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RESEARCH ARTICLE

Crustacean zooplankton community in relation to physicochemical factors and phytoplankton of 13 waterbodies located in the Yangtze River delta

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Abstract – The relationship between crustacean zooplankton community and environmental factors remains a hot topic in eutrophication bio-monitoring subject. Most water bodies in the Yangtze River delta are mesotrophic/eutrophic, which has attracted much attention from ecologists. Nevertheless, previous studies on crustacean zooplankton community in this region only focused on their relation to physicochemical factors excluding phytoplankton. In this study, the crustacean zooplankton abundance and environmental factors (physicochemical factors and phytoplankton abundance) were investigated in Spring, Summer, Autumn and Winter in 13 waterbodies (8 lakes and 5 reservoirs) located in the Yangtze River delta. Results showed that NO_2^-–N and TN in Spring, SD and TP in Summer had significant difference ($P < 0.05$) between 8 lakes and 5 reservoirs. That may be related to microbial communities and macrophytes. All 13 studied water bodies were dominated by cyanophyta, whose Microcystis may determine the dominance of *Bosmina fatalis*. Moreover, eutrophic level should be in relation to the significant difference of plankton between 8 lakes and 5 reservoirs. Finally, five factors (cyanophyta, SD, WT, pH and DO) were significantly correlated with crustacean zooplankton abundance. That indicated the metabolism, reproduction, development and competitors of crustacean zooplankton were affected by these five factors. This research provided basic data of the 13 water bodies and studied the relationship between zooplankton and physicochemical factors as well as phytoplankton, providing scientific basis for the monitoring of eutrophic waterbodies located in the Yangtze river delta.

Keywords: Crustacean zooplankton / physicochemical factors / phytoplankton / lakes and reservoirs / the Yangtze River delta

1 Introduction

Crustacean zooplankton including cladocerans and copepods are one of the most important organisms in aquatic ecosystem, because they occupy an intermediate position between microorganisms (microalgae, rotifers, protozoa, and bacteria) and larger organisms (*e.g.*, fish) (Sarma *et al.*, 2006; Berggren *et al.*, 2014). Meanwhile, crustacean zooplankton is sensitive to individual stressors. Therefore, the relationship between crustacean zooplankton and environmental factors have been studied intensively in water monitoring studies (Wei *et al.*, 2017; del Arco *et al.*, 2019; Nichun *et al.*, 2009). As eutrophication is a significant problem to many freshwater ecosystems around the world (Verschuren *et al.*, 2002; Paerl and Fulton, 2006; Paerl and Huisman, 2008), more and more researchers began to study the relationship between crustacean zooplankton community and environmental factors in eutrophic waterbodies. They hope to provide bioindicator references for eutrophication monitoring (Nichun *et al.*, 2009; Perbiche-Neves *et al.*, 2016; Zhang *et al.*, 2016).

The Yangtze River delta is one of the most densely populated and economically developed areas, where most waterbodies need to be monitored (Lingzhen *et al.*, 2003). Accordingly, Chinese ecologists stated to focus on the relationship between crustacean zooplankton and environmental factors in the region. Previous studies have greatly helped us to understand the crustacean zooplankton communities in the Yangtze River delta. Nevertheless, they focused more attention on the impact of physicochemical factors, while less attention was paid to phytoplankton (Wang *et al.*, 2007; Kun *et al.*, 2018). In this paper, we presented a snapshot of crustacean zooplankton communities again. Based on previous study regarding to physicochemical factors, we added the investigation of phytoplankton. By this means, we hope to obtain more details on crustacean zooplankton communities

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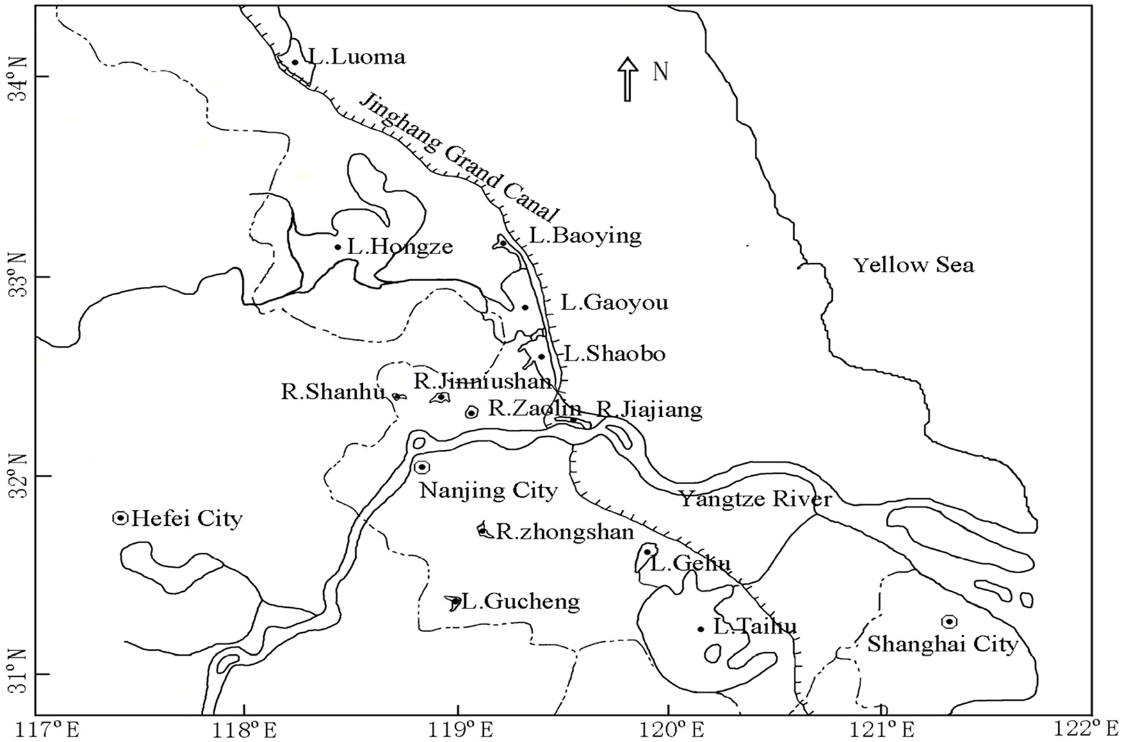

Fig. 1. Distribution of sampling sites in the Yangtze River delta region. ① L. Lm; ② L. Hz; ③ L. By; ④ L. Gy; ⑤ L. Sb; ⑥ L. Gh; ⑦ L. Th; ⑧ L. Gc; ⑨ R. Sh; ⑩ R. Jns; ⑪ R. Zl; ⑫ R. Jj; ⑬ R. Zs).

Table 1. List of 13 waterbodies (8 lakes and 5 reservoirs) and their basic information.

	L. Lm	L. Hz	L. By	L. Gy	L. Sb	L. Gh	L. Th	L. Gc	R. Sh	R. Jns	R. Zl	R. Jj	R. Zz
Area (km^2)	375	2069	140	648	10	167	2338	39	3.33	4.25	3.28	5.32	1.22
Depth (m)	5.5	5.5	4.3	5.6	4.6	2.5	2.9	7	2.3	1.6	2.1	1.3	2.5
TSI	56.73	59.22	44.07	60.34	57.25	62.46	65.7	50.32	52.97	46.81	49.63	55.88	51.21
Trophic level	Eutro	Eutro	Meso	Eutro	Eutro	Eutro	Eutro	Meso	Meso	Meso	Meso	Eutro	Meso

Note: Limits of T.S.I.: Oligotrophic < 44; Mesotrophic between 44–54; Eutrophic > 54.

and their relations to physicochemical factors as well as phytoplankton, providing scientific basis for the monitoring of eutrophic waterbodies in the Yangtze River delta.

2 Materials and methods

2.1 Study site

The Yangtze River delta is characterized by a subtropical East Asian monsoon climate with a hot and wet summer as well as a cold and dry winter. The annual average air temperature is about 15–20 °C with a minimum temperature of 0–4 °C in the winter and a maximum of 27–30 °C in the summer (Huang *et al.*, 2011). 13 mesotrophic/eutrophic waterbodies (8 lakes and 5 reservoirs), located in the Yangtze River delta region, were selected for this study (Fig. 1) including Luoma (L. Lm), Hongze (L. Hz), Baoying (L. By), Gaoyou (L. Gy), Shaobo (L. Sb), Gehu (L. Gh), Taihu (L. Th), Gucheng (L. Gc) and reservoirs are Shanhu (R. Sh), Jinniushan (R. Jns), Zaolin (R. Zl), Jiajiang (R. Jj), Zhongshan (R. Zz). These waterbodies ranged from 1.22 to 2338 km^2 in area and from 1.3 to 7.0 m in mean depth (Tab. 1).

2.2 Sampling and analysis

Sampling was carried out on four occasions: May (Spring), August (Summer), November (Autumn) 2014 and February (Winter) 2015. Sampling site was only at the center of each water bodies. Firstly, Secchi depth visibility (SD) were obtained *in situ* using Secchi disk (Beijing Yiqi Co.). Then, water sampled from the top (*i.e.* 0.5 m below the water surface) and bottom (*i.e.* 0.5 m above the bottom of the waterbody) was combined and taken for subsequent analyses (*i.e.* physico-chemical index determination and plankton analyses). Values of water temperature (WT) and dissolved oxygen (DO) were

Table 2. Mean values for the physicochemical composition, and *P* values for *t*-tests between lakes and reservoir (95% confidence interval).

	Spring			Summer			Autumn			Winter		
	Lakes	Reservoirs	*P* value	Lakes	Reservoirs	*P* value	Lakes	Reservoirs	*P* value	Lakes	Reservoirs	*P* value
WT (°C)	22.61 ± 2.58	24.16 ± 0.88	0.23	29.60 ± 1.43	27.96 ± 1.71	0.09	12.24 ± 5.58	13.74 ± 3.87	0.61	6.56 ± 2.04	5.44 ± 0.97	0.48
DO (mg/L)	8.31 ± 1.57	8.08 ± 1.17	0.79	7.21 ± 2.30	9.3 ± 2.01	0.12	9.34 ± 9.44	8.32 ± 2.40	0.34	9.44 ± 1.64	9.78 ± 1.30	0.72
pH	8.25 ± 0.64	8.32 ± 0.15	0.83	8.00 ± 0.41	8.52 ± 0.49	0.07	7.88 ± 7.97	7.95 ± 0.41	0.73	7.97 ± 0.24	7.86 ± 0.10	0.07
SD (m)	0.73 ± 0.47	1.04 ± 0.35	0.23	0.59 ± 0.25	1.41 ± 0.40	0.02	0.64 ± 0.56	0.87 ± 0.33	0.42	0.78 ± 0.56	0.88 ± 0.28	0.71
NH_4^+–N(mg/L)	0.44 ± 0.24	0.32 ± 0.16	0.34	0.41 ± 0.15	0.29 ± 0.06	0.13	0.20 ± 0.14	0.11 ± 0.04	0.25	0.21 ± 0.02	0.18 ± 0.07	0.12
NO_2^-–N (mg/L)	0.02 ± 0.01	0.01 ± 0.00	0.01	0.02 ± 0.02	0.02 ± 0.02	0.63	0.02 ± 0.02	0.02 ± 0.01	0.85	0.02 ± 0.01	0.02 ± 0.00	0.77
TN (mg/L)	0.77 ± 0.37	0.25 ± 0.08	0.01	0.57 ± 0.30	0.35 ± 0.20	0.17	0.66 ± 0.26	0.45 ± 0.14	0.13	0.87 ± 0.32	0.68 ± 0.16	0.94
TP (mg/L)	0.08 ± 0.04	0.05 ± 0.02	0.17	0.16 ± 0.05	0.04 ± 0.02	0.01	0.11 ± 0.05	0.07 ± 0.04	0.19	0.09 ± 0.06	0.05 ± 0.01	0.13
Chl *a* (μg/L)	1.81 ± 1.16	1.23 ± 0.64	0.33	3.01 ± 1.54	2.67 ± 1.21	0.69	1.98 ± 1.29	2.35 ± 1.62	0.66	1.78 ± 0.87	0.92 ± 0.65	0.14

Note: In lake cases $n = 8$ lakes × 4 seasons; in reservoir cases $n = 5$ reserviors × 4 seasons.

measured using FG4-FK (Mettler Toledo Co., Greifensee, Switzerland), pH was measured using FG2-FK (Mettler Toledo Co.). Other hydrochemical parameters were determined in the laboratory: ammonia nitrogen (NH_4^+–N, with the Nessler method), nitrite nitrogen (NO_2^-–N, with sulphanilic acid), total nitrogen (TN, after Potassium persulfate desgestion), total phosphorus (TP, after persulphate digestion) and chlorophyll a (Chl a, with acetone). These analyses were carried out following the methods described in detail by Huang (1999). Trophic status of water was estimated using the Trophic State Index as described by Carlson (1977), where the average values (TSI) of total phosphorus (TSITP), chlorophyll a (TSIChl) and water transparency (TSISD) were taken into consideration. A range of TSI indices for trophic stages were taken from Perbiche-Neves *et al.* (2016).

A 1-L phytoplankton sample was preserved in acetic Lugol's solution (1% v/v) and settled for 48 h prior to counting with Olympus CX21 microscope, and taxonomic identification was performed according to their morphology (Hu and Wei, 2006). The identification and quantification of phytoplankton were performed to the species level as far as possible. Meanwhile, quantitative samples of crustaceans were collected by 50 L composite samples as stated above through a 64 μm plankton net and preserved with 5% formalin. The sample containing crustaceans was then placed in a counting chamber and examined under an Olympus CX21 microscope according to their morphology (Chiang and Du, 1979).

2.3 Statistical analysis

An independent-samples *t*-test was used to compare the difference of physical-chemical parameters and plankton between the lakes and reservoirs using SPSS ver. 16.0 software (SPSS, Inc., Chicago, IL, USA). Then, the data (except for pH) was transformed into $\log_{10}(x+1)$ before following analysis. We performed stepwise multiple regression analysis with forward selection to identify the important environmental variables explaining total crustacean abundance. Environmental variables included physicochemical factors and phytoplankton abundance of different phylum. Variables were selected in the multiple regression only if $P < 0.05$. Thirdly, to assess the effects of environmental variables (physicochemical factors and phytoplankton abundance) on the crustacean community as a whole, redundancy analysis (RDA) within CANOCO 4.5 (Ter Braak and Smilauer, 2004) was used as the lengths of the detrended correspondence analaysis (DCA) axis were short (2.163). Dependent variables considered were the abundance of Cladocera, Calanoida and Cyclopoida. After a forward selection, the final RDA ordination only included significant independent variables ($P < 0.05$). Lastly, to gain further understanding of the linkage between crustacean zooplankton community (Cladocera, Calanoida and Cyclopoida) and the important environmental variables (which were significant variables in both stepwise multiple regression and RDA analysis), we employed linear regression analysis in SPSS.

3 Results

3.1 Physicochemical factors

The trophic level of studied waterbodies was mesotrophic or eutrophic according to TSI. (44.07–65.70), which is a classic trophic state index (Carlson, 1977) (Tab. 1). The mean of the physicochemical composition, and *P*-values for *t*-tests between the lakes and reservoirs were listed in Table 2. Most physicochemical parameters determined in the four season was not significantly different between the lakes and reservoirs ($P > 0.05$). However, significant difference ($P < 0.05$) was detected for NO_2^-–N ($P = 0.01$) and TN ($P = 0.01$) in Spring, SD ($P = 0.02$) and TP ($P = 0.01$) in Summer.

3.2 Phytoplankton community

Of all the phytoplankton species observed in the four season, a total of 122 algal species were identified in the waterbodies including seven taxonomic categories: cyanophyta (21 taxa), cryptophyta (3), pyrrophyta (2), chrysophyta (6), bacillariophyta (24), euglenophyta (9) and chlorophyta (57). No matter what season, the abundance of cryptophyta, pyrrophyta, chrysophyta, bacillariophyta, euglenophyta, chlorophyta in the lakes were similar with those in the reservoirs, while the cyanophyte abundance showed significant difference between the lakes and reservoirs ($P < 0.05$) (Fig. 2). In addition, the most genus of cyanophyte was Microcystis in both lakes and reservoirs (Fig. 3).

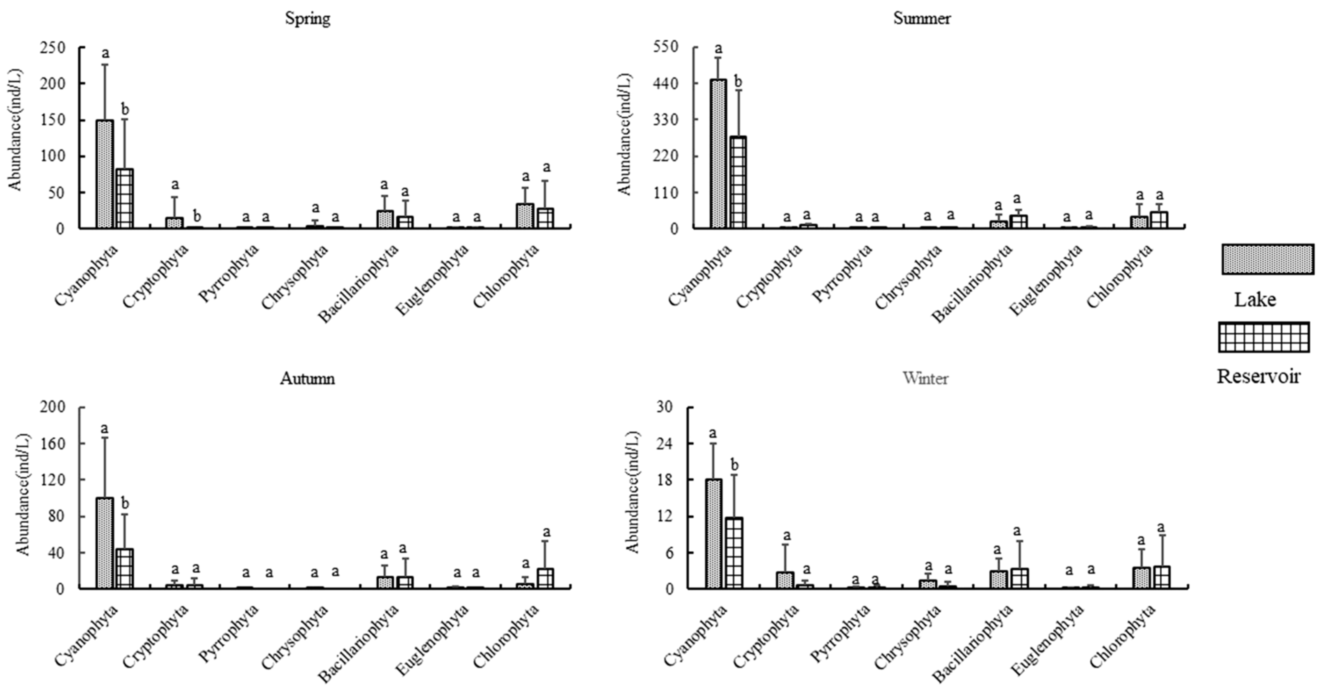

Fig. 2. The phytoplankton abundance of studied waterbodies in four seasons. In lake cases $n = 8$ lakes × 4 seasons; in reservoir cases $n = 5$ reserviors × 4 seasons.; a and b means $P < 0.05$, whereas a and a means $P > 0.05$.

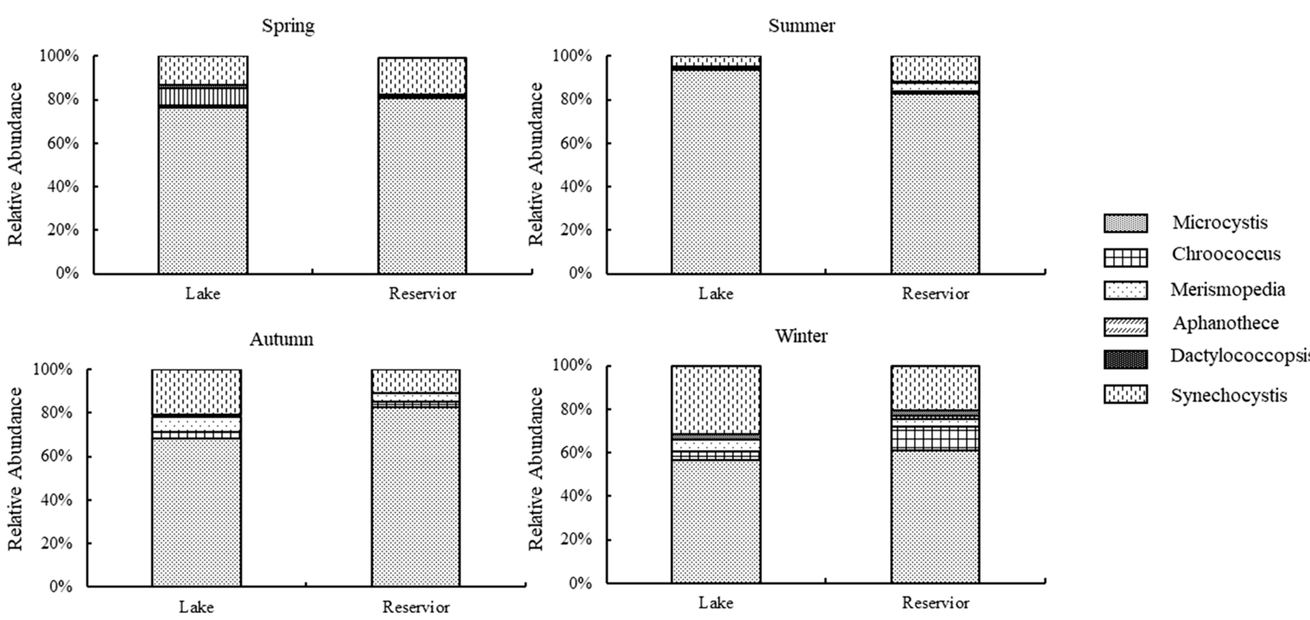

Fig. 3. The relative abundance of cyanophyte in the 8 lakes and 5 reservoirs in four seasons.

3.3 Crustacean zooplankton community

A total of 21 species of crustacean zooplankton were collected, including 10 species of cladoceran (belonging to 8 genera) and 4 species of calanoida (belonging to 3 genera) as well as 7 species of cyclopoida (belonging to 7 genera). In Spring and Summer, the abundance of all crustacean zooplankton in lakes were significantly higher than those in reservoirs ($P < 0.05$). However, except for cladoceran in Winter, the differences of crustacean zooplankton abundance between the lakes and reservoirs were not significant in Autumn and Winter ($P > 0.05$) (Fig. 4). No matter what season, the highest relative abundance species of cladoceran was *Bosmina fatalis*, which had the most proportion (almost 50%) in the lakes and reservoirs (Fig. 5).

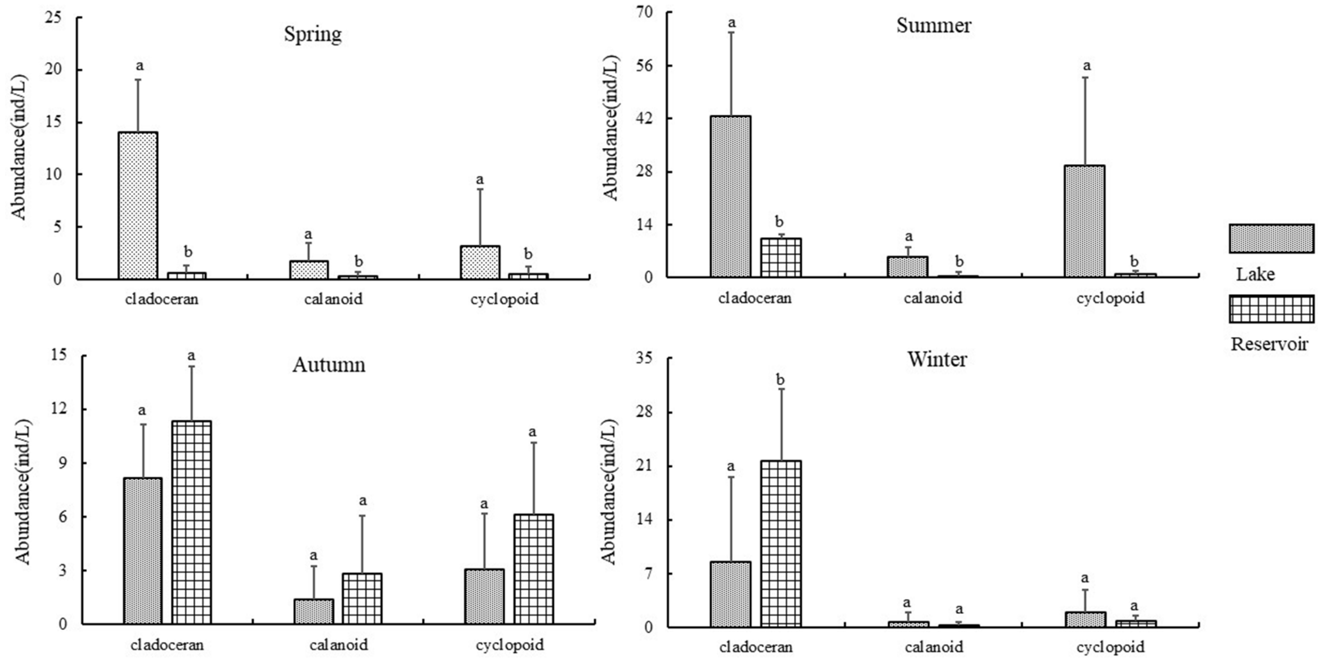

Fig. 4. The crustacean abundance of studied waterbodies in four seasons. In lake cases $n = 8$ lakes $\times$ 4 seasons; in reservoir cases $n = 5$ reserviors $\times$ 4 seasons; a and b means $P < 0.05$, whereas a and a means $P > 0.05$.

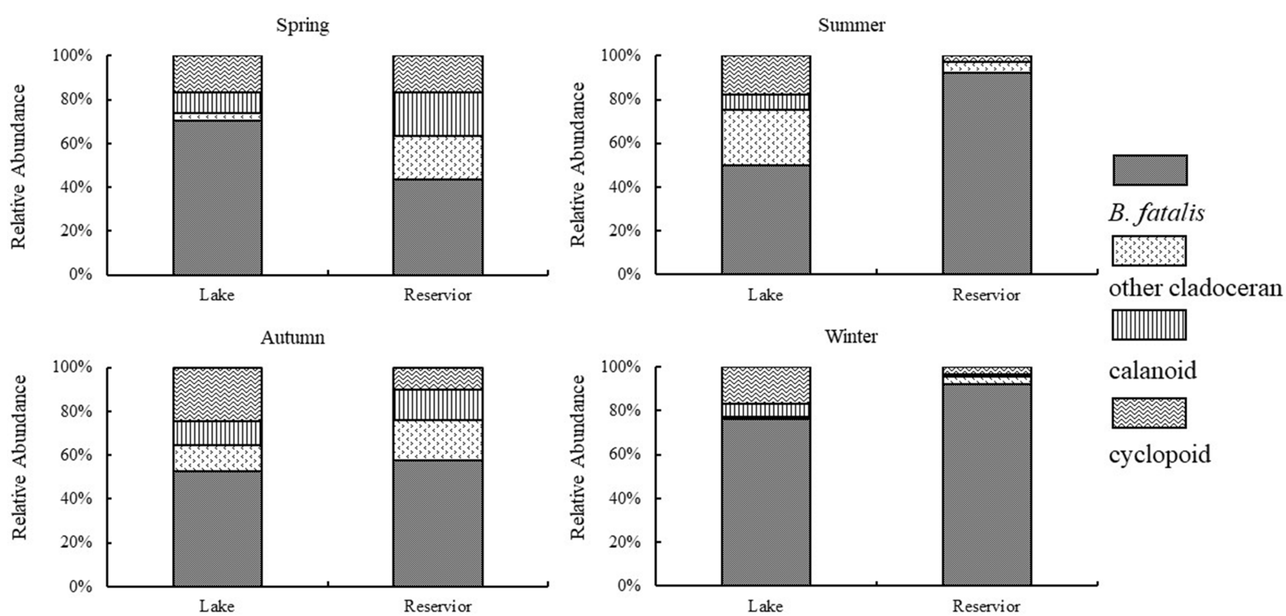

Fig. 5. The relative abundance of crustacean in the 8 lakes and 5 reservoirs in four seasons.

3.4 Relationships between crustacean abundance and environmental factors

Multiple regression analysis showed that crustacean abundance was correlated with four environmental variables. Cyanophyta accounted for most of the variation in crustacean abundance followed by SD, WT and pH (Tab. 3). After a forward selection, the final RDA ordination only included three significant ($P < 0.05$) independent variables (cyanophyta, SD and DO) which explained 51.5% of

Table 3. Stepwise multiple regression model details for crustacean zooplankton abundance and environmental variables.

Variables entered	R^2 adj	P-value
Cyanophyta	0.332	<0.001
SD	0.436	<0.001
WT	0.476	<0.001
pH	0.511	<0.001

Note: Residual $df = 51$.

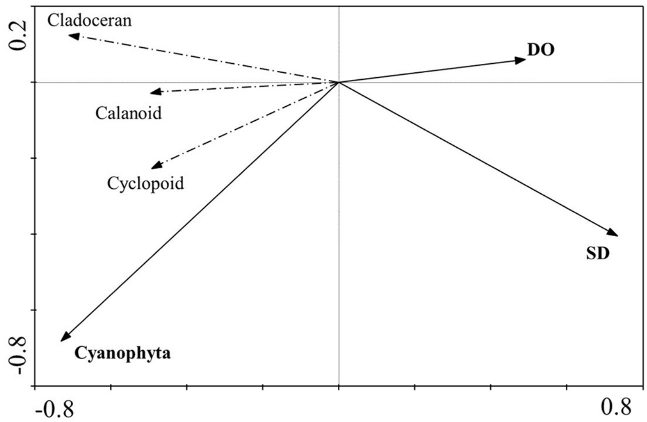

Fig. 6. Redundancy analysis (RDA) biplot based on crustacean zooplankton data and environmental variables of 13 waterbodies.

variance in the zooplankton community data (Fig. 6). Both cyanophyta and SD were important factors affecting the crustacean zooplankton community (Tab. 3 and Fig. 6). The positive relationship was observed between the cyanophyta abundance and every crustacean community abundance (Cladocera; $r^2=0.18$ $P=0.020$, Calanoida; $r^2=0.13$ $P=0.009$, Cyclopoida; $r^2=0.27$ $P<0.001$) (Fig. 7A). However, the relationship between the SD and every community abundance was negative (Cladocera; $r^2=0.09$ $P=0.020$, Calanoida; $r^2=0.09$ $P=0.033$, Cyclopoida; $r^2=0.08$ $P=0.036$) (Fig. 7B).

4 Discussion

4.1 The significant difference of physicochemical parameters

Most physicochemical parameters were similar between lakes and reservoirs, while NO_2^--N and TN in Spring, SD and TP in Summer was significantly different in this study. On the one hand, microbial communities in the aquatic ecosystems play a key role help in the nitrogen cycle (N-cycle), which involves nitrogen fixation, ammonification, nitrification and denitrification processes carried out by different microorganisms (e.g. ammonifying bacteria and denitrifying bacteria) (Altmann, 2003). Also, their effects on N-cycle vary greatly with different season (Liu and Luo, 2002). The different microbial communities between the lakes and reservoirs may be the reason for their significant difference of NO_2^--N and TN in Spring (Zhao *et al.*, 2015). On the other hand, in Summer, macrophytes may significantly enhance water residence time, and TP were retained by increasing deposition of particulate organic matter. The increase of TP will promote the growth of phytoplankton, which will lead to the decline of SD (Schulz *et al.*, 2003). 8 lakes are rich in macrophytes, while there are few macrophytes in the 5 reservoirs (Wenzhi Wei, personal observation). That may be explained for the significant difference of SD and TP between lakes and reservoirs in Summer in this study.

4.2 The structure of plankton

It is understandable that cyanophyta is the dominant genus among the 13 mesotrophic/eutrophic water bodies in this study. In addition, the high proportion of Microcystis in cyanophyta may provide an explanation to the dominance of *B. fatalis* in our studied waterbodies (Figs. 3 and 5). It is well-known that with increasing cyanobacterial biomass, large-sized crustaceans are replaced by smaller species. This can be explained by the competitive dominance achieved by smaller cladocerans that are less inhibited by toxic cyanobacteria (Ghadouani *et al.*, 2003; Guo and Xie, 2006). Consequently, crustacean communities were dominated by small cladocerans (Haberman *et al.*, 2007; Sun *et al.*, 2012). Whereas the co-occurring small-bodied cyclopoid and cladoceran species have markedly different algal diets and the cladocera represent the main trophic link transferring cyanobacterial carbon to the food web in eutrophic systems (Tõnno *et al.*, 2016). As a group of small and competitive cladoceran (Hanazato and Yasuno, 1987; Watanabe *et al.*, 2010), *B. fatalis* can digest and absorb Microcystis better than other crustacean species. So *B. fatalis* is the most dominant species in many mesotrophic or eutrophic waterbodies (Adamczuk, 2016). It is also not surprised that *B. fatalis* became dominant species in these 13 waterbodies, where have abundant Microcystis (Figs. 3 and 5).

Although the dominance of plankton in 13 waterbodies (8 lakes and 5 reservoirs) is consistent, their abundances have some significant difference between lakes and reservoirs. In sampled waterbodies, 7 of 8 lakes were eutrophic, while 4 of 5 reservoirs were mesotrophic (Tab. 1). Eutrophic lakes provided more nutrients for the growth of cyanobacteria than mesotrophic reservoirs (Chengxue and Hongxian, 2013). So, the cyanophyte abundance in lakes was significantly higher than that in reservoirs in this study (Fig. 2). Moreover, it seems that trophic level also affected crustacean zooplankton communities. Especially in spring and summer, eutrophic lakes can provide more food resources for the growth and development of crustacean zooplankton (Burns and Schallenberg, 1996). That may be the reason for significant difference of crustacean community (cladoceran, calanoid and cyclopoid abundance) between lakes and reservoirs (Fig. 4).

4.3 Relationships between crustacean abundance and environmental factors

Both stepwise multiple regression analysis and RDA revealed that the importance of cyanophyta and SD in structuring crustacean zooplankton community (Tab. 3 and Fig. 6). SD can provide an estimator of the volume of the phytoplankton biomass (Wu *et al.*, 2015). And cyanophyta was the most dominant phytoplankton in 13 studied waterbodies. Therefore, the significant relationship between SD and crustaceans may be an indirect influence of cyanophyta on crustaceans in this study. A few studies suggested that cyanophyta (is also known as cyanobacteria) may be the main food for cladocerans in eutrophic systems (Nanazato and Yasuno, 1985; Hanazato, 1991). As demonstrated in many studies, cyanobacteria do provide good growth conditions for bacteria (Hoppe, 1981; Heinänen *et al.*, 1995). It is commonly

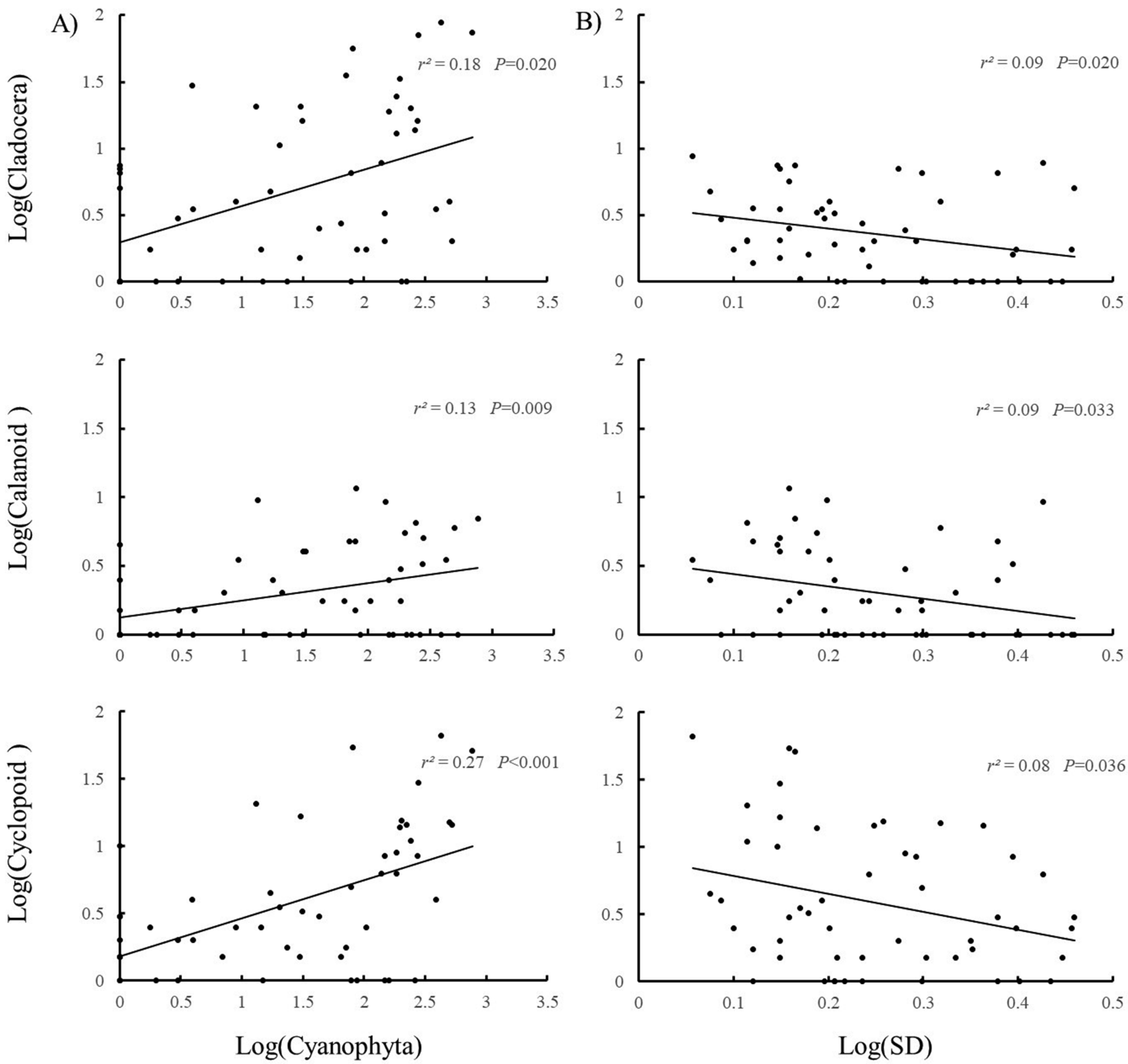

Fig. 7. Regression analysis of crustacean structure abundance and cyanophyta (A) as well as SD (B).

accepted that protozoa can transfer the carbon and energy of bacteria to zooplankton (Pomeroy, 1974; Azam *et al.*, 1983). Cladocerans and copepods have been observed to consume bacteria (Sanders *et al.*, 1989; Brucet *et al.*, 2008). Our results also indicated that the increase of cyanobacteria provided more food for zooplankton, increasing their abundance (Fig. 7A). In addition, zooplankton excretion may also stimulate the growth of cyanobacteria (Kozak *et al.*, 2019). Due to negative relationship between cyanobacteria and SD, crustacean zooplankton had negative relationship with SD (Fig. 7B).

Except for cyanophyta and SD, stepwise multiple regression analysis also showed that WT and pH affected crustacean zooplankton abundance significantly (Tab. 3). In natural water bodies, the hatch of dormant eggs (or winter eggs) of most crustacean zooplankton was accelerated by the rise of water temperature. At the same time, the increase of water temperature also promoted the blooms of algae, which provide sufficient food for crustacean zooplankton (Guangrong *et al.*, 2008). Therefore, the effect of WT on crustacean zooplankton abundance was significant in this study. On the other hand, previous study showed that pH has an important impact on the metabolism, reproduction, and development of crustacean zooplankton (Chunhou and Xiangfei, 1992). So, pH was another significant factor in stepwise multiple regression analysis. In addition, RDA showed that DO was also a significant variable that affect crustacean zooplankton. Higher DO may increase other creature, which competes with crustacean zooplankton (Vad *et al.*, 2013). That may be responsible for the significant effect of DO on crustacean zooplankton.

5 Conclusion

The present study investigated physicochemical factors, phytoplankton and crustacean zooplankton in 13 waterbodies (8 lakes and 5 reservoirs) located in the Yangtze River delta. At same time, we analyzed that which environmental variables (physicochemical factors and phytoplankton abundance) were related to crustacean zooplankton community. NO_2^{-}–N and TN in Spring, SD and TP in Summer was significantly different between 8 lakes and 5 reservoirs. That may be related to microbial communities and macrophytes. 13 mesotrophic/eutrophic water bodies were dominant by cyanophyta, which can facilitate small cladocerans. And the high proportion Microcystis of in cyanophyta may provide an explanation to the dominance of *B. fatalis*. Furthermore, eutrophic level should be in relation to the significant difference of plankton between 8 lakes and 5 reservoirs. In addition, cyanophyta, SD, WT, and pH may affect metabolism, reproduction, and development of crustacean zooplankton. While DO may increase the competitors of crustacean zooplankton. Therefore, the five factors (cyanophyta, SD, WT, pH and DO) were significantly correlated with crustacean zooplankton abundance. This research studied the relationship between zooplankton and environmental factors of 13 waterbodies located in the Yangtze River delta, providing scientific basis for the monitoring of eutrophication in the region.

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