

# RELATIONSHIP BETWEEN ENVIRONMENTAL FACTORS AND METAZOOPLANKTON COMMUNITY STRUCTURE FROM ZHALONG NATIONAL NATURE RESERVE IN HEILONGJIANG PROVINCE, NORTHEASTERN CHINA

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**Abstract.** In the Zhalong National Nature Reserve in Heilongjiang Province of China, 24 sampling sites were set up in spring (May), summer (August) and autumn (October) of 2019. According to the hydrological conditions and sampling feasibility of the study area, metazooplankton, environmental factors and the correlation between metazooplankton functional groups and environmental factors were analysed and discussed. Results show that, a total of 52 species of metazooplankton were identified, including 36 species of rotifers belonging to 17 genera, 8 species of Cladocera and 2 orders of copepods. The metazooplankton can be divided into 7 functional groups: Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and cladocera filter feeders (SCF), Middle copepods and cladocera filter feeders (MCF), Middle copepods and cladocera carnivore (MCC), Large copepods filter feeders (LCF) and Large copepods carnivore (LCC). The functional groups showed a seasonal succession pattern of RF/RC/SCF → RF/RC/MCC → RF/RC/LCC. Redundancy Analysis (RDA) showing that The main water environmental factors affecting the distribution of metazooplankton functional groups were water temperature (WT), biological oxygen demand (BOD<sub>5</sub>), ammonia nitrogen (NH<sub>3</sub>-N), total phosphorus (TP) and chloride (Cl<sup>-</sup>).

**Keywords:** *Zhalong, wetland, zooplankton, impact, RDA*

## Introduction

Wetland covers only 6% of the earth's surface, which provides a living environment for 20% of the known species on the earth (Andrea et al., 2020; Biervliet et al., 2020; Lettoof et al., 2020; Tsai et al., 2020). It has irreplaceable ecological functions, so it enjoys the reputation of "kidney of the earth" (Chavan and Mutnuri, 2020; Cuthbert et al., 2020). A wetland ecosystem is a unique ecosystem formed by the interaction of land and water. It is one of the three most important ecosystems on earth (Taddeo and Dronova, 2020). It is composed of wetland plants, animals, microorganisms and environmental factors. It is also an important habitat for animals and plants and one of the most diverse ecological landscapes in nature (Duek et al., 2020). Wetland is located between water and land, because of its unique transitional nature in the ecosystem, it has eight ecological functions: natural reservoir, flood regulation and storage, groundwater supplement, coastal protection and erosion control, natural air conditioner and humidifier, carbon sink and carbon source, natural sewage treatment plant and cradle of life (Mahlatini et al., 2020). Due to economic development, urban expansion, industrial and agricultural pollution and human activities, wetland areas were rapidly reduced in size, causing serious ecological damage (Jabońska et al., 2020).

Plankton is one of the main biological groups in wetland ecosystems. Because of their wide distribution and strong fecundity, they are the basis of other biological productivity in the water area (Brasil et al., 2020). Zooplankton, as the secondary producer of water ecosystem, plays an important role in the material cycle, energy flow and information transmission of the ecosystem. Its species composition, abundance, biomass and other community structure characteristics are important indicators for water environment quality evaluation (Sordino et al., 2020). As an indicator of water pollution, plankton plays an important role in water environmental biological monitoring; some plankton species play an important role in water environment biological monitoring. Because of its ability to enrich radioisotopes, the substance can be used as an indicator of water contamination by radioisotopes (Flores et al., 2019). Therefore, the study of plankton is of great significance to evaluate the water quality of wetland, protect the biodiversity of wetland and ecological restoration of polluted water area. Therefore, it may become the main food source of the future world (Kosiba et al., 2018).

Metazooplankton is an important link in the circulation of freshwater ecosystem elements (Yuzhan et al., 2018). The up-down effect of metazooplankton fully reflects the feedback and negative feedback mechanism among the components of the aquatic ecosystem, and also determines the stability and balance of other trophic levels in the aquatic ecosystem (Palijan and Balkic, 2018). In the process of material circulation and energy flow, the changes of metazooplankton will directly or indirectly affect the horizontal distribution of other aquatic organisms in the same aquatic ecosystem, and play an important role in the material regulation of aquatic ecosystem (Velip and Rivonker, 2018). Eutrophication is one of the main reasons for the change of metazooplankton community structure, and the change of nutrients has a significant impact on its vertical distribution (Zhang et al., 2020). The species composition, community structure and species abundance of metazooplankton in a certain stage can reflect the quality of aquatic ecological environment. Therefore, metazooplankton is often used as one of the indicators of water environment monitoring and evaluation (Setubal and Riccardi, 2020). It is of great significance to improve water productivity, and has a close relationship with freshwater fish culture (Wu et al., 2008).

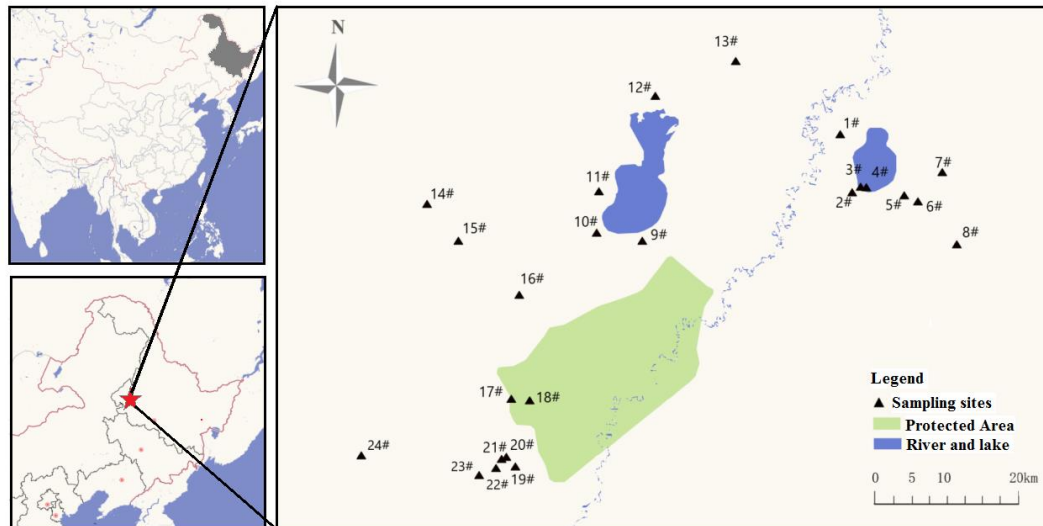
Zhalong National Nature Reserve, a wetland ecosystem nature reserve, affected by natural and human factors such as climate change, population increase, reclamation and expansion, factory sewage discharge and other natural and human factors (Wu et al., 2014; Zou et al., 2010). The precipitation in the reserve and its surrounding basins has been reduced, the water environment has deteriorated, and the water quality in the reserve has also been polluted to a certain extent (Han et al., 2007). Therefore, this study discussed the composition and seasonal variation characteristics of metazooplankton functional groups, and the interaction between them and the physical and chemical factors of water environment, combined with the physical and chemical indexes and biological indexes, the water quality of the reserve was evaluated. In order to provide basic data and scientific basis for water environment and biodiversity protection and rational development and utilization of Zhalong Nature Reserve.

## Materials and methods

### *Study area*

Zhalong National Nature Reserve (46°52'N-47°32'N, 123°47'E-124°37'E) is located in the west of Heilongjiang Province and the lower reaches of Wuyuer River in

Songnen Plain. It crosses Fuyu County, Tailai County, Tiefeng District, Ang'angxi District of Qiqihar City, and Lindian county and Duerbert Mongolian Autonomous County of Daqing City, with a total area of 2100 km<sup>2</sup> Theme of rare birds and wetland ecological types of National Nature Reserve. In 1992, Zhalong National Nature Reserve was listed in the list of important wetlands in the world (*Fig. 1*).



**Figure 1.** Map of sampling sites in Zhalong National Nature Reserve

### **Environmental factors data sampling**

We collected all samples three times from 24 sampling sites in Zhalong National Nature Reserve in May, July and October periods for spring, summer and autumn in 2019 (*Fig. 1*; *Table 1*). At each sampling site, water temperature (WT), conductivity (EC), pH, chloride (Cl<sup>-</sup>) and dissolved oxygen (DO) measured in the field using a portable multi-probe (YSI 6600, YSI Inc.). The concentration of ammonium nitrogen (NH<sub>3</sub>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>) turbidity (TUR), total nitrogen (TN), total phosphorus (TP) and biological oxygen demand (BOD<sub>5</sub>) were measured according to the standard methods for China (MEP, 2002).

**Table 1.** Sampling sites coordination in Zhalong National Nature Reserve

Sampling sites	Coordination (E, N)	Sampling sites	Coordination (E, N)
1#	124°13'29", 47°12'24"	13#	124°18'55", 47°16'47"
2#	124°13'5", 47°10'44"	14#	124°17'7", 47°18'9"
3#	124°14'15", 47°12'22"	15#	124°19'27", 47°20'47"
4#	124°7'16", 47°10'50"	16#	124°28'12", 47°18'15"
5#	124°12'9", 47°10'17"	17#	124°27'58", 47°18'17"
6#	124°13'39", 47°10'32"	18#	124°27'7", 47°19'44"
7#	124°13'17", 47°10'48"	19#	124°27'37", 47°18'8"
8#	124°12'51", 47°10'29"	20#	124°29'46", 47°18'3"
9#	124°9'59", 47°17'48"	21#	124°31'21", 47°18'41"
10#	124°11'18", 47°16'47"	22#	124°30'20", 47°17'52"
11#	124°13'49", 47°15'17"	23#	124°31'57", 47°16'41"
12#	124°17'2", 47°17'0"	24#	124°22'47", 47°21'45"

### ***Metazooplankton data sampling***

We collected three random 20 L water at each sampling sites for metazooplankton samples and filtered through plankton net (Beijing Purity Instrument Co., LTD) with 64 mm mesh. Then subsamples fixed with formaldehyde solution with 4% concentration and transported to the laboratory for identification. In the laboratory, all samples were identified and counted with a light stereomicroscope (Leica Microsystems, Germany) following the species keys (Chiang and Du, 1979; Research Group of Carcinology, 1979; Wang, 1961). The metazooplankton biomass was calculated by using dry weight obtained from length-weight relationship of the filtered water volume (Sun et al., 2019). All samples were divided into 7 functional groups (FGs) according to Sun et al. (2019): Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claochera filter feeders (SCF), Middle copepods and claochera filter feeders (MCF), Middle copepods and claochera carnivore (MCC), Large copepods filter feeders (LCF) and Large copepods carnivore (LCC) (*Table 2*).

### ***Data analysis***

Statistical analyses were carried out using the SPSS 19.0 software. Variation and correlation of environmental factors and metazooplankton FGs biomass in different sampling sites were analyzed by using One-way ANOVA. Relationship between metazooplankton FGs biomass and environmental factors was done using CANOCO 4.5 software. Before analysis, all data (except pH) were transformed by  $\lg(x + 1)$  to satisfy the normal distribution. The 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 metazooplankton FGs biomass in the RDA.

## **Results and discussion**

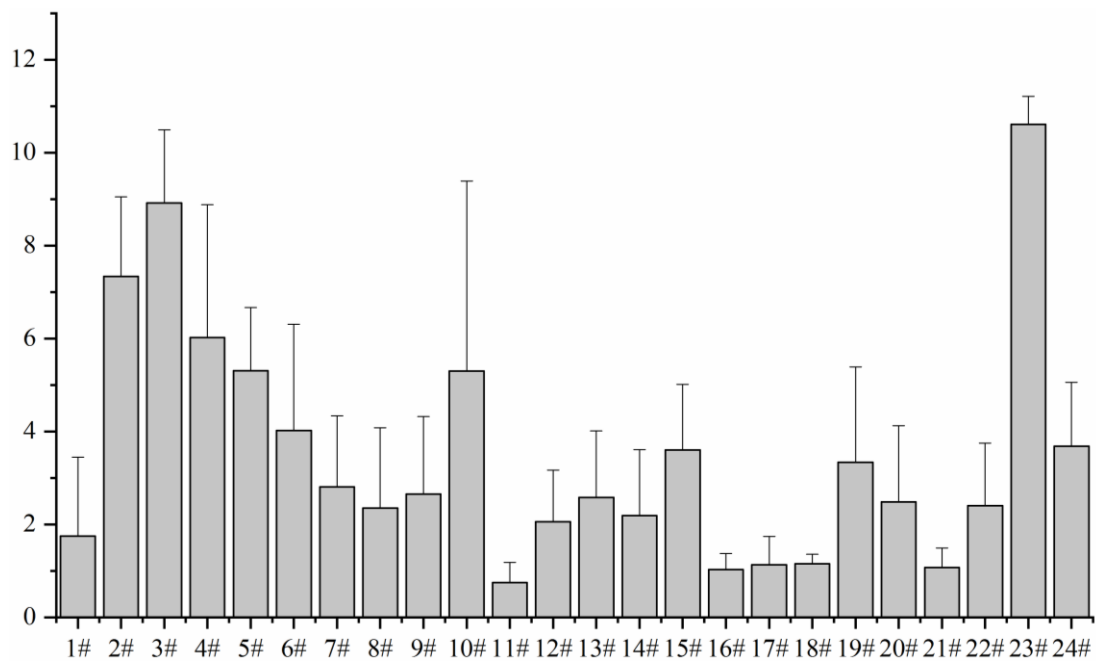
### ***Environmental factors data characteristics***

Environmental factors of Zhalong National Nature Reserve were shown in *Table 3*. WT, pH, NH<sub>3</sub>-N, NO<sub>3</sub><sup>-</sup> and TP were extremely significant differences ( $P < 0.01$ ), and Cl<sup>-</sup> showed significant difference ( $P < 0.05$ ). WT in autumn ( $8.84 \pm 2.06$ ) was significantly lower than that in spring and summer. The pH in summer ( $9.26 \pm 0.39$ ) was significantly lower than that in spring and autumn. TP in spring ( $3.61 \pm 5.28$ ) was significantly higher than that in summer and autumn. Cl<sup>-</sup> and NO<sub>3</sub><sup>-</sup> were showed an increasing trend, while NH<sub>3</sub>-N decreased significantly from spring to autumn. Besides, Cond, TUR, DO, TN and BOD<sub>5</sub> were no differences ( $P > 0.05$ ). In the aquatic ecosystem, the water environment affects the biological population and community structure. Meanwhile, it plays a decisive role in the change of water quality (Hou et al., 2019; Divya et al., 2020; Munawar et al., 2020; Ozumchelouei et al., 2020).

### ***Metazooplankton data characteristics***

Meatzooplankton is an important part of aquatic ecosystem and plays an important role in ecosystem services (Tapia and Genzano, 2019; Cabrera et al., 2019; Uzundumlu et al., 2020). The dominant species have a high degree of ecological adaptability, which often determines the environmental conditions within the

community to a large extent, so it has a great impact on the survival and growth of other species (Naumenko and Telesh, 2019; Pinheiro-Silva et al., 2020). In Zhalong National Nature Reserve, we found 9 dominant metazooplankton species, including 7 rotifers (*Asplanchna brightwelli*, *Asplanchna priodonta*, *Brachionus angularis*, *Brachionus calyciflorus*, *Keratella cochlearis*, *Trichocerca pusilla* and *Polyarthra trigla*) and 2 copepods (*Nauplii* and *Thermocyclops hyalinus*) (Table 4). In Figure 2, mean metazooplankton biomass is 1.79 mg/L, the highest biomass observed at 23# sampling site, and the lowest at 11# sampling site. The results of one-way ANOVA showed that there was significant seasonal variation of metazooplankton biomass in Zhalong Nature Reserve ( $P = 0.001$ ).



**Figure 2.** Biomass (mg/L) of metazooplankton in Zhalong National Nature Reserve. Error bars mean standard error

**Table 2.** Descriptor metazooplankton functional groups (FGs) in freshwater ecosystem

FGs	Body size (mm)	Feeding habits
Rotifers filter feeders (RF)		A filter eater that feeds on bacteria, algae, and organic matter
Rotifers carnivore (RC)		A predator that feeds on protozoa, other rotifers, and small crustaceans
Small copepods and claochera filter feeders (SCF)	<0.7	A filter eater that feeds on bacteria, algae, organic matter, and protozoa
Middle copepods and claochera filter feeders (MCF)	0.7-1.5	A filter eater that feeds on bacteria, algae, organic matter, and protozoa
Middle copepods and claochera carnivore (MCC)	0.7-1.5	A predator that feeds on rotifers, clades, diptera (chironomid larvae), and oligochaetes
Large copepods filter feeders (LCF)	>1.5	A filter eater that feeds on bacteria, algae, organic matter, and protozoa
Large copepods carnivore (LCC)	>1.5	A predator that feeds on rotifers, clades, diptera (chironomid larvae), and oligochaetes

**Table 3.** The values (mean  $\pm$  SD) of environmental factors among sampling sites. Water temperature (WT), conductivity (EC), pH, chloride (Cl<sup>-</sup>), ammonium nitrogen (NH<sub>3</sub>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>), turbidity (TUR), dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP) and biological oxygen demand (BOD<sub>5</sub>), p values from One-way ANOVA tested by post-hoc test using Tukey HSD ANOVA

	Spring	Summer	Autumn	p
WT (°C)	17.89 $\pm$ 2.21	23.68 $\pm$ 2.81	8.84 $\pm$ 2.06	0.000**
Cond (ms/m)	0.5 $\pm$ 0.51	0.65 $\pm$ 0.7	0.37 $\pm$ 0.32	0.205
pH	9.46 $\pm$ 0.38	9.26 $\pm$ 0.39	9.63 $\pm$ 0.42	0.008**
Cl <sup>-</sup> (mg/L)	38.22 $\pm$ 21.12	73.27 $\pm$ 113.52	114.24 $\pm$ 130.78	0.044*
NH <sub>3</sub> -N (mg/L)	60.1 $\pm$ 34.65	31.84 $\pm$ 17	16.37 $\pm$ 9.67	0.000**
NO <sub>3</sub> <sup>-</sup> (mg/L)	17.93 $\pm$ 9.24	50.23 $\pm$ 25.63	269.42 $\pm$ 287.42	0.000**
TUR (NTU)	8.28 $\pm$ 9.16	14.32 $\pm$ 19.06	13.55 $\pm$ 16.79	0.365
DO (mg/L)	6.9 $\pm$ 2.16	7.63 $\pm$ 2.41	9.79 $\pm$ 1.9	0.107
TN (mg/L)	2.18 $\pm$ 1.27	1.25 $\pm$ 1.15	2.48 $\pm$ 1.34	0.239
TP (mg/L)	3.61 $\pm$ 5.28	0.52 $\pm$ 0.5	0.57 $\pm$ 0.45	0.001**
BOD <sub>5</sub> (mg/L)	1.56 $\pm$ 0.24	2.29 $\pm$ 0.2	2.79 $\pm$ 0.21	0.166

\*p < 0.05

\*\*p < 0.01

We totally identified 3 taxonomic and 52 species of metazooplankton consist of 36 rotifers (69%), 8 cladocera (15.5%) and 8 copepods (15.5%). Metazooplankton were divided into predators and filter feeders according to their feeding habits (Shi et al., 2015; Mwagona et al., 2018; Ma et al., 2019; Gholizadeh et al., 2019). Combined with body size and the interaction between metazooplankton, seven functional groups in freshwater ecosystem was divided into Rotifer filter feeders (RF), Rotifer carnivore (RC), Small copepods and cladocera filter feeders (SCF), Middle copepods and cladocera filter feeders (MCF), Middle copepods and cladocera carnivore (MCC), Large copepods filter feeders (LCF) and Large copepods carnivore (LCC) (Table 5).

Zooplankton is an important part of aquatic ecosystem, and its biomass research is of great significance (Jiang et al., 2020; Romero et al., 2020; Rocha et al., 2019; Cabrera et al., 2019).

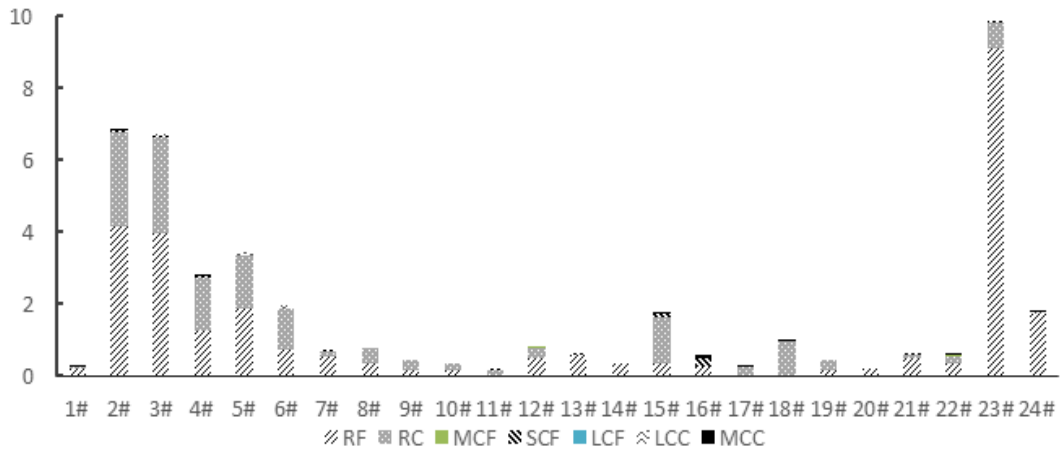
**Table 4.** Dominant species and their dominances from Zhalong National Nature Reserve

Dominant species	Dominance (Li et al., 2019)		
	Spring	Summer	Autumn
<i>Asplanchna brightwelli</i>	0.03	0.02	-
<i>Asplanchna priodonta</i>	0.07	0.07	0.1
<i>Brachionus angularis</i>	0.18	0.14	0.18
<i>Brachionus calyciflorus</i>	-	0.04	0.09
<i>Keratella cochlearis</i>	0.06	0.06	0.08
<i>Trichocerca pusilla</i>	0.04	0.03	0.04
<i>Polyarthra trigla</i>	-	0.04	0.05
<i>Nauplii</i>	0.14	0.09	-
<i>Thermocyclops hyalinus</i>	0.03	0.04	-

**Table 5.** Composition of metazooplankton functional groups in Zhalong National Nature Reserve. Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claochera filter feeders (SCF), Middle copepods and claochera filter feeders (MCF), Middle copepods and claochera carnivore (MCC), Large copepods filter feeders (LCF) and Large copepods carnivore (LCC)

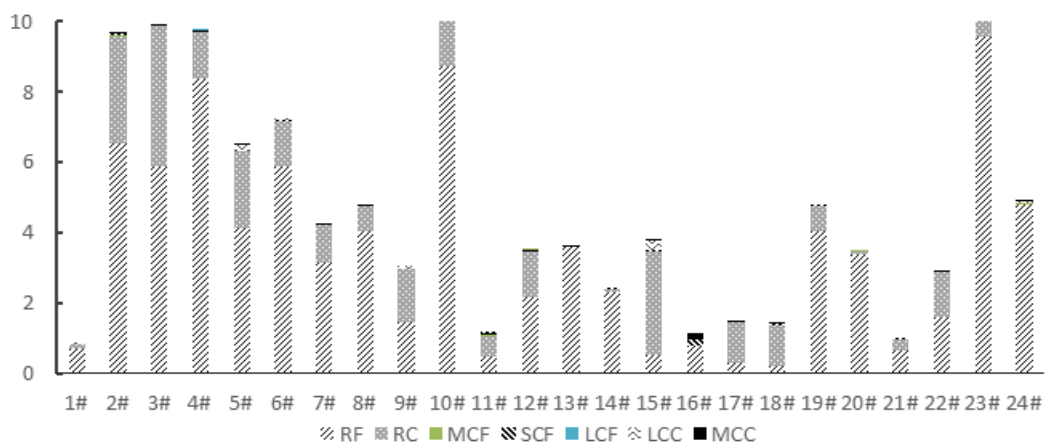
Taxonomic	Species	FGs
Rotifera	<i>Rotaria neptunia</i>	RF
	<i>Asplanchna brightwel</i>	RC
	<i>Asplanchna priodonta</i> Gosse	RC
	<i>Colurella obtusa</i>	RF
	<i>Colurella adriatica</i>	RF
	<i>Conochilus unicornis</i>	RF
	<i>Brachionus angularis</i>	RF
	<i>Brachionus calyciflorus</i> Pallas	RF
	<i>Brachionus forficula</i>	RF
	<i>Brachionus leydigi</i>	RF
	<i>Brachionus diversicornis</i>	RF
	<i>Brachionus quadridentatus</i>	RF
	<i>Keratella cochlearis</i>	RF
	<i>Keratella valga</i>	RF
	<i>Keratella quadrata</i>	RF
	<i>Notholon sqwmoda</i>	RF
	<i>Lecane buna</i>	RF
	<i>Lecane ungulata</i>	RF
	<i>Monostyla lunaris</i>	RF
	<i>Monostyla bulla</i>	RF
	<i>Monostyla closterocerca</i>	RF
	<i>Monostyla pyriformis</i>	RF
	<i>Scaridium longicaudum</i>	RF
	<i>Trichocerca bicristata</i>	RF
	<i>Trichocerca longiseta</i>	RF
	<i>Trichocerca capucina</i>	RF
	<i>Trichocerca pusilla</i>	RF
	<i>Diurella weberi</i>	RF
	<i>Chromogaster ovalis</i>	RF
	<i>Polyarthra trigla</i>	RC
	<i>Gastropus hytopus</i>	RF
<i>Filinia longiseta</i>	RF	
<i>Pompholyx sulcata</i>	RF	
<i>Pompholyx complanata</i>	RF	
<i>Collotheca pelagica</i>	RF	
<i>Euchlanis lyra</i> Hudson	RF	
Cladocera	<i>Diaphanosoma leuchtenbergianum</i>	MCF
	<i>Daphnia longispina</i>	LCF
	<i>Daphnia hyalina</i>	MCF
	<i>Moina micrura</i>	LCF
	<i>Bosmina longirostris</i>	MCF
	<i>Alona rectangular</i> Sars	SCF
	<i>Alona quadrangularia</i>	SCF
	<i>Chydorus sphaericus</i>	SCF
Copepoda	<i>Sinocalanus tenellus</i>	MCF
	<i>Cyclops vicinus</i>	MCF
	<i>Nauplii</i>	SCF
	<i>Eucyclops serrulatus</i>	MCF
	<i>Sinodiaptomus sarsi</i>	LCC
	<i>Eucyclops speratus</i>	MCF
	<i>Thermocyclops hyalinus</i>	MCC
<i>Thermocyclops dybowskii</i>	MCC	

In spring, the biomass of metazooplankton functional groups was higher in sampling sites 23#, 2# and 3#. Among the sampling sites 1#, 13#, 14# and 20#, the functional group RF was dominant; the functional group RC was dominant at 9#, 10#, 11# and 18# sampling sites; SCF and RF were dominant in 16# sampling sites; RF and RC were dominant in other sampling sites (Fig. 3).



**Figure 3.** Metazooplankton functional groups biomass (mg/L) of Zhalong National Nature Reserve in spring. Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claochera filter feeders (SCF), Middle copepods and claochera filter feeders (MCF), Middle copepods and claochera carnivore (MCC), Large copepods filter feeders (LCF), and Large copepods carnivore (LCC)

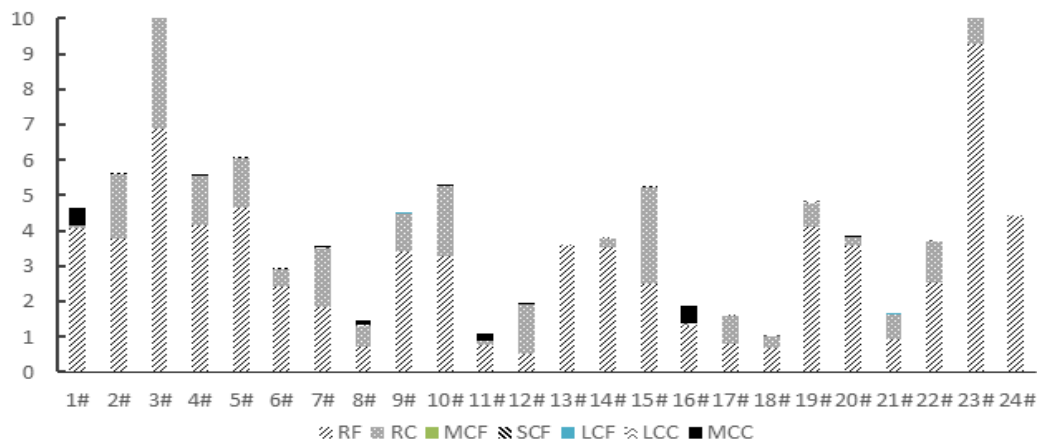
In summer, the biomass of metazooplankton functional groups was higher in sampling sites 10#, 23#, 2#, 3# and 4#. Functional groups RF were dominant in 1#, 13#, 14#, 20# and 24# sampling sites, RC was dominant in 11#, 17# and 18# sampling sites, RF, RC and LCC were dominant in 15# sampling sites, RF, SCF and MCC were dominant in 16# sampling sites, and RF and RC were dominant in other sampling sites (Fig. 4).



**Figure 4.** Metazooplankton functional groups biomass (mg/L) of Zhalong National Nature Reserve in summer. Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claochera filter feeders (SCF), Middle copepods and claochera filter feeders (MCF), Middle copepods and claochera carnivore (MCC), Large copepods filter feeders (LCF), and Large copepods carnivore (LCC)



In autumn, the biomass of metazooplankton functional groups was higher in sampling sites 3# and 23#. Among the 12# sampling sites, the functional group RC was dominant, while the functional group RF was dominant in 13#, 20#, 23# and 24# sampling sites, RF and MCC were dominant in 1# and 16# sampling sites, and RF and RC were dominant in other sampling sites (Fig. 5).

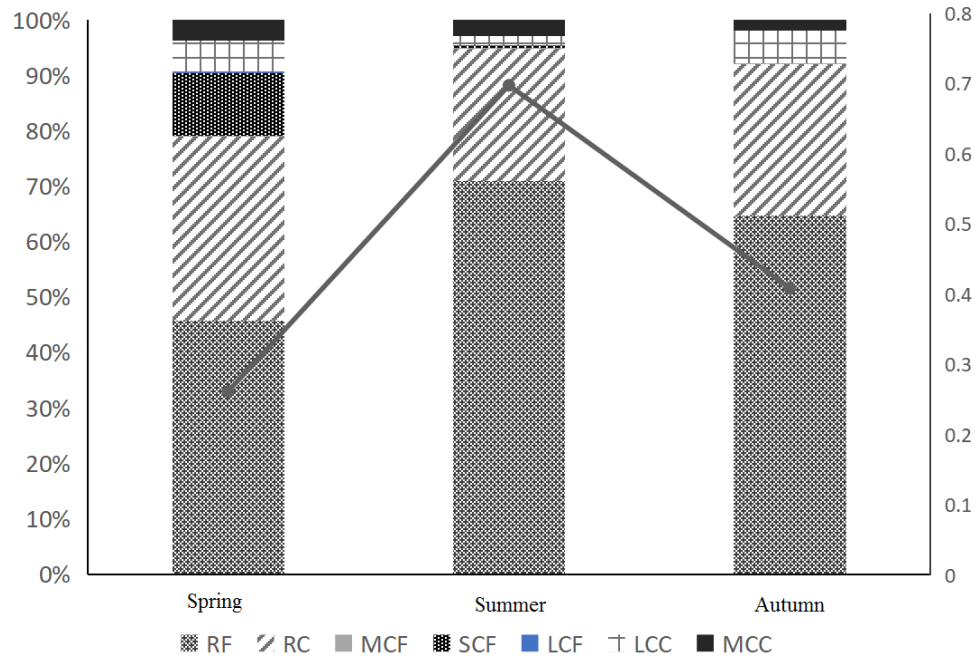


**Figure 5.** Metazooplankton functional groups biomass (mg/L) of Zhalong National Nature Reserve in autumn. Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claochera filter feeders (SCF), Middle copepods and claochera filter feeders (MCF), Middle copepods and claochera carnivore (MCC), Large copepods filter feeders (LCF), and Large copepods carnivore (LCC)

If the biomass of metazooplankton functional group is more than 10% of the total biomass, it is called the dominant functional group. As the dominant species aggregation in the survey period, the dominant functional group can best reflect the water environment of the study area (Zaghloul et al., 2020; Sharma and Sharma, 2020; Macmillan et al., 2019). In spring, RF, RC and SCF were dominant, accounting for 45.76%, 33.2% and 11.41% respectively. In summer, RF, RC and MCC were dominant, accounting for 71%, 23.78% and 2.81% respectively. In autumn, RF, RC and LCC were dominant, accounting for 64.76%, 27.35% and 5.83% respectively. The average biomass of metazooplankton functional groups in Zhalong Nature Reserve was arranged from high to low in different seasons. The results showed that summer (0.70 mg/L) > autumn (0.41 mg/L) > spring (0.26 mg/L) (Fig. 6).

### Correlation analysis between metazooplankton FGs biomass and environmental factors

Environmental conditions have a great influence on the growth of metazooplankton (Kang et al., 2008; Qing et al., 2010; Tutası and Escribano, 2019; Meng and Li, 2020). It can be seen from Table 6 that the biomass of metazooplankton functional groups has obvious correlation with water environmental factors except RC. RF was negatively correlated with WT ( $P < 0.01$ ) and TN ( $P < 0.05$ ), while positively correlated with BOD5 ( $P < 0.01$ ). MCF and LCC were both negatively correlated with WT ( $P < 0.05$ ). SCF was positively correlated with  $\text{NH}_3\text{-N}$  ( $P < 0.01$ ) and TP ( $P < 0.01$ ), while negatively correlated with  $\text{NO}_3^-$  ( $P < 0.05$ ) and DO ( $P < 0.05$ ). LCF was negatively correlated with WT ( $P < 0.05$ ) and TN ( $P < 0.05$ ). MCC was positively correlated with  $\text{Cl}^-$  ( $P < 0.01$ ).



**Figure 6.** Metazooplankton functional groups relatively biomass (%) and average biomass (mg/L) among seasons of Zhalong National Nature Reserve. Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claochera filter feeders (SCF), Middle copepods and claochera filter feeders (MCF), Middle copepods and claochera carnivore (MCC), Large copepods filter feeders (LCF), and Large copepods carnivore (LCC)

**Table 6.** Pearson correlation analysis of between metazooplankton FGs biomass and environmental factors. Metazooplankton FGs: Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claochera filter feeders (SCF), Middle copepods and claochera filter feeders (MCF), Middle copepods and claochera carnivore (MCC), Large copepods filter feeders (LCF) and Large copepods carnivore (LCC). Environmental factors: Water temperature (WT), conductivity (Cond), pH, chloride (Cl<sup>-</sup>), ammonium nitrogen (NH<sub>3</sub>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>) turbidity (TUR), dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP) and biological oxygen demand (BOD<sub>5</sub>)

	WT	Cond	Cl <sup>-</sup>	NH <sub>3</sub> -N	NO <sub>3</sub> <sup>-</sup>	NTU	DO	TN	TP	BOD <sub>5</sub>	pH
RF	<b>-.328**</b>	0.219	-0.022	-0.103	0.002	0.092	-0.202	<b>-.254*</b>	-0.114	<b>.342**</b>	-0.042
RC	-0.225	0.134	-0.019	-0.057	0.014	0.156	-0.104	-0.214	-0.084	0.181	0.03
MCF	<b>-.264*</b>	0.218	-0.193	0.025	-0.219	0.033	-0.217	-0.208	0.085	0.051	-0.03
SCF	-0.071	0.111	-0.163	<b>.334**</b>	<b>-.260*</b>	-0.017	<b>-.258*</b>	-0.019	<b>.408**</b>	-0.125	-0.085
LCF	<b>-.250*</b>	0.135	0.009	-0.028	-0.087	0.091	-0.192	<b>-.251*</b>	0.03	0.134	-0.117
LCC	<b>-.267*</b>	0.214	-0.102	0.071	-0.031	0.066	-0.194	-0.167	0.023	0.028	-0.148
MCC	0.032	-0.09	<b>.338**</b>	-0.127	0.145	0.107	0.146	0.225	-0.036	0.216	-0.003

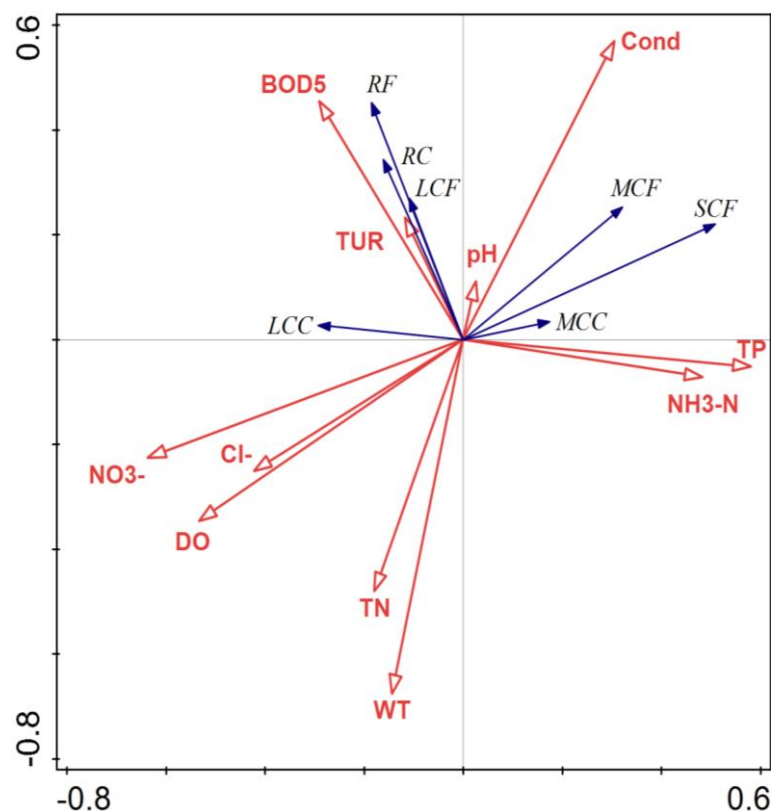
\*  $p < 0.05$

\*\*  $p < 0.01$

First of all, after detrend correspondence analysis (DCA) of metazooplankton functional group biomass data, it was found that the maximum gradient of ordination axis was 1.77, less than 3. Therefore, linear model sequencing analysis (RDA) was carried out for metazooplankton functional groups and environmental factors (Wang et

al., 2016; Sun et al., 2019; Wu et al., 2019; Saler et al., 2019). From Table 7, eigenvalues of Axis1 and Axis2 were 0.076 and 0.061, respectively. FGs-environment correlations of Axis1 and Axis2 were 0.581 and 0.511, respectively. The first two axes account for 13.7% of FGs data relation.

In aquatic ecosystem, the community structure and its changes of metazooplankton are the result of the action of many environmental factors in time series (Cryer et al., 1986; Tavernini et al., 2005; Li et al., 2013; Huo et al., 2020; Zamora-Barrios et al., 2019). Figure 7 shows the RDA sequence diagram of the metazooplankton functional groups and water environmental factors in Zhalong National Nature Reserve, where the environmental factors are represented by the red solid line with a hollow arrow, and the functional group is represented by a solid line with a solid arrow. The most relevant positive environmental factors of Axis 1 were TP (0.5763), and the negative environmental factors were DO (-0.5382) and Cl<sup>-</sup> (-0.3944). The positive environmental factors related to axis 2 were Cond (0.5728) and BOD<sub>5</sub> (0.4849), while the negative environmental factors were TN (-0.4739) and WT (-0.6397). RF, RC and LCF were positively correlated with TUR, Cond and BOD<sub>5</sub>, negatively correlated with TN and WT; MCF, SCF and MCC were positively correlated with TP, NH<sub>3</sub>-N, Cond, and NO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup> and DO. LCC was positively correlated with NO<sub>3</sub><sup>-</sup> and TUR, and negatively correlated with TP and NH<sub>3</sub>-N.



**Figure 7.** RDA biplot of metazooplankton FGs and environmental factors. Metazooplankton FGs: Rotifers filter feeders (RF), Rotifers carnivore (RC), Small copepods and claocera filter feeders (SCF), Middle copepods and claocera filter feeders (MCF), Middle copepods and claocera carnivore (MCC), Large copepods filter feeders (LCF) and Large copepods carnivore (LCC). Environmental factors: Water temperature (WT), conductivity (EC), pH, chloride (Cl<sup>-</sup>), ammonium nitrogen (NH<sub>3</sub>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>) turbidity (TUR), dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP) and biological oxygen demand (BOD<sub>5</sub>)

**Table 7.** RDA results of metazooplankton FGs biomass

Axes	1	2	3	4
Eigenvalues	0.076	0.061	0.033	0.026
FGs-environment correlations	0.581	0.511	0.436	0.381
Cumulative percentage variance of FGs data	7.6	13.7	17.0	20.0
Cumulative percentage variance of FGs-environment relation	35.9	64.3	79.8	91.6

## Conclusion

In our study, we found 52 species of metazooplankton from Zhalong National Nature Reserve, including 36 species of Rotifera, eight species of Cladocera and eight species of Copepoda. They belong to seven functional groups (RF, RC, MCF, SCF, LCF, LCC and MCC), and seasonal succession is RF/RC/SCF→RF/RC/MCC→RF/RC/LCC. Environmental factors of WT, pH, NH<sub>3</sub>-N, NO<sub>3</sub><sup>-</sup> and TP were extremely significant differences ( $P < 0.01$ ), and Cl<sup>-</sup> showed significant difference ( $P < 0.05$ ). The main environmental factors are WT, BOD<sub>5</sub>, NH<sub>3</sub>-N, TP and Cl<sup>-</sup>. We strongly suggest that the monitoring of these environmental factors should be strengthened to ensure the stability of zooplankton community structure in the future study.

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