Section III

Plankton and Periphyton Studies

Chapter 5

Collection, Preservation and Quantification (Plankton)

5.1 What are Plankton

Plankton are free floating organisms without powers of movement or with very feeble locomotion abilities. *Net plankton* are planktonic organisms which are caught in a fine meshed net and *nanoplankton* are organisms too small to be caught in nets and must be extracted from water.

The two wide categories of plankton are animal plankton (zooplankton) and plant plankton (phytoplankton). However, depending on the size, following classification has been made:

Ultra nanoplankton	below 2 µ
Nanoplankton	2-20 µ
Microplankton	20-200 µ
Mesoplankton	200-2000 µ
Megaplankton	above 2000 µ

5.2 Foreworks of Plankton Sampling

(1) Preliminary Preparation

Before plankton sampling, following fore works are to be done.

- Determine sampling location (also called ‘station’), its depth and prepare a

brief note.

- Label sample container with date, time, serial number, sampling station, study area, type of sample, depth and if surface plankton are concerned record surface temperature.
- If samples are to be preserved, make fixative ready so that it can be used immediately after collection.
- In the field record book, note sample location, depth, type, time, meteorological conditions, turbidity, water temperature, conductivity. Use leak proof vials or containers and waterproof labels.

5.3 Preservation of Plankton

(1) Preparation of Fixatives

Most commonly used preservatives are

1. 2-5% formalin
2. Lugol's solution
3. Formalin acid-alcohol (FAA)
4. Formal alcohol
5. 80% methyl alcohol

(a) 2-5% Formalin solution: Commercially available 40% formalin is a saturated solution for formaldehyde gas in water. This is suitably diluted with water for preservation of plankton.

(b) Lugol's solution: Water samples containing nano-plankton (especially zooplankton) are best fixed and preserved in lugol's iodine to which acetic acid or acetate has been added. Iodine fixes, preserves and colours the plankton while acetic acid preserves the flagella and cilia. To about 100ml of water sample containing nano-plankton add 2-3 drops of lugol's iodine solution. Keep the bottles tightly closed and store in dark. Such complex will keep good for a very long time. Allow at least 24 hrs for the nano-plankton to settle down by sedimentation.

BOX 2

Preparation of x% formalin

$$S_1V_1=S_2V_2$$

Here, S_1= x S_2 = 40
V_1 = 100 V_2 = ?

Since $S_1V_1=S_2V_2$, $V_2 = S_1V_1/ S_2 = 100x/40 = 2.4x$
If x = 5, 2.5X5 =12.5

Add 87.5ml distilled water to it. This is 5% formalin.

Preparation of Lugol's solution: Prepare lugol's solution by dissolving 20g potassium iodide (KI) and 10g iodine crystals in 200ml distilled water containing 20ml glacial acetic acid.

(c) Formalin Acid Alcohol (FAA): FAA is a good preserving and killing agent when the material is to be used for cytological studies of planktonic algae.

(d) Preparation of FAA: FAA is prepared by mixing together 50ml 95% alcohol, 5ml glacial acetic acid or 10ml 40% formalin and 35ml distilled water.

(e) Formal alcohol: It is prepared by equal parts of 5% formalin and 70% alcohol.

(f) 80% methyl alcohol: Methyl alcohol is diluted to make 80% to be used for preservation of plankton.

5.4 Plankton Sampling

5.4.1 Plankton Samplers

(1) Selection of Plankton Sampler

Samplers usually are referred to as 'surface' or 'depth' (or 'subsurface') sampler. The later can collect samples from some 'stated depth'. Samplers are specifically designed to collect samples from surface desired depth. Moreover, standardized samplers are required for qualitative and quantitative study of plankton. Depending on the objective and depth, plankton samplers can be of two types:

1. Water sampler bottles
2. Plankton net

(2) Water Sampler Bottles

Water sampler bottles are used for quantitative and qualitative study. It consists of a cylindrical tube or bottle of known volume with stoppers at each end. It is fixed to a graduated tube and sent down to the required depth. Then a weight called 'messengers' is released which slides down the supporting rope and performs the closing mechanism. The commonly used sampler on this principle is Kemmerer sampler.

(3) Plankton Net

Plankton nets are preferred where plankton are few and plankton samples are required for qualitative study. However, modifications are required for quantitative work. A typical plankton net is a bolting cloth constructed in a conical shape with circular mouth. The bolting cloth is made of silk, nylon or other synthetic fibres and is available in a variety of mesh sizes.

(4) Classification of Plankton Net Depending on the Mesh Size

Plankton nets are classified to different numbers (Silk No.) depending on the mesh size. This is important to know the mesh size of the plankton net since it affects the size of plankton collected. Table 2 gives a detail characteristic of commonly used plankton nets with the type of plankton can be collected.

Table 2. *Classification of plankton nets on their mesh sizes.*

Silk No	Mesh Size(μm)	Mesh^{-cm} (app.)	App. open area(%)	Type of plankton to be collected
000	1024	9	58	Largest zooplankton and Ichthypoplankton
00	752	11	54	Largest zoo- and ichthyoplankton
0	569	15	50	Largest zoo- and ichthyoplankton
2	366	21	46	Large microcrustacea
6	239	29	44	Microcrustacea
10	158	43	45	Microcrustacea and rotifers
20	76	68	45	Net phyto and zooplankton
25	64	79	33	Nannoplankton

APHA, 1998

BOX 3

How to determine unknown mesh size of a plankton net

The simple procedure to determine mesh size of a plankton net is as follows:

- With a millimeter scale, count the units of a plankton net up to an accurate whole number. Magnifying glass can be used for counting such units.
- Find the size of one unit in μm scale.

For example

Let 20 smaller units of plankton net = 2mm in scale

$$1 \text{ smaller unit} = \frac{2}{20} \times 1000\ \mu \quad (1\text{mm} = 1000\ \mu\text{m})$$

$$= 100\ \mu$$

The mesh size of the plankton net is 100μ. Therefore, only micro plankton (Table 1) can be collected by this plankton net.

5.4.2 Plankton Sampling Methods from Surface Water

Usually plankton are collected from the surface of water body. The commonly used tool for plankton collection from surface water body is plankton net.

(1) Equipment

1. A no. 5 or 20 plankton net
2. Plastic mug of accurately one litre capacity
3. Collecting vials (preferable 25ml)
4. Record book and label
5. Pen/pencil

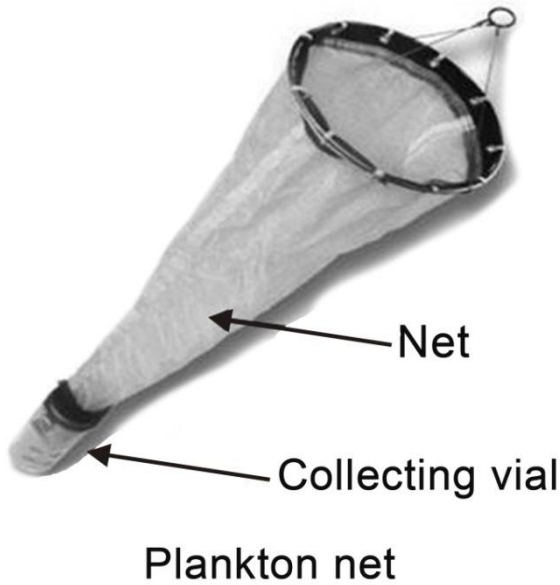

Fig. 3. *A simple plankton net for collection of plankton from surface water.*

(2) Reagents

Formalin (determine with a dropper the amount to be added to 25ml water to make 4%) or lugol's solution.

(3) Procedure

- Select sites for plankton collection.
- Collect water samples from selected sites with a mug of known capacity in volume.
- Collect full mug of water every time and count the number of full-mug water collection.
- Strain every mug of water through plankton net immediately after collection.
- Transfer collected volume of sample to collecting vial and pour required drops of formalin to make 4%. Lugol's solution can be used to make 1%. It will give a light tea colour.

(4) Precaution

- Before collection, the water surface should not be disturbed. A disturbance makes water turbid which block the collecting vial attached to net.

- Do not forget to label the vials with (i) time of collection, (ii) Date of collection (iii) Number of collection or total number in litre (volume) filtered (iv) Site of collection (v) Temperature at the time of collection.

(5) Sedimentation of Sample

Though there are different techniques of sedimentation of plankton, most commonly used technique is by centrifugation. In centrifugation, a pre-processing is done by allowing all preserved samples to settle down keeping the collecting vials on a plan surface for at least 1hr. This settlement is followed by gentle removal of approximately 10ml of water from the top of the sample bottle. The sample is then transferred to centrifuge for further sedimentation. However, this step can be skipped if a centrifuge tube of greater than 15ml capacity is used as collecting vial. In this case, the centrifuge tube that is used as collecting vial can directly be placed on to a centrifuge for further sedimentation. The rotor is balanced and centrifugation is done at 1000-1500 rpm for 15-20 minutes. After centrifugation, the volume in centrifuged tube can be reduced further to desired level. All processed centrifuge tube must be transferred to good quality glass vials with approximate volume of fixative. Labeling with initial volume (in L when sampled from surface water) and final volume (in ml after centrifugation) must be done for quantification.

5.5 Quantitative Analysis of Plankton

(1) Preparation of Slides

1. Agitate the settled sample after centrifugation or stored in vials.
2. With the help of a calibrated pipette, withdraw a required amount (See counting technique, Lackys' drop count) on the slide.
3. Calibrated pipette can be replaced by a standardized dropper. For this, a dropper with capacity 1.0ml is used. One can count the number of drops in 1.0ml and thereby volume of single drop can be calculated out. One drop of the sample placed on the glass slide is counted for plankton and then converted to 1.0ml subsequently.
4. Add one to two drops of glycerin to the slide. This prevents the organisms from drying after water evaporation.
5. Place a cover slip gently so that no air bubble forms.
6. Allow the slide to stand for few minutes. It helps in settling organisms for study.

(2) Counting Technique

Two counting techniques are widely used:

(1) The Sedgwick-Rafter (S-R) cell counting and

(2) Lacky's drop count.

5.5.1 The Sedgwick Rafter (S-R) Cell Counting

S-R cell is a device approximately of 50ml long by 20mm wide by 1mm deep. The total bottom area is approximately 1000mm or 1ml.

(1) Procedure

- Place the cover glass diagonally across the cell.
- Transfer sample (1ml) with a large-bore pipette or dropper. Mix thoroughly the sample before transfer.
- Allow cover slip to rotate slowly with a fine needle.
- Allow the S-R cell to stand for at least 15minutes to settle plankton.
- Now, count plankton on the bottom of S-R cell.

(2) Strip Count in S-R Cell

- Strip counting is done when few plankton are preserved.
- A strip is approximately 50mm long, 1mm deep, and the width of the total whipple grid.
- More strip count will give closer to the real value of plankton present.
- The number of plankton per ml can be derived from the following equation.

$$\text{Number/ml} = \frac{\text{C}\times 1000\ \text{mm}^3}{\text{L}\times\text{D}\times\text{W}\times\text{S}}\ (\text{APHA}, 1998)$$

Where,

C = Number of organisms counted;

L = Length of each strip (S-R cell length, 50mm) in mm;

D = Depth of strip (S-R cell length, mm) in mm;

W = Width of a strip (whipple grid width) in mm and;

S = Number of strips counted.

Number of plankton per litre of water can be determined as:

$$\text{Number/ml} = \frac{\text{Number/ml} \times 1000}{\text{Concentration factor (cf)}} \text{ (APHA, 1998)}$$

where

$$\text{cf} = \frac{\text{Volume of pond water filtered (ml)}}{\text{Volume of concentrate}}$$

(3) Field Count in SR Cell

- Field counting is performed for samples containing large number of plankton in a field.
- In this method, count plankton in random fields each consisting of one whipple-grid. More field selection gives more accuracy to the count.
- The number of plankton per milliliter can be determined as:

$$\text{Number/ml} = \frac{\text{C} \times 1000\ \text{m}^3}{\text{A} \times \text{D} \times \text{F}} \text{ (APHA, 1998)}$$

Where,

C = Number of organisms counted;

A = Area of a field (S-R cell depth) in mm;

F = Number of fields counted;

D = Depth of the field (1mm deep) in mm.

Number/ml can be converted to number/L following the conversion formula described for strip counting.

5.5.2 Lacky's Drop Count Method

This is a simple method for counting dense plankton population. Nano plankton can also be enumerated by Lacky's drop count method.

(1) Procedure

- Take one drop of sample by a pre-standardized pipette or dropper from a homogenized sample.
- Place a cover slip gently to avoid any overflow or air bubble.
- Allow 15 minutes to settle down all organisms.
- Start counting through strips.
- Calculate number of organisms per milliliter as follows:

$$\text{Number/ml} = \frac{C \times A_C}{A_S \times S \times V} \text{ (APHA, 1998)}$$

Where,

C = Number of organisms counted;

A_c = Area of cover slip in mm^2;

A_s = Area of one strip, mm^2;

S = Number of strips and;

V = Volume of sample under cover slip in ml.

Note: An easier method is to count the whole cover slide and present the organisms as number/drop. Since each drop is pre standardized before counting, the total count in a drop can be converted to any desired volume for quantitative expression of plankton analysis.