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Advances in zooplankton studies- an overview

Avances en los estudios de zooplancton- una revisión.

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ABSTRACT

Zooplankton are floating or drifting animals that have many ecological importance in both fresh water and marine ecosystems. Many are considered to be bio indicators and have undeniable role in energy transfer through food chains and biogeochemical cycling. To know about different aspects about zooplankton the care should be taken from the level of collection and further in to their preservation, identification, sorting, enumeration and their analysis through different scientific procedures. A nutshell of on site as well as laboratory wise procedures involving different techniques and instrumentation in zooplankton studies and advancements that have been made and currently followed by the researches are included in this review article.

Keywords: Zooplankton, Bio indicators, Food chains, Biogeochemical cycling

RESUMEN

El zooplancton son animales flotantes o a la deriva que tienen mucha importancia ecológica tanto en ecosistemas marinos como de agua dulce. Muchos se consideran bioindicadores y tienen un papel innegable en la transferencia de energía a través de las cadenas alimentarias y los ciclos biogeoquímicos. Para conocer los diferentes aspectos del zooplancton se debe tener cuidado desde el nivel de recolección y más allá hasta su preservación, identificación, clasificación, enumeración y su análisis a través de diferentes procedimientos científicos. En este artículo de revisión se incluye una breve descripción de los procedimientos en el sitio y en el laboratorio que involucran diferentes técnicas e instrumentación en los estudios de zooplancton y los avances que se han realizado y seguido actualmente por las investigaciones.

Palabras clave: Zooplancton, Bioindicadores, Cadenas tróficas, Ciclos biogeoquímicos.

INTRODUCTION

Zooplankton are found in the sunlit zone as they drift in the water column where food resources are most abundant in ocean and fresh water bodies. They play an important role in food web by connecting the primary producers and higher trophic levels. The major fresh water zooplankton are Protozoa, Rotifers, Cladocerans, Copepods and Ostracods (Ferdous and Muktadir, 2009). Unicellular protozoans which are planktonic in nature is either flagellated or ciliated organisms. Picoplankton, nano flagellates or small nano phytoplankton are the main food sources of planktonic protozoans. Rotifers are the soft bodied metazoans and the most important fact is that they have short life cycle among the plankton. There are a lot of factors influence their life cycle but the three most important factors are temperature, food and photoperiod. For the members that belongs to the higher trophic level, cladocerans represent the most useful and nutritive group (Ferdous and Muktadir ,2009). They feed on smaller zooplankton, bacterioplankton and algae (Murugan *et al.,*1998). Cladocerans react against even low concentration of pollutants. Copepods have toughest exoskeleton and classified in to three orders: Cyclopoid, Calanoid, Harpacticoid (Ferdous and Muktadir,2009). Copepods which belong to order cyclopoid feed on algae, bacteria and detritus. The calanoid copepods on the other hand is omnivorous in nature. They feed on ciliates, rotifers, algae, bacteria and detritus. Harpacticoid copepods are purely benthic. Ostracods are bottom dwellers of lakes and habitats on dead phytoplankton (Ferdous and Muktadir, 2009). Ostracods forms the main food source of fish and benthic macroinvertebrates (Chakrapani *et al.,*1996).

Plankton are the prominent bio indicators to monitor the aquatic ecosystems and integrity of water (Beaugrand *et al*.,2000, Li *et al.,*2000) since its influence on abiotic and biotic factors (Christou 1998, Escribano and Hidalgo 2000, Beyst *et al.,*2001). The distribution and growth of zooplankton are influenced by some abiotic factors such as temperature, salinity, stratification and pollutants. Biotic parameters such as food limitation, predation and competition also influence them. Studies have proved that zooplankton communities were significantly impacted by excessive loading of nutrients (Wang *et al*., 2010, Duggan *et al*.,2019). Factors like presence of microplastics (Scherer *et al*., 2018) pesticides (Hanazato *et al*.,2001), pharmaceuticals and personal care products (Garric 2013) negatively affects the zooplankton communities.

Growth of zooplankton is influenced by the changes in the concentrations of different factors such as dissolved oxygen, temperature, total alkalinity, total nitrogen, phosphate and pH (Sarkar and Chowdhury,1999). Micro zooplankton are the group of organisms which are found in all aquatic habitats. They have major role in pelagic food webs. Their importance in pelagic food webs has been stimulated by the recognition of the importance of very small algal cells called picoplankton (Stockner and Shortreed, 1989) and the role of the microbial loop (Azam *et al.,* 1983). Zooplankton have prominent role in biological pump and to transfer energy to organisms belong to higher trophic levels and thus their role in biogeochemical cycle is inevitable (Ward *et al*., 2012, Turner 2015). There are different methods for the sampling, collection and identification of zooplankton and advances that have been made in that area.

Characteristics of zooplankton sampling

a) Collection of plankton samples

Non-conventional methods for collecting the sample usually bring errors. For collection, it is important to consider the mesh size of the zooplankton net that suits the proposed study. In the case of towed plankton nets having small mesh size, larger and

better swimming organisms sense the pressure wave in front of a small mesh and thus avoid the entering in to the net. The smaller zooplankton will be extruded through the mesh in the case of larger mesh size and this phenomenon is referred as net extrusion (Suthers and Rissik, 2008). According to UNESCO, the standard mesh size for zooplankton sampling is 200µm (Harris *et al.,* 2000). The accurate way of collecting zooplankton is by slowly towing the net horizontally at a constant speed of around 1-2 meters per second. The change in the speed of the net movement affects the collection of plankton sample. Faster the speed, higher will be the extent of extrusion and slower may increase the chance of avoidance. To determine the number or biomass of zooplankton per cubic meter it is important to determine the volume of water filtered (Suthers and Rissik,2008). Continuous Automated Litter and Plankton Sampler (CALPS) functions continuously under the sea condition that estimates the volumetric abundance of particles at pump depth and thus helps to assess aggregated distributions and it can use up to six nets of different mesh sizes (Pitois *et al*., 2016). A similar existing system is Continuous Underway Fish Eggs Sampler (CUFES) (Checkley *et al*.,1997) is a good sampler for small zooplankton (Sono *et al*.,2009).

b) Fixation and preservation of plankton samples

Preservative such as alcohol reduces or stops decomposition without chemically fixing the tissue. 2% formaldehyde is used to preserve micro zooplankton. Macro zooplankton preservation is usually done with 5% buffered formaldehyde (37% formaldehyde with sodium tetraborate or hexamine). For the purpose of long-term preservation, it should be transferred to 70% alcohol (Suthers and Rissik, 2008). 4% solution of formaldehyde is also used to preserve macro zooplankton. It is prepared by adding 10ml of 40% commercial or concentrated grade in 90ml sea water or fresh water. 1 or 2% formaldehyde that use to preserve micro zooplankton is made up from 25 or 50 ml of 40% concentrated formaldehyde and made up to 1 litre (Steedman 1976). It is important not to squeeze too much plankton in to a sample jar. The plankton to solution ratio should be always 1:9 (Steedman 1976). It has been reported that acetone is more efficient at preserving DNA in samples with high water content which is more important in the preservation of bulk plankton material (Fukatsu 1999). Ethanol is widely used as a preservative because it has long been known to yield high molecular weight (HMW) DNA. But it is also reported that DNA degradation in ethanol preserved samples over long storage times at warmer storage temperatures and in high water content (Reiss *et al.,* 1995) (Holzmann and Pawlowski 1996, Fukatsu 1999). The bulk ethanol preservation of planktonic copepods at a storage temperature of -20^{0}C for almost 41 days also revealed decrease in DNA copies (Jungbluth *et al*., 2013).

Methods for extracting and sequencing DNA from formalin fixed samples have been studied especially in medical field. The major breakthrough is the procedure for extracting DNA from formalin-fixed-paraffin embedded (FFPE) samples (Goelz *et al*.,1985, Gilbert *et al*.,2007, Pairedar *et al.,* 2013). However, studies related to extraction of DNA from formalin fixed samples stored in museums and laboratories is going through (Schander and Halanych 2003, Bucklin and Allen 2004, Ruane and Austin 2017). The difference of museum and laboratory samples from FFPE samples is that most of them are stored in buffered formalin solution. Storage in formalin solution results in ongoing cross linking over time and in the other hand FFPE samples exclude formalin prior paraffin embedding so further crosslinking is reduced. There has been reported some successful extraction

and recovery of DNA from samples preserved in formalin for long periods (Bucklin and Allen 2004, Ripley *et al.,* 2008, Ruane and Austin, 2017). Plankton communities collected by plankton nets, sediment traps and continuous plankton recorders in the field of oceanography and limnology have been routinely preserved by formalin-fixation (UNESCO 1994, Reid *et al.,*2003, Mills 2012). Neutral 10% Lugol's iodine solution is a useful preservative for molecular analysis of marine plankton (Sano *et al.,*2020). There are other fixatives that are used which is mentioned in Table 1 (Suthers and Rissik,2008).

Table 1: List of possible plankton fixatives

Plankton	Fixatives
Phytoplankton	30% methylated spirits 5% glutaraldehyde Lugol's solution Tincture of iodine Acid Lugol's 2% formaldehyde
Micro zooplankton	2% formaldehyde
Macro zooplankton	5% buffered formaldehyde. Use 70% alcohol for long term preservation.

c) Sorting and subsampling of plankton samples

Firstly, to remove the formaldehyde solution and grass and sticks the sample should be first rinsed in a sieve which of the same or smaller mesh size of the plankton net. Rinsing with cold fresh water is perfect choice for preserved plankton. At this stage gelatinous zooplankton should be counted and removed and record the data. Later on, rinse the plankton from the sieve in to a beaker or a volumetric cylinder (Suthers and Rissik, 2008). While dealing with bulky sample especially with detritus it is important to let the plankton to settle. Choose a uniform time period and record the approximate displacement volume. Displacement volume is the approximate volume in millilitres of zooplankton when normally zooplankton is added to the water. Detritus tends to sink slower and sand grains, if any will sink faster enabling to estimate the actual zooplankton biomass. After recording the displacement volume thoroughly mix the zooplankton and while still swirling remove an accurate 2ml or 4ml subsample using a pipette. In this way 2% or 4% of the total sample is removed such that multiple the zooplankton counts by 50 or 25 to get the estimate of total number (Suthers and Rissik,2008).

Subsampling is an important step in zooplankton counting and samples contain more organisms should be enumerated by following subsampling procedures. It is possible to count the entire samples with low zooplankton numbers (<200 zooplankters) without subsampling. Before the subsampling procedures care must be taken to remove and

specify all large uncommon organisms such as fish larvae (APHA 2012). There are two methods of subsampling: pipet method and splitting method. In pipet method, using a graduated cylinder or Imhoff cone adjust sample to a convenient volume (APHA 2012).

In splitting method different splitting device should be used and Folsom plankton splitter is the best known (Longhurst and Seibert, 1967). First step is to level the splitter and then place the sample in the splitter and divide in to sub splits. (APHA 2012). Epson Perfection 4990 photo scanner having VueScan Professional Edition 8.4.77 software can be used to digitize the subsamples at a resolution of 1200 dpi and result thus developed can be processed using software ZooImage 1 version 1.2-1 (http://www.sciviews.org/zooimage) (Grosjean and Denis, 2007).

d) Identification of zooplankton

Species identification is a rigorous activity that requires time, microscopic activity of samples that are normally preserved, subsampling, counting and identification of individuals in taxonomic groups (Benfield *et al*., 2007). The important factor for the purpose of environment conservation and monitoring is biodiversity assessment which involves describing community taxonomic composition at different trophic levels (Lodge *et al.,*2012). Protocols by the International Council for the Exploration of the sea (ICES) has been used to morphologically identify marine zooplankton (Roger *et al.,* 2000). Different taxonomic keys (https://wgimt.net/morphological/keys and http://cfb.unh.edu/cfbkey/html/history.htm) and different manuals (https://www.mrcmekong.org/assets/Publications/tech-No45-handbook-freshwater.pdf are available online for the identification of zooplankton. DNA barcoding is one of the most widely applied molecular method for identifying plankton (Webb *et al*.,2006, Bucklin *et al*., 2007, Lin *et al*.,2009). DGGE (Denaturing Gradient Gel Electrophoresis) and T-RFLP (Terminal Restriction Fragment Length Polymorphism) are the two other methods for analyzing the finger prints of natural communities using DNA (Caron *et al*.,2004). But Savin *et al*., (2009) reported that comparison of microscopic examination of plankton samples with DGGE showed high levels of diversity.

e) Enumeration and analysis of plankton samples

In the case of larger zooplankton like mature microcrustacean use a counting chamber holding 1 to 5 ml. A Sedgwick rafter is unsuitable for larger zooplankton because of its size. An open counting chamber with dimensions of 80× 50 × 2mm deep is desirable but the problem is that an open chamber is difficult to move without jarring and disrupting the count. It is recommended to use a mild detergent solution in the chamber before counting to reduce the organism movements or use special counting trays with parallel or circular grooves or partitions (Dodson and Thomas 1964, Gannon 1971). There are some automated quantitative tools for plankton counting such as flow cam, microscopy-imaging, zooscan for the laboratory use and for the field study fluorescence probes, cytobuoy, flowcytobot, imaging cameras have been used (Lombard *et al.,* 2019).

For the characterization of zooplankton biodiversity there are a lot of traditional methods like visual surveys which are laborious but it can be environmentally destructive (Wheeler *et al.,*2004, Wheeler and Valdecasas, 2005). Recently metabarcoding techniques have been discovered which revealed that most plankton are morphologically indistinguishable yet highly diverse (De Vargas *et al.,* 2015, Ibarbalz *et al.,* 2019). DNA shed by organisms present in a given environment represents environmental DNA (eDNA).

Genetic analysis of eDNA offers a high throughput, cheaper more sensitive and less destructive methods for the characterization of biodiversity (Davy *et al.,* 2015, Flynn *et al.*, 2015, Harvey *et al.,* 2017). Large scale biodiversity analysis can be done by metabarcoding with next generation sequencing (Shokralla *et al.,* 2012). Analysis of diversity of mixed zooplankton tissue samples for the 18srRNA, COI (Cytochrome Oxidase I) and 28srRNA genetic loci have been successfully done by metabarcoding (Lindeque *et al.,* 2013). Dual Scripps Plankton Camera (DPSC) is a new initiative for automated in situ monitoring of phytoplankton as well as zooplankton based on dual magnification dark field imaging microscopy (Merz *et al.,* 2021).

There are many size-based plankton such as coulter counter, flow cytometry and HIAC particle counters but these are specialised instruments operating from laboratory. The size categories must be then cross referenced with some typical taxa. For the purpose of counting and sizing of zooplankton in the 0.3-3mm size range, one of the major field instruments used is the optical plankton counter (OPCs) (Suthers and Rissik,2008). Flow cytometry that has assisted by imaging is used to analyse the dynamics of single species of phytoplankton and micro zooplankton (Hunter *et al.,* 2016). In optical plankton counter, as samples passes through a small sampling tunnel it counts and sizes plankton and the flow interrupt a thin red light. The instrument records the decrease in light intensity through the aid of a sensor as particle and converts to an area and thus an equivalent spherical diameter. The size is converted to biomass using the volume of the sphere and thus assuming the density of water. The sensor must receive a constant illumination especially in the case of turbid water. Thus, the light output must be increased which is recorded as light attenuance. Using OPCs one records count, size and turbidity.

Laser optical plankton counter (LOPC) is a next generation optical plankton counter which use a narrow beam and new sampling geometry. It offers the measurement of speed of the flow through the sampling tunnel by making statistical estimates of the particle time- of -transit (Herman *et al.,*2004). Automated Imaging Flow cytometry (Flow CAM) combines flow cytometer with a camera and microscope (Alvarez *et al.,* 2001). The major important application of flow CAM is that, it is possible to distinguish between copepods and phytoplankton in a mixed sample (Ide *et al.,* 2008).

Planktometrix method (PMX) is a regular Mac application. Microscope equipped with a digital camera as well as a Macintosh computer is essential for using PMX. And altogether it represents the hardware components. It provides services like counting, measuring sizes, entering data, computations and storage in database which forms all the steps of conventional microscope-based zooplankton analyses. It simultaneously offers the production of higher quality data in less time with fewer typing errors and lower user fatigue (Zohary *et al.*,2017).

f) Estimation of zooplankton biomass

The better understanding of physiological process such as ingestion, growth, respiration and egestion as well as the precise estimation of their biomass are very important. Semi-automated Image Based System (IBS) have been developed for the purpose of estimation of biomass since the estimation based on dry weight (Love grove 1966) leads to the destruction of some of the samples (Grosjean *et al.,* 2004, Benfield *et al.*,2007, Gislason and Silva 2009, MacLeod *et al.,* 2010). Estimation of biomass using dry weight can be done by firstly separating the zooplankton based on the size and dry them at 60^0C for 24h and weighs on a microbalance (Love grove 1966). Estimation of biomass

using IBS involves the sample should be sieved in to two size fractions using a 1000µm to separately analyze large and small zooplankton. The size based separated fractions should be introduced in a flask where organisms should be homogenously distributed. Depending on the zooplankton density, aliquots of 5ml should be taken with a pipette and should be poured on to polystyrene plates (90 ×130mm). This procedure promises to best represent the zooplankton diversity with minimum overlap of the animals (Garijo and Hernandez-Leon, 2015). Subsamples then should be digitalized using an Epson perfection 4990 photo scanner (Grosjean and Denis, 2007). Organisms then should be enumerated, measured and weighed and categorize based on different taxa (Garijo and Hernandez-Leon, 2015).

Significance of zooplankton study

Zooplankton is the connecting link between phytoplankton and fish and it plays an important role in keeping the integrity of ecosystem intact. Every member in a trophic food chain contributes to their surrounding which eventually sums up at the higher trophic level. Zooplankton ranges from microscopic to large jelly fish in oceans but regardless of their size each one of the zooplankton is important for the survival of organisms connected with them. Studies about zooplankton is going all around the world and advances that have made in this field helped the zooplankton ecologists for the better understanding of the physiological process and their contribution to the ecosystem. Zooplankton feeds on phytoplankton which is known as the producer of aquatic ecosystem. It has a major role in the purpose of proper functioning and the productivity of aquatic ecosystems through its influence on the dynamics of nutrients and its prominent position in the food webs (Trishala *et al.,* 2016, Ismali and Adnan 2016, Gianuca *et al.,* 2016, D'Alelio *et al.,* 2016, Yang *et al.,* 2017). Aquatic productivity directly correlates the fish population and thus help to maintain the integrity of aquatic ecosystem.

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