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An Overview of Recent Developmental Studies in Zooplanktons

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

In both saltwater and freshwater environments, zooplankton can be found floating in the sunlit zone, where food sources are plentiful. They are crucial to the functioning of the food web because they mediate between primary producers and higher trophic levels. Current methods and equipment for studying and characterizing zooplankton are discussed. Similarly, zooplankton are highly attuned to their surroundings and exhibit measurable responses to shifts in water chemistry, temperatures, and other hydrographic factors. Though zooplankton have been shown to be useful as bio-indicators of eutrophication and water quality status, widespread application and development of such indicators are still relatively new and face several challenges. In this review article traditional and modern approaches used for zooplankton analysis are well discussed and the major focus area is the characterization of zooplankton sampling. In order to cover all bases in zooplanktonic studies, a holistic approach is used.

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1. INTRODUCTION

Zooplankton are aquatic animals with limited swimming ability that float in the water column of oceans, seas, or freshwater bodies to travel large distances [1]. Zooplankton are an essential biotic component of aquatic ecosystems because they serve as an intermediary between phytoplankton and fish and play a significant part in the cycling of organic materials in an aquatic environment. Zooplankton communities are particularly sensitive to environmental changes and, hence, have a high potential for value as water quality indicators. Zooplankton association, richness, abundance, variation, and diversity may be utilized for water quality evaluation and management strategies [2]. The zooplankton community is tightly linked to upper and lower levels of the trophic web due to its important role in aquatic settings. They can be impacted by phytoplankton blooms during bottom-up processes and respond swiftly, or they can exert pressure in top-down management and dictate phytoplankton composition and abundance [3]. Because of their central functions in aquatic food webs, zooplankton play critical roles in energy transmission and nutrient cycling in running water habitats. Furthermore, zooplankton have been identified as one of the first and most sensitive taxonomic groups during environmental change processes such as trophic status dynamics in aquatic environments [4]. In addition to regulating the population of algae and bacteria as grazers, zooplankton also provides nitrogen and phosphorus to phytoplankton in a closed-loop nutrient recycling system. In particular, zooplankton may alter the concentration of prey populations and predator populations (by consumption), thereby affecting fish biomass. Specific zooplankton species have been identified as bioindicators sensitive to changes in natural ecosystems. As a result, they are frequently referred to as "sentinels of environmental changes and stresses" [5]. Zooplanktons play a pivotal role in ecosystems due to their ability to act as a link between primary production and higher levels while also serving as a useful indicator of water quality, pollution, and eutrophication. As an added bonus, zooplankton are crucial participants in the water's natural cycle of carbon and other elements. Many marine hatcheries rely on zooplankton as the primary appropriate food for the larval stages of fish and shellfish species [1]. The variety of zooplankton implies a chronic problem with water contamination. Zooplankton is critical for the survival of commercially important fish populations. The study of zooplankton diversity, abundance, and the influence of seasonal fluctuations on them is important in fisheries planning and management. The most important factors impacting planktonic biomass production were physicochemical conditions and water nutrition status [6].

The biodiversity of zooplankton is important for keeping our ecosystem healthy since each species has a distinct job (recycling nutrients, providing food for others, and preserving soil fertility) in the ecosystem, and certain species may allow natural ecosystems to function in a healthy manner [7]. As passive drifters, zooplanktons are bound to be impacted by environmental conditions, and organisms may be moved towards or away from the coast depending on the prevailing tides and currents. Zooplankton are animal plankton that travels at the whim of ocean currents.

They're accountable for devouring millions of tiny algae that can grow to an uncontrollable size. An inadequate understanding of plankton and their dynamics is a major impediment to gaining a better understanding of the biological system of sparkling water bodies. Numerous health stressors are wreaking havoc on aquatic environments, threatening biodiversity. In the future, the loss of biodiversity and its implications are predicted to be worse in aquatic environments than in terrestrial environments. Zooplankton species have unique life histories that are influenced by seasonal changes in biotic components, feeding ecology, and predation strain. Primary consumers (which eat phytoplankton) and secondary consumers (which eat other zooplankton) are both part of the zooplankton community (which feeds upon other zooplankton). They serve as a direct connection between the top producers and the higher tropic levels, where animals like fish live. During their larval stages, almost all fish depend on zooplankton for food, and some fish even eat zooplankton as adults. The physicochemical properties of water have a significant impact on the abundance and variety of zooplankton in aquatic ecosystems [8]. Zooplankton can respond to changes in water quality by altering species composition, abundance, and morphological abnormalities [9]. Zooplankton are tiny aquatic creatures, with sizes ranging from a

few microns to a millimeter or more. They comprise individuals from practically every taxon of the animal world (Goswami 2004) who spend all (holoplankton) or a portion (meroplankton) of their lives as plankton (Lindeque et al. 2013). The rise in the phytoplankton population is most beneficial to the expansion of the zooplankton population (Kumar et al. 2011). Zooplankton are especially significant in biomonitoring systems because they may respond quickly to anthropogenic and natural environmental changes (Vieira et al. 2011; Mano and Tanaka, 2016). As a result, the functional approach may increase knowledge of the role of zooplankton communities in these processes [10].

2. CHARACTERISTICS OF ZOOPLANKTON SAMPLING

2.1 Collection of the Sample

Water is filtered through a net, collected in bottles or water samplers, or pumped through a system to collect zooplankton. The right equipment, mesh size of the netting material, time of collection, water depth of the study area, and sampling strategy are crucial to the success of the sampling. Larger and more advanced swimming organisms can feel the pressure wave in front of a small mesh in towed plankton nets and avoid entering the net as a result. Net extrusion describes the process by which smaller zooplankton are pushed through a larger mesh [11]. The standard mesh size for zooplankton sampling is 200 m, as recommended by UNESCO [12]. Slowly towing the net horizontally at a constant speed of about 1–2 meters per second is the most effective method for collecting zooplankton. The rate of plankton sample collection is sensitive to variations in net movement speed. The greater the velocity, the greater the degree of extrusion; the slower the velocity, the greater the possibility of avoidance. The volume of water filtered is an important factor in calculating the number of zooplankton per cubic meter as well as the biomass of these tiny organisms [11]. A net is the most common tool for catching zooplankton. More water is filtered, and the equipment can be used for either qualitative or quantitative research. There is a wide range of sizes and styles of plankton nets in use. Two main types of nets can be distinguished: open nets, which are used for horizontal and oblique hauls, and closed nets with messengers, which are used to collect vertical samples at specific depths. There are two samplers named CALPS and CUFES. In order to help evaluate aggregated distributions, the continuous automated litter and plankton sampler (CALPS) can use up to six nets of varying mesh sizes and operates continuously under sea conditions that estimate the volumetric abundance of particles at pump depth [13]. The continuous underway fish eggs sampler (CUFES) (Checkley et al. 1997) is a good sampler for small zooplankton [14], and it works in a similar way.

2.2 Fixation and Preservation

Decomposition can be slowed or stopped with a preservative like alcohol, but the tissue is not chemically fixed. Micro-zooplankton are preserved in formaldehyde at a 2% concentration. 5 percent formaldehyde buffered with sodium tetraborate or hexamine is the standard method for preserving macro-zooplankton. Transfer to 70% alcohol for long-term storage [11]. Additionally, macro-zooplankton can be preserved in a formaldehyde solution at a concentration of 4%. To make it, mix 10 milliliters of the 40% commercial or concentrated grade with 90 milliliters of saltwater or freshwater. Preserving micro-zooplankton requires 25 to 50 ml of a 40% concentrated solution of formaldehyde, which is then diluted to make 1 liter of 1 or 2% formaldehyde [15]. Try not to cram too much plankton into a small container. Keep the plankton-to-solution ratio at 1:9 [15]. Acetone has been reported to be more effective at preserving DNA in samples with a high-water content, which is more important when preserving bulk plankton material. In order to preserve DNA, ethanol is commonly used due to the fact that it produces HMW DNA. Although DNA degradation in ethanol-preserved samples has not been observed under normal conditions, reports of DNA degradation at higher storage temperatures and in high-water-content samples have been published [16]. DNA copy number reduction was also observed in planktonic copepods that were preserved in bulk ethanol at -20^0C for nearly 41 days [17].

Allow at least 10 days for things to settle down. After the zooplankton are fixed, they are moved to airtight containers with enough preservatives to keep them alive. Care should be taken to make sure that no part of the zooplankton sample is lost while it is being moved. There are many different kinds of preservatives. Most of the time, 4 to 5% buffered formalin is used as both a fixative and a preservative. Either 70% ethanol or 40% isopropanol is also used as a preservative.

Ethanol is used to store museum items, but it is expensive and easy to break. Glycerin is often added to formalin to keep specimens from shrinking or drying out and to help keep the colors of zooplankton. If you want the zooplankton samples to last longer, you should change the preservative within the first 6 months. The zooplankton samples should be kept in a well-ventilated room with a temperature of less than 25°C. The samples should be stored in glass jars with wide mouths. Labels with the name of the collector, the preservative and fixative used, and other field information should be put on the jars so that they are easy to find when the samples are being analyzed. Some studies [18-20] have reported the successful extraction and recovery of DNA from samples preserved in formalin for long periods. In the fields of oceanography and limnology, formalin-fixation has become standard practice for preserving plankton communities collected with sediment traps, plankton nets, and continuous plankton recorders (UNESCO 1994, [21], Mills 2012). To preserve marine plankton samples before analyzing them molecularly, a neutral 10% Lugol's iodine solution is a great choice [22].

3. IDENTIFICATION AND ENUMERATION

Scientists utilize a wide variety of equipment, including nets, pumps, and water bottles, to collect specimens and construct a gatherer information of species composition and abundance [23,24,25]. This conventional method is valued for its ability to detect and count microplankton, providing important data for determining species [23,25]. The scanning electron microscope (SEM) has advanced greatly as an analytical tool since the introduction of morphological identification using electron microscopes. The resolution of specimen identification and the detection of taxonomic keys are both greatly improved by the use of SEM. Using SEM, researchers were able to distinguish various rotifer species with specialized trophi [26] that otherwise appeared to share a common morphology. Using both optical and scanning electron microscopes, [27] revised the taxonomic classification of crustacean zooplankton in a Philippine lake. [28] used SEM for high-resolution species identification to investigate the biogeography of freshwater ciliates (protozoa) in Florida, USA. Because of this, SEM has emerged as a crucial supplementary technique for morphology-based monitoring of zooplankton, especially for debated microtaxa.

Several fully automatic or semiautomatic tools have been developed to speedily evaluate zooplankton samples since the advent of digital photography and scanning technology and these methods are illustrated in Fig. 1. Examples are the ZOOSCAN system [25] and the Flow Cytometer and Microscope (FlowCAM) [23]. To quickly and (semi-)automatically examine zooplankton samples, ZOOSCAN captures digital images of the samples and compares them to databases. The ZOOSCAN system is able to capture and classify digital zooplankton photos from collected zooplankton communities through the integration of ZooProcess and the Plankton Identifier software. For quantitative studies of zooplankton samples, the ZOOSCAN system can assess the biomass and precise body size of each species. However, most taxa of Rotifera and protozoa zooplankton in freshwater habitats are outside the size range of ZOOSCAN's body detectors (200 m to several cm). ZOOSCAN systems are widely used now in marine habitats for biodiversity monitoring due to their ability to swiftly estimate mesozooplankton biomass and size distribution [29].

Automated imaging flow cytometer FlowCAM is built on the foundation of fluid imaging technology. The laser detection system in FlowCAM allows for the digital imaging of particles/organisms in a fluid. Digital image analysis allows very precise estimates of population number and diversity [30]. Depending on the configuration of the instrument, FlowCAM is capable of detecting organisms with body sizes ranging from 3 to 3000 m. This includes virtually all species found in zooplankton communities in freshwater ecosystems, even in polluted ecosystems where smaller-sized species predominate. While FlowCAM was initially developed for studying phytoplankton [31,32], more recent research has employed sophisticated FlowCAM for quantitatively studying zooplankton [33].

Recently, a variety of molecular approaches for unambiguous species determination have been developed. Numerous of these methods are founded on DNA sequence analysis. DNA barcoding study of the mitochondrial cytochrome, C oxidase subunit I gene (COI) fragment is one way [34-37]. Several studies have employed COI barcoding to identify copepods in marine plankton [36,37,38]. Other research reported

using polymerase chain reaction (PCR) techniques for fast identification of copepods [39].

Another technique is protein profiling using MALDI-TOF MS, which uses matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Metazoans were also identified using MALDI-TOF MS protein profiling [40-43]. Three species of freshwater copepods and North Sea calanoid copepods [44] have both shown that this method can be used to identify different zooplankton species [45]. MALDI-TOF MS protein profiling is based on comparing the protein profiles of whole cells or organisms to those in a reference database. Without the need for DNA sequence expertise, it enables the quick and precise identification of unknown species. The resulting MALDI-TOF mass spectra can be evaluated using clustering techniques like principal component analysis to shed light on the diversity of a community (PCA). PCA can reveal the minute differences between samples that add up to significant variation [46]. Dual scripps plankton camera (DPSC) is a new endeavor for automated in situ phytoplankton and zooplankton monitoring based on dual magnification dark field image microscopy [47].

3.1 Analysis of Zooplankton

The following techniques are used to estimate biomass. 1. The volumetric method, which uses displacement and settling volumes. 2.Gravimetric (ash-free dry weight, wet weight, and dry weight) method 3. Use of chemicals. Larger zooplankters like ctenophores, medusae, salps, fish larvae, and siphonophores should be expulsed from the zooplankton sample, and their biomass should be measured separately before determining biomass. The biomass of the larger forms plus the biomass of the remaining zooplankton would make up the total biomass. Different strategies of zooplankton analysis are depicted in Fig. 2.

It is suggested that an aliquot subsample be taken for the common taxa in order to conduct a faunal enumeration. However, for the rare categories, it is important to conduct complete sample counts. Ten percent to twenty-five percent of a sample is typically analyzed for zooplankton counts. However, the amount of zooplankton in the sample will dictate whether the aliquot percentage is increased or decreased. When exposed to a fixative or preservative, zooplankton respond with a series of spasmodic, jerking movements and a shrinking of the body and appendages. For some species, this can make identification difficult. A brief anesthetic is used to keep this under control, and the specimens are allowed to recover after the necessary tests have been performed. Chloroform, carbonated water, methyl alcohol, and magnesium chloride (about 7g of $MgCl_2$ dissolved in 100 ml of distilled water) are all viable narcotic solution options [48]. Species can only be properly identified using specialized equipment like dissecting needles, a stereoscopic dissecting microscope, glass slides, pipettes, coverslips, fine forceps, and chemical reagents. Preparing slides, cleaning, dissecting, and staining are all part of the process.

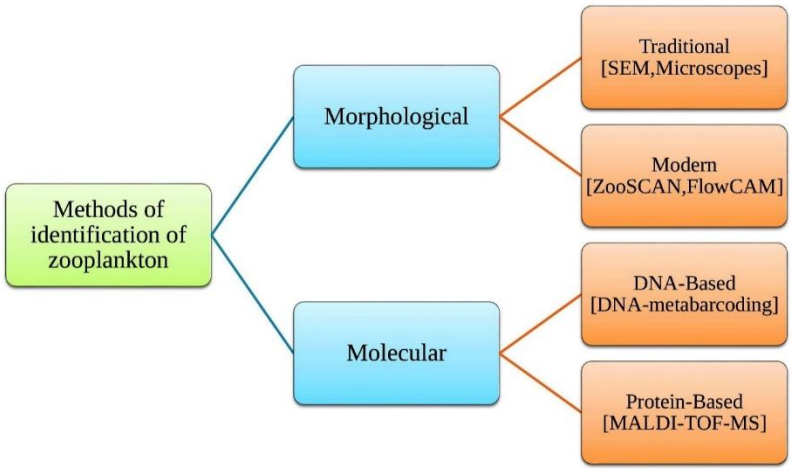

Fig. 1. Study framework for identification of zooplankton

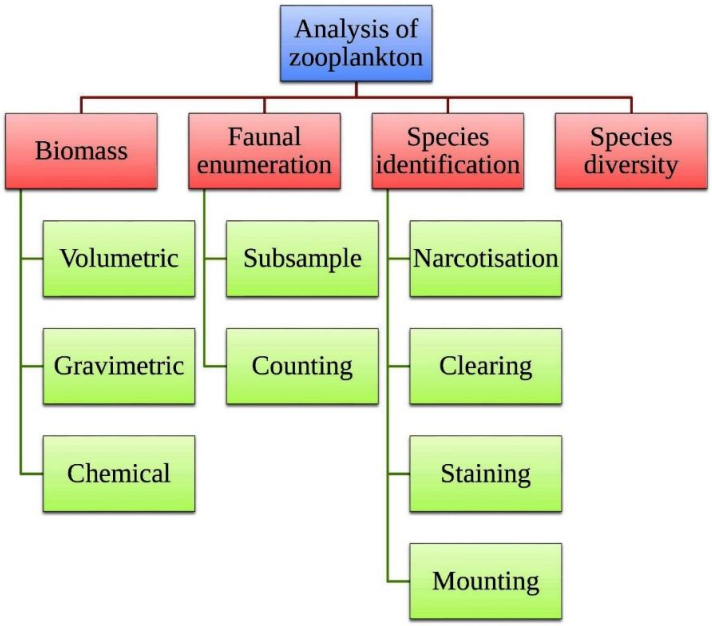

Fig. 2. Analytical study of zooplankton and their preservation

3.2 Significance of Zooplanktonic Studies

As the link between fish and phytoplankton, zooplankton plays a crucial role in maintaining the health of the ecosystem as a whole. In a trophic food chain, each organism affects the environment around it, which adds up at the top. There is a wide spectrum of zooplankton sizes in the oceans, from tiny bacteria to enormous jellyfish. Progress in the study of zooplankton has allowed ecologists to gain a deeper understanding of the organisms' physiological processes and the role they play in the ecosystem. Aquatic ecosystem producers, or phytoplankton, are a primary food source for zooplankton. Through its influence on nutrient dynamics and its prominent position in food webs, it plays a significant role in the health and productivity of aquatic ecosystems [49,50]. Fish populations are directly correlated with aquatic productivity, which helps to protect the health of the aquatic ecosystem as a whole.

4. CONCLUSION

We can draw the conclusion that physicochemical factors and hydrological regimes influence the species richness and species composition of zooplankton in the riverine system. Depending on the technique used to collect the samples, the total number of zooplankton in a given area can vary greatly. The various methods, from the more familiar to the more modern, for collecting zooplankton are discussed. This review provides a synthesis of previous work on zooplanktonic organisms. In terms of aquatic biology, fisheries, and aquatic monitoring, zooplankton are crucial.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alprol AE, Heneash AMM, Soliman AM, Ashour M, Alsanie WF, Gaber A et al. Assessment of water quality, eutrophication, and zooplankton community in lake Burullus, Egypt. Diversity. 2021;13(6):268. Available:https://doi.org/10.3390/d13060268
2. Singh S, Sharma RC. Zooplankton diversity and potential indicator species for assessment water quality of high altitude wetland. India: Dodi Tal of Garhwal Himalaya. Academia Arena. 2020;12(5): 1-6. Available:https://doi.org/10.7537/marsaaj120520.01
3. Muñoz-Colmenares ME, Soria JM, Vicente E. Can zooplankton species be used as indicators of trophic status and ecological potential of reservoirs? Aquat Ecol. 2021;55(4):1143-56.

Available: https://doi.org/10.1007/s10452-021-09897-8

4. Xiong W, Ni P, Chen Y, Gao Y, Li S, Zhan A. Biological consequences of environmental pollution in running water ecosystems: A case study in zooplankton. Environ Pollut. 2019;252(B):1483-90.
Available:https://doi.org/10.1016/j.envpol.2019.06.055
5. Lomartire S, Marques JC, Gonçalves AMM. The key role of zooplankton in ecosystem services: A perspective of interaction between zooplankton and fish recruitment. Ecol Indic. 2021;129:107867.
Available:https://doi.org/10.1016/j.ecolind.2021.107867
6. Chattopadhyay D, Panda S. Establishment of Significant Correlation between Seasonal Physicochemical parameters and Zooplankton Diversity in Saheb Bandh, at Purulia, West Bengal. International Journal of Scientific Research in Research Paper. J Biol Sci. 2022; 9(2):17-23.
Available: www.isroset.org
7. Manickam N, Bhavan PS, Santhanam P, Bhuvaneswari R, Muralisankar T, Srinivasan V et al. Impact of seasonal changes in zooplankton biodiversity in Ukkadam Lake, Coimbatore, Tamil Nadu, India, and potential future implications of climate change. J Basic Appl Zool. 2018;79(1):15.
Available: https://doi.org/10.1186/s41936-018-0029-3
8. Prasannatha A, Neeraja DB, Kamraju M. Quantitative analysis of zooplanktons of fresh water ecosystem in dindi reservoir, Telangana, India. Int J Res Anal Rev. 2019;6(2):4621-61.
9. Telesh IV. Plankton of the Baltic estuarine ecosystems with emphasis on Neva Estuary: a review of present knowledge and research perspectives. Mar Pollut Bull. 2004;49(3):206-19.
Available:https://doi.org/10.1016/j.marpolbul.2004.02.009
10. Gomes LF, Pereira HR, Gomes ACAM, Vieira MC, Martins PR, Roitman I et al. Zooplankton functional-approach studies in continental aquatic environments: a systematic review. Aquat Ecol. 2019;53(2): 191-203.
Available: https://doi.org/10.1007/s10452-019-09682-8
11. Rissik D. Plankton Suthers IM, editor. CSIRO Publishing; 2009.
Available:https://doi.org/10.1071/9780643097131
12. Harris RP, Wiebe J, Lenz HRS, MHM. ICES zooplankton methodology manual. Elsevier; 2000.
Available: https://doi.org/10.1016/B978-0-12-327645-2.X5000-2
13. Pitois SG, Bouch P, Creach V, van der Kooij J. Comparison of zooplankton data collected by a continuous semi-automatic sampler (CALPS) and a traditional vertical ring net. J Plankton Res. 2016;38(4):931-43.
Available:https://doi.org/10.1093/plankt/fbw044
14. Sono S, Moloney CL, van der Lingen CD. Assessing the utility of a continuous, underway fish egg sampler (CUFES) for sampling zooplankton. Afr J Mar Sci. 2009;31(2):181-9.
Available:https://doi.org/10.2989/AJMS.2009.31.2.6.878
15. Steedman HF. Zooplankton fixation and preservation. Monographs on Oceanographic Methodology, UNESCO Press. 1976;4:182-3.
16. Reiss RA, Schwert DP, Ashworth AC. Field preservation of Coleoptera for molecular genetic analyses. Environ Entomol. 1995; 24(3):716-9.
Available:https://doi.org/10.1093/ee/24.3.716
17. Jungbluth MJ, Goetze E, Lenz PH. Measuring copepod naupliar abundance in a subtropical bay using quantitative PCR. Mar Biol. 2013;160(12): 3125-41.
Available: https://doi.org/10.1007/s00227-013-2300-y
18. Bucklin A, Allen LD. MtDNA sequencing from zooplankton after long-term preservation in buffered formalin. Mol Phylogenet Evol. 2004;30(3):879-82.
Available:https://doi.org/10.1016/j.ympev.2003.11.002
19. Ripley SJ, Baker AC, Miller PI, Walne AW, Schroeder DC. Development and validation of a molecular technique for the analysis of archived formalin-preserved phytoplankton samples permits retrospective assessment of Emiliania huxleyi communities. J Microbiol Methods. 2008;73(2):118-24.
Available:https://doi.org/10.1016/j.mimet.2008.02.001
20. Ruane S, Austin CC. Phylogenomics using formalin-fixed and 100+ year-old

intractable natural history specimens. Mol Ecol Resour. 2017;17(5):1003-8.
Available: https://doi.org/10.1111/1755-0998.12655

21. Reid PC, Colebrook JM, Matthews JBL, Aiken J. The Continuous Plankton Recorder: concepts and history, from Plankton Indicator to undulating recorders. Prog Oceanogr. 2003;58(2-4):117-73.
Available:https://doi.org/10.1016/j.pocean.2003.08.002
22. Sano M, Makabe R, Kurosawa N, Moteki M, Odate T. Effects of Lugol's iodine on long-term preservation of marine plankton samples for molecular and stable carbon and nitrogen isotope analyses. Limnol Oceanogr Methods. 2020;18(11): 635-43.
Available:https://doi.org/10.1002/lom3.10390
23. Álvarez E, López-Urrutia Á, Nogueira E, Fraga S. How to effectively sample the plankton size spectrum? A case study using FlowCAM. J Plankton Res. 2011;33(7):1119-33.
Available:https://doi.org/10.1093/plankt/fbr012
24. Andújar C, Arribas P, Yu DW, Vogler AP, Emerson BC. Why the COI barcode should be the community DNA metabarcode for the metazoa. Mol Ecol. 2018;27(20):3968-75.
Available:https://doi.org/10.1111/mec.14844
25. Gorsky G, Ohman MD, Picheral M, Gasparini S, Stemmann L, Romagnan J-B et al. Digital zooplankton image analysis using the ZooScan integrated system. J Plankton Res. 2010;32(3):285-303.
Available:https://doi.org/10.1093/plankt/fbp124
26. Segers HH. A reappraisal of the Scaridiidae (Rotifera, Monogononta). Zool Scripta. 1995;24(2):91-100.
Available: https://doi.org/10.1111/j.1463-6409.1995.tb00394.x
27. Donne Papa RS, Tordesillas DT, Mamaril AC, Taal L. An updated taxonomic account of limnetic crustacean zooplankton in. Philipp J Sci. 2012;141(2).
28. Hines H. The biogeography, phylogeny, and dispersal of freshwater and terrestrial free-living ciliates in Florida, USA. Bournemouth University; 2019.
29. Schultes S, Lopes RM. Laser Optical Plankton Counter and Zooscan intercomparison in tropical and subtropical marine ecosystems. Limnol Oceanogr Methods. 2009;7(11):771-84.
Available:https://doi.org/10.4319/lom.2009.7.771
30. Álvarez E, Moyano M, López-Urrutia Á, Nogueira E, Scharek R. Routine determination of plankton community composition and size structure: a comparison between FlowCAM and light microscopy. J Plankton Res. 2014;36(1):170-84.
Available:https://doi.org/10.1093/plankt/fbt069
31. Buskey EJ, Hyatt CJ. Use of the Flow CAM for semi-automated recognition and enumeration of red tide cells (Karenia brevis) in natural plankton samples. Harmful Algae. 2006;5(6):685-92.
Available:https://doi.org/10.1016/j.hal.2006.02.003
32. Poulton NJ. FlowCam: quantification and classification of phytoplankton by imaging flow cytometry; 2016. p. 237-47.
Available: https://doi.org/10.1007/978-1-4939-3302-0_17
33. Le Bourg B, Cornet-Barthaux V, Pagano M, Blanchot J. FlowCAM as a tool for studying small (80-1000 µm) metazooplankton communities. J Plankton Res. 2015;37(4):666-70.
Available:https://doi.org/10.1093/plankt/fbv025
34. Radulovici AE, Archambault P, Dufresne F. DNA barcodes for marine biodiversity: moving fast forward? Diversity. 2010;2(4):450-72.
Available:https://doi.org/10.3390/d2040450
35. Hebert PDN, Cywinska A, Ball SL, deWaard JR. Biological identifications through DNA barcodes. Proc Biol Sci. 2003;270(1512):313-21.
Available:https://doi.org/10.1098/rspb.2002.2218
36. Bucklin A, Hopcroft RR, Kosobokova KN, Nigro LM, Ortman BD, Jennings RM et al. DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition. Deep Sea Res II. 2010a;57(1-2):40-8.
Available:https://doi.org/10.1016/j.dsr2.2009.08.005
37. Bucklin A, Ortman BD, Jennings RM, Nigro LM, Sweetman CJ, Copley NJ et al. A Rosetta Stone for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (northwest Atlantic

Ocean). Deep Sea Res II. 2010b;57(24-26):2234-47.
Available:https://doi.org/10.1016/j.dsr2.2010.09.025

38. Chen G, Hare MP. Cryptic diversity and comparative phylogeography of the estuarine copepod Acartia tonsa on the US Atlantic coast. Mol Ecol. 2011;20(11): 2425-41.
Available: https://doi.org/10.1111/j.1365-294X.2011.05079.x
39. Hill R, Allen L, Bucklin A. Multiplexed species-specific PCR protocol to discriminate four N. Atlantic Calanus species, with an mtCOI gene tree for ten Calanus species. Mar Biol. 2001;139(2):279-87.
Available:https://doi.org/10.1007/s002270100548
40. Feltens R, Görner R, Kalkhof S, Gröger-Arndt H, von Bergen M. Discrimination of different species from the genus Drosophila by intact protein profiling using matrix-assisted laser desorption ionization mass spectrometry. BMC Evol Biol. 2010;10(1):95.
Available: https://doi.org/10.1111/j.1365-2915.2010.00927.x
41. Kaufmann C, Ziegler D, Schaffner F, Carpenter S, Pflüger V, Mathis A. Evaluation of matrix-assisted laser desorption/ionization time of flight mass spectrometry for characterization of *Culicoides nubeculosus* biting midges. Med Vet Entomol. 2011;25(1):32-8.
Available: https://doi.org/10.1111/j.1365-2915.2010.00927.x
42. Mazzeo MF, de Giulio BD, Guerriero G, Ciarcia G, Malorni A, Russo GL et al. Fish authentication by MALDI-TOF mass spectrometry. J Agric Food Chem. 2008;56(23):11071-6.
Available:https://doi.org/10.1021/jf8021783
43. Volta P, Riccardi N, Lauceri R, Tonolla M. Discrimination of freshwater fish species by Matrix- Assisted Laser Desorption/ ionization- time of flight mass spectrometry (MALDI-TOF MS): a pilot study. J Limnol. 2012;71(1):17.
Available:https://doi.org/10.4081/jlimnol.2012.e17
44. Laakmann S, Gerdts G, Erler R, Knebelsberger T, Martínez Arbizu P, Raupach MJ. Comparison of molecular species identification for North S ea calanoid copepods (C rustacea) using proteome fingerprints and DNA sequences. Mol Ecol Resour. 2013;13(5): 862-76.
Available: https://doi.org/10.1111/1755-0998.12139
45. Riccardi N, Lucini L, Benagli C, Welker M, Wicht B, Tonolla M. Potential of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for the identification of freshwater zooplankton: a pilot study with three Eudiaptomus (Copepoda: Diaptomidae) species. J Plankton Res. 2012;34(6):484-92.
Available:https://doi.org/10.1093/plankt/fbs022
46. Hynek R, Kuckova S, Cejnar P, Junková P, Přikryl I, Říhová Ambrožová J. Identification of freshwater zooplankton species using protein profiling and principal component analysis. Limnol Oceanogr Methods. 2018;16(3): 199-204.
Available:https://doi.org/10.1002/lom3.10238
47. Merz E, Kozakiewicz T, Reyes M, Ebi C, Isles P, Baity-Jesi M et al. Underwater dual-magnification imaging for automated lake plankton monitoring. Water Res. 2021;203:117524.
Available:https://doi.org/10.1016/j.watres.2021.117524
48. Raju P, Moorthy K, Selvaraju A. Techniques in fixation and preservation of marine copepods.
Available:https://www.researchgate.net/publication/328064551.
49. Parmar TK, Rawtani D, Agrawal YK. Bioindicators: the natural indicator of environmental pollution. Front Life Sci. 2016;9(2):110-8.
Available:https://doi.org/10.1080/21553769.2016.1162753
50. Yang J, Zhang X, Xie Y, Song C, Zhang Y, Yu H et al. Zooplankton community profiling in a eutrophic freshwater ecosystem-lake Tai Basin by DNA metabarcoding. Sci Rep. 2017;7(1):1773.
Available: https://doi.org/10.1038/s41598-017-01808-y