

## SEASONAL VARIATIONS OF ZOOPLANKTON FUNCTIONAL GROUPS AND RELATIONSHIP WITH ENVIRONMENTAL FACTORS IN A EUTROPHIC RESERVOIR FROM COLD REGION

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**Abstract.** In this study, the concept of functional feeding groups was used to classify zooplankton community into functional groups among three seasons (spring, summer and autumn). A total of 30 zooplankton species were sampled in the Tuanjie Reservoir and identified into seven functional groups. Environmental factors and zooplankton functional group biomass were both varied in spatially and seasonally. Water transparency (SD), dissolved oxygen (DO), pH, N:P and dissolved iron (Fe<sup>3+</sup>) were higher in autumn while total phosphorus (TP), chemical oxygen demand (COD<sub>Mn</sub>) and dissolved copper (Cu<sup>2+</sup>) were higher in spring, and temperature (T) and ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N) were higher in summer. Zooplankton functional groups biomass was higher in summer, followed by spring and autumn. In spring, zooplankton functional group only dominated by group PF, while in summer and autumn dominated by group PF and RF. Spearman and RDA results showed that SD, conductivity (COND), DO, NH<sub>4</sub><sup>+</sup>-N, nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N) and COD<sub>Mn</sub> were the major factors influencing zooplankton functional groups in Tuanjie Reservoir.

**Keywords:** *seasonal dynamics, zooplankton functional groups, abundance and biomass, northeast of China*

### Introduction

Zooplankton is found in almost all kinds of waterbodies. Compared with other aquatic animals, they are small in size, numerous in numbers and have strong metabolic activities. They feed on phytoplankton, bacteria, fragments and other organisms. Zooplankton also participates in the decomposition and circulation of organic matter in the water ecosystem through excretion and secretion, energy transfer from the primary producers to higher consumers (Wetzel, 2001). Variation of zooplankton can influence the structure of other nutrient levels in water ecosystems (Beaugrand et al., 2000; David et al., 2005; Lobry et al., 2008; Steinberg and Condon, 2009). The zooplankton community structure, their abundance and biomass is influenced by top-down and bottom-up control (Jeppesen et al., 2003), and is one of determinants for water quality (Jeppesen et al., 2011). This is due to the interaction between biological organisms and environmental factors (Ejsmont-Karabin and Karabin, 2013).

Traditional methods of zooplankton classification are usually based on the taxonomy system. However, it is difficult to reflect the aquatic ecological function. Therefore, ecologists have put forward the concept of functional groups, species characteristics of the functional group more closely in touch with environment, through the study of functional groups, can more directly reflect the ecological process of ecological

environmental impact of aquatic biological communities (Hood et al., 2006). In recent years, the use plankton functional groups have been applied in several studies of biogeochemical models (Anderson, 2005). Zooplankton is divided into three functional groups: micro-, meso- and macrozooplankton (Quéré et al., 2010). By using the concept of functional groups, zooplankton community composition can be used to model the ecosystem process of the aquatic ecosystem and it is also conducive to analyzing the seasonal variation of zooplankton community (Sun et al., 2010).

Tuanjie Reservoir was built in 1981 with the aim of supplying drinking water to the local village people. The main sources water of the reservoir is Muling River, which is the largest tributary of the Ussuri River on the left bank of the border between China and Russia. In this study the concept of functional feeding groups was used to reveal the seasonal variation of zooplankton functional groups in relation to environmental factors in Tuanjie Reservoir. Understanding the variation of zooplankton functional groups biomass in relation to environmental factors is very important to better understand the environmental factors that significantly structure the zooplankton community in the reservoir. This study aims at: (1) identifying and classifying zooplankton species of Tuanjie Reservoir into functional group, and (2) to determine the seasonal and spatial dynamics of the biomass of zooplankton functional groups and their relationship to environmental factors. We hypothesized that seasonal and spatial change in biomass of zooplankton functional groups will be strongly influenced by environmental factors.

## Materials and methods

### *Study area*

Tuanjie Reservoir (130°8'-130°11'E, 44°01'-44°04'N) is located in the southeast of Heilongjiang Province Northeastern China (*Fig. 1*). The reservoir was built in 1981 in order to control floods, provide water for irrigation, fish farming, power generation and for aesthetic value. Tuanjie Reservoir has a surface area of 445 km<sup>2</sup>, a capacity of  $8.63 \times 10^7$  m<sup>3</sup> and it shaped like big "Y". The region where located the reservoir is influenced by temperate continental monsoon. The average annual evaporation and precipitation of the reservoir are 950 mm and 534 mm, respectively. The annual mean temperature is 1 °C, which ranged between -44.1 °C to 37.6 °C. In winter, the surface water of the reservoir is covered by ice.

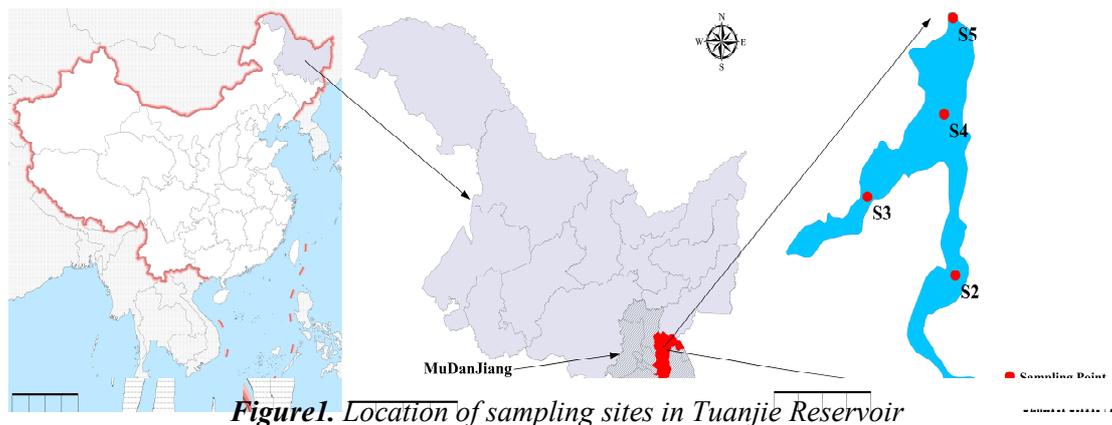
### *Field sampling and laboratory analysis*

Based on the reservoir shape, 5 sampling sites (*Table 1*) were selected for sampling on spring (May), summer (July) and autumn (September). Samples were collected from top (0.5 m from the surface water), middle and bottom (0.5 m from the waterbed) of water column according to the different water depth among sites inside the Tuanjie Reservoir (*Fig. 1*). At every sampling site, water temperature (T), pH, conductivity (COND), dissolved oxygen (DO) was measured in the field using a portable multi-probe (YSI 6600, YSI Inc.). Water transparency (SD) and depth (D) were measured using Secchi disk and longline method. Triplicate water samples for chemical analyses were collected at each sampling sites and put on acid-washed plastic bottles, placed in ice box and transported to laboratory for analysis. The concentration of total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), chemical oxygen demand (COD<sub>Mn</sub>) and dissolved iron (Fe<sup>3+</sup>) and dissolved

copper ( $\text{Cu}^{2+}$ ) were measured according to the standard methods for China (MEP, 2002).

**Table 1.** Five sampling sites coordinates in Tuanjie Reservoir

Sampling sites	Latitude	Longitude
S1	N44°01'48"	E130°11'24"
S2	N44°03'36"	E130°10'48"
S3	N44°03'00"	E130°09'36"
S4	N44°04'12"	E130°10'36"
S5	N44°04'48"	E130°10'48"



**Figure 1.** Location of sampling sites in Tuanjie Reservoir

Zooplankton samples (20 L water filtered through 64 mm mesh size) were fixed with formaldehyde solution (4% concentration). Protozoa and rotifera samples were obtained by taking 1 L subsamples to form the 20 L pooled sample. The samples were preserved with Lugol's iodine and formaldehyde and allowed to sediment in 1 L jar for at least 48 h. The supernatant water was carefully removed and the residue was then collected and made to a known volume of 30 mL (Thompson et al., 2013). Identification and counting of the zooplankton specimen were using an inverted microscope at 400× magnification following the species keys (Chen, 1974; Chiang and Du, 1979; Yeatman, 1959). We calculated zooplankton functional group biomass by using dry weight (mg) obtained from length-weight relationship of the filtered water volume (L) (Zuo et al., 2003).

### **Zooplankton functional groups classification**

Zooplankton species in Tuanjie Reservoir were classified into seven functional groups mainly according to body size/length and feeding mode (Benedetti et al., 2018; Ma et al., 2019; Mwagana et al., 2018). The seven functional groups are: group PF (protozoa filter feeders), group RF (rotifera filter feeders), group SCF (small copepods and cladoceran filter feeders, body size < 0.7 mm), group MCC (middle copepods and cladoceran carnivore, body size 0.7~1.5 mm), group MCF (middle copepods and cladoceran filter feeders, body size 0.7~1.5 mm), group LCC (large copepods and cladoceran carnivore, body size > 1.5 mm) and group LCF (large copepod filter feeders, body size > 1.5 mm).

## Data analysis

Statistical analyses were carried out using the SPSS 19.0 software. Variation and correlation of environmental factors and biomass of zooplankton functional groups in different seasons were analyzed by using One-way ANOVA and Tukey's honesty significant difference (HSD) tests, and Spearman analysis, respectively. Relationship between zooplankton functional group biomass and environmental factors was done using CANOCO 4.5 software (Microcomputer Power, New York, USA). Before analysis, the biological and abiotic data were transformed by  $\log_{10}(x+1)$  to satisfy the normal distribution. We found that the detrended corresponding analysis (DCA) of the largest gradient length of the four axes was 1.889 ( $< 3$ ). Therefore, linear ordination method of the redundancy analysis (RDA) was used to reveal the relationship. Monte Carlo simulations with 499 permutations were used to test the significance of the environmental factors in explaining the biomass of zooplankton functional groups data in the RDA.

## Results

### Seasonal variation of environmental factors

The mean values of environmental factors presented among seasons are shown in Table 2. Most of the factors varied significantly (One-way ANOVA and Tukey HSD test). The mean values of D, COND, TN and  $\text{NO}_3^-$ -N were not statistically significant difference among the seasons ( $P > 0.05$ ). While the mean values of DO, pH,  $\text{NH}_4^+$ -N,  $\text{COD}_{\text{Mn}}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ , T, TP, N:P and SD varied significantly with seasons ( $P < 0.05$ ). The minimum mean values of DO, pH, N:P and  $\text{Fe}^{3+}$  were observed in summer, while their maximum values were observed in autumn except for  $\text{COD}_{\text{Mn}}$ . On the contrary, the maximum mean values of T and  $\text{NH}_4^+$ -N were observed in summer and minimum presented in autumn.

**Table 2.** The seasonal variations (mean  $\pm$  SE,  $n = 32$ ) of environmental factors: water transparency (SD), depth (D), conductivity (COND), dissolved oxygen (DO), pH, water temperature (T), total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen ( $\text{NH}_4^+$ -N), nitrate nitrogen ( $\text{NO}_3^-$ -N), chemical oxygen demand ( $\text{COD}_{\text{Mn}}$ ) and dissolved iron ( $\text{Fe}^{3+}$ ) and dissolved copper ( $\text{Cu}^{2+}$ ), P-value from One-way ANOVA and a, b, c mean differences between the seasons were tested by post-hoc test using Tukey HSD ANOVA

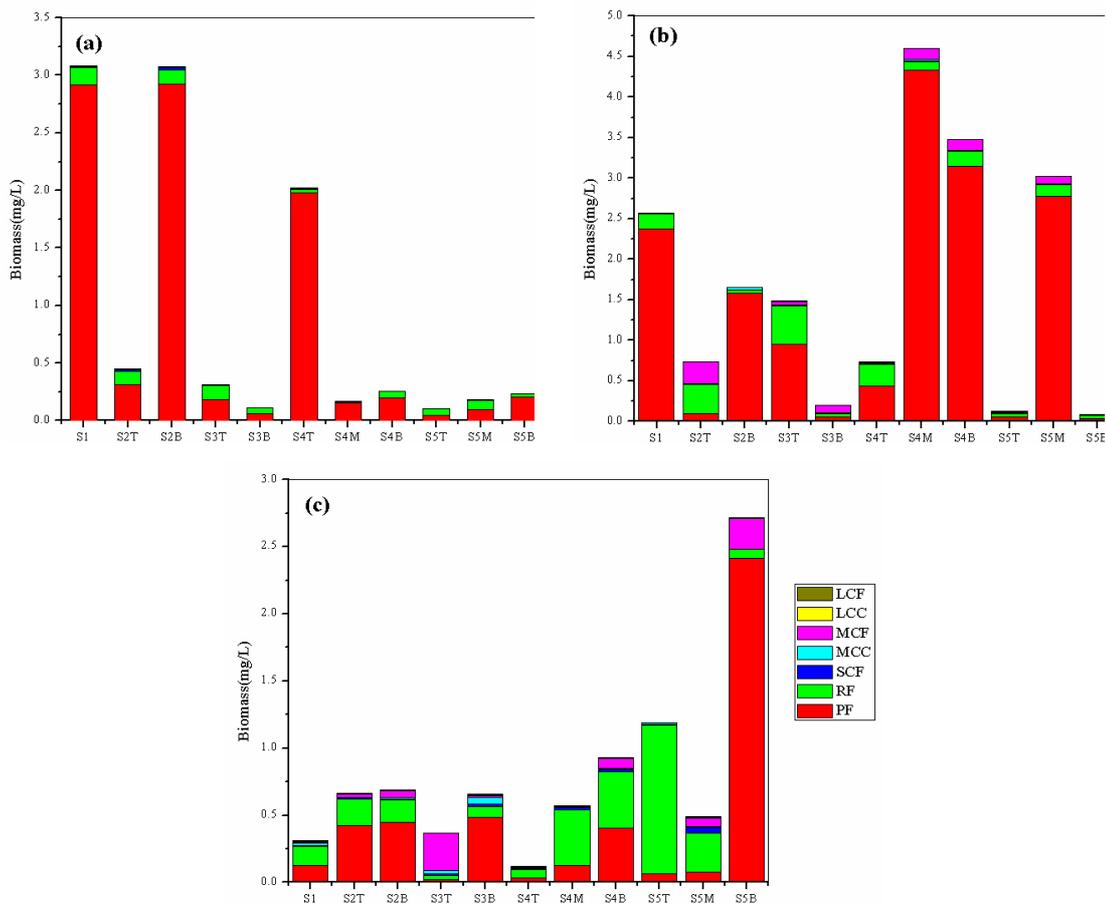
	Spring	Summer	Autumn	P-value
SD (m)	0.76 $\pm$ 0.12 <sup>a</sup>	1.01 $\pm$ 0.16 <sup>b</sup>	1.11 $\pm$ 0.12 <sup>b</sup>	0.037
D (m)	8.48 $\pm$ 3.26 <sup>a</sup>	10.93 $\pm$ 3.99 <sup>a</sup>	10.57 $\pm$ 3.70 <sup>a</sup>	0.716
COND (ms/cm)	0.11 $\pm$ 0.04 <sup>a</sup>	0.15 $\pm$ 0.05 <sup>a</sup>	0.12 $\pm$ 0.03 <sup>a</sup>	0.603
DO (mg/L)	7.66 $\pm$ 0.24 <sup>a</sup>	7.39 $\pm$ 0.43 <sup>a</sup>	8.91 $\pm$ 0.20 <sup>b</sup>	0.000
pH	7.44 $\pm$ 0.10 <sup>a</sup>	7.17 $\pm$ 0.15 <sup>a</sup>	7.80 $\pm$ 0.10 <sup>b</sup>	0.000
T ( $^{\circ}$ C)	17.3 $\pm$ 0.90 <sup>a</sup>	22.94 $\pm$ 0.45 <sup>b</sup>	10.23 $\pm$ 0.74 <sup>c</sup>	0.002
TN (mg/L)	0.99 $\pm$ 0.20 <sup>a</sup>	0.87 $\pm$ 0.08 <sup>a</sup>	0.91 $\pm$ 0.09 <sup>a</sup>	0.277
TP (mg/L)	0.74 $\pm$ 0.16 <sup>a</sup>	0.52 $\pm$ 0.05 <sup>ab</sup>	0.36 $\pm$ 0.06 <sup>b</sup>	0.002
N:P	1.97 $\pm$ 0.67 <sup>a</sup>	1.67 $\pm$ 0.04 <sup>a</sup>	2.92 $\pm$ 0.56 <sup>b</sup>	0.005
$\text{NH}_4^+$ -N (mg/L)	0.13 $\pm$ 0.01 <sup>a</sup>	0.23 $\pm$ 0.04 <sup>b</sup>	0.12 $\pm$ 0.02 <sup>a</sup>	0.000
$\text{NO}_3^-$ -N (mg/L)	0.36 $\pm$ 0.06 <sup>a</sup>	0.28 $\pm$ 0.07 <sup>a</sup>	0.20 $\pm$ 0.05 <sup>a</sup>	0.069
$\text{COD}_{\text{Mn}}$ (mg/L)	4.44 $\pm$ 0.07 <sup>a</sup>	3.78 $\pm$ 0.12 <sup>b</sup>	4.24 $\pm$ 0.04 <sup>a</sup>	0.000
$\text{Fe}^{3+}$ (mg/L)	0.32 $\pm$ 0.04 <sup>a</sup>	0.21 $\pm$ 0.07 <sup>b</sup>	0.42 $\pm$ 0.01 <sup>a</sup>	0.000
$\text{Cu}^{2+}$ (mg/L)	0.21 $\pm$ 0.04 <sup>a</sup>	0.16 $\pm$ 0.03 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>b</sup>	0.000

### Variation of zooplankton functional groups in Tuanjie Reservoir

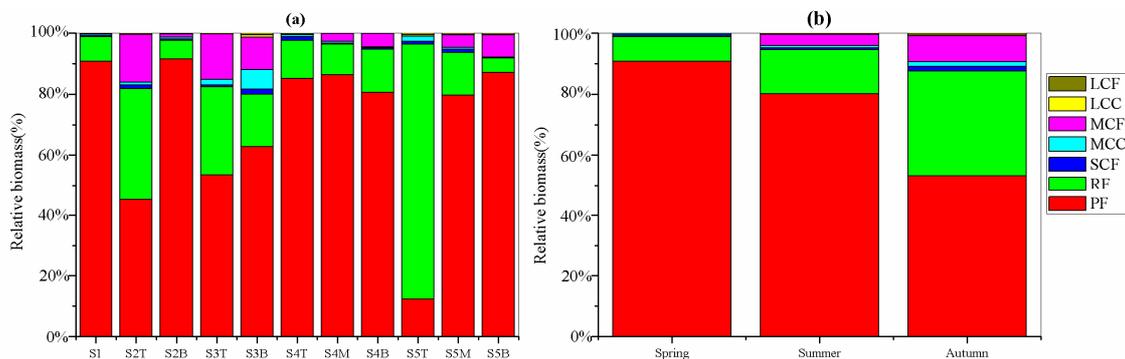
A total of 30 zooplankton species belonging to 25 genera and four taxonomic groups including protozoa (33.33%), rotifera (36.67%), cladoceran (16.67%) and copepods (13.33%) were identified in the Tuanjie Reservoir (Table 3). The biomass of zooplankton functional groups showed seasonal and spatial variation (Figs. 2 and 3). Summer recorded the highest number of zooplankton species (20) followed by spring (19) and autumn (17). In summer, the dominant zooplankton functional group was PF mainly comprised by *Paramecium* (43.81%) and *Didinium nasutum* (37.55%), and RF which was presented by *Keratella cochlearis* (3.27%) and *Polyarthra trigla* (2.03%). While in spring, the dominant zooplankton functional group PF was presented only by *Paramecium* (74%). In autumn, the dominant zooplankton functional group PF was contributed by *Paramecium* (44.82%) and that of group RF was *Keratella cochlearis* (26.89%).

**Table 3.** Zooplankton functional groups and biomass (%) in Tuanjie Reservoir, PF (protozoa filter feeders), RF (rotifera filter feeders), SCF (small copepods and cladoceran filter feeders), MCC (middle copepods and cladoceran carnivore), MCF (middle copepods and cladoceran filter feeders), LCC (large copepods and cladoceran carnivore), LCF (large copepod filter feeders)

Taxonomic group	Species	Functional groups	Spring	Summer	Autumn
Protozoa	<i>Lagynophrya conifera</i>	PF	2.43	-	-
	<i>Strombidium viride</i>	PF	2.97	-	2.79
	<i>Strobilidium velox</i>	PF	1.35	1.88	2.69
	<i>Strobilidium gyrans</i>	PF	0.72	-	-
	<i>Tetrahymena priformis</i>	PF	3.67	0.69	1.61
	<i>Vorticella campanula</i>	PF	0.46	0.88	1.26
	<i>Paramecium sp.</i>	PF	74.00	43.81	44.82
	<i>Didinium balbianii nanum</i>	PF	4.49	-	-
	<i>Didinium nasutum</i>	PF	-	37.55	-
	<i>Diffugia avellana</i>	PF	0.60	-	-
Rotifera	<i>Polyarthra trigla</i>	RF	2.47	2.03	4.55
	<i>Filinia longiseta</i>	RF	0.50	-	-
	<i>Keratella cochlearis</i>	RF	-	3.27	26.89
	<i>Keratella quadrata</i>	RF	2.34	-	-
	<i>Trichocerca Lamarck</i>	RF	1.05	1.20	2.90
	<i>Trichocerca porcellus</i>	RF	-	0.21	-
	<i>Lecane luna</i>	RF	1.78	-	-
	<i>Lecane tenuiseta</i> Harring	RF	-	1.77	-
	<i>Pompholyx sulcata</i> Hudson	RF	-	0.55	-
	<i>Colurella uncinata</i>	RF	-	0.08	-
	<i>Brachionus diversicornis</i>	RF	-	0.96	-
Cladoceran	<i>Bosmina longirostris</i>	SCF	0.43	-	0.05
	<i>Diaphanosoma leuchtenbergianum</i>	MCF	-	-	1.21
	<i>Leptodora kindti</i>	LCC	-	0.05	0.44
	<i>Daphnia cristata</i> Sars	SCF	-	0.13	1.09
	<i>Daphnia magna</i>	MCF	-	4.09	7.24
Copepoda	<i>Cyclops strenuus</i>	MCC	0.26	0.35	0.60
	<i>Calanioda.sp</i>	LCF	0.12	0.16	0.45
	<i>Thermocyclops dybowskii</i>	MCC	0.19	0.25	1.00
	<i>Microcyclops javanus</i>	SCF	0.18	0.09	0.41



**Figure 2.** Zooplankton functional group biomass of spring (a), summer (b) and autumn (c), S2T (Sampling site 2 top water layer), S4M (Sampling site 4 middle water layer), S2B (Sampling site 2 bottom water layer), PF (protozoa filter feeders), RF (rotifera filter feeders), SCF (small copepods and cladoceran filter feeders), MCC (middle copepods and cladoceran carnivore), MCF (middle copepods and cladoceran filter feeders), LCC (large copepods and cladoceran carnivore), LCF (large copepod filter feeders)



**Figure 3.** Relative biomass of zooplankton functional groups among sampling sites (a) and seasons (b), S2T (Sampling site 2 top water layer), S4M (Sampling site 4 middle water layer), S2B (Sampling site 2 bottom water layer), PF (protozoa filter feeders), RF (rotifera filter feeders), SCF (small copepods and cladoceran filter feeders), MCC (middle copepods and cladoceran carnivore), MCF (middle copepods and cladoceran filter feeders), LCC (large copepods and cladoceran carnivore), LCF (large copepod filter feeders)

### Correlation of zooplankton functional groups and environmental factors

The Spearman correlation analysis of zooplankton functional group biomass and environmental factors were listed in *Tables 4* and *5*, respectively. Group PF influenced RF, and group LCC influenced SCF and LCF. The feeding relationship of zooplankton functional groups has been proved by the results. Meanwhile, most environmental factors were correlated with zooplankton functional groups. Like group LCC, was significantly influenced by SD ( $r = 0.374$ ), T ( $r = 0.347$ ),  $\text{NH}_4^+\text{-N}$  ( $r = 0.494$ ) and  $\text{COD}_{\text{Mn}}$  ( $r = -0.541$ ). Groups MCF was negatively correlated with  $\text{NO}_3^-\text{-N}$  ( $r = -0.419$ ) and  $\text{COD}_{\text{Mn}}$  ( $r = -0.541$ ). Other side, COND was positively correlated with group PF ( $r = 0.407$ ), RF ( $r = 0.392$ ) and SCF ( $r = 0.477$ ). However, the DO and  $\text{NH}_4^+\text{-N}$  was only significant positively correlated with group MCC ( $r = 0.386$ ) and LCF ( $r = 0.410$ ) respectively.

**Table 4.** Spearman correlation of zooplankton functional group biomass. PF (protozoa filter feeders), RF (rotifera filter feeders), SCF (small copepods and cladoceran filter feeders), MCC (middle copepods and cladoceran carnivore), MCF (middle copepods and cladoceran filter feeders), LCC (large copepods and cladoceran carnivore), LCF (large copepod filter feeders)

	PF	RF	SCF	MCC	MCF	LCC	LCF
PF	1	.253	.112	.010	.080	-.044	.071
RF	.253	1	.313	.197	.344*	.296	.035
SCF	.112	.313	1	.178	.065	.421*	.225
MCC	.010	.197	.178	1	.273	.261	-.100
MCF	.080	.344*	.065	.273	1	.340	.011
LCC	-.044	.296	.421*	.261	.340	1	.356*
LCF	.071	.035	.225	-.100	.011	.356*	1

\*\* $p < 0.01$ , \* $p < 0.05$

**Table 5.** Spearman correlation between zooplankton functional group biomass and environmental factors, water transparency (SD), depth (D), conductivity (COND), dissolved oxygen (DO), pH, water temperature (T), total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ), chemical oxygen demand ( $\text{COD}_{\text{Mn}}$ ) and dissolved iron ( $\text{Fe}^{3+}$ ) and dissolved copper ( $\text{Cu}^{2+}$ ). PF (protozoa filter feeders), RF (rotifera filter feeders), SCF (small copepods and cladoceran filter feeders), MCC (middle copepods and cladoceran carnivore), MCF (middle copepods and cladoceran filter feeders), LCC (large copepods and cladoceran carnivore), LCF (large copepod filter feeders)

	SD	D	COND	DO	pH	T	TN	TP	N:P	$\text{NH}_4^+\text{-N}$	$\text{NO}_3^-\text{-N}$	$\text{COD}_{\text{Mn}}$	$\text{Fe}^{3+}$	$\text{Cu}^{2+}$
PF	-.075	.121	.407*	.152	.302	-.178	.040	-.074	.041	-.272	-.157	.220	.164	-.050
RF	.099	-.154	.392*	-.126	.095	.102	.100	-.109	-.050	.196	-.119	-.306	-.021	-.137
SCF	-.004	-.179	.477**	-.247	-.201	.262	-.061	-.068	-.086	.149	.034	-.147	-.286	.140
MCC	-.011	-.332	.007	.386*	.189	-.092	.083	-.017	.038	-.125	-.149	-.294	.257	-.291
MCF	.339	.174	.182	-.015	.049	.107	-.144	.032	-.203	.067	-.419*	-.541**	-.133	-.262
LCC	.374*	.160	.148	-.312	-.193	.347*	-.171	.031	-.187	.494**	-.160	-.541**	-.324	-.013
LCF	.209	-.071	-.033	-.128	-.118	-.052	-.054	-.153	.154	.410*	.179	.089	-.122	-.048

\*\* $p < 0.01$ , \* $p < 0.05$

### ***RDA analysis between zooplankton functional groups and environmental factors***

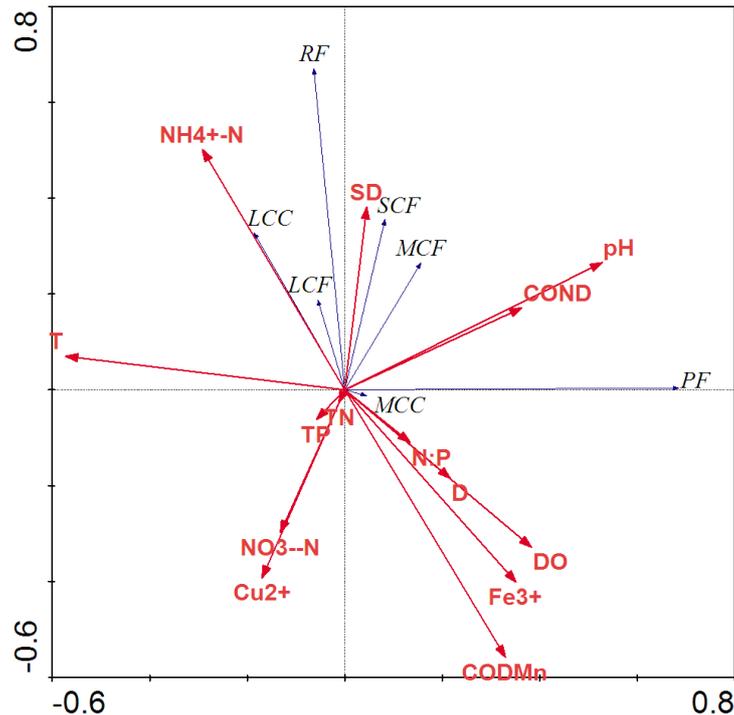
The results of Monte Carlo test showed that the first canonical axis and all canonical axes were significant ( $F = 13.521$ ,  $p = 0.002$ ;  $F = 1.130$ ,  $p = 0.002$ ; 499 random permutations). The eigenvalues of the four axes were 0.429, 0.033, 0.015, 0.004, respectively (Table 6). The functional groups-environment correlations for Axis 1 and Axis 2 were 0.686 and 0.691, respectively. The first two axes account for 46.2% of functional groups-environment variables relation (axis 1: 42.9%, axis 2: 3.3%) and 98.8% of the functional groups variables (axis 1: 91.7%, axis 2: 7.1%). Axis 1 was mainly positive correlated with pH ( $r = 0.728$ ) and DO ( $r = 0.683$ ) and negatively correlated with T ( $r = -0.672$ ). Axis 2 was positively correlated with  $\text{NH}_4^+\text{-N}$  ( $r = 0.602$ ), SD ( $r = 0.481$ ) and negatively correlated with  $\text{COD}_{\text{Mn}}$  ( $r = -0.658$ ),  $\text{Fe}^{3+}$  ( $r = -0.501$ ). Groups PF was positively related with pH, COND and  $\text{COD}_{\text{Mn}}$ , and negatively related with T,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and  $\text{Cu}^{2+}$ . Group RF, SCF, MCF, LCF and LCC were positively related with  $\text{NH}_4^+\text{-N}$  and SD, and negatively related with  $\text{COD}_{\text{Mn}}$ ,  $\text{Fe}^{3+}$ , DO,  $\text{Cu}^{2+}$  and  $\text{NO}_3^-\text{-N}$ . Nevertheless, the group MCC was not related with most of environmental factors (Fig. 4).

**Table 6.** RDA results of zooplankton functional group biomass

Axes	Eigenvalues	Species-environment correlations	Cumulative percentage variance of species data	Cumulative percentage variance of species-environment relation
1	0.429	0.686	42.9	91.7
2	0.033	0.691	46.2	98.8
3	0.015	0.563	46.8	100
4	0.004	0.468	46.8	100

### **Discussion**

Zooplankton, as an important part of the food chain in the aquatic ecosystem, is mainly composed of free-living protozoa, rotifera, cladocera and copepod. Zooplankton is very sensitive to environmental changes and hence considered as good indicator. Many previous studies focused on zooplankton community structure in relation to environmental factors (Bachiller et al., 2018; Coz et al., 2018; Jeppesen et al., 2011; Li et al., 2014; Matsuzaki et al., 2018; Neumann-Leitão et al., 2018). Recently, using functional groups to reveal the reaction of environmental factors are considered as a new method (Ma et al., 2019; Mwagona et al., 2018; Shi et al., 2015; Sun et al., 2010). Functional groups are species with similar morphological and physiological traits (Reynolds et al., 2002). In marine ecosystems, six functional groups have been classified according to zooplankton species individual size and feeding habits from the yellow sea (Sun et al., 2010). In Western English channel, the researchers analyzed the food web using three zooplankton functional groups (Araújo et al., 2006). On the other hand, in freshwater ecosystems, six and eight zooplankton functional groups have been confirmed in Small Xingkai Wetland Lake and Xiquanyan Reservoir in Heilongjiang Province, Northeast of China, respectively (Ma et al., 2019; Mwagona et al., 2018). In our study, we identified 30 species belonging to seven zooplankton functional groups in Tuanjie Reservoir, including group PF, RF, SCF, MCC, MCF, LCC and LCF.



**Figure 4.** RDA biplot of zooplankton functional groups and environmental factors, water transparency (SD), depth (D), conductivity (COND), dissolved oxygen (DO), pH, water temperature (T), total nitrogen (TN), total phosphorus (TP), N:P ratio (N:P), ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrate nitrogen ( $\text{NO}_3^-\text{-N}$ ), chemical oxygen demand ( $\text{COD}_{\text{Mn}}$ ) and dissolved iron ( $\text{Fe}^{3+}$ ) and dissolved copper ( $\text{Cu}^{2+}$ ). PF (protozoa filter feeders), RF (rotifera filter feeders), SCF (small copepods and cladoceran filter feeders), MCC (middle copepods and cladoceran carnivore), MCF (middle copepods and cladoceran filter feeders), LCC (large copepods and cladoceran carnivore), LCF (large copepod filter feeders)

### Composition and variations of zooplankton functional groups in Tuanjie Reservoir

Seasonal and spatial variations of zooplankton biomass were demonstrated in aquatic ecosystems by Dube et al. (2017). In this study, the biomass of zooplankton functional groups varied significantly differences among seasons and sites. The mean values of biomass reported in spring (0.91 mg/L), summer (1.70 mg/L) and autumn (0.79 mg/L) were higher than those recorded by Mwagona et al. (2018) in the oligotrophic Xiquanyan Reservoir during spring (54.25  $\mu\text{g/L}$ ), summer (245.81  $\mu\text{g/L}$ ) and autumn (196.54  $\mu\text{g/L}$ ) and also higher than those found by Ma et al. (2019) in Small Xingkai Lake during spring (0.28 mg/L) and autumn (0.72 mg/L) in the same province. Mwagona et al. (2018) found that low nutrients level, which unable to meet the demand of the growth of zooplankton. While, Ma et al. (2019) noted that nutrient-rich level could promote the zooplankton breeding, and high turbid with low transparency also may contribute to zooplankton avoid predators.

As shown in Figure 2, during spring and summer seasons, zooplankton biomass was both dominated by protozoa filter feeders (group PF) accounting for 90.68% and 84.8% of total biomass, respectively. In spring, group PF mainly composed of *Paramecium sp.* (74%), while in summer mainly composed of *Paramecium sp.* (43.81%) and *Didinium nasutum* (37.55%). Group PF (protozoa filter feeders) are filter feeding on bacteria, algae and organic detritus and found in all sampling sites and water layer. We found

that the biomass of group PF in summer was significantly higher than that in spring probably due to higher temperature and nutrients contributed to algae growth of their food resources (Duggan et al., 2001; Špoljar et al., 2010), and higher water transparency help them locate prey more clearly and predation activities more easily (Ma et al., 2019). Besides, *Didinium nasutum* could use their flagella enhance swimming ability in favor of avoiding predators and harvesting suitable light and food for growth, therefore became an dominant species (Bovo-Scomparin and Train, 2008). In autumn, zooplankton biomass was dominated by protozoa filter feeders (group PF) and rotifera filter feeders (group RF) accounted for 53.17% and 34.34% of total biomass, respectively. Group PF mainly composed of *Paramecium sp.* (44.82%) and group RF mainly composed of *Keratella cochlearis* (26.89%). This could be due to higher temperature in summer was suitable for rotifera breeding in large numbers (Galkovskaja, 1987), so they formed a dominant species in autumn. Another reason could be some environmental factors such as nutrients and turbidity can influence rotifera community structure in autumn (Armengol et al., 1998; Ma et al., 2019), and finally, it leads to the change of the structure and composition of zooplankton community. Moreover, other zooplankton functional groups preyed by their predators such as *Hypophthalmichthys molitrix* and *Aristichthys nobilis*, and some competitive interactions between those functional groups lead to relative low biomass among sampling sites and seasons in the reservoir during study (Fig. 3a and b).

#### ***Driving factors of zooplankton functional groups in Tuanjie Reservoir***

There are so many driving factors influence the zooplankton community structure in aquatic ecosystems (Li et al., 2014; Ma et al., 2019; Mwagana et al., 2018; Shi et al., 2015; Sun et al., 2010). Among zooplankton functional groups, group MCF was positively correlated with group RF, and group LCC were both positively correlated with group SCF and LCF, which could due to intraspecific competition (Table 4). However, between environment and zooplankton, water depth did not affect the distribution of zooplankton functional groups (Table 5). Similarly, there was seemingly no significant variation in the abundance patterns of the dominant zooplankton taxa in response to changing pH value (Morgan, 1985a). The Spearman correlation results showed that pH was not related with the biomass of zooplankton functional groups in Tuanjie Reservoir (Table 5). The fluctuation of pH value in the freshwater ecosystem was primarily the result of enhanced primary productivity (Morgan, 1985b). Further studies also proved that the density of zooplankton individuals is influenced by the primary producers phytoplankton biomass (Trevisan and Forsberg, 2007).

On the other hand, this present study showing that nitrogen and phosphorus can usually be considered the main limiting nutrient (Fasham et al., 1990; Shumka et al., 2018). While in our study, both Spearman correlation and RDA results showing that the zooplankton functional groups were not affected by TN, TP and N:P ratio in Tuanjie Reservoir (Table 5; Fig. 4). That could be due to trophic level weakened their effect on plankton community in the reservoir, because an increase in trophic state can caused an increase in the total numbers of crustaceans (Ejsmont-Karabin and Karabin, 2013). The Spearman correlation and RDA results both showed that  $\text{NH}_4^+\text{-N}$  was positively influence group LCC and LCF, and  $\text{NO}_3^-\text{-N}$  was negatively influence group MCF (Table 5; Fig. 4).

Metallic element concentrations were strongly correlated with plankton structure and biomass composition (Long et al., 2018). Such as iron (Fe) considered as important

nutrient affecting the growth of phytoplankton (Alderkamp et al., 2015). Some phytoplankton species can take up Fe through their specialized transport mechanisms (Morel et al., 2008). Some researchers also found that zooplankton could directly obtain enough Fe to maintain growth (Baines et al., 2016). Similarly, copper (Cu) plays an important role in the physiology of natural plankton communities (Semeniuk et al., 2009). In Tuanjie Reservoir, there was no relationship among metal ion ( $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ) with the biomass of zooplankton functional groups, which results consistent with RDA (Table 5, Fig. 4). We suspect that could be the zooplankton functional groups affected by the top-down and bottom-up control in the food webs (Carvalho, 1994; Prowe et al., 2012; Sinistro, 2010; Stephen, 2010; Sun et al., 2013). This proof confirmed a complex interaction between Fe and Cu physiology in plankton communities (Semeniuk et al., 2016). Besides, the primary correlated factors included chlorophyll a levels, cyanobacterial toxin levels, and temperature, suggesting that both phytoplankton biomass and composition play important roles in zooplankton dynamics (Srifá, 2010).

In addition, turbulence and turbidity of water column intensified the competition among zooplankton species (Zhou et al., 2018). These environmental factors should be considered as the new influencing variables in Tuanjie Reservoir for further studies in the future.

## Conclusion

In this study, a total of 30 zooplankton species belonging to protozoa, rotifera, cladoceran and copepod were identified and classified into seven functional groups including PF, RF, SCF, MCC, MCF, LCC and LCF. Both environmental factors and zooplankton functional groups biomass in Tuanjie Reservoir varied seasonally and spatially. SD, DO, pH, N:P and  $\text{Fe}^{3+}$  were significant higher in autumn, while TP and T was higher in spring and summer. Zooplankton functional group biomass was higher in summer (0.62 mg/L), followed by spring (0.33 mg/L) and autumn (0.29 mg/L). In spring group PF was the dominant functional group, while in summer and autumn group PF and RF were both the dominant functional group. SD, COND, DO,  $\text{NH}_4^+\text{-N}$ ,  $\text{NO}_3^-\text{-N}$  and  $\text{COD}_{\text{Mn}}$  considered as the major factors influence zooplankton functional groups in Tuanjie Reservoir. Group PF was positively correlated with pH and COND while negatively correlated with T. Groups RF, SCF and LCC were positively influenced by  $\text{NH}_4^+\text{-N}$  and SD, while negatively influenced by  $\text{COD}_{\text{Mn}}$ ,  $\text{Fe}^{3+}$ , DO,  $\text{Cu}^{2+}$  and  $\text{NO}_3^-\text{-N}$ .

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