

Article

# Spatial and Temporal Dynamics of Potentially Toxic Cyanobacteria in the Riverine Region of a Temperate Estuarine System Altered by Weirs

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**Abstract:** The effects of weirs on fish and other biological communities have garnered considerable study, whereas the effects of weirs on community composition of toxic cyanobacteria have not yet been well documented. In this study, temporal and spatial variations in species composition and the abundance of potentially toxic cyanobacteria were investigated in the riverine regions of the temperate Youngsan River estuary, where two weirs have recently been constructed. Four stations were sampled 0.5 m below the surface monthly along the channel of the upper river from May 2014 to April 2015 to explore cyanobacterial composition and abundance, while physicochemical and biological parameters were measured to elucidate possible mechanisms controlling these dynamics. Two stations were located upstream at free-flowing sites, and the other stations were located downstream at impounded sites near the weirs. Twenty-eight cyanobacterial species were identified, seven of which were potentially toxic: *Microcystis* sp., *M. aeruginosa*, *M. flos-aquae*, *Dolichospermum* sp., *Aphanocapsa* sp., *Oscillatoria* sp. and *Phormidium* sp. *Microcystis* sp. was the most abundant in June 2014 at the lowest station near the weir. Meanwhile, *Phormidium* sp. occurred at low abundance throughout the study period, except during the winter months, when its abundance was elevated. The interactive forward selection method highlighted dissolved inorganic nitrogen and zooplankton abundance as explanatory variables for this observed variation, but their effects on cyanobacterial growth are unclear. However, temperature was the major determinant for the temporal variation in cyanobacterial populations. Cluster analysis showed that the downstream stations near the weirs had a high similarity of potentially toxic cyanobacteria. Significantly higher abundance, especially of *Microcystis* sp., was also recorded at the impounded sites suggesting that the presence of weirs might affect variations in toxic cyanobacterial communities.

**Keywords:** toxic cyanobacteria; weir; *Microcystis*; *Phormidium*; temperature

## 1. Introduction

Cyanobacteria are one of the most primal organisms on earth, dating back approximately 3500 million years [1]. They are naturally pervasive in both freshwater and marine environments. Their widespread adaptability is credited to their proficient internal characteristics, such as sheath pigments that absorb UV light for radical scavenging [2], buoyancy regulation for advantageous access to light and atmospheric CO<sub>2</sub> [3], and their capacity to undergo spontaneous pre-selective mutations [4]. Despite their high adaptability, cyanobacterial abundance and diversity are still influenced by

certain environmental conditions such as light intensity [5], temperature [6,7], turbidity [6,8], grazer populations [9], and nitrogen and phosphorus availability, as well as their ecophysiological characteristics (e.g., growth rate) [9]. These factors are crucial in understanding bloom formation, although eutrophication is generally a major cause of cyanobacterial blooms [6,10,11]. Blooms impact ecosystems by depleting oxygen and light supply in the water, but more importantly, certain species of cyanophytes produce toxins that can be transferred to higher trophic levels. Cyanobacteria also exude chemicals with foul tastes or odours (geosmin and 2-methylisoborneol) [12,13] that alter drinking water palatability.

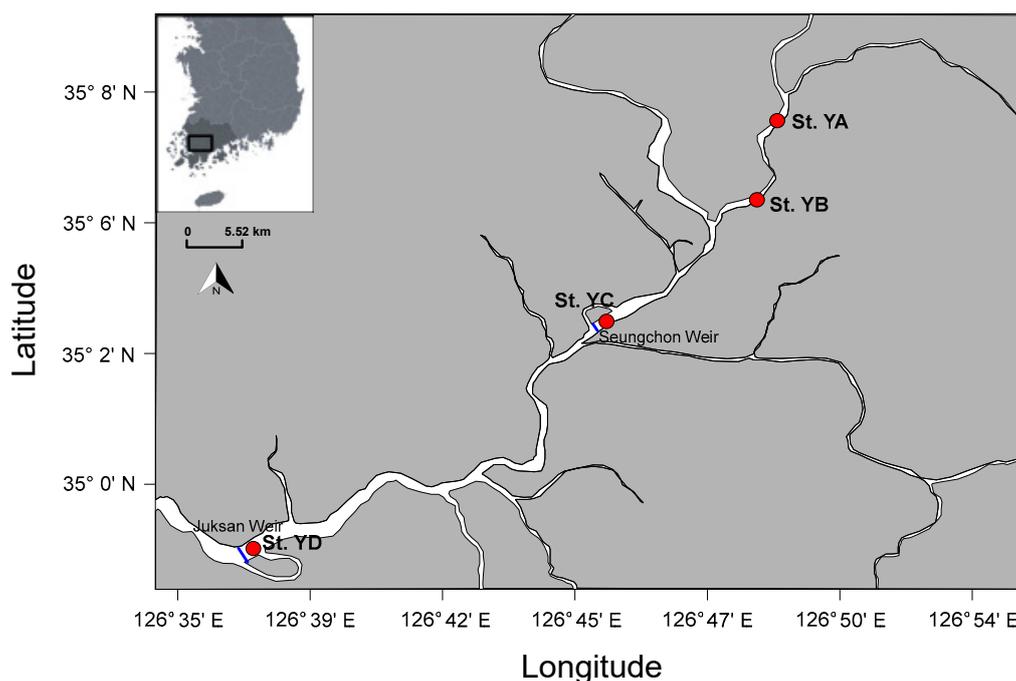
The aforementioned factors can be affected by hydrological modifications in aquatic ecosystems, including riverine zones of estuaries. The natural conditions of lotic environments are especially modified when man-made structures are introduced. These structures can change riverine systems to lacustrine conditions [10,14]. When riverine waters become impounded, changes in water quality and biota are detected [15,16]. For example, the Murray River in Australia has been observed to be more favourable to cyanobacterial growth in areas where weirs were installed, as opposed to free-flowing areas [17]. The study pointed out that the presence of weirs was advantageous for cyanobacteria over other phytoplankton species because they are capable of buoyancy. Nutrient accumulation, particularly dissolved inorganic nitrogen (DIN) and phosphorus (DIP), aggravated by the presence of weirs, sequentially contributed to bloom formation [7,18,19]. A hydrological–ecological model revealed that changes in hydrology promote algal biomass proliferation [20]. This suggests that modifications in water dynamics can stimulate shifts in cyanobacterial taxonomic composition, but these effects on species composition are not fully understood.

In Korea, multiple weirs have been constructed in the lotic zones of major rivers as part of the Four Major Rivers Restoration Project. This project was designed to regulate problems related to flooding and drought in the Youngsan River, one of the four major rivers connected to an estuary. Two weirs, the Seungchon Weir and Juksan Weir, were constructed transecting a stretch of the Youngsan River. Although an increase in algal blooms has been reported since the construction of the weirs in the river [21], the temporal and spatial patterns of outbreaks of cyanobacteria that are potentially capable of producing toxins have not yet been explored. We hypothesised that the construction of weirs may have contributed to the increased blooms, particularly those of toxic cyanobacteria. The objective of this study was to compare cyanobacterial composition and abundance, particularly of potentially toxic cyanobacteria, in the free-flowing and impounded sites of the riverine region of the Youngsan River estuary. Physicochemical and biological parameters were measured to determine the major factors controlling variability in cyanobacterial composition and abundance.

## 2. Materials and Methods

### 2.1. Study Site and Field Sampling

The Youngsan River, located in southwest Korea, is about 129.5 km in length and has a basin area of 3455 km<sup>2</sup> [22]. It serves as the lifeline of the Jeollanam province, catering to the agricultural, industrial, water consumption and leisure needs of the residents [23]. Four sampling stations, designated YA, YB, YC and YD, with depths ranging from 1.3 to 4.5 m, were monitored along the channel of the upper region of the estuary (Figure 1) on a monthly basis from May 2014 to April 2015. The first two stations (control; YA and YB) were situated far from the weirs, whereas the other two sites (treatment; YC and YD) were sampled just upstream of the weir structures. Water samples were collected 0.5 m below the surface at the sampling stations using Niskin bottles and stored in 2 L amber polycarbonate bottles in a dark and cool environment (<24 °C) until further analysis. Sample collection was based on the methods of Hötzel and Croome [24].



**Figure 1.** The sampling stations situated far from the weirs (YA, YB) and near the weirs (YC, YD) in the upper Youngsan River estuary, South Korea.

## 2.2. Determination of Physicochemical Parameters

In situ water temperature ( $^{\circ}\text{C}$ ) and turbidity (NTU) were measured using the YSI Model 6600 Multi-parameter Water Quality Sonde (YSI, Inc., Yellow Springs, OH, USA). A photosynthetically active radiation (PAR;  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) Quantum Radiometer (LICOR, Lincoln, NE, USA) was used to measure light intensity from 0 cm (surface level) to 50 cm water depth (at 10 cm intervals). Light attenuation coefficient ( $k_d$ ;  $\text{m}^{-1}$ ) values were derived from the Beer-Lambert equation [25]:

$$I_z = I_0 (e^{-k_d \cdot z}),$$

where

$I_z$  = irradiance at depth,

$I_0$  = irradiance at surface,

$k_d$  = light attenuation coefficient,

and

$z$  = depth.

No PAR data are available for the months of October and November at station YD owing to technical problems during sampling. Dissolved inorganic nutrients ( $\mu\text{M}$ ) were measured by filtering 15 mL of each water sample using Whatman 25 mm GF/F glass microfiber filters (0.7  $\mu\text{m}$  pore size; Whatman, Buckinghamshire, UK). The filtrates were immediately stored at  $-75^{\circ}\text{C}$  until further analysis. Nutrient concentration analysis was conducted with a QuAatro Autoanalyser (SEAL Analytical, Inc., Norderstedt, Germany). The detection limit for DIN is 0.02–0.03  $\mu\text{M}$ , while that for DIP is 0.006  $\mu\text{M}$ . DIN was calculated as the sum of ammonium ( $\text{NH}_4^+$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ); DIP was equal to the soluble reactive phosphate ( $\text{PO}_4^{3-}$ ) concentration [26].

### 2.3. Determination of Chlorophyll *a*

The water samples (100 mL) were filtered under  $\leq 100$  mmHg pressure using Whatman 25 mm GF/F glass microfiber filters (0.7  $\mu\text{m}$  pore size). The filters were placed inside 8 mL amber glass bottles pre-filled with 90% acetone solution and stored at 4 °C for 12 h. Chlorophyll (Chl) extracts were measured using a 10-AU fluorometer (Turner Designs, Sunnyvale, CA, USA). The method used for Chl *a* measurement was based on Arar and Collins [27].

### 2.4. Determination of Zooplankton Abundance

Zooplankton were collected from 20 to 50 L water samples filtered with a 60- $\mu\text{m}$  net. Samples were preserved by adding 10% formalin (4% final concentration). Large zooplankton were counted using an inverted microscope at 25 or 50 $\times$  magnification, and smaller zooplankton were counted at 100 or 400 $\times$  magnification. Identification was made to the genus or species level using Voigt [28], Smirnov and Timms [29] and Einsle [30] as references.

### 2.5. Cyanobacteria Identification and Enumeration

Water samples were placed into opaque 1 L bottles pre-filled with 3 mL iodine potassium-iodide (IKI) solution [31] and stored at room temperature for identification. The bottles were allowed to stand until the phytoplankton settled to the bottom, after which the water above was removed, while the remaining 50 mL of the sample containing the phytoplankton was transferred to a conical tube [24]. Cyanobacteria were counted in a 1 mL Sedgewick–Rafter counting chamber using a Zeiss microscope (Zeiss® Axiolab, Jena, Germany). For identification of species, the following references were used: Hirose et al. [32], Komárek and Anagnostidis [33,34].

### 2.6. Statistical Analyses

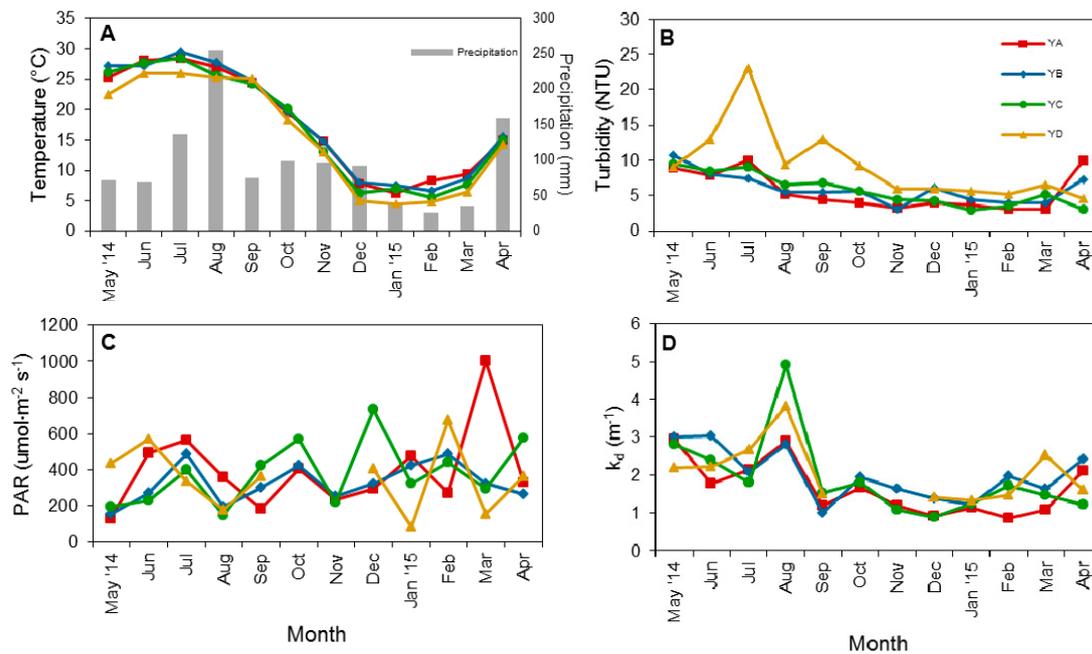
Using CANOCO software ver. 5 (Microcomputer Power, Ithaca, NY, USA), ecological ordination analysis was applied to elucidate the relationships between cyanobacterial communities, environmental parameters and biotic factors (zooplankton abundance and Chl *a*). An unconstrained unimodal detrended correspondence analysis (DCA) was initially performed to determine the homogeneity of the data. The first DCA axis determines the method to use, and in this study, redundancy analysis (RDA) was employed. To test the significance of the relationships, a permutation test (significant at  $p < 0.05$ ) was applied. Subsequently, interactive forward selection method was further employed to detect changes in variables that may influence cyanobacterial communities and abundance. The false discovery rate (FDR)  $p$ -value correction method was applied to prevent inclusion of unnecessary predictors. Monthly means of the potentially toxic species were used in the analyses. To determine the similarity among sites, hierarchical cluster analysis was performed in Primer 6 (Quest Research Limited, Auckland, New Zealand). The total abundances of the potentially toxic cyanobacteria were used to determine similarity. The complete linkage method of distance clustering was used. Based on the cluster analysis results, the study sites were categorised into two groups: free-flowing (stations YA and YB; control) and impounded (stations YC and YD; treatments). The paired  $t$ -test (significant at  $p < 0.05$ ) was used to compare environmental parameters and toxic cyanobacterial abundances between the groups. The data were transformed to  $\log(x + 1)$  values prior to the statistical analysis.

## 3. Results

### 3.1. Physical Factors and Inorganic Nutrient Concentrations

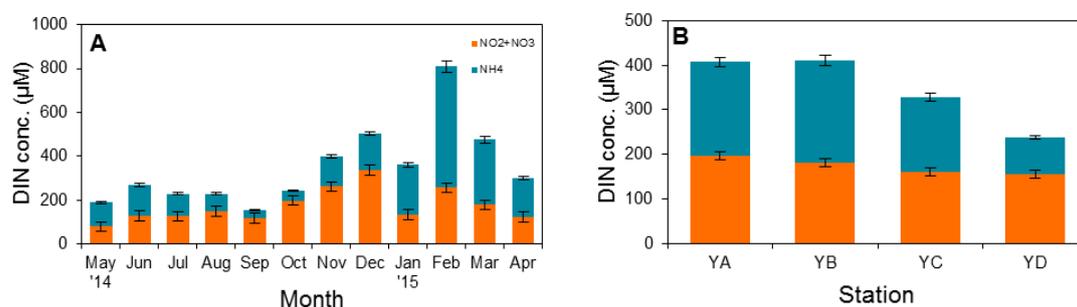
Surface water temperatures followed a general seasonal trend: highest during the summer and lowest in winter (Figure 2A). The highest average precipitation was observed in the summer months of August and July, as well as in April (spring). Turbidity was relatively constant from August to April for stations YA–YC (Figure 2B), whereas turbidity at station YD gradually increased until it peaked in

July, then abruptly dropped in August. The turbidity at YD was already stable and low by the end of autumn. PAR at the surface (0.5 m water depth) fluctuated from month to month or every other month (Figure 2C). A peak in the light attenuation coefficient ( $k_d$ ) was recorded during the month of August for all sampling sites, with the highest peak at station YC. The rest of the months were stable, as with the trend in turbidity (Figure 2D).

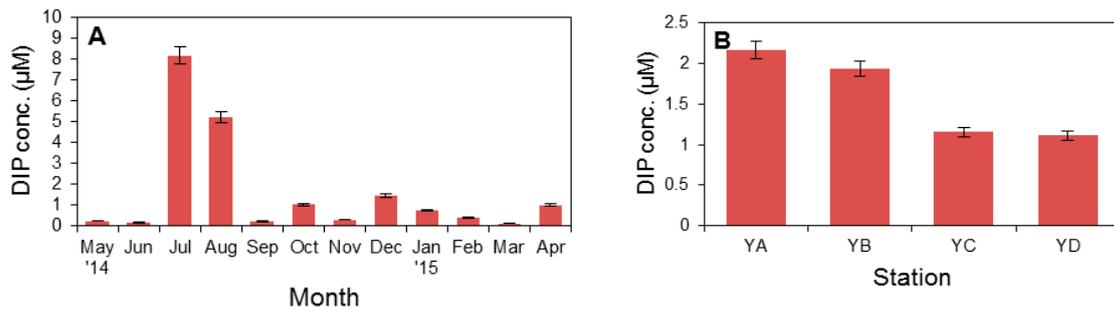


**Figure 2.** Monthly and spatial variations of physical variables. (A) Temperature and precipitation, (B) Turbidity, (C) photosynthetically active radiation (PAR), and (D) light attenuation coefficient at stations YA, YB, YC, and YD in the upper Youngsan River estuary.

On a monthly basis, DIN was consistently between 200 and 400  $\mu\text{M}$ , but increased from October to December and peaked in February (Figure 3A). DIN was lowest at station YD and highest at YA and YB (Figure 3B), decreasing in concentration along the downstream course of the river system. DIP concentration (Figure 4A) was highest in July (DIP = 8.17  $\mu\text{M}$ ) and August and lowest in June (DIP = 0.17  $\mu\text{M}$ ). The concentrations at YA and YB, and YC and YD, were similar (Figure 4B).



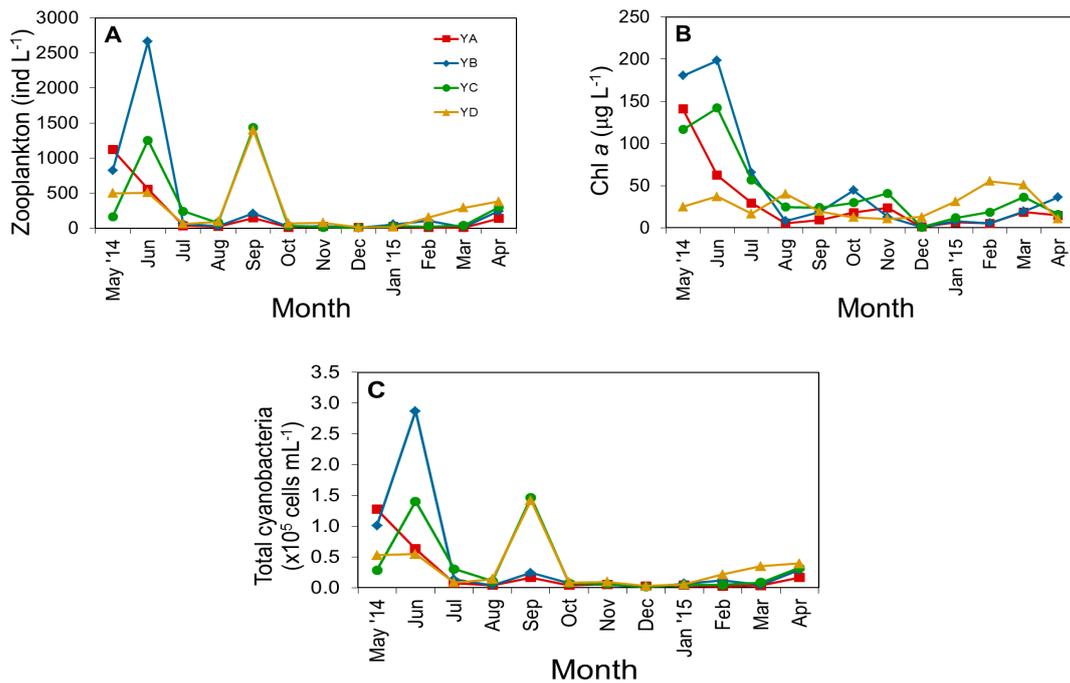
**Figure 3.** Average (A) monthly and (B) spatial variations in dissolved inorganic nitrogen (DIN) concentrations in the upper Youngsan River estuary. Error bars represent standard deviations.



**Figure 4.** Average (A) monthly and (B) spatial variations of dissolved inorganic phosphate (DIP) concentrations in the upper Youngsan River estuary. Error bars represent standard deviations.

3.2. Biological Parameters

Zooplankton was most abundant in June at station YB (2661 ind·L<sup>-1</sup>), followed by YC. The second peak of abundance was observed in September at stations YC (1433 ind·L<sup>-1</sup>) and YD (1395 ind·L<sup>-1</sup>). Zooplankton species at these stations proliferated either during the onset of summer or in early autumn (Figure 5A). The concentration of Chl *a* was also prominently high during the early summer at all stations except YD. Lower concentrations of less than 50 µg·L<sup>-1</sup> were maintained throughout the remaining months (Figure 5B).



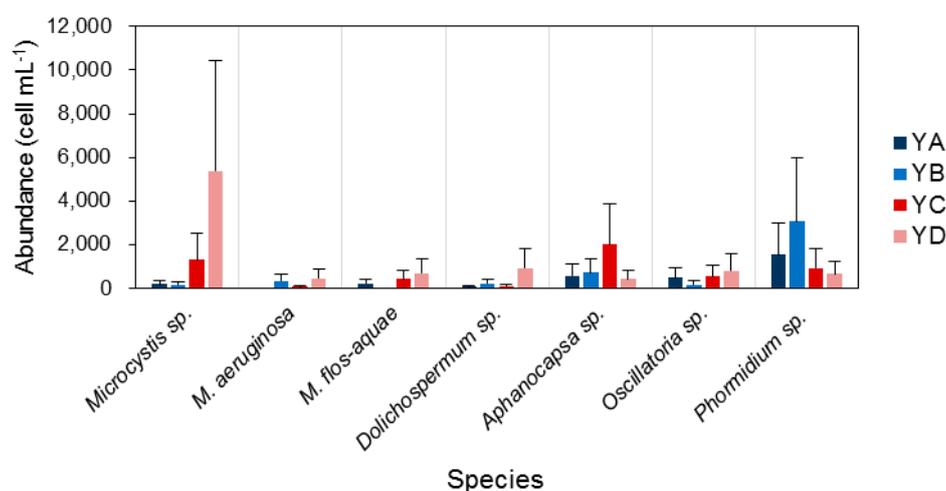
**Figure 5.** Monthly and spatial variations of biological variables: (A) zooplankton abundance, (B) chlorophyll *a*, and (C) total cyanobacterial count at stations YA, YB, YC and YD in the upper Youngsan River estuary.

Cyanobacterial count rose during June, with the highest population at station YC, followed by YB (Figure 5C). Abundant cyanobacteria were also observed in September at YC, and a similar trend was confirmed for zooplankton abundance. Overall, there was a clear concurrent trend between stations YB and YC during June. PAR and turbidity values during this month were similar at both stations.

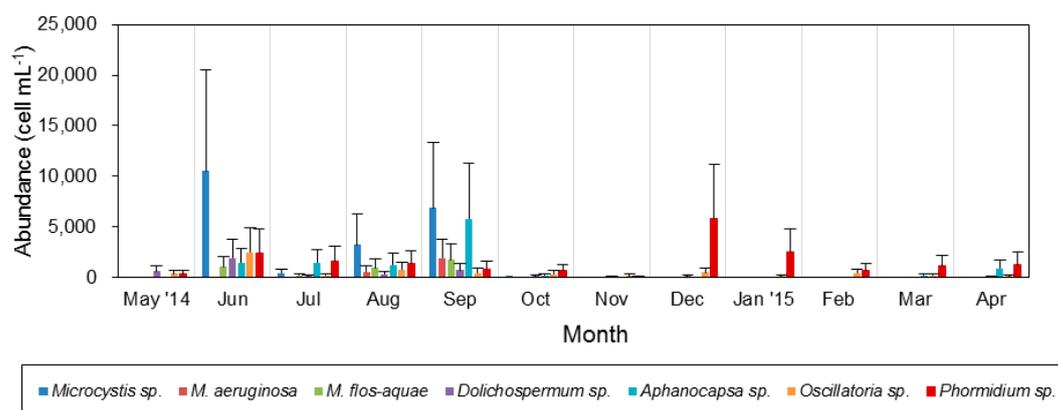
### 3.3. Spatiotemporal Variation of Potentially Toxic Cyanobacteria

Out of the 28 cyanobacterial species identified (Table S1 in Supplementary Materials), seven were potentially toxic strains classified in previous studies: *Microcystis* sp., *M. aeruginosa*, *M. flos-aquae*, *Dolichospermum* sp., *Aphanocapsa* sp., *Oscillatoria* sp. and *Phormidium* sp.

Figure 6 displays the abundance of the seven genera and species at different sites. Among the potentially toxic species, *Microcystis* sp. was the most abundant at station YD, followed by YC, with cell counts of 5365 cells·mL<sup>-1</sup> and 1298 cells·mL<sup>-1</sup>, respectively (Figure 6). *Aphanocapsa* sp. was primarily found at station YC. The cell count of *Phormidium* sp. was higher in the upper part of the freshwater zone and steadily decreased downriver. The other species were recorded in the summer months and early autumn (Figure 7). However, the occurrence of *Phormidium* sp. was more evident during the coldest months of the year.



**Figure 6.** Variation in spatial abundance of the potentially toxic species in the upper part of the Youngsan River estuary. Bars in blue hues indicate upstream stations away from weirs (stations YA and YB), while red bars indicate stations near the weirs (stations YC and YD).



**Figure 7.** Variation in temporal abundance of the potentially toxic species in the upper regions of the Youngsan River estuary.

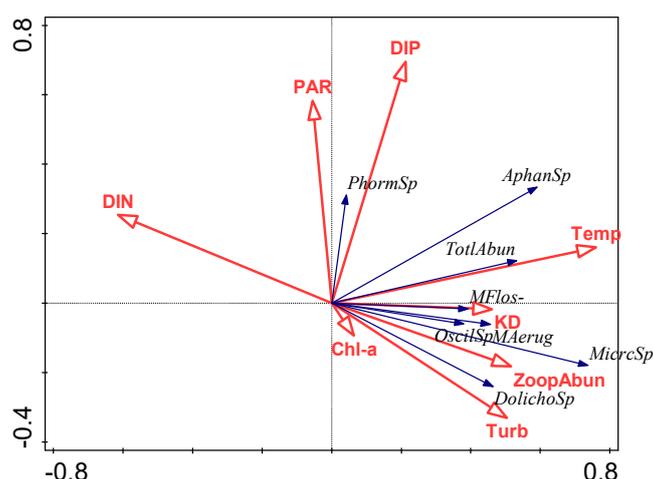
### 3.4. Relationship between Environmental Factors and Cyanobacterial Species

The gradient length of the first axis was <4, suggesting that the cyanobacterial abundance data were homogeneously distributed (Table 1), and therefore, linear methods were used.

**Table 1.** Detrended correspondence analysis results for the homogeneity of variables.

Statistic	Axis 1
Eigenvalues	0.3528
Explained variation (cumulative)	32.34
Gradient length	2.34

The RDA biplot (Figure 8) summarises the relationships among environmental factors and potentially toxic cyanobacteria. The percentages explained by each axis are as follows: Axis 1 = 77.53%, Axis 2 = 14.09%, Axis 3 = 5.70%, and Axis 4 = 2.69%. The *Microcystis* genus was closely associated with *Dolichospermum* sp. and *Oscillatoria* sp. in the RDA analysis. These species were highly positively correlated with turbidity and zooplankton abundance, while displaying negative relationships with DIN. Surface water temperature and the light attenuation coefficient also demonstrated strong relationships with the aforementioned cyanobacterial species. Surface water DIP and PAR were positively related to the abundance of *Phormidium* sp. Total abundance was highly positively correlated with temperature. The explained variation accounted for 39.3% of the total variability in potentially toxic cyanobacterial abundance (adjusted explained variation = 25.8%). Furthermore, the permutation test (significant at  $p < 0.05$ ;  $p = 0.028$ ) confirmed the significance of these relationships.



**Figure 8.** RDA biplot of the environmental variables and potentially toxic species. Total abundance (TotlAbun) and potentially toxic cyanobacteria species (*Microcystis* sp. = MicrcSp; *M. aeruginosa* = MAerug; *M. flos-aquae* = MFlos-; *Dolichospermum* sp. = DolichoSp; *Oscillatoria* sp. = OscilSp; *Aphanocapsa* sp. = AphanSp; *Phormidium* sp. = PhormSp) are represented by blue lines, whereas environmental factors are represented by red lines.

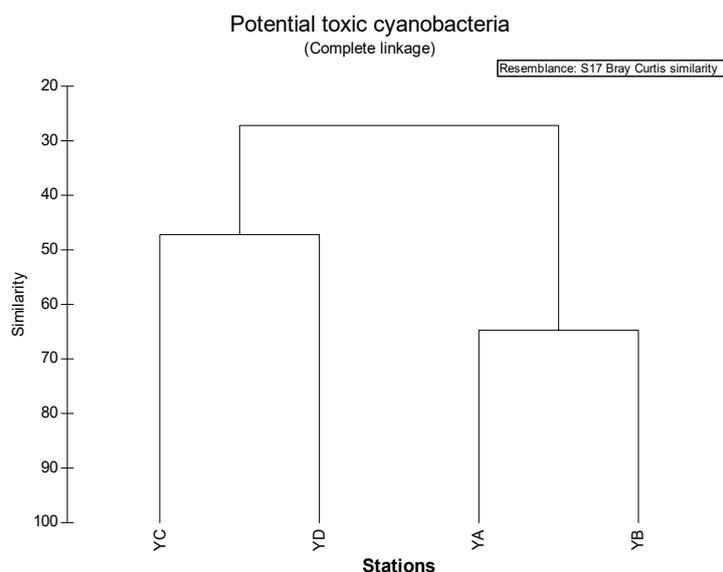
The interactive forward selection analysis considered all variables in explaining 39.3% of the total variation, the same as that computed by RDA. Of the eight variables considered, three variables appeared to contribute to the overall variation. Table 2 shows the significant explanatory variables governing the abundance of the toxic cyanobacteria species.

**Table 2.** Explanatory variables for the abundance of potentially toxic cyanobacterial species.

Environmental Variable	Contribution %	P-adj
Temperature	45.2	0.008
DIN	32.1	0.008
Zooplankton abundance	22.0	0.028

### 3.5. Similarity among Sampling Sites

Hierarchical cluster analysis among sampling stations (Figure 9) suggested that stations YA and YB were the most similar (Bray Curtis Index (BCI) = 64.64), followed by YC and YD (BCI = 47.16). Integrating the stations into clusters, two groups were composed in which the similarity was very low (BCI = 27.17), indicating that stations near the weirs and those far from the weirs differed in terms of potentially toxic cyanobacterial abundance.



**Figure 9.** Cluster analysis of the sampling sites. Two clusters were formed: stations further away from the weirs (YA, YB) and stations near the weirs (YC, YD).

The *t*-test results (Table 3) also showed that the abundances of *Microcystis* sp., a dominant genus in the river, and *Phormidium* sp. were significantly different between the free-flowing (stations YA and YB) and impounded (stations YC and YD) sites. Environmental variables, such as DIN, water temperature, turbidity and zooplankton abundance, were also significantly different. DIN and temperature were higher, whereas turbidity and zooplankton were lower, at the free-flowing than the impounded sites.

**Table 3.** Paired *t*-test results for the environmental variables and abundances (cell·mL<sup>-1</sup>) of potentially toxic cyanobacteria collected from free-flowing (stations YA and YB; control) and impounded (stations YC and YD; treatment) sites. The data were transformed to  $\log(x + 1)$  values prior to the statistical analysis.

Variables	Control (Mean ± SD) *	Treatment (Mean ± SD) *	<i>p</i> -Values (One-Tailed)
<u>Environmental</u>			
Temperature	17.97 ± 8.42	16.61 ± 8.73	0.00026
DIN	409.27 ± 223.73	284.0 ± 149.56	9.0386 × 10 <sup>-5</sup>
Turbidity	5.80 ± 2.36	7.52 ± 4.23	0.01451
Zooplankton abundance	264 ± 570	298 ± 428	0.01098
<u>Toxic cyanobacteria</u>			
<i>Microcystis</i> sp.	164 ± 477	3331 ± 9234	0.00752
<i>Phormidium</i> sp.	2300 ± 3867	776 ± 1000	0.00324

Note: \* Means and standard deviations prior to transformation to  $\log(x + 1)$  values.

## 4. Discussion

### 4.1. Temporal and Spatial Dynamics of Cyanobacteria

Temperature, light and nutrient concentration are the major determinants of cyanobacterial temporal variation [35]. Cyanobacteria generally thrive from midsummer to early autumn due to favourably high temperatures and high light intensities [36]. Blooms occur during the period from July to September in tropical settings [37], and summer months (June–August) are the peak season for blooms in temperate regions [38]. This study confirmed that most potentially toxic cyanobacteria occur during the summer months, extending until the onset of autumn. On a spatial scale, the presence of cyanobacterial species varied. *Microcystis* spp., the most common cyanobacterial species, appear mainly in the upper, freshwater zones of estuaries [39,40]. Their photosynthetic efficiency diminishes while moving down the estuary [41]; therefore, the abundance of the toxic species decreases downstream [21]. However, in this study, the abundance of *Microcystis* sp. was more evident at station YD. Because YD is near Juksan weir, the weir may contribute to *Microcystis* abundance by manipulating hydrological factors. In terms of the water column, toxic *Microcystis* cells are abundant in surface water [42], which may be attributed to their efficient buoyancy regulation relative to other algal species [43].

Potentially toxic *Dolichospermum* cells have been documented since 1993 in the Swan-Canning River and numerous wetlands in Perth, Australia [44]. This species is known to grow in freshwater to brackish regions [45], suggesting its wide range of adaptability to the different salinity gradients of estuaries. In this study, the abundance of *Dolichospermum* sp. was most prominent at station YD, close to the Juksan weir. According to Sin and colleagues [46], *Dolichospermum* sp. dominated in the upper portions of the Youngsan River but was also detected in the seawater zone.

*Aphanocapsa* sp. is observed in turbid, low water levels [47,48], such as littoral zones of saline lakes [49]. This species thrived well at station YC. The *Oscillatoria* genus is documented to settle in freshwater areas [50]. Not much difference in abundance was observed among the stations in this study. *Phormidium* sp. has a competitive advantage in that the species can thrive in the oligotrophic portions or riverine zones [15]. This species was more abundant at stations without weirs.

According to the Korean National Institute of Environmental Research [51], the weirs in the Youngsan River were predicted to increase the residence time of water 1.7–38.8 times. Romo et al. [52] reported that a longer water residence time contributes to cyanobacterial bloom outbreaks. In this context, cyanobacterial blooms may be affected by the presence of weirs in the Youngsan River. Toxic species in this study were mostly observed during the summer season, suggesting that there is probable thermal stratification of the water system, which further promotes propagation of cyanobacterial abundance. The cluster analysis results support the hypothesis that the presence of weirs may affect cyanobacterial abundance, because stations close to the weirs were similar, and those distant from the weirs were grouped together. The *t*-test also showed that the abundance of *Microcystis* sp. was significantly higher at the impounded sites ( $p < 0.05$ ), supporting the hypothesis. On the other hand, the proliferation of *Phormidium* sp. at the upriver stations rather than the latter stations contradicts the hypothesis about the impact of the presence of weirs on this species, suggesting that the effects of weir construction on spatial variations may be species dependent.

### 4.2. Effect of Environmental Variables on Cyanobacteria and Potentially Toxic Species

The spatial and temporal variations of algal species are primarily due to the physical and chemical characteristics governing a particular system [35,46]. In this study, temperature, inorganic nitrogen, and zooplankton abundance were associated with variation in the cyanobacterial species based on the forward selection method. Temperature accounted for 45.2% of the total variability in potentially toxic cyanobacterial species in the upper riverine region of the Youngsan River estuary. The Kruskal-Wallis test confirmed a significant difference in cyanobacterial abundance among the different seasons ( $p < 0.05$ ; Table S2). A positive relationship between blue-green algae and water temperature has been widely documented. The optimal growth rate of cyanobacteria usually occurs in warm conditions

around 20–30 °C [53,54]. *Microcystis* blooms in the Youngsan River estuary developed in the range of 24–27 °C, conforming to the established findings. Unlike most cyanobacteria, *Phormidium* sp. thrived during the colder months. *Phormidium* sp. is known to grow well at temperatures ranging from 4 to 20 °C [55]. Temperature is a crucial element to emphasise because of the ongoing issue of global climate change. The Korea Ministry of Environment [56] reported the proliferation of harmful cyanobacterial cells in mid-May 2015, earlier than their usual appearance. One reason for this was the slight increase in temperature over the past years. Continuous temperature elevation over the years may promote the occurrence of cyanobacteria, especially toxic strains. The temperature effect is correlated with toxin formation. In mixed cultures, the abundance of toxic strains was often higher than non-toxic strains when exposed to higher temperatures [57,58]. Increases in temperature concurrent with increases in phosphorus concentration intensify the occurrence of toxic *Microcystis* populations [59].

For nutrient concentrations, the RDA biplot illustrated the negative relationship of DIN to most species. DIN was also significantly higher at the free-flowing than at the impounded sites, whereas *Microcystis* sp. abundance was lower. It is inferred that nitrogen may contribute to increases in cyanobacterial species. Cyanobacterial occurrence can be influenced by the concentrations of nitrogen or phosphorus, or both [60]. Although there was a significant negative correlation, the amount of DIN was still high for a riverine system (200–800 µM); therefore, nitrogen may not be a limiting nutrient. The DIP concentration was also too high to be considered a limiting nutrient.

Zooplankton abundance and most of the toxic cyanobacterial species in this study were linked in a positive relationship. A similar pattern was reported by Jia et al. [61]. Zooplankton and *Microcystis* sp. abundances were also higher at the impounded sites near the weirs than at the free-flowing sites, suggesting that when a high abundance of cyanobacteria was recorded, enough food was supplied for zooplankton communities. Another possible explanation is the presence of other phytoplankton species as food sources.

Because the environmental variables explained only 39% of the variability of cyanophytes in the RDA analysis, other possible regulatory factors should be explored, such as hydrodynamics [62], and other biological communities (e.g., bacteria) [63].

## 5. Conclusions

Seven potentially toxic cyanobacteria were identified, including *Microcystis* sp., *M. aeruginosa*, *M. flos-aquae*, *Dolichospermum* sp., *Aphanocapsa* sp., *Oscillatoria* sp. and *Phormidium* sp. A high incidence of *Microcystis* spp. was observed in the early summer, whereas *Phormidium* sp. thrived during the colder months. Although variables such as zooplankton and DIN contributed to the overall variations, their effects on cyanobacterial abundance remain unclear. Therefore, temperature is the major determinant of potentially toxic cyanobacteria variation (especially temporal). Sampling stations near the weirs had higher similarity with each other and higher abundances of potentially toxic cyanobacteria than did the sites with no weirs, suggesting that the presence of weirs might have an effect on potentially toxic cyanobacterial species, especially *Microcystis* spp. Further studies incorporating a time series before and after weir construction should be conducted to strengthen our understanding of the effects of weirs on cyanobacteria.

**Supplementary Materials:** The following are available online at [www.mdpi.com/2073-4441/9/11/819/s1](http://www.mdpi.com/2073-4441/9/11/819/s1), Table S1: Cell abundance (mean ± SD, cell·mL<sup>-1</sup>) of the cyanobacteria species observed during sampling (n = 12). Potentially toxic species are marked by (\*), Table S2: Kruskal-Wallis test of the variation of potentially toxic cyanobacterial abundance among the seasons. Monthly data were grouped into four seasons.

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