Cyanobacteria in a tributary backwater area in the Three Gorges Reservoir, China

Yan Xiao,1 Zhe Li,1,3* Jinsong Guo,1 Jing Liu,1,2 and Yang Huang1,2

1 Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Shuitu Town, Beibei District, Chongqing, China
2 College of Urban Construction & Environmental Engineering, Campus B, Chongqing University, Chongqing, China
3 China Three Gorges Corporation, Beijing, China

* Corresponding author: Lizhe@cigit.ac.cn

Received 12 October 2014; accepted 8 September 2015; published 22 January 2016

Abstract

The ecological processes leading to cyanobacterial dominance in reservoirs differ somewhat from those in lakes and rivers. To explore the development of cyanobacteria in response to large-scale reservoir operation, a 2-year, biweekly monitoring program was implemented from 2007 to 2009 in the Pengxi River Backwater Area, a typical tributary of the Yangtze River in the Three Gorges Reservoir. The data were divided into 3 subsets based on the seasonal operations of the reservoir. During the low-water operation period, temperature stratified in the water column, and intensive rainfall brought suspended solids and nutrients into the Pengxi River Backwater Area. These drivers led to relatively high levels of total particulate matter and low levels of euphotic depth in the water column compared with both the high-water operation period and reservoir discharge period. During these 2 periods, increases in water temperature stimulated the growth of most cyanobacteria. We concluded that the large-scale operation caused structurally different habitats in the Pengxi River Backwater Area, related to water depth, reservoir volume, and nutrient dynamics. These impacts were closely linked to seasonal effects, such as rainfall, river flow, and water temperature, which resulted in differences in habitat among reservoir operation stages.

Key words: cyanobacteria, environmental factors, reservoir operation, Three Gorges Reservoir, tributary backwater area

Introduction

In freshwaters, cyanobacteria dominance and the occurrence of cyanobacterial blooms significantly reduce water quality and cause serious ecological damage and economic losses (Oliver and Ganf 2002, Becker et al. 2008). Over the past 3 decades, many studies have investigated the ecological behavior and regulating factors of cyanobacteria communities in aquatic systems (Takamura et al. 1985, Reynolds 1989, Yamamoto 2009). High phosphorus concentrations and low intensity turbulent mixing in the water column are known forces driving the dominance of cyanobacteria (Paerl et al. 2001); however, the ecological processes leading to cyanobacteria dominance vary greatly among waterbodies (Xie 2007). Studies conducted by Reynolds (1989), Oliver and Ganf (2002), and Becker et al. (2008) suggested that, in the summer, weak disturbance from wind stress and the continuous high temperature increases the light ratio between the euphotic layer and mixed layer ($Z_{eu}/Z_{in} > 1$) in deep lakes. Cyanobacteria with suspension growth mechanisms can move to more appropriate layers in the water column and form blooms (Humphries and Lyne 1988, Zohary and Breen 1989, Ibelings and Maberly 1998). Sherman et al. (1998), Mitrovic et al. (2003), and Davis and Koop (2006) reported that river discharge...
strongly influenced the formation of *Anabaena* blooms. Under low river flow, turbulence at the bottom of the water column was higher than at the surface, which allowed temporary, strong temperature stratification, conditions that might induce *Anabaena* blooms when they last >5 days (Ibelings and Maberly 1998). Paerl et al. (2001) summarized that tolerance to extreme environmental conditions; greater competitiveness for nitrogen (N), phosphorus (P), and other nutrients; superior buoyancy; and predator avoidance can result in a specific cyanobacteria dominating inland waters. Nevertheless, the specific physical, chemical, or biological attributes of distinct waterbodies contribute different mechanisms to induce cyanobacteria blooms (Kong and Gao 2005, Ploug 2008).

Reservoirs on dammed rivers are typical transitional ecosystems (Thornton et al. 1990, Wetzel 2001). Due to the submergence of the terrestrial ecosystem, the first few years of impoundment may trigger an algae boost, known as a trophic upsurge (Ostrofsky and Duthie 1980). The hydrodynamic conditions of reservoirs are affected by both river runoff and artificial reservoir operation, leading to distinctive habitats (Li et al. 2009). In most reservoirs, however, the effect of these 2 drivers on environmental factors and their subsequent effect on cyanobacteria remain uncertain (Zheng et al. 2006). Lin and Han (2007) found that hydraulic retention time was a major factor influencing cyanobacteria blooms in reservoirs, with a sharp increase in filamentous cyanobacteria occurring when the hydraulic retention time was >14 days. Similarly, Ha et al. (2002) confirmed a strong influence of river discharge on the occurrence of *Microcystis* blooms. Although physical controls in reservoirs are crucial for cyanobacterial blooms, the link between large-scale reservoir operation and cyanobacteria growth has not been clearly documented.

The Three Gorges Project is one of the world’s greatest hydro-projects, providing multiple functions including flood control, hydropower, and navigation. After the Yangtze River was blocked by the construction of the Three Gorges Dam (TGD) in 2002, the Three Gorges Reservoir (TGR) formed, with a length >600 km and a width of 1–2 km. The dam has significantly changed the limnological features of the Yangtze River, and its tributaries in the TGR range from lotic systems to nearly lentic river–reservoir hybrid systems. Artificial operation of the TGR in the summer for flood control and in the winter for hydropower generation causes large seasonal water level fluctuations (>30 m) and creates distinctive habitats for cyanobacteria. Over the past few decades, serious algal blooms have been reported, especially cyanobacterial blooms, in some tributaries of the Yangtze River in the TGR, regarded as key visible ecological responses to reservoir operation. Previous studies have shown that suspended sediments and hydrodynamic conditions impacted by river flow and artificial operation are the main factors regulating the succession of phytoplankton, leading to light limitation in this meso-eutrophic reservoir ecosystem. Phosphorus concentration and the N/P ratio were also found to potentially affect the growth of cyanobacteria in the reservoir (Zhang et al. 2009). A detailed analysis indicated that the N/P ratio potentially regulated phytoplankton growth during specific reservoir operating stages, which might create cyanobacterial dominance in the aquatic ecosystem (Li et al. 2012).

In the current study, we hypothesized that the large-scale seasonal operation of the TGR structured distinctive habitats in the tributary backwater area that regulated the growth and dominance of cyanobacteria. To test the hypothesis, a 2-year field research program was carried out from 2007 to 2009 in the Pengxi River Backwater Area (PBA), a typical tributary of the Yangtze River in the TGR. We evaluated the effect of variations in environmental factors on cyanobacteria, with a focus on the mechanisms associated with the development and dominance of cyanobacteria in the context of large-scale reservoir operations.

**Study site**

The initial impoundment of the TGR began in 2003. Three years after dam construction and river flow regulation, the water level in the TGR reached 156 m for the first time in October 2006 and the TGR began its preliminary operating stage, during which water levels were 145 m in the summer flood season and 156 m or higher in the winter (Fig. 1). In November 2009, the water level in the TGR reached 175 m for the first time, indicating it had reached fully functional operation. Since 2009, under the optimal operation strategy, the TGR begins impoundment in the autumn and retains a 175 m high water level during the winter. The dam generates hydropower from the end of winter to the end of spring. The water level in the TGR is kept at 145 m before the flood season, preparing for storage of 22 billion m$^3$ (Fig. 1).

The Pengxi River (Fig. 2) is one of the largest tributaries of the Yangtze River, located at 31°00’N, 107°56’E to 31°42’N, 108°54’E in the mid-reach of the TGR region. The river covers a watershed area of 5172.5 km$^2$ under subtropical monsoon climate conditions (Fig. 2). The main stream length of the Pengxi River is about 182 km, with an average channel slope of 0.125%. The annual precipitation in the watershed of the Pengxi River is 1100–1500 mm, and the annual discharge is 118 m$^3$ s$^{-1}$. Following impoundment of the TGR at a water level of 145 m, the Pengxi River formed a backwater area ~60 km from its conflux with the Yangtze River in the town of Shuangjiang, extending upstream to the town of Yanglu.
Methods

Sampling program

To fully reflect the water environment of the PBA, 5 sampling sites were set up in the main channel of the backwater area (Fig. 2); Quma (QM: 31°07′50.8″N, 108°37′13.9″E), Gaoyang (GY: 31°5′48.2″N, 108°40′20.1″E), Huangshi (HS: 31°00′29.4″N 108°42′39.5″E), Shuangjiang (SJ: 30°56′51.1″N, 108°41′7.5″E), and Hekou (at the conflux with the Yangtze River; HK: 30°57′03.8″N, 108°39′30.6″E). Sampling frequency was twice a month for both cyanobacteria and other physiochemical parameters. Water samples were collected in polyethylene bottles from depths of 0.5, 1.0, 2.0, 3.0, 5.0, and 8.0 m using a 3 L Kitahara’s water sampler. Sampling was conducted between 0930 and 1630 h, and all chemical analyses were completed within 48 h of sampling.

Measurement and data processing

Field environmental variables included the water temperature profile (WT) and underwater photosynthetic active radiation (PAR). The PAR gradients in the water column were measured using a LI-192 SA photon flux meter (Li-Cor Bioscience, Lincoln, IL, USA). Euphotic depth ($Z_{eu}$) was estimated according to the Beer-Lambert
law. Temperature profiles were used to estimate the mixing layer in the water column \((Z_m)\). The \(Z_m/Z_{eu}\) ratios were then estimated as an environmental factor affecting cyanobacteria in the PBA.

Mixed water samples were filtered through a Whatman GF/F fiber membrane before chemical analysis for ammonium \((NH_4-N)\), nitrate \((NO_3-N)\), nitrite \((NO_2-N)\), dissolved total nitrogen \((DTN)\), soluble reactive phosphate \((SRP)\), and dissolved total phosphorus \((DTP)\). Chemical analyses were conducted according to APHA (1995). Mixed water samples without filtration pretreatment were directly digested for total phosphorus \((TP)\) and total nitrogen \((TN)\) measurements. The total suspended solids \((TSS)\) were determined based on difference in the weight of a GF/F membrane before and after filtration of 500 mL mixed water samples \((APHA 1995)\).

Cyanobacteria samples were immediately preserved on site with 1% Lugol’s solution. A 48 h sedimentation method was used for taxon identification and counting \((Eker et al. 1999)\). Cyanobacteria was identified and cell density was counted under a microscope \((Olympus BX 51, Japan)\) using the appropriate magnification and a Fuchs-Rosenthal slide. Before counting, the whole sample was gently mixed. Taxonomic identification was conducted in accordance with Hu and Wei \((2006)\). Cyanobacteria identification and counting were conducted at the species level, and quantitative cyanobacteria data were processed by genus.

The water level at HK in the PBA was estimated according to the water surface gradients between the TGD dam site \((http://www.ctgpc.com.cn)\) and the Wanxian hydrology monitoring station \((http://www.cqwater.gov.cn)\). Daily rainfall data were downloaded from the China Meteorological Data Sharing System \((http://cdc.cma.gov.cn)\). The daily discharge of PBA was modeled from a distributed hydrologic model in the Xiaojiang Watershed \((Long et al. 2009a, 2009b, Wu et al. 2010)\). The values of rainfall and river flow were homogenized in this study. Equation 1 was applied to calculate the average value of rainfall and discharge:

\[
\text{Ave } J = \frac{1}{d} \sum_{i=1}^{d} J_i
\]

where \(d\) was the time interval between 2 sampling events and \(J\) was the daily rainfall or river discharge. Therefore, Ave \(J\) is denoted as AveRain or AveFlow for the average rainfall and flow discharge, respectively.

All the datasets were divided into 3 stages \((Fig. 2)\) according to the operation cycle of the TGR; low-water operation \((LW: \text{Jun–Sep})\), high water operation \((HW: \text{Oct–Feb})\), and the discharge period \((DS: \text{Mar–May})\).

Statistical analysis of the 3 subsets was carried out using the SPSS 13.0 package. Nonparametric correlation analyses were used to determine relationships among cyanobacterial cell density and major environmental factors. Ordination analysis was performed using CANOCO 4.5 \((ter Braak and Šmilauer 2002)\). Canonical correspondence analysis \((CCA)\) was performed to get an approximate ordering of the cyanobacteria species’ optima for the environmental variables.

Results

Variations in environmental factors

Daily river flow and water levels in the PBA during the 2-year study \((Fig. 3)\) show that in 2007, the water level was maintained at 156 m during the winter drought season and 145 m during the summer flood season. At the end of October 2008, the water level in the TGR was impounded at 172.3 m, approaching the designated water level of 175 m. Seasonal changes in rainfall resulted in changes in the river flow in the PBA. The maximum river flow during the study was 907.09 m\(^3\) s\(^{-1}\) recorded on 20 June 2007. An epilimnion layer gradually developed in the water column as the air temperature rapidly increased from late spring \((May)\) to the end of summer \((Sep)\), reaching a maximum of \(~10\ m\) below the water surface \((Fig. 4)\); however, during LW, incoming floods disturbed stable stratification. This pulse effect frequently occurred in second half of July during the study. At the end of September, water column stratification diminished as the water level in the PBA increased rapidly due to reservoir impoundment. During HW, no stratification occurred in the PBA.
Generally, the PBA was a monomictic system, similar to most inland waterbodies under these climatic conditions.

The TN, TP, and total particulate matter (TPM) in the PBA varied, and their concentrations started to increase from March, reaching a maximum level in May. Conversely, $Z_{eu}$ started to decrease from February and reached a minimum level at the end of August. The $Z_{eu}/Z_{m}$ ratio was highest from March to May (Fig. 5). Concentrations of TN and TP were among the highest during DS compared with those during LW and HW, and the TN/TP ratios were among the highest during the LW period (Fig. 6). Major soluble forms of N and P (i.e., NO$_3$-N, DIN, and SRP) were significantly higher during HW and DS than during LW. Nevertheless, NH$_4$-N was relatively higher during LW compared with HW and DS. Euphotic depth was among the highest during HW and the lowest during LW, whereas the $Z_{eu}/Z_{m}$ ratio was highest during DS. Conversely, TPM was among the highest during LW and the lowest during HW. ANOVA analysis indicated significant differences in environmental factors among the 3 reservoir operation stages ($P < 0.05$).

**Variation of cyanobacteria cell density and its relative abundance**

The cell density of cyanobacteria and its relative abundance in phytoplankton (Fig. 7) indicates that the mean cyanobacterial cell density during the study period was $46.50 \times 10^5$ cells L$^{-1}$, which is ~31.8% of the total density of phytoplankton. In general, large numbers of cyanobacteria appeared in April, with the highest levels attained in May, remaining high until August. The cell density then gradually decreased from September to February, reaching its lowest value in February or March.

Cyanobacterial cell density was clearly much lower during HW than during LW and DS, as indicated by the average values of $4.72 \times 10^5$ cells L$^{-1}$, $39.77 \times 10^5$ cells L$^{-1}$, and $100.13 \times 10^5$ cells L$^{-1}$, respectively (Table 1).

**Correlation analysis of cyanobacteria and major environmental factors**

Spearman correlation analyses of cyanobacteria cell density and major environmental factors at LW, HW, and DS (Supplementary Tables S1–S3) show that at LW, the cell density of cyanobacteria was negatively correlated with NH$_4$-N and TN ($P < 0.05$). Although there were no significant correlations between cyanobacteria cell density and P ($P > 0.05$), the TN/TP ratio showed a negative correlation with cyanobacteria cell density ($P < 0.05$). Moreover, the increase in $Z_{eu}/Z_{m}$ ratio and water level in the PBA also positively correlated with the increase in cyanobacteria cell density ($P < 0.05$).

During HW, cyanobacteria cell density was negatively correlated with NH$_4$-N and TN ($P < 0.05$) as well as TP.
Moreover, increases in water temperature, rainfall, and river flow contributed to the increase in cyanobacteria cell density during HW. Increases in TPM and decreases in euphotic depth also contributed to the increase in cyanobacteria during HW. We found no significant correlation between the $Z_{eu}/Z_m$ ratio and cyanobacteria ($P > 0.05$).

Unlike during LW and HW, there was no significant correlation between NH$_4$-N and cyanobacteria, whereas NO$_3$-N, TN, and SRP showed negative correlations with cyanobacteria during DS ($P < 0.05$). TP was not significantly correlated with cyanobacteria. As in the HW period, increases in water temperature, rainfall, and river flow contributed to the increase in cyanobacteria during DS. Additionally, there was a weak but significant positive correlation between the $Z_{eu}/Z_m$ ratio and cyanobacteria ($P < 0.05$).

### Canonical correspondence analysis

During LW, axis 1 had a species–environment correlation of 0.875 and axis 2 a correlation of 0.735. Specifically, axis 1 explained 14.9% and axis 2 explained 20.7% of the variation in the species data. The 4 axes could explain 89.2% of the variance in species–environmental relations. Water temperature, water level, and NH$_4$-N were the most influential environmental factors affecting the distribution of cyanobacteria species during LW (Fig. 10). During LW, water level explained 36% of cyanobacteria species variations, and water temperature and NH$_4$-N explained

![Fig. 5. Seasonal variations in key environmental factors at 5 sampling locations in the PBA from 2007 to 2009 during the 2-year survey. (a) Total nitrogen, TN; (b) total phosphorus, TP; (c) TN/TP; (d) total particulate matter, TPM; (e) euphotic depth, $Z_{eu}$; and (f) $Z_{eu}/Z_m$ ratio.](image-url)
Table 1. Cell density of cyanobacteria and its relative abundance in phytoplankton at 5 sampling locations of the PBA from 2007 to 2009 during different operation strategy periods of the Three Gorges Reservoir.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Parameter</th>
<th>2007–2009</th>
<th>Low water operation</th>
<th>High water operation</th>
<th>Discharge period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell density ((×10^5 \text{cells} \cdot \text{L}^{-1}))</td>
<td>Mean value</td>
<td>46.50 ± 8.24</td>
<td>39.77 ± 5.60</td>
<td>4.72 ± 0.88</td>
<td>100.13 ± 29.96</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0–1132.02</td>
<td>0.28–224.58</td>
<td>0–40.48</td>
<td>0–1132.02</td>
</tr>
<tr>
<td>Relative abundance in phytoplankton(%)</td>
<td>Mean value</td>
<td>31.8 ± 1.90</td>
<td>47.27 ± 2.69</td>
<td>16.37 ± 2.04</td>
<td>37.60 ± 5.09</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0–99.45</td>
<td>1.54–92.24</td>
<td>0–68.95</td>
<td>0–99.45</td>
</tr>
</tbody>
</table>

Data are mean ± SD (Low water operation, n = 80; High water operation, n = 100; Discharge period, n = 55)

Fig. 6. Key environmental factors at 5 sampling sites of the PBA from 2007 to 2009 at different operation stages of the TGR. Data are shown as mean ± SD (low water operation, n = 80; high water operation, n = 100; discharge period, n = 55). (a) Forms of nitrogen: NH\(_4\)-N, NO\(_3\)-N, DIN, TN; (b) forms of phosphorus: SRP, PP, TP; (c) total particulate matter TPM and TN/TP; and (d) euphotic depth: \(Z_{eu}\) and \(Z_{eu}/Z_{m}\).

Fig. 7. Variations in cell density of cyanobacteria and its relative abundance in phytoplankton communities at 5 sampling sites in the PBA from 2007 to 2009.
Fig. 8. Frequency distribution and variations in dominant phytoplankton densities at 5 sampling sites in the PBA from 2007 to 2009 during the 2-year survey.

Fig. 9. (a) Frequency distribution and (b) average cell density of each genus at 5 sampling sites in the PBA from 2007 to 2009 in different operation strategy periods of the Three Gorges Reservoir.

Fig. 10. Biplots of CCA results between environmental variables and cyanobacteria cell density in different operation strategy periods (LW = low water; HW = high water; DS = discharge period) of the Three Gorges Reservoir. CCA analysis was based on CANOCO for Windows (v4.51). Monte-Carlo permutations (199 runs) were applied to determine the significance and to validate the CCA model as well as to analyze the conditional and marginal effects of environmental variables. In all the models above, the first canonical axis and all canonical axes were significant ($P < 0.05$).
Seasonal variations in cyanobacteria

Table 2. List of Cyanobacteria species recorded at 5 sampling sites in the PBA from 2007 to 2009.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabaena</td>
<td>A. azotica, A. circinalis, A. flos-aquae, A. oscillarioides, A. spiroides</td>
</tr>
<tr>
<td>Aphanizomenon</td>
<td>A. flos-aquae</td>
</tr>
<tr>
<td>Aphanocapsa</td>
<td>A. banaresensis, A. elachista, A. koordersi, A. rivularis, A. incerta</td>
</tr>
<tr>
<td>Aphanothece</td>
<td>A. castagnei, A. clathrata, A. saxicola, A. minutissima</td>
</tr>
<tr>
<td>Aulosira</td>
<td>A. laxa</td>
</tr>
<tr>
<td>Chroococcus</td>
<td>C. limneticus, C. minor, C. minutus, C. turgidus, C. varius</td>
</tr>
<tr>
<td>Cyanobium</td>
<td>C. parvum</td>
</tr>
<tr>
<td>Cylindrospermum</td>
<td>C. stagnale</td>
</tr>
<tr>
<td>Gomphosphaeria</td>
<td>G. aponina</td>
</tr>
<tr>
<td>Leptolyngbya</td>
<td>L. foveolarana, L. valderiana</td>
</tr>
<tr>
<td>Lyngbya</td>
<td>L. gardheri, L. perelegans, L. subconervoides,</td>
</tr>
<tr>
<td>Merismopedia</td>
<td>M. minima, M. punctata, M. tenuissima</td>
</tr>
<tr>
<td>Microcystis</td>
<td>M. aeruginosa, M. flos-aquae</td>
</tr>
<tr>
<td>Oscillatoria</td>
<td>O. cortiana, O. fracta, O. princeps, O. subcontorta</td>
</tr>
<tr>
<td>Phormidium</td>
<td>P. acutissimum, P. tenue</td>
</tr>
<tr>
<td>Planktolyngbya</td>
<td>P. subtilis</td>
</tr>
<tr>
<td>Raphidiopsis</td>
<td>R. curvata, R. sinensis</td>
</tr>
<tr>
<td>Synechococcus</td>
<td>S. elongatus</td>
</tr>
<tr>
<td>Synechocystis</td>
<td>S. minuscula</td>
</tr>
<tr>
<td>Chroococcus</td>
<td></td>
</tr>
</tbody>
</table>

>20%. Although the ratio of $Z_{eu}/Z_{m}$ was also a significant environmental factor regulating the distribution of cyanobacteria species, it only explained 7% of the species variation during LW in the CCA model. The dominance of the bloom-forming cyanobacteria (e.g., *Anabaena, Microcystis*) mainly related to NH$_4$-N, $Z_{eu}$, TP, and TN, whereas WT and AveRain had significant effects on 71.9% of the genera, including *Gomphosphaeria, Merismopedia,* and *Aphanocapsa.*

During HW, axis 1 had a species–environment correlation of 0.834 whereas that of axis 2 was 0.763. The 2 main axes explained 72% of cyanobacteria species variation in the CCA model. Rainfall, TN, and $Z_{eu}$ at HW were the main environmental factors regulating the distribution of cyanobacteria species and explained >60% of cyanobacteria species distribution during HW in the CCA model. *Anabaena* was also clearly influenced by TN, TP, and SRP, whereas *Merismopedia* and *Planktolyngbya* were clearly affected by WT and SRP, respectively. *Microcystis* responded positively to $Z_{eu}$.

During DS, axis 1 and axis 2 explained 66.2% of variances in species–environmental relations. The 4 axes in the CCA model explained 91.4% of the variances. Monte Carlo permutation tests indicated that water level and water temperature were the most influential environmental factors regulating cyanobacteria species distributions; however, biplots of the CCA models during DS showed weak explanatory power for the selected environmental gradients on the distribution of cyanobacteria. *Anabaena* and *Oscillatoria* were mainly related to AveLevel, AveRain, and SRP. *Chroococcus* were obviously affected by TN and TP, but there were no significant relationship between most other cyanobacteria species and environmental variables (Fig. 10).

**Discussion**

According to the hydropower generation operation strategy of the Three Gorges Reservoir, water level decreases to 145 m during the flood season for flood control and increases to the high water level operation stage after the flood season. This operation strategy is called “clear water impounding and muddy flow releasing” because water in the Yangtze River is muddy with high amounts of suspended solids during the flood season, becoming clear after the floods due to the sedimentation of suspended solids. From our study, it was evident that this pattern of reservoir operation structured distinctive habitats for the growth of cyanobacteria and potentially regulated variations in cyanobacteria in tributaries of the Yangtze in the TGR; therefore, this study aimed to find a link among reservoir operation, habitat, and cyanobacteria cell density in the PBA.

The analysis indicates significant differences in environmental factors among the 3 reservoir operation stages. During LW, concentration levels of N and P were generally sufficient for cyanobacteria. Although temperature stratified in the water column during this warm or even hot season, intensive rainfall during LW created an increase in river flow and water level in the PBA, bringing suspended solids and nutrients into the PBA at LW and resulting in...
relatively high levels of TPM, TN, TP and low levels of $Z_{eu}$ in the water column in LW compared with HW and DS. These findings were also supported by the significant correlation among river flow, water level, TPM, and $Z_{eu}$ (Supplementary Table S1). This hydrological characteristic during LW has 2 effects: (1) water temperature stratification was temporally disturbed by increases in river flow and water level; and (2) the pulse effect of the river flow extensively increased the mixing layer in water column. The ratio of $Z_{eu}/Z_{m}$ varied dramatically and was frequently <1 during LW, indicating that underwater light conditions might limit the growth of cyanobacteria during this period. Because the CCA results indicated that decreases in water level and rainfall at LW favored several bloom-forming cyanobacteria (e.g., *Microcystis* sp., *Anabaena* sp.), we inferred that formation of cyanobacteria blooms was mainly driven by the river hydrological regime during LW. Similar studies found blooms of *Microcystis* in the Nakdong River, South Korea (Jeong et al. 2007), and *Anabaena* in the Murray River, Australia (Bowling et al. 2013), occurring under comparable climatic conditions, with a decrease in rainfall leading to prolonged periods of low water level and low flow in rivers. Moreover, Yoshinaga et al. (2006) found that low hydraulic turnover rates led to *Microcystis* blooms in field measurements. Chung et al. (2014) also indicated that, under stable thermal stratification, a shallow mixed layer depth relative to the euphotic depth provided a perfect physical habitat for the dominance of these cyanobacteria relative to other species due to their buoyancy control capability. Further, during LW, the abundance of some specific species (e.g., *Gomphosphaeria*, *Merismopedia*, *Oscillatoria*) were significantly correlated with water temperature, a finding consistent with previous research (Burford and O’Donohue 2006, Mahar et al. 2009) that recorded high densities during the summer months and small numbers of colonies during the winter.

Habitat during HW was driven by an increase in water level after the summer floods in the PBA. At least 4 effects structured habitat during HW by increasing the water level: (1) hydraulic retention time increased substantially (Li et al. 2009); (2) sedimentation of suspended solids after the flood season significantly increased the euphotic depth in the water column (Li et al. 2012); (3) temperature stratification rapidly disappeared as water level increased at the beginning of the HW (Fig. 5); and (4) soluble nutrients potentially leached from the newly flooded nearshore riparian zone (Zheng et al. 2006). DIN and SRP were among the highest in the year cycle. Together with simultaneous decreases in air temperature associated with seasonal changes, the cyanobacteria habitat was totally different during HW compared with LW, with LW characterized as a “calm period.”

During HW, cyanobacteria cell density in the water column and their relative abundance in the phytoplankton community decreased. Increased water temperature, rainfall, and river flow at HW were conditions preferred by cyanobacteria. Cyanobacteria species in the PBA varied with gradients in different environmental factors; however, the $Z_{eu}/Z_{m}$ ratio did not show explanatory power for cyanobacteria growth and species distribution. Increased water temperature (e.g., *Anabaena* sp.), $Z_{eu}$ and NH$_4$-N (e.g., *Microcystis* sp.) were preferred by the bloom-forming cyanobacteria in the PBA. Increases in rainfall and water level during HW favored the development of *Oscillatoria* and *Raphidiopsis*. These phenomena were also reported by Fonseca and Bicudo (2008) and Su et al. (2014), indicating that *Raphidiopsis* was one of the most important taxa in the eutrophic reservoir during the wet season (Jan–Mar and Sep–Dec). High *Oscillatoria* biomass was only observed in late September, which was related to the Secchi depth/local water depth ratio.

Interestingly, the maximum water level in 2007 was 156 m, and the N$_2$-fixing *Aphanizomenon* had the highest density during the HW period. In contrast, in 2008, maximum water level was 172 m, and the cell density of *Planktolyngbya*, which cannot fix N$_2$, was higher during HW compared with any other cyanobacteria. Noges et al. (2002) found that, in a lake with a large water level change, *Planktolyngbya limnetica* reached its maximum in the low-water period in the mid-1970s, whereas *Aphanizomenon skujae* increased during the low-water period in the 1990s. The increased dominance of filamentous blue-greens among phytoplankton followed changes in the water level caused by changes in light and nutrient availability in the fully mixed environment.

The DS is a transition period in the full cycle of annual reservoir operation. We hypothesized that river discharge in the PBA, due to decreases in water level, might reduce cyanobacteria cell density in the PBA. Water level in the PBA decreased gradually during the TGR’s operation, however, accompanied by increases in solar radiation and water temperature as the weather warmed. Temperature stratification began at the end of the DS but did not show strong evidence to support our hypothesis that low water would decrease cell density. Cell density of cyanobacteria was significantly positively correlated with water temperature, rainfall, and river flow, and weather conditions, and hydrological characteristics seemed to be the main driving forces for growth in both the DS and LW periods. Nevertheless, biplots from the CCA model during DS showed weak explanatory power for elucidating changes in cyanobacteria species. We attributed this finding to the following hypothesis: decreases in water level might have contributed to the physical transport of cyanobacteria from upstream to downstream, and as the water level gradually, but not
Seasonal variations in cyanobacteria

rapidly, decreased, opportunities arose for cyanobacteria transportation to downstream reaches. No literature reporting similar situations to support this hypothesis was found, however, and further research to track cyanobacteria transport and growth in this river–reservoir hybrid system is therefore required.

In summary, although the habitat was distinctive among LW, HW, and DS periods, water temperature and hydrological characteristics in the PBA were the main environmental factors that potentially regulated changes in cyanobacteria, which were approximately the same in all reservoir operating stages. This finding does not mean, however, that large-scale reservoir operations did not affect the growth of cyanobacteria in the PBA; we concluded that the large-scale operation created structurally different habitats in the PBA related to water depth, reservoir volume, and nutrient dynamics, and these impacts were closely linked with seasonal effects (e.g., rainfall, river flow, water temperature), resulting in differences in habitats among reservoir operation stages. Because the life cycle of cyanobacteria cells is relatively short, direct impacts of reservoir operation on the ecology of cyanobacteria were not evident in the present study.

Acknowledgements

This work was supported by the National Natural Science Foundation (Program No. 51179215, 51309220). The authors are also grateful for the Western Action Research Program grant from the Chinese Academy of Science (KZCX2-XB3-14) and funding provided by the Chongqing Natural Science Program (CSTC2012JJB20004). We also thank Jie Chen, Jinping Sheng, Chao Zhang, and Yang He, who participated in field sampling in the Three Gorges Reservoir.

References


DOI: 10.5268/IW-6.1.803

Inland Waters (2016) 6, pp. 77-88
between climate change and phytoplankton composition in a large shallow temperate lake. Hydrobiologia. 506:257–263.


**Supplementary Material**

Supplementary Material is available for download via the Inland Waters website, https://www.fba.org.uk/journals/index.php/IW:

Supplementary tables S1–S3.