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A Study of Polluted fresh Water Ecosystem in Zooplankton Biodiversity

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ABSTRACT-
Rotifers, crustaceans, and other micro-eukaryotes are abundant in freshwater ecosystems, and these various taxonomic groups are crucial to the health and operation of the ecosystem. Unfortunately, many environmental stresses, especially those resulting from intensive human activities like chemical pollution, pose a threat to freshwater ecosystems and the species that resides within them. Significant efforts have been made over the last few decades to stop the loss of biodiversity and restore the functions and services of freshwater ecosystems. The first and most important stage in identifying the effects of pollution on ecosystems and developing conservation strategies is biodiversity monitoring. However, because of numerous technical difficulties with micro-zooplankton, including their minuscule size, hazy morphological traits, and extremely high biodiversity, bio-monitoring of ubiquitous micro-eukaryotes is incredibly difficult. Here, we examine existing techniques for managing damaged freshwater ecosystems by monitoring zooplankton biodiversity. We examine the advancement of DNA-based approaches like metabarcoding and real-time quantitative PCR as well as conventional morphology-based identification techniques like scanning electron microscopy (SEM), ZOOSCAN, and FlowCAM automatic systems. We also list the benefits and drawbacks of using these techniques to monitor damaged habitats, and we suggest doable DNA-based monitoring procedures for researching the biological effects of environmental contamination in freshwater ecosystems. Finally, we suggest potential fixes for current technical problems to increase the precision and effectiveness of DNA-based biodiversity monitoring.

I. Introduction

1.1. Biodiversity loss in freshwater ecosystems

The significance of biodiversity has been widely acknowledged, and biodiversity protection has garnered a lot of attention since the term "biodiversity" was originally proposed at the United Nations Conference on Environment and Development in 1992 [1]. Freshwater environments, among other types of ecosystems, offer special habitats that support a high amount of biodiversity. Only over 0.8% of the Earth's surface is covered by freshwater environments, but they are home to almost 6% of all known species. For instance, more than 10,000 species of fish, or 40% of all known fish species worldwide, are found in freshwater habitats. Additionally, freshwater ecosystems offer incomparable benefits to humans in the form of food, water for drinking and irrigation, food production, and microclimate adjustment. However, over the past few decades, a number of causes, especially those brought on by manmade activity like water pollution and invasive species, have significantly damaged freshwater ecosystems. One of the most endangered ecosystems on the planet is the freshwater environment, which includes rivers and inland lakes. Freshwater habitats experience biodiversity loss far more quickly than their terrestrial counterparts as a result. Even worse, despite much effort being made to preserve or restore biodiversity in freshwater habitats, biodiversity loss in threatened freshwater ecosystems has not slowed down recently. Due to frequent disruption brought on by expanding anthropogenic activities and understanding gaps about biodiversity in freshwater environments, these attempts have mostly been fruitless. Since biological response to disturbance in freshwater ecosystems is not fully understood, especially on the common but hidden microscopic taxa like zooplankton, biodiversity loss in freshwater ecosystems is likely far more serious than we have realised. Scientific studies have shown how human activity has caused macro-eukaryotes including fish, amphibians, mollusks, and crustaceans to lose some of their variety. However, research on the dynamics of micro-eukaryotic biodiversity loss is scarce. Better-known macro-eukaryotes have received greater attention in terms of monitoring and conservation priority than smaller micro-eukaryotes, like tiny zooplankton. In fact, the tiny zooplankton play important ecological roles in aquatic food webs, such as connecting phytoplankton and bacteria to higher trophic levels like fish. The preservation of biodiversity at high trophic levels, as well as the integration and operation of freshwater ecosystems, are largely determined by the protection and recovery of unnoticed microscopic zooplankton species.

1.2. Potential indicative roles of zooplankton in freshwater ecosystems

Zooplankton include diverse taxa such as protists, rotifers, copepods and cladocerans, many of which are microscopic. Multiple studies have made a consistent and crucial realization that zooplankton taxa are rapid responders to many environmental stressors, such as hydrological changes, climate changes and anthropogenic activity-induced water pollution. Specifically, previous laboratory or field studies have indicated that zooplankton communities were significantly impacted by excessive loading of nutrients and also negatively affected by microplastics, pesticides, and pharmaceuticals and personal care products (PPCPs). As such, researchers have identified their usefulness as ecological indicators to water pollution. For instances, rotifers are used to diagnose ecological impacts of freshwater toxicants, such as endocrine disruptors, bioconcentration of lead, and nanoparticles toxicity. Some scientists zooplankton communities could be used to predict ecological thresholds of ammonia nitrogen. Some listed and recommended seven key reasons for the use of protists as good bio-indicators in aquatic ecosystems. Others showed that zooplankton communities played a complementary role to macroinvertebrates in indicating variation of the trophic status of waters. Thus, bio-monitoring zooplankton communities has become a widely accepted and irreplaceable aspect in ecological conservation and management of aquatic ecosystems.

1.3 Our goals,

This study seeks to provide an overview of the known techniques for monitoring the biodiversity of eukaryotic zooplankton, including both morphology-based and DNA-based approaches, in order to advance management of degraded freshwater ecosystems. Additionally, we go into technological challenges, their root causes, and how they affect biodiversity monitoring. As a takeaway, we also suggest a useful monitoring workflow for researching the biological effects of environmental contamination in aquatic environments.

II. Morphology-based methods for zooplankton biodiversity monitoring

2.1. Traditional morphological methods

The acquisition of biodiversity data has historically been based on morphological characterization of species. Researchers use many tools\ such as nets, pumps or water bottles to collect specimens and gatherer formation of composition and abundance of species, and then collected specimens are subjected for identification by taxonomists under a microscope

This traditional technique is considered to be useful for the identification and enumeration of micro plankton and thus providing invaluable information on species identification .

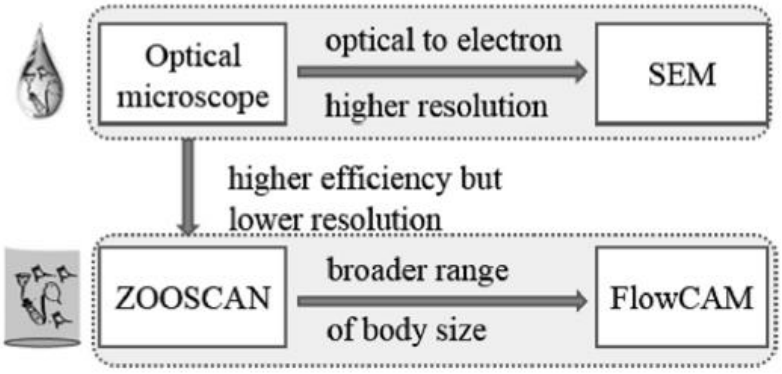

Fig Morphology-based methods for zooplankton biodiversity monitoring

2.2. New morphological identification methods

It is evident that SEMs and conventional microscopes can both identify biological features in great detail, but they are both unable to quickly analyse a huge number of species. Since the advent of digital imaging and scanning technology, numerous automatic or mostly automatic tools have been developed to quickly examine zooplankton samples. The two exemplary examples are the ZOOSCAN system and Flow CtometerAnd Microscope (FlowCAM). ZOOSCAN swiftly and (semi-)automatically takes digital pictures of zooplankton samples and analyses pictures using widely available databases. Combining ZooProcess and Plankton Identifier software, the ZOOSCAN system can gather and categorise digital zooplankton photographs from collected zooplankton populations by comparing scanned images of each object with a reference library. The ZOOSCAN system has the ability to calculate each species' specific body size and biomass for a quantitative examination of zooplankton samples. However, because ZOOSCAN's range of permissible organisms is only 200 m to several

centimetres, the majority of rotifera and protozoa zooplankton species in freshwater habitats are not suitable for the system. In order to swiftly calculate mesozooplankton biomass and size distribution for biodiversity monitoring, ZOOSCAN devices are currently employed extensively in maritime ecosystems.

Fluid imaging technology was used to produce FlowCAM, an automated imaging flow cytometer. Using laser detection, FlowCAM captures digital images of the particles and organisms in a fluid. Image analysis of collected digitised images enables precise estimation of the quantity and size of organisms as well as automatic classification of creatures. Nearly all species in zooplankton communities in freshwater settings, especially in polluted ones where smaller-sized species predominate, may be identified by FlowCAM, which can detect organisms with body sizes ranging from 3 to 3000 m. More studies have employed enhanced FlowCAM to quantitatively examine zooplankton even though it was originally created to analyse phytoplankton. Using FlowCAM, low-abundance sample issues can be resolved. Overall, it is evident that rapid and automatic zooplankton counting in mixed-species samples is useful for assessing biodiversity in contaminated freshwater ecosystems. FlowCAM can also identify low abundance species, such as endangered or recently introduced nonnative invasive species, for risk assessment and conservation management.

2.3. Technical challenges

The use of direct observation methods, such as using a microscope or scanning electron microscope (SEM) to directly observe morphological traits of zooplankton organisms or indirect methods like ZOOSCAN and FlowCAM, is a common practise in traditional approaches for monitoring freshwater biodiversity. We should be aware of any technological issues that still need to be handled since new strategies like genetic techniques can't totally replace these methods.

SEM has the potential to greatly improve the accuracy of traditional morphological methods. Of course, qualified taxonomists should carefully review and confirm the specific features, notably the new taxonomic keys. However, they are unlikely to be appropriate for broad surveys for ecological studies, such as the causes and effects of environmental pollution in freshwater habitats. Such in-depth analyses can assist in resolving taxonomy issues. By automating and digitising samples, ZOOSCAN and FlowCAM significantly increase the effectiveness of sample processing. However, FlowCAM and ZOOSCAN both degrade the resolution of species identification. Images from ZOOSCAN or FlowCAM are frequently categorised to high taxonomic levels, such as genus or above. ZOOSCAN also works well for species with body sizes between 200 millimetres and a few centimetres. In polluted freshwater, small animals like rotifers and ciliates typically control zooplankton groups. Since libraries for the taxonomic categorization of digital pictures were built from zooplankton species in seas, reference libraries derived from freshwater ecosystems must make considerable effort during this time. FlowCAM has the same problems as ZOOSCAN (specifically, reference libraries), although being more adapted to zooplankton monitoring in polluted freshwater settings. Additionally, automatic sample classification is prone to error because collected zooplankton samples contain a range of items, including artefacts. Therefore, validation or data quality control is required to avoid errors caused by automatic procedures.

III. Sample collection and DNA isolation/capture

In order to monitor biodiversity, there are two zooplankton sampling techniques. One method is collecting bulk specimen samples (community DNA metabarcoding), which entails filtering the same amount of water at each sampling site with plankton nets in order to quantitatively capture and enrich zooplankton. The samples can either be stored directly in anhydrous alcohol for preservation or further filtered using 5-m microporous filter membranes before being stored at -20°C for eventual DNA extraction. The alternative technique is ambient DNA metabarcoding sampling. All species living in aquatic settings have environmental DNA (eDNA), which is the DNA that has been suspended from skin cells, organelles, gametes, or even extracellular DNA. By filtering 1-2 L of water in a lab or the field, environmental DNA can be collected onto filter membranes and stored in anhydrous alcohol.

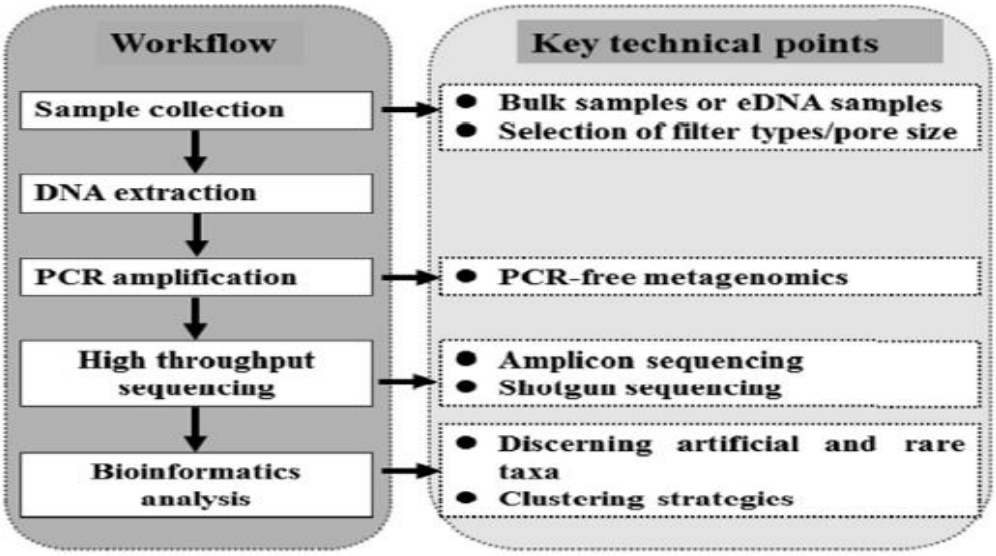

Fig. Work flow and key technical points of meta barcoding in biodiversity monitoring of zooplankton.

To date, there is still a debate on the selection of filers and associated parameters including type, pore size, pre-filtration and filter preservation strategy during the eDNA capture process. The most commonly used filter for eDNA capture is 0.45-μm cellulose nitrate membrane, followed by 0.75/1.2-μm glass microfiber filers or polyethersulfone.. Besides, in order to avoid false negatives caused by random sampling of rare taxa, it is important to conduct repeated sampling for at least three times at each sampling site.

It is important to keep in mind when we collect eDNA samples for biodiversity monitoring that 1) the stability, dispersal and degradation rate of eDNA are substantially influenced by various environmental factors such as temperature, pH, light intensity and water chemistry. Currently, most studies relating to zooplankton biodiversity analysis used bulk specimen samples However, when using eDNA to perform biodiversity monitoring, sampling will be easier and less invasive. Assessed the potential of eDNAmetabarcoding to monitoring zooplankton biodiversity, by comparing method of eDNAmetabarcoding to bulk sample metabarcoding and morphological methods, and they suggested that eDNAmetabarcoding should be able to provide complementary insights in biodiversity monitoring of zooplankton.

3.1.2. Selection of robust genetic markers and universal primers

A DNA sequence fragment known as a "DNA metabarcode" is utilised for the taxonomic identification of many species found in a mixed sample taken from a community or environment. The number of detected species, the make-up of taxonomic groups, and the precision of species identification will all be significantly impacted by the choice of DNA metabarcode. Therefore, selecting the right genetic producers is crucial for DNA metabarcoding analysis. It is expected that a desired genetic marker for PCR primer design will simultaneously contain both evolutionarily conserved and hypervariable regions, of which conserved regions were used to design universal primers for amplifying a wide range of species or taxonomic groups (versatility), and hypervariable regions in the amplicons were used to accurately distinguish the closely related species (high resolution).

3.1.3. Bioinformatics analysis

High throughput sequencing (HTS) in DNA metabarcoding analysis produces enormous short sequence reads, especially after processing a large number of samples gathered from very different geographic scales. The ensuing data processing steps face enormous problems as a result of such large data volumes. Filtering raw data, clustering operational taxonomic units (OTUs), and taxonomy assignment are all parts of a broad data analysis process. The research available demonstrates that sequencing mistakes have a significant impact on biodiversity estimations. Determining which sequencing errors are caused by genuine uncommon taxa and which are errors is difficult, making the removal of sequencing errors the first and one of the most crucial procedures. Low number of sequences (for example, low abundance OTUs) typically recover both rare species and artificial reads, and the removal of artificial reads is likely to filter out a significant fraction of rare species in samples. The clustering technique for OTUs may be another crucial step in employing DNA metabarcoding to evaluate community biodiversity, depending on the research goals. While a predetermined similarity threshold (such as 97%) used in the clustering step may split one traditional species into two or more OTUs or sequences for

different taxa were clustered into the same OTUs, these clustered molecular OTUs were assigned to traditional taxonomic species in biodiversity monitoring.

3.2. Indicator species detection based on metabarcoding

Biological indicator species are essential in determining the impacts of pollution on aquatic ecosystems and serving as early indicators of environmental changes due to their high sensitivity to environmental stressors. Instead of conducting a standard qualitative investigation of every species in biological communities, identifying indicator species offers a practical and appealing method for environmental monitoring, conservation, and management. The ubiquitous application of DNA barcoding in the detection of indicator species in biodiversity monitoring activities has increased the sensitivity and effectiveness of species-level identification. Numerous studies have demonstrated the effectiveness of DNA barcoding for identifying species, including fish, copepods, crustaceans, and molluscs. Due to high-throughput sequencing (HTS), the use of the DNA barcoding approach has recently been greatly expanded and now allows for the identification of indicator species from mixed-species samples or populations.

3.3. Rare species detection based on metabarcoding and qPCR

Aquatic ecosystems typically consist of a small number of dominating species and a large variety of low abundance species. In reaction to environmental disturbance or change, the uncommon biosphere taxa may move to new abundant members of a community, potentially providing a source of genetic and functional variety to preserve ecosystem functioning. Native rare species and recently introduced non-indigenous species (NIS) make up the majority of the rare biosphere in running water habitats. Native uncommon species may be in danger of becoming extinct and becoming endangered due to environmental pollution or demographic stochasticity. Furthermore, recently established NIS typically maintain low population densities in communities for a considerable amount of time, even in situations where they eventually become dominant to have significant detrimental consequences. Determining native vulnerable rare species for conservation and recently introduced NIS for biosecurity should therefore be a priority while exploring and monitoring the rare biosphere. However, because of their minute body size, variety of species, complicated community structure, and confusing physical features, microscopic organisms in freshwater environments continue to provide significant technical obstacles to the detection of uncommon species.

3.4. Technical difficulties and possible solutions

Even though DNA-based approaches to biodiversity assessment clearly have their benefits, technical obstacles and constraints still need to be overcome before biodiversity monitoring programmes can be put into place. As was already indicated, PCR-free strategies can get around some of the drawbacks of PCR-based techniques. These techniques, however, did not perform well in complicated real-community data and had low sensitivity to find unusual species. We concentrate on the widely applied PCR-based techniques in this study.

3.4.2. Reference database

One of our main goals when utilising DNA-based techniques to monitor biodiversity is to recover the taxonomic mix from sequence data. Sequences must be compared to a metabarcode reference dataset that contains taxonomy information in order to complete this phase. The accuracy of DNA metabarcoding is directly influenced by the completeness and high quality of taxonomic reference databases. Despite the fact that many DNA sequences have been uploaded to public databases, an important problem is the uneven representation of various taxonomic groupings in these databases. When compared to commercially significant or better investigated species, the number of available sequences for poorly studied species in public databases remains low. Therefore, extensive partnerships and sharing between various research groups, especially those with their own taxa of interest, should be encouraged in order to create and enhance community-level reference libraries. Additionally, the majority of the sequencing data in these databases came from conventional molecular makers like COI and ribosomal RNA genes, which is insufficient to detect complex communities and closely related species. Therefore, more sequence data from various molecular manufacturers should be gathered in order to create comprehensive reference libraries. Outlooks for the future

IV. CONCLUSION

In conclusion, metabarcoding is revolutionising the study of freshwater biodiversity and offers a potent tool for finding "hidden diversity" below the surface of the water. We suggest that researchers and managers embrace DNA-based metabarcoding because of its inherent useful and relevant qualities, which can mainly resolve technical challenges in biodiversity evaluation in polluted freshwater environments. In fact, a number of international organisations, including the European Water Framework Directive of the European Union, have approved DNA-based metabarcoding. However, there are still a lot of technical problems with the use of

metabarcoding-based biomonitoring in freshwater environments. To allow taxonomic retrieval of metabarcoding results, we should first keep building taxonomically complete reference databases based on various genetic markers. More variable gene areas should be screened in order to enable accurate identification, discrimination, and detection of closely related or cryptic species. Alternately, the use of short-gun sequencing method to sequence meta-DNA isolated from bulk samples might raise the resolution of taxonomical identification as HTS becomes more widely available and less expensive. Shot-gun sequencing is also a PCR-free technique, which is advantageous for quantifying taxon abundance. In metabarcoding analyses, spiking the standard DNA into various samples is a useful way to advance quantification. Finally, despite the fact that there are still several technical problems with morphology-based methods, it is clear that conventional methods based on morphology cannot be abandoned. To enable precise and speedy zooplankton species identification and to advance the analysis of causes and effects of biodiversity loss in contaminated freshwater environments, both conventional morphology-based and DNA-based approaches must be cross-referenced.

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