

# Article

# Spatial and Temporal Variations in Environmental Variables in Relation to Phytoplankton Community Structure in a Eutrophic River-Type Reservoir

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Abstract: This study assesses spatial and temporal variation in environmental variables in relation to phytoplankton community size and composition in a typically eutrophic river reservoir (Hai River, northern China). The aim is to identify environmental parameters governing spatial and temporal differences in phytoplankton density and composition. Physicochemical parameters, including nutrient concentrations, were determined in monthly surface water samples from 2015. The average concentration of key eutrophication indexes (i.e., total phosphorous (TP:  $0.24 \pm 0.11 \text{ mg} \cdot \text{L}^{-1}$ ), total nitrogen (TN:  $2.96 \pm 1.60 \text{ mg} \cdot \text{L}^{-1}$ ), and Chlorophyll a (Chl a:  $38.5 \pm 11.5 \text{ mg} \cdot \text{m}^{-3}$ )) substantially exceeded threshold values for eutrophic streams. Moreover, the eutrophication increased significantly downstream along the river reservoir as a consequence of an increasing fraction of agricultural and industrial land-use in the watershed. 103 phytoplankton species were identified, of which Chlorophyta was the dominated phylum (47 species), followed by Bacillariophyta (23 species) and Cyanophyta (18 species). No spatial difference in species distribution (ANOVA, p > 0.05) were found, while the temporal differences in species composition exhibited significant heterogeneity (ANOVA, p < 0.001). Phytoplankton abundance was highest in early summer (June and July), with maximum values increasing from  $1.78\times10^8$  and  $2.80\times10^8$  cells  $L^{-1}$  in upstream and middle reaches, respectively, to  $4.18 \times 10^8$  cells  $L^{-1}$  furthest downstream. *Cyanophyta*, also known as Cyanobacteria and commonly referred to as blue-green algal, are known to constitute algae bloom in eutrophic systems. Common species are Microcystis marginata, Microcystis flos-aquae, and Oscillatoria sp. This was the dominant phyla during summer months, especially in the middle and lower reaches of the stream reservoir where it accounted for 88.9% of the phytoplankton community. Shannon weaver index (H') and Pielous's evenness index (J') were extremely low (1.91–2.43 for H' and 0.39-0.45 for J') in samples collected from the lower part of the stream during the period of algal bloom, indicating an imbalance in the phytoplankton communities. Canonical correspondence analysis (CCA) indicated that water temperature (WT) and possible pH, along with nitrate (NO<sub>3</sub>-N) and nitrite (NO<sub>2</sub>-N), were the most important explanatory parameters in regard to phytoplankton composition. This research provides an understanding of the role of physicochemical water quality parameters in governing algal blooms and phytoplankton composition in river reservoirs.



**Keywords:** river-type reservoir; eutrophication; environmental factors; phytoplankton; canonical corresponding analysis (CCA)

## 1. Introduction

Eutrophication, manifested by severe algae blooms, is globally the most pervasive water quality challenge [1–3]. It perturbs ecosystem services of the aquatic systems and decreases biodiversity [3–5]. The total amount of phytoplankton in a sample is a good biological indicator of the trophic state since algae are ubiquitous, abundant and respond quickly to environmental changes in the ecosystem [6–9]. But the total amount of algal provides little information regarding the processes governing their spatial and temporal distribution. In order to understand the conceptual mechanisms governing eutrophication there is a need for qualitative information of phytoplankton community structures.

River reservoirs, being both a river and a reservoir, are commonly used for water supply, flood control, irrigation as well as hydroelectric power generation [10,11]. These dammed rivers are generally characterized by great lengths, slow water flow velocity and thus long hydraulic residence time [12,13]. Their dams impose a substantial influence on sediment transport, thermal conditions and chemical cycling, and hence have strong impact on water quality and the aquatic ecosystems in the river [14]. Wang et al found that 21.4% of 14 river reservoirs in the Yangtze basin were eutrophic [15].

Hai River in Tianjin city is a typical river reservoir in northern China. Physical and chemical characteristics of its water quality has been documented by several studies, but there is a scarcity in systematic studies of its phytoplankton composition [16–19].

In this paper, a time series of environmental and phytoplankton variables have been studied. Temporal and spatial differences in phytoplankton composition, abundance, and diversity and their relations with environmental conditions are assessed. The objective of this study is to improve the knowledge basis for early warning systems for algal blooms as well as strategies for remediation of eutrophication in similar types of river reservoirs.

## 2. Materials and Methods

### 2.1. Study Area

Hai River reservoir (Figure 1) winds 72 kilometers through the urbanized area of Tianjin city, draining a catchment area of 2066 km<sup>2</sup> before it feeds into the Bohai Sea [20]. It is an important reservoir as it serves several functions including flood control, water supply, irrigation, and recreation. It thus exerts a great influence on the economic and cultural development of Tianjin megacity. In recent years the water flow of Hai River has become practically stagnant due to reduced water influx and gate-dam constructions. Moreover, Hai River suffered severe eutrophication due to diffuse losses of nutrients from intensive agriculture and industry, as well as leaks in sewage system from a dense urban population, in the watershed [19–21].

## 2.2. Sampling

Water samples from Hai River (Figure 1) were collected at a depth of 20–30 cm from 10 sampling sites, including two main tributaries (S1 & 2), junction (S3), and along Hai River (S4–10). Sampling was conducted in 2015 at weekly intervals between May and August, and monthly during the period from September to November.



**Figure 1.** Map of the locations of sampling sites. ● refers sampling sites. ★ denotes dams.

## 2.3. Measurements

Water Temperature (WT), pH, Dissolved Oxygen (DO), Oxidation Reduction Potential (ORP), and Conductivity (Cond) were measured in situ with a multi-parameter sonde (YSI Inc.©, Yellow Springs, OH, USA). Chemical Oxygen Demand (COD), Total Nitrogen (TN), Ammonia Nitrogen (NH<sub>3</sub>-N), Nitrite (NO<sub>2</sub>-N), Nitrate (NO<sub>3</sub>-N), Total Phosphorus (TP), Phosphate (PO<sub>4</sub>-P), Suspended Solids (SS), Salinity (SAL), and Chlorophyll a (Chl a) were analyzed by standard methods issued by the Chinese State Enviroemntal Protection Administration [22].

For taxonomic identification and algal cell numbers, a polyethylene bottle was filled with 1000 L sample and fixed immediately with 5‰ Lugol solution. After sedimentation for 48 h, the bulk of water was decanted off, leaving 20–30 mL of sedimented algae which was collected for analysis [23,24]. Counting was performed using microscopy (Algacount S300 (Hangzhou Xunshu Technology Co., Ltd., Hangzhou, China)).

Data from the sample sites S1–10 were grouped into three sections and averaged, with sites 1–3, 4–7, and 8–10 belonging to the upper, middle and lower reaches of the streams, respectively. Data from weekly samples from May to August were averaged to monthly values.

## 2.4. Species Distribution and Diversity

Dominant species were determined using Equation (1) [25],

$$Y = \left(\frac{n_i}{N}\right) f_i \tag{1}$$

where  $n_i$  denotes the number of algae of species *i*, *N* is the total number of algae in the sample,  $f_i$  denotes the frequency of the species *i* appearing in the sample, and *Y* > 0.02 indicates dominant species.

The Shannon-Weaver index (H'), which is used to estimate species diversity in categorical data, was computed using the Equations (2) and (3) [26],

$$H' = -\sum_{i=1}^{S} P_i log_2 P_i \tag{2}$$

$$P_i = \frac{n_i}{N} \tag{3}$$

where  $n_i$  denotes the number of algae of species *i*, *N* is the total number of algae in the sample, and  $P_i$  is the proportion of species in a given family.

The Pielou index (J'), adopted as the Evenness index, was calculated using Equation (4) [27].

$$J' = \frac{H'}{\log_2 S} \tag{4}$$

where *S* denotes total number of species in the samples.

#### 2.5. Statistical Analysis

Spatial and temporal variation in the composition of phytoplankton species was assessed by analysis of variance (ANOVA) using SPSS 19. Differences were considered significant at  $p \le 0.05$  (95% confidence level).

Canonical correspondence analysis (CCA) (Canoco for Windows 4.5 software) [28,29] was used to examine empirical relationships between environmental factors and phytoplankton species. All measured environmental parameters (Section 2.3) except Chl a were included in the analysis after  $log_{10}(x + 1)$  transformation (except pH). Phytoplankton species that were included in the CCA satisfied the following two criteria [30,31]: (1) the chosen species appeared more than 3 times among all sampling sites; (2) the relative population density of this species exceeded 1% in at least one sampling site.

### 3. Results and Discussion

#### 3.1. Environmental Factors

Monthly data from April to November 2015 on the physicochemical parameters measured in Hai River are given in Figure 2. Average pH is alkaline, which is typical for eutrophic waters (Figure 2a) [32,33]. The COD varied from 23 mg·L<sup>-1</sup> to 42 mg·L<sup>-1</sup> (Figure 2b). This ranks between Grade III and V of the Environmental Quality Standards for Surface Water in China (GB3838-2002) [34]. Average TP (0.23 mg·L<sup>-1</sup>), TN (2.8 mg·L<sup>-1</sup>) and Chl a (38.5 mg·m<sup>-3</sup>) substantially exceed threshold values for eutrophic state (TP of 0.075 mg·L<sup>-1</sup>, TN of 1.5 mg·L<sup>-1</sup>, Chl a of 30 mg·m<sup>-3</sup>) proposed by Odds et al. [35] (Figure 2c–e). It is thus clear that Hai River suffers severe eutrophication. Moreover, measured values generally increased from the upper to the lower reaches of the stream, except for WT, ORP, and SS. Especially, nutrient levels in the lower reaches of the stream were much higher than in the other two sections, especially in the fall (Figure 2c–i). The spatial trend in eutrophication along Hai River is likely due to differences in land use. The watershed of the upper and middle reaches is mainly recreation areas that are not strongly influenced by human activities, while the catchment of the lower part of the stream was strongly affected by local agriculture and industry. Anthropogenic activities such as uncontrolled sewage discharge and surface runoff drainage contributed to the high eutrophication of downstream [18].

Temporally, pH remained  $\geq 9$  in the lower reaches, while in the middle and upper parts of the stream the pH was in range 8.3–8.9 (Figure 2a). WT varied according to seasons, with minimum and maximum values of 18 °C and 29 °C recorded in November and August, respectively (Figure 2j). Cond and SAL decreased from spring to summer and increased again in fall (Figure 2k,l). SS increased through the year from around 5 mg·L<sup>-1</sup> in the spring to a maximum of 38 mg·L<sup>-1</sup> in the fall (Figure 2m). ORP was high only in the early spring (April) and dropped to relatively low level during June to August (Figure 2n), likely due to increased respiration of dead organic matter from the algae bloom. DO fluctuated but generally decreased from spring to fall (Figure 2o). TP and PO<sub>4</sub>-P were relatively low during spring and summer, increased through late summer, and declined in the late fall (November), especially in the lower section of the river (Figure 2c,i). This is also the case for TN and NO<sub>3</sub>-N (Figure 2d,f). Chla was low in spring, high during the summer, and declined again in the fall, basically following the same trends as WT. During the summer period, from May to September, the Chl a concentration frequently exceeded 30 mg/m<sup>3</sup> and occasionally even exceeded



 $60 \text{ mg/m}^3$ . Periods with Chl a concentration above  $30 \text{ mg/m}^3$  are considered as algal bloom in several studies [36,37].

Figure 2. Spatial and temporal variations of environmental factors. The environmental factors are: (a) pH; (b)  $\text{COD}_{Mn}$ ; (c) TP; (d) TN; (e) Chl a; (f) NO<sub>3</sub>-N; (g) NO<sub>2</sub>-N; (h) NH<sub>3</sub>-N; (i) PO<sub>4</sub>-P; (j) T; (k) Cond; (l) SAL; (m) SS; (n) ORP; (o) DO.

# 3.2. Phytoplankton Community Structure

## 3.2.1. Phytoplankton Composition

A total of 103 phytoplankton species (including the varieties and forms), belonging to 8 phyla (Table S1 in Supplementary Materials and Figure 3), were found in the samples from Hai river reservoir. *Chlorophyta* was by far the most species-abundant group (47 species, accounting for 46% of the total number of species), followed by *Bacillariophyta* (23 species, 22%) and *Cyanophyta* (18 species, 17%).

46%



4%

2% 2% 2%

Figure 3. Phytoplankton species composition.

17%

Distribution of phytoplankton groups at the three parts of the river and during different months are shown in Figure 4a,b. The relative contribution of the dominant phyla *Chlorophyta, Bacillariophyta,* and *Cyanophyta* were not significantly different between the three surveyed stream sections (ANOVA, p > 0.05). However, there was significant temporal heterogeneity (ANOVA, p < 0.001) in the distribution of phytoplankton groups. Highest phytoplankton biodiversity was found in June (8 phyla, 93 species) and lowest in May (5 phyla, 20 species). The contribution of *Chlorophyta* to the total number of species remained practically constant throughout the sampling months while *Bacillariophyta* reached its maximum (23%) and minimum (13%) contribution in May and June, respectively. The relative amount of *Cyanophyta* (*Cyanobacteria*) increased from less than 10% in May to 26% in July, thereafter remaining stable around 20% for the rest of the year.



Figure 4. Cont.



Figure 4. (a) Relative abundancy of species within different segment of the stream; (b) During different months.

# 3.2.2. Phytoplankton Abundance

Absolute amount of phytoplankton and relative abundance of *Cyanobacteria* tended to increase from upper through middle to lower reaches of the stream (Figure 5a–c). Since similar trends were also found for bioavailable nutrients (Figure 2f–i), it is inferred that the spatial differences in levels of phytoplankton, and especially *Cyanobacteria*, were mainly governed by the levels of nutrients, as also reported by many other studies [31,38].



Figure 5. Cont.



**Figure 5.** Spatial and temporal variations of phytoplankton and *Cyanophyta* abundance in upper, middle and lower reaches of the stream: (**a**) Upper reaches; (**b**) Middle reaches; (**c**) Lower reaches.

Temporally, phytoplankton levels were low in spring (May), though they grew rapidly during early summer, reaching a maximum within 1–2 months. This is likely mainly due to increase in WT (Figure 2j), as well as a concurrent increase in daylight. Average phytoplankton levels in June and July were  $1.78 \times 10^8$ ,  $2.80 \times 10^8$ , and  $4.18 \times 10^8$  cells·L<sup>-1</sup> in upstream, middle, and lower reaches, respectively. *Cyanobacteria* was during summer the main phyla, comprising on average 54.2%, 74.5%, and 88.9% of the total phytoplankton in the three sections, respectively, through the summer and fall algae bloom decreased gradually. With a drop in WT from 29 °C in August to 18 °C in November, the relative contribution of *Cyanophyta* to total phytoplankton abundance decreased by between 94% to 96%. It appears thus clear that the decrease in WT and daylight especially inhibited the growth of *Cyanophyta* relative to the other phytoplankton groups.

## 3.2.3. Dominant Species

In order to identify the dominant species before and during the algal bloom, samples collected in May and August were selected for investigation. As shown in Table 1, a clear difference in the dominant species was observed between May and August (Table 1). Phytoplankton species in the *Cyanophyta*, *Chlorophyta*, and *Bacillariophyta* groups dominated ( $Y \ge 0.02$ ) the alga community. In May, the number of dominant species was slightly higher than in August, with a maximum biodiversity recorded in lower parts of the stream (9 species), followed by upper reaches of the stream (7 species). The dominant species in May were mainly from the phylum *Chlorophyta* and *Bacillariophyta*, including *Scenedesmus quadricauda*, *Scenedesmus acuminatus*, *Chlorogonium elongatum*, *Coccomyxa* sp., *Gonatozygon* sp., *Melosira italica*, *Diatoma vulgare*, and *Cyclotella bodanica*. *Cyanophyta* was only represented with 2 dominant species. This indicates that *Cyanobacteria* were not common in the relatively cool river water (~17 °C) before algal bloom.

	May				August	
Latin Name	Upper Reaches	Middle Reaches	Lower Reaches	Upper Reaches	Middle Reaches	Lower Reaches
Cyanophyta						
Chroococcus minutus Chroococcus turgidus Microcustis marcinata	0.025		0.079		0.042	0.102
Microcystis flos-aayae					0.042	0.102
Oscillatoria sp. Spirulina sp.				0.071	0.125	0.108 0.028
Merismopedia sp.				0.072	0.051	
Chlorophyta						
Scenedesmus quadricauda		0.136	0.186	0.046		
Scenedesmus acuminatus			0.043			
Chlorogonium elongatum	0.093	0.096	0.057			
Coccomyxa sp. Tetrastrum glabrum	0.027		0.024 0.043			
Gonatozygon sp. Pediastrum simplex	0.061	0.091		0.132	0.062	
Bacillarionhuta					0.037	
Malacina italiaa	0.000	0.101	0.070			
Melosira italica Diatoma zulgara	0.293	0.121	0.079			
Cuclotella hodanica	0.080	0.136	0.236			
Melosira granulata	0.02/	0.120	0.107			0.038

In August, most of the dominant species found in May had disappeared. Instead, *Cyanobacteria* became overwhelmingly dominant in middle and lower reaches, constituting 4 out of the 6 dominant species in the middle part, and 4 of 5 species in the lower reaches of the stream. Dominant species in August were *Microcystis marginata*, *Microcystis flos-aquae*, and *Oscillatoria* sp., which are all renowned as problematic bloom-forming species in eutrophic systems.

## 3.2.4. Species Diversity

Species diversity is a key indicator of water quality as it is closely related to the trophic state of the water body [8,39]. Moreover, several studies have shown that a high diversity index indicates that the ecosystem is in a healthy and stable state, while a low value suggests a less healthy or degraded ecosystem [8,40]. The Shannon species diversity index (H') and Pielous's evenness index (J') were therefore used to estimate the eco-system stability of Hai River.

*H'* differed between the upper, middle, and lower sections of the river (Figure 6a), with relatively medium (2.78), higher (3.15), and lower (2.33) average values, respectively. *J'* followed the same

spatial pattern, with average values of 0.73, 0.78, and 0.51, respectively (Figure 6b). In the upper part of the stream, low phytoplankton abundance gave moderate values of diversity and evenness. With conditions more suitable for algae growth in middle reaches, both H' and J' reached their maximal values. The indexes varied also less through the year in the middle section, reflecting a more stable system. In the lower stream section, with the largest algal bloom, competition between phytoplankton species for resources was tough [41], resulting in a significantly lower biodiversity. Lowest monthly values of H' and J' were thus usually found in the samples from the lower part of the stream. Similar trends were also found in Salto Grande reservoir [42].



Figure 6. Spatial and temporal variations of Shannon–Wiener: (a) The Shannon species diversity index; (b) Pielous's evenness index.

In the lower part of the stream both H' and J' declined sharply from May to June as maximum phytoplankton abundance was attained. Low H' (1.91–2.43) and J' (0.39–0.45) were maintained during the summer period from June to September, with only minor parallel temporal fluctuations, reflecting the strong sensitivity of these diversity indices to the predominance of a few *Cyanobacteria* 

species. That Cyanobacteria blooms causes decreased H' and J' has been thoroughly documented in many studies [6,43].

## 3.3. Relationships between Phytoplankton Community and Physicochemical Parameters

CCA has been found useful for qualitative assessment of the interactions between environmental factors and phytoplankton community in highly complex systems [28,29,44]. In this study, CCA was conducted between the 13 dominant phytoplankton species (Table 2) and all of the 14 measured physicochemical parameters. The statistical relationships between population density of phytoplankton species and physico-chemical parameters in Hai River are shown in Figure 7.

Table 2. Codes of phytoplankton species used in canonical correspondence analysis CCA.

Codes	Phytoplankton			
S1	Microcystis marglnata			
S2	Microcystis flos-aquae			
S3	<i>Oscillatoria</i> sp.			
S4	Anabeana sp.			
S5	Merismopedia sp.			
S6	Spirulina sp.			
S7	Pediastrum simplex			
S8	Pediastrum simplex var.			
S9	Scenedesmus quadricauda			



**Figure 7.** Species-environment biplot from CCA. The points represent the individual phytoplankton species and the arrows represent each environmental variable pointing in the direction of its maximum change across the diagram during the study. The species are denoted with codes referring to Table 2.

The horizontal axis (SPE AX1) is a linear combination of environmental variables that best explains the variation in a matrix of species abundance, while the orthogonal axis (SPE AX2) explains the remaining variance in the community data [45,46]. The eigenvalues of SPE AX1 and SPE AX2 were 0.320 and 0.195, respectively, which accounted for 64.8% of the cumulative variance in the species data.

The explained variance of SPE AX1 and SPE AX2 was 40.3% and 24.5%, respectively. The correlation coefficient between SPE AX1 and AX2 was zero, indicating that the analysis results were reliable [28].

SPE AX1 was mainly negatively correlated with pH (-0.883), WT (-0.806), TP (-0.743), and TN (-0.739), whereas SPE AX2 was mainly positively correlated with NO<sub>3</sub>-N (0.731) and NO<sub>2</sub>-N (0.747). These loadings indicate that the main variation in the species density is related to the parameters pH, WT, TP, and TN. Of these, only WT is an explanatory factor as pH, TP, and TN are all parameters that, to a large extent, are governed by the alga density itself rather than governing the species density. Photosynthesis affects the carbon equilibrium via carbon fixation and tends to increase water pH values [32]. On the other hand, pH is reported to be closely related to photosynthetic primary productivity [32]. Studies found that alkaline water environment could act as a trap for atmospheric carbon dioxide and thereby benefit photosynthesis [47]. Therefore, the high alkaline conditions (especially in the lower part) may represent positive feedback mechanisms leading to a strong relationship with phytoplankton activity. Moreover, WT is reported as the most important factor influencing community composition and distribution via controlling the multiplication rate and standing stock of phytoplankton population in many studies [31,48,49]. It is also strongly related to the hours of daylight. SPE AX2 represents the variance of NO<sub>3</sub>-N and NO<sub>2</sub>-N. Essential macronutrients for algal growth, PO<sub>4</sub>-P and No<sub>x</sub>-N, are found to have different effect on phytoplankton community composition (Figure 7). This is due to the fact that different phytoplankton species have unlike requirements [50]. The N-fixating Cyanothyta Microcystis marginata (S1) have competitive advantages where PO<sub>4</sub>-P is high and NO<sub>3</sub>-N is low, while Chlorophyta Pediastrum simplex (S7), Pediastrum simplex.var (S8), and Scenedesmus quadricauda (S9) showed the opposite phenomenon.

# 4. Conclusions

Spatial differences in phytoplankton density along the Hai River reservoir in Tianjin, China, were found to be strongly related to bioavailable nutrient levels. *Cyanophyta* constituted a large part of phytoplankton composition and abundance during the summer months. Species diversity was the lowest in the nutrient-rich part of the river with high phytoplankton density. This reflects a more unstable water ecosystem due to the rapid growth of some *Cyanobacteria* species. Furthermore, WT (as well as the concurrent duration of daylight) and, possibly, pH were identified as the most important environmental factors contributing to the temporal fluctuations in phytoplankton community structure in the Hai River. These discoveries provided a better insight into the estimation of the eutrophic state in the Hai River trunk stream. In this study, water samples were collected from 20 cm to 30 cm below the surface. Further work should be directed towards exploring the vertical distribution of phytoplankton communities in response to thermal stratification in a eutrophic river reservoir. In addition, athough 103 species were identified in the community, only 13 taxa that constituted the highest number of species were chosen for CCA analysis on account of simplicity. In the follow-up work, more intensive and long-term sampling work should be carried out for more accurate CCA analysis between more taxa and the environmental variables.

**Supplementary Materials:** The following are available online at www.mdpi.com/2073-4441/9/10/754/s1, Table S1: Phytoplankton species composition in Haihe river trunk stream.

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