

## Diving into the Microcosm: Zooplankton Collection and Identification

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### SUMMARY

This abstract provides an overview of the methodology employed for the collection and subsequent identification of zooplankton, essential components of aquatic ecosystems. The study of zooplankton is pivotal in understanding the dynamics and health of aquatic environments, as these minute organisms are key indicators of ecosystem stability and environmental changes. The methodology for zooplankton collection involves the utilization of various sampling devices, such as plankton nets, pumps, and sediment traps, designed to capture these organisms at different water depths. The collected samples are then carefully preserved and transported to a laboratory for further analysis. Identification of zooplankton entails a multi-step process that typically includes sorting, preservation, and microscopic examination. Specialized taxonomic keys, microscopes, and image analysis software aid in species identification. Morphological characteristics, such as body shape, appendages, and size, are used to classify different zooplankton species. The accurate identification of zooplankton is crucial for assessing water quality, ecosystem health, and ecological changes. It provides valuable insights into the impact of environmental factors, such as temperature, nutrient levels, and pollution, on these organisms and their role in aquatic food webs. Zooplankton data also contribute to the broader understanding of ecosystem dynamics and inform management and conservation efforts in aquatic environments.

### INTRODUCTION

Zooplankton, deriving its name from the Greek words "Zoon" (meaning animal) and "planktos" (meaning wandering), comprises a diverse array of floating and drifting organisms with limited locomotive abilities. The majority of zooplankton consists of microscopic, single-celled, or multicellular organisms, varying in size from a few microns to a millimeter or even larger. Beyond size distinctions, these organisms exhibit differences in morphology and taxonomic classification. Zooplankton play a significant role in the exploration of faunal biodiversity within aquatic ecosystems. They encompass representatives from virtually every animal taxon and can be found in the pelagic environment as either adult organisms (referred to as holoplankton) or in the form of eggs and larvae (known as meroplankton). Due to their sheer abundance, occurring at various depths, zooplankton serve as valuable indicators for assessing energy transfer at secondary trophic levels. They feed on phytoplankton, facilitating the conversion of plant matter into animal tissue, and subsequently serve as a fundamental food source for higher animals, notably fish, including their larvae. Additionally, those zooplankton with calcareous or siliceous shells contribute to the composition of bottom sediments. Compared to phytoplankton, zooplankton exhibit greater diversity, with their variability in any given aquatic ecosystem being primarily influenced by factors such as patchiness, diurnal vertical migration, and seasonal changes.

### Methods of collection:

The zooplankton collection involves primarily the filtration of water by net, collecting the water in bottles/ water samplers or by pumps.

### Bottles / water samplers

This method is used mainly for collecting smaller forms or microzooplankton. The water is collected at the sampling site in bottles or water samplers of 5 to 20 litre capacity. The sterile bottles should be preferred. 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 obtaining samples from the desired depths. The microzooplankton are then concentrated by allowing them to settle, centrifuging or fine filtration

**Pumps**

The gear is normally used on board the vessel/boat. The sampling can also be carried out from a pier. In this method, the inlet pipe is lowered into the water and the outlet pipe is connected to a net of suitable meshsize. The net is particularly submerged in a tank of a known volume. This prevents damage to the organisms. The zooplankton is filtered through the net. A meter scale on the pump records the volume of water filtered. This method is used for quantitative estimation and to study the small scale distribution of plankton. The frictional resistance of the sampled water in the hose can cause turbulence; damaging the larger plankton especially the gelatinous forms viz. medusae, ctenophores and siphonophores etc.

**Nets**

The most common method of zooplankton collection is by a net. The amount of water filtered is more and the gear is suitable both for qualitative and quantitative studies. The plankton nets used are of various sizes and types. The different nets can broadly be put into two categories, the open type used mainly for horizontal and oblique hauls and the closed nets with messengers for collecting vertical samples from desired depths. Despite minor variations, the plankton net is conical in shape and consists of ring (rigid/flexible and round/square), the filtering cone and the collecting bucket for collection of organisms

**Fixation**

The necessity of proper fixation and preservation of zooplankton needs no emphasis. The poorly fixed and preserved samples would render their subsequent analysis difficult. The whitish precipitate and ruptured exoskeletons can be seen in the improper fixed samples. The zooplankton deteriorates rapidly in tropics. After the sampling, the fixation of samples should be carried out, as early as possible, at least within 5 minutes after the collection to avoid damage to animal tissue by bacterial action and autolysis. An ideal fixative should be cheap and which kills the animals quickly. Again it should be non-corrosive or toxic in nature. The most common fixing and preserving reagent is (4-5%) formaldehyde (formalin). It is the cheapest fixative and the zooplankton samples can be stored for number of years. The other fixatives occasionally used are ethanol, picric acid, acetic acid etc. The dilution is in the ratio of 1 part formalin and 9 parts of fresh water or seawater. The pH of the fixative should be around 8.0. It is advisable to use buffered formalin. The commonly used buffers are borax (sodium tetraborate) or hexamethylene tetramine. The buffers are added in an amount of 200 g to one litre of concentrated formalin. The fixative usually renders the zooplankton body tissues hard and brittle. The additives viz. propylene phenoxetal and propylene glycerol (2 to 5 %) are added to fixatives for flexibility of specimens, resistance to bacteria and moulds.

**Preservation**

Allow 10 days as the minimum fixation periods. After fixation, the zooplankton are transferred and stored in airtight containers with sufficient quantity of preservative. While transferring, due care should be taken so that no part of the zooplankton sample is lost. Various types of preservatives are available. The buffered formalin (4 to 5%) is mostly used both as fixative and as the preservative. The other preservative used is 70% ethanol or 40% isopropanol. The ethanol is used for preserving museum specimens but it is costly and volatile. Glycerin is often added to formalin to prevent shrinkage of specimens, drying of the material and to facilitate retaining colours of zooplankters. For better shelf life of the zooplankton samples, the preservative should be changed within the first 6 months.

**Analysis of the samples.**

The basic analysis consists of measurements of biomass (standing stock), enumeration of common taxa and species.

**Biomass**

The term biomass denotes the live weight or the amount of living matter present in the zooplankton sample. The value obtained is used to evaluate the secondary productivity and fishery potentials of the study area. The biomass is estimated by the following methods.

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

The total biomass would be the biomass of bigger forms plus the biomass of the rest of the zooplankton

### Faunal enumeration

Information on the faunal composition and the relative abundance of different zooplankton taxa and their species is obtained by counting the plankters present in the samples. The enumeration of specimens in the total sample is laborious, time consuming and mostly impractical. The number of common zooplankton groups and their species observed in the samples may vary from tens to thousands. For enumeration it is recommended that the subsample or an aliquot is taken for the common taxa. However, for the rare groups, the total counts of the specimens in the samples should be made. For enumeration of zooplankton the subsample or aliquot of 10 to 25% is usually examined. However, the percentage of aliquot can be increased or decreased depending on the abundance of zooplankton in the sample.

1. Subsample (aliquot) - Folsom plankton splitter is widely used.
2. Counting: After splitting, the next step in the analysis is to sort and count the specimens

### Species identification

Species is defined as a group of individuals capable of interbreeding. Correct species identification is prerequisite for understanding distributional pattern, seasonal variability and community structure of zooplankton in an aquatic ecosystem. It is a specialized work and requires patience, experience and sufficient published literature. The initial identification of common species could be done with the help of illustrated checklists. The taxonomic experts should later confirm the identification. The identified and labelled specimens should be kept properly for further reference. For identification of species a stereoscopic dissecting microscope, good quality glass slides, coverslips, stainless steel fine forceps, dissecting needles, pipettes and chemical reagents are required. It involves various steps such as cleaning of specimens, staining, dissection and slide preparation.

1. Narcotisation- initial reactions of zooplankters to any fixative and preservative are rapid and jerky movements, contraction of body and appendages
2. Clearing - The fixed specimens must be cleared of any attached material such as detritus or precipitate
3. Staining and dissection: Light staining of the specimens is carried out by adding a few drops of rose Bengal, lignin pink, chlorazol black E and methylene blue added to the lactic acid.
4. Mounting: Permanent glass slides are made by using the natural or synthetic resins. Canada balsam, gum chloral, glycerin jelly and lactophenol are used as mounting agents.

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