RELATIONSHIP BETWEEN ZOOPLANKTON COMMUNITY STRUCTURE AND ENVIRONMENTAL FACTORS IN MUDFLATS WETLANDS IN QINGTONGXIA RESERVOIR AREA WETLAND NATURE RESERVE, CHINA

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Abstract. In order to investigate the relationship between zooplankton community structure and environmental factors in different mudflats in Qingtongxia Reservoir Area Wetland Nature Reserve (QRAWNR), Ningxia Hui Autonomous Region (NHAR), China, the study conducted a survey of zooplankton and water environmental factors in natural and restored mudflats in March (spring) and June (summer) in 2021. A total of 39 zooplankton species were found, including 19 species of rotifers, 14 species of copepods, 5 species of cladicornis, and only 1 species of protozoa. *Cyclops strenuous* is a common dominant species in both natural and restored mudflats. In spring, the average density of zooplankton in natural mudflats (490.25 ind./L) was higher than that in repaired mudflats (343.25 ind./L), while the average biomass of zooplankton in natural mudflats (4.45 mg/L) was lower than that in restored mudflats (7.328 mg/L). In summer, the average density and biomass of zooplankton in natural mudflats were 118.25 ind./L and 2.27 mg/L, respectively, which were lower than those in restored mudflats (229.50 ind./L and 5.91 mg/L). Univariate analysis of variance (ANOVA) and Turkey test showed that there were significant differences in water temperature, pH and conductivity between natural and restored mudflats ($p < 0.05$) but no significant differences occurred in zooplankton community structure ($p > 0.05$). The results of RDA analysis and Mantel test are synthetical: pH, total phosphorus (TP), ammonium ion (NH_4^+), and chemical oxygen demand (COD) are the main environmental factors in the community structure of the zooplankton.
Keywords: *wetlands ecosystems, zooplankton, community structure, physical and chemical factors of water, redundancy analysis*

Introduction

Due to their small size, high metabolic activity, strong reproductive capacity, and sensitivity to changes in the aquatic environment, zooplankton are often regarded as biological indicators of water quality (Sun et al.,2024; Li et al., 2024). The community structure of zooplankton varies with different environmental and hydrological conditions, with influencing key environmental factors including pH levels, nutrient contents, water level fluctuations, and climate changes (Hobbs et al., 2021; Afonina et al., 2019; Dorak et al., 2019; Wang et al., 2024; Tan et al., 2022; Chen et al., 2022).

The Qingtongxia Reservoir Area Wetland Nature Reserve (QRAWNR) in Ningxia Hui Autonomous Region (NHAR), China, is the only reserve encompassing natural forest vegetation, precious migratory birds, and aquatic biota, making it the largest wetland reserve in NHAR (Xu, 1990). In April 1967, the completion of the Qingtongxia water conservancy hub significantly disrupted the natural sediment balance, leading to continuous rises in water levels and increased sedimentation, forming sandwiched

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mudflats of varying heights. Local human activities such as diversion for fish farming, dike construction by deforestation, pond excavation, and other interventions, along with changes in hydrological factors, have severely damaged the aquatic ecological health and balance of the reserve (Duan, 2020). The “2018 Green Shield Action” (Duan, 2020). legally enforced the removal of all aquaculture enterprises within the reserve and promptly carried out ecological restoration, including ecological water replenishment, improvement of wetland infrastructure, and wetland restoring projects. These measures have restored the ecological environment of the QRAWNR, effectively improving the ecological environment of the mudflat wetlands.

Our team conducted sampling during each of the three seasons: spring, summer, and autumn. However, the characteristics of the zooplankton community in autumn were not as distinct as those in spring and summer. Additionally, the region′s climate, characterized by drought and limited rainfall, had a lesser impact on the zooplankton community structure compared to other regions. Therefore, we ultimately selected the data from spring and summer for our research, which is sufficient to demonstrate the experiment’s completeness.

This study investigates the community structure of zooplankton and physicochemical factors of the water in the natural and restored mudflats within the QRAWNR. It aims to clarify the characteristics of plankton community structure in different mudflats. It examines the relationship between zooplankton community structure and environmental factors through redundancy analysis and Mantel tests, providing a scientific basis for the restoration of aquatic biodiversity and the healthy development of aquatic ecosystems in the reserve.

Materials and methods

Study area overview

The Qingtongxia Reservoir Area Wetland Nature Reserve (QRAWNR), is located at the southern border of Wuzhong City and the northern border of Zhongning County in NHAR, with geographical coordinates ranging from 105°47′30″E to 106°0′11″E and from 37°33′14″N to 37°53′22″N, covering an area of approximately 196.96 km^2 (*Fig. 1*). The reserve includes seasonal mudflats and lakes formed by long-term sedimentation of the Yellow River, such as Bird Island, Sulphur Island, and Central Lake. The primary protection targets are typical inland wetlands and endangered, precious, rare species of flora and fauna, along with their habitats (Zhang et al., 2019). The climate is a mid-temperature arid, typical of continental monsoon climates, characterized by dryness, scarce rainfall, significant annual and diurnal temperature variations, and high evaporation levels. In recent years, the average temperature in the reserve has been 10.15°C, with annual precipitation of 225.00 mm and annual evaporation of 1925.00 mm (Zhang et al., 2019; Xu et al., 2023).

Sampling sites

In March (spring) and June (summer) of 2021, four sampling sites were set up in the natural and restored mudflats of the QRAWNR (*Table 1*). As shown in *Figure 2*, sampling sites N1-N4 are located in the natural mudflats formed by the Yellow River flowing through the reserve in its middle and lower reaches, while sites R1-R4 are located in the restored mudflat wetlands.

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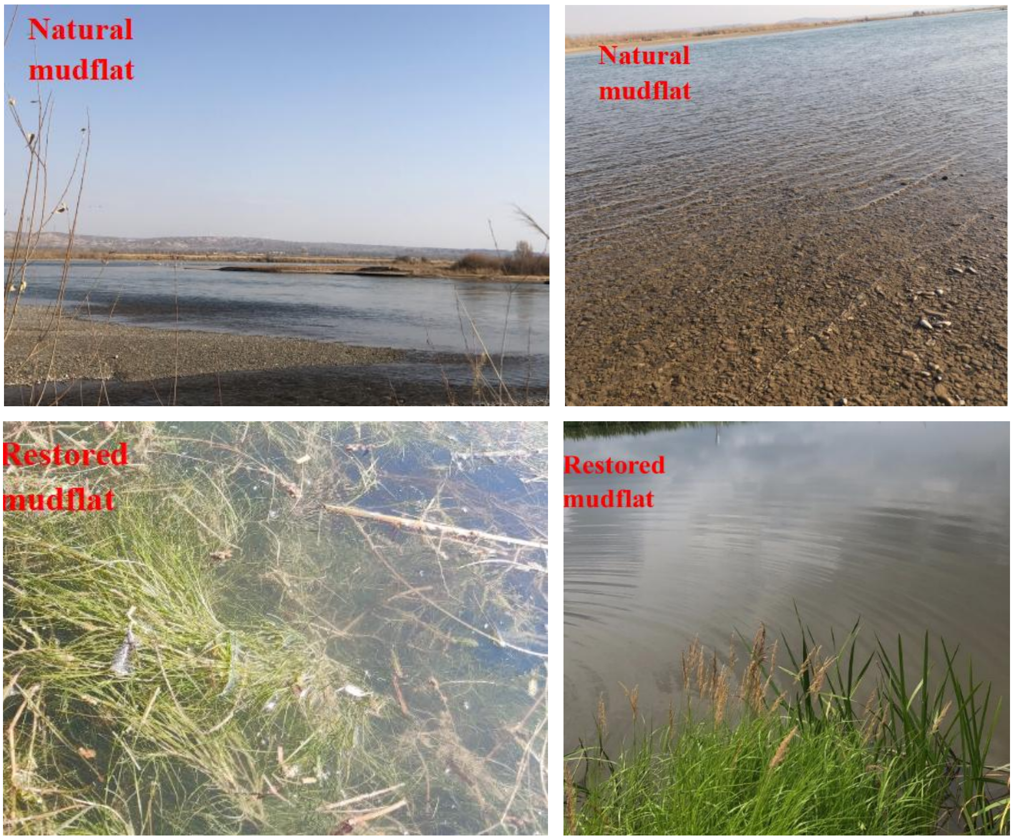

***Figure 1.** The sampling sites and habitats*

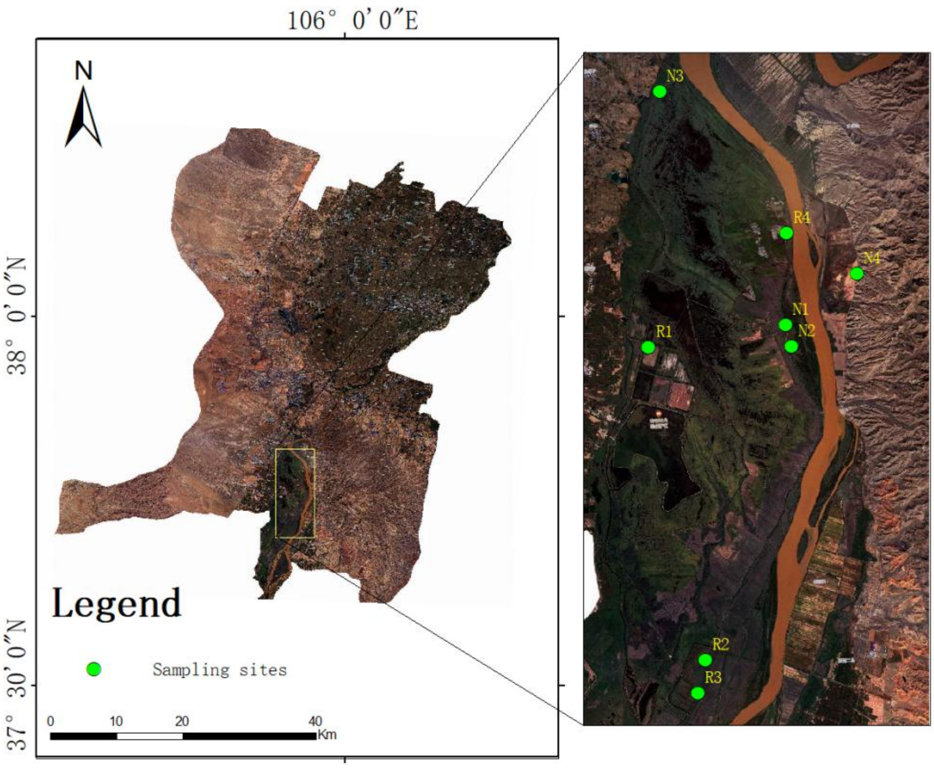

***Figure 2.** Sampling sites of Qingtongxia Reservoir Area Wetland Nature Reserve (QRAWNR)*

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Table 1. *Basic information on sampling sites*

Site No.	Latitude	Longitude	Water depth (m)	
			Spring	Summer
N1	37°46′30.14″	105°56′37.53″	0.56 m	0.53 m
N2	37°46′14.08″	105°56′40.14″	0.89 m	0.92 m
N3	37°49′8.91″	105°55′1.67″	0.64 m	0.60 m
N4	37°47′2.32″	105°57′38.79″	0.84 m	0.80 m
R1	37°46′19.95″	105°54′42.51″	1.09 m	1.05 m
R2	37°42′50.06″	105°55′19.43″	0.75 m	0.35 m
R3	37°42′28.79″	105°55′10.32″	0.69 m	0.30 m
R4	37°47′31.20″	105°56′42.00″	1.22 m	1.20 m

Sample collection and measurement

Collection and measurement of zooplankton

For collecting cladocerans and copepods, 20 L of mixed water samples were filtered through a No.13 plankton net, concentrated to 50 ml, and fixed on-site with a 3% formaldehyde solution. Protozoans and rotifers were collected using a No. 25 plankton net at a depth of 0.5 m, stored in 1000 ml polyethylene bottles, and fixed on-site with 10-15 ml of Lugol’s solution before labelling and transporting to the laboratory. After settling for 48 h, the samples were siphoned and concentrated to 30 ml. Counts and identifications were carried out using a pipette and a MoticBA400 (China) optical microscope. A 5 ml sample was placed in a counting chamber for counting under a microscope at a 4×10 magnification, used for to identify *cladocerans* and copepods; the 1 ml sample was counted in a counting chamber under a microscope at a 10×10 magnification for identifying rotifers; for protozoans, 0.1 ml of the sample was first placed in a counting chamber and then counted under a microscope at a 20×10 magnification. Identification references included the *Aquatic Biology* (Zhao, 2016), *Chinese Freshwater Rotifers* (Wang, 1961), *Chinese Fauna Crustacea Cladocera Freshwater* (Jiang et al., 1979), and *Chinese Fauna Crustacea Copepoda Freshwater* (Crustacean Research Group, Institute of Zoology, Chinese Academy of Sciences, 1979).

The formula for calculating plankton abundance is:

$$N = (Vs \times n) / (V \times Va) \quad \text{(Eq.1)}$$

where N is the number of plankton observed per liter of water; Vs is the volume of the concentrated sample; n is the number of plankton observed under the microscope; V is the volume sampled; and Va is the counting volume. Using this formula, plankton abundance is calculated, and plankton, biomass can be determined with wet weight data.

Collection and measurement of environmental factors

Alongside plankton sampling, water quality parameters such as pH, water temperature (Temp), electrical conductivity (COND), and ammonium ion (NH_4^+) were measured using a portable water quality analyzer (YSI6600-02 USA). Water samples were brought back to the laboratory for analysis of total phosphorus (TP), total nitrogen (TN), and chemical oxygen demand (COD) using Hach water quality testing equipment (DR1900, HACH).

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Data analysis and processing

Using Excel 2021, statistical calculations were performed on the biomass, density, and dominant plankton species. Dominant species were determined based on the dominance value (Y), with a threshold of $Y \geq 0.02$ signifying dominance (Xu et al., 1989). The PAST (326b) software was used to calculate the Shannon-Wiener diversity index (*H'*) (Shannon and Weaver, 1949), Pielou evenness index (*J*) (Sun and Liu, 2003), and Margalef richness index (*d*) (Margalef, 1951). The biological condition of the reservoir area was assessed and graded based on biodiversity using the guidelines from *Monitoring and Evaluation of Aquatic Organisms in Lakes and Reservoirs* (Ministry of Ecology and Environment of the People's Republic of China, 2023).

Water quality assessment standards: Using the *Surface Water Environmental Quality Standards* (GB3838-2002) (State Environmental Protection Administration, 2002), the leading water quality indicators were evaluated. The ggplot2 packages in R (R.3.6.1) software was employed to perform box plot analysis, one-way ANOVA, and Turkey tests to explore the spatio-temporal heterogeneity of the zooplankton community structure and environmental factors in the QRAWNA.

Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA) was conducted using Canoco5.0 software. An initial Detrended Correspondence Analysis (DCA) was performed on the biodiversity indices data of zooplankton. If the maximum gradient length (SD) in the ordination axis was less than 3, RDA was applied; if $3 < SD < 4$, both analyses were possible; and if $SD > 4$, CCA was implemented to analyze further the environmental factors affecting the community structure of plankton in the reserve. The vegan, corrplot, ggcor and tidyverse packages in R software were utilized to perform the Mantel test to verify the correlation between the plankton community structure and environmental factors.

Results and analysis

Physicochemical characteristics of mudflats wetland water bodies

During the spring, the pH values of the different mudflats ranged from 7.01 to 8.46; in the summer, they ranged from 8.12 to 8.93, showing a slight variation but maintaining an overall weak alkalinity. The water temperature in the natural mudflats was higher than in the restored mudflats across both seasons. In the spring, except for lower average values of total phosphorus (TP), total nitrogen (TN), and chemical oxygen demand (COD) in the natural mudflats compared to the restored ones, all other water environmental factors were higher. In the summer, aside from the electrical conductivity (COND), pH, water depth (WD), and COD being lower in the natural mudflats, other water environmental factors were higher than those in the restored mudflats.

According to the "*Surface Water Environmental Quality Standards* (GB3838-2002)" (State Environmental Protection Administration, 2002), the reserve's water quality is relatively poor. TN and TP values reach Class IV and V water standards, respectively, and COD levels are Class III and above. Dissolved oxygen (DO) levels at all sampling sites reached Class I water standards.

The box plot analysis and one-way ANOVA (ANOVA, $df = 1$, $P < 0.05$) of the physicochemical factors of the water environments in both mudflats across different seasons are illustrated in *Figure 3.* Significant differences were observed in water

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temperature (Temp), pH, and COND, whereas no significant differences were found in other environmental factors (ANOVA, *df* = 1, *P* > 0.05).

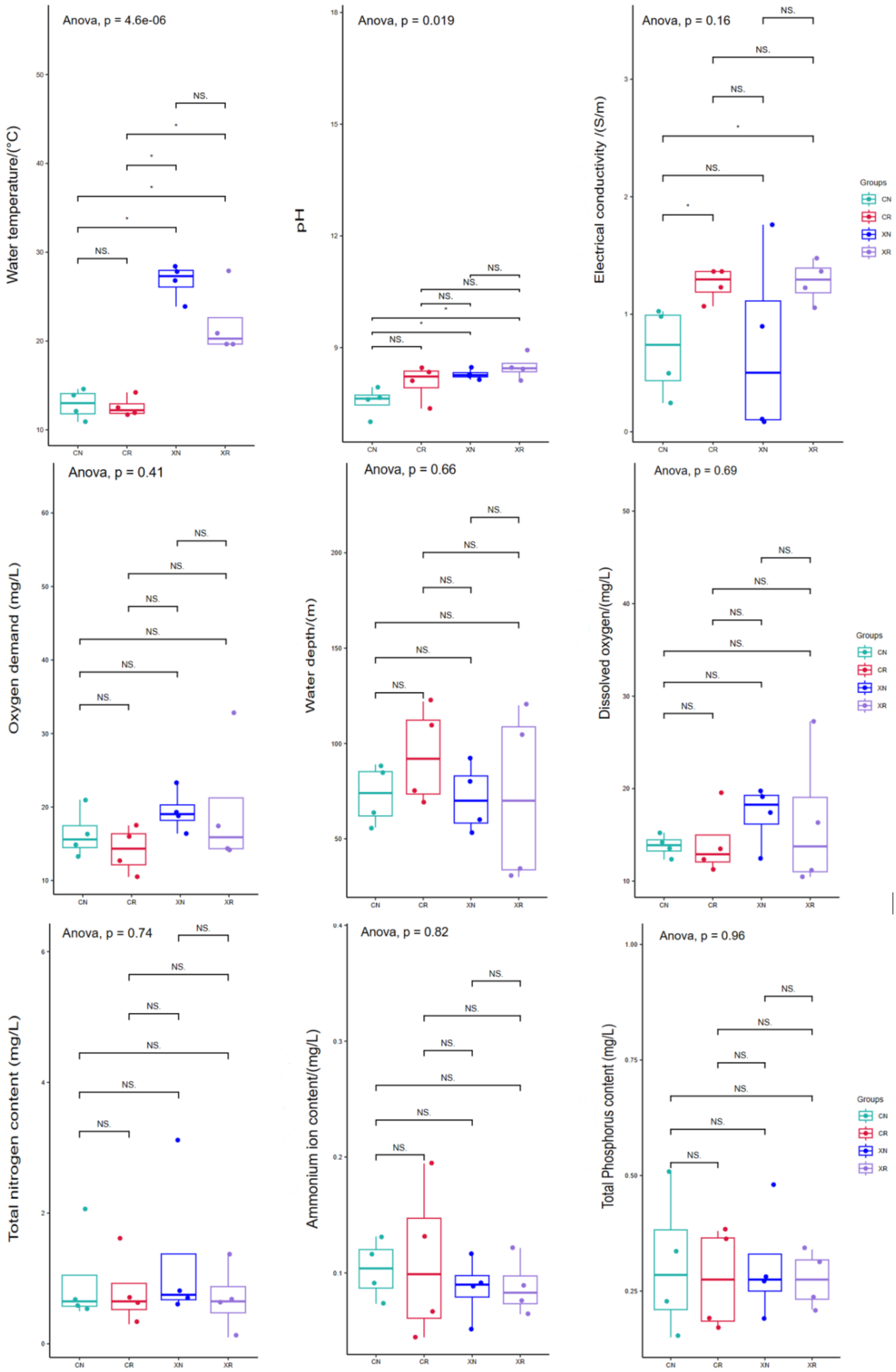

Figure 3. *Temporal and spatial changes of physicochemical factors in Qingtongxia Reservoir Area Wetland Nature Reserve. CN: natural mudflats in spring; CR: restored mudflats in spring; XN: natural mudflats in summer; XR: restored mudflats in summer. * Means the significant difference between groups (t-test, p ≤ 0.05), ns means the insignificant difference between groups*

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Composition of zooplankton species and dominant species

In this survey 39 plankton species were identified across four categories (*Table 2*): 1 species of protozoans, 19 species of rotifers, 6 species of cladocerans, and 14 species of copepods which accounted for 2.6%, 48.7%, 15.4%, and 33.3%, respectively. Both in spring and summer, the species richness in the restored mudflats was higher than in the natural mudflats. *Cyclops strenuous*, a copepod, emerged as a common dominant species in both the natural and restored mudflats.

Table 2. *Number of zooplankton species by season*

Species/region	Natural mudflats in spring	Restored mudflats in spring	Natural mudflats in summer	Restored mudflats in summer
Protozoon	1	0	0	0
Rotifera	6	9	3	5
Cladocerans	1	2	3	3
Copepoda	5	6	4	5
Total	13	17	10	13

During the spring season, 13 species were identified in the natural mudflats, with rotifers being the most abundant (9 species), accounting for 46.2% of the total plankton species identified. Dominant species in the natural mudflats included *Strombidium viride*, *Polyarthra trigla* and *Asplanchna priodonta*. In the restored mudflats, 17 species were identified, with rotifers also being the most abundant (9 species), representing 52.9% of the total zooplankton species. Dominant species in the restored mudflats include *Keratella quadrata*, *Notholca acuminata*, and *Alona quadrangularis*, while *Cyclops strenuous* was a common dominant species shared between the natural and restored mudflats.

In the summer, 10 species were identified in the natural mudflats, with copepods being the most numerous (4 species), making up 40% of the total plankton species identified. Dominant species in the natural mudflats included *Brachionus calyciflorus*, *Asplanchna priodonta*, and *Chydorus ovalis*. In the restored mudflats, 13 species were identified, with rotifers and copepods dominating (5 species), each accounting for 38.5% of the total plankton species. Dominant species in the restored mudflats included *Brachionus urceus*, *Alona quadrangularis*, and *Mesocyclops leuckarti*. Common dominant species shared between the natural and restored mudflats included *Asplanchna priodonta*, *Alona quadrangularis*, *Chydorus ovalis*, *Cyclops strenuus*, *Cyclops vicinus vicinus*, and *Mesocyclops leuckarti* (*Table 3*).

Plankton density, biomass, and biodiversity

Box plot analysis and one-way ANOVA (ANOVA, $df = 1$, $P > 0.05$) results, as shown in *Figure 4*, reveal no significant spatio-temporal differences in the density, biomass, species number, and diversity indices of plankton between the natural and restored mudflats. This indicates that the plankton community structure and distribution are similar in both mudflats without significant differences.

In the spring, the average plankton density in the natural mudflats (490.25 ind./L) was higher than in the restored mudflats (343.25 ind./L). In contrast, the average

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biomass was lower in the natural mudflats (4.45 mg/L) compared to the restored mudflats (7.328 mg/L). The densities in the natural and restored mudflats ranged between 10 and 1001 ind./L and 10 and 1122 ind./L, respectively, dominated by rotifers, which accounted for 97.9% and 89.6% of the total density, respectively. The biomass ranged between 0.20–3.76 mg/L in the natural mudflats and 0.20–3.76 mg/L in the restored mudflats, predominantly copepods, accounting for 97.3% and 98% of the total biomass, respectively.

Table 3. *Dominant species and degree of zooplankton dominance in QRAWNR*

Dominant species	Spring		Summer	
	Natural mudflats	**Restored mudflats**	**Natural mudflats**	**Restored mudflats**
Strombidium viride	0.250	-	-	-
Polyarthra trigla	0.633	-	-	-
Keratella quadrata	-	0.024	-	-
Notholca acuminata	-	0.085	-	-
Brachionus calyciflorus	-	0.030	0.039	-
Brachionus urceus	-	0.182	-	0.040
Schizoccrca diversicornis	-	-	0.096	-
Asplanchna priodonta	0.039	-	0.230	0.136
Filinia maior	-	-	-	0.046
Alona quadrangularis	-	0.125	0.357	0.333
Alona diaphana	0.250	-	-	-
Simocephalus exspinosus	-	-	0.036	-
Chydorus ovalis	-	0.125	0.035	0.111
Daphnia longispina	-	-	-	0.028
Cyclops strenuus	0.080	0.2606	0.686	0.519
Cyclops vicinus vicinus	-	-	0.038	0.029
Mesocyclops leuckarti	-	0.049	0.028	0.042
Canthocamptus carinatus	0.033	-	-	-

“-” indicates that the species is not dominant

In the summer, average densities and biomass were lower in the natural mudflats (118.25 ind./L and 2.27 mg/L, respectively) than in the restored mudflats (229.50 ind./L and 5.91 mg/L). The densities ranged between 10–448 ind./L in the natural mudflats and 86–529 ind./L in the restored mudflats, primarily consisting of rotifers, which accounted for 82.4% and 71.9% of the total density, respectively. The biomass ranged between 0.63–4.99 mg/L in the natural mudflats and 0.40–13.67 mg/L in the restored mudflats, predominantly consisting of copepods, which accounted for 96.9% and 98.7% of the total biomass, respectively.

Using the grading method from *Monitoring and Evaluation of Aquatic Organisms in Lakes and Reservoirs* (Ministry of Ecology and Environment of the People’s Republic of China, 2023), with the Shannon-Wiener diversity index (*H’*) and Pielou’s evenness index (*J*) as criteria, the biological condition of the reserve was evaluated. In spring, the *H’* values ranged between 0.8–1.3.

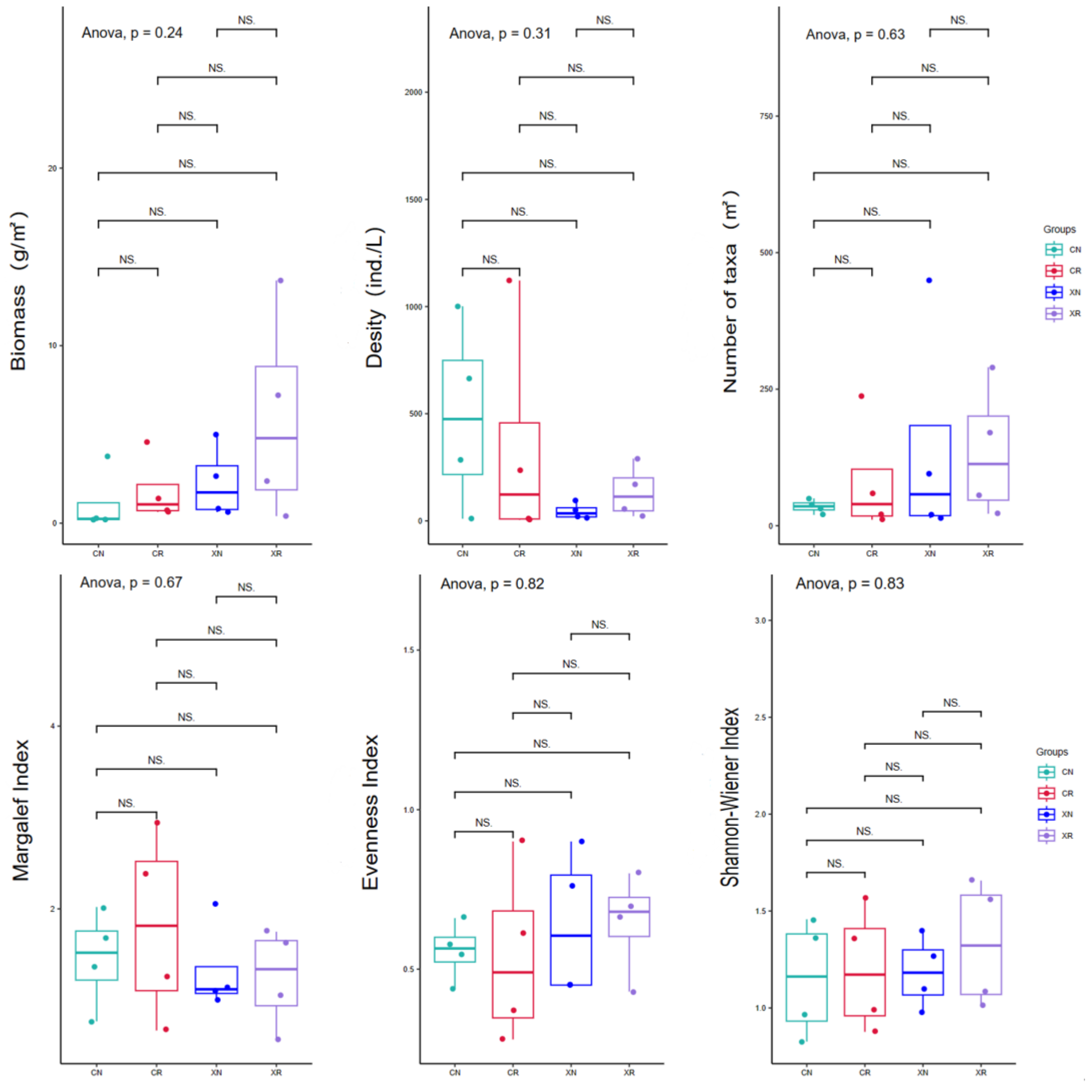

Figure 4. *Spatial and temporal distribution of zooplankton community structure and biodiversity index. CN: natural mudflats in spring; CR: restored mudflats in spring; XN: natural mudflats in summer; XR: restored mudflats in summer. * Means the significant difference between groups (t test, p ≤ 0.05), ns means the insignificant difference between groups*

Relationship between plankton community structure and water environmental factors

Figure 5 depicts the redundancy analysis (RDA) of the relationship between zooplankton community structure and environmental factors in the mudflats of the QRAWNR during different season. In the spring, the explanatory power of the first and second axes was 95.51% and 3.97%, respectively, jointly accounting for 99.48% of the total variance. The analysis showed a positive correlation between the evenness index (*J*) and ammonium (NH_4^+) and between the Shannon-Wiener diversity index (*H'*).

The Mantel test results showing the relationship between the zooplankton community structure and environmental factors in the different mudflats of the QRAWNR are illustrated in *Figure 6*: In spring, there was no significant correlation between plankton biodiversity indices (*H', J, d*) and all environmental factors

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($P > 0.05$). In summer, there was a significant correlation between the Shannon-Wiener diversity index (*H'*) and ammonium (NH_4^+) ($P < 0.05$) and between the evenness index (*J*) and total phosphorus (TP) ($P < 0.05$).

Discussion

This study indicates that rotifers dominate the zooplankton community in the mudflat of QRAWNR, while cladocerans and copepods (larger plankton) are relatively less abundant. This finding aligns with research on lakes and reservoirs (Zhang et al., 2024; Chen et al., 2024; Gu et al., 2024; Habib et al., 1997), where, typically, rotifers are numerous in cleaner water conditions but fewer in species. Conversely, in severely polluted waters, some pollution-tolerant rotifers become dominant (Hou et al., 2020; Agasild, et al., 2013), such as *carapace rotifers* and *brachionus rotifers*, which are known for their high pollution tolerance (Zhang et al., 2022). This study found that the dominant plankton species in the reserve include *Brachionus urceus*, *Brachionus calyciflorus*, *Keratella quadrata*, and *Asplanchna priodonta*, with a focus on rotifers. The water quality in the reserve is generally poor to moderate, and the variation in the types and abundance of rotifers correlates closely with water quality, contributing to the relatively high diversity and abundance of rotifers in the mudflats of the QRAWNR.

Typically, biomass and density are directly proportional; as density increases, biomass also increases (Chen et al., 2010; Ju et al., 2016). In this study, the average plankton density in the natural mudflats was higher in spring than in the restored mudflats. However, the average biomass was lower in the natural mudflats, attributed to numerous small-bodied *Polyarthra trigla*, which contribute minimally to total biomass (Ju et al., 2016). One-way ANOVA showed no significant spatio-temporal differences in zooplankton community structure in the reserve. However, the density of zooplankton ranged widely from 10 to 1122 ind./L across sampling sites, mainly due to critical environmental factors like total phosphorus (TP) and ammonium (NH_4^+). Environmental factors can affect phytoplankton (Zhao et al., 2024), the primary food source for filter-feeding zooplankton, thus indirectly impacting zooplankton's the density and community structure (Huang et al., 2024).

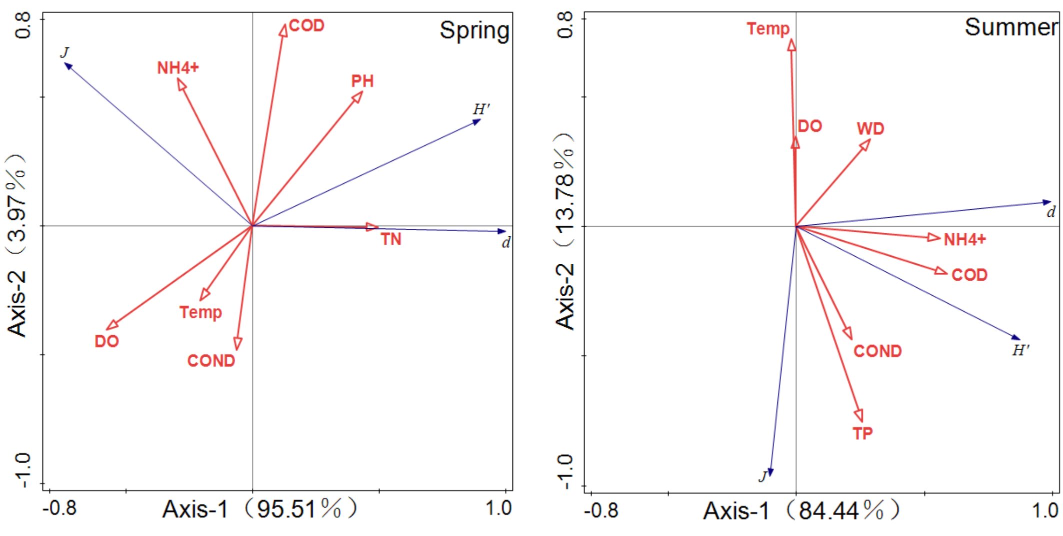

Figure 5. *Redundancy analysis of zooplankton community structure and environmental factors in spring and summer*

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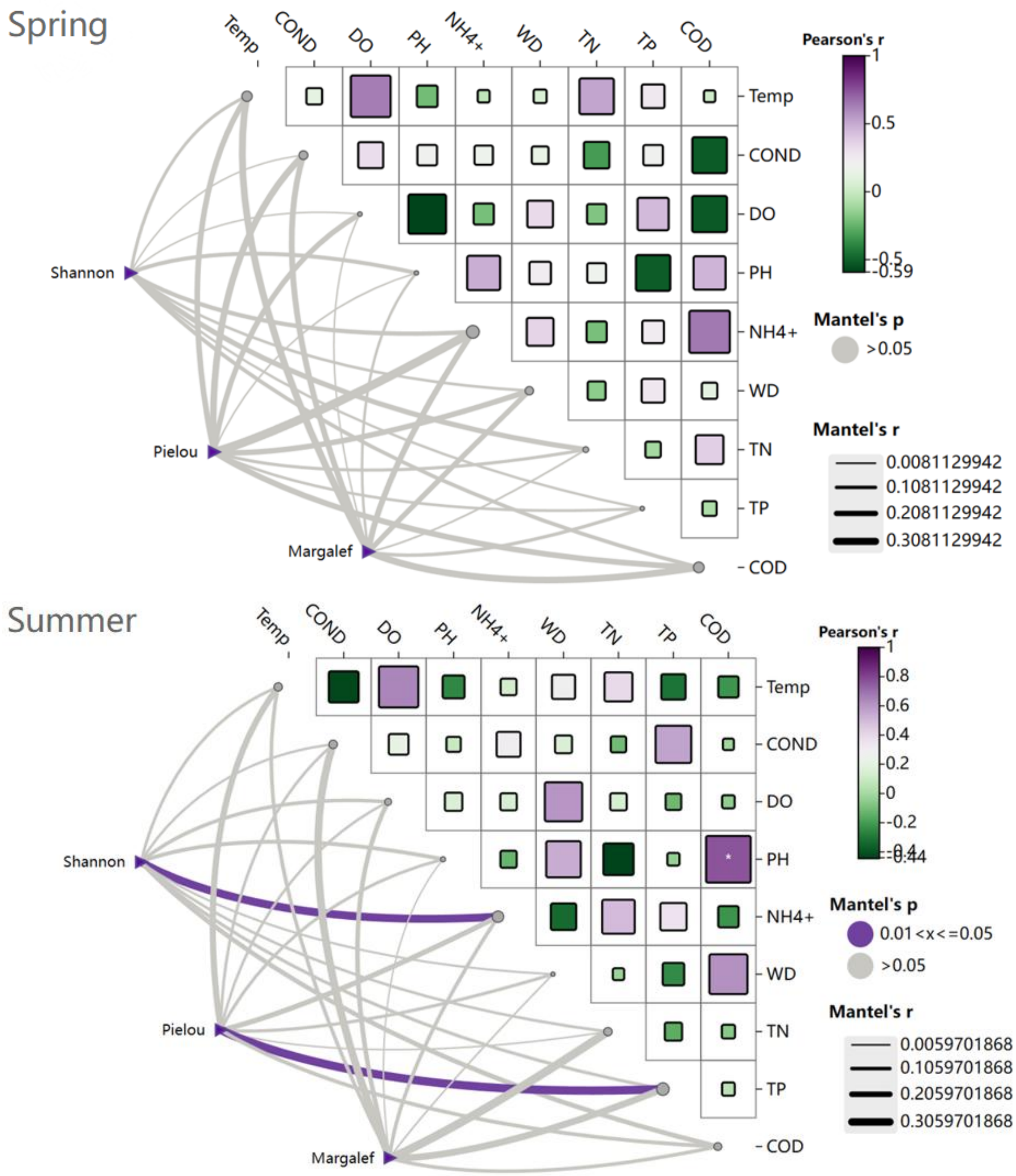

Figure 6. *Mantel test of zooplankton community structure and water environmental factors in spring and summer*

Aside from significant spatio-temporal differences in pH, water temperature, and conductivity, changes in water environmental factors between natural and restored mudflats were insignificant. The spatial differences in water environmental factors in the mudflats of the QRAWNR are primarily influenced by the physicochemical properties of the sediments and activities such as ecological restoration projects from the “2018 Green Shield Action” (Duan, 2020). These projects effectively improved the heterogeneity of restored mudflats, reducing organic pollution. They altered the hydrological connectivity of wetlands and nutrient status, which are associated with the gradual improvement of water conditions in the restored mudflats. Submerged plants

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reduce nutrient concentration in the water by minimizing wind-wave induced resuspension of sediments, absorbing excess nutrients, secreting substances that inhibit algal growth, protecting zooplankton, and enhancing predation on phytoplankton, thereby maintaining clean water quality (Barko and Smart, 1981; Barko et al., 1991).

The research showed that the density of submerged plants in the restored mudflats is significantly higher than in the natural mudflats, and the dissolved oxygen levels at all sampling sites met Class I water standards, further indicating effective improvement in the water environment of the restored mudflats, this is consistent with previous studies (Ekoko et al., 2022), indicating that the results of this study are reliable.

Considering the physicochemical indicators and planktonic evaluation indices, the overall water quality of the mudflats in the QRAWNR remains mildly polluted. Although the water quality of the restored mudflats has improved to some extent, continuous protection and necessary restoration measures should still be strengthened to support the recovery and sustainable use of the wetland ecosystem in the reserve.

Conclusion

A total of 39 species of zooplankton s were identified in the mudflats of the QRAWNR, with 20 species in the natural mudflats and 19 in the restored mudflats, among which *Cyclops strenuous* is a common dominant species in both. The structure and biodiversity of the zooplankton communities showed no significant spatio-temporal differences between the two types of mudflats. Other water quality factors showed no significant variations besides temperature, pH, and conductivity, which exhibit significant spatio-temporal difference. The water quality of the reserve was generally in a state of mild pollution, and pH, TP, NH_4^+, and COD are the main environmental factors influencing the zooplankton community structure.

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