

Ecosystem Health Assessment of Caohai Lake (Guizhou, China) Based on a Phytoplankton-based Index of Biotic Integrity

Yan Ren^{1,2}, Tao Lin^{1,2} and Lan Zhang^{1,2}

1. Key Laboratory for Information System of Mountainous Areas and Protection of Ecological Environment, Guizhou Normal University, Guiyang 550025, China

2. China Guizhou Key Laboratory of Plateau Wetland Conservation and Restoration, Guiyang 550025, China

Abstract: Caohai Lake, a typical freshwater lake on the southwestern plateau of China, is threatened by drainage projects, domestic sewage, and agricultural pollution, which jeopardize its ecological functions. This study aimed to assess the ecological health of Caohai Lake and to develop a phytoplankton-based Index of Biotic Integrity (P-IBI) adapted to its ecological characteristics. From July to November 2024, fourteen sampling sites were established across the lake, and samples were collected monthly to analyze physicochemical water parameters and phytoplankton community structure, followed by the construction of the P-IBI. A total of 88 phytoplankton species belonging to six phyla were identified, with *Chlorophyta* being the most species-rich group (46 species, 52.27%) and *Cyanophyta* as the dominant group. Nutrient concentrations, including total nitrogen (TN) and total phosphorus (TP), were higher in July-August and gradually decreased from September to November. The mean P-IBI of Caohai Lake was 3.851, corresponding to a “sub-healthy” ecological status. Key metrics, including the percentage of *Bacillariophyta* cell density (M16), percentage of cell density contributed by the three dominant species (M18), Shannon-Wiener diversity index (M19), and the overall P-IBI, were significantly correlated with water temperature (WT), chlorophyll-a (Chl.a), and nutrient concentrations. These results provide a scientific basis for the environmental management and ecological protection of Caohai Lake.

Key words: Caohai Lake, phytoplankton, Index of Biotic Integrity (IBI), ecosystem health, water quality assessment.

1. Introduction

Lakes are central to terrestrial freshwater ecosystems, fulfilling indispensable roles in maintaining regional ecological balance, conserving biodiversity, and sustaining socioeconomic development worldwide [1]. However, intensified urbanization and watershed development have markedly increased nutrient inputs, driving widespread eutrophication and functional degradation in many lakes, ultimately threatening the provision of freshwater ecosystem services worldwide [2, 3]. Although the total surface area of lakes in China accounts for less than 1% of the national land area, they sustain exceptionally high levels of biodiversity and ecosystem service provision, rendering changes in lake health of

profound ecological and societal importance [4]. Consequently, comprehensive assessments of lake ecological health have become a central task in aquatic ecosystem management, particularly in the context of rapidly declining ecosystem service values.

Ecological health assessment is a key diagnostic approach for evaluating ecological conditions and identifying environmental stressors. Its theoretical foundation is rooted in the concept of biotic integrity, originally proposed by Karr, which quantifies integrated environmental effects through biological community attributes and was later formalized as the multi-metric Index of Biotic Integrity (IBI) [5]. By integrating physical, chemical, and biological metrics, the IBI

Corresponding author: Tao Lin, Ph.D., senior experimentalist, research field: lake eutrophication and ecological restoration.

offers a more comprehensive assessment of ecosystem condition than single indicators and has been widely applied in freshwater health monitoring worldwide [6, 7]. For example, fish-based IBIs have been standardized across numerous freshwater systems in North America and are commonly used as decision-support tools for water resource management and ecological restoration planning [8, 9]. Within modern ecological assessment frameworks, the IBI is used both to diagnose ecosystem health and to track biological responses to restoration, serving as a key link between ecological science and water management policy.

Compared with fish and benthic macroinvertebrates, phytoplankton exhibit rapid environmental responses and high sensitivity to eutrophication, making them efficient indicators of aquatic ecological status [10, 11]. These traits make phytoplankton community structure highly sensitive to eutrophication and environmental stress, rendering it a valuable indicator of aquatic ecosystem condition [12, 13]. Phytoplankton-based indices of biotic integrity (P-IBI) have been widely applied in lakes, rivers, and reservoirs, effectively linking biological metrics to environmental pressures and capturing gradients of ecological degradation [11]. Previous studies have shown that P-IBI scores are strongly correlated with conventional water quality parameters and exhibit high spatial and seasonal discriminatory power, underscoring the effectiveness of phytoplankton-based metrics for ecological health diagnosis [14-16]. Thus, the application of phytoplankton-derived IBIs not only expands the methodological toolbox for ecological assessment but also provides a practical and sensitive approach for rapid monitoring of aquatic ecosystem health.

Caohai Lake, a representative freshwater wetland on the southwestern Chinese Plateau, has experienced increasing ecological degradation driven by historical drainage and intensified watershed disturbances. In response to the absence of regionally adapted phytoplankton-based integrity indices for plateau lakes, this study constructs a phytoplankton Index of Biotic

Integrity (P-IBI) to evaluate ecosystem health and inform conservation and restoration strategies.

2. Materials and Methods

2.1 Study Area and Sample Collection

The study area is located in the southwestern part of Weining Yi, Hui and Miao Autonomous County, Guizhou Province, China (26°47'32"-26°52'52" N, 104°10'16"-104°20'40" E). It is a typical plateau wetland and one of the representative natural freshwater lakes on the Yunnan-Guizhou Plateau. The region is characterized by a subtropical plateau monsoon climate, with a mean annual air temperature of approximately 10.9 °C and a distinct seasonal precipitation pattern, in which rainfall is mainly concentrated between May and August. Caohai Lake has a surface water area of about 22.39 km² and exhibits pronounced spatial heterogeneity in habitat conditions. The lake supports diverse aquatic vegetation communities, including extensive stands of emergent and submerged macrophytes, which play a key role in regulating nutrient cycling and providing habitats for aquatic organisms. Field investigations were conducted from July to November 2024, during which a total of 14 sampling sites were established across the lake (Fig. 1) to represent different habitat types and environmental gradients, ensuring comprehensive coverage of the spatial variability within the study area.

At each sampling site, 1.5 L of water was collected using a water sampler and immediately transported to the laboratory for physicochemical analysis. Phytoplankton samples were collected following standard freshwater protocols [17]. For qualitative sampling, a 25- μ m plankton net was used to gently tow near the surface to 0.5 m depth at 20-30 cm s⁻¹ in an "∞"-shaped pattern for approximately 2 min; the collected concentrate was transferred to a sampling bottle and preserved with 5% formalin. For quantitative sampling, 1.5 L of surface water was collected with a Plexiglas sampler, preserved with 5% formalin, and transported to the laboratory for further processing.

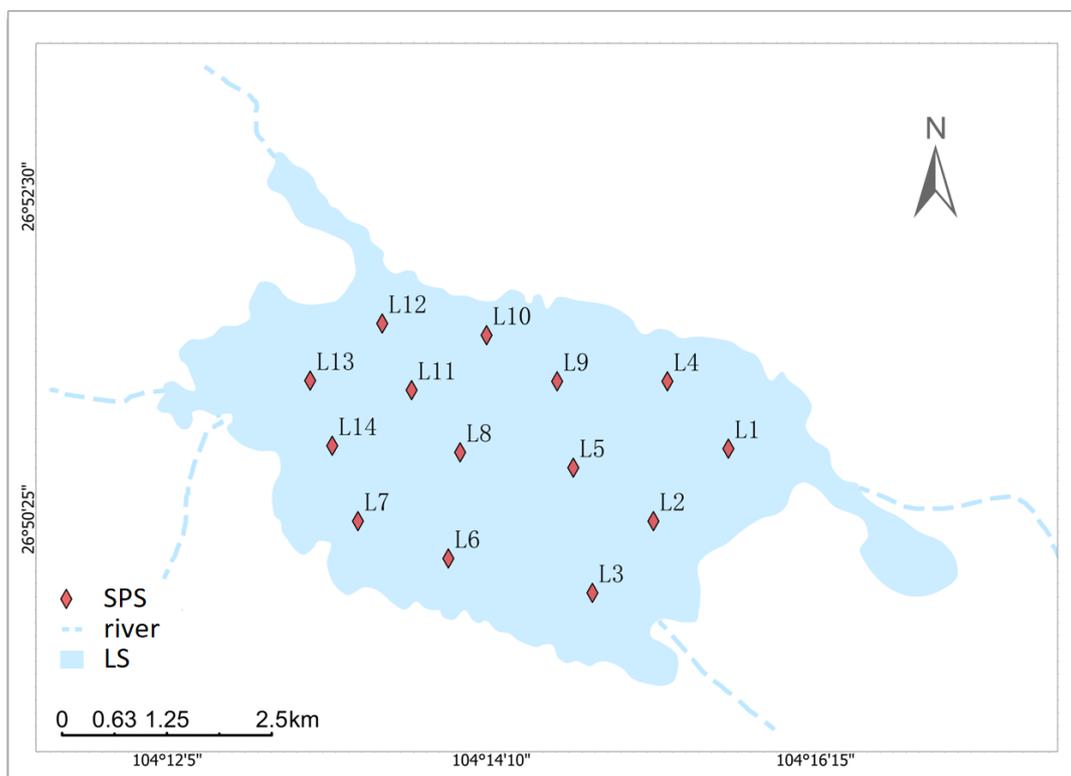


Fig. 1 Map showing the distribution of sampling points in Caohai.

2.2 Sample Collection and Processing

Water quality parameters were measured following the *Environmental Quality Standards for Surface Water* (GB 3838-2002). Total phosphorus (TP) was determined using the ammonium molybdate spectrophotometric method, total nitrogen (TN) by alkaline potassium persulfate digestion followed by UV spectrophotometry, ammonium nitrogen ($\text{NH}_4^+\text{-N}$) via the Nessler reagent colorimetric method, and permanganate index (COD_{Mn}) according to GB 11892-89. Chlorophyll a (Chl.a) concentration was measured spectrophotometrically, pH and water temperature (WT) were recorded in situ using a YS multiparameter probe, and Secchi disk depth (SD) was used to assess water transparency. pH was measured using a portable water quality analyzer (WTW, Germany; Lei Xi, China). Phytoplankton species identification and enumeration were conducted microscopically: samples were homogenized, 0.1 mL was placed in a counting chamber, and organisms were identified under a $\times 400$

microscope. Each sample was counted independently twice; if the difference between counts was $\leq 15\%$, the average was used; otherwise, a third count was performed and the mean of all three counts was reported. Taxonomic identification followed *Freshwater Algae of China: Systematics, Taxonomy and Ecology* [18].

2.3 P-IBI Establishment

2.3.1 Calculation and Evaluation Criteria of the P-IBI Index

Species dominance was calculated using the dominance index (Y):

$$Y = P_i + f_i \quad (1)$$

$$P_i = \frac{n_i}{N} \quad (2)$$

The number of individuals of the i -th species is denoted as n_i , and N represents the total number of individuals across all species in the sample. f_i refers to the frequency of the i -th species across sampling sites. Species with a Y value ≥ 0.02 are considered dominant species [19].

Ecosystem Health Assessment of Caohai Lake (Guizhou, China) Based on a Phytoplankton-based Index of Biotic Integrity

Shannon–Wiener diversity index (H'):

$$H' = - \sum \left(\frac{N_i}{N} \right) \log_2 \left(\frac{N_i}{N} \right) \quad (3)$$

where N_i is the number of individuals of species i and N is the total number of individuals. According to commonly accepted criteria, $H' < 1$ indicates heavily polluted conditions, $1 \leq H' < 3$ indicates moderately polluted conditions, and $H' \geq 3$ indicates slightly polluted or unpolluted conditions [10].

To standardize metric scores, the ratio method was applied. For metrics that decrease with increasing

disturbance, scores were calculated as:

$$\text{Score} = \frac{A_i}{A_{95\%}} \quad (4)$$

For metrics that increase with disturbance, scores were calculated as:

$$\text{Score} = \frac{A_{\max} - A_i}{A_{\max} - A_{5\%}} \quad (5)$$

where A_i is the observed metric value, $A_{95\%}$ and $A_{5\%}$ represent the 95th and 5th percentile values, respectively, and A_{\max} is the maximum observed value. Values exceeding 1 were assigned a score of 1 [20].

Table 1 Candidate phytoplankton-based biological metrics for the assessment of biotic integrity.

Indicator code	Candidate metric	Metric description	Response to disturbance
Species composition			
M1	Total number of phytoplankton genera	Total number of phytoplankton genera	Decrease
M2	Total number of <i>Cyanophyta</i> genera	Total number of <i>Cyanophyta</i> genera	Decrease
M3	Total number of <i>Chlorophyta</i> genera	Total number of <i>Chlorophyta</i> genera	Decrease
M4	Total number of <i>Bacillariophyta</i> genera	Total number of <i>Bacillariophyta</i> genera	Decrease
M5	Total number of non- <i>Bacillariophyta</i> genera	Total number of non- <i>Bacillariophyta</i> genera	Decrease
M6	Total number of <i>Bacillariophyta</i> + <i>Chlorophyta</i> genera	Total number of <i>Bacillariophyta</i> + <i>Chlorophyta</i> genera	Decrease
M7	Total algal density	Total number of algal cells per unit area/volume	Decrease
M8	Density of <i>Cyanophyta</i>	Total number of <i>Cyanophyta</i> cells per unit area/volume	Decrease
M9	Density of <i>Chlorophyta</i>	Total number of <i>Chlorophyta</i> cells per unit area/volume	Decrease
M10	Density of <i>Bacillariophyta</i>	Total number of <i>Bacillariophyta</i> cells per unit area/volume	Decrease
Relative abundance metrics			
M11	Percentage of <i>Cyanophyta</i> genera	(<i>Cyanophyta</i> genera / total genera) × 100%	Decrease
M12	Percentage of <i>Chlorophyta</i> genera	(<i>Chlorophyta</i> genera / total genera) × 100%	Decrease
M13	Percentage of <i>Bacillariophyta</i> genera	(<i>Bacillariophyta</i> genera / total genera) × 100%	Decrease
M14	Percentage of <i>Cyanophyta</i> cell density	(<i>Cyanophyta</i> cell density / total algal cell density) × 100%	Decrease
M15	Percentage of <i>Chlorophyta</i> cell density	(<i>Chlorophyta</i> cell density / total algal cell density) × 100%	Decrease
M16	Percentage of <i>Bacillariophyta</i> cell density	(<i>Bacillariophyta</i> cell density / total algal cell density) × 100%	Decrease
M17	Percentage of <i>Bacillariophyta</i> + <i>Chlorophyta</i> cell density	((<i>Bacillariophyta</i> + <i>Chlorophyta</i>) cell density / total algal cell density) × 100%	Decrease
M18	Percentage of cell density contributed by the three dominant species	(Cell density of the three dominant species / total algal cell density) × 100%	Decrease
Community diversity			
M19	Shannon–Wiener Diversity Index	$H' = - \sum (N_i/N) \log_2 (N_i/N)$ N: total number of individuals; N_i : total number of individuals of species i	Decrease

2.3.2 Selection of Candidate Metrics

Based on an extensive review of relevant international studies [21-23], and taking data availability and feasibility into consideration [21], a total of 19 phytoplankton-based biological metrics were initially selected as candidate indicators (Table 1). These metrics were designed to reflect key ecological attributes of phytoplankton communities and were classified into three categories: species composition, relative abundance, and community diversity.

Candidate metrics were first evaluated using box-and-whisker plots to assess their discrimination ability between reference and impaired sites based on interquartile range (IQ) criteria [24]. Metrics with $IQ \geq 2$ were retained for further analysis. Pearson correlation analysis was then conducted to examine redundancy among metrics; when the absolute correlation coefficient $|r| > 0.75$, one of the correlated metrics was removed. The remaining metrics were selected as core indicators for P-IBI construction.

Each metric value was standardized by dividing it by the 95th percentile of that parameter across all sampling sites. For metrics that increase with increasing disturbance, the 5th percentile of the parameter across all sites was defined as the optimal reference value, and the metric score was calculated as (maximum value—observed value at a given site)/(maximum value—optimal reference value). The P-IBI value for each site was obtained by summing the scores of all individual metrics. Following this procedure, all metric scores were constrained to the range of 0-1, with values greater than 1 assigned a value of 1 [25]. Finally, the 25th percentile of P-IBI values at reference sites was used as the threshold for defining “healthy” conditions. Values below this threshold were evenly divided into four categories, representing “poor”, “average”, “sub-healthy”, and “healthy” ecological status, respectively.

2.4 Data Analysis

Statistical analyses, including correlation analysis for metric screening, were performed using SPSS 27.

Spatial distribution maps of sampling sites were produced using ArcGIS 10.8, and figures were generated using Origin 2024.

3. Results

3.1 Physicochemical Characteristics of the Water Body

Water quality parameters of Caohai Lake from July to November 2024 are shown in Fig. 2. In July, WT was 22.87 °C and pH was 8.13, while mean values of SD, COD_{Mn}, Chl.a, TP, TN, and NH₄⁺-N were 1.19 m, 7.18 mg/L, 6.78 µg/L, 0.10 mg/L, 0.89 mg/L, and 0.45 mg/L, respectively. In August, WT decreased to 21.68 °C and pH increased to 8.60; SD increased to 1.37 m, COD_{Mn} remained at a level similar to July, and mean concentrations of Chl.a, TP, TN, and NH₄⁺-N were 6.21 µg/L, 0.09 mg/L, 0.96 mg/L, and 0.42 mg/L, respectively. From September to November, WT continuously decreased to 11.11 °C, while pH ranged from 7.99 to 8.72. Mean SD increased to 1.50 m, COD_{Mn} increased to values above 8 mg/L, and TP, TN, and NH₄⁺-N exhibited an overall decreasing trend. During the same period, Chl.a concentrations fluctuated between 7 and 9 µg/L.

3.2 Phytoplankton Community Structure Characteristics

3.2.1 Phytoplankton Community Composition and Abundance

During the study period, a total of 88 phytoplankton species belonging to six phyla were identified in Caohai Lake (Fig. 3). *Chlorophyta* was the dominant phylum in terms of species richness, comprising 46 species (52.27%), followed by *Bacillariophyta* with 19 species (21.59%) and *Cyanophyta* with 16 species (18.18%). *Dinophyta* and *Euglenophyta* each included three species (3.41%), whereas *Chrysophyta* was represented by a single species (1.14%). Monthly species richness ranged from 70 to 74 species, with all six phyla present in each month. In July, 73 species were recorded, of which *Chlorophyta* accounted for 53.42%, followed by *Bacillariophyta* (20.55%) and *Cyanophyta* (17.81%). In August, 74 species were

Ecosystem Health Assessment of Caohai Lake (Guizhou, China) Based on a Phytoplankton-based Index of Biotic Integrity

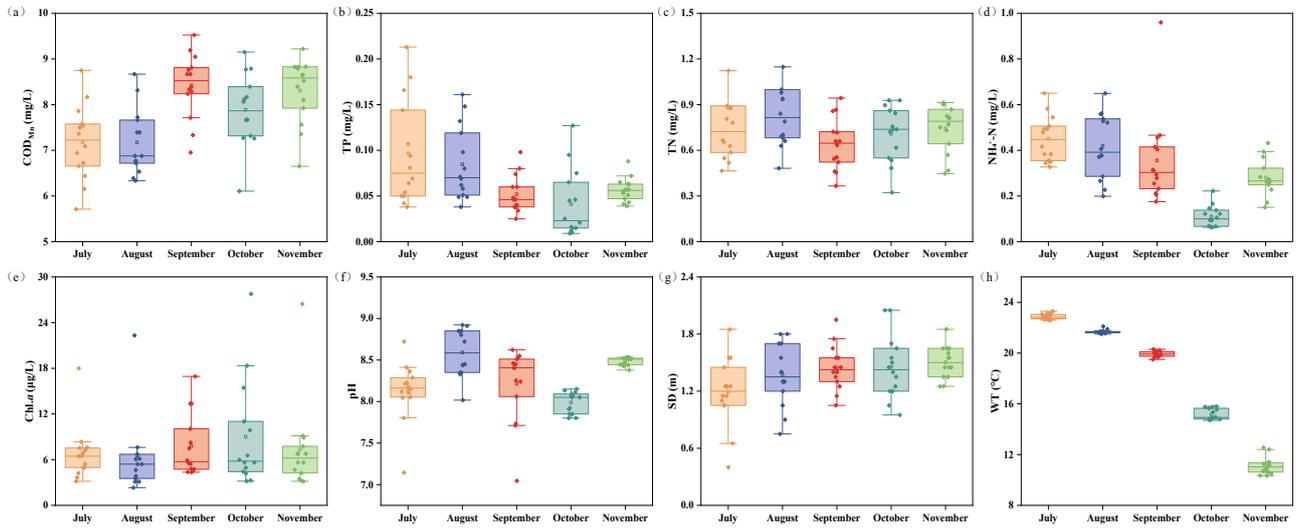


Fig. 2 Physicochemical Properties of Water from July to November. COD_{Mn}: permanganate index, TP: total phosphorus, TN: total nitrogen, NH₄⁺-N: ammonium nitrogen, Chl.a: chlorophyll *a*, pH: hydrogen ion concentration index, SD: Secchi disk depth, WT: water temperature.

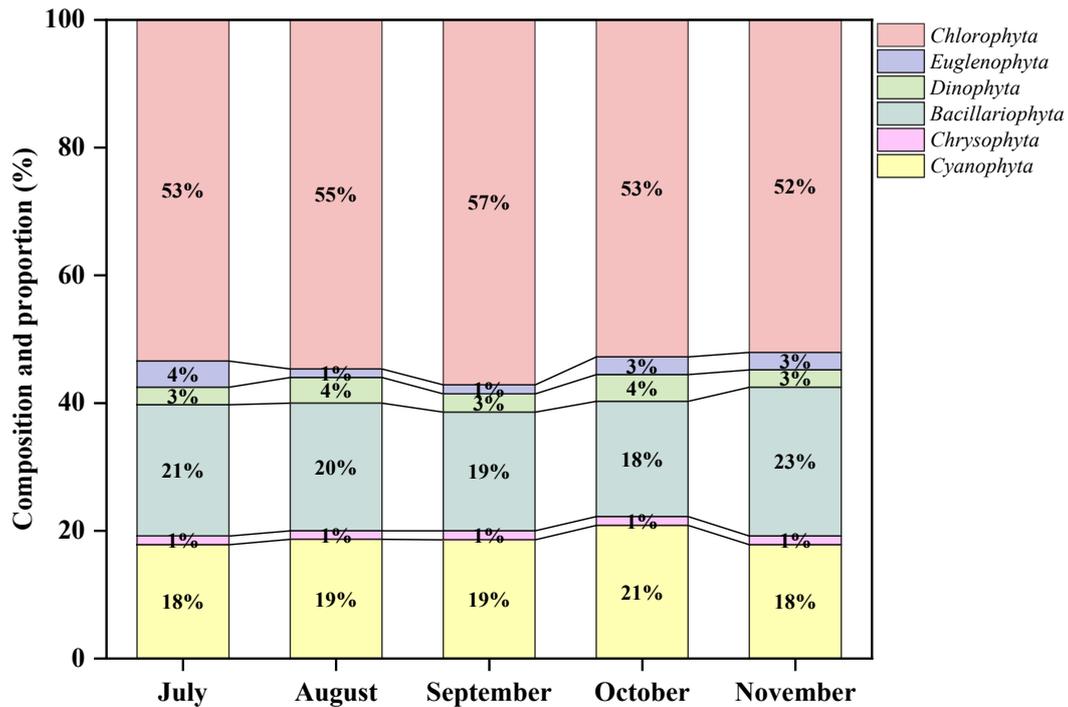


Fig. 3 Monthly species composition of phytoplankton.

identified, with *Chlorophyta* remaining dominant (54.67%). In September, the total number of species decreased to 70, and *Chlorophyta* contributed 57.14%, while *Bacillariophyta* and *Cyanophyta* each accounted for 18.57%. In October and November, 72 and 73 species were recorded, respectively, with *Chlorophyta* consistently representing more than 52% of the total

species composition. *Chrysophyta* was limited to one species in all months.

Phytoplankton abundance showed pronounced variation among months and sampling sites (Fig. 4). The highest phytoplankton density was observed in August, with a maximum value of 49.31×10^6 cells/L at site L1, which substantially exceeded densities at

other sites during the same month. In July, phytoplankton densities across sites ranged from 0.40 to 3.55×10^6 cells/L. In September, overall abundance declined, with site-specific values generally below 1.31×10^6 cells/L. In October, an increase in abundance was recorded at site L3, reaching 4.69×10^6 cells/L, while other sites remained below 2.60×10^6 cells/L. In November, phytoplankton densities ranged from 0.16 to 1.92×10^6 cells/L, with relatively higher values occurring at sites L7, L10, and L11. Overall, phytoplankton abundance varied substantially among months and sites, whereas species composition remained relatively consistent throughout the study period.

3.2.2 Dominant Algal Species

The dominant phytoplankton taxa in Caohai Lake varied among months, while taxa belonging to *Cyanophyta* consistently dominated throughout the study period. In July, the dominant genera were mainly cyanobacteria, including *Merismopedia*, *Aphanocapsa*,

Microcystis, *Pseudanabaena*, and *Oscillatoria*, accompanied by the diatom *Cyclotella* and the green alga *Scenedesmus*. In August, dominance was restricted to cyanobacterial genera, with only *Microcystis*, *Pseudanabaena*, and *Oscillatoria* remaining dominant. In September, the dominant assemblage comprised *Microcystis* and *Oscillatoria* (Cyanophyta), together with *Cyclotella* (Bacillariophyta) and *Pediastrum* (Chlorophyta). October exhibited the highest richness of dominant taxa; in addition to cyanobacteria (*Microcystis*, *Pseudanabaena*, *Oscillatoria*) and diatoms (*Cyclotella*), the chrysophyte *Dinobryon* and the green algae *Pediastrum* and *Scenedesmus* also became dominant. In November, a total of ten dominant genera were identified, including cyanobacteria (*Merismopedia*, *Microcystis*, *Pseudanabaena*, *Oscillatoria*), diatoms (*Cyclotella*, *Synedra*, *Navicula*), chrysophytes (*Dinobryon*), and green algae (*Scenedesmus*), indicating a more complex phytoplankton dominance structure.

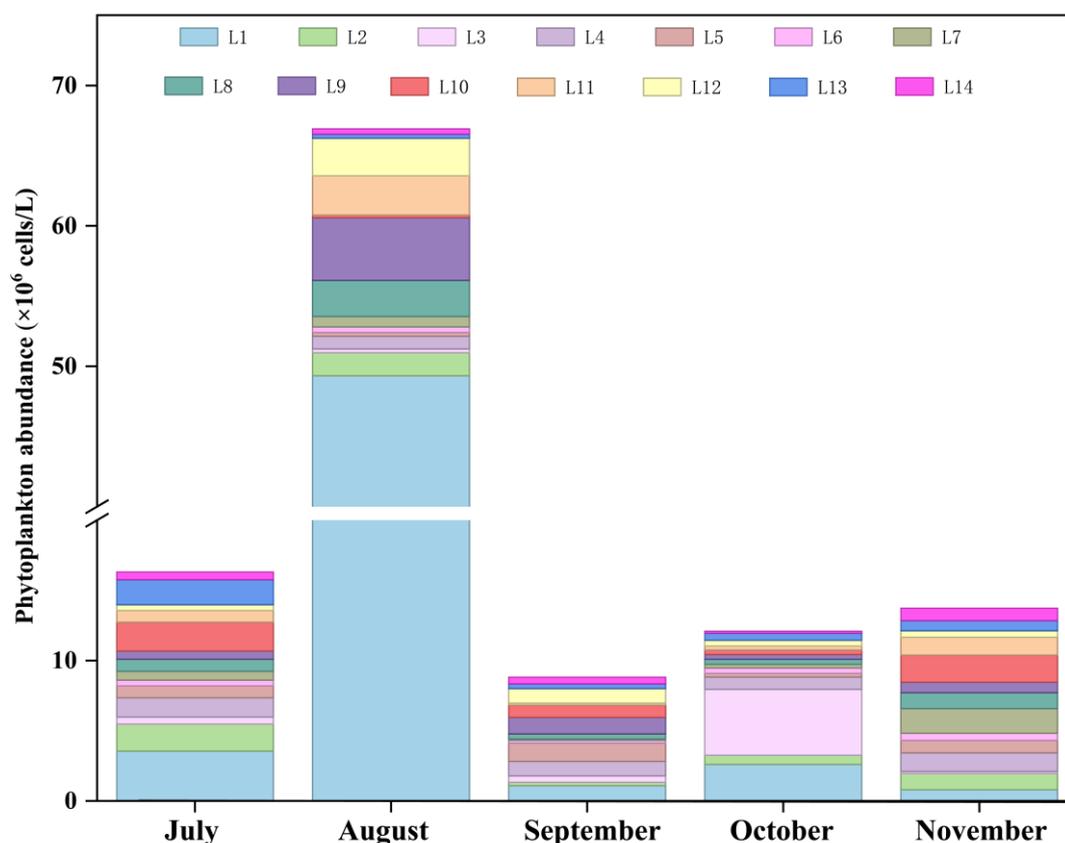


Fig. 4 Monthly algal abundance of phytoplankton.

Ecosystem Health Assessment of Caohai Lake (Guizhou, China) Based on a Phytoplankton-based Index of Biotic Integrity

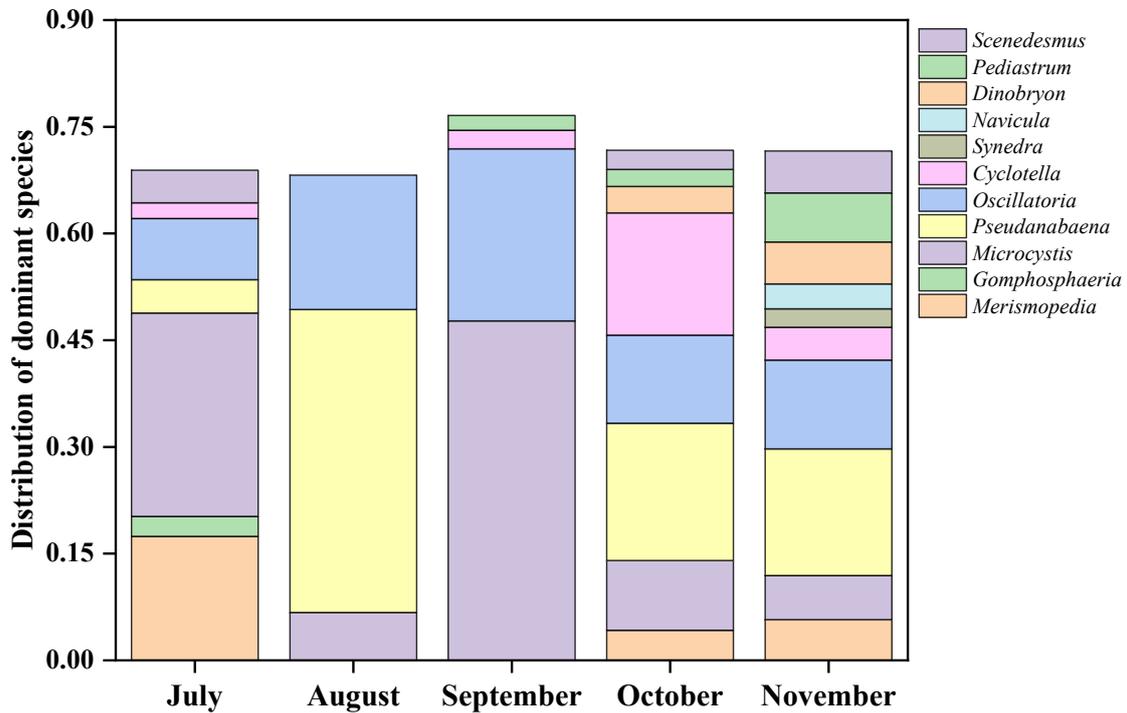


Fig. 5 Distribution of phytoplankton dominance in different months.

Table 2 Matrix Pearson correlation analysis among the candidate parameters.

	M4	M14	M15	M16	M17	M18	M19
M4	1						
M14	-0.130	1					
M15	-0.245	-0.663**	1				
M16	0.228	-0.784**	0.162	1			
M17	-0.026	-0.945**	0.788**	0.735**	1		
M18	0.074	0.651**	-0.465**	-0.419*	-0.581**	1	
M19	-0.088	-0.473**	0.416*	0.336	0.495**	-0.640**	1

Table 3 P-IBI selected parameter feature values.

	Biological metric	Minimum	Maximum	25th percentile	75th percentile
M4	Total number of <i>Bacillariophyta</i> genera	4.00	14.00	6.50	10.50
M14	Percentage of <i>Cyanophyta</i> cell density	5.44	78.18	45.09	62.91
M15	Percentage of <i>Chlorophyta</i> cell density	12.22	60.22	18.73	35.74
M16	Percentage of <i>Bacillariophyta</i> cell density	2.10	45.45	6.39	19.41
M17	Percentage of <i>Bacillariophyta</i> + <i>Chlorophyta</i> cell density	18.94	82.80	30.46	51.51
M18	Percentage of cell density contributed by the three dominant species	3.63	63.64	22.12	49.75
M19	Diversity index	3.02	4.33	3.39	3.83

3.3 Phytoplankton Biotic Integrity Assessment

3.3.1 Selection of Reference Sites

Reference and impaired sites were identified based on the Shannon-Wiener diversity index. In July, three

sites (L1, L2, and L3) were classified as reference sites. In August, seven sites (L2, L3, L4, L5, L6, L7, and L10) were identified as reference sites. In September, seven sites (L2, L3, L4, L6, L7, L11, and L13) were classified as reference sites. In October, four sites (L5, L10, L12,

and L14) were identified as reference sites. In November, twelve sites (L1, L2, L3, L4, L5, L6, L9, L10, L11, L12, L13, and L14) were classified as reference sites.

3.3.2 Screening and Selection of Candidate Metrics

The discriminatory power of the 19 candidate metrics was assessed using boxplot analysis based on the interquartile range (IQ; 25th-75th percentiles) to compare reference and impaired sites. Based on this analysis, seven metrics were selected for further evaluation: total number of diatom genera (M4), percentage of cyanobacterial density (M14), percentage of chlorophyte density (M15), percentage of diatom density (M16), combined percentage of diatom and chlorophyte density (M17), percentage of cell density contributed by the three dominant species (M18), and the diversity index (M19). Pearson correlation analysis was subsequently conducted among these metrics (Table 2). The results indicated that M4, M18, and M19 exhibited low correlations with other metrics ($|r| < 0.75$)

and were therefore retained directly. Strong correlations were observed between M14 and M16, as well as between M15 and M17 ($|r| > 0.75$); consequently, M15 and M16 were retained. Through this stepwise screening procedure, five metrics (M4, M15, M16, M18, and M19) were ultimately identified as the core indicators for constructing the P-IBI (Table 3).

3.3.3 P-IBI Assessment Results

The P-IBI assessment of Caohai is presented in Fig. 6. In July, the P-IBI values ranged from 2.70 to 4.95, with six sampling sites classified as “sub-healthy” and eight sites as “moderate”, yielding a mean value of 3.67. In August, the index ranged from 2.44 to 5.25, including one “healthy” site, six “sub-healthy” sites, six “moderate” sites, and one “poor” site, with a mean of 3.87 (sub-healthy). In September, values ranged from 1.98 to 4.51, with seven sites classified as “sub-healthy”, four as “moderate”, and three as “poor”, resulting in a mean of 3.51 (moderate). In October, the index ranged from 2.36 to 5.34, comprising one “healthy” site, six “sub-healthy”

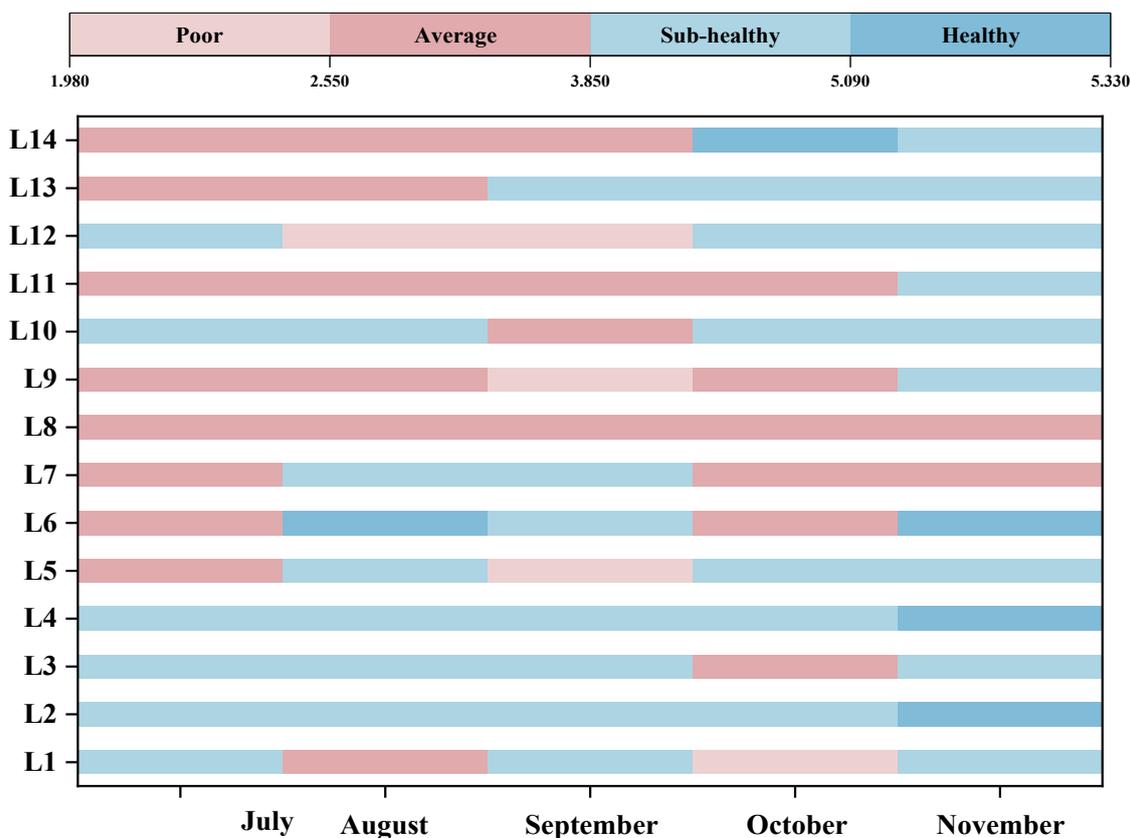


Fig. 6 P-IBI evaluation results for each sampling point.

Ecosystem Health Assessment of Caohai Lake (Guizhou, China) Based on a Phytoplankton-based Index of Biotic Integrity

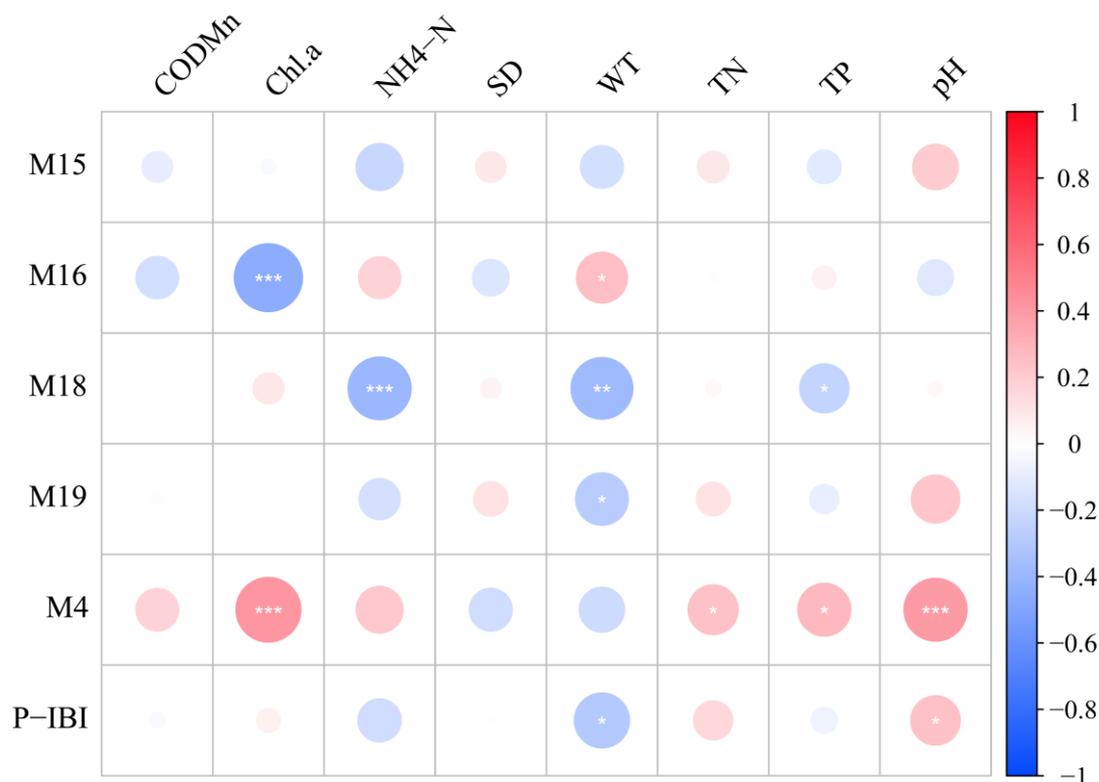


Fig. 7 Spearman correlation analysis between environmental factors and key P-IBI parameters (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.005$).

sites, six “moderate” sites, and one “poor” site, with a mean of 3.75 (moderate). In November, values ranged from 3.20 to 5.10, including one “healthy” site, eleven “sub-healthy” sites, and two “moderate” sites, yielding a mean of 4.46. Across all sampling sites, the P-IBI ranged from 1.98 to 5.34, with an overall assessment of “sub-healthy,” consistent with the results observed in August and November.

2.3.4 Correlation Between P-IBI and Environmental Factors

Correlation analysis revealed distinct relationships between the selected metrics and environmental variables (Fig. 7). Specifically, M16 was highly negatively correlated with Chl-a ($p < 0.005$) and positively correlated with water temperature (WT) ($p < 0.05$). M18 showed a strong negative correlation with $\text{NH}_4^+\text{-N}$ ($p < 0.005$), as well as significant negative correlations with WT ($p < 0.01$) and total phosphorus (TP) ($p < 0.05$). M4 was highly positively correlated with Chl-a and pH ($p < 0.005$), and also significantly

positively correlated with total nitrogen (TN) and TP ($p < 0.05$). Both M19 and P-IBI were positively correlated with WT ($p < 0.05$), and P-IBI was additionally positively correlated with pH ($p < 0.05$). In contrast, M15 showed weak and non-significant correlations with all measured environmental factors. Overall, key metrics such as M4, M16, and M18 were sensitive indicators of variations in Chl-a, nutrients, WT, and pH.

3. Discussion

3.1 Phytoplankton Community Composition and Structural Stability Under Environmental Gradients

The phytoplankton community of Caohai Lake was characterized by persistently high species richness and a relatively stable taxonomic framework dominated by *Chlorophyta*, *Bacillariophyta*, and *Cyanophyta*. Such compositional stability across seasons, despite marked fluctuations in cell density, is increasingly recognized as a common feature of shallow and plateau lakes experiencing moderate nutrient enrichment and

strong environmental filtering. Previous studies have demonstrated that when nutrient concentrations exceed limiting thresholds, phytoplankton assemblages tend to stabilize in terms of species pools, while biomass becomes more sensitive to short-term drivers such as temperature, light availability, and hydrodynamic disturbance [26-28]. In Caohai Lake, the dominance of *Chlorophyta* in species richness reflects their broad ecological amplitude and high functional redundancy, allowing coexistence under fluctuating nutrient and thermal regimes [29, 30]. Meanwhile, the consistent presence of *Cyanophyta* among dominant taxa suggests that nutrient stoichiometry and elevated summer temperatures favor taxa with efficient phosphorus acquisition strategies and buoyancy regulation, even when total nutrient concentrations decline seasonally [31, 32].

The relatively invariant community composition observed from July to November further indicates that Caohai Lake may currently be in a transitional ecological state, where environmental pressures regulate community structure through environmental filtering rather than species turnover. Similar patterns have been reported in other shallow lakes where internal nutrient cycling and sediment–water interactions buffer phytoplankton communities against rapid compositional shifts [33, 34]. The persistence of *Bacillariophyta* such as *Cyclotella* and *Navicula* across seasons further highlights the importance of mixing intensity and silica availability in maintaining diatom populations under declining temperatures [35, 36]. Collectively, these findings suggest that phytoplankton community composition in Caohai Lake is shaped by long-term nutrient legacies and physical constraints, providing a stable biological template upon which environmental fluctuations primarily modulate abundance and dominance strength rather than taxonomic identity.

3.2 Mechanistic Links between P-IBI Core Metrics and Nutrient-thermal Dynamics

The selected P-IBI core metrics (M4, M15, M16,

M18, and M19) exhibited distinct and mechanistically meaningful responses to key environmental gradients, underscoring their ecological relevance. The positive relationships between the total number of diatom genera (M4) and nutrients (TN and TP) indicate that moderate nutrient availability can enhance niche differentiation within *Bacillariophyta*, promoting higher taxonomic resolution rather than competitive exclusion. This pattern aligns with the subsidy-stress framework, which predicts increased diversity under intermediate nutrient enrichment [37, 38]. Diatoms possess high nutrient uptake efficiency and rapid growth rates, allowing them to exploit transient nutrient pulses derived from sediment resuspension and internal loading [39]. Moreover, the significant positive correlation between M4 and Chl.a suggests that diatom diversity responds not only to nutrient concentration but also to overall phytoplankton productivity, reflecting their contribution to primary production in meso-eutrophic systems [40].

In contrast, dominance-related metrics such as M18 (percentage of cell density contributed by the three dominant species) were negatively correlated with $\text{NH}_4^+\text{-N}$, TP, and WT, highlighting the destabilizing effects of nutrient enrichment and thermal stress on community evenness. Elevated ammonium concentrations have been shown to preferentially stimulate fast-growing cyanobacteria, leading to competitive dominance and reduced community balance [41, 42]. However, the observed negative association between M18 and WT suggests that warming can also weaken dominance hierarchies by enhancing metabolic rates and competitive interactions among multiple taxa [43, 44]. This mechanism supports the idea that thermal variability may partially counteract nutrient-driven homogenization by increasing temporal niche overlap, thereby modulating dominance intensity. Such nonlinear responses emphasize the necessity of incorporating multiple functional metrics into P-IBI frameworks to capture complex ecosystem responses to interacting stressors.

3.3 Integrated Interpretation of P-IBI Responses and Implications for Ecosystem Health Assessment

The overall “sub-healthy” classification of Caohai Lake derived from P-IBI reflects the cumulative effects of moderate nutrient enrichment, seasonal thermal variability, and phytoplankton structural adjustment. Notably, the positive correlations between P-IBI, WT, and pH suggest that biological integrity improves under warmer and slightly alkaline conditions, likely due to enhanced metabolic activity and increased photosynthetic efficiency [14, 45]. Alkaline conditions can increase phosphorus bioavailability by altering sorption equilibria at the sediment-water interface, indirectly supporting diverse phytoplankton assemblages [46]. The sensitivity of P-IBI to these physicochemical gradients confirms its effectiveness in integrating structural and functional attributes of phytoplankton communities, consistent with previous IBI-based assessments in shallow lakes worldwide [47].

From a mechanistic perspective, the P-IBI framework captures ecosystem health by linking community composition (diatoms and green algae), dominance structure (cyanobacterial prevalence), and diversity patterns to environmental forcing. This integrative capacity is particularly valuable in plateau lakes, where strong seasonal contrasts and internal nutrient cycling complicate traditional chemical assessments [48]. By emphasizing biologically meaningful metrics, P-IBI transcends snapshot water quality measurements and reflects cumulative ecological responses. Recent studies advocate for combining phytoplankton-based IBIs with high-resolution biogeochemical monitoring to disentangle legacy effects and short-term disturbances [49, 50]. Therefore, the present study not only validates the applicability of P-IBI in Caohai Lake but also highlights its potential as a robust tool for long-term ecosystem health assessment and adaptive lake management under ongoing climate change and nutrient stress.

4. Conclusions

(1) A total of 88 phytoplankton species belonging to

six phyla were identified in Caohai Lake, Guizhou. During July-August, the dominant species were mainly from *Cyanophyta*, whereas from September to November, additional dominant taxa from *Bacillariophyta* (*Cyclotella*) and *Chrysophyta* (*Dinobryon*) appeared. Overall, *Chlorophyta* was the most species-rich group (46 species, 52.27%), while *Cyanophyta* remained the core dominant group. In terms of density, sites near the shore (L1, L2, L3) had higher average phytoplankton densities, whereas the site near the outflow (L12) exhibited the lowest density.

(2) Correlation analyses indicated that the main environmental drivers influencing the P-IBI of Caohai Lake were water temperature (WT), nutrients (TN, TP, $\text{NH}_4^+\text{-N}$), and Chl.a, with nutrient inputs and WT fluctuations identified as the key factors. Specifically, the total number of *Bacillariophyta* genera was significantly positively correlated with Chl.a, $\text{NH}_4^+\text{-N}$, TN, TP, and pH. The percentage of *Bacillariophyta* density was extremely negatively correlated with Chl.a and significantly positively correlated with WT. Overall, P-IBI was significantly positively correlated with WT and pH.

(3) From July to November 2020, the mean P-IBI of Caohai Lake was 3.851, ranging from 1.98 to 5.34, indicating an overall “sub-healthy” status. The proportion of sampling sites by ecological class was: healthy 4.29%, sub-healthy 51.43%, general 37.14%, and poor 5.71%. Spatially, sites near the shore (L1, L2, L3) were mostly classified as “general,” whereas the site near the outflow (L12) showed relatively better conditions. Temporally, the lake was generally “sub-healthy” in August and November, and “general” in July, September, and October.

References

- [1] Zhang, Y., Zhu, H., Li, B., Yang, G., and Wan, R. 2021. “Aquatic Ecosystem Health Assessment of Poyang Lake Through Extension Evaluation Method.” *Water* 13 (2): 211, <https://doi.org/10.3390/w13020211>.
- [2] Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z., Knowler, D. J., Lévêque, C., and Naiman, R. J., et al. 2006. “Freshwater Biodiversity: Importance,

- Threats, Status and Conservation Challenges.” *Biol. Rev. Camb. Philos. Soc.* 81 (2): 163-182. <http://dx.doi.org/10.1017/s1464793105006950>.
- [3] Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., and Glidden, S., et al. 2010. “Global Threats to Human Water Security and River Biodiversity.” *Nature* 467 (7315): 555-561. <http://dx.doi.org/10.1038/nature09440>.
- [4] Vári, Á., Podschun, S. A., Erős, T., Hein, T., Pataki, B., Iojă, I. C., Adamescu, C. M. et al. 2022. “Freshwater Systems and Ecosystem Services: Challenges and Chances for Cross-Fertilization of Disciplines.” *Ambio* 51 (1): 135-151. <http://dx.doi.org/10.1007/s13280-021-01556-4>.
- [5] Karr, J. R. 1981. “Assessment of Biotic Integrity Using Fish Communities.” *Fisheries* 6 (6): 21-27. [https://doi.org/10.1577/1548-8446\(1981\)006%3C0021:A0BIUF%3E2.0.CO;2](https://doi.org/10.1577/1548-8446(1981)006%3C0021:A0BIUF%3E2.0.CO;2).
- [6] Huang, X., Xu, J., Liu, B., Guan, X., and Li, J. 2022. “Assessment of Aquatic Ecosystem Health with Indices of Biotic Integrity (IBIs) in the Ganjiang River System, China.” *Water* 14 (3): 278. Doi: <http://dx.doi.org/10.3390/w14030278>.
- [7] Zhu, H., Zhang, Y. Z., Peng, Y. C., Shi, B. C., Liu, T., Dong, H. B., and Wang, Y., et al. 2023. “Assessing the Ecological Health of the Qingyi River Basin Using Multi-Community Indices of Biotic Integrity.” *Ecol. Indic.* 156: 111160. Doi: <https://doi.org/10.1016/j.ecolind.2023.111160>.
- [8] Cooper, M. J., Lamberti, G. A., Moerke, A. H., Ruetz, C. R., Wilcox, D. A., Brady, V. J., and Brown, T. N. et al. 2018. “An Expanded Fish-Based Index of Biotic Integrity for Great Lakes Coastal Wetlands.” *Environ. Monit. Assess.* 190 (10): 580. Doi: <http://dx.doi.org/10.1007/s10661-018-6950-6>.
- [9] Souza, G. B. G., and Vianna, M. 2020. “Fish-based Indices for Assessing Ecological Quality and Biotic Integrity in Transitional Waters: A Systematic Review.” *Ecol. Indic.* 109: 105665. Doi: <https://doi.org/10.1016/j.ecolind.2019.105665>.
- [10] Essa, D. I., Elshobary, M. E., Attiah, A. M., Salem, Z. E., Keshta, A. E., and Edokpayi, J. N. 2024. “Assessing Phytoplankton Populations and Their Relation to Water Parameters as Early Alerts and Biological Indicators of the Aquatic Pollution.” *Ecol. Indic.* 159: 111721. Doi: <https://doi.org/10.1016/j.ecolind.2024.111721>.
- [11] Ugya, A. Y., Yan, C., Chen, H., and Wang, Q. 2025. “Unravelling the Eco-Monitoring Potential of Phytoplankton Towards a Sustainable Aquatic Ecosystem.” *Mar. Pollut. Bull.* 216: 118021. Doi: <http://dx.doi.org/10.1016/j.marpolbul.2025.118021>.
- [12] Han, Y., Zhang, K., Lin, Q., Huang, S., and Yang, X. 2023. “Assessing Lake Ecosystem Health from Disturbed Anthropogenic Landscapes: Spatial Patterns and Driving Mechanisms.” *Ecol. Indic.* 147: 110007. Doi: <https://doi.org/10.1016/j.ecolind.2023.110007>.
- [13] Ruaro, R., and Gubiani, É. A. 2013. “A Scientometric Assessment of 30 Years of the Index of Biotic Integrity in Aquatic Ecosystems: Applications and main flaws.” *Ecol. Indic.* 29: 105-110. Doi: <https://doi.org/10.1016/j.ecolind.2012.12.016>.
- [14] Qin, M., Fan, P., Li, Y., Wang, H., Wang, W., Liu, H., and Messyas, B., et al. 2023. “Assessing the Ecosystem Health of Large Drinking-Water Reservoirs Based on the Phytoplankton Index of Biotic Integrity (P-IBI): A Case Study of Danjiangkou Reservoir.” *Sustainability* 15 (6): 5282. Doi: <http://dx.doi.org/10.3390/su15065282>.
- [15] Wu, Z., Kong, M., Cai, Y., Wang, X., and Li, K. 2018. “Index of Biotic Integrity Based on Phytoplankton and Water Quality Index: Do They Have a Similar Pattern on Water Quality Assessment? A Study of Rivers in Lake Taihu Basin, China.” *Sci. Total Environ.* 658: 395-404. Doi: <https://doi.org/10.1016/j.biortech.2017.07.081>.
- [16] Zhao, K., Dong, A., Wang, S., and Yu, X. 2022. “Ecological Health Status of the Yitong River, China, Assessed with the Planktonic Index of Biotic Integrity.” *Water* 14 (19): 3191. Doi: <http://dx.doi.org/10.3390/w14193191>.
- [17] Zhang, Z., and Huang, X. 1991. *Methods for Studying Freshwater Phytoplankton*.
- [18] Hu, H., and Wei, Y. 2006. *Freshwater Algae of China: Systematics, Ecology, and Taxonomy*. Beijing: Science Press.
- [19] Abonyi, A., Ács, É., Hidas, A., Grigorszky, I., Várbíró, G., Borics, G., and Kiss, K. T. 2018. “Functional Diversity of Phytoplankton Highlights Long-Term Gradual Regime Shift in the Middle Section of the Danube River Due to Global Warming, Human Impacts and Oligotrophication.” *Freshwater Biol.* 63 (5): 456-472. Doi: <http://dx.doi.org/10.1111/fwb.13084>.
- [20] Wei, H., Wang, Z., Du, L., and Wang, B. 2025. “Diversity and Index of Biotic Integrity Assessment of Macroinvertebrates in the Liujiang River.” *Journal of Guangxi Normal University* 43 (04): 224-238. Doi: <https://doi.org/10.16088/j.issn.1001-6600.2024072901>.
- [21] Hill, B., Herlihy, A., Kaufmann, P., Stevenson, R., McCormick, F., and Johnson, C. 2000. “Use of Periphyton Assemblage Data as an Index of Biotic Integrity.” *J. N. Am. Benthol. Soc.* 19: 50-67. Doi: <https://doi.org/10.2307/1468281>.
- [22] Tan, X., Ma, P., Bunn, S. E., and Zhang, Q. 2015. “Development of a Benthic Diatom Index of Biotic Integrity (BD-IBI) for Ecosystem Health Assessment of Human Dominant Subtropical Rivers, China.” *J. Environ. Manage.* 151: 286-294. Doi: <https://doi.org/10.1016/j.jenvman.2015.07.044>.

- jenvman.2014.12.048.
- [23] Yongo, E., Mutethya, E., Zhang, P., Lek, S., Fu, Q., and Guo, Z. 2023. "Comparing the Performance of the Water Quality Index and Phytoplankton Index of Biotic Integrity in Assessing the Ecological Status of Three Urban Rivers in Haikou City, China." *Ecol. Indic.* 157, 111286. Doi: <https://doi.org/10.1016/j.ecolind.2023.111286>.
- [24] Zhu, H., Hu, X. D., Wu, P. P., Chen, W. M., Wu, S. S., Li, Z. Q., and Zhu, L., et al. 2021. "Development and Testing of the Phytoplankton Biological Integrity Index (P-IBI) in Dry and Wet Seasons for Lake Gehu." *Ecol. Indic.* 129: 107882. Doi: <https://doi.org/10.1016/j.ecolind.2021.107882>.
- [25] Li, Z., Ma, C., Sun, Y., Lu, X., and Fan, Y. 2022. "Ecological health evaluation of rivers based on phytoplankton biological integrity index and water quality index on the impact of anthropogenic pollution: A case of Ashi River Basin." *Front. Microbiol.* 13. Doi: <https://doi.org/10.3389/fmicb.2022.942205>.
- [26] Hautier, Y., Seabloom, E. W., Borer, E. T., Adler, P. B., Harpole, W. S., Hillebrand, H., and Lind, E. M. et al. 2014. "Eutrophication Weakens Stabilizing Effects of Diversity in Natural Grasslands." *Nature* 508 (7497): 521-525. Doi: <https://doi.org/10.1038/nature13014>.
- [27] Tang, Y., Xu, F., Yang, Q., and Zhang, T. 2025. "Phytoplankton Community Assembly and Health Assessment in the Middle-Lower Jialing River via High-Throughput Sequencing." *Water Res.* X 30: 100470. Doi: <https://doi.org/10.1016/j.wroa.2025.100470>.
- [28] Yu, S., Cao, X., Chen, P., Liu, Y., Duan, G., Qi, W., and Peng, J. et al. 2025. "Climate Warming and Nutrient Enrichment Destabilize Plankton Network Stability Over the Past Century." *Commun. Earth Environ.* 6 (1): 247. Doi: <https://doi.org/10.1038/s43247-025-02206-3>.
- [29] Cao, H., Zhang, K., Deng, D., Qi, H., Li, J., Cao, Y., and Jin, Q. et al. 2023. "Environmental Heterogeneity Affecting Spatial Distribution of Phytoplankton Community Structure and Functional Groups in a Large Eutrophic Lake, Lake Chaohu, China." *Environ. Sci. Pollut. Res.* 30 (32): 79001-79014. Doi: <https://doi.org/10.1007/s11356-023-28043-5>.
- [30] Maberly, S. C., Chao, A., and Finlay, B. J. 2022. "Seasonal Patterns of Phytoplankton Taxon Richness in Lakes: Effects of Temperature, Turnover and Abundance." *Protist* 173 (6): 125925. Doi: <http://dx.doi.org/10.1016/j.protis.2022.125925>.
- [31] Cao, J., Wu, Y., Li, Z. K., Hou, Z. Y., Wu, T. H., Chu, Z. S., and Zheng, B. H. et al. 2024. "Dependence of Evolution of Cyanobacteria Superiority on Temperature and Nutrient Use Efficiency in a Meso-Eutrophic Plateau Lake." *Sci. Total Environ.* 927: 172338. Doi: <http://dx.doi.org/10.1016/j.scitotenv.2024.172338>.
- [32] Li, J., and Murdock, J. 2025. "Nutrient Concentration, Stoichiometry, and Timing of Delivery Can Regulate Cyanobacterial Dominance and Microcystin Production in Rivers." *J. Environ. Manage.* 377: 124714. Doi: <http://dx.doi.org/10.1016/j.jenvman.2025.124714>.
- [33] Zhang, K., Li, T., Chai, Y., Dai, B., Pan, Q., Wu, J., and Zhou, Q. et al. 2025. "Internal Cycling Influences Nutrient Changes Leading to Altered Nutrient Limitation in Eutrophic Lake." *Water* 17 (17): 2604. Doi: <https://doi.org/10.3390/w17172604>.
- [34] Zhao, L., Su, Y., Gao, W., Zhang, K., and Wu, J. 2025. "Delayed Water Quality Response to External Nutrient Loading Reduction: The Role of Internal Nutrient Loading in a Shallow Eutrophic Lake Over 20 Years." *Ecol. Indic.* 177: 113731. Doi: <https://doi.org/10.1016/j.ecolind.2025.113731>.
- [35] Burdis, R. M., Ward, N. K., and Manier, J. T. 2025. "Phytoplankton Assemblage Dynamics in Relation to Environmental Conditions in a Riverine Lake." *Aquat. Ecol.* 59 (2): 467-485. Doi: <https://doi.org/10.1007/s10452-025-10174-1>.
- [36] Szczerba, A., Rzodkiewicz, M., and Tylmann, W. 2023. "Modern Diatom Assemblages and Their Association with Meteorological Conditions in Two Lakes in Northeastern Poland." *Ecol. Indic.* 147: 110028. Doi: <https://doi.org/10.1016/j.ecolind.2023.110028>.
- [37] Griffiths, K., Duda, M. P., Antoniadis, D., Smol, J. P. and Gregory-Eaves, I. 2024. "Diatom Species Responses Along Gradients of Dissolved Inorganic Carbon, Total Phosphorus, and Lake Depth from Lakes Across Canada." *J. Phycol.* 60 (4): 834-852. Doi: <http://dx.doi.org/10.1111/jpy.13464>.
- [38] Yuan, L. L., Mitchell, R. M., Pollard, A. I., Nietch, C. T., Pilgrim, E. M., and Smucker, N. J. 2023. "Understanding the Effects of Phosphorus on Diatom Richness in Rivers and Streams Using Taxon-Environment Relationships." *Freshw Biol.* 68 (3): 473-486. Doi: <https://doi.org/10.1111/fwb.14040>.
- [39] Lee, J. A., Vineis, J. H., Poupon, M. A., Resplandy, L., and Ward, B. B. 2025. "Phytoplankton Community Succession and Biogeochemistry in a Bloom Simulation Experiment at an estuary-ocean interface." *Biogeosciences* 22 (18): 4743-4761. Doi: <http://dx.doi.org/10.5194/bg-22-4743-2025>.
- [40] Rzodkiewicz, M., Zawiska, I., and Sobczak, M. 2026. "Diatoms as Indicators of Eutrophication: Relationship With Transparency, Total Phosphorus and Chlorophyll—A Concentration in Central European Lakes." *Global Ecol. Conserv.* 65: e04025. Doi: <https://doi.org/10.1016/j.gecco.2025.e04025>.
- [41] Liu, X., Zhang, J., Wu, Y., Yu, Y., Sun, J., Mao, D., and Zhang, G. 2024. "Intensified Effect of Nitrogen Forms on

- Dominant Phytoplankton Species Succession by Climate Change.” *Water Res.* 264: 122214. Doi: <http://dx.doi.org/10.1016/j.watres.2024.122214>.
- [42] Silva, F. S., Moura, A. N., and Amorim, C. A. 2025. “Eutrophication Drives Functional and Beta Diversity Loss in Epiphytic Cyanobacteria.” *Hydrobiologia* 852 (17): 4459-4474. Doi: <https://doi.org/10.1007/s10750-025-05870-w>.
- [43] Beng, K. C., Cerbin, S., Monaghan, M. T., and Wolinska, J. 2025. “Long-term Changes to Plankton Communities in Artificially Heated Lakes.” *Limnol. Oceanogr.* 70 (10): 3029-3042. Doi: <http://dx.doi.org/10.1002/lno.70192>.
- [44] Hao, X., Shi, X., Zhao, S., Yu, H., Kang, R., Han, Y., and Sun, Y. et al. 2024. “Impacts of Temperature and Nutrient Dynamics on Phytoplankton in a Lake: A Case Study of Wuliangshuai Lake, China.” *Sustainability* 16 (24): 11195. Doi: <https://doi.org/10.3390/su162411195>.
- [45] Cao, J. L., Liang, H. Y., Zhang, Y.-H., Du, S.-L., Zhang, J., Tao, Y. 2024. “Development and Evaluation of the Plankton Biological Integrity Index (P-IBI) in Dry and Wet Seasons for Dianchi Lake.” *Ecologies* 5: 68-82. Doi: <https://doi.org/10.3390/ecologies5010005>.
- [46] Ding, Y., Yi, Q., Jia, Q., Zhang, J., Zhou, Z., and Liu, X. 2023. “Quantifying Phosphorus Levels in Water Columns Equilibrated with Sediment Particles in Shallow Lakes: From Algae/Cyanobacteria-Available Phosphorus Pools to pH Response.” *Sci. Total Environ.* 868: 161694. Doi: <https://doi.org/10.1016/j.scitotenv.2023.161694>.
- [47] Zou, Y., Liu, L., Jiang, Y., and Yang, C. 2025. “Research on the Gradient of Aquatic Ecological Integrity of Phytoplankton in Regional River Segments of Jiangsu Province and Its Driving Mechanism.” *Water* 17 (11): 1645. Doi: <https://doi.org/10.3390/w17111645>.
- [48] Zhang, H., Duan, Z., Wang, Z., Zhong, M., Tian, W., Wang, H., and Huang, H. 2019. “Freshwater Lake Ecosystem Health Assessment and Its Response to Pollution Stresses Based on Planktonic Index of Biotic Integrity.” *Environ. Sci. Pollut. Res.* 26 (34): 35240-35252. <https://doi.org/10.1007/s11356-019-06655-0>.
- [49] Cai, G., Ge, Y., Dong, Z., Liao, Y., Chen, Y., Wu, A., Li, Y., et al. 2024. “Temporal Shifts in the Phytoplankton Network in a Large Eutrophic Shallow Freshwater Lake Subjected to Major Environmental Changes Due to Human Interventions.” *Water Res.* 261: 122054. Doi: <https://doi.org/10.1016/j.watres.2024.122054>.
- [50] Eyring, S., Reyes, M., Merz, E., Baity-Jesi, M., Ntetsika, P., Ebi, C., and Dennis, S., et al. 2025. “Five Years of High-Frequency Data of Phytoplankton Zooplankton and Limnology from a Temperate Eutrophic Lake.” *Sci. Data* 12 (1): 653. Doi: <https://doi.org/10.1038/s41597-025-04988-9>.