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The Effects of Water Level Fluctuation on Zooplankton Communities in Shahu Lake Based on DNA Metabarcoding and Morphological Methods

Xuemei Qiu 1,2, Quanfeng Lu 1, Chenchen Jia 1, Yuting Dai 1, Shan Ouyang 1 and Xiaoping Wu 1,*

1 School of Life Science, Nanchang University, Nanchang 330036, China; qiuxuem2i165@126.com (X.Q.); qfl89509@163.com (Q.L.); bb960301@163.com (C.J.); dyt18270925406@163.com (Y.D.); ouyangshan@ncu.edu.cn (S.O.)
2 School of Life Science, Jiangxi Science and Technology Normal University, Nanchang 330013, China
* Correspondence: xpwu@ncu.edu.cn

Simple Summary: The water level in Shahu Lake varies greatly during an annual cycle: ~4 m deep to nearly dry. Over the course of one year we studied the relationship between zooplankton diversity and the water level in Shahu Lake using DNA metabarcoding. The morphology method was compared with the DNA metabarcoding method to see whether the results were replicable. The results were highly consistent for α-diversity and the community composition of zooplankton using both methods; both methods also showed a significant relationship between the zooplankton community composition and water level. Our research contributes to the application of the DNA metabarcoding method and aquatic ecological investigations.

Abstract: Background: The water level of Poyang Lake (China) fluctuates seasonally. Shahu Lake, a smaller body of water connected to Poyang Lake during the wet season, is separated in the dry season. Due to a special fishing method termed ‘lake enclosed in autumn’, the water level is lowered and reaches its lowest point in January, which is <0.5 m deep in the middle of the lake. Our research investigated the effect of water level changes on the zooplankton community composition in Shahu Lake. Methods: We used both DNA metabarcoding method (MBC) (18S rRNA gene V4 region) and morphological method (MOI) to track the zooplankton community structure over four seasons in Shahu Lake (China). Results: Totals of 90 and 98 species of zooplankton were detected by MOI and MBC, respectively, with rotifers being the main zooplankton component. The α-diversity index of both methods increased from spring to summer and decreased from summer to autumn, reaching the lowest value in winter. NMDS and a cluster analysis showed that all zooplankton communities detected by MOI and MBC were significantly separated by season. The zooplankton community in winter was separated from that of the other three seasons, but the summer and autumn communities were more similar. Conclusions: Changes in the water level had significant effects on the zooplankton community composition. We found that MBC was more able to detect the differences in the zooplankton composition than MOI. MBC also had more advantages in copepod recognition. In our study, 37 species of copepods were detected by MBC, but only 11 species were detected by MOI. We concluded that MBC should be used to research the seasonal variations of zooplankton.

Keywords: China; DNA metabarcoding; morphological method; Poyang Lake; zooplankton

1. Introduction

The largest freshwater lake in China, Poyang Lake (115°55′–116°03′ E, 29°05′–29°15′ N), is located in northern Jiangxi Province. However, this lake is a floodplain–wetland complex, comprising many sub-lakes that have been formed by varied processes including hydrodynamics, sediment erosion and deposition, and artificial modification [1]. The result is a variety of depressions varying in size and shape. During floods, the sub-lakes merge...
with the main lake, but during periods of low water, these sub-lakes are relatively isolated from Poyang Lake. The sub-lakes have ecological values such as a large vegetation biomass, an abundant species diversity, and excellent migratory bird habitats, all of which play an important role in maintaining the wetland biodiversity and ecosystem integrity [2]. Shahu Lake is one of the main sub-lakes of the Poyang Lake National Nature Reserve. Local fishermen harvest fish from that lake using a method known as ‘lake enclosed in autumn’. In this process, a gate is opened to drain water from the sub-lake in the middle of October. Thus, the lake is basically drained until January of the next year when the gate is closed. Draining causes the surface of the lake to drop sharply to 0.3–0.4 m [1]. Due to the importance of this lake to the local fishery, we studied the spatial and temporal distribution of zooplankton in sub-lake Shahu Lake during a yearly cycle of water level changes [3].

Water level fluctuation is a key factor affecting biodiversity and ecosystem functions in aquatic habitats [4,5]. Due to the small size and short lifespan of zooplankton, the structure of their community is sensitive to environmental changes, especially in lakes with significant water level fluctuations such as flooding or water disturbance [6]. Zooplankton are an integral part of the food web, connecting phytoplankton and fish [7]; thus, they represent a major food source for fish [8] and, ultimately, the zooplankton community influences the fish density [9]. Zooplankton also are important indicators of ecological conditions [10,11] and can be used to control the standing biomass of cyanobacteria. Thus, they can contribute to ecological restoration and improved water quality [12,13].

As a result, monitoring zooplankton community structures provides an insight into ecosystem functions. Unfortunately, monitoring zooplankton using morphological methods to obtain qualitative and quantitative information about the zooplankton composition is both laborious and time-consuming; it requires considerable human and material support [14–17]. For example, copepods cannot be identified in their early stages of life (copepods nauplii) [15]; thus, overcoming the difficulties of identifying species and reducing the cost of classification are important problems to be solved.

With the development of second-generation sequencing technology and the concomitant reduction of sequencing costs, DNA metabarcoding has become an important tool for biodiversity investigations. It has an efficient processing speed as well as a sensitive detection efficiency [18,19]. Studies have shown that DNA metabarcoding technology can effectively characterize the species composition of environmental DNA or a large number of biodiversity samples. As a result, this technique has become an important tool to make distinct contributions to ecological studies [20,21]. It can also be a useful tool for assessing zooplankton diversity in marine and freshwater environments [22–24] because DNA barcoding sequences can identify morphologically unrecognizable larval stages [25,26]. Studies have shown that the zooplankton community structure has obvious seasonal characteristics [5] with water level fluctuation being a main driving factor for zooplankton diversity [4,27]. However, there are few reports on the effects of water level fluctuations on zooplankton using DNA metabarcoding.

We studied the impact of water level changes on the zooplankton diversity and community structure in a sub-lake (Shahu Lake) based on DNA metabarcoding technology and morphology methods. This comparative study provides theoretical support for the planning and management of water resources for biodiversity conservation and an important scientific basis for the protection and sustainable utilization of lake ecosystem biodiversity resources.

2. Materials and Methods

2.1. Sample Collection

Zooplankton samples were collected from five sampling sites in Shahu Lake at four different times during a single hydroperiod cycle: April 2019 (spring); July 2019 (summer); October 2019 (autumn); and January 2020 (winter). For each sample, 10 L of water was filtered through a plankton net with a mesh size of 64 µm, preserving three replicates in 4%
formalin for the morphological methods (MOI) [5] and two replicates in 95% ethanol for the DNA extraction (MBC) (Figure 1).

**Figure 1.** Sites sampled in the sub-lake Shahu Lake of the floodplain–wetland complex of Poyang Lake (Northern Jiangxi Province, China). The sampling sites are numbered 1, 2, 3, 4, and 5.

### 2.2. Physiochemical Analysis of the Water

At each sampling, we measured several environmental factors. A YSI 650MDS (YSI) multiparameter meter was used to measure the water temperature (°C), dissolved oxygen (mg/L), pH, salinity (mg/L), and turbidity (NTU+). The chlorophyll A concentration (mg/L) was measured by a chlorophyll meter (PCH-800); the water velocity was measured by a velocity meter (FP111, Global Water, 0.1 m/s accuracy). We used a digital sonar system (H22px handheld sonar system) to measure the water depth (m). We collected water samples for each site, preserved them in sulfuric acid (H₂SO₄), and refrigerated them before measuring the total nitrogen (TN, mg/L) and total phosphorus (TP, mg/L) using ultraviolet spectrophotometry.

### 2.3. Morphological and Molecular Research Methods

We stained the morphological samples with Rose Bengal sodium salt for 24 h. Species identification and counting were conducted under an anatomical microscope (Leica, S9I) and a compound microscope (Leica, DM500). The species were identified to the species level or genus level. The larvae of the copepods could not be identified to the species level so they were all counted as one species. Zooplankton were then identified [28–35]. The
method used for zooplankton counting was volume sampling based on Zhang and Huang (1995) [36].

Disposable 50 mL plastic boxes and nitrile gloves were used to collect the samples to prevent DNA contamination. In the field, plankton nets were thoroughly triple-rinsed with river water between the sample sites [37]. Each sample tube was sealed and the samples were stored at 4 °C until the DNA extraction. The DNA was extracted within two weeks. The DNA extractions and PCR amplification were conducted in a fume hood and all the disposable pipes and liquid-transferring suckers were high-temperature sterilized in advance. The DNA was extracted using a marine fish tissue DNA extraction kit (TIANamp Marine Animals DNA Kit) and performed according to the instructions of the kit.

The PCR amplification system (25 µL) was 5 × 5 µL reaction buffer, 5 × 5 µL GC buffer, 2 µL dNTP (2.5 mM), 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 2 µL DNA template, 8.75 µL ddH2O, and 0.25 µL Taq DNA Polymerase.

The primer sequences were as follows [38]:

Uni18S: AGGGCAAKYCTGGTGCCAGC;
Uni18SR: GRCGGTATCTRATCGYCTT.

2.4. High-Throughput Sequencing and Bioinformatics

The PCR amplification products were sequenced using the Illumina MiSeq platform from Shanghai Personalbio Technology Co., Ltd. (Shanghai, China). The libraries were prepared using the TruSeq Nano DNA LT Library Prep Kit of Illumina and then the PCR amplification products were pooled to form a library for sequencing. The equimolar PCR products from each sample were used to ensure an equal contribution of each community in the final sequencing library. An Illumina MiSeq platform (San Diego, CA, USA) was used using a paired-end run of about 430 bp sequence reads after the library preparation.

Raw FASTQ files were demultiplexed and quality filtered using QIIME 1.17 and reads of a low quality (mean quality < 20; scanning window = 50; contained ambiguous ‘N’; sequence length: ≥150 bp) were discarded. UCLUST was used to cluster the operational taxonomic units (OTUs) with a 97% similarity threshold and QIIME1.17 was used to generate rarefaction curves. The Statistical Assignment Package (SAP) version 1.3.2 was used to assign the representative sequence from each OTU to a specific taxonomic group according to a reference database (the NCBI nucleotide database in GenBank).

2.5. Analytical Method

The dominant species were calculated by the formula below:

\[ Y = \frac{n_i \times f_i}{N} \]

where Y is the species dominance, \( n_i \) is the number of individuals of species \( i \), \( N \) is the total number of individuals for all species, and \( f_i \) is the frequency of occurrence of the species. When the species’ \( Y \geq 0.02 \), it was recognized as the dominant species. For MOI, we used the density of the zooplankton for the calculation and for MBC, we used the zooplankton species and the reads.

NMDS was drawn with Primer5. SPSS 25 was used for the ANOVA analysis of the diversity index. We used R package Vegan for the ANOSIM statistics as well as for mapping and calculating the α-diversity index (the Shannon–Wiener Index, Simpson’s Diversity Index, and Pielou Evenness Index). We used R package ggvenn for the Venn diagrams. The line chart was drawn using Origin8. The image processing used Adobe Illustrator CC 2019 and the other analyses were performed using Excel 2010 for statistics and analyses.

A redundancy analysis (RDA) with 499 Monte Carlo permutations was performed using CANOCO version 4.5 to evaluate the correlation between the environmental factors and the community composition of the zooplankton. All environmental factors and the community composition of the zooplankton were log10-transformed (\( X + 1 \)) to meet the assumptions of multivariate normality and to moderate the influence of extreme data [39].
3. Results

3.1. Sequence Classification Composition and Richness

A total of 464,563 sequences and 3080 OTUs were detected by MBC. The results of the sequence alignment showed that the sequence mainly belonged to Arthropoda and rotifers; the sequence of the Arthropoda accounted for 43.43% and OTUs accounted for 33.12% whereas the sequence of the rotifers accounted for 25.90% and OTUs accounted for 16.79%. The sequence length was mostly about 428 bp (Figure 2).

![Sequence length composition diagram.](image)

3.2. Taxon and Species Composition

Rotifers were the main zooplankton detected by the morphological method (MOI) and DNA metabarcoding method (MBC) (Figure 3). A total of 90 species of zooplankton were detected by MOI (Table A1); they belonged to 49 genera and 22 families. A total of 98 species of zooplankton belonging to 30 families and 66 genera were detected by MBC. We detected 66 species of rotifers (26 genera) by MOI; this accounted for 73.3% of the total zooplankton species. There were 11 species of copepods (11 genera), which accounted for 12.2% of the total zooplankton species, and 13 species of cladocerans (10 genera), which accounted for 14.4% of the total zooplankton species. The results of MBC showed that 58 rotifers (39 genera) accounted for 59.2% of the total zooplankton species, 37 copepods (23 genera) accounted for 37.8% of the total zooplankton species, and 3 cladocerans belonging to 3 genera accounted for 3.1% of the total zooplankton species.

There were 21 species (12.6%), 22 genera (25.6%), and 16 families (41%) detected by both methods (MOI, MBC). As observed from the various monitored zooplankton groups, the total number of rotifer species detected by MOI and MBC was 108 and 16 were species detected by both methods, accounting for 14.8%. For copepods, a total of 45 species were detected including 5 species in both methods, accounting for 11.1%. A total of 16 cladocerans and 2 species were detected in both methods, accounting for 12.5% (Figure 4).
Figure 3. Three zooplankton species were detected by MOI and MBC. A: autumn; SP: spring; SU: summer; W: winter.

Figure 4. Venn diagram comparing the assessment of species composition in Shahu Lake (China). (a) Venn diagrams of zooplankton composition at the species, genus, and family level by MOI and MBC. (b) Venn diagrams of species composition of Rotifer, Copepod, and Cladoceran by MOI and MBC.

The cluster heat map showed that summer and autumn and spring and winter were clearly separated by the MBC analysis. A greater number of taxa were identified in summer and autumn and fewer in winter and spring. In contrast, MOI identified more species
in summer and autumn, but only autumn had a distinct community from the rest of the seasons (Figure 5).

Figure 5. Cluster heat maps of family by MOI and MBC. (a) MBC; (b) MOI. A: autumn; SP: spring; SU: summer; W: winter. Numbers 1, 2, 3, 4, and 5 are the sample sites.

3.3. The $\alpha$–Diversity Index

The variation trend in the zooplankton $\alpha$–diversity index of MOI and MBC was consistent. The $\alpha$–diversity index increased from spring to summer and was significantly higher in summer than in the other seasons; the diversity index decreased from summer to autumn and reached the lowest in winter (Figure 6).

For the MOI, the Shannon–Wiener Index was significantly different between spring and summer (ANOVA, $p = 0.000$), spring and winter (ANOVA, $p = 0.026$), summer and autumn (ANOVA, $p = 0.000$), and summer and winter (ANOVA, $p = 0.000$). The Simpson Index for summer was significantly different from the other three seasons (ANOVA, $p = 0.029$, $p = 0.002$, and $p = 0.001$). Additionally, the Pielou Evenness Index was significantly different in spring and autumn (ANOVA, $p = 0.003$), spring and winter (ANOVA, $p = 0.008$), summer and winter (ANOVA, $p = 0.002$), and summer and autumn (ANOVA, $p = 0.001$) whereas the Shannon–Wiener Index, the Simpson Index, and the Pielou Evenness Index showed no difference among all the sampling points detected by MOI (ANOVA, $p > 0.05$).

For the MBC, the Shannon–Wiener Index was significantly different between spring and summer (ANOVA, $p = 0.000$), spring and autumn (ANOVA, $p = 0.026$), spring and winter (ANOVA, $p = 0.002$), summer and autumn (ANOVA, $p = 0.001$), summer and winter (ANOVA, $p = 0.000$), and autumn and winter (ANOVA, $p = 0.000$). The Simpson Index was significantly different between spring and summer (ANOVA, $p = 0.016$), spring and winter (ANOVA, $p = 0.001$), summer and winter (ANOVA, $p = 0.000$), and autumn and winter (ANOVA, $p = 0.000$). The Pielou Evenness Index was significantly different in spring and summer (ANOVA, $p = 0.005$), spring and winter (ANOVA, $p = 0.000$), summer and autumn (ANOVA, $p = 0.003$), summer and winter (ANOVA, $p = 0.000$), and autumn and winter (ANOVA, $p = 0.000$). As with MOI, the Shannon–Wiener Index, the Simpson Index, and the Pielou Evenness Index showed no difference among all the sampling points detected by MBC (ANOVA, $p > 0.05$).
Figure 6. Seasonal variation of zooplankton $\alpha$–diversity. (a) MBC; (b) MOI, including the Shannon–Weiner Index, Simpson’s Index, and Pielou Evenness Index. SP: spring; SU: summer; A: autumn; W: winter. a, b, c, d are marked letter, the difference is insignificant if there is a same marked letter, otherwise significant if there is a different marked letter. o is the outliers.

3.4. Community Feature

The NMDS analysis showed that all the zooplankton communities detected by MOI and MBC were significantly separated by season (Figure 7). The cluster analysis showed that the zooplankton community in winter was separate from that of the other three seasons; the summer and autumn communities were more similar and gathered into one branch. The ANOSIM results showed that the R values of both MOI and MBC were greater than 0, indicating that the seasonal differences of the zooplankton in Shahu Lake were greater than the intra–seasonal differences. The $p$–values of both methods were 0.001, indicating that there were extremely significant differences.

The RDA results (Figure 8, Table A2, Table A3) showed that the cumulative percentage difference between the zooplankton and the environment was 53.7% (MOI) and 73.1% (MBC). MOI showed that the main environmental factors affecting the zooplankton community structure were temperature (T), pH, water depth (WD), and salinity (Sal) whereas MBC showed the main environmental factors were total nitrogen (TN), water depth (WD), velocity (V), pH, and salinity (Sal).
Figure 7. NMDS sorting diagram based on multidegree data and a similarity clustering analysis diagram. (a) MBC; (b) MOI. Sampling sites 1–5. SP: spring; SU: summer; A: autumn; W: winter.

Figure 8. RDA of zooplankton family composition with environmental factors. (a) MBC; (b) MOI.

4. Discussion

Changes in the water levels led to significant seasonal changes in the zooplankton community (Figure 3). There were 36 (spring), 71 (summer), 63 (autumn), and 35 (winter) species of zooplankton on average detected by MOI and MBC. Rotifer had 19 (spring), 56 (summer), 39 (autumn), and 23 (winter) among them. The number of rotifers increased at first and then decreased from spring to winter. When the water level was higher in summer and autumn, there were more rotifers. The average number of rotifer species detected by MOI and MBC was 47 in summer and autumn; the number of rotifer species then decreased in spring and winter due to low water levels. The average number of rotifer species detected by the two methods was 21 in spring and winter; the number of rotifer species decreased by 55.3% from the wet season to the dry season. The result was consistent
with the research of Novotny who, by analyzing the diversity of trophic niches, found that the smaller sized rotifer had population peaks during summer [40]. Other research using morphological methods has also reported similar results [41–43].

We posited that variations in the lake water level caused a significant seasonal change in the zooplankton diversity [44] (Figure 6). The Shannon–Weiner Index, the Simpson Index, and the evenness index of the zooplankton showed a trend of first increasing and then decreasing from spring to winter in both MOI and MBC. The index value was the highest in summer and lowest in winter. The zooplankton diversity was highest when the water level was high and lowest when the water level was low.

Changes in the water levels caused the dominant species to change with the seasons (Table 1). Copepods were predominant in spring, autumn, and winter; rotifers were predominant in summer and the dominant rotifers were fewer in spring and winter due to low water levels. MOI showed the dominant species of rotifers totaled 5 in spring, 13 in summer, 4 in autumn, and 3 in winter. The dominant copepods in the four seasons were nauplii; Microcyclops varicans was dominant in spring and Mesocyclops leuckarti was dominant in autumn. MBC showed no dominant rotifers in spring, but 10 in summer, 5 in autumn, and 1 in winter. There were 6 dominant species of copepods in spring, 3 in summer, 4 in autumn, and 2 in winter. No dominant cladocerans were recognized by either MOI or MBC. Studies have shown that zooplankton communities are strongly influenced by climate warming and nutrient load; therefore, eutrophication and climate warming can change the zooplankton community structure and increase the dominance of small crustaceans [43]. In this study, we found that the low density of cladocerans in Shahu Lake could be related to the high degree of eutrophication in the sub-lake.

Table 1. Seasonal variation of dominant species found in Shahu Lake (China) in which morphological method (MOI) and DNA metabarcoding method (MBC) were used.

| Group   | Species                        | SP | SU    | A    | W   |
|---------|--------------------------------|----|-------|------|-----|
| Rotifers| Anuraeopsis fissa (MOI)         |    | 0.04  |      | 0.12|
|         | Ascomorpha ovalis (MBC)         |    | 0.03  |      |     |
|         | Asplanchna prionota (MOI)       |    | 0.02  |      |     |
|         | Asplanchnopus dahlgreni (MBC)   |    | 0.04  | 0.04 |     |
|         | Asplanchna brightwellii (MBC)   |    | 0.04  | 0.04 |     |
|         | Brachionus angularis (MOI)      | 0.06|    |      |     |
|         | Brachionus calyciflorus (MOI)/MBC| 0.14|    | 0.02 |     |
|         | Brachionus budapestiensis (MOI) |    | 0.03  |      |     |
|         | Brachionus falcatus (MOI)       |    | 0.04  |      |     |
|         | Brachionus diversicornis (MOI)  |    | 0.03  | 0.08 |     |
|         | Brachionus urceolaris (MBC)     |    | 0.07  | 0.04 |     |
|         | Brachionus sp. 1 (MBC)          |    | 0.02  |      |     |
|         | Cephalodella gibba (MOI)        |    | 0.03  |      |     |
|         | Colloetheca tenuilobata (MBC)   |    | 0.04  |      |     |
|         | Filinia longiseta (MOI)         | 0.04|    | 0.04 |     |
|         | Hexarthra intermedia (MBC)      |    | 0.06  |      |     |
|         | Keratella valga (MOI)           |    | 0.09  | 0.11 |     |
|         | Keratella cochlearis (MOI)      |    | 0.15  | 0.24 |     |
|         | Keratella quadrata (MBC)        |    | 0.11  | 0.05 |     |
|         | Lecane sp. 1 (MOI)              | 0.02|    |      |     |
|         | Polyarthra dolichoptera (MOI)/MBC| 0.15|    | 0.06 |     |
|         | Polyarthra vulgaris (MOI)       |    | 0.07  |      |     |
|         | Polyarthra remata (MBC)         |    | 0.02  |      |     |
|         | Ptygura libera (MBC)            |    | 0.04  |      |     |
|         | Rotaria neptunia (MOI)          |    | 0.02  |      |     |
|         | Synchaeta tremula (MOI)/MBC     |    | 0.06 | 0.03 |     |
|         | Trichocerca cylindrica (MOI)    |    | 0.02  |      |     |
|         | Trichocerca capucina (MOI)      |    | 0.05  |      |     |
|         | Trichocerca lophoessa (MOI)     |    | 0.04  |      |     |
Table 1. Cont.

| Group   | Species                        | SP  | SU  | A    | W    |
|---------|--------------------------------|-----|-----|------|------|
| Copepods| Copepod nauplii (MOI)          | 0.32| 0.08| 0.33 | 0.32 |
|         | Eucyclops serrulatus (MBC)     | 0.02|     |      |      |
|         | Eucyclops dumonti (MBC)        | 0.06|     |      |      |
|         | Mesocyclops leuckarti (MOI)    |     |     | 0.03 |      |
|         | Microcyclops varicans (MOI)    | 0.04|     |      |      |
|         | Mesocyclops pehpeiensis (MBC)  | 0.02| 0.03|      |      |
|         | Mesocyclops dissimilis (MBC)   | 0.3 | 0.05| 0.06 |      |
|         | Neodiaptomus schmackeri (MBC)  | 0.31|     |      |      |
|         | Pseudodiaptomus inopinus (MBC) |     |     | 0.2  |      |
|         | Sinocalanus sinensis (MBC)     | 0.08|     | 0.31 | 0.08 |
|         | Thermocyclops sp. 1 (MBC)     |     |     |      | 0.74 |
|         | Thermocyclops czessus (MBC)    |     | 0.05| 0.12 |      |
|         | Thermocyclops decipiens (MBC)  |     |     |      | 0.03 |

1 SP, SU, A, and W are spring, summer, autumn, and winter, respectively. —: the species was not dominant in the season. MOI: the species was the dominant species in MOI. MBC: the species was the dominant species in MBC. MOI/MBC: the species was the dominant species in both MOI and MBC.

A variation in the water levels of Poyang Lake drives environmental heterogeneity, with the main abiotic factors being the water level, water temperature, electrical conductivity, total nitrogen, nitrates, and total phosphorus [45,46]. Water level fluctuations are a key factor affecting aquatic biodiversity in seasonally submerged freshwater ecosystems including in floodplain wetlands; the biomass and individual size of zooplankton are the lowest in seasonally submerged floodplain habitats [4,47]. A few studies have shown that the α-diversity of zooplankton in floodplains changes with the water level [27,46]. Our study found that when Shahu Lake was connected to the main lake in summer, its α-diversity also reached the highest value. The water level of Shahu Lake was relatively high in summer and autumn and its species diversity was higher in these seasons than in spring and winter (Figure 6). The NMDS and cluster analysis showed that the zooplankton communities clustered together in summer and autumn (Figure 7); in spring and winter, the zooplankton communities gathered separately, also indicating that the zooplankton community was correlated with the water level. The reason may relate to the change of the phytoplankton. Using DNA metabarcoding to study trophic interactions, Zamora-Terol [48] found that the spring phytoplankton bloom, a dominance of diatom and dinoflagellate trophics with links to copepods, and summer zooplankton showed a more diverse diet dominated by cyanobacteria and heterotrophic prey. A five-year study of the phytoplankton succession in Poyang Lake by Qian et al. (2021) found that water level fluctuations greatly influenced phytoplankton succession and hydrology fluctuations had an indirect impact on a decrease of the Cyanophyta biomass. Several other studies have also showed the positive correlation between zooplankton abundance and phytoplankton biomass [49–51]. Although a change in the water levels did not stop the growth of zooplankton, it changed the community structure of the zooplankton; copepods were dominant in spring, autumn, and winter and rotifers were dominant in summer.

5. Conclusions

The effective identification of freshwater zooplankton to the species level is an important step toward understanding the structural richness and diversity of zooplankton populations [18,52–54]. MOI is a classic method that has been used for some time, but MBC is rapidly becoming a valuable research method. Previous studies have shown that MOI and MBC provide complementary information in biological surveys. When properly implemented, these two methods can be reliable, efficient, and low-cost in assessing the environmental impact of the marine industry [55,56]. Many studies have shown that MBC data provide the ability to identify correlations between the community structure and environmental parameters comparable with, or superior to, MOI [57]. In our study, we also
found a consistency between MBC and MOI when studying the zooplankton diversity and community structure. A few researchers argue that MBC could provide wider coverage and a better resolution of taxa when compared with MOI; that outcome would strengthen biological investigations of freshwater plankton communities [38,59]. Nevertheless, generating accurate species lists from MBC data is challenging [60]. Thus, the availability of reference sequences linked to known species needs to be developed [17,61]. Several researchers have found that MBC was superior to MOI in single-sample comparisons [62,63]. In our study, the number of zooplankton species detected by MBC was higher than MOI (Table S1), but MBC was highly consistent with MOI in the study of α-diversity. However, there were also inconsistencies between MBC and MOI. For example, the species list determined by MBC was different from that of MOI. We found that three dominant species were detected by both methods, but, at the genus level, there were five dominant rotifer genera detected by both methods. This accounted for 50% of the total. The differences between the results obtained by MOI or MBC may partly be attributed to an erroneous taxonomic assignment and/or cryptic species as well as species that are hard to detect by visual means [17]. This ignores the possibility of errors in the reference database (the NCBI nucleotide database in GenBank). Nevertheless, that database is improving and recent analyses have shown that metazoan identifications in GenBank are accurate with an error rate probably < 1% at the genus level and can, therefore, be used reliably [64]. These issues will, no doubt, be improved by subsequent analyses. It seems appropriate that researchers should combine MBC with MOI at this stage, but increase the research on MBC, especially the corresponding relationship between the reads and OTU numbers generated by MBC and the density and biomass of MOI. We believe that the MOI and MBC complement each other and provide a more accurate and efficient means of freshwater ecosystem biodiversity assessments when used in combination.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani12080950/s1, Table S1: The mean density of zooplankton detected by MOI, and the reads of zooplankton detected by MBC.

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Conflicts of Interest: The authors declare no conflict of interest.
### Appendix A

#### Table A1. The species list of MBC and MOI.

| Group       | Family       | MBC Genus | Species                  | Group       | Family       | MOI Genus | Species                  |
|-------------|--------------|-----------|--------------------------|-------------|--------------|------------|--------------------------|
| Rotifers    |              | Rotifers  | Asplanchna brightwellii  | Rotifers    | Asplanchna  | Asplanchna brightwellii  |
| Asplanchna  |              | Asplanchna| Asplanchna brightwellii  | Asplanchna  | Asplanchna  | Asplanchna girodi        |
| Asplanchnus |              | Asplanchnus| Asplanchnus dahlgrei    | Asplanchna  | Asplanchna  | Asplanchna priodonta     |
| Brachionidae| Brachionus   | Brachionus| Brachionus calyciflorus  | Brachionidae| Brachionus  | Brachionus multiceps     |
|             |              | Brachionus| Brachionus plicatilis    |             | Brachionus  | Anuraeopsis fissa        |
|             |              | Brachionus| Brachionus sp. 1         |             | Brachionus  | Brachionus angularis     |
|             |              | Brachionus| Brachionus urceolaris    |             | Brachionus  | Brachionus budapestensis |
|             |              | Epiphanes | Epiphanes senta          |             | Brachionus  | Brachionus calyciflorus  |
|             |              | Euchlanis | Euchlanis dilatata       |             | Brachionus  | Brachionus diversicornis |
|             |              | Keratella | Keratella quadrata       |             | Brachionus  | Brachionus faicalis      |
|             |              | Lepadella | Lepadella rhomboides     |             | Brachionus  | Brachionus forficula     |
|             |              | Mytilina  | Mytilina mucronata       |             | Brachionus  | Brachionus leydigii      |
|             |              | Platirus  | Platironus patulus       |             | Brachionus  | Brachionus quadridentatus |
| Collothecidae| Collotheca   | Collotheca| Collotheca campanulata   | Collothecidae| Collotheca| Brachionus urceus        |
|             |              |          | Collotheca tensilobata   |             |             | Brachionus urceus        |
| Conochilidae| Conochilus   | Conochilus| Conochilus coeobasis     | Conochilidae| Conochilus| Keratella valga           |
|             |              |          | Keratella coelebris      |             | Keratella  | Keratella coelebris      |
|             |              |          | Keratella quadrata       |             | Keratella  | Keratella quadrata       |
|             |              |          | Keratella valga          |             | Keratella  | Keratella valga          |
| Dicranophoridae| Dicranophorus| Dicranophorus| Dicranophorus forcipatus| Dicranophoridae| Dicranophorus| Dicranophorus forcipatus|
| Encentrum   |              | Encentrum| Encentrum astride        | Dicranophoridae| Dicranophorus| Dicranophorus forcipatus|
| Floscularia | Floscularia  | Floscularia| Floscularia armata       | Gastropodidae| Ascomorpha| Ascomorpha ecaudis       |
| Lacinularia | Lacinularia  | Lacinularia| Lacinularia flosculosa   |             | Ascomorpha  | Ascomorpha ovalis        |
|              |              |          | Limnias melicerta        | Gastropodidae| Ascomorpha| Ascomorpha ovalis        |
|              |              |          | Limnias ceratophylli     |             | Ascomorpha  | Ascomorpha ovalis        |
| Pentatrocha | Pentatrocha  | Pentatrocha| Pentatrocha gigantea     |             | Ascomorpha  | Ascomorpha ovalis        |
| Ptgura      | Ptgura       | Ptgura   | Ptgura libera           |             | Ascomorpha  | Ascomorpha ovalis        |
| Sinantherina| Sinantherina | Sinantherina| Sinantherina ariprepes   | Gastropus    | Gastropus  | Gastropus leukeni        |
|             |              |          | Sinantherina semibullata |             | Ascomorpha  | Gastropus leukeni        |
|             |              |          | Sinantherina socialis    |             | Ascomorpha  | Gastropus leukeni        |
| Gastropodidae| Ascomorpha   | Ascomorpha| Ascomorpha ovalis        | Lecanidae   | Lecane      | Lecane bulla             |
| Lecanidae   | Lecane       | Lecane   | Lecane bulla            |             | Lecane      | Lecane corruta           |
| Group       | MBC Genus | Species          | Group       | MOI Genus | Species          |
|------------|-----------|------------------|------------|-----------|------------------|
| Notommatidae | Lecane inermis | Monostyla sp.  1 | Cephalodella | Lecane luna | Lecane inermis   |
|            | Lecane ungulata | Monostyla sp.  1 | Lecane sp.  1 | Lecane luna | Lecane inermis   |
|            | Monommata sp.  1 | Cephalodella forficula | Lecane inermis | Lecane sp.  1 | Lecane inermis   |
|            | Notommatidae | Monommata maculata | Cephalodella | Lecane sp.  1 | Lecane inermis   |
|            | Notommatidae | Notommatida allantos | Cephalodella | Lecane sp.  1 | Lecane inermis   |
|            | Notommatidae | Notommatida codonella | Cephalodella | Lecane sp.  1 | Lecane inermis   |
| Philodinidae | Anomopus telphusae | Philodina megalotrocha | Notommatidae | Eothinia elongata | Eothinia elongata |
|            | Philodina | Rotaria rotatoria | Notommatidae | Eothinia elongata | Eothinia elongata |
|            | Philodina | Notommatidae | Notommatidae | Eothinia elongata | Eothinia elongata |
| Proalidae   | Proales doliaris | Macracteaeus sp. | Synchaetidae | Ploesoma hudsoni | Ploesoma hudsoni |
| Synchaetidae | Macrocheateus sp. | Ploesoma hudsoni | Synchaetidae | Ploesoma hudsoni | Ploesoma hudsoni |
|            | Ploesoma | Ploesoma truncatum | Synchaetidae | Ploesoma hudsoni | Ploesoma hudsoni |
|            | Polycytha | Polycytha dolichoptera | Synchaetidae | Ploesoma hudsoni | Ploesoma hudsoni |
|            | Synchaeta | Synchaeta pectinata | Testudinellidae | Filinia longiseta | Filinia longiseta |
| Scaridiidae | Scaridium longicauda | Hexarthra sp.  1 | Testudinellidae | Filinia passa | Filinia passa |
|            | Scaridium longicauda | Hexarthra mira | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Scaridium longicauda | Testudinella patina | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Scaridium longicauda | Testudinella sp.  1 | Testudinellidae | Hexarthra mira | Hexarthra mira |
| Trichocercidae | Trichocerca elongata | Trichocerca elongata | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Trichocerca | Trichocerca tenuior | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Trichocerca | Trichocerca capucina | Testudinellidae | Hexarthra mira | Hexarthra mira |
| Trichotriidae | Trichotria tetractis | Trichocerca | Testudinellidae | Hexarthra mira | Hexarthra mira |
| Copepods   | - | Cyclops sp.  1 | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Acantholeberis | Acantholeberis curvirostris | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Acantholeberis | Attheyella crassa | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Acantholeberis | Sinocalanus sinensis | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Acantholeberis | Sinocalanus tenellus | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Acantholeberis | Limnocalanus macrurus | Testudinellidae | Hexarthra mira | Hexarthra mira |
| Cyclipidiae | Acanthocyclops | Acanthocyclops bicuspispidatus | Testudinellidae | Hexarthra mira | Hexarthra mira |
|            | Acanthocyclops | Acanthocyclops galbinus | Testudinellidae | Hexarthra mira | Hexarthra mira |
## Table A1. Cont.

| Group          | MBC   | Genus                        | Species                  | Group     | Family        | Genus     | Species                  |
|----------------|-------|------------------------------|--------------------------|-----------|---------------|-----------|--------------------------|
| Diacyclops     | Diacyclops jasnitskii | Copepods                  | -                        | Centropagidae | Sinocalanus  | Copepod nauplii          |
|                | Diacyclops sp. 1     |                            |                          | Cyclopidae  | Cyclopidae    | Copepod nauplii          |
|                |                    |                            |                          | Eucyclops   | Eucyclops     | Copepod nauplii          |
| Ectocyclops    | Ectocyclops polyplinosa |                            |                          | Cletodidae  | Limnocletodes | Copepod nauplii          |
| Eucyclops      | Eucyclops dumonti   |                            |                          | Eucyclops   | Eucyclops     | Copepod nauplii          |
|                | Eucyclops serrulatus |                            |                          | Limnocletodes | Limnocletodes | Copepod nauplii          |
| Eucyclops      | Eucyclops speratus  |                            |                          | Mesocyclops  | Mesocyclops   | Copepod nauplii          |
|                | Eucyclops macruroides |                            |                          | Microcyclops | Microcyclops  | Copepod nauplii          |
| Megacyclops    | Megacyclops viridis |                          |                          | Thermocyclops | Thermocyclops | Copepod nauplii          |
| Mesocyclops    | Mesocyclops dissimilis |                          |                          | Diaptomidae  | Neodiaptomus  | Copepod nauplii          |
|                | Mesocyclops leuckarti |                          |                          | Ectocyclops  | Neodiaptomus  | Copepod nauplii          |
| Microcyclops   | Microcyclops varics  |                          |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Neodiaptomus   | Neodiaptomus schmackeri |                          |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Thermocyclops  | Thermocyclops sp. 1 |                          |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Tropocyclops   | Tropocyclops sp. 1   |                          |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Paracyclops    | Paracyclops sinbrutus |                          |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Diaptomidae    | Acanthodiaptomus     |                            |                          | Leptodoridae | Leptodora     | Leptodora kindii         |
|                | Arctodiaptomus       |                            |                          | Macrothricidae | Ilyocryptus | Ilyocryptus sordidus     |
|                | Arctodiaptomus stephanidesi |                        |                          | Moinidae     | Moina         | Moina micrura            |
|                | Paracyclopina        |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Sinodiaptomus  | Sinodiaptomus sars    |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Ergasilidae    | Ergasilus            |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
|                | Neoergasilus         |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
|                | Pseudergasilus       |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
|                | Sinergasilus         |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Lernaeidae     | Lernaea              |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Pseudodiaptomida | Pseudodiaptomus inopinus |                        |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
| Cladocerans    | Chydoridae           |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
|                | Sididae              |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |
|                | Diaphanosoma         |                            |                          | Cladocerans  | Cladocerans   | Copepod nauplii          |

*: the genus and family names are unknown. Sp. 1: a species of the genus or class.
Table A2. The Family of MBC and MOI and their abbreviations.

| Family of MBC | Abbreviation | Family of MOI | Abbreviation |
|---------------|--------------|---------------|--------------|
| Acantholeberis| ACAN         | Asplanchnidae | ASPL         |
| Asplanchnidae | ASPL         | Brachionidae  | BRAC         |
| Brachionidae  | BRAC         | Bosminidae    | BOSM         |
| Centropagidae | CENT         | Centropagidae | CENT         |
| Chydoridae    | CHYD         | Chydoridae    | CHYD         |
| Collothecidae | COLL         | Conochilidae  | CONO         |
| Conochilidae  | CONO         | Cyclopidae    | CYCL         |
| Cyclopidae    | CYCO         | Daphnidae     | DAPH         |
| Diaptomidae   | DIAP         | Dicranophoridae| DICR        |
| Dicranophoridae| DICR        | Diaptomidae   | DIAP         |
| Ergasilidae   | ERGA         | Gastropoda    | GAST         |
| Flosculariidae| FLOS         | Lecanidae     | LECA         |
| Gastropoda    | GAST         | Leptodoridae  | LEPT         |
| Lecanidae     | LECA         | Macrothricidae| MACR         |
| Notommatidae  | NOTO         | Moinidae      | MOIN         |
| Philodinidae  | PHIL         | Notommatidae  | NOTO         |
| Proalidae     | PROA         | Philodinidea  | PHIL         |
| Pseudodiaptomidae| PSEU    | Sibididae     | SIDI         |
| Scaridiidae   | SCAR         | Synchaetidae  | SYNC         |
| Sibididae     | SIDI         | Testudinellida| TEST         |
| Synchaetidae  | SYNC         | Trichocercidae| TRIC         |
| Testudinellida| TEST         |               |              |
| Trichocercida | TRIC         |               |              |
| Trichotriidae | TRIH         |               |              |

Table A3. The seasonal environment factors.

|       | WD       | V        | Turb | T     | Sal     | DO      | Chl a   | pH     | TN    | TP    |
|-------|----------|----------|------|-------|---------|---------|---------|--------|-------|-------|
| SP    | 1.28 ± 0.05 | 0.11 ± 0.00 | 13.00 ± 1.86 | 22.29 ± 0.1 | 0.03 ± 0.00 | 10.21 ± 1.13 | 17.93 ± 0.65 | 7.10 ± 0.08 | 1.23 ± 0.06 | 0.13 ± 0.01 |
| SU    | 3.38 ± 0.08 | 0.17 ± 0.01 | 13.84 ± 1.46 | 30.03 ± 0.13 | 0.11 ± 0.00 | 8.4 ± 0.09 | 16.32 ± 0.31 | 7.28 ± 0.01 | 1.22 ± 0.02 | 0.2 ± 0.01  |
| A     | 1.46 ± 0.04 | 0.1 ± 0.00  | 49.64 ± 3.52 | 16.6 ± 0.26  | 0.02 ± 0.00 | 9.53 ± 0.16  | 17.93 ± 0.66  | 7.46 ± 0.06  | 1.28 ± 0.07 | 0.20 ± 0.01 |
| W     | 0.86 ± 0.04 | 0.1 ± 0.00  | 129.92 ± 8.31 | 8.81 ± 0.13  | 0.03 ± 0.00 | 8.07 ± 0.09  | 16.54 ± 0.89  | 6.69 ± 0.04  | 3.63 ± 0.15 | 0.33 ± 0.05 |

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