05 Collection and estimation of zooplankton

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Introduction

Zooplankters are the diverse, delicate and often very beautiful assemblages of animals that drift in the waters of the world oceans. The name zooplankton is derived from the Greek: Zoon, animal; planktos, wandering. They play a key role in the marine food web by transferring the organic energy produced by the unicellular algae to higher trophic levels such as pelagic stocks. Because of their critical role as food source for larval and juvenile fish, the dynamics of zooplankton populations, their reproductive cycles, growth and survival rates are all important factors influencing recruitment of fish stocks and thereby the magnitude of fishery. Majority of them are microscopic, unicellular or multicellular forms with size ranging from a few microns to a millimeter or more. In addition to size variations, there are differences in morphological features and taxonomic position. The zooplankton plays an important role to study the faunal bio-diversity of aquatic ecosystems. They include representatives of almost every taxon of the animal kingdom and occur in the pelagic environment. The zooplankton are more varied as compared to phytoplankton, their variability in any aquatic ecosystem is influenced mainly by patchiness, diurnal vertical migration and seasons.

Zooplankters are classified in four ways based on different criteria. Firstly, they are divided into Holoplankton and Meroplankton. Species spending their whole life in the pelagic realm are termed holoplankton (eg. copepods, chaetognaths, salps etc) and those drift in the sea only for a part of their life cycle are called meroplankton (larvae of benthic mollusks, barnacles etc). Secondly, zooplankters are divided into Protozoa and Metazoa. Among the protozoan group, the ciliates form an ecologically important group. They rapidly multiply and are often the first grazers during algal blooms (diatom blooms). Metazooplankton have comparatively longer life span ranging from several days (eg. rotifers) and few weeks (eg. small crustaceans) to several years (eg. large euphausiids in polar regions). Thirdly, zooplankters are classified according to their size. Depending on the size of plankton, several attempts have been made to classify them

Plankton	Size of plankton	Types of plankton found in the specific size group (words in bold indicate major occurrence)	Commonly found zooplankton
Nano plankton	2 - 20 μm	Bacterio- plankton, **Myco-plankton**, **Phytoplankton**, Protozooplankton	Heterotrophic nanoflagellates feeding on bacteria
Micro plankton	20 - 200 μm	Myco-plankton, Phytoplankton, Protozooplankton, Metazooplankton	Most protozoans especially ciliates, eggs and early larval stages of crustacean plankton and meroplanktonic larvae
Meso plankton	0.2 – 2 mm	**Phytoplankton**, **Protozooplankton**, Metazooplankton	Small hydro medusae, ctenophores, chaetognaths, appendicularians, doliolids, fish eggs and larvae together with older stages of crustacean plankton and meroplanktonic larvae
Macro plankton	2mm -20 cm	Phytoplankton, Protozooplankton, **Metazooplankton**	Larger specimens of hydromedusae, siphonophores, scyphomedusae, ctenophores, mysids, amphipods, euphausiids, salps, eel larvae etc
Mega plankton	20- 200 cm	**Metazooplankton**	Jellyfish , siphonophores, scyphozoan, pelagic tunicates, chain forming salps etc

and the present system followed is that described by Sieburth *et al.*, 1978 (Harris *et al.*, 2006).which is given below after incorporating some modifications.

Fourthly, zooplankters are classified into Neritic and Oceanic. Neritic plankton inhabits inshore waters upto about 200 m depth. Beyond that oceanic plankton prevails. In oceanic regime, they are again subdivided into epipelagic (0-200 m), mesopelagic (200- 1000 m) and beyond 1000 m depth. Of these, the epipelagic and mesopelagic are the main domain of zooplankton.

Methods of zooplankton collection

Collection of zooplankton was carried out by using water bottles, pumps or nets over the past years. Water Bottles are used mainly for collecting smaller forms or microzooplankton. Water is collected at the sampling site in water samplers of 5 to 20 litre capacity. Surface water can be collected by scooping water into the bottle of suitable size. While collecting the water samples, there should be minimum disturbance of water to prevent avoidance reaction by plankton. The Von Dorn bottles or water samplers with closing mechanisms are commonly used for collecting samples from the desired depths. These bottles, named after Dr. William Van Dorn of Scripps Institute of Oceanography can be used to obtain composite samples from several depths or to pool samples from one depth and thus can be used for both horizontal and vertical sampling. Horizontal bottles are often used for sampling at the thermocline, at other stratification levels, or just above the bottom. Because they collect whole water samples, all size classes of plankton are obtained. Zooplankton collected in the bottle are concentrated by allowing them to settle, centrifuging or through fine filtration. The advantage of this method is that it is easy to operate and sampling depths are accurately known. The disadvantages are that the amount of water filtered is less, the macro zooplankton and rare forms are usually not collected by this method and so it is unsuitable for qualitative and quantitative estimations.

Pumps are normally used on board the vessel/boat. The inlet pipe is lowered into the water and the outlet pipe is connected to a net of suitable mesh size. The zooplankton is filtered through the net. This method is used for quantitative estimation and to study the small scale distribution of plankton. The advantage of this method is that the volume of the water pumped is known and continuous sampling is possible. However, the sampling depth is limited to a few meters and it is difficult to obtain samples from deeper layers. Disadvantage is that larger plankton especially the gelatinous forms like the medusae, ctenophores and siphonophores etc can be damaged.

Plankton Nets are the most common method of zooplankton collection. The plankton nets used are of various sizes and types and can be broadly categorized as the open type used mainly for horizontal and oblique hauls and closed nets with messengers for collecting vertical samples from desired depths. Despite minor variations, the plankton net which is usually made of bolting silk, nylon or other synthetic material is conical in shape consisting of a ring (rigid/flexible and round/square), the filtering cone and a collecting bucket. The collecting bucket should be strong and easily removable from the net. In this method the amount of water filtered is more and the gear is suitable both for qualitative and quantitative studies. The mesh size of the netting material will influence the type of zooplankton collected. Different mesh sizes are available from the finest to the coarse pore sizes. The mesh size of 0.2 mm of monofilament nylon is usually used for collecting zooplankton for taxonomic and productivity studies. In addition to the mesh size, the type, length and mouth area of the net, towing speed, time of collection and type of haul will determine the quality and quantity of zooplankton collected.

The zooplankton collections can be made by horizontal, oblique or vertical hauls. In horizontal sampling the net is towed at a slow speed (1.0 to 2.0 knots) usually for 10 minutes. In oblique hauls, the net is usually towed above the bottom. The disadvantage of this method is that the sampling depth may not be accurately known. The net may be damaged if it touches the substratum. Vertical haul is made to sample the water column. The plankton net is lowered to the required depth and hauled slowly upwards collecting the zooplankton sample from the water column transversed by the net. Closing mechanisms are generally used to study zooplankton abundance at different depths.

Most of the zooplankters migrate vertically in response to light conditions. Their occurrence is poor in upper layers during daytime. For better quantitative and qualitative zooplankton collections, the suitable time for horizontal zooplankton sampling would be before dawn, after dusk or night. The net should be submerged in water. The horizontal collections are mostly carried out for the surface and subsurface layers.

There are a number of continuous net samplers and multiple net zooplankton samplers.

Continous net samplers are based on the principle of collecting animals on a continuous ribbon of netting and include the continuous Plankton Recorder (Hardy, 1939), the Longhurst Hardy Continuous Plankton Recorder (Longhurst *et al.*, 1966), the Autosampling and Recording Instrumental Environmental Sampling System (Dunn *et al*, 1933a, 1993b) and the high-speed Gulf-III OCEAN sampler (Nellen and Hempel 1969).

The second group of samplers, the multiple net samplers are based on the principle of opening and closing a series of individual plankton nets in succession. To sample deep areas, Multiple Opening and Closing Sampler with 5 to 10 nets which can collect zooplankton simultaneously at different depths are used. The nets are closed by means of messengers

before retrieval of samplers.

Multiple net systems now routinely carry sensors to measure water properties such as temperature, pressure/depth, conductivity/salinity, phytoplankton fluorescence/biomass and beam attenuation/total particulate matter. They also measure net properties such as volume of water filtered, net speed, and altitude from the bottom, as well as net function such as an alarm to tell when a net closes.

Flow meter reading and calculations

A flow meter has to be fitted in the middle of the frame of the zooplankton net to understand the quantity of water filtered through the net for quantitative estimation of plankton collected. Flow meter is a small device with a propeller at one end and there is a small window on one side where the revolutions of the propeller are indicated in numbers. For the purpose of calibrating the flow meter, the net fitted with flow meter has to be towed for a known distance either vertically or horizontally. The number of revolutions made by the flowmeter during the haul has to be noted from the flowmeter. The volume of the water column through which the net travelled is then calculated using the formula $\pi r^2 h$, where r is the radius of the mouth ring and h is the known depth or the horizontal distance. By using the volume of water column and the number of revolutions made by the flowmeter, the volume of water which can be filtered in one revolution is found out. This is the calibration factor which can be used to multiply the number of revolutions made at each haul for a particular station to calculate the volume of water filtered by the net.

Fixation and Preservation

Zooplankton for taxonomic study should be fixed and preserved immediately after collection to prevent degradation due to bacterial action, cannibalism or chemical deterioration. Fixation is done to kill an organism by maintaining its morphological characteristics and preservation is done for maintenance of the fixed condition for long periods of time. The most common fixing and preserving reagent is formaldehyde (4-5%) and the zooplankton samples can be stored for several years. It is advisable to use buffered formalin. The commonly used buffers are borax (sodium tetraborate) or hexamethyene teteramine. The buffers are added in an amount of 200 g to one litre of concentrated formalin.

Estimation of zooplankton

Quantitative estimation

Zooplankton biomass can be estimated by the following three methods (Goswami, 2004).

1. Volumetric (displacement volume and settling volume) method
2. Gravimetric (wet weight and dry weight) method
3. Chemical method

Larger zooplankters (macrozooplankters) such as medusae, ctenophores, fish larvae, salps and siphonophores should be separated from the zooplankton sample and their biomass taken separately. The total biomass would be the biomass of bigger forms plus the biomass of the rest of the zooplankton and should be indicated in the analysis sheet.

In the volumetric method, the total zooplankton volume is determined by the displacement volume method and is expressed as ml per m^3. The displacement volume can be estimated by two methods. One is by using an volume determiner. The volume determiner is a transparent cylindrical plastic apparatus of 100 ml capacity with both ends open. One end is then fixed with a piece of netting of the same mesh size used for the plankton net and can be fixed water tight over its base made of plastic. On the other end is a removable lid of plastic with a side hole. From the centre of the lid hangs a metallic pointer which when the lid is fixed over the cylinder would reach up to the distance where 50 ml mark is made on the cylinder. The preserved plankton is poured into the volume determiner. The water filters out and the interstitial water remaining in the plankton is removed by placing the cylinder over a blotting paper repeatedly till the water gets completely run out. The cylinder with the plankton is fixed water tight over its base. Adequate quantity of 5% formaldehyde solution is slowly let out from a 50 ml burette without any air bubbles till the water level just touches the pointed needle of the lid. The level of solution remaining in the burette is equivalent to the volume of plankton in the cylinder. Another method is by filtering zooplankton through a netting material having a mesh size of equal or smaller than the net used for collecting plankton. Then, the interstitial water between the organisms is removed with blotting paper and the plankton has to be transferred to a measuring cylinder with a known volume of 4% buffered formalin. Thus the difference in levels of solution in the measuring cylinder is equivalent to the volume of plankton.

The volume of plankton is also determined by noting the settled volume after the plankton sample allowed settling for at least 24 hours. The volume of plankton per m^3 of water filtered can be estimated by calculating the quantity of water filtered by the net during sampling as described earlier.

In the Gravimetric method, wet weight and dry weight can be taken. The wet weight can be taken after filtering and removing interstitial water on a preweighed filter paper or aluminium foil. The wet weight is expressed in grams. The dry weight is determined by drying an aliquot of the zooplankton sample in a predried and preweighed filter paper in an electric oven at a constant temperature of 60ºC for 24 hrs. The dry weight is expressed in milligram. The weight of plankton is expressed as g per m^3 or mg per m^3.

In the Chemical method, measurement of elements such as

carbon, nitrogen, phosphorus and biochemical elements viz. protein, lipid and carbohydrates are being carried out.

Qualitative estimation

In the qualitative estimation, the individuals in the sample will be identified and enumerated. Enumeration of specimens in the whole sample is mostly not practical as most of the samples contain numerous individuals. Hence, a subsample or an aliquot of 10 to 25% is usually taken for enumeration. However, the percentage of aliquot can be increased or decreased depending on the abundance of zooplankton in the sample. Folsom plankton splitter is widely used for subsampling. By this, the sample can be divided into two equal halves at a time. This dividing process has to be continued till a suitable subsamble is obtained for counting. For counting, a Sedgwick Rafter Counting Cell can be used which is kept under a stereoscopic microscope. The counts in the subsample have to be raised to the total volume. The numbers have to be expressed in per $m^3$of water by considering the volume of water filtered by the net during sampling.

Suggested reading

Goswami. S.C. 2004 Zooplankton Methodology, collection and Identification- a field manual. National Institute of Oceanography. 16pp

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