Species diversity and community structure of crustacean zooplankton in highland small waterbodies in Northwest Yunnan, China

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Small waterbodies are a unique aquatic ecosystem and have gained increasing recognition for their important role in maintaining regional biodiversity and delivering ecosystem services. However, small waterbodies in Northwest Yunnan, one of the most concerned global biodiversity hot-spots, remain largely unknown because of limited research. In this study, we investigated the community structure of crustacean zooplankton and their relationships with environmental factors in the highland small waterbodies in Northwest Yunnan in both the rainy (October 2015) and dry (June 2016) seasons. A total of 38 species of crustacean zooplankton were identified in our study, which is significantly higher than many other waterbodies in the Yunnan-Guizhou plateau and the Yangtze River basin. This suggests that highland small waterbodies are critical in maintaining regional zooplankton diversity in Northwest Yunnan. Meanwhile, we found the species composition in the dry and rainy seasons are quite different. Further canonical correspondence analysis showed that NO_3N, pH, Cond, and DO were the main environmental factors determining the spatiotemporal variation of the crustacean zooplankton community structure in the highland small waterbodies. Our study revealed the diversity and community structure of crustacean zooplankton in the highland small waterbodies in Northwest Yunnan and highlighted the importance of small waterbodies in maintaining regional biodiversity.

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Abstract

Small waterbodies are a unique aquatic ecosystem and have gained increasing recognition for their important role in maintaining regional biodiversity and delivering ecosystem services. However, small waterbodies in Northwest Yunnan, one of the most concerned global biodiversity hot-spots, remain largely unknown because of limited research. In this study, we investigated the community structure of crustacean zooplankton and their relationships with environmental factors in the highland small waterbodies in Northwest Yunnan in both the rainy (October 2015) and dry (June 2016) seasons. A total of 38 species of crustacean zooplankton were identified in our study, which is significantly higher than many other waterbodies in the Yunnan-Guizhou plateau and the Yangtze River basin. This suggests that highland small waterbodies are critical in maintaining regional zooplankton diversity in Northwest Yunnan. Meanwhile, we found the species composition in the dry and rainy seasons are quite different. Further canonical correspondence analysis showed that NO_3N, pH, Cond, and DO were the main environmental factors determining the spatiotemporal variation of the crustacean zooplankton community structure in the highland small waterbodies. Our study revealed the diversity and community structure of crustacean zooplankton in the highland small waterbodies in Northwest Yunnan and highlighted the importance of small waterbodies in maintaining regional biodiversity.

Keywords: Northwest Yunnan; Small waterbodies; Biodiversity; Canonical correspondence analysis; Crustacean zooplankton

Introduction

Northwest Yunnan, located in Southwest China, has been designated as a global biodiversity hot-spot by World Wildlife Fund (WWF) and International Union for Conservation of Nature (IUCN) because of the rich biodiversity, unique and diverse highland landscape (*Mackinnon et al., 1996; Xu & Wilkes, 2004; Trizzino et al., 2014*). This region is in the upper stream of the Yangtze (Jinsha) River, the Mekong (Lancang) River, the Salween (Nujiang) River, and the Irrawaddy (Dulongjiang) River, attracting extensive attention of local and international communities (*Xu & Wilkes, 2004*). Currently, most ecological and biodiversity-related studies in this region are focusing on the terrestrial vegetation and endangered wild animals (*Xu & Wilkes, 2004; Li et al., 2014*), yet still few studies addressed the aquatic ecosystems, especially for small waterbodies in this region.

Small waterbodies are a unique aquatic ecosystem and have a wide distribution in Northwest Yunnan (*Liu et al., 2018*). Small waterbodies are critical for regional biodiversity and are increasingly recognized for their role in the ecosystem service delivery (*Williams et al., 2004; Biggs, von Fumetti & Kelly-Quinn, 2017; Kuczyńska-Kippen, 2020*). For this reason, there is a growing interest in understanding the role of small waterbodies in Europe as well as in North America (*Biggs, von Fumetti & Kelly-Quinn, 2017*). However, small waterbodies in Northwest Yunnan, one of the most concerned global biodiversity hot-spots, have still received relatively little research attention.

Crustacean zooplankton is an important group in freshwater ecosystems because they occupy a central position in aquatic food webs, transferring energy to higher trophic levels (*Sommer et al., 1986; Fussmann, 1996*). Besides, crustacean zooplankton is sensitive to climate and environmental change (*Keller et al., 2002; Jones & Gilbert, 2016*). For quite a long time, the studies on crustacean zooplankton are mainly carried out in lakes (*Barbiero et al., 2019*) and reservoirs (*Liu et al., 2020*). Yet, the ecology of crustacean zooplankton in highland small waterbodies remains seldom addressed.

In this study, we focus on the community structure and species diversity of crustacean zooplankton in highland small waterbodies in Northwest Yunnan, China. ~~As far as our knowledge, the ecology of crustacean zooplankton in highland small waterbodies of Northwest Yunnan remains largely unknown. Therefore~~, the ~~main~~ aims of our study are to understand: i) the diversity and community structure of crustacean zooplankton in highland small waterbodies in Northwest Yunnan, ii) the difference of community structure of crustacean zooplankton in dry and rainy season ~~in this region~~, iii) which environmental factors determining the spatiotemporal variations of diversity and community structure of the crustacean zooplankton in highland small waterbodies.

Materials & Methods

Study sites and field sampling

The study sites were distributed on the east and west sides of a high mountain ridge located in Gong-shan Country, Yunnan province, China (Fig. 1). The altitude of the ridge is 3700 m above sea level. The east side of the ridge was defined as "area E" and the west side was defined as "area W". There was a disused road lying across the "area E", which separated this area into Upstream (EU) and Downstream (ED) subgroups. The arrows represent the connectivity and direction of water flow between different small waterbodies. The average elevation and area of small waterbodies 3131 m and 9.9 m^2 for the area W, 3328 m and 13 m^2 for the area EU, and 3274 m and 41 m^2 for the area ED, respectively.

Two ~~field~~ samplings were carried out in the dry (October 2015) and rainy (June 2016) season. A total of 30 and 32 small waterbodies were sampled in the dry and rainy season, respectively. For each sampling site, the conductivity (Cond), dissolved oxygen (DO), pH, and water temperature (WT) were measured by a portable multi-parameter device (YSI Professional plus, USA) ~~*in situ*~~. And 350 ml water sample was collected at each site for ammonia nitrogen (NH_3N), nitrate nitrogen (NO_3N), total nitrogen (TN), phosphate (PO_4P), total phosphorus (TP), dissolved silicate (DSi), and dissolved organic carbon (DOC). The above chemical variables were analyzed by the segmented flow analyzer (Skalar SAN++, Netherlands), according to the user manual of Skalar. Meanwhile, 350 ml water sample was filtered through a micro-filter (~1.2 µm, GF/C Waterman) for the measurement of chlorophyll *a* (Chl-*a*). The concentration of Chl-*a* was measured with a spectrophotometer (Shimadzu UV-1800, Japan) with the standard method of APHA (*1998*). Crustacean zooplankton samples were collected by filtering 20 L water sample with a plankton net (64 µm in mesh size). All crustacean zooplankton samples were preserved in 5% formalin solution.

Zooplankton counting and identification

Crustacean zooplankton was counted and identified under the stereoscope (Zeiss Stereo Discovery V20, German). Specifically, all samples were screened under the stereoscope because of the low density of the crustacean zooplankters. The major reference books for identification were Chiang and Du (*1979*), Shen (*1979*), and Błędzki and Rybak (*2016*).

Statistical analysis

Detrended correspondence analysis (DCA) was used to select the proper ordination model (*Rautio, 1998*). Pre-analysis showed that the axis length of DCA1 and DCA2 were 5.58 and 3.33 respectively. This suggested that canonical correspondence analysis (CCA) is a suitable model in the ordination because both axis lengths of DCA are large than 3.0 (*Lepš & Šmilauer, 2003*). For the above reason, CCA was used in our study to explore the relationship between environmental factors and the crustacean zooplankton community structure.

Specifically, the environmental factors including TN, NO_3N, NH_3N, TP, PO_4P, DSi, DOC, Cond, WT, pH, DO, and Chl-*a*, were selected as candidate environmental factors affecting zooplankton community in the CCA. To make environmental factors achieve normally distributed, all the above environmental factors were log(x+1) transformed before analysis. Meanwhile, to remove the bias from rare species, the crustacean zooplankton species with a relative abundance of less than 1% were removed in the CCA (*MacLeod, Keller & Paterson, 2018*). As a result, sixteen species were included in the CCA. Following the suggestion of Cottenie (*2001*), all zooplankton densities were square-root transformed to minimize the effect of sampling variability in zooplankton. In the CCA, the Monte Carlo permutation tests (999 permutations) were used to select the significant variables of each forward selected variable. The DCA and CCA were carried out by the package "*vegan*" in R (*R Core Team, 2017*).

Results

Community composition

A total of 38 crustacean zooplankton taxa, including 20 Cladocera species and 18 Copepoda species, were identified in all sampled small waterbodies in northwest Yunnan (Table 1 and 2). In the rainy season (Table 1), the most common species in this region were *C. vicinus*, *M. leuckarti*, *A. affinis*, *M. varicaricans*, *M. irrasa, C. strenuuss*, and *E. phaleratus* which occurred in more than 50% of the surveyed small waterbodies. And in the dry season (Table 2), *C. ovalis*, *M. varicaricans*, *T. prasinus*, *C. laticaudata*, and *A. exigua* had a relative occurrence above 50% in this region.

For each small waterbody in the dry and rainy seasons, the species richness ranged from 3 to 18, with an average value of 10.6 (Fig. 2). Besides, average species richness in the dry season was 11.3, which was significantly higher than the average species richness (8.0) in the rainy season (Mann-Whitney U test, $p < 0.05$). The density ranged from 0.1 to 38.3 ind./L, with a mean value of 8.8 ind./L. The average density in the rainy season and dry season were 12.4 ind./L and 8.5 ind./L respectively. Mann-Whitney U test showed that the density in the rainy season significantly higher than the dry season ($p < 0.05$).

Crustacean community in relation to environmental factors

CCA revealed general patterns of crustacean zooplankton and identified the major environmental factors determining the spatiotemporal variations of crustacean zooplankton of highland small waterbodies in Northwest Yunnan (Fig 3). Specifically, the first three axes explained 19.19% of the variance in community (CCA1 = 14.45%, CCA2 = 3.66%, CCA3 = 1.08%). The species with the strongest positive loadings on CCA1 were *A. exigua* (1.24), *C. laticaudata* (1.14), *T. prasinus* (1.13), *C. ovalis* (0.95) and *C. quadrangula* (0.68). The species with strongest negative loadings on CCA1 were *M. leuckarti* (-0.95), *A. affinis* (-0.90), *C. strenuussand* (-0.79), *C. vicinus* (-0.78) *N. lacustri* (-0.76) and *M. irrasa* (-0.74). The species with strong positive loadings on CCA2 was *A. exigua* (0.93). The species with strongest negative loadings on CCA2 were *C. quadrangula* (-1.31) and *L. sinensis* (-0.77). There are no species with strong positive or negative loadings on CCA3.

CCA suggested that NO_3N, pH, DO and Cond were the most important environmental factors in explaining the variation of crustacean zooplankton community (Fig. 3). Specifically, the density of *M. leuckarti*, *A. affinis*, *C. strenuussand*, *C. vicinus*, and *N. lacustri* were positively associated with NO_3N. *C. quadrangula* and *L. sinensis* were positively associated with pH. *C. laticaudata*, *T. prasinus*, and *C. ovalis* had a significant positive relationship with Cond, but negatively associated with NO_3N. *A. exigua* was positively associated with DO.

CCA also found that the sampling sites in the dry season and the rainy season are well separated in the ordination (Fig. 4). Most sites in the left quadrant were sampled in the rainy season. These sites were characterized by a higher concentration of NO_3N and lower values of DO, Cond, and pH, by comparing to the samples in the dry season. Correspondingly, *M. leuckarti*, *A. affinis*, *C. strenuussand*, *C. vicinus*, *N. lacustri* and *M. irrasa* had a higher abundance in the rainy season than the dry season (Fig. 3). Contrarily, samples collected in the dry season were generally distributed in the right quadrant (Fig. 4). They were associated with comparatively high values of DO, Cond, and pH, but a low concentration of NO_3N. *A. exigua*, *C. laticaudata*, *T. prasinus*, and *C. ovalis* had higher abundance in the dry season samples than in the rainy season samples.

Discussion

As far as ~~our knowledge~~, ~~our study first reported~~ the crustacean zooplankton community of highland small waterbodies in Northwest Yunnan. ~~A total of 38 species of crustacean zooplankton was observed in the dry and rainy seasons.~~ We found the species composition in the dry and rainy seasons are quite different. Specifically, *C. ovalis*, *M. varicaricans*, *T. prasinus*, *C. laticaudata*, *A. exigua* were the most common species in the dry season. However, in the rainy season, the common species shifted to *C. vicinus*, *M. leuckarti*, *A. affinis*, *M. varicaricans*, *M. irrasa, C. strenuuss*, *E. phaleratus*. Among these species, we found 2 species (*T. hebereri* and *N. mariadvigae*) ~~belong to the~~ endemic ~~species in~~ the Yunnan-Guizhou plateau (Shen, 1979). Meanwhile, we also found 9 species (e.g. *C. vicinus*, *M. leuckarti*, *A. affinis*, *M. irrasa*) in the Yangtze River basin (*Chiang & Du, 1979*).

One interesting finding of our study is that the species richness in the small waterbodies in our study area is significantly higher than many other reported waterbodies in the Yunnan-Guizhou plateau as well as in the Yangtze River basin (Table 3). For example, Guo (*2009*) identified a total of 36 crustacean zooplankton species in 13 different lakes in the Yunnan-Guizhou plateau with areas ranged from 10.7 to 297.9 km^2. Another similar research carried out in the plateau lake (Erhai Lake) in Yunnan province only recorded 11 crustacean zooplankton species for 12 field stations with one-year continuous monthly monitoring (*Yang et al., 2014*). Comparing to the lakes and other waterbodies, small waterbodies usually have a high habitat heterogeneity which can support more diverse species and maintain a high diversity community (*Williams et al., 2004*).

Our study found that NO_3N is the most important nutritional factor determining the community structure of crustacean zooplankton in highland small waterbodies (Fig. 3, 4). Nitrogen is one of the most fundamental chemical elements for aquatic ecosystems. Many studies showed that increasing the nitrogen can boost phytoplankton biomass, which provides more available food sources for zooplankton and therefore affects its community structure (*Elser et al., 2007; Sitta et al., 2018*). In our study, we found that the mean concentration of NO_3N in the rainy season is 0.102 ± 0.063 mg/L, which is significantly higher ($P < 0.05$) than that in the dry season (Fig.. 5). Accordingly, we found a quite different community structure of crustacean zooplankton in the dry and rainy seasons. Specifically, we found that most dominant species such as *M. leuckarti*, *A. affinis*, *C. strenuussand*, and *C. vicinus* in the dry season were positively correlated with NO_3N, and most dominant species such as *C. laticaudata*, *T. prasinus*, and *C. ovalis* in the dry season was negatively correlated with NO_3N.

In addition to the nutritional factors, other factors such as pH and DO were also important in our system. Many previous studies have shown that pH is an important factor affecting the zooplankton community, and low pH condition generally decreases the species richness and abundance because of acidification (*Havens, Yan & Keller, 1993; Keller et al., 2002*). In our study, we found that a similar pattern that a lower abundance of crustacean zooplankton was observed in the dry season with lower pH. DO affects the crustacean zooplankton community structure may because different zooplanktons have different sensitivity to the DO (*Aka et al., 2000; Bertilsson et al., 1995; Marcus 2001*). For example, the optimum DO concentration of *P. trigonellus* ranged from 17 - 64 mg/L, *P. laevis* ranged from 11 - 37 mg/L (*Chiang & Du, 1979*).

We should add a caveat that not all potential environmental factors affecting the crustacean zooplankton communities were examined in our study due to the limited data. For example, it is well known that fish predation could dramatically impact on the zooplankton community, especially in small and shallow waterbodies (*Keller & Conlon, 1994*). Meanwhile, some researches also suggested that macrophytes cover is important to maintain zooplankton diversity because macrophytes provide shelter and concealment from predators(*Celewicz-Goldyn & Kuczynska-Kippen, 2017*). Future works in discovering the factor shaping zooplankton community in small waterbodies could focus on the fish predation or the effect of macrophyte which are probably important to affect zooplankton species assemblages (*Feniova et al., 2019; dos Santos et al., 2020*).

Conclusions

In this study, we reported the crustacean zooplankton community and their relationships with environmental factors in the highland small waterbodies in Northwest Yunnan for both the dry and rainy seasons. We identified a total of 38 species of crustacean zooplankton in the highland small waterbodies, which is significantly higher than many other waterbodies in the Yunnan-Guizhou plateau as well as in the Yangtze River basin. This suggests that small waterbodies are a biodiversity hotspot and are important in maintaining regional zooplankton diversity in Northwest Yunnan. Meanwhile, we found that the sampling sites in the dry and rainy season are well separated in the CCA ordination, which suggests that the species composition in the dry and rainy seasons are quite different. Further analysis showed that NO_3N, pH, Cond, and DO were the main environmental factors determining the spatiotemporal variation of the crustacean zooplankton community structure in the highland small waterbodies. This study improved our understanding of the diversity and community structure of crustacean zooplankton in the highland small waterbodies in Northwest Yunnan and highlighted the importance of small waterbodies for biodiversity conservation and research.

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Table 1 (on next page)

Relative occurrence of crustacean zooplankton species in highland small waterbodies in Northwest Yunnan in the rainy season.

Table 1 Relative occurrence of crustacean zooplankton species in highland small waterbodies in Northwest Yunnan in the rainy season.

Species	% of all area	% of area E	% of area ED	% of area EU	% of area W
Cyclops vicinus	71.2	63.6	69.2	55.6	90.0
Mesocyclops leuckarti	71.2	63.6	84.6	33.3	90.0
Alona affinis	65.6	72.7	84.6	55.6	50.0
Microclops varicaricans	62.5	50.0	53.8	44.4	90.0
Moina irrasa	56.3	54.5	76.9	22.2	60.0
Ectocyclops phaleratus	59.4	38.5	38.5	55.6	90
Cyclops strenuuss	56.3	45.5	53.8	33.3	80.0
Limnoithona sinensis	46.7	36.4	46.2	11.1	80.0
Nitocra lacustri	43.8	54.5	38.5	77.8	20.0
Chydorus ovalis	40.6	31.8	38.5	22.2	60.0
Sinodiaptomus sarsi	43.8	31.8	38.5	22.2	70.0
Eucyclops serrulatus	37.5	40.9	23.1	66.7	30.0
Diaphanosoma sp.	31.3	31.8	38.5	22.2	30.0
Bosmina coregoni	21.9	27.3	15.4	44.4	10.0
Sinocalanus dorrii	21.9	13.6	7.7	22.2	40.0
Onychocamptus mohammed	21.9	27.3	23. 8	33.3	10.0
Neutrodiaptomus mariadvigae	15.6	13.6	15.4	11.1	20.0
Alona guttata	12.5	18.2	8.0	11.1	0.0
Bryocamptus sp.	9.4	13.6	15.4	11.1	0.0
Tropodiaptomus hebereri	6.3	9.1	7.7	11.1	0.0

Table 2(on next page)

Relative occurrences of crustacean zooplankton species in highland small waterbodies in Northwest Yunnan in the dry season.

Table 2 Relative occurrences of crustacean zooplankton species in highland small waterbodies in Northwest Yunnan in the dry season.

Species	% of all area	% of area E	% of ED area	% of area EU	% of area W
Chydorus ovalis	93.3	90.1	92.3	88.9	100.0
Microclops varicaricans	90.0	86.4	92.3	77.8	100.0
Tropocyclops prasinus	66.7	63.6	69.2	55.6	75.0
Ceriodaphnia laticaudata	53.3	45.5	53.8	33.3	75.0
Alonella exigua	53.3	59.1	69.2	44.4	37.5
Onychocamptus mohammed	46.7	27.3	23.1	33.3	100.0
Alona karua	30.0	31.8	15.4	55.6	25.0
Limnoithona sinensis	26.7	4.5	0.0	11.1	87.5
Alona rectangula	23.3	22.7	15.4	33.3	25.0
Graptoleberis testudinaria	20.0	18.2	30.8	0.0	25.0
Ceriodaphnia quadrangula	20.0	9.1	15.4	0.0	50.0
Moina rectirostris	16.7	13.6	15.4	11.1	25.0
Alonella globulosa	16.7	13.6	23.1	0.0	25.0
Eucylops serrulatus	16.7	22.7	7.7	44.4	0.0
Paracyclops fimbriatus	16.7	18.2	7.7	33.3	12.5
Tropodiaptomus hebereri	13.3	0.0	0.0	0.0	50.0
Ceriodaphnia reticulata	13.3	18.2	30.8	0.0	0.0
Diaphanosoma sp.	12.5	9.9	7.7	0.0	0.0
Alona quadrangularis	10.0	13.6	7.7	22.2	0.0
Sinocalanus dorrii	10.0	4.5	7.7	0.0	25.0
Schmackeria inopinus	10.0	4.5	7.7	0.0	25.0
Neutrodiaptomus mariadvigae	10.0	0.0	0.0	0.0	37.5
Paracyclops affinis	10.0	9.1	15.4	0.0	12.5
Ectocyclops phaleratus	6.7	9.1	0.0	22.2	0.0
Alonella sp.	6.7	9.1	15.4	0.0	0.0
Chydorus barroisi	6.7	9.1	15.4	0.0	0.0
Bryocamptus sp.	3.3	4.5	0.0	11.1	0.0
Cyclops strenuuss	3.3	0.0	0.0	0.0	12.5
Bosmina coregoni	3.3	4.5	7.7	0.0	0.0

Alona sp.	3.3	0.0	0.0	0.0	12.5
Alona guttata	3.3	4.5	7.7	0.0	0.0
Alonella nana	3.3	0.0	0.0	0.0	12.5

Table 3(on next page)

A comparison of the species richness in the highland small waterbodies in Northwest Yunnan with other reported waterbodies in Yunnan-Guizhou plateau and Yangtze River basin, *n* indicates the number of total samples in the reported case.

Table 3 A comparison of the species richness in the highland small waterbodies in Northwest Yunnan with other reported waterbodies in Yunnan-Guizhou plateau and Yangtze River basin, *n* indicates the number of total samples in the reported case.

Reported waterbodies	Province	Area (km^2)	Species richness	References
Thirteen lakes in Yunnan and Guizhou (*n* = 112)	Yunnan and Guizhou	10.7~297.9	36	*Guo et al., 2009*
Gaoyou Lake (*n* = 26)	Jiangsu	674	26	*Wei et al., 2017*
Chaohu Lake (*n* = 228)	Anhui	780	23	*Deng et al., 2008*
Lugu Lake (*n* = 36)	Yunnan	57.7	23	*Dong et al., 2014*
Fuxian Lake (*n* = 220)	Yunnan	211	8	*Pan et al., 2009*
Erhai (*n* = 144)	Yunnan	249	11	*Yang et al., 2014*
Our study (*n* = 66)	Yunnan	<0.001	38	Our study

Figure 1

Location of the research area and the spatial distribution of highland small waterbodies.

The research area located in the Gongshan county, Northwest Yunnan province, China. The arrows represent the connectivity and water flow direction.

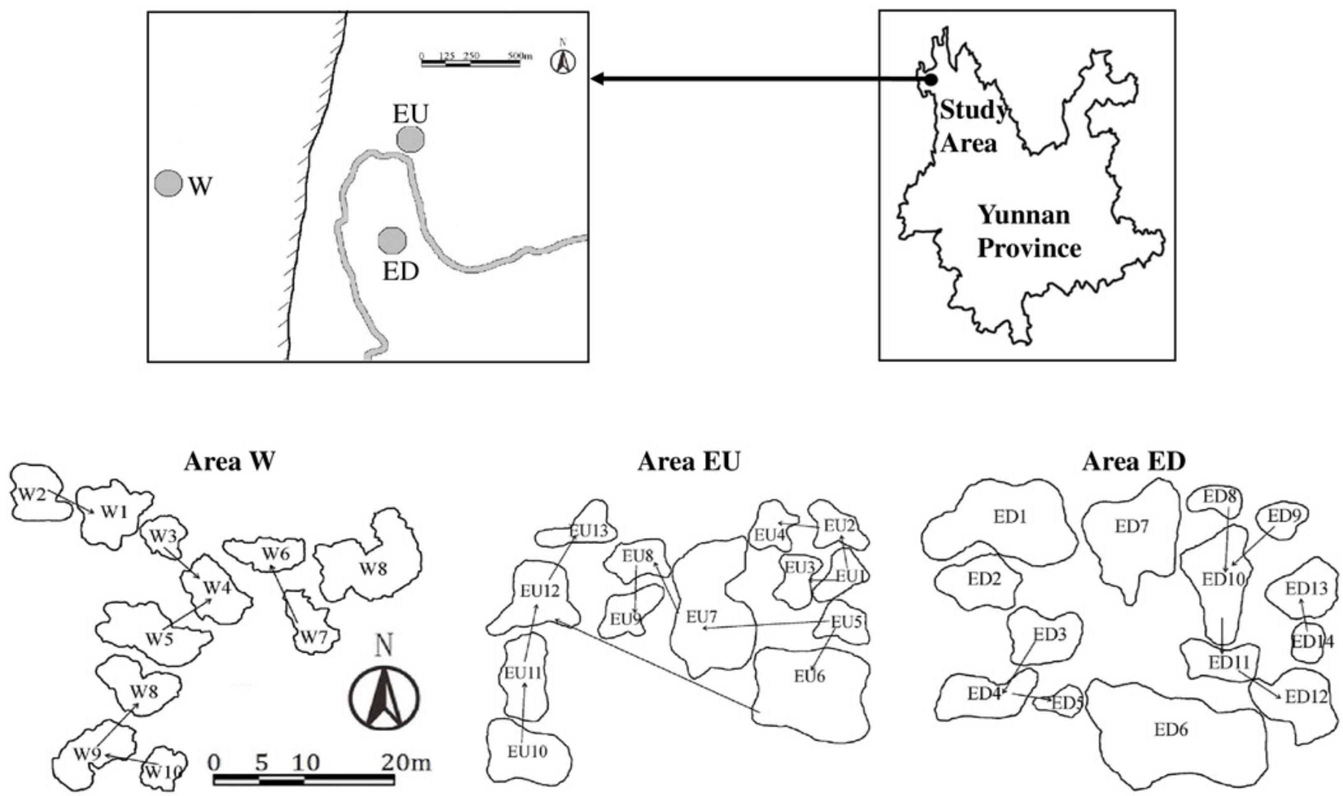

Figure 2

Boxplot showing the species richness and density of crustacean zooplankton in highland small waterbodies in Northwest Yunnan in the rainy season and the dry season.

The dark horizontal lines represent the median, the box encloses the 25th and 75th percentiles, the whiskers represent the 5th and 95th percentiles, and circles represent the outliers.

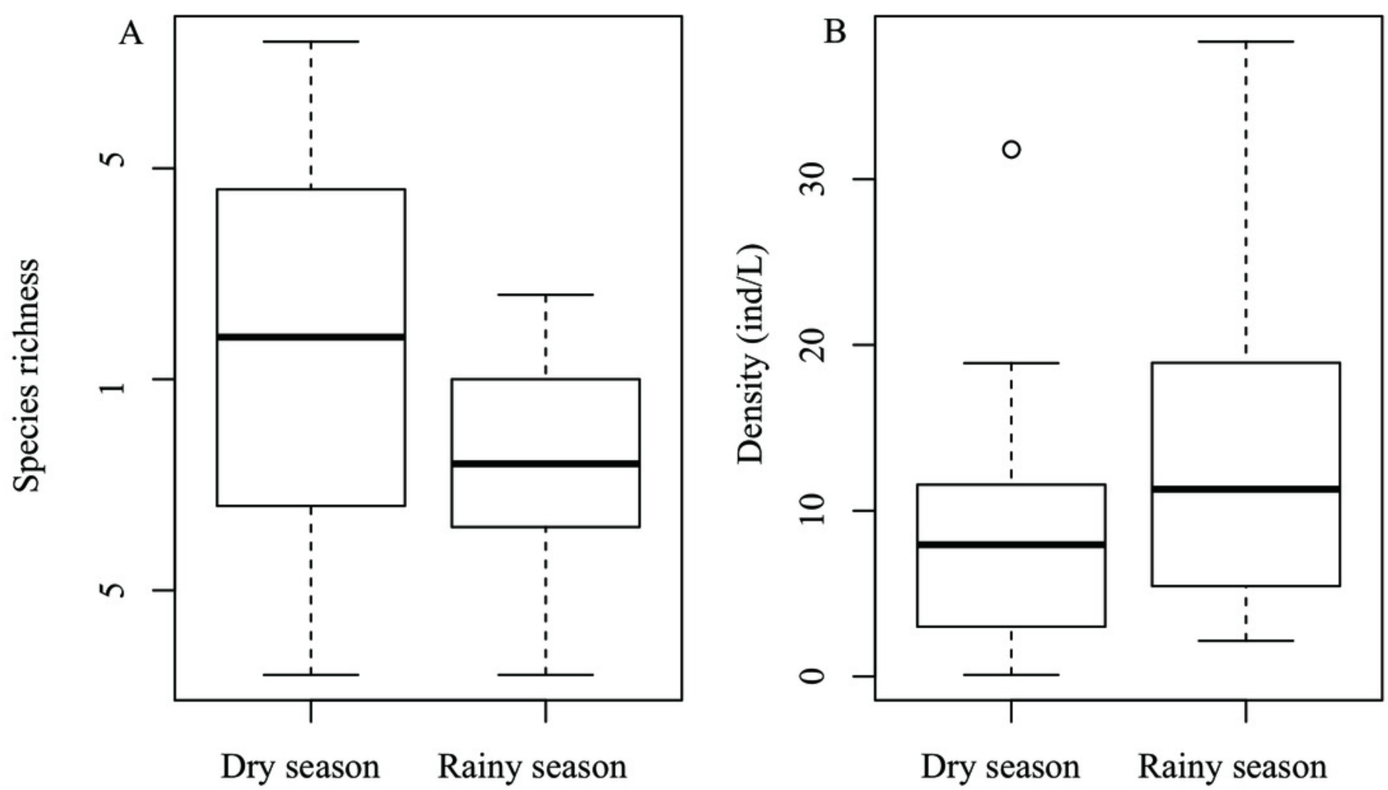

Figure 3

Canonical correspondence analysis (CCA) of crustacean zooplankton species and environmental factors in highland small waterbodies in Northwest Yunnan.

Only the environmental factors which have significant effects on crustacean zooplankton community were plotted in the figure.

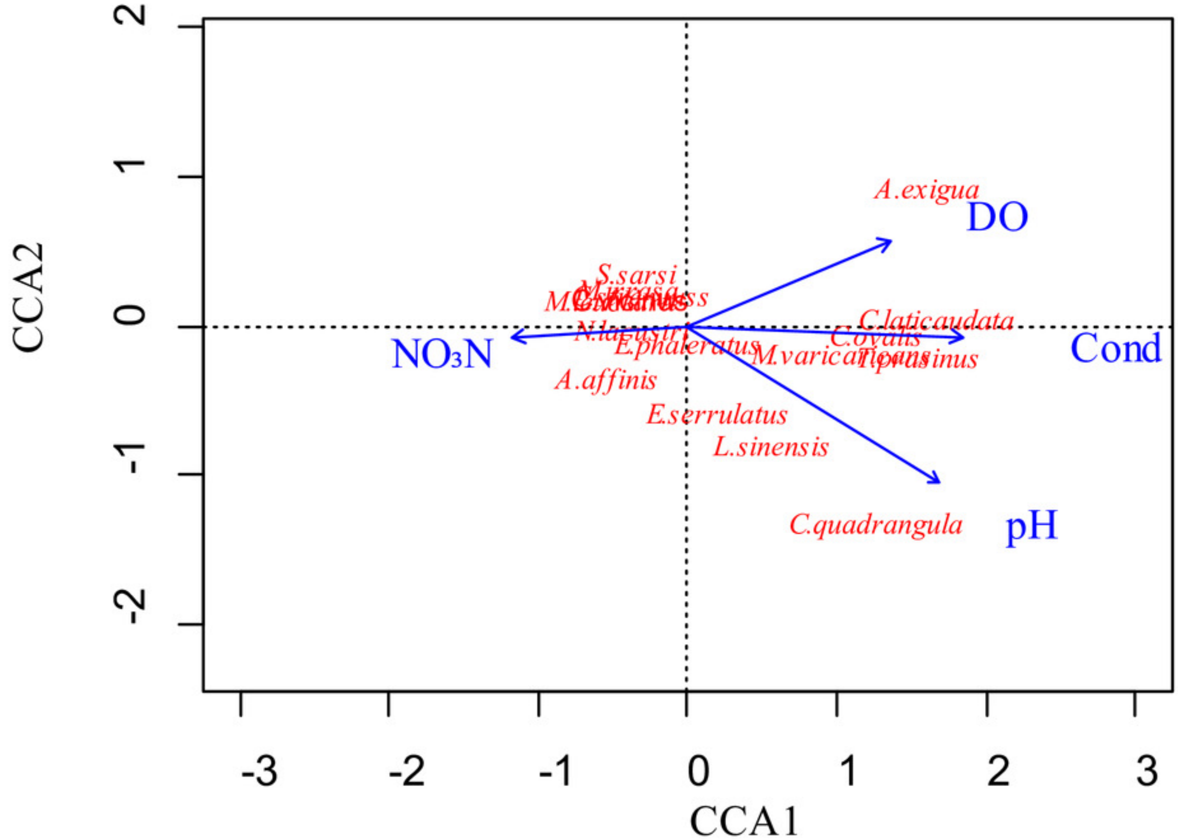

Figure 4

Canonical correspondence analysis (CCA) of small waterbodies ordination by the rainy season (red dots) and the dry season (black dots).

The sampling sites in the dry and rainy seasons are well separated in the CCA ordination. Only the environmental factors which have significant effects on crustacean zooplankton community were plotted in the figure.

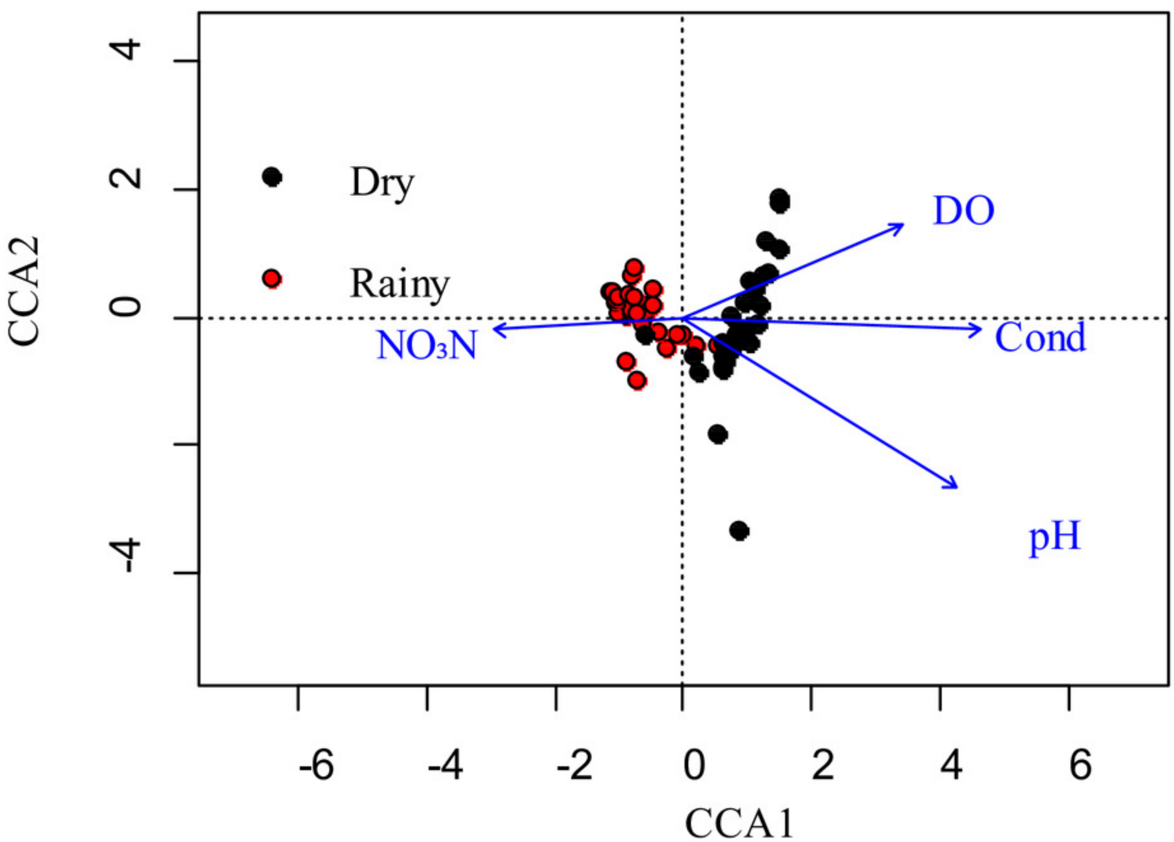

Figure 5

Boxplot showing the concentration of nitrate nitrogen (NO_3N) of highland small waterbodies in Northwest Yunnan in the rainy season is significant higher than that in the dry season.

The dark horizontal lines represent the median, the box encloses the 25th and 75th percentiles, the whiskers represent the 5th and 95th percentiles, and circles represent the outliers.