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Zooplankton Compositions in the Danjiangkou Reservoir, a Water Source for the South-to-North Water Diversion Project of China

Mantang Xiong [1,2], Ruojing Li [1,2], Tanglin Zhang [1], Chuansong Liao [1], Gongliang Yu [1], Jing Yuan [1], Jiashou Liu [1] and Shaowen Ye [1,*]

[1] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[2] College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
* Correspondence: yeshw@ihb.ac.cn; Tel.: +86-68788862

Abstract: The Danjiangkou Reservoir (DJKR) serves as the water source for the world's biggest water diversion project, the Middle Route of the South-to-North Water Diversion Project (MR-SNWDP) in China, and this project concerns the water security of tens of millions of people in northern China. Hence, the maintenance of ecosystem health and optimization of management necessitate studies to assess the composition and dynamics of key aquatic living resources. Zooplankton represent a critical component of the reservoir ecosystem and are sensitive to environmental changes and anthropogenic disturbances. In this study, the zooplankton compositions in DJKR were quantified and compared in May, August, and November 2017. Simultaneously, the effects of water trophic states on the zooplankton community structure were analyzed at three levels (overall, taxonomic, and functional groups). A total of 65 zooplankton taxa were recorded, with the taxonomic richness of Rotifera (28 taxa) being the highest among taxonomic groups, which were further classified into 10 functional groups. The community was characterized by low diversity and high evenness. Compared with historical studies, the biomass had increased remarkably, while the abundance showed a decreasing trend in DJKR, and there were more large-bodied zooplankton in this study. The multivariate analysis revealed that zooplankton compositions changed significantly among the three sampling months without distinguishable spatial variations. Moreover, the zooplankton compositions at all three levels correlated significantly with total nitrogen, water transparency, and permanganate index in most situations, as verified by db-RDA and Mantel's test. However, the contributions of chlorophyll *a* and total phosphorus were only significant for the LCF group, implying that the bottom-up effects of phytoplankton on zooplankton were weak in DJKR. Therefore, analysis based on functional groups may reflect a more accurate snapshot of the relationships. Our findings will contribute to enriching the long-term fundamental ecological knowledge of the DJKR and the MR-SNWDP, as well as provide key taxonomic information for ecosystem assessment and management.

Keywords: drinking water reservoir; zooplankton; community composition; ecological assessment; multivariate statistical analysis

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1. Introduction

Reservoirs, formed by the construction of dams on natural rivers at enormous environmental and socioeconomic costs [1–3], are an essential human initiative for flood control and compensating for freshwater scarcity through anthropogenic regulation and impoundment, and play a crucial role in fulfilling human demands for food, water, and energy [4]. A thorough analysis of the key taxonomic variations and their influencing factors is necessary for the long-term viability of ecosystem functions and is a precondition for accurate assessment and scientific management of complex ecosystems. The environment in reservoirs, with characteristics of both rivers (away from the dam) and lakes (close to the dam), and couplings of natural (temperature, rainfall, etc.) and anthropogenic (regulation of water levels) factors, is more complicated than those in natural waters. Consequently, the driving forces determining community composition in reservoirs are more difficult to clarify and possess a higher degree of specificity [5]. In order to obtain adequate knowledge of the composition patterns in key taxa and environmental factors, more detailed relevant research is needed [6].

Zooplankton are one of the key biological components in aquatic ecosystems. Feeding mainly on algae, bacteria, and organic detritus (bottom-up effects, [7]), zooplankton are high-quality forage organisms for filter-feeding fish (top-down effects, [8]), and simultaneously promote the decomposition and recycling of organic matter through excretion and secretion. Therefore, zooplankton play a linkage role in the energy flow and material cycle of aquatic ecosystems [9]. Since zooplankton, especially large-sized *Daphnia* species, are efficient filter feeders and grazers of algae [10–12], they have been the focus of research in ecological restoration and biomanipulation, including algal bloom control and eutrophication remediation [13,14]. Furthermore, zooplankton, with their particular characteristics that include a short life cycle, wide distribution range, poor or nearly no swimming ability, and sensitivity to environmental changes, can be easily influenced by changes in water quality with respect to their species composition, abundance, biomass, community structure, and so on. Thus, zooplankton may be a useful biological indicator for assessing water quality and ecosystem health [15–19]. Hence, adequate knowledge of zooplankton compositions and responses of zooplankton communities to environmental changes is required, particularly in complicated ecosystems, whether for the purpose of conserving fisheries resources, maintaining ecosystem stability, effectively regulating the ecological environment, or accurately monitoring the water environment.

Among multiple environmental drivers, nutrients can affect zooplankton through bottom-up effects on both food quality and quantity. Meanwhile, an understanding of the relationships between zooplankton and nutrient levels could also provide vital information about the eutrophication risk of the ecosystem. However, owing to the fact that zooplankton are simultaneously affected by bottom-up and top-down effects as well as climatic conditions and anthropogenic disturbances, their relationships with water quality are not consistent across ecosystems. The role of the trophic state on zooplankton compositions has not yet been clearly ascertained, and there is controversy as to whether zooplankton can be used as an effective indicator [20–22].

The Danjiangkou Reservoir (DJKR) is one of the largest reservoirs in Asia, with its main functions being water supply, flood control, electric power generation, irrigation, and aquaculture. In terms of water supply, DJKR, the water source for the Middle Route of the South-to-North Water Diversion Project (MR-SNWDP), has delivered a total of 44.1 billion cubic meters of water to northern China since the official supply of water to the north commenced in December 2014. It supplies 24 large and medium-sized cities, including Beijing and Tianjin, and more than 190 counties (urban areas) with a population of 79 million residents benefit along the route. Considering the fact that the water quality and ecosystem health of DJKR are of critical importance, it has been designated as a National Water Source Protection Zone. Moreover, except for the common features of large reservoirs, such as unnatural water level fluctuations, DJKR has a unique reservoir morphology, constituted by the relatively independent and closely linked two reservoir zones, which possess different hydrodynamics and morphologies. Simultaneously, DJKR is also influenced by a large water diversion project, making it an ideal research site for exploring the responses of aquatic communities to the couplings of anthropogenic regulations and spatiotemporal environmental variations.

To date, there are relatively abundant studies on water quality assessment in DJKR [23–26], but ecological investigations and quantitative studies on aquatic organisms, including zooplankton, are still insufficient. Detailed studies on zooplankton in the reservoir were conducted before the construction of the dam [27] and after the completion of the dam in 1986–1987 [28] and 1992–1993 [29]. However, in recent years, there have been fewer relevant studies in the reservoir [30], especially after the dam was raised; only Wang et al. [31] have conducted preliminary studies on the community structure of planktonic crustaceans. Moreover, since the reservoir is in the early stage of water storage operation at a high water level [32], coupled with substantial fluctuations in water levels, zooplankton compositions could be affected by the changes in hydrological conditions and physicochemical environments. Accordingly, given the importance and peculiarity of the ecosystem, which is accompanied by uncertainties and controversies in the sustainability of ecosystem services [33–35], research on the zooplankton community patterns of DJKR need to be further strengthened.

Therefore, we conducted a reservoir-wide investigation and quantitative study at different water levels, focusing on the four broad taxonomic groups of zooplankton, i.e., Protozoa, Rotifera, Cladocera, and Copepoda and investigated physicochemical parameters of water trophic states in parallel. We hypothesized that (1) the abundance and biomass of zooplankton would increase considerably compared with historical studies; and (2) zooplankton compositions would correlate significantly with water trophic states (changing as the water level rose). Our findings are expected to deepen the understanding of zooplankton compositions in DJKR or analogous waters, which is critical to minimizing unintended consequences of reservoir regulations and providing basic support for surface water quality protection, ecosystem health maintenance, and management optimization.

2. Materials and Methods

2.1. Study Area and Sampling Design

DJKR (110°59′–111°49′ E, 32°33′–33°48′ N), completed in 1973, is located on the border of Henan and Hubei Provinces in China (Figure 1) and has a catchment area of 95,200 km^2. It currently has a total surface area of 1050 km^2 and a total storage capacity of 29.05 billion m^3 after the dam was raised in 2012, and the normal water level increased from 157 m to 170 m. The reservoir is located in the subtropical monsoon climate region with four distinct seasons and an average annual temperature of 15–16 °C. The rainfall is concentrated in summer, with an average annual value of 881 mm, and the multi-year mean inflow is 39.48 billion m^3. In addition, the land coverage types around the reservoir are mainly forest, cropland, urban land, and grassland, accounting for 59.8%, 18.0%, 4.4%, and 3.2% of the total watershed area, respectively [36]. Contrary to cropland, the percentage of urban land shows an increasing trend, whereas the proportion of cropland is still second only to forest [37].

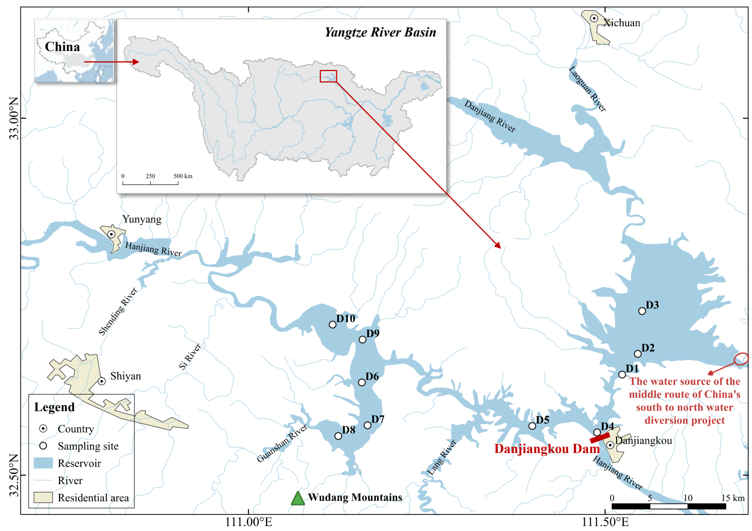

Figure 1. Geographical location and contour map of DJKR, showing the distribution of the sampling sites (D1~D10). The inset map indicates the location of DJKR in the Yangtze River Basin, China.

In this study, a total of 10 sampling sites were selected in the main lacustrine zones according to the morphological characteristics of the reservoir, including sites D1–D3 in the Danjiang reservoir zone (DR), site D4 in the proximity of Danjiangkou dam (BD), and sites D5–D10 in the Hanjiang reservoir zone (HR) (Figure 1). The sampling schedule was mainly based on the annual variations of water level and rainfall in the reservoir (Figure 2). During the sampling period, after dropping to the lowest in early March, the water level in the reservoir started to rise gradually and reached its highest value in October when the water storage filling was complete. The rainfall was mainly concentrated in June to October, with the maximum in September (89% higher than the multi-year average rainfall in that month) [32]. Accordingly, field samplings at each site were conducted during the early parts of May, August, and November in 2017.

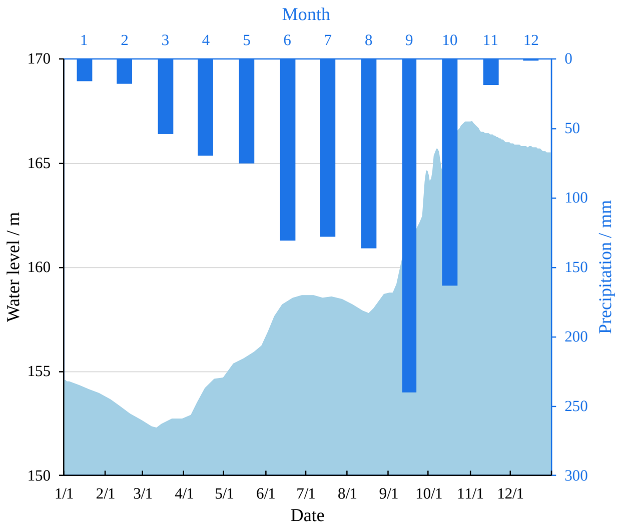

Figure 2. Temporal variation of water levels and precipitation in DJKR during the sampling periods in 2017. The area plot with the bottom *x*-axis and left *y*-axis represents the changes in water level. The histogram with the top *x*-axis and right *y*-axis represents the accumulated precipitation in each month. The sampling months of this study are indicated with grey bars.

2.2. Sampling Methods and Analytical Procedures

The quantitative samples (volume, 1 L) of small-bodied zooplankton (Protozoa, Rotifera, and Copepoda nauplii) were collected from the surface layer (0.5 m underwater, the same below) using a 5 L polycarbonate water sampler, then transferred to plastic bottles and fixed with 10 mL of Lugol's solution. The samples were then concentrated to 30 mL after standing for 36 h. Meanwhile, 20 L quantitative samples of Cladocera and Copepoda juveniles and adults were collected from the surface layer and concentrated by a plankton net (mesh size 64 µm). Therefore, the zooplankton compositions mentioned in this study refer to those in the surface water. The specimens were preserved with a final concentration of 4% formalin solution. Thereafter, all samples were identified to the lowest possible taxonomic level according to Wang [38], Shen [39], Jiang and Du [40], and Zhou et al. [41], and counted with the aid of a light microscope (Olympus BX51, Tokyo, Japan) following the specific procedure given by Zhang and Huang [42]. The density of zooplankton was approximated to that of water (1 g/cm^3). Then biomass (mg/L) was estimated by the volumetric method [42]. Furthermore, the identified taxa were classified into different functional groups based on the body size/length and feeding habits ([43,44], Table A1).

At each site, physicochemical parameters for water quality were simultaneously measured based on the Chinese Standard Methods for Monitoring Lake Eutrophication. Water transparency (Secchi disk depth, SD) was measured in situ by using a weighted Secchi disk (20 m). The potassium persulfate digestion method was used to measure total nitrogen (TN) and total phosphorus (TP). The permanganate index (COD_{Mn}) was determined by the acidic potassium permanganate method, and chlorophyll *a* (Chl.*a*) was analyzed with 90%

acetone extraction followed by spectrophotometry. In general, these parameters are the main indicators used to examine water trophic levels in China. The water trophic state was further assessed using the comprehensive trophic level index (TLI_c), a weighted sum based on the correlations between Chl.*a* and other sub-indices, as recommended by the Chinese National Environment Monitoring Center. All the five parameters mentioned above were used to calculate the TLI_c, and detailed formulas are supplied in Table A2.

The Margalef richness index (D_m) [45], Shannon–Wiener diversity index (H'_N) [46] and Pielou evenness index (J'_N) [47] were chosen as indices to describe the biodiversity characteristics, with formulas being $D_m = (S - 1)/\ln N$, $H'_N = -\sum(P_i \times \ln P_i)$ and $J'_N = H'_N/\ln S$, respectively, where S is the taxonomic richness, N is the total zooplankton abundance (ind./L) and P_i denotes the percentage of taxon i abundance to the total zooplankton abundance (%).

2.3. Statistical Analyses

Two-way ANOVA analysis or non-parametric test (Scheirer–Ray–Hare test) was performed to determine the significant difference in the zooplankton abundance, biomass, biodiversity indices, etc., across the sampling months (i.e., May, August, and November in 2017) or reservoir areas (i.e., HR, DR, BD), according to whether the data satisfied normality and homogeneity of variance among groups. The corresponding multiple comparison methods were Tukey's Honest Significant Differences and Dunn's Kruskal–Wallis Multiple Comparisons (p-value was corrected by the "Bonferroni" method), respectively.

Hierarchical Clustering (hclust, using a "UPGMA" linkage algorithm) and Nonmetric Multidimensional Scaling (NMDS) were conducted to show overall variations in the community composition [48]. Analysis of Similarities (ANOSIM) was used to test whether the differences were significant [49]. Furthermore, Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted to analyze variations in the composition of each taxonomic group across different sampling months and areas [50], which were further revealed by Principal Coordinate Analysis (PCoA). Similarity Percentage Analysis (SIMPER) was implemented to identify the "responsible taxa" in each group with contributions to the variation > 5% and $p < 0.05$ [49].

To ascertain associations between zooplankton and water quality, distance-based Redundancy Analysis (db-RDA, also known as canonical analysis of principal coordinates) was performed [51]. The Monte Carlo permutation test [52] was used to examine whether the significance level was reached. The hierarchical partitioning method, which is an unordered assessment method of importance, was used to determine the explanation rate of each parameter and its significance [53].

Mantel's test [54] was carried out to explore the relationships of each functional group with the water quality parameters and trophic state. The Euclidean distance matrix of both biomass data ("hellinger" transformed in advance) of each functional group and explanatory variables were used for the analysis. Moreover, Spearman's correlation was implemented between each pair of water quality parameters.

The overall multivariate analysis was based on the presence/absence data to avoid the effects of different orders of magnitudes among the abundance of taxonomic groups, and abundance data was used for the analysis of each taxonomic group. Before the multivariate statistical methods were employed in this study, the abundance data was transformed by the ln(x + 1) algorithm to remove the effects of rare taxa and extreme values, and the environmental parameters were scaled to zero means and unit variances. If not specified, the Bray–Curtis dissimilarity matrix of the data was used for the multivariate analysis. The number of permutations was 999, and the significance level was 0.05. All the statistical analyses and data visualizations were performed in R 4.1.0 [55], using the packages "car" [56], "rcompanion" [57], "FSA" [58], "vegan" [59], "rdacca.hp" [53], "linkET" [60], "eulerr" [61], "ggplot2" [62], "ggtree" [63], "aplot" [64], and "ggpubr" [65].

3. Results

3.1. Zooplankton Composition and Biodiversity Indices

A total of 65 zooplankton taxa were identified in the study, including 28 taxa of Rotifera, 17 taxa of Protozoa, 10 taxa of Cladocera, and 10 taxa of Copepoda (Table S1). The number of taxa found in May, August, and November was 39, 35, and 26, respectively. The number of unique taxa occurring in May, August, and November was 21, 17, and 4, respectively, with 12 shared taxa in all the three sampling periods (Figure 3a). There were 22 taxa shared amongst the sampling areas, and HR possessed a higher total number of taxa as well as unique taxa (Figure 3b). Moreover, the mean taxa number in May (19.86 ± 1.44) was significantly higher than that in November (13.00 ± 1.79) (p = 0.021), whereas there were no apparent differences among the sampling areas ($p > 0.05$).

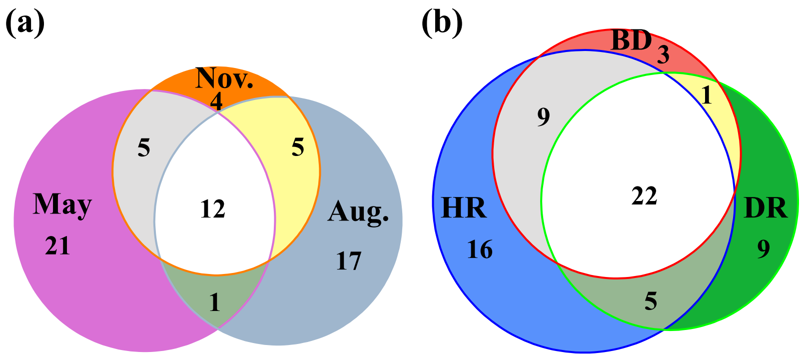

Figure 3. Changes in zooplankton taxa compositions among different sampling months and areas in DJKR. Number of shared taxa (overlapping parts of the circles) and unique taxa (parts of the circles with no overlaps) among the sampling months and areas are shown with Venn diagrams (**a**) and (**b**), respectively.

The average abundance of zooplankton in the three investigations was 3.84 × 10^3 ind./L, with Protozoa possessing the highest proportion (93.47%), followed by Rotifera (4.27%). The maximum and minimum values occurred in November at site D9 (1.41 × 10^3 ind./L) and site D4 (0.17 × 10^3 ind./L), respectively. Temporally, the mean zooplankton abundance reached a minimum in August (1.94 ± 0.26 × 10^3 ind./L) and a maximum in November (4.95 ± 2.61 × 10^3 ind./L). Spatially, the mean abundance was highest in HR (4.25 ± 1.24 × 10^3 ind./L). Overall, no significant temporal and spatial differences ($p > 0.05$) in zooplankton abundance were detected by the Scheirer–Ray–Hare test (Figure 4a). In terms of taxonomic groups, only the mean abundance of Copepoda in May was significantly lower than that in August (p = 0.006) and in November (p = 0.003).

Likewise, there were no significant spatiotemporal differences concerning zooplankton biomass ($p > 0.05$). The mean biomass of zooplankton in the three samples was 3.40 mg/L, with Copepoda, Cladocera, Rotifera and Protozoa accounting for 83.64%, 7.92%, 3.16% and 5.28%, respectively. The biomass was highest in November (6.42 ± 2.55 mg/L) among different sampling months, and regarding the sampling areas, it was highest in HR (4.45 ± 1.42 mg/L). The maximum value was observed at site D8 (14.13 mg/L) in November, with the minimum value at site D6 (0.42 mg/L) in May (Figure 4b). From the perspective of taxonomic groups, the mean biomass of Copepoda was consistently highest in the three sampling months and areas. None of the four taxonomic groups displayed significant spatiotemporal differences ($p > 0.05$).

The ranges of the three biodiversity indices, namely D_m, H'_N and J'_N, were 1.27–3.18, 0.41–2.38 and 0.17–0.72, respectively, with averages of 1.99, 1.46 and 0.53 (Table 1). No significant spatial differences were found in any indices ($p > 0.05$). Only H'_N was significantly higher in August (1.77 ± 0.11) than in November (1.03 ± 0.21) (p = 0.020).

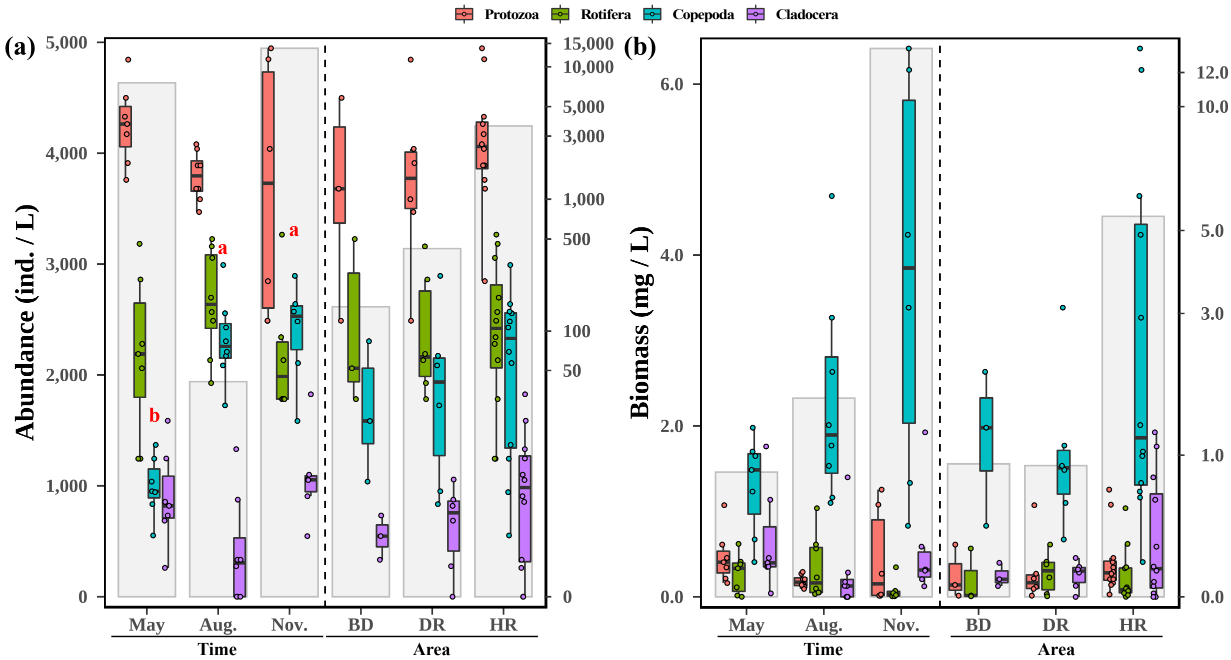

Figure 4. Zooplankton (**a**) abundance and (**b**) biomass among different sampling months and areas in DJKR. Mean values (left y-axis) of zooplankton are represented by the grey histogram. Values (right y-axis) for each group (Protozoa, Rotifera, Copepoda, Cladocera) are represented by boxplots with original data points, and different letter labels indicate a significant difference ($p < 0.05$) between the months or the areas. To decrease the effects of the existing maximum value and improve the visual appearance, the right y-axes of the graphs are shown with ln(x + 1)-transformed scales. HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam.

Table 1. Changes in the zooplankton biodiversity indices among different sampling months and areas in DJKR.

Biodiversity Indices	Month					Area					Month: Area		Analysis Method
	May (N = 7)	Aug. (N = 8)	Nov. (N = 6)	F/H	p Value	BD (N = 3)	DR (N = 6)	HR (N = 12)	F/H	p Value	F/H	p Value	
Margalef richness index (D_m)	1.71~3.18 (2.30 ± 0.18) ^{a}	1.43~2.94 (2.04 ± 0.19) ^{a}	1.27~2.13 (1.64 ± 0.14) ^{a}	5.71	**0.018**	1.94~2.94 (2.32 ± 0.31) ^{a}	1.32~2.35 (1.67 ± 0.15) ^{a}	1.27~3.18 (2.11 ± 0.15) ^{a}	4.63	**0.032**	1.19	0.363	Anova
Shannon–Wiener diversity index (H')	0.61~2.38 (1.58 ± 0.20) ^{ab}	1.40~2.23 (1.77 ± 0.11) ^{a}	0.41~1.68 (1.03 ± 0.21) ^{b}	6.72	**0.011**	1.43~2.23 (1.78 ± 0.24) ^{a}	0.61~1.80 (1.34 ± 0.16) ^{a}	0.41~2.38 (1.50 ± 0.18) ^{a}	2.09	0.167	1.49	0.266	Anova
Pielou evenness index (J'_N)	0.22~0.72 (0.53 ± 0.06) ^{a}	0.58~0.71 (0.64 ± 0.02) ^{a}	0.17~0.70 (0.42 ± 0.09) ^{a}	5.30	0.071	0.48~0.71 (0.63 ± 0.07) ^{a}	0.22~0.61 (0.52 ± 0.06) ^{a}	0.17~0.72 (0.52 ± 0.06) ^{a}	1.30	0.522	4.70	0.320	Scheirer–Ray–Hare test

Notes: The indices are represented by the minimum and maximum values with the mean and standard error in parentheses. The H statistic is derived from the Scheirer–Ray–Hare test, and the F statistic is produced by the Two-way Anova. Different letters and p values in bold indicate a significant difference ($p < 0.05$) between the months, the areas, or their interactions. HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam.

3.2. Changes in Zooplankton among Different Sampling Months and Areas

The result of NMDS based on the presence/absence of data of all the taxa in the four taxonomic groups showed that taxa composition was very dissimilar across the sampling months with no distinct spatial variations (Stress = 0.123 < 0.20) (Figure 5a). When the sites were clustered into three categories, Hierarchical Clustering using a "UPGMA" linkage algorithm demonstrated that sites in the same sampling month were essentially gathered into the same group (Figure 5b). Moreover, ANOSIM with multiple comparisons revealed statistically significant differences in zooplankton composition across the sampling months (R = 0.894; p = 0.001), whereas the spatial difference was not remarkable (R = 0.115; p = 0.105). This variation pattern was further supported by PERMANOVA and presented by PCoA on the four taxonomic groups, in which spatial differences were always indistinguishable and clear variations among sampling months were ascertained (Figure A1).

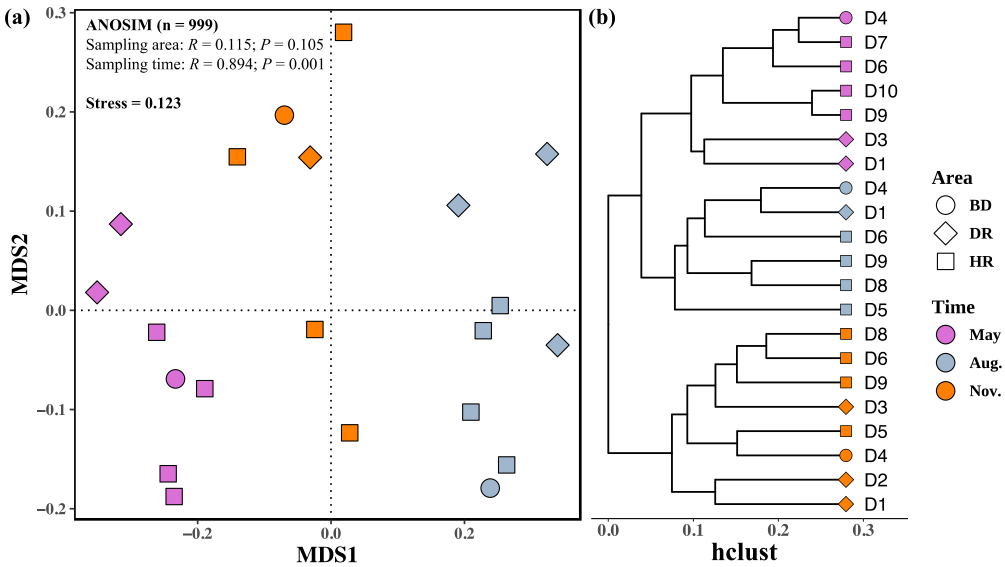

Figure 5. Plots of multivariable analysis results for the zooplankton in DJKR. (**a**) Nonmetric Multidimensional Scaling (NMDS) analysis (Stress < 0.2), and results of Analysis of Similarities (ANOSIM) are shown in the top and left corner of the plot. (**b**) Hierachical clustering (hclust) analysis on the samples over different sampling months and areas. HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam.

Additionally, the results of SIMPER considering each taxonomic group indicated that *Halteria cirrifera*, undetermined ciliates, *Difflugia globulosa*, *Strobilidium gyrans*, and *Strombidium viride* were the most influential taxa in Protozoa based on their contributions, with the mean dissimilarity among the three sampling months being 62.38% (May vs. Aug.), 64.96% (May vs. Nov.), and 53.99% (Aug. vs. Nov.), respectively. The mean dissimilarities in Rotifera were 95.91% between May and August, 96.89% between May and November, and 83.56% between August and November, respectively, which were mainly attributed to *Trichocerca rousseleti*, *Ascomorpha saltans*, and *Collotheca pelagica*. In Cladocera, the dissimilarities among the sampling months primarily resulted from *Bosmina coregoni*, *Diaphanosoma dubium*, and *Daphnia cucullata*. As to the variations in Copepoda among the three months, the responsible taxa were principally Copepoda nauplii, Cyclopoida copepodids, *Sinocalanus dorrii*, and *Microcyclops varicans* (Table A3).

3.3. Zooplankton Functional Groups Composition

The zooplankton taxa identified in DJKR were classified into 10 functional groups according to their body size/length and feeding mode (Figure 6, Table S1). Only the mean biomass of the LCF, SCF, and SCC functional groups changed significantly among the sampling months as determined by the Scheirer–Ray–Hare test ($p < 0.05$). The relative value of average biomass in the LCF group, represented by *S. dorrii*, *D. cucullata*, *Daphnia hyalina*, and *Daphnia galeata*, fell remarkably from 45.28% (May) to 0.27% (August) and recovered to 49.69% in November. In contrast, the relative values of mean biomass in the SCF (dominated by Calanoida copepodids, Copepoda nauplii, and *B. coregoni*) and SCC (only including Cyclopoida copepodids) groups showed the opposite trend across the three sampling months.

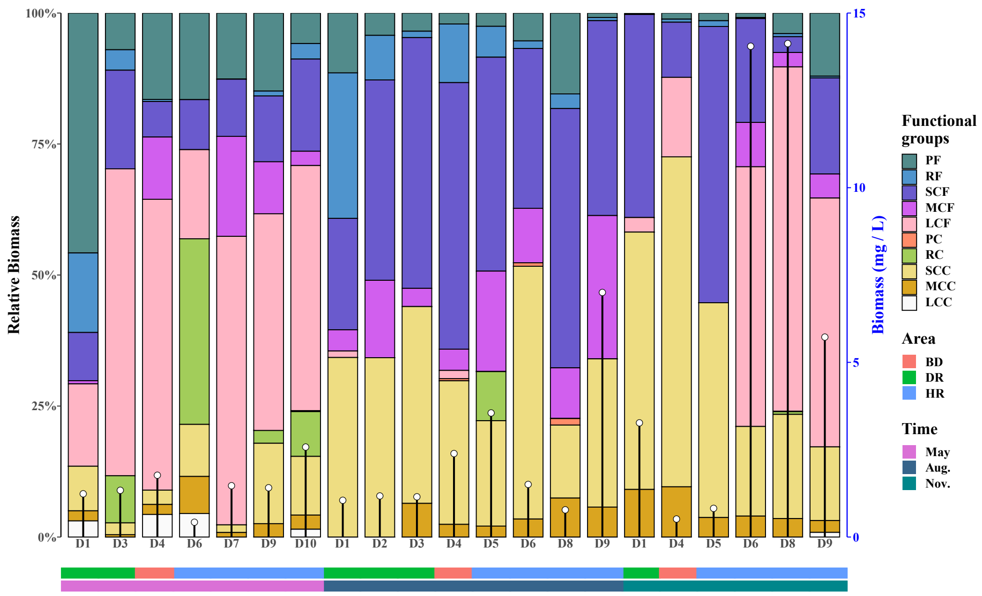

Figure 6. The composition of zooplankton functional groups. The relative biomass of functional groups and total biomass of zooplankton at each site in different sampling months are shown using a stacked histogram (left *y*-axis) and lollipop chart (right *y*-axis), respectively. HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam.

3.4. Relationships between Zooplankton and Water Trophic States

The physicochemical features in DJKR during the sampling periods are provided in Table 2. All the parameters except TP showed clear temporal differences ($p < 0.05$), whereas the spatial variations were not significant ($p > 0.05$). In general, the water quality was relatively poor during the period of high water levels (referring to November in this study) and in HR, with SD being lower and other parameters being higher. The mean TLI_c among the three sampling months was 37.11, characterizing DJKR as mesotrophic, whereas the trophic state at site D8 in November was abnormally higher, reaching 61.53 (medium-eutrophic).

Table 2. Variations in water quality parameters in DJKR with the comparison results between different sampling time and areas.

Parameters	Month					Area					Month: Area	
	May (N = 7)	Aug. (N = 8)	Nov. (N = 6)	H	p Value	BD (N = 3)	DR (N = 6)	HR (N = 12)	H	p Value	H	p Value
SD (m)	6.10~10.20 (7.75 ± 0.57) ^{a}	2.70~4.30 (3.71 ± 0.21) ^{b}	0.90~4.50 (2.48 ± 0.59) ^{b}	13.97	**<0.001**	3.50~7.40 (5.07 ± 1.19) ^{a}	3.90~7.30 (5.11 ± 0.60) ^{a}	0.90~10.20 (4.42 ± 0.90) ^{a}	2.12	0.346	2.14	0.711
Chl.*a* (μg/L)	1.28~2.68 (1.96 ± 0.17) ^{b}	1.66~5.91 (4.18 ± 0.60) ^{ab}	3.31~91.29 (21.42 ± 14.10) ^{a}	9.86	**0.007**	1.84~4.74 (2.81 ± 0.96) ^{a}	1.61~5.58 (2.96 ± 0.61) ^{a}	1.28~91.29 (12.46 ± 7.25) ^{a}	3.42	0.181	1.44	0.837
TN (mg/L)	1.45~1.95 (1.79 ± 0.06) ^{a}	0.83~1.19 (1.05 ± 0.04) ^{b}	1.50~2.69 (1.91 ± 0.18) ^{a}	12.82	**0.002**	1.12~1.86 (1.52 ± 0.22) ^{a}	0.96~1.73 (1.32 ± 0.12) ^{a}	0.83~2.69 (1.65 ± 0.16) ^{a}	2.34	0.310	2.02	0.732
TP (mg/L)	0.01~0.04 (0.02 ± 0.00) ^{a}	0.01~0.04 (0.03 ± 0.00) ^{a}	0.02~0.10 (0.05 ± 0.01) ^{a}	4.36	0.113	0.02~0.03 (0.02 ± 0.00) ^{a}	0.01~0.04 (0.02 ± 0.00) ^{a}	0.01~0.10 (0.04 ± 0.01) ^{a}	3.34	0.188	3.79	0.436
COD_{Mn} (mg/L)	1.63~1.80 (1.72 ± 0.03) ^{b}	2.14~2.77 (2.45 ± 0.07) ^{a}	1.82~5.48 (2.72 ± 0.56) ^{a}	14.07	**<0.001**	1.79~2.68 (2.21 ± 0.26) ^{a}	1.68~2.40 (2.06 ± 0.13) ^{a}	1.63~5.48 (2.42 ± 0.30) ^{a}	0.45	0.800	1.20	0.878
TLI_c	26.99~33.86 (30.95 ± 0.90) ^{b}	30.12~38.85 (36.08 ± 1.04) ^{a}	33.75~61.53 (44.29 ± 4.06) ^{a}	10.68	**0.005**	30.85~38.24 (34.30 ± 2.15) ^{a}	28.72~38.32 (33.22 ± 1.56) ^{a}	26.99~61.53 (39.07 ± 2.63) ^{a}	3.80	0.150	1.07	0.899

Notes: SD: Secchi disk depth, Chl.*a*: chlorophyll *a*, TN: total nitrogen, TP: total phosphorus, COD_{Mn}: permanganate index. HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam. The parameters in each group are represented by the minimum and maximum values, with the mean and standard error in parentheses. The H statistic was obtained by the Scheirer–Ray–Hare test. Different letters and p values in bold indicate a significant difference ($p < 0.05$) between the months, the areas, or their interactions.

The results of Distance-based Redundancy Analysis (db-RDA) demonstrate that the selected environmental parameters explained 46.08% (Adjusted R^2) of the total variation in the zooplankton based on the presence/absence data of all the taxa, and the statistical significance was verified by the Monte Carlo permutation test (pseudo-F: 3.32, p = 0.001). The canonical axes were further analyzed, and only the first axis reached significance levels (pseudo-*F*: 12.66, $p < 0.05$), explaining 29.30% of the variance (Figure 7). When we implemented db-RDA to the abundance data considering each of the four taxonomic groups, all the groups showed a significant correlation with the explanatory variables ($p < 0.05$, Figure A2). The adjusted R^2 was 0.48 in Protozoa, 0.14 in Rotifera, 0.56 in Copepoda, and 0.49 in Cladocera, respectively.

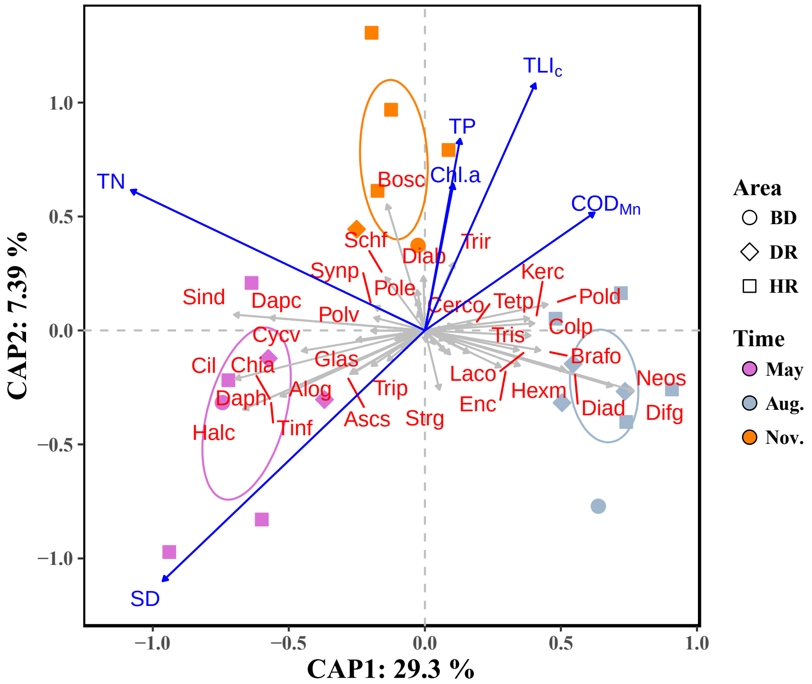

Figure 7. Results of Distance-based redundancy analysis (db-RDA) on the relationship between zooplankton (based on the presence/absence data of all the taxa) and environmental parameters (scaling = 2). Confidence intervals for the sites in different sampling months are represented by the ellipses (confidence level: 0.95). SD: Secchi disk depth, Chl.*a*: chlorophyll *a*, TN: total nitrogen, TP: total phosphorus, COD_{Mn}: permanganate index. HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam. The full names of taxa codes are listed in Table S1.

Specifically, the sampling sites in May were located in the negative direction of the first axis, with higher SD and TN but lower COD_{Mn}, Chl.*a*, TP, and TLI_c (Figures 7 and A2). At these sites, *H. cirrifera*, *Chilodonella algivora*, *Tintinnidium fluviatile*, and undetermined ciliates in Protozoa, *A. saltans* and *Trichocerca pusilla* in Rotifera, *D. cucullate*, and *D. hyalina* in Cladocera were more abundant. This was in contrast to the environmental characteristics of sampling sites in August, with *D. globulosa*, *S. gyrans*, and *Lacrymaria olor* in Protozoa, *Keratella cochlearis*, *Polyarthra dolichoptera*, *Hexarthra mira*, *Encentrum* sp., *C. pelagica*, *Brachionus forficula*, and *Trichocerca similis* in Rotifera, Copepoda nauplii and Calanoida copepodids in Copepoda, and *D. dubium* in Cladocera dominating at these sites. The sampling sites in November had intermediate results compared with those of May and August, with *T. rousseleti* in Rotifera, Copepoda nauplii, Cyclopoida copepodids, *S. dorrii*, and *M. varicans* in Copepoda, and *B. coregoni* in Cladocera prevailing at these sampling sites.

The individual effects of environmental parameters using hierarchical partitioning analysis indicated that among the selected parameters, TN, SD, and COD_{Mn} were important and significant explanatory variables for the differences in zooplankton composition based on the presence/absence data of all the taxa, with TN explaining the largest variance (18.07%, Figure 8). When it comes to the four taxonomic groups, consistent orders of importance were only obtained in Cladocera, whereas SD explained most of the variations in Protozoa, Rotifera, and Copepoda. Moreover, TP and Chl.*a* were always insignificant regardless of the overall analysis or separate analysis.

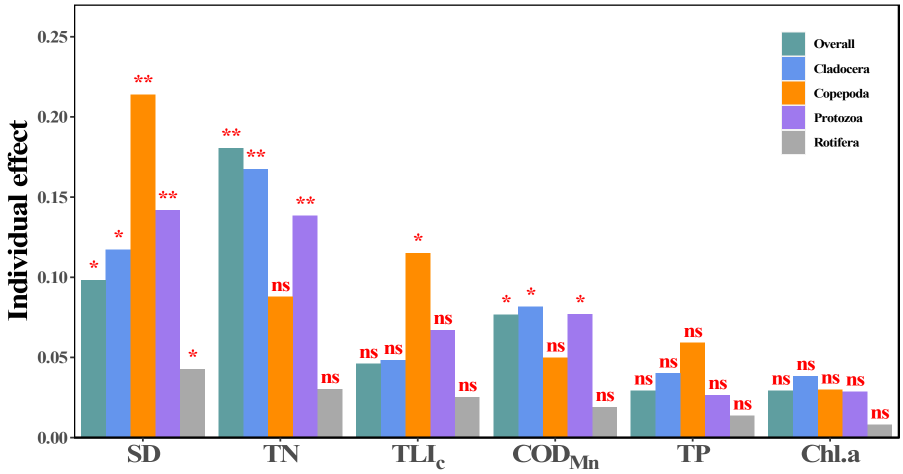

Figure 8. The individual effects of environmental factors on zooplankton composition. The histogram shows the results of hierarchical partitioning for the variables' explanation rate in the overall analysis (based on the presence/absence data of all the taxa) and separate analysis (based on the abundance data of each taxonomic group). **: $p < 0.01$, *: $p < 0.05$, ns: not significant. SD: Secchi disk depth, Chl.*a*: chlorophyll *a*, TN: total nitrogen, TP: total phosphorus, COD_{Mn}: permanganate index, HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam.

Mantel's test showed that SD correlated significantly with most of the zooplankton functional groups, followed by TN and COD_{Mn} ($p < 0.05$, Figure 9). Moreover, significant correlations of the LCF group with Chl.*a* and TP were revealed ($p < 0.05$), which was not detected in the aforementioned analyses.

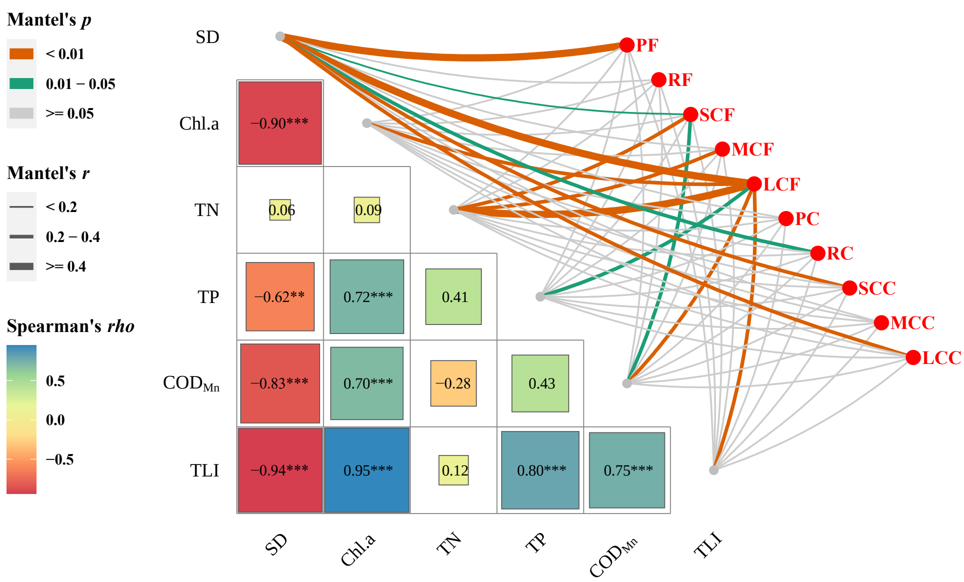

Figure 9. Correlations between functional groups and environmental parameters. The color gradient denotes Spearman's correlation coefficients between each pair of the environmental factors. The label in each of the squares indicates the specific value of the correlation coefficient (***: $p < 0.001$, **: $p < 0.01$). The composition of all functional groups (based on the Euclidean distance of "hellinger" transformed biomass data) was related to each environmental factor (based on the Euclidean distance) by Mantel's test. Edge width corresponds to the Mantel's r statistic for the correlation of the two corresponding distance matrices, and edge color indicates the statistical significance based on 999 permutations. SD: Secchi disk depth, Chl.*a*: chlorophyll *a*, TN: total nitrogen, TP: total phosphorus, COD_{Mn}: permanganate index.

4. Discussion

4.1. Characteristics of the Zooplankton Composition and Biodiversity

Despite the great importance of DJKR, limited field studies on zooplankton, especially targeting the considered four taxonomic groups, have been published (Table S2). Our study indicated that the abundance and biomass of zooplankton increased remarkably after the reservoir was impounded, as predicted by experts before the construction of the dam [27]. This may be related to the environmental changes (i.e., decreased water flow, increased hydrological retention time, nutrients, and transparency; [66]) after river damming, which are more suitable for the survival of Cladocera and Copepoda compared with before damming [67]. This has also been observed in other studies [68]. After water storage in the reservoir, Alfonso et al. [68] suggested that in the absence of disturbance, planktonic communities tended to become more complex and to increase their species richness and abundance over time. However, the occurrence of disturbances may mask the age effect on zooplankton that leads to a decrease in the species richness and abundance with the aging of the system. Consequently, there could be a maximum of the richness and abundance, at least for Cladocera and Copepoda. Changes in zooplankton in DJKR were partly consistent with this phenomenon (Table S2), but the total abundance of zooplankton in this study showed a decreasing trend, in spite of not varying considerably, compared with Yang et al. [28] and Han et al. [29]. This was probably because the increase in Rotifera and crustacean zooplankton led to a decrease in the abundance of Protozoa through predation and competition.

The zooplankton biomass was roughly five times more than that in the two historical studies [28,29]. An increase of the large-bodied zooplankton was implied by the ratio of biomass/abundance (an approximation of the community abundance-weighted mean for body size trait). However, it was Copepoda that contributed to the increased biomass, whereas the biomass of Cladocera decreased to nearly half of that in Han et al. [29] and Cladocera's ratio of biomass/abundance was lower in our study compared with the historical studies (Table S2). Since large-bodied zooplankton are more vulnerable to fish predation and Copepoda are less affected by filter-feeding fish than Cladocera [10,69], this phenomenon might be caused by high fish predation as a result of the continuous and large-scale stocking of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) in DJKR. Moreover, the extensive outbreak of icefish (*Neosalanx taihuensis*) population in DJKR, which predominantly feeds on Cladocera and Copepoda [70], may also contribute to suppressing the growth of macrozooplankton populations [71]. The size structure of zooplankton populations can be changed by the feeding selectivity of planktivorous fish through reducing the population of large-bodied species and increasing the dominance of smaller ones [72,73], especially in the fish growth season [74], which accounts for the scarcity of large-bodied Cladocera. In addition, the change of food quality could be another driver, as implied by the increase in the relative abundance of Cyanobacteria [75], indicating that inedible algae in DJKR became more abundant.

Furthermore, compared with recent studies on other taxa, the biodiversity of fish [76], phytoplankton [75], and zoobenthos [77] in DJKR were all present at low levels, indicating relatively simple biotic community structures. According to Dodson et al. [78], species would accumulate in a newly formed ecosystem over time until the available space is saturated. In addition, mature systems usually have higher biodiversity [79]. As DJKR is a man-made ecosystem mainly for water supply and flood control, the frequent anthropogenic disruptions, such as water storage regulation, fishing activities, and agricultural production around the reservoir, may continuously revert the assemblages to an earlier developmental stage and impede them from reaching a more mature state [68,80,81].

4.2. Changes of Zooplankton among the Sampling Months and Areas

Generally, zooplankton are sensitive to environmental changes and habitat heterogeneity, which can be partly reflected in the temporal and spatial dynamics of zooplankton taxonomic composition [5,82,83]. In this study, we observed a distinct shift in the zooplankton composition of DJKR between the three sampling months. Similar observations were made in previous studies [84,85], mainly due to seasonal changes in abiotic factors (i.e., water trophic state, temperature) and biotic factors (i.e., food resources availability and fish predation pressure). November became the most productive period compared with May and August, concerning the mass propagation of zooplankton (Figure 4). The increased nutrients (associated with increased rainfall, surface runoff, and the rising water level; Figure 2 and Table 2), suitable water temperatures, and the decreasing predation pressure due to fish harvesting may be responsible for the variation [27].

At the reservoir scale, damming is expected to cause biotic differentiation through increasing the heterogeneity of the environment with a spatially hydrological gradient that supports the colonization and population growth of different zooplankton species [83,86]. The environment close to the dam would reach a lacustrine state over time, as a nearly realized dam usually has an environment closer to the riverine state. Regarding the spatial dynamics in the main lacustrine zones of DJKR, the two surveys conducted in 1986–1987 [28] and 1992–1993 [29,87] revealed that the richness and abundance of zooplankton in HR were higher than those in DR, whereas the biomass of zooplankton in HR was lower (namely, there were fewer large-bodied zooplankton in HR than in DR). Compared with DR, HR had environmental conditions more similar to a natural river. In this study, the richness, abundance, and biomass of zooplankton in HR, or the four specific taxonomic groups, were all higher than those in DR, except for the biomass of Rotifera. Similar phenomena were observed in the historical studies on Rotifera [30] and crustacean zooplankton [31] in DJKR, which may be related to the higher water trophic state in HR (Table 2), indicating a higher availability of food resources. Meanwhile, the environment in HR is gradually approaching that of natural lakes, which makes it more suitable for the growth of large-bodied zooplankton than before [67]. However, the differences in zooplankton community structure between the two reservoir zones of DJKR, either in terms of the overall analysis or multivariate statistical analysis of taxon composition and biodiversity, were not statistically significant. The corresponding environmental parameters were also not significantly different. The limited sampling sites, only covering the main lacustrine zones, could be an important aspect not to be neglected in the insignificant results.

4.3. Relationships of Zooplankton with Water Trophic States

Relationships between zooplankton and water trophic levels may be influenced by biotic factors, abiotic factors, and their interactions. In this study, zooplankton taxa composition correlated notably with the factors related to water trophic states, as determined by the db-RDA analyses, and the selected parameters had an explanation rate of more than 45%, except for Rotifera (only 13.90%). The lower correlation between the factors and Rotifera could be caused by top-down pressure: the increase of Copepoda may have resulted in a high level of predation on Rotifera (Figure 4).

Moreover, hierarchical partitioning analysis revealed that the contributions of Chl.*a* and TP were always low and insignificant regardless of the overall analysis or separate analysis in this study. Mantel's test based on the biomass data of functional groups detected that only the LCF group correlated significantly with Chl.*a* and TP. The results imply that analysis based on functional groups of zooplankton could provide a more accurate result about the relationship between zooplankton and water trophic levels (at least the related parameters). In addition, the results also indicated a weak bottom-up effect of phytoplankton on zooplankton compositions in DJKR.

TP, a main limiting factor for the growth of algae, could influence the zooplankton community through bottom-up effects. The value of Chl.*a*, as an index of food resources availability for zooplankton, is important in shaping zooplankton community structure [88]. Three possible processes may lead to the weak bottom-up effect: (i) the increase of inedible algae mentioned above [75]; (ii) crustacean zooplankton is dominated by Copepoda that are less efficient grazers than large-bodied Cladocera (Figure 4); and (iii) the potentially strong top-down effects on zooplankton. In waterbodies where top-down effects dominate, the zooplankton community composition, size distribution, and biodiversity are more likely to be influenced by fish predation, resulting in a weak relationship between zooplankton and water quality [21]. The relationship between zooplankton and phytoplankton could also be weakened, and even collapse [21,22,89].

The potentially strong top-down effects could be supported by the following phenomena. As far as we know, major fish harvests in DJKR were dominated by zooplanktivorous fish (silver carp, bighead carp, and icefish) ([71]; Table S3), with commercial fish harvest production reaching up to about 75 kg/ha. Furthermore, reductions of Cladocera abundance and biomass at the time of study (in August) were consistent with the massive outbreak of young-of-the-year juveniles of zooplanktivorous fish, e.g., *Culter* spp., icefish, and *Hemiculter leucisculus*; moreover, those of Cladocera increased after the commercial fish harvesting (Figure 4; [90]). Additionally, there was a shift from the LCF group (in May) to the SCC and SCF groups (in August) in functional groups of zooplankton. Given that this is not always the case [20,91,92], and TP did not differ statistically across the sampling areas or time in our study, more targeted and specific studies are needed.

4.4. Implications for Reservoir Ecological Conservation and Future Research

Last but not least, the zooplankton compositions in this study only covered the surface water in the main lacustrine zones of DJKR, which was insufficient to justify the correlations discovered for the entire reservoir. Considering the complexity and heterogeneity of large freshwater ecosystems, studies at a whole reservoir scale are needed to obtain more comprehensive knowledge of zooplankton compositions to ensure the sustainable development of ecosystem service functions in DJKR. Moreover, functional groups of zooplankton could be taken into consideration when evaluating the relationships between zooplankton and water trophic levels. In addition, given that large-bodied zooplankton are more effective in controlling algae [10], in the future management of DJKR, it is recommended that the fish stocking strategy be adjusted to increase the stocking of native piscivorous fish and control species similar to *N. taihuensis*, and thereby allow the proportion of large-bodied zooplankton to increase gradually [73].

5. Conclusions

In this study, we reported the zooplankton composition targeting three levels (overall, taxonomic, and functional groups) and their relationships with the environmental factors related to the trophic levels in DJKR. We identified 65 zooplankton taxa, including 28 taxa of Rotifera, 17 taxa of Protozoa, 10 taxa of Cladocera, and 10 taxa of Copepoda, which were further classified into 10 functional groups. The lower level of diversity indices and higher evenness demonstrated that the zooplankton community structure was relatively simple. The zooplankton composition varied significantly over the three sampling months but showed no distinct spatial differences in the main lacustrine zones of DJKR. Significant correlations between zooplankton and water trophic states could be only detected in some of the main indicators of water trophic states and zooplankton compositions. Moreover, analysis based on functional groups could provide a more accurate snapshot of the relationships. This study improved our understanding of zooplankton composition patterns and highlighted the different performances of methods when exploring relationships between zooplankton and water trophic levels in the surface layer of DJKR.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14203253/s1, Table S1: A list of zooplankton taxa and abbreviations in DJKR, and their occurrence in each sampling month and area; Table S2: Records on the number of taxa (NS), mean abundance (D, ind./L), and mean biomass (B, mg/L) of the four zooplankton taxonomic groups in DJKR investigated in this study and documented in the literature, as well as water quality parameters for each period; Table S3: A list of fish species composition and major characteristics in DJKR, compiled from our survey data during 2013–2018.

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Appendix A

Table A1. The classification method of zooplankton functional groups in Danjiangkou Reservoir.

Functional Group	Abbreviation	Feeding Habits	Body Size/Length
Protozoan filter feeders	PF	Filter-feeding, feeding on bacteria, phytoplankton, and organic detritus	
Protozoan carnivores	PC	Carnivorous-feeding, feeding on small protozoans	
Rotifer filter feeders	RF	Filter-feeding, feeding on bacteria, phytoplankton, and organic detritus	
Rotifer carnivores	RC	Carnivorous-feeding, feeding on protozoans, other rotifers, and small crustacean	
Small copepod and cladoceran filter feeders	SCF	Filter-feeding, feeding on bacteria, phytoplankton, organic detritus, and protozoans	<0.7 mm
Small copepod and cladoceran carnivores	SCC	Carnivorous-feeding, feeding on rotifers, cladocerans, dipster (chironomidae larvae), and oligochaeta	<0.7 mm
Middle copepod and cladoceran filter feeders	MCF	Filter-feeding, feeding on bacteria, phytoplankton, organic detritus, and protozoans	0.7–1.5 mm
Middle copepod and cladoceran carnivores	MCC	Carnivorous-feeding, feeding on rotifers, cladocerans, dipster (chironomidae larvae), and oligochaeta	0.7–1.5 mm
Large copepod and cladoceran filter feeders	LCF	Filter-feeding, feeding on bacteria, phytoplankton, organic detritus, and protozoans	>1.5 mm
Large copepod and cladoceran carnivores	LCC	Carnivorous-feeding, feeding on rotifers, cladocerans, dipster (chironomidae larvae), and oligochaeta	>1.5 mm

Table A2. The formulas used to calculate the comprehensive trophic level index and its assessment criteria.

Equation	Meaning of Abbreviation	Notes	Assessment Criteria
$TLIc = \sum_{j=1}^{m} (r_{ij}^2 / \sum_{j=1}^{m} r_{ij}^2) \times TLI_j$ (1) TLI(Chl.*a*) = 10(2.5 + 1.086lnChl.*a*) (2) TLI(TP) = 10(9.436 + 1.624lnTP) (3) TLI(TN) = 10(5.453 + 1.694lnTN) (4) TLI(SD) = 10(5.118 − 1.94lnSD) (5) TLI(COD_{Mn}) = 10(0.109 + 2.661lnCOD_{Mn}) (6)	m: number of indicators r_{ij}: coefficients of each parameters *j* TLI_j: sub-index of TLIc Chl.*a*: chlorophyll *a* (µg/L) TP: Total phosphorus (mg/L) TN: Total nitrogen (mg/L) SD: Secchi disk depth (m) COD_{Mn}: Permanganate index (mg/L)	r_{ij}: Chl.*a*, 1; TP, 0.84; TN, 0.82; SD, −0.83; COD_{Mn}, 0.83	Oligotrophic: <30 Mesotrophic: 30–50 Eutrophic: >50 (light-eutrophic: 50–60; medium-eutrophic: 60–70; hyper-eutrophic: >70)

Table A3. Results of SIMPER analysis for taxa with contribution to the variation among the three sampling months > 5% and $p < 0.05$, based on the four taxonomic groups.

Taxonomic Groups	Months	Taxa Code	Average Dissimilarity	Standard Deviation	Contribution	*p*
Protozoa	May vs. Aug.	Halc	0.1197	0.0317	0.1919	0.001
		Cil	0.0950	0.0187	0.1523	0.001
		Difg	0.0910	0.0164	0.1459	0.009
		Chia	0.0636	0.0440	0.1019	0.002
		Tinf	0.0582	0.0377	0.0933	0.001
	May vs. Nov.	Halc	0.1457	0.0465	0.2243	0.001
		Cil	0.0922	0.0417	0.1419	0.003
		Chia	0.0759	0.0543	0.1168	0.001
		Tinf	0.0691	0.0456	0.1063	0.001
		Glas	0.0369	0.0467	0.0568	0.01
	Aug. vs. Nov.	Difg	0.1604	0.0365	0.2970	0.001
		Strg	0.0904	0.0698	0.1675	0.003
		Strv	0.0858	0.0719	0.1589	0.005
		Laco	0.0583	0.0709	0.1079	0.017
		Tetp	0.0375	0.0671	0.0695	0.034
Rotifera	May vs. Aug.	Colp	0.0864	0.1095	0.0901	0.048
		Brafo	0.0670	0.0744	0.0698	0.01
		Hexm	0.0646	0.0868	0.0674	0.012
		Enc	0.0550	0.0910	0.0573	0.043
	May vs. Nov.	Trir	0.1788	0.1889	0.1845	0.002
		Ascs	0.1011	0.0976	0.1043	0.005
		Trip	0.0854	0.1497	0.0882	0.02
		Synp	0.0605	0.0841	0.0625	0.037
		Polv	0.0487	0.0658	0.0503	0.037
	Aug. vs. Nov.	Colp	0.0943	0.1116	0.1129	0.025
		Brafo	0.0700	0.0772	0.0838	0.007
		Hexm	0.0675	0.0893	0.0808	0.023
		Enc	0.0577	0.0937	0.0690	0.049
		Tris	0.0553	0.0591	0.0661	0.038
Cladocera	May vs. Aug.	Dapc	0.3049	0.1408	0.3372	0.003
		Diad	0.2099	0.1373	0.2321	0.001
		Daph	0.1732	0.1262	0.1915	0.002
	May vs. Nov.	Bosc	0.2718	0.1475	0.4133	0.018
	Aug. vs. Nov.	Bosc	0.2996	0.2189	0.3782	0.014
		Diad	0.1787	0.1152	0.2255	0.007
		Cerco	0.0514	0.0795	0.0649	0.021
Copepoda	May vs. Aug.	Nau	0.2721	0.0801	0.4690	0.001
		Cycc	0.1116	0.0544	0.1923	0.003
	May vs. Nov.	Nau	0.2237	0.0803	0.3703	0.001
		Cycc	0.1287	0.0524	0.2131	0.002
		Sind	0.0789	0.0478	0.1306	0.005
		Micv	0.0542	0.0370	0.0897	0.009

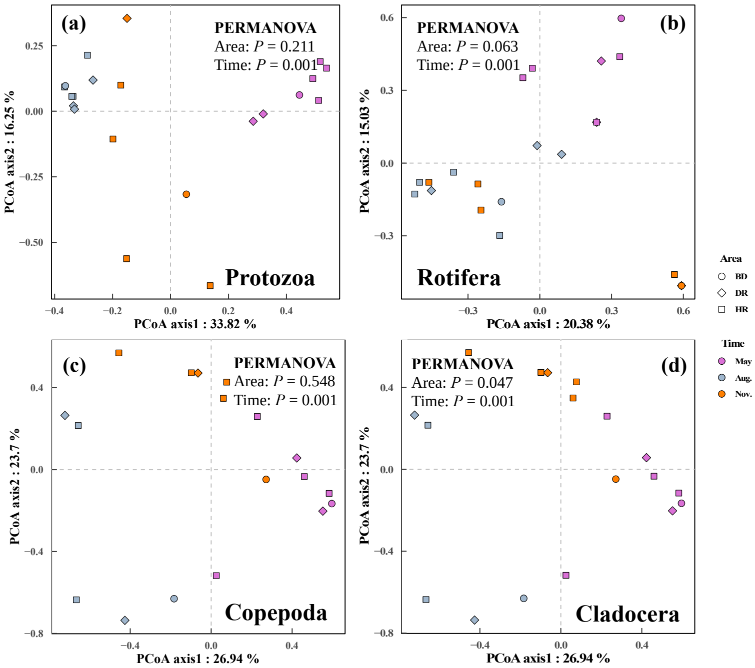

Figure A1. Plots of Principal Coordinate Analysis (PCoA) of the composition in the four taxonomic groups, with results of Permutational Multivariate Analysis of Variance (PERMANOVA) shown in top and right (or left) corner. (**a**) Protozoa, (**b**) Rotifera, (**c**) Copepoda, (**d**) Cladocera.

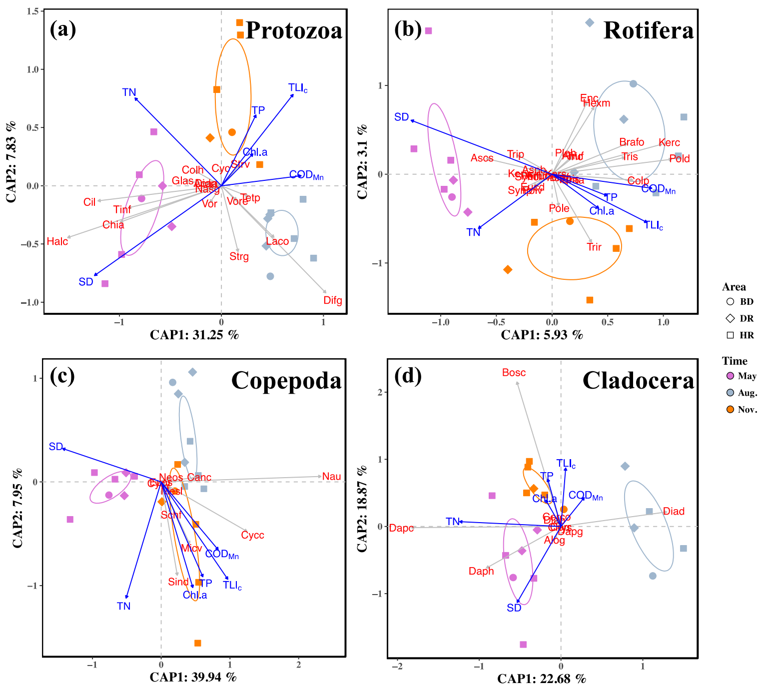

Figure A2. Plots of distance-based Redundancy Analysis (db-RDA) on the relationships between each taxonomic group (based on the abundance data) and environmental parameters (scaling = 2). Confidence intervals for the sites in different sampling months are represented by the ellipses (confidence level: 0.95). (**a**) Protozoa, (**b**) Rotifera, (**c**) Copepoda, (**d**) Cladocera. SD: Secchi disk depth, Chl.*a*: chlorophyll *a*, TN: total nitrogen, TP: total phosphorus, COD_{Mn}: permanganate index. HR: Hanjiang reservoir area, DR: Danjiang reservoir area, BD: the area close to the dam. The full names of taxa codes are listed in Table S1.

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