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The Monitoring of Sediment Protists in Lake and River Basins Based on Digital Holographic Microscopy

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Abstract: The community structure of protists reflects the environmental conditions during the period when the living environment was sampled. Biodiversity index, species composition and abundance can reflect various changes in the environment, indicating the quality of water quality during this period. Protists are ideal target species for environmental monitoring and can be used as indicator organisms. However, due to the few morphological characteristics of protists, they are easily affected by environmental pollutants and change their own form. The manual monitoring method has the possibility of human error, and long-term monitoring requires a lot of time and energy. The work caused great difficulties. Therefore, a monitoring technology that can efficiently monitor the protist community has important application value for environmental monitoring and biodiversity conservation. The digital holographic microscopic imaging technology has the characteristics of three-dimensional imaging, and can observe the community structure of protists. In this paper, this technology is used to monitor the community of protists in the sediments of the lake and river basins. Quantitative sequencing technology was used to analyze the diversity of protists, and the calculated alpha diversity index of the protist community was used to reflect the quality of water quality.

1. Introduction

Water pollution can cause biodiversity decline in aquatic ecosystems. Due to the long-term pollution of agricultural and industrial sewage in the lake basin, the aquatic ecosystem has been seriously degraded. Sediment protists are an important part of aquatic ecosystems. The traditional ecological monitoring method for protists is the PFU method, which is greatly affected by water quality. Therefore, this paper adopts digital holographic microscopy imaging technology to monitor sediment protists.

At present, there are many protist monitoring technologies used to monitor sediments in lake and river basins. For example, molecular biology methods have found ecological information of many unculturable micro-eukaryotes in anaerobic sediments. Several neolineages of anisoflagellates were found in anaerobic environments, which may represent true anaerobic or hypoxia-tolerant biological species, rather than invaders from other environments, which is in agreement with previous studies of anisoflagellates. The conclusions of Neozoic plankton taxa from distant ocean surface waters are different [1]. A scholar used the PRIMER software package to study the temporal and spatial changes of the community structure of benthic microeukaryotes, ciliates and dinoflagellates in seagrass beds through non-metric multidimensional scaling (NMDS) analysis, and found that benthic microeukaryotes, ciliates and beetles. The samples in the algal grass area are grouped into one group, and the samples in the grass-free area are grouped into a group, indicating that the community structure in the grass area and the grass-free area is different [2]. A scholar sampled protists on the surface of hot springs, and the relative abundance of each group in the sample points showed that there were many types of dominant groups of protists in surface sediments, including ciliates, sporozoites, Filopoda and flagellates [3]. Although there are many monitoring technologies, few scholars use digital holography to analyze the protist community, so it is necessary to further explore the application of digital holography in protist monitoring in the future.

In this paper, three traditional sediment protist community monitoring technologies are firstly proposed, and then the concept of digital holographic microscopy imaging technology is introduced. Through digital holography technology, the imaging image of the protist community structure can be obtained, and then the lake and river basin are sampled to obtain Protist sequences, and finally the differences in alpha diversity of different water types were analyzed.

2. Related Technologies

2.1. Traditional Sediment Protist Community Monitoring Technology

(1) mRNA-based cDNA library

Due to the stability of DNA and even amplification in dead cells or extracellular, DNA-based analysis of microbial community structure often ignores its viability or metabolic activity [4]. One of the main difficulties in molecular ecology research is that it is impossible to determine whether the research object really survives in the environment. This problem is very important for studying the impact of environmental chemical composition on microbial diversity and the changes in microbial community structure at spatial scales [5]. For example, in anaerobic water bodies with distinct oxygen and hydrogen sulfide concentration gradients, researchers found many of the same phylotypes in samples from different concentration gradients, but based on the DNA sequence information obtained, they were unable to determine their relationship between oxygen and sulfide. It is also impossible to tell whether the microorganisms detected in the bottom water body are actually alive in samples with such a wide difference in hydrogen concentration. Also in the study of benthic microbial community structure analysis, we cannot determine whether the detected microorganisms are true microbial community components, or just come from cells, cysts, or even extracellular DNA settled in the upper water body [6- 7].

(2) High-throughput sequencing technology

With the continuous development of sequencing technology, high-throughput sequencing technology has greatly developed microbial ecology and played an increasingly important role in solving biological problems. This technology makes it possible to gain a deep understanding of the rare biosphere and complete analysis of microbial communities [8]. High-throughput sequencing

technology has been applied to study the diversity of protists in a variety of ecosystems, including surface and deep seawater, marine sediments, lake water, soil, and metazoan hosts. However, this sequencing technology includes a one-step PCR reaction, and DNA polymerase and primers are prone to amplification bias, which affects the final amplification ratio and negatively affects subsequent analysis and research [9-10].

(3) Metagenome sequencing

Metagenomics is a microbial research method to study microbial diversity, community structure and function, evolutionary relationship and interaction with the environment [11]. The advantage of metagenomics is that it skips the PCR amplification reaction and is no longer limited to pure culture. In metagenomic sequencing, tag sequencing can capture low-abundance groups, and miTags can accurately reveal dominant groups with less preference. The combination of the two provides an efficient research tool for microbial research [12-13].

2.2. Digital Holographic Microscopy Imaging Technology

Digital holography refers to recording the hologram on the electro-coupled optical disc device CCD instead of the traditional hard disk, and re-creates the hologram through optical diffraction by the computer to capture the 3D image of the measured object [14].

The holographic wavefront is reproduced in the coaxial holography, which has a DC term, a constant term, and a conjugate image [15]. The DC term represents an undiffracted light field propagating parallel to the optical axis. The existence of the DC term will affect the quality of the reproduced image, and we can adopt some algorithms to suppress the influence of the DC term. The constant term represents the reference light intensity distribution and contains the light field we need to extract, which is multiplied by a constant factor. The conjugate image is similar to the incident light field recorded on the holographic dry plate, but has the opposite curvature, which is out of focus. The existence of the DC term and the conjugate image introduces the interference term to the holographic reconstruction, which affects the quality of the holographic reconstructed image [16-17].

3. Materials and Methods

3.1. Sample Collection

The Peteson mud grabber was used for sampling. During the sampling process, the disturbance of the water body was avoided, and the sediment samples on the surface were taken into the water. There are 15 sampling points in total.

3.2. Bioinformatics Analysis Methods

High-throughput sequencing results need to be pre-processed (quality analysis) to remove sequences containing six or more consecutive identical nucleotide sequences, or more than one mismatch in DNA samples, and more than 4 sequences in PCR primers. The 18srDNAV9 region retains a sequence length of about 100-200bp. Barcodes were removed from the sequencing results and annotated according to the alignment database [18]. The OTUs obtained from the annotations need to be compared for quality control according to the USEARCHV7 script, clustered according to the 97% similarity, and the OTU chimeras obtained from the clustering results are removed, the sequence of a single copy is removed, and the random errors generated in the previous steps are

removed [19]. The OTU sequence obtained after the final quality control can be used for further analysis.

3.3. Data Processing and Statistical Analysis

DNA extraction from lake sediment samples was performed using the NovoGene DNA extraction kit. Agarose gel electrophoresis was used to check the quality of the extracted DNA. The 18SrDNAV9 hypervariable region was amplified using polymerase chain reaction (PCR) with primers 1391f (GTACACCGCCCGTC) and 1510r (TGATCCTTCTGCAGGTTCACCTAC). PCR amplification in a 50uL reaction system contains 5μL 10xPCR buffer, 1.5uL dNTP primers, 1.5puL (10uM) for both forward and reverse, 0.5μL Taq DNase, 2puL template DNA (5-30ng), 1μL bovine serum albumin Protein (BSA), 37pL ddH2O. The PCR program was 94 ℃ for 1 min, 94 ℃ for 20s, 57 ℃ for 25s, 30 cycles of 68 ℃ for 45s, and a final extension at 68 ℃ for 10 min. The amplified products were sequenced and analyzed on the Ion S5XL technology sequencing platform.

3.4. Alpha Diversity

Alpha diversity calculates the alpha diversity index (Richness, Shannon, Simpson, Pielou, Chao1 index, and ACE index) of the protist community in the sediments of the lake river basin. The Richness index represents the species richness, which is the number of species in the ecosystem, That is, the number of OTU species cannot represent the relative abundance. The Shannon index was used to estimate the level of community diversity. The higher the Shannon index, the better the sample homogeneity. The Pielou index represents the evenness of the species. The Richness index, Shannon index, and Pielou index were used for statistical testing, and the non-independent sample T value was used to test whether there was a significant difference in the α diversity index between the two samplings.

The Shannon index is calculated as:

$$X = -\sum(Ni) \times (\ln Ni) \tag{1}$$

Pielou index calculation formula:

$$P = X / \ln Y \tag{2}$$

In the formula, Ni represents the ratio of the number of individuals to the population, X is the Shannon index, S represents the number of species, and P is the evenness.

4. Analysis of Experimental Results

4.1. Monitoring Results

Table 1 shows the statistical results of data screening in the sampling results in March and September. The number of OTU sequences is 238,745, of which 125,162 OTU sequences are found in postflagellate organisms, accounting for 52.43%; there are 37,485 OTU sequences in

anisoflagellate organisms sequence, accounting for 19.89%; protists have 31267 OTU sequences, accounting for 17.28%; primitive chromophore has 10535 OTU sequences, accounting for 10.40%.

Table 1. The proportion of protist sequences

	Postflagellate organisms	Anisoflagellate	Protists	Primitive chromophore
Sequence number	125162	47485	41267	24831
Proportion	52.43%	19.89%	17.28	10.40%

4.2. Sequencing of Sediment Protists

Figures 1 and 2 show the alpha diversity index in sediments. In the protist community, the Richness index from 3.6 to 81.2, the Simpson index from 0.64 to 65.3, the Shannon index from 0.12 to 0.75, the Pielou index from 0.13 to 0.66, the Chao1 index from 9.4 to 65.3, the ACE index from 4.6 to 67.1, and the sequencing depth index It indicates that the protist sequences in the samples have a high probability of being detected.

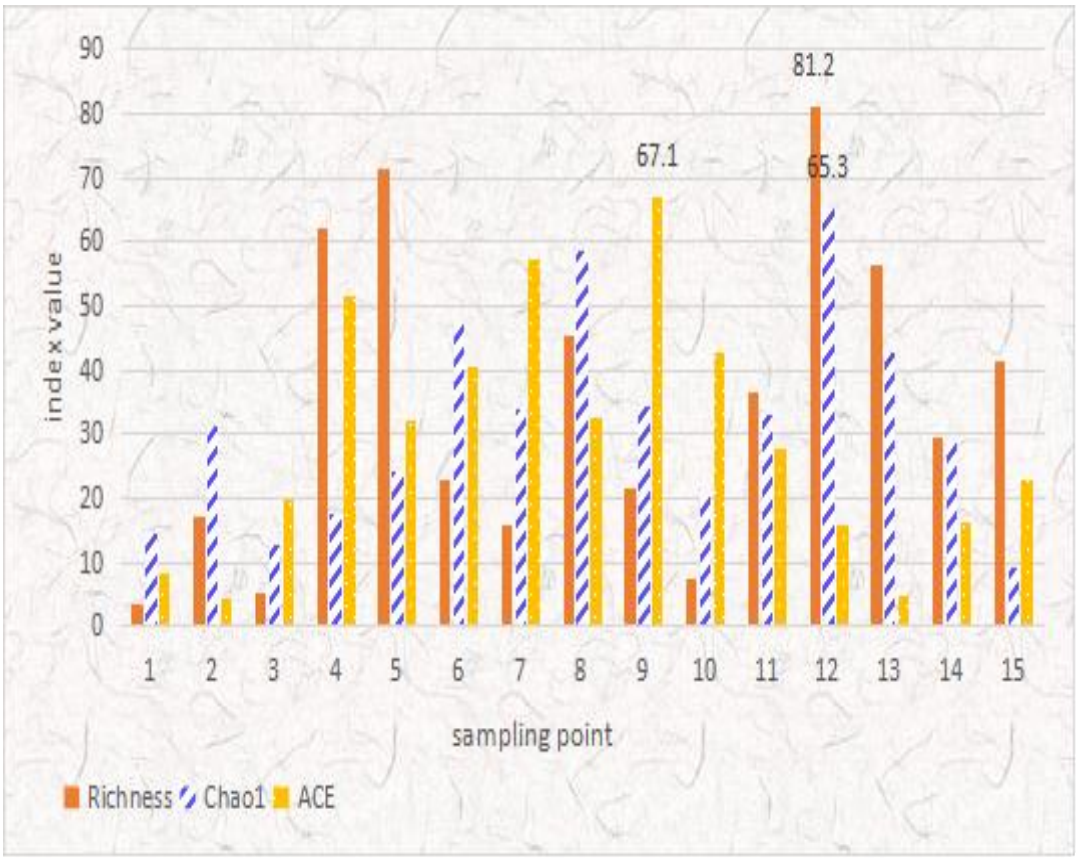

Figure 1. Richness index, Chao1 index, ACE index

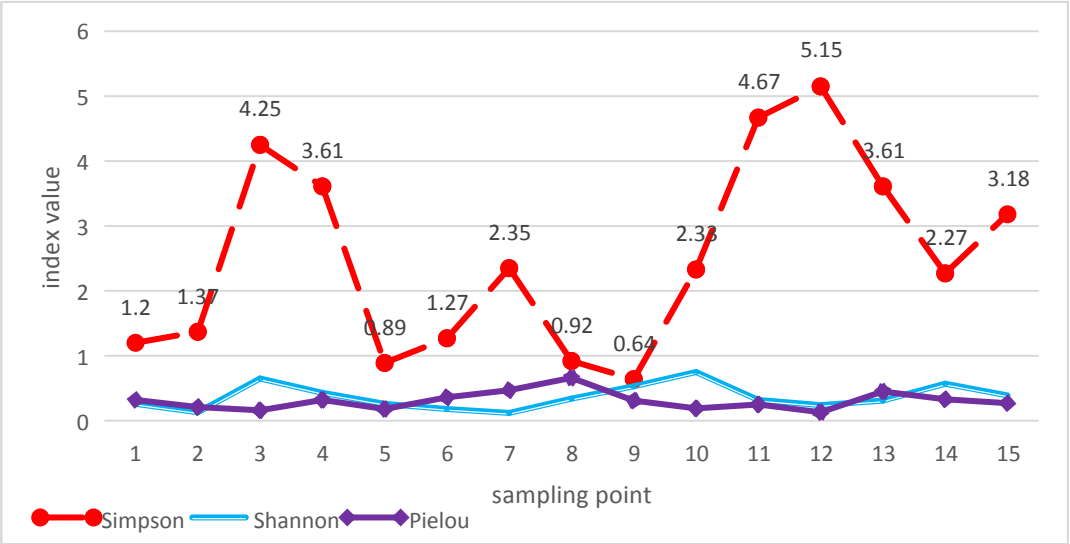

Figure 2. Simpson index, Shannon index, Pielou index

4.3. Differences in Alpha Diversity of Protists in Different Water Types

The samples are divided into three types, namely river sites, reservoir sites, and lake swing sites. According to the normality test analysis results, the groups are all subject to normal distribution. R statistical language was used for analysis, TukeyHSD test was performed on the results of one-way analysis of variance (ANOVA), and pairwise comparisons between groups were performed. The results are as follows.

The Richness index, Shannon index, and Pielou index were statistically tested, and the differences in the OTU level of the sampling sites in March are shown in Table 2. The statistical analysis results in March showed that there were significant differences in the Shannon index and evenness of rivers and reservoirs in the α diversity index at the OTU level selected between the three species of sampling sites. (Note: * represents significant difference)

Table 2. March diversity (OUT level) ANOVA analysis significance test graph

	River	Lake swing	Reservoir
Richness	315	267	282
Shannon	6.2*	6.9	7.3*
Pielou	0.57*	0.67	0.81*

Table 3. September diversity (OUT level) ANOVA analysis significance test graph

	River	Lake swing	Reservoir
Richness	227	235	264
Shannon	5.8	6.3*	6.9*
Pielou	0.65	0.62*	0.75*

The differences in the OTU level of the sampling sites in September are shown in Table 3. There are significant differences in the Shannon index and evenness index between the lake swing site and the reservoir site in the OTU level between the three types of sampling sites. Based on the above analysis, it can be seen that there are significant differences in the biodiversity of each sampling

point in the Lake River Basin. Because of this, the biological species of protists are different, so they can be used as indicator species to monitor water quality.

5. Conclusion

Protists are a class of single-celled organisms with simple and primitive structures that play an important role in ecosystems. Protists reproduce extremely fast and are extremely sensitive to changes in the living environment, reflecting rapid changes in population and morphology. For example, there is a huge difference in biological abundance between ciliates in clear water lakes and in heavily eutrophic lakes. Therefore, protists can be used as target species for monitoring biodiversity, which can be used to assess the environment. This paper studies the application of community monitoring of sediment protists in the Lake River Basin. The results show that the monitored protist sequences account for 17.28%, and there are significant differences in the alpha diversity index at the OTU level selected between the sampling sites.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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