Test

Principle: In the absence of fermentable glucose or lactose, some microorganisms are capable of using citrate as a carbon source for their energy. This ability depends on the presence of a citrate permease that facilitates the transport of citrate in the cell. Citrate is the

first major intermediate in the Krebs cycle, and is produced by the condensation of active acetyl with oxaloacetic acid. Citrate is acted on by the enzyme citrase, which produces oxaloacetic acid and acetate. These products are then enzymatically converted to pyruvic acid and carbon dioxide. During this reaction, the medium becomes alkaline—the carbon dioxide that is generated combines with sodium and water to form sodium carbonate, an alkaline product. The presence of sodium carbonate changes the bromthymol blue indicator incorporated into the medium from green to deep Prussian blue. The below figure illustrates the chemistry of this reaction. Following incubation, citrate-positive cultures are identified by the presence of growth on the surface of the slant, which is accompanied by blue coloration, as seen with *E. aerogenes*. Citrate negative cultures will show no growth, and the medium will remain green.



Enzymatic degradation of citrate

Materials: Test tubes, beaker, Simmon's Citrate Agar.

Procedure:

1. Using aseptic technique, inoculate each organism into its appropriately labelled tube by means of streak inoculation. The last tube will serve as a control.

2. Incubate all cultures for 24 to 48 hours at 37°C.

3. Examine all agar slant cultures for the presence or absence of growth and coloration of the medium.

Interpretation: Test tube with green color changed to blue is an indication of positive result.

Cultivation of Fungi

Principle: Because the structural components of molds are very delicate, even simple handling with an inoculating loop may result in mechanical disruption of their components. The following slide culture technique is used to avoid such disruption. A deep concave slide containing a suitable nutrient medium with an acidic pH, such as Potato dextrose agar (PDA), is covered by a removable coverslip. Mold spores are deposited in the surface of the agar and

incubated in a moist chamber at room temperature. Direct microscopic observation is then possible without fear of disruption or damage to anatomical components. Molds can be identified as to spore type and shape, type of sporangia, and type of mycelium.

Materials: Cultures, Potato dextrose agar (PDA), Petridish, Lactophenol cotton blue, slide, cover slip.

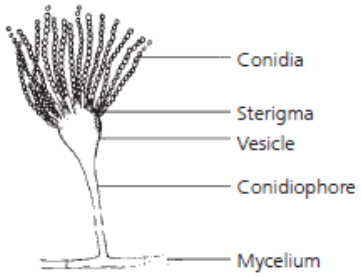
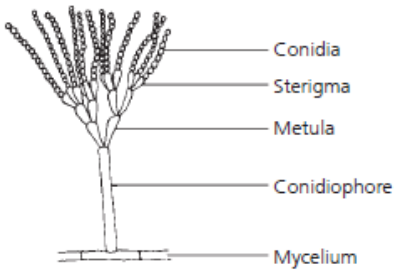
Procedure:

1. Following aseptic technique and with the help of a sterile inoculating loop, inoculated PDA plates with the supplied samples.

2. Incubate all plates at room temperature, 30°C, for 2 to 5 days. Note: Do not invert the plates.

3. 1. Examine each mold plate under the low and high power of a dissecting microscope by following LCB mount.

Observations and Result:

DIAGRAM	COLONIAL MORPHOLOGY	MICROSCOPIC APPEARANCE
 *Aspergillus:* Plant and animal pathogens; some species used industrially	White colonies become greenish-blue, black, or brown as culture matures	Single-celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophore, the vesicle; long conidiophores arise from a septate mycelium
 *Penicillium:* Antibiotic-producing citrus fruit contaminant; soil inhabitant	Mature cultures usually greenish or blue-green	Single-celled spores (conidia) in chains develop at the end of the sterigma arising from the metula of the conidiophore; branching conidiophores arise from a septate mycelium

LCB Mount

Principle: The lactophenol cotton blue (LCB) wet mount preparation is the most widely used method of staining and observing fungi. It has the following constituents: 1) Phenol kills fungus; 2) Lactic acid acts as a clearing agent and helps preserve the fungal structures, 3)

Cotton blue is an aniline dye that stains the chitin in the fungal cell walls which adds colour to the fungal preparation thereby enhancing and contrasting the structures; and 4) Glycerol is a viscous substance that prevents drying of the prepared slide specimen.

Materials: Microscope slide and cover glass, and Microscope.

Procedure:

1) After grease free of the glass slide, a drop of LCB was placed on the slide.

2) Aseptically add the supplied sample mixed to the dye and placed a cover glass on it.

3) Observed under microscope.

Results and Interpretation: Lactophenol cotton blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. Fungal spores, hyphae, and fruiting structures stain blue while the background stains pale blue.

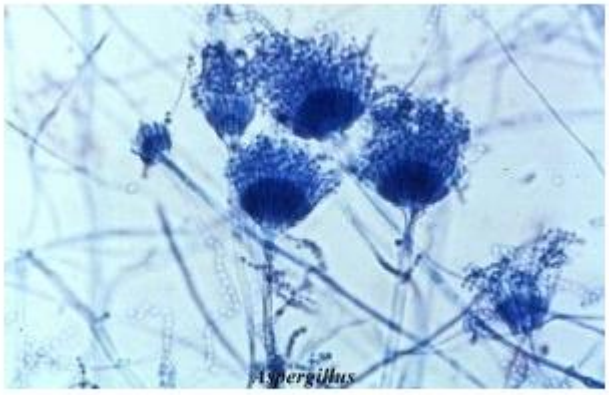


Fig: *Aspergillus* spp in LPCB Mount

Observation of Blue-Green algae

Anabaena

Domain: Bacteria

Phylum: Cyanobacteria

Class: Cyanophyceae

Order: Nostocales

Family: Nostocaceae

Genus: Anabaena

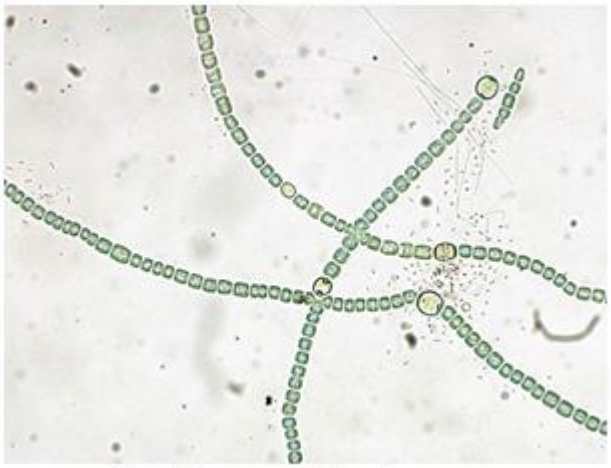


Fig.- Structure of *Anabaena* sp.

1. Anabaena has uniseriate, straight, curved, or coiled trichomes that may be constricted at the cell walls.

2. The blue-green to yellow-green colored cells may be spherical, ellipsoidal, cylindrical, or bent, but overall look much like a string of beads.

3. Some species have soft and colorless mucilage.

Others important characters are-

1. Groups of gas vesicles provide bouyancy in eutrophic or turbid waters for planktonic species.

2. Intercalary, solitary heterocysts for nitrogen fixation are spaced fairly regularly along the filament.

3. The akinetes are also intercalary, solitary or in groups of 2-5, are spherical, ellipsoidal, cylindrical, or curved in shape, and are sometimes found adjacent to the heterocysts.

4. Anabaena is similar in morphology to Nostoc, but has looser, more indistinct mucilage, filaments that are less constricted, akinetes in different locations, more motile hormogonia, and a different habitat.

Candida albicans

Division: Deuteromycotina

Class: Blastomycetes

Order: Cryptococcales

Family: Cryptococcaceae

Genus: *Candida*

Species: *albicans*

(i) *Candida albicans* is small, oval, and yeast like unicellular fungus.
(ii) It shows pseudomycelial growth and multilateral budding. The cell elongates and develops into pseudomycelium.
(iii) The genus comprises of imperfect forms of ascomycetous and basidiomycetous yeasts of various genera.
(iv) *C. albicans* is the imperfect state of Syringospora, which is a member of basidiomycotina.
(v) They form chlamydospores and produce spherical clusters of blastospores.
(vi) The colonies are moderate in size, smooth and pasty. The older colonies have honey comb like appearance in the centre and develop radical furrows.
(vii) The possess a capsular form of polysaccharide that shows pyrogenic activity.
(viii) Examples are *C. albicans*, *C. utilis*, etc.

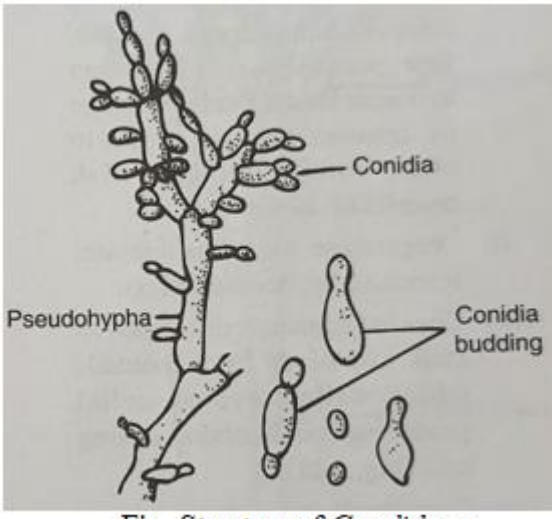


Fig.-Structure of *Candida* sp.

Aspergillus

Class: Deuteromycetes

Order: Moniliales

Family: Moniliaceae

Genus: *Aspergillus*

(i) Colonies on Czapek Dox agar are white (*A. versicolor*) on white at first and becoming yellowish (*A. flavipes*), blue green (*A. sydowi*), lime green (*A. flavus*), cinnamon to deeper brown shades with age (*A. terreus*), blackish brown to black with slight yellowish mycelia (*A. niger*).
(ii) Vegetative mycelim septate branched hyphae colourless.
(iii) Conidial apparatus developed as stalk and heads from footcells (thick-walled hyphal cells) producing conidiophores at long axis.
(iv) Conidiophores septate or un-septate, broadening into elliptical, hemispherical or globose fertile vesicles.
(v) Vesicles bear phialides in one series (uniseriate), or two series (biseriate).

(vi) Phialides clustered in terminal groups or radiating from entire surface.

(vii) Conidia (conidia bearing cells) elliptical, globose, smooth walled, rough or spinulose walls produced in chains.

(viii) Some species produce cleistothecia e.g. *A. versicolor*, *A. ruber*, some strains produce sclerotia e.g. *A. niger*; some species produce irregularly globose, ovoid or elongated heavily walled abundant hulls cells e.g. *A. granulosis*.

(ix) They play a significant role in production of amylase (a. niger), diastase (*A. flavipes*, *A. parasiticus*), otomycosis in humans (*A. niger*), etc.

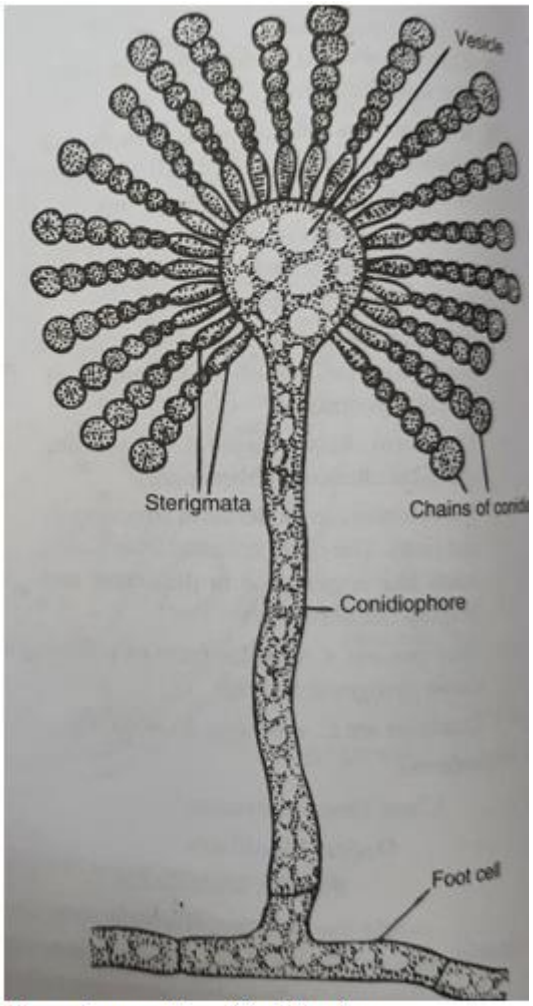
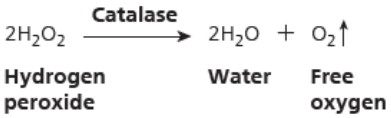


Fig.-*Aspergillus:* Conidiophore and chains of conidia

Catalase Test

Principle: During aerobic respiration, microorganisms produce hydrogen peroxide and, in some cases, an extremely toxic superoxide. Accumulation of these substances will result in death of the organism unless they can be enzymatically degraded. These substances are produced when aerobes, facultative anaerobes, and microaerophiles use the aerobic respiratory pathway, in which oxygen is the final electron acceptor, during degradation of carbohydrates for energy production. Organisms capable of producing catalase rapidly degrade hydrogen peroxide as illustrated:



Aerobic organisms that lack catalase can degrade especially toxic superoxides using the enzyme superoxide dismutase; the end product of a superoxide dismutase is H_2O_2, but this is less toxic to the bacterial cells than are the superoxides. The inability of strict anaerobes to synthesize catalase, peroxidase, or superoxide dismutase may explain why oxygen is poisonous to these microorganisms. In the absence of these enzymes, the toxic concentration of H_2O_2 cannot be degraded when these organisms are cultivated in the presence of oxygen. Catalase production can be determined by adding the substrate H_2O_2 to an appropriately incubated Trypticase soy agar slant culture. If catalase is present, the chemical reaction mentioned is indicated by bubbles of free oxygen gas $O_2\uparrow$. This is a positive catalase test; the absence of bubble formation is a negative catalase test.

Note: With the increasing worry about methicillin-resistant strains of *Staphylococcus* in hospitals, the catalase test is a quick and easy way to differentiate *S. aureus*, which may be methicillin-resistant *S. aureus* (MRSA), from other *Staphylococcus* species that have exhibited lower incidences of methicillin resistance.

Requirements: Slide, Hydrogen peroxide, Inoculating loop, Cultures.

Procedure:

1. Label slides with the names of the organisms.

2. Using a sterile loop, collect a small sample of the first organism from the culture tube and transfer it to the appropriately labelled slide.

3. Place the slide in the Petri dish.

4. Place one drop of 3% hydrogen peroxide on the sample. Do not mix. Place the cover on the Petri dish to contain any aerosols.

5. Observe for immediate presence of bubble formation.

Observations and Result: The supplied sample generated bubbles, therefore, it was catalase positive.

Gram Staining

Principle: The most important differential stain used in bacteriology is the Gram stain, named after Dr. Hans Christian Gram. It divides bacterial cells into two major groups, gram positive and gram negative, which makes it an essential tool for classification and differentiation of microorganisms. Differential staining requires the use of at least four chemical reagents that are applied sequentially to a heat-fixed smear. The first reagent is called the primary stain. Its function is to impart its color to all cells. The second stain is a mordant used to intensify the color of the primary stain. In order to establish a color contrast,

the third reagent used is the decolorizing agent. Based on the chemical composition of cellular components, the decolorizing agent may remove the primary stain from the entire cell or only from certain cell structures. The final reagent, the counterstain, has a contrasting color to that of the primary stain. Following decolorization, if the primary stain is not washed out, the counterstain cannot be absorbed, and the cell or its components will retain the color of the primary stain. If the primary stain is removed, the decolorized cellular components will accept and assume the contrasting color of the counterstain. In this way, cell types or their structures can be distinguished from each other on the basis of the stain that is retained.

The Gram stain reaction is based on the difference in the chemical composition of bacterial cell walls. Gram-positive cells have a thick peptidoglycan layer, whereas the peptidoglycan layer in gram-negative cells is much thinner and surrounded by outer lipid-containing layers. Peptidoglycan is a polysaccharide composed of two chemical subunits found only in the bacterial cell wall. These subunits are N-acetylglucosamine and N-acetylmuramic acid. With some organisms, as the adjacent layers of peptidoglycan are formed, they are cross-linked by short chains of peptides by means of a transpeptidase enzyme, resulting in the shape and rigidity of the cell wall. In the case of gram-negative bacteria and several of the gram-positive, such as the Bacillus, the cross-linking of the peptidoglycan layer is direct because the bacteria do not have short peptide tails. The Gram stain uses four different reagents.

Procedure:

I. A clean glass slide was obtained.

II. The smear was prepared by placing a drop of culture by using sterile inoculating loop.

III. The smear was allowed to air dry and then heat fixed by using Bunsen-burner.

IV. The smear was covered with several drops of crystal violet and incubated for 30 seconds to 1 minute.

V. The slide was gently washed with drops of tap water.

VI. The smear was then flooded with the Gram’s iodine and incubated for one minute.

VII. The slide was gently washed with drops of tap water.

VIII. The slide was then decolourized with 90% ethyl alcohol.

IX. The slide was air dried followed by counter staining with safranin for 45 seconds.

X. The slide was gently washed with drops of tap water.

XI. The slide was air dried and observed under microscope.

Result: Under microscope, the supplied sample bacterium seen as rod shaped and violet color; hence, it was a Gram-positive bacterium.

Negative Staining

Principle: Negative staining requires the use of an acidic stain such as India ink or nigrosin. The acidic stain, with its negatively charged chromogen, will not penetrate the cells, because of the negative charge on the surface of bacteria. Therefore, the unstained cells are easily discernible against the colored background. The practical application of negative staining is twofold. First, since heat fixation is not required and the cells are not subjected to the distorting effects of chemicals and heat, we can see their natural size and shape. Second, we can observe bacteria that are difficult to stain, such as some spirilla. Because heat fixation is not done during the staining process, keep in mind that the organisms are not killed and slides should be handled with care.

The principle application of negative staining is to determine if an organism possesses a capsule (a gelatinous outer layer that makes the microorganism more virulent), although it can also be used to demonstrate spore formation. The technique is frequently used in the identification of fungi such as Cryptococcus neoformans, an important infectious agent found in bird droppings that is linked to meningeal and lung infections in humans.

Materials: Nigrosin, 48 h old culture, glass slide, cover slip.

Procedure:

1. Place a small drop of nigrosin close to one end of a clean slide.

2. Using aseptic technique, place a loopful of inoculum from the supplied culture in the drop of nigrosin and mix.

3. Place a slide against the drop of suspended organisms at a 45° angle and allow the drop to spread along the edge of the applied slide.

4. Push the slide away from the drop of suspended organisms to form a thin smear and air dried.

Observations: Colorless cells were observed against the dark field background.

Spore Stain (Schaeffer-Fulton Method)

Principle: Members of the anaerobic genera *Clostridium* and *Desulfotomaculum* and the aerobic genus *Bacillus* are examples of organisms that have the capacity to exist either as metabolically active vegetative cells or as highly resistant, metabolically inactive cell types called spores. When environmental conditions become unfavourable for continuing vegetative cellular activities, particularly with the exhaustion of a nutritional carbon source, these cells have the capacity to undergo sporogenesis and give rise to a new intracellular structure called the endospore, which is surrounded by impervious layers called spore coats. As conditions continue to worsen, the endospore is released from the degenerating vegetative cell and becomes an independent cell called a free spore. Because of the chemical composition of spore layers, the spore is resistant to the damaging effects of excessive heat, freezing, radiation, desiccation, and chemical agents, as well as to the commonly employed

microbiological stains. With the return of favourable environmental conditions, the free spore may revert to a metabolically active and less resistant vegetative cell through germination. Note that sporogenesis and germination are not meaning of reproduction but merely mechanisms that ensure cell survival under all environmental conditions.

Malachite green (Primary Stain): Unlike most vegetative cell types that stain by common procedures, the free spore, because of its impervious coats, will not accept the primary stain easily. For further penetration, we must apply heat. After we apply the primary stain and heat the smear, both the vegetative cell and spore appear green.

Water (Decolorizing Agent): Once the spore accepts the malachite green, it cannot be decolorized by tap water, which removes only the excess primary stain. The spore remains green. On the other hand, the stain does not demonstrate a strong affinity for vegetative cell components; the water removes it, and these cells will be colorless.

Safranin (Counterstain): This contrasting red stain is used as the second reagent to color the decolorized vegetative cells, which will absorb the counterstain and appear red. The spores retain the green of the primary stain.

Materials: 24 h grown culture, malachite green, glass slide.

Procedure:

1. Obtain two clean glass slides.

2. Make individual smears in the usual manner using aseptic technique.

3. Allow smear to air-dry, and heat fix in the usual manner.

4. Flood the smears with malachite green and place on top of a water bath, allowing the preparation to steam for 2 to 3 minutes.

Note: Do not allow stain to evaporate; replenish stain as needed. Prevent the stain from boiling by adjusting the hot plate temperature.

5. Remove the slides from the hot plate, cool, and wash under running tap water.

6. Counterstain with safranin for 30 seconds.

7. Wash with tap water.

8. Blot dry with bibulous paper and examine under oil immersion.

Observations: Only pink color rod cells appeared under the microscope, therefore, the supplied sample is endospore negative.

MPN Test

Principle: The three basic tests to detect coliform bacteria in water are presumptive, confirmed, and completed. The tests are performed sequentially on each sample under

analysis. They detect the presence of coliform bacteria (indicators of fecal contamination), through the fermentation of lactose that will produce acid and gas that is detectable following a 24-hour incubation period at 37°C.

The Presumptive Test: The presumptive test is specific for detection of coliform bacteria. Measured aliquots of the water to be tested are added to a lactose fermentation broth containing an inverted gas vial. Because these bacteria are capable of using lactose as a carbon source (the other enteric organisms are not), their detection is facilitated by the use of this medium. In this experiment, you will use lactose fermentation broth containing an inverted Durham tube for gas collection. Tubes of this lactose medium are inoculated with 10-ml, 1-ml, and 0.1-ml aliquots of the water sample. The series consists of at least three groups, each composed of five tubes of the specified medium. The tubes in each group are then inoculated with the designated volume of the water sample, as described under "Procedure: Lab One." The greater the number of tubes per group, the greater the sensitivity of the test. Development of gas in any of the tubes is presumptive evidence of the presence of coliform bacteria in the sample. The presumptive test also enables the microbiologist to obtain some idea of the number of coliform organisms present by means of the most probable number (MPN) test. The MPN is estimated by determining the number of tubes in each group that show gas following the incubation period.

The Confirmed Test: The presence of a positive or doubtful presumptive test immediately suggests that the water sample is nonpotable. Confirmation of these results is necessary because positive presumptive tests may be the result of organisms of noncoliform origin that are not recognized as indicators of fecal pollution. The confirmed test requires that selective and differential media (e.g., eosin–methylene blue (EMB) or Endo agar) be streaked from a positive lactose broth tube obtained from the presumptive test. The nature of the differential and selective media was discussed in Experiment 14 but is reviewed briefly here. Eosin–methylene blue contains the dye methylene blue, which inhibits the growth of gram-positive organisms. In the presence of an acid environment, EMB forms a complex that precipitates out onto the coliform colonies, producing dark centers and a green metallic sheen. The reaction is characteristic for *Escherichia coli*, the major indicator of fecal pollution. Endo agar is a nutrient medium containing the dye fuchsin, which is present in the decolorized state. In the presence of acid produced by the coliform bacteria, fuchsin forms a dark pink complex that turns the *E. coli* colonies and the surrounding medium pink.

The Completed Test: The completed test is the final analysis of the water sample. It is used to examine the coliform colonies that appeared on the EMB or Endo agar plates used in the confirmed test. An isolated colony is picked up from the confirmatory test plate, inoculated into a tube of lactose broth, and streaked on a nutrient agar slant to perform a Gram stain. Following inoculation and incubation, tubes showing acid and gas in the lactose broth and presence of gram-negative bacilli on microscopic examination is further confirmation of the presence of *E. coli*, and they are indicative of a positive completed test.

Materials: Lactose broth, MacConkey agar, nutrient agar, test tubes, Petridish, slide, crystal violet, safranin, gram's iodine, 95% ethanol.

Procedure:

Presumptive Test

1. Examine the tubes from your presumptive test after 24 and 48 hours of incubation. Your results are positive if the Durham tube fills 10% or more with gas in 24 hours, doubtful if gas develops in the tube after 48 hours, and negative if there is no gas in the tube after 48 hours.

2. Determine the MPN using standard table, and record your results in the Lab Report.

Confirmed Test

1. Using a positive 24-hour lactose broth culture from the sewage water series from the presumptive test, streak the surface of one EMB or one Endo agar plate, to obtain discrete colonies.

2. Incubate all plate cultures in an inverted position for 24 hours at 37°C.

3. Examine all the plates from your confirmed test for the presence or absence of *E. coli* colonies.

4. Based on your results, determine whether each of the samples is potable or nonpotable. The presence of *E. coli* is a positive confirmed test, indicating that the water is nonpotable. The absence of *E. coli* is a negative test, indicating that the water is not contaminated with fecal wastes and is therefore potable.

Completed Test

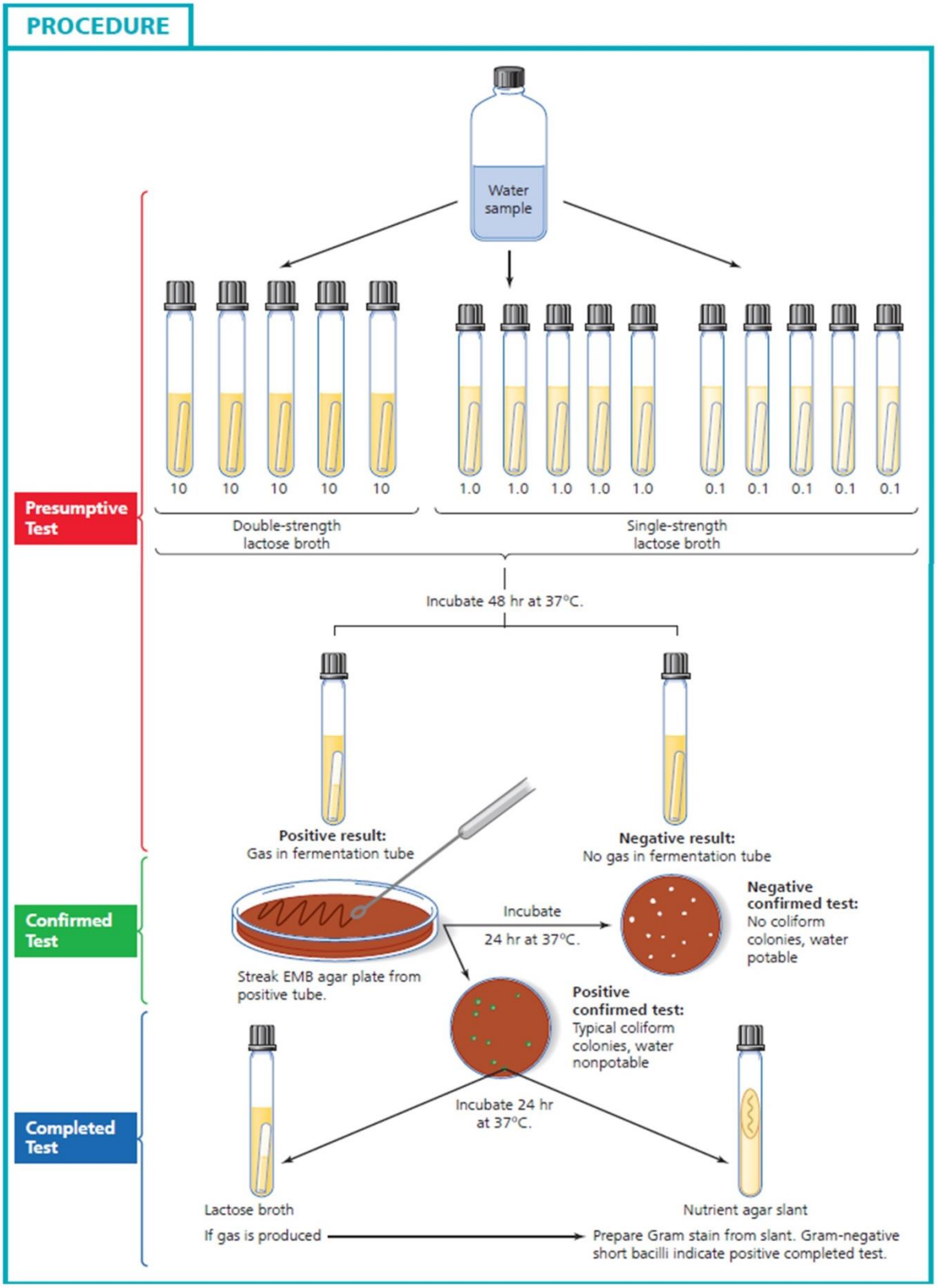
1. Label each tube of nutrient agar slants and lactose fermentation broths with the source of its water sample.

2. Inoculate one lactose broth and one nutrient agar slant with a positive isolated *E. coli* colony obtained from each of the experimental water samples during the confirmed test.

3. Incubate all tubes for 24 hours at 37°C.

4. Prepare a Gram stain, using the nutrient agar slant cultures of the organisms that showed a positive result in the lactose fermentation broth.

5. Examine the slides microscopically for the presence of gram-negative short bacilli, which are indicative of *E. coli* and thus nonpotable water.



Observation and Result: MPN index

NUMBER OF TUBES WITH POSITIVE RESULTS						NUMBER OF TUBES WITH POSITIVE RESULTS					
FIVE OF 10 ML EACH	FIVE OF 1 ML EACH	FIVE OF 0.1 ML EACH	MPN INDEX PER 100 ML	95% CONFIDENCE LIMITS		FIVE OF 10 ML EACH	FIVE OF 1 ML EACH	FIVE OF 0.1 ML EACH	MPN INDEX PER 100 ML	95% CONFIDENCE LIMITS	
				LOWER	UPPER					LOWER	UPPER
0	0	0	<2	0	6	4	2	1	26	7	67
0	0	1	2	<0.5	7	4	3	0	27	9	78
0	1	0	2	<0.5	7	4	3	1	33	9	78
0	2	0	4	<0.5	11	4	4	0	34	11	93
1	0	0	2	0.1	10	5	0	0	23	7	70
1	0	1	4	0.7	10	5	0	1	31	11	89
1	1	0	4	0.7	12	5	0	2	43	14	100
1	1	1	6	1.8	15	5	1	0	33	10	100
1	2	0	6	1.8	15	5	1	1	46	14	120
2	0	0	5	<0.5	13	5	1	2	63	22	150
2	0	1	7	1	17	5	2	0	49	15	150
2	1	0	7	1	17	5	2	1	70	22	170
2	1	1	9	2	21	5	2	2	94	34	230
2	2	0	9	2	21	5	3	0	79	22	220
2	3	0	12	3	28	5	3	1	110	34	250
3	0	0	8	2	22	5	3	2	140	52	400
3	0	1	11	4	23	5	3	3	180	70	400
3	1	0	11	5	35	5	4	0	130	36	400
3	1	1	14	6	36	5	4	1	170	58	400
3	2	0	14	6	36	5	4	2	220	70	440
3	2	1	17	7	40	5	4	3	280	100	710
3	3	0	17	7	40	5	4	4	350	100	710
4	0	0	13	4	35	5	5	0	240	70	710
4	0	1	17	6	36	5	5	1	350	100	1100
4	1	0	17	6	40	5	5	2	540	150	1700
4	1	1	21	7	42	5	5	3	920	220	2600
4	1	2	26	10	70	5	5	4	1600	400	4600
4	2	0	22	7	50	5	5	5	≥2400	700	– – –

Isolation of *Rhizobium* from Root Nodules

Principle: Rhizobia (*Rhizobium*, *Bradirhizobium*, and *Azorhizobium* spp.) are the Gram-negative and aerobic bacteria that symbiotically form nodules with roots of leguminous plants. They fix atmospheric nitrogen and render it into combined forms resulting in high amount of proteins in roots. Eighty percent nitrogen produced from the symbiosis is involving leguminous plants and Rhizobiaceae. The proteins are transported along the plants and also secreted in rhizosphere region. In recent years, rhizobia are used as biofertilizers for selected crops.

Materials: Root nodules, YEM (yeast extract-mannitol) agar medium, Test tube with nylon mesh, Petri dishes, 0.1% acidified $HgCl_2$(1 g $HgCl_2$, 5 ml conc. HCI, 1 litre distilled water), Sterile tap water, Nichrome blade.

YEM agar medium composition: K_2HPO_4 (0.5 g), $MgSO_4$, $7H_2O$ (0.2 g), NaCl (0.1 g), Mannitol (10.0 g), Yeast extract (1.0 g), Distilled water (1 lit), Agar (20.0 g), pH 6.8.

Procedure:

(i) Procure healthy root nodules of a young leguminous plant by cutting with blade.

(ii) Wash the nodules thoroughly first with tap water and then with sterile distilled water keeping over the nylon mesh under aseptic conditions so as to remove contaminants and adhering soil particles.

(iii) Thereafter, immerse them in 0.1% acidified $HgCl_2$ for 5 minutes.

(iv) Transfer nodules in a sterile beaker containing 10 ml of 95% ethanol and wait for 2-3 minutes.

(v) Wash the nodules thoroughly for 5 times with sterile tap water, and blot dry by using sterile blotting paper.

(vi) Aseptically crush the nodules with glass rod or dissect the nodules by using nichrome blade and prepare dilutions.

(vii) Pour 1 ml suspension on YEM agar plates.

(viii) Incubate the inoculated plates at 28°C for 48 hours.

Results: After incubation it was observed that the bacterial colonies were gummy, translucent or white opaque in appearance and Gram-negative. Colonies of above features ensured the presence of *Rhizobium*.

Isolation of *Azotobacter* from Soil

Principle: Soil contains several microorganisms beneficial to plants. These microorganisms may live freely as well as in association with some plants. Among the free-living microorganisms, *Azotobacter* spp. fix atmospheric nitrogen into ammonia utilized by the plants as nitrogenous fertilizers. The presence of Azotobacter in soil can be checked by isolation and its identification. It is a Gram-negative, motile, rod shaped, pleomorphic aerobic bacterium which produces catalase.

Ashby's Mannitol Agar are formulated as described by Subba Rao. It is used for isolation of *Azotobacter*, a nonsymbiotic nitrogen fixing bacteria which uses mannitol as a carbon source and atmospheric nitrogen as nitrogen source.

Materials: Soil, Incubator, Petri dish, inoculating loop, Sieve 2 mm, Ashby's medium.

Ashby's agar medium composition: K_2HPO_4 (0.2 g), $MgSO_4$, $7H_2O$ (0.2 g), NaCl (0.2 g), Mannitol (20.0 g), K_2SO_4 (0.1 g), $CaCO_3$ (0.5 g), Distilled water (1 lit), Agar (20.0 g), pH 7.4.

Procedure:

I. Pour Ashby's medium into sterile Petri plates and allow them to solidify.

II. Add the other 10 g soil sample into the 90 ml water blank, shake for 20-25 min on the magnetic shaker.
III. Make serial dilutions of this sample though sterile water blanks as men-tioned under bacteria.
IV. Add 1 ml of each dilution on to the agar plates, rotate the plates for even spread-ing of inoculum and incubate at 28°C for 3-4 days.

Results: After incubation, it was observed that the bacterial colonies were flat, soft, mu-coid and milky in appearance and Gram-negative. Colonies of above features ensured the presence of *Azotobacterium*.

Isolation of *Azospirillum* from Soil/Root

Principle: *Azospirillum* species occur as free-living in soil or in association with the roots of cereal crops, grasses and tuber plants. *Azospirillum* species are plant-associated diazotrophs (able to grow without external sources of fixed nitrogen) of the alpha subclass of Proteobacteria. Malic acid is used as the carbon source. *Azospirillum* species grow well in presence of Malic acid and are not overgrown by other nitrogen fixers. Dipotassium phosphate provides buffering effect and other inorganic salt ingredients provide necessary growth nutrients. Agar at 0.17% concentrations provides microaerophillic conditions necessary for nitrogen fixation by *Azospirillum* species.

Materials: Soil, Incubator, Screw cap bottles, inoculating loop, Sieve 2 mm, *Azospirillum* medium.

***Azospirillum* medium composition:**

Part A - Malic acid (5.0 g), Dipotassium hydrogen phosphate (0.5 g), Ferrous sulphate (0.5 g), Manganese sulphate (0.01 g), Magnesium sulphate (0.2 g), Sodium chloride (0.1 g), Bromo thymol blue (0.002), Sodium molybdate (0.002 g), Calcium chloride (0.02 g), Agar (1.75 g).

Part B - Potassium hydroxide (4.0 g), Final pH 6.8.

Mixing Protocol: Suspend 8.0 g of dehydrated Part A in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add required quantity of 4.0 g potassium hydroxide (Part B) dissolved in 50 ml of sterile distilled water to obtain pH of 6.8.

Procedure:

I. Collect plant roots/soil sample from field.
II. Cut 0.5 cm long root pieces and wash it with sterile distilled water.
III. After cooling down of *Azospirillum* medium, it pours down into small screw cap bottles and put small pieces of washed roots on it.
IV. Incubated the plates for two days at 28-30°C. Pellicles of *Azospirillum* were seen 1-2 mm below the upper surface of the medium. The bacteria grow in the semi-solid

media at low partial pressure of oxygen which favours the organisms to grow at a state of nitrogen fixation.

Results: Development of white, dense, and undulating fine pellicle on the semi-solid malate medium was characteristic of *Azospirillum*.

Antibiotic Sensitivity Assay (Kirby-Bauer Method)

Principle: The available chemotherapeutic agents vary in their scope of antimicrobial activity. Some have a limited spectrum of activity, effective against only one group of microorganisms. Others exhibit broad-spectrum activity against a range of microorganisms. The drug susceptibilities of many pathogenic microorganisms are known, but it is sometimes necessary to test several agents to determine the drug of choice.

A standardized diffusion procedure with filter-paper discs on agar, known as the Kirby-Bauer method, is frequently used to determine the drug susceptibility of microorganisms isolated from infectious processes. This method allows the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. In this procedure, filter-paper discs of uniform size are impregnated with specified concentrations of different antibiotics and then placed on the surface of an agar plate that has been seeded with the organism to be tested. The medium of choice is Mueller-Hinton agar, with a pH of 7.2 to 7.4, which is poured into plates to a uniform depth of 5 mm. The plates are then heavily inoculated with a standardized inoculum by means of a spreader to ensure the confluent growth of the organism. The discs are aseptically applied to the surface of the agar plate at well-spaced intervals. Once applied, each disc is gently touched with a sterile applicator stick to ensure its firm contact with the agar surface. Following incubation, the plates are examined for the presence of growth inhibition, which is indicated by a clear zone surrounding each disc. The susceptibility of an organism to a drug is assessed by the size of this zone, which is affected by other variables such as the following:

1. The ability and rate of diffusion of the antibiotic into the medium and its interaction with the test organism.

2. The number of organisms inoculated.

3. The growth rate of the organism.

Requirements: Muller-Hinton Agar, Antibiotics disc, Forceps, Inoculating loop, Cultures, Spreader.

Procedure:

1. Label the bottom of each of the agar plates with the name of the test organism to be inoculated.

2. Using aseptic technique, inoculate all agar plate with the test organism and properly spread it over the plates.

3. Allow all culture plates to dry for about 5 minutes.

4. Using sterilized forceps the antibiotic discs were placed over the agar surface and pressing the disc slightly.

5. Examine all plate cultures for the presence or absence of a zone of inhibition surrounding each disc and carefully measure each zone of inhibition in millimetre scale.

Result and Observation: A measurement of the diameter of the zone of inhibition in millimeters is made, and its size is compared with that contained in a standardized chart. Based on this comparison, the test organism is determined to be resistant, intermediate, or susceptible to the antibiotic.

Table 42.2 Zone Diameter Interpretive Standards for Organisms Other Than *Haemophilus* and *Neisseria gonorrhoeae*

		ZONE DIAMETER, NEAREST WHOLE MM		
ANTIMICROBIAL AGENT	DISC CONCENTRATION	RESISTANT	INTERMEDIATE	SUSCEPTIBLE
Ampicillin				
when testing gram-negative bacteria	10 μg	≤13	14–16	≥17
when testing gram-positive bacteria	10 μg	≤28	—	≥29
Carbenicillin				
when testing *Pseudomonas*	100 μg	≤13	14–16	≥17
when testing other gram-negative organisms	100 μg	≤19	20–22	≥23
Cefoxitin	30 μg	≤14	15–17	≥18
Cephalothin	30 μg	≤14	16–17	≥18
Chloramphenicol	30 μg	≤12	13–17	≥18
Clindamycin	2 μg	≤14	15–20	≥21
Erythromycin	15 μg	≤13	14–22	≥23
Gentamicin	10 μg	≤12	13–14	≥15
Kanamycin	30 μg	≤13	14–17	≥18
Methicillin when testing staphylococci	5 μg	≤9	10–13	≥14
Novobiocin	30 μg	≤17	18–21	≥22
Penicillin G				
when testing staphylococci	10 units	≤28	—	≥29
when testing other bacteria	10 units	≤14	—	≥15
Rifampin	5 μg	≤16	17–19	≥20
Streptomycin	10 μg	≤11	12–14	≥15
Tetracycline	30 μg	≤14	15–18	≥19
Tobramycin	10 μg	≤12	13–14	≥15
Trimethoprim/sulfamethoxazole	1.25/23.75 μg	≤10	11–15	≥16
Vancomycin				
when testing enterococci	30 μg	≤14	15–16	≥17
when testing *Staphylococcus* spp.	30 μg	—	—	≥15
Sulfonamides	250 or 300 μg	≤12	—	≥17
Trimethoprim	5 μg	≤10	—	≥16

Source: Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk Susceptibility Tests*, Tenth Edition, 2008.

Biofilm Test

Principle: Bacterial biofilms are important because they can be helpful (in water purification and nutrient cycling), but also harmful (persistent infections in the human body and build up on medical equipment/prosthetic devices). In nature, majority of bacteria live together in large complex sessile communities termed as biofilms. Within biofilm community, compared with their planktonic state, the physiological behavior of biofilm members is deeply altered, leading to gaining resistance to various environmental stresses, such as desiccation, antimicrobial agents and host immune response. As final stage of biofilm maturation, biofilm dispersal is also accepted as a critical step in bacterial lifestyle allowing dissemination of bacteria from the original site of infection in the host or the environment and increasing virulence of pathogenic bacteria. Microbial biofilms are extremely robust in terms of resistance to various chemicals and antimicrobials and are relevant in more than half of infectious diseases globally.

Reagents: Brain-heart Infusion broth, Test tubes, Crystal violet, Inoculating Loop, Cultures.

Procedure:

1. Sterile test tubes containing 10 mL of Brain Heart Infusion broth was inoculated with the cultures and incubated for 2-5 days.

2. After the incubation period, discard the broth from test tubes and kept the test tube at incubator for drying for 1 h.

3. Stained the test tubes with the crystal violet and wait for 2 min and wash with water.

4. Inside of the test tubes which were seen as gloomy, confirmed the formation of biofilm.

Observation and Results: Test tubes with cloudy appearance from their inside confirmed positive result.

Course: BFSC-105: Fundamentals of Bio- Chemistry

Normality and Molarity

Normality:

Normality is a measure of concentration equal to the gram equivalent weight per liter of solution. Gram equivalent weight is the measure of the reactive capacity of a molecule. The solute's role in the reaction determines the solution's normality. Normality is also known as the equivalent concentration of a solution.

Normality Equation:

- **Normality (N)** is the **molar concentration 'ci'** divided by an **equivalence factor 'feq'**:

N = ci / feq

- Another common equation is **normality (N)** is equal to the **gram equivalent weight (g)** divided by **liters of solution (L)**:

N = gram equivalent weight/liters of solution

(often expressed in g/L)

- Or it might be the **molarity (M)** multiplied by the **number of equivalents (n)**:

N = M * n

Key Takeaways:

1. Normality is a unit of concentration of a chemical solution expressed as gram equivalent weight of solute per liter of solution. A defined equivalence factor must be used to express concentration.
2. Common units of normality include N, eq/L, or meq/L.
3. Normality is the only unit of chemical concentration that depends on the chemical reaction being studied.
4. Normality is not the most common unit of concentration, nor is its use appropriate for all chemical solutions. Typical situations when you might use normality include acid-base chemistry, redox reactions, or precipitation reactions. For most other situations, molarity or molality are better options for units.

Units of Normality:

The capital letter N is used to indicate concentration in terms of normality. It can also be expressed as eq/L (equivalent per liter) or meq/L (milliequivalent per liter of 0.001 N, typically reserved for medical reporting).

Examples of Normality:

For acid reactions, a 1 M H2SO4 solution will have a normality (N) of 2 N because 2 moles of H+ ions are present per liter of solution.

For sulfide precipitation reactions, where the SO4- ion is the important part, the same 1 M H2SO4 solution will have a normality of 1 N.

Example Problem:

Find the normality of 0.1 M H2SO4 (sulfuric acid) for the reaction:

$H_2SO_4 + 2\ NaOH \rightarrow Na_2SO_4 + 2\ H_2O$

According to the equation, 2 moles of H+ ions (2 equivalents) from sulfuric acid react with sodium hydroxide (NaOH) to form sodium sulfate (Na_2SO_4) and water. Using the equation:

N = molarity x equivalents

N = 0.1 x 2

N = 0.2 N

Don't be confused by the number of moles of sodium hydroxide and water in the equation. Since you've been given the molarity of the acid, you don't need the additional information. All you need to figure out is how many moles of hydrogen ions are participating in the reaction. Since sulfuric acid is a strong acid, you know it completely dissociates into its ions.

Potential Issues Using N for Concentration:

Although normality is a useful unit of concentration, it can't be used for all situations because its value depends on an equivalence factor that can change based on the type of chemical reaction of interest. As an example, a solution of magnesium chloride ($MgCl_2$) might be 1 N for the Mg^{2+} ion, yet 2 N for the Cl^- ion.

While N is a good unit to know, it's not used as much as molality in actual lab work. It has value for acid-base titrations, precipitation reactions, and redox reactions. In acid-base reactions and precipitation reactions, 1/feq is an integer value. In redox reactions, 1/feq might be a fraction.

How Normality Can Change:

Because normality references concentration with respect to the reactive species, it's an ambiguous unit of concentration (unlike molarity). An example of how this can work may be seen with iron(III) thiosulfate, $Fe_2(S_2O_3)_3$. The normality depends on which part of the redox reaction you're examining. If the reactive species is Fe, then a 1.0 M solution would be 2.0 N (two iron atoms). However, if the reactive species is S_2O_3, then a 1.0 M solution would be 3.0 N (three moles of thiosulfate ions per each mole of iron thiosulfate).

(Usually, the reactions aren't this complicated and you'd just be examining the number of H+ ions in a solution.)

Molarity:

In chemistry, molarity is a concentration unit, defined to be the number of moles of solute divided by the number of liters of solution. It is one of the most common concentration units, so it's important to understand how it's calculated and when to use it versus when to use another unit. Here's what you need to know.

Molarity Key Features:

1. Molarity (M) is a unit of the concentration of a chemical solution.

2. It is the moles of solute per liters of solution. Note this is different from the liters of solvent (a common mistake).
3. While molarity is a valuable unit, it does have one main disadvantage. It does not remain constant when temperature changes because temperature affects the volume of a solution.
4. Since you don't physically measure solute in moles, usually you convert grams of solute to moles and then divide this number by liters of solution.

Units of Molarity:

Molarity is expressed in units of moles per liter (mol/L). It's such a common unit, it has its own symbol, which is a capital letter M. A solution that has the concentration 5 mol/L would be called a 5 M solution or said to have a concentration value of 5 molar.

Molarity Examples:

1. There are 6 moles of HCl in one liter of 6 molar HCl or 6 M HCl.
2. There are 0.05 moles of NaCl in 500 ml of a 0.1 M NaCl solution. (The calculation of moles of ions depends on their solubility.)
3. There are 0.1 moles of Na+ ions in one liter of a 0.1 M NaCl solution (aqueous).

Example Problem:

- Express the concentration of a solution of 1.2 grams of KCl in 250 ml of water.

In order to solve the problem, you need to convert the values into the units of molarity, which are moles and liters. Start by converting grams of potassium chloride (KCl) into moles. To do this, look up the atomic masses of the elements on the periodic table. The atomic mass is the mass in grams of 1 mole of atoms.

mass of K = 39,10 g/mol
mass of Cl = 35.45 g/mol

So, the mass of one mole of KCl is:

mass of KCl = mass of K + mass of Cl
mass of KCl = 39.10 g + 35.45 g
mass of KCl = 74.55 g/mol

You have 1.2 grams of KCl, so you need to find how many moles that is:

moles KCl = (1.2 g KCl)(1 mol/74.55 g)
moles KCl = 0.0161 mol

Now, you know how many moles of solute are present. Next, you need to convert the volume of solvent (water) from ml to L. Remember, there are 1000 milliliters in 1 liter:

liters of water = (250 ml)(1 L/1000 ml)
liters of water = 0.25 L

Finally, you're ready to determine molarity. Simply express the concentration of KCl in water in terms of moles solute (KCl) per liters of solute (water):

molarity of solution = mol KC/L water

molarity = 0.0161 mol KCl/0.25 L water

molarity of the solution = 0.0644 M (calculator)

Since you were given mass and volume using 2 significant figures, you should report molarity in 2 sig figs also:

molarity of KCl solution = 0.064 M

Advantages and Disadvantages of Using Molarity:

There are two big advantages of using molarity to express concentration. The first advantage is that it's easy and convenient to use because the solute may be measured in grams, converted into moles, and mixed with a volume.

The second advantage is that the sum of the molar concentrations is the total molar concentration. This permits calculations of density and ionic strength.

The big disadvantage of molarity is that it changes according to temperature. This is because the volume of a liquid is affected by temperature. If measurements are all performed at a single temperature (e.g., room temperature), this is not a problem. However, it's good practice to report the temperature when citing a molarity value. When making a solution, keep in mind, molarity will change slightly if you use a hot or cold solvent, yet store the final solution at a different temperature.

When to Use Molarity and Normality:

For most purposes, molarity is the preferred unit of concentration. If the temperature of an experiment will change, then a good unit to use is molality. Normality tends to be used most often for titration calculations.

Converting From Molarity to Normality:

You can convert from **molarity (M)** to **normality (N)** using the following equation:

N = M*n

where **'n'** is the **number of equivalents**

Note that for some chemical species, **N and M are the same (n is 1)**. The conversion only matters when ionization changes the number of equivalents.

Buffer, buffering capacity, properties of good buffer and role of buffers

Buffer:

Buffers are defined as solutions which resists small change in pH by adding small amount of acid or base. A buffer usually consists of a weak acid and its salt (fore eg, acetic acid and sodium acetate) or a weak base and its salt (for eg, ammonium hydroxide and ammonium chloride).

The buffer solution is a solution able to maintain its Hydrogen ion concentration (pH) with only minor changes on the dilution or addition of a small amount of either acid or base. Buffer Solutions are used in fermentation, food preservatives, drug delivery, electroplating, printing, the activity of enzymes, blood oxygen carrying capacity need specific hydrogen ion concentration (pH).

Solutions of a weak acid and its conjugate base or weak base and its conjugate acid are able to maintain pH and are buffer solutions.

Types of Buffer Solution:

The two primary types into which buffer solutions are broadly classified into are acidic and alkaline buffers.

Acidic Buffers:

As the name suggests, these solutions are used to maintain acidic environments. Acid buffer has acidic pH and is prepared by mixing a weak acid and its salt with a strong base. An aqueous solution of an equal concentration of acetic acid and sodium acetate has a pH of 4.74.

- pH of these solutions is below seven
- These solutions consist of a weak acid and a salt of a weak acid.
- An example of an acidic buffer solution is a mixture of sodium acetate and acetic acid (pH = 4.75).

Alkaline Buffers:

These buffer solutions are used to maintain basic conditions. Basic buffer has a basic pH and is prepared by mixing a weak base and its salt with strong acid. The aqueous solution of an equal concentration of ammonium hydroxide and ammonium chloride has a pH of 9.25.

- The pH of these solutions is above seven
- They contain a weak base and a salt of the weak base.
- An example of an alkaline buffer solution is a mixture of ammonium hydroxide and ammonium chloride (pH = 9.25).

Mechanism of Buffering Action:

In solution, the salt is completely ionized and the weak acid is partly ionized.

- $CH_3COONa \rightleftharpoons Na^+ + CH_3COO^-$
- $CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$

Addition of Acid and Base:

1. On addition of acid, the released protons of acid will be removed by the acetate ions to form an acetic acid molecule.

$H^+ + CH_3COO^-$ (from added acid) ⇌ CH_3COOH (from buffer solution)

2. On addition of the base, the hydroxide released by the base will be removed by the hydrogen ions to form water.

$HO^- + H^+$ (from added base) ⇌ H_2O (from buffer solution)

Preparation of Buffer Solution:

If the dissociation constant of the acid (pK_a) and of the base (pK_b) are known, a buffer solution can be prepared by controlling the salt-acid or the salt-base ratio.

As discussed earlier, these solutions are prepared by mixing the weak bases with their corresponding conjugate acids, or by mixing weak acids with their corresponding conjugate bases.

An example of this method of preparing buffer solutions can be given by the preparation of a phosphate buffer by mixing HPO_4^{2-} and H_2PO^{4-}. The pH maintained by this solution is 7.4.

Buffering capacity:

The efficiency of a buffer in maintaining a constant pH on addition of acid or base is referred to as buffering capacity. It mostly depends on the concentration of buffering component. The maximum buffering capacity is achieved by keeping same concentration of acid and its salt or base and its salt.

The number of millimoles of acid or base to be added to a litre of buffer solution to change the pH by one unit is the Buffer capacity of the buffer.

B = millimoles /(ΔpH)

Properties of good buffer:

- The buffer should be non-toxic
- The buffer should be able to penetrate cell membrane and should not absorbs light at UV or visible region
- Buffer should have adequate buffering capacity
- Buffer should not form insoluble complex with any anions or cations in the reaction.

Role of buffer in vitro:

- Acid-base buffer helps in tissue or organ preservation (eg phosphate buffer)
- In tissue culture, the optimum pH is maintained by buffers. For example HEPES buffer is widely used in cell culture because it is better at maintaining physiological pH despite change in CO2 concentration in media.
- HEPES also maintains enzyme structure and function at low temperature

- Buffer are used in fermentation process
- It is also used for setting correct condition for dyes used in coloring fabrics
- Buffer is used to calibrate pH meter

Role of buffer in vivo:

There are three buffer system in human body to maintain acid-balance.

1. Bicarbonate buffer
2. Phosphate buffer
3. Protein buffer

Bicarbonate buffer:

- Sodium bicarbonate and carbonic acid constitute bicarbonate buffer. It is the most predominant buffer system in plasma.
- It is extracellular buffer
- It helps in maintaining blood pH 7.4

Phosphate buffer:

- Sodium dihydrogen phosphate and disodium hydrogen phosphate constitute phosphate buffer.
- It is intracellular buffer

Protein buffer:

- The plasma protein and hemoglobin together constitute protein buffer system of blood.
- Th ebuffering capacity of protein buffer depends upon pKa of amino acids.
- The imidazole group of histidine (pKa= 6.7) is the most effective contributor of protein buffer.
- Hemoglobin of RBCs is also an important buffer. Hb binds to H+ ions and helps to transport CO2 as HCo3- ion with minimum change in pH. In lungs Hb combines with O2 then H+ ion is released and combine with HCO3- to form carbonic acid (HCO3-). Later carbonic acid dissociates to release CO2.

Lysis buffer for blood DNA extraction:

Two different combinations of solutions are used for lysis buffer preparation, especially for the blood samples. The major components of the lysis buffer for blood DNA extraction are Tris, EDTA, MgCl2, KCl, NaCl and SDS.

Solution – I (For 250ml)

10mM	Tris	(0.061	gm)
10mM	KCl	(0.186	gm)
10mM	MgCl2	(0.238	gm)

Make-up final volume with D/W and set pH 7.6

Solution- II (50ml)

10mM	Tris	(0.061gm)
10mM	KCl	(0.037gm)
10mM	MgCl2	(0.048gm)
0.5M		NaCl(1.461gm)
2mM		EDTA(0.037gm)

Mix all ingredients in sterile D/W and set pH 7.6
Autoclave it and wait to come at room temperature.
add 0.5% SDS (0.250gm).

Lysis buffer for plant DNA extraction:

CTAB (hexadecyltrimethylammonium bromide) is the major ingredient for plant DNA extraction. Pectin present in the plant cell wall makes it harder and so it is also difficult to digest it. Along with some other ingredients like Tris, EDTA, NaCl, PVP, Beta-mercaptoethanol and ascorbic acid, CTAB lysis the plant cell wall effectively.

Here is the lysis buffer recipe for plant DNA extraction.

Solution A (200ml)

2% CTAB (4.0 g)
100 mM Tris (pH 8.0) (20 ml)
20 mM EDTA (2 ml)
1.4 M NaCl (16.4 g)
4% polyvinylpyrrolidone (PVP) (8.0 g)
0.1% ascorbic acid (0.2 g)
10 mM β-mercaptoethanol (140 µL)

Solution B (200ml)

100 mM Tris-HCl (pH 8.0) (20 mL)
50 mM EDTA (10 mL)
100 mM NaCl (0.12 g)
10 mM β-mercaptoethanol (140 µL)

Lysis buffer for bacterial DNA extraction:

The cell structure of bacteria is totally different from the plant, here a smooth cell membrane is present instead of a hard cell wall. And therefore lysing bacterial cell membranes is an easy task comparing to plant.

Only the TE buffer is sufficient to lyse bacterial cell membrane. However, a pinch of SDS along with the TE buffer increases the yield. The composition of the lysis buffer for the bacterial cell is as followed,

Lysis buffer (100 ml):
10% SDS (10 ml)
90 ml TE buffer

Recipe for 1X TE buffer:

DNA extraction needs a specialized buffer system to protect the DNA from harmful chemical degradation. One such buffer is the TE (Tris- EDTA) buffer system. Tris and EDTA are used throughout the DNA extraction protocol as components of lysis buffer, elution buffer and washing buffer and helps to achieve our final goal that is to get the pure DNA.

10mM Tris HCl

1mM EDTA

Adjust the pH using the HCl and so add only the half amount of the D/W and adjust the pH of the solution with HCl until the pH reaches nearby 8.0.

TAE/TBE buffer for agarose gel electrophoresis:

Two important functions performed by gel electrophoresis buffer,

1. It maintains the pH of reaction nearly neutral. The weak acid and base in buffer keep pH in the desired range.
2. By maintaining neutral pH, it controls the net charge of molecules which helps in proper migration and separation of the molecule.

Tris is a strong base and borate is an acid, combination of both maintains the pH nearly neutral range of 8 to 8.5. Under this alkaline condition, DNA is protected and can separate properly. EDTA has some important role to play in this combination. EDTA is a chelating agent. It chelates the Mg^{2+} ion which is required for enzyme DNAse as a cofactor. So by addition of EDTA, our DNA is protected from the enzymatic activity. Further, the buffer will neutralize the charge of a water molecule.

Preparation of 1X TBE buffer(for 250ml):

- Tris- 90mM
- Boric Acid- 89mM
- EDTA- 0.2mM
- Add distilled water to make the final volume of 250ml.
  Set pH nearly 8.0 to 8.5.

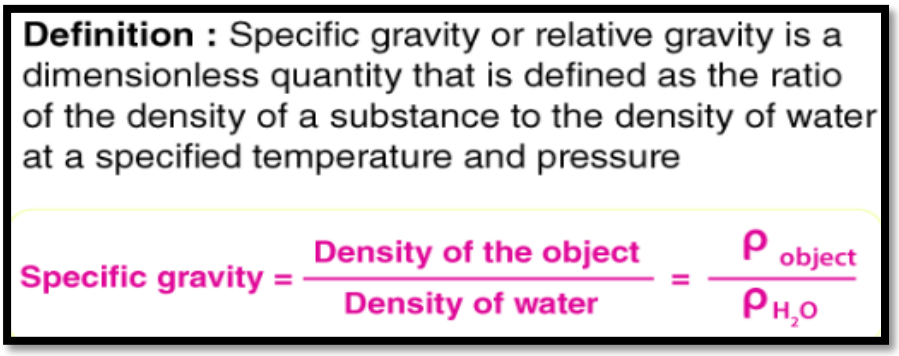
Preparation of 1X TAE buffer(for 250ml):

- 40mM Tris
- 20mM acetic acid
- 1mM EDTA
- Add D/W to make the final volume of 250ml

Specific Gravity & API Gravity

A specific gravity scale developed by the American Petroleum Institute (API) for measuring the relative density of various petroleum liquids, expressed in degrees. API gravity is graduated in degrees on a hydrometer instrument and was designed so that most values would fall between 10° and 70° API gravity. The arbitrary formula used to obtain this effect is presented in the following paragraphs.

Specific Gravity (SG):
Specific gravity is a dimensionless value that compares all liquids to fresh water and all gases to air. Certain liquid physical values can be calculated by multiplying the specific gravity of that liquid by the value for fresh water. For gases, the values are calculated by multiplying the specific gravity of the gas by the value for air. Freshwater and air have a specific gravity of one. Liquids and gases with a specific gravity greater than one are heavier than water or air.



Specific Gravity or relative gravity is a dimensionless quantity that is defined as the ratio of the density of a substance to the density of the water at a specified temperature and is expressed as

$SG = \rho_{substance} / \rho_{H2O}$

It is common to use the density of water at 4 ^{o}C as a reference point as water at this point has the highest density of 1000 kg/m^3.

Specific Gravity of Oil:

$SG_{oil} = \rho_{oil} / \rho_{water}$
= /
=

API Gravity:
Oil density is often reported in terms of API gravity. API gravity can be converted to specific gravity using the API to Specific Gravity Conversion equation, while specific gravity can be converted to API gravity using the Specific to API Gravity Conversion equation. Both equations are presented below:

API to Specific Gravity Conversion:

$$Sp.Gr. = \frac{141.5}{131.5 + API}$$

Specific to API Gravity Conversion:

$$°API = \frac{141.5}{SG} - 131.5$$

Composite Specific Gravity (SG_{comp}):

The composite specific gravity of oil and water mixture can be calculated if the individual fluid's specific gravities and percentages are known. The specific gravity of this mixture is calculated as shown in the following equation:

$SG_{comp} = [SG_o \times (1 - WC)] + (SG_w \times WC)$
Where:

WC = Water Cut (fraction)
SG_o = Oil specific gravity
SG_w = Water specific gravity

Example:
Knowing the oil gravity, water gravity and water cut, calculate the composite SG of the produced fluids:

- Oil Gravity: 35 API,
- Water Cut: 90%,
- Water Gravity: 1,01 rel-H2O,

Using the API to Specific Gravity Conversion formula, the oil specific gravity is calculated:

SG(oil) = 141.5 / (°API+131.5) = 141.5 / (35+131.5) = 0.845.

SG_o = 0.845
Then the mixture SG can be calculated:

SG= 0.9×1.01+(1-0.9)×0.845= 0.9935

SG_{comp} = 0.9935
NB: *The following Table summarizes the ranges of data used for oil gravity and gas SG:*

Property	Minimum	Maximum	Average
Crude oil gravity, °API	6.0	63.7	34.1
Gas specific gravity	0.511	3.445	1.005

Determination of Saponification Value

Saponification is the process by which the fatty acids in the glycerides of the oil are hydrolyzed by an alkali. Saponification value is the amount (mg) of alkali required to saponify a definite quantity (1g) of an oil or fat. This value is useful for a comparative study of the fatty acid chain length in oils.

Principle:

A known quantity of oil is refluxed with an excess amount of alcoholic KOH. After saponification, the remaining KOH is estimated by titrating it against a standard acid.

Materials:

» Hydrochloric acid 0.5N, accurately standardized.
» Alcoholic KOH – Dissolve 40g KOH in one liter of distilled alcohol keeping the temperature below 15.5oC while the alkaline is being dissolved. This solution should remain clear.
» Phenolphthalein Indicator – 1% in 95% alcohol.
» Air Condenser.

Procedure:

1. Melt the sample if it is not already liquid and filter through paper to remove any impurities and the last traces of moisture. The sample must be completely moisture-free.

2. Weigh 4-5g sample into the flask. Add 50mL of alcoholic KOH from burette by allowing it to drain for a definite period of time.

3. Prepare a blank also by taking only 50mL of alcoholic KOH allowing it to drain at the same duration of time.

4. Connect air condenser to the flasks and boil them gently for about 1h.

5. After the flask and condenser get cooled, rinse down the inside of the condenser with a little distilled H2O and then remove the condenser.

6. Add about 1mL of indicator and titrate against 0.5N HCl until the pink color just disappears.

Calculation:

Saponification value = 28.05 x (titer value of blank – titer value of sample) / Weight of sample (g)

Notes:

1. Alcohol is inflammable. Use electrical heating.
2. Alcohol should not get dried up during saponification. Effective cooling of alcohol vapor is essential.
3. Clarity and homogeneity of the test solution are indicators of complete saponification.

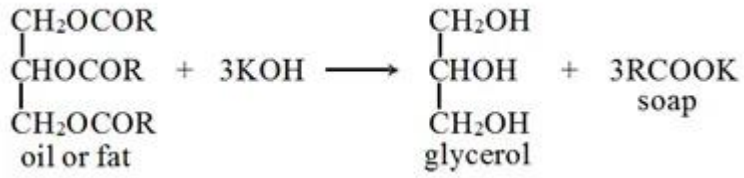
Alternative Procedure: 1

Principle:

Saponification value is defined as the number of milligrams of KOH required to completely hydrolyse (saponify) one gram of the oil/fat. In practice a known amount of the oil or fat is refluxed with excess amount of standard alcoholic potash solution and the unused alkali is titrated against a standard acid.

Aim: To determine saponification value of the given oil/fat.

Reaction:



Requirements:

Sample: Oil or fat

Apparatus:

1. Conical flask
2. Reflux and condenser

3. Water bath

4. Burette and Pipette

Chemical:

1. 0.5 N HCl

2. Phenolphthalein

3. 0.5 N alcoholic potassium-hydroxide solution

Procedure:

About 2 g of the given oil or fat is taken in a conical flask but weighed accurately (w g). The oil/fat is dissolved in 25 ml of 0.5 N alcoholic potassium-hydroxide solution. Then the reaction mixture is refluxed using a water condenser on a water-bath for half an hour. The resulting solution is cooled and titrated against a 0.5 N HCl solution adding 1 ml of phenolphthalein. The number of ml of acid required is noted (a). An exactly identical blank experiment (leaving the fat or oil) is performed. Number of ml of hydrochloric acid required is noted (b).

Observation:

	Samples No.	**Initial Burette Reading (ml)**	**Final Burette Reading (ml)**	**Difference (ml)**	**Average (ml)**
For Excess KOH remaining	**1**				**a =**
	2				
For Blank	**1**				**b =**
	2				

Calculation:

$$\text{Saponification value} = \frac{\text{Volume of acid required to neutralise remaining KOH x Equivalent Factor x 1000}}{w}$$

$$= \frac{(b - a) \times 0.02805 \times 1000}{w}$$

Conclusion:

Saponification value of the given oil/fat is …….

Alternative Procedure: 2

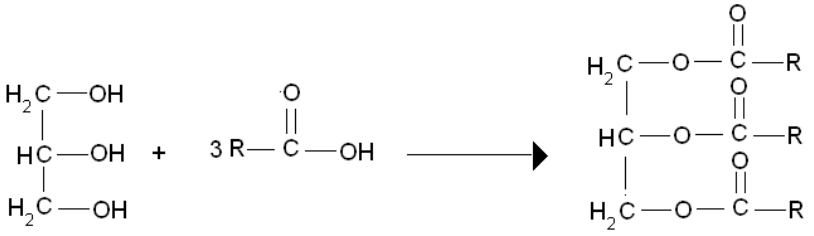
Objective:

To Estimate the Saponification value of oils.

Theory:

Fats and oils are the principle stored forms of energy in many organisms. They are highly reduced compounds and are derivatives of fatty acids. Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons, they can be saturated or unsaturated. The simplest lipids constructed from fatty acids are triacylglycerols or triglycerides. Triacylglycerols are composed of three fatty acids each in ester linkage with a single glycerol. Since the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkages, triacyl glycerols are non polar, hydrophobic molecules, which are insoluble in water.

Saponification is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid.

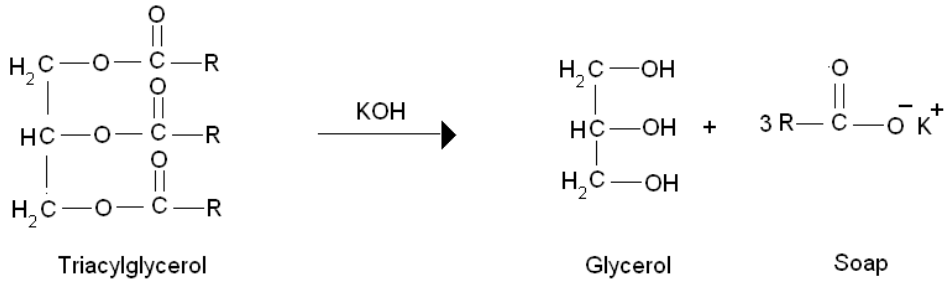


Saponification literally means "soap making". It is important to the industrial user to know the amount of free fatty acid present, since this determines in large measure the refining loss. The amount of free fatty acid is estimated by determining the quantity of alkali that must be added to the fat to render it neutral. This is done by warming a known amount of the fat with strong aqueous caustic soda solution, which converts the free fatty acid into soap. This soap is then removed and the amount of fat remaining is then determined. The loss is estimated by subtracting this amount from the amount of fat originally taken for the test.

The saponification number is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat . It gives information concerning the character of the fatty acids of the fat- the longer the carbon chain, the less acid is liberated per gram of fat hydrolysed. It is also considered as a measure of the average molecular weight (or chain length) of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat and therefore high molecular weight .

Principle:

Fats (triglycerides) upon alkaline hydrolysis (either with KOH or NaOH) yield glycerol and potassium or sodium salts of fatty acids (soap) .



Procedure:

Materials Required:

1) Fats and Oils [coconut oil, sunflower oil]
2) Conical Flask
3) 100ml beaker
4) Weigh Balance
5) Dropper
6) Reflux condenser
7) Boiling Water bath
8) Glass pipette (25ml)
9) Burette

Reagents Required:

1) Ethanolic KOH(95% ethanol, v/v)

2) Potassium hydroxide [0.5N]

3) Fat solvent

4) Hydrochloric acid[0.5N]

5) Phenolphthalein indicator

Procedure:

1) Weigh 1g of fat in a tared beaker and dissolve in about 3ml of the fat solvent [ethanol /ether mixture].

2) Quantitatively transfer the contents of the beaker three times with a further 7ml of the solvent.

3) Add 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser.

4) Set up another reflux condenser as the blank with all other reagents present except the fat.

5) Place both the flasks in a boiling water bath for 30 minutes .

6) Cool the flasks to room temperature .

7) Now add phenolphthalein indicator to both the flasks and titrate with 0.5N HCl .

8) Note down the endpoint of blank and test .

9) The difference between the blank and test reading gives the number of millilitres of 0.5N KOH required to saponify 1g of fat.

10) Calculate the saponification value using the formula :

Saponification value or number of fat = mg of KOH consumed by 1g of fat.

Weight of KOH = Normality of KOH * Equivalent weight* volume of KOH in litres

Volume of KOH consumed by 1g fat = [Blank – test]ml

Determination of iodine value of oil:

The iodine value is a measure of the degree of unsaturation in an oil. It is constant for a particular oil or fat. Iodine value is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid.

Principle:

The oils contain both saturated and unsaturated fatty acids. Iodine gets incorporated into the fatty acid chain wherever the double bond exist. Hence, the measure of the iodine absorbed by an oil, gives the degree of unsaturation. Iodine value/number is defined as the 'g' of iodine absorbed by 100g of the oil.

Materials:

» Hanus Iodine Solution

Weigh 13.6g of iodine and dissolve in 825mL glacial acetic acid by heating, and cool. Titrate 25mL of this solution against 0.1N sodium thiosulphate. Measure another portion of 200mL of glacial acetic acid and add 3mL of bromine to it. To 5mL of this solution add 10mL of 15% potassium iodide solution and titrate against 0.1N sodium thiosulphate. Calculate the value of bromine solution, to double halogen content of the remaining 800mL of the above iodine solution as follows:

» X = B/C, where X = mL of bromine solution required to double the halogen content, B = 800 x thiosulphate equivalent of 1mL of iodine solution and C = thiosulphate equivalent of one mL of bromine solution.

» 15% Potassium Iodide Solution

» 0.1% Sodium Thiosulphate

» 1% Starch

Procedure:

1. Weigh 0.5 or 0.25g of oil into an iodine flask and dissolve in 10mL of chloroform.

2. Add 25mL of Hanus iodine solution using a pipette, draining it in a definite time. Mix well and allow to stand in dark for exactly 30min with occasional shaking.

3. Add 10mL of 15% KI, shake thoroughly and add 100mL of freshly boiled and cooled water, washing down any free iodine on the stopper.

4. Titrate against 0.1N sodium thiosulphate until yellow solution turns almost colorless.

5. Add a few drops of starch as indicator and titrate until the blue color completely disappears.

6. Towards the end of titration, stopper the flask and shake vigorously so that any iodine remaining in solution in CHCl3 is taken up by potassium iodide solution.

7. Run a blank without the sample.

Calculation:

The quantity of thiosulphate required for blank minus the quantity required for sample gives thiosulphite equivalent of iodide adsorbed by the fat or oil taken for analysis.

Iodine number = (B – S) x N x 12.69 / Weight of sample (g)

where,

B = mL thiosulphate for blank

S = mL thiosulphate for sample

N = normality of thiosulphate solution

Amount of fat/oil taken should be adjusted such that the excess iodine in the added 25mL of Hanus iodine solution has about 60% of excess iodine of the amount added, i.e., if (B – S) is greater than B/2, repeat the smaller amount of sample.

Alternative Procedure: 1

Principle:

Iodine value of an oil or fat is the number of grams of iodine, which is absorbed by 100 g of the substance (oil or fat) under described conditions. It can be determined by the following method.

Aim:

To determine Iodine value of the sample (oil or fat)

Requirements:

Chemical:

1. Carbon tetrachloride,
2. Iodine monochloride
3. 0.1 M sodium thiosulphate,
4. Starch solution

Apparatus:

1. Volumetric flask
2. Pipette
3. Burette

Procedure:

(Iodine Monochloride (ICl) Method or Wijs Method)

An accurately weighed quantity (w g) of the sample (oil or fat) is taken in a 500 ml iodine flask. 10 ml of carbon tetrachloride (CCl4) is added to dissolve it. 20 ml of iodine monochloride (ICl) solution is added. The mix solution is allowed to stand in the dark at a temperature between 15° – 25° C for 30 minutes, inserting the stopper. Then 15 ml of potassium iodide solution is added. The flask and the stopper are ringed with 100 ml of water, shaken and titrated with 0.1 M sodium thiosulphate solution using freshly prepared starch solution as indicator, which is added towards the end of the titration. The number of ml required is noted as (a). The procedure is repeated without the sample and the number of ml required is noted as (b). Iodine value is calculated from the observed data.

Observation:

	No. of Observation	**Initial Burette Reading (ml)**	**Final Burette Reading (ml)**	**Difference (ml)**	**Average (ml)**
For Test	**1**				**a =**
	2				
For Blank	**1**				**b =**
	2				

Calculation:

Iodine value = 1.269 (b – a)/w
Here, w = weight in grams of the sample.
[N.B.- The approx. weight in grams of the sample to be taken can be calculated by dividing 20 by the highest expected iodine value. If more than half of the halogen is absorbed, the test must be repeated with a lesser quantity of sample.]

Conclusion:

Iodine value of the given sample of oil/fat is …….

Alternative Procedure: 2

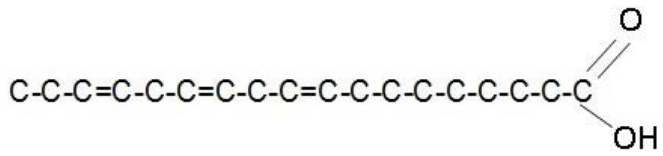
Objective :

To determine the iodine value of fats and oils and thus estimate the unsaturation of the fats and oils.

Theory:

Fats and oils are a mixture of triglycerids. Triglycerides are made up of three fatty acids linked to glycerol by fatty acyl esters. Fatty acids are long chain hydrocarbons with carboxyl groups (COOH groups). These fatty acids can be classified into saturated or unsaturated based on the number of double bonds present in the fatty acid. Saturated fatty acids contain only single bond between the carbon atoms and are tend to be solids at room temperature. Unsaturated fatty acids contain double bonds between the carbon atom in addition to the single bonds present in the fatty acid chain. They are likely to exists as liquids at room temperature. The double bonds present in the naturally occurring unsaturated fats are in the Cis form. Trans fatty acids are associated with health problems and cardiovascular diseases.

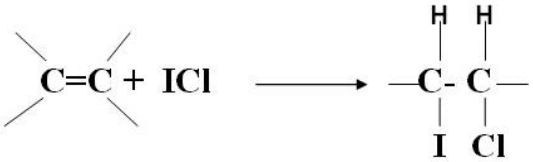
Unsaturated fatty acids can be converted into saturated by the process of hydrogenation. Depending upon the degree of unsaturation, the fatty acids can combine with oxygen or halogens to form saturated fatty acids. So it is important to know the extend to which a fatty acid is unsaturated. There are different methods for checking the unsaturation level in fatty acids, one among them is by determining the iodine value of fats. Iodine value or number is the number of grams of iodine consumed by 100g of fat. A higher iodine value indicates a higher degree of unsaturation.



Unsaturated fatty acids

Principle:

Fatty acids react with a halogen [iodine] resulting in the addition of the halogen at the C=C double bond site. In this reaction, iodine monochloride reacts with the unsaturated bonds to produce a di-halogenated single bond, of which one carbon has bound an atom of iodine.



After the reaction is complete, the amount of iodine that has reacted is determined by adding a solution of potassium iodide to the reaction product.

ICl + KI ----------> KCl + I2

This causes the remaining unreacted ICl to form molecular iodine. The liberated I2 is then titrated with a standard solution of 0..1N sodium thiosulfate.

$$I2 + 2\ Na2S2O3 \text{ ----------> } 2\ NaI + Na2S2O4$$

Saturated fatty acids will not give the halogenation reaction. If the iodine number is between 0-70, it will be a fat and if the value exceeds 70 it is an oil. Starch is used as the indicator for this reaction so that the liberated iodine will react with starch to give purple coloured product and thus the endpoint can be observed.

Important note:

Iodine monochloride is caustic. So handle the reagent with gloves.

For better results, perform the experiments without any time gap during addition of reagents as the liberated iodine is susceptible to oxidation by light.

Materials Required:

- Iodine Monochloride Reagent
- Potassium Iodide
- Standardized 0.1 N Sodium thiosulphate
- 1% Starch indicator solution
- Reagent bottle
- Chloroform
- Fat sample in chloroform
- Iodination flask
- Burette and burette stand with magnetic stirrer
- Glass pipette
- Measuring cylinder
- Distilled water

Method:

1. Arrange all the reagent solutions prepared and the requirements on the table.

2. Pipette out 10ml of fat sample dissolved in chloroform to an iodination flask labeled as “TEST".

3. Add 20ml of Iodine Monochloride reagent in to the flask. Mix the contents in the flask thoroughly.

4. Then the flask is allowed to stand for a half an hour incubation in dark.

5. Set up a BLANK in another iodination flask by adding 10ml Chloroform to the flask.

6. Add to the BLANK, 20ml of Iodine Monochloride reagent and mix the contents in the flask thoroughly.

7. Incubate the BLANK in dark for 30 minutes.

8. Mean while, Take out the TEST from incubation after 30 minutes and add 10 ml of potassium iodide solution into the flask.

9. Rinse the stopper and the sides of the flask using 50 ml distilled water.

10. Titrate the "TEST" against standardized sodium thiosulphate solution until a pale straw colour is observed.

11. Add about 1ml starch indicator into the contents in the flask, a purple colour is observed.

12. Continue the titration until the color of the solution in the flask turns colourless.

13. The disappearance of the blue colour is recorded as the end point of the titration.

14. Similarly, the procedure is repeated for the flask labeled 'Blank'.

15. Record the endpoint values of the BLANK .

16. Calculate the iodine number using the equation below:

Volume of Sodium thiosulphate used = [Blank- Test] ml

$$Iodine\ No.of\ fat = \frac{Equivalent\ Wt.of\ Iodine \times Volume\ of\ Na_2S_2O_3\ used \times Normality\ of\ Na_2S_2O_3 \times 100 \times 10^{-}}{Weight\ of\ fat\ sample\ used\ for\ analysis(g)}$$

Equivalent Weight of Iodine = 127

Normality of sodium thiosulphate ($Na_2S_20_3$) = 0.1

Estimation of Crude Protein in Fishery Products by Micro-kjeldahl Method

Nutritionally, protein is the most important constituent of fish. The content of protein, on an average is about 19% and the range is 15-20%.

Objective:

After performing this experiment, you will be able to:

- Estimate the Crude Protein content in Fishery Products by Micro-Kjeldahl Method.

Principle:

The nitrogen in the compound is converted into ammonium sulphate by digesting the compound with concentrated sulphuric acid. Ammonium sulphate thus obtained is decomposed by alkali and the liberated ammonia is absorbed in boric acid and titrated against standard sulphuric acid/ hydrochloric acid.

Requirements:

- **Boric acid:** A 2% solution is prepared by dissolving 2g of boric acid in 100 ml of water.
- **Sodium hydroxide:** A 40% solution is prepared by dissolving 40g sodiumhydroxide in 100 ml water.
- **Mixed indicator:** A 0.2% solution is prepared by dissolving 150 mg methylene blue and 50 mg methyl red in 100 ml absolute alcohol.
- **Digestion mixture:** Copper sulphate and potassium sulphate are mixed in the ratio of 1:5 by weight.
- **Standard 0.05N H_2SO_4 :** An approximately 0.1 N solution of H_2SO_4 is prepared by diluting 2.8 ml of concentrated sulphuric acid to 1 litre. It is standardized against standard alkali and diluted to give an exactly 0.05 N H_2SO_4.

Procedure:

1) 50 to 200 mg of dry sample or about 1 g of wet sample is weighed into a Kjeldahl's digestion flask.

2) 10ml concentrated sulphuric acid and a pinch of digestion mixture (Potassiumsulphate: copper sulphate: selenium dioxide in the ratio of 100:10:2.5) is added to the flask and the flask is heated on a Kjeldahl digestion rack. A glass bead is added to reaction mixture to prevent bumping.

3) The mixture is digested for 6-8 hrs, or until the digest becomes clear or colorless to ensure complete conversion of the nitrogen in the sample into ammonium sulphate. After cooling, the volume is made up to 100ml with distilled water.

4) From this solution, 2 ml is taken for distillation along with 10ml of 40%sodium hydroxide, added to the inner chamber in Kjeldahl distillation apparatus.

5) Liberated ammonia is steam distilled into a receiver flask containing 10 ml of 2% boric acid solution to which 2-3 drops of mixed indicator is added.

6) The indicator is red in boric acid and turns green when ammonia is absorbed. The distillation is carried out for 5 minutes and the content of the receiver flask (ammonia absorbed in boric acid) is titrated against standard.0.05N H_2SO_4 using a microburette until the original red colour is obtained as compared with a blank.

The nitrogen present in the sample is calculated using the nitrogen equivalence viz., 1 ml of 0.05 N H_2SO_4 = 0.0007g of nitrogen. The protein value is obtained by multiplying the N content by 6.25.

Calculation:

Let 'X' ml of 0.05 N of H_2SO_4 be required to titrate the ammonia liberated from 5ml of the made up solution (titre value-blank)

1 ml of 0.05 N H_2SO_4 = 0.0007g N_2

X ml of 0.05 N H_2SO_4 = 0.0007 × Xg of N_2

. . . 5 ml of sample contains = 0.0007 × Xg of N_2

100 ml of sample contains = 0.0007 × X × 100g N / 5

Let "W" be the weight of the sample taken

"W" g of sample contains = 0.0007 × X × 100g N / 5

100 g of the sample contains = 0.0007 × 100 × X × 100g of N / 5

Protein percentage = 0.0007 × 100 × X × 100 × 6.25g / 5

Result Crude protein% of the given sample is ..... %

Estimation of free fatty acids

A small quantity of free fatty acids is usually present in oils along with the triglycerides. The free fatty acid content is known as acid number/acid value. It increases during storage. The keeping quality of oil therefore relies upon the free fatty acid content.

Principle:

The free fatty acid in oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required to neutralize the free fatty acids present in 1g of sample. However, the free fatty acid content is expressed as oleic acid equivalents.

Materials:

» 1% phenolphthalein in 95% ethanol.

» 0.1N potassium hydroxide

» Neutral Solvent: Mix 25mL ether, 25mL 95% alcohol and 1mL of 1% phenolphthalein solution and neutralize with N/10 alkali.

Procedure:

1. Dissolve 1-10g of oil or melted fat in 50mL of the standard solvent in a 250mL conical flask.
2. Add a few drops of phenolphthalein.
3. Titrate the contents against 0.1N potassium hydroxide.
4. Shake constantly until a pink color which persists for fifteen seconds is obtained.

Calculation:
Acid value (mg KOH/g) = Titrate value x Normality of KOH x 56.1 / Weight of sample (g)
The free fatty acid is calculated as oleic acid using the equation
1mL N/10 KOH = 0.028g oleic acid

Note:

To find out the exact strength of KOH, prepare 0.1N oxalic acid solution (630mg in 100mL water) and titrate against KOH with phenolphthalein as indicator. Calculate the strength of KOH by the formula V1N1 = V2N2.

Alternative Procedure: 1

Objectives:

After attending to this experiment, we shall be able to:

• learn to perform determination of free fatty acids and acid value in oils and fats.

Introduction:

The Acid value has wide implication in the oil refining industry. It conveys not only the quality of oil but also total quantity of alkali needed to neutralize the acidity in a particular batch for making it suitable for the purpose of hydrogenation or marketing of refined oil or fat of very low acidity. Acid value is a measure of the hydrolytic rancidity present in the sample.

Principle:

The acid value is determined by directly titrating the material in an alcoholic medium with aqueous sodium or potassium hydroxide solution. Acid value is the number of mg of KOH required to neutralize the free fatty acids present in 1 g of the oil or fat. Free fatty acid is calculated as oleic, lauric, ricinoleic or palmitic acids.

Requirements:

Reagents:

Ethyl Alcohol - 95%v/v, neutral to phenolphthalein indicator.

Phenolphthalein Indicator Solution - Dissolve 1 g of phenolphthalein in 100 ml of ethyl alcohol.

Note : When testing oils or fats which give dark coloured soap solution, the observation of the end point of the titration may be facilitated either (a) by using thymolphthalein or alkali blue 6B in place of phenolphthalein, or (b) by adding 1 ml of a 0.1%, w/v solution of methylene blue in water to each 100 ml of phenolphthalein indicator solution before the titration.

Standard Aqueous Potassium Hydroxide or Sodium Hydroxide Solutions - 0.1 N or 0.5 N.

Procedure:

Mix the oil or melted fat thoroughly before weighing. Weigh accurately a suitable quantity of the cooled oil or fat in a 200 ml conical flask. The weight of the oil or fat taken for the test and the strength of the alkali used for the titration shall be such that the volume of alkali required for the titration does not exceed 10 ml. Add 50 to 100 ml of freshly neutralized hot ethyl alcohol, and about 1 ml of phenolphthalein indicator solution. Boil the mixture for about five minutes and titrate while as hot as possible with standard aqueous alkali solution, shaking vigorously during titration.

Calculation:

Acid value = 56.1 × N × V / W

Where,

V = volume in ml of standard KOH/NaOH solution used,

N = normality of standard KOH/NaOH solution, and

W = weight in g of the material taken for the test.

Results And Inference:

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst (repeatability) shall not exceed 0.1. The acid value of commonly used edible oils is given as below.

Type of Oil	Acid Value	Type of Oil	Acid Value
Coconut oil	0.5	Safflower oil	2.0
Cottonseed oil	0.3	Sunflower oil	0.5
Groundnut oil	0.5	Soybean oil	0.5
Mustard oil	0.5	Rice bran oil	0.5
Sesame oil	0.5	Palm oil	0.5

Precautions:

• The formation of two layers should be avoided by vigorous shaking so that the free acids do not get transferred into the ethanolic layer.

• The freshly neutralized alcohol must also be hot at the time of addition.

• The weight of the oil or fat taken for acidity determination and the strength of NaOH should be such that the volume of alkali used does not exceed 10 ml.

Alternative Procedure: 2

Revised Method For Determination Of Acid Value In Oils And Fats

Definition:

The acid value is defined as the number of milligrams of Potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of triglycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid, lauric, ricinoleic and palmitic acids.

Principle:

The acid value is determined by directly titrating the oil/fat in an alcoholic medium against standard potassium hydroxide/sodium hydroxide solution.

Analytical Importance:

The value is a measure of the amount of fatty acids, which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and/or lipolytic enzyme lipase.

Apparatus:

250 mL conical flasks

Reagents:

a) Phenolphthalein indicator solution: Dissolve one gram of phenolphthalein in 100mL of ethyl alcohol.

b) Alkali Blue 6B indicator solution: When testing rice bran oil or rice bran oil based blended oils or fats, which give dark colored soap solution, the observation of the end point of the titration may be facilitated, by using Alkali Blue 6B in place of Phenolphthalein.

Preparation: (2%) Extract 2gm of alkali blue 6B with rectified spirit in a Soxhlet apparatus at reflux temperature. Filter the solution if necessary and dilute to 100Ml with rectified spirit. Alkali blue 6B indicator to be stored in closed Ambered colored bottle to avoid oxidation of dye.

c) Ethyl alcohol:
1) Ninety-five percent alcohol or rectified spirit neutral to phenolphthalein indicator.
2) Ninety-five percent alcohol or rectified spirit neutral to Alkali blue 6B indicator in case of rice bran oil or rice bran oil based blended oil or fats.

d) Standard aqueous Potassium hydroxide or sodium hydroxide solution 0.1 or 0.5 N. The solution should be colourless and stored in a brown glass bottle. For refined oils, the strength of the alkali should be fixed to 0.1 N.

Procedure:
Mix the oil or melted fat thoroughly before weighing. The mass of the test sample shall be taken based on the colour and expected acid value.

Expected Acid Value	Mass of Test portion (gm)	Accuracy of weighing of test portion (gm)
<1	20	0.05
1 to 4	10	0.02
4 to 15	2.5	0.01

15 to 75	0.5	0.001
>75	0.1	0.0002

a) Weigh accurately appropriate amount of the cooled oil sample as mentioned in the above table in a 250 mL conical flask.

b) Add 50 mL of freshly neutralised hot ethyl alcohol and about one ml of phenolphthalein indicator solution. In case of rice bran oil or RBO based blends, add about 1mL of Alkali blue indicator.

c) Heat the mixture for about fifteen minutes in water bath (75-80°C) In case of Rice bran oil or RBO based blended oils or fats, add 1mL of Alkali blue indicator after heating.

d) Titrate while hot against standard alkali solution shaking vigorously during the titration.

e) End point using phenolphthalein indicator shall be from colourless to light pink (Persisting for 15 sec.)

f) End point using Alkali blue 6B indicator shall be disappearance of blue colour which developed during addition of indicator.

Note: Noting burette reading after "obtaining dark pink colour OR Orangish red" as end point should be avoided as it will lead to erroneous result

g) The weight of the oil/fat taken for the estimation and the strength of the alkali used for titration shall be such that the volume of alkali required for the titration does not exceed 10mL.

Calculation:

Acid value = 56.1 × V×N / W

Where,

V = Volume in mL of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution; and

W = Weight in gm of the sample

Determination Of Free Fatty Acid (FFA)

Introduction:

The FFA figure is usually calculated as oleic acid by dividing the acid value by 2. With most oils the acidity begins to be noticeable to the palate when the FFA calculated as oleic acid is about 0.5-1.5%.

When the FFA cannot be estimated in terms of oleic acid, it can be calculated from the saponification value.

Calculation:

1. Determination of Free Fatty Acid from Acid Value

   FFA (%) = acid value x mol. wt. of oleic acid x 100 / mol. wt. of KOH x 1000
   = acid value x 282.27 x 1 / 56.11 x 10
   = acid value x 1 / 2

2. Determination of Free Fatty Acid from Acid Value And Saponification Value Expressed as mg Number per 100 g Meat

   FFA (m g/100 g) = acid value x total lipid x 100 / saponification value

Tests for Known Sample:

1. Tests for Carbohydrates

Experiment	Observation	Inference
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4 slowly along the side of the test tube.	A red violet (purple) ring is formed at the junction of two layers.	Presence of carbohydrate.
Benedict's test: Take 1-2 ml of aqueous solution of the sample in a test tube and add 1-2 ml of Benedict's reagent. Keep the test tube in a boiling water bath.	A reddish precipitate is formed.	Presence of a reducing sugar.
Barfoed's test: Take 2 ml of Barfoed's reagent in a test tube. Add 2 ml of the test solution to the above test tube. Mix the solutions and hold the test tube on flame and boil for minutes. Allow to cool at	Red precipitates are formed after the first 5 minutes	Presence of a monosaccharide.

room temperature.		
Seliwanoff's test: Take 3 ml of Seliwanoff's reagent in a test tube. Add 1 ml of test solution in the above test tube. Hold the test tube on flame and allow to boil for 30 seconds. Allow to cool at room temperature	Cherry red colour forms in the test tube upon cooling.	Presence of a keto-sugar.
Iodine test: Take 2 ml of the given solution in a test tube. Add 2-3 drops of iodine reagent in the above test tube. Wait for some time	When the iodine is added to the solution, the colour of the solution turns blue.	Presence of a polysaccharide.

2. Tests for Proteins

Experiment	Observation	Inference
Biuret test: Take a cleaned and dried test tube and add the sample into the test tube. Add 2ml of sodium hydroxide and 5 to 6 drops of copper sulfate solution to it. Shake the test tube gently to mix the ingredients thoroughly and allow the mixture to stand for 4 – 5 minutes.	A bluish- violet colour appears.	Presence of protein.
Xanthoproteic test: Take a cleaned and dried test tube and add the sample into the test tube. Add a few drops of concentrated sulfuric acid (H_2SO_4) and shake the test tube. Heat the test tube gently on a Bunsen burner.	A yellow precipitate forms.	Presence of protein.
Ninhydrin test: Take 1 ml. of test solution in a test tube. Add 10 drops of Ninhydrin solution in the above test tube. Hold the test tube on flame. Boil the solution.	Bluishpurple color formed in the solution.	Presence of free alpha amino acids.
Solubility test:	The given powder is	Presence insoluble protein.

Take 10 ml. distilled water in a test tube. Add the given powder into the water. Shake the test tube.	insoluble in water.	
Isoelectric pH test: Take 3 ml. test solution in a test tube. Add 3 drops of indicator (bromocresol green). Add 1% acetic acid solution to the above test tube drop by drop. Keep adding acetic acid until a light green color appears indicating isoelectric pH. Allow it to stand.	A curdy green precipitate is formed at the top of the test tube.	Presence of milk protein.

3. Tests for Lipids

Experiment	Observation	Inference
Acrolein test: Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.	A pungent irritating odour or smell of acrolein is felt.	Presence of lipids.
Sudan III test: Take 0.5 ml ether or chloroform in a test tube. Add 0.5 ml sample-drop by drop till the sample is fully dissolves. Add one drop of Sudan III reagent.	Red colour appears.	Presence of lipids.
Solubility Test: Take five test tubes marking A, B, C, D, E. Put 5 ml—water, absolute alcohol, ether, chloroform and benzene one in each test tube respectively. Add 3 to 4 drops of sample in each test tube, shake thoroughly, allow to stand.	In test tube A drops of the sample are seen floating on the surface of water. In test tube B sample drops settle at the bottom of alcohol. In test tubes C, D and E the sample is mixed.	Presence of lipids.

Tests for Unknown Sample:

1. Tests for Glucose (Reducing Monosaccharide, Aldose)

Experiment	Observation	Inference
Biuret test: **(Alternative - Xanthoproteic test, Millions test, Ninhydrin test)** Take 2 ml. of the solution to be tested in a test tube. Add 2 ml. of 5% sodium hydroxide solution. Mix the solutions. Add two drops of 1% copper sulphate solution	No violet or purple colour formed in the solution.	The sample doesn't contain any form of protein.
Acrolein test: **(Alternative - Sudan III test, Paper spot test)** Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4 slowly along the side of the test tube.	A red violet (purple) ring is formed at the junction of two layers.	Presence of carbohydrate (Glucose, Fructose, Lactose, Maltose, Sucrose, Starch) is confirmed.
Iodine test: Take 2 ml of the given solution in a test tube. Add 2-3 drops of iodine reagent in the above test tube. Wait for some time	When the iodine is added to the solution, the colour of the solution doesn't change. No blue colour appears.	No polysaccharide (Starch) is present in the sample solution.
Benedict's test: **(Alternative - Fehling's test, Tollen's test)** Take 1-2 ml of aqueous solution of the sample in a test tube and add 1-2 ml of Benedict's reagent. Keep the test tube in a boiling water bath.	A reddish precipitate is formed.	Reddish precipitate indicates the presence of a reducing sugar (Glucose, Fructose, Lactose, Maltose).
Barfoed's test: Take 2 ml of Barfoed's reagent in a test tube. Add 2 ml of the test solution to the above test tube. Mix the	Red precipitates are formed after the first 5 minutes	Formation of red precipitates indicates the presence of a monosaccharide (Glucose, Fructose).

solutions and hold the test tube on flame and boil for minutes. Allow to cool at room temperature.		
Seliwanoff's test: Take 3 ml of Seliwanoff's reagent in a test tube. Add 1 ml of test solution in the above test tube. Hold the test tube on flame and allow to boil for 30 seconds. Allow to cool at room temperature	No cherry red colour forms in the test tube upon cooling.	The given solution contains an aldo-sugar (Glucose).

2. Tests for Fructose (Reducing Monosaccharide, Ketose)

Experiment	Observation	Inference
Biuret test: (Alternative - Xanthoproteic test, Millions test, Ninhydrin test) Take 2 ml. of the solution to be tested in a test tube. Add 2 ml. of 5% sodium hydroxide solution. Mix the solutions. Add two drops of 1% copper sulphate solution	No violet or purple colour formed in the solution.	The sample doesn't contain any form of protein.
Acrolein test: (Alternative - Sudan III test, Paper spot test) Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4 slowly along the side of the test tube.	A red violet (purple) ring is formed at the junction of two layers.	Presence of carbohydrate (Glucose, Fructose, Lactose-Maltose, Sucrose, Starch) is confirmed.
Iodine test: Take 2 ml of the given solution in a test tube. Add 2-3 drops of iodine reagent in the above test tube. Wait for	When the iodine is added to the solution, the colour of the solution doesn't change. No blue colour appears.	No polysaccharide (Starch) is present in the sample solution.

some time		
Benedict's test: **(Alternative - Fehling's test, Tollen's test)** Take 1-2 ml of aqueous solution of the sample in a test tube and add 1-2 ml of Benedict's reagent. Keep the test tube in a boiling water bath.	A reddish precipitate is formed.	Reddish precipitate indicates the presence of a reducing sugar (Glucose, Fructose, Lactose-Maltose).
Barfoed's test: Take 2 ml of Barfoed's reagent in a test tube. Add 2 ml of the test solution to the above test tube. Mix the solutions and hold the test tube on flame and boil for minutes. Allow to cool at room temperature.	Red precipitates are formed after the first 5 minutes	Formation of red precipitates after the initial first 5 minutes indicates the presence of a monosaccharide (Glucose, Fructose).
Seliwanoff's test: Take 3 ml of Seliwanoff's reagent in a test tube. Add 1 ml of test solution in the above test tube. Hold the test tube on flame and allow to boil for 30 seconds. Allow to cool at room temperature	A cherry red colour forms in the test tube upon cooling.	The given solution contains a keto-sugar (Glucose).

3. Tests for Sucrose (Non-reducing Disaccharide)

Experiment	Observation	Inference
Biuret test: **(Alternative - Xanthoproteic test, Millions test, Ninhydrin test)** Take 2 ml. of the solution to be tested in a test tube. Add 2 ml. of 5% sodium hydroxide solution. Mix the solutions. Add two drops of 1% copper sulphate solution	No violet or purple colour formed in the solution.	The sample doesn't contain any form of protein.
Acrolein test: **(Alternative - Sudan III test, Paper spot test)** Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.

change in odour.		
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H_2SO_4 slowly along the side of the test tube.	A red violet (purple) ring is formed at the junction of two layers.	Presence of carbohydrate (Glucose, Fructose, Lactose, Maltose, Sucrose, Starch) is confirmed.
Iodine test: Take 2 ml of the given solution in a test tube. Add 2-3 drops of iodine reagent in the above test tube. Wait for some time	When the iodine is added to the solution, the colour of the solution doesn't change. No blue colour appears.	No polysaccharide (Starch) is present in the sample solution.
Barfoed's test: Take 2 ml of Barfoed's reagent in a test tube. Add 2 ml of the test solution to the above test tube. Mix the solutions and hold the test tube on flame and boil for minutes. Allow to cool at room temperature.	No red precipitate is formed after the first 5 minutes.	Absence of red precipitate indicates the absence of a monosaccharide (Glucose, Fructose). Presence of disaccharide (Lactose, Maltose, Sucrose)
Benedict's test: (Alternative - Fehling's test, Tollen's test) Take 1-2 ml of aqueous solution of the sample in a test tube and add 1-2 ml of Benedict's reagent. Keep the test tube in a boiling water bath.	No reddish precipitate is formed.	Absence of a reducing sugar (Lactose, Maltose). Presence of non-reducing sugar (Sucrose).
Seliwanoff's test: Take 3 ml of Seliwanoff's reagent in a test tube. Add 1 ml of test solution in the above test tube. Hold the test tube on flame and allow to boil for 30 seconds. Allow to cool at room temperature.	A cherry red colour forms in the test tube upon cooling.	The given solution contains an aldo-keto sugar (Sucrose).
Hydrolysis and Benedict's test: Take 2ml of sample in a test tube and add a few drops of HCl and boil the test tube gently for one to two minutes. Make the solution alkaline with NaOH. Now perform Benedict's test with	Orange or brick red ppt is observed in the test tube. Sucrose sugar is a "non-reducing sugar" and thus, will give a negative result (bluish clear solution) with this test. Hydrolysis of sucrose causes the generation of "reducing	Sample contains non-reducing disaccharide (Sucrose).

the solution.	sugars" (fructose and glucose), and give a positive result for Benedict's test.)	

4. Tests for Lactose (Reducing Disaccharide)

Experiment	Observation	Inference
Biuret test: **(Alternative - Xanthoproteic test, Millions test, Ninhydrin test)** Take 2 ml. of the solution to be tested in a test tube. Add 2 ml. of 5% sodium hydroxide solution. Mix the solutions. Add two drops of 1% copper sulphate solution	No violet or purple colour formed in the solution.	The sample doesn't contain any form of protein.
Acrolein test: **(Alternative - Sudan III test, Paper spot test)** Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4 slowly along the side of the test tube.	A red violet (purple) ring is formed at the junction of two layers.	Presence of carbohydrate (Glucose, Fructose, Lactose, Maltose, Sucrose, Starch) is confirmed.
Iodine test: Take 2 ml of the given solution in a test tube. Add 2-3 drops of iodine reagent in the above test tube. Wait for some time	When the iodine is added to the solution, the colour of the solution doesn't change. No blue colour appears.	No polysaccharide (Starch) is present in the sample solution.
Benedict's test: **(Alternative - Fehling's test, Tollen's test)** Take 1-2 ml of aqueous solution of the sample in a test tube and add 1-2 ml of Benedict's reagent. Keep the test tube in a boiling water bath.	A reddish precipitate is formed.	Reddish precipitate indicates the presence of a reducing sugar (Glucose, Fructose, Lactose, Maltose).

Barfoed's test: Take 2 ml of Barfoed's reagent in a test tube. Add 2 ml of the test solution to the above test tube. Mix the solutions and hold the test tube on flame and boil for minutes. Allow to cool at room temperature.	No red precipitate is formed.	No change indicates the absence of monosaccharide (Glucose, Fructose). Presence of reducing disaccharide (Lactose, Maltose)
Osazone test: Take 5 ml of test solution in a test tube, add 3 pinches of osazone mixture. Mix thoroughly and boil for five minutes. Check every 5 minutes to ensure yellow crystals are not missed	Cotton ball-shaped crystals are formed within 30 minutes.	Cotton ball-shaped crystals indicate the presence of Lactose in the sample.

5. Tests for Maltose (Reducing Disaccharide)

Experiment	Observation	Inference
Biuret test: (Alternative - Xanthoproteic test, Millions test, Ninhydrin test) Take 2 ml. of the solution to be tested in a test tube. Add 2 ml. of 5% sodium hydroxide solution. Mix the solutions. Add two drops of 1% copper sulphate solution	No violet or purple colour formed in the solution.	The sample doesn't contain any form of protein.
Acrolein test: (Alternative - Sudan III test, Paper spot test) Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4 slowly along the side of the test tube.	A red violet (purple) ring is formed at the junction of two layers.	Presence of carbohydrate (Glucose, Fructose, Lactose, Maltose, Sucrose, Starch) is confirmed.
Iodine test:	When the iodine is added to	No polysaccharide (Starch) is

Take 2 ml of the given solution in a test tube. Add 2-3 drops of iodine reagent in the above test tube. Wait for some time	the solution, the colour of the solution doesn't change. No blue colour appears.	present in the sample solution.
Benedict's test: **(Alternative - Fehling's test, Tollen's test)** Take 1-2 ml of aqueous solution of the sample in a test tube and add 1-2 ml of Benedict's reagent. Keep the test tube in a boiling water bath.	A reddish precipitate is formed.	Reddish precipitate indicates the presence of a reducing sugar (Glucose, Fructose, Lactose, Maltose).
Barfoed's test: Take 2 ml of Barfoed's reagent in a test tube. Add 2 ml of the test solution to the above test tube. Mix the solutions and hold the test tube on flame and boil for minutes. Allow to cool at room temperature.	No red precipitate is formed.	No change indicates the absence of monosaccharide (Glucose, Fructose). Presence of reducing disaccharide (Lactose, Maltose)
Osazone test: Take 5 ml of test solution in a test tube, add 3 pinches of osazone mixture. Mix thoroughly and boil for five minutes. Check every 5 minutes to ensure yellow crystals are not missed.	Sunflower shaped crystals are formed within 30 to 40 minutes.	Sunflower shaped crystals indicate the presence of Maltose in the sample.

6. Tests for Starch (Polysaccharide)

Experiment	Observation	Inference
Biuret test: **(Alternative - Xanthoproteic test, Millions test, Ninhydrin test)** Take 2 ml. of the solution to be tested in a test tube. Add 2 ml. of 5% sodium hydroxide solution. Mix the solutions. Add two drops of 1% copper sulphate solution	No violet or purple colour formed in the solution.	The sample doesn't contain any form of protein.
Acrolein test: **(Alternative - Sudan III test, Paper spot test)** Take the sample to be tested	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.

in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.		
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4 slowly along the side of the test tube.	A red violet (purple) ring is formed at the junction of two layers.	Presence of carbohydrate (Glucose, Fructose, Lactose, Maltose, Sucrose, Starch) is confirmed.
Benedict's test: **(Alternative - Fehling's test, Tollen's test)** Take 1-2 ml of aqueous solution of the sample in a test tube and add 1-2 ml of Benedict's reagent. Keep the test tube in a boiling water bath.	No reddish precipitate is formed.	Absence of a reducing sugar (Lactose, Maltose). Presence of non-reducing sugar (Sucrose) or polysaccharide (Starch).
Hydrolysis and Benedict's test: Take 2ml of sample in a test tube and add a few drops of HCl and boil the test tube gently for one to two minutes. Make the solution alkaline with NaOH. Now perform Benedict's test with the solution.	Orange or brick red ppt is observed in the test tube. (Sucrose is a "non-reducing sugar" and Starch is a "non-reducing polysaccharide" and thus, will give negative results (bluish clear solution) with the Benedict's test. Hydrolysis of Sucrose and Starch causes the generation of "reducing sugars" (Sucrose into Fructose and Glucose and Starch into Glucose), and give a positive result for Benedict's test.)	Sample contains non-reducing sugar (Sucrose) or polysaccharide (Starch).
Iodine test: Take 2 ml of the given solution in a test tube. Add 2-3 drops of iodine reagent in the above test tube. Wait for some time	When the iodine is added to the solution, the colour of the solution turns blue.	Polysaccharide (Starch) is present in the sample solution.

7. Tests for Albumin (Protein)

Experiment	Observation	Inference
Molisch's test:	No red violet (purple) ring is	The sample doesn't contain

Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4 slowly along the side of the test tube.	formed at the junction of two layers.	any form of carbohydrate.
Acrolein test: **(Alternative - Sudan III test, Paper spot test)** Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.
Biuret test: **(Alternative - Xanthoproteic test, Millions test, Ninhydrin test)** Take a cleaned and dried test tube and add the sample into the test tube. Add 2ml of sodium hydroxide and 5 to 6 drops of copper sulfate solution to it. Shake the test tube gently to mix the ingredients thoroughly and allow the mixture to stand for 4 – 5 minutes.	A bluish- violet colour appears.	Appearance of bluish- violet colour indicates the presence of protein in the sample.
Xanthoproteic test: Take a cleaned and dried test tube and add the sample into the test tube. Add a few drops of concentrated sulfuric acid (H_2SO_4) and shake the test tube. Heat the test tube gently on a Bunsen burner.	A yellow precipitate forms.	Formation of yellow precipitate confirms the presence of protein in the sample.

8. Tests for Peptone (Denatured Protein)

Experiment	Observation	Inference
Molisch's test: Take about 2 ml of aqueous solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H2SO4	No red violet (purple) ring is formed at the junction of two layers.	The sample doesn't contain any form of carbohydrate.

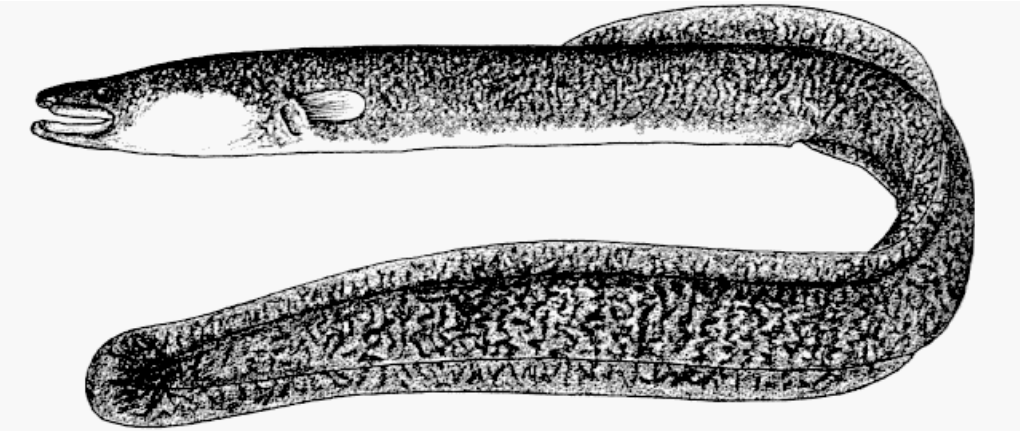
slowly along the side of the test tube.		
Acrolein test: **(Alternative - Sudan III test, Paper spot test)** Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate (KHSO4) to it. Heat the mixture and observe the change in odour.	There is no pungent irritating odour or smell of acrolein.	The sample doesn't contain any form of fat or oil.
Biuret test: **(Alternative - Xanthoproteic test, Millions test, Ninhydrin test)** Take a cleaned and dried test tube and add the sample into the test tube. Add 2ml of sodium hydroxide and 5 to 6 drops of copper sulfate solution to it. Shake the test tube gently to mix the ingredients thoroughly and allow the mixture to stand for 4 – 5 minutes.	A bluish- violet colour appears.	Appearance of bluish- violet colour indicates the presence of protein in the sample.
Xanthoproteic test: Take a cleaned and dried test tube and add the sample into the test tube. Add a few drops of concentrated sulfuric acid (H_2SO_4) and shake the test tube. Heat the test tube gently on a Bunsen burner.	A yellow precipitate forms.	Formation of yellow precipitate confirms the presence of protein in the sample.

9. Tests for Fats and Oils (Lipid)

Experiment	Observation	Inference
Biuret test: **(Alternative - Xanthoproteic test, Millions test, Ninhydrin test)** Take 2 ml. of the solution to be tested in a test tube. Add 2 ml. of 5% sodium hydroxide solution. Mix the solutions. Add two drops of 1% copper sulphate solution	No violet or purple colour formed in the solution.	The sample doesn't contain any form of protein.
Molisch's test: Take about 2 ml of aqueous	No red violet (purple) ring is formed at the junction of two	The sample doesn't contain any form of carbohydrate.

solution of the sample in a test tube and add few drops of Molisch's reagent into it. Pour 1 ml conc. H_2SO_4 slowly along the side of the test tube.	layers.	
Acrolein test: **(Alternative - Sudan III test, Paper spot test)** Take the sample to be tested in a test tube. Add few crystals of potassium bisulfate ($KHSO_4$) to it. Heat the mixture and observe the change in odour.	A pungent irritating odour or smell of acrolein is felt.	Pungent irritating odour of acrolein indicates the presence of fat in the sample.
Sudan III test: Take 0.5 ml ether or chloroform in a test tube. Add 0.5 ml sample-drop by drop till the sample is fully dissolves. Add one drop of Sudan III reagent	Red colour appears.	Appearance of red colour confirms the presence of fat in the sample.

Specimen: 1



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Elopomorpha
Order – Anguilliformes
Family – Anguillidae
Genus – *Anguilla*

Species – *bengalensis*

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

- 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 pectoral fins.
- 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 ventral surface
- Lips are thick and well developed
- Jaws are equal
- Caudal fin continued round the end of the tail

Hence, Genus – *Anguilla*

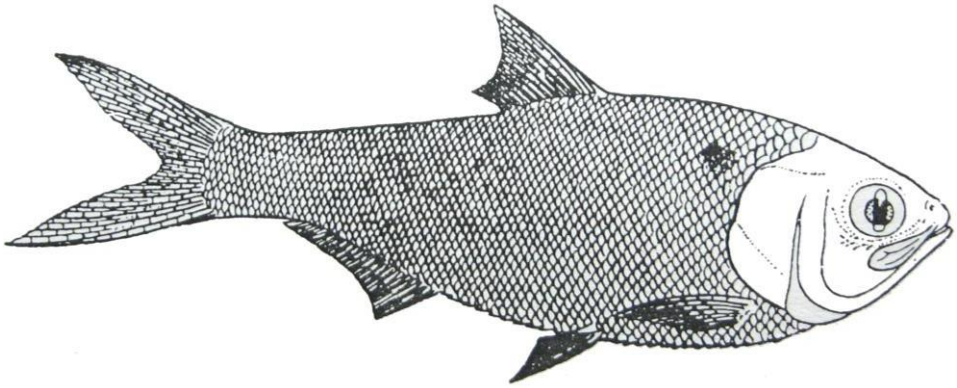
- Villiform teeth are present in the jaws and palate
- Dorsal fin inserted midway between gill opening and origin of anal fin with 220-305 rays and no spine
- Anal fin long with 200 – 250 rays
- Colouration of adult is variegated

Hence, species – *bengalensis*

Hence, the provided specimen is *Anguilla bengalensis*

Distribution - Throughout India, Pakistan, Bangladesh, Sri Lanka, Burma and Malay

Specimen: 2



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Clupeomorpha
Order – Clupeiformes
Family – Clupeidae
Genus – *Gudusia*
Species – *chapra*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Branchiostegals numbering as high as 15, usually fewer
- Mesocoracoid arch invariably present
- Usually no lateral line possess on trunk

Hence, Super Order – Clupeomorpha

- Body short, oblong, not eel like, covered with minute cycloid scales, scales are absent in head regions
- Abdomen with keeled scutes along the ventral line
- Radiating cutaneous canals on opercular bones

Hence, Order – Clupeiformes

- Maxillaries composed of three pieces and not composed with together
- Teeth when present, is rudimentary and deciduous
- Barbells absent
- Gill membranes free
- Opercular pieces four

Hence, Family – Clupeidae

- Body compressed and oblong
- Abdomen serrated, with 18 – 19 pre pelvic and 8 – 10 post pelvic scutes
- Head short and highly compressed
- Snout rounded
- Mouth slightly upturned, terminal, cleft not extending to the orbit
- Eyes large, lateral, in anterior part of the head and not visible from the ventral surface with broad adipose eyelid
- Lips thin
- Caudal fin forked

Hence, Genus – *Gudusia*

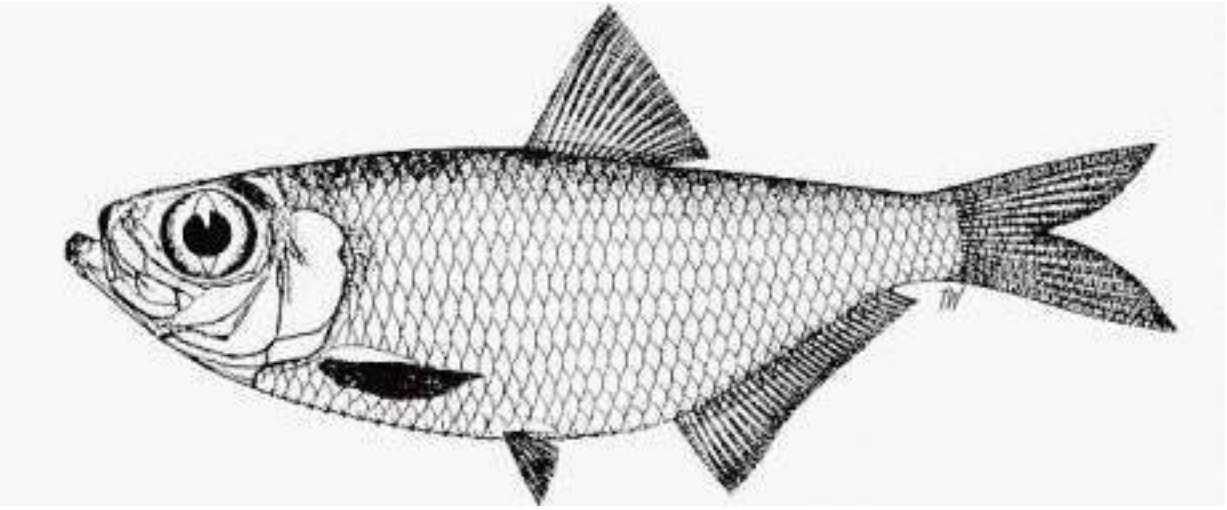
- Body without any cross bars on sides
- Dorsal fins inserted in above pelvic origin, with 16 rays
- Anal fin with 19 – 21 rays
- Caudal fin forked
- Lateral line absent, 80 – 120 scales in the lateral series

Hence, species – *chapra*

Hence, the provided specimen, *Gudusia chapra*

Distribution – India, Pakistan, Bangladesh, China and Burma, Nepal, Malaya

Specimen: 3



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Clupeomorpha
Order – Clupeiformes
Family – Clupeidae
Genus – *Pellona*
Species – *ditchela*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Branchiostegals numbering as high as 15, usually fewer
- Mesocoracoid arch invariably present
- Usually no lateral line possesses on trunk

Hence, Super Order – Clupeomorpha

- Body short, oblong, not eel like, covered with minute cycloid scales, scales are absent in head regions
- Abdomen with keeled scutes along the ventral line
- Radiating cutaneous canals on opercular bones

Hence, Order – Clupeiformes

- Maxillaries composed of three pieces and not composed with together
- Teeth when present, is rudimentary and deciduous
- Barbells absent
- Gill membranes free
- Opercular pieces four

Hence, Family – Clupeidae

- Body compressed and elongated
- Abdomen serrated, with 18 – 20 pre pelvic and 8 – 9 post pelvic scutes
- Head long and compressed
- Snout blunt
- Mouth upturned, cleft not reaching to the orbit
- Eyes large, lateral, in the middle of the head and not visible from the ventral surface with narrow adipose eyelid
- Lips thin
- Lower jaw longer than upper

Hence, Genus – *Pellona*

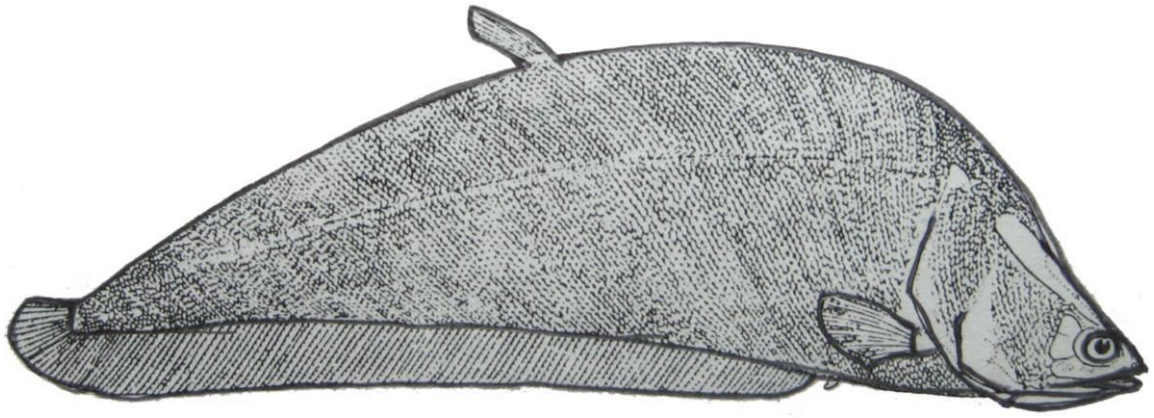
1. Dorsal fins inserted in above the tip of the pectoral fin or slightly behind, with 17 - 18 rays
2. Anal fin with 30 – 40 rays
3. Lateral line absent, 40 – 45 scales in the lateral series

Hence, species – *ditchela*

Hence, the provided specimen, *Pellona ditchela*

Distribution – India, Pakistan, Bangladesh, China and Burma, Nepal, Malaya, Thailand, Vietnam, South Africa, and Madagascar

Specimen: 4



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Osteoglossomorpha
Order – Osteoglossiformes
Family – Notopteridae
Genus – *Notopterus*

Species – *chitala*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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*

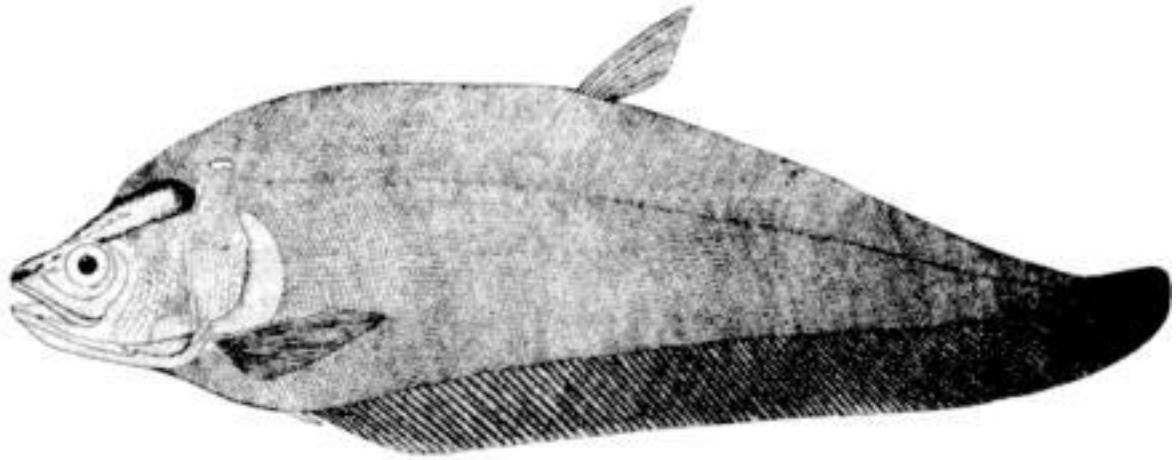
- Dorsal fin small, tuft like, inserted near the middle of the body, with 9 – 10 rays
- Anal fin is very low, ribbon like, 110 – 135 rays, confluent with caudal fin
- Pelvic fin rudimentary with 6 rays
- Lateral line is more or less with 180 rays
- Gape of the mouth not extending beyond the hind edge of orbit
- Silvery on sides, copper brown on back, having 15 silvery bars dorsally

Hence, species – *chitala*

Hence, the provided specimen is *Notopterus chitala*

Distribution – India: Northern India, Pakistan, Bangladesh, and Burma

Specimen: 5



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Osteoglossomorpha
Order – Osteoglossiformes
Family – Notopteridae
Genus – *Notopterus*

Species – *notopterus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 upto 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*

- Dorsal fin small, tuft like, inserted near the middle of the body, with 7 – 9 rays
- Anal fin is very low, ribbon like, 100 – 110 rays, confluent with caudal fin
- Pelvic fin rudimentary with 5-6 rays
- Lateral line is more or less with 225 rays
- Gape of the mouth extending beyond the hind edge of orbit
- Silvery white on sides, Gray on back

Hence, species – *chitala*

Hence, the provided specimen is *Notopterus notopterus*

Distribution – India: Northern India, Pakistan, Bangladesh, and Burma

Specimen: 6

Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Protacanthopterygii
Order – Salmoniformes
Family – Salmonidae
Genus – *Salmo*

Species – *gairdnerii*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- A wide spread trend towards the exclusion by Premaxilla of the maxillae from the gape and development of Premaxillary possess
- Branchiostegals numerous

Hence, Super Order – Protacanthopterygii

- Body elongated, to sub cylindrical covered with cycloid scales, head without scales
- Abdomen non-keeled and non-serrated
- Psedobranchiae present

Hence, Order – Salmoniformes

- Body compressed, with fine scales
- Dorsal fin two, an adipose dorsal fin present
- Cleft of the mouth

Hence, Family Salmonidae

- Body elongated and sub cylindrical, with round abdomen
- Head moderate and snout obtuse
- Mouth wide and cleft of the mouth oblique, extending up to front border of the eye
- Eyes large and superior, in the middle of the head, not visible from below the ventral surface
- Lips thin and jaws equal

Hence, Genus – *Salmo*

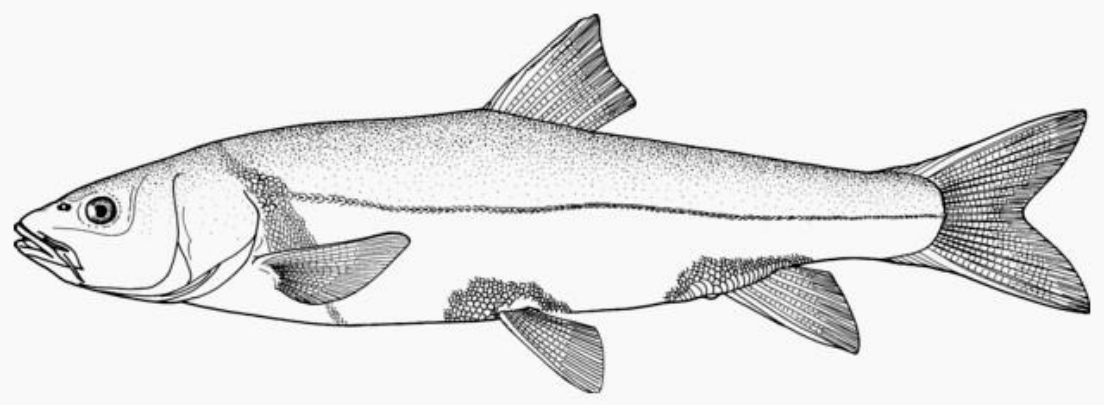
- Rayed Dorsal fin inserted above the pelvic fin, with 12 – 14 rays
- Adipose dorsal fin smooth, small inserted above mid anal
- Anal fin short, 10 – 13 rays
- Caudal fin forked
- Body without spots, bellow the lateral line and Lateral line straight with 127 - 160 rays
- Colour of the body is still blue

Hence, species – *gairdnerii*

Hence, the provided specimen is *Salmo gairdnerii*

Distribution – India: hilly regions, Pakistan, Sri Lanka, Bangladesh, S. Africa, England and North America

Specimen: 7



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Schizothorax*

Species – *kumoanensis*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 elongated and sub cylindrical, with round abdomen
- Head large and pointed anteriorly, snout rounded
- Mouth inferior and transverse
- Eyes large laterally placed, not visible from below the ventral surface
- Lips thick and fleshy, lower lip with a free posterior edge forming a sucker
- Barbells two pairs, one each rostral and other is maxilla

Hence, Genus – *Schizothorax*

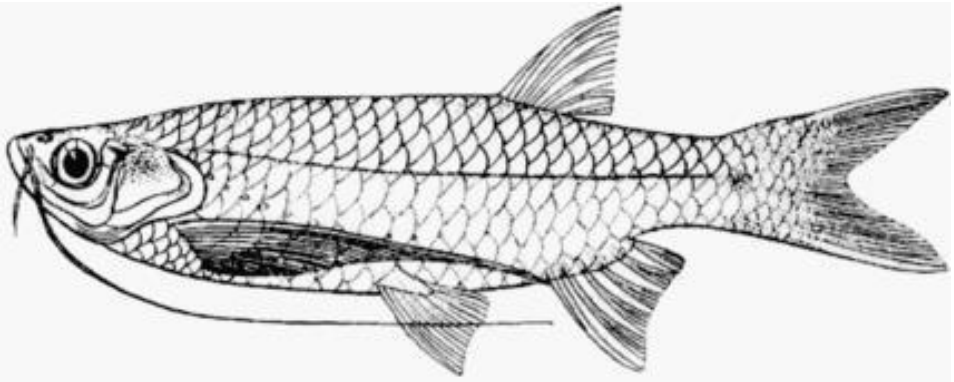
- Dorsal fin inserted slightly ahead pf pelvic fin, with 11 – 12 rays and with spine which may be articulated, smooth or serrated
- Anal fin short, 7 – 8 rays
- Caudal fin forked
- Lateral line complete with 98 - 100 rays
- Body uniformly silvery without black spots (alcohol preserved specimen)

Hence, species – *kumoanensis*

Hence, the provided specimen is *Schizothorax kumoanensis*

Distribution – India: hilly regions, Pakistan, Sri Lanka, Bangladesh, S. Africa, England and North America

Specimen: 8



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Esomus*
Species – *danricus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 elongated strongly compressed, with round abdomen
- Head small and blunt, snout blunt
- Mouth small, obliquely directed upwards
- Eyes inferiorly placed, visible from below the ventral surface
- Lips thin
- Barbells two pairs, maxillary pair is long, extending up to the anal fin

Hence, Genus – *Esomus*

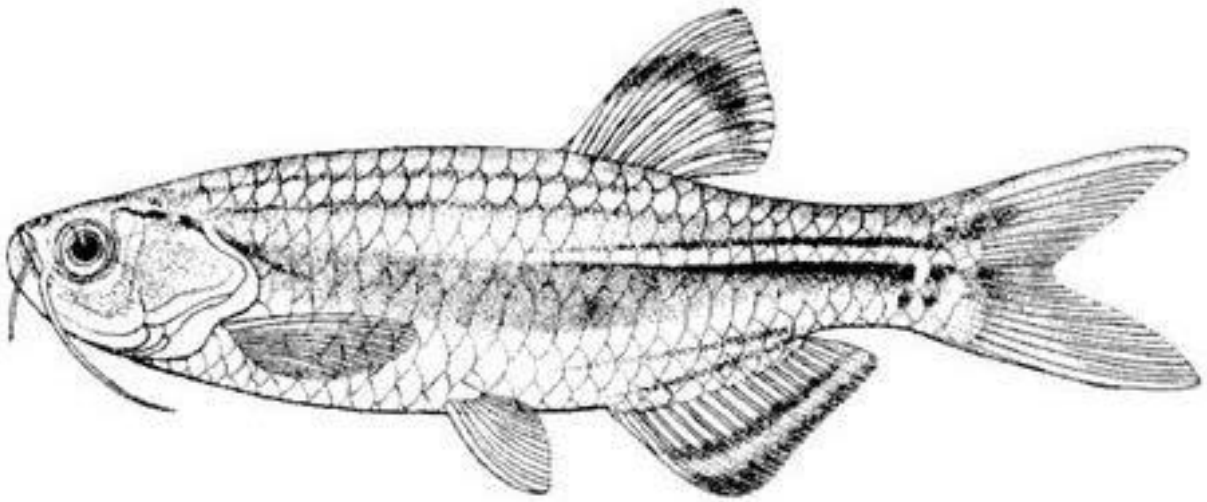
- Dorsal fin inserted in the interspaces between anal and pelvic fin, with 6 branched rays and without spine
- Anal with 5 branched rays
- Caudal fin forked
- No pre caudal spots are present, sides with broad lateral band, 14 scale round the caudal peduncle

Hence, species – *danricus*

Hence, the provided specimen is *Esomus danricus*

Distribution – India, Pakistan, Nepal and Bangladesh, Sri Lanka, Thailand, Malay

Specimen: 9



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Danio*
Species – *dangila*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 elongated compressed, sub cylindrical with round abdomen
- Head moderate and blunt, snout obtuse
- Mouth anterior, cleft of the mouth not protractile
- Eyes large centrally placed, not visible from below the ventral surface
- Lips thin and simple
- One or two pairs of barbells, rudimentary or none

Hence, Genus – *Danio*

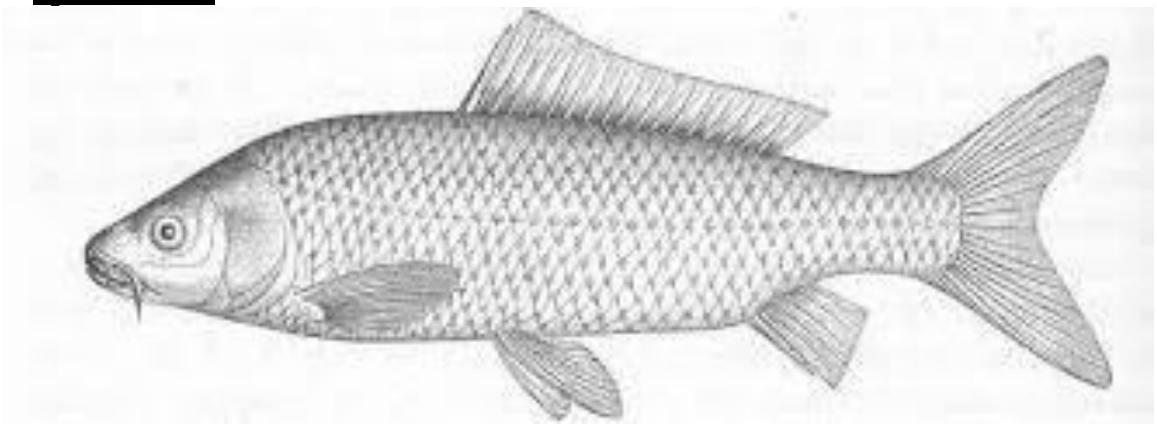
- Dorsal fin inserted in the interspaces between anal and pelvic fin, with 12 – 16 branched rays and without spine
- Anal with 13 – 20 rays
- Caudal fin forked
- Both pairs of barbells much longer than eye diameter
- Lateral line scales – 36 to 42

Hence, species – *dangila*

Hence, the provided specimen is *Danio dangila*

Distribution – India: Bihar, Bengal, Eastern Himalaya, Bangladesh, Nepal and Burma

Specimen: 10



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Cyprinus*
Species – *carpio*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 ventral surface
- Lips fleshy
- Barbells two pairs, one pair each of rostral and maxillary

Hence, Genus – *Cyprinus*

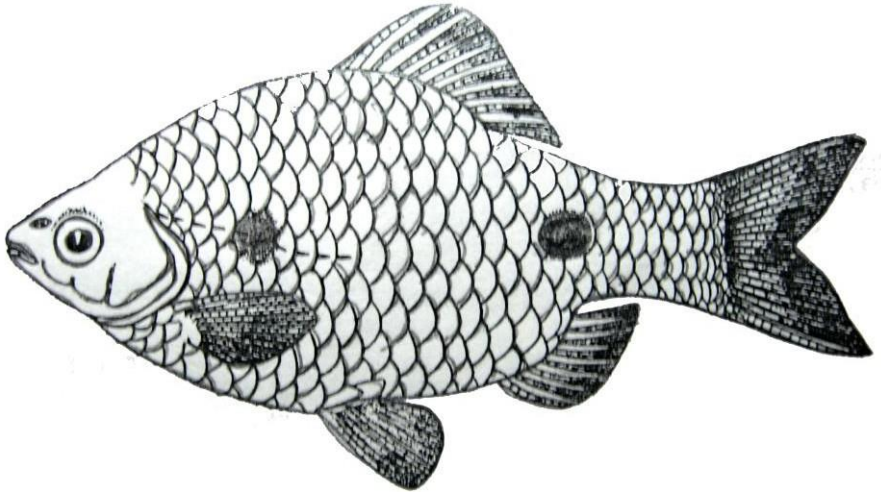
- Dorsal fin very long inserted above the tip of pectoral fin, with 3 spines and 17 rays, 3rd spine strongest and serrated
- Anal with short with 3 spines and 5 rays
- Caudal fin deeply emarginated and lobs pointed
- Lateral line straight with 36 scales

Hence, species – *carpio*

Hence, the provided specimen is *Cyprinus carpio*

Distribution – China, Korea, Japan, Taiwan, Europe, America, Introduced in India in 1939

Specimen: 11



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Puntius*
Species – *ticto*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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, jaws simple covered by lips

Hence, Genus – *Puntius*

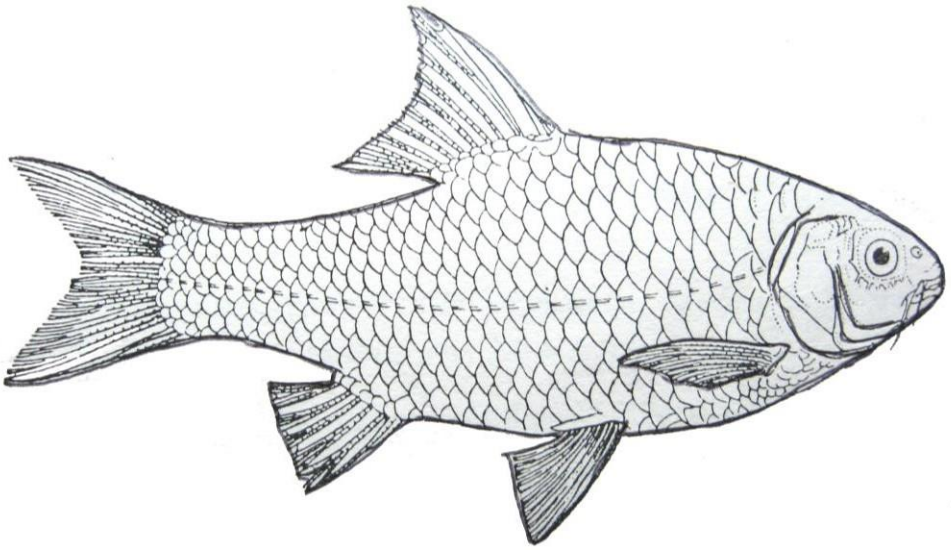
- Dorsal fin short inserted nearly opposite of the pelvic fin, with 3 to 4 soft spines and 8 rays
- Anal with short with 2-3 spines and 5 rays
- Pectoral fin with single soft spine and 12 – 14 rays
- Pelvic fin with single soft spine and 8 rays
- Lateral line straight with 23 - 25 scales

Hence, species – *ticto*

Hence, the provided specimen is *Puntius ticto*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 12



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Puntius*
Species – *sarana*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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, jaws simple covered by lips

Hence, Genus – *Puntius*

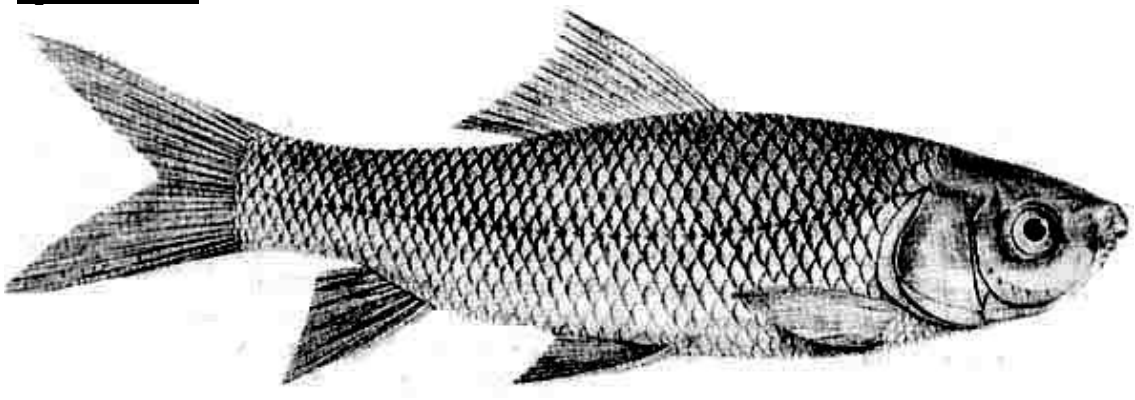
- Dorsal fin short inserted nearly opposite of the pelvic fin, with 3 to 4 soft spines and 8 rays
- Anal with short with 3 spines and 5 rays
- Pectoral fin with single soft spine and 14 – 16 rays
- Pelvic fin with single soft spine and 8 rays
- Lateral line straight with 30 - 33 scales

Hence, species – *sarana*

Hence, the provided specimen is *Puntius sarana*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 13



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Labeo*
Species – *rohita*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 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 the head, not visible from below the ventral surface
- Lips thick and fleshy, fringed, covering both jaws, continuous at angle of mouth forming labial fold.
- Barbells two pairs, one pair or none.

Hence, Genus – *Labeo*

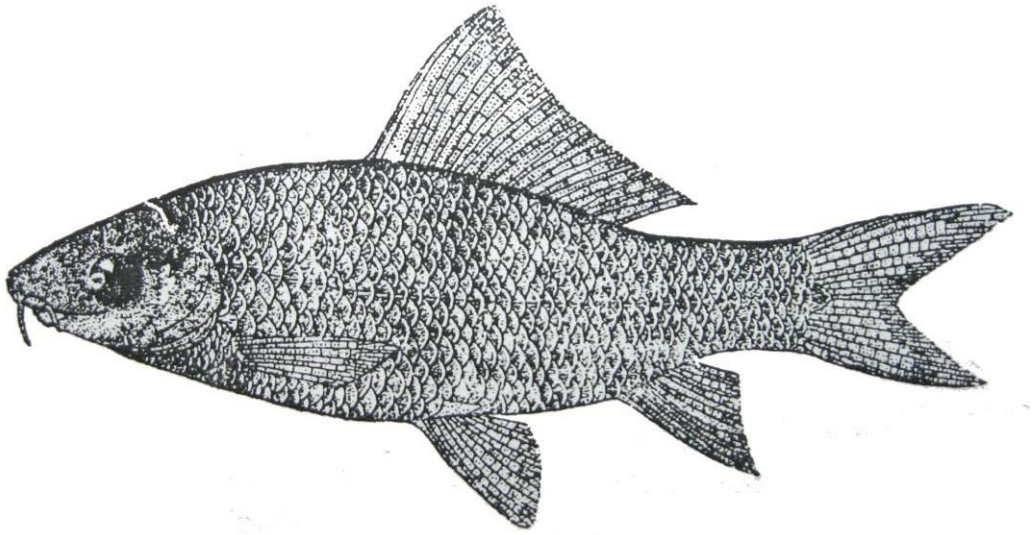
- Dorsal fin inserted above pelvic fin, and 12 - 14 rays,
- Anal fin short with 7 - 8 rays (two or three simple)
- Pectoral fin with 16 – 18 rays
- Lateral line straight with 40 - 44 scales
- Scales between lateral and pelvic fin base – 6 to 7

Hence, species – *rohita*

Hence, the provided specimen is *Labeo rohita*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 14



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Labeo*
Species – *calbasu*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 the head, not visible from below the ventral surface
- Lips thick and fleshy, fringed, covering both jaws, continuous at angle of mouth forming labial fold.

Hence, Genus – *Labeo*

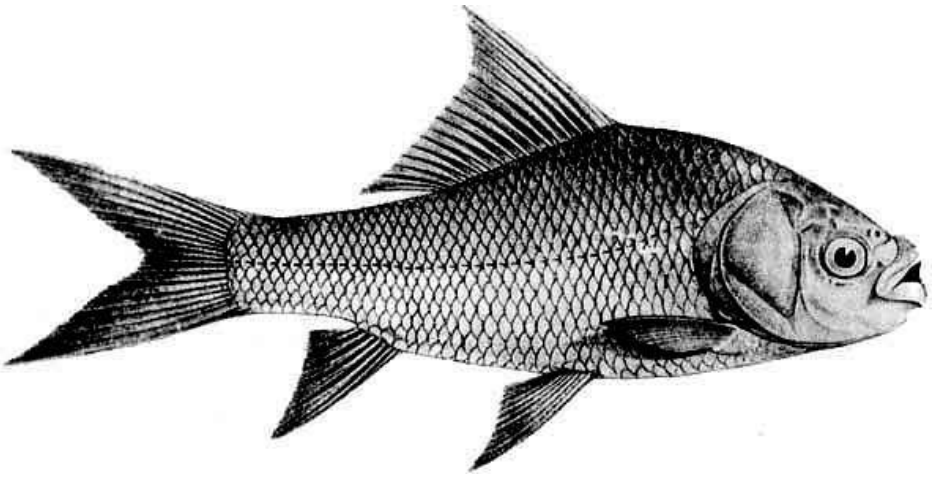
- Dorsal fin inserted above pelvic fin, and 16 - 18 rays,
- Anal fin short with 5 rays (two or three simple)
- Pelvic fin with 8 rays
- Lateral line straight with 40 - 44 scales
- One pair each rostral and maxillary barbells are present
- Colour of the body black, including fins

Hence, species – *calbasu*

Hence, the provided specimen is *Labeo calbasu*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 15



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Catla*
Species – *catla*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 short and deep
- 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

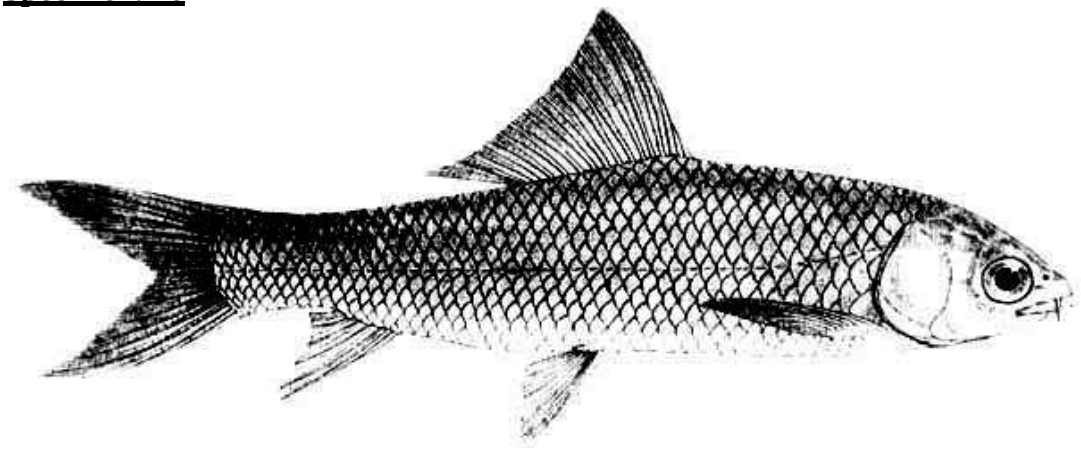
- Body attains a length of about 120 cm in three years
- Dorsal fin long, inserted above the tip of the pectoral fin with 17 to 19 rays (3/4 simple)
- Anal fin short with 8 rays (5 branched)
- Lateral line covered with 40 – 43 scales
- Caudal fin deeply forked

Hence, species – ***catla***

Hence, the provided specimen is *Catla catla*

Distribution – India, Pakistan, Nepal, Bangladesh, Thailand

Specimen: 16



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Cirrhinus*
Species – *mrigala*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 the ventral surface
- Upper lip fringed or entire, not continuous with lower
- Lower jaw sharp with a small tubercle at the symphysis, without any cartilaginous covering inside the jaw

Hence, Genus – *Cirrhinus*

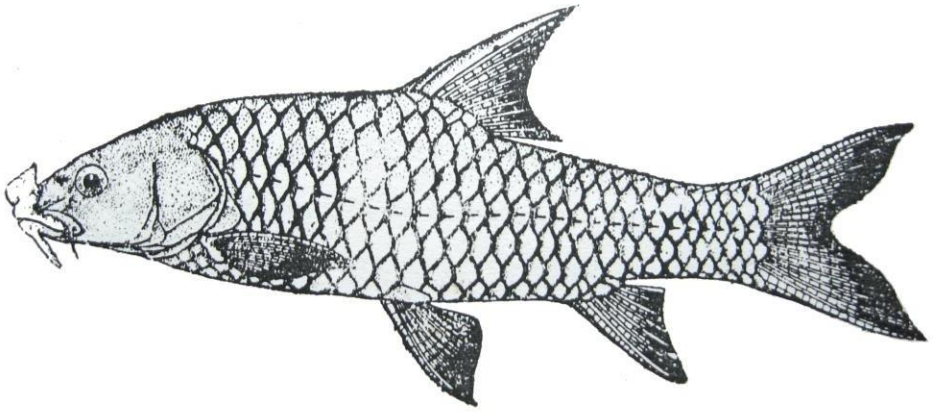
- Body attains a length of about 99 cm in three years
- Dorsal fin long, inserted ahead of the pelvic fin with 15 to 16
- Anal fin short with 7 or 8 rays (2/3 simple)
- Lateral line covered with 40 – 45 scales
- Caudal fin deeply forked or lunate

Hence, species – ***mrigala***

Hence, the provided specimen is *Cirrhinus mrigala*

Distribution – India, Pakistan, Nepal, Bangladesh, Thailand, Burma

Specimen: 17



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Tor*

Species – *tor*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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, elongated moderately compressed with round abdomen
- Head small, broadly pointed, snout angularly rounded
- Mouth inferior and arched
- Eyes far forward and not visible from below the ventral surface
- Lips fleshy, continuous at angles of mouth, posterior lip with or without a median lobe and post labial groove continuous, lip condition variable
- Pharyngeal teeth in three rows, 5, 3, 2
- A scaly sheathe present at the base of the dorsal fin

Hence, Genus – *Tor*

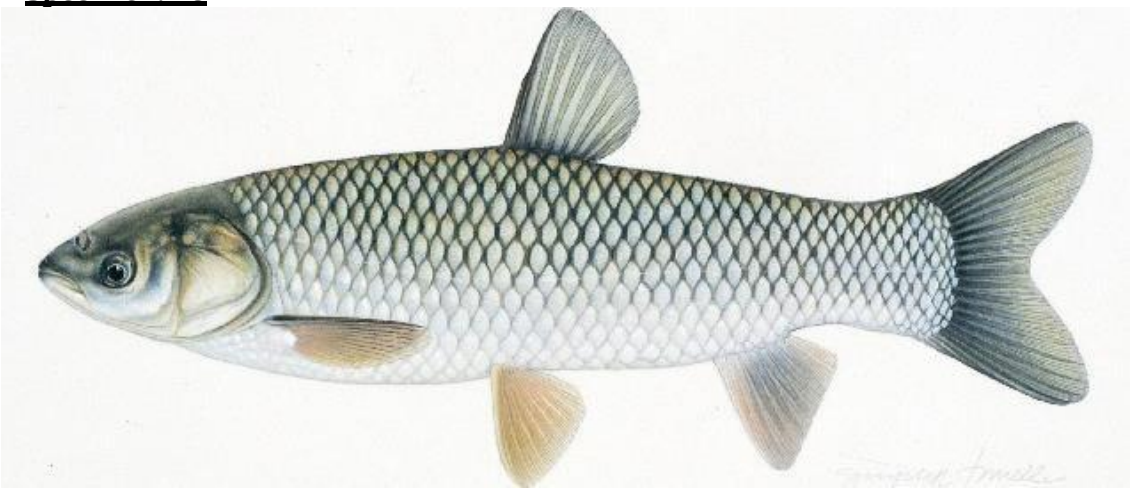
- Body attains a length of about 15 cm
- Ventral profile is more ahead than dorsal
- Dorsal fin, inserted above of the pelvic fin with 12 to 13 rays (8 – 9 branched)
- Anal fin with 5 rays (2/3 simple)
- Lateral line covered with 22 – 27 scales
- Caudal fin deeply forked

Hence, species – ***tor***

Hence, the provided specimen is *Tor tor*

Distribution – India, Pakistan, Nepal, Bangladesh, Thailand, Burma

Specimen: 18



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Ctenopharyngodon*

Species – *idella*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 round abdomen
- 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 the ventral surface
- Lips thin without any lobes
- Upper jaw slightly longer than lower and protractile

Hence, Genus – *Ctenopharyngodon*

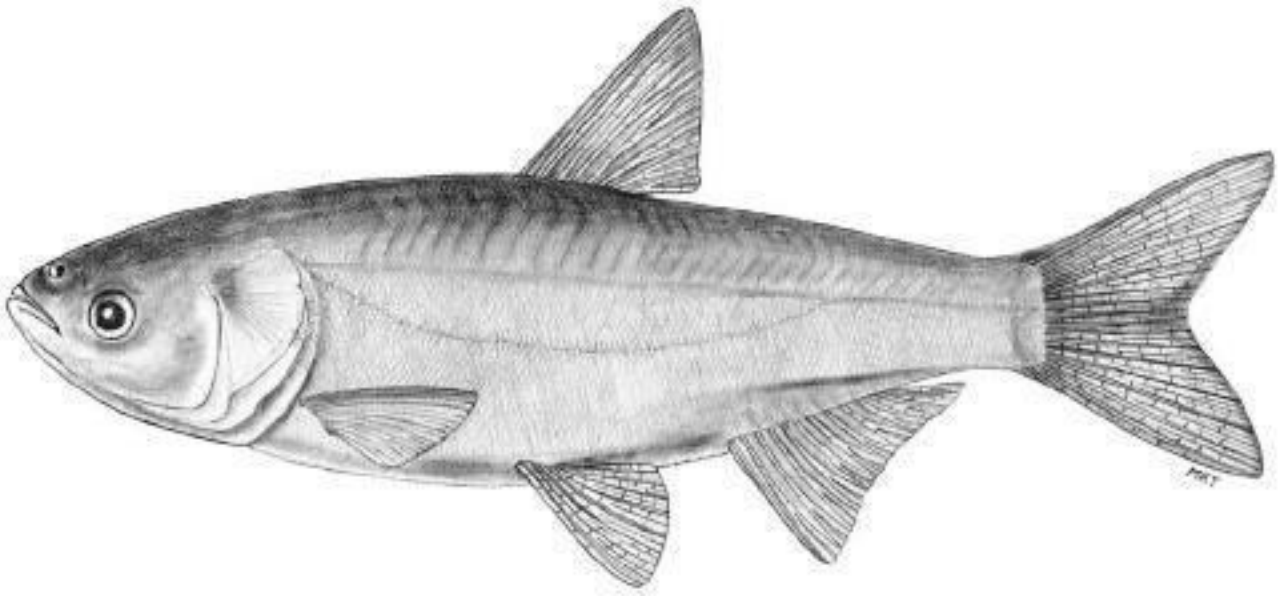
- Body attains a length of about 86 cm in three years
- Dorsal fin inserted slightly ahead of the pelvic fin with 10 rays
- Anal fin short with 10 rays (8 branched)
- Lateral line continuous slightly curved covered with 40 – 42 scales
- Caudal fin deeply forked

Hence, species – ***idella***

Hence, the provided specimen is *Ctenopharyngodon idella*

Distribution – Naturally found in Amur region Siberia, North China to South China, U.S.S.R, introduced in India in 1959

Specimen: 19



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Hypophthalmichthys*
Species – *molitrix*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 abdomen to 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 from below the ventral surface
- Lips thin
- Upper jaw slightly protruded upward a little longer than lower

Hence, Genus – *Hypophthalmichthys*

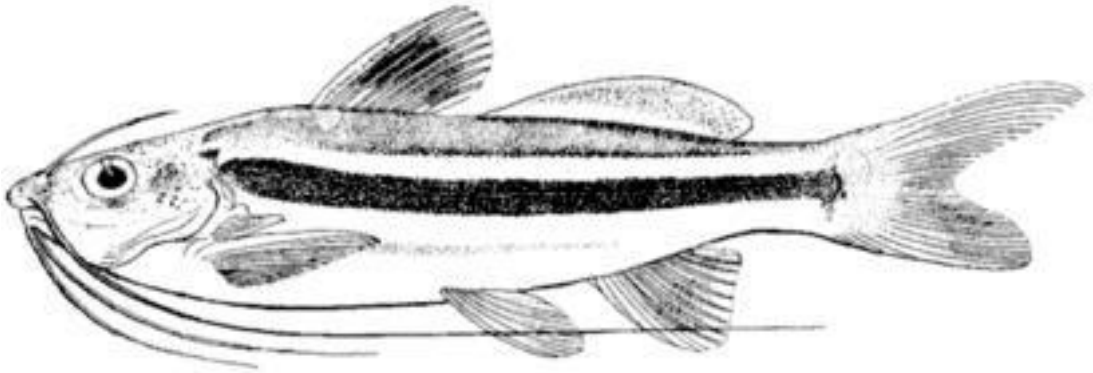
- Body attains a length of about 82 cm in three years
- Dorsal fin inserted behind the pelvic fin or above the tip of the pectoral fin with 10 rays
- Anal fin short with 14 – 17 rays (12 – 14 branched)
- Lateral line continuous slightly curved covered with 110 – 115 scales
- Caudal fin deeply forked

Hence, species – ***molitrix***

Hence, the provided specimen is *Ctenopharyngodon idella*

Distribution – Naturally found in Amur region Siberia, North China to South China, U.S.S.R, introduced in India in 1959

Specimen: 20



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Bagridae
Genus – *Mystus*
Species – *vittatus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 and more or less elongated fish
- Nostrils widely separated
- Barbell six or eight, generally well developed

Hence, Family – Bagridae

- Body short or moderately elongated, compressed with round abdomen
- Head moderate in size and compressed
- Mouth terminal, transverse and moderately wide
- Eyes moderately large, supra lateral, in anterior part of the head not visible from bellow the ventral surface
- Lips thin
- Jaws sub equal
- Barbells four pairs, one each of maxillary, nasal, and two mandibular, generally longer than head

Hence, Genus – *Mystus*

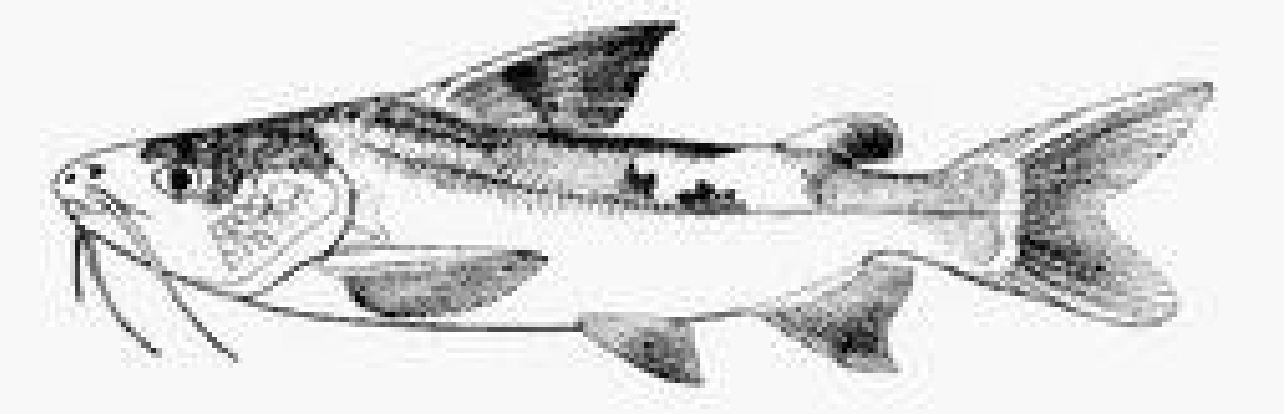
- Rayed dorsal fin inserted above the last quarter of the pectoral fin with 7 rays and a spine
- Adipose dorsal fin low of varying length
- Pectoral fin with 9 rays
- Body with 3 – 4 longitudinal colour bands above and bellow the lateral line
- A dark shoulder spot present
- No spot at the base of caudal fin

Hence, Species – *vittatus*

Hence, the provided specimen is *Mystus vittatus*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 21



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Siluridae
Genus – *Ompok*
Species – *pabda*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 of eyes, snout bluntly rounded, depressed
- Eyes small, ventral border on the level with corner of mouth, visible from under side of the head
- Lips thin, Jaws sub equal, lower jaw prominent
- Barbells two pairs, one pair each of maxillary and mandibular, latter occasionally small or rudimentary

Hence, Genus – *Ompok*

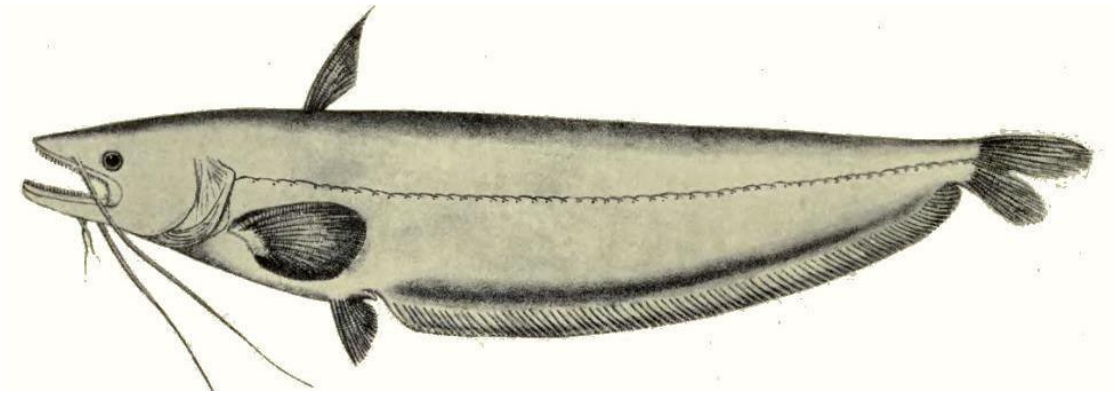
- Body attains 17 cm
- Rayed dorsal fin inserted above the last half of the pectoral fin with 4-5 rays and without any spine
- Adipose dorsal fin absent
- Pectoral fin with 11-14 rays
- Anal fin with 22-56 rays, very long, close to caudal fin, free from it
- Caudal fin forked

Hence, Species – *pabda*

Hence, the provided specimen is *Ompok pabda*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 22



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Siluridae
Genus – *Wallago*
Species – *attu*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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*

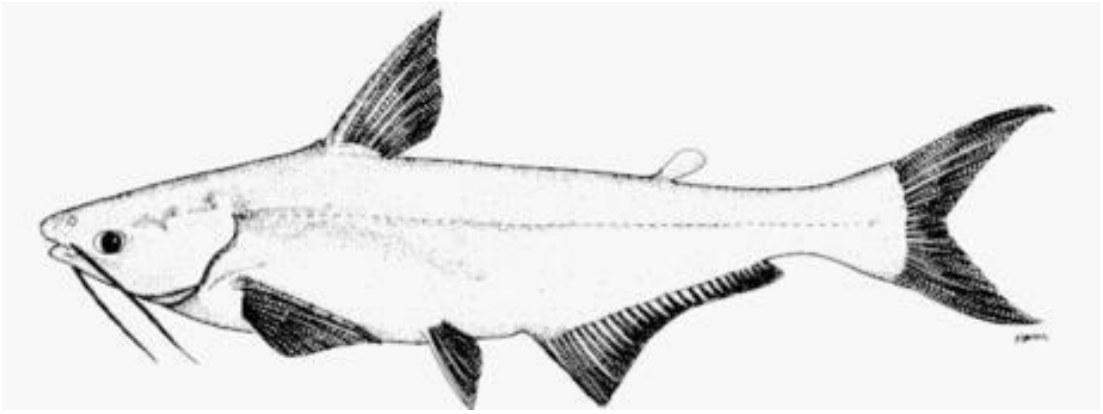
- Rayed dorsal fin inserted above half of the pectoral fin with 5 rays and without any spine
- Adipose dorsal fin absent
- Pectoral fin with 13-15 rays, and feeble smooth spine
- Anal fin long with 86-89 rays, free from caudal fin
- Caudal fin forked with rounded lobes
- Lateral line complete, well marked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

Hence, Species – *attu*

Hence, the provided specimen is *Wallago attu*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 23



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Pangasiidae
Genus – *Pangasius*

Species – *pangasius*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 fish with compressed body
- Nostrils widely separated, anterior ones wide, situated along the front border of the snout
- Barbell four, moderately developed

Hence, Family – Pangasiidae

- Body elongated, compressed with round abdomen
- Head moderate, exceptionally granulated and blunt. snout prominent, rounded
- Mouth sub terminal, horizontal or slightly ascending
- Eyes large, behind the corner of the mouth, visible bellow the ventral surface
- Lips thin, Jaws sub terminal, upper jaw slightly longer
- Barbells two pairs, one pair each of maxillary and mandibular

Hence, Genus – *Pangasius*

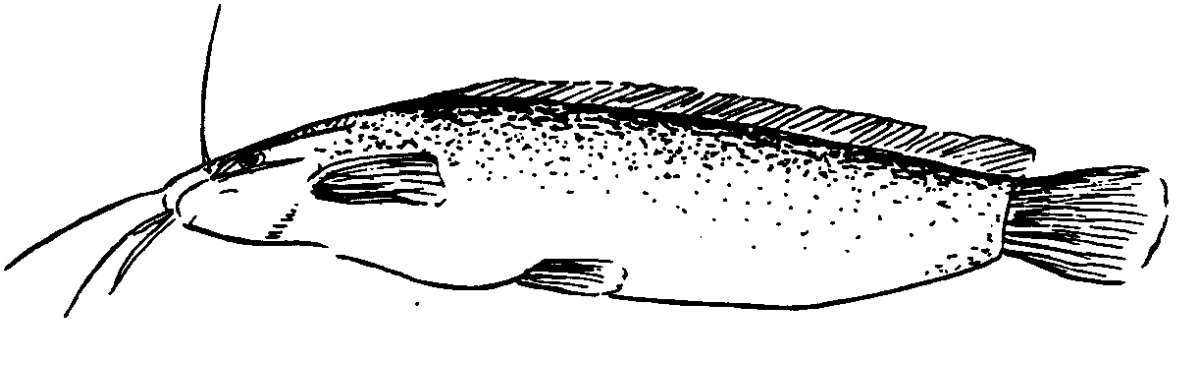
- Rayed dorsal fin inserted above last quarter of the pectoral fin with 6-7 rays and a spine
- Adipose dorsal fin absent, short and posteriorly free
- Pectoral fin with 9-12 rays, and a strongly serrated spine
- Anal fin long with 30-34 rays, free from caudal fin
- Caudal fin forked
- Lateral line complete, and simple

Hence, Species – *pangasius*

Hence, the provided specimen is *Pangasius pangasius*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka, Viet-Nam, Indonesia

Specimen: 24



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Clariidae
Genus – *Clarias*
Species – *batrachus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 latterly forming 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*

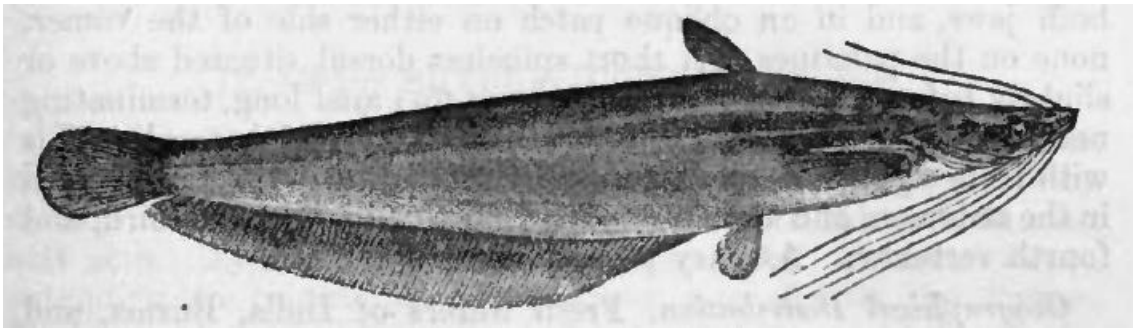
- Rayed dorsal fin long with 62-77 rays without any spine
- Adipose dorsal fin absent,
- Pectoral fin with 7-11 rays, and a strongly serrated spine, enveloped in a skin
- Anal fin long with 45-63 rays
- Caudal fin almost rounded
- Lateral line complete, and simple

Hence, Species – *batrachus*

Hence, the provided specimen is *Clarias batrachus*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka, Viet-Nam, Indonesia

Specimen: 25



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Heteropneustidae
Genus – *Heteropneustes*
Species – *fossilis*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

- Moderate sized elongated fish with compressed body
- Nostrils widely separated, anterior produced into short tube on the tip of the snout
- Barbells 8, well developed

Hence, Family – Heteropneustidae

- Body elongated, compressed with round abdomen
- Head moderate, greatly depressed, covered with thin skin, Snout flat
- Mouth terminal, transverse and narrow
- Eyes small, lateral in anterior part of the, not visible bellow ventral surface
- Lips fleshy and papillated, jaws sub equal
- Barbells four pairs, one pair each of maxillary nasal and 2 of mandibular

Hence, Genus – *Heteropneustes*

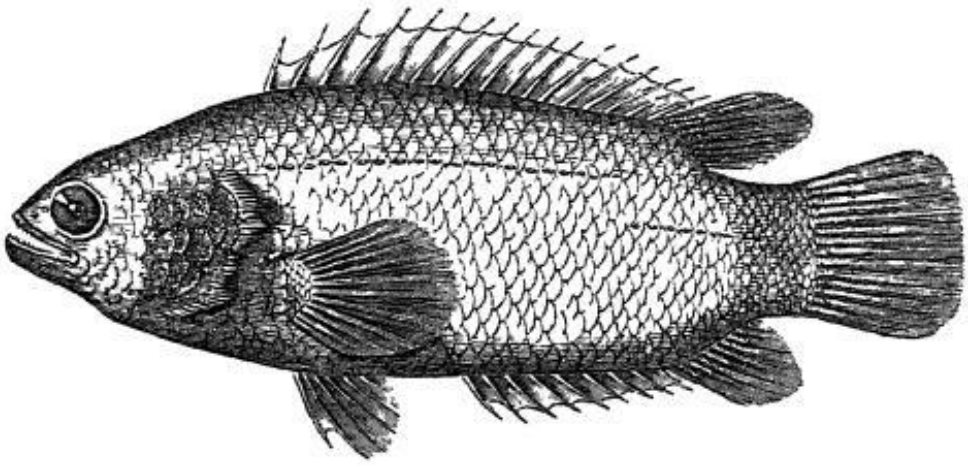
- Rayed dorsal fin short inserted above the tip of the pectoral fin with 6-8 rays without any spine
- Adipose dorsal fin absent, or represented by low adipose ridge, along the posterior third of the caudal region
- Pectoral fin with 7-8 rays, and a strong spine, serrated along the inner edge
- Anal fin long with 60-79 rays, separated from the caudal fin with deep notch
- Caudal fin almost rounded
- Pelvic fin with 6 rays
- Lateral line complete, and simple

Hence, Species – *fossilis*

Hence, the provided specimen is *Heteropneustes fossilis*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 26



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Acanthopterygii
Order – Perciformes
Family – Anabantidae
Genus – *Anabas*
Species – *testudineus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

- Moderately elongated compressed body,
- Head and body covered with ctenoid scales

Hence, Family – Anabantidae

- Body oblong, compressed with round abdomen
- Head moderate, compressed, Snout slightly conical
- Mouth small, terminal, oblique, cleft not wide
- Eyes large, lateral in anterior part of the head, not visible bellow the ventral surface
- Lips thin, jaws equal

Hence, Genus – *Anabas*

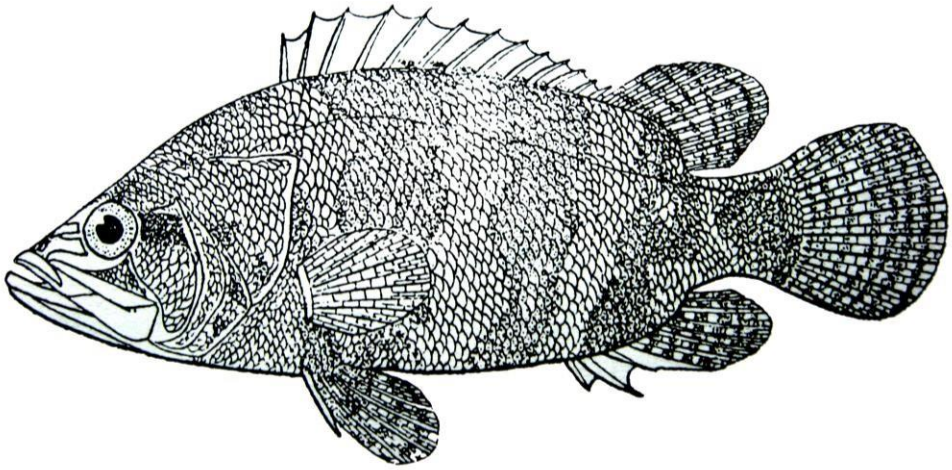
- Single dorsal fin inserted above the pectoral base, with 17-18 spine & 8-10 rays, number of spine variable
- Anal fin with 8-10 spines and 9-11 rays,
- Caudal fin rounded
- Lateral interrupted, with 21-29 scales

Hence, Species – *testudineus*

Hence, the provided specimen is *Anabas testudineus*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 27



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Acanthopterygii
Order – Perciformes
Family – Nandidae
Genus – *Nandus*
Species – *nandus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

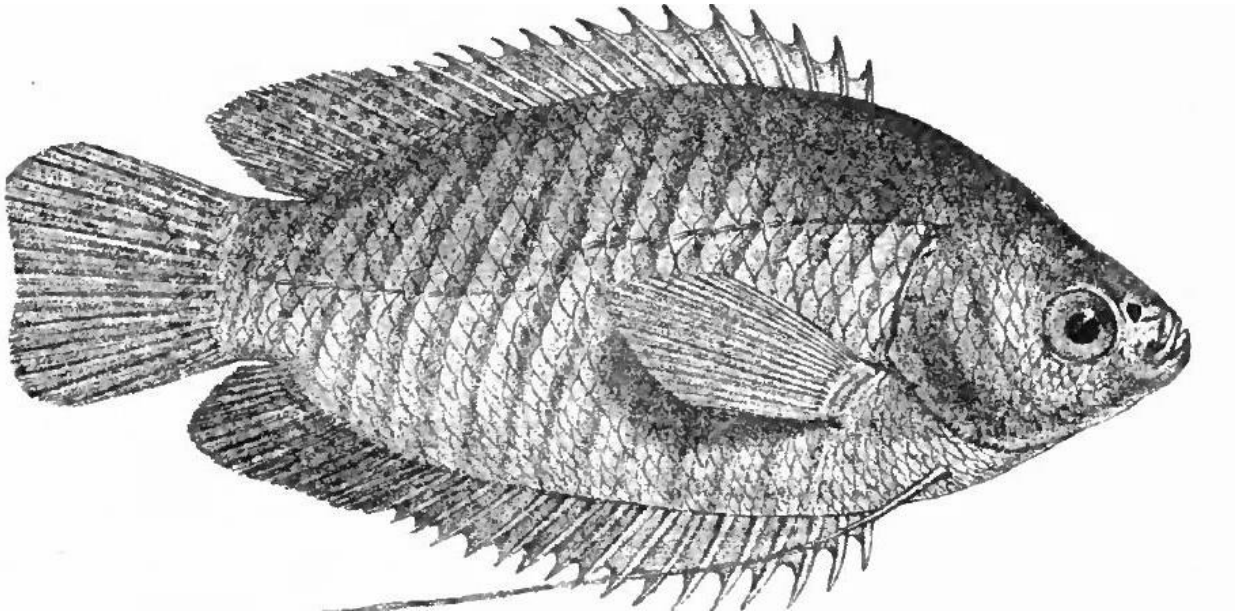
- Body oblong, elevated, compressed with moderate sized ctenoid scales
- Head and body covered with ctenoid scales
- Teeth on jaws and palate
- Operculum armed with spine
- A single dorsal fin with spine and soft portion

Hence, Family - Nandidae

- Body oblong and compressed
- Mouth terminal and very protractile
- Opercle with one spine, preopercle, pre orbit, sub and inter opercle serrated or more or less entire.
- Dorsal fin with 12-14 spines and 7-9 rays.
- Lateral line interrupted at about 36^{th} scales, 46-57 scales along with the lateral line.

Hence the Specimen is *Nandus nandus*

Specimen: 28



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Acanthopterygii
Order – Perciformes
Family – Belontidae
Genus – *Colisa*
Species – *fasciata*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

Hence, Family – Belontidae

- Body elevated, compressed
- 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*

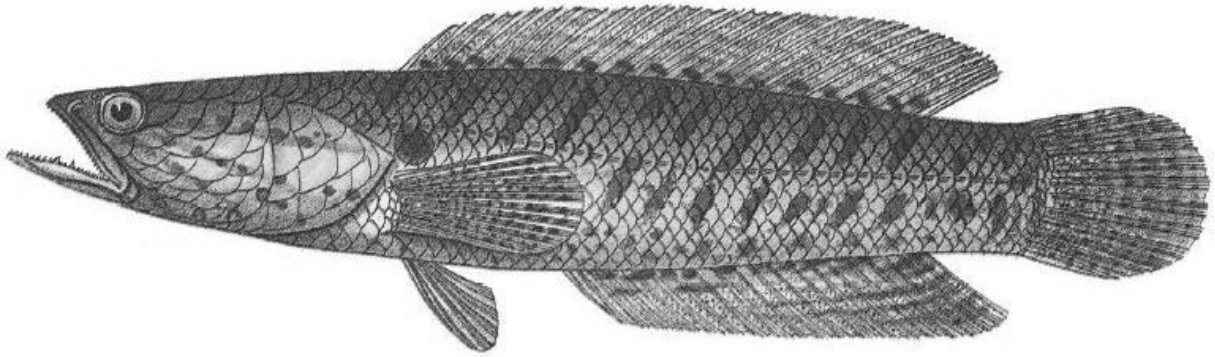
- Single dorsal fin commencing above from near pectoral base, with 15-18 spine & 7-13 rays, number of spine variable
- Anal fin with 15-20 spines and 11-19 rays, number of spines in anal and dorsal fin is variable
- Pelvic fin form, single elongated, filliform ray
- Caudal fin slightly emarginated or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

Hence, Species – *fasciata*

Hence, the provided specimen is *Colisa fasciata*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma,

Specimen: 29



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Channiformes
Family – Channidae
Genus – *Channa*
Species – *punctatus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

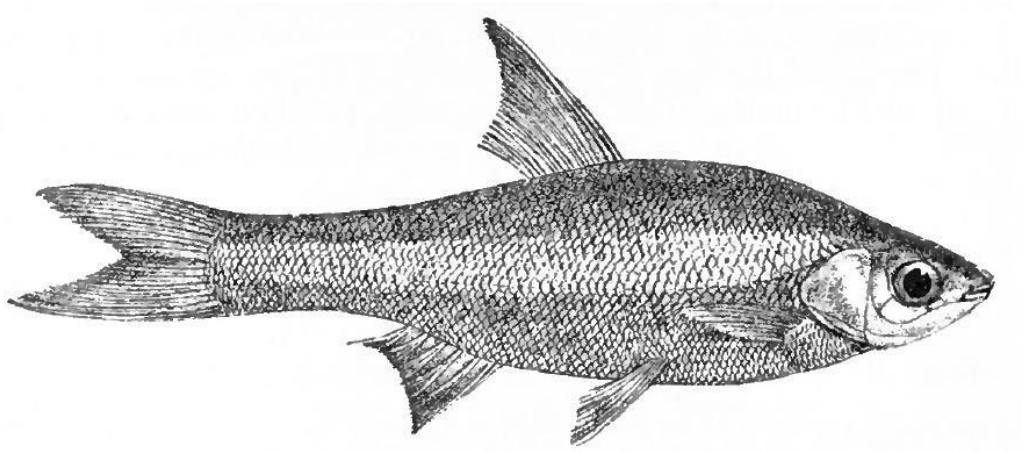
Hence, Genus – ***Channa***

- 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*

Specimen: 30



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Cypriniformes
Family – Cyprinidae
Genus – *Amblypharyngodon*
Species – *mola*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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 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 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*

Specimen: 31

Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Mastacembeliformes
Family – Mastacembelidae
Genus – *Mastacembelus*
Species – *pancalus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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 and 50-90 rays.
- Anal fins with 3 spines and 31-98 rays.
- Caudal fin round

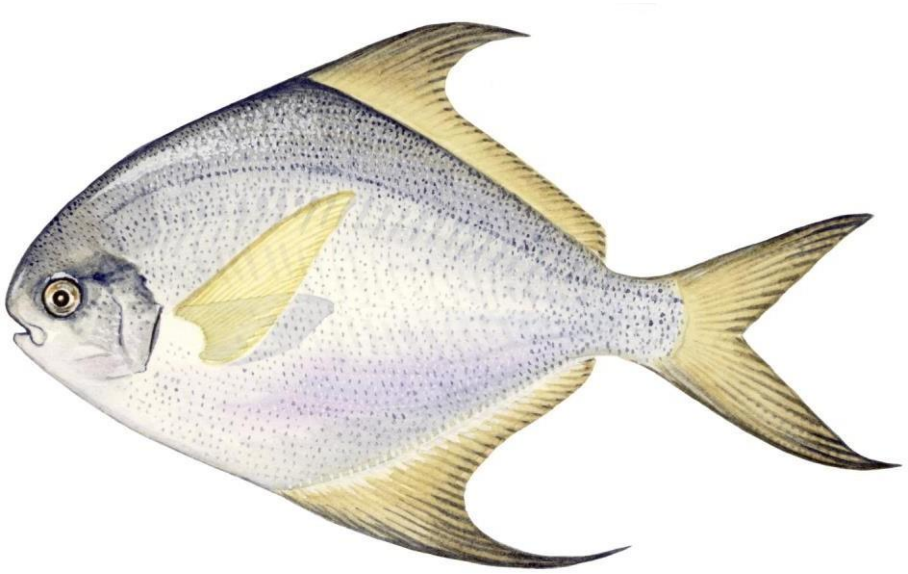
Hence, Genus – *Mastacembelus*

- Dorsal fin with 24 – 31 spines, 30 – 42 rays.

Hence, species – pancalus

Hence, the specimen is *Mastacembelus pancalus*

Specimen: 32



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Perciformes
Family – Stromateidae
Genus –
Species –

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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.

- 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

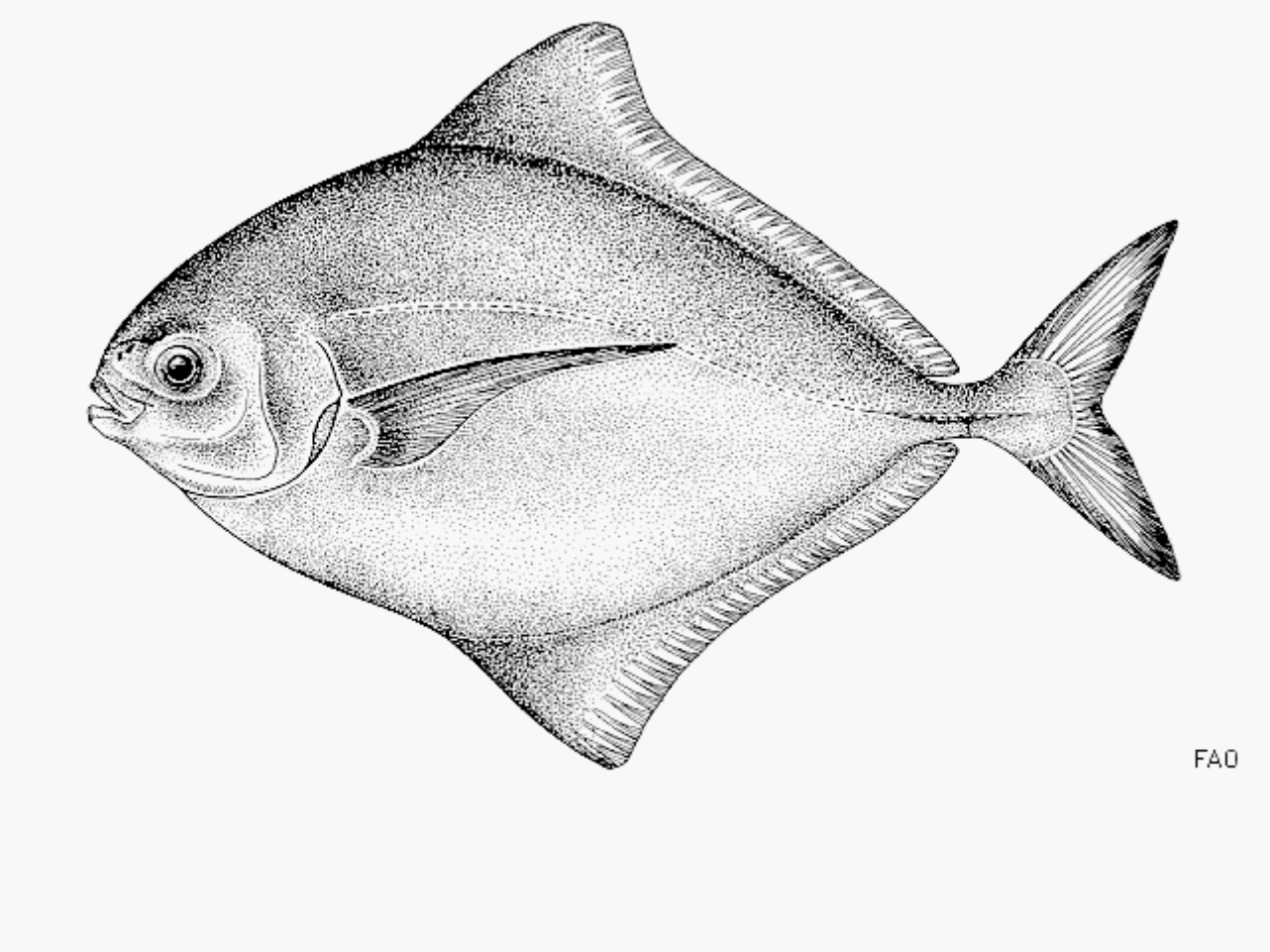
- Medium-sized fishes with a deep, compressed body of a bluish or silvery colour.
- Eye and mouth small. Dorsal fin single and long-based, longer than the similar anal fin; anterior rays longer than those which follow, but fins not falcate.
- Pectoral fins broad and wing-like, but not prolonged; pelvic fins absent (except possibly in very small specimens).
- Scales small, cycloid, easily shed, extending onto the bases of dorsal and anal fins; top of head naked.

Hence, Family – Stromateidae

- Dorsal spines (total): 0; dorsal soft rays (total): 37-43; Vertebrae: 34 - 37.
- Body firm, very deep, oval, and compressed. Operculum absent; gill opening reduced to a vertical slit on the side of the body; gill membrane broadly united to isthmus.
- Dorsal and anal fins preceded by a series of 5 to 10 blade-like spines with anterior and posterior points.
- Pelvic fins absent. Caudal fin deeply forked, the lower lobe longer than the upper.
- Colour is grey above grading to silvery white towards the belly, with small black dots all over the body. Fins are faintly yellow; vertical fins with dark edges.

Hence, the Specimen - *Pampus argenteus*

Specimen: 33



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Perciformes
Family – Stromateidae
Genus – *Parastromateus*
Species – *Parastromateus niger*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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.

- 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

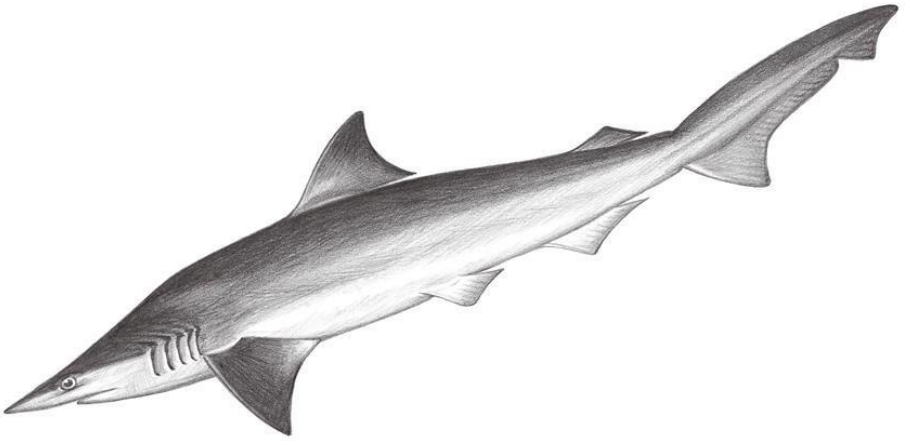
- Medium-sized fishes with a deep, compressed body of a bluish or silvery colour.
- Eye and mouth small. Dorsal fin single and long-based, longer than the similar anal fin; anterior rays longer than those which follow, but fins not falcate.
- Pectoral fins broad and wing-like, but not prolonged; pelvic fins absent (except possibly in very small specimens).
- Scales small, cycloid, easily shed, extending onto the bases of dorsal and anal fins; top of head naked.

Hence, Family – Stromateidae

- Dorsal spines (total): 2 - 6; Dorsal soft rays (total): 41-46; Anal spines: 2;
- Anal soft rays: 35 - 40; Vertebrae: 24. Deep-bodied and strongly compressed fishes.
- Lateral line ends in weakly-developed scutes on the caudal peduncle.
- Pelvic fins lost in individuals over 9 cm.
- Color is brown above, silvery-white below.
- The anterior parts of the dorsal and anal fins bluish-gray. The other fins yellowish.

Hence, the Specimen - *Parastromateus niger* (Bloch, 1795), Black pomfret

Specimen: 34



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Carcharhiniformes
Family – Carcharhinidae
Genus – *Scoliodon*
Species – *laticaudus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- nictitating eyelids present
- mouth behind front of eyes
- five pairs of gill slits
- both dorsal fins without spines
- anal fin present

Hene, Order – Carcharhiniformes

- Most members of this family are medium to large in size, usually ranging from about 1 - 3m, though some smaller members are less than a metre in length and larger ones may be over 7m.
- Eyes are round and have an internal nictitating membrane for protection.
- The sharks have arched mouths with blade-like teeth. The teeth of the upper jaw are often broader than those in the lower jaw.
- The pectoral fins are situated behind the gill slits. The first dorsal fin is bigger than the second and usually set well ahead of the pelvic fins. The upper lobe of the caudal fin is bigger than the lower. One anal fin present.

Hence, Family – Carcharhinidae

- *Scoliodon* has an elongated, spindle-shaped, body tapered at the ends, making it a very fast swimmer.
- The trunk and tail are laterally compressed, while the head region is dorsoventrally compressed.
- The entire body is covered by an exoskeleton of placoid scales. The mouth is located on the ventral side and is bound on both sides by jaws.
- It has two rows of homodont or polyphyodont teeth, which are homologous to the placoid scales covering the body.

Hence, specimen - *Scoliodon laticaudus*

Specimen: 35

Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Carcharhiniformes
Family – Sphyrnidae
Specimen – *Sphyrna* sp.

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- Members of this order are characterized by the presence of a nictitating membrane over the eye,
- Two dorsal fins, an anal fin, and five gill slits.

Hence, Order – Carcharhiniformes

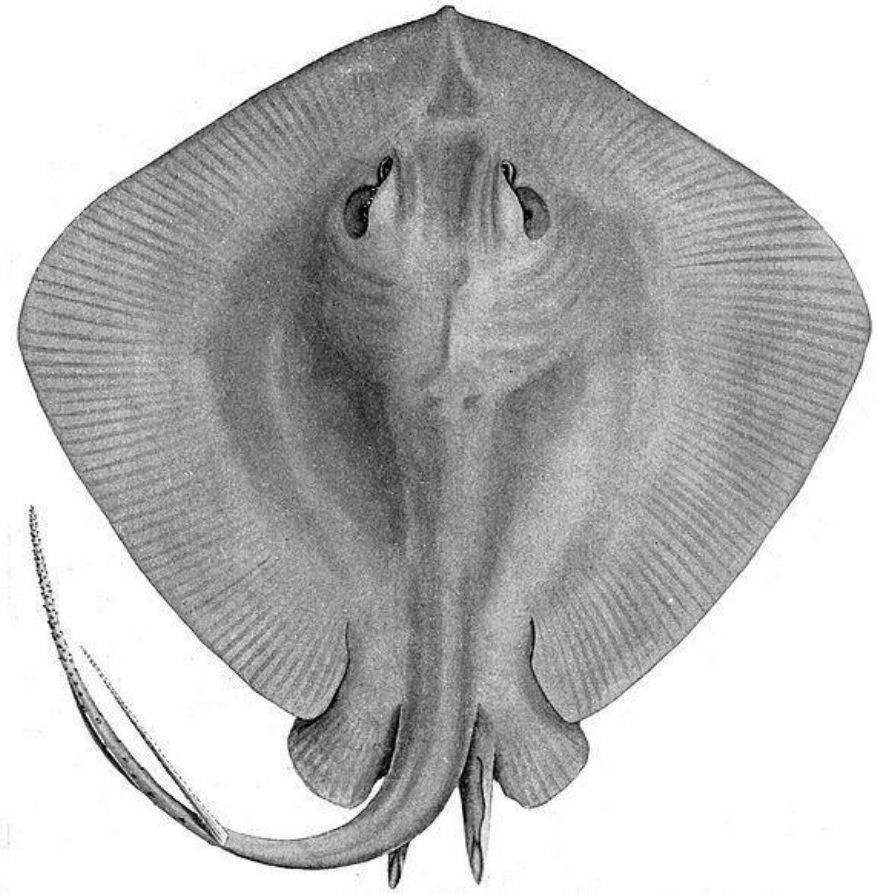
- They are usually light gray and have a greenish tint to them.
- Their bellies are white which allows them to blend into the ocean when viewed from the bottom and sneak up on their prey.
- Their heads have lateral projections which give them a hammer-like shape.

Hence, Family – Sphyrnidae

- Head variably spade, mallet or axe-shaped in dorsoventral view and moderately broad, width across head about 17 to 33% of total length;
- lateral blades of head broad, not winglike; nostrils short, their widths 7 to 14 in internarial width and less than half mouth width;
- No bumps along anterior margin of head.
- Upper precaudal pit transverse and crescentic.

Hence, Genus – *Sphyrna* sp.

Specimen: 36



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Myliobatiformes
Family – Dasyatidae
Specimen – *Dasyatis* sp.

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- The disc is strongly depressed and varies from oval longitudinally to much broader than long;
- the tail is well marked off from the body sector, very short to long and whiplike, and equipped with a poisonous spine in some species;
- the pectoral rays are either continuous along the side of the head or separate from the head and modified to form rostral lobes or finlike rostral appendages (cephalic fins);
- The dorsal fin, if present, is near the base of the tail; and development is ovoviviparous.
- Sometimes an individual ray may have two or three, very rarely four, tail spines rather than the usual one.

Hence, Order – Myliobatiformes

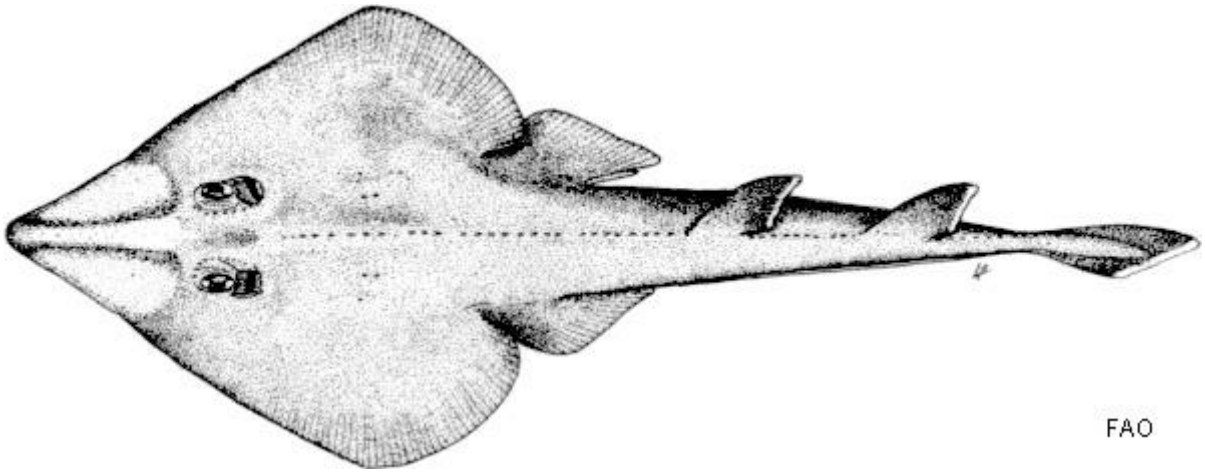
- Medium to large rays (disc width to 2.1 m), the disc rhomboid to oval, its width ranging from greater to less than its length;
- snout obtuse and little produced to acute and moderately produced, head not elevated from disc; tail distinct from disc, slender and whip-like, equal to or much longer than distance from snout to cloaca, with one or several serrated spines on top near base, keels or membranous folds along upper or lower sides (or both) present in some species.
- Nostrils separated from mouth, but front margins greatly expanded to reach back and join each other. Mouth almost straight or arched, with a transverse row of bulbous papillae along floor, teeth small and numerous.
- Spiracles well developed, without tentacle-like processes. Dorsal and caudal fins absent, pectoral fins joined to side of head, the finrays beginning at tip of snout. Upper surfaces naked or covered with tubercles, thorns or thornlets.
- Benthic on soft bottoms, generally in shallow tropical and warm temperate waters, but also to depths of 200 m.

Hence, Family – Dasyatidae

- Snout obtuse and little produced to acute and strongly produced;
- disc more or less rhomboid, but not circular; tail whip-like,
- Filamentous near tip, with or without a membranous fold or ridge above, but with a fold below (beginning at level of spine).
- Upper surfaces with or without bucklers, tubercles and thorns with conical cusps.

Hence, Specimen – *Dasyatis* sp.

Specimen: 37



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Rajiformes
Family – Rhinobatidae
Specimen – *Rhinobatos productus*.

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- Rajoids typically have a dorsoventrally flattened body.
- The snout is slender and pointed and the wide mouth, often covered with a fleshy nasal flap, is on the underside of the head.
- The eyes and well-developed spiracles are located on the top of the head. In most species, the spiracles are large and are the main means of drawing water in for respiration.
- There is no nictitating membrane and the cornea is continuous with the skin surrounding the eyes.
- The gill slits are on the ventral surface just behind the head and there are five in all species except the sixgill stingray (*Hexatrygon bickelli*).

Hence, Order – Rajiformes

- They have a body form intermediate between those of sharks and rays.
- The tail has a typical shark-like form, but in many species, the head has a triangular, or guitar-like shape, rather than the disc-shape formed by fusion with the pectoral fins found in other rays.

Hence, Family – Rhinobatidae

- Shovelnose guitar fish most resemble sharks in posterior body shape, with a flattened anterior like a ray.
- Their snouts are pointed and shovel-like, and they have broad pectoral fins.
- Their dorsal surfaces are smooth except for rows of small thorns around the eyes and tail. The tail is rather thick with a rounded caudal fin (characteristic of a benthic fish), lacking the lower lobe that most other sharks possess.
- Shovelnose guitarfish have two equally-sized dorsal fins positioned close to the end of the tail. This species' body colour ranges from sandy brown to olive, with a white underside; the distal end of the snout is partially translucent.
- Their teeth are small and rounded, and range in number from 102 to 112.
- Females usually grow larger than males, reaching up to 137 centimetres when fully gown, while males may reach up to 120 centimetres.

Hence, Specimen - *Rhinobatos productus*

QUALITATIVE TEST FOR PROTEINS

Introduction

Proteins are sequences of amino acids, its contain C, H, O, and Nitrogen, Common structure: Central C, with a H, amino group (NH2), and an acid group (COOH), and a side group, proteins made up of about 20 different amino acids; Unique Side Groups, Differ in size, shape, electrical charge. Proteins are probably the most important class of biochemical molecules, although of course lipids and carbohydrates are also essential for life. Proteins are the basis for the major structural components of animal and human tissue. It can be hydrolyzed by acids, bases or specific enzymes. Amino acids are organic compounds that contain amino and carboxyl groups. The R- in the above formula stands for different chemical groups (may be aliphatic, aromatic or heterocycylic) and this determines the characteristics of the amino acids. The color tests have frequently been used for qualitative detection of amino acids. Not all amino acids contain the same reactive groups. For this reason the various color

tests yield reactions varying in intensity and type of color according to the nature of groups contained in the particular amino acid under examination.

Millon's reaction

Principle

The reaction is due to the presence of the hydroxyphenyl group, C_6H_5OH in the amino acid molecule; and any phenolic compound which is unsubstituted in the 3,5 positions such as tyrosine, phenol and thymol will give the reaction. Solutions of nitric acid containing mercuric nitrate reacts with phenols, producing red colors or yellow precipitates which react with nitric acid to form red solution. The reaction probably depends on the formation of a nitro compound; which then reacts with phenol.

Procedure

Add 3 to 4 drops of Millon's reagent to 5 ml of test solution. Mix and bring the mixture gradually to a boiling point by heating over a small flame. Development of red color is due to the presence of protein. Excess of reagent should however be avoided since it may produce a yellow color which is not a positive reaction.

Xanthoproteic reaction

Principle

This reaction is due to the presence in the amino acid molecule of the phenyl group $–C_6H_5$, with which the nitric acid forms certain nitro modifications. The particular amino acids which are of especial importance in this connection are those of tyrosine and tryptophan. Phenylalanine does not respond to this test as it is ordinarily preformed.

Materials

1. Conc. HNO_3
2. Ammonium hydroxide
3. Sodium hydroxide

Method

Add 1 ml of conc. Nitric acid to 2 to 3 ml of test solution in a test tube. A white precipitate forms, which upon heating turns yellow and finally dissolves, imparting to the solution a yellow color, cool the solution and carefully add ammonium hydroxide or sodium hydroxide in excess. Note that the yellow color deepens into an orange.

Biuret test

Principle

The Biuret test is given by those substances whose molecules contain two cabamyl ($-CONH_2$) groups

joined either directly or through a single atom of nitrogen or carbon. Similar substances which contain- $CSNH_2$,

-$C(NH)NH_2$, or – CH_2NH_2 in place of the –$CONH_2$ group also respond to the test. It follows from this fact that substance which are non-protein in character but which contain the necessary groups will respond to the biuret test.

Protein responds positively since there are pairs of CONH groups in the molecule. A copper coordination complex with the ring structure is probably produced. Short chain polypeptides give a pinkish violet color, longer one including proteins a more purple blue. The amino acid histidine gives a pink color, which depends on the peptide linkage, does not vary greatly in intensity from protein to protein. Several procedure based on this method have been suggested for quantitative determination of milk proteins, but it is not in general use in dairy research.

Materials

1. 10% NaOH
2. 0.5% $CuSO_4$

Method

To 2 to 3 ml of test solution in a test tube add an equal volume of 10% sodium hydroxide solution, mix thoroughly, and add a 0.5% copper sulphate solution drop by drop, mixing between drops, until a purplish-violet or pinkish-violet color is produced. The color depends upon the nature of the protein, proteoses and peptones give a decided pink; the color produced with gelatin is not far removed from a blue.

Ninhydrin reaction

Principle

This test gives positive results with proteins, peptones, peptides, amino acids and other primary amines, including ammonia. Proline and hydroxyproline give yellow color with ninhydrin, while other acids give blue to purple color.

Materials

1. 0.1% Ninhydrin
2. pH paper

Method

To 5 ml of dilute test solution, which must be approximately between pH 5 and pH 7 (a few drops of pyridine or a few crystals of sodium acetate may be used to adjust the pH), add 0.5 ml of 0.1 % ninhydrin, heat to boiling for one to two minutes, and allow to cool. A blue color develops if the test is positive.

Add 5ml of the alkaline solution to 1 ml of the test solution. Mix thoroughly and allow to stand at room temperature for 10 mins. Add 0.5 ml diluted Folin-Ciocalteau reagent rapidly with immediate mixing. Observe for development of color after 30 mins. Development of characteristic blue color

indicates presence of indolyl or phenol group.

Sakaguchi test
Principle

Arginine and other guanidyl derivatives (glycocyamine, methylgyanidine etc) react with hypo bromide and alpha napthol to give a red colored product.

Materials

1. Sodium hydroxide solution (40%)
2. Alpha napthol solution (1% in alcohol)
3. Bromine water (a few drops of bromine in 100 ml distilled water)

Method

Mix 1 ml of sodium hydroxide with 3 ml of test solution and add 2 drops of alpha napthol. Mix thoroughly and add 4 to 5 drops of bromine water. Note the color formed. Formation of a red color indicates presence of guanidine group. This is a very sensitive and specific test.

Nitroprusside test

Principle

Sodium nitroprusside reacts with compounds containing sulphahydryl groups produce an intensely red but somewhat unstable color.

Materials

1. Sulphur amino acids (1.0% cystine, cysteine and methionine)
2. Sodium nitroprusside (2% prepared fresh)
3. Ammonium hydroxide

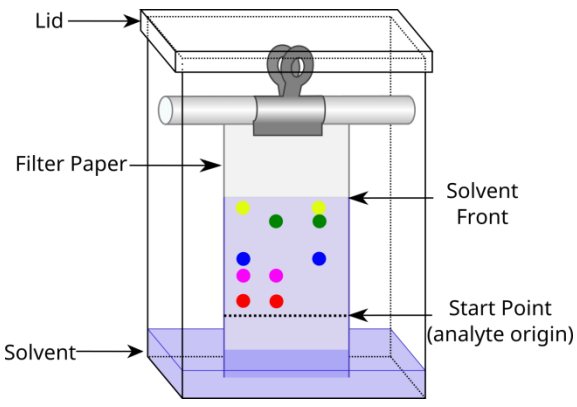
Method

Mix 0.5 ml of a fresh solution of sodium nitropruside with 2 ml of the test solution and add 0.5 ml of ammonium hydroxide.

Paper Chromatography of Amino Acids

Introduction

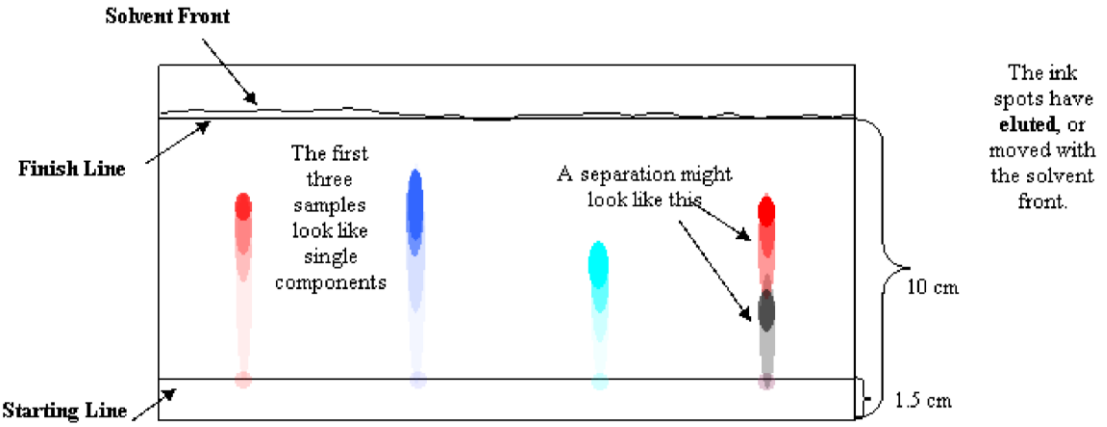
Chromatography is a collective term for a set of analytical techniques used to separate mixtures. Chroma means color and graph means to write or draw. Paper chromatography is an analytical technique used to separate mixtures of chemicals (sometimes colored pigments) using a partitioning method. The paper in this method is called the stationary phase because it does not move and serves as a substrate or surface for the separation. Analytes (substances being analyzed) are separated from each other based on a differential affinity to a solvent. The solvent dissolves and carries the analytes along the matrix of the stationary phase. Since the solvent moves through a wicking action, it is called the mobile phase.



The figure above shows a chamber containing mobile phase and a prepared paper stationary phase. A line drawn at the bottom edge of the paper is the starting point/line. The starting line and sample dots must be above the level of the mobile phase when the paper is placed inside the chamber. If the starting line is below the liquid level, the samples will wash out into the mobile phase rather than elute up the stationary phase. Another line is drawn about 10 cm above the bottom edge of the paper. This is the finish line. Its location was chosen for this experiment because when the eluting solution reaches that line, any inks that are mixtures should be clearly separated.

When the solvent front reaches the finish line, the paper should be removed immediately from contact with the mobile phase.

The figure below shows a typical paper chromatogram of the separation of pen inks. There are a few difficulties commonly encountered in the elution process. One problem is that spots tend the spread out as they elute, and can bleed into each other as they proceed up the paper. This can be confusing when interpreting the chromatogram. To avoid this problem, space the spots of sample far apart and make repeated, tiny applications of sample to prevent spreading. Another problem is an uneven solvent front. This can happen if the beaker is nudged – if the mobile phase sloshes inside, the elution trails may travel diagonally, which makes interpretation very difficult. This can also happen if the two edges of the chromatogram are allowed to touch when they are stapled or taped together to form a cylinder.



A component with a given solubility travels along with the mobile phase at one rate, regardless of what other components are present in the sample. If the red part of purple ink travels at the same rate as pure red ink, and both stop in the same place, the two should be the same red ink. The two red spots should have the same Retention Factor, Rf. The Rf is the distance, D, traveled by the spot divided by the distance traveled by the eluting solution, or Solvent Front, F.

Rf= frac DF

Comparing the Rf values allows the confirmation of a component in multiple samples because unique components have unique Rf values.

Experimental Procedure

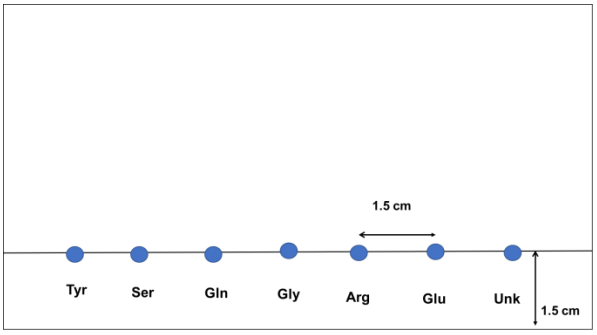
Materials and Equipment

Materials: chromatography paper, amino acids (1% solutions): tyrosine, serine, glutamine, glycine, arginine, glutamic acid and unknown, eluting solution (isopropyl alcohol, 0.5 M NH4OH; 0.2% ninhydrin spray).

Equipment: 600-mL beaker, pencil, ruler, evaporating dish, toothpicks, hair dryer, stapler and paper towels.

Preparation of Chromatography Paper

1. Wash your hands thoroughly to remove excess oils from your skin. Obtain a ruler and a piece of chromatography paper from the fume hood. Handle the paper only on the edges to avoid leaving fingerprints, as these may hinder the elution process.
2. Place the chromatography paper on a sheet of clean notebook paper or paper towel to avoid picking up dirt or contaminants from the bench top. Orient the paper into a "landscape" position and write your name on the top edge of the paper in one corner. Using a pencil and ruler to measure accurately, draw a straight line across the paper, about 1.5 cm above the bottom edge. This is the starting line. At the top of the paper draw a straight line across the paper about 2.0 cm from the top, this is finish line.
3. On the starting line, measure in from one side about 2.5 cm and lightly draw a small "X" centered on the starting line. Draw six more, 1.5 cm apart.
4. In the center of each X, make a small spot of amino acid sample in this order: tyrosine, serine, glutamine, glycine, arginine, glutamic acid and unknown.
5. When you have finished, you should have something that looks like ?????? below.
6. Go back over each amino acid spot a second time to ensure there is enough amino acid in the spot.
7. Gently curl the paper into a cylinder, with the spots on the outside. Staple the ends together near the top and bottom, taking care that the two edges of the paper do not touch. If they do touch, the eluent will creep on a diagonal, and the spots will run together, or not in straight lines.



Acquisition of Chromatogram

1. Take a 600-mL beaker and pour 10-mL of 0.5 M NH_4OH and 20 mL of isopropyl alcohol (eluting solution) into the beaker. Obtain your evaporating dish and use it to cover the beaker.

2. Gently place the paper cylinder into the beaker and cover the top with the plastic wrap. Remember that the spots must be above the liquid level for the experiment to work. Watch the eluent creep up the paper until it is about 2 cm from the top. It will take about 45-60 minutes for the solvent front to reach the finish line.
3. When the solvent front reaches the finish line, remove the paper from the beaker, being careful to touch only the top. Let excess eluent drip into the beaker. Gently remove the staple and lay the chromatogram on a piece of paper towel. Use a hair dryer to dry the chromatogram completely. Pour the eluting solution in the organic waste container under the fume hood.
4. Working in the fume hood, spray the chromatogram lightly with the ninhydrin solution. Dry the sprayed chromatogram with a hair dryer, distinct colored spots will appear as a result of the ninhydrin reacting with the amino acids.
5. CAUTION: Use the ninhydrin inside the fume hood. Do not breathe the fumes or get spray on your skin.

Interpretation of Chromatogram

1. Circle around each color spot.
2. Use a ruler and draw a plus sign in the center of each spot. Measure the distance from the starting line to each plus sign. Record this distance for each spot on your lab report. These are the DD values, in cm.
3. Measure the distance between the starting line and the finish line or, the farthest up that the solvent front reached. Record this distance. This is the FF value, in cm.
4. Calculate the retention factor (Rf) for each spot and record the values in your lab report.
5. You and your lab partner will hand in your lab reports at the same time, with the paper chromatogram stapled to one of the lab reports.

Quantitative estimation of Lowry Methods.

INTRODUCTION:

Measurement of the quantity of the protein present in the solution is common and fundamental methods used in all laboratories. Generally these are of two types-

a. Direct spectophotometric method
b. Colorimetric method

Colorimetric method such as Biuret method, Lowery method, Bradford method etc are used extensively. These are the destructive method of protein analysis because the protein being measure cannot be recovered after such analysis. In this colorimetric method protein concentrations are measured by taking absorbance of visible wed length of light.

PRINCIPLE:

The "Lowery or Folin-Ciocalteau method" combines the copper reaction of the biuret method and the Folin-Ciocalteau reagent which reacts with tyrosine residues in proteins. When alkaline copper sulphate solution containing Sodium-Potassium Tartarate reacts with a protein solution, the copper ions forms a co-ordination complex with four NH peptide bond groups. In addition the phosphor molybdate present in the Folin-Ciocalteau reagent is reduced by tyrosine and tryptophan (aromatic amino acid) present in the protein, produce a dark blue/ purple colour complex, with maximum absorbance at 700nm. The intensity of the colour depends on the amount of these aromatic amino acids present which thus very with the different proteins present in the test sample.

CHEMICALS:

1. Alkaline sodium carbonate solution(20g/liter Na_2Co_3 in 0.1 mol/ltr NaOH)
2. Copper sulphate sodium Patasium tartarare solution(5g/ltr $CuSo_4,5H_2O$ in 10g/ltr Na-K tartarate).
3. Alkaline solution, prepared freshly by using 50ml of solution1 and 1ml of solution 2.
4. Folin-ciocalteau reagent(containing solution of sodium tungstate and sodium molybolate in phosphoric and hydrochloric acids and it is commercially available). Diluted the commercially available reagent with an equal of water just before use.
5. Standard sample preparation:- BSA standard solution are prepared by dissolved BSA in DH_2O. The concentration of new stock sample is 0.1mg/ml.
6. At first 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml, 0.9ml of protein sample is taken in dry test tube from 2nd stock or diluted stock solution of protein(BSA). Then the volume make upto 1ml by adding distilled water in test tube and prepared different concentration of protein solution(20, 40, 60, 80μg/ml).

OTHER REAGENTS:

i. Test tube
ii. Test tube rack
iii. Tissue paper
iv. Graph paper
v. Reagent bottle
vi. Beakers
vii. Measuring cylinders
viii. Posteur pipettes
ix. Notepad
x. Calculator

PROCEDURE:

1. previously prepared BSA stock solutions are used(10μg/ml, 20μg/ml, 30μg/ml, 40μg/ml, 50μg/ml, 60μg/ml, 70μg/ml, 80μg/ml, 90μg/ml).
2. 1ml distilled water is taken in a dry test tube and marked as “Blanked” there is no protein solution is present.
3. 1ml alkaline solution is added into each and every test tube.
4. Solution are incubated for 15 min at room temperature.
5. 0.1ml Folin reagent is added into each test tube.
6. Next the complete mixture are allowed for incubation at room temperature for 30 min. This incubation should be in dark.
7. Last the OD are measure in colorimeter a 700nm and graph is plotted based on the OD value.

PREPARATION OF BSA STANDARD CURVE:

SAMPLE NO.	BSA CONCENTRATION (μg)	BSA TAKEN (μl)	DISTILLED WATER (μl)	OD VALUE	CORRECT OD VALUE
1.	10	100	900	0.07	0.06
2.	20	200	800	0.15	0.14
3.	30	300	700	0.24	0.23
4.	40	400	600	0.28	0.27
5.	50	500	500	0.37	0.36
6.	60	600	400	0.38	0.37
7.	70	700	300	0.51	0.50
8.	80	800	200	0.64	0.63
9.	90	900	100	0.78	0.77
BLANK	0	0	1000	0.01	

SAMPLE	PROTEIN TAKEN (μl)	DISTILLED WATER (μl)	OD VALUE	CORRECTED OD VALUE
UNKNOWM	400	600	1.06	1.05

ELECTROPHORETIC STUDY

Demonstration of proteins separation by 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%)
Stacking gel (5%)

Distilled water 4.0 ml 30% acrylamide mix 3.3 ml
1.5M Tris pH8.8 2.5 ml
10% SDS 0.1 ml
10% ammonium persulfate 0.1 ml
TEMED 0.004ml

Distilled water 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

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.

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.

CHROMOSOME PREPARATION .

The uses of chromosome cytologic information are many, including karyotypic evolution, cytotaxonomy and phylogenetic relationships in plants, insects, and mammals among other organisms. In fish, chromosome analysis based on variations in chromosome number and morphology are typically used to conduct evolutionary and genetic questions such as stock identification, study of hybrids or induced polyploidy. Most fishes possess relatively large numbers of small chromosomes that can be easily viewed with a light microscope at the metaphase stage of mitosis.

The techniques for chromosome preparation are based on obtaining sources of dividing cells to produce high quality metaphase spreads with good chromosome definition. These procedures use specific chemicals, one of the most used reagents is colchicine, to inhibit the formation of the mitotic huse and thus, to block dividing cells at metaphase. Subsequently, a hypotonic treatment is used to separate the metaphase chromosomes from each other in the cells. Next, cells are fixed for staining. Commonly used methods of chromosome preparations are based on direct chromosome preparation from solid-tissues or cell cultures (Thorgaard and Disney, 1990). Accordingly, mitotic chromosome preparations can be successfully made using several tissues collected at different life stages of fish (Baksi and Means, 1988). These include embryos and fish fry and alternatively, the head and gill arches. If adult fish are used for chromosome preparation and analysis, dividing cells are obtained from sources showing a high proportion of actively dividing cells. These are head kidneys, gills, intestines, spleens, testes, scale epithelium or regenerating fin tissues.

Procedure

(i) Place the newly hatched larvae (n~12) into a 250-ml beaker containing 0.007% colchicine solution prepared in seawater and allow them to swim for 4 hours. Seawater may be aerated, if necessary.

(ii) Chop each larvae individually into 2-3 mm small pieces using a clean razor bladder.

(iii) Expose the pieces to a hypotonic solution of 0.4% KCl for 20-30 minutes.

(iv) Discard the hypotonic solution and fix the tissues by washing the chopped pieces twice in a freshly made cold mixture of 3:1 ethanol:acetic acid for at least 30 minutes each wash. Tissue may be stored in fixative for several months at 4ºC.

(v) Take the pieces out from the fixative and dry the excess fixative on a paper towel.

(vi) Place the pieces on an excavated micro slide and add 2-3 drops of 50% acetic acid.

(vii) Chop the pieces until a cell suspension is formed.

(viii) Clean micro slides using a mixture of 1:1 ether:ethanol and warm them on a heat plate at 40-50ºC.

(ix) Take the cell suspension with a pasteur pipette and let this suspension fall from a distance of ~12 cm on a precleaned and prewarmed micro slide.

(x) Repeat this process several times forming 2-3 rings of cells of ~1 cm of diameter.

(xi) Allow chromosome preparation to dry for 10-15 minutes.

(xii) Stain chromosome preparation with 15% Giemsa for 45 minutes.

(xiii) Wash chromosome preparation with distilled water and allow it to air dry for 10-15 minutes.

(xiv) Wash it with xylen for 10 minutes and add a cover glass using 2-3 drops of DPX.

(xv) Use a light microscope to view the chromosome preparation. The use of a 20x objective is recommended for a fast localization of chromosome metaphases that usually appear in the

periphery of the rings formed.

Materials and equipment

- Razor blades for chopping larvae
- Tweezers
- Excavated glass micro slides
- Clean glass slides
- Paper towel
- Heat plate
- Glass pasteur pipette
- Cover glass
- Light microscope
- Gloves and lab coat

Reagents and solutions

- 0.007% colchicine solution prepared in seawater. Colchicine is highly toxic and it should be handled with care. The operator should wear gloves during the procedure since colchicine is a hazardous chemical that may cause cancer and heritable genetic damage.
- Hypotonic solution of 0.4% KCl prepared in distilled water
- Fixer solution of 3:1 ethanol: acetic acid
- Staining solution of 15% Giemsa prepared in phosphate buffer (0.01 M, pH 7.0)
- Distilled water
- Xylen
- DPX

Course: BFSC-106: Taxonomy of finfish

Course: BFSC-106: Taxonomy of finfish 3 (1+2)

Theory

Principles of taxonomy. Nomenclature, types. Classification and interrelationships. Criteria for generic and specific identification. Morphological, morphometric and meristic characteristics of taxonomic significance. Major taxa of inland and marine fishes up to family level. Commercially important freshwater and marine fishes of India and their morphological characteristics. Introduction to modern taxonomic tools: karyo taxonomy, DNA barcoding, protein analysis and DNA polymorphism.

Practical

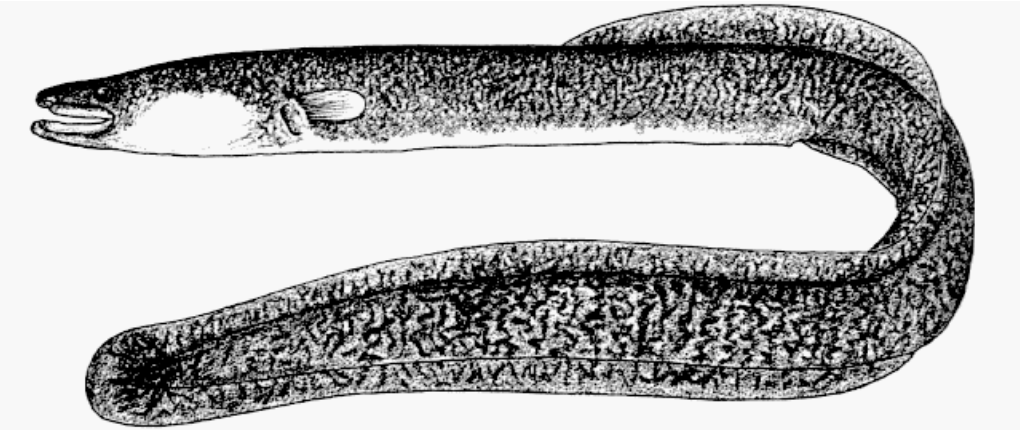
Collection and identification of commercially important inland and marine fishes. Study of their external morphology and diagnostic features. Modern taxonomic tools - Protein analysis and electrophoretic studies; Karyo taxonomy - chromosome preparation and identification. DNA barcoding, DNA polymorphism; Visit to fish landing centres to study commercially important fishes and catch composition.

Reference Books

Nelson, J.S. 1994. *Fishes of The World. 3rd Edition*. John Wiley & Sons, Inc., New York.

2. Day, F. 1989. *Fishes – Vol.I.* Today and Tomorrow's Printers and Publishers, New Delhi.

Specimen: 1



Phylum – Chordata

Subphylum – Vertebrata

Super Class – Gnathostomata

Class – Osteichthyes

Super order – Elopomorpha

Order – Anguilliformes

Family – Anguillidae

Genus – *Anguilla*

Species – *bengalensis*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 pectoral fins.
- 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 ventral surface
- Lips are thick and well developed
- Jaws are equal
- Caudal fin continued round the end of the tail

Hence, Genus – *Anguilla*

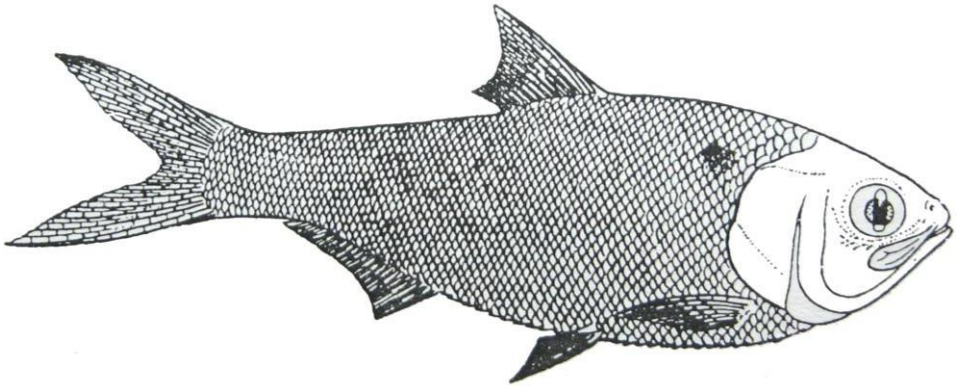
- Villiform teeth are present in the jaws and palate
- Dorsal fin inserted midway between gill opening and origin of anal fin with 220-305 rays and no spine
- Anal fin long with 200 – 250 rays
- Colouration of adult is variegated

Hence, species – *bengalensis*

Hence, the provided specimen is *Anguilla bengalensis*

Distribution - Throughout India, Pakistan, Bangladesh, Sri Lanka, Burma and Malay

Specimen: 2



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Clupeomorpha
Order – Clupeiformes
Family – Clupeidae
Genus – *Gudusia*
Species – *chapra*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Branchiostegals numbering as high as 15, usually fewer
- Mesocoracoid arch invariably present
- Usually no lateral line possess on trunk

Hence, Super Order – Clupeomorpha

- Body short, oblong, not eel like, covered with minute cycloid scales, scales are absent in head regions
- Abdomen with keeled scutes along the ventral line
- Radiating cutaneous canals on opercular bones

Hence, Order – Clupeiformes

- Maxillaries composed of three pieces and not composed with together
- Teeth when present, is rudimentary and deciduous
- Barbells absent
- Gill membranes free
- Opercular pieces four

Hence, Family – Clupeidae

- Body compressed and oblong
- Abdomen serrated, with 18 – 19 pre pelvic and 8 – 10 post pelvic scutes
- Head short and highly compressed
- Snout rounded
- Mouth slightly upturned, terminal, cleft not extending to the orbit
- Eyes large, lateral, in anterior part of the head and not visible from the ventral surface with broad adipose eyelid
- Lips thin
- Caudal fin forked

Hence, Genus – *Gudusia*

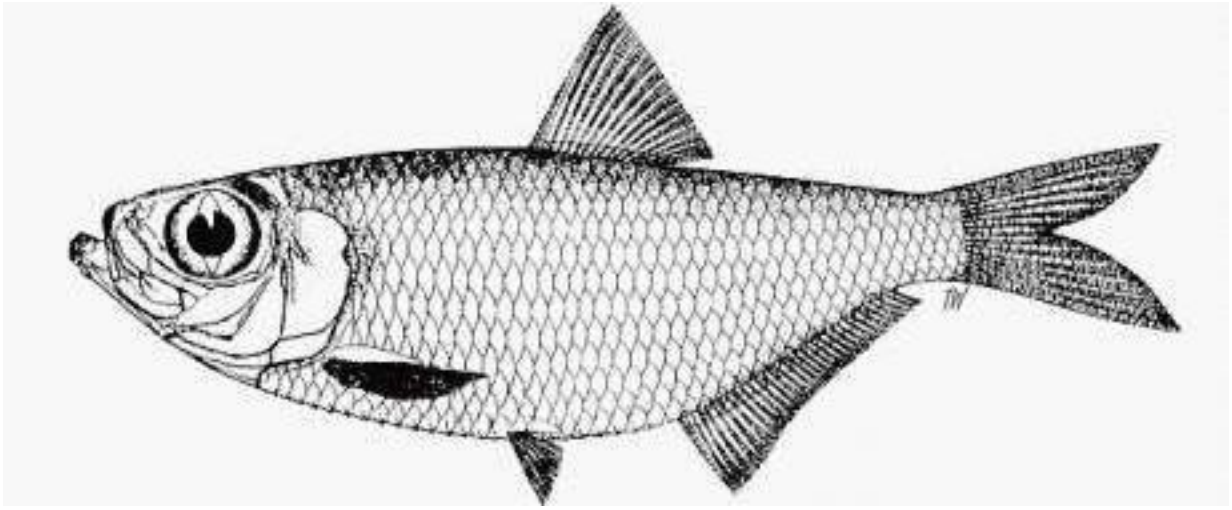
- Body without any cross bars on sides
- Dorsal fins inserted in above pelvic origin, with 16 rays
- Anal fin with 19 – 21 rays
- Caudal fin forked
- Lateral line absent, 80 – 120 scales in the lateral series

Hence, species – *chapra*

Hence, the provided specimen, *Gudusia chapra*

Distribution – India, Pakistan, Bangladesh, China and Burma, Nepal, Malaya

Specimen: 3



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Clupeomorpha
Order – Clupeiformes
Family – Clupeidae
Genus – *Pellona*
Species – *ditchela*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Branchiostegals numbering as high as 15, usually fewer
- Mesocoracoid arch invariably present
- Usually no lateral line possesses on trunk

Hence, Super Order – Clupeomorpha

- Body short, oblong, not eel like, covered with minute cycloid scales, scales are absent in head regions
- Abdomen with keeled scutes along the ventral line
- Radiating cutaneous canals on opercular bones

Hence, Order – Clupeiformes

- Maxillaries composed of three pieces and not composed with together
- Teeth when present, is rudimentary and deciduous
- Barbells absent
- Gill membranes free
- Opercular pieces four

Hence, Family – Clupeidae

- Body compressed and elongated
- Abdomen serrated, with 18 – 20 pre pelvic and 8 – 9 post pelvic scutes
- Head long and compressed
- Snout blunt
- Mouth upturned, cleft not reaching to the orbit
- Eyes large, lateral, in the middle of the head and not visible from the ventral surface with narrow adipose eyelid
- Lips thin
- Lower jaw longer than upper

Hence, Genus – *Pellona*

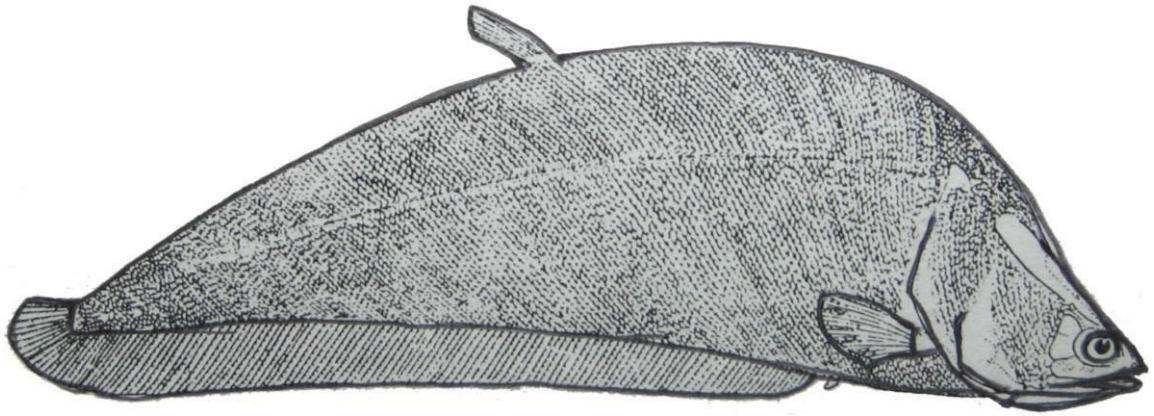
4. Dorsal fins inserted in above the tip of the pectoral fin or slightly behind, with 17 - 18 rays
5. Anal fin with 30 – 40 rays
6. Lateral line absent, 40 – 45 scales in the lateral series

Hence, species – *ditchela*

Hence, the provided specimen, *Pellona ditchela*

Distribution – India, Pakistan, Bangladesh, China and Burma, Nepal, Malaya, Thailand, Vietnam, South Africa, and Madagascar

Specimen: 4



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Osteoglossomorpha
Order – Osteoglossiformes
Family – Notopteridae
Genus – *Notopterus*

Species – *chitala*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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*

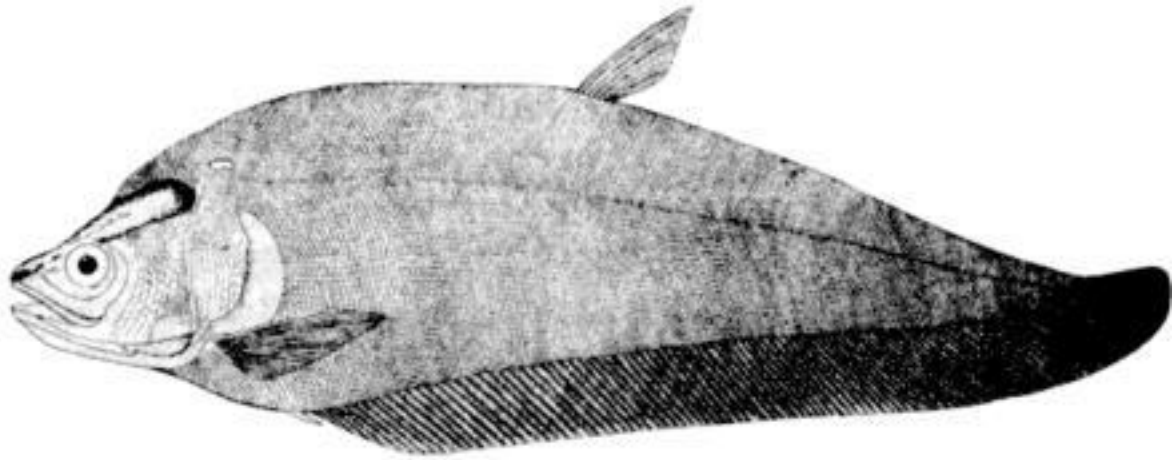
- Dorsal fin small, tuft like, inserted near the middle of the body, with 9 – 10 rays
- Anal fin is very low, ribbon like, 110 – 135 rays, confluent with caudal fin
- Pelvic fin rudimentary with 6 rays
- Lateral line is more or less with 180 rays
- Gape of the mouth not extending beyond the hind edge of orbit
- Silvery on sides, copper brown on back, having 15 silvery bars dorsally

Hence, species – *chitala*

Hence, the provided specimen is *Notopterus chitala*

Distribution – India: Northern India, Pakistan, Bangladesh, and Burma

Specimen: 5



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Osteoglossomorpha
Order – Osteoglossiformes
Family – Notopteridae
Genus – *Notopterus*

Species – *notopterus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 upto 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*

- Dorsal fin small, tuft like, inserted near the middle of the body, with 7 – 9 rays
- Anal fin is very low, ribbon like, 100 – 110 rays, confluent with caudal fin
- Pelvic fin rudimentary with 5-6 rays
- Lateral line is more or less with 225 rays
- Gape of the mouth extending beyond the hind edge of orbit
- Silvery white on sides, Gray on back

Hence, species – *chitala*

Hence, the provided specimen is *Notopterus notopterus*

Distribution – India: Northern India, Pakistan, Bangladesh, and Burma

Specimen: 6

Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Protacanthopterygii
Order – Salmoniformes
Family – Salmonidae
Genus – *Salmo*

Species – *gairdnerii*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- A wide spread trend towards the exclusion by Premaxilla of the maxillae from the gape and development of Premaxillary possess
- Branchiostegals numerous

Hence, Super Order – Protacanthopterygii

- Body elongated, to sub cylindrical covered with cycloid scales, head without scales
- Abdomen non-keeled and non-serrated
- Psedobranchiae present

Hence, Order – Salmoniformes

- Body compressed, with fine scales
- Dorsal fin two, an adipose dorsal fin present
- Cleft of the mouth

Hence, Family Salmonidae

- Body elongated and sub cylindrical, with round abdomen
- Head moderate and snout obtuse
- Mouth wide and cleft of the mouth oblique, extending up to front border of the eye
- Eyes large and superior, in the middle of the head, not visible from below the ventral surface
- Lips thin and jaws equal

Hence, Genus – *Salmo*

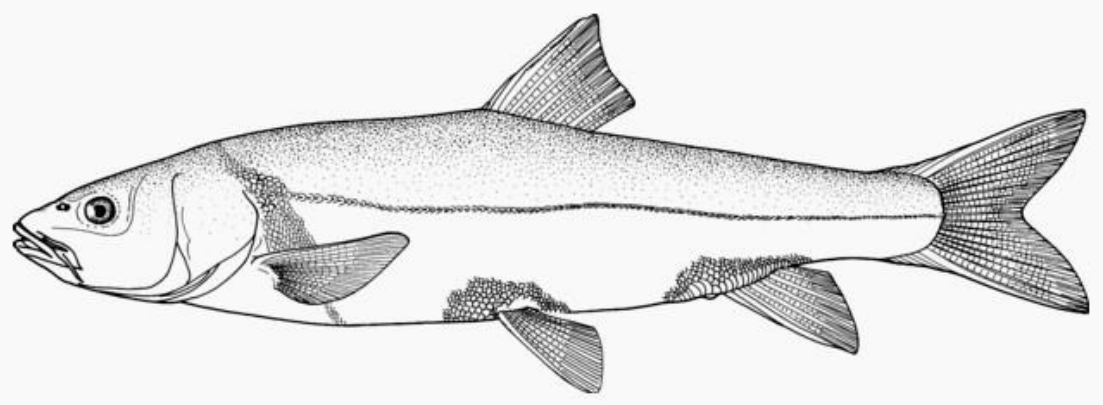
- Rayed Dorsal fin inserted above the pelvic fin, with 12 – 14 rays
- Adipose dorsal fin smooth, small inserted above mid anal
- Anal fin short, 10 – 13 rays
- Caudal fin forked
- Body without spots, bellow the lateral line and Lateral line straight with 127 - 160 rays
- Colour of the body is still blue

Hence, species – *gairdnerii*

Hence, the provided specimen is *Salmo gairdnerii*

Distribution – India: hilly regions, Pakistan, Sri Lanka, Bangladesh, S. Africa, England and North America

Specimen: 7



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Schizothorax*

Species – *kumoanensis*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 elongated and sub cylindrical, with round abdomen
- Head large and pointed anteriorly, snout rounded
- Mouth inferior and transverse
- Eyes large laterally placed, not visible from below the ventral surface
- Lips thick and fleshy, lower lip with a free posterior edge forming a sucker
- Barbells two pairs, one each rostral and other is maxilla

Hence, Genus – *Schizothorax*

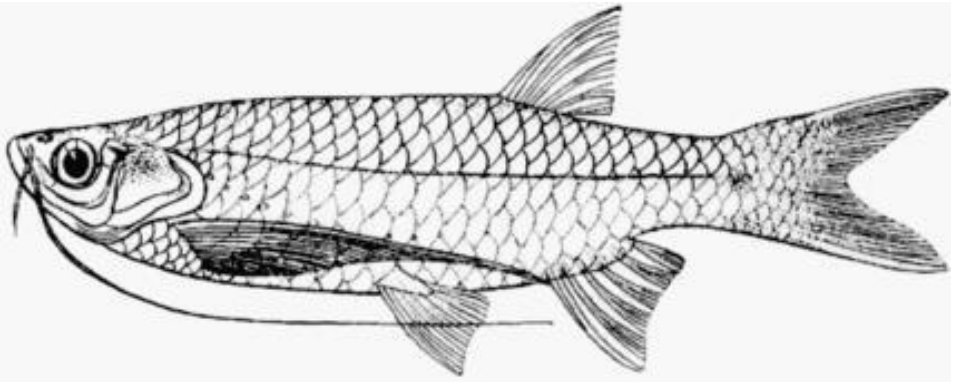
- Dorsal fin inserted slightly ahead pf pelvic fin, with 11 – 12 rays and with spine which may be articulated, smooth or serrated
- Anal fin short, 7 – 8 rays
- Caudal fin forked
- Lateral line complete with 98 - 100 rays
- Body uniformly silvery without black spots (alcohol preserved specimen)

Hence, species – *kumoanensis*

Hence, the provided specimen is *Schizothorax kumoanensis*

Distribution – India: hilly regions, Pakistan, Sri Lanka, Bangladesh, S. Africa, England and North America

Specimen: 8



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Esomus*
Species – *danricus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 elongated strongly compressed, with round abdomen
- Head small and blunt, snout blunt
- Mouth small, obliquely directed upwards
- Eyes inferiorly placed, visible from below the ventral surface
- Lips thin
- Barbells two pairs, maxillary pair is long, extending up to the anal fin

Hence, Genus – *Esomus*

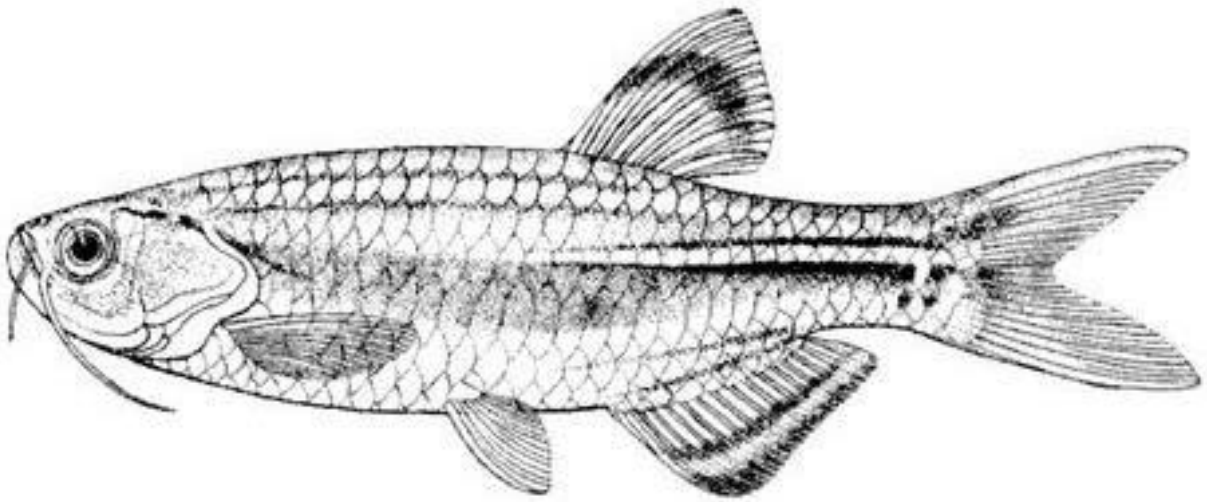
- Dorsal fin inserted in the interspaces between anal and pelvic fin, with 6 branched rays and without spine
- Anal with 5 branched rays
- Caudal fin forked
- No pre caudal spots are present, sides with broad lateral band, 14 scale round the caudal peduncle

Hence, species – *danricus*

Hence, the provided specimen is *Esomus danricus*

Distribution – India, Pakistan, Nepal and Bangladesh, Sri Lanka, Thailand, Malay

Specimen: 9



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Danio*
Species – *dangila*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 elongated compressed, sub cylindrical with round abdomen
- Head moderate and blunt, snout obtuse
- Mouth anterior, cleft of the mouth not protractile
- Eyes large centrally placed, not visible from below the ventral surface
- Lips thin and simple
- One or two pairs of barbells, rudimentary or none

Hence, Genus – *Danio*

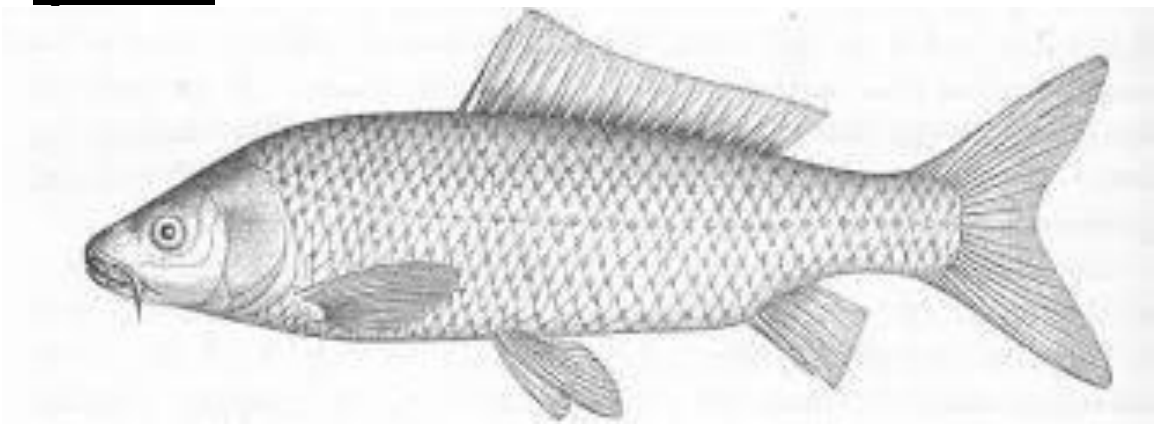
- Dorsal fin inserted in the interspaces between anal and pelvic fin, with 12 – 16 branched rays and without spine
- Anal with 13 – 20 rays
- Caudal fin forked
- Both pairs of barbells much longer than eye diameter
- Lateral line scales – 36 to 42

Hence, species – *dangila*

Hence, the provided specimen is *Danio dangila*

Distribution – India: Bihar, Bengal, Eastern Himalaya, Bangladesh, Nepal and Burma

Specimen: 10



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Cyprinus*
Species – *carpio*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 ventral surface
- Lips fleshy
- Barbells two pairs, one pair each of rostral and maxillary

Hence, Genus – *Cyprinus*

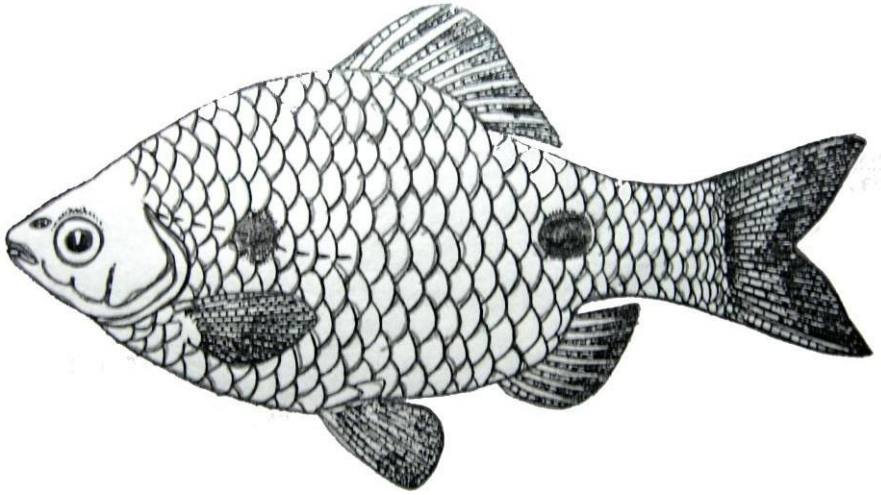
- Dorsal fin very long inserted above the tip of pectoral fin, with 3 spines and 17 rays, 3rd spine strongest and serrated
- Anal with short with 3 spines and 5 rays
- Caudal fin deeply emarginated and lobs pointed
- Lateral line straight with 36 scales

Hence, species – *carpio*

Hence, the provided specimen is *Cyprinus carpio*

Distribution – China, Korea, Japan, Taiwan, Europe, America, Introduced in India in 1939

Specimen: 11



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Puntius*
Species – *ticto*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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, jaws simple covered by lips

Hence, Genus – *Puntius*

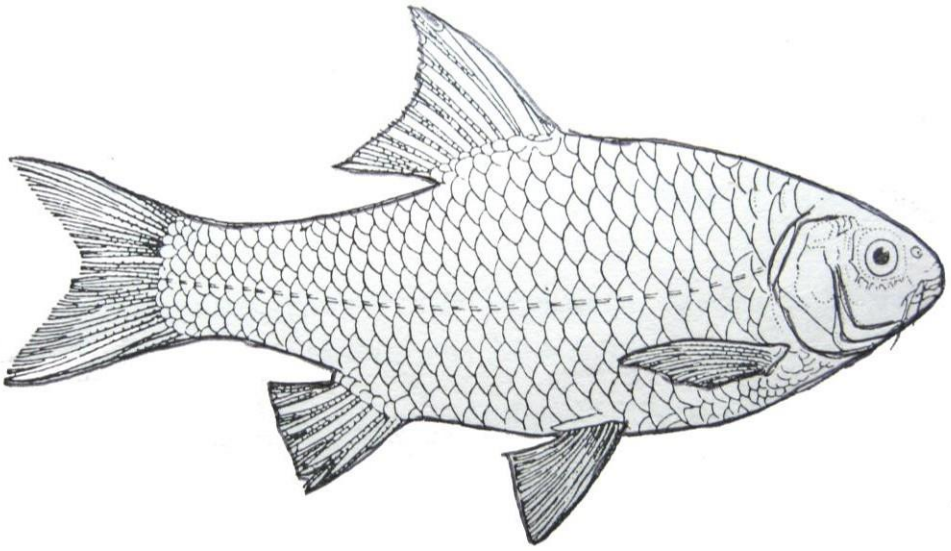
- Dorsal fin short inserted nearly opposite of the pelvic fin, with 3 to 4 soft spines and 8 rays
- Anal with short with 2-3 spines and 5 rays
- Pectoral fin with single soft spine and 12 – 14 rays
- Pelvic fin with single soft spine and 8 rays
- Lateral line straight with 23 - 25 scales

Hence, species – *ticto*

Hence, the provided specimen is *Puntius ticto*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 12



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Puntius*
Species – *sarana*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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, jaws simple covered by lips

Hence, Genus – *Puntius*

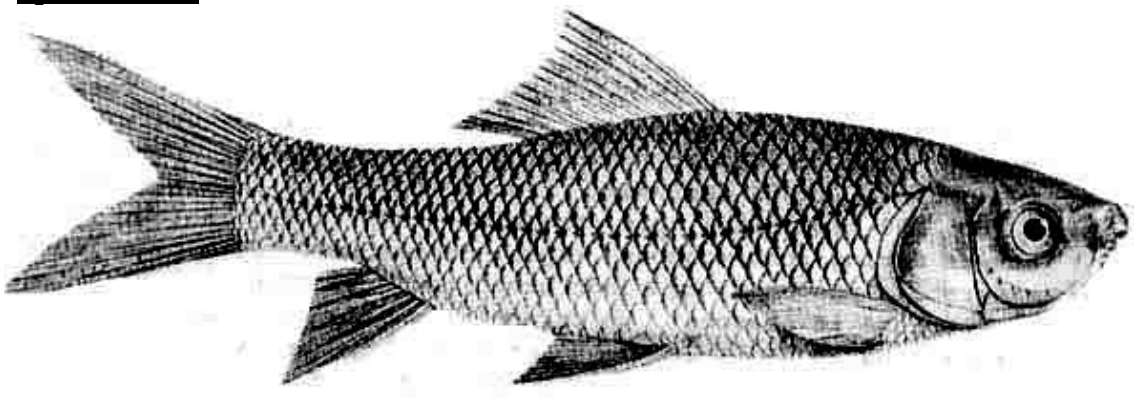
- Dorsal fin short inserted nearly opposite of the pelvic fin, with 3 to 4 soft spines and 8 rays
- Anal with short with 3 spines and 5 rays
- Pectoral fin with single soft spine and 14 – 16 rays
- Pelvic fin with single soft spine and 8 rays
- Lateral line straight with 30 - 33 scales

Hence, species – *sarana*

Hence, the provided specimen is *Puntius sarana*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 13



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Labeo*
Species – *rohita*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 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 the head, not visible from below the ventral surface
- Lips thick and fleshy, fringed, covering both jaws, continuous at angle of mouth forming labial fold.
- Barbells two pairs, one pair or none.

Hence, Genus – *Labeo*

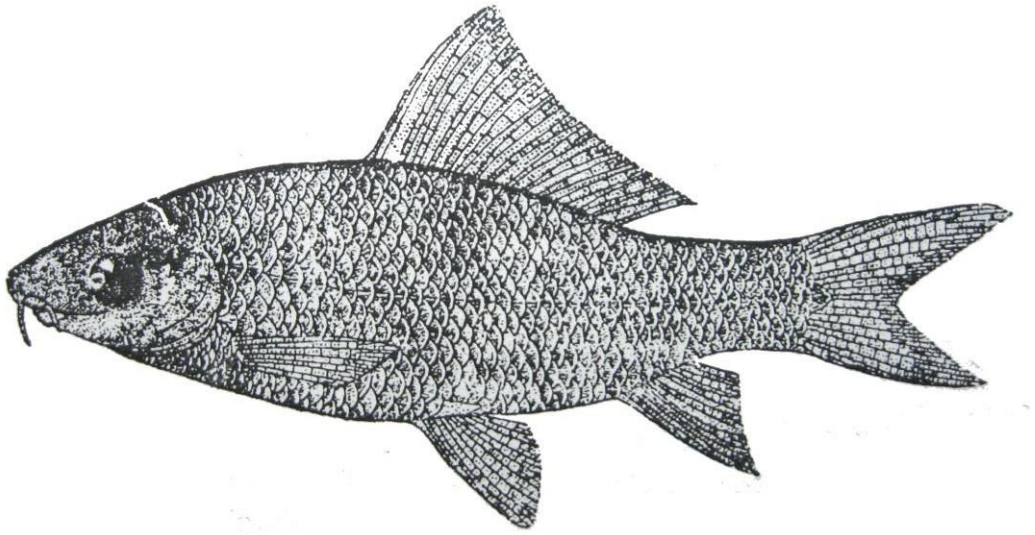
- Dorsal fin inserted above pelvic fin, and 12 - 14 rays,
- Anal fin short with 7 - 8 rays (two or three simple)
- Pectoral fin with 16 – 18 rays
- Lateral line straight with 40 - 44 scales
- Scales between lateral and pelvic fin base – 6 to 7

Hence, species – *rohita*

Hence, the provided specimen is *Labeo rohita*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 14



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Labeo*
Species – *calbasu*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 the head, not visible from below the ventral surface
- Lips thick and fleshy, fringed, covering both jaws, continuous at angle of mouth forming labial fold.

Hence, Genus – *Labeo*

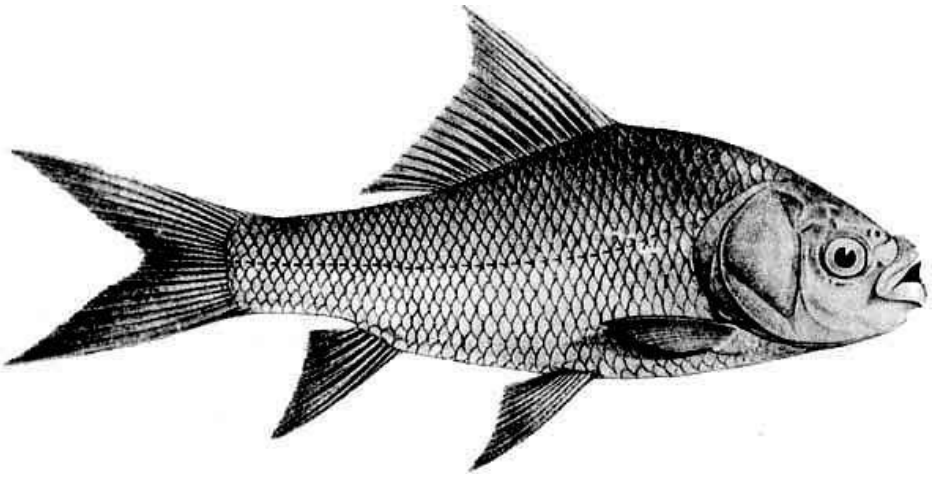
- Dorsal fin inserted above pelvic fin, and 16 - 18 rays,
- Anal fin short with 5 rays (two or three simple)
- Pelvic fin with 8 rays
- Lateral line straight with 40 - 44 scales
- One pair each rostral and maxillary barbells are present
- Colour of the body black, including fins

Hence, species – *calbasu*

Hence, the provided specimen is *Labeo calbasu*

Distribution – India, Pakistan, Nepal, Bangladesh, Sri Lanka, Burma, Malay

Specimen: 15



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Catla*
Species – *catla*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 short and deep
- 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

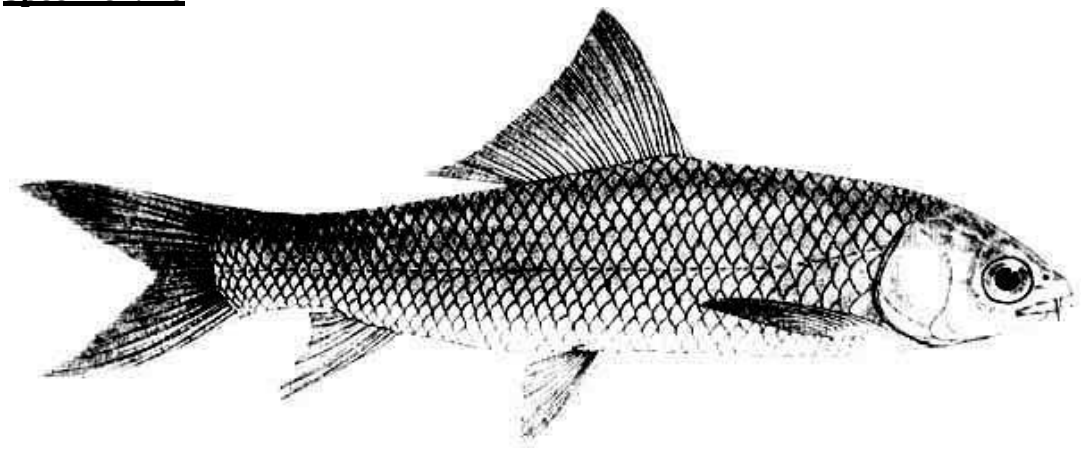
- Body attains a length of about 120 cm in three years
- Dorsal fin long, inserted above the tip of the pectoral fin with 17 to 19 rays (3/4 simple)
- Anal fin short with 8 rays (5 branched)
- Lateral line covered with 40 – 43 scales
- Caudal fin deeply forked

Hence, species – ***catla***

Hence, the provided specimen is *Catla catla*

Distribution – India, Pakistan, Nepal, Bangladesh, Thailand

Specimen: 16



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Cirrhinus*
Species – *mrigala*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 the ventral surface
- Upper lip fringed or entire, not continuous with lower
- Lower jaw sharp with a small tubercle at the symphysis, without any cartilaginous covering inside the jaw

Hence, Genus – *Cirrhinus*

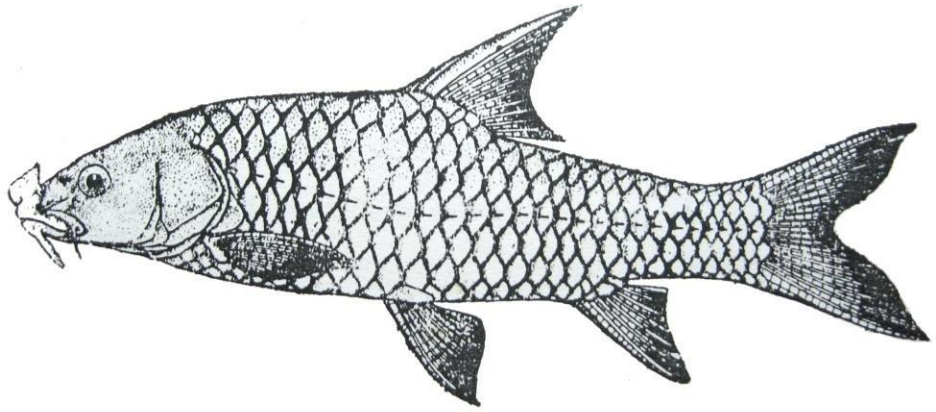
- Body attains a length of about 99 cm in three years
- Dorsal fin long, inserted ahead of the pelvic fin with 15 to 16
- Anal fin short with 7 or 8 rays (2/3 simple)
- Lateral line covered with 40 – 45 scales
- Caudal fin deeply forked or lunate

Hence, species – *mrigala*

Hence, the provided specimen is *Cirrhinus mrigala*

Distribution – India, Pakistan, Nepal, Bangladesh, Thailand, Burma

Specimen: 17



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Tor*

Species – *tor*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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, elongated moderately compressed with round abdomen
- Head small, broadly pointed, snout angularly rounded
- Mouth inferior and arched
- Eyes far forward and not visible from below the ventral surface
- Lips fleshy, continuous at angles of mouth, posterior lip with or without a median lobe and post labial groove continuous, lip condition variable
- Pharyngeal teeth in three rows, 5, 3, 2
- A scaly sheathe present at the base of the dorsal fin

Hence, Genus – *Tor*

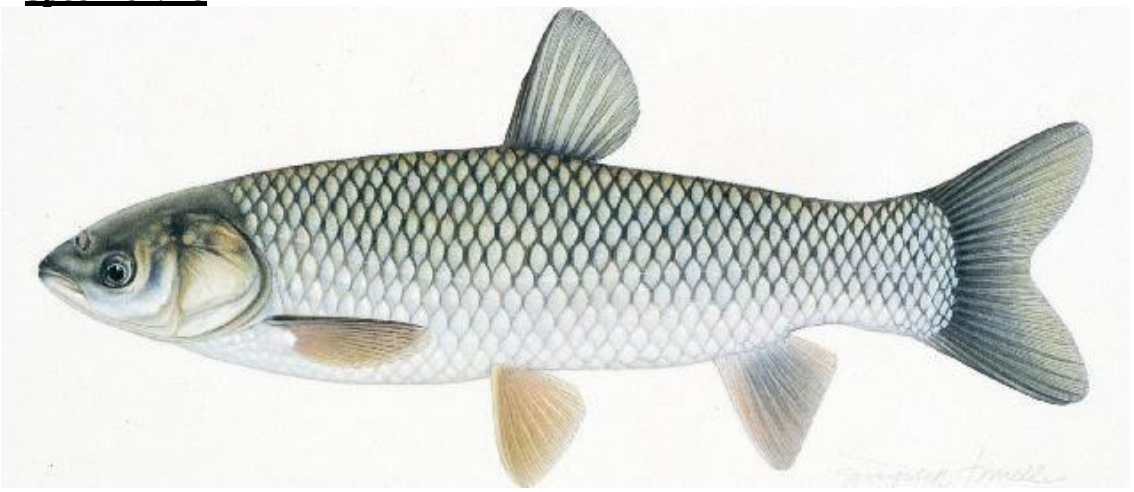
- Body attains a length of about 15 cm
- Ventral profile is more ahead than dorsal
- Dorsal fin, inserted above of the pelvic fin with 12 to 13 rays (8 – 9 branched)
- Anal fin with 5 rays (2/3 simple)
- Lateral line covered with 22 – 27 scales
- Caudal fin deeply forked

Hence, species – ***tor***

Hence, the provided specimen is *Tor tor*

Distribution – India, Pakistan, Nepal, Bangladesh, Thailand, Burma

Specimen: 18



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Ctenopharyngodon*

Species – *idella*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 round abdomen
- 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 the ventral surface
- Lips thin without any lobes
- Upper jaw slightly longer than lower and protractile

Hence, Genus – *Ctenopharyngodon*

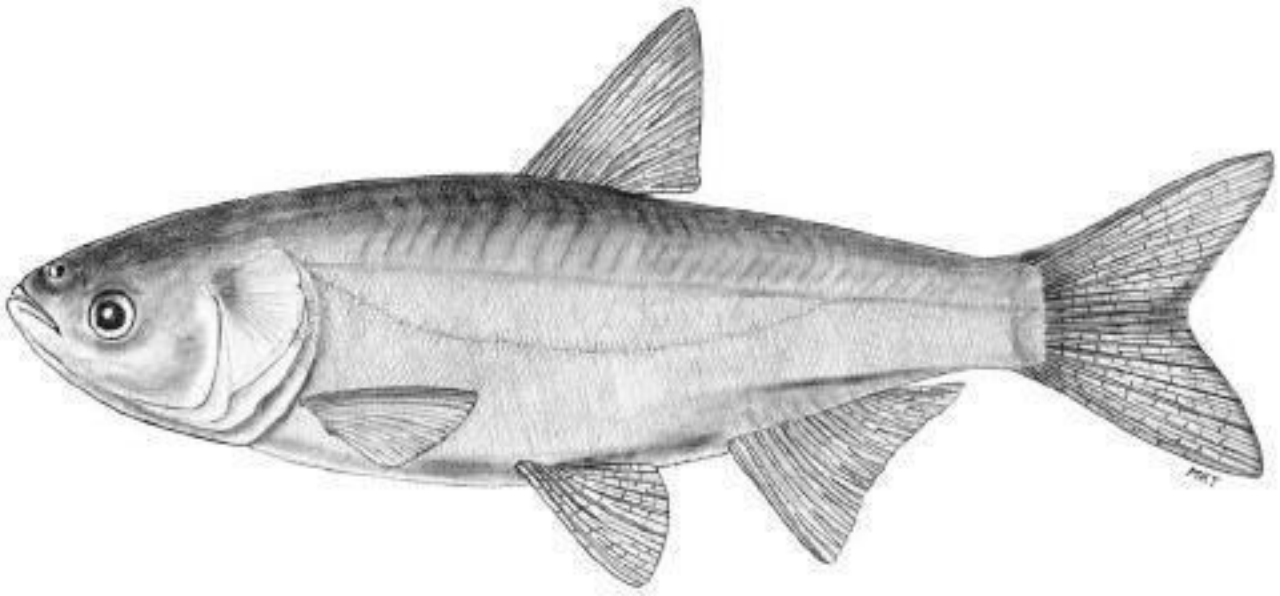
- Body attains a length of about 86 cm in three years
- Dorsal fin inserted slightly ahead of the pelvic fin with 10 rays
- Anal fin short with 10 rays (8 branched)
- Lateral line continuous slightly curved covered with 40 – 42 scales
- Caudal fin deeply forked

Hence, species – ***idella***

Hence, the provided specimen is *Ctenopharyngodon idella*

Distribution – Naturally found in Amur region Siberia, North China to South China, U.S.S.R, introduced in India in 1959

Specimen: 19



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Cypriniformes
Family – Cyprinidae
Genus – *Hypophthalmichthys*
Species – *molitrix*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 abdomen to 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 from below the ventral surface
- Lips thin
- Upper jaw slightly protruded upward a little longer than lower

Hence, Genus – *Hypophthalmichthys*

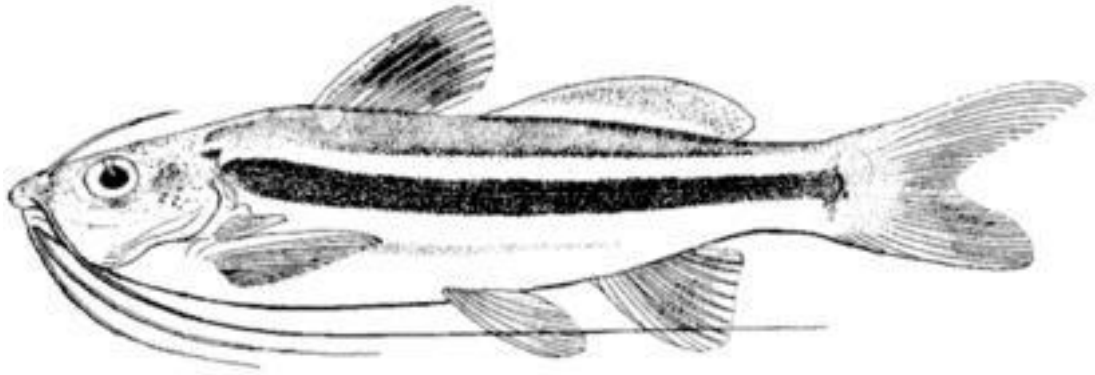
- Body attains a length of about 82 cm in three years
- Dorsal fin inserted behind the pelvic fin or above the tip of the pectoral fin with 10 rays
- Anal fin short with 14 – 17 rays (12 – 14 branched)
- Lateral line continuous slightly curved covered with 110 – 115 scales
- Caudal fin deeply forked

Hence, species – ***molitrix***

Hence, the provided specimen is *Ctenopharyngodon idella*

Distribution – Naturally found in Amur region Siberia, North China to South China, U.S.S.R, introduced in India in 1959

Specimen: 20



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Bagridae
Genus – *Mystus*
Species – *vittatus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 and more or less elongated fish
- Nostrils widely separated
- Barbell six or eight, generally well developed

Hence, Family – Bagridae

- Body short or moderately elongated, compressed with round abdomen
- Head moderate in size and compressed
- Mouth terminal, transverse and moderately wide
- Eyes moderately large, supra lateral, in anterior part of the head not visible from bellow the ventral surface
- Lips thin
- Jaws sub equal
- Barbells four pairs, one each of maxillary, nasal, and two mandibular, generally longer than head

Hence, Genus – *Mystus*

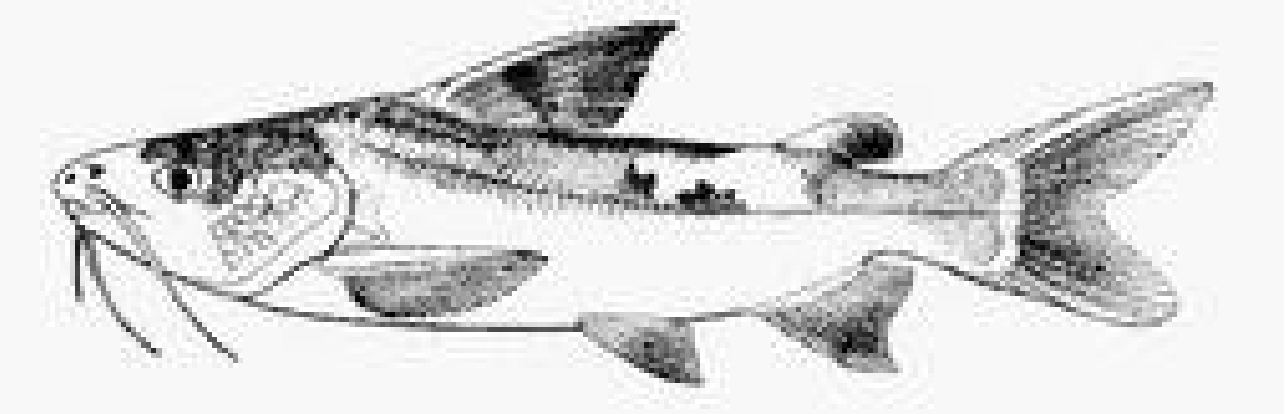
- Rayed dorsal fin inserted above the last quarter of the pectoral fin with 7 rays and a spine
- Adipose dorsal fin low of varying length
- Pectoral fin with 9 rays
- Body with 3 – 4 longitudinal colour bands above and bellow the lateral line
- A dark shoulder spot present
- No spot at the base of caudal fin

Hence, Species – *vittatus*

Hence, the provided specimen is *Mystus vittatus*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 21



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Siluridae
Genus – *Ompok*
Species – *pabda*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 of eyes, snout bluntly rounded, depressed
- Eyes small, ventral border on the level with corner of mouth, visible from under side of the head
- Lips thin, Jaws sub equal, lower jaw prominent
- Barbells two pairs, one pair each of maxillary and mandibular, latter occasionally small or rudimentary

Hence, Genus – *Ompok*

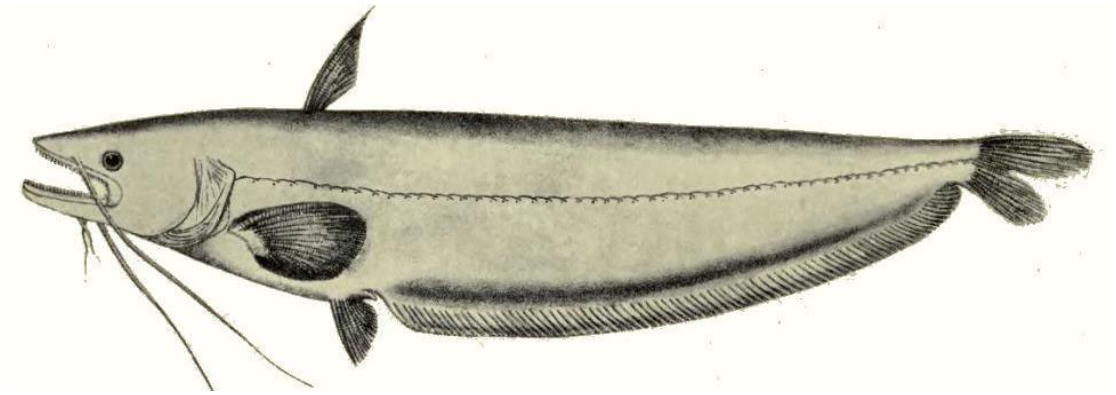
- Body attains 17 cm
- Rayed dorsal fin inserted above the last half of the pectoral fin with 4-5 rays and without any spine
- Adipose dorsal fin absent
- Pectoral fin with 11-14 rays
- Anal fin with 22-56 rays, very long, close to caudal fin, free from it
- Caudal fin forked

Hence, Species – *pabda*

Hence, the provided specimen is *Ompok pabda*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 22



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Siluridae
Genus – *Wallago*
Species – *attu*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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*

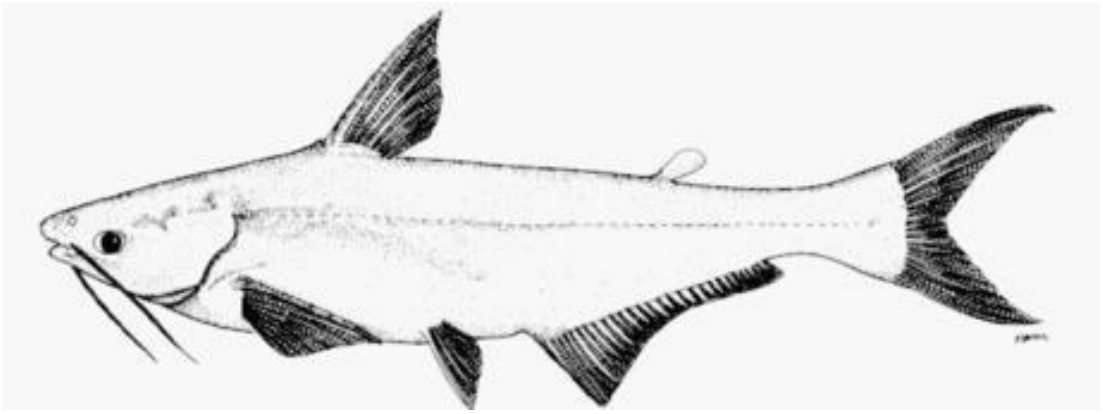
- Rayed dorsal fin inserted above half of the pectoral fin with 5 rays and without any spine
- Adipose dorsal fin absent
- Pectoral fin with 13-15 rays, and feeble smooth spine
- Anal fin long with 86-89 rays, free from caudal fin
- Caudal fin forked with rounded lobes
- Lateral line complete, well marked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

Hence, Species – *attu*

Hence, the provided specimen is *Wallago attu*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 23



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Pangasiidae
Genus – *Pangasius*

Species – *pangasius*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 fish with compressed body
- Nostrils widely separated, anterior ones wide, situated along the front border of the snout
- Barbell four, moderately developed

Hence, Family – Pangasiidae

- Body elongated, compressed with round abdomen
- Head moderate, exceptionally granulated and blunt. snout prominent, rounded
- Mouth sub terminal, horizontal or slightly ascending
- Eyes large, behind the corner of the mouth, visible bellow the ventral surface
- Lips thin, Jaws sub terminal, upper jaw slightly longer
- Barbells two pairs, one pair each of maxillary and mandibular

Hence, Genus – *Pangasius*

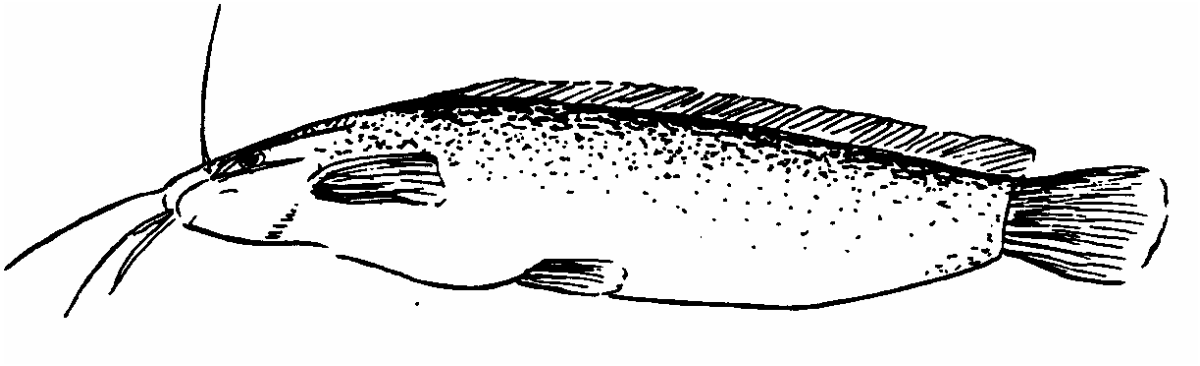
- Rayed dorsal fin inserted above last quarter of the pectoral fin with 6-7 rays and a spine
- Adipose dorsal fin absent, short and posteriorly free
- Pectoral fin with 9-12 rays, and a strongly serrated spine
- Anal fin long with 30-34 rays, free from caudal fin
- Caudal fin forked
- Lateral line complete, and simple

Hence, Species – *pangasius*

Hence, the provided specimen is *Pangasius pangasius*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka, Viet-Nam, Indonesia

Specimen: 24



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Clariidae
Genus – *Clarias*
Species – *batrachus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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 latterly forming 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*

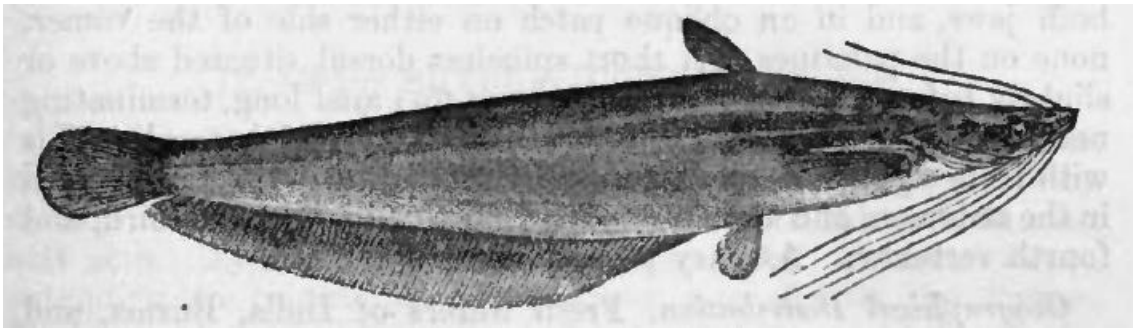
- Rayed dorsal fin long with 62-77 rays without any spine
- Adipose dorsal fin absent,
- Pectoral fin with 7-11 rays, and a strongly serrated spine, enveloped in a skin
- Anal fin long with 45-63 rays
- Caudal fin almost rounded
- Lateral line complete, and simple

Hence, Species – *batrachus*

Hence, the provided specimen is *Clarias batrachus*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka, Viet-Nam, Indonesia

Specimen: 25



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Ostariophysi
Order – Siluriformes
Family – Heteropneustidae
Genus – *Heteropneustes*
Species – *fossilis*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

- Moderate sized elongated fish with compressed body
- Nostrils widely separated, anterior produced into short tube on the tip of the snout
- Barbells 8, well developed

Hence, Family – Heteropneustidae

- Body elongated, compressed with round abdomen
- Head moderate, greatly depressed, covered with thin skin, Snout flat
- Mouth terminal, transverse and narrow
- Eyes small, lateral in anterior part of the, not visible bellow ventral surface
- Lips fleshy and papillated, jaws sub equal
- Barbells four pairs, one pair each of maxillary nasal and 2 of mandibular

Hence, Genus – *Heteropneustes*

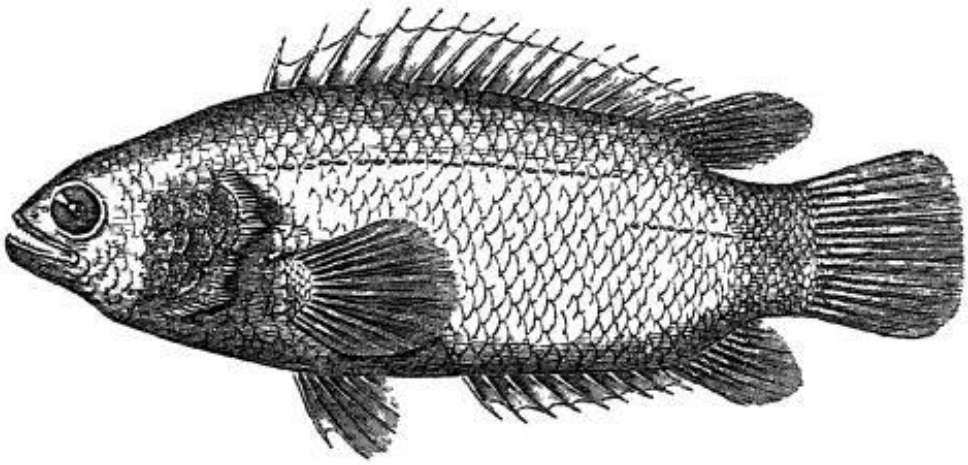
- Rayed dorsal fin short inserted above the tip of the pectoral fin with 6-8 rays without any spine
- Adipose dorsal fin absent, or represented by low adipose ridge, along the posterior third of the caudal region
- Pectoral fin with 7-8 rays, and a strong spine, serrated along the inner edge
- Anal fin long with 60-79 rays, separated from the caudal fin with deep notch
- Caudal fin almost rounded
- Pelvic fin with 6 rays
- Lateral line complete, and simple

Hence, Species – *fossilis*

Hence, the provided specimen is *Heteropneustes fossilis*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 26



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Acanthopterygii
Order – Perciformes
Family – Anabantidae
Genus – *Anabas*
Species – *testudineus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

- Moderately elongated compressed body,
- Head and body covered with ctenoid scales

Hence, Family – Anabantidae

- Body oblong, compressed with round abdomen
- Head moderate, compressed, Snout slightly conical
- Mouth small, terminal, oblique, cleft not wide
- Eyes large, lateral in anterior part of the head, not visible bellow the ventral surface
- Lips thin, jaws equal

Hence, Genus – *Anabas*

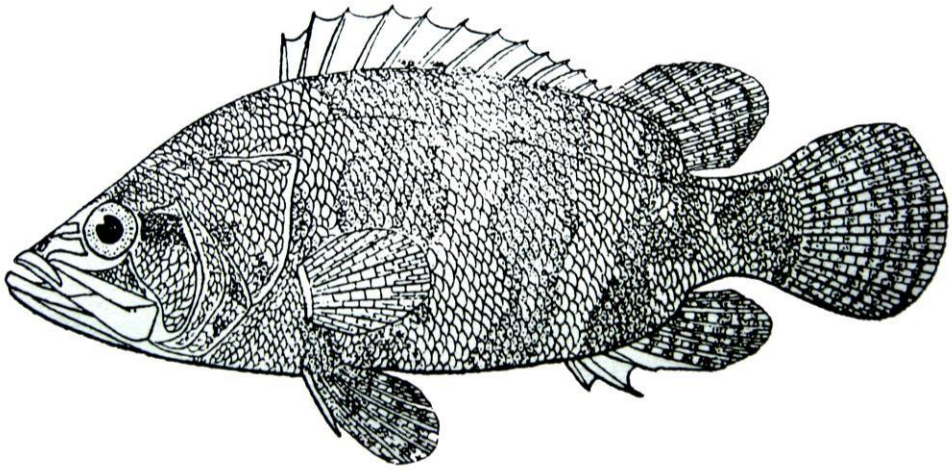
- Single dorsal fin inserted above the pectoral base, with 17-18 spine & 8-10 rays, number of spine variable
- Anal fin with 8-10 spines and 9-11 rays,
- Caudal fin rounded
- Lateral interrupted, with 21-29 scales

Hence, Species – *testudineus*

Hence, the provided specimen is *Anabas testudineus*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma, Thailand, China, Sri Lanka

Specimen: 27



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Acanthopterygii
Order – Perciformes
Family – Nandidae
Genus – *Nandus*
Species – *nandus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

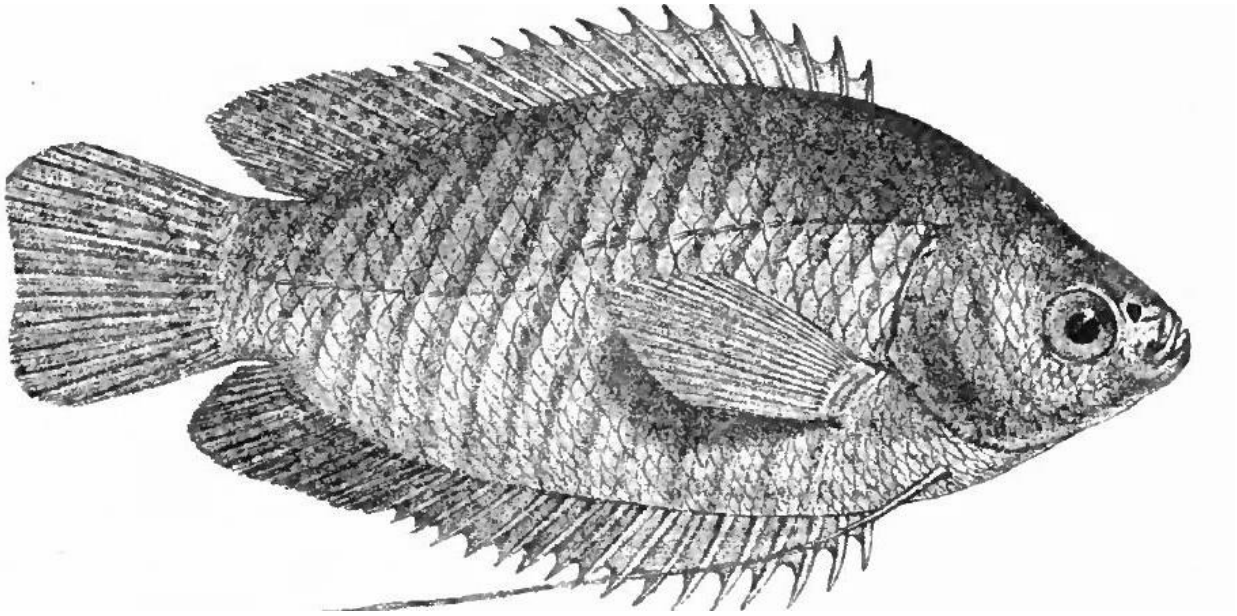
- Body oblong, elevated, compressed with moderate sized ctenoid scales
- Head and body covered with ctenoid scales
- Teeth on jaws and palate
- Operculum armed with spine
- A single dorsal fin with spine and soft portion

Hence, Family - Nandidae

- Body oblong and compressed
- Mouth terminal and very protractile
- Opercle with one spine, preopercle, pre orbit, sub and inter opercle serrated or more or less entire.
- Dorsal fin with 12-14 spines and 7-9 rays.
- Lateral line interrupted at about 36^{th} scales, 46-57 scales along with the lateral line.

Hence the Specimen is *Nandus nandus*

Specimen: 28



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Super order – Acanthopterygii
Order – Perciformes
Family – Belontidae
Genus – *Colisa*
Species – *fasciata*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- 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

Hence, Family – Belontidae

- Body elevated, compressed
- 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*

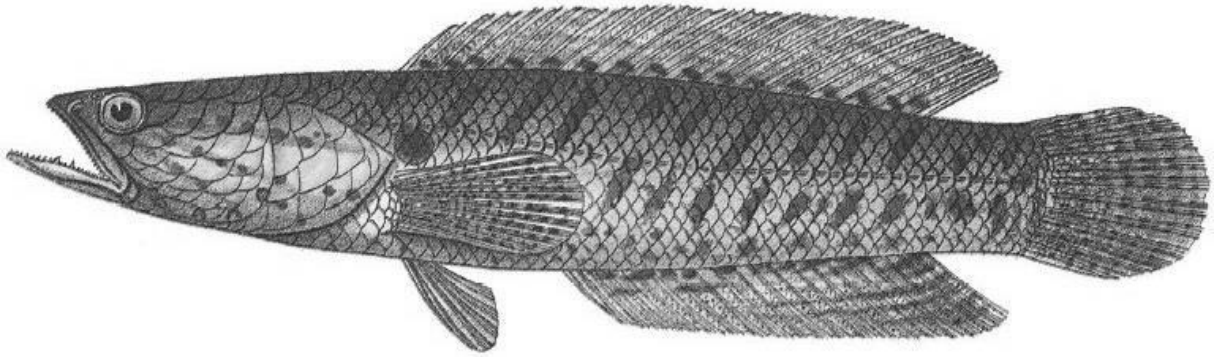
- Single dorsal fin commencing above from near pectoral base, with 15-18 spine & 7-13 rays, number of spine variable
- Anal fin with 15-20 spines and 11-19 rays, number of spines in anal and dorsal fin is variable
- Pelvic fin form, single elongated, filliform ray
- Caudal fin slightly emarginated or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

Hence, Species – *fasciata*

Hence, the provided specimen is *Colisa fasciata*

Distribution: India, Nepal, Pakistan, Bangladesh, Burma,

Specimen: 29



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Channiformes
Family – Channidae
Genus – *Channa*
Species – *punctatus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

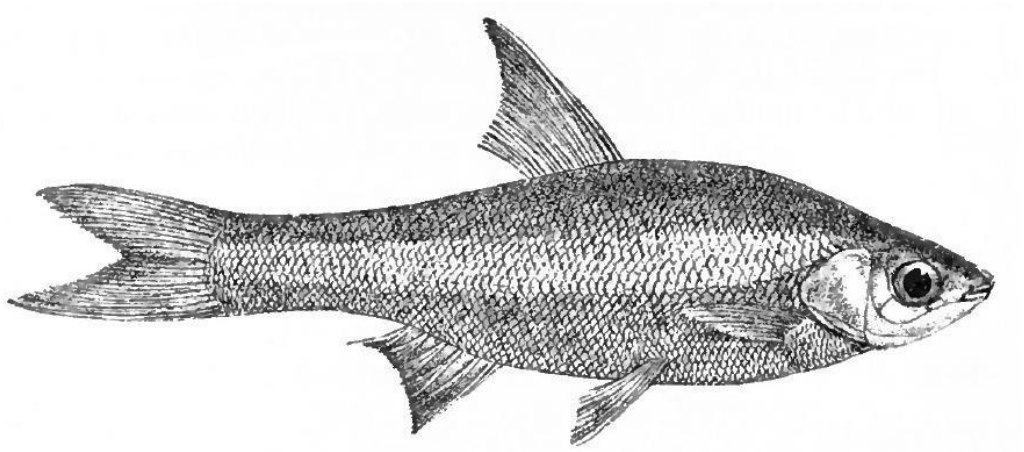
Hence, Genus – ***Channa***

- 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*

Specimen: 30



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Cypriniformes
Family – Cyprinidae
Genus – *Amblypharyngodon*
Species – *mola*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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 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 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*

Specimen: 31

Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Mastacembeliformes
Family – Mastacembelidae
Genus – *Mastacembelus*
Species – *pancalus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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 and 50-90 rays.
- Anal fins with 3 spines and 31-98 rays.
- Caudal fin round

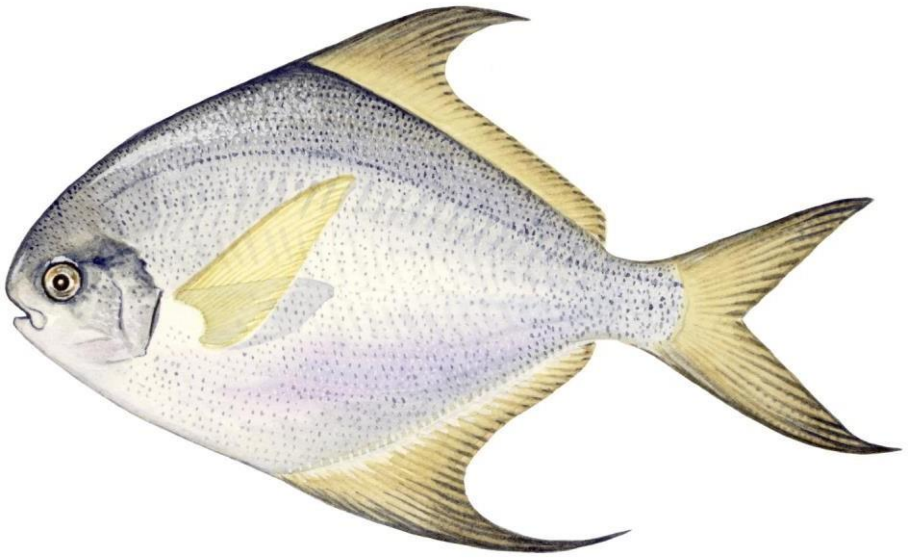
Hence, Genus – *Mastacembelus*

- Dorsal fin with 24 – 31 spines, 30 – 42 rays.

Hence, species – pancalus

Hence, the specimen is *Mastacembelus pancalus*

Specimen: 32



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Perciformes
Family – Stromateidae
Genus –
Species –

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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.

- 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

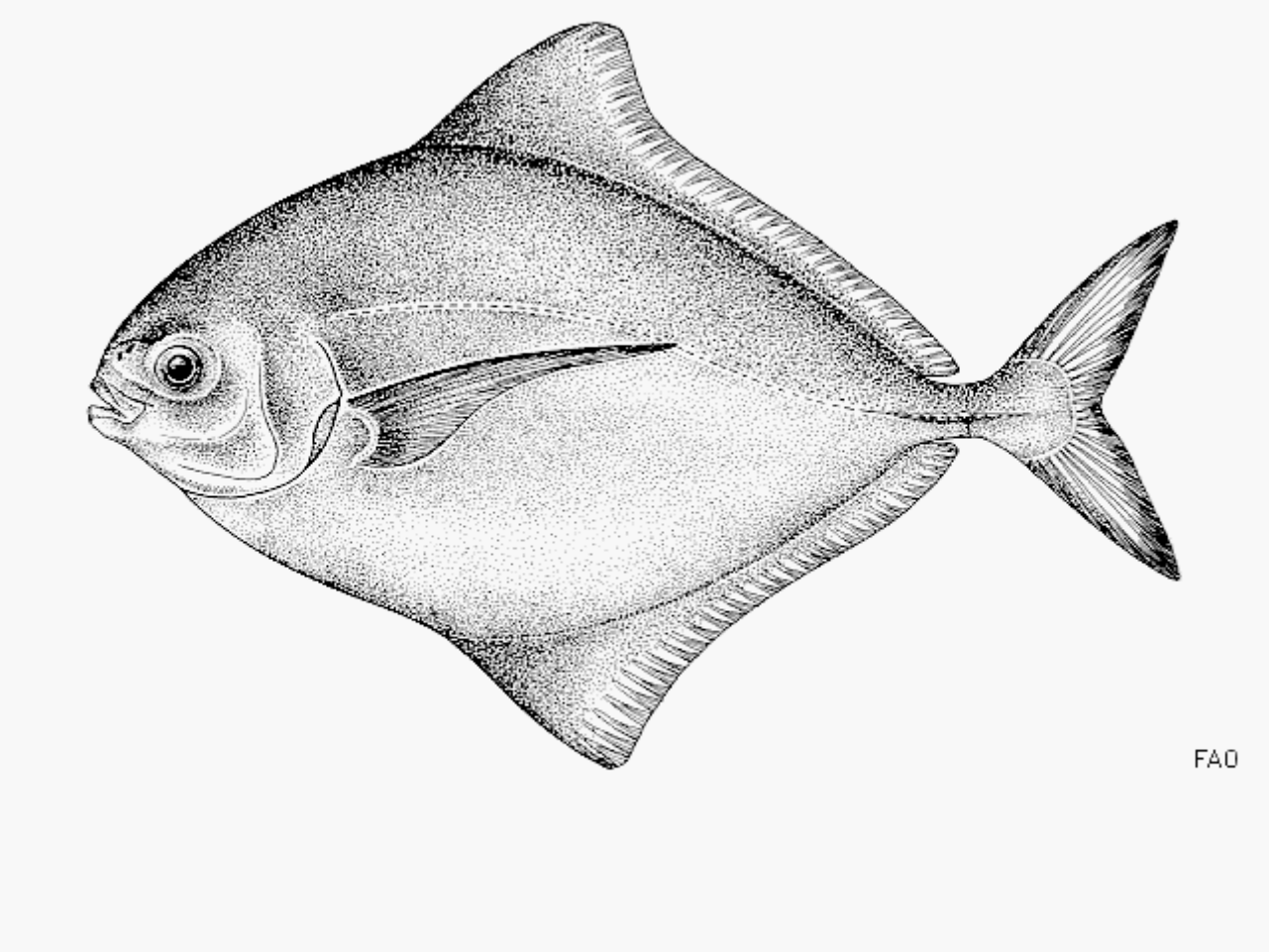
- Medium-sized fishes with a deep, compressed body of a bluish or silvery colour.
- Eye and mouth small. Dorsal fin single and long-based, longer than the similar anal fin; anterior rays longer than those which follow, but fins not falcate.
- Pectoral fins broad and wing-like, but not prolonged; pelvic fins absent (except possibly in very small specimens).
- Scales small, cycloid, easily shed, extending onto the bases of dorsal and anal fins; top of head naked.

Hence, Family – Stromateidae

- Dorsal spines (total): 0; dorsal soft rays (total): 37-43; Vertebrae: 34 - 37.
- Body firm, very deep, oval, and compressed. Operculum absent; gill opening reduced to a vertical slit on the side of the body; gill membrane broadly united to isthmus.
- Dorsal and anal fins preceded by a series of 5 to 10 blade-like spines with anterior and posterior points.
- Pelvic fins absent. Caudal fin deeply forked, the lower lobe longer than the upper.
- Colour is grey above grading to silvery white towards the belly, with small black dots all over the body. Fins are faintly yellow; vertical fins with dark edges.

Hence, the Specimen - *Pampus argenteus*

Specimen: 33



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Osteichthyes
Order – Perciformes
Family – Stromateidae
Genus – *Parastromateus*
Species – *Parastromateus niger*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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.

- 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

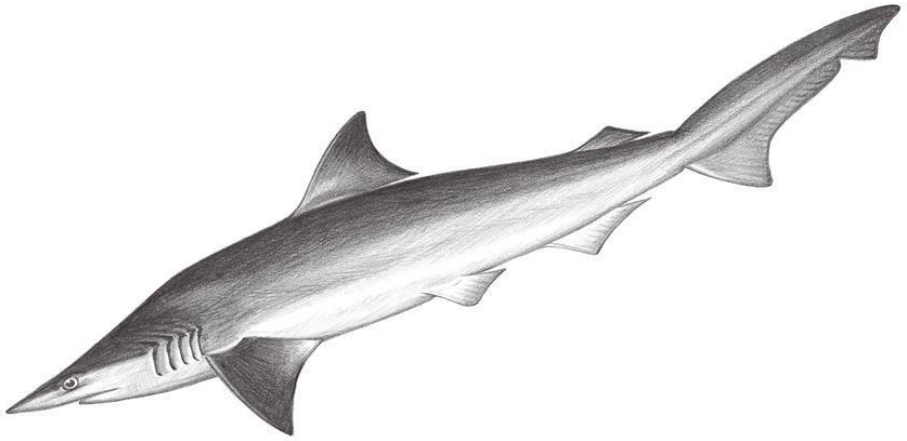
- Medium-sized fishes with a deep, compressed body of a bluish or silvery colour.
- Eye and mouth small. Dorsal fin single and long-based, longer than the similar anal fin; anterior rays longer than those which follow, but fins not falcate.
- Pectoral fins broad and wing-like, but not prolonged; pelvic fins absent (except possibly in very small specimens).
- Scales small, cycloid, easily shed, extending onto the bases of dorsal and anal fins; top of head naked.

Hence, Family – Stromateidae

- Dorsal spines (total): 2 - 6; Dorsal soft rays (total): 41-46; Anal spines: 2;
- Anal soft rays: 35 - 40; Vertebrae: 24. Deep-bodied and strongly compressed fishes.
- Lateral line ends in weakly-developed scutes on the caudal peduncle.
- Pelvic fins lost in individuals over 9 cm.
- Color is brown above, silvery-white below.
- The anterior parts of the dorsal and anal fins bluish-gray. The other fins yellowish.

Hence, the Specimen - *Parastromateus niger* (Bloch, 1795), Black pomfret

Specimen: 34



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Carcharhiniformes
Family – Carcharhinidae
Genus – *Scoliodon*
Species – *laticaudus*

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- nictitating eyelids present
- mouth behind front of eyes
- five pairs of gill slits
- both dorsal fins without spines
- anal fin present

Hene, Order – Carcharhiniformes

- Most members of this family are medium to large in size, usually ranging from about 1 - 3m, though some smaller members are less than a metre in length and larger ones may be over 7m.
- Eyes are round and have an internal nictitating membrane for protection.
- The sharks have arched mouths with blade-like teeth. The teeth of the upper jaw are often broader than those in the lower jaw.
- The pectoral fins are situated behind the gill slits. The first dorsal fin is bigger than the second and usually set well ahead of the pelvic fins. The upper lobe of the caudal fin is bigger than the lower. One anal fin present.

Hence, Family – Carcharhinidae

- *Scoliodon* has an elongated, spindle-shaped, body tapered at the ends, making it a very fast swimmer.
- The trunk and tail are laterally compressed, while the head region is dorsoventrally compressed.
- The entire body is covered by an exoskeleton of placoid scales. The mouth is located on the ventral side and is bound on both sides by jaws.
- It has two rows of homodont or polyphyodont teeth, which are homologous to the placoid scales covering the body.

Hence, specimen - *Scoliodon laticaudus*

Specimen: 35

Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Carcharhiniformes
Family – Sphyrnidae
Specimen – *Sphyrna* sp.

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- Members of this order are characterized by the presence of a nictitating membrane over the eye,
- Two dorsal fins, an anal fin, and five gill slits.

Hence, Order – Carcharhiniformes

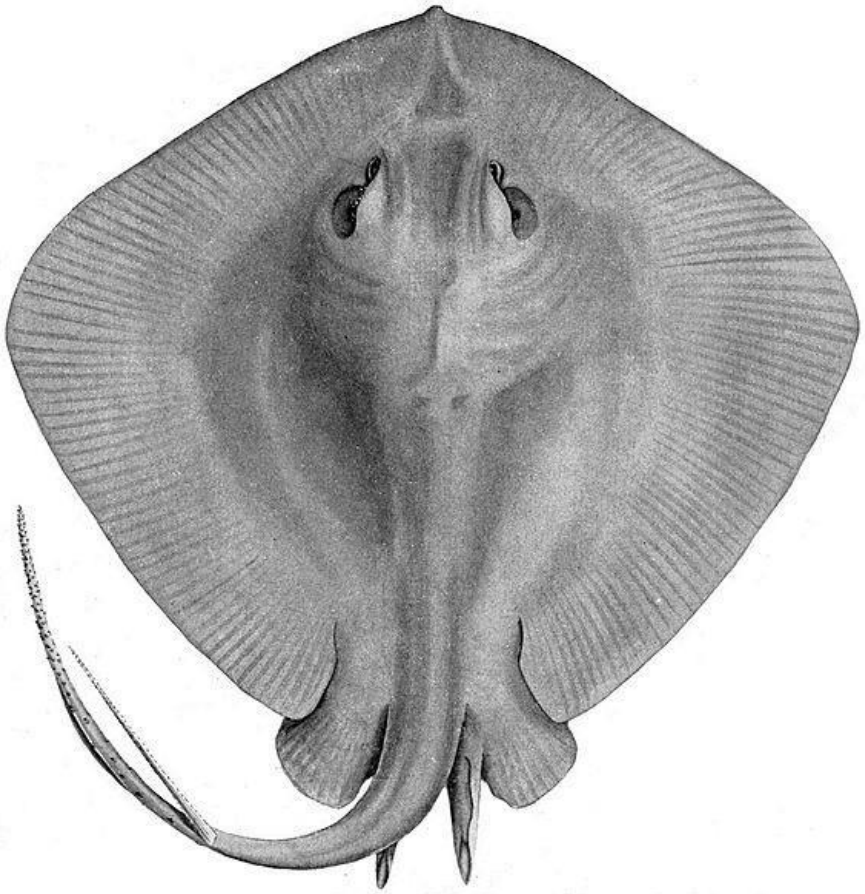
- They are usually light gray and have a greenish tint to them.
- Their bellies are white which allows them to blend into the ocean when viewed from the bottom and sneak up on their prey.
- Their heads have lateral projections which give them a hammer-like shape.

Hence, Family – Sphyrnidae

- Head variably spade, mallet or axe-shaped in dorsoventral view and moderately broad, width across head about 17 to 33% of total length;
- lateral blades of head broad, not winglike; nostrils short, their widths 7 to 14 in internarial width and less than half mouth width;
- No bumps along anterior margin of head.
- Upper precaudal pit transverse and crescentic.

Hence, Genus – *Sphyrna* sp.

Specimen: 36



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Myliobatiformes
Family – Dasyatidae
Specimen – *Dasyatis* sp.

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- The disc is strongly depressed and varies from oval longitudinally to much broader than long;
- the tail is well marked off from the body sector, very short to long and whiplike, and equipped with a poisonous spine in some species;
- the pectoral rays are either continuous along the side of the head or separate from the head and modified to form rostral lobes or finlike rostral appendages (cephalic fins);
- The dorsal fin, if present, is near the base of the tail; and development is ovoviviparous.
- Sometimes an individual ray may have two or three, very rarely four, tail spines rather than the usual one.

Hence, Order – Myliobatiformes

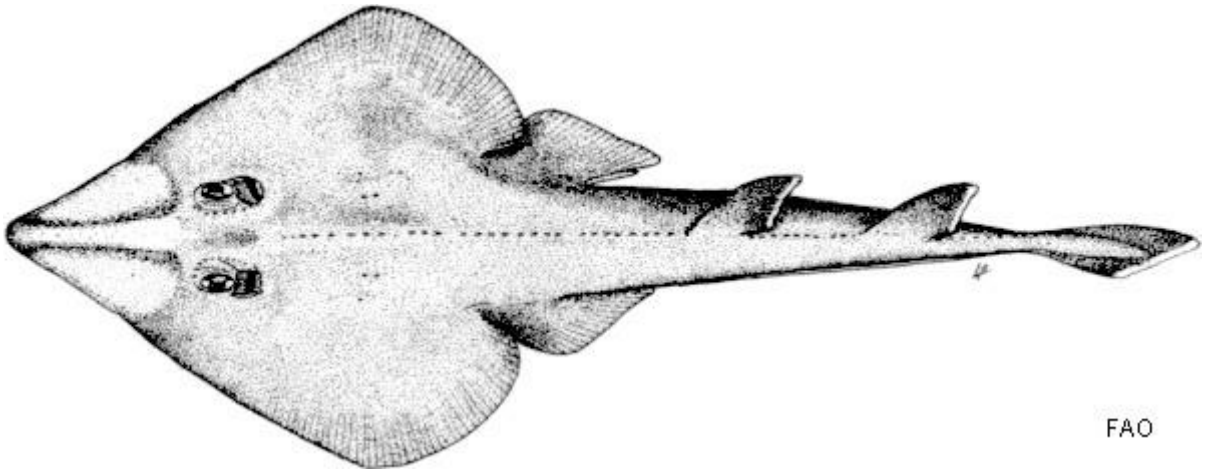
- Medium to large rays (disc width to 2.1 m), the disc rhomboid to oval, its width ranging from greater to less than its length;
- snout obtuse and little produced to acute and moderately produced, head not elevated from disc; tail distinct from disc, slender and whip-like, equal to or much longer than distance from snout to cloaca, with one or several serrated spines on top near base, keels or membranous folds along upper or lower sides (or both) present in some species.
- Nostrils separated from mouth, but front margins greatly expanded to reach back and join each other. Mouth almost straight or arched, with a transverse row of bulbous papillae along floor, teeth small and numerous.
- Spiracles well developed, without tentacle-like processes. Dorsal and caudal fins absent, pectoral fins joined to side of head, the finrays beginning at tip of snout. Upper surfaces naked or covered with tubercles, thorns or thornlets.
- Benthic on soft bottoms, generally in shallow tropical and warm temperate waters, but also to depths of 200 m.

Hence, Family – Dasyatidae

- Snout obtuse and little produced to acute and strongly produced;
- disc more or less rhomboid, but not circular; tail whip-like,
- Filamentous near tip, with or without a membranous fold or ridge above, but with a fold below (beginning at level of spine).
- Upper surfaces with or without bucklers, tubercles and thorns with conical cusps.

Hence, Specimen – *Dasyatis* sp.

Specimen: 37



Phylum – Chordata
Subphylum – Vertebrata
Super Class – Gnathostomata
Class – Chondrichthyes
Order – Rajiformes
Family – Rhinobatidae
Specimen – *Rhinobatos productus*.

Identifying Character:

- All members possess either in adult or larval stage a notochord, which forms the axial skeleton 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

- Skeletons made of cartilage rather than bone.
- Chondrichthyans have tough skin covered with dermal teeth.
- Chondrichthyans have toothlike scales called dermal denticles or placoid scales.
- All chondrichthyans breathe through five to seven pairs of gills, depending on the species.

Hence, Class - Chondrichthyes

- Rajoids typically have a dorsoventrally flattened body.
- The snout is slender and pointed and the wide mouth, often covered with a fleshy nasal flap,is on the underside of the head.
- The eyes and well-developed spiracles are located on the top of the head. In most species,the spiracles are large and are the main means of drawing water in for respiration.
- There is no nictitating membrane and the cornea is continuous with the skin surrounding theeyes.
- The gill slits are on the ventral surface just behind the head and there are five in all speciesexcept the sixgill stingray (*Hexatrygon bickelli*).

Hence, Order – Rajiformes

- They have a body form intermediate between those of sharks and rays.
- The tail has a typical shark-like form, but in many species, the head has a triangular, or guitar-like shape, rather than the disc-shape formed by fusion with the pectoral fins found inother rays.

Hence, Family – Rhinobatidae

- Shovelnose guitar fish most resemble sharks in posterior body shape, with a flattened anterior like a ray.
- Their snouts are pointed and shovel-like, and they have broad pectoral fins.
- Their dorsal surfaces are smooth except for rows of small thorns around the eyes and tail. The tail is rather thick with a rounded caudal fin (characteristic of a benthic fish), lackingthe lower lobe that most other sharks possess.
- Shovelnose guitarfish have two equally-sized dorsal fins positioned close to the end of the tail. This species' body colour ranges from sandy brown to olive, with a white underside; thedistal end of the snout is partially translucent.
- Their teeth are small and rounded, and range in number from 102 to 112.
- Females usually grow larger than males, reaching up to 137 centimetres when fully gown, while males may reach up to 120 centimetres.

Hence, Specimen - *Rhinobatos productus*

QUALITATIVE TEST FOR PROTEINS

Introduction

Proteins are sequences of amino acids, its contain C, H, O, and Nitrogen, Common structure: Central C, with a H, amino group (NH2), and an acid group (COOH), and a side group, proteins made up of about 20 different amino acids; Unique Side Groups, Differ in size, shape, electrical charge. Proteins are probably the most important class of biochemical molecules, although of course lipids and carbohydrates are also essential for life. Proteins are the basis for the major structural components of animal and human tissue. It can be hydrolyzed by acids, bases or specific enzymes. Amino acids are organic compounds that contain amino and carboxyl groups. The R- in

the above formula stands for different chemical groups (may be aliphatic, aromatic or heterocyclic) and this determines the characteristics of the amino acids. The color tests have frequently been used for qualitative detection of amino acids. Not all amino acids contain the same reactive groups. For this reason the various color tests yield reactions varying in intensity and type of color according to the nature of groups contained in the particular amino acid under examination.

Millon's reaction

Principle

The reaction is due to the presence of the hydroxyphenyl group, C_6H_5OH in the amino acid molecule; and any phenolic compound which is unsubstituted in the 3,5 positions such as tyrosine, phenol and thymol will give the reaction. Solutions of nitric acid containing mercuric nitrate reacts with phenols, producing red colors or yellow precipitates which react with nitric acid to form red solution. The reaction probably depends on the formation of a nitro compound; which then reacts with phenol.

Procedure

Add 3 to 4 drops of Millon's reagent to 5 ml of test solution. Mix and bring the mixture gradually to a boiling point by heating over a small flame. Development of red color is due to the presence of protein. Excess of reagent should however be avoided since it may produce a yellow color which is not a positive reaction.

Xanthoproteic reaction

Principle

This reaction is due to the presence in the amino acid molecule of the phenyl group $–C_6H_5$, with which the nitric acid forms certain nitro modifications. The particular amino acids which are of especial importance in this connection are those of tyrosine and tryptophan. Phenylalanine does not respond to this test as it is ordinarily preformed.

Materials

1. Conc. HNO_3
2. Ammonium hydroxide
3. Sodium hydroxide

Method

Add 1 ml of conc. Nitric acid to 2 to 3 ml of test solution in a test tube. A white precipitate forms, which upon heating turns yellow and finally dissolves, imparting to the solution a yellow color, cool the solution and carefully add ammonium hydroxide or sodium hydroxide in excess. Note that

the yellow color deepens into an orange.

Biuret test

Principle

The Biuret test is given by those substances whose molecules contain two cabamyl (-$CONH_2$) groups joined either directly or through a single atom of nitrogen or carbon. Similar substances which contain- $CSNH_2$,

-$C(NH)NH_2$, or – CH_2NH_2 in place of the –$CONH_2$ group also respond to the test. It follows from this fact that substance which are non-protein in character but which contain the necessary groups will respond to the biuret test.

Protein responds positively since there are pairs of CONH groups in the molecule. A copper coordination complex with the ring structure is probably produced. Short chain polypeptides give a pinkish violet color, longer one including proteins a more purple blue. The amino acid histidine gives a pink color, which depends on the peptide linkage, does not vary greatly in intensity from protein to protein. Several procedure based on this method have been suggested for quantitative determination of milk proteins, but it is not in general use in dairy research.

Materials

1. 10% NaOH
2. 0.5% $CuSO_4$

Method

To 2 to 3 ml of test solution in a test tube add an equal volume of 10% sodium hydroxide solution, mix thoroughly, and add a 0.5% copper sulphate solution drop by drop, mixing between drops, until a purplish-violet or pinkish-violet color is produced. The color depends upon the nature of the protein, proteoses and peptones give a decided pink; the color produced with gelatin is not far removed from a blue.

Ninhydrin reaction

Principle

This test gives positive results with proteins, peptones, peptides, amino acids and other primary amines, including ammonia. Proline and hydroxyproline give yellow color with ninhydrin, while other acids give blue to purple color.

Materials

1. 0.1% Ninhydrin
2. pH paper

Method

To 5 ml of dilute test solution, which must be approximately between pH 5 and pH 7 (a few drops

of pyridine or a few crystals of sodium acetate may be used to adjust the pH), add 0.5 ml of 0.1 % ninhydrin, heat to boiling for one to two minutes, and allow to cool. A blue color develops if the test is positive.

Add 5ml of the alkaline solution to 1 ml of the test solution. Mix thoroughly and allow to stand at room temperature for 10 mins. Add 0.5 ml diluted Folin-Ciocalteau reagent rapidly with immediate mixing. Observe for development of color after 30 mins. Development of characteristic blue color indicates presence of indolyl or phenol group.

Sakaguchi test

Principle

Arginine and other guanidyl derivatives (glycocyamine, methylgyanidine etc) react with hypo bromide and alpha napthol to give a red colored product.

Materials

1. Sodium hydroxide solution (40%)
2. Alpha napthol solution (1% in alcohol)
3. Bromine water (a few drops of bromine in 100 ml distilled water)

Method

Mix 1 ml of sodium hydroxide with 3 ml of test solution and add 2 drops of alpha napthol. Mix thoroughly and add 4 to 5 drops of bromine water. Note the color formed. Formation of a red color indicates presence of guanidine group. This is a very sensitive and specific test.

Nitroprusside test

Principle

Sodium nitroprusside reacts with compounds containing sulphahydryl groups produce an intensely red but somewhat unstable color.

Materials

1. Sulphur amino acids (1.0% cystine, cysteine and methionine)
2. Sodium nitroprusside (2% prepared fresh)
3. Ammonium hydroxide

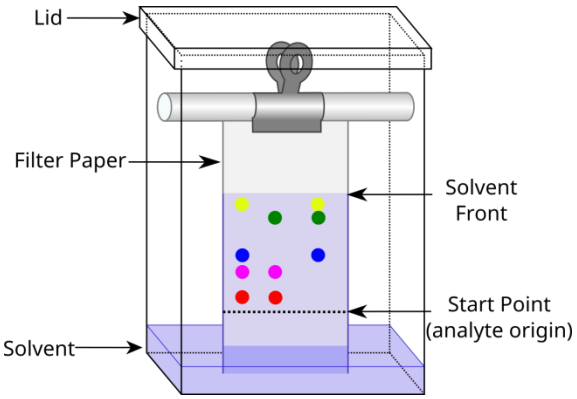
Method

Mix 0.5 ml of a fresh solution of sodium nitropruside with 2 ml of the test solution and add 0.5 ml of ammonium hydroxide.

Paper Chromatography of Amino Acids

Introduction

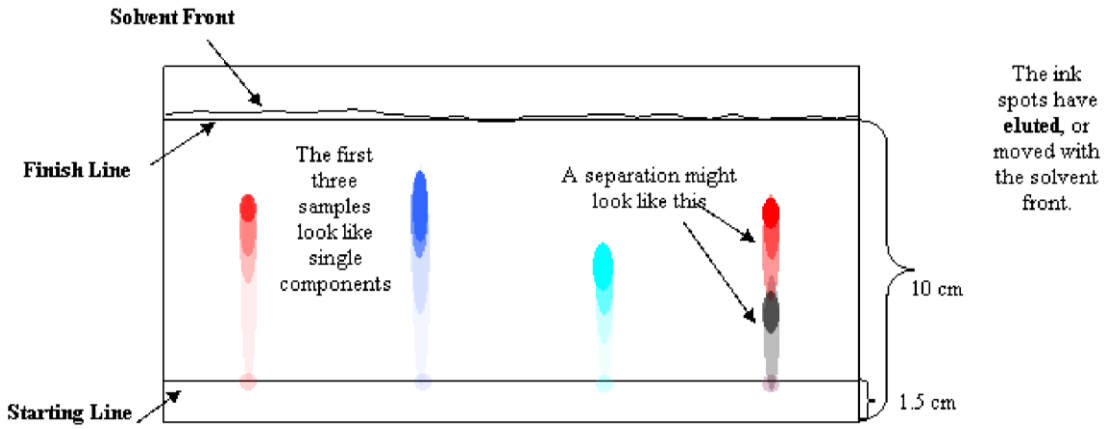
Chromatography is a collective term for a set of analytical techniques used to separate mixtures. Chroma means color and graph means to write or draw. Paper chromatography is an analytical technique used to separate mixtures of chemicals (sometimes colored pigments) using a partitioning method. The paper in this method is called the stationary phase because it does not move and serves as a substrate or surface for the separation. Analytes (substances being analyzed) are separated from each other based on a differential affinity to a solvent. The solvent dissolves and carries the analytes along the matrix of the stationary phase. Since the solvent moves through a wicking action, it is called the mobile phase.



The figure above shows a chamber containing mobile phase and a prepared paper stationary phase. A line drawn at the bottom edge of the paper is the starting point/line. The starting line and sample dots must be above the level of the mobile phase when the paper is placed inside the chamber. If the starting line is below the liquid level, the samples will wash out into the mobile phase rather than elute up the stationary phase. Another line is drawn about 10 cm above the bottom edge of the paper. This is the finish line. Its location was chosen for this experiment because when the eluting solution reaches that line, any inks that are mixtures should be clearly separated.

When the solvent front reaches the finish line, the paper should be removed immediately from contact with the mobile phase.

The figure below shows a typical paper chromatogram of the separation of pen inks. There are a few difficulties commonly encountered in the elution process. One problem is that spots tend the spread out as they elute, and can bleed into each other as they proceed up the paper. This can be confusing when interpreting the chromatogram. To avoid this problem, space the spots of sample far apart and make repeated, tiny applications of sample to prevent spreading. Another problem is an uneven solvent front. This can happen if the beaker is nudged – if the mobile phase sloshes inside, the elution trails may travel diagonally, which makes interpretation very difficult. This can also happen if the two edges of the chromatogram are allowed to touch when they are stapled or taped together to form a cylinder.



A component with a given solubility travels along with the mobile phase at one rate, regardless of what other components are present in the sample. If the red part of purple ink travels at the same

rate as pure red ink, and both stop in the same place, the two should be the same red ink. The two red spots should have the same Retention Factor, Rf. The Rf is the distance, D, traveled by the spot divided by the distance traveled by the eluting solution, or Solvent Front, F.

Rf= frac DF

Comparing the Rf values allows the confirmation of a component in multiple samples because unique components have unique Rf values.

Experimental Procedure

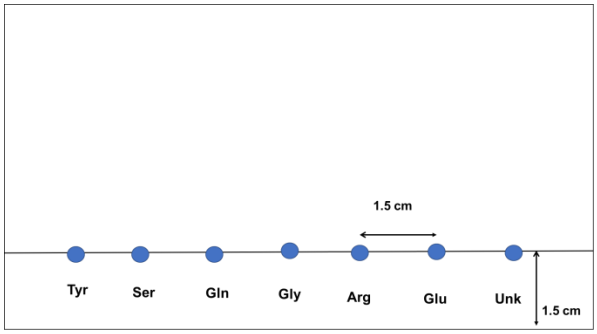
Materials and Equipment

Materials: chromatography paper, amino acids (1% solutions): tyrosine, serine, glutamine, glycine, arginine, glutamic acid and unknown, eluting solution (isopropyl alcohol, 0.5 M NH4OH; 0.2% ninhydrin spray).

Equipment: 600-mL beaker, pencil, ruler, evaporating dish, toothpicks, hair dryer, stapler and paper towels.

Preparation of Chromatography Paper

8. Wash your hands thoroughly to remove excess oils from your skin. Obtain a ruler and a piece of chromatography paper from the fume hood. Handle the paper only on the edges to avoid leaving fingerprints, as these may hinder the elution process.
9. Place the chromatography paper on a sheet of clean notebook paper or paper towel to avoid picking up dirt or contaminants from the bench top. Orient the paper into a “landscape” position and write your name on the top edge of the paper in one corner. Using a pencil and ruler to measure accurately, draw a straight line across the paper, about 1.5 cm above the bottom edge. This is the starting line. At the top of the paper draw a straight line across the paper about 2.0 cm from the top, this is finish line.
10. On the starting line, measure in from one side about 2.5 cm and lightly draw a small “X” centered on the starting line. Draw six more, 1.5 cm apart.
11. In the center of each X, make a small spot of amino acid sample in this order: tyrosine, serine, glutamine, glycine, arginine, glutamic acid and unknown.
12. When you have finished, you should have something that looks like ?????? below.
13. Go back over each amino acid spot a second time to ensure there is enough amino acid in the spot.
14. Gently curl the paper into a cylinder, with the spots on the outside. Staple the ends together near the top and bottom, taking care that the two edges of the paper do not touch. If they do touch, the eluent will creep on a diagonal, and the spots will run together, or not in straight lines.



Acquisition of Chromatogram

6. Take a 600-mL beaker and pour 10-mL of 0.5 M NH_4OH and 20 mL of isopropyl alcohol (eluting solution) into the beaker. Obtain your evaporating dish and use it to cover the beaker.
7. Gently place the paper cylinder into the beaker and cover the top with the plastic wrap. Remember that the spots must be above the liquid level for the experiment to work. Watch the eluent creep up the paper until it is about 2 cm from the top. It will take about 45-60 minutes for the solvent front to reach the finish line.
8. When the solvent front reaches the finish line, remove the paper from the beaker, being careful to touch only the top. Let excess eluent drip into the beaker. Gently remove the staple and lay the chromatogram on a piece of paper towel. Use a hair dryer to dry the chromatogram completely. Pour the eluting solution in the organic waste container under the fume hood.
9. Working in the fume hood, spray the chromatogram lightly with the ninhydrin solution. Dry the sprayed chromatogram with a hair dryer, distinct colored spots will appear as a result of the ninhydrin reacting with the amino acids.
10. CAUTION: Use the ninhydrin inside the fume hood. Do not breathe the fumes or get spray on your skin.

Interpretation of Chromatogram

6. Circle around each color spot.
7. Use a ruler and draw a plus sign in the center of each spot. Measure the distance from the starting line to each plus sign. Record this distance for each spot on your lab report. These are the DD values, in cm.
8. Measure the distance between the starting line and the finish line or, the farthest up that the solvent front reached. Record this distance. This is the FF value, in cm.
9. Calculate the retention factor (Rf) for each spot and record the values in your lab report.
10. You and your lab partner will hand in your lab reports at the same time, with the paper chromatogram stapled to one of the lab reports.

Quantitative estimation of Lowry Methods.

INTRODUCTION:

Measurement of the quantity of the protein present in the solution is common and fundamental methods used in all laboratories. Generally these are of two types-

c. Direct spectophotometric method
d. Colorimetric method

Colorimetric method such as Biuret method, Lowery method, Bradford method etc are used extensively. These are the destructive method of protein analysis because the protein being measure cannot be recovered after such analysis. In this colorimetric method protein concentrations are measured by taking absorbance of visible wed length of light.

PRINCIPLE:

The "Lowery or Folin-Ciocalteau method" combines the copper reaction of the biuret method and the Folin-Ciocalteau reagent which reacts with tyrosine residues in proteins. When alkaline copper sulphate solution containing Sodium-Potassium Tartarate reacts with a protein solution, the copper ions forms a co-ordination complex with four NH peptide bond groups. In addition the phosphor molybdate present in the Folin-Ciocalteau reagent is reduced by tyrosine

and tryptophan (aromatic amino acid) present in the protein, produce a dark blue/ purple colour complex, with maximum absorbance at 700nm. The intensity of the colour depends on the amount of these aromatic amino acids present which thus very with the different proteins present in the test sample.

CHEMICALS:

7. Alkaline sodium carbonate solution(20g/liter Na_2Co_3 in 0.1 mol/ltr NaOH)
8. Copper sulphate sodium Patasium tartarare solution(5g/ltr $CuSo_4$,$5H_2O$ in 10g/ltr Na-K tartarate).
9. Alkaline solution, prepared freshly by using 50ml of solution1 and 1ml of solution 2.
10. Folin-ciocalteau reagent(containing solution of sodium tungstate and sodium molybolate in phosphoric and hydrochloric acids and it is commercially available). Diluted the commercially available reagent with an equal of water just before use.
11. Standard sample preparation:- BSA standard solution are prepared by dissolved BSA in DH_2O. The concentration of new stock sample is 0.1mg/ml.
12. At first 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml, 0.9ml of protein sample is taken in dry test tube from 2nd stock or diluted stock solution of protein(BSA). Then the volume make upto 1ml by adding distilled water in test tube and prepared different concentration of protein solution(20, 40, 60, 80μg/ml).

OTHER REAGENTS:

xi. Test tube
xii. Test tube rack
xiii. Tissue paper
xiv. Graph paper
xv. Reagent bottle
xvi. Beakers
xvii. Measuring cylinders
xviii. Posteur pipettes
xix. Notepad
xx. Calculator

PROCEDURE:

8. previously prepared BSA stock solutions are used(10μg/ml, 20μg/ml, 30μg/ml, 40μg/ml, 50μg/ml, 60μg/ml, 70μg/ml, 80μg/ml, 90μg/ml).
9. 1ml distilled water is taken in a dry test tube and marked as “Blanked” there is no protein solution is present.
10. 1ml alkaline solution is added into each and every test tube.
11. Solution are incubated for 15 min at room temperature.
12. 0.1ml Folin reagent is added into each test tube.
13. Next the complete mixture are allowed for incubation at room temperature for 30 min. This incubation should be in dark.
14. Last the OD are measure in colorimeter a 700nm and graph is plotted based on the OD value.

PREPARATION OF BSA STANDARD CURVE:

SAMPLE NO.	BSA CONCENTRATION (μg)	BSA TAKEN (μl)	DISTILLED WATER (μl)	OD VALUE	CORRECT OD VALUE
1.	10	100	900	0.07	0.06
2.	20	200	800	0.15	0.14
3.	30	300	700	0.24	0.23
4.	40	400	600	0.28	0.27
5.	50	500	500	0.37	0.36
6.	60	600	400	0.38	0.37
7.	70	700	300	0.51	0.50
8.	80	800	200	0.64	0.63
9.	90	900	100	0.78	0.77
BLANK	0	0	1000	0.01	

SAMPLE	PROTEIN TAKEN	DISTILLED WATER	OD VALUE	CORRECTED OD VALUE

	(μl)	(μl)		
UNKNOWM	400	600	1.06	1.05

ELECTROPHORETIC STUDY

Demonstration of proteins separation by 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%)	Stacking gel (5%)
Distilled water	4.0 ml	
30% acrylamide mix	3.3 ml	
1.5M Tris pH8.8	2.5 ml	

10% SDS 0.1 ml
10% ammonium persulfate 0.1 ml
TEMED 0.004ml

Distilled water 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

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.

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.

CHROMOSOME PREPARATION .

The uses of chromosome cytologic information are many, including karyotypic evolution, cytotaxonomy and phylogenetic relationships in plants, insects, and mammals among other organisms. In fish, chromosome analysis based on variations in chromosome number and morphology are typically used to conduct evolutionary and genetic questions such as stock identification, study of hybrids or induced polyploidy. Most fishes possess relatively large numbers of small chromosomes that can be easily viewed with a light microscope at the metaphase stage of mitosis.

The techniques for chromosome preparation are based on obtaining sources of dividing cells to produce high quality metaphase spreads with good chromosome definition. These procedures use specific chemicals, one of the most used reagents is colchicine, to inhibit the formation of the mitotic huse and thus, to block dividing cells at metaphase. Subsequently, a hypotonic treatment is used to separate the metaphase chromosomes from each other in the cells. Next, cells are fixed for staining. Commonly used methods of chromosome preparations are based on direct chromosome preparation from solid-tissues or cell cultures (Thorgaard and Disney, 1990). Accordingly, mitotic chromosome preparations can be successfully made using several tissues collected at different life stages of fish (Baksi and Means, 1988). These include embryos and fish fry and alternatively, the

head and gill arches. If adult fish are used for chromosome preparation and analysis, dividing cells are obtained from sources showing a high proportion of actively dividing cells. These are head kidneys, gills, intestines, spleens, testes, scale epithelium or regenerating fin tissues.

Procedure

(xvi) Place the newly hatched larvae (n~12) into a 250-ml beaker containing 0.007% colchicinesolution prepared in seawater and allow them to swim for 4 hours. Seawater may be aerated, if necessary.

(xvii) Chop each larvae individually into 2-3 mm small pieces using a clean razor bladder.

(xviii) Expose the pieces to a hypotonic solution of 0.4% KCl for 20-30 minutes.

(xix) Discard the hypotonic solution and fix the tissues by washing the chopped pieces twice in a freshly made cold mixture of 3:1 ethanol:acetic acid for at least 30 minutes each wash. Tissue may be stored in fixative for several months at 4ºC.

(xx) Take the pieces out from the fixative and dry the excess fixative on a paper towel.

(xxi) Place the pieces on an excavated micro slide and add 2-3 drops of 50% acetic acid.

(xxii) Chop the pieces until a cell suspension is formed.

(xxiii) Clean micro slides using a mixture of 1:1 ether:ethanol and warm them on a heat plate at 40-50ºC.

(xxiv) Take the cell suspension with a pasteur pipette and let this suspension fall from a distance of ~12 cm on a precleaned and prewarmed micro slide.

(xxv) Repeat this process several times forming 2-3 rings of cells of ~1 cm of diameter.

(xxvi) Allow chromosome preparation to dry for 10-15 minutes.

(xxvii) Stain chromosome preparation with 15% Giemsa for 45 minutes.

(xxviii) Wash chromosome preparation with distilled water and allow it to air dry for 10-15 minutes.

(xxix) Wash it with xylen for 10 minutes and add a cover glass using 2-3 drops of DPX.

(xxx) Use a light microscope to view the chromosome preparation. The use of a 20x objective is recommended for a fast localization of chromosome metaphases that usually appear in the periphery of the rings formed.

Materials and equipment

- Razor blades for chopping larvae
- Tweezers
- Excavated glass micro slides
- Clean glass slides
- Paper towel
- Heat plate
- Glass pasteur pipette
- Cover glass
- Light microscope

- Gloves and lab coat

Reagents and solutions

- 0.007% colchicine solution prepared in seawater. Colchicine is highly toxic and it should be handled with care. The operator should wear gloves during the procedure since colchicine is a hazardous chemical that may cause cancer and heritable genetic damage.

- Hypotonic solution of 0.4% KCl prepared in distilled water

- Fixer solution of 3:1 ethanol: acetic acid

- Staining solution of 15% Giemsa prepared in phosphate buffer (0.01 M, pH 7.0)

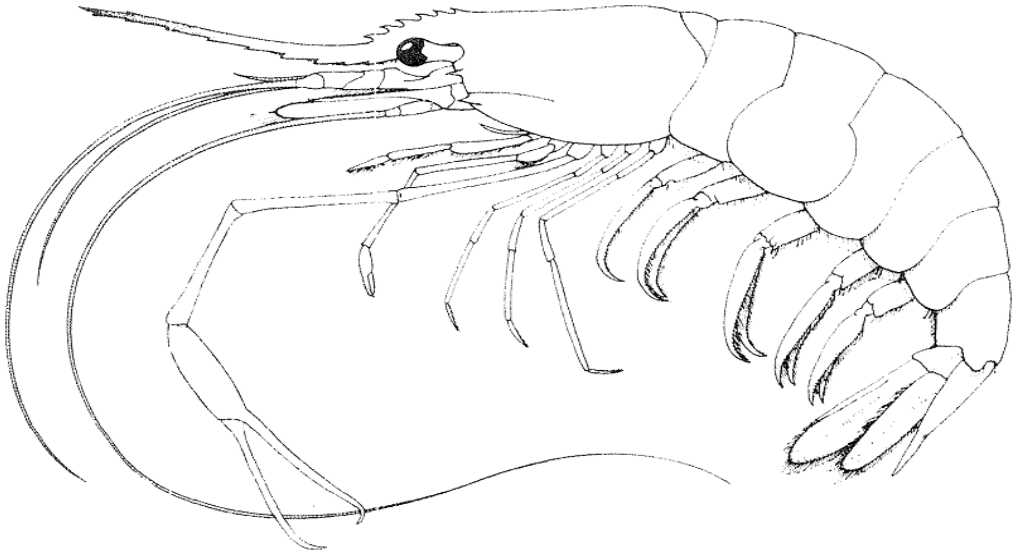
- Distilled water

- Xylen

- DPX

Course: BFSC-108: Taxonomy of Shell Fishes

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.

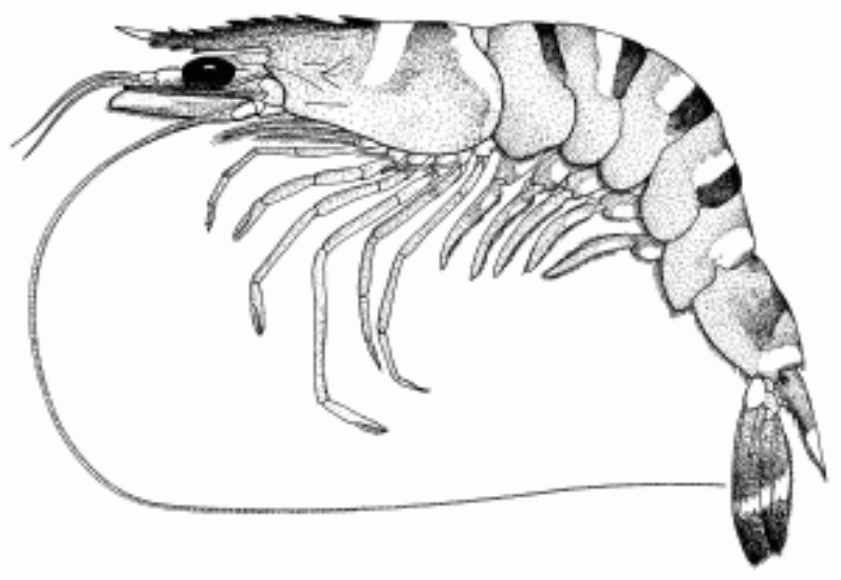
7. Males with an appendix masculine and an appendix interna on the endopods of second pleopods.

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



Systematic Position

Kingdom: Animalia
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.
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. 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.

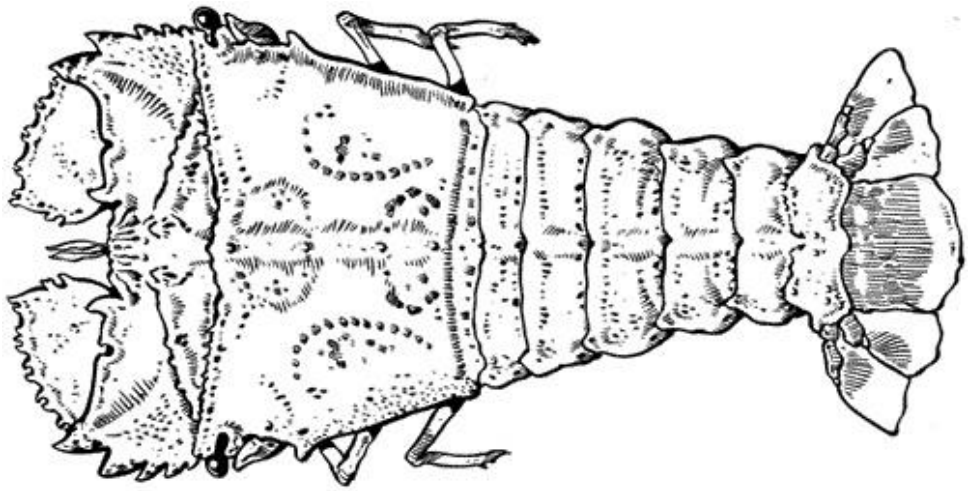
Hence, Family – Penaeidae

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.

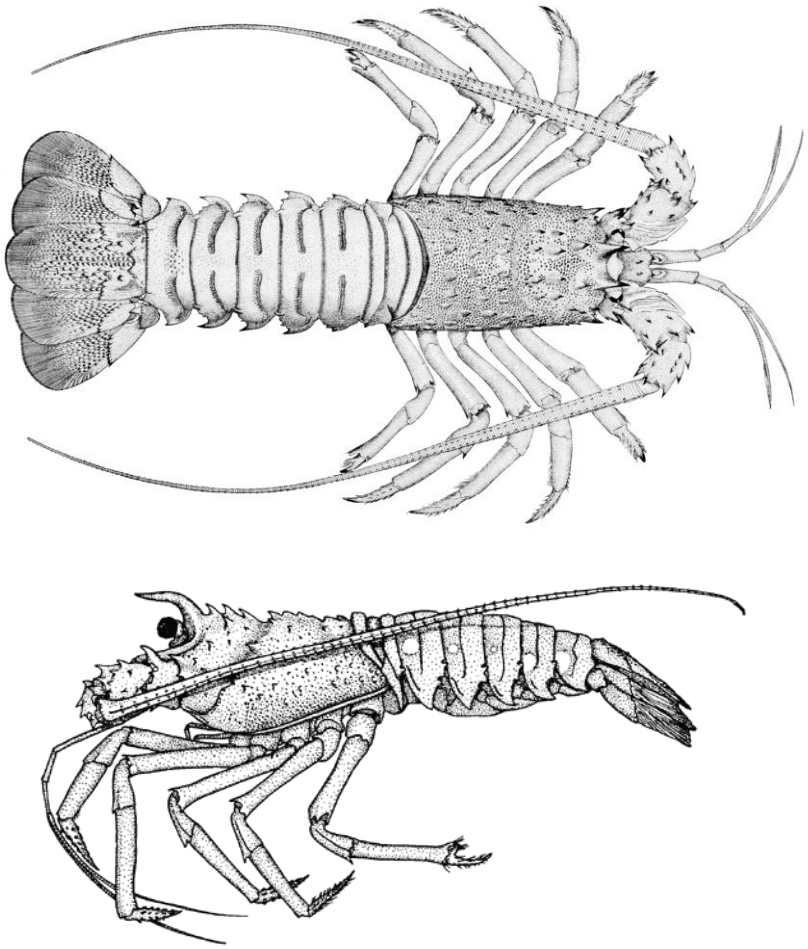
Hence, Family – Scyllaridae

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).

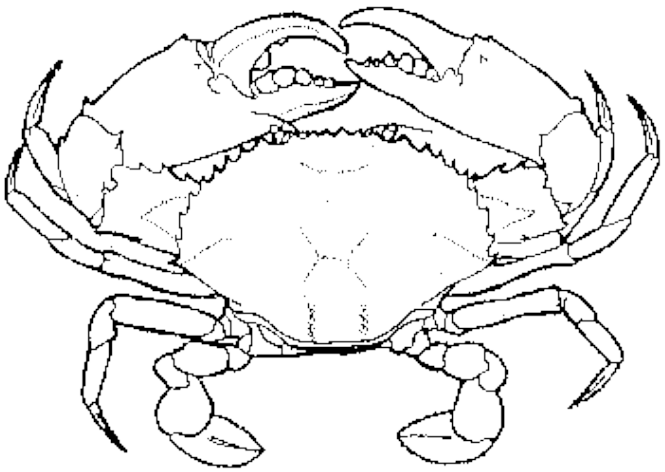
Hence, Family – Palinuridae

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

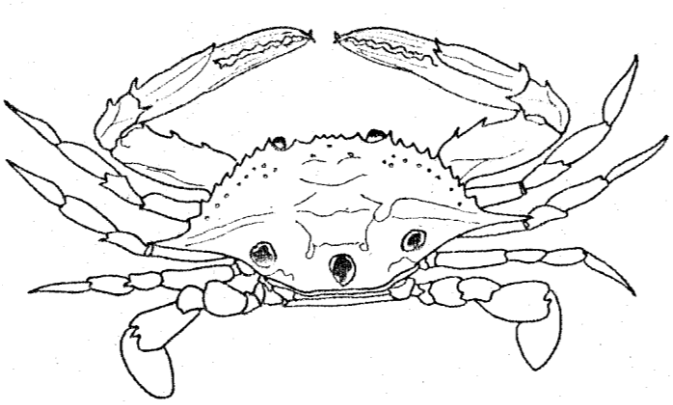
1. Portunid crabs are characterized by the flattening of the fifth pair of legs into broad paddles, which are used for swimming.
2. This ability, together with their strong, sharp claws, allows many species to be fast and aggressive predators.

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.

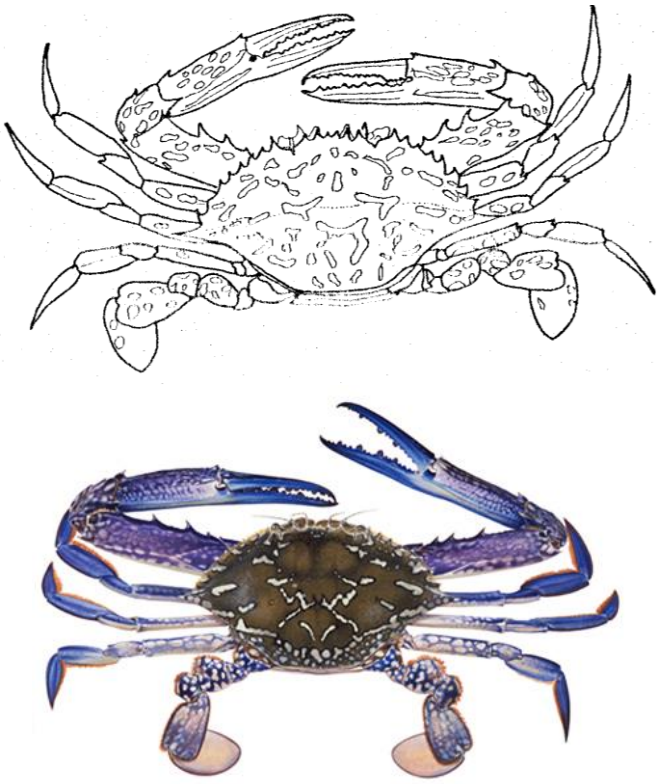
Hence, Family – Portunidae

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.

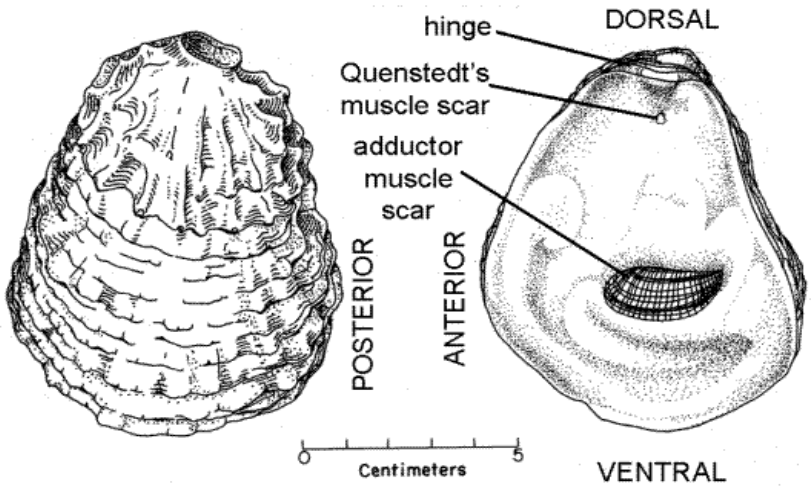
2. This ability, together with their strong, sharp claws, allows many species to be fast and aggressive predators.

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

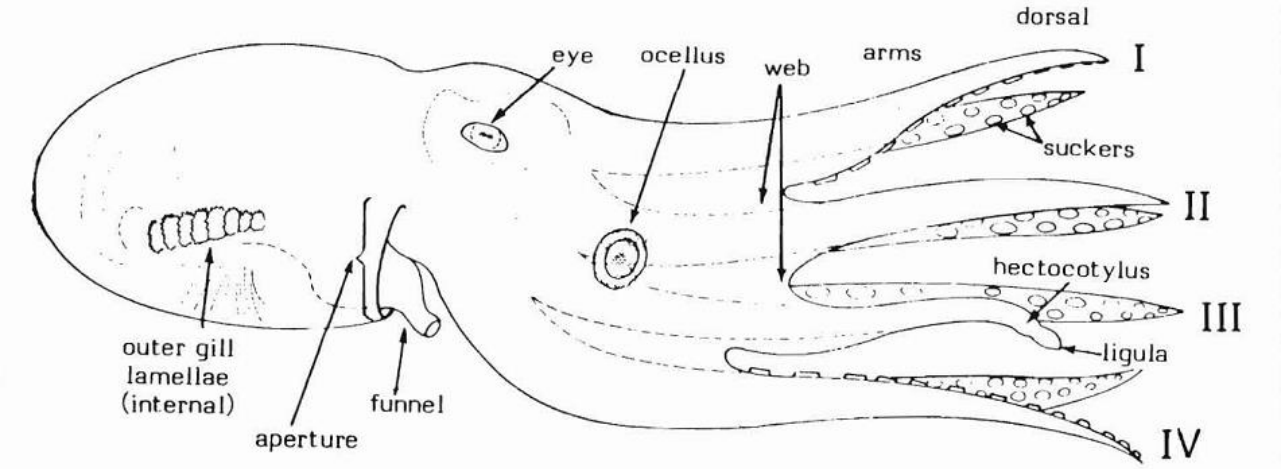
1. Shell thick, rugose; inequivalve,
2. Left (lower) valve convex, often forming a deep bowl and frequently overlapping the right valve, usually cemented to the substratum when small, right valve flat or slightly concave.
3. Inequilateral, umboes anterior to midline.
4. Juveniles with small taxodont 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

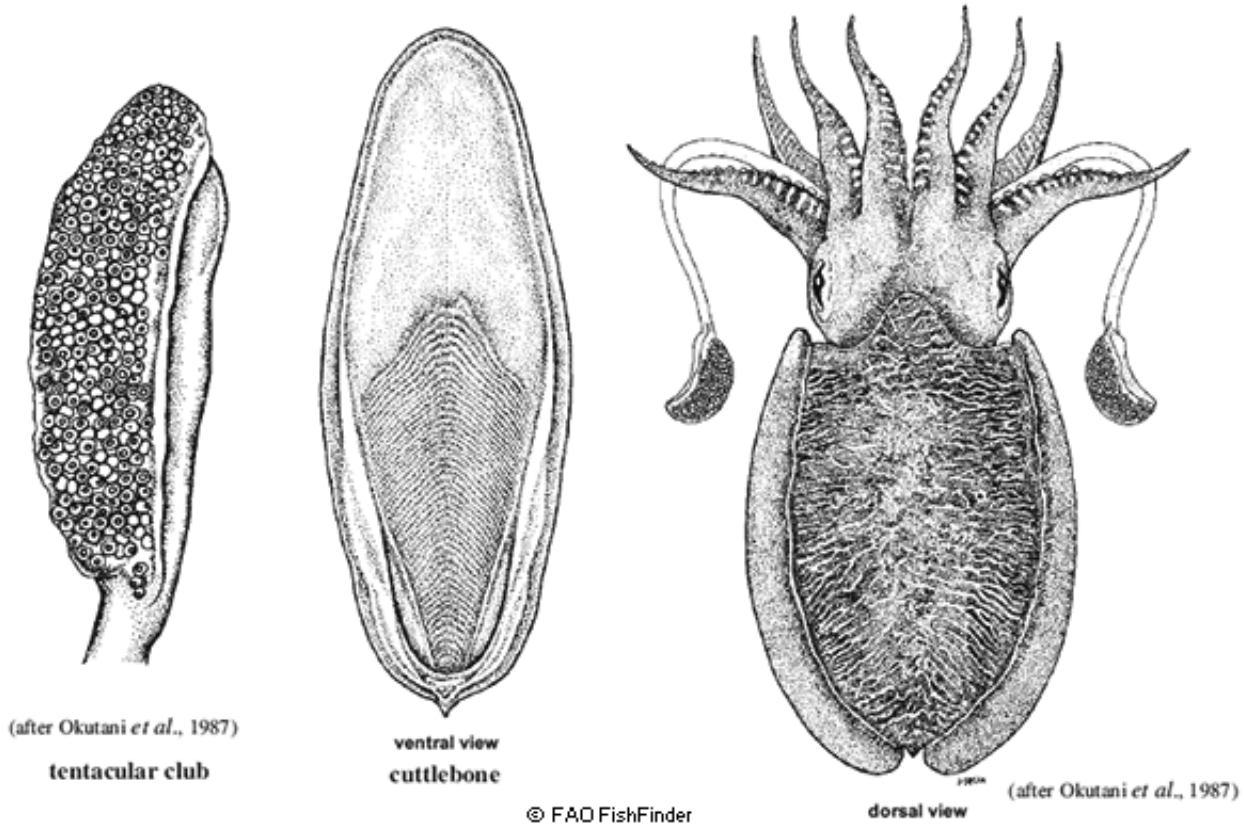
1. Presence of eight circumoral arms.
2. Absence of tentacles.
3. Arm sucker arranged in two rows, without horny rings and stalks.
4. Third arm tip of male spoon shaped.

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

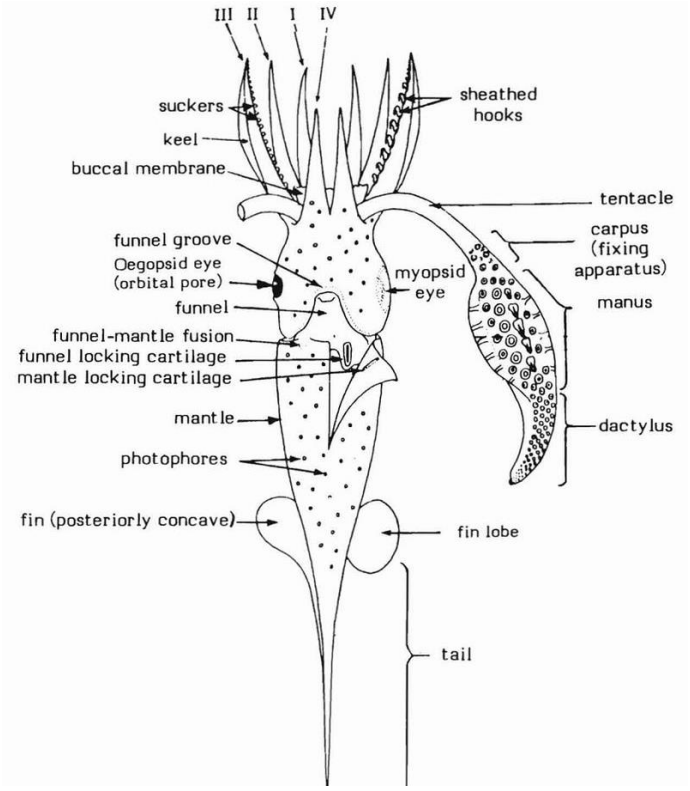
1. These animals have flattened arms, tentacles that can retract into a pocket of the body,
2. An eye pore inside of a ventral eyelid, narrow fins, and the shell of a cuttlebone.

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*

Course: BFSC-107: Information and Communication Technology

1. **How to open Ms word?**

Start → search Ms word on the search bar → open Ms word → create blank document.

2. **How to insert a table?**

Go to insert tab → click on the table menu → now select the number row and column → the table will be inserted.

3. **Step to insert chart and graph in document?**

Go to insert tab → click on any one chart →modify or edit the chart .

4. **How to insert image or graphics file to the document?**

Go to insert tab→ picture→ select the picture from any location .

5. **How to insert number and bullet in a document?**

Go to insert tab→ paragraph comment group → number and bullet.

6. **Make a simple bio data about your self by using Ms word?**

BIODATA

Photo

Candidate Name

Contact No.:

e-mail-id:

PERSONAL DETAILS:

DATE OF BIRTH :

FATHER'S NAME :

ADDRESS :

E-MAIL ID :

CONTACT NO :

NATIONALITY :

RELIGION :

MARITAL STATUS :

GENDER :

LANGUAGES KNOWN :

AREA OF INTEREST :

ACADEMIC QUALIFICATION:

-
-
-

WORK EXPERIENCE:

-
-

DATE:

PLACE:

Your Name

7. how to colour the whole document?

Go to insert tab→click on the page colour →select any colour from the colour pallet → the page will be coloured.

8. How to add water mark in a document?

Go to design tab →click on water mark → click on custom water mark →now type the text in the text box → choose the colour → choose the layout (horizontal or vertical) → ok.

9. How to start mail merge?

Go to mailing tab→start mail merge →step by step mail merge →select the document type →select the recipients list →create the recipients list →check the recipients list where you want to sent the email→ click on the sent mail button.

10. How to add header and footer to the document?

Go to insert tab → click on the header or footer to comment group →now select header or footer style from the list.

MS POWERPOINT

1. What is slide?

A slide is **a single page of a presentation**. Collectively, a group of slides may be known as a slide deck. A slide show is an exposition of a series of slides or images in an electronic device or in a projection screen.

2. Write down the step creative blank's presentation about five slide?

Create a presentation

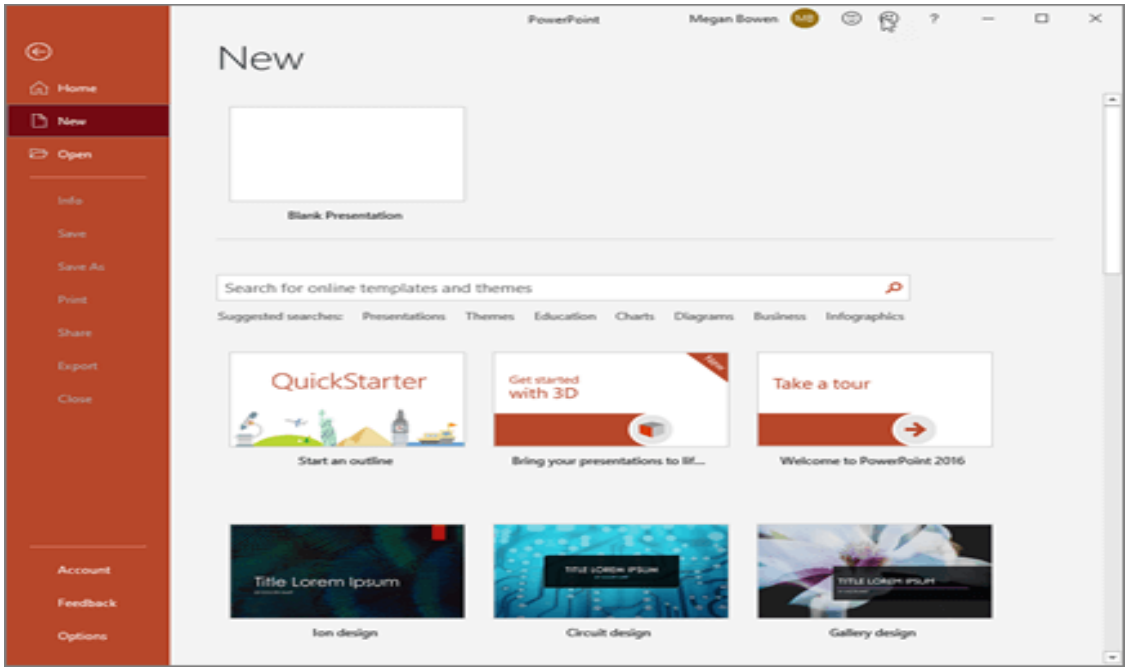
1. Open PowerPoint.
2. In the left pane, select **New**.
3. Select an option:
    - To create a presentation from scratch, select **Blank Presentation**.
    - To use a prepared design, select one of the templates.

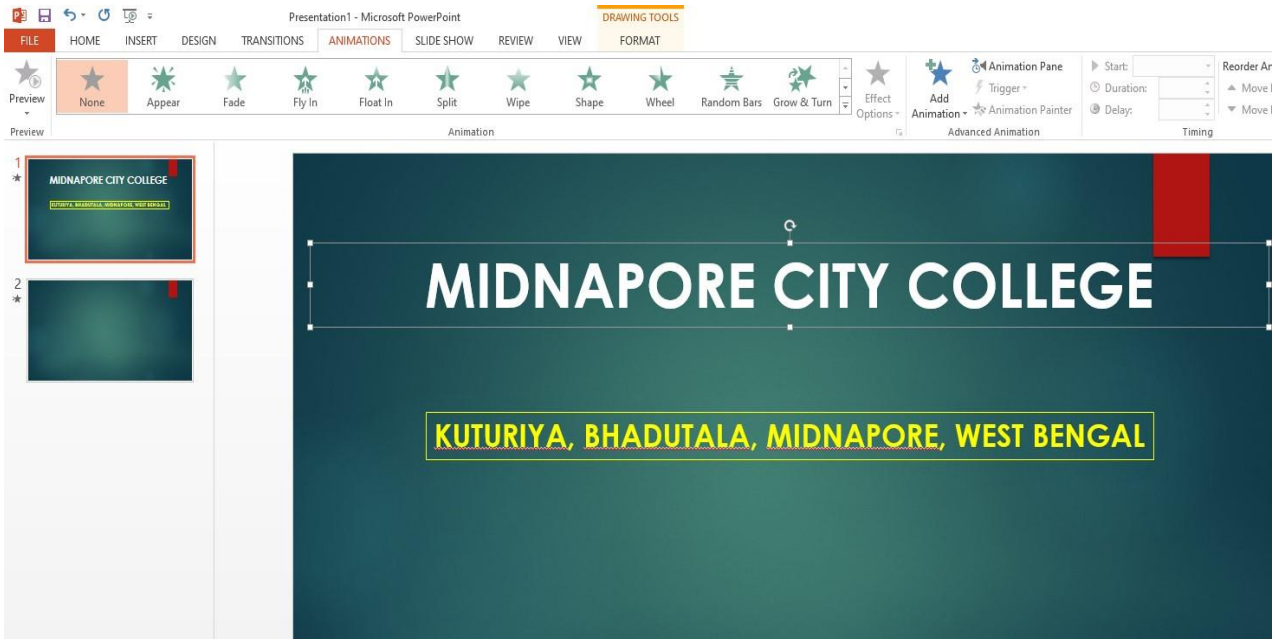
- To see tips for using PowerPoint, select **Take a Tour**, and then select **Create**, .

3. How to add a transition in a slide?

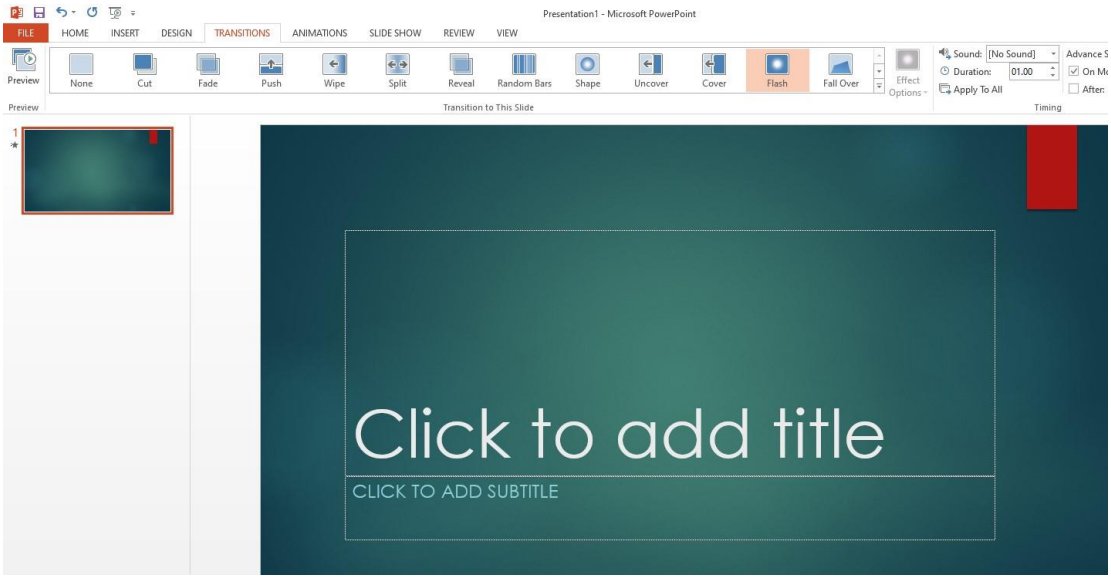
Slide transitions are the animation-like effects that happen when you move from one slide to the next during a presentation.

1. Select the slide you want to add a transition to.
2. Select the **Transitions** tab and choose a transition.
3. Select a transition to see a preview.
   Select **Effect Options** to choose the direction and nature of the transition.
   Note: Not every transition has **Effect Options**.
4. Select **Preview** to see what the transition looks like.





5. To remove a transition, select **Transitions** > **None**.



4. How to add animation effect in each slide?

Open the Animation Pane

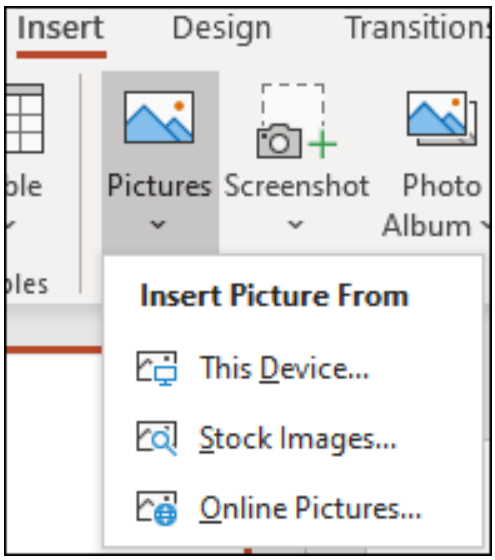
1. Select the object on the slide that you want to animate.
2. On the Animations tab, click Animation Pane.
3. Click Add Animation, and pick an animation effect.
4. To apply additional animation effects to the same object, select it, click Add Animation and pick another animation effect.

5. Write down the step to image and graphics file following the slide ?

Insert a picture in PowerPoint

 Click where you want to insert the picture on the slide.

 On the **Insert** tab, in the **Images** group, click **Pictures** and then click **This Device**.



 In the dialog box that opens, browse to the picture that you want to insert, click that picture, and then click **Insert**.

6. What are the advantages of using PowerPoint presentation ?

- Visual Impact. Making your presentation more interesting through the use of multimedia can help to improve the audience's focus. ...
- Collaboration. PowerPoint allows you to work with other people in a collaborative manner. ...
- Content Sharing. ...
- Flexibility.

7. Step to insert theme to the whole presentation ?

Apply a theme to all slides

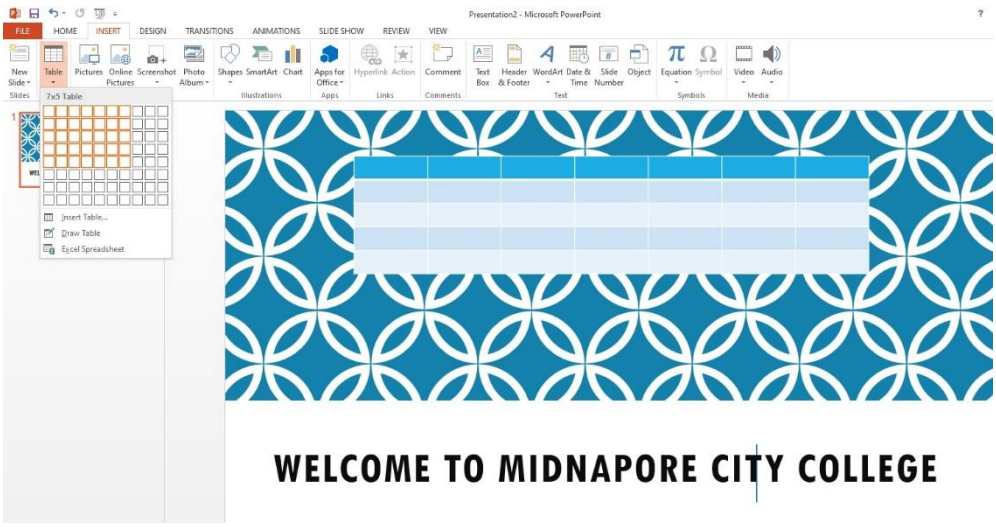
1. In the slide thumbnail pane on the left, select a slide.
2. On the Design tab, in the Themes group, click the More button (illustrated below) to open the entire gallery of themes:
3. Point the mouse at the theme you want to apply. Right-click it, and then select Apply to All Slides.

8. How to insert the time slab in each slide ?

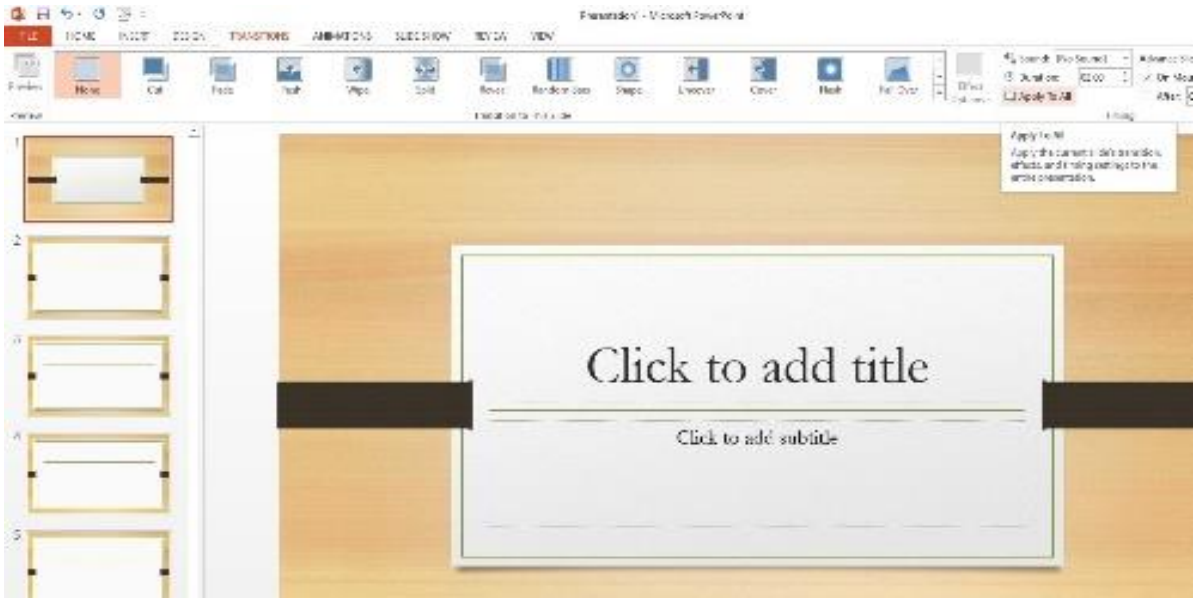
In Normal view, click the slide that you want to set the timing for. **On the Transitions tab, in the Timing group, under Advance Slide, select the After check box, and then enter the number of seconds that you want the slide to appear on the screen**. Repeat the process for each slide that you want to set the timing for.

9. Write down the steps to insert table In a slide?

1. Select the slide that you want to add a table to.



2. On the Insert tab, select Table.
3. In the Insert Table dialog box, do one of the following: Use the mouse to select the number of rows and columns that you want. ...
4. To add text to the table cells, click a cell, and then enter your text.



10. What is the Short cut key to play the slide?

Keyboard Shortcut Function **F5** Start a slide presentation from the beginning with the push of a button.

MS EXCEL

1. What is spread sheet software? Give example. How many rows and column's present in MS excel (in a sheet)?

A spreadsheet is **a computer program that can capture, display and manipulate data arranged in rows and columns**. Spreadsheets are one of the most popular tools available with personal computers. A spreadsheet is generally designed to hold numerical data and short text strings.

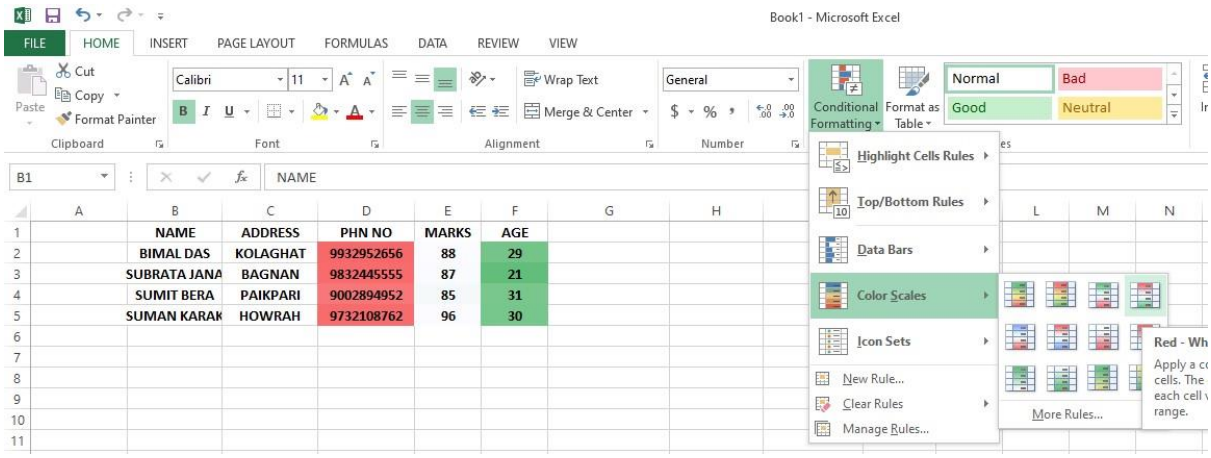
Examples include: **Lotus 1-2-3, Microsoft Works Spreadsheet, Open Office Calc and Google Drive Spreadsheet**.

Total number of rows and columns on a worksheet	**1,048,576 rows by 16,384 columns**

2. What is conditional formatting ?make a table in MS excel and apply it the conditional formatting future ?

Conditional formatting **makes it easy to highlight certain values or make particular cells easy to identify**. This changes the appearance of a cell range based on a condition (or criteria). You can use conditional formatting to highlight cells that contain values which meet a certain condition.

1. On the Home tab, in the Styles group, click Co nditional formatting → New Rule…
2. In the New Formatting Rule window, select Use a formula to determine which cells to format.
3. Enter the formula in the corresponding box.



4. Write down the formula of addition , average , maximum, minimum? Give also a table to make operation.

FUNCTION NAME	FORMULA
SUM	=SUM(No 1,No 2)
AVERAGE	=AVERAGE(No 1,No 2)
MAX	=SUM(No 1,No 2)
MIN	=SUM(No 1,No 2)

NAME	ADDRESS	PHN NO	BENGALI	ENGLISH	FUNDAMENTAL	SYSTEM PROG	TOTAL	AVERAGE	MAX	MIN
BIMAL DAS	KOLAGHAT	9932952656	88	29	56	77	250	62.5	88	29
SUBRATA JANA	BAGNAN	9832445555	87	21	45	85	238	59.5	87	21
SUMIT BERA	PAIKPARI	9002894952	85	31	74	96	286	71.5	96	31
SUMAN KARAK	HOWRAH	9732108762	96	30	65	63	254	63.5	96	30

5. Make a Mark shit including a column total , average , grade , percent ?(also including formula applying the table)

To create a table in Microsoft Excel with a column total, average, grade, and percent, follow these steps:

1. **Open a new workbook in Excel.**
2. **Click on the "Insert" tab in the ribbon.**
3. **Click on the "Table" button in the Tables group. This will bring up the "Create Table" dialog box.**
4. **In the "Table Name" field, enter a name for your table. This will be used to refer to the table in formulas.**

5. **In the "Column Labels" field, enter the names for the columns in your table. In this case, you can enter "Total," "Average," "Grade," and "Percent."**
6. **Click on the "My table has headers" checkbox to indicate that the first row of your table will contain column labels.**
7. **Click on the "OK" button to create your table.**
8. **Enter the data for your table in the rows below the column labels.**
9. **To calculate the total for a column, you can use the SUM function. For example, to calculate the total for the "Total" column, you can use the formula "=SUM(Table1[Total])", where "Table1" is the name of your table.**
10. **To calculate the average for a column, you can use the AVERAGE function. For example, to calculate the average for the "Average" column, you can use the formula "=AVERAGE(Table1[Average])".**

11.To calculate the grade for a column, you can use the IF function and specify a range of values for different grades. For example, to calculate the grade for the "Percent" column, you can use a formula like this: "=IF(Table1[Percent]>=90, "A", IF(Table1[Percent]>=80, "B", IF(Table1[Percent]>=70, "C", IF(Table1[Percent]>=60, "D", "F"))))". This formula will assign an "A" grade to values greater than or equal to 90, a "B" grade to values greater than or equal to 80, and so on.

12.To format the cells in the "Grade" column as text, you can select the cells and then click on the "Home" tab in the ribbon. In the "Number" group, click on the "General" button and then choose "Text" from the dropdown menu. This will ensure that the grades are displayed as text and not interpreted as numbers.

5.What is the deference between auto copy and auto fill ?

AutoFill is a feature in Microsoft Excel that allows you to quickly enter a series of data into a range of cells. For example, if you want to enter the numbers 1 through 10 in a column, you can select the first cell, enter 1, and then drag the fill handle (the small black square at the bottom-right corner of the cell) down to the last cell in the range to automatically fill in the rest of the series.

AutoCopy is a feature that allows you to quickly copy the contents of a cell or range of cells by simply selecting the cells and then dragging the fill handle to the destination cells. When you release the mouse button, the contents of the cells are copied to the destination cells.

So, in summary, AutoFill is used to enter a series of data into a range of cells, while AutoCopy is used to copy the contents of cells to a different location.

Name	Add	Phn	Beng	Bfsc	Geo	Sum	Average	Max	Min
Kuhu Das	Midnapore	9993952654	85	96	85	266	88.66667	96	85
Nilakhi Bera	Kgp	9832725025	74	89	52	215	71.66667	89	52

6.Create a table, fill the data including pie chart and graph chart in to the table ?

1) **To create a table in Microsoft Excel, follow these steps:**
2) **Open a new or existing worksheet in Excel.**
3) **Select the cells that you want to include in the table.**
4) **Click the "Insert" tab on the ribbon.**
5) **Click the "Table" button in the Tables group.**
6) **In the Create Table dialog box, verify that the range of cells is correct and that the My table has headers checkbox is selected if the first row of your range contains column headings.**
7) **Click "OK". Excel will create the table and apply a default table style to it.**

To fill data into the table, simply select a cell within the table and enter the data as you would in a normal worksheet.

To add a pie chart to the table, follow these steps:

Select the cells that contain the data you want to include in the pie chart.

1) **Click the "Insert" tab on the ribbon.**
2) **Click the "Pie" button in the Charts group.**
3) **Click the desired pie chart layout in the dropdown menu.**
4) **Excel will insert the pie chart into the worksheet.**

To add a graph chart to the table, follow these steps:

Select the cells that contain the data you want to include in the graph chart.

1. **Click the "Insert" tab on the ribbon.**
2. **Click the "Line" button in the Charts group.**
3. **Click the desired graph chart layout in the dropdown menu.**
4. **Excel will insert the graph chart into the worksheet.**

7.How to insert a sheet to the following work book ? How to delete the sheet ? How to rename the sheet?

To insert a new sheet in a workbook in Microsoft Excel, follow these steps:

1. **Open the workbook in Excel.**
2. **Click the "Home" tab on the ribbon.**
3. **Click the "New Sheet" button in the Cells group.**

Excel will insert a new sheet into the workbook, with the default name "Sheet2".

To delete a sheet in Excel, follow these steps:

1) **Right-click the sheet tab at the bottom of the workbook that you want to delete.**
2) **Click "Delete" on the context menu.**
   Excel will delete the sheet.

To rename a sheet in Excel, follow these steps:

1) **Right-click the sheet tab at the bottom of the workbook that you want to rename.**
2) **Click "Rename" on the context menu.**
3) **Type the new name for the sheet and press "Enter**

8.Write down the Step to marge and center the cell in a work sheet ?

1) **Select the cells that you want to merge.**
2) **Click the "Home" tab on the ribbon.**
3) **Click the "Merge & Center" button in the Alignment group.**

Excel will merge the selected cells and center their contents. If the selected cells contain multiple rows or columns, Excel will merge all of the cells into a single cell that spans the entire range.

Note: If the selected cells contain data in multiple rows or columns, only the data in the top-left cell will be retained after the cells are merged. The data in the other cells will be lost.