

Article

# Effect of Water Column Stability on Surface Chlorophyll and Time Lags under Different Nutrient Backgrounds in a Deep Reservoir

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**Abstract:** Hydrodynamic conditions are considered to be very important in the control of algal blooms. Weekly or daily measurements may miss some important events in the hydrodynamic process, resulting in inaccurate evaluations of the impacts of hydrodynamics on phytoplankton. In this study, high-frequency (15-min interval) measurements were used to analyze the effect of water column stability on surface chlorophyll *a* (Chl *a*) and lag time under different nutrient backgrounds during a cyanobacterial bloom in the Three Gorges Reservoir, China. Cross-correlation analysis between the relative water column stability (RWCS) and Chl *a* was performed at different stages. The results showed that the RWCS above the euphotic depth influenced the surface Chl *a* concentration most significantly. A lower RWCS (<20) limited the increase in the Chl *a* concentration, and a higher RWCS caused a significant increase in Chl *a* only when nutrients were not limited (TN/TP < 29) and light and temperature conditions were suitable. It took a short time for a higher RWCS to significantly increase the surface Chl *a* concentration compared with a lower RWCS. When the waterbody had a very low Chl *a* concentration (almost 0), approximately 2 days were needed to significantly increase the Chl *a* concentration, while approximately only half an hour was needed when the background concentration of Chl *a* was slightly higher. During the bloom period, a decline in the RWCS significantly decreased the Chl *a* in a very short time (approximately half an hour). Reducing the water column stability could be a good approach to control cyanobacterial blooms.

**Keywords:** hydrodynamics; time lag; automated high-frequency monitoring; cyanobacteria; Three Gorges Reservoir

## 1. Introduction

Environmental factors such as nutrients, light, and hydrodynamic conditions [1–3] impact the general composition of phytoplankton through their synergistic effects. High concentrations of nutrients are critical for the formation of algal blooms but are not always the determining factor [4]. The mixing regime or the stratification of the waterbody plays a very important role in the bloom outbreak and in the control processes [5,6]. In some areas, algal blooms always occur or reach a peak in response to increased water temperature and the presence of stratification [7,8], especially

cyanobacterial blooms [9–12]. Some studies have reported that blooms do not break out in the littoral zone with high levels of nutrients, but rather in the pelagic zone with weak water disturbance [13]. These researchers concluded that nutrients in the water column will not stimulate strong algal blooms unless vertical diffusivity is very low.

The water mixing regime, water retention time, and water column stability are usually used to describe the hydrodynamic conditions when investigating the relationship between algal blooms and hydrodynamics [14]. Becker et al. found that, in a deep Mediterranean reservoir, an increase in water column stability during spring stratification led to an increase in phytoplankton biomass due to the dominance of small flagellate functional groups [15]. Jones and Elliott found that a shorter water retention time under a fixed nutrient load resulted in a reduced chlorophyll concentration; a longer water retention time caused the spring bloom to start earlier and the autumn bloom to persist longer [16].

In reservoirs that are usually frequently artificially regulated, water level fluctuations (WLF) enhance the mixing regime of the water column and reduce the water stability, and also influence the plankton community [17,18]. However, most previous studies have used weekly or daily data to assess the hydrodynamic conditions [19,20], which may overlook important events during the physical processes involved in algal bloom formation, e.g., deepening of stratification, because in some reservoirs, hydrodynamic conditions change very frequently due to WLF, especially in very large and deep reservoirs (e.g., the Three Gorges Reservoir in China). Additionally, organisms do not respond immediately to environmental changes. There are specific time lags between environmental changes and phytoplankton responses [21,22]. Low-frequency monitoring may miss these types of time lags or may not yield accurate results. In recent years, high-frequency auto-monitoring devices have been widely used [23–25]. Sondes are placed at a fixed site for sampling at a frequency of minutes, instead of days or weeks as used with bottle methods. High-frequency data collection can yield more accurate results than bottle methods.

Algal blooms are a widespread problem. Many researchers and reservoir managers seek to find effective methods to control the blooms. Changing the hydrodynamic condition by regulating the water level of the reservoir was recognized as a potential possible approach. In this study, chlorophyll *a* (Chl *a*) was used to represent the algae biomass, as many other researchers have done in other studies [16], and then we measured the water temperature profile and Chl *a* concentration using a high-frequency automated device to accurately analyze the quantitative relationships between the hydrodynamic parameters and surface Chl *a* concentration. Here, we hypothesized that the water column stability plays an important role in algal bloom dynamics only when the resources (light, temperature and nutrients) are sufficient. To test our hypothesis, we divided the study period into several stages according to the availability of these resources, and then analyzed the correlations between water column stability and surface Chl *a* concentration at different stages, and we determined the time lags in these stages. Although zooplankton grazing may be very important to the composition of the phytoplankton community, we did not consider it in this study because it is also influenced by water column stability [26].

## 2. Materials and Methods

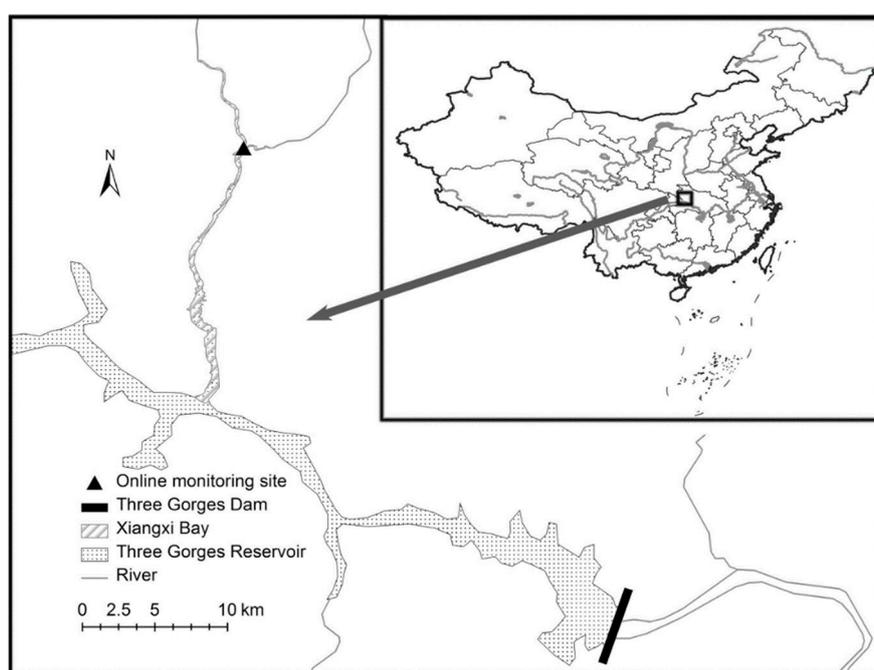
### 2.1. Study Site

The sampling site is located in the middle region of Xiangxi Bay in the Three Gorges Reservoir (TGR) in China (Figure 1). The TGR is the largest man-made reservoir in China, with flood control as its most important task. During the flood season, a lower water level must be maintained to prevent flooding. However, the inflow discharge is always very high during this period, which leads to dramatic water level fluctuations (WLF). The Xiangxi River is the largest tributary of the TGR near the dam. Since the impoundment of the TGR in June 2003, the lower reaches of the river have evolved as Xiangxi Bay. As water velocity decreases and the water retention time increases, the risk of

eutrophication increases. Consequently, algal blooms have occurred more frequently and vigorously, especially in the middle region of the bay [27,28]. This region belongs to the lacustrine zone according to the zonation theory of Straskraba and Tundisi [29]. The stable nature of this zone has contributed greatly to the outbreak of algal blooms.

## 2.2. Field Sampling and Data Analysis

A survey was carried out in Xiangxi Bay during the flood season of 2008. Cyanobacterial blooms occurred from 1 June to 23 July, 2008 [30]. Consequently, this period was selected as our study period. The monitoring site is located at the Xiangxi Ecosystem monitoring station of the Chinese Academy of Sciences/China Three Gorges Corporation (Figure 1). During the study period, the water level of the TGR fluctuated between 144.66 and 146.84 m. The depth of the study site ranged from 12.3 to 14.5 m and was generally lower than 13.0 m. Therefore, we considered 12 m as the depth of the whole water column.



**Figure 1.** Location of the study site.

Water samples were collected (0.5 m beneath the surface) every day, except from 5 to 18 June when weekly samples were collected, for water chemistry analysis (total nitrogen (TN), total phosphorus (TP)). The samples were stored in a pre-cleaned plastic bottle and were acidified to  $\text{pH} < 2$  using sulfuric acid. The water chemistry was measured using a segmented flow analyzer (Skalar San++, Breda, The Netherlands). In conjunction with the water sampling, transparency (Sd) was measured using a 20-cm-diameter Secchi disk. Photosynthetically active radiation (PAR) was monitored using a quantum sensor (Li-Cor 192SA, Lincoln, NE, USA) at 5-min intervals from 6:00 to 19:00 during the daytime. We aggregated PAR data to a daily average value for analysis to show the variations of PAR during the study period. The depth of the euphotic zone was calculated as 2.7 times the transparency [31]. The water-level data of the TGR were provided by the China Three Gorges Project Company.

The surface Chl *a* concentration was measured using a multi-parameter water quality sonde (YSI, EDS 6600V2, Brannum Lane, OH, USA), and the water temperature profile measurements were carried out using thermistor chains; both sets of measurements were conducted at 15-min intervals. The thermistors were placed at 1 m intervals from 1 m to 12 m. The YSI and thermistor chains were joined to a data logger device (EcoTech Umwelt-Meßsysteme, GmbH, Bonn, Germany) such that synchronous measurements could be made.

The relative water column stability (RWCS) was used to describe the hydrodynamic conditions [32]:

$$\text{RWCS} = \frac{D_b - D_5}{D_4 - D_5} \quad (1)$$

where  $D_b$  is the density of the bottom water;  $D_s$  is the density of the surface water (1 m); and  $D_4$ ,  $D_5$  are the water density at 4 °C and 5 °C, respectively. High values of stability indicate water column stratification while low stability signifies mixing.

To identify the depth that could have a significant effect on the surface Chl *a* concentration, we calculated the RWCS of each depth from 2 m to 12 m at 1-m intervals. For example, the relative stability of the column above 3 m was referred to as the 3-m RWCS ( $D_b$  is the density of the water at 3 m) whereas the relative stability of the column above 12 m was referred to as the 12-m RWCS ( $D_b$  is the density of the water at 12 m).

Cross-correlation analysis was used to show the relationship between the time series over a selected range of time differentials (lags), using SPSS 16.0 software. The 15-min-interval data were used; therefore, a lag number of 1 represents 15 min. In the analysis, we set the maximum lag number to 400 and compared the cross-correlation coefficients (CCFs) between RWCS and Chl *a* under different lag times.

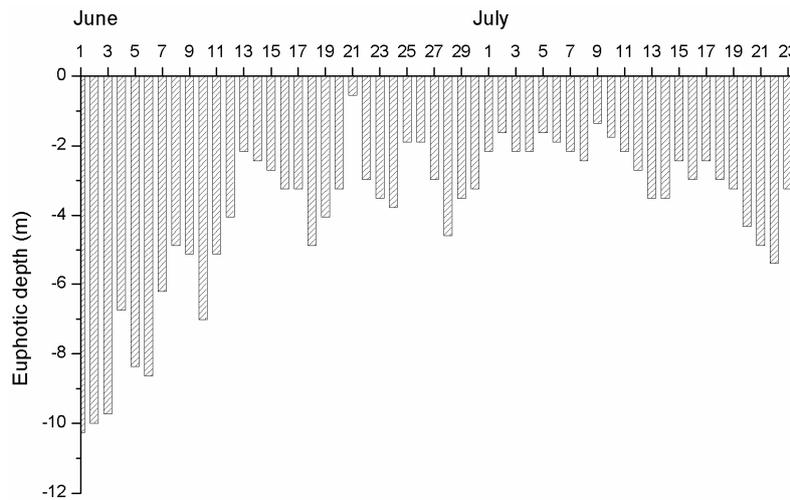
To confirm the hypothesis that we proposed in the Introduction, we divided the whole study period into several stages according to the availability of the resources. Light is an essential condition for phytoplankton growth, but it is not a limiting factor for algal blooms. Therefore, we considered that the PAR measured in Xiangxi Bay during the study period did not limit phytoplankton growth. Cyanobacteria generally grow better at higher temperature (often above 25 °C) due to their competitive advantage over other phytoplankton species such as diatoms and green algae [2,33]. This competitive advantage is attributed to the thermal stability of the DNA, and the protein synthesis and photosynthetic systems of the cyanobacterial cells. However, it is not necessarily inevitable that cyanobacteria will grow to “bloom” proportions in aquatic ecosystems [34]. Consequently, we considered that the water temperature during the study period was not a limiting factor for cyanobacterial growth.

The research of Smith indicated that cyanobacteria tend to be dominant in low TN/TP ratio waters [35]. TN/TP ratios greater than 29 limit cyanobacterial dominance. Therefore, in this study, the whole survey period was divided into 4 stages according to a TN/TP ratio of 29: S1 (1–17 June); S2 (18 June–1 July); S3 (2–14 July); and S4 (15–23 July). Among the 4 stages, weekly monitoring of the water chemistry was performed from 6 to 17 June during S1; we considered this time period as one stage according to the overall changing trend of the TN/TP ratio.

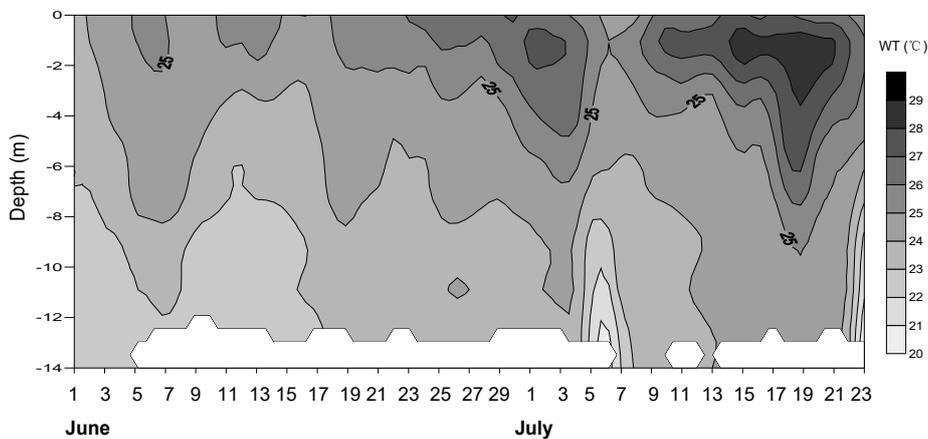
### 3. Results

#### 3.1. Physical and Chemical Conditions

Maximum daily PAR was measured on 26 June and 8 July (Figure 2), and measured 1161 and 1172  $\mu\text{mol}\cdot\text{s}^{-1}\text{ m}^{-2}$ , respectively. However, the instantaneous maximum occurred between 11:00–12:00 on 23 June, when it measured more than 2420  $\mu\text{mol}\cdot\text{s}^{-1}\text{ m}^{-2}$ . The PAR fluctuation was not remarkable, except on 7–9 June, 20–22 June and 22 July, when the PAR was less than 300  $\mu\text{mol}\cdot\text{s}^{-1}\text{ m}^{-2}$ . The euphotic zone was very deep at the beginning of the study period and became shallower after 11 June (Figure 2). The average euphotic depth was 3.83 m (range: 0.54–10.26 m), and it typically ranged between 2 and 5 m. The surface water temperature in Xiangxi Bay fluctuated between 23.06 and 30.75 °C and generally exceeded 25 °C. Stratification was evident, especially when the water temperature increased (Figure 3).

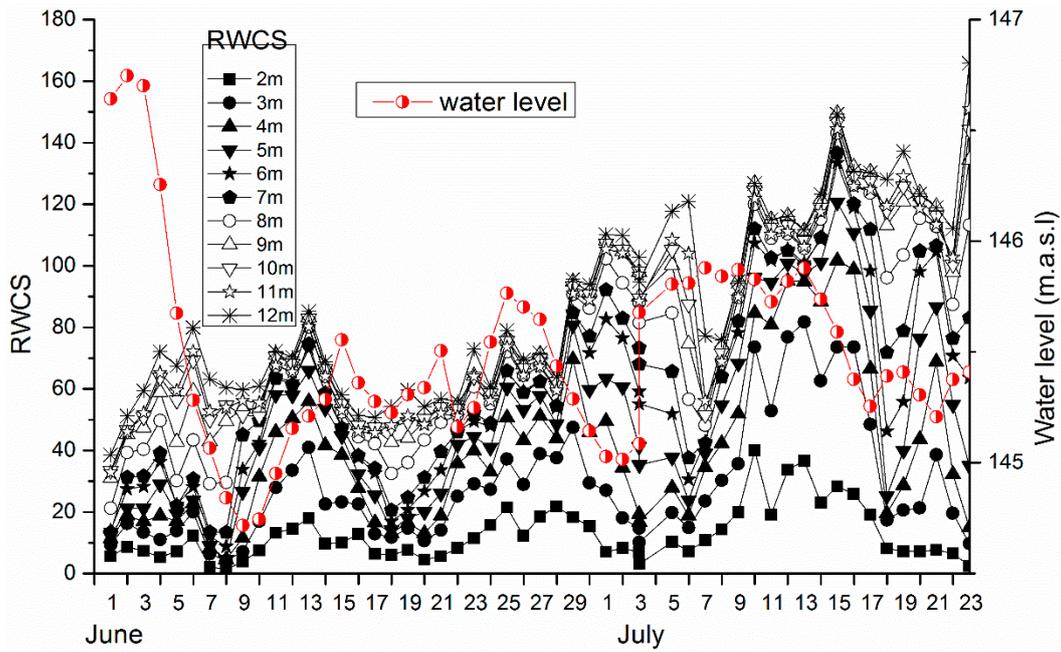


**Figure 2.** Variations in the daily photosynthetically active radiation (PAR) and the euphotic depth of Xiangxi Bay from 1 June to 23 July, 2008.



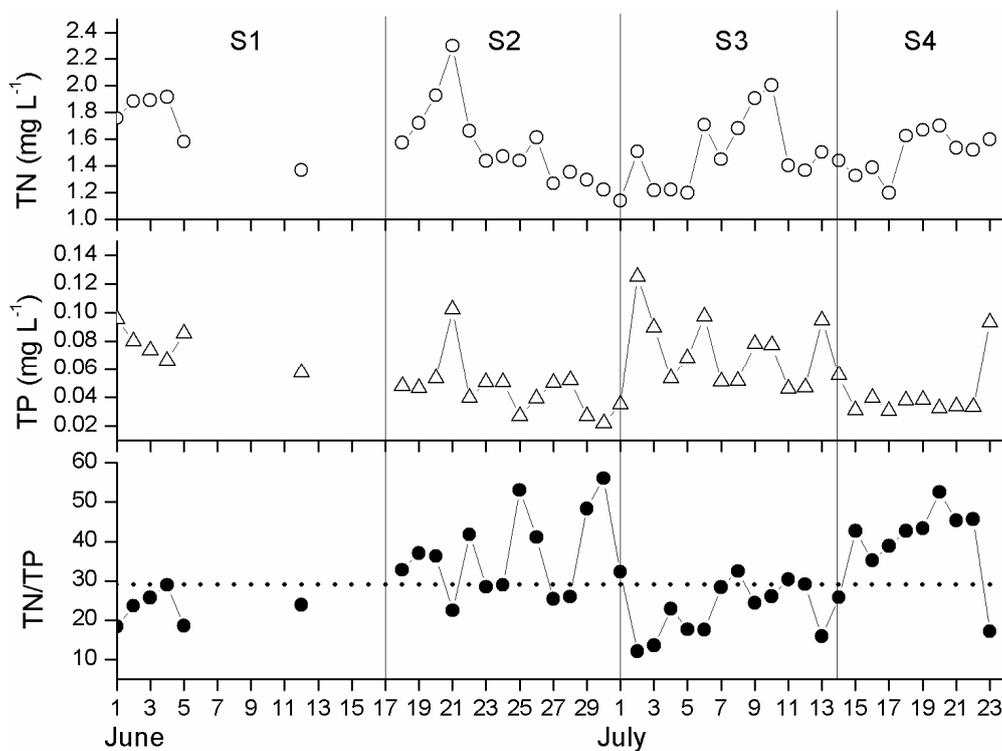
**Figure 3.** Isolines of the daily average water temperature (WT) profile of Xiangxi Bay from 1 June to 23 July, 2008. The white area is the bottom of the bay.

Corresponding to the water stratification, the RWCS increased as the stratification became more evident (Figure 4). The RWCS of the upper water column was much lower than that of the whole water column (12 m), and it showed marked fluctuations, implying frequent water exchange in the upper water column. The RWCS was much lower before 9 June, especially that of the upper water column, due to the continuous dramatic water level decrease. The RWCS in different depths showed that a mixing event happened in 8 and 9 June, with very low RWCS values in the upper 7 m water column. From 10 June, RWCS gradually increased as the water level increased and reached a peak on 14 June; however, the increase in the magnitude of the 2 m RWCS was very small. Subsequently, several obvious fluctuations were observed. In most cases, the increase or decrease in RWCS was accompanied by an increase and decrease in water level.



**Figure 4.** Fluctuations of the daily average relative water column stability (RWCS) of different water columns (2-m water column to 12-m water column, 1-m intervals) in Xiangxi Bay, and the water level (m above sea level, m a.s.l.) variations in Three Gorges Reservoir from 1 June to 23 July 2008.

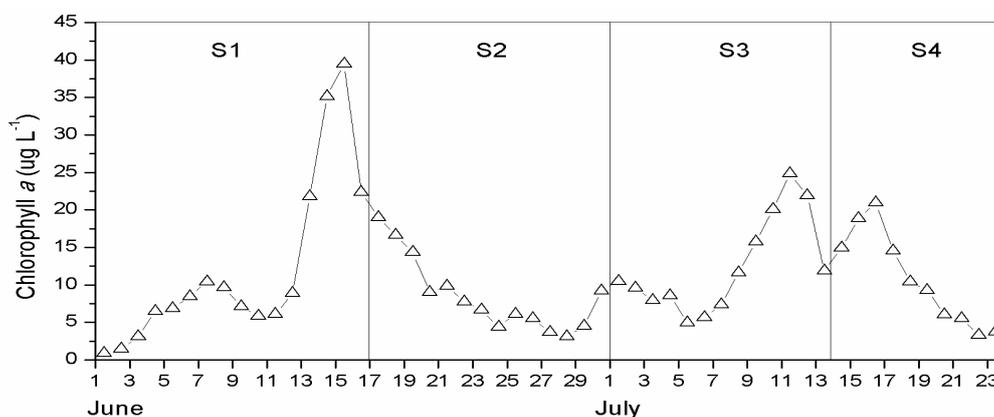
The TN content ranged from 1.14 to 2.30 mg/L, and TP ranged from 0.022 to 0.125 mg/L (Figure 5). TP showed a temporal pattern of “high-low-high-low”, with an evident decrease at the end of June and July, which caused fluctuations in the TN/TP ratio.



**Figure 5.** Daily variations of the nutrients in the surface water of Xiangxi Bay from 1 June to 23 July 2008. The dotted line in the figure shown in TN/TP ratio represents TN/TP = 29. S1: TN/TP < 29; S2: TN/TP > 29; S3: TN/TP < 29; S4: TN/TP > 29.

### 3.2. Surface Chlorophyll *a*

Two phases were observed based on the fluctuations of the daily average value of Chl *a*: 1–29 June and 30 June–23 July. The daily average values ranged between 0.92 and 39.47  $\mu\text{g/L}$ , but based on the data monitored at 15-min interval, the maximum Chl *a* concentration in the first phase was 75.30  $\mu\text{g/L}$  and was observed at 18:30 on 14 June, compared with 46.70  $\mu\text{g/L}$  at 15:00 on 16 July in the second phase (Figure 6).



**Figure 6.** Variations in the daily average Chl *a* concentration in Xiangxi Bay from 1 June to 23 July 2008. S1–S4 were divided according to the value of TN/TP = 29. S1: TN/TP < 29; S2: TN/TP > 29; S3: TN/TP < 29; S4: TN/TP > 29.

### 3.3. Cross-Correlation Analysis between Water Column Stability and Chlorophyll *a*

Cross-correlation analysis was carried out between the RWCS above specific depths and the Chl *a* concentration measured in different stages (S1–S4 and the whole period). We extracted the highest CCF and the relative lag number (Table 1).

**Table 1.** Cross-correlation coefficient (CCF) and the lag number between the RWCS of each layer and the surface chlorophyll *a* concentration. A: all data during the study period. (a lag number of 1 represents 15 min).

		RWCS										
		2 m	3 m	4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m
A	CCF	0.28	0.32	0.34	0.32	0.28	0.25	0.21	0.18	0.17	0.14	0.13
	lag	195	101	103	104	97	97	97	98	97	97	97
S1	CCF	0.49	0.61	0.69	0.70	0.69	0.66	0.64	0.60	0.54	0.47	0.44
	lag	202	202	202	200	200	200	199	199	200	200	200
S2	CCF	-0.31	-0.40	-0.48	-0.49	-0.46	-0.41	-0.38	-0.37	-0.37	-0.37	-0.37
	lag	-109	-112	-112	-112	-112	-111	-125	-207	-207	-207	-312
S3	CCF	0.43	0.54	0.62	0.64	0.63	0.58	0.51	0.46	0.40	0.34	0.30
	lag	0	0	3	2	2	2	2	2	2	2	2
S4	CCF	0.42	0.60	0.51	0.39	0.30	0.34	0.32	0.27	0.19	0.11	0.04
	lag	0	2	3	2	2	2	2	2	2	3	3

When the data of the whole study period were used in the analysis, the relationship between RWCS and Chl *a* was significant, but the CCF (A) was very low. In S1, a lag number of 200 (50 h) was recorded for the response of Chl *a* to changes in RWCS. The highest CCF was recorded between 5-m RWCS and Chl *a*, indicating that the stability of the water column above 5 m caused the most significant effect on the surface Chl *a* concentration. In S2, all lag numbers were negative, indicating no significant effect of RWCS on Chl *a*. In S3, the lag numbers between 2-m RWCS and Chl *a* and between 3-m RWCS and Chl *a* were both 0, implying that Chl *a* responded rapidly following the disturbance of

the water column above 2 and 3 m. The response time of Chl *a* to the RWCS of other water columns was 30 min (a lag number of 2). The highest CCF was also recorded between 5-m RWCS and Chl *a*. This indicated that a disturbance in the upper 3 m of the water column influenced the surface Chl *a* concentration, but the effect of the RWCS above 5 m was the most prominent. In S4, similar to S3, changes in the 2-m RWCS significantly and rapidly affected the Chl *a* concentration, but the influence of the 3-m RWCS was the most remarkable, with a lag delay of 30 min (a lag number of 2).

Because the effects of the RWCS above the 3- and 5-m water columns were usually the most prominent, we showed the relationships between the 3-m RWCS and Chl *a*, 5-m RWCS and Chl *a*, and 12-m RWCS and Chl *a* in different stages (S1–S4 and the whole period (All)) (Figure 7). It was evident that the CCF was much lower when all data were analyzed (the first row). Different relationships were obtained at different stages. In S1 and S3, the concentration of Chl *a* showed periodic fluctuation. In S4, no periodic changes were observed because the Chl *a* concentration decreased over a short period of time, and therefore, the CCF was also not periodic.

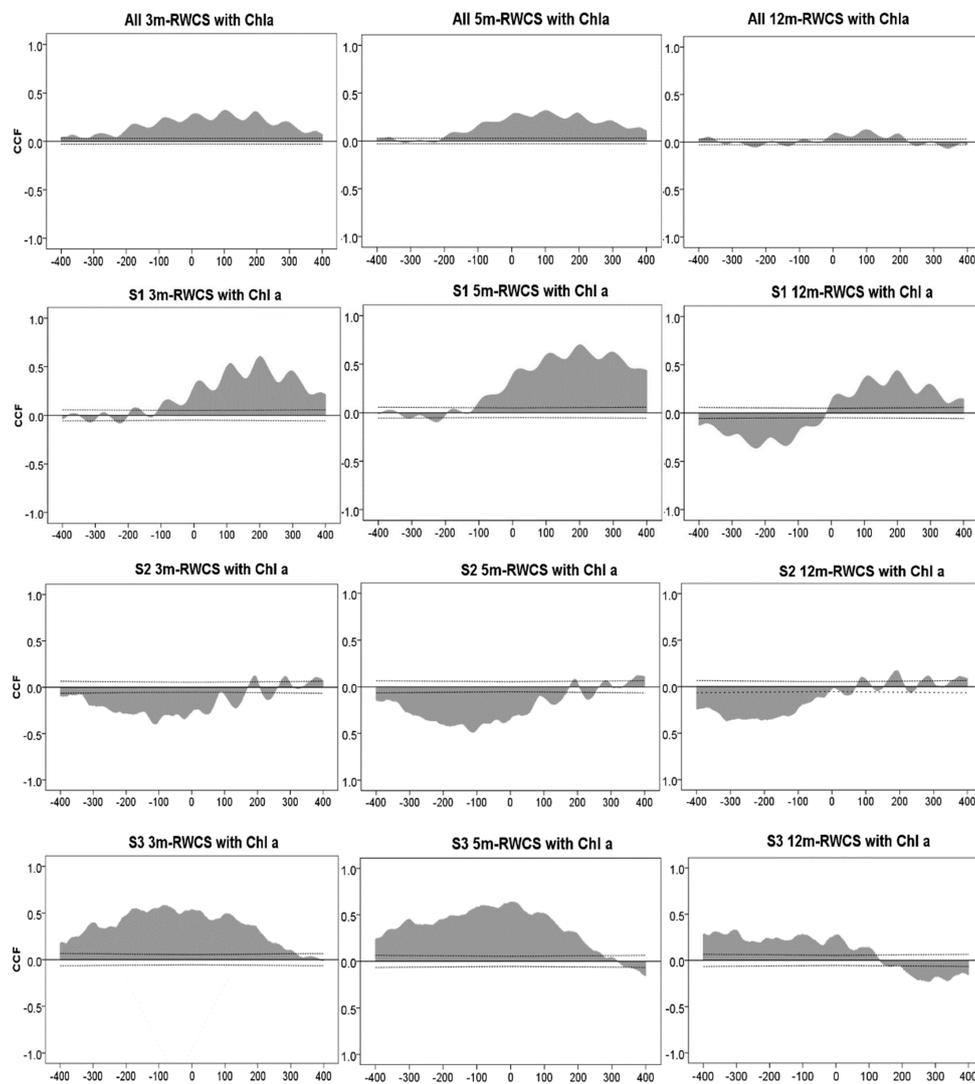
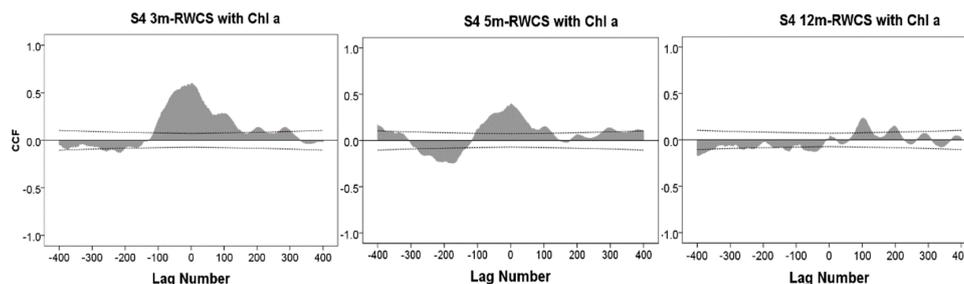


Figure 7. Cont.



**Figure 7.** Cross-correlations between the relative stability of different water columns (3-m RWCS, 5-m RWCS, and 12-m RWCS) and Chl *a* at different stages according to the TN/TP ratio (the dotted line represents the confidence limit). RWCS represents the relative water column stability.

#### 4. Discussion

Light can influence the vertical distribution of phytoplankton biomass by influencing the photosynthesis in the water column [15,36,37]. Ait Hammou et al. found high concentrations of microcystin-producing cyanobacteria in the first five meters of the water column [4]. This is similar to our results. In our study, after 11 June, the average residence depth of cyanobacteria (the dominant species during the study period) fluctuated between 2 and 5 m [30], which was proved to be the euphotic depth, indicating the importance of light. Additionally, in S1 and S3, the surface Chl *a* concentration was most significantly influenced by the stability of the water column above 5 m, which helped us to conclude that only the water stability in the upper water column affects the algal blooms. This has also been proved by several other researchers. For example, Scofield et al. found that a deep chlorophyll layer formed once the water column stratified, and remained until the epilimnion deepened beyond the euphotic zone [38].

Usually, high nutrient concentration and a low TN/TP ratio are identified as the most important reasons for increased cyanobacterial biomass [39–41]. However, at the beginning of S1 in our study, the Chl *a* concentration was very low even though the nutrient contents and TN/TP ratio were suitable. This might be caused by the low water column stability. Stability is one of the most important factors influencing the phytoplankton, especially the prevalence of cyanobacteria [5,12,34]. Cyanobacteria can benefit from vertical migration in stratified water by regulating their buoyancy, which gives them a competitive advantage for nutrients and light [33,42]. However, in regions with high river flow or short residence time, intensive water disturbance can limit phytoplankton biomass [43,44]. During our study period, at the beginning of S1, the RWCS was usually lower than 20, especially in the water column above 5 m. Therefore, we concluded that a lower RWCS (<20) in the water column restricted the growth of the phytoplankton and limited the cyanobacterial bloom.

In some stages, the Chl *a* concentration increased as the RWCS increased under the background of sufficient nutrients. However, it decreased in S2 although the RWCS was similar to that in S1. This might be caused by the limitation caused by a high TN/TP ratio [35]. This confirmed our hypothesis that a high RWCS can influence algal blooms only when nutrients, water temperature and light are not limiting factors.

Our results indicated that there was an obvious time lag between the changes in Chl *a* and water column stability. Time lags, or delays, are common in many systems. Volpe et al. found that phytoplankton biomass and surface heat content were significantly correlated based on an approximate 5-month lag time [45]. A study conducted by Nezlin and Li in Santa Monica Bay indicated that chlorophyll biomass was significantly correlated with air temperature with a lag time of 5 days [46]. The effect of a toxic phytoplankton bloom on zooplankton mortality also occurs after a time lapse [47]. However, these studies usually considered only one environmental factor and neglected the synergistic effect caused by other factors. In our study, if we did not consider the effect of nutrients and we used all the experimental data to perform the analysis, a significant correlation and the related lag time could also be obtained, but with a lower CCF and an inaccurate lag time. The analysis based

on the data of different stages, however, indicated different lag times under different backgrounds. The correlation was positive in both S1 and S3, while the response time of Chl *a* to RWCS was different, i.e., 50 h and 30 min, respectively. The cyanobacterial bloom occurred during these two stages, S1 and S3. Usually, at the end of the bloom stage, the phytoplankton density decreases due to nutrient exhaustion. However, numerous phytoplankton cells are still present in the water compared to clean water. Therefore, following the re-enrichment of nutrients, a second phytoplankton bloom breaks out in a relatively shorter time [48]. Additionally, the relatively higher water column stability in S3 could be another factor that led to the shorter response time in S3.

Water column stability is usually strongly related to WLF. Valdespino-Castillo et al. found that low water levels tend to intensify boundary-mixing events, and that water level manipulation could be a useful management tool to promote phytoplankton groups other than cyanobacteria [18]. In our study, a decreased water level was accompanied by low water column stability, which might be effective for controlling the algal blooms, especially cyanobacteria blooms. Although we did not undertake a quantitative analysis between the water level and water column stability, their variation trends were compared. In the future, choosing a good WLF parameter (e.g., one-day WLF, two-day WLF, or half-day WLF) to study the quantitative influence of hydrodynamic conditions on Chl *a* or phytoplankton, would be a good approach to investigate how to accurately manipulate water levels to control algal blooms.

## 5. Conclusions

As evident from the above results, we concluded that short-term variations in the stability of the upper part of the water column significantly impacted the surface Chl *a* concentration. When nutrients, water temperature and light are sufficient, a stable water environment can cause a significant increase in Chl *a* within approximately 2 days. The higher the stability, the faster the algal bloom forms. We also concluded that lower stability (less than 20) can limit phytoplankton growth, which provides a good approach for controlling algal blooms. Lower water column stability usually depended on a decrease in the water level. Therefore, during an algal bloom period, especially a bloom with a high prevalence of cyanobacteria, reducing the water column stability by decreasing the water level could prevent or control algal blooms.

**Author Contributions:** M.Z. performed the field investigation, did the analysis and prepared the manuscript; Z.N. made the review of the manuscript, English corrections, and contributed to manuscript preparation; Q.C. performed the field investigation and provided the funding. Y.X. did the field investigation and laboratory work. X.Q. supervised the research and gave suggestions during the review and editing process.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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