Factors related to aggravated Cylindrospermopsis (cyanobacteria) bloom following sediment dredging in an eutrophic shallow lake

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A R T I C L E   I N F O

Article info
Received 15 October 2019
Received in revised form 17 November 2019
Accepted 29 November 2019

Keywords:
Bloom control
Cylindrospermopsis
Environmental variables
Sediment dredging

A B S T R A C T

In recent years, Cylindrospermopsis raciborskii blooms have been widely found worldwide. Topics dealing with the mitigation of C. raciborskii bloom is of great importance for toxins produced could threaten public health. The paper first investigated C. raciborskii dynamics over three years following sediment dredging in a shallow eutrophic Lake Dongqian (China). Based on rpoC1 gene copies, C. raciborskii bloom formed with average density of $1.30 \times 10^7$ cells/L on July 2009. One year later after sediment dredging, C. raciborskii cell density decreased below $1.17 \times 10^5$ cells/L or under detected limits during summer days on 2010. While two years later, the C. raciborskii bloom period was returned with markedly increased cell density reaching up to $4.15 \times 10^7$ cells/L on October 2011, and the maximum peak density was shown at 20.3 °C that was much lower than reported optimal growth temperature. Inferred from Spearman correlation analysis, linear regression showed C. raciborskii density was significant and positive with pH and SD, whereas they were significant and negative with TP and DO. Multiple regression analysis further demonstrated that TN, TP, SRP, pH and DO provided the best model and explained 53.1% of the variance in C. raciborskii dynamics. The approaches managing nutrients reduction might not control C. raciborskii bloom as extremely low TN (avg. 0.18 mg/L) and TP concentrations (avg. 0.05 mg/L) resulted in the highest C. raciborskii cell density after sediment dredging.

1. Introduction

A wider distribution and aggravated C. raciborskii blooms have been found in tropical and temperate regions around the world due to global warming and eutrophication in lakes and reservoirs [1–3]. Much attention has been paid to C. raciborskii for its potential to produce various kinds of toxins, including cylindrospermopsin (CYN), paralytic shellfish poisons and anatoxin-a [4,5]. C. raciborskii bloom has been found in Guangdong, Taiwan and Macau freshwaters [2,6,7]. Through a survey of over 100 freshwaters from 2006 to 2017 in China, we found C. raciborskii has been widely distributed in tropical, sub-tropical and temperate zones, and blooms were found in many lakes, reservoirs and ponds. The distribution of C. raciborskii in China will likely expand in the future with global warming since high temperature promotes its growth [8,9].

Increasing concern and monitoring of C. raciborskii occurrence and dynamics should be carried out for bloom formation and control. Measures developed to control C. raciborskii bloom are of the utmost importance in that toxins produced could endanger public health.

Long-term nutrient loading to many lakes has resulted in excessive accumulation of phosphorus (P) and nitrogen (N) in sediment, which are considered two key regulators in algal bloom forming [10–12]. Sediment dredging, the controversial technology for eutrophication control, could remove the nutrient-rich sediment surface layer, has been widely applied to reduce the internal nutrient loading in shallow lakes [13,14]. Yet, opposite results have been obtained in previous studies. In the years after sediment dredging, cyanobacterial biomass and lower chlorophyll-a and TP concentrations were observed in Sweden and UK lakes [15,16]. However, the algal biomass returned to the same magnitude in Lake Trehörningen of Norway two years later following dredging with nitrate-N concentrations increasing tenfold [17].

There are few studies, if any, that have documented whether sediment dredging could control C. raciborskii bloom. In this study,
the potential of sediment dredging to control *C. raciborskii* bloom and the factors influencing *C. raciborskii* dynamics following sediment dredging were investigated in Lake Dongqian.

2. Materials and methods

2.1. Studying area

Lake Dongqian, a state-level scenic spot in Ningbo city, China, is an urban shallow lake (22 km²; mean depth, 2.2 m) and a natural lagoon formed through geological movement in late Quaternary. It is the largest freshwater lake in Zhejiang Province (up to 339 million m³ storage volume), and the main drinking water supply source for Ningbo City. It is 85 km from north to south and 65 km from east to west. With its subtropical monsoon climate, Lake Dongqian is highly suitable for *C. raciborskii* bloom formation, which may explain its dominance in recent years.

2.2. Dredging project and sampling

A suction dredging project was conducted to remove the sediment surface, thus to wipe off internal nutrient loading. The dredging started from July 2009 and ended at January 2012 (Fig. 1). Sediment in the lake and along the bank was removed respectively with environmental protection type 4010 cutter 121 suction dredger and 0.3 m² grab dredger equipped with mud barge. Dredging characteristics are presented in Table S1. Aluminum potassium sulfate was used for flocculating and sinking suspended solids and leaked sediments.

2.3. Imaging of microalgae

The phytoplankton were concentrated with 20-μm-mesh plankton net and examined using a Nikon eclipse 80i light microscope (Nikon, Japan). Microphotograph of *C. raciborskii* was taken using a MicroPublisher 50 real time viewing charge-coupled device camera equipped with differential interference contrast. Unialgal cultures of *C. raciborskii* were isolated and maintained at the group of the “Biology of Harmful Algae” (HAB), Institute of Hydrobiology, the Chinese Academy of Sciences.

2.4. Sampling method and environmental parameters

Water samples were collected quarterly at a depth of 10 cm below the surface at 9 sampling sites from April 2009 to January 2012 (Fig. 1). 100–300 mL water determined by algal concentration were filtered through 0.22-μm polycarbonate membrane filters (Millipore), and then immediately frozen at −20 °C until processing. TN, TP, SRP (soluble reactive phosphorus) and TDP (total dissolved phosphate) were measured following Chinese standard methods [18]. T (water temperature), DO (dissolved oxygen), and pH were obtained via a multi-parameter meter (YSI 6820, Yellow Spring Instruments, USA). SD (Secchi depth) was measured with a 20-cm diameter black and white Secchi disk.

2.5. DNA extraction and qPCR

The total genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method by Ref. [19]. Primers cyl2 (5'-GGCATTCCTAGTTATATTGCCAT-3') and cyl4 (5'-GCCGTTTTTGTCCTTTGCAT-3') specific to *C. raciborskii* were
used to quantitatively detect cell density [20]. Primers cyt2 and cyt4 designed targeting 305 bp rpoC1 gene fragments were selected due to only a single copy existed in the cyanobacterial genome [21], suggesting one rpoC1 gene copy represents one cell of C. raciborskii.

Amplification and quantification were performed in an ABI Prism 7000 real-time PCR detection (Applied Biosystems, USA) equipped with the ABI Prism 7000 SDS fluorescence detection system and software (version 11). The genomic DNA from C. raciborskii strain CHAB 158 isolated from a pond in Yunnan Province, China, was used as external standard to determine environmental C. raciborskii rpoC1 gene copy numbers. A tenfold dilution series from 1 × 10^{1} to 1 × 10^{6} gene copies were prepared and used for real-time PCR analyses. A linear regression equation could be obtained between the gene copy numbers and the cycle threshold (Ct) values (Efficiency = 96.9%, R² = 99.9%) indicating good performance of standard curves.

All qPCR reactions were carried out in a total volume of 20 μL that contained 10 μL 2× SYBR Green real-time PCR Master Mix (Toyobo, Osaka, Japan), 0.5 pmol each primer, and 1 μL DNA from the standards or samples and replenished to 20 μL with sterile ultra-pure water. Each PCR reaction was run in triplicates and three negative controls without DNA were added. Amplifications were performed as follows: an initial denaturation of 3 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 30 s at 58 °C, and 30 s at 72 °C, then by fluorescent melting curve analysis with the temperature gradually increasing from 72 °C to 95 °C at a rate of 0.1 °C/s.

2.6. Statistical analyses

The normality of environmental variables and C. raciborskii density was tested with Kolmogorov-Smirnov test. When data failed to pass through Kolmogorov-Smirnov test (p < 0.05), then Spearman correlation coefficients were used to explore the relationship between environmental variables and C. raciborskii dynamics. The stepwise multiple regression analysis with forward selection of variables was further performed to explore the most important environmental variables explaining C. raciborskii dynamics after sediment dredging in Lake Dongqian. Statistical analysis was performed with SPSS 20.0 (SPSS Inc., USA). Non-metric multidimensional scaling (nMDS) ordination was used to investigate divergence in C. raciborskii dynamics between different sampling periods based on Bray-Curtis similarities performed with PRIMER v7 [22]. Before nMDS analysis, environmental variables and C. raciborskii density were log- and fourth root transformed, respectively, to meet homogeneity and normality of variance.

3. Results

3.1. Morphology of C. raciborskii

Two morphotypes of C. raciborskii were found in Lake Dongqian with characteristics of straight or screwed coiled filaments (Fig. S1). Trichomes solitary, free floating, slightly tapering towards ends and constricted at the cross-walls. Cells cylindrical, with gas vesicles, apical cells narrowed, conically rounded or rounded at the ends. Heterocytes located at one or both filaments’ ends, drop-like, rounded-conical at the ends. Akinetes were cylindrical in straight morphotype and kidney-shaped in coiled type.

3.2. Nutrients and environmental variables

T ranged from 3.2 °C to 31.3 °C, and was highest on July and lowest on January every year. The pH values were highest on April 2009, and continued to decrease until July 2011, then slightly increased till October 2011. SD varied between 0.20 m and 1.98 m, and was relatively lower before April 2011, but showed the peak values on July 2011. DO showed the lowest values from April to July, and the highest values from October to January in the following year. TN ranged from 0.062 mg/L to 2.121 mg/L, and exhibited peak values on April 2011 and valley values on October 2011. TP ranged between 0.010 mg/L and 0.183 mg/L, and presented the highest values on January 2011. The TN and TP ratio values varied from 5.1 to 130.29, and presented the highest values on April and July 2011. The level of TDP had a range from 0.005 mg/L to 0.142 mg/L, and exhibited peak values on April 2010. The SRP values continued to decrease from April 2009 to October 2010, and had peak values ranging from 0.021 mg/L to 0.043 mg/L on January 2011, then declined until January 2012 (Fig. 2).

3.3. Dynamics of C. raciborskii based on qPCR

Based on the qPCR assay, C. raciborskii cells were detected in all sampling periods except on April 2010. Cells were detected in all sampling sites during July 2009 and October 2011 but were under detection limits in most sampling sites on other sampling period. The highest cell abundance occurred on October 2011, ranging from 8.43 × 10^{3} to 4.15 × 10^{5} cells/L (avg. 1.47 × 10^{5} cells/L). Another peak values were shown on July 2009 varying from 1.04 × 10^{6} to 4.75 × 10^{5} cells/L (avg. 1.30 × 10^{5} cells/L). Cells were relatively lower than 2.99 × 10^{5} cells/L during other sampling period except in sampling site A on November 2009 of 1.37 × 10^{5} cells/L. Inferred from nMDS ordination plot, C. raciborskii reached the highest abundance at 20.3 °C on October 2011, whereas lower density was shown with high temperature over 30 °C on July 2010 and 2011. An exception was observed in July 2009 with the temperature ranging to 28 °C. C. raciborskii tended to have higher density in low temperature compared with that on high temperature (Fig. 4).

3.4. Environmental variables related to C. raciborskii dynamics

As shown in Table 1, C. raciborskii presented a significant and positive linear correlation with pH (p = 0.198) and SD (p = 0.251), conversely, TP (p = 0.193) and DO (p = 0.309) exhibited the significant and opposite trend with C. raciborskii dynamics. The multiple regression analysis suggested TN, TP, SRP, pH and DO had significant impacts on C. raciborskii dynamics, and they explained 53.1% of the total variation of the C. raciborskii density (R² = 0.531, P = 0.000, Table 2). TN was the best driver of C. raciborskii dynamics and explained 15.4% of the variation (R² = 0.154, P = 0.000), TP (R² = 0.056, P = 0.008), pH (R² = 0.061, P = 0.006), and DO (R² = 0.067, P = 0.010) explained 5.6%, 6.1% and 6.7% variation of C. raciborskii dynamics.

4. Discussion

In the study, C. raciborskii dynamics were investigated in shallow Lake Dongqian for three years after sediment dredging to investigate whether dredging could mitigate C. raciborskii bloom. Quantification of C. raciborskii cells were carried out with qPCR technique for C. raciborskii filaments were not or slightly constricted at the cross walls, which were hard to count cell numbers by microscope. C. raciborskii reached up to 4.75 × 10^{5} cells/L (avg. 1.30 × 10^{5} cells/L) on July 2009. After sediment dredging in areas S6, S7, S8 for nearly one year, C. raciborskii was under detection limits across the whole lake on April 2010 while it was ranging from 1.19 × 10^{4} to 2.56 × 10^{5} cells/L in most sampling sites on April 2009. On July 2010, density ranging from 2.94 × 10^{2} to 1.17 × 10^{4} cells/L was shown in half of sampling sites, and on October 2010, only 1.61 × 10^{4} cells/L was detected in sampling site A (Fig. 3). The results indicated that C. raciborskii blooms were alleviated or even
eliminated in Lake Dongqian one year after sediment dredging. Surprisingly, two years later following dredging, a bloom returned on October 2011 with up to 1004 times higher densities ranging from $8.43 \times 10^5$ to $4.15 \times 10^7$ cells/L (avg. $1.47 \times 10^7$ cells/L) than that on July 2009. The sharp and robust results suggested sediment dredging might not be effective to mitigate *C. raciborskii* blooms in Lake Dongqian.

A similar result was also demonstrated by Ref. [17] who found algal biomass returned to the same magnitude two years following sediment dredging in Lake Trehorningen. In contrast, in the years following sediment removal, dredging resulted in a decrease in cyanobacterial biomass the water quality [15,16]. The conflicting performance by sediment dredging might be caused by different dredging pattern, such as dredging at different time and depth could generate the adverse results. Dredging in winter might alleviate or suppress the occurrence of black blooms, while dredging in summer might even induce it in Lake Taihu [23]. Black blooms occurred in the un-dredged, 7.5 cm dredged, and 12.5 cm dredged treatments but did not occur in the 22.5 cm dredged treatment under laboratory simulation test of Lake Taihu [24]. Dredging depth ranging from 0.30 to 0.80 m and dredging through the whole year in Lake Dongqian were performed. Perhaps, dredging time and depth were not appropriate for mitigating *C. raciborskii* bloom in Lake Dongqian. Pilot experiment based on simulation testing

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**Fig. 2.** Quarterly dynamics of environmental variables from April 2009 to January 2012 in Lake Dongqian. (a), temperature (T); (b), pH; (c), secchi depth (SD); (d), dissolved oxygen (DO); (e), soluble reactive phosphorus (SRP); (f), total dissolved phosphorus (TDP); (g), total nitrogen (TN); (h), total phosphorus (TP); (i), TN and TP ratio. The bottom, middle, and upper of each box represent 25%, 50%, 75% of the dataset. The bottom and upper rhombus represent the minimum and maximum values of the dataset. The grey and blue bands represent the sampling periods of July and October, respectively.

**Table 1** Spearman correlation between *C. raciborskii* dynamics and environmental variables in Lake Dongqian.

| Density | TN  | TP   | TN/TP | SDP | SRP | pH   | T   | DO   |
|---------|-----|------|-------|-----|-----|------|-----|------|
| Density | -0.174 | 0.054 | 0.004 | 0.460** | -0.793** | -0.019* | 0.494** | 0.252* |

Note: * and ** indicate significant levels at 0.05 and 0.01.

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should be carried out ahead of time to find appropriate dredging depth and time in Lake Dongqian. Moreover, dredging individually might not reach ideal result and should be combined with other managing strategies since [14] found that only combining sediment dredging and Phoslock® addition could result in a gradual decrease of Microcystis aeruginosa (Cyanobacteria) until below the detection level. Aggravated C. raciborskii bloom two years later following dredging could also be explained by its competitive physiochemical advantages that assisted in overcoming adverse conditions.

C. raciborskii is primarily considered to be confined in tropical regions, but has been found more recently to be widely distributed in subtropical and temperate freshwaters in Europe, America, Australia and Asia [25]. Previous studies have reported the ecological success of C. raciborskii attributed to many factors such as floatability, the preference for high water temperatures, superior shade tolerance, tolerance of high salinity and high phosphorus and ammonium uptake rate and high phosphorus-storage capacity [25]. Vegetative cells differentiated into heterocysts could fix N₂ in the atmosphere under low nitrogen condition, and akinetes could allow survival through unfavorable environmental conditions and also allowed easy dispersal by birds, ballast [26]. Moreover, a toxic secondary metabolite (CYN) has been demonstrated to allow its resistance to grazing, and C. raciborskii exudates showed allelopathic interference to inhibit growth of phytoplankton in the surroundings [27]. These factors likely supported the wide distribution and expansion of C. raciborskii, and to even allow its dominance in various kinds of freshwaters worldwide.

In cultures, the optimal growth temperature of C. raciborskii is relatively high, exceeding over 25 °C [8,9]. As well, C. raciborskii cell density exhibited a strong positive relationship with average epilimnetic temperature in a large man-made water impoundment Lake Julius in Australia’s semi-arid tropics. In temperatures between 28 °C and 32 °C, peak concentrations greater than 5 × 10⁷ cells/L occurred [28]. In Lake Dongqian, it was noteworthy that the highest C. raciborskii cell density occurred on October 2011 under a lower temperature 20.3 °C (Figs. 2 and 3).

### Table 2

| Regression variables | Partial regression coefficients | Standard error | Standardized coefficients | P       |
|----------------------|--------------------------------|----------------|---------------------------|---------|
| Density (model R² adj = 0.531, P = 0.000) |                           |                |                          |         |
| Constant             | -8.162                         | 9.321          | 0.384                     |         |
| TN                   | -3.392                         | 0.714          | -0.430                    | 0.000   |
| TP                   | -2.876                         | 0.884          | -0.445                    | 0.002   |
| SRP                  | 2.057                          | 0.850          | 0.249                     | 0.018   |
| pH                   | 19.356                         | 3.935          | 0.474                     | 0.000   |
| DO                   | -9.214                         | 2.334          | -0.567                    | 0.000   |

Notes: Environmental variables investigated in the regression analysis contained water temperature, dissolved oxygen, pH, total nitrogen, total phosphorus, total nitrogen and total phosphorus ratio, soluble reactive phosphorus, and total dissolved phosphate. Environmental variables were selected only if P < 0.05 and were listed in the order of entry into the model.
intriguing finding is that high C. raciborskii densities varied from $4.54 \times 10^4$ to $2.99 \times 10^5$ cells/L occurred during winter periods on January 2012 with temperature around 5 °C (Fig. 3). The abnormal result indicated that C. raciborskii bloom formed under far less than optimal growth temperature, and C. raciborskii tended to flourish under much lower temperature. C. raciborskii was capable of growing at low temperature of 11 °C [29], and 24 Thailand and Japan C. raciborskii strains exhibited better growth at 17.5 °C [30]. High temperature was a key factor to facilitate C. raciborskii growth during the germination process at the beginning of the vegetative season [25,31], once populations were developed, growth could continue even at relatively low temperature [32]. This could be associated with the occurrence of C. raciborskii different ecotypes adapting to low temperature [1]. With global warming, the warm season days at temperatures over 20 °C will increase, and period of blooms of C. raciborskii could also increase in that different ecotypes exhibited optimal growth at wide temperature tolerance. Moreover, another peak values were shown with the temperature over 27.9 °C on July 2009 at the start of sediment dredging, while the highest peaks values were shown at 20.3 °C on October 2011 two years later after sediment dredging, we suggested the contrasting phenomenon could be interfered by sediment dredging.

C. raciborskii was always present in turbid freshwaters due to its superior shade tolerance that could enable them to survive under the low light condition [33,34]. In Dongguan reservoirs, C. raciborskii biomass exhibited a significant negative correlation with Secchi depth, indicating that the higher density could be reached when the water transparency was low [2]. Based on CCA

![Non-metric multidimensional scaling (MDS) ordination based on the Bray-Curtis similarity calculated from fourth root transformed C. raciborskii density. Number in circles represent temperature on sampling periods, and sampling periods with temperature exceeding 20 °C were labeled with blue circles. Stress = 0.01.](image-url)
Phosphorus was hypothesized to play a key role in the occurrence of cyanobacterial bloom [12]. C. raciborskii cell density was significantly and negatively correlated with TP in Lake Dongqian (Table 1). A relatively low TP concentrations ranging from 0.01 to 0.12 mg/L (avg. 0.05 mg/L) were observed in October 2011 when C. raciborskii reached the highest densities (Figs. 2 and 3). A negative correlation also existed between C. raciborskii cell density and the dissolved phosphorus concentration (avg. 0.05 mg/L) shown two years following dredging. Much higher TP concentration was observed compared to TN, that might contribute to the C. raciborskii bloom in Lake Dongqian. Sediment dredging in Lake Dongqian did not effectively control TP concentration, and this might be explained by phosphorus release from sediments by high pH or moderate phosphorus concentrations, suggesting the approaches managing nutrient control like sediment dredging might not be effective in mitigating C. raciborskii blooms. It is TP, rather than TN, that might contribute to the C. raciborskii bloom in Lake Dongqian, China.

5. Conclusion and perspective

In the study, C. raciborskii blooms were alleviated or even eliminated one year after dredging, but much higher densities were shown two years later following dredging. Sediment dredging may not effectively control C. raciborskii blooms. Sediment dredging increased nutrients as lower TN (avg. 0.18 mg/L) and TP concentrations (avg. 0.05 mg/L) were shown two years following dredging. Much higher TP concentration was observed compared to TN concentration in the lake when the blooms formed after dredging. Furthermore, Spearman correlation analysis showed that C. raciborskii density correlated with decreasing TP. These results demonstrated TP but not TN contributed more to C. raciborskii bloom in Lake Dongqian. Sediment dredging in Lake Dongqian did not effectively remove TP concentration, and this might be explained by phosphorus release from sediments by high pH or perhaps without nutrients control from runoff in the bank. Furthermore, C. raciborskii bloom period was pushed back after sediment dredging as aggravated blooms were formed in autumn with temperature under 20.3 °C, which was far less than optimal growth temperature of C. raciborskii. Much lower TP concentration may be sustained with global warming to remove C. raciborskii blooms, but it will be a harder task for TP reduction in the future.

Acknowledgements

The National Key Research and Development Program of China (2017YFA0605003), the National Natural Science Foundation of China (51922010, 91751114, 41521003, 31700404), supported this study. Great appreciations were contributed to Pro. Jian tong Liu for providing environmental variables data in the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2020.100014.

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