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Resuscitation of Dormant Microcystis Cells and Microbial Community Dynamics in the Surface Sediment of Chongtian Lake [Postprint]

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Abstract

Surface sediment and overlying water samples were collected from Chongtian Lake in West Dongting, which experiences frequent *Microcystis* blooms, to detect and analyze the abundance of *Microcystis* resting stages and bacterial concentration in the surface sediment, *Microcystis* cell abundance and bacterial concentration in the overlying water, as well as some physicochemical properties, combined with laboratory simulation experiments. The results showed that from February to June, the total bacterial concentration in both the surface sediment and overlying water of Chongtian Lake increased significantly ($P < 0.05$), with the total bacterial concentration in the surface sediment being significantly higher than that in the overlying water ($P < 0.05$). The dominant bacterial groups were *Exiguobacterium*, *Pseudomonas*, and *Bacillus*. In April, *Microcystis* resting stages in the surface sediment began to recruit and their abundance decreased, with the abundance in June being significantly lower than that in April-May ($P < 0.05$), while the *Microcystis* cell abundance in the overlying water increased, being significantly higher in June than in April-May ($P < 0.05$). The dominant recruiting algae were *Microcystis aeruginosa*, *Microcystis flos-aqua*, and *Microcystis wesenbergii*. During the recruitment period, the concentration of dominant bacterial groups promoting resting stage recruitment increased significantly, while the dissolved oxygen concentration and TN/TP ratio at the sediment-water interface decreased significantly ($P < 0.05$). This suggests that the dominant bacterial groups in the surface sediment and overlying water of Chongtian Lake may influence *Microcystis* resting stage recruitment by altering the physicochemical environment of the surface sediment.

Full Text

Relationship between Recruitment of *Microcystis* Dormant Cells in Sediment and Annual Dynamics of Bacterial Flora in Lake Chongtian

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Abstract: The recruitment of *Microcystis* dormant cells from surface sediment represents a critical stage in the life history of *Microcystis* and plays an important role in bloom formation. However, little is known about the benthic bacterial communities associated with this recruitment process. To investigate the relationship between *Microcystis* dormant cell recruitment and bacterial flora dynamics, we conducted a year-long sampling and analysis of surface sediment and overlying water in Lake Chongtian, a lake in western Dongting Lake region that has experienced frequent *Microcystis* blooms in recent years. We measured bacterial density, *Microcystis* dormant cell abundance, and several physicochemical parameters, complemented by laboratory simulation experiments. Results showed that total bacterial density in both surface sediment and overlying water gradually increased from January to May, remained stable from June to September, and gradually decreased from October to December. However, total bacterial density in sediment was significantly higher than in the overlying water column each month. From April to June, total bacterial density increased significantly ($p < 0.05$) while *Microcystis* dormant cell density decreased significantly ($p < 0.05$) in the surface sediment, indicating that recruitment began in April. During this period, dissolved oxygen concentration and the TN/TP ratio also decreased significantly in the overlying water. In May, total bacterial density in sediment reached 7.32×10^7 colony-forming units (cfu)/mL, significantly higher than in April, and the proportion of dominant flora increased remarkably (up to 61%). *Microcystis* cell density in the overlying water column increased significantly to 180×10^3 cells/mL in June, while dormant cell density in surface sediment reached its minimum value of 3.71×10^3 cells/mL. *Microcystis aeruginosa* was the dominant species at this time. In July, total bacterial density in surface sediment showed no significant difference from May and June, while the proportion of dominant flora decreased to 40–42%, which was the average value for other months. Meanwhile, *Microcystis* dormant cell density in sediment increased significantly ($p < 0.05$). In August, total bacterial density in surface sediment was 8.89×10^7 cfu/mL, and the proportion of dominant flora

increased significantly (up to 57%). Consequently, in September, dormant cell density in surface sediment again decreased significantly, while *Microcystis* cell density in the overlying water column increased significantly ($p < 0.05$). This study also revealed that the dominant *Microcystis* species in Lake Chongtian were *M. aeruginosa*, *M. flos-aquae*, and *M. wesenbergii*, though their relative proportions differed among months. The dominant bacterial flora in sediment and overlying water were *Exiguobacterium*, *Pseudomonas*, and *Bacillus*, all of which can promote, to some extent, the recruitment of *M. flos-aquae*, *M. wesenbergii*, and *M. aeruginosa* dormant cells from surface sediment. These results have important implications in that dominant bacterial flora in surface sediment may exert significant effects on the recruitment of *Microcystis* dormant species.

Keywords: *Microcystis*; dormant cells; dominant flora; recruitment; sediment; overlying water

1 Introduction

Freshwater *Microcystis* blooms frequently occur because *Microcystis* cells can form vegetative dormant cells when subjected to environmental stress, allowing them to survive harsh growth periods. When environmental conditions become suitable, these dormant cells initiate recruitment, leading to population outbreaks and bloom formation [1-3]. The algal cells or seed sources during the initial stages of *Microcystis* blooms mainly originate from *Microcystis* dormant cell populations in sediment [4-5]. Many environmental scientists have been investigating the mechanisms of *Microcystis* dormant cell recruitment to find methods to control *Microcystis* blooms at their source. Previous ecological and physiological research on *Microcystis* dormant cell recruitment has focused primarily on temperature [6-8], light intensity [8-9], nutrients [10-13], and hydrodynamics [14-16], with few studies examining the influence of biological factors, particularly the impact of surface sediment bacterial communities on *Microcystis* dormant cell recruitment. The surface sediment environment is extremely complex, and bacterial communities in surface sediment and overlying water are important components of aquatic ecosystems, forming intricate relationships with numerous biological and environmental factors. Whether these bacterial communities are associated with *Microcystis* dormant cell recruitment and bloom outbreaks remains unknown. This study addresses this question through year-long sampling of sediment and overlying water in the Yongfengyuan area of Lake Chongtian in western Dongting Lake, Hunan Province, where *Microcystis* blooms occur frequently. By analyzing monthly dynamics of algae and bacteria in sediment and overlying water, combined with laboratory simulation experiments, we explored the relationship between *Microcystis* dormant cell recruitment and bacterial flora dynamics from an algae-bacteria interaction perspective.

2 Materials and Methods

2.1 Study Site and Sampling Locations

Lake Chongtian is a typical freshwater lake located northeast of Shigongqiao Town, Dingcheng District, Changde City, Hunan Province, covering an area of 15.7 km². It belongs to the western Dongting Lake water system. Due to intensive artificial pearl aquaculture development, Lake Chongtian has become severely eutrophic, with frequent cyanobacterial blooms. The average water depth is 1.35 m during dry seasons and 1.80 m during wet seasons. Sediment and water sampling sites were established at three locations in the Yongfengyuan area of Lake Chongtian: Site A (111°90 059 E, 29°14 386 N), Site B (111°90 462 E, 29°13 963 N), and Site C (111°90 922 E, 29°13 881 N) [Figure 1: see original paper].

2.2 Sample Collection and Processing

2.2.1 Sediment Collection and Processing From January to December 2015, sediment samples were collected monthly from the three sampling sites using a portable sediment corer (KC-Denmark). The surface 0-10 cm of sediment cores were collected, filtered through a 125 μ m sieve for pretreatment, placed in sterilized numbered sample bags, and stored in a portable incubator for transport to the laboratory.

2.2.2 Microcystis Traps Microcystis traps (radius: 20 cm; height: 180 cm) are specialized transparent cylinders with open bottoms fixed to the sediment surface. The lower portion of the trap walls has small holes (10 μ m mesh) that allow water exchange while eliminating interference from existing *Microcystis* cells in the overlying water. Traps were deployed at sites A, B, and C. Immediately after deployment, water inside the traps was extracted using a small pump through a rubber stopper at the top. The extracted water was filtered through a 10 μ m mesh to remove existing algae. For sampling, 250 mL of water was collected from inside each trap, mixed thoroughly, and divided into two sterilized bottles (one with Lugol's solution for algal concentration measurement), then stored in a portable incubator for transport.

2.2.3 Overlying Water Collection Outside Traps Overlying water outside the traps was collected simultaneously using the same method as for water inside the traps.

2.3 Bacterial Culture Media and Cultivation

Freshwater bacteria-specific medium was used (composition: [details would be here but were truncated in original]). The medium was prepared at pH 7.3, sterilized at 121°C for 20 min.

2.3.1 Sediment Bacteria Cultivation In the laboratory, sediment samples from each site were placed in sterilized 250 mL bottles containing 100 mL sterile distilled water, mixed thoroughly, and serially diluted to 10^{-6} . Aliquots of 0.1 mL from appropriate dilutions were spread on freshwater medium plates for cultivation and colony counting.

2.3.2 Bacteria Cultivation from Trap Water Water samples (1 mL) from inside and outside the traps were diluted serially in sterile distilled water to 10^{-6} . Aliquots of 0.1 mL were spread on freshwater medium plates for cultivation.

2.4 Bacterial Isolation and Identification

2.4.1 Isolation Single colonies were randomly selected from plates and streaked on new plates for purification until pure strains were obtained. Pure strains were preserved in glycerol saline solution at -80°C .

2.4.2 Molecular Identification Bacterial genomic DNA was extracted using standard procedures. The 16S rDNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTACCTTGTTACGACTT-3'). PCR products were sequenced by Shanghai Sangon Biotech, and sequences were analyzed for identification.

2.5 Microcystis Identification and Counting

2.5.1 Microcystis in Trap Water *Microcystis* cells in water samples were identified according to reference [18] and counted using a hemocytometer to calculate abundance [19].

2.5.2 Microcystis Dormant Cells in Sediment Sediment samples were processed with Percoll gradient centrifugation, and dormant cells were examined and counted under an inverted fluorescence microscope [9].

2.6 Physicochemical Parameter Measurement

Temperature, pH, and dissolved oxygen (DO) were measured on-site using a portable multi-parameter water quality meter (FG4-B). Total nitrogen (TN) was measured by alkaline potassium persulfate digestion-UV spectrophotometry (GB11893-89), and total phosphorus (TP) by ammonium molybdate spectrophotometry (GB11894-89).

2.7 Laboratory Simulation Experiment

Sediment was sterilized by autoclaving at 121°C for 20 min, then evenly distributed in special graduated glass cylinders (diameter: 15 cm). Separated dominant bacterial strains and dormant *Microcystis* cells were mixed and embedded

in culture medium, then slowly added along the cylinder walls to avoid disturbing the sediment (control group received no bacteria). Bacterial concentration was set at 10 cfu/mL and dormant cell abundance at 10 cells/mL, matching field conditions. Cylinders were incubated at 24°C with 12:12 h light:dark cycle at $10 \text{ E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (equivalent to average light intensity at 180 cm depth in summer). *Microcystis* abundance in water was measured every 4 days for 28 days using the method described in Section 2.5.1.

2.8 Statistical Analysis

All measurements were performed in triplicate. Data are presented as means \pm SD. Statistical analysis was conducted using Excel 2007 and SPSS 13.0. One-way ANOVA with Duncan's multiple comparison test was used to analyze monthly dynamics, with $p < 0.05$ considered statistically significant.

3 Results

3.1 Dynamics of Physicochemical Parameters in Overlying Water

Monthly average water temperature gradually increased from January, reaching $(25.75 \pm 1.82)^\circ\text{C}$ in July, with significant differences between March-June and other months ($p < 0.05$). The water was alkaline ($\text{pH} > 8.0$) from April to September. Dissolved oxygen concentrations in April, May, and June were (2.32 ± 0.38) mg/L, (2.28 ± 0.26) mg/L, and (2.21 ± 0.38) mg/L respectively, significantly lower than other months ($p < 0.05$). The TN/TP ratio was also significantly lower in April-June (4.33-4.58) compared to other months ($p < 0.05$).

3.2 Total Bacterial Concentration in Surface Sediment and Trap Water

Total bacterial concentration in surface sediment increased significantly from January to May, showed no significant difference between May and September ($p > 0.05$), and decreased gradually from October to December. The minimum concentration occurred in February (1.16×10^6 cfu/mL), while the maximum occurred in September (8.89×10^6 cfu/mL). Total bacterial concentration in sediment was significantly higher than in overlying water each month ($p < 0.05$). The monthly dynamics of total bacterial concentration inside and outside the traps were essentially consistent with those in surface sediment, with no significant difference between inside and outside trap water ($p > 0.05$) [Figure 2: see original paper].

3.3 Abundance of Microcystis Dormant Cells in Sediment and Vegetative Cells in Water

There was no significant difference in *Microcystis* dormant cell abundance in surface sediment from January to March ($p > 0.05$). Abundance decreased significantly from April to June ($p < 0.05$), then increased sharply in September to

36.46×10^6 cells/mL ($p < 0.05$), the highest value recorded. No *Microcystis* cells were detected in trap water in March, but low abundance was detected outside the traps. Cells were detected in both inside and outside trap water from April onward, with abundance increasing gradually. Total *Microcystis* cell abundance increased rapidly in June both inside and outside the traps, with no significant difference between them ($p > 0.05$) but significant differences from other months ($p < 0.05$). Cell abundance decreased significantly in August ($p < 0.05$) [Figure 3: see original paper].

3.4 Bacterial Flora Dynamics in Sediment and Water

A total of 13 bacterial genera were isolated from sediment, with *Exiguobacterium*, *Bacillus*, and *Pseudomonas* being dominant. Ten genera were isolated from overlying water, with *Pseudomonas*, *Exiguobacterium*, and *Bacillus* as dominant flora. The dominant bacterial genera were the same in both sediment and water

In May, the proportion of *Exiguobacterium*, *Pseudomonas*, and *Bacillus* in surface sediment reached 61% of total flora, significantly higher than the ~40% observed in other months. In overlying water, these three dominant genera accounted for 54% in May, with *Pseudomonas* proportion higher and *Bacillus* proportion lower than in sediment. In August, the dominant flora proportion in sediment increased again to 57% [Figure 4: see original paper].

3.5 Dominant Microcystis Species and Proportions

In June and September, the dominant *Microcystis* species in overlying water were *M. aeruginosa*, *M. flos-aquae*, and *M. wesenbergii*, with no significant difference in proportions between inside and outside traps ($p > 0.05$). In June, these three species accounted for 92% of total *Microcystis* cells, with *M. aeruginosa* comprising 58%. In September, they accounted for 89% of cells, with *M. aeruginosa* at 51%. The proportion of *M. aeruginosa* showed no significant difference from *M. flos-aquae* in June ($p > 0.05$) but was significantly different from other species ($p < 0.05$) [Figure 5: see original paper].

3.6 Effects of Dominant Bacterial Genera on Microcystis Recruitment

All three dominant bacterial genera (*Exiguobacterium*, *Pseudomonas*, and *Bacillus*) significantly promoted the recruitment of *M. aeruginosa* dormant cells. The promoting effects of *Exiguobacterium* and *Bacillus* were significantly stronger than that of *Pseudomonas* ($p < 0.05$). For *M. wesenbergii* dormant cells, the promoting effects of the three genera were weaker than for *M. aeruginosa*, with no significant difference among them ($p > 0.05$), but all were significantly stronger than the control ($p < 0.05$). *Pseudomonas* showed significantly stronger promotion of *M. flos-aquae* recruitment than *Exiguobacterium* and *Bacillus* ($p < 0.05$), while the latter two showed no significant difference ($p > 0.05$) [Figure 6: see original paper].

4 Discussion

No *Microcystis* cells were detected in trap water in March, while low abundance was detected outside the traps. From April to June, *Microcystis* cells were detected in both inside and outside trap water, with abundance increasing gradually but showing no significant difference between them. This indicates that *Microcystis* dormant cells in Lake Chongtian surface sediment began recruiting in April, and that sediment dormant cells are the primary seed source for overlying water *Microcystis* populations. The source of *Microcystis* seeds in eutrophic lake water has long been debated. Preston et al. [4] used isotopic tracing to study the *Microcystis* life cycle in Blelham Tarn and found that seed sources in summer water primarily came from sediment-overwintering dormant cells. Brunberg and Blomqvist [9] found that cells participating in bloom formation originated from sediment dormant cells in Swedish lakes. Thomas and Walsby [20] demonstrated that bloom-forming *Microcystis* came from sediment recruitment. Boström et al. [15] even suggested that in eutrophic waters, the number of sediment *Microcystis* dormant cells could exceed the maximum total *Microcystis* biomass in water. Our results are similar to these findings.

In April, the average water temperature in Lake Chongtian was $(11.58 \pm 0.62)^\circ\text{C}$, meeting temperature requirements for sediment *Microcystis* dormant cell recruitment [21–22]. Dissolved oxygen concentrations reached minimum values in April–June, and the TN/TP ratio decreased significantly. Tsujimura et al. [16] studied seasonal variations of *Microcystis* in Lake Biwa sediments and found that low DO environments could promote or trigger dormant cell recruitment. Brunberg and Blomqvist [9] reached similar conclusions in their study of *Microcystis* overwintering in lake sediments, suggesting that low DO levels benefit overwintering and recruitment of *Microcystis* dormant cells. In our study, total bacterial concentration in surface sediment increased significantly from April to June, with dominant flora mainly consisting of *Exiguobacterium*, *Pseudomonas*, and *Bacillus*. The rapid growth and proliferation of these dominant bacterial groups consumed oxygen at the sediment–water interface [23], likely creating the low DO environment that promoted *Microcystis* dormant cell recruitment. This result aligns with Tsujimura et al. [16] and Brunberg and Blomqvist [9].

TN, TP, and the TN/TP ratio are nutritional factors for *Microcystis* dormant cell recruitment and bloom formation. Verspagen et al. [25] demonstrated that reducing the TN/TP ratio promotes *M. aeruginosa* recruitment. Berg et al. [24] and Katri et al. [2] reached similar conclusions. However, Misson et al. [26] suggested that the TN/TP ratio is only a necessary condition rather than a triggering factor. Anne et al. [27] showed that small-scale bioturbation promotes growth and metabolism of sediment *Microcystis* dormant cells, supported by Li and Xiao [28]. Su et al. [29] argued that physical disturbance by benthic organisms has less impact than low DO. In our study, the TN/TP ratio was significantly lower in April–June, possibly due to increased dominant bacterial density, particularly denitrifying bacteria within *Exiguobacterium* (*E. abietanum*, *E. sibiricum*) and other species with strong nitrogen removal capacity. The mech-

anism by which decreased TN/TP ratio promotes recruitment remains unclear.

Laboratory simulation experiments showed that *Exiguobacterium*, *Pseudomonas*, and *Bacillus* all promoted recruitment of *M. aeruginosa*, *M. flos-aquae*, and *M. wesenbergii* dormant cells, though with species-specific differences. Studies on algicidal and algicidal bacteria from sediment are rare. Ukeles and Bishop [30] isolated a bacterium from shallow sea sediment that promoted diatom growth. Imai et al. [31] and Spilling et al. [32] demonstrated that some environmental bacteria can specifically promote growth of certain algae. Hernandez et al. [33] isolated *Bacillus pumilus* from shrimp ponds that promoted *Chlorella vulgaris* growth. In our study, dominant sediment bacteria promoted *Microcystis* recruitment, but the significant differences among genera may explain seasonal variations in dominant algae proportions in Lake Chongtian. The dominant *Microcystis* species were *M. aeruginosa*, *M. flos-aquae*, and *M. wesenbergii*, but their proportions varied monthly. Seasonal variations in algal dominance are common in other water bodies [34].

Our study only measured algal and bacterial concentrations and some physicochemical parameters. Besides dominant bacterial growth potentially altering the sediment environment to affect recruitment, the specific mechanisms—such as allelopathic or interspecies competitive relationships—require further investigation.

5 Conclusion

Microcystis dormant cells in Lake Chongtian surface sediment are the primary seed source for overlying water blooms. The dominant species are *M. aeruginosa*, *M. flos-aquae*, and *M. wesenbergii*. Total bacterial concentration in surface sediment increases gradually from January, with dominant flora being *Exiguobacterium*, *Pseudomonas*, and *Bacillus*. *Microcystis* dormant cell recruitment begins in April, with decreasing dormant cell abundance in sediment and increasing vegetative cell abundance in overlying water. Variations in species proportions, TN/TP ratio, and DO concentration are closely related to rapid proliferation of dominant bacterial flora.

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