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eDNA of zooplankton reveals the ecological community thresholds for key environmental factors in the Baiyangdian Lake aquatic ecosystem

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Abstract

Background The drastic change in an ecosystem as a threshold phenomenon caused by abrupt changes in environmental conditions is a focus of current ecological research. However, the study of ecological thresholds has generally been limited to estimating the threshold values of single factors. Using eDNA metabarcoding technology, we collected zooplankton data from Baiyangdian Lake, the largest freshwater lake in the North China Plain, to explore the zooplankton community distribution characteristics and the relevant environmental factors. We used Threshold Indicator Taxa Analysis (TITAN) to determine the thresholds of key environmental factors and to identify the factors influencing biological diversity.

Results By comparing previous studies, we found that the zooplankton community composition based on eDNA metabarcoding was similar to that based on morphological methods, and that the data could be used to estimate ecological thresholds and assess risk conditions. Temperature (T), electrical conductivity (EC), and turbidity were the major environmental factors affecting the zooplankton community structure. The composition and structure of zooplankton communities in rivers and lakes were significantly different due to the influence of specific environmental factors. The results of TITAN analysis showed that there were different indicator species for T and EC in rivers and lakes. The protection thresholds of zooplankton in rivers were $T = 19.0\text{ }^{\circ}\text{C}$ and $\text{EC} = 795\text{ }\mu\text{S/cm}$, whereas the protection thresholds of zooplankton in lakes were $T = 14.3\text{ }^{\circ}\text{C}$ and $\text{EC} = 1920\text{ }\mu\text{S/cm}$. The overall values for the Baiyangdian watershed were $T = 15.5\text{ }^{\circ}\text{C}$ and $\text{EC} = 1073\text{ }\mu\text{S/cm}$. Compared with the field monitoring results, approximately 50% of the water quality index values at the sampling points in the Baiyangdian watershed exceeded the negative response threshold, indicating that Baiyangdian Lake was disturbed.

Conclusions The validity of eDNA technology in biodiversity analysis was confirmed by the zooplankton community data from Baiyangdian Lake. The ecological thresholds derived by combining eDNA technology with Threshold Indicator Taxa Analysis (TITAN) are beneficial to the biological conservation of the region.

Keywords Baiyangdian, Zooplankton, eDNA, Ecological threshold, The Threshold Indicator Taxa Analysis (TITAN)

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Background

Zooplankton are important for maintaining the richness and stability of the aquatic food web [1]. Zooplankton are sensitive to changes in the environment, and their community structure will be affected by changes in water temperature, pH, and other environmental factors. This sensitivity is conducive to the evaluation of environmental water quality, and thus zooplankton are often used as indicators in environmental monitoring [2–6]. In aquatic ecosystems, species are usually identified by traditional morphological methods. However, the rich diversity and complexity of zooplankton require researchers to have extensive identification experience and knowledge reserves; otherwise, there will be species identification errors. In addition, due to the small sizes of zooplankton individuals, the collection and identification process requires significant technical requirements. Therefore, the traditional methods have limitations in practical application. Environmental DNA (eDNA) refers to the method of directly extracting target gene fragments from environmental samples (e.g., soil, sediments, and water bodies) and using various molecular technologies for qualitative or quantitative analyses [7]. As a new biological identification method [8], eDNA metabarcoding technology has been widely used in research on plankton [9–12], fish [13] and other taxa to explore the structure and spatial changes of biological communities. Compared with traditional morphological methods, the advantages of eDNA metabarcoding technology are that eDNA sample collection is non-destructive and can be analyzed without naked eye observation. The methods can be used to detect and identify a wide range of species, including invasive species. In addition, the technology can better characterize biodiversity, evaluate endangered species, assess biomass, and measure species diversity. These advantages enable the use of eDNA technology to better understand the state of the entire ecosystem [14]. Takahara et al. [15] confirmed that the method can estimate species biomass in the natural environment more easily and quickly than traditional methods such as tagging and mark–recapture. It should be noted that in order to improve the accuracy of the eDNA macro-barcode technique, future research should also focus on collecting more field data and comparing the technique with other estimation methods. In addition, eDNA metabarcoding technology can be used to interpret the influence of environmental factors on the plankton community [16] and to estimate the ecological thresholds of environmental factors such as BOD₅ and NH₃-N [17].

An ecological threshold is defined as the limit of disturbance that an ecosystem can withstand before being altered or degraded to a different system. The concept was first proposed by May [18] in the 1970s. They defined

a threshold as the breakpoint between two stable states of the system. The ecological threshold is a point or interval in which the ecosystem rapidly changes from one state to another, and the driving force for this change comes from small additional changes in one or more key ecological factors [19]. Different environmental factors have various ecological thresholds [20], and studying of the relationship between the two is crucial for protecting biodiversity and maintaining the stability of the ecosystem [21]. At present, studies on the threshold values of grasslands [22], lakes [21], rivers [23] and other ecosystems have been carried out. A large number of nonlinear statistical methods such as nonparametric analysis, Bayesian analysis, and piecewise regression analysis have been used to deduce the ecological thresholds of various environmental factors [24, 25]. Other studies have found that ecological genome methods based on genetic diversity can also be used to determine the ecological thresholds of factors and establish water quality standards, for example location-specific water quality standards (WQC) for ammonia in Taihu Basin [21]. In addition, Threshold Indicator Taxa Analysis (TITAN) is regarded as an effective method of estimating an ecological threshold. This method was proposed by Baker and colleagues and features combining the two methods of inflection point analysis and indicator species analysis. The positive and negative response directions of indicator species to environmental disturbances are identified, and ecological thresholds of environmental factors are then derived [26, 27]. In recent years, TITAN methods have been widely used to study the responses of benthic organisms [26, 28] macroinvertebrates [29], zooplankton [30] and algae [31] to changes in different environmental factors.

Baiyangdian Lake (115°45′–116°07′E, 38°44′–38°49′N) is the largest natural freshwater lake in the North China Plain [32]. The water body of Baiyangdian Lake has undergone large changes throughout its history. After 2000, the environment was improved through water replenishment. The water quality was improved, and ecological functions were gradually restored [5, 33]. In order to reveal the impact of environmental factors on biodiversity change in the Baiyangdian watershed, this study used eDNA metabarcoding technology to analyze the biodiversity of zooplankton in the lake area; the results were then used to determine the thresholds of environmental factors and to characterize the status of water quality in this region. We hypothesized that (1) variation in environmental factors would lead to significant differences in community composition and distribution among different water types (rivers, lakes) or at different points in rivers; (2) when the environmental factors exceed the response thresholds, the zooplankton community structure will be disturbed to some extent.

Materials and methods

Field sampling

In April 2019, sample collection was carried out at 15 points of seven rivers entering Baiyangdian Lake (the Nanjuma River, the Ping River, the Nanbeipu River, the Cao River, the Fu River, the Tang River, and the Xiaoyi River) and at 15 points of Baiyangdian Lake (Fig. 1).

Three parallel surface water samples (20 L) were collected from each sampling site and were combined. The plankton nets (20 μm) were cleaned with sterile water and 60 L of surface water, and then used to collect zooplankton. After the plankton samples were diluted to 1 L in sterile water, they were further filtered through 142-mm-diameter and 5-μm-aperture microporous membranes of filtration (BHLM, CHN). The filter membranes intercepted the plankton and were stored in a cryostat (liquid nitrogen) and transferred to a refrigerator at −80 °C until DNA extraction. In addition, negative controls were set to avoid the unreliability of experimental results due to sample contamination and other factors.

Measurement of environmental variables

The index environmental factors measured from water samples included temperature (T), electrical conductivity (EC), dissolved oxygen (DO), pH, turbidity (Turb), oxidation–reduction potential (ORP), and chlorophyll a (Chl-a). In addition, Baiyangdian Lake is in a mild state of

pollution according to the 2019 "Ecological Environment Condition Bulletin" issued by the Hebei provincial environmental protection department. The main water pollution index is chemical oxygen demand, and thus we used the potassium dichromate (K₂Cr₂O₇) oxidation method to determine chemical oxygen demand (COD_{Cr}) as an environmental factor.

Specifically, water T, EC, pH, turbidity, and ORP were measured in situ by a multi-parameter water quality instrument probe (MYRON Company, USA), and DO and Chl-a were measured by a portable dissolved oxygen meter (HACH Company, USA) and a handheld fluorometer (Turner Designs, USA) for in situ determination. The COD_{Cr} was measured according to the 'Determination of Water Quality Chemical Oxygen Demand' method (HJ 828-2017).

Metabarcoding analysis of the zooplankton community

eDNA extraction, amplification, and sequencing

Before the total DNA of the zooplankton community was extracted, the samples were stored in 100 ml of 100% alcohol. Then, 10 mL was extracted from each sample and filtered using a 20-μm silk sieve. This procedure was repeated three times, and the samples were combined for total DNA extraction. Total DNA was extracted from zooplankton samples using a DNeasy Blood and Tissue Kit (Qiagen Canada Inc., ON, Canada) according to the manufacturer's instructions. The concentration and mass

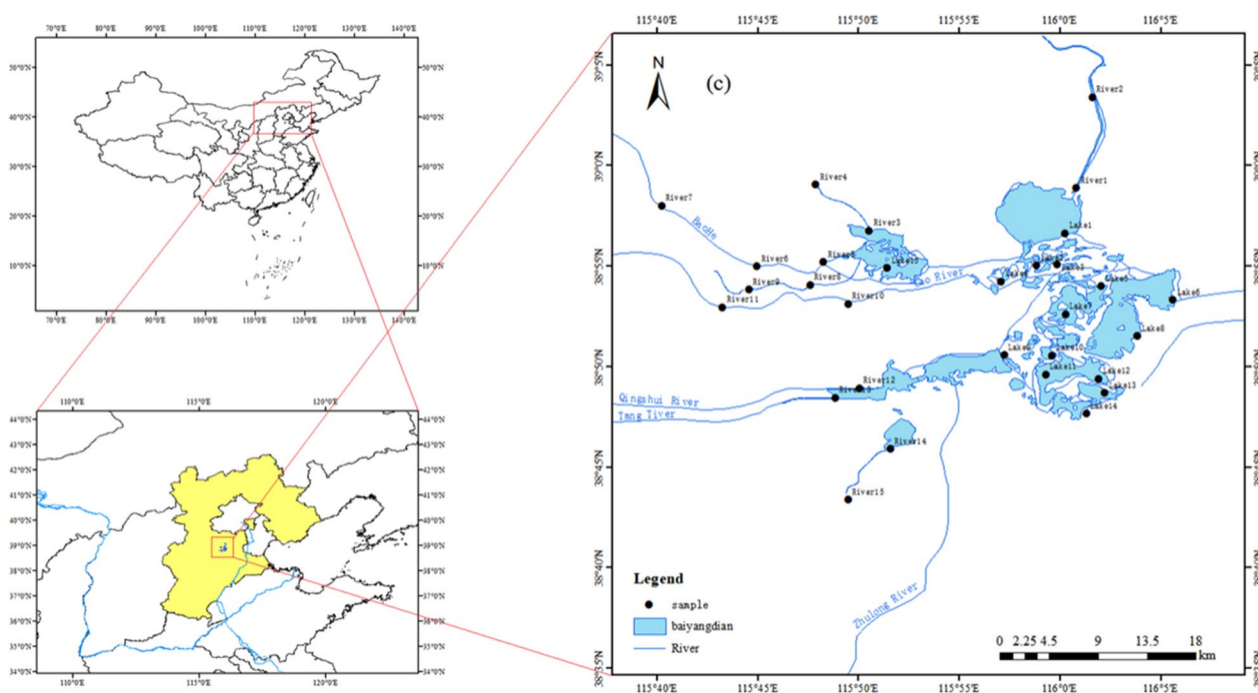


Fig. 1 Sampling sites in the Baiyangdian Lake area

of total DNA in the samples were determined using a UV spectrophotometer (NanoDrop, Thermo Fisher Scientific Inc., USA). The primers used in this study were 5-AGG GCAAAYCTGGTGCCAGC-3 (Uni18S) and 5-GRC GGTATCTRATCGYCTT-3 (Uni18SR) that were used to amplify the V4 region of nuclear small subunit ribosomal DNA (18S rDNA) of the zooplankton [34]. In order to construct a sequencing library from the 30 collected samples, this study added 8-base sequence tags (barcodes/tags) [35] that uniquely corresponded to the sample sites at the 5'-ends of the upstream and downstream primers, and PCR amplification was performed on the total DNA of each sample by using the primers with barcodes. PCR amplification was performed eight times for each sample site to avoid the influence of random biased amplification on community structure analysis. All PCR reactions were carried out with 15 μ L of Phusion[®] High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μ M of forward and reverse primers, and about 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s followed by a final extension at 72 °C for 5 min. An equal volume of IX loading buffer (containing SYB Green) was combined with the PCR products and subjected to electrophoresis on 2% agarose gels for detection. Eight PCR products from each sample site were combined in equal density ratios. The mixed PCR products were then purified using a Qiagen Gel Extraction Kit (Qiagen, Germany). Purified PCR products from the 30 zooplankton communities were then combined, and sequence libraries were generated using a TruSeq[®]DNA PCR-free sample preparation kit (Illumina, USA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit[®]2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina NovaSeq platform, and 250-bp paired-end reads were generated.

Bioinformatic analysis

The UPARSE algorithm was used to analyze the raw data from the high-throughput sequencing results. First, data errors were filtered out; the criteria were (1) sequence tags or sequences with mismatched primers (python scripts, UPARSE), sequences containing N bases, sequences with Q scores (Phred scores) < 30, and those with a maximum expected error threshold less than 0.75. Secondly, the sequences were concatenated together. In order to avoid inconsistency in lengths of sequences, the process was repeated as necessary. The filtered and concatenated sequences were processed repeatedly to reduce the amount of subsequent data processing. Finally,

a cluster analysis was carried out. In this study, the UPARSE-OTU algorithm was used to cluster the non-duplicated sequences based on 97% similarity, and OTUs along with the corresponding sequence information of each OTU were organized in an OTU table and subjected to blast comparison analysis. The BLASTn function embedded in Seed was used to conduct sequence alignment for all OTUs obtained based on the GenBank database, and the classification boundary element of each OTU was identified. The screening criteria for comparison results were an E value < 10^{-80} , minimum query coverage > 80%, and similarity > 85%. OTUs the comparison results of which were vertebrates or algae were rejected and did not participate in subsequent analysis [34–36].

The operational taxonomic units (OTUs) clustering and species classification analysis were conducted based on valid data. Representative sequences of each OTU were annotated to obtain the corresponding species information and species-based abundance distributions, and the required zooplankton OTUs were screened. At the same time, the abundance and diversity of OTUs were calculated to obtain information on the richness and evenness of species in the samples as well as the common and specific OTUs among different groups.

Statistical analysis

Changes in the zooplankton community composition

To explore the richness and diversity of the zooplankton communities at the sampling points, alpha diversity indices were computed, and a rarefaction curve was drawn [37, 38]. The distribution characteristics of the zooplankton community in Baiyangdian Lake were obtained by non-metric multidimensional scaling (NMDS) sorting analysis, and a similarity analysis based on Bray–Curtis distance (ANOSIM) was used to test for differences in dominant taxa and to further explore the differences in community structure. NMDS and ANOSIM analyses were implemented by the software PRIMER 5.0 [39, 40]. In addition, a similarity analysis (SIMPER) was used to select the main OTUs contributing to community gradient changes [40]. The $\log(x+1)$ transformation of environmental variables except for pH was performed for species relative abundance data to improve normality.

Community environment interaction analysis

Redundancy analysis (RDA) was used to analyze the relationships between zooplankton community structure and environmental factors. Before selecting redundancy analysis, we first conducted a detrended correspondence analysis (DCA) for the communities, and the results showed that the highest gradient of difference between the communities was less than 4, indicating that the zooplankton community and environmental variables

showed a linear corresponding relationship; RDA was thus considered as more suitable to reflect the relationships between the zooplankton community and environmental factors. DCA and RDA were performed using CANOCO4.5 software [41, 42].

Ecological threshold analysis

The thresholds obtained in this study were calculated by the TITAN method [26]. TITAN monitors the response patterns of species with environmental pressure gradients. The performance of individual species with varying pressure changes is measured by the IndVal score, and the reliability of the calculated thresholds is tested by bootstrapping. The calculated fraction of each species to environmental pressure is finally summed to determine the response threshold of the community to pressure, and this is labeled as the sum-z. The $\log(x+1)$ transformation of species abundance data was performed before data analysis to exclude species with a frequency less than 3 and thereby reduce the impact of rare species. After the initial mutation point of species was obtained by TITAN, the threshold and the reliability of the corresponding species were verified based on uncertainty ($P < 0.05$), purity (purity ≥ 0.95) and reliability (reliability ≥ 0.90). TITAN was conducted in R-3.6.2 software using the TITAN2 software package.

Results

Zooplankton community composition

A total of 7,596,296 raw sequences (NCBI SRA No.: PRJNA984715) were obtained from the flux sequencing results for 30 sample communities. After quality filtering and algae and vertebrate sequence removal and screening, the final effective dataset retained a total of 5,875,456 sequences (accounting for 77.3% of the original sequence data). The rarefaction curve of samples tended to be flat, indicating that the sequencing depth was able to reflect the diversity of the zooplankton in the samples (Additional file 1: Fig. S1).

A total of 3553 OTUs were obtained, of which 1293 were special to Baiyangdian Lake and 1253 were special to Baiyangdian rivers, with 1007 common OTUs. Most zooplankton sequences in the water samples belonged to *Rotifera* and *Arthropoda*, accounting for 92.40%. The remaining sequences belonged to *Mollusca*, *Annelida*, *Platyhelminthes*, *Nematoda*, *Apicomplexa*, *Bryozoa*, and *Gastrotricha* (Additional file 1: Fig. S2).

Differences in zooplankton community composition

NMDS and ANOSIM analyses of the Baiyangdian watershed showed that there were significant differences in zooplankton communities between Baiyangdian lakes and rivers, and between different rivers (Fig. 2). In the β -diversity analysis, NMDS (stress < 0.05) showed a relatively clear separation of zooplankton communities in the Baiyangdian area. ANOSIM ($R = 0.349$, $P < 0.01$) confirmed that there were significant differences between

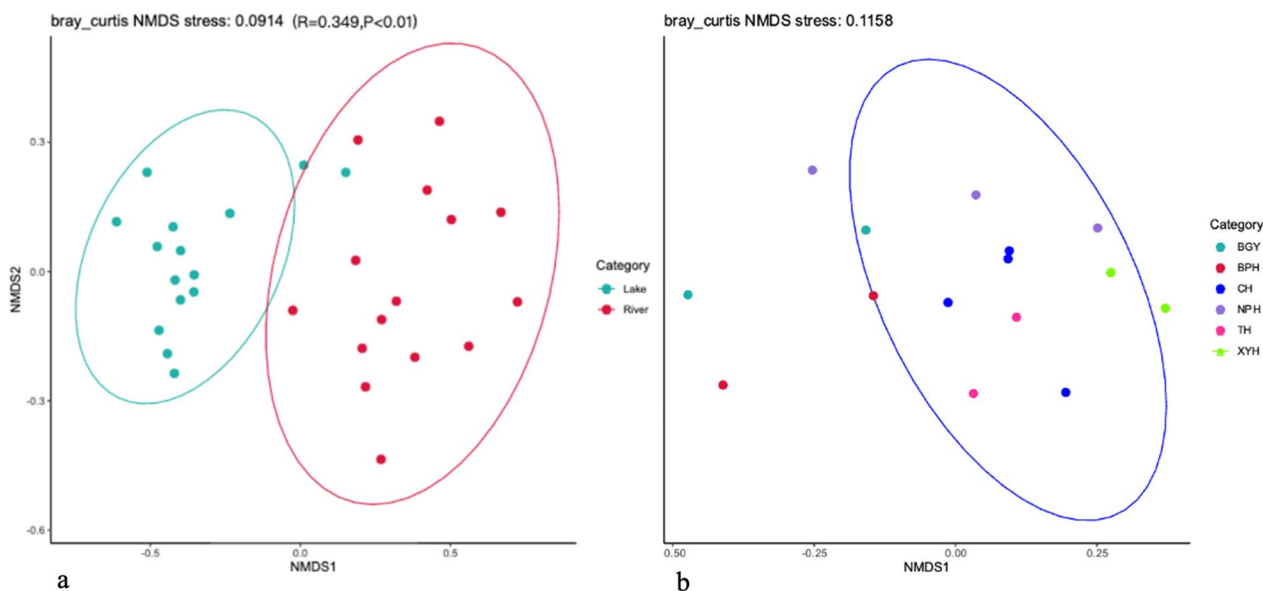


Fig. 2 Analysis based on Bray–Curtis non-metric multidimensional scale (NMDS) sorting and similarity analysis (ANOSIM). The degree of difference is shown between lakes and rivers (a) and between different rivers (b)

Table 1 Contributions of different species to the changes in communities analyzed by similarity percentage (SIMPER)

Species	Average abundance		SD	Contributions (%)
	River	Lake		
<i>Brachionus calyciflorus</i>	963.74	2202.71	0.08	31.44
<i>Thermocyclops</i> sp.	5980.73	1842.86	0.09	25.21
<i>Megacyclops viridis</i>	354.37	150.04	0.04	7.69
<i>Aeolosoma</i> sp.	2.53	190.57	0.03	4.69
<i>Corbicula fluminea</i>	147.07	302.92	0.03	4.68
<i>Eucyclops serrulatus</i>	257.92	2202.70	0.03	3.58
<i>Philodina megalotrocha</i>	22.42	424.10	0.02	3.21
<i>Sinocalanus sinensis</i>	299.97	1188.38	0.03	2.82
<i>Mesocyclops pehpeiensis</i>	1109.03	393.71	0.02	2.25
<i>Cephalodella forficula</i>	467.73	686.20	0.01	2.17
<i>Thermocyclops crassus</i>	429.03	244.59	0.01	2.12
<i>Synchaeta tremula</i>	688.20	653.73	0.01	2.06

The corresponding average abundance, standard deviation (SD) across sites in each group. The table above shows OTUs contributing (contribution > 2%)

lakes and rivers. Among different points in rivers, ANOSIM ($R > 0$, $P = 0.024$) confirmed that the difference between groups was greater than the difference within groups.

The SIMPER similarity percentage analysis showed that the relative abundances of *Brachionus calyciflorus* and *Eucyclops serrulatus* in Baiyangdian Lake were much higher than in the inflow rivers, while the relative abundances of *Thermocyclops* sp. and *Mesocyclops pehpeiensis* in Baiyangdian Lake were lower than that in the inflow rivers. These groups contributed significantly to the variation among zooplankton communities (Table 1). The alpha diversity analysis showed that the species richness of zooplankton in Baiyangdian rivers was higher than in the lake (Additional file 1: Tables S1 and S2).

Relative abundances of 13 genera of zooplankton detected in Baiyangdian Lake exceeded 1% (Fig. 3). These were *Brachionus*, *Thermocyclops*, *Eucyclops*, *Synchaeta*, *Cephalodella*, *Mesocyclops*, *Sinocalanus*, *Megacyclops*, *Filinia*, *Corbicula*, *Philodina*, *Cyclops*, and *Aeolosoma*. In Baiyangdian Lake, the most abundant genera were

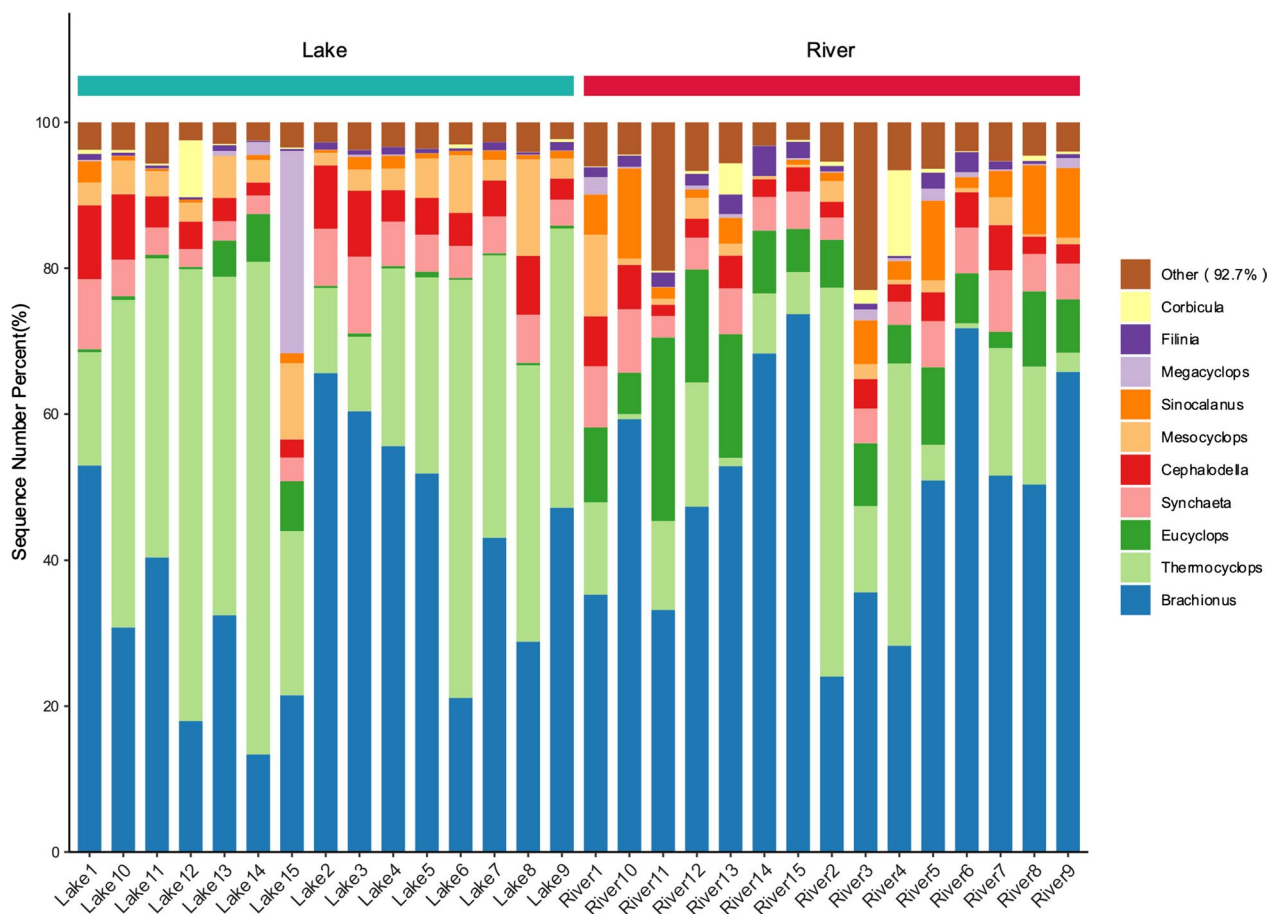


Fig. 3 Zooplankton community composition at different sampling sites in the Baiyangdian watershed using relative abundance of different taxa at the genus level

Brachionus, *Thermocyclops*, *Synchaeta*, *Cephalodella*, *Mesocyclops*, and *Megacyclops*. Relative abundances of *Brachionus*, *Thermocyclops*, *Eucyclops*, *Mesocyclops*, *Sinocalanus*, *Corbicula*, *Philodina* and *Aeolosoma* were higher in inflow rivers.

Relationship between zooplankton community and environmental factors

DCA analysis of the zooplankton communities in the Baiyangdian watershed showed that the maximum values of the first four axes of the lengths of gradient were less than 3.0, and thus RDA could be used to assess the relationships between environmental factors and zooplankton communities at different sampling points. The RDA ranking diagram showed that the first two RDA axes accounted for 56.63% of the zooplankton community structure. Among the eight measured environmental variables, T, turbidity, and EC were significantly correlated with the difference in zooplankton communities in Baiyangdian Lake (Fig. 4a). The sampling points of the inflow rivers were mainly located in the first and fourth quadrants, and the communities were closely related to T, turbidity, and EC. The lake sampling points were largely distributed in the second quadrant, and the communities were closely related to ORP, DO, and COD_{Cr} . Environmental variables played an important role in the distribution of zooplankton communities (Fig. 4a). Species such as *Mesocyclops pehpeiensis*, *Cyclops* sp., *Thermocyclops* sp., *Thermocyclops crassus*, *Itunella muelleri*, *Brachionus calyciflorus*, *Sinocalanus sinensis*, *Keratella quadrata*, *Physocypria* cf., *Lampsilis cardium*, *Aeolosoma* sp., *Macrocyclus albidus*, *Dero borellii*, *Eucyclops serrulatus*, and *Eucyclops speratus* showed significant correlations with the above six environmental variables (Fig. 4b). In the RDA analysis of rivers, T, EC, and DO were significant environmental factors affecting community structure (Fig. 4c), and species such as *Eucyclops serrulatus*, *Thermocyclops* sp., and *Brachionus calyciflorus* were strongly affected by these factors (Fig. 4d).

Ecological thresholds of key environmental factors influencing factors for zooplankton

We explored the main environmental factors affecting zooplankton using RDA analysis. The changes in community structure reflected the effects of environmental variables on the relative abundances of species. The factors T, turbidity, and EC were significantly correlated with the distribution of zooplankton in the entire Baiyangdian basin. To investigate the mechanisms by which zooplankton responded to various environmental factors and their differences in rivers and the lake, we performed a Threshold Indicator Taxa Analysis (TITAN). Turbidity was not an environmental factor with a significant influence in

the RDA analysis of rivers, and thus we did not apply turbidity in the subsequent comparative analysis.

Indicator species and thresholds of water quality indexes

The TITAN method was used to analyze the responses of zooplankton to T and EC, key environmental factors in the Baiyangdian watershed. The OTUs in Baiyangdian rivers were analyzed, and TITAN determined 27 OTUs as sum ($z-$) individual indicator taxa that decreased with increased temperature from 18.4 °C to 21.2 °C, while 11 OTUs were identified as sum ($z+$) taxa, indicating that individual taxa increased with temperature from 19.0 °C to 21.3 °C (Fig. 5, Table 2). Most OTU change points overlapped in the range of 18.4–21.2 °C. The screening of indicator species for T showed that with the increase of temperature, the maximum inflection point of sum ($z-$) taxa occurred at 19.0 °C, and the species that were good indicators included *Synchaeta tremula*, *Brachionus calyciflorus*, and *Synchaeta pectinata*. All three species occurred in all samples. The maximum inflection point of sum ($z+$) occurred at 21.1 °C, and *Brachionus urceolaris* was the main indicator species of this point. Similarly, in Baiyangdian Lake, *Synchaeta tremula* and *Cyclops* sp. were indicators for the T inflection point of sum ($z-$), and *Megacyclops viridis* and *Eucyclops serrulatus* were indicators for the sum ($z+$) point (Fig. 6, Table 2).

TITAN determined 63 OTUs as sum ($z-$), indicating a classification group that decreased with EC in the range of 747–1603 $\mu\text{S}/\text{cm}$, while only six OTUs were determined as sum ($z+$), indicating increasing with EC in the range of 882.1–1864 $\mu\text{S}/\text{cm}$. Most OTU change points overlapped in the range of 747–1603 $\mu\text{S}/\text{cm}$. The screening of indicator species for EC via TITAN showed that with the increase of EC, the change point of sum ($z-$) was 795 $\mu\text{S}/\text{cm}$, and the species that were good indicators for this point were *Fabaeformiscandona subacuta*, *Sinocalanus sinensis*, and *Diplogasteroides luxuriosae*, while the change point of sum ($z+$) was 1928 $\mu\text{S}/\text{cm}$ (Fig. 5, Table 2). Similarly, in Baiyangdian Lake, *Sinocalanus sinensis* and *Synchaeta tremula* were indicators of EC inflection point sum ($z-$), and *Thermocyclops* sp. was an indicator of sum ($z+$) (Fig. 6, Table 2).

Forty indicator species related to water temperature and 18 indicator species related to EC were screened out by TITAN (Fig. 7, Table 2). For the response of zooplankton to environmental variable T in Baiyangdian, nine water temperature-related indicator species were identified as sum ($z-$) indicator groups that decreased with increasing T between 14.8 °C and 21.0 °C, while 31 species were identified as sum ($z+$) indicator groups increasing with T ranging from 14.8 °C to 20.0 °C. The indicator species of T were screened, and sum ($z-$) reached the peak at 15.5 °C with the increase of temperature, and then

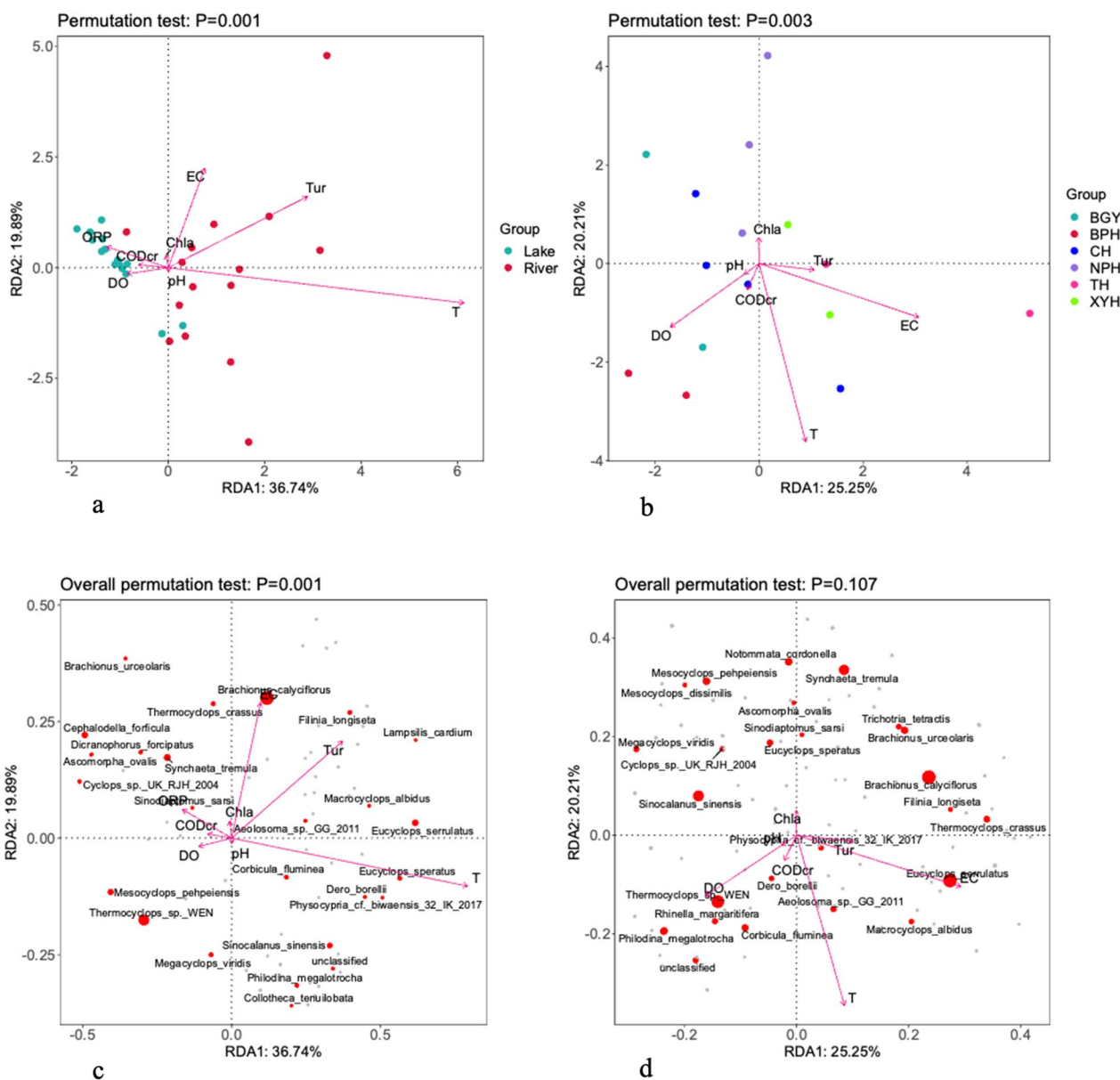


Fig. 4 RDA analysis of sample–environment relationships **a** and species–environment relationships **b** between lakes and rivers. RDA analysis of sample–environment relationships **c** and species–environment relationships **d** between streams. $P < 0.05$ indicated that the variance was significantly indigenous, and each point represented a species. The larger the point was, the higher the corresponding species abundance was. The gray point represented the species with low abundance

decreased rapidly (Fig. 7). *Cyclops* sp. and *Thermocyclops* sp. were the best indicators of this point. There was no clear peak in the species with positive response to T, and the cumulative species fraction of sum (z+) remained stable above 18.0 °C and then decreased slightly, thus determining the community threshold level at 16.0 °C. Species that were good indicators for this point included *Prodorylaimus* sp., *Lampsilis cardium*, *Physocypria* cf., and *Cypridopsis uenoi*.

By analyzing the response of zooplankton in the Baiyangdian basin to the environmental variable EC, TITAN identified 15 species as sum (z-) indicator groups that decreased with the increasing EC gradient ranging from 710 $\mu\text{S}/\text{cm}$ to 1208 $\mu\text{S}/\text{cm}$, while three species were identified as sum (z+) indicator groups, with the EC gradient ranging from 1312 $\mu\text{S}/\text{cm}$ to 1864 $\mu\text{S}/\text{cm}$. After screening the indicator species for EC, the maximum inflection point of sum (z-) occurred at 1073 $\mu\text{S}/\text{cm}$,

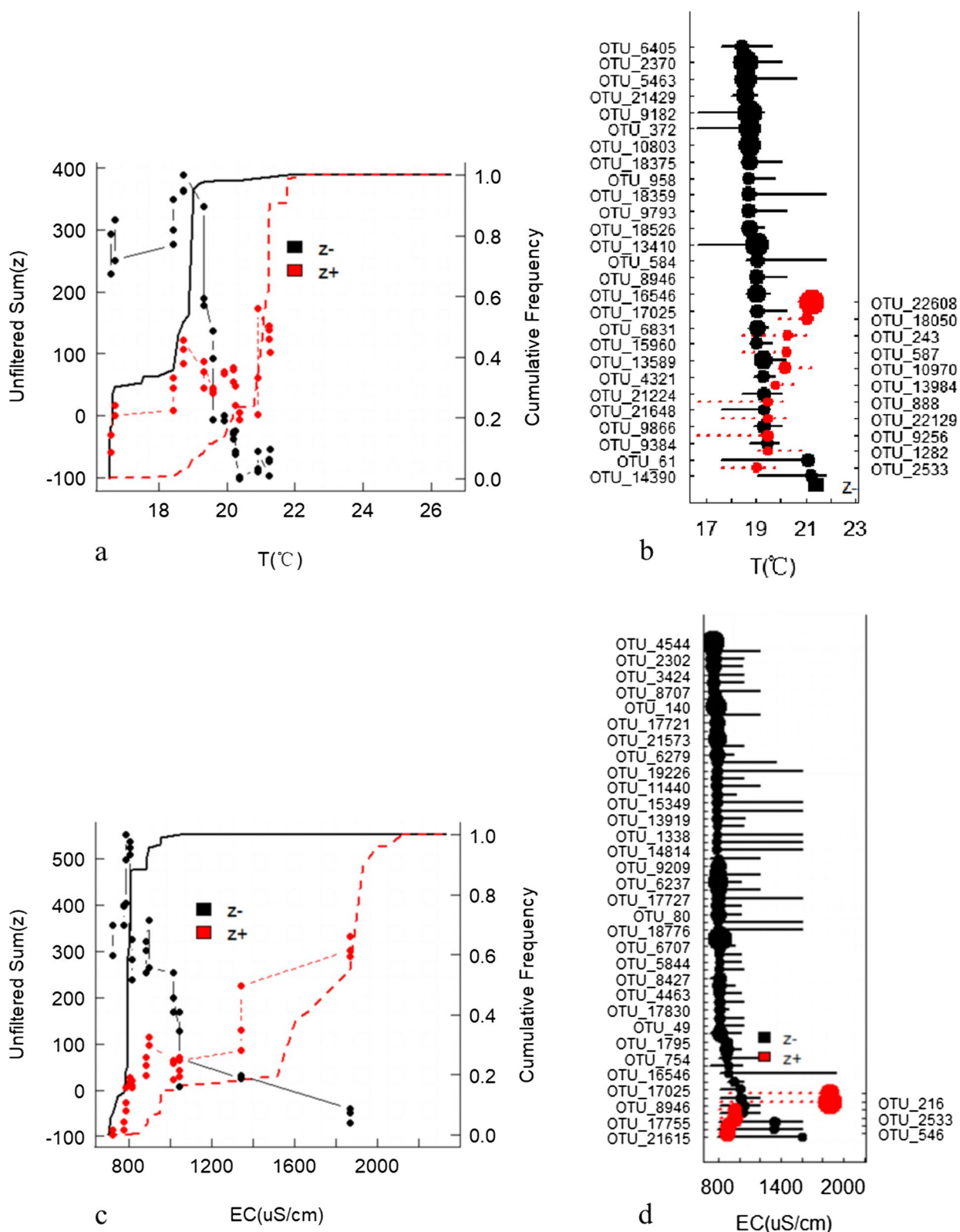


Fig. 5 The T response curve (a), T indicator species (b), EC response curve (c), and EC indicator species (d) in Baiyangdian rivers identified by TITAN. z-, zooplankton negative response species; z+, zooplankton positive response species. In the indicator species figure (left), the size of circle indicates the response intensity of species. The black solid line and red dot represent the cumulative frequency distribution of sum (z-) and sum (z+), respectively.

Table 2 Community-level thresholds of zooplankton groups to T and EC based on TITAN

Water body	Item	Sum	Env.cp	5%	10%	50%	90%	95%
Baiyangdian	T (°C)	Sum (z-)	15.5	15.4	15.5	16.0	18.2	21.3
		Sum (z+)	16.0	15.4	15.5	16.0	16.7	18.4
	EC (μS/cm)	Sum (z-)	1073	886	1027	1073	1073	1105
		Sum (z+)	1864	1341	1780	1864	1928	1992
Lake	T (°C)	Sum (z-)	14.3	14.1	14.1	14.3	16.7	16.7
		Sum (z+)	15.4	14.8	14.8	15.4	16.7	16.7
	EC (μS/cm)	Sum (z-)	920	759	824	920	1118	1178
		Sum (z+)	1363	1312	1312	1423	1615	1615
River	T (°C)	Sum (z-)	19.0	16.6	16.6	18.7	19.0	19.0
		Sum (z+)	21.1	19.0	19.5	21.1	21.3	21.8
	EC (μS/cm)	Sum (z-)	795	710	747	795	889	896
		Sum (z+)	1928	896	956	1864	1992	1992

and the species that had indicator effects on this point included *Philodina megalotrocha*, *Megacyclops viridis*, and *Diplogasteroides luxuriosae*. The maximum inflection point of sum (z+) occurred at 1864 μS/cm, and *Brachionus urceolaris* was the main indicator species of this point (Fig. 7, Table 2).

Discussion

Effects of environmental factors on the zooplankton community distribution in the Baiyangdian watershed

Due to the difference in flow structure and the ecological environment between rivers and lakes, the physical and chemical properties of turbidity, water temperature, and DO will vary [43], and the different water conditions of lakes and rivers will also affect the structure of the zooplankton community [44]. The present study focused on a single area, as relatively few studies have considered the effects of the same ecosystem on the distribution of plankton under different water conditions. Therefore, studies of rivers and lakes in the same climate and geographical area taking into account their environmental characteristics and examining the response of zooplankton to environmental factors are of practical importance for ecosystem conservation. In previous studies of the Baiyangdian watershed, the dominant genus of copepods was *Thermocyclops*, and the dominant genus of rotifers was *Brachionus* [45]. This result was consistent with the results in this study, indicating that the dominant groups of zooplankton in the Baiyangdian area have changed little in recent years. However, the spatial variation of zooplankton in different regions of rivers and lakes was a result of environmental impacts. The different regional characteristics of the lakes and rivers in Baiyangdian affected the abundance of zooplankton. In this study, the relative abundances of *Brachionus*, *Thermocyclops*,

Synchaeta, *Cephalodella*, *Mesocyclops* and *Megacyclops* in Baiyangdian Lake were higher, while *Brachionus*, *Thermocyclops*, *Eucyclops*, *Mesocyclops*, *Sinocalanus*, *Corbicula*, *Philodina* and *Aeolosoma* were the most abundant genera in the inflow rivers. This phenomenon indicated that in the same season and the same study area, rivers and lakes may have different biological distributions due to their intrinsic differences.

T, turbidity, and EC were identified as the main environmental factors that influenced the variation in the structure of zooplankton communities in the Baiyangdian watershed. Wang et al. [46] pointed out that zooplankton are highly sensitive to water temperature changes, a finding that was in accordance with our research results. In addition, it is also known that water temperature and EC are important environmental factors affecting the growth and abundance of phytoplankton, and thus can indirectly affect the community structure of zooplankton by directly acting on the phytoplankton. For example, *Microcystis* easily forms colonies, and some *filamentous cyanobacteria* have relatively large particle sizes, making them less easy to be preyed on, thereby affecting the growth of zooplankton [47]. In addition, there are few studies on the correlation between zooplankton community structure and turbidity. It has been speculated that the abundance of zooplankton in Baranagua Bay being extremely high may be due to the fact that the "maximum turbidity area" around the bay hinders the movement of zooplankton and aggregates the zooplankton in the high turbidity area [48]. Other studies have shown that summer turbidity in the Bohai Sea is higher than winter turbidity, resulting in a more significant correlation between zooplankton abundance and summer turbidity. Researchers have speculated that the contribution

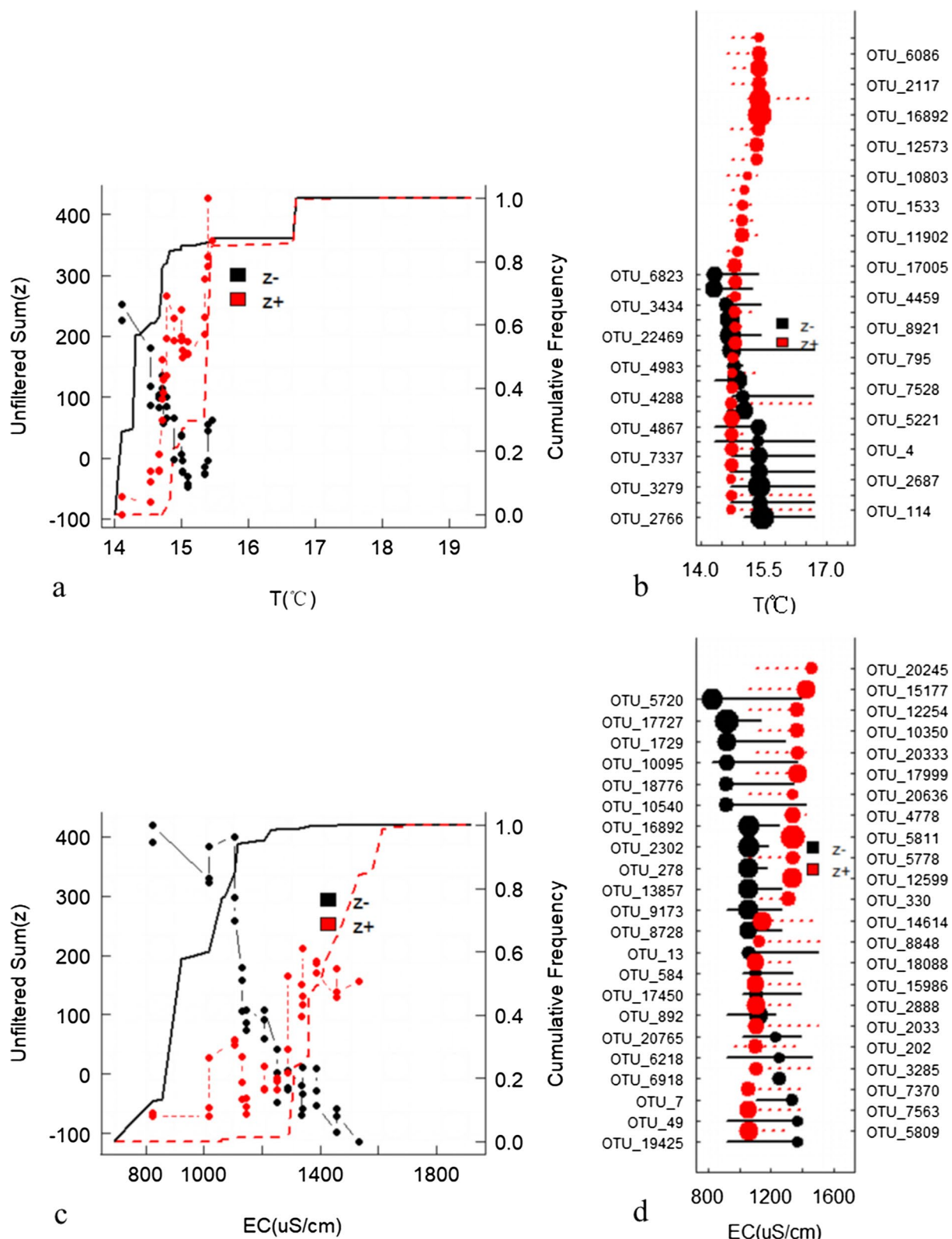


Fig. 6 The T response curve (a), T indicator species (b), EC response curve (c), and EC indicator species (d) in Baiyangdian Lake identified by TITAN. z-, zooplankton negative response species; z+, zooplankton positive response species. In the indicator species figure (left), the size of circle indicates the response intensity of species. The black solid line and red dot represent the cumulative frequency distribution of sum (z-) and sum (z+), respectively.

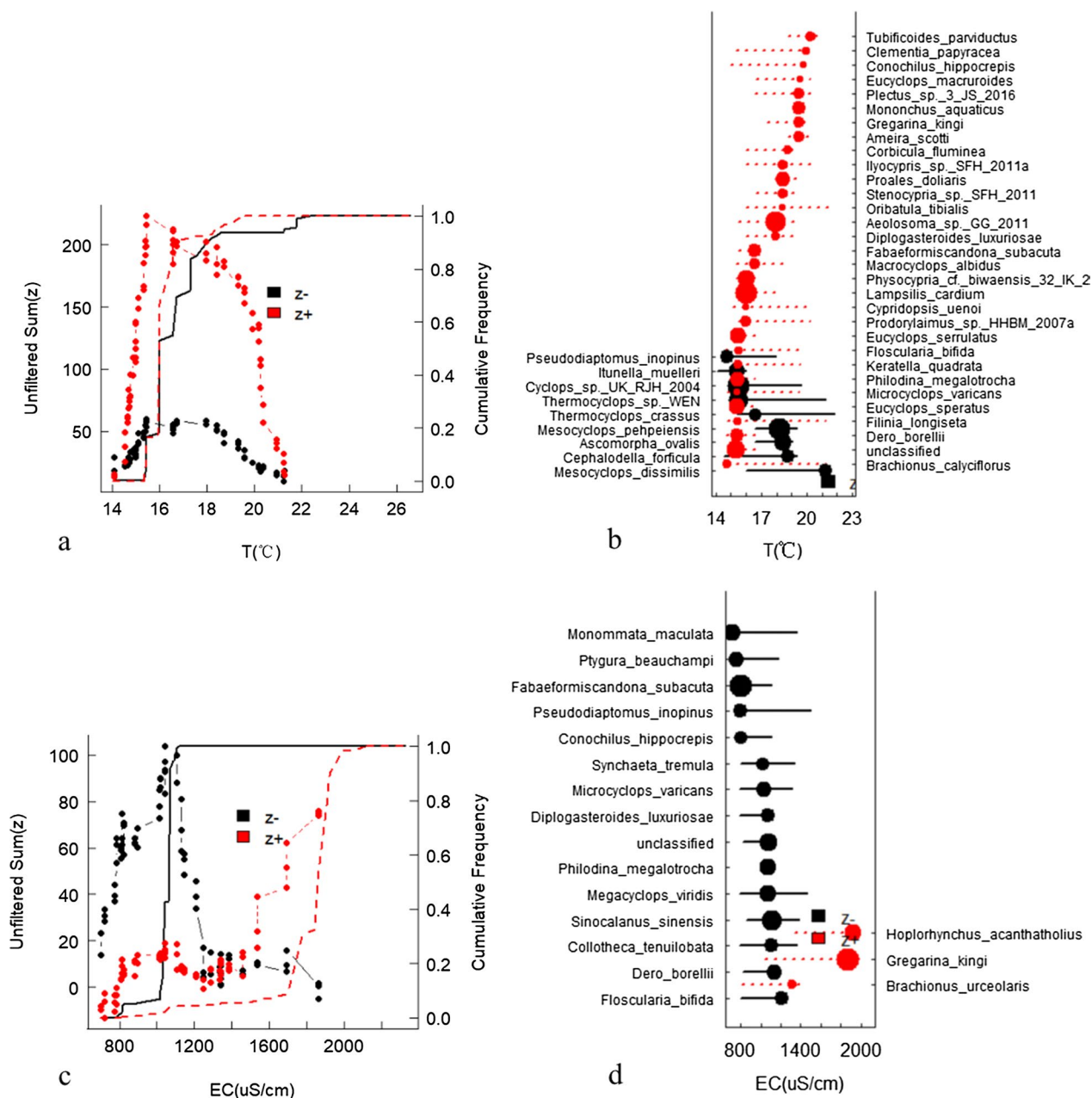


Fig. 7 The T response curve (a), T indicator species (b), EC response curve (c), and EC indicator species (d) in the Baiyangdian watershed obtained by TITAN. z−, zooplankton negative response species; z+, zooplankton positive response species. In the indicator species figure (left), the size of circle indicates the response intensity of species. The black solid line and red dot represent the cumulative frequency distribution of sum (z−) and sum (z+), respectively.

of zooplankton density to turbidity in the study area is more significant, and the abundance of zooplankton in summer is much higher than in winter, hence the contribution to turbidity is higher, resulting in a more significant correlation of zooplankton with turbidity [49]. Therefore, the reason for the significant influence of turbidity as a factor in this study could be that

seasonality affects zooplankton density, resulting in an enhanced correlation between organisms and turbidity.

Indicator species and environmental thresholds

Screening of indicator species is important for analyzing the relationships between biological populations and the environment as well as for environmental monitoring

and management. At present, there are no studies on indicator species in the Baiyangdian watershed. In this study, the TITAN analysis of zooplankton communities was performed based on environmental DNA technology. More than 80% of the indicator groups were in a strong indicator state for significant environmental factors, confirming the efficient indicator properties of zooplankton. The threshold of the water quality index in Baiyangdian watershed was analyzed using the TITAN method. When the water quality index exceeds the negative response threshold, the community structure of zooplankton begins to be disturbed, indicating a decrease in the density of sensitive species. When the water quality index exceeds the positive response threshold, some resistant species also reach the tolerance limit and begin to decline. The structure of the zooplankton community will then change significantly. Therefore, the negative response threshold can be used as the minimum value to trigger a change in the zooplankton community, and the positive response threshold can be regarded as the tolerance limit value of the zooplankton community [19, 50]. By comparing the number and distribution characteristics of zooplankton species in rivers and lakes in the Baiyangdian watershed, we found that the biodiversity was highest in areas (rivers) with high temperature and low electrical conductivity. The results suggested that the decrease in temperature and the increase in electrical conductivity reduced the frequency of zooplankton-sensitive species. Comparing different water quality index thresholds with the field sampling data, we found that the water quality index values of more than 56.7% of the sampling points in the Baiyangdian watershed exceeded the negative response threshold for temperature, and 53.3% of the sampling points exceeded the corresponding positive response threshold. In addition, 46.7% of the sampling points exceeded the corresponding negative threshold for electrical conductivity, and 6.7% of the sampling points exceeded the positive response threshold.

The community thresholds of T and EC were analyzed in different regions of the Baiyangdian watershed. Among the species indicated by the EC of the river, the period of gradual change in the range of the species composition due to the increase of the EC was the factor that led to the greatest change in the community structure. The negative response for EC reached a peak at 795 $\mu\text{S}/\text{cm}$ and then decreased sharply. The community threshold of most positive response species was within 1100 $\mu\text{S}/\text{cm}$, and the upper limit threshold sum ($z+$) was much higher than this (1928 $\mu\text{S}/\text{cm}$). Therefore, the community thresholds for tolerant species were inconsistent. The negative response species to EC in the lake decreased sharply at 920 $\mu\text{S}/\text{cm}$, and no clear peak was observed for the tolerant species. The cumulative species fraction ($z+$)

increased by about 500 $\mu\text{S}/\text{cm}$ and remained stable above the value of 1350 $\mu\text{S}/\text{cm}$, then showed a slight decrease, thus determining the threshold level of tolerant species at 1363 $\mu\text{S}/\text{cm}$. The temperature-sensitive species in the river decreased sharply after reaching the peak at 19.0 $^{\circ}\text{C}$, and the temperature-tolerant species decreased slightly after 21.0 $^{\circ}\text{C}$; the community threshold of temperature-tolerant species was therefore determined. In lakes, due to the gradual change in the species composition caused by the increase in T , there was no overlap in the change points of many species with similar concentrations, and thus it was impossible to propose indicator species for the change points of T .

At present, most studies generally focus on the derivation of the nutrient threshold. There have been few studies on the ecological thresholds for other water quality indicators using the TITAN method. Richards et al. [29] used the TITAN method to analyze stream invertebrates in Idaho. The temperature change point for macroinvertebrates decreasing with the increase of temperature was about 20.5 $^{\circ}\text{C}$, a value that was similar to our threshold results; however, the temperature change point of groups that increased with the increase of temperature was about 11.5 $^{\circ}\text{C}$, far lower than our results. Schröder et al. [31] used TITAN to describe the changes in the macroinvertebrate community composition between 800 and 1000 $\mu\text{S}/\text{cm}$ and obtained the community threshold levels of sensitive species ($\text{EC}=926 \mu\text{S}/\text{cm}$) and tolerant species ($\text{EC}=1416 \mu\text{S}/\text{cm}$). This was similar to our results. We may conclude that the differences in threshold values between studies may arise from variation in environmental factors and the distribution of biological groups in the study area. In addition, differences in environmental factors such as water bodies can affect the threshold derivation results. It should be noted that EC is highly susceptible to pollutants, and thus 1073 $\mu\text{S}/\text{cm}$ can only be used as a reference value for EC biological protection in the Baiyangdian watershed. Specific analysis should also be combined with the results of nearby areas and laboratory toxicology tests.

In the present study, we also found that there were some differences in the indicator species for the ecological thresholds of the water quality indicators in different regions. Different indicator species have been analyzed and compared to the dominant genera in the Baiyangdian area. *Brachionus calyciflorus* and *Synchaeta pectinata* were widely distributed and had high frequencies of occurrence, and thus could be used as negative response indicator species of river regional temperature. *Sinocalanus sinensis* could be used as a negative response indicator species of regional river EC, while *Synchaeta tremula* could be used as an indicator species of regional lake T . *Sinocalanus sinensis* could be used as indicator

species of regional lake EC. Among the EC indicator species in Baiyangdian Lake, the negative response species *Collotheca tenuilobata*, *Floscularia bifida*, and *Synchaeta tremula* and the positive response species *Brachionus urceolaris* had the highest occurrence frequencies, while the other indicator species occurred at relatively low frequencies. Among the change points corresponding to the indicator species of the EC, only one species was above the positive response threshold for EC, while the others were basically between the positive and negative response thresholds. This indicated that when the EC concentration in the water exceeded 1864 $\mu\text{S}/\text{cm}$, although only a small number of species would show changes in density, most zooplankton will have exceeded the EC tolerance limit, and the zooplankton community would not produce a significant threshold response. In addition, although the EC indicator species *Monommata maculata*, *Ptygura beauchampi* and *Gregarina kingi* appeared less frequently, they were highly indicative, emphasizing that the determination of an EC threshold cannot ignore the influence of rare species. The sum (z^-) for T reached a peak at 15.5 °C with the increase of temperature, and then decreased rapidly. The species that were good indicators for this point included *Cyclops* sp. and *Thermocyclops* sp. There was no clear peak in the species with positive response to T, and the cumulative species fraction sum (z^+) remained stable above 18.0 °C and then decreased slightly, thereby determining the threshold level of tolerant species as 16.0 °C. The negative response species *Cyclops* sp., *Thermocyclops* sp., *Thermocyclops crassus*, *Mesocyclops pehpeiensis*, *Ascomorpha ovalis*, *Cephalodella forficula* and the positive response species *Brachionus calyciflorus*, *Dero borellii*, *Eucyclops speratus*, *Filinia longiseta*, *Eucyclops serrulatus* and *Floscularia bifida* appeared in all samples. *Pseudodiaptomus inopinatus*, *Mesocyclops dissimilis*, *Philodina megalotrocha*, *Microcyclops varicans*, *Keratella quadrata*, *Macrocyclus albidus*, *Corbicula fluminea* and *Conochilus hippocrepis* were among the species with relatively high occurrence. The occurrence of indicator species was less than 38% in all samples. The data showed that the threshold of T was 15.5 °C, and the threshold of EC was 1073 $\mu\text{S}/\text{cm}$. This means that 50.0% of the sensitive species could be protected when T was less than 15.5 °C, and 65.8% of the sensitive species could be protected when EC was less than 1073 $\mu\text{S}/\text{cm}$.

Conclusion

In this study, we combined eDNA metabarcoding technology and the TITAN method to explore the response relationships between zooplankton communities and environmental factors in the Baiyangdian watershed and to determine the relevant ecological thresholds.

Through comparison with previous studies, we found that the community composition of zooplankton based on eDNA metabarcoding technology was similar to that based on morphological methods. Under the influence of environmental factors, the community structure in different regions (rivers and lakes) was significantly different. The TITAN method identified positive and negative response relationships between indicator species and environmental factors, and the data could be used to emphasize different ecological thresholds. At the same time, the threshold values for different environmental factors varied among research areas. Therefore, we believe that the thresholds derived by combining eDNA technology and the TITAN method are significant for regional protection.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12302-023-00761-0>.

Additional file 1: Fig. S1. Rarefaction curves of zooplankton OTU at each point in Baiyangdian Lake. **Fig. S2.** Composition of zooplankton OTUs. **Table S1.** a Diversity analysis of each point in Baiyangdian Lake. **Table S2.** Range and average value of field environmental variables in three different areas of Baiyangdian.

Acknowledgements

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Author contributions

JC and SW conceived, designed, and performed the experiments. JC, SW, ZY conducted the bioinformatic and statistical analysis. XZ and ZY were major contributors in methodology and funding acquisition. JC, SW and ZY wrote the article and made a critical revision of the manuscript. We also acknowledge MF, JW, XZ and QZ for their assistance with the experimental sampling and data analyses. All authors read and approved the final manuscript.

Funding

This work was financially supported by the National Key R&D Program of China (Grant No. 2021YFC3201005) and the Key Research and Development Program of Hebei Province (Grant No. 20374204D).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 15 October 2022 Accepted: 1 July 2023

Published online: 22 July 2023

References

- Yang Y, Chen H, Yang ZF (2012) Integration of water quantity and quality in environmental flow assessment in wetlands. *Procedia Environ Sci* 13:1535–1552. <https://doi.org/10.1016/j.proenv.2012.01.146>
- Ismail AH, Lim CC, OMAR WMM (2019) Evaluation of spatial and temporal variations in zooplankton community structure with reference to water quality in Teluk Bahang Reservoir, Malaysia. *Trop Ecol* 60:186–198
- Picapedra PHS, Fernandes C, Lansac-Toha FA (2017) Zooplankton community in the Upper Parnaíba River (Northeastern, Brazil). *Braz J Biol* 77:402–412
- Portinho JLO, Nogueira MG (2017) Does artificial drawdown affect zooplankton structure in shallow lakes? A short-term study in a tropical reservoir. *Hydrobiologia* 797:303–318
- Tang C, Yi Y, Yang Z, Zhou Y et al (2019) Planktonic indicators of trophic states for a shallow lake (Baiyangdian Lake, China). *Limnologia* 1(78):125712
- Isibor PO, Imoobe TO, Dedeke GA, Adagunodo TA, Taiwo OS (2020) Health risk indices and zooplankton-based assessment of a tropical rainforest river contaminated with iron, lead, cadmium, and chromium. *Sci Rep* 10(1):16896
- Ficetola GF, Miaud C, Pompanon F et al (2008) Species detection using environmental DNA from water samples. *Biol Lett* 4(4):423–425
- Wang S, Yan Z, Hanfling B et al (2021) Methodology of fish eDNA and its applications in ecology and environment. *Sci Total Environ* 755:142622
- Djurhuus A, Pitz K, Sawaya NA, Rojas-Márquez J, Michaud B, Montes E, Muller-Karger F, Breitbart M (2018) Evaluation of marine zooplankton community structure through environmental DNA metabarcoding. *Limnol Oceanogr Methods* 16(4):209–221
- Bucklin A, Ortman BD, Jennings RM et al (2010) A “Rosetta Stone” for metazoan zooplankton: DNA barcode analysis of species diversity of the Sargasso Sea (Northwest Atlantic Ocean). *Deep Sea Res Part II* 57:2234–2247
- Hirai J, Yasuike M, Fujiwara A et al (2015) Effects of plankton net characteristics on metagenetic community analysis of metazoan zooplankton in a coastal marine ecosystem. *J Exp Mar Biol Ecol* 469:36–43
- Suter L, Polanowski AM, Clarke LJ et al (2021) Capturing open ocean biodiversity: comparing environmental DNA metabarcoding to the continuous plankton recorder. *Mol Ecol* 30:3140–3157
- Xie Y, Ni X, Zeng D et al (2016) Effect of complex nutritional preparation on the plankton community structure and water regulation. *J Agric Biotechnol* 24:738–746
- Fan J, Wang S, Li H et al (2020) Modeling the ecological status response of rivers to multiple stressors using machine learning: a comparison of environmental DNA metabarcoding and morphological data. *Water Res* 183:116004
- Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata ZI (2012) Estimation of fish biomass using environmental DNA. *PLoS ONE* 7(4):e35868
- Sun Y, Liu Y, Wu C, Fu X, Guo C, Li L, Sun J (2021) Characteristics of eukaryotic plankton communities in the cold water masses and nearshore waters of the south yellow sea. *Diversity* 13(1):21
- Li F, Wang S, Zhang Y et al (2022) DNA metabarcoding reveals human impacts on macroinvertebrate communities in polluted headwater streams: Evidence from the Liao River in northeast China. *Environ Pollut* 300:118929
- MAY RM (1977) Thresholds and breakpoints in ecosystems with a multiplicity of stable states. *Nature* 269:471–477
- Groffman P, Baron J, Blett T et al (2006) Ecological thresholds: The key to successful environmental management or an important concept with no practical application? *Ecosystems* 9:1–13
- Huggett AJ (2005) The concept and utility of “ecological thresholds” in biodiversity conservation. *Biol Cons* 124:301–310
- Yang J, Zhang X, Xie Y et al (2017) Ecogenomics of zooplankton community reveals ecological threshold of ammonia nitrogen. *Environ Sci Technol* 51:3057–3064
- Hoffmann WA, Geiger EL, Gotsch SG, Rossatto DR, Silva LC, Lau OL, Haridasan M, Franco AC (2012) Ecological thresholds at the savanna-forest boundary: how plant traits, resources and fire govern the distribution of tropical biomes. *Ecol Lett* 15(7):759–768
- Daily JP, Hitt NP, Smith DR et al (2012) Experimental and environmental factors affect spurious detection of ecological thresholds. *Ecology* 93:17–23
- Qian SS, King RS, Richardson CJ (2003) Two statistical methods for the detection of environmental thresholds. *Ecol Model* 166:87–97
- Smith AJ, Thomas RL, Nolan JK et al (2013) Regional nutrient thresholds in Wadeable streams of New York State protective of aquatic life. *Ecol Ind* 29:455–467
- Baker ME, King RS (2010) A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods Ecol Evol* 1:25–37
- Baker ME, King RS (2013) Of TITAN and straw men: an appeal for greater understanding of community data. *Freshwater Science* 32:489–506
- King RS, Richardson CJ (2003) Integrating bioassessment and ecological risk assessment: an approach to developing numerical water-quality criteria. *Environ Manag* 31:795–809
- Richards DC, Lester G, Pfeiffer J, Pappani J (2018) Temperature threshold models for benthic macroinvertebrates in Idaho Wadeable streams and neighboring ecoregions. *Environ Monit Assess* 190(3):120
- Yang Y, Ni P, Gao Y et al (2018) Geographical distribution of zooplankton biodiversity in highly polluted running water ecosystems: validation of fine-scale species sorting hypothesis. *Ecol Evol* 8:4830–4840
- Schroder M, Sondermann M, Sures B et al (2015) Effects of salinity gradients on benthic invertebrate and diatom communities in a German lowland river. *Ecol Ind* 57:236–248
- Chen CY, Pickhardt PC, Xu MQ et al (2008) Mercury and arsenic bioaccumulation and eutrophication in Baiyangdian Lake, China. *Water Air Soil Pollut* 190:115–127
- Li DK, He P, Liu CQ et al (2021) Evaluation of eutrophication level changes in Baiyangdian Lake based on multiple biological groups. *Ying Yong Sheng Tai Xue Bao* 32:4488–4498
- Zhan A, Hulak M, Sylvester F et al (2013) High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. *Methods Ecol Evol* 4:558–565
- Parameswaran P, Jalili R, Tao L, Shokralla S, Gharizadeh B, Ronaghi M, Fire AZ (2007) A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. *Nucleic Acids Res* 35(19):e130
- Vetrovsky T, Baldrian P (2013) Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED. *Biol Fertil Soils* 49:1027–1037
- Tuomisto H (2010) A consistent terminology for quantifying species diversity? Yes, it does exist. *Oecologia* 164:853–860
- Wang Y, Sheng H-F, HE Y et al (2012) Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags. *Appl Environ Microbiol* 78:8264–8271
- Clarke KR, Gorley RN 2001. PRIMER Version 5.0: User Manual/Tutorial.
- Clarke KR, Warwick RM (1994) Similarity-based testing for community pattern: the two-way layout with no replication. *Mar Biol* 118:167–176
- Etten EV (2005) Multivariate analysis of ecological data using CANOCO. *Austral Ecol* 30:486–487
- Braak T, Smilauer P. Canoco reference manual and user's guide: software for ordination, version 5.0. Ithaca USA: Microcomputer Power, 2012.
- Faggotter SJ, Webster IT, Burford MA (2013) Factors controlling primary productivity in a wet-dry tropical river. *Mar Freshw Res* 64:585–598
- Gorski K, Collier KJ, Duggan IC et al (2013) Connectivity and complexity of floodplain habitats govern zooplankton dynamics in a large temperate river system. *Freshw Biol* 58:1458–1470
- Wang YZ, Luo Y, Zhou XS, Zhang SL, Cui WY (2015) Zooplankton biodiversity and water ecological evaluation in Baiyangdian lake. *J Water Resour Water Eng.* 26:94–100
- Wang P, Yan Z, Yang S et al (2019) Environmental DNA: an emerging tool in ecological assessment. *Bull Environ Contam Toxicol* 103:651–656

47. Xushen Z, Na L, Bowen S (2021) Seasonal variation of plankton community structure in the Lake Baiyangdian and the relationship with environmental factors. *Water Resour Hydropower Res.* 52(8):1–14 **(in Chinese)**
48. Salvador B, Bersano JGF (2017) Zooplankton variability in the subtropical estuarine system of Paranagua Bay, Brazil, in 2012 and 2013. *Estuar Coast Shelf Sci* 199:1–13
49. Bu YQ, Zhu LY, Cheng X, Dong HH, Sui Y, Wang C (2019) Community structure of zooplankton and its relationship with environmental factors in the Bohai and the north Huanghai Sea in summer and winter. *Period Ocean Univ China* 49(02):59–66 **(in Chinese)**
50. Tang T, Ren Z, Tang T et al (2016) Total nitrogen and total phosphorus thresholds for epilithic diatom assemblages in inflow tributaries of the Three Gorges Reservoir, China. *Ying Yong Sheng Tai Xue Bao* 27:2670–2678

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