LIMNOLOGY PRACTICAL WRITE-UP

Practical Manual

Limnology

B. F. Sc 2nd Semester

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Practical 1 Morphometry of Lakes, Ponds and Streams

Morphometry is the measurement of external form or shape of a selected water body.

a. Location : Trace the correct name of the locality and its latitude, longitude and altitude from authentic sources such as government records and publications, maps and topo-sheets of the Survey of India etc.

b. History : It is important to know the history of the area, land use of surroundings, formations or excavation of the basin, past glaciations or tectonic activities if any and other relevant information available from published or unpublished work, district gazetteer and by interviewing the local inhabitants.

c. Area: Surface area is an extremely important dimension for it is at the surface that the solar energy enters into the lake and marks the beginning of the lacustrine energy exchanges. As a result many limnological data related to productivity and heat budget etc. are generally given as unit area of the lake surface, making it possible to compare meaningfully the limnological characteristic of different sized water bodies.

Requirements: Stakes, measuring tape, graph paper, planimeter

Bathymetric map (Contour map). It can be prepared by recording the depth at several points at equidistance in the lake; the number of points will depend on the size of the size of the water body.

a. Maximum Length (L): It is distance between two most distant points on the surface of the lake without land interruptions.

b. Depth (Z): Depth is the minimum vertical distance between the surface and the underlying bottom of the lake at any point, along with the area it gives an idea of the volume of water in the lakes.

Result: Express the depth in meters (m).

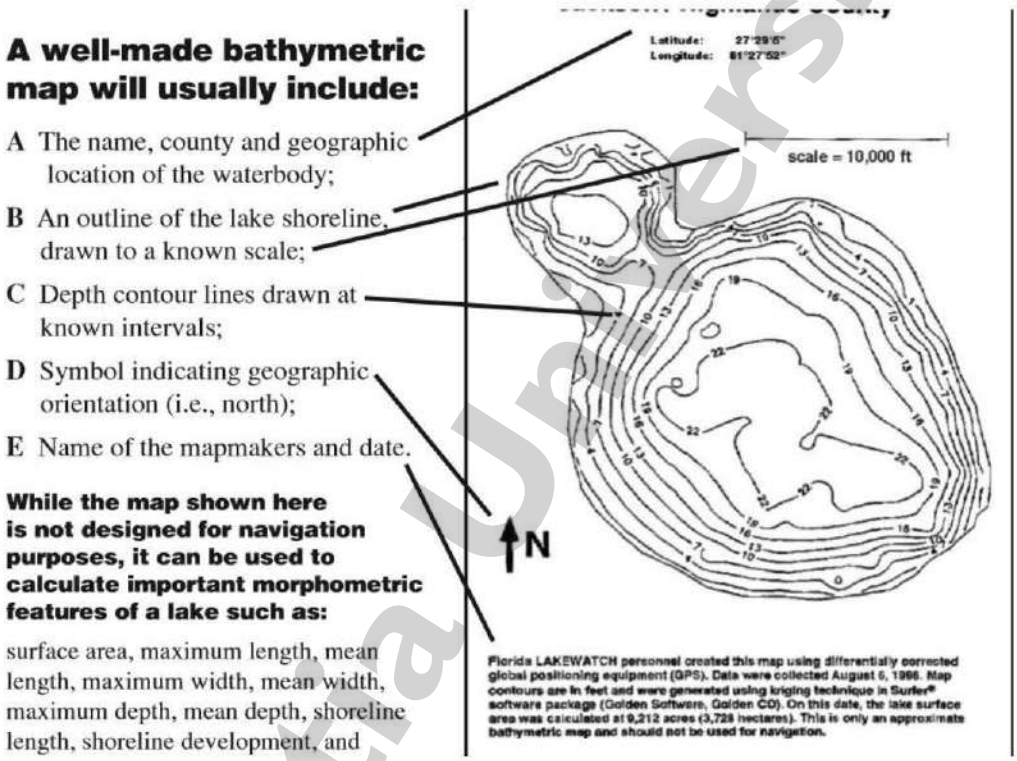
c. Maximum Depth (Zm): It is the measured at the deepest point of the lake.

d. Volume (V): Volume of the basin is the integral of the areas at successive close depth.

e. Mean depth (Z): Mean depth is calculated by dividing the volume of the lake by its surface area (Z/A).

f. Geology: This includes the geomorphological, pedological (origin and development of soil), edaphological (soil characters) and topographical information, which can be

collected from reliable sources like local and regional offices of survey of India, Geological survey of India, District Gazetteer and relevant published Literature. In case, no such information is available the help of a Geologist should be taken.



https://www.youtube.com/watch?v=AWPLG-XwClY

https://www.youtube.com/watch?v=SItsTdHSGus

Practical 2 Determination of physical (water surface, water color, temperature, Transparency) characteristics of inland waters

a) **Water surface**

Visual changes in the nature of water surface are mainly due to the wind action and to a certain extent are governed by the topography of the surrounding area. This phenomenon is responsible for the localization and dispersion of the algal bloom and other particulate organic matter. Therefore, observe the water surface and record its condition as mirror smooth, rippled, wavy and highly wavy and so on.

b) **Water colour**

Light coming from the lake surface yields an apparent colour which is the result of the true colour of water (due to materials in solution), seston (due to living and non-living particulate matter) and the reflections of surface and sub surface objects. The colour is best judged by observing through a water telescope or also by the standard empirical colour scale (or even by visual observations).

c) **Temperature**

The surface water temperature was measured by using a mercury centigrade thermometer (0 to 50^0C) with 0.10^0C graduation at station itself (Adoni *et al.*, 1985).

REAGENT PREPARATION: NIL

Apparatus required:

1. Thermometer

- **Principle:**
- Generally temperature of a water body is determined by the help of centigrade thermometer, graduated in 0.10C scales or for more accurate results in 0.010C or by instrument which display the temperature on digital meter.

Procedure:

- Dip the thermometer directly in the sample.
- Read the mercury level in the thermometer.
- Read the temperature of deep water system by means of a reversible thermometer.

Calculation:

- Note the reading of the thermometer.

d) Transparency of water

The turbidity of water is directly related to light penetration and visibility or transparency which can be measured by Secchi disc. This disc was devised by an Italian scientist, Secchi (1865) for studying the transparency of aquatic bodies. The Secchi disc is a metallic plate of 20 cm diameter with four (alternate black and white) quadrants on the upper surface and a hook in the centre to tie a graduated rope.

Principle: The transparency is inversely proportional to the turbidity of water, which in turn is directly proportional to the amount of suspended organic and inorganic matters. When the disc is gradually lowered in water it remains visible in the euphotic zone, only to that lower level where light is about 15% of the radiation at the surface.

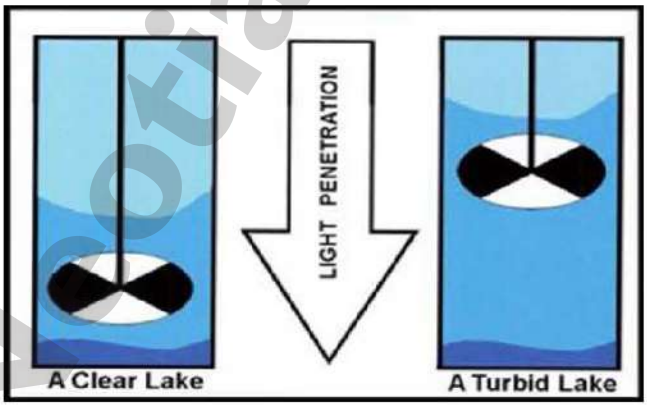
Requirement: Secchi disc, measuring tape, graduated nylon rope etc.

Method:

a. Lower the disc in water and note the depth (in cm) at which it disappeared

b. Now slowly raise the disc upward and note the depth at which it reappears.

c. Take the average value of Secchi disc depth (SDD) or transparency



https://www.youtube.com/watch?v=wLB5hNe_IBY

https://www.youtube.com/watch?v=J2M4McUmU1s

Practical 3 Determination of Chemical (pH, DO, Free CO_2) characteristics of inland waters

a) PH

Principle: The pH of water samples were measured by a digital pH meter (Systronics: model no.MK-VI) following the electrometric method described by Adoni *et al.*, 1985.

Reagent preparation: Nil

Apparatus required:

1. pH paper

pH meter

Range: 7.5 to 8.5

b) DO

Principle:

Manganous sulphate ($MnSO_4$) reacts with potassium or sodium hydroxide (NaOH or KOH) to give a white precipitate of manganous hydroxide [$Mn^{2+}(OH)_2$].

In the presence of oxygen, brown manganic basic oxide [$Mn^{4+}O(OH)_2$] is formed.

$$Mn^{2+}SO_4 + 2KOH \longrightarrow Mn^{2+}(OH)_2 + K_2SO4$$

$$2Mn_2{+}(OH)_2 + O_2 \longrightarrow 2Mn^{+4}O(OH)_2.$$

Sulphuric acid dissolves the brown basic manganic oxide yielding manganic sulphate, which reacts instantly with sodium or potassium iodide to yield iodine.

$$2Mn^{+4}O(OH)_2 + 2H_2SO_4 \longrightarrow Mn^{+4}(SO_4)_2 + 3H_2O$$

$$Mn^{+4}(SO_4)_2 + KI \longrightarrow Mn^{2+}SO_4 + K_2SO_4 + I_2$$

In effect, oxygen oxidizes Mn^{2+} to Mn^{+4} oxidizes to I_2

Iodine is then determined titrimetrically with starch as an indicator which blue colour changes to colourless.

Sampling and preservation

Collect the sample in a 300 ml BOD bottle,

(Taking precaution: avoid dissolution of atmospheric oxygen).

Samples to be analyzed in the laboratory are to be fixed in the field.

Add 2 ml manganous sulphate (MnSO4).

Add 2 ml alkaline-iodide azide solution.

Shake well and allow to settle the precipitation and then store in a cool place.

Reagent preparation:

Manganous sulphate solution: (for 250 ml) (Winkler's A)

Take 480 gm of $MnSO_4$, $4H_2O$ or 400 gm $MnSO_4$,$2H_2O$ or 364 gm of $MnSO_4$,H_2O is dissolved in distilled water.

Alkaline- Iodine Azide solution: (for 250 mL) (Winkler's B)

Take 500 gm of Sodium hydroxide (NaOH), 135 gm of sodium iodide (NaI) or 700 gm of potassium hydroxide and 150 gm of potassium iodide are dissolved in distilled water and the solution is diluted to 1000 ml. Mix separately in DW and then together make up to 250 ml. then add 10 gm NaN3 (sodium azide) dissolved in 40 ml distilled water

Con. H_2SO_4 (Sulphuric acid)

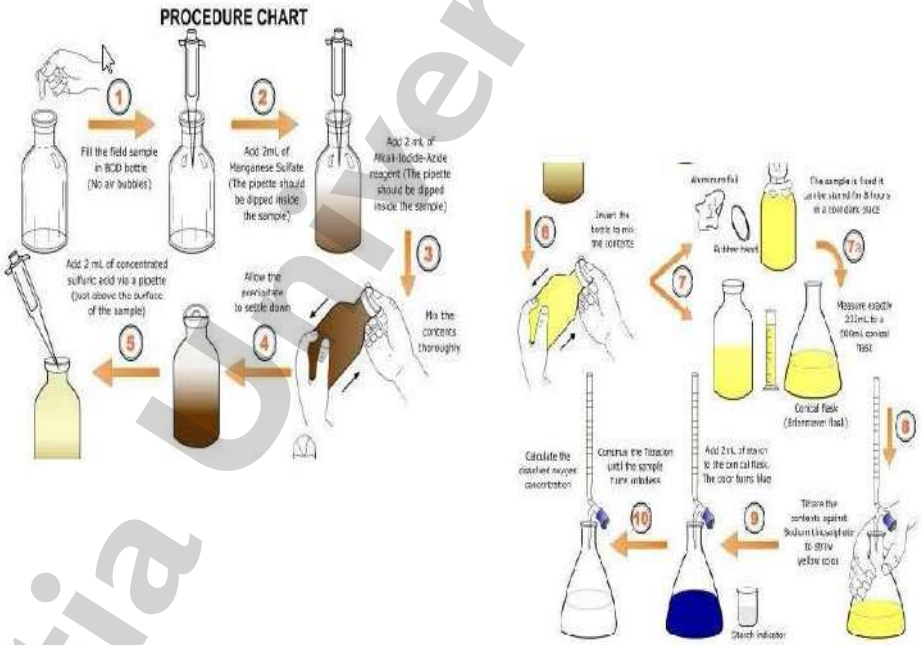
Starch Indicator (1%):

Take 1 gm of starch powder and dissolve in 100 ml of boiled water and stir.

Sodium thiosulphate: (N/40 or 0.025 N)

Take 6.250 gm of Sodium thiosulphate ($Na_2S_2O_3$) in previously boiled distilled water and make up to 1000 ml

Apparatus required:



(1litre) volumetric flask.

Pipettes -2 ml

BOD bottles-300 ml

burette -25ml and stand

conical flasks-250 ml

measuring cylinder-100 ml

PROCEDURE STEPS:

Collect sample in 350 ml BOD bottle.

Add 2ml of each Winkler's A and Winkler's B to the sample.

Shake well and allow to settle the precipitation and store in a cool place.

Then wait for sufficient precipitation and not to expose of direct sunlight.

Brown color precipitation is dissolved by adding 2 ml of Conc. H_2SO4.

Take 100 ml of that sample in 250 ml conical flask

Add 2-3 drop of starch indicator.

Dark blue colour appears.

Titrate with 0.025 N of Sodium thiosulphate.

End point blue color to color less.

CALCULATION OF DO:

T X N X 8 X 1000/ V

Where, T= ml of titrant

N= Normality of the titrant

V= Volume of the sample

Express the result as mg/l

c) FREE CARBON DIOXIDE (CO2)

Aim: To estimate Free Carbon Dioxide (CO_2) content in the given water sample.

Principle: Free carbon dioxide reacts with sodium hydroxide to form sodium carbonate.

Aqueous solution of sodium carbonate reacts with more free carbon dioxide to form sodium carbonate.

Completion of the reaction is indicated by the development of the pink color characteristics of phenolphthalein indicator at pH 8.3.

$$2NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

Reagent preparation:

Phenolphthalein indicator: Dissolve 0.5 g Phenolphthalein in 50 ml of 95% ethyl alcohol. Make up the volume to 100 ml with recently boiled distilled water.

Sodium hydroxide solution (1N): 40g sodium hydroxide dissolved into distilled water and volume make up to 1000 ml.

Sodium hydroxide solution (N/44): 22.7 ml of 1N sodium hydroxide solution diluted to 1000 ml with distilled water.

Apparatus required:

1. Auto-zero burette- 25ml capacity

2. Conical flask -150 ml capacity

3. Measuring cylinder-50 ml capacity.

PROCEDURE STEPS:

Take 50 ml of sample in a conical flask.

Add 2-3 drops of Phenolphthalein indicator

If pink colour appears then no CO_2 is there.

Then, not titrate the sample with 0.0227N Sodium hydroxide.

End point: color less to pink color.

CALCULATION OF CO2:

Free CO2= TXNX44X1000/V

Where, T= ml of titrant, N= Normality of the titrant and V= Volume of the sample

https://www.youtube.com/watch?v=vwY-xWMam7o

https://www.youtube.com/watch?v=FKdzbgHaQQM

Practical 4 Determination of Chemical characteristics (Alkalinity, Hardness) of inland waters

d) ALKALINITY (APHA, 2005)

Aim: To estimate alkalinity content in the given water sample.

Principle: phenolphthalein alkalinity is determined by titration of a sample aliquot with a standard solution of strong acid to a pH 8.3. in this stage, when the water sample is titrated with phenolphthalein indicator, its carbonate content is converted into bicarbonates.

$$H_2SO_4 \longrightarrow H^+ + HSO_4$$
$$CO_3^{2-} + H^+ \longrightarrow HCO3-$$

Total alkalinity or Methyl orange alkalinity is determined in water by titration of a sample aliquot with a standard solution of strong acid using methyl orange indicator which determines the equivalent point of pH of the titration. During this titration all the bicarbonates (HCO3-) are converted into carbon dioxide and water.

$$H_2SO_4 \longrightarrow H^+ + HSO_4^-$$
$$HCO_3 + H^+ \longrightarrow H_2O + CO_2$$

REAGENT PREPARATION:

Phenolphthalein indicator: Readymade phenolphthalein indicator solution is available for laboratory use.

Methyl orange indicator: Readymade Methyl Orange indicator solution is available for laboratory use.

Standard H_2SO_4 or Sulphuric acid (N/50 or 0.02N)

0.56 ml concentration sulphuric acid diluted to 1000 ml with distilled water.

Apparatus required:

Measuring cylinder- 50 ml

Auto-zero burette- 25ml capacity

Conical flasks- 50 ml capacity

PROCEDURE STEPS:

Take 20 ml of sample in a conical flask.

Add 2-3 drops of Phenolphthalein indicator.

If pink colour appears (pH >8.3) then titrate with 0.02N H_2SO_4 and note down reading as "A"

If sample remains colour less after adding Phenolphthalein indicator then add 2-3 drops of Methyl Orange indicator.

Then titrate with 0.02N H_2SO_4 and note down reading as "B"

End point colour changes from Orange to pink colour (pH comes down to 4.5).

CALCULATION OF ALKALINITY:

Phenolphthalein alkalinity: $\frac{\text{Vol. of Sulphuric acid used (A) X 1000}}{\text{Vol. of sample taken}}$

Methyl Orange alkalinity: $\frac{\text{Total vol. of Sulphuric acid used (A+B) X 1000}}{\text{Vol. of sample taken}}$

e) HARDNESS

Aim: To estimate hardness content in the given water sample.

Principle: The concentration of Ca and Mg expressed as equivalent $CaCO_3$ is considered as a measure of total hardness. The Ca^{++} and Mg^{++} ions of the sample are titrated with Ethylene Diamine Tetra acetic acid (EDTA) disodium salt to form the stable Ca-EDTA and Mg-EDTA. A small quantity of Eriochrome Black-T (EBT) added to the water sample and buffered at pH 10 would lead to a soluble wine red complex with some of the Ca^{2+} and Mg^{2+} ions. During titration the EDTA will first complex of all of the free Ca2+ and Mg2+ ions and the solution turns blue.

The Ca2+ and Mg2+ ions would then dissociate from their complexes with Eriochrome black-T to form more stable complexes with EDTA

$$Ca2+ + Mg2+ + EBT\ (Blue) \longrightarrow Ca\text{-}EBT + Mg\text{-}EBT\ (wine\ red)$$

$$Ca\text{-}EBT + Mg\text{-}EBT\ (wine\ red) + EDTA \longrightarrow Ca\text{-}EDTA + Mg\text{-}EDTA + EBT\ (Blue)$$

REAGENT PREPARATION

1. Standard EDTA solution (0.01N):

Take 3.723g of EDTA (EDTA-Na_2, $2H_2O$), dissolved in DW and make up to 1000 ml. Store in polythene or Pyrex containers

2. Eriochrome black-T indicator: Dissolve 5 g of Eriochrome black-T in 100 mL of 95% methyl alcohol or readymade EBT indicator solution is available for laboratory use.

3. Ammonium buffer solution (Ready made solution/ Dissolve 16.9 g of Ammonium Chloride (NH_4Cl) in 142 ml. Conc. Ammonium Hydroxide (NH_4OH). Add 1.25 g magnesium salt of EDTA and dilute to 250 ml with distilled water)

Apparatus required.

1. Pipettes (1,2,and 5 mL.)

2. Measuring cylinder- 50 ml

3. Auto-zero burette- 25ml capacity

4. Conical flasks- 50 ml capacity

PROCEDURE STEPS:

Take 50 mL of sample in a conical flask.

Add 1-2 mL of Ammonium buffer solution

Add a pinch of Eriochrome black-T indicator.

Titrate against EDTA.

Add the last few drops at 3-5 second interval.

End point colour change is from wine red to ink blue.

CALCULATION OF HARDNESS:

$$\frac{\text{Vol. (mL) of EDTA used X 1000}}{\text{Vol. (mL) of sample taken}}$$

https://www.youtube.com/watch?v=pQCPqCXY01U

https://www.youtube.com/watch?v=ljNNZvxOw0g

Practical 5 Collection of fresh water plankton

Plankton

The term 'plankton' was coined by Victor Henson in 1887 to designate the heterogeneous assemblage of suspended microscopic materials, minute organisms and detritus in water which wander at mercy of winds, currents and tides. However, the use of the term has been confined to designate only the microscopic, free-floating organisms; which depending on their nature are divided in two major groups, namely, phytoplankton and zooplankton.

Collection of nano plankton

The nano plankton compared to net plankton has less number of species flagellates diatoms which have a size range of 5-20µm contribute more than 90% in the phytoplankton biomass.

Methods of collection

Bottle sampler

Bottle samplers are ideal for small quantitative phytoplankton collection. It is mainly used for the collection of water samples from any desired depth of shallow ecosystem from a stationary vessel – near shore waters, estuaries and mangroves. Surface water can be obtained by gently scooping water in to a container of suitable size from the leeward side of the ship. Subsurface water can be obtained by using sampler like Mayer's Water Sampler, Friedenger's Water Sampler, Nansen reversing water sampler, Vaan Dorn water sampler, Niskin water bottle, NIO water bottle, Universal water sampler, etc. Samplers are sent to a desired depth on the rope in an open condition.

Collection of net (micro) plankton

Plankton of more than 50µm size can be collected by ordinary net sampling. This method could preferably used for quantitative plankton collections, as large quantity of water is filtered. Net is

towed vertically, horizontally, or obliquely. Shape of nets commonly used is conical, conico-cylindrical and conical with a mouth reducing cone. Rectangular shaped nets have been designed. The net is attached to the wire directly with a bridle. At the cod end of the plankton net, a sampling bucket is attached.

Preservation of plankton

After the collection of plankton, the samples should be preserved immediately. For determination of chemical composition, specimens should be fixed within 10 min after collection. Add 2-3 drops of 2.5% formalin to each 100ml of sample.

Storage of sample

Container such as glass bottles with wide mouth, polypropylene with plastic screw on lid, polycarbonate containers, whirl pack – polypropylene bag are commonly used.

- Store the sample in a dust free dark and cool place
- Maintain the pH (6.5 to 7.5)
- Periodic checking for colour and pH is required

Labels

Water resistant papers are used for external and internal labeling with following information in each container. External label should have bottle no, station no, date of sampling, day/night, sky, time, depth of sampling, type of net, mesh aperture, type of haul, flow meter reading, collector name etc. and internal label should have station no, date of sampling, sampling depth, type of net, mouth size and mess size, type of haul, number of turns in flow meter, collector's name, etc.

https://www.discovercayugalake.org/plankton-sampling

Practical 6 Enumeration and Biomass estimation (Qualitative analysis) of Freshwater phyto & zoo plankton

1. **Qualitative estimation of plankton:**

Qualitative analysis of plankton involves three steps, they are **Splitting, Sorting, Counting**

a. Splitting:

• Transfer well mixed sample in to the Folsom's plankton splitter.

• Circular drum is moved back and forth to homogenize and to divide the sample into equal parts.

• The zooplankton sample to be sub sampled is poured into the drum and the drum is rotated slowly back and forth.

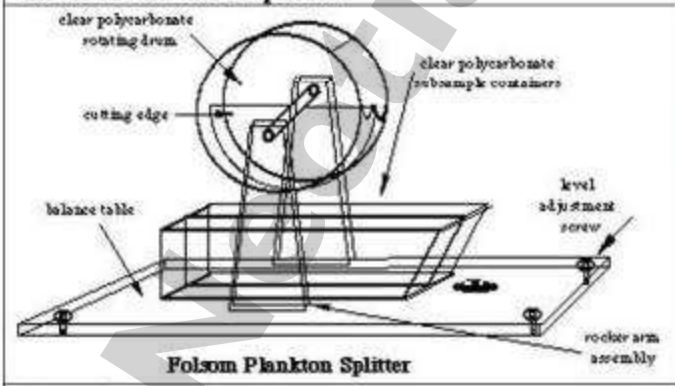
• Internal partitions divide the samples into equal fractions.

• The fraction may be poured again into the drum for further splitting.

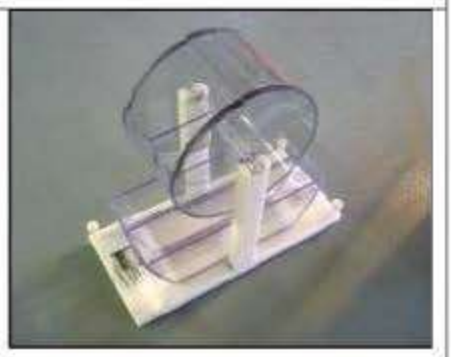
• The process is repeated until a suitable subsample is obtained for counting.

• The splitter is thoroughly rinsed to recover the organisms, which may be sticking onto the wall of the drum.

Folsom Plankton Splitter



Folsom Plankton Splitter



General Procedures:

- level table with adjusting screws
- pour sample into console and mix thoroughly
- rotate sample drum on rocker arm

sample is split into two compartments that drain into separate holding trays when rotation is complete

b. Sorting:

• Well mixed representative sample is poured into a clean petridish.

• Place the petridish on the dark background

• Use hand lenses or eye pieces (10 x) to identify the bigger organisms.

• Individual specimens belonging to same genera or species are grouped together and preserved separately.

• Identify the specimens to generic or species level.

c. Counting:

• Remove all macro zooplankton in the sample.

• Measure the total volume of water and mix well

• Transfer 1 ml of water sample into **Sedgwick rafter counting cell** by using wide mouthed pipette.

• Place the cover slip diagonally across the counting cell and introduce the sample from the corner. The cover slip moves into its proper position by capillary action.

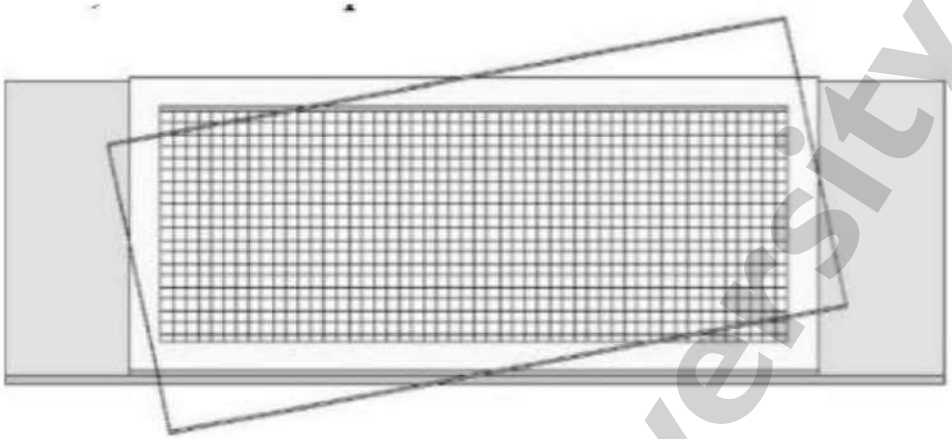
• The prepared cell is placed under microscope and allowed for sedimentation.

• The cell is moved horizontally or vertically along the first row of squares and the organisms in each square are counted.

• Organisms are identified to genera/species and counted accordingly.

• If one lack sufficient time to count all the squares in the counting cell, count just a few transects considering the homogenous settlement of the plankton.

• The total number of organisms are then computed by multiplying the number of individuals counted in these transects with the ratio of the whole chamber area to the area of intercepted transects.



Sedgwick rafter counting cell

Calculation:

The total number of plankton present in a litre is given by N= n × v / V Where: N = total number of plankton per litre n = average number of plankton in 1 ml v = volume of plankton concentrates (ml) V = volume of total water filtered

https://www.youtube.com/watch?v=PMvzK5G-G7M

Practical 7 Enumeration and Biomass estimation (Qualitative analysis) of Freshwater

2. **Quantitative estimation of plankton**

i. Volumetric analysis:

a. Settlement volume method:

• The plankton is allowed to settle by gravity and the space occupied by the settled material is taken as settled volume.

• Concentrate the sample using a net. A known volume of sample is transferred into a graduated cylinder or sedimentation / centrifuge tube.

• Mix well and allow the sample to settle for 1-2 days.

• The settled volume of the sample is recorded (cc).

• The volume of the sample s then calculated in m^3

b. Displacement method:

• The space occupied by the plankton is measured in terms of the equivalent volume of liquid displaces.

• The sample is filtered through plankton net of known mesh size.

• The concentrated plankton is carefully removed and transferred to 25ml measuring jar.

• Pour the water from 25ml burette into the measuring jar till the 25ml marks is reached.

• Record the volume remained in the burette (cc).

• Displaced volume is equivalent to the plankton present in the sample.

ii. Gravimetric method:

It is the total weight of planktonic organisms concentrated from the known volume of water.

a. Wet weight

It is the raw weight of the planktonic organism with their natural body fluids.

• Plankton sample is screened through filters.

• Adhering water is blotter off with filter paper.

• Transfer the plankton matter to a pre-weighed aluminium foil.

• Weigh the sample near to a milligram.

b. Dry weight

It is the raw weight of the planktonic organism without water content.

- The plankton concentrate for which the wet weight is already known is placed in pre-weighed platinum dish or silicon crucible.
- Dry in an electric oven at 60°C until all the water is evaporated
- The weight of the dish and contents are then weighed.

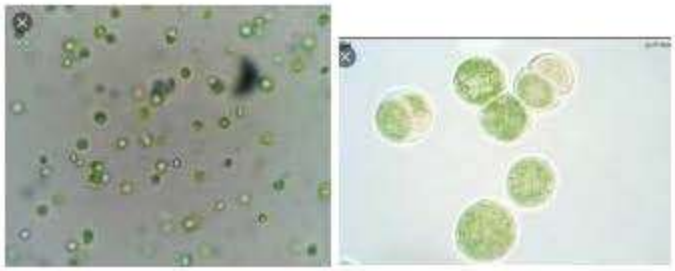
The difference between the final weight and dish weight gives the dry weight.

Note: The best expression of plankton biomass is dry weight preferably ash free dry weight or carbon weight. It is important to indicate the method of measurement along with results.

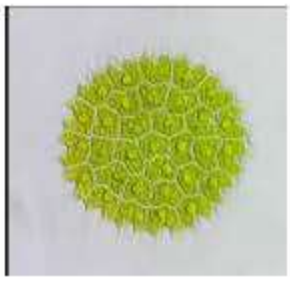
https://www.youtube.com/watch?v=PMvzK5G-G7M

Practical 8 Identification of freshwater Phytoplankton

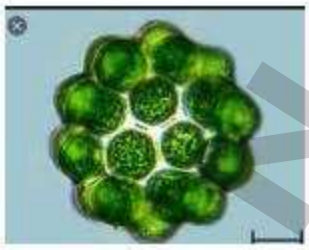
1. Chlorella: Cells spherical to ellipsoidal, solitary or aggregated, small smooth walled, cup-shaped reproduction by autospores, free living or symbiotic.



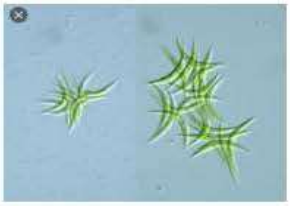
2. Pediastrum: Colonies stellate to disc-shaped, cells 4 to 128, polygonal, marginal cells mostly with one, two or four processes, cells multinucleate.



3. Coelastrum: Colony hallow sphere, rarely polygonal to pyramidal, cells 4 to 128, radially arranged, spherical, ovoid or pyramidal, chloroplast cup-shaped, reproduction by autocolonies.



4. Ankistrodesmus: Cells acicular or cresent shaped, solitary or in small loose groups, cells straight or curved, often twisted around one another, wall smooth with gradually tapering ends, spines lacking, chloroplast single, reproduction by autospores, planktonic.



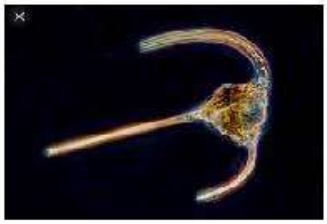
5. Selenastrum: Colonies without an outer mucilaginous envelope, consist of 4, 8 or 16 cells, chloroplast single, parietal, lying along the convex wall, reproduction by autospores, planktonic.

6. Ulothrix: Simple unbranched filaments of indefinite length, cells uninucleate, riddle shaped parietal band.

7. Spirogyra: Filaments long, unbranched, cells as long as broad or several times the breadth, chloroplast 1 to 16, ribbon shaped with half to 3 or rarely 8 left hand spirals.

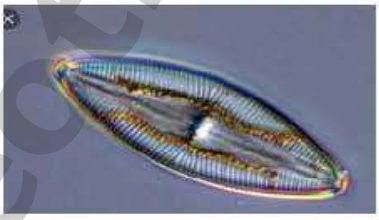
8. Euglena: Cells uniflagellate, fusiform to acicular, flexible, constantly change their shape, posterior end more or less pointed, gullet and eye spot anterior. Chloroplasts many, discoid to band shaped.

9. Ceratium: Cells broadly fusiform, 2 antapical plates which terminate in posterior horns.

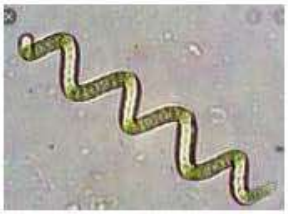


10. Synedra: needle shaped in both views or with slightly capitates poles, straight to curved.

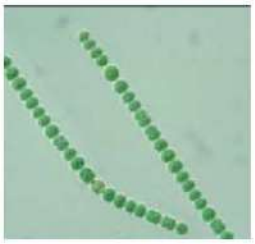
11. Navicula: rectangular in gridle view, latter is narrow and without any expansion.



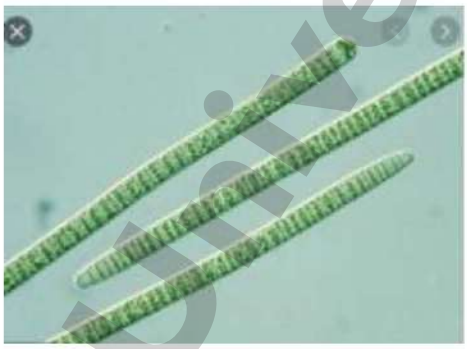
12. Spirulina: Cells terminals round, usually not tapering, regularly spirally coiled, spirals broad or narrow, planktonic.



13. Anabaena: Trichomes solitary or aggregated in a soft amorphous mucilaginous mass, thickness of trichome usually the same throughout, trichome with watery and inconspicuous sheath.



14. Oscillotoria: Trichome unbranched and without a distinct sheath, solitary and scattered or form expanded masses, mostly straight or in irregular spirals, ends distinctly marked.



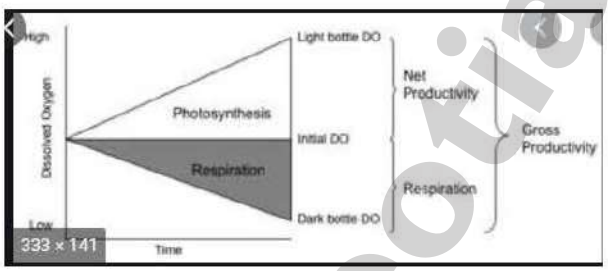
15. Amoeba: Body shape irregular, asymmetrical and changes constantly, pseudopodi and body with ecto and endoplasm, lobopodia many, indeterminate a directing locomotion, without shell or pellicle, covered with plasmalemma usually uni or binucleate, single contractile and food vacuoles conspicuous.

https://www.sepro.com/stewards-of-water/archive/algae-corner-how-to-identify-different-algae-types

Practical 9 Estimation of Primary Productivity in freshwater bodies

The primary production in the aquatic ecosystem starts with the synthesis of organic compounds from the inorganic constituents of water by the activity of plants / phytoplankton in the presence of sunlight. The inorganic constituents which form the raw material for this synthesis are water, carbon dioxide, nitrate ions, phosphate ions and various other chemical substances. The products are mainly carbohydrates and proteins and fats in very small quantities. Organic production by plants is the first step in tapping energy by living beings from non-living natural resources and hence called primary productivity.

The method of estimating primary productivity by **dark and light bottle method** was introduced by Garder and Gran (1930). In this method, the water samples are incubated for a certain period in light and dark bottles which are then suspended at the same depths from where the samples are taken. In light bottles, oxygen is released as a result of photosynthesis and a part of oxygen is used for community respiration. In the dark bottles, only oxygen consumption takes place as a result of respiration. The amount of oxygen liberated by phytoplankton during photosynthesis is considered as a measure of primary production.



Materials required

1. BOD bottle (2 light / transparent and 1 dark)
2. Nylon or Jute ropes
3. Burettes,
4. Reagents (Manganous sulphate solution, Alkaline iodide azide solution, Sodium thiosulphate, Concentrated Sulphuric acid, Starch indicator solution) etc.

Procedure:

a. Fill three BOD bottles with water sample in round stopper bottles (1 Light bottle, 1 dark bottle and 1 control light bottle) avoiding air bubbles.

b. Water sample in the control bottle is immediately fixed by using Winker's fixatives.

c. The dark bottle is wrapped with aluminum foil and kept in a black bag to protect from light.

d. Use one of the light bottles for estimating the initial dissolved oxygen As control)

e. Suspend both light and dark bottles exactly at the depth from where the sample was drawn are then suspended on to a raft and anchored.

f. The bottles are normally incubated for a period of 3-4 hrs between dawn to midday or sunset in the respective depths

g. At the end of incubation period, the bottles are retrieved and fixed with oxygen fixatives.

h. The oxygen content in the sample is determined by using Winkler's method.

Calculation

IB=Initial oxygen level
DB= Final oxygen level in dark bottle
LB=Final oxygen level in light bottle
LB – IB= Net oxygen production
IB – DB=Oxygen consumed for respiration
LB – DB=Gross production of oxygen
T=Incubation period
0.375: Ratio of weight of C and O
PQ (Photosynthetic co-efficiency) =1.2

Gross primary productivity= **GPP**

Net primary productivity=**NPP**

$$\textbf{G.P.P} = \frac{(L.B - D.B) \times 0.375 \times 1000}{T \times PQ} \text{ mg C/m3/hour}$$

$$\textbf{N.P.P} = \frac{(L.B - I.B) \times 0.375 \times 1000}{T \times PQ} \text{ mg C/m3/hour}$$

$$\textbf{Respiration rate} = \frac{IB - DB \times 1000 \times 1 \times 0.375}{t} \text{ mg C/m}^3\text{/hour}$$

https://www.youtube.com/watch?v=ibeR7Y_smec

Practical 10 Collection of freshwater Zooplankton

Zooplankton, the microscopic free-swimming animal components of aquatic systems, are represented by a wide array of taxonomic groups; of which the members belonging to protozoa, rotifer, cladocera and copepod are most common and often dominate the entire consumer communities. They are endowed with many remarkable features and are often armoured with spines, which hamper their predation by higher organisms. The ability of movement not only provide them an effective defense measure but also enable them to actively search and feed upon the phytoplankton. Their high and rapid rate of parthenogenetic reproduction usually overcomes the predation losses and enables them to exploit algal blooms. They constitute important link between primary producers and consumers of higher order in aquatic food webs. Therefore, the population dynamics of zooplankton with reference to system provides key information for the management practices.

Preparation of sample

• Collect known amount of water sample eg. 25 litre and filter through a plankton net of bolting silk of No. 25

• Transfer the net plankton in 50ml bottle and preserve in 5% formalin. Add few drops of glycerin to it.

• If further concentration is required allow the sample to stand for a day.

• Practically all the zooplankton will settle down at the bottom of the bottle.

• Remove supernatant plankton-free water with the help of pipette and reduce the sample to the designed volume.

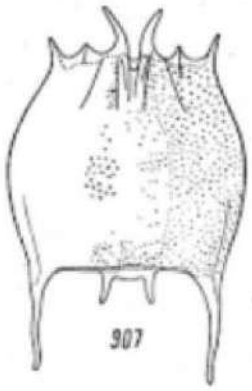
https://www.youtube.com/watch?v=n7Ggmh_uSaQ

Practical 11 Identification of freshwater Zooplankton

Qualitative and quantitative analysis:

Identify the zooplankton in the sample using keys and monographs. fresh water Rotifer

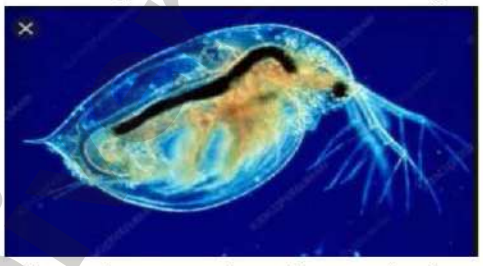
1. Brachionus: Dorso-ventrally flattened, anterior end with 2, 4 or 6 spines, posterior end angled, rounded or with 1 to 2 spines, foot opening posterior, foot long, worm like, wrinkled annulated, flexible, sharply marked off from body.



2. Keratella: thick, dorsally curved and ventrally flattened or concave, dorsal plate, anterior spines – 6, symmetric, posterior spines mostly present, foot and toes absent.

Cladocera (Water fleas)

3. Daphnia: Body compressed, valve surface squarish or rhomboidal, dorsal and ventral margins rounding over towards each other, posterior part provided with a sharp caudal spine, head not separated from body by a dorsal notch, females with well-marked and pointed rostrum, small antennules, 3-4 abdominal processes-anterior one bent forward, tongue shaped and long, males with large antennules and first leg with hook and long flagellum, lack rostrum.



4. Moina: head large, thick, rounded in front and bent downwards, without a beak, rostrum lacking, in female abdominal projection horse-shoe shaped, post abdomen wide, claw small.

Copepoda

5. Cyclops: Anterior part fatter and antennae comparatively shorter, first antennae 17 segmented, first 4 pair of legs 3-segmented, fifth pair of legs 2-segmented, the dorsal segment small, narrow eggs sacs two.

https://www.youtube.com/watch?v=n7Ggmh_uSaQ

Practical 12 Collection of Fresh water Benthos

The heterogenous assemblage or organisms attached or resting on the bottom or living in the bottom sediments of a body of water are known as benthos.

Phyto-benthos and zoo-benthos are the terms used for benthic plants and animals respectively. The term benthos is widely referred to flora and fauna which are intimately associated with sediments in an aquatic system.

Benthic environment represents bacteria, plants and animals including bottom living fishes from all phyla and their sizes widely varied. Benthic organisms are, in general sessile and slow moving in nature.

About 75% of benthic animals live on firm substrates (rocks, corals), 20% occur in sandy / muddy bottoms and only 15% of the total are planktonic.

Most of the benthic organisms are detritivores and form an important life in the food chain on account of their ability to convert low quality and low energy detritus into better quality food for higher organisms in the food web. By virtue of being relatively stationary, they are constantly exposed to changes in the mud-water interface and respond very well to it. Therefore, several pollution indices have been proposed using qualitative and quantitative change in benthic populations.

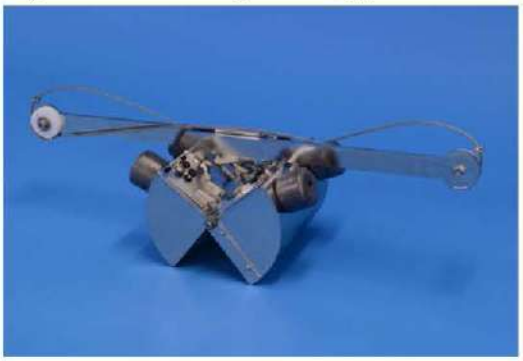
In the collection of benthos the samplers should be such that they penetrate well into the sediments to a sufficient depth in order to capture the organisms inhabiting in that area. The benthic samplers are of mainly two types, viz., grabs and core samplers.

Peterson grab : It is consisting of two hinged pincer like buckets which are sent down to the sediments in open condition. As the drawing line slackness, the release mechanism is activated. In retrieval, the two buckets come together and thus a semi-circular section of sediment is cut and entrapped. The drawing line is then pulled and the grab which is now in a closed condition is made open in a tray or bucket.

Ekman- Birge grab : This is the commonest grab devised for use in muddy bottoms by Ekman (1911) and Birge (1922). The two shovels which are kept open against very strong spring action by means of two chains are closed from the above by means of a weight (metallic messenger). Immediately after this operation, one can pull the grab out of the bottom and finally out of the water column. It is very heavy and made of brass in order to avoid rusting in the water. The upper portion is box shaped and it is closed by two movable covers which fall in under the pressure of the water when t ısal surface of this grab is about 250cm^2.

Van Veen's grab : It is also a very convenient and reliable grab devised by Van Veen (1936). The working principle of this grab is more or less similar to Ekman-Birge. However, it is held open by a small bar and is not operated by a metallic messenger. During operation, the grab is sent down the bottom when the two shovels out so that the bar is released automatically. The draw rope is attached in such a way that with the pull from above, the two shovels of the grab are

made to close tightly with mud sample entrapped in it. The basal area of this grab may be $1/20m^2$.



Core sampler : It consists of metallic tube of 50 cm length and 3cm diameter and is loaded at the top with a heavy lead weight. A valve present at its upper end allows the water in the tube to escape upwards when the corer is sent down to the bottom and it closes again when the tube is pulled up. The lead weight drives the tube strongly in to the mud, so that a profile of the bottom sediment is cut out. The sample is later removed from the tube with a rod.

https://www.youtube.com/watch?v=LYRudRHmRgA

https://www.youtube.com/watch?v=nmfJRg6f2HQ

Practical 13 Identification of Fresh water Benthos

Oligochaeta

1. **Chaetogaster**

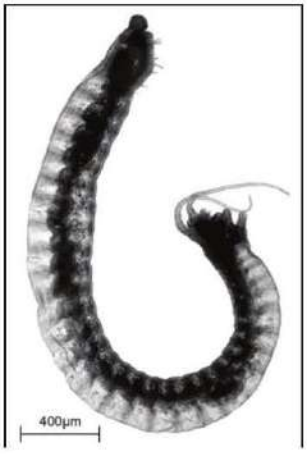
Body very transparent, anterior part broader, postomium well developed, in some bluntly pointed with stiff sensory hairs, dorsal setae totally absent, 2 bundles of hooked setae on the ventral side of each segment, no bristles in the segments 3 to 5, blood colourless, commonly associated with tubes of insect larvae.

3. **Nais**

Head distinct, both dorsal and ventral setae present, dorsal setae long, hair like, start at segment 6, not serrate, similar in length, ventral setae short and with cleft ends, of segments 2 to 5 mostly well differentiated than the posterior segments, posterior end not forming retractile appendages, blood yellow or red, only anterior body segments with lateral commissural blood vessels, spermatothecae in the same segments where testes are situated, body somewhat transparent.

3. **Dero**

Setae similar to Nais, posterior end modifies into a ciliated gill bearing retractile respiratory organ, the branchial area without long process or palps, blood reddish, eyes absent often in tubes.



4. Aulophorus

Dorsal and ventral setae as in Nais, posterior end modified into the respiratory organ, the branchial area, ventral margin of branchial area with long processes or palps.

5. **Stylaria**

Prostomium long, tentacles like, forming a long conspicuous narrow proboscis, a pair of eye spots present, setae an in Nais, dorsal setae begin in 5th or 6th segment.

6. **Lumbriculus**

Worms usually red or brown in colour, prostomium not elongated into a proboscis, paired setae on both surfaces of the segments, all of one form, forked at the end, distal tooth smaller than the proximal, dorsal blood vessels with paired contractile blind appendages, two pairs of sperm ducts with a pair of openings.

7. **Branchura**

Body may be reddish, fairly stout, very contractile, prostomium bluntly conical, ventral setae cleft, dorsal bundles comprised of 1-3 hairs, 5-8 needles forked in anterior region, anterior segments smaller, increasing posteriorly, with small at the tip, posterior segments with a dorsal and a ventral gill, gills non-ciliated, last segment without gill, live in tubes.

8. **Tubifex**

Worms reddish, coils into balls by slight disturbance, anterior end embedded in mud, waves the posterior end in water for aeration, segments clearly demarketed, prostomium short, triangular, tips pointed, eye spot and cilia absent, dorsal bundles with forked and usually hair setae, setae on ventral bundles usually forked, two lateral teeth of dorsal pectinate setae widely divergent, live in tubes.

9. **Tipula**

Larvae large, about 3 cm or more when extended, head retractile, head capsule broad and massive, non-sclerotized posteriorly and often also ventrally, last abdominal segment has rectangular plate surrounded by 6 to 8 lobes, anal gills not pinnately branched, pupa with long breathing horns.

10. **Antocha**

Head capsule slender, spiracular disc with 2 ventral elongated lobes and the rest reduced or vestigial, spiracles lacking or vestigial, larva and pupa enclosed in silken case, pupal respiratory tube 6 to 8 branched.

11. **Psychoda**

Body more or less cylindrical, without sucker discs, intermediate body segments without spiracles, thoracic and abdominal segments secondarily divided, at least terminal abdominal segments with sclerotized plates, preanal or prosternal plates absent, adanal region with a transverse plate, dorsal plates usually less than 26.

Mollusca.

12. **Lymnaea**

Shell thin with a prominent acute spirae, dextral, large often flaring aperture, columella hoisted, tentacles flattened on pair with eyes at their base, lip simple, acute, inner lip of aperture smooth, radula composed of 3 pieces, one large transversely elongated and two small, foot rounded behind.

13. **Gyraulus**

Shell discoid, small, apparently dextral, orbicular above, flat beneath, whorls few, rounded to carinate, rapidly increasing, shell with rounded periphery, shell compressed vertically so that aperture is much greater in breadth than height (oblique), somewhat deflected, tentacles cylindrical, jaws in 3-segments, radula with numerous teeth.

14. **Pisidium**

Shell small, oval to round thin, greenish or yellowish,3 cardinals, one cardinal in the right and 2 in the left wall, siphon two, short consolidated into a single tube, umbo back of the middle, directed backwardly, foot flattened, tongue shaped, capable of great extension.

15. **Corbicula**

Shell triangular, thick, equilateral, having the beaks central, outer surface with rather concentric ridges, lateral teeth serrated, 2 cardianal teeth in each valve.

16. **Lamellidens**

Shell nacreous, equivalve, oval, anterior end rounded posterior more or less pointed, umbo towards the anterior end, hinge teeth consisting of cardinals and posterior lateral, siphones short, complete, adductor muscles two, equal in size, foot large, byssus in young, cosmopolitan, freshwater in habit.

https://www.youtube.com/watch?v=nmfJRg6f2HQ

Practical 13 Collection of aquatic insects

- Insects are the most diverse and successful group of animals
- Nearly 80% of the world fauna are insects
- Quite a large number of insects spend at least part of their life in water
- 11 orders inhabit water – out of these only beetles and bugs spend their whole life in water
- They are dependent on atmospheric air for respiration
- Hence, more prevalent in shallow waters
- They are generally desirable on fish ponds since they are important part of the aquatic food web
- However, in nursery ponds they are undesirable, since they feed on spawn and fry.
- They also compete with spawn for food and space.

Major aquatic orders

- Hemiptera
- Coleoptera
- Odonata

Minor orders

- Ephemeroptera
- Trichoptera
- Lepidoptera
- Diptera

Practical 14 Identification of Aquatic insects

Materials required-

- rubber boots, hip or chest waders
- a long handled net or kitchen sieve
- a shallow white pan or bottom of a white plastic pail
- an assortment of small liquid proof vials and jars
- forceps
- "pooter"
- field notebook
- 90% to 100% alcohol for specimens collected from water
- 70% to 80% alcohol for specimens collected in aerial sweeps
- field bag to carry things such as the vials, forceps, notebook etc.

Procedure-

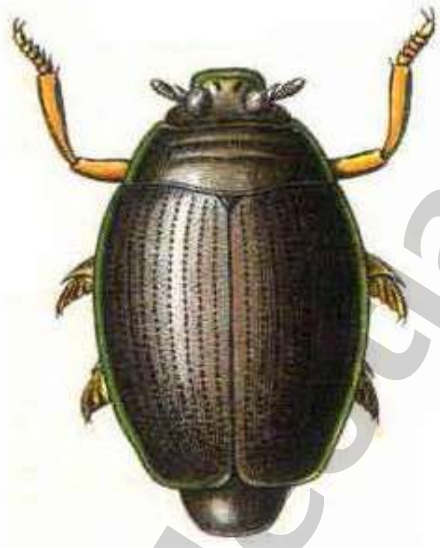
- To make collections in ponds and along lake shores the net is passed through the weeds and open water.
- In streams and rivers the net can be held downstream of the feet and the feet shuffled to disturb pebbles and small stones. Macro invertebrates on the stones will fall off and be carried by the current into the net.
- The net is then swirled in the water to remove fine silt and mud. The contents of the net are then dumped into the pan with about 2 cm of water in it. The macro invertebrates will be seen crawling out of vegetation, sticks and pebbles and can be easily picked up with forceps or a small net and transferred to a vial or small jar with preservative. The macro invertebrates can be found from submerged logs and stones by hand.

- Aerial net sweeps of the shoreline vegetation will collect adult aquatic insects and swarming adults.
- The net contents are examined and the insects removed using a pair of forceps dipped in alcohol.
- Many adult insects are attracted to lights, especially black lights, so checking lighted windows at night or looking around street lights can result in many interesting specimens.
- For each collection a label should be made indicating the location, date, and collector's name. I have found an HB Pencil or art pens are alcohol permanent. If mass produced labels are needed it is best to test the printer ink in alcohol for a number of weeks to ensure it does not run or fade.

Examples of Aquatic insects include-:

1. ***Gyrinus sp.***

- Order coleoptera
- Commonly called whilrgig beetle
- Bluish black in colour
- Antenna short, middle and hind legs flattened
- Feeds on vegetable and animal matter
- Harmful to fry



2. ***Dytiscus sp.***

- Order coleoptera
- Great diving beetle
- Grows to 2.5 cm
- Legs fringed with hair-like structures
- Hind legs flattened

- Feeds on spawn and fry

3. *Hydrophilus sp.*

- Order coleoptera
- Silver beetle
- Feeds on spawn and fry
- Carries water droplets at the top of hind legs which appear as silver globules

4. *Notonecta sp.*

- Ord. Hemiptera
- Body slightly cylindrical
- Anterior pair of legs leathery at the base

- Hind legs flattened and fringed for swimming
- Found in large numbers in ponds fertilized with organic manure
- Feeds on spawn and small fry
- Common name back-swimmer

Corixa sp

- Ord.Hemiptera
- Dorso-ventrally flattened body
- Tail end blunt
- Forelegs have flat structures
- Body dark greenish in colour marked with dark spots
- Harmful to spawn and fry
- Commonly called water boatman

Gerris sp

- Ord. Hemiptera
- Common name pond skater

- Longer and thinner legs
- Body thin
- Skates on water film
- Not so predatory

Lethocerus sp.

- Giant water bug
- Not so common in culture ponds
- Body flat, oval; brown or dull green
- Forelegs smaller, others long and ciliated
- Highly predatory, feeds on spawn fry and even fingerlings

Ranatra sp. (water stick insect)

- Body much elongated
- Legs long and slender
- Breathing tube long
- First pair of legs used for holding prey

Nepa sp.

- Water scorpion
- Body oval, prothorax broad
- Long spine like structure at the tail which act as breathing tube

Dragon fly nymphs

- Order odonata
- 2 types: short bodied and long bodied
- Found crawling at the bottom of the pond
- Small projections at tail region with mechanism to close and open
- Expulsion of air through pores helps them to propel in water
- Feed on spawn and fry

https://www.youtube.com/watch?v=rSDwu6f-wDo

Practical 15 Collection of aquatic plants from different freshwater body

The plants vary greatly in the degree to which they have become truly aquatic and present in an interesting series of gradations from those which are little more than amphibious, living at the edge of the water in very moist or water saturated soil. Aquatic plants are those unwanted and undesirable vegetation which reproduce and grow in water and if left unchecked may choke the entire body of water posing a serious menace to pisciculture. Another definition is that the surplus growth of a plant that influences adverse physical, chemical and biological effects on a water body with its resultant economic and aesthetic losses.

Collection of Aquatic plants

The aquatic plants can be collected using a long handled hook, nets or by hand. For quantification of sample in a given area the floating or sinking type of quadrates of known size namely (1m x 1m or 0.5m x 0.5m) made up of PVC pipes or wood are used. These quadrates are placed to mark the area from which sample is to be taken. After collection, these plants are brought to the laboratory for identification. Before identification of these plants, they must be classified based on their habitat into the following classifications, they are :

i. Floating macrophytes
ii. Marginal macrophytes
iii. Submerged macrophytes
iv. Emergent macrophytes

https://www.youtube.com/watch?v=UaAO2aDhtBg

Practical 16 Identification of aquatic plants from different freshwater body

Identified of Plants using the following keys:

i. Floating macrophytes

1. **Eichhornia sp (Water hayacinth or blue devil)**

Class : Angiosperm

Family : Pontederiacea

It is native of Brazil, accidentally brought to India and released in West Bengal, one of the most damaging aquatic weeds, inhabits stagnant and slow moving rivers.

Leaves broad with swollen stalks filled with air to enable them to float on water surface, dense leathery roots, flower pinkish in colour, multiplication by vegetative propagation, dries off in winter and spourts during summer.

2. **Salvinia (water fern velvet)**

Family : Salviniaceae

This plant has got rhizome, stalk or stem is delicate, oblong or hemispherical leaves, actual roots absent, leaves sessile with short stalk, leaves in two or more whorls, second whorl is either lateral and floating, third one submerged in water which looks like roots, lateral leaves sometimes filled with air which aids in floating.

3. **Pistia (water lettuce)**

Family : Araceae

A free floating perennial plant, plant body comprise a shell like rosette of tongue shaped leaves, reduced stem, sessile leaves and numerous branching roots, leaves form common cup shaped structure, leaves ovate and surrounded at the base by membranous sheath.

4. Lemna (duck weed)

Light green in colour, occurs in group of one to three, no distinct stem, leaves have flattened, minute leaf-like fronds, vegetative reproduction is rapid, often forming a scum over the surface, flowers are rare and so small that they are invisible to naked eye, appear as small weeds.

5**. Azolla (water velvet)**

Family : Azollaceae

Smaller plant, found in stagnant water bodies, leaves lobed, scale like, thick and about 0.5 mm in length, the entire plant is 1.5 – 2.0 cms in length, impart reddish green colour to water surface by covering it, it fixes atmospheric nitrogen.

ii**. Marginal macrophytes**

1. Colocasia

Family : Araceae

This plant covers large areas of the water body, leaves ovate, 6-20 inches long and 3-12 inches wide, leaf margin dark green in colour, base of stem triangular, petiole long up to 3-4 inches, colour of petiole green, violet or purple.

2. **Typha (Cat tail or Elephant grass)**

Family : Typhaceae

Common in margins of ponds, lakes, rivers and canals, perennial, creeping rhizome with leaves growing up to 2 m height and leaves have sheath at the base. Leaves bi-serrate, thick and spongy, secreting organ present at the leaf base, flower numerous and cylindrical.

4. **Marsilea (water shamrock)**

Family : Marsiliaceae

It inhabits ponds, rooted in shallow and stagnant waters, roots slender, stalks slender and thin, roots burrowed into the ground, petiole long with four cloves like or sharp pointed leaflets.

4. **Scirpus (Bullrush)**

Family : Cyperaceae

Annual herb, triangular in cross section, stem bears sheath at the base but sometimes leafy and naked, spiklets numerous with one or more long leaves from the base of branch, spiklets are usually with more flowers.

5. **Cyperus (Flat sedges)**

Family : Cyperaceae

Perennial herb with a single stem, cylindrical in cross section and hallow. The stem has sheath at the base and with one or more leaves on top forming a cluster, flowers or spikltes are present at the top.

iii. **Submerged macrophytes (Rooted)**

1. **Hydrilla**

Family : Hydrocharitacea

It is found to occur in almost all water bodies in India like ponds, lakes tanks etc. Leaves linearly arranged in whorls while stem is slender, grows up to 45 cms, has got fibrous roots, multiplies very rapidly by spores and vegetative propagation, infestation density is 20-30 kg per square meter, broken parts of this plant develops into a new plant by attaching themselves with the help of roots, provides shelter to young fish in aquaria offer a substrate for attachment of spawn of common carp.

2. **Chara (stonewort)**

Occurs in all types of freshwater bodies, stem has got erect branches and are gregarious in habit, nodes and internodes can be easily distinguished, grow up to 15 – 30 cm in length, remains unattached to the bottom, plant is rough to touch.

3. **Vallisneria (eel grass / tape grass)**

Plant with long ribbon like leaves measuring 0.5 – 1 m width, female flowers are long, thread like, twisted and appear at stalks, propagation is by offshoots, it can tolerate temperature of 25 – 30°C and medium water hardness.

6. **Ceratophyllum (Horn wort) - (Non-rooted)**

It has got a fragile algal like structure, grows to about 80 cms in length, roots are lacking, leaf branches are sometimes modified into rhizoids, lower part of stem serves as an anchore and helps in the absorption of nutrients, leaves are set in whorls, repeatedly forked with minute teeth on the side of the segment.

7. **Cobamba (Fan wort)**

Leaves are opposite, cut into thread like regions, stem slender with a gelatinous lining; plant provides shade and shelter for small organisms and forms a beautiful aquarium plant.

iv. Emergent macrophytes

1. Nymphaea (Water lily / Nilkamal)

Found in ponds, lakes, canals and also in water up to 1.5 m depth, perennial herb, petiole with lower end of leaflet, leaf round, veins radiating from the centre, leaves float on the surface of water, flower white or pink and solitary.

2. Nelumbo (Lotus)

Perennial herb, inhabiting tanks, ponds, lakes and other stagnant water bodies, leaves almost brown and are raised well above the water surface when mature, petiole attached to the centre of leaf, veins prominently radiate from the centre, flower large pinkish red leaf diameter ranging from 30 to 90 cms.

3. Trapa (Water chestnut / Singhara)

A perennial herb, occurs commonly in wild waters, leaves floating, solitary, branched or rhomboidal in shape, petiole with spongy swelling, flowers are solitary projecting over water surface, nuts with two or four sharp spines.

4. **Myriophyllum (Parrot head / Water milfoil)**

Found in stagnant and slow moving waters especially in places which are sheltered from wind, plants with slender, sparingly branched floating system mostly rooting freely at lower nodes, leaves opposite or whorled, the emergent leaves are horn like, flowers are very small and sessile and found in the axis of upper, emergent leaves grows to moderate height.

Other emergent type of plants are Nymphoides (Floating or Tringed water lily), Nuphar (Yellow lily or Cow lily) etc.

https://www.youtube.com/watch?v=UaAO2aDhtBg