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## Method for screening water physicochemical parameters to calculate water quality index based on these parameters' correlation with water microbiota

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## ABSTRACT

Water quality index (WQI) plays a crucial role in guiding water resource management. However, WQI calculation methods are not uniform, especially the selection of water parameters and the weighting given to each water parameter (P<sub>i</sub>). To optimize WQI calculation, 132 water samples from seven rivers and from Chaohu Lake (33 sampling sites in Chaohu Lake Basin) in four seasons were collected, and the water parameters and microbiota composition were analyzed using high-throughput sequencing of 16 S rDNA. The correlation coefficient  $R^2$  between water parameters and microbiota composition using redundancy analysis with the Monte Carlo method were calculated, and the water parameters that significantly correlated with the microbiota composition were selected to calculate WQI<sub>min</sub>. The results showed that TP, COD, DO, and Chl a correlated significantly with water microbiota composition. WQI<sub>b</sub> calculated by substituting  $R^2$  for  $P_i$  was more consistent with the similarity between the microbiota compositions. WQI<sub>minb</sub> calculated using TP, COD, and DO was consistent with WQI<sub>b</sub>. The results of WQI<sub>b</sub> and WQI<sub>minb</sub> were more consistent than those of WQI and WQI<sub>min</sub>. These results imply that using  $R^2$  instead of  $P_i$  could help obtain a more stable WQI<sub>b</sub> that could better reflect the biological characteristics of the Chaohu Lake Basin.

## 1. Introduction

Rivers and lakes are important components of the Earth's ecosystem, acting as primary links between terrestrial and ocean habitats [1]. Rivers and lakes provide important water resources for irrigation, and for industrial and domestic purposes [2,3]. Therefore, rivers and lakes play an essential role in maintaining the geochemical cycle and human civilization [4]. Serious water quality degradation events occur commonly worldwide due to numerous stresses, including natural processes, and anthropogenic activities [3,5,6]. Although water quality assessment in rivers and lakes is important [6], and water quality index (WQI) and minimum WQI (WQI<sub>min</sub>) have been widely used to indicate water quality [3,7–12], when calculating WQI, there is no unified weighting factor for each water physicochemical parameter, leading to nonuniformity of conclusions. For instance, regarding the calculation of WQI, Mathuthu et al.

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Abbreviations: WQI, water quality index; WT, water temperature; ZGR, Zhegao River; NFR, Nanfei-Dianbu River; PR, Pai River; HBR, Hangbu-Fengle River; BSSR, Baishishan River; ZR, Zhao River; YXR, Yuxi River; RDA, redundancy analysis; WCL, West Chaohu Lake; ECL, East Chaohu Lake.

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[7] set weights for DO, fecal coliforms, pH, BOD,  $NO_3^-$ ,  $PO_4^{3-}$ , temperature, suspended solids, and total solids to 0.17, 0.15, 0.12, 0.10, 0.10, 0.10, 0.10, 0.08, and 0.08, respectively. Debels et al. [13] set weights for DO, BOD5, ammonia, nitrates, nitrites, orthophosphate, temperature, pH, conductivity, and COD to 0.18, 0.17, 0.13, 0.07, 0.07, 0.12, 0.10, 0.10, 0.06, and 0.17, respectively. Pesce and Wunderlin [14] introduced a subjective constant k, and when calculating the subjective WQI, set weights for DO, total solids, and surfactants to the maximum value (4); ammonia nitrogen, BOD-5, COD, and total coliforms to 3; nitrates, nitrites, oil and grease, dissolved solids, sulfates, and turbidity to 2; and calcium, chloride, hardness, magnesium, pH, phosphorus, and temperature to 1. Therefore, establishment of a unified calculation method of the weighting factor for each water physicochemical parameter is of great significance for optimizing calculation of WQI and WQI<sub>min</sub> and avoiding artificial deviation.

The microbiota in rivers and lakes is a diverse functional assemblage of microorganisms, including viruses, prokaryotes, and microeukaryotic phototrophs and heterotrophs that influence key processes in river and lake nutrient cycles [15,16]. These microbes are vital to the river food web, and changes in their composition can vary temporally, resulting in unintended consequences for river ecosystem health [17]. Seasonal fluctuations in flow, water temperature (WT), input of inorganic compounds, and suspension and deposition of allochthonous and autochthonous organic materials have the potential to alter the structure of microbial communities in rivers and lakes [18,19], thereby affecting the circulation of geochemical substances and the flow of energy through water microorganisms. By analyzing the correlation between environmental factors and the microbiota in water, the weights of environmental factors can be calculated, potentially increasing the environmental indicative value and objectivity of WQI and WQI<sub>min</sub>.

The Chaohu Lake Basin ( $30^{\circ}52'25''-32^{\circ}7'53''N$ ,  $116^{\circ}23'59''-118^{\circ}22'5''E$ ) with  $1.35 \times 10^4$  km<sup>2</sup> of drainage area belongs to the water system on the left bank of the downstream region of the Yangtze River. The area has a subtropical humid monsoon climate, with four distinct seasons [2]. There are 33 rivers in the Chaohu Lake Basin that belong to 7 river systems, namely Zhegao River (ZGR), Nanfei-Dianbu River (NFR), Pai River (PR), Hangbu-Fengle River (HBR), Baishishan River (BSSR), Zhao River (ZR), and Yuxi River (YXR). In this system, YXR flows out of the lake and connects with the Yangtze River, and the other six rivers are tributaries to the lake [20]. Zhang et al. [21] divided the seven rivers into different river types according to the landscape composition of the river basin: HBR is a forest river, NFR and PR are urban rivers, BSSR and ZR are agricultural rivers, ZGR is a mixed type river, the YXR estuary and upstream region of the river are mixed type rivers, while its midstream and downstream regions are forest rivers.

To verify that WQI and WQI<sub>min</sub> can be calculated based on reliable correlations between microbiota and physicochemical parameters, the water physiochemical parameters of the rivers and lake water samples collected in the Chaohu Lake Basin over a year were measured, and the water microbiota composition were analyzed through high-throughput sequencing of 16 S rDNA amplification. The correlation coefficient  $R^2$  between each physicochemical parameter and the microbiota composition were calculated, and the physicochemical parameters that significantly correlated with microbiota composition were identified using redundancy analysis (RDA) and Monte Carlo methods. WQI<sub>b</sub> and WQI<sub>minb</sub> were calculated based on  $R^2$  and the significantly correlated physicochemical parameters. These results provide a methodological reference for the subsequent optimization of river and lake water quality assessments, and assessment of the impact of water quality on the composition and metabolism of freshwater microbiota.

#### 2. Materials and methods

#### 2.1. Sample collection and determination of water physicochemical properties

A total of 33 fixed sampling sites were chosen in Chaohu Lake (eight sampling sites including four sampling sites in West Chaohu Lake [WCL], four sampling sites in East Chaohu Lake [ECL], and 25 sampling sites in the seven rivers). The sampling sites were set in the upstream, midstream, downstream, and estuary segments of the NFR, HBR, ZR, and YXR and in the midstream, downstream, and estuary segments of the PR, BSSR, and ZGR. All sampling sites were located using GPS [20].

Water samples were collected on October 14, 2019 (autumn), January 3 (winter), April 24 (spring), and July 10, 2020 (summer) as previously described [20]. Water was collected every 0.5 m depth using a 1-L water collector, and 1000 mL of the mixed water sample was filtered using a GF/C filter membrane with 0.22 µm pore size for collecting microorganisms. Water physicochemical properties were measured according to a previously described [20].

#### 2.2. DNA extraction and high-throughput sequencing of 16 S rDNA

Water microbiota DNA was extracted using a DNeasy PowerSoil kit (QIAGEN, Hilden, Germany). The V4–V5 hypervariable region of prokaryotic 16 S rDNA was amplified using primers 515 F and 909 R, and high-throughput sequencing was performed using a HiSeq 3000 platform (Illumina, San Diego, CA, USA) at Guangdong Meilikang Bio-Science Ltd. (Dongguan, China), as previously described [22,23].

Sequenced raw reads were merged into tags using FLASH 1.2.8 [24]. Low-quality tags (length <300 bp, containing an ambiguous base or an average base quality score <30) were filtered to obtain high-quality tags using QIIME 1.9.0 [25]. Subsequently, chimeric sequences were identified and removed from the high-quality tags to get effective tags using the UCHIME algorithm for further analysis [26]. Effective tags were clustered into operational taxonomic units (OTUs) at 97% similarity using UPARSE [27]. Taxonomic assignment of each OTU was determined using the RDP classifier [28]. Subsequently, all samples were randomly resampled using the same effective tags for further analysis [29].



**Fig. 1.** Phylum composition of water microbiota (A) and RDA profile illustrating the correlation between water physicochemical properties and microbiota composition (B) in Chaohu Lake Basin. \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001.

#### 2.3. WQI calculation

The objective water quality index, WQI was calculated as follows:

$$WQI = \frac{\sum_{i} C_{i} P_{i}}{\sum_{i} P_{i}}$$
(1)

where  $C_i$  is the value assigned to each parameter after normalization, and  $P_i$  is the relative weight assigned to each parameter [13,14]. To ensure that  $P_i$  is closer to the impact of various water parameters on microbiota, the correlation coefficient R<sup>2</sup> between the water parameters and microbiota compositions were calculated used RDA and Monte Carlo methods, and WQI<sub>b</sub> was calculated using R<sup>2</sup> instead of  $P_i$  and compared with the WQI.

To simplify the water parameters used for the WQI calculation, the water parameters that significantly correlated with the microbiota compositions obtained using RDA with the Monte Carlo method were screened to calculate  $WQI_{min}$  and  $WQI_{minb}$ , and compared them with WQI and  $WQI_{b}$ .

## 2.4. Data analysis

Regression analysis was conducted using R 4.2.0 software with the basicTrendline package. RDA with the Monte Carlo method was performed using R 4.2.0 software with vegan and ade4 packages.

## 3. Results

To obtain water microbiota composition information in the Chaohu Lake Basin, 12, 044, 556 high-quality sequences in 132 samples collected over the four seasons were measured. Next, 29,606 sequences were randomly selected from each sample for subsequent analyses. These sequences were clustered into 236,280 OTUs, of which 185,130 (78.35%) were singletons. Except for a few OTUs, most OTUs were divided into 75 phyla (three archaeal phyla and 72 bacterial phyla), in which Acidobacteria, Actinobacteria, Bacteroidetes, Chlorobi, Chloroflexi, Cyanobacteria, Firmicutes, Fusobacteria, Gemmatimonadetes, Nitrospirae, OD1, Planctomycetes, Proteobacteria, Synergistetes, TM7, and Verrucomicrobia dominated (Fig. 1A). Furthermore, these OTUs were divided into 2076 prokaryotic genera, including 408 dominant genera (Table S2).

The results of RDA using the Monte Carlo method based on water parameters and genus composition of the microbiota showed that TP, COD, DO, and Chl a correlated significantly with water microbiota composition (Fig. 1B). Simultaneously,  $R^2$  for each water parameter was calculated (Table 1). The WQI was calculated according to  $P_i$  and  $C_i$  described by Pesce and Wunderlin [14], and WQI<sub>b</sub> was calculated using  $R^2$  instead of  $P_i$ . Pearson correlation analysis of the water parameters showed that COD correlated significantly with Chl a (Fig. S1). Therefore, TP, COD, and DO were used as water parameters for calculating WQI<sub>min</sub> and WQI<sub>minb</sub>, and compared these parameters with WQI and WQI<sub>b</sub>. Although the fluctuation trends of the four WQI were basically the same, and the WQIs of NFR and PR were lower than those of the other rivers, WCL, ECL, and WQI<sub>min</sub> were higher than the other three WQI values in most samples. The results for WQI<sub>b</sub> and WQI<sub>minb</sub> were more consistent than those for WQI and WQI<sub>min</sub> (Fig. 2A). Regression analysis also showed that the consistency between WQI<sub>b</sub> and WQI<sub>minb</sub> was the best, followed by that between WQI and WQI<sub>b</sub>. Consistency between WQI and WQI<sub>min</sub> was the worst (Fig. 2B–G).

## 4. Discussion

The WQI plays a crucial role in water resource management and is widely used to assess surface water and groundwater [3,11,12,

Table 1

Correlation between water physicochemical properties and microbiota composition. \*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001.

Water physicochemical properties	RDA1	RDA2	$R^2$	Pr (>r)
WT	-0.03918	0.99923	0.0264	0.185
DO	0.7306	-0.68281	0.0444	0.049*
рН	0.73764	0.67519	0.0291	0.144
Cond	0.73575	-0.67726	0.0315	0.118
Trans	-0.46087	-0.88747	0.0256	0.223
TN	0.87299	0.48774	0.0071	0.61
TP	0.2606	0.96545	0.0803	0.001***
PO <sub>4</sub> –P	0.69768	0.7164	0.0121	0.475
NH <sub>4</sub> –N	-0.84026	0.54218	0.0004	0.979
NO <sub>3</sub> -N	0.89462	-0.44683	0.0212	0.229
NO <sub>2</sub> -N	0.96885	-0.24764	0.0121	0.433
COD	0.54291	0.83979	0.0429	0.042*
BOD <sub>5</sub>	0.17524	0.98453	0.0179	0.308
Chla	0.4597	0.88807	0.0656	0.004**



**Fig. 2.** Differences in WQI values obtained by different calculation methods (A) and correlations between WQI values obtained by different calculation methods (B–G) in Chaohu Lake Basin. (B)–(G) show correlations between WQI and WQI<sub>b</sub>, WQI and WQI<sub>min</sub>, WQI and WQI<sub>min</sub>, WQI<sub>b</sub> and w

30]. However, the methods used to calculate WQI lack uniformity. These differences are mainly reflected in the fixing of weight values  $P_i$  for different water parameters and the number of water parameters used to calculate WQI [2,10,13,31,32]. Given that water parameters that exert the greatest impact on aquatic organisms should be given maximum weight when calculating WQI and water parameters that have no obvious impact on aquatic organisms should be given minimum weight [14], it is reasonable to directly analyze the correlation between water parameters and aquatic organisms and weight these water parameters according to their correlation coefficients. In this study, the correlations between water parameters and bacterioplankton community compositions were analyzed using RDA with the Monte Carlo method, and calculated their correlation coefficients  $R^2$ . Then  $R^2$  were used instead of  $P_i$  to optimize the calculation of WQI to obtain WQI<sub>b</sub> and WQI<sub>min</sub> and WQI<sub>minb</sub> were calculated according to the screened water parameters that significantly correlated with the microbiota structure. In the present study, the results showed that WQI<sub>b</sub> and WQI<sub>minb</sub> were more stable than WQI and WQI<sub>minb</sub> better reflect the water microbiota structure than WQI and WQI<sub>min</sub>.

Transparency and chlorophyll *a* concentration are two important water parameters that are closely related to river and lake primary productivity [33–36], and are used to assess the degree of eutrophication of rivers and lakes [37–40]. However, they are usually excluded when calculating WQI [14,32,40]. Although using the trophic level index calculated using chlorophyll *a*, total phosphorus, total nitrogen, transparency, and permanganate index can compensate for their absence in WQI calculation, including the two water parameters, especially transparency, in the WQI calculation possibly enables WQI to better reflect water's biological characteristics. In the present study, the results showed that transparency had no significant correlation with other water parameters, while chlorophyll *a* concentration exhibited a very significant correlation with COD (Fig. S1). Therefore, the impact of chlorophyll *a* concentration on aquatic organisms may be partially represented by the COD during data analysis.

Although it was confirmed in this study that using the correlation coefficient R<sup>2</sup> between water parameters and microbiota

composition instead of  $P_i$  could help obtain a more stable WQI<sub>b</sub> that could better reflect the biological characteristics of rivers and lakes, it was still failed to improve the standard conversion method for each water parameter in this study. The standard conversion method should be optimized according to the impact of the water parameters on aquatic organisms. In addition, in this study, the compositions of prokaryotic communities were used as a biological characteristic for analysis. Although prokaryotic communities play an important role in the biological metabolism and energy flow of rivers and lakes, the inclusion of eukaryotic communities in the evaluation system may improve the indicative value of the WQI for the biological characteristics of the river or lake ecosystem.

Furthermore, in this study, we provide a method to optimize the calculation of WQI rather than a WQI standard that can be used in different scenarios. In different application scenarios of WQI, the biological community information used in this method should also be different. For example, if WQI is concerned about the indication of water self-purification capacity, it is recommended to use planktonic bacterial community as biological community information, because the bacterioplankton community has the major impact on the water self-purification capacity [41]. If the WQI is concerned about its indicative effect on aquatic vertebrates, it is recommended to use aquatic vertebrate communities as biological community information. Another issue should be clarified is that we had adopted the DNA high-throughput sequencing technology of 16 S rRNA gene to analyze the bacterioplankton community (such as [1, 42–45]. However, this does not mean that other analysis methods of plankton community, such as morphological identification [20, 46,47], are not applicable to the calculation of WQI<sub>b</sub> and WQI<sub>minb</sub> that described in this study.

#### 5. Conclusion

Using the correlation coefficient  $R^2$  between water parameters and microbiota composition instead of  $P_i$  could yield a more stable WQI<sub>b</sub> that could better reflect the biological characteristics.

#### Author contribution statement

Li Wu: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper.

Yan Zhang:analyzed and interpreted the data; contributed reagents, materials, analysis tools or data.

Ziving Wang: conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools or data.

Ming Geng: conceived and designed the experiments; performed the experiments.

Yajun Chen: performed the experiments.

Fangyan Zhang: performed the experiments.

## Data availability statement

Data associated with this study has been deposited at NCBI Sequence Read Archive database under the accession number PRJNA868422.

## Additional information

Supplementary content related to this article has been published online at [URL].

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#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16697.

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