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Effects of Seasonal and Diel Variations in Thermal Stratification on Phytoplankton in a Regulated River

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Abstract: Thermal stratification is an important driver shaping phytoplankton community and their habitat condition in freshwater ecosystems. However, studies on river stratification have been restricted to rivers below dams or reservoirs affected by their water release and lacked examination of diel stratification and its impact on phytoplankton, in particular. Therefore, this study aimed to determine the degree of thermal stratification, its environmental drivers, and the response of water quality and phytoplankton community against stratification in the mid-lower reach of the Nakdong River, whose morphology has been highly modified, including the construction of eight weirs. We implemented vertical temperature profiling at three study sites, both seasonally and diurnally. From this data, we calculated three stratification indices: relative water column stability (RWCS), Schmidt stability (S), and maximum temperature gradient (Max). These indices showed that most sites experienced diel stratification during summer (mean = RWCS 74.3, S 41.5 J m^{-2}, Max 0.9 °C m^{-1}). Principal component analysis showed that stratification significantly led to seasonal and diel variations in the water environment. Solar radiation and air temperature were positive controllers, while a negative controller (in this case, the river flow rate) existed only for diel variation in the stratification. The seasonal shifts in phytoplankton community structure were either insensitive or showed a limited response to the stratification indices. In summer, *Microcystis* cell abundance and accumulation into the surface water was positively affected by the diel variations in the stratification indices and thermocline instead of with other temperature and nutrient variables. Overall, the results suggest that the river has summer stratification, which is involved in amplifying cyanobacterial bloom intensity. Without a suppressing factor, summer stratification is expected to be recurrent in the river, and thus mitigating the developed stratification is needed by promptly regulating the river flow.

Keywords: river stratification; diel stratification; thermal stratification index; vertical distribution; cyanobacterial bloom; phytoplankton community

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1. Introduction

Stratification generally degrades aquatic ecosystems, causing harmful cyanobacterial blooms on surfaces, and oxygen depletion and nutrient elution in bottom water are the most severe stratification outcomes [1,2]. To manage freshwater ecosystems, it is urgent to evaluate thermal stratification in possible outbreak areas. Previously, rivers were not seriously considered in stratification research because the water flow generated sufficient turbulence. However, river alterations such as dredging and flow regulation by artificial structures, including dams and weirs, have gradually increased the number of cases where stratification was observed across decades [3]. The thermal stratification of rivers has been reported in several weir pools and rivers below dams and reservoirs [4–6]. River stratifications possess different characteristics (e.g., thermal structure, intensity, and period) than the typical lake-based stratification, which forms multiple thermal layers, with temperature gradients (thermoclines) in between these layers.

These features of stratification and their diel variations make them a major driver shaping water quality and phytoplankton communities in freshwater ecosystems, especially by controlling the mixing depth, light availability, and mixing time interval, which hinders cell accumulation in the surface layer during the daytime [7,8]. Whether or not the river water column is mixed at least once on a diurnal basis has great significance for the distribution of the slowly floating cyanobacterial genera *Anabaena* [8]. If the population was uniformly dispersed through the water column at sunrise, then during daylight hours, the population would not accumulate significantly in the near-surface euphotic zone where photosynthesis occurs. However, several studies have reported that *Microcystis* colonies remain largely in the epilimnion during summer, where they take up nutrients [9,10]. Cyanobacteria, including *Microcystis*, have enhanced growth rates under stratification [11] and often form massive blooms.

In Republic of Korea, the Nakdong River was drastically altered by the Four Major Rivers (FMR) Project in 2011 [12]. After the project, severe water quality deterioration and excessive cyanobacterial proliferation previously restricted to the downstream areas of the river are now frequently reported in midstream areas [13]. These effects are often considered a consequence of the formation of stratification. However, few studies have dealt with stratification or vertical profiles in rivers, with the exception of Kim et al. [14]. Because the Nakdong River experiences severe blooms of diatoms in winter and cyanobacteria in summer [15], investigating the river will illuminate the underlying process of river stratification involved in algal bloom dynamics.

Therefore, the purpose of this study was to (i) diagnose the stratification degree and identify the stratification characteristics in the Nakdong River, (ii) examine the relationships between the stratification degree with hydrometeorological variations and vertical nutrient patterns, and (iii) identify the phytoplankton changes in community composition and vertical cell distribution associated with stratification variability. We hypothesized that the river stratification would have different relationships with environmental variables and effects on phytoplankton at seasonal and diel scales, and then analysed them separately.

2. Materials and Methods

2.1. Study Area

The Nakdong River, the second largest river in the nation, has a length of 511 km and a catchment area of 23,690 km^2 (Figure 1). The river has a typical temperate monsoon climate, with a mean annual precipitation of approximately 1500 mm.

The Nakdong River is famous for its naturally sluggish riverbed and minimal slope (>1/10,000) (Figure 1). The FMR project in 2011 led to the river having a more stagnant flow regime than before. A total of 334 km of the total river length (525 km) was altered by eight serial weirs and 4.4 billion m^3 of dredging. The average river depth increased up to 6–12 m. Mid-downstream of the river was investigated, and the three sampling sites were located 20, 69, and 94 km upstream from the estuarine barrage. The salinity of the downstream sampling site was below <0.2 psµ, indicating that there was no saltwater intrusion into the sampling site.

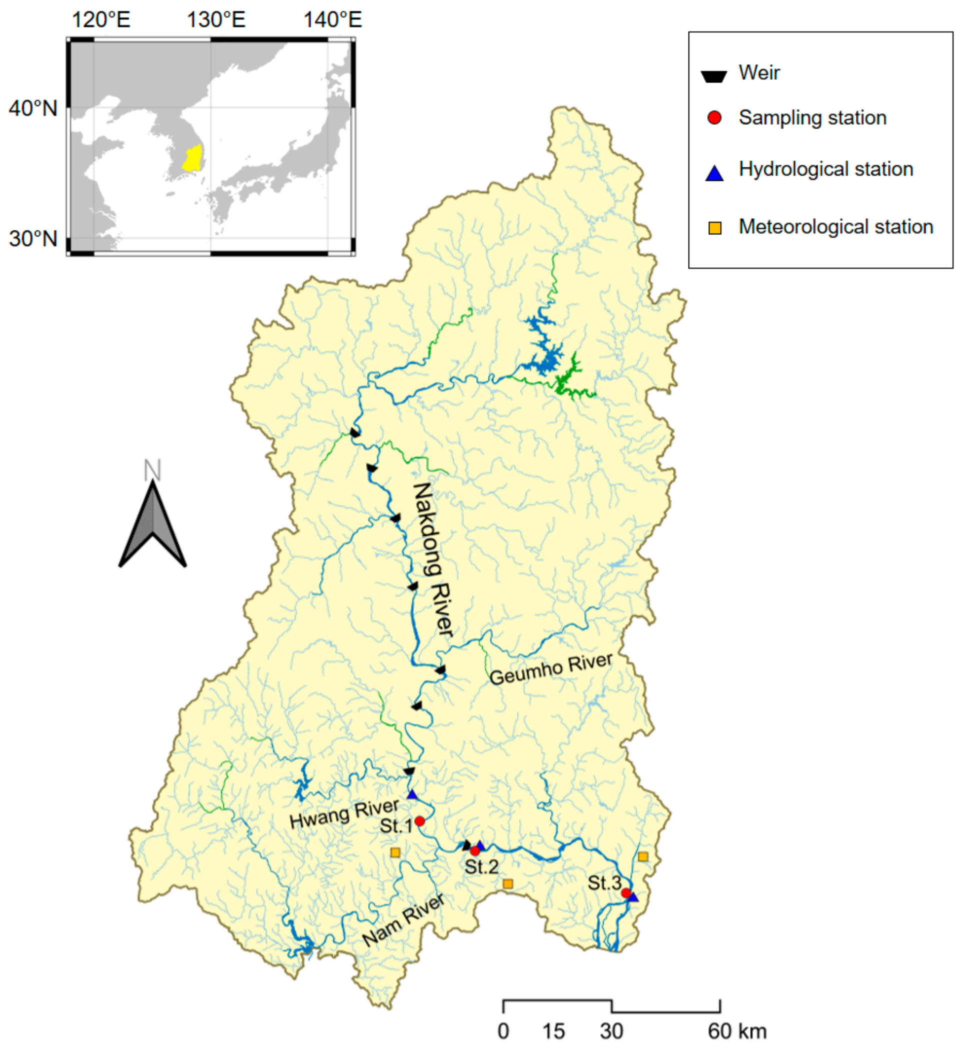

Figure 1. Map of the location of the sampling stations, hydrological stations, and meteorological stations in the Nakdong River basin.

2.2. Field Sampling and Data Collection

From 2017 to 2018, we conducted five seasonal surveys in offshore of the river using a boat at the up-, mid-, and downstream sampling sites. At noon, we measured the water temperature at one-meter intervals using a portable water quality monitoring device (M-4000, Technology & Environment Corp., Seoul, Republic of Korea). Samples for chemical and phytoplankton analyses were taken at three vertical water layers (depths of 0.5 m and 3 m from the surface water and 0.5 m above the river bottom) by using a Van Dorn sampler and samples for phytoplankton analyses were fixed with Lugol's solution. In August, diel surveys were additionally conducted at two stratified sites for 24 h at three-hour intervals. Filtered water for the concentrations of nitrate (NO_3^--N), orthophosphate (PO_4^--P), and silica (SiO_2) was analysed via spectrophotometry with glass microfiber filters (Whatman GF/C, 0.45 μm). Chlorophyll a concentration was determined after the quantitative concentration, following a previously published extraction method (Wetzel and Likens, 2000). The concentrations of nutrient and chlorophyll a were determined using a UV-VIS spectrophotometer (UV-1601, Shimadzu Corp., Tokyo, Japan), in accordance with standard methods for examining water and wastewater [16]. Phytoplankton samples from three vertical water layers were identified by light microscopic observations to the level of species and sometimes to the level of genus [17–19]. We examined the cell density of dominant and sub-dominant species for each of the three major phytoplankton phyla: chlorophytes, bacillariophytes, and cyanobacteria. Quantitative assessment of the phytoplankton was performed by counting independent cells under ×200 and ×400 magnification using a Sedgewick–Rafter (S-R) chamber with a light microscope (Axioskop 40, Carl Zeiss Inc., Göttingen, Germany). Other environmental data that corresponded to our survey date and time were collected. Meteorological (air temperature, irradiance, and wind speed) and hydrological (flow rate and water elevation) data were collected from the local stations of the Korean Meteorological Administration and the Nakdong River Flood Control Office (Figure 1).

2.3. Thermal Stratification Indices

To identify the existence and intensity of thermal stratification in the Nakdong River, three indices (i) RWCS, relative water column stability, (ii) S, Schmidt stability, and (iii) Max, maximum temperature gradient were calculated (Table 1). They do not include hydrological nor meteorological terms in their calculation then could be analysed with those environmental parameters. A number of studies have evaluated the overall thermal structure to study its response to external forces, such as air temperature, wind, and rainfall. For this purpose, the Schmidt index [20–22] and RWCS [23,24] have been widely adopted. At present, the gradient criterion is mostly used to identify thermal layers, as it distinguishes a region with a temperature gradient greater than a specific threshold as the thermocline, and the remaining two layers are then determined. This method is concise and practical, but the threshold is given empirically and varies from 0.2 $^{\circ}C \cdot m^{-1}$ [25], 1.0 $^{\circ}C \cdot m^{-1}$ [26,27], and 2.0 $^{\circ}C \cdot m^{-1}$ [28] with the environment. Most research on phytoplankton response against stratification in freshwater ecosystems has used these indices [23,24,29–33]. We compared our findings with other stratification cases based on these indices.

First, the relative water column stability (RWCS) is the ratio of the difference in density between the surface (ρ_s) and the bottom (ρ_b) layers to the density difference between 4 °C (ρ_4) and 5 °C (ρ_5) water, which provides an overall assessment of the mixing status along the water column (Equation (1)). We calculated the water densities ($kg \cdot m^3$) using a multidimensional equation of water temperature [34].

Second, the Schmidt stability index (S) indicates the mixing energy demand per unit area for complete mixing (Equation (2)). We used the rLakeAnalyzer v1.11.4.1 package to calculate the index. This package needs vectors for water temperature, cross-sectional areas, and depths of measurement. Because of the lack of river areas across the entire depth, we assumed water columns of 1 m^2 area. g is the gravitational acceleration, A_s is the surface area of the waterbody, A_z is the cross-sectional area at depth z, z_b is the maximum depth, and z_v is the depth to the center of volume.

Finally, we calculated the maximum temperature gradient (Max) using temperature difference (ΔT) between adjacent depths (Δd) in the water column to assess the extent and location of water layer separation, which was regarded as the thermocline in this study. To assess the formation of thermal stratification, the values of each indicator were compared with the previously suggested criteria [20,23,26,35–38].

Table 1. Three stratification indices with abbreviations, equations, and low and high threshold values from works of literature.

Index	Abbrev.	Equation	Low Threshold	High Threshold
Relative Water Column Stability [23]	RWCS	(1) $\text{RWCS} = \frac{\rho_b - \rho_s}{\rho_4 - \rho_5}$	30 [35]	50 [23]
Schmidt stability [20]	S	(2) $\text{S} = \frac{g}{A_s} \int_0^{z_b} (z - z_v)\, \rho_z\, A_z\, \partial_z$	30 J m^{-2} [36]	100 J m^{-2} [37]
Maximum temperature gradient [26]	Max	Maximum slope within the water column	0.25 °C m^{-1} [38]	1 °C m^{-1} [26]

2.4. Data Analysis

Two raw-data-based multivariate analyses were used to assess the relationships (i) among environmental variables and (ii) between environmental variables and phytoplankton variables. Considering that the relationships would vary depending on the time scale of the analyses, the seasonal and diel datasets were analysed separately. The environmental datasets consisted of 13 variables: the stratification indices, hydrological and meteorological factors, and water quality parameters labelled with three sampling depths (0.5 m (0), 3 m (3), and bottom (B)). Each environmental variable, except for the water quality parameters, was scaled from 0 to 1 to eliminate differences in their units of measure. Three variables of the same water quality parameters with different depth labels were scaled together unless their vertical differences were removed. For the phytoplankton, we assumed that three phyla (Bacillariophyta, Cyanophyta, and Chlorophyta) at three different depths constituted an assemblage at a sampling time. Therefore, phytoplankton datasets consisted of nine variables from the three major phyla with three depth labels, which were the summed cell densities of their dominant and sub-dominant species, and they were log (x + 1) transformed.

To examine the correlation structure among environmental variables, including stratification indices, a principal component analysis (PCA) was applied to describe the correlation structure between environmental variables. For the respective PCA, we identified two environmental gradients (principal components), along which the variation in the data was maximal. The PCA yielded coordinates for the respective variables against the first two PCs, with eigenvalues greater than 1. An eigenvalue > 1 indicates that PCs account for more variance than one of the original variables and are considered significant [39]. The percentages of the variance explained by the two PCs are presented. The PCA biplots were produced using the coordinates, and the angles among the variables' vectors reflect their correlation. Bartlett's test of sphericity was performed to examine the suitability of the data for the PCA. All the environmental datasets showed Bartlett's significance level lower than 0.001, indicating that there were significant relationships among variables, and PCA was useful.

A redundancy analysis (RDA) was used to delineate the effects of significant environmental drivers of thermal stratification on variations in phytoplankton community composition. Before the RDA, detrended correspondence analyses (DCA) were performed to determine whether the variables in the phytoplankton datasets followed a unimodal or linear response model. All DCA resulted in the first ordination axes (scaled in units of standard deviation, SD) with a length between 3 SD and 4 SD, which indicated an intermediate response.

Both the PCA and RDA were followed by Monte Carlo permutation tests to test the significance level of the first two axes and determine the parameters that significantly contributed to the axes [40].

The Kruskal–Wallis ANOVA and Spearman's rank-order correlation were used to study the vertical differences and correlation structures between variables. All statistical analyses and visualizations were performed using the FactoMineR v2.4, vegan v2.5.7, stats v4.0.3, and ggplot2 v3.3.3 packages within R v4.0.3 (R Core Team, 2021, Vienna, Austria) and RStudio (Version 1.3.959; RStudio Inc., Boston, MA, USA).

Environmental variables in the environmental datasets with the same time scale were selected based on the PCA results [41]. Only those variables showing a significant correlation ($p < 0.05$) with PC1 and PC2 were considered. All combined variables in the PCA were eliminated from the RDA because they would promote information redundancy and analysis distortion [42,43]. The RDA is much more robust when only a few environmental variables are required to identify species distribution [44]. Consequently, five and six environmental variables were used in the present RDA at the seasonal and diel scales, respectively.

3. Results

3.1. Stratification Pattern

We found that the three stratification indices had similar seasonal variations, with the highest values (i.e., strong stratification) in August, a gradual increase in May, and a sharp decrease in September (Figure 2). Spatial variations in RWCS and Max were similar, and thus their spatio-temporal variations were highly correlated with each other (Spearman's $r^2 = 0.872$, $p < 0.01$, $n = 15$). However, S exhibited a different pattern: it showed the highest values midstream.

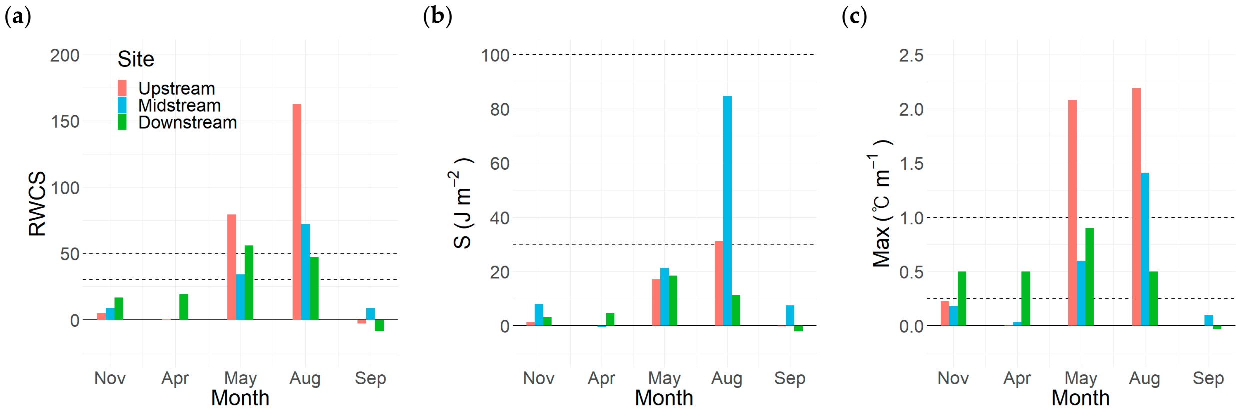

Figure 2. Seasonal variation in three stratification indices of three sites during the study period (**a**) RWCS, relative water column stability, (**b**) S, Schmidt's stability, and (**c**) Max, maximum temperature gradient, dashed lines: high and low thresholds for each index).

We identified thermal stratification events exceeding the threshold values as follows: the higher thresholds of RWCS and Max (RWCS = 50, Max = 1 °C m) coincidently suggested that the upstream stratified in May and August, but the midstream stratified in August only. The lower threshold of S (S = 30 J m^{-2}) suggests that the upstream and midstream regions were stratified in August. Strong stratification upstream and midstream in August was further investigated diurnally to determine whether it would decrease below the thresholds.

For the higher criteria of RWCS and Max (RWCS = 50, Max = 1 °C m^{-1}), the upstream remained stratified for most of the day and reached its peak at 12:00 (RWCS = 163, Max = 2.19 °C m^{-1}), but the midstream experienced destratification during the night (Figure 3). For the lower criterion (S = 30 J m^{-2}), the midstream remained stratified and reached its peak at 15:00 (S = 97.6 J m^{-2}), but the upstream remained below the criterion, except at 12:00. Both the stratified sites had steep thermoclines (Figure 4). In the midstream, as the surface of the water body warmed up, a steep thermocline developed at a depth of 3–4 m from 12:00 to 15:00. As the surface cooled and the heat was transferred downwards, the thermocline weakened and shifted deeper. A similar diel migration of the thermocline was identified upstream.

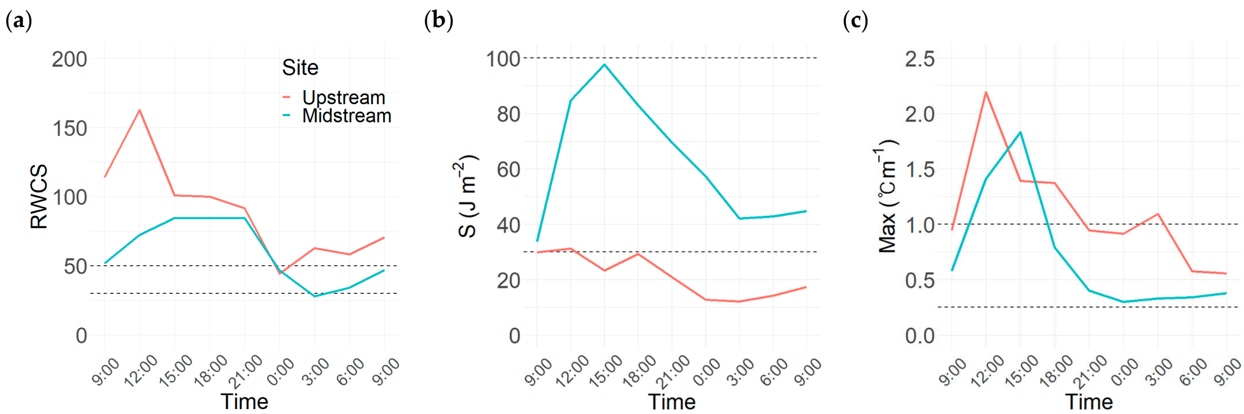

Figure 3. Diel variations in three stratification indices of two stratified sites in August ((**a**) RWCS, (**b**) S, and (**c**) Max, dashed lines: high and low thresholds for each index).

(a)

(b)

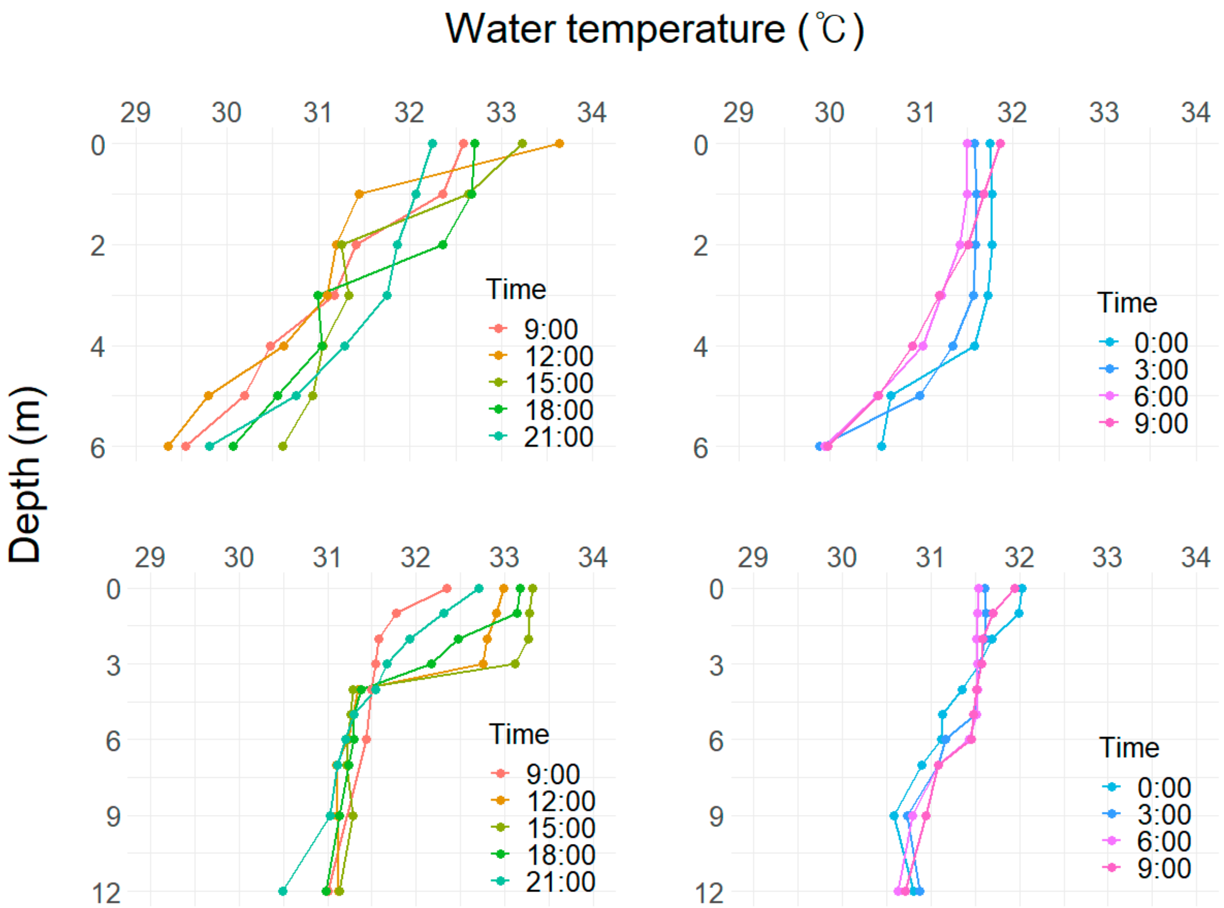

Figure 4. Diel migration of thermoclines at two stratified sites, (**a**) upstream and (**b**) midstream in August.

3.2. Environmental Conditions and Relationship with Stratification Indices

The hydrological variables indicate that the river was physically calm (Table S1). The concentrations of nutrients and chlorophyll a indicated that the river was eutrophic. None of the water quality variables were significantly different among the three water depths of 0 m, 3 m, and bottom (Kruskal Wallis test, $p \geq 0.05$, $n = 15$). However, the diel survey in August showed that water temperature and chlorophyll a varied significantly with depth (Kruskal Wallis test, $p < 0.01$, $n = 18$). Dunn post hoc tests revealed that the differences between WT0, WT3, and WTB were all significant, but the ChlB was only significantly different from Chl0 and Chl3 ($p < 0.05$, $n = 18$).

The two PCA applications (seasonal and diel) successfully revealed the relationships between all environmental variables, including the three stratification indices. The first two PCs explained variances of each PCA model by 62.9% and 67.1%, respectively (Figure 5). All stratification indices had significant contributions to both ordination models ($p < 0.05$), implying that stratification was one of the major drivers of water environment characteristics (Table S1). Some environmental variables, such as FR, WL, WV, and Chl3,B, significantly contributed only to diel PCA.

In the seasonal PCA, the observations of the water environment were separated more by season than by site (Figure 5a). The water environments of the three sampling sites were clearly separated mainly along PC2, whereas both PC1 and PC2 were involved in the separation of the five seasons. PC2 was associated with RWCS, Max, SR, and SI0,3,B, and PC1 was associated with S, AT, WT0,3,B, Chl0, PO0,3, and B at $p < 0.05$. Furthermore, strong relationships were found among the environmental variables. The RWCS and Max were positively correlated with SR. S was positively correlated with Ch0 and AT. Some water quality parameters, such as SI, PO, and WT, were correlated with the values measured at different depths. Finally, S, Max, SI3, PO3, and WT0 were selected for the RDA, considering the interrelated variables in the PCA.

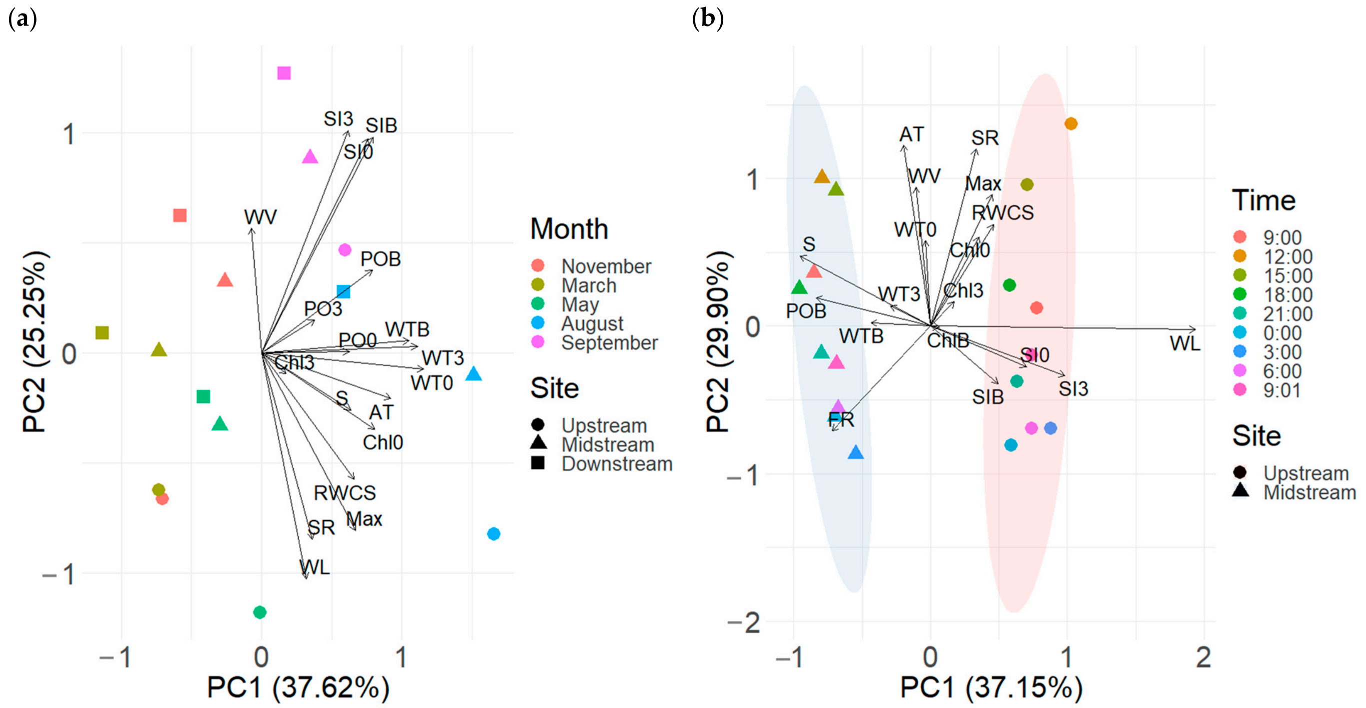

Figure 5. Principal component analysis ordinations of the relationships among the environmental variables at (**a**) seasonal and (**b**) diel scales (n = 15: seasonal, 18: diel). The environmental variables, marked with measured water depth (0, 3, and B), are as follows: AT, air temperature; Chl, chlorophyll a concentration; FR, flow rate; Max, maximum temperature gradient; NO, nitrate; PO, orthophosphate; RWCS, relative water column stability; S, Schmidt's stability; SI, silica; SR, solar radiation; WL, water level; WT, water temperature; WV, wind velocity. Variables displayed at $p < 0.05$.

In the diel PCA, the upstream and midstream were clearly separated along PC1, associated with S, WL, WT3,B, POB, SI0,3,B, and ChlB, and these variables were highly site-dependent (Figure 5b). Each site had a diel cycle driven by PC2, associated positively with Max, RWCS, SR, WV, AT, WT0, and Chl0,3, and negatively with FR. In particular, Chl0,3 was positively correlated with RWCS and negatively correlated with FR. S, AT, Max, WL, FR, and SI3 were selected for the RDA.

3.3. Phytoplankton Structure and Relationship with Stratification Indices

The phytoplankton community exhibited a seasonal shift at the phylum level (Figure 6). Massive cyanobacterial blooms were observed in November, August, and September, with high cell densities concentrated in surface waters. The blooming cyanobacterial genera varied by season, with *Microcystis* for August, the strongest stratification period, and *Aphanizomenon* for November and September, when the water bodies mixed well, and their proliferation was independent of stratification (Table S2). In May, Bacillariophytes dominated, and in April and September, co-domination of Bacillariophytes and Chlorophytes was detected. Unlike cyanobacteria, the cell densities of Bacillariophytes and Chlorophytes exhibited less vertical variation. For all the phytoplankton phyla, cell densities were not significantly different among the three water depths (0 m, 3 m, and bottom) (Kruskal–Wallis test, $p \geq 0.05$, $n = 15$). However, the diel survey in August showed that all the phyla showed significant differences in cell density among water depths (Kruskal–Wallis test, $p < 0.05$, $n = 18$) (Table S3). Dunn post hoc tests revealed the water depths with significantly higher cell densities for each phylum: Cya0, Cya3, BacB, and ChlB ($p < 0.05$, $n = 18$).

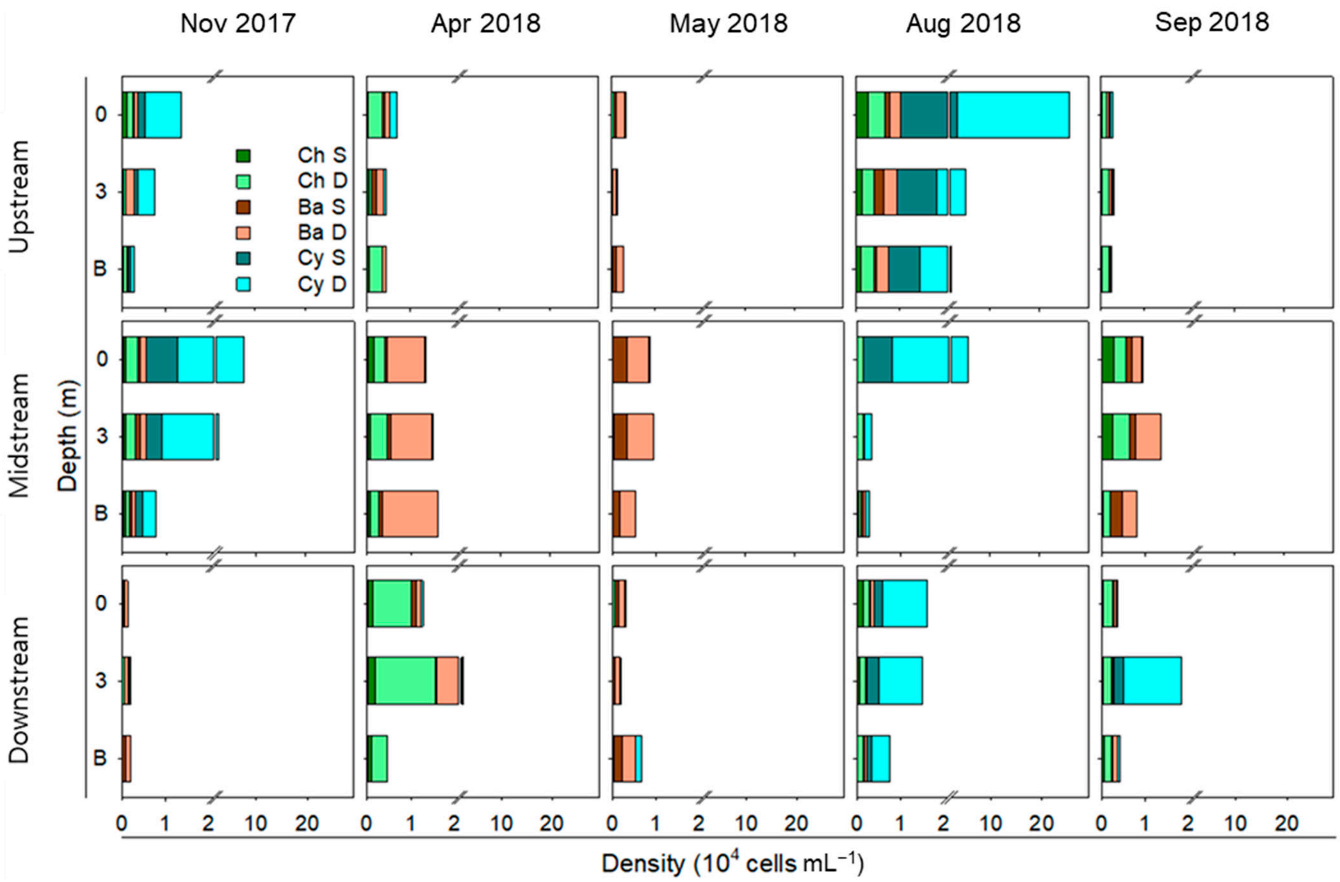

Figure 6. Vertical distributions of cell densities for dominant and subdominant species from Chlorophyta, Bacillariophyta, and Cyanophyta (i.e., Ch S: subdominant chlorophyte species).

During summer, *M. wesenbergii* dominated all the study sites (Figure 6 and Table S3). However, their surface (0.5 m depth) cell densities and relative abundance at the surface layer were much higher in the stratified upstream (22.55 × 10^4 cells mL^{-1}, 83.6%) and midstream (5.44 × 10^4 cells mL^{-1}, 95.2%), coinciding with the spatial variation of stratification. In the relatively mixed downstream, the species showed lower density (1.02 × 10^4 cells mL^{-1}) and surface accumulation (50.6%). An identical vertical distribution pattern was observed for the subdominant cyanobacterial species, *M. aeruginosa* and *Microcystis* sp. For comparison with the genus, in August, five species of Bacillariophytes and Chlorophytes were simultaneously detected at the three vertical water depths in both stratified and unstratified sites. They showed much less vertical variation in their cell densities in both stratified and unstratified sites, with low relative standard deviations (0.216 ± 0.082; n = 5). In addition, they were not concentrated in the surface water (1985 ± 539 cells mL^{-1}, 38.2 ± 2.8%; n = 5). We further explored the effect of diel stratification on the cyanobacterial distribution. When strong thermoclines (>1 °C m^{-1}) developed in the water columns (Figure 4), cyanobacterial densities at both stratified sites showed their maximum in the surface waters and minimum in the bottom waters (Figure 7). Upstream, a shallow thermocline floated from 1–2 m depth to the water surface, and then the cell density became concentrated in the upper water from 9:00 to 12:00. The high surface cell density decreased rapidly as the thermocline deepened. In the midstream, a shallow thermocline developed at 9:00, earlier than in the upstream, and the cell density showed its maximum value, followed by a decrease.

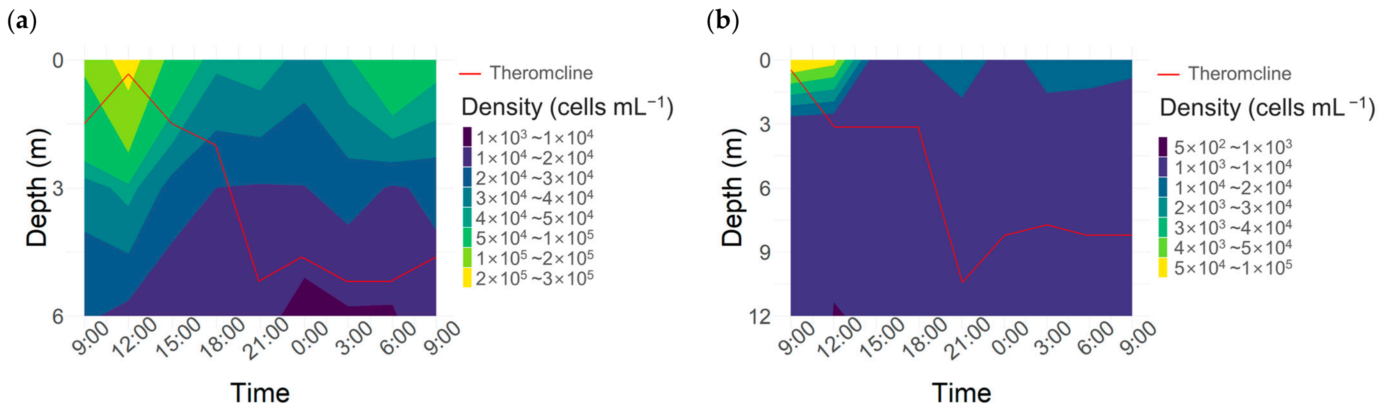

Figure 7. Diel variations of cyanobacterial cell density and thermocline depth at two stratified sites, (**a**) upstream and (**b**) midstream in August.

Species scores were largely separated along the first axis by the phytoplankton phylum rather than by the water depth in the seasonal RDA (Figure 8a). The separation of species scores by water depth was the largest in Cyanophyta, followed by Bacillariophyta and Chlorophyta. However, in the diel RDA, species scores overlapped among the three phyla and were separated by water depth to a similar extent. The community composition and vertical structure of phytoplankton were dependent on the values of S (F = 22.23, p = 0.001), WL (F = 10.28, p = 0.008), Max (F = 2.03, p = 0.011), RWCS (F = 1.67, p = 0.031), and TD (F = 1.59, p = 0.031) for the diel RDA, but there were no significant variables for the seasonal RDA. Species scores of Cyanophyta were closely ordinated to the site scores of stratification events (RWCS > 50) in both RDAs. In the diel RDA, cya 3 and cya B were positively correlated with RWCS and WL values, and cya 0 had a negative relationship with S.

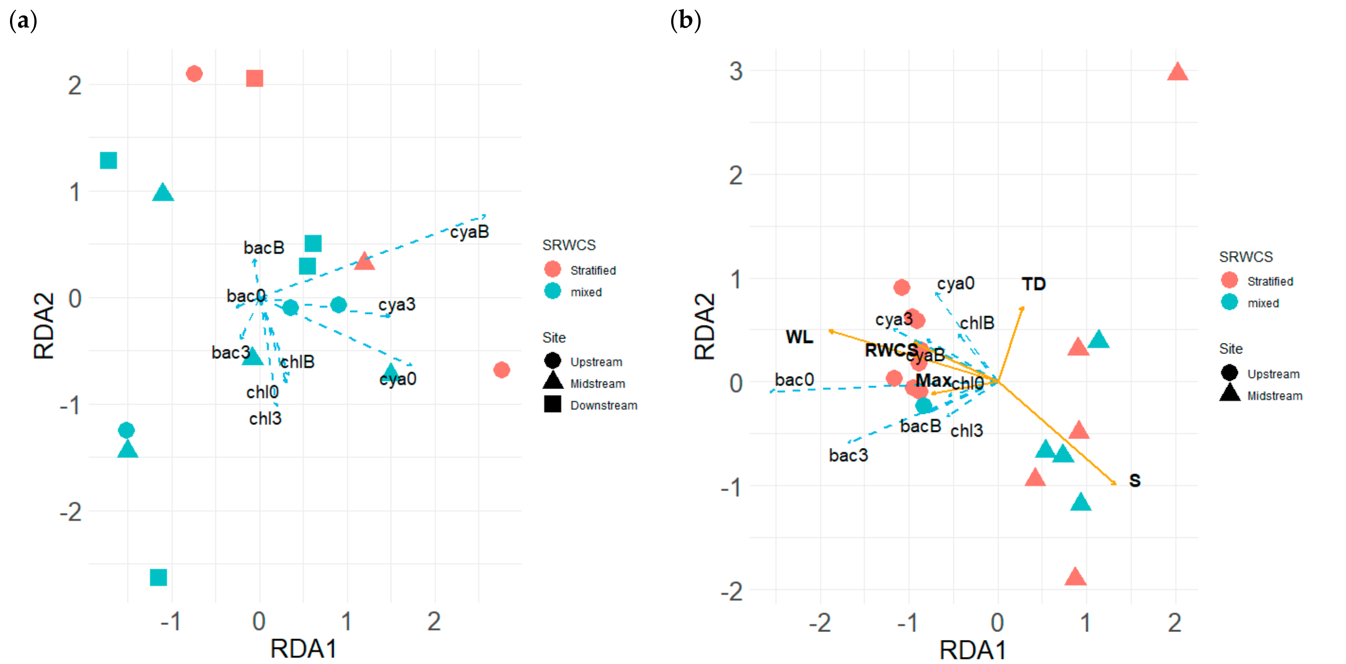

Figure 8. Triplot diagram for the redundancy analysis of the relationship between phytoplankton phyla (blue dashed line) and constraining environmental variables based on the PCA results (yellow solid line) at (**a**) seasonal and (**b**) diel scales (n = 15: seasonal, 18: diel). The phytoplankton phyla and environmental variables, marked with measured water depth (0, 3, and B), are as follows: chl, Chlorophyta cell density; bac, Bacillariophyta; cya, Cyanophyta; Max, maximum temperature gradient; RWCS, relative water column stability; S, Schmidt's stability; TD, thermocline depth; WL, water level.

4. Discussion

4.1. Stratification Assessment

Schmidt stability (S) showed a different spatial variation to the other two stratification indices because of the dependence of its equation on the water depth. This indicates that the choice of indices can influence stratification evaluation when multiple sites are included. Yang et al. [35] utilized S and relative water column stability (RWCS) for the long-term monitoring of a lake, and their interchange was possible. The maximum thermocline gradient (Max) directly represents the vertical partitioning of the water layers, which is important for the distribution and transfer of materials, and thus, may be the most precise term for assessing stratification independent of spatial heterogeneity. The RWCS does not require an entire temperature profile, and its simplicity allows many phytoplankton researchers to explore its role in phytoplankton ecology [23,24,29–33]. Our finding of similarity between Max and RWCS in seasonal and diel variations may provide a bridge between studies that use these indices.

Overestimation of the stratification in the midstream by S compared to the other indices indicates that the waterbody of the midstream required more turbulent energy to mix than expected for its entire stability and thermocline strength, especially in August. Moreover, hydrological factors and wind velocity exhibited greater spatial variability, indicating spatial heterogeneity in the susceptibility to thermal stratification formation. This makes the upstream site more favourable for stratification in terms of its lower flow rate, lower wind velocity, and higher water level.

4.2. Characteristics of Stratification in the Nakdong River

Thermal stratification has become a general phenology of regulated rivers and reservoirs in the global trend of river regulation [4,45,46]. However, the stratification impact can be expressed differently depending on where it occurs and ecological characteristics. The intensity of the summer stratification in the river was weak (mean: RWCS 74.3, S 41.5 J m^{-2}, Max 0.9 °C m^{-1}) compared to that of a temperate lake with a maximum depth of 21 m (mean S 92 J m^{-2} [47]) and many deep Arctic lakes at the beginning of the warming season (S = 100 J m^{-2} [22]). Because of its weak intensity, river stratification appears to form a single thermocline at shallow depths smaller than 60% of the river depth [48]. The thermocline exhibited diel migration and nighttime destratification, as seen in other studies on unstable stratification [49,50]. This diurnal thermocline is found in warm polymictic lake systems [51] and develops on top of persistent thermoclines in the case of multiple thermoclines [52]. The thermocline in the river could be differentiated from the lake stratification because it moved along the entire water column and reached the riverbed by diel migration.

4.3. Environmental Drivers of Stratification

The PCA ordinations revealed that thermal stratification is one of the most important drivers of water environments in the Nakdong River, largely accounting for seasonal and diel variations. Air temperature and hydraulic residence time are well-known major drivers of stratification [53]. In the Nakdong River, solar radiation and air temperature were the major drivers of stratification, increasing surface water temperature and resulting in vertical temperature differences. The strong association of the stratification indices with air and water temperatures and solar radiation coincides with previous findings of strong relationships between these variables [2,54]. The dependence of variations in stratification on these meteorological variables supports the current increasing trend of its occurrence and intensity in a changing climate [55]. It is problematic that there is currently no factor suppressing the seasonal variation of stratification at our study sites, although we tested commonly known factors inducing vertical mixing of water bodies, such as flow and wind velocity [56,57]. In summary, the intensification of summer stratification is inevitable in rivers. The strong relationships between flow rate, stratification indices, and chlorophyll a concentration from the diel-scale analysis suggest that the possibility of flow management from relevant research [5,58] could be adopted to mitigate the adverse impact of stratification on the river ecosystem by operating weirs. This is supported by the discharge-growth hypothesis [8], which depends on three components: the relationship between discharge and stratification, the relationship between the vertical distribution of phytoplankton and stratification, and the competitive advantage of a buoyant population under stratified conditions. Moreover, hydrological factors and wind velocity exhibited greater spatial variability, indicating spatial heterogeneity in the susceptibility to thermal stratification formation. This makes the upstream site more favorable for stratification in terms of its lower flow rate, lower wind velocity, and higher water level.

4.4. Stratification Effect on Phytoplankton Community

A phylum-level shift between seasons was the major variation in the phytoplankton community and was not associated with stratification indices. Moreover, stratification timing is important for plankton seasonality and peak abundance [21,59,60]. The impacts of thermal stratification on the community, which was obvious for buoyant cyanobacteria in the river, could be selective, depending on phytoplankton groups that possess different adaptations and functional traits [61]. Unlike cyanobacteria, the vertical cell distribution of Chlorophytes and Bacillariophytes remained uniform over the five seasons, even during the stratification period, providing further information on stratification. In the absence of sufficient turbulent mixing, most phytoplankton species do not have a buoyancy-regulation sink [62]. Under stratification, deep-water populations of these phyla are affected more by nutrient and light availability than by water stability itself [25,30]. Our stratification was too unstable and brief to have a vertical gradient of nutrients, and insufficient for these phyla to possess any vertical distribution. In addition, blooms of the cyanobacteria *Aphanizomenon* during fall–winter tolerance of water temperature in the range of 5–15 °C in the river [63] hindered the detection of a significant relationship between stratification and cyanobacterial density on a seasonal scale. Thus, the summer period and cyanobacteria are of high priority for determining the effects of river stratification on phytoplankton.

Two harmful cyanobacterial species, *M. wesenbergii* and *M. aeruginosa*, were enhanced by summer stratification in terms of their abundance and cell accumulation in the surface water. They are two of the most common bloom-forming *Microcystis* species in many countries, and in the Nakdong River, and they dominate successively from summer to autumn [64–67]. In particular, monocultures of large colonies of *Microcystis* can be explained by buoyancy control, which accommodates diel fluctuations in stratification and mixing in low-latitude lakes [68]. Diel fluctuations in their biomass, with high values of chlorophyll a in the surface water which did not persist as the thermocline weakened and deepened, were primarily associated with stratification indices and less associated with other variables, such as water temperature and nutrients (Figure 5b). Before the river was modified in 2011, Ha et al. [69] reported an accumulation of the same genus in the surface water during a calm night downstream in the river, which could explain the intensification of cyanobacterial proliferation in the river after the river modification. Thus, we suggest that stratification involves maintaining cyanobacteria in surface water once they occur and amplifying bloom intensity.

One comparable river stratification occurs in the regulated river, the Saar [4]. Stratification increased phytoplankton abundance and vertical differences only when the abundance was low. However, in this study, summer stratification was positively associated with phytoplankton abundance and vertical differences when cyanobacterial cell density was abundant. One abundant motile alga showed striking and regular vertical migrations in the lake, moving below the thermocline at night and returning to surface waters in the early morning. These migrations took cells across a 10 °C temperature gradient. The non-motile phytoplankton showed a constant vertical distribution. Two stratification events, followed by the deepening of the thermocline, occurred during the study period and led to changes in the vertical distribution of phytoplankton populations.

5. Conclusions

This study analysed the degree of thermal stratification, its environmental drivers, and the response of water quality and phytoplankton community to stratification in a river after intensive river channel modification, including the construction of eight weirs. Three indices for assessing the degree of stratification showed that most of the sites experienced diel stratification during summer. The PCA results showed that stratification led to significant seasonal and diel variations in the water environment. Unlike other phytoplankton phyla, buoyant cyanobacteria changed their cell density vertically in the water column. Blooms of two cyanobacterial genera were observed, and the stratification effect on *Microcystis* was further assessed as it bloomed during the stratification period. Higher abundance and surface cell accumulation of the genus were observed at the stratified sites, and the diel variations in its biomass (chlorophyll a) in the surface water were primarily associated with the stratification indices compared to other temperature and nutrient variables. The findings of this study suggest that stratification involves maintaining cyanobacteria in surface water once they occur and amplifying the bloom intensity. Concerning the seasonal and diel variations in stratification, solar radiation, and air temperature were the positive controllers, while a negative controller (river flow rate) existed only for diel variation in the stratification. Without a suppressing factor, summer stratification is expected to be recurrent in the river, and thus mitigation of the developed stratification is needed by promptly regulating river flow. Future research will extend this study by focusing on the stratification duration and destratification interval during the entire summer.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su152316330/s1, Table S1: Depth-averaged water characteristics and environmental factors of three sites during the study period (n = 3, mean ± S.E.). Table S2: Pearson correlation coefficient of determination (r^2) and p-values based on random permutations between the environmental variables and the PC coordinates at seasonal and diel scales (n = 15: seasonal, 18: diel). The bold values statistical significance ($p < 0.05$). Table S3. Vertical distributions of cell densities for dominant and subdominant species from Chlorophyta, Bacillariophyta and Cyanobacteria.

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