B.Sc. ZOOLOGY LAB MANUAL

1st Semester

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PREFACE TO THE FIRST EDITION

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

ACKNOWLEDGEMENT

We are really thankful to our students, teachers and non teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

C1 P1 –Non-Chordates I (Lab)

List of Practical

1. Study of whole mount of *Euglena*, *Amoeba* and *Paramoecium*

2. Identification of *Amoeba*, *Euglena*, *Entamoeba*, *Opalina*, *Paramecium*, *Plasmodium vivax* and *Plasmodium falciparum* (from the prepared slides)

3. Identification of *Sycon*, Neptune's Cup, *Obelia*, *Physalia*, *Millepora*, *Aurelia*, *Tubipora*, *Corallium*, *Alcyonium*, *Gorgonia*, *Metridium*, *Pennatula*, *Fungia*, *Meandrina*, *Madrepora*

4. Identification and significance of adult *Fasciola hepatica*, *Taenia solium* and *Ascaris lumbricoides*

5. Staining/mounting of any protozoa/helminth from gut of cockroach

C2 P2 –Ecology (Lab)

List of Practical

1. Study of life tables and plotting of survivorship curves of different types from the hypothetical/real data provided

2. Determination of population density in a natural/hypothetical community by quadrate method and calculation of Shannon-Weiner diversity index for the same community

3. Study of an aquatic ecosystem: Phytoplankton and zooplankton, Measurement of area, temperature, turbidity/penetration of light, determination of pH, and Dissolved Oxygen content (Winkler's method), Chemical Oxygen Demand and free CO_2

4. Report on a visit to National Park/Biodiversity Park/Wild life sanctuary

Sycon sp.

1. Body wall contains numerous pores called **ostia** through which water enters in the body.
2. Water enters in the body through a canal system into the central body cavity, called **spongocoel**.
3. Body also contains one or more openings called **oscula** through which water passes out from the body.
4. Sponges have an exoskeleton which is made up of either **spongin fibers** or **calcareous/siliceous spicules** or a combination of both.

-------Hence, the specimen belongs to **Phylum Porifera**.

1. Skeleton consists mainly of calcareous spicules.

-------Hence, the specimen belongs to **Class Calcarea**.

1. It has a vase-shaped cylindrical body measuring about 20-30 mm in length.
2. Each cylinder opens to the exterior by an **osculum.**
3. Osculum is encircled by a fringe of **monoaxon spicules.**
4. Body surface contains numerous pores called **ostia.**

-------Hence, the specimen seems to be **Sycon sp**.

Neptune's cup

Same upto **Phylum Porifera**.

1. Skeleton consists mainly of spongin fibres which may be in combination with spicules.
2. Canal system is leuconoid type.

-------Hence, the specimen belongs to **Class Demospongiae**.

1. Large sponge resembling a **cup** or wine **glass.**
2. The stalk is embedded in the ground with 'roots'.
3. Colours white to yellow.

-------Hence, the specimen seems to be **Poterion sp**.

Obelia sp.

1. Presence of gastrovascular cavity or coelenterons.
2. The body has a single opening called hypostome urrounded by sensor y tentacles.
3. Diploblastic, outer ectoderm and inner endoderm. Mesogloea separates these two layer.
4. Organ for capturing and paralyzing pray, present in tentacles called nematocysts.

-------Hence, the specimen belongs to **Phylum Cnidaria**.

1. Asexual Polyps are dominant form.
2. Medusa possess true velum.
3. Mesogloea is simple and acellular.

-------Hence, the specimen belongs to **Class Hydrozoa**.

1. These are small, transparent, solitary, free swimming saucer-shaped or bell-shaped zooids.
2. These are the reproductive zooids which produce the sex cells.
3. The inner concave side of the body is known as sub-umbrella and outer convex as ex-umbrella.
4. A short, hollow, quadrangular projection, the manubrium, hangs down from the middle of the sub umbrella surface.

-------Hence, the specimen seems to be medusa of **Obelia sp**.

<u>Physalia</u> sp.

Same upto **Class Hydrozoa**.

1. It has a single, highly distinguishable, gas filled float (the pheumatophore).
2. It has contractile tentacles.
3. The crest of the purple float has a shot of pink running through it.

-------Hence, the specimen seems to be **<u>Physalia</u> sp**.

<u>Aurelia</u> sp.

Same upto **Phylum Cnidaria**.

1. Medusa is dominant and it is Large bell or umbrella shaped.
2. Mesogloea is usually cellular.
3. Polyps is short lived or absent.

-------Hence, the specimen belongs to **Class Scyphozoa**.

1. It has a smooth, flattened saucer-shaped bell (the umbrella) with eight simple marginal lobes.
2. The umbrella is colourless, while the radial canals, oral arms and gonads are typically mauve, violet, reddish, pink or yellowish in colour.
3. The umbrella is quite thick, thinning towards the edge, with numerous short, hollow tentacles.

-------Hence, the specimen seems to be **<u>Aurelia</u> sp**.

Metridium sp.

Same upto **Phylum Cnidaria**.

1. Medusa stage is absent.
2. Mesogloea contains fibrous connective tissue and amoeboid cells.

-------Hence, the specimen belongs to **Class Anthozoa**.

1. It has a short, cylindrical body, radially symmetrical and divisible into three distinct regions, viz., oral disc, column and pedal or basal disc.
2. The upper free end is the flat, circular oral disc or peristome.
3. The pedal or basal disc is expanded and is used for fixing the animal to rocks or shells.

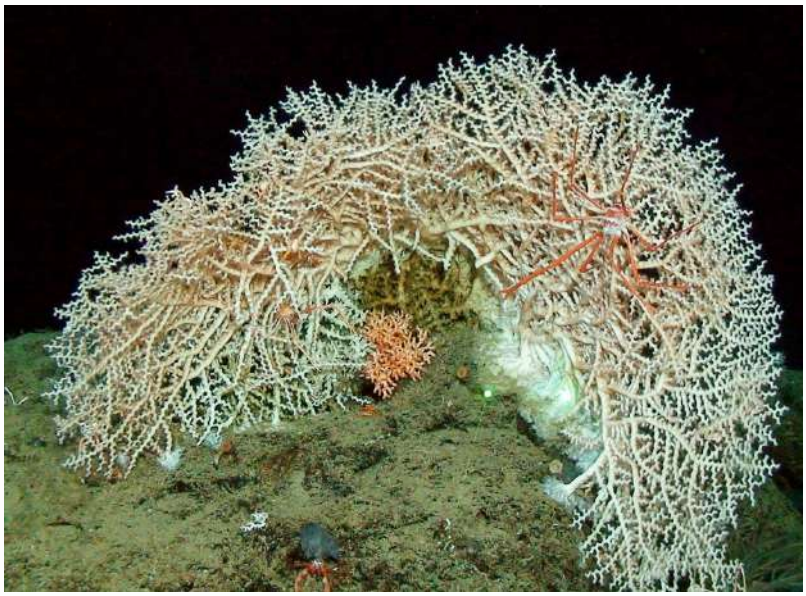
-------Hence, the specimen seems to be **Metridium sp**.

<u>Pennatula</u> sp.

1. The colony is elongated, dimomorphic, feather-like and is differentiated into a lower peduncle or stalk and an upper rachis.
2. The peduncle is dialated at its lower tip into an end bulb, which remains burried in mud or sand at the sea bottom and is devoid of zoids.
3. The rachis is narow at two ends, dialated in the middle and bears two rows of lateral branches—the pinnules.

-------Hence, the specimen seems to be **<u>Pennatula</u> sp**.

Madrepora sp.



Same upto **Class Anthozoa**.

1. It has thick skeletal parts that grow in a lamellar pattern.
2. Its skeleton is fragile and unable to sustain a large framework.
3. It is bushy, growing in small colonies that form thickets, creating matrices that are fan-shaped.
4. Colony is branched with small polyps in cylindrical cups separated by perforated coenosare.

-------Hence, the specimen seems to be **Madrepora sp.**

Gorgonia sp.

Same upto **Class Anthozoa**.

1. Colonies are large, in a single plane, and fan shaped.
2. The outline of the colony is an interconnected network of thin branches.
3. The branches are round or slightly compressed in the plane of the fan.
4. The apertures are very small pores located in two rows along the edges of the branches.

-------Hence, the specimen seems to **Gorgonia sp.**

<u>Fasciola</u> sp.

1. They are triploblastic, with three germ layers.
2. They do not have a body cavity and are acoelomate.
3. The <u>excretory system</u> has protonephridia with the flame.
4. The anus is absent.
5. The <u>digestive system</u> is incomplete or absent.

-------Hence, the specimen belongs to **Phylum Platyhelminthes**.

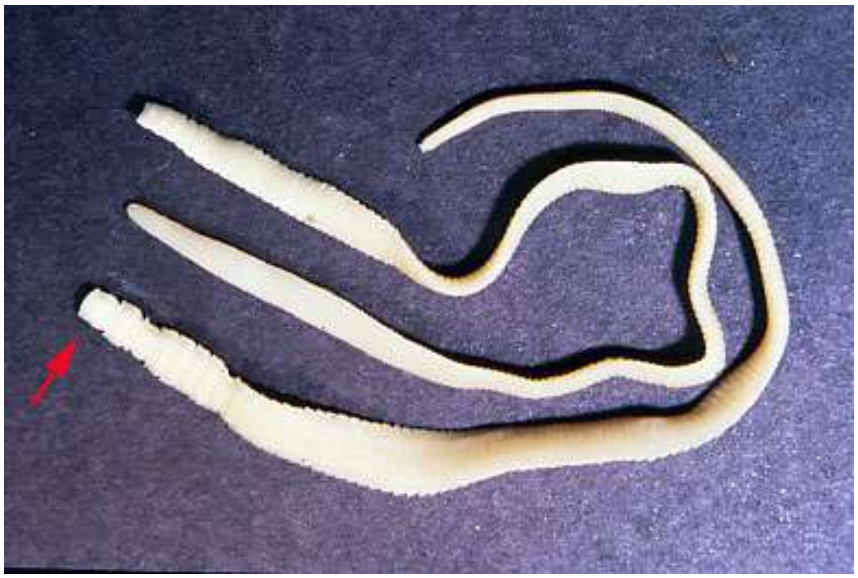
1. Body is covered by tegument.
2. Anterior end is a scolex.
3. Body segments called proglottids.
4. Endoparasitic.

-------Hence, the specimen belongs to **Class Cestoda**.

1. The adult worm has a very **characteristic** leaf shape with the anterior end being broader than the posterior end and an anterior cone-shaped projection.
2. The fluke possesses a powerful oral sucker at the end the anterior cone and a ventral sucker at the base of the cone.
3. Each worm possesses ovaries and testes which are highly branched.

-------Hence, the specimen seems to be **<u>Fasciola</u> sp.**

<u>Taenia</u> sp.



Same upto **Class Cestoda**.

1. The body of the worm is long, dorsoventrally flattened, narrow, ribbon-like.
2. The colour of the body is opaque-white.
3. Body consists of scolex, neck and strobila or body segments.

-------Hence, the specimen seems to be **<u>Taenia</u> sp.**

Ascaris sp.

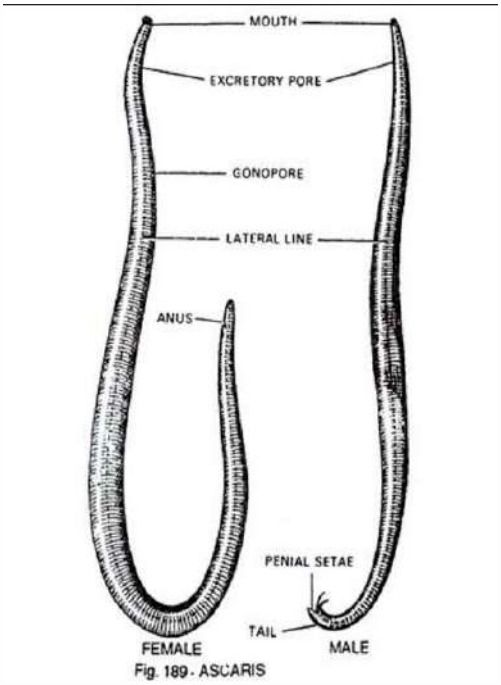


Fig. 189. ASCARIS

1. They have a pseudocoeloem, where the body cavity is not lined by the mesodermal layer.
2. They are bilaterally symmetric. The body is cylindrical or thread like with elongated, slender worm-like appearance and tapering at both ends.
3. The digestive system is complete, with a mouth and anus.
4. Amphids and papillae are the main sensory organs.

-------Hence, the specimen belongs to **Phylum Aschelminthes**.

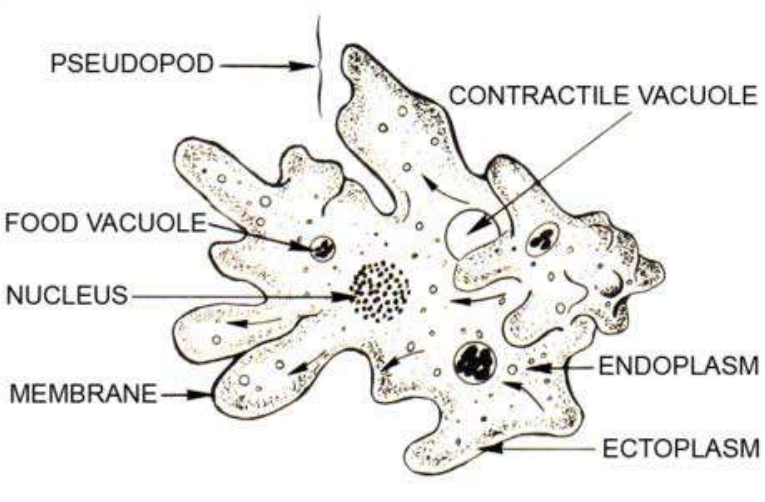
1. They are devoid of the circulatory system and respiratory system.
2. Their cuticle moults periodically.
3. The body-wall muscles are longitudinal.
4. They exhibit tissue level organization.
5. They possess amoeboid sperm cells.

-------Hence, the specimen belongs to **Class Nematoda**.

1. They are white or pink and are tapered at both ends.
2. It is the largest intestinal roundworm.
3. Body wall is composed of cuticle, epidermis and musculature.
4. Presence of a false body pseudocoelom not lined by epithelium.
5. Digestive system is complete.
6. Respiration by simple diffusion.

-------Hence, the specimen seems to be **Ascaris sp.**

Amoeba sp.



1. Locomotory organs are either pseudopodia, flagella, cilia or myoneme fibrils.
2. Acellular or non-cellular organism.
3. Single cell performs all the vital activities thus the single cell acts like a whole body.

-------Hence, the specimen belongs to **Subkingdom Protozoa**.

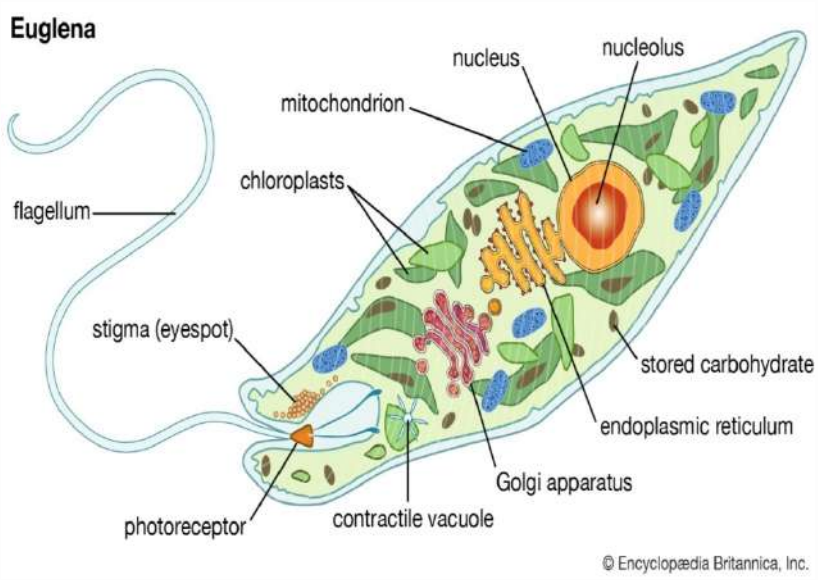
1. Locomotory organ either pseudopodia or flagella or both.
2. Spore formation is absent.
3. Reproduction asexual , but when sexually it is essentially by **syngamy.**

-------Hence, the specimen belongs to **Phylum Sarcomastigophora**.

1. Very small and contain a single nucleus.
2. They constantly change their body shape due to ameboid movement.
3. They engulf their prey with their pseudopodia, forming food vacuoles.

-------Hence, the specimen seems to be **Amoeba sp.**

Euglena sp.

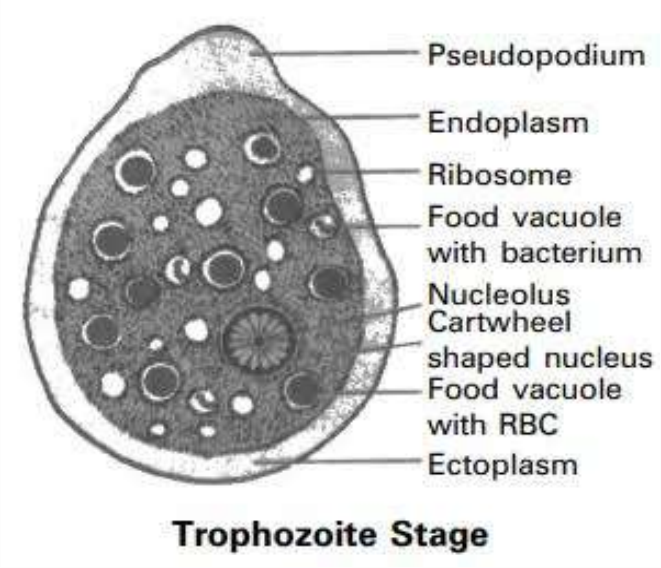


Same upto **Phylum Sarcomastigophora**.

1. Single-celled flagellated microorganisms that feature both plant and animal characteristics.
2. Unicellular with a **characteristic** whip-like tail known as a flagellum.
3. Lacks a rigid cellulose wall and has a flexible pellicle (envelope) that allows them to change shape.

-------Hence, the specimen seems to be **Euglena sp.**

Entamoeba sp.



Trophozoite Stage

Same up to **Phylum Sarcomastigophora.**

1. Cells are small, with a single nucleus and typically a single lobose pseudopod taking the form of a clear anterior bulge.
2. It divides by simple binary fission to form two smaller daughter cells.
3. Depending on the species, these can have one, four or eight nuclei and are variable in size.

-------Hence, the specimen seems to be **Entamoeba sp.**

<u>Plasmodium</u> sp.

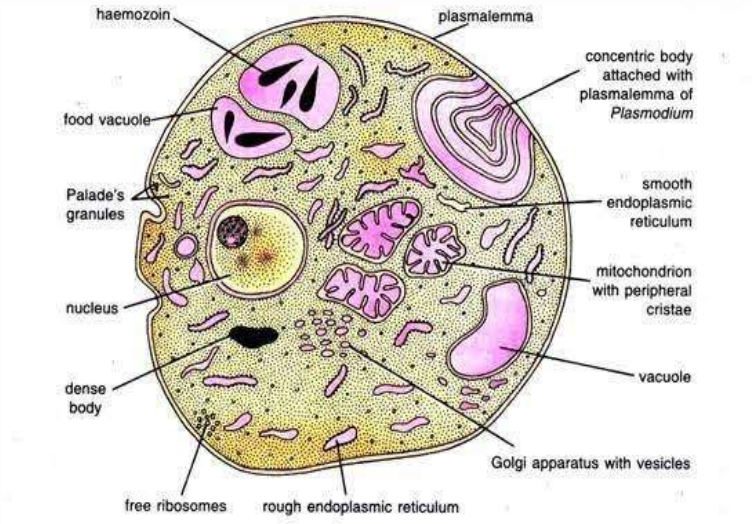


Fig. 19.1. *Plasmodium*. Ultrastructure of trophozoite in R.B.C. as seen in electron microscope.

Same up to **Subkingdom Protozoa**.

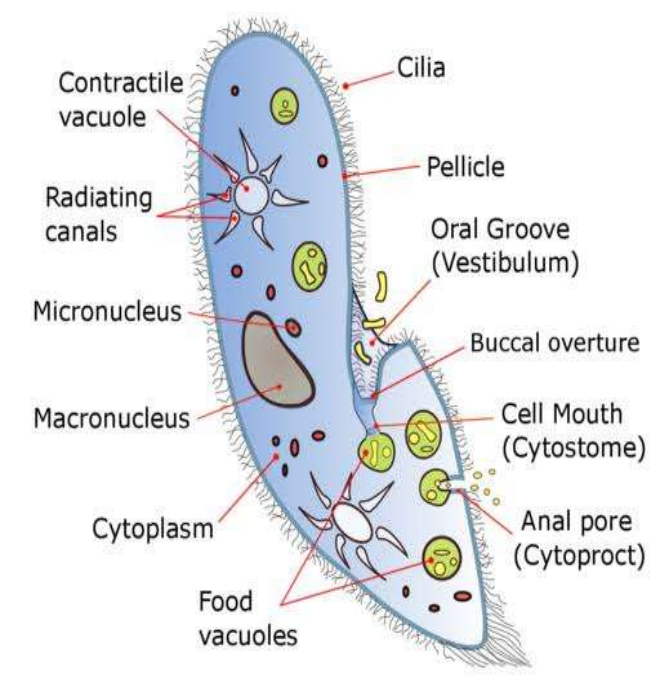
1. They possess a unique form of organelle called an apicoplast.
2. They possess an apical complex structure.

-------Hence, the specimen belongs to **Phylum Apicomplexa.**

1. Microgametocytes have a larger more diffuse nucleus while macrogametocytes have darker-staining cytoplasm.
2. Liver schizonts appear as clusters of small basophilic bodies.
3. The oocysts produce thousands of thin elongate sporozoites.

-------Hence, the specimen seems to be **<u>Plasmodium</u> sp.**

<u>Paramoecium</u> sp.



Same up to **Subkingdom Protozoa**.

1. Possesses <u>cilia</u> in at least one stage of their life cycle.
2. Has two different types of nuclei: one <u>macronucleus</u> and one or more <u>micronuclei</u>.

-------Hence, the specimen belongs to **Phylum Ciliophora.**

1. Body covered by a protective pellicle that functions like skin.
2. It has an oral groove that functions like a mouth.
3. It uses cilia to move and obtains its food.
4. **Conjugation** is the form of sexual reproduction.

-------Hence, the specimen seems to be **<u>Paramoecium</u> sp.**

POPUPALTION DENSITY BY QUADRAT METHOD

Real Lab Procedure

1. In the selected site of study, hammer the nails firmly without damaging the vegetation.
2. Fix four nails to make a square.
3. Tie each end of the nails using a thread, to make a 1 m X 1 m quadrat.
4. Similarly make nine more quadrats randomly in the site of study.
5. Count the number of individuals of a species “A” present in the first quadrat.
6. Record the data in the table.
7. Similarly count the number of individuals of the species “A” in other quadrats respectively and record the data in the table.
8. Count the number of individuals of a species “B” present in the all quadrat.
9. Record the data in the table.
10. Repeat the same procedure for species C and record the data in the table.
11. We can calculate the density of plant population by this equation:
12. Density =Total number of individuals of the species in all the sampling unit (S) / Total number of sampling units studied (Q)

Observations

Plant Species	Number of individuals in Each quadrats										Total Number of Individuals (S)	Total Number of Quadrats Studied (Q)	Density D= (S/Q)
	I	II	III	IV	V	VI	VII	VIII	IX	X			
A	2	0	5	7	10	0	0	0	0	3	27	10	2.7
B	1	0	4	0	8	0	3	0	0	2	20	10	2.0
C	4	0	0	3	0	6	0	0	1	2	19	10	1.9

The density value thus obtained is then expressed as number of individuals per unit area.

STATISTICS - SHANNON WIENER DIVERSITY INDEX

In the literature, the terms species richness and species diversity are sometimes used interchangeably. We suggest that at the very least, authors should define what they mean by either term. Of the many species diversity indices used in the literature, the Shannon Index is perhaps most commonly used. On some occasions it is called the Shannon-Wiener Index and on other occasions it is called the Shannon-Weaver Index. We suggest an explanation for this dual use of terms and in so doing we offer a tribute to the late Claude Shannon (who passed away on 24 February 2001).

Shannon-Wiener Index is defined and given by the following function:

$$H = \sum[(p_i) \times ln(p_i)]$$

Where –

- p_i = proportion of total sample represented by species i . Divide no. of individuals of species i by total number of samples.
- S = number of species, = species richness
- $H_{max} = ln(S)$ = Maximum diversity possible
- E = Evenness = $\frac{H}{H_{max}}$

ExampleExample

Problem Statement:
The samples of 5 species are 60,10,25,1,4. Calculate the Shannon diversity index and Evenness for these sample values.
Sample Values (S) = 60,10,25,1,4 number of species (N) = 5
First, let us calculate the sum of the given values.
Sum = (60+10+25+1+4) = 100

Species (i)	No. in sample	p_i	$ln(p_i)$	$p_i \times ln(p_i)$
Big bluestem	60	0.60	-0.51	-0.31
Partridge pea	10	0.10	-2.30	-0.23
Sumac	25	0.25	-1.39	-0.35
Sedge	1	0.01	-4.61	-0.05
Lespedeza	4	0.04	-3.22	-0.13
S = 5	Sum = 100			Sum = -1.07

$H = 1.07$

$H_{max} = ln(S) = ln(5) = 1.61$

$E = \frac{1.07}{1.61} = 0.66$

$Shannon\ diversity\ index(H) = 1.07$

$Evenness = 0.66$

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

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

STUDY OF AN AQUATIC ECOSYSTEM: PHYTOPLANKTON AND ZOOPLANKTON

Introduction

A microscopic community of plants (phytoplankton) and animals (zooplankton) found usually free floating, swimming with water currents. Planktons are of great importance as food and in the natural purification of polluted water.

Planktons, particularly phytoplanktons, have long been used as indicator of water current because of their short life span and quick response to environmental changes.

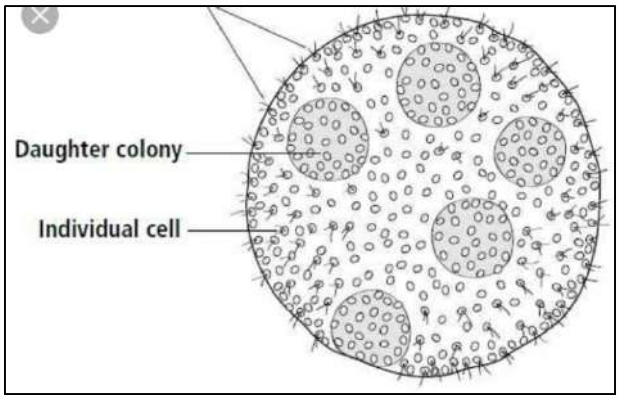
Zooplanktons, like phytoplantons, are also recently used as indicators.

Materials and methods

The study site was visited at an interval of 15 days. Samples were collected from water surface of three water bodies between 8 am to 10 am. The samples were collected in separate vials and fixed with 1 ml of 40% formalin. 10 ml of well mixed sample was centrifuged at 200 rpm for 10 minutes. To calculate the density of plankton mathematical methods were used but for qualitative study they are studied individually.

Phytoplankton

A. Systematic position



Kingdom- Plantae
Division- Chlorophyta
Class- Chlorophyceae
Order- Chlamydomonadales
Family- Volvocaceae
Genus- Volvox

Identifying Characters

1. Found in temporary and permanent ponds, lakes, pools and ditches.

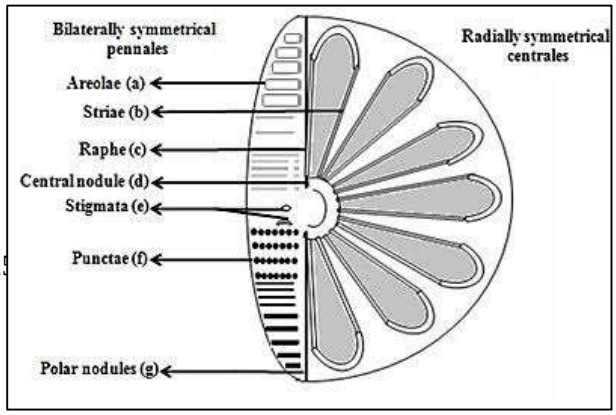
2. A multi colonial algae which forms a colony known as ‘coenobium’.

3. Each somatic cells of colony feature two flagella, several contractile vacuoles, a single chloroplast and an eyespot used for light reception.

4. Free floating, fresh water green algae.

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

B. Systematic position



Kingdom- Eukaryota
Division- Chromalveolata

Class- Bacillariphycaea
Order- Centrales, Pennales

Identifying Characters

1. Microscopic cells are of different shapes.

2. Plant bodies are either bilateral or radially symmetrical.

3. Vegetative cells are diploid.

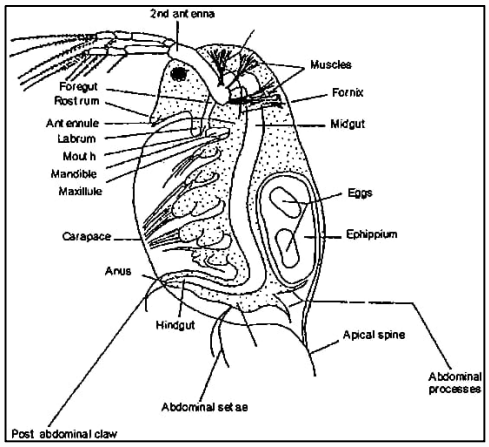
4. The walls have secondary structure like spines, bristles etc.

Hence, the specimen seems to be Diatom.

Zooplankton

A. Systematic position

Phylum- Arthropoda
Subphylum- Mandibulata
Class- Crustacea
Subclass- Brachiopoda



Identifying Characters

1. Bilaterally compressed body, enclosed in bivalved carapace, ending into sharp caudal pointed rostrum.
2. Rounded head bears large, sessile, single, distinct eye.
3. Presence of five pairs of leaf-like thoracic appendages and abdomen is devoid of appendages.
4. Presence of brood pouch in females.

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

B. Systematic position

Phylum- Arthropoda
Subphylum- Mandibulata
Class- Crustacea
Subclass- Copepoda

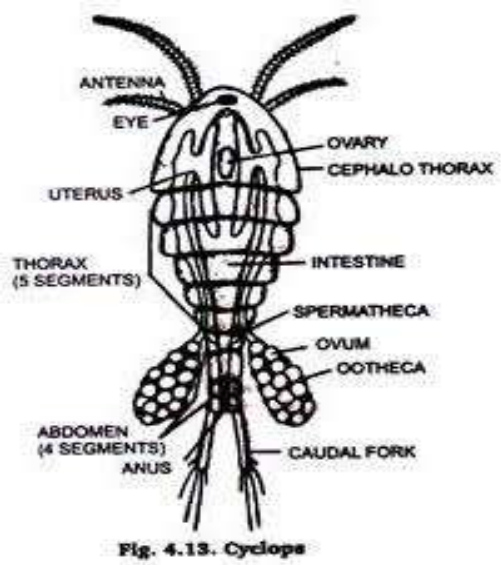


Fig. 4.13. Cyclops

Identifying Characters

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

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

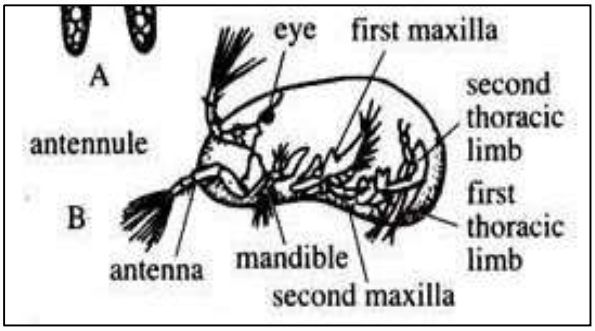
3. A median eye is present near the anterior end.

4. A pair of caudal styles having plumose setae is present.

5. Females carry two lateral egg sacs attached to the abdomen.

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

C. Systematic position



Phylum- Arthropoda
Subphylum- Mandibulata
Class- Crustacea
Subclass- Ostracoda

Identifying Characters

1. The body is entirely enclosed in a bivalve carapace.
2. Well developed antennae used for swimming.
3. Gills enclosed within the valves.
4. Head bears four pairs of thoracic appendages.
5. Presence of three pairs of thoracic appendages.

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

ESTIMATION OF CHEMICAL OXYGEN DEMAND FROM WATER SAMPLE

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

Principle

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

Materials required

Reagents

1. **0.25N Potassium dichromate solution**: 12.259g of $K_2Cr_2O_7$ is dissolved in water to make 1L of solution
2. **0.025N Potassium dichromate solution**: 0.25N $K_2Cr_2O_7$ is diluted 10 times.
3. **0.1N Ferrous ammonium sulphate:** 39.2g of $Fe(NH_4)_2(SO_4).6H_2O$ is dissolved in water, adding 20ml of concentrated H_2SO_4 to make it 1L of solution.
4. Ferroin indicator
5. Concentrated H_2SO_4
6. $HgSO_4$
7. $AgSO_4$

Glassware

1. COD flask
2. Conical flask
3. Beaker
4. Glass pipette and glass rod
5. Glass burette with stand

Procedure

1. 20 ml of sample is taken in a 250-500ml COD flask.
2. If the sample is expected to have COD more than 50mg/L, 10ml of 0.025N Potassium dichromate solution is added to the sample.
3. A pinch of $HgSO_4$ and $AgSO_4$ is added.
4. 30ml of sulphuric acid is added to it.
5. The total solution is refluxed for 2 hours on a water bath or hot plate and the refluxed water is made to a final volume of 140ml.
6. 2-3 drops of Ferroin indicator is added and mixed thoroughly and titrated with 0.1N Ferrous ammonium sulphate.
7. A blank is run with distilled water using same quantity of the chemicals.

Calculation

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

a= ml of titrant with sample
b= ml of titrant with blank

ESTIMATION OF DISSOLVED OXYGEN IN WATER

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

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

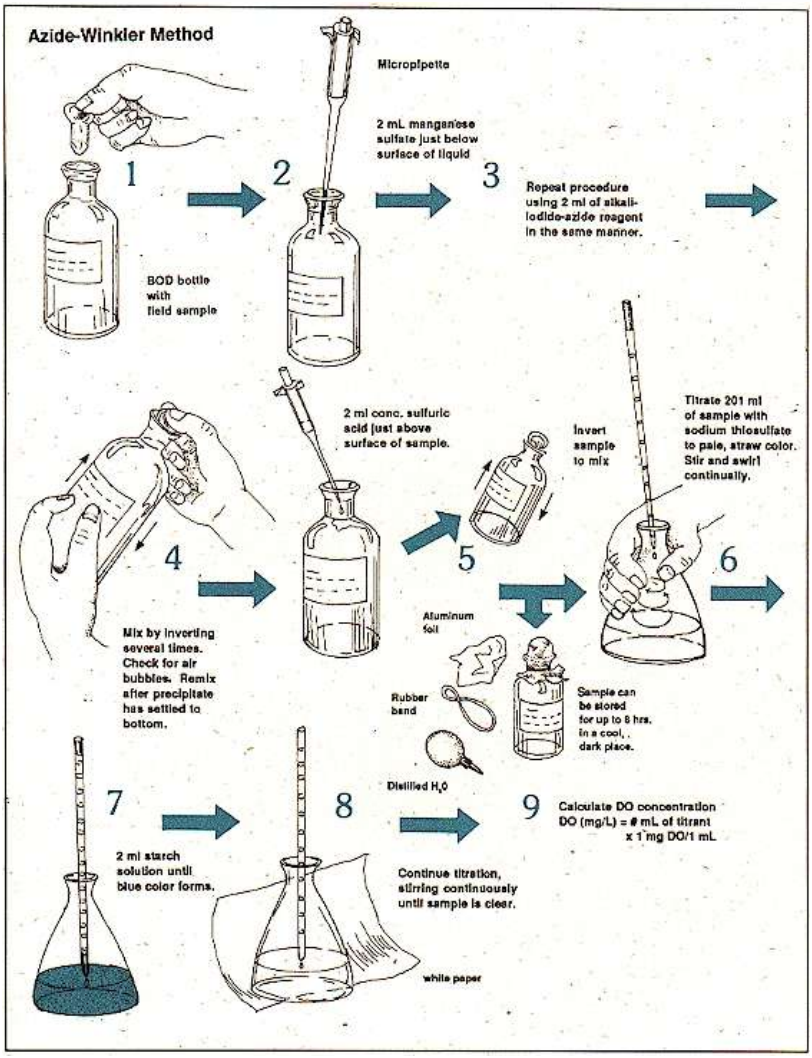
These reagents are available in dissolved oxygen field kits, such as those made by the Hach Company. Please use caution when using these reagents, as they can be hazardous to one's health.

Procedure:

1. Carefully fill a 300-mL glass Biological Oxygen Demand (BOD) stoppered bottle brim-full with sample water.
2. Immediately add 2mL of manganese sulfate to the collection bottle by inserting the calibrated pipette just below the surface of the liquid. (If the reagent is added above the sample surface, you will introduce oxygen into the sample.) Squeeze the pipette slowly so no bubbles are introduced via the pipette.
3. Add 2 mL of alkali-iodide-azide reagent in the same manner.
4. Stopper the bottle with care to be sure no air is introduced. Mix the sample by inverting several times. Check for air bubbles; discard the sample and start over if any are seen. If oxygen is present, a brownish-orange cloud of precipitate or floc will

appear. When this floc has settle to the bottom, mix the sample by turning it upside down several times and let it settle again.

5. Add 2 mL of concentrated sulfuric acid via a pipette held just above the surface of the sample. Carefully stopper and invert several times to dissolve the floc. At this point, the sample is "fixed" and can be stored for up to 8 hours if kept in a cool, dark place. As an added precaution, squirt distilled water along the stopper, and cap the bottle with aluminum foil and a rubber band during the storage period.
6. In a glass flask, titrate 201 mL of the sample with sodium thiosulfate to a pale straw color. Titrate by slowly dropping titrant solution from a calibrated pipette into the flask and continually stirring or swirling the sample water.
7. Add 2 mL of starch solution so a blue color forms.
8. Continue slowly titrating until the sample turns clear. As this experiment reaches the endpoint, it will take only one drop of the titrant to eliminate the blue color. Be especially careful that each drop is fully mixed into the sample before adding the next. It is sometimes helpful to hold the flask up to a white sheet of paper to check for absence of the blue color.
9. The concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used. Each mL of sodium thiosulfate added in steps 6 and 8 equals 1 mg/L dissolved oxygen.



CALCULATION:

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

V_1=volume of titrant

N = Normality of titrant

V_2= vol. of sample bottle

V_3= vol.of reagent added

V_4= vol.of sample titrated

ESTIMATION OF FREE CARBON DIOXIDE (CO_2):

Principle:
Carbon Dioxide is present in water in the form of a dissolved gas. Calcium and magnesium combine with carbon dioxide to form carbonates and bicarbonates. Aquatic plant life depends upon carbon dioxide and bicarbonates in water for growth. Free CO_2 of water samples can be measured by titrating the test sample against a strong alkali (pH- 8.3) where all free CO_2 molecules in the test sample is converted in to bicarbonates giving a faint pink color.

Materials Required:
Reagents-
1. N/44 NaOH solution (1N)
2. Phenolphthalein indicator
3. Sample water

Glasswares-
1. Conical Flask
2. Beaker

Reaction:
$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$

Procedure:
1. 50 ml sample water was taken in a conical flask.
2. Add 4 drops of phenolphthalein indicator.
3. If there is no change of colour, it is confirmed that free CO_2 is present.
4. The water sample was then titrated against N/44 NaOH taken in a burette till a faint pink colour appears.
5. Three readings were taken and the mean volume of the NaoH was recorded.

Observation:
Data Sheet:

Sample index	Observation No.	Vol. of sample (ml)	Burette reading (ml)		Vol. of NaOH	Mean vol. of NaOH used (ml)
			Initial	Final		

Calculation:

$^{N}/_{44}$ NaOH is equal to $^{40}/_{44}$, i.e 0.9 gm of NaOH.
1000 ml of water sample contains 0.99 gm of NaOH.
1 ml of CO_2 ≅ 1 mole of NaOH.
44 gm of CO_2 ≅ 40 gm of NaOH.

1000 ml of 1(N) NaOH ≅ 40 gm of NaOH ≅ 44 gm of CO_2
1000 ml of 1(N) NaOH ≅ 44000 mg of CO_2
1 ml of 1(N) NaOH ≅ $^{44000}/_{1000}$ = 44 mg of CO_2
1 ml of $^{N}/_{44}$ NaOH ≅ $^{44}/_{44}$ mg of CO_2 = 1 mg of CO_2

Therefore,
x ml of $^{N}/_{44}$ NaOH ≅ ***x*** mg of CO_2
50 ml of water sample contains ***x*** mg of CO_2
1 ml of water sample contains $^{x}/_{50}$ mg of CO_2
1000 ml of water sample contains $^{x}/_{50}$ X 1000 mg of CO_2 = 20***x*** mg of CO_2.

Hence,
1. The amount of free CO_2 present in sample water (pond) is 20***x*** X ____ mg/L = ____ mg/L.
2. The amount of free CO_2 present in sample water (tap) is 20***x*** X ____ mg/L = ____ mg/L.

Comment:
Carbon dioxide in water regulates photosynthetic process essential for aquatic life. The ideal range of free CO_2 in water should be less than 10 mg/L. Greater tyhan 30 mg/L is not good for aquatic organisms specially fishes.
The CO_2 content of the evaluated water sample from pond is _____ mg/L which is suitable for fish and the CO_2 content of the evaluated water sample from tap is _____ mg/L which is not suitable for fish.

pH DETERMINATION OF WATER

Principle

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

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

Materials required

a. pH meter
b. Distilled water
c. Sample water
d. Buffer tablets (pH 4, 7 and 9) and corresponding buffer solutions.

Procedure

1. Buffer solution of pH 4.0, 7.0 and 9.0 are prepared by using standard buffer tablets.
2. Electrodes are connected to pH meter.
3. Electrodes are dipped in a buffer solution of pH 7.0.
4. Temperature control of the pH meter is set as per the temperature of the sample water.
5. pH is recorded. The meter gives a reading near 7.0.
6. The electrodes are then removed from buffer solutions and washed with distilled water and dried. The electrodes are dipped to buffer of pH 4.0 and 9.0 consecutively. The meter should correspondingly show a reading of 4.0 and 9.0 respectively.
7. Next the electrodes are dipped in the water sample (test sample). The sample is gently swirled twice and pH is seen in the digital display or in the meter of the instrument. Three such replicates are taken.
8. Mean pH value is taken.

Observation

No. of Observations	pH value	Mean pH value

Inference

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

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

ESTIMATION OF TRANSPARENCY OF WATER BY SECCHI DISC METHOD

Principle

Turbidity in the water reduces light penetration. Light penetration in fact, depends partly on the light flux and mainly on the optical properties of water.

Light penetration in a body of water can be obtained by immersing secchi disc, which is a circular disc of metal of 20 cm in diameter painted matt white. Sometimes, a disc painted alternatively black and white in a radial fashion is also used. It has got a weight at the lower face so as to avoid a drift during lowering in water. A string is attached to it for lowering which may be marked in centimeters.

In conditions of bright light and calm water irradiance at the depth of secchi disc penetration is about 15% to that of just below the surface. The compensation level (photosynthesis and respiration) or the euphotic limit at which irradiance is 1% to that of the surface is nearly 2.5 times secchi disc depth. The vertical alternation co-efficient is about 1.9 times secchi disc depth.

Procedure

1. Lower the secchi disc in the water with the help of the string tied to it, until it just disappears. Note the depth by marking on the string.
2. Now uplift the secchi disc and note the depth at which it reappears again.
3. Repeat the procedure two times more at different points of the same pond.
4. For better result, measurement should be made during the middle of a sunny day.

Calculation

$$\text{Secchi disc penetration} = \frac{\text{point of disappearance + point of reappearance}}{2}$$

MEASUREMENT OF TEMPERATURE

Temperature affects aquatic organisms in a variety of ways. The body temperature of most aquatic organisms in the same as the surrounding water and adapted to live in a narrow temperature range and they die when the temperature becomes too low or too high. Temperature affects their metabolism, reproduction and emergence. Temperature affects rate of photosynthesis of aquatic plants, the base of the aquatic food web. Populants can become more toxic at higher temperature. The amount of dissolved oxygen becomes lower as the water becomes warmer. Temperature is measured in Degree, Fahrenheit or Celsius (Centigrade).

Materials:

Water Quality data sheet dial

OR

Digital thermometer.

Procedure (Dial Thermometer):

1. Remove thermometer from its protective cover. Push the end of the thermometer through the loop or hole in the side of the cover. This turns the cover into a handle- do not hole the thermometer rod itself. Hold with dial facing up so you can read it.

2. Air temperature: Hold the thermometer by the handle away from your body above the water. Wait at least 1minute. Read the temperature (each line is 2^oC).

3. Water temperature: Hold the thermometer by the handle and place the end in the water. Wait at least 1 minute. Read the temperature (each line 2^oC) repeat 2 more times.

Observation:

At the depth of 0 cm (surface) = _____$^\circ$C

At the depth of ***x*** cm = _____$^\circ$C

At the depth of ***y*** cm = _____$^\circ$C

***For the difference of _____cm the temperature of water changes = (____$^\circ$C – ____$^\circ$C) =____$^\circ$C