ZOOPLANKTON COMPOSITIONS AND ITS RELATIONSHIP WITH SURVIVABILITY OF THE YANGTZE FINLESS PORPOISE IN THE NANJING YANGTZE DOLPHIN NATURE RESERVE

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Abstract. To understand the community structure of zooplankton and assess the survivability of the Yangtze finless porpoise (YFP), a field investigation of seasonal variation of zooplankton and environmental parameters was conducted in the Nanjing Yangtze Dolphin Nature Reserve (NYDNR) from October 2021 to August 2022. The results showed that: (1) a total of 54 species were identified, and the number of species belonging to protozoa, rotifer, cladocera, and copepods was 19, 12, 11 and 12, respectively; (2) the total zooplankton density was 21571.65 ind./L, dominated by protozoa (89.7%), and the total biomass was 6.6762 mg/L, dominated by rotifer (28.0%) and copepods (48.2%); (3) the assessment results of water quality showed that the NYDNR was classified as mesotrophic level, it was indicated that the water quality of the NYDNR satisfied the living requirement for YFP; (4) the fish productivity in the NYDNR is about 43139.34 kg, which could meet the nutrition requirements of 28 YFPs, and fish potential productivity estimation through zooplankton showed that food sources of YFP were being threatened. The results of this study not only improved the understanding of the community structure of zooplankton, but also have important implication to the protection of YFP in the NYDNR.
Keywords: *taxonomic richness, density, fishing potential, heterogeneity, environmental factors*

Introduction

Globally, the rich biological resources in freshwater ecosystems are dramatically declining, because of freshwater ecosystems face multiple anthropogenic stressors (Dudgeon et al., 2006). The Yangtze River is the third largest river in the world and the fishes in the Yangtze River are essential for the sustainable development of freshwater fisheries and the conservation of aquatic biodiversity in China (Fu et al., 2003). However, the fishery resources in the Yangtze River Basin have shown rapid decline due to various human activities. Protected areas are a cornerstone of biological conservation with functions such as biodiversity conservation, scientific research, education, ecological environment maintenance and ecotourism (Giakoumi et al., 2017). The nature reserves in the middle reach of the Yangtze River focus mainly on the conservation of rare and economically critical aquatic animals and their habitats, such as *Acipenser sinensis*, *Lipotes vexillifer*, *Yangtze finless porpoise* (YFP, *Neophocaena asiaeorientalis asiaeorientalis*), and other rare fish (Zhu et al., 2018), of

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which the YFP is the key protected object. The YFP is currently the only cetacean in the Yangtze River Basin and is an important indicator of the health of the Yangtze River ecosystem. As flagship species of the Yangtze River, YFP is the symbol of healthy water ecosystems (Richard, 2010; Turvey et al., 2012). However, due to increasing serious human activities (such as overfishing, river-lake disconnection, and shipping), the habitat of freshwater dolphins is deteriorating gradually, and death events occur frequently (Mei et al., 2014). Since the 1980s, the number of the YFP in the Yangtze River has declined rapidly, the YFP is listed as a key wild animal for national protection in 2021. Therefore, strengthening the protection of Yangtze finless porpoise is of great urgency.

The Nanjing Yangtze Dolphin Nature Reserve (NYDNR) (118°28’39.14”-118°44’38.35”E, 31°46’34.83”-32°7’3.81”N) is located on the Yangtze River in Nanjing City, Jiangsu Province, adjacent to Ma’anshan City, Anhui Province. The total area of the reserve is 86.92 km^2, and is divided into three parts: the core zone, buffer zone, and experimental zone. The total area of the core zone, buffer zone and experimental zone is 30.25 km^2, 23.66 km^2, and 33.01 km^2, accounting for 34.80%, 27.22% and 37.98% of the total area of the reserve, respectively (Shan et al., 2021). Since the late 20th century, with the deterioration of ecological environment in the Yangtze River basin (Xu et al., 2017), the NYDNR has become one of the main habitats of YFP in the middle and lower reaches of the Yangtze River.

Zooplankton are an integral part of aquatic ecosystems and play a linkage role in the material transformation, energy flow and information transfer of aquatic food web (Persson et al., 2007). Zooplankton mainly feed on algae, bacteria, and organic detritus (bottom-up effects) (Taipale et al., 2009), and they are also the food source for filter-feeding fish and other aquatic animals (top-down effects) (Guo et al., 2003; Jayasinghe et al., 2015). Due to their short life cycle and wide distribution range, and sensitivity to environmental changes, zooplankton species composition, abundance, and biomass can be easily influenced by changes in water quality. Thus, zooplankton has been advocated as a biological indicator for assessing water quality and ecosystem health (Meshram et al., 2018; Ochocka, 2021; Stamou et al., 2019, 2021). A habitat is a collection of various biotic and abiotic factors in a certain place, characterized by temporal and spatial heterogeneity (Partridge, 1978). Water quality and fishery resources are important factors affecting the survival of finless porpoise (Zhang et al., 2018). Through studying the zooplankton community structure, the water quality in the finless porpoise reserve can be evaluated, and the survival status and population size of finless porpoise can be estimated by the bottom-up effect of zooplankton on fish population. To date, studies on the zooplankton community structure had been carried in Xijiang River, Wanhe estuary and Zhenjiang Reserve (Dai et al., 2011; Tan et al., 2021, 2022; Zhang et al., 2018), but no study of the temporal and spatial variations in zooplankton communities and survival assessment of the finless porpoise in the NYDNR have been reported. In this study, we conducted an annual survey on zooplankton and environmental variables in the NYDNR with the following objectives: (1) to understand the temporal and spatial patterns of zooplankton communities in the NYDNR; (2) to evaluate the water quality of the NYDNR; (3) to estimate the productivity of zooplankton community available to fish and assess the survival status of the YFP. The results of this study can provide scientific basis for the environmental protection and sustainable utilization of resources in the NYDNR.

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Materials and methods

Sampling sites and sample collection

Four sample collections were conducted on October 6, 2021 (autumn), December 28, 2021 (winter), May 5, 2022 (spring) and August 7, 2022 (summer). A total of 41 sample sites were set up in the river zone (RZ), core zone (CZ), experimental zone (EZ) and buffer zone (BZ) of the reserve (*Fig. 1*), including 9 in the river zone (sample sites 1-3, 36-41), 10 in the experimental zone (sample sites 4, 5, 7, 18-21, 32-34), 8 in the core zone (sample sites 6, 8-11, 26-28), and 14 in the buffer zone (sample sites 12-17, 22-25, 29- 31, 35) (*Fig. 1*). Referring to the standards for zooplankton collection and analysis by Zhang and Huang (1991), the procedures for zooplankton collection were as follows: (1) one liter quantitative samples of protozoa and rotifer were collected from the surface layer (0.5 m underwater, the same below) using a 2.5 L polycarbonate water sampler, preserved with 1.5% Lugol's iodine solution, which was then concentrated to 50 mL for protozoa and rotifer samples after being settled for 48 h based on the concentrated water sample method; (2) 20 L quantitative samples of cladocera and copepods were collected from the surface layer, a plankton net of 64 µm mesh size was used to filter one mixed 20 L water sample and 0.5 mL Lugol's iodine solution was added to fix the filtered fluid in a 50 mL vial for the determination of cladocera and copepods.

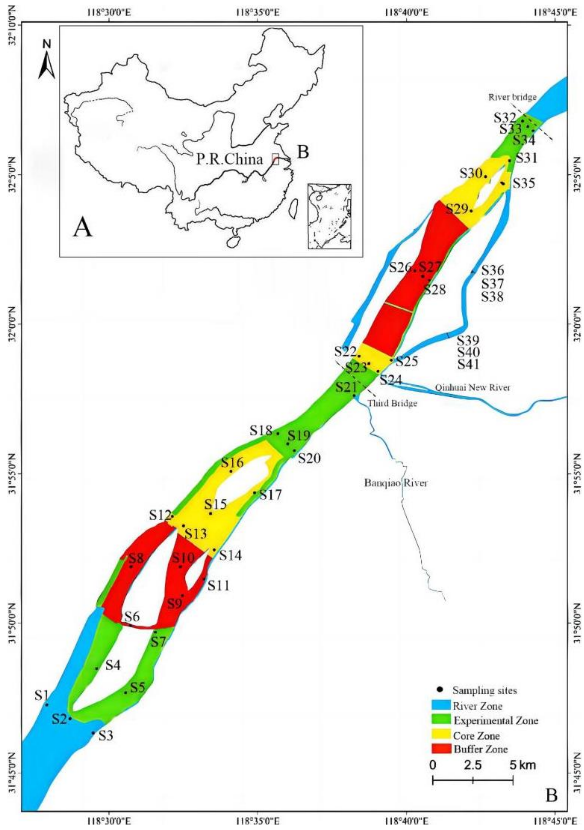

***Figure 1.** Sampling sites in the Nanjing Yangtze Dolphin Nature Reserve*

At each site, environmental variables for water quality were simultaneously measured according to the "Analytical Methods for Water and Wastewater Monitoring" (2002). Dissolved oxygen (DO), water temperature (WT) and pH were measured in situ

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by using a YSI multi-parameter water quality analyzer (YSI6600-V2, USA), water depth (WD), water transparency (Secchidisk depth, SD) and turbidity (Turb) were measured in situ by using a weighted Secchi disk (20 m) and a Hash 2100Q portable turbidimeter, respectively. 1.5 L water samples from each site were collected at least 20 cm below the water surface to eliminate the air, and transported to the laboratory for further analyzing chlorophyll *a* (Chl *a*) content and water parameters, including total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), chemical oxygen demand (COD), orthophosphate (PO_4-P).

Species identification and sample analysis

The concentrated protozoa and rotifer samples were taken to the laboratory for identification under an Axioplan 2 Imaging microscope (Zeiss, Jena, Germany) (magnification 400×). A 0.1 mL and 1 mL plankton counting chamber was used to count the abundance of protozoa and rotifer, respectively, which was expressed as ind./L. Each sample from each section was counted twice and the mean value of the two numbers was selected as the result. Samples for cladocera and copepods analysis were studied under a compound microscope. 20 L cladocera and copepods filtered samples were counted entirety. Zooplanktonic organisms were identified at the species and genus levels where even possible following the methods according to Chiang and Du (1979), Shen et al. (1990), Wang (1961), Zhang and Huang (1991).

Data analysis

The following analytical measures were used:

(1) The dominance of zooplankton (*Y*):

$$Y = \frac{N_i}{N} f_i \quad \text{(Eq.1)}$$

where N_i is the density of the i^{th} species; *N* is the total zooplankton density, f_i is the frequency at each site; the dominant species were based on dominance > 0.02 (Zhang et al., 2018).

(2) Three diversity indices were used to analyze zooplankton communities, including: Shannon-Wiener diversity index (*H'*) (Shannon and Wiener, 1963), Margalef richness index (*D*) (Margalef, 1968), and Pielou evenness index (*J*) (Pielou, 1966).

$$H' = -\sum_{i=1}^{S} P_i \log_2 P_i \quad \text{(Eq.2)}$$

$$D = (S - 1) / \log_2 N \quad \text{(Eq.3)}$$

(Eq.4)

where *S* is the number of species, P_i is the relative abundance of each species (N_i/N).

(3) Prior to the ANOVAs, all data were log (x + 1) transformed to meet the conditions of normality and homogeneity of variance conditions. Significant differences

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in environmental variables, zooplankton density, zooplankton biomass, the TLI, and the WQI at the spatial and seasonal scales were evaluated with One-way ANOVAs (If there was no significant difference in homogeneity test for variance) or Kruskal-Wallis H test (a non-parametric test) (If there was a significant difference in homogeneity test for variance). A statistical difference was considered significant when $P < 0.05$.

(4) Canonical correspondence analysis (CCA) was chosen to test the relationships among species assemblages and environmental variables because the gradient length of the first DCA (detrended correspondence analysis) axis performed using species data was 4.6 (Lepš and Šmilauer, 2003). Individual taxa chosen for analyses had a total relative abundance > 1.0% when all samples were summed (Wu et al., 2012). Log (x + 1) transformation was performed and forward selection and Monte Carlo permutations (999 iterations) were used to examine whether the significance level was reached. DCA and CCA were implemented in CANOCO 4.5 software.

Evaluation method of nutritional status

The zooplankton biomass, three diversity indices, including *H'*, *D*, and *J*, comprehensive trophic level index (TLI) and water quality index (WQI) were used to evaluate the water quality of the NYDNR.

(1) Based on the *H'* value and *D* value, trophic status is classified into five grades: polysaprobic (0-1), α-mesosaprobic (1-2), β-mesosaprobic (2-3), oligosaprobic (3-4), clean (>4) (Zhang et al., 2018). Based on the *J* value, trophic status is classified into three grades: heavy pollution (0~0.3), moderate pollution (0.3~0.5), light pollution or no pollution (0.5~0.8) (Shen et al., 1990). Based on the zooplankton biomass, trophic status is classified into three grades: oligotrophic (<1.0 mg/L), mesotrophic (1-3 mg/L), oligosaprobic conditions (>3 mg/L) (Zhang et al., 2018).

(2) Calculation of TLI: According to "Lakes (Reservoirs) Eutrophication Assessment Methods and Classification Technology Requirements" (2001), TLI is a weighted sum based on the correlations between Chl *a* and other parameters (including TP, TN, SD, and COD). The TLI equation was calculated as follows:

$$\text{TLI}(\Sigma) = \sum_{j=1}^{m} W_j \times \text{TLI}(j) \quad \text{(Eq.5)}$$

$$\text{W}_j = r_{ij}^2 \Big/ \sum_{j=1}^{m} r_{ij}^2 \quad \text{(Eq.6)}$$

where W_j is correlative weight for trophic level index of *j*; TLI(*j*) is trophic level index of *j*; r_{ij} is the correlation coefficients between the reference Chl *a* and each parameter *j* (Chl *a*, 1; TP, 0.84; TN, 0.82; SD, 0.83; COD, 0.83), and *m* is the number of indicators.

The calculation formulas of each trophic state index (TLI (*j*)) were established as follows:

$$\text{TLI (Chl } a) = 10\ (2.5 + 1.086 \ln \text{Chl } a) \quad \text{(Eq.7)}$$

$$\text{TLI (TP)} = 10\ (9.436 + 1.624 \ln \text{TP}) \quad \text{(Eq.8)}$$

$$\text{TLI (TN)} = 10\ (5.453 + 1.694 \ln \text{TN}) \quad \text{(Eq.9)}$$

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$$\text{TLI (SD)} = 10\,(5.118 - 1.94 \ln \text{SD}) \quad \text{(Eq.10)}$$

$$\text{TLI (COD)} = 10\,(0.109 + 2.661 \ln \text{COD}) \quad \text{(Eq.11)}$$

where the units of Chl *a* and SD are μg/L and cm, respectively; the units of TP, TN and COD are mg/L, respectively.

The TLI ranges from 0 to 100, with high values representing high eutrophication levels. Based on the TLI value, trophic status is classified into five grades: oligotrophic ($TLI(\sum) < 30$), mesotrophic ($30 \leq TLI(\sum) \leq 50$), light-eutrophic ($50 < TLI(\sum) \leq 60$), mid-eutrophic ($60 < TLI(\sum) \leq 70$) and hyper-eutrophic ($TLI(\sum) > 70$) (He et al., 2021).

(3) Calculation of WQI: The WQI method was proposed by Pesce and Wunderlin (Horton, 1965). In the current study, twelve water quality parameters including WT, DO, pH, TN, TP, PO_4-P, NH_4-N, NO_2-N, PO_4-P and COD were used to calculate the WQI, and their measured values were used for normalization. The WQI was calculated as follows:

$$\text{WQI} = \frac{\sum_{i=1}^{n} C_i P_i}{\sum_{i=1}^{n} P_i} \quad \text{(Eq.12)}$$

where n is the total number of parameters included in the study, C_i is the normalized value of parameter i, and P_i is the weight of parameter i. The P_i value used in the study ranged from 1 to 4. These values have been verified in the literatures (Horton, 1965; Kocer and Sevgili, 2014; Qu et al., 2020). Based on the WQI score, the water quality was classified into five grades: very poor (0-25), poor (26-50), moderate (51-70), good (71-90), and excellent (91-100). The higher WQI values indicating better overall water quality condition.

Evaluation of the potential fish productivity

In order to evaluate the capacity of the porpoise in the reserve, based on the biomass of zooplankton and the average water depth to obtain the standing stock of zooplankton per hectare at each site, and to calculate the potential fish productivity (*F*):

$$F = (b \times P / B \times a) \quad \text{(Eq.13)}$$

where b is the biomass of zooplankton; P/B is the ratio between the standing stock and biomass of zooplankton; a is the available coefficient of zooplankton by fish, K is the bait coefficient of zooplankton feeding by fish. Referring to the literature (Dai et al., 2011), it was determined that the P/B coefficient was 40, a was 30%, and K was 10.

Results

Hydrographic conditions

The 15 environmental variables reflected the water quality and habitat gradients and showed a wide range of values (*Fig. 2*). Except for WD, the environmental variables of the reserve were significantly different in four seasons ($P < 0.05$). The water in the

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reserve was slightly alkaline, with the highest TDN and NH_4-N in spring, TN and WT in summer, TP in autumn, and Chl *a* in winter; TDP and PO_4-P were higher in spring and winter than in summer and autumn; COD and DO were highest in winter in river zone, experimental zone and core zone (*Fig. 2*).

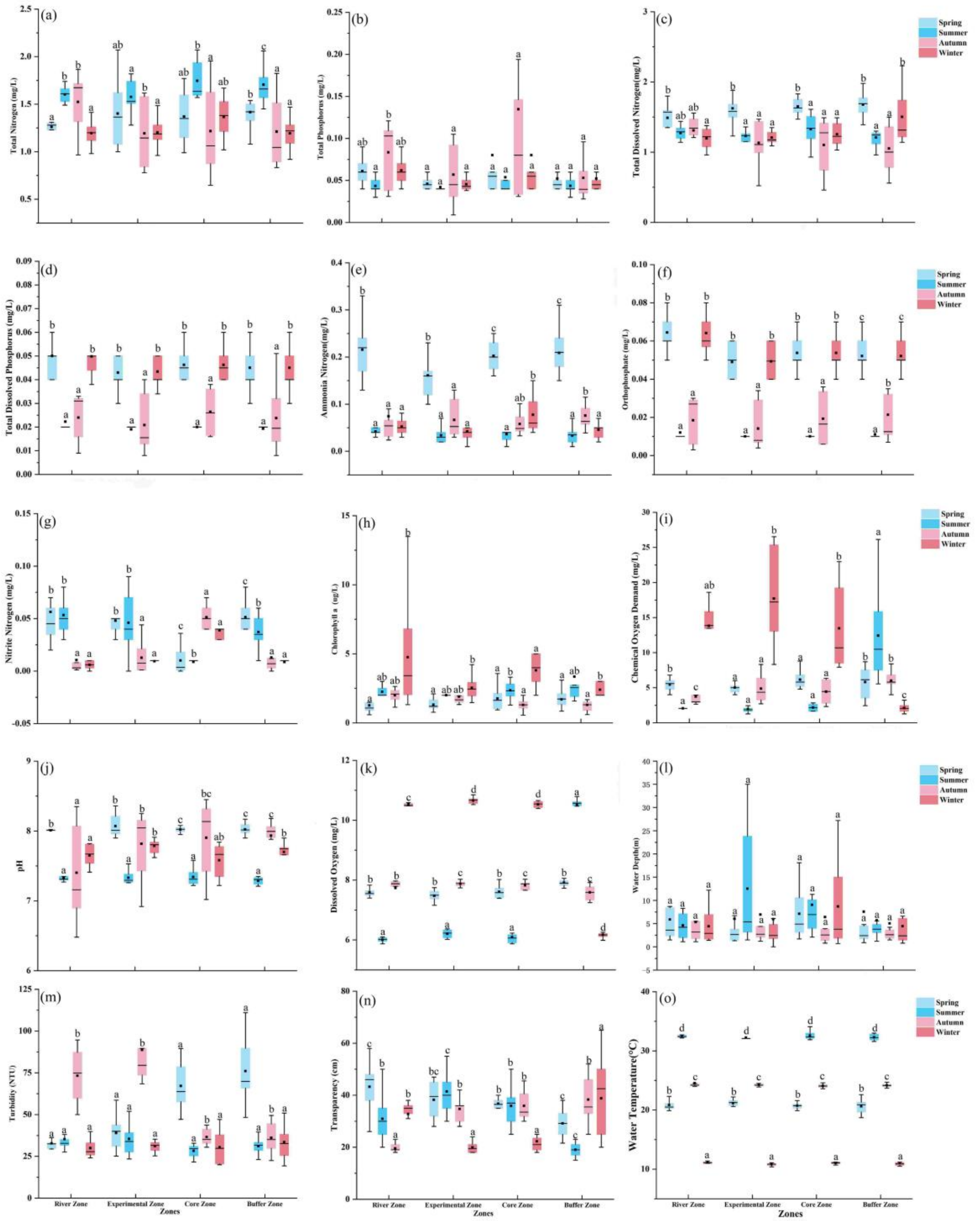

Figure 2. *Seasonal patterns of (a) total nitrogen, (b) total phosphorus, (c) total dissolved nitrogen, (d) total dissolved phosphorus, (e) ammonia nitrogen, (f) orthophosphate, (g) nitrite nitrogen, (h) chlorophyll a, (i) chemical oxygen demand, (j) pH, (k) dissolved oxygen, (l) water depth, (m) turbidity, (n) transparency, and (o) water temperature in the regions. Note: bars with different lowercase letters indicate significant differences among seasons*

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Zooplankton community structure

A total of 54 zooplankton species were identified in the four surveys, including 19, 12, 11, and 12 species of protozoa, rotifer, cladocera and copepods, accounting for 35.2%, 22.2%, 20.4% and 22.2% of the total species, respectively. Temporally, the number of zooplankton species found in spring, summer, autumn, and winter was 35, 14, 22 and 7, respectively. Spatially, the experimental zone had the highest species number (32), followed by buffer zone (25), river zone (23) and core zone (23) (*Table 1*).

Table 1. *Zooplankton species composition in the NYDNR*

Taxa	Genus	Code	Species	Spring	Summer	Autumn	Winter	RZ	EZ	CZ	BZ
Protozoa	*Arcella*	S1	*Arcella discoides*	+							+
	Arcella	S2	*Arcella hemiisphaerica*				+		+		
	Centropyxis	S3	*Centropyxis aculeata*				+	+	+	+	+
	Centropyxis	S4	*Centropyxis* sp.	+	+			+		+	+
	Difflugia	S5	*Difflugia acuminata*	+					+		
	Difflugia	S6	*Difflugia biwae*	+				+	+		
	Difflugia	S7	*Difflugia levanderi*				+		+		+
	Difflugia	S8	*Difflugia limnetica*	+				+			
	Proteus	S9	*Proteus* sp.	+				+			
		S10	Ciliate		+				+		+
	Epistylis	S11	*Epistylis urceolata*	+				+			
	Holosticha	S12	*Holosticha kessleri*	+				+			
	leprotintinnus	S13	*Leprotintinnus fluviatile*	+				+	+	+	+
	Paramecium	S14	*Paramecium caudatum*	+				+	+		
	Tintionnopsis	S15	*Tintinnopsis entzii*	+				+			
	Tintionnopsis	S16	*Tintinnopsis kiangsuensis*	+			+	+	+		
	Tintionnopsis	S17	*Tintinnopsis longus*	+				+			
	Tintionnopsis	S18	*Tintinnopsis wangi*	+			+		+	+	+
	Vorticella	S19	*Vorticella* sp.	+				+			+
Rotifer	*Asplachna*	S20	*Asplanchna* sp.	+				+	+		
	Brachionus	S21	*Brachionus angularis*	+					+		
	Brachionus	S22	*Brachionus calyciflorus*				+			+	
	Brachionus	S23	*Brachionus diversicornis*	+						+	
	Brachionus	S24	*Brachionus forficula*		+				+		
	KeratelIa	S25	*Keratella cochlearis*		+	+	+	+	+		
	KeratelIa	S26	*KeratelIa valaa*	+		+	+	+	+		
	Ploesoma	S27	*Ploesoma truncatum*	+					+		
	Polyarthra	S28	*Polyarthra trigla*	+	+				+	+	+
	Pompholyx	S29	*Pompholyx sulcata*								
	Trichocerca	S30	*Trichocerca cylindrica*		+						+
	Trichocerca	S31	*Trichocerca* sp.			+					+
Cladocera	*Bosmina*	S32	*Bosmina longirostris*	+	+	+	+	+	+	+	+
	Ceriodaphnia	S33	*Ceriodaphnia cornuta*		+				+		
	Chydorus	S34	*Chydorus ovalis*				+		+	+	+
	Chydorus	S35	*Daphnia hyalina*	+			+		+	+	
	Chydorus	S36	*Daphnia longispina*	+			+		+	+	+
	Chydorus	S37	*Daphnia psittacea*				+			+	
	Diaphanosoma	S38	*Diaphanosoma brachyurum*	+	+	+		+	+	+	+
	Moina	S39	*Moina micrura*	+	+				+		+
	Sida	S40	*Sida crystallina*				+			+	
	Simocephalus	S41	*Simocephalus vetulus*				+			+	
	Scapholeberis	S42	*Scapholeberis mucronata*	+						+	

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Copepods		S43	Copepodid	+	+	+	+	+	+	+	+
		S44	Copepod nauplii		+		+	+	+	+	+
	Eucyclops	S45	*Eucyclops macruroides*	+			+	+	+	+	+
	Eucyclops	S46	*Eucyclops serrulatus*				+			+	
	Limnoithona	S47	*Limnoithona sinensis*	+	+				+		+
	Macrocyclops	S48	*Macrocyclops* sp.	+							+
	Schmackeria	S49	*Schmackeria forbesi*	+	+				+	+	+
	Schmackeria	S50	*Schmacheria inopinus*	+				+			
	Schmackeria	S51	*Schmackeria* sp.			+	+	+	+	+	+
	Sinocalanus	S52	*Sinocalanus dorrii*	+			+		+	+	+
	Sinodiaptomus	S53	*Sinodiaptomus* sp.				+				+
	Thermocyclops	S54	*Thermocyclops dybowskii*	+					+		
			Species number	35	14	7	22	23	32	23	25

"+" indicates the presence of the species. RZ: river zone; CZ: core zone; EZ: experimental zone; BZ: buffer zone

The total zooplankton density of 41 sample sites in four seasons was 21571.65 ind./L, with a mean value of 131.53 ind./L, dominated by protozoa (89.7%), and rotifer, cladocera and copepods only accounted for 8.0%, 0.2% and 2.1%, respectively; the total zooplankton biomass was 6.6762 mg/L, with a mean value of 0.041 mg/L, dominated by rotifer (28.0%) and copepods (48.2%), protozoa and cladocera accounted for 14.5% and 9.3%. Mean zooplankton densities were significantly different among the four seasons ($P < 0.05$), with higher densities in spring (341.77 ind./L) and winter (158.74 ind./L) than in summer (21.92 ind./L) and autumn (3.71 ind./L); mean zooplankton biomass was also significantly different among the four seasons ($P < 0.05$), with higher densities in winter (0.095 mg/L) was higher than spring (0.04 mg/L), summer (0.017 mg/L) and autumn (0.01 mg/L). In terms of taxonomic groups, the mean densities and biomass of protozoa and copepods were significantly different in four seasons ($P < 0.05$), while the mean densities and biomass of rotifer and cladocera were not significantly different in four seasons ($P > 0.05$) (*Table 2*).

The mean zooplankton densities were higher in the river zone (183.75 ind./L) and experimental zone (182.11 ind./L) than core zone (84.13 ind./L) and buffer zone (88.94 ind./L); the mean zooplankton biomass were highest in the core zone (0.096 mg/L) than river zone (0.022 mg/L), experimental zone (0.043 mg/L) and buffer zone (0.02 mg/L). There were no significant spatial differences concerning zooplankton density and biomass ($P > 0.05$). Likewise, none of the four taxonomic groups displayed significant spatial differences ($P > 0.05$) (*Table 2*).

A total of 31 dominant species were recorded throughout the whole year, including 18 protozoa, 10 rotifera, 1 cladocera and 2 copepods. The dominant species composition differed between seasons. The most dominant species in spring were 11 species, with the highest dominance of *Leprotintinnus fluviatile* (0.28) and *Tintinnopsis kiangsuensis* (0.18); the dominant species in winter and summer were 6 species, with the highest dominance of *Centropyxis aculeata* (0.80) and *Centropyxis* sp. (0.45); the dominant species in autumn were 5 species, with higher dominance of *KeratelIa valaa* (0.26), *Keratella cochlearis* (0.26), *Trichocerca* sp. (0.26) and *Schmackeria* sp. (0.18). The dominant species in spring, summer and winter were mainly protozoa that prefer a relatively oligotrophic state, whereas the dominant species in autumn were indicative of a higher eutrophic status. Spatially, the river zone and experimental zone had the most dominant species, with 14 and 10 species, respectively, while the core zone and buffer

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zone had 8 and 6 species, respectively. The dominant species in the river zone, experimental zone and buffer zone were mainly protozoa that occurring in clean water, *Centropyxis aculeata* and *Leprotintinnus fluviatile* were the common dominant species of the four zones (*Table 3*).

Diversity indexes of zooplankton community

The average values of the Shannon-Wiener diversity index (*H'*), Margalef richness index (*D*) and Pielou evenness index (*J*) for the four seasons and four zones were showed in *Table 2*. The ranges of *H'*, *D*, and *J* were 0.33-0.45, 0.86-2.68, and 0.25-0.43, respectively, with averages of 0.39, 1.76 and 0.36 in four seasons. The value variations of *H'*, *D* and *J* in the four zones ranged from 0.33 to 0.44, 0.88 to 3.24 and 0.31 to 0.41, respectively, with averages of 0.38, 1.69 and 0.36. No significant spatiotemporal differences were found in any indices ($P > 0.05$).

Table 2. *The density, biomass and diversity indices of zooplankton at different seasons and different zones in the NYDNR (average ± SD)*

		Spring	**Summer**	**Autumn**	**Winter**	***P***
Density	Protozoa	320.00 ± 624.05^{b}	13.66 ± 53.37^{a}	0.00 ± 0.00^{a}	138.54 ± 189.34^{a}	0.000
	Rotifer	21.46±56.64	7.80±24.03	2.93±10.55	9.76±26.50	0.237
	Cladocera	0.05±0.08	0.30±0.77	0.11±0.14	0.29±0.65	0.071
	Copepods	0.26 ± 0.43^{a}	0.16 ± 0.19^{a}	0.66 ± 0.68^{a}	10.16 ± 34.72^{b}	0.000
	Zooplankton	341.77 ± 630.84^{c}	21.92 ± 59.47^{ab}	3.71 ± 10.56^{a}	158.74 ± 211.20^{b}	0.000
Biomass	Protozoa	0.016 ± 0.031^{b}	0.001 ± 0.003^{a}	0.000 ± 0.000^{a}	0.007 ± 0.009^{a}	0.000
	Rotifer	0.021±0.05	0.009±0.029	0.004±0.013	0.012±0.032	0.191
	Cladocera	0.001±0.002	0.006±0.015	0.002±0.003	0.006±0.013	0.081
	Copepods	0.002 ± 0.003^{a}	0.001 ± 0.001^{a}	0.005 ± 0.005^{a}	0.071 ± 0.243^{b}	0.021
	Zooplankton	0.04 ± 0.06^{ab}	0.017 ± 0.032^{a}	0.01 ± 0.014^{a}	0.095 ± 0.253^{b}	0.021
Diversity indices	*H'*	0.44±0.50	0.45±0.46	0.33±0.31	0.34±0.39	0.63
	D	2.68±9.85	1.23±1.65	2.26±3.89	0.86±1.07	0.17
	J	0.35±0.40	0.43±0.43	0.41±0.38	0.25±0.30	0.16
		River zone	**Experimental zone**	**Core zone**	**Buffer zone**	***P***
Density	Protozoa	175.56±547.22	156.00±320.49	65.00±159.27	84.29±277.30	0.441
	Rotifera	7.78±23.07	25.00±57.78	7.50±23.69	3.57±15.77	0.149
	Cladocera	0.07±0.11	0.16±0.31	0.17±0.31	0.30±0.80	0.203
	Copepods	0.35±0.66	0.95±2.11	11.45±39.28	0.78±1.93	0.436
	Zooplankton	183.75±546.04	182.11±347.37	84.13±190.27	88.94±276.86	0.407
Biomass	Protozoa	0.009±0.027	0.008±0.016	0.003±0.008	0.004±0.014	0.441
	Rotifera	0.009±0.028	0.025±0.052	0.009±0.028	0.004±0.019	0.107
	Cladocera	0.001±0.002	0.003±0.006	0.003±0.006	0.006±0.016	0.199
	Copepods	0.002±0.005	0.007±0.015	0.08±0.275	0.005±0.014	0.440
	Zooplankton	0.022±0.038	0.043±0.062	0.096±0.287	0.02±0.033	0.191
Diversity indices	*H'*	0.33±0.44	0.40±0.44	0.36±0.36	0.44±0.43	0.64
	D	0.91±1.43	3.24±10.10	0.88±1.35	1.73±2.98	0.35
	J	0.31±0.36	0.33±0.38	0.37±0.40	0.41±0.40	0.60

The different superscripts in the table indicate significant differences. *H'*: Shannon-Wiener diversity index; *D*: Margalef richness index; *J*: Pielou evenness index

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Table 3. *Zooplankton dominance of the NYDNR*

Taxa	Species	Spring	Summer	Autumn	Winter	RZ	EZ	CZ	BZ
Protozoa	*Centropyxis aculeata*				0.80	0.11	0.31	0.51	0.18
	Centropyxis sp.	0.05	0.45			0.04		0.03	0.15
	Difflugia biwae	0.03				0.04	0.03		
	Difflugia limnetica	0.04				0.09			
	Proteus sp.	0.07				0.16			
	Epistylis urceolata					0.04			
	Holosticha kessleri					0.04			
	Leprotintinnus fluviatile	0.28				0.11	0.14	0.12	0.37
	Paramecium caudatum	0.05				0.04	0.05		
	Tintinnopsis entzii					0.04			
	Tintinnopsis kiangsuensis	0.18				0.21	0.18		
	Tintinnopsis longus					0.04			
	Vorticella sp.	0.05				0.04			0.08
	Difflugia acuminata	0.04					0.08		
	Tintinnopsis wangi	0.05			0.02		0.03	0.12	0.06
	Arcella discoides	0.03							0.08
	Ciliate		0.18						
	Difflugia levanderi				0.02				
Rotifer	*KeratelIa valaa*			0.26	0.02	0.03	0.03		
	Keratella cochlearis		0.09	0.26	0.02				
	Asplanchna sp.						0.03		
	Ploesoma truncatum						0.03		
	Brachionus calyciflorus							0.03	
	Brachionus diversicornis							0.03	
	Polyarthra trigla		0.09					0.03	
	Brachionus forficula		0.09						
	Trichocerca cylindrica		0.09						
	Trichocerca sp.			0.26					
Cladocera	*Bosmina longirostris*			0.03					
Copepods	Copepodid				0.06			0.13	
	Schmackeria sp.			0.18					
	Species number	11	6	5	6	14	10	8	6

RZ: river zone; CZ: core zone; EZ: experimental zone; BZ: buffer zone

Relationship between zooplankton community structure and environmental parameters

Species-environment correlations provided insights into the factors driving zooplankton assemblage structure and their distributions. The results of Canonical correspondence analysis (CCA) demonstrate that the selected environmental parameters explained 46.5% of the total variation in the zooplankton density, and the statistical significance was verified by the Monte Carlo permutation test (pseudo-F: 3.42, p = 0.001). The canonical axes were further analyzed, and the first axis and second axis reached significance levels (pseudo-F: 5.07, p = 0.002 and pseudo-F: 2.04, p = 0.016), explaining 28% and 18.5% of the variance. CCA analysis showed that Chl *a*, WT, SD, pH, TN, TDN, NH_4-N and NO_2-N were the environmental variables that were strongly correlated with the zooplankton community structure ($P < 0.05$). The distributions of zooplankton in the four zones in spring and in core and buffer zones in summer were mainly positively correlated with WT, SD, pH, TN, TDN, NH_4-N, NO_2-N; the distributions of zooplankton in four zones in autumn and winter and in experimental and

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river zones in summer were mainly positively related to Chl *a* (*Fig. 3*). There is a positive correlation between Chl *a* and *Centropyxis aculeata*, *Keratella cochlearis*, *KeratelIa valaa*, and Copepodid, indicating that these species were suitable for living in water with high algae abundance. *Arcella discoides*, *Centropyxis* sp., *Difflugia biwae*, *Leprotintinnus fluviatile*, *Tintinnopsis wangi*, *Asplanchna* sp., *Ploesoma truncatum* and *Polyarthra trigla* were positively correlated with TN and TDN, indicating that these species preferred occurring in high-nutrient conditions; *Difflugia levanderi*, *Difflugia limnetica*, *Proteus* sp. and *Epistylis urceolata*, *Holosticha kessleri*, *Paramecium caudatum*, *Tintinnopsis entzii*, *Tintinnopsis kiangsuensis*, *Tintinnopsis longus* and *Vorticella* sp. were positively correlated with WT, SD, pH, NH_4-N, and NO_2-N, indicating that these species had similar ecological habits and preferred alkaline water with high temperature, transparency and nitrogen nutrient (*Fig. 3*).

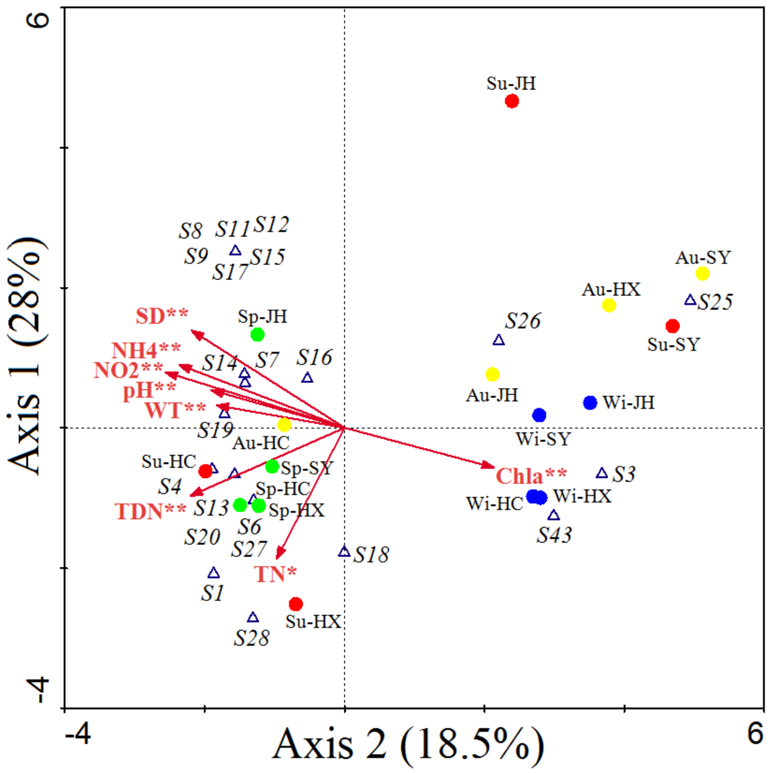

Figure 3. *CCA of the species-environment relationships (see Table 1 for species codes)*

Water quality evaluation

The variation ranges of TLI index and WQI index in the four seasons were 33.75-48.05 and 73.55-83.90, with mean values of 41.56 and 79.76, respectively; the variation ranges of TLI index and WQI index in the four zones were 40.22-41.49 and 79.36-79.92, respectively. The TLI and WQI indexes were significantly different in four seasons ($P < 0.05$) (*Fig. 4*). Based on the annual mean values of *H’* (0.39), *D* (1.76), *J* (0.36), zooplankton biomass (1.669 mg/L), TLI (41.56) and WQI (79.76), the trophic status of the reserve was considered to be polysaprobic, α-mesosaprobic, moderate pollution, mesotrophic, mesotrophic and good, respectively. The result of the

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zooplankton biomass was more consistent with TLI and WQI, and the water quality of the NYDNR was considered as mesotrophic level.

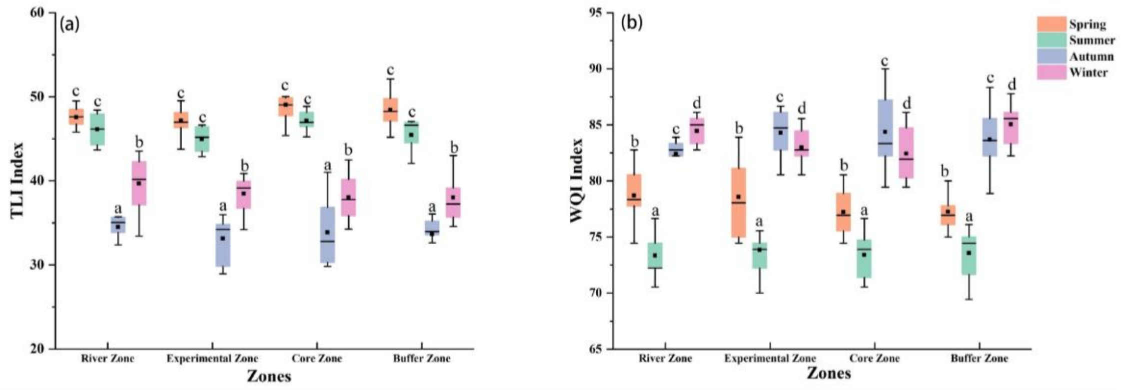

***Figure 4.** Seasonal patterns of (a) TLI index and (b) WQI index in the regions. Note: bars with different lowercase letters indicate significant differences among seasons*

The potential fish productivity

The fish production potential of four zones in four seasons were showed in *Table 4*. The average value of fish productivity in the core zone, experimental zone and buffer zone were 9.86 kg/hm^2, 3.62 kg/hm^2 and 1.40 kg/hm^2, respectively, and the total area of the reserve was 86.92 km^2. The average value of fish productivity in the core, buffer zone and experimental zone was 4.96 kg/hm^2, the total fish productivity of zooplankton-eating fish in the reserve was 43139.34 kg. If the annual food consumption of each adult YFP is 1500 kg, the fish productivity of the reserve could meet the nutrition requirements of 28 YFPs, theoretically.

***Table 4.** Fish potential production estimation of different seasons in the NYDNR*

Seasons	Sample sites	Average water depth (m)	P/B coefficient	Available coefficient (%)	Bait coefficient	Average zooplankton biomass		Zooplankton actual yield (kg/hm^2)	Zooplankton fishing productivity (kg/hm^2)
						Monitoring biomass (mg/L)	Existing biomass (kg/hm^2)		
Autumn	RZ	5.32	40	30	10	0.014388889	0.765808648	30.63	0.92
	EZ	6.94	40	30	10	0.013105	0.909487	36.38	1.09
	CZ	6.41	40	30	10	0.0047625	0.305395313	12.22	0.37
	BZ	5.04	40	30	10	0.009292857	0.467961728	18.72	0.56
Winter	RZ	4.42	40	30	10	0.027766667	1.227903718	49.12	1.47
	EZ	6.61	40	30	10	0.054635	3.611980556	144.48	4.33
	CZ	8.66	40	30	10	0.3445375	29.84556094	1193.82	35.81
	BZ	4.44	40	30	10	0.02535	1.126264286	45.05	1.35
Spring	RZ	5.86	40	30	10	0.042761111	2.503900611	100.16	3
	EZ	6.02	40	30	10	0.081865	4.928273	197.13	5.91
	CZ	7.13	40	30	10	0.0182375	1.299421875	51.98	1.56
	BZ	7.56	40	30	10	0.022184615	1.676523048	67.06	2.01
Summer	RZ	4.59	40	30	10	0.002555556	0.117271625	4.69	0.14
	EZ	12.18	40	30	10	0.02161	2.632098	105.28	3.16
	CZ	9.03	40	30	10	0.01579375	1.425385938	57.02	1.71
	BZ	5.94	40	30	10	0.023632143	1.402736488	56.11	1.68

RZ: river zone; CZ: core zone; EZ: experimental zone; BZ: buffer zone

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Discussion

Characteristics of the zooplankton community

The river ecosystem is characterized by the interaction between flowing water, temperature, organic matter, inorganic matter, energy and river organisms (Zhou, 2007). In order to adapt to the river habitat, river zooplankton are mainly benthic species, and achieve much lower densities in rivers than in stagnant waters (Zhou, 2007), especially the river current with high flow velocity and sediment content is not suitable for reproduction and feeding of zooplankton (Ekwu and Udo, 2014). A total of 54 zooplankton species were identified in the NYDNR in four seasons, among which there were more protozoan species, and the species number of rotifer was more consistent with cladocera and copepods. The dominant species were mainly algal-feeding protozoa. Despite the differences in sampling frequency, sampling section and identification level, the zooplankton community structure in this study was consistent with other studies in the YFP reserve (Dai et al., 2011; Tan et al., 2021, 2022), further verifying that the river zooplankton has a unique community system (Speirs and Gurney, 2001)

The zooplankton species number and dominant species number were the highest in spring, and the density was also higher than that of the other three seasons, which was consistent with the zooplankton structure of the Wanhe estuary (Tan et al., 2022), but different from the results of the higher zooplankton density and biomass in summer and autumn in the Xijiang River (Zhang et al., 2018) and the Zhenjiang River (Tan et al., 2021), which may be attributed to the environmental factors affecting the zooplankton community in different rivers. The results showed that the zooplankton density and biomass in summer and autumn were higher than those in spring and winter in Zhenjiang. Tan et al. (2021) pointed out that the differences in density and biomass among the core zone, buffer zone and main channel of Zhenjiang reserve were not significant, and the differences in density and biomass of zooplankton and four groups in the four zones of the NYDNR in this study were also not significant ($P > 0.05$), which was consistent with the results of the above study, indicating that the spatial heterogeneity of river zooplankton communities was not obvious due to the influence of water flow.

Correlations between zooplankton community structure and environmental variables

Environmental factors affecting zooplankton community structure in rivers and lakes have been the focus of research in freshwater zooplankton ecology. The zooplankton community structure is affected by a complex combination of abiotic factors, such as temperature, light, pH and nutrients, as well as biotic factors, such as food quality, competition and predation (Wang, 2008). CCA analysis showed that WT, SD, pH, Chl *a*, TN, TDN, NH_4-N, NO_2-N were the environmental factors with strong correlation with zooplankton community structure in NYDNR ($P < 0.05$). Temperature is generally considered to be the most important factor affecting the seasonal variations of zooplankton species composition and stocks (Lin et al., 2014), 20-22°C, 26-32°C and 20-30°C are the optimal growth temperature ranges of protozoa (Feng et al., 2017), rotifer (Zhang and Huang, 1991), cladocera and copepods (Jin et al., 1991), respectively. Some studies suggest that nitrogen and phosphorus nutrients are important environmental factors affecting zooplankton distribution (Feng et al., 2017; Qiu et al., 2012), and the nutrient status of the waterbodies may affect zooplankton abundance, community structure, body size and productivity (Gutierrez et al., 2020; Wang et al., 2021). In this study, compared with summer and winter, both spring and autumn temperatures were

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more suitable for zooplankton growth, but TN, TDN, TDP, PO_4-P, and NO_2-N were higher in spring than autumn, indicating that temperature and nutrient concentration in spring were more suitable for zooplankton survival and reproduction in the NYDNR. As an index of food resources availability for zooplankton, phytoplankton is important in shaping zooplankton community structure (Liu et al., 2020; Nie et al., 2019). In this study, Chl *a* was the highest in winter, and the positive correlation between Chl *a* and zooplankton densities showed the positive effect of Chl *a* on zooplankton density.

Water quality evaluation

At present, the diversity index of zooplankton has been widely used to evaluate the water quality of various water bodies (rivers, lakes, reservoirs, aquaculture ponds, etc.). The diversity index can objectively reflect the comprehensive cumulative effect of the water environment on the species and quantity of zooplankton, and is an important indicator of community structure characteristics (Li et al., 2015; Lin et al., 2018). It is generally believed that the species diversity is low in extremely poor waters due to the scarcity of food resources for zooplankton, and some pollution-sensitive species disappear in eutrophic waters (Dussart et al., 1984), whereas the zooplankton community structure is complex and diversity is high in mesotrophic waters (Qian et al., 2007). Xie et al. (2005) pointed out that the Margalef index and Shannon-Wiener diversity index failed to reflect the current situation and change trend of water quality in the Jinjiang River basin. TLI is a commonly used method for evaluating the eutrophication state of lakes and reservoirs in China. It is mainly based on physical and chemical indexes such as total nitrogen (TN), total phosphorus (TP), water transparency (SD), chemical oxygen demand (COD) and biological index chlorophyll *a* (Chl *a*) concentration (He et al., 2021). WQI has been widely used in river water quality assessment because WQI can effectively integrate multiple physicochemical parameters into a single value, and detect the overall water quality status and water quality trends over time and space (Qu et al., 2020). Compared with static water bodies such as lakes and reservoirs, rivers are linear habitats with a single flow direction. Many conditions such as flow state, hydrological condition, shoreline type, etc. will have an important impact on the formation of river zooplankton communities (Speirs and Gurney, 2001). Pace et al. (1992) found a close correlation between river flow status and zooplankton abundance in the Hudson River, and the difference in the retention time of different rivers will lead to the difference in the abundance of zooplankton, even in different reaches of the same river, the abundance of zooplankton community is not completely the same. Therefore, the zooplankton density and biomass of rivers are lower than those of static water bodies. In this study, compared with three diversity indices, the result of the zooplankton biomass was more consistent with TLI and WQI, our study concluded that zooplankton biomass, TLI index and WQI index were more suitable for river water quality evaluation than diversity index, and the water quality of the NYDNR was considered as mesotrophic level. Referring to the water quality evaluation results of other finless porpoise reserves (Dai et al., 2011; Zhang et al., 2018), the water quality of the NYDNR was suitable for the survival of the finless porpoise.

Potential of fish production and porpoise capacity

Fish resources are one of the important factors affecting the survival of the finless porpoise, and the finless porpoise can only consume small pelagic fish less than 6 cm in

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length with no obvious hard spines, such as *Hemiculter leucisculus* and *Hemiculter bleekeri*, due to the structural characteristics of the throat (Zhang et al., 2015). As one of the main habitats of the YFP population in the middle and lower reaches of the Yangtze River, the NYDNR is increasingly short of fishery resources due to the excessive exploitation of human resources, and the food of the Yangtze finless porpoise is increasingly reduced. As the basic link of the food chain and productivity of the aquatic ecosystem, zooplankton can affect the structure and distribution of the fish community, and then affect the population distribution and scale of the YFP through the bottom-up effect (Zhang et al., 2018; Zhao et al., 2008). Therefore, zooplankton can be used to assess the fish productivity of the reserve and to make a preliminary assessment of the survival of the finless porpoise (Dai et al., 2011; Zhang et al., 2018). In this study, the fish productivity of the NYDNR can meet the nutrition requirements of 28 YFPs, however, there were about 60 YFPs in the NYDNR in the simultaneous survey, the fish productivity estimation through zooplankton showed that food sources of YFP were being threatened. However, in this study, fish productivity was estimated only based on zooplankton, and aquatic vascular plants, phytoplankton, organic detritus and zoobenthos could also affect fish resources (Gong et al., 2019). Therefore, in the future studies, on the premise of adhering to the ten-year fishing ban policy of the Yangtze River, small fish that are preferentially fed by the YFP should be bred and released in the upper and middle layers in the NYDNR, and regularly monitor and evaluate the aquatic organisms and environmental factors in the reserve, so as to more accurately assess the survival status of the YFP in the NYDNR.

Conclusions

In this study, a total of 54 zooplankton species were identified, and the total density and biomass of zooplankton were 21571.65 ind./L and 6.6762 mg/L, respectively. According to the zooplankton biomass, comprehensive trophic level index (TLI) and water quality index (WQI), the water quality in NYDNR was mesosaprobic, and the water quality of the NYDNR satisfied the living requirement for YFP. WT, SD, pH, Chl *a*, TN, TDN, NH_4-N, NO_2-N were vital factors affecting the zooplankton community. The fish productivity in the NYDNR could meet the nutrition requirements of 28 YFPs, and fish potential productivity estimation through zooplankton showed that food sources of YFP were being threatened. Ultimately, these results add to our growing understanding of the zooplankton community structure in the NYDNR, and has certain reference significance for the management and protection of YFP in the NYDNR.

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