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IDENTIFYING SUITABLE METHODOLOGY FOR WATER SAMPLE COLLECTION TO STUDY ZOOPLANKTONS

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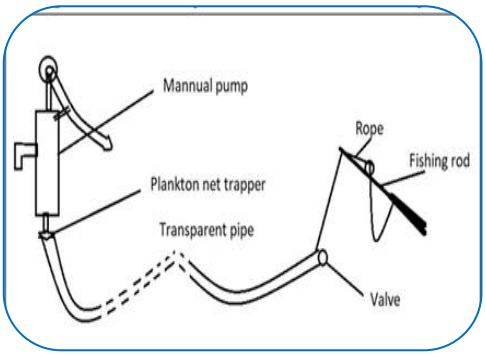
ABSTRACT:



The choice of water sampling techniques depends on the goals of the research, whether it targets specific species, examines diversity, or looks at community structure and composition. Although the bucket method captures the most diverse zooplankton, its reliability is compromised due to disturbances and considerable variability during sample collection. The effectiveness of plankton nets varies with mesh size and towing methods, with a 200-mesh net being the most efficient in horizontal tows, although it shows lower diversity than vertical tows. Vertical tows are better suited for studies focused on Cladocera, while horizontal tows provide a greater diversity of rotifers but yield fewer Cladocera. In comparison to other techniques, plankton nets are less efficient for collecting Copepods and Cladocera. The pump method enhances efficiency by allowing samples to be taken from different water layers, making it ideal for studies on community structure and taxonomy. Although the trapper pipe method is labor-intensive, it is effective for collecting benthic plankton, particularly Cladocera and Copepods.

KEYWORDS: *water sampling techniques , collecting Copepods and Cladocera.*

INTRODUCTION

It is challenging to decide correct method of water sampling for the study of zooplanktons and phytoplanktons. Even after several references it is quite difficult select suitable method, because lakes are unique in terms of its location, surrounding physiography, depth etc. It becomes further difficult where annual fluctuation of water volume is more. In this research we collected water samples for study of seasonal variation in zooplankton community, in which we employed various methods to decide upon the course of further collection. We used open water collection of bucket method, plankton net of different mesh size with horizontal and vertical tow, a simple Manual pump and trapper pipe. Open water collection of bucket method is crude method and not at all suitable for research. As samples collected by this method were not congruent with other method. It is not possible to collect epi, meta and hypolimnion water with this method. We found use of plankton net is not suitable for seasonally dry lakes were it is quite difficult to collect water samples with vertical tow, while horizontal tow has

clogging problem. Manual pumps are suitable for collection of water from required depth at cheaper cost. It is most suitable to study plankton community with respect to different thermo strata of water body. The trapper pipe method was bit tedious but found most suitable method for collection of benthic sample. The advantage of this method is zooplanktons are collected without any damage. With the advancement of technology robotic traps are most useful but it's cost is much higher than other methods mentioned above.

The conditions of sampling are different in oceans than freshwater because of various factors like location, quantity of water, depth of lake and amount of contamination while sampling etc. In freshwater the conditions further differ from lotic to lentic habitat. Where researcher has to adapt suitable method of sample collection for accurate results. Each lake is unique in terms of its geographical location, its bottom topography, depth, volume of water and seasons. Selection of the suitable method become more challenging when lake is almost dry having water level up to knee in dry season and more than 20 feet depth in rainy season. On one hand researcher needs suitable method to cope with all seasons, because change in sampling method depending upon water level may alter the estimates or results. Here are the few methods tried for the research on zooplankton collection, research aspirants can choose suitable method depending on available resources and objective of research because every lake is unique. The most popular techniques of sample collection are use of nets and traps (Wetzel et.al, 1979).

Many of research scholars worked over different sampling methods. Schindler described two devices for vertical sample collection in which one is inexpensive syringe method and other is transparent trap which is found to be more efficient than other popular methods or devices (D. W. Schindler, 2011). Some others describe efficiency of pump method is far better than trap or plankton net method. In a similar study on distribution of zooplankton in vertical strata pump method was used by Minna Rahkola, who says that plankton density obtained with pump method is far better than plankton net and tube sampler (Minna Rahkola et al., 1994). Waite and his colleagues designed a new submersible filter pump apparatus which is suitable for lotic and lentic habitats and also for cooling lakes. The advantage of the device was it can collect samples continuously with simultaneous filtration. This method has combined features of pump and tow net. (Waite, S.W. et al., 1980).

In the present study, we are going to analyse the different low cost methods of sample collection, which include open water collection by bucket method, plankton net of different mesh size with horizontal and vertical tow, a simple Manual pump and trapper pipe. All these methods are used and studied by different scholars, but the purpose of this research is to analyse efficiency of these methods. Though net is still popular method but it is having numerous constraints. The sample collection may be influenced by net design , haul speed, stall speed and zooplankton community structure which integrated to affect net filtration efficiency of plankton net (Donald J. McQueen et al., 1993). We analysed pump method of sample collection, which was found to be most efficient and convenient to collect water sample from three strata. Similar opinion was found with research done by Stephane Masson, in which he compared nets and pumps as sampling gears as in our case. According to their research pump system captured higher densities of animals per taxa as compared to cantilever and Wisconsin nets. The cantilever has advantage that it can collect mobile plankton more efficiently than pump and Wisconsin net (Stéphane Masson et al., 2004). Pump method has disadvantage that due to friction jointed appendages may damage thus misleading data analysis, therefore pump should be designed properly by using net. (Charles B. Miller & David C. Judkins .,1981).

In an experiment at Baltic lake the efficiency of plankton net was tested against trap method, in which it was found that plankton net was only 75% efficient that of trap method. Plankton net and trap were found to be equally efficient for collection of nauplius larva but plankton net is in efficient to capture adult copepod and Cladocera. The main findings of this research was only 65% of the total zooplankton biomass retained in the trap was collected by the net which concluded that net is not suitable method for collection of zooplanktons for quantitative analysis (Paula Kankaala 1984).

In a study of Canadian Shield lake, for collection of water samples from stratified lake that is epi-, meta- and hypolimnion water samples, two sampling methods were tested that is Plankton Net

and Pump. They found highest variation due to lake water layer effect. Within the same lake selection of different sampling methods explained more variation than that of lake effect for some zooplanktons which lead to alter interpretation of zooplankton abundance. This study found that pumping system is highly efficient in capturing high density of planktons per taxa while cantilever net was more efficient in capturing mobile taxa but its efficiency varied among layers so making difficult for definite conclusion. (Stéphane Masson et.al., 2004).

The advancement of technology can also be used in sample collection, like use of robotics, computer operated devices. But these are bit expensive because of involvement of technology. One of such technology is use of ROV (Remotely Operated Vehicle). It has useful features as to locate patches of species, a large number of sample can be studied in term of video or images which enables in situ observations of planktons similarly it is having disadvantages like it is difficult to study rare organisms, inadequate image resolutions and planktons responds toward ROV (Peter C et al., 1995). In this experiment we have also designed a new trap method. Entire experiment was carried on Kurnur reservoir of Solapur district, Maharashtra, India.

This study is related with analysis of seasonal variation in zooplankton community structure in which sampling problems at fresh water lakes which have far different condition than marine water like bottom topography of lake, lotic or lentic ecosystem and seasonal avalibility of water and so on. As a case study a Bori River dam is selected from India, in Solapur district of state Maharashtra. It is a government built Mini Porject by name "*Bori Laghu Prakalpa*" popularly called as Kurnur Dam which is also known as Bori Dharan locally. The detailed information about the dam is already provided in above paragraphs.

The monsoonal rainfall is un predictable therefore the reservoir water volume is also un predicable. In rainy season the reservoir is full when water over flow through gates and during summer in month of April and May, the water level reaches below dead storage that is not even up to knee. This provided the opportunity to study different water sampling technique for the study of planlkton.

MATERIAL AND METHODS

The sampling time is very important. Prior to the start with actual research work it was essential to identify the correct and suitable sampling method, therefore for water sample collection May month was selected because of maximum catch was found during summer season in various studies. Water samples were collected from four sites P1, P2, P3 and P4 in Kurnur dam. We employed following methods to study possible results of different water sampling methods. It include open water collection of bucket method, plankton net of different mesh size with horizontal and vertical tow, a simple Manual pump and trapper pipe.

A) Bucket method:

A hanging bucket of manageable size was selected and calibrated for its over flow volume which is measured to be average 5243 ml. To collect water sample, specified persons entered bare foot in water from margin up to depth of two feet from the firm water bottom to surface of water. At this depth the water level touches knees, which is safe and comfortable to collect water. With the help of swinging bucket the surface water is collected with horizontal tow by partly dipping and dragging bucket on water surface with the help of both arms. Water samples from different directions can be collected in this way which is filtered in plankton net of 200 mesh size. The plankton net is tied with sampling bottle at the bottom. This step was followed for ten times so as to filter near about 50 litters of water and each time over flow level from bucket is maintained.

A hanging can also be used for vertical tow. The bucket is dipped in water and its position is inverted and pulled rapidly to collect bottom water sample that is hypolimnion sample. The bucket can be made aperture at the bottom. The planktons from the entire vertical column can be collected by tying the net of 200 mesh size to the bottom of bucket and dipping inverted bucket without closing its aperture, in the bucket is reverted and pulled entire vertical column of water pass through bucket which is filtered through plankton net.

Advantage of this method are, water can be collected from any direction , water sample can be collected from epi, meta and hypolimnion. The undamaged planktons can be collected. The method is cost effective and eco friendly.

There are several disadvantage of this method like contamination of water due to physical disturbance, Planktons that avoid littoral area cant be collected like pelagic and benthic copepod and cladocera, clogging problem of plankton net from bottom samples collected in bucket. The planktons may escape due to disturbance.

B) Plankton net method:

Plankton nets of 80, 125 and 200 mesh size were used in this research. Mesh size is an aperture or eye size in net. Sample are collected through two methods that is horizontal tow and vertical tow. One need to enter in water through boat trawler to collect water sample. The handling of plankton nets from margin has possibility of damage and clogging. The surface water sample is collected through horizontal tow by tying floaters on end of the ring and heavy material at other end of the ring. Volume of water that flow through net can be calculated by formula as mentioned below.

It is very easy to collect water sample through vertical tow as ring at mouth of net is heavy it can dip deep into water. The depth of net can be measured with help of plastic rope tied with ring and marked with scales, which is used to calculate the amount of water flow through net during vertical tow.

Advantages of this method are, it is not required to get in to water, easy to collect samples. Benthic undamaged planktons can be collected. It is cost effective and eco friendl. There are few disadvantage, like clogging of plankton net, physical damage of net during tow, it can be properly operated in deep water and not suitable for shallow water. The planktons may escape due to disturbance

C) Manual pump:

A plastic Manual pump is available in market as shown in figure 2**,** The pumping action create vacuum in pump which can suck water from distant, through pipe. The biggest problem of pump is damage of planktons due to frictional force, which may pave the way for wrong inferences. So as to collect undamaged planktons with the help of pump we invented unique technique. The long flexible transparent pipe of 50 feet (1524 cm) length and 2.54 cm inner diameter pipe is used to collect water sample. One end of this pipe is tied to inlet end of pump and other end is fitted with valve to prevent back flow of water. The pipe is heavy itself therefore when free end is thrown into water it automatically dip in water and reach the bottom. The tip level of pipe can be maintained with the help of stick and rope marked with scales (Cantileaver). One can tie the tip with floaters or dippers. Therefore with this method water samples from epi, meta and hypolimnion can be collected without damaging planktons. Pumping force sucks water into the pipe from mouth of valve which prevent back flow of water. One can collect bottom clog with this technique. When the pipe is full with water by pumping force, it is withdrawn from the water body and its valve is opened in a bucket to collect water, which is filtered in plankton net of 200 mesh size to reduce the sample size. Another small modification can be done by placing filter of plankton at the junction of two pipes near the pump that filters only water and zooplanktons to plankton net at the junction of pipe and pump inlet. Later the free end of pipe is lifted from water and emptied in a bucket. The procedure is repeated seven times to collect near about 50 litters of water (each time 7725.3 litters). If the length of pipe is more than 50 feet it requires more pressure to be exerted by the pump and sample collection become hectic job. The free end of pipe can be tied with floater and plastic rope marked with scale. The floater and rope enables the tip of pipe to maintain particular depth from the surface of water.

Figure 1.12 Trapper pipe for water sample collection.

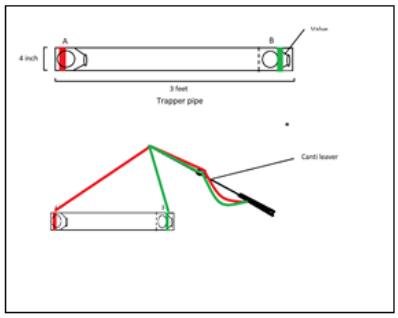
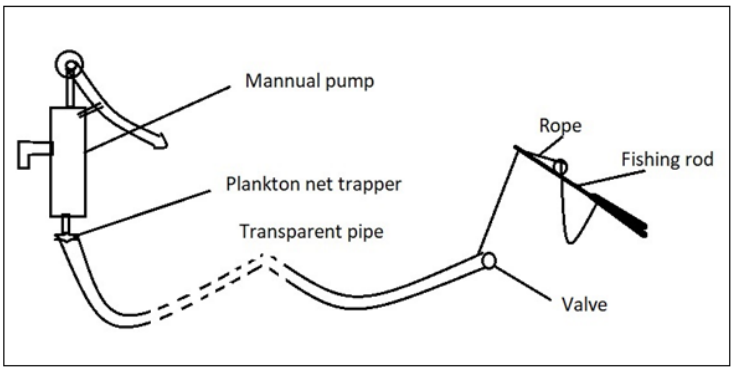


Figure. 1.11 Manual Pump method for collection of water sample.



There are some advantages of this method. One can collect water sample from epi, meta and hypolimnion. Bottom samples can be collected which is not possible in other methods. Water sample can be collected from margin of water body or boats. Undamaged zooplanktons can be collected from water trapped in pipe. Cost effective and ecofriendly.

There are some disadvantages of this method, like lot of strength is required for manual pump. Disturbance in water lead to escape of planktons but can be rectifiable by waiting to settle down the pipe.

D) Trapper pipe

The trap pipe is designed by us is very simple and cost effective. It consist of 4 inch pipe (10.16 cm) diameter of 3 feet length (91.44 cm) which can store 7416.317 litters of water. It has two ends A and B, and both ends are having valve and two different coloured nylon rope marked with scales. The A end is tied with red rope and B end is tied with green rope. Both of them are tied with fishing rod with pulley. When pipe is vertically dipped making A end towards bottom and B end towards water surface by regulating green rope, it allows the water to enter in the pipe. The green rope is liberated for required depth with the help of pulley. The pipe can reach the bottom because of its weight. The level of depth can be adjusted with help of above mentioned nylon rope with scales. The water is allowed to settle by waiting for few minutes. When there is no disturbance, escaped planktons return and may enter in pipe along with water. After few minutes the red which is tied with another fishing rod is pulled with help of pulley rapidly. This makes A end of pipe upward towards water surface and B end towards bottom. This rapid inversion blocks the valve and prevent exit of trapped water. As the length of pipe is three feet, it provides least opportunity for planktons to escape from pipe. The red wire is pulled with help of pulley to collect the pipe out of water. The rod for red rope must be sufficiently strong for to sustain near about 8 kg weight of pipe (Weight of pipe + Valves +water). The pipe is reverted in a bucket to collect trapped water and filtered through plankton net of 200 mesh size to reduce the sample volume. It is best method to collect water samples from hypolimnion without damaging planktons.

After collection of water sample either any of above methods, filtration with plankton nets is essential to reduce the sample size to 100 ml, therefore 100 ml plastic bottles are tied at free end of undamaged plankton net. After filtration and collection of water sample, immediately preserved by adding formalin powder. Small packets of 4 gm formaldehyde powder and 3 gm borax powder sachets are added to 100 ml bottles containing sample water. The another method of preservation is use of alcohol but we employed formaldehyde for preservation. The samples bottles are carried to laboratory for analysis of collection efficiency and to study suitability of methods of sampling.

Sample volume is further reduced to 20 ml by using small piece of plankton nets with 200 mesh size. The reduced volume is easy for further analysis with Sedgewick-Rafter Counting chamber. It is plastic chamber available in market. We used standard Sedgewick-Rafter Counting chamber with 50mm long x 20mm wide and 1mm deep Chamber that can withstand 1ml of sample volume. The fine

grid lines of 100 x 1mm squares makes easy for calculation. Number of organisms belong to Rotifera, Cladocera, Copepoda and Ostracoda are recorded in individual table. Further statistical part is applied as mentioned in result and discussion.

RESULT ANALYSIS AND DISCUSSION

The findings of various methods of water collection are discussed as follows. Water samples were collected from different sites at Kurnur Dam with ten different methods to select suitable method for further course of research. The Table. No. 1.1.0 mentions about the amount of water volume filtered through net. For horizontal and vertical tow, flow rate is considered to calculate the volume of water filtered.

Table. No. 1.1.0 Sampling methods and total water volume collected for analysis

Sr.No	Sampling Method	Volume of water sample collected for analysis in litters
1	Hanging bucket method horizontal tow	50
2	Hanging bucket method vertical tow	50
3	Plankton net method 80 mesh (Horizontal tow)	65
4	Plankton net method 125 mesh (Horizontal tow)	73
5	Plankton net method 200 mesh (Horizontal tow)	68
6	Plankton net method 80 mesh (Vetical tow)	66
7	Plankton net method 125 mesh (Vertical tow)	67
8	Plankton net method 200 mesh (Vertical tow)	62
9	Manual pump	50
10	Trapper pipe	50

Among zooplanktons following Rotifera, Copepod, Cladocera and Ostracoda were studied which are mentioned in Table. No 1.1.2 to 1.1.4

Table. No.1.1.2 List of Rotifer found in collected water sample.

Asplanchna sp.	*Cephalodella exigna*	*Keratella tecta*
Brachionus angularis	*Cephalodella forficula*	*Keratella tropica*
Brachionus calyciflorus	*Colurella adriatica*	*Kertella valga*
Brachionus caudatus	*Dicranophorus dolerus*	*Lecane bidentata*
Brachionus diversicornis	*Euchlanis dilatata*	*Lecane bulla*
Brachionus falcatus	*Filinia longiseta*	*lecane depressa*
Brachionus folculus	*Filinia opoliensis*	*Lecane pyriformis*
Brachionus forficula	*Keratella cochlearis*	*Lepadella ovalis*
B. quadridentatus	*Keratella procura*	*Lepadella patella*
Brancionous calciflorus	*Keratella quadrata*	*Monostyella sp.*
Notholca acuminata	*Testudinella patina*	*Tripleuchlanis spp*
Notomata copeus	*Testudinella sp.*	*Rotaria spp*
Proales decipiens	*Trichocerca tigris*	*Synchaeata spp*
Polyarthra spp		

Table. No. 1.1.3 List of Copepod found in collected water sample.

Mesocyclop sps	*Paracyclops spp.*	*Mesocyclops leuckarti*
Nauplius larvae	*Eudiaptomus gracilis Sars*	*Paracyclops fimbriatus*
Cyclops viridis	*Heliodiaptomus contortus*	*Trophocyclops prascinus*
Megacyclops sp.	*Diaptamus spp.*	

Table. No. 1.1.4 List of Cladocera found in collected water sample.

Alona	*Ceriodaphnia pulchella Sars*	*Bosmina longirostris*
Alona guttata Sars	*Cypris*	*Trophocyclops*
Biapertura	*Daphnia sp*	*Macrothrix spinosa* (King)
Biapertura affinis (Leydig)	*Daphnia cucullata Sars*	*Moina mircura*
Bosmina	*Flurcularia sp*	*Scapholeberis kingi*
Ilyocryptus sordidus (Lievin)	*Grimaldina brazzai (Richard)*	*Macrothrix goeldii* (Richard)

Table. No. 1.1.5 List of Ostracoda found in collected water sample.

Candocypris spp.	*Cyprinotus*	*Limnocythere*
Candona	*Cypris spp.*	*Metacypris*
Centrocypris	*Darwinula*	*Potamocypris*
Stenocypris spp.	*Ilyocypris*	*Cyprides*

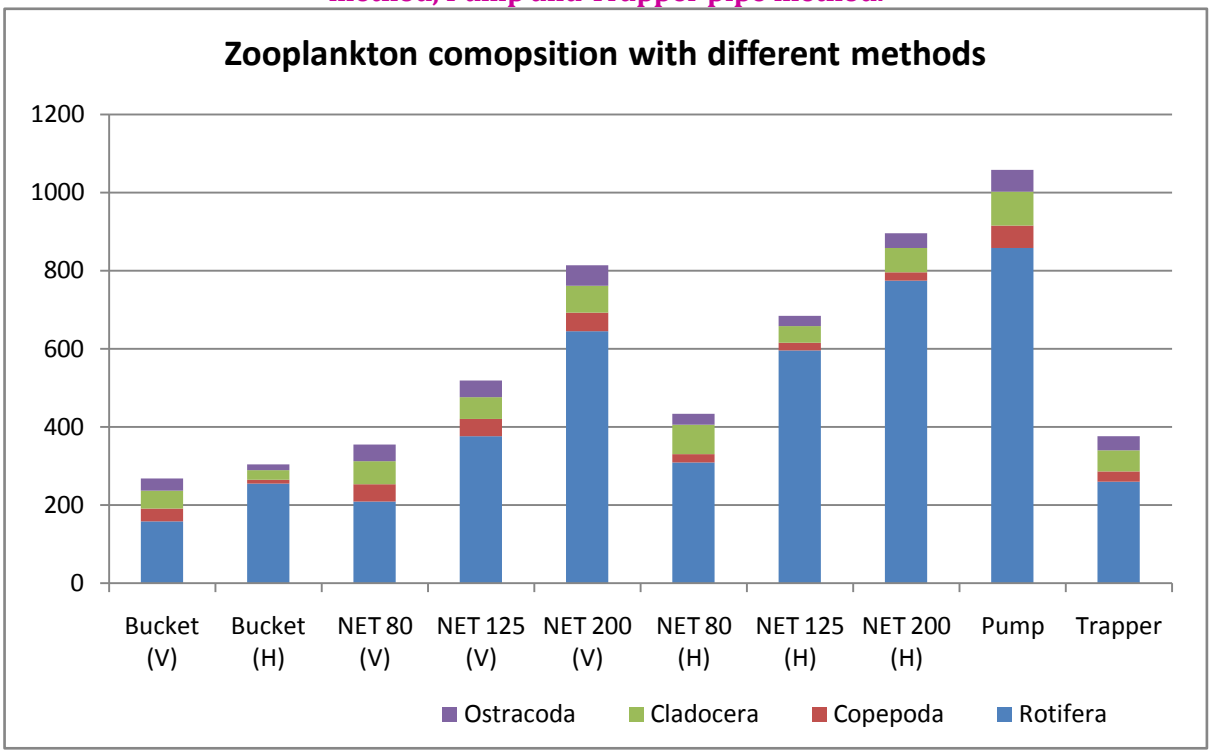
The data was collected and analysed in the laboratory. Two popular statistical technique Simpson index and Shanon index were applied to the collected data. The highest diversity among collected samples were found with vertical methods rather than horizontal tow methods because horizontal tow is dominated by Rotifera community while vertical tow collected samples from benthic part of the lake which include more numbers of Copepod and Cladocera. But the diversity doesn't give the clear picture of community composition, population density, as the community is dominated by Rotifera. From the graph, it is clear that Pump method has highest efficiency of collection by collecting maximum numbers of species.

Table. No.1.2.1 Simpson index and Shanon index

Indices	Bucket (V)	Bucket (H)	NET 80 (V)	NET 125 (V)	NET 200 (V)	NET 80 (H)	NET 125 (H)	NET 200 (H)	Pump	Trapper
Simpson Index (D)	0.41	0.71	0.4	0.55	0.64	0.55	0.76	0.76	0.67	0.51
Simpson reciprocal index (1/D)	2.46	1.4	2.48	1.82	1.56	1.83	1.31	1.32	1.49	1.96
Shanon Index (H)	1.12	0.61	1.12	0.89	0.74	0.87	0.52	0.53	0.69	0.94

Note: V- vertical, H- Horizontal, NET- Plankton Net, Pump- Manual pump, Trapper- Trapper pipe.

Figure. 1.13 Zooplankton Composition of water samples collected with Bucket method, Net method, Pump and Trapper pipe method.



CONCLUSION

Methods of water sample collection depends upon the purpose or objective of the study, therefore it varies with objective of species specific study, diversity study, study of community structure, composition study, population study or study of any particular effect. Bucket method is crude method of study though it has highest diversity among zooplanktons collection methods, in which one has to enter in the water which may create disturbance and allows escape of zooplanktons. Therefore it is quite difficult rely on Bucket method of sampling. Therefore there is high degree of variability in five samples collected with this method. The vertical tow of bucket has poor collection and high diversity over horizontal tow because of collection of benthic species. The plankton collection with help of plankton net has shown different result. The plankton net with 200 mesh size his highly efficient in terms of collection which yielded maximum collection and low diversity in horizontal tow rather than vertical tow as well as when compared to net of mesh size 125 and 80. Vertical tow of net is better for study of Cladocera which has shown high count as compared to horizontal tow. Horizontal tow of plankton net yielded high diversity of rotifer but low number of Cladocera which do not give the correct picture of community structure. The overall observation of plankton nets when compared to bucket method, pump method and trapper method they have low efficiency for copepod and Cladocera collection. Besides its efficiency varies with different layers of water. Pump method has high efficiency as compared to other methods. Its advantage is that water samples can be collected from any layers with the help of hand held cantilever or binding with floaters and dippers. It also allows horizontal move in particular layer that combines the property of vertical as well as horizontal tow of net method. The collection of sample yielded good count and diversity of planktons. It is suitable for study of community structure as well as taxonomic study. Trapper pipe method is time consuming and good for benthic sample collection. It gives high count for cladocera and copepod but low for rotifer. Therefore trapper pipe is suitable for specific benthic plankton study.

The methodology that involves collection of samples, fixation, preservation, analysis and computation of data (Goswami, 2004). The plan of water samples collection depends on the volume of

water in the reservoir. Bottrell et al. (1976), the relative efficiency of the sampling procedure utilized is determined with regard to the most efficient one. This assumes, as did Bottrell et al.,(1976) that the most efficient sampling is the one which catches the greatest number of individuals.

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