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Detection of chlorophyll fluorescence as a rapid alert of eutrophic water

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ABSTRACT

An in-situ detection of chlorophyll fluorescence was used in an innovative manner to detect eutrophic water, and the results were compared with the Carlson's Trophic State Index (CTSI) indicators. Eutrophication was due to climate warming and anthropogenic activities. The turbidity and chromaticity showed a strong linear relationship ($R^2 = 0.85$) of the Bi Lake in Taipei city. Both the swimming area and the bridge are popular with the general public and had the worst turbidity (35.80–44.00 NTU) and chromaticity (495.37–552.27 Pt). The CTSI had a stronger linear relationship with the phycocyanin (PC) concentration ($R^2 = 0.605$) than with any other three CTSI factors like chlorophyll-a concentration, total phosphorus (TP) concentration, and transparency. The TP pollution had a potential to cause an increase in PC concentration found in this study ($R^2 = 0.86$). The absorbances of the water samples represented that the environment is PC (cyanobacteria) dominant in winter. The PC concentration in Bi Lake ranged from 75.55 to 80.24 μg/L and was higher with lower water temperature. Measurement of in-situ chlorophyll fluorescence is similar to lab-scale spectrophotometer ($R^2 > 0.92$). The real-time detection of PC concentration could be the basis of a rapid alert system for biological threats to waters.

Key words: chlorophyll, chromaticity, cyanobacteria, eutrophication, fluorescence

HIGHLIGHTS

- Total phosphorus had a stronger linear relationship with the phycocyanin concentration.
- Phycocyanin concentration had higher correlation than a single CTSI indicator.
- The environment is cyanobacteria dominant in winter but changes to chlorophyll-a in summer.
- Measurement of in-situ chlorophyll fluorescence is verified by lab-scale spectrophotometer.

LIST OF ABBREVIATIONS

CA	chlorophyll-a
CTSI	Carlson's Trophic State Index
DO	dissolved oxygen
EPA	Environmental Protection Agency
PC	phycocyanin
TP	total phosphorus

1. INTRODUCTION

Due to rapid agricultural and industrial intensification, a great amount of wastewater and municipal sewage is discharged into water bodies, resulting in eutrophication of lakes, streams, rivers, and reservoirs. Eutrophication is a serious consequence of water pollution and severely threatens biodiversity and habitat quality in aquatic ecosystems. Nineteen of the 20 main reservoirs monitored by the Taiwan Environmental Protection Agency (EPA) have been eutrophic for a long period (Taiwan EPA 2020). An Organization for Economic Cooperation and Development (OECD) study concluded that 80% of water eutrophication was attributable to phosphorus. Indeed, increasing urbanization in a region can be inferred from increasing concentrations of total phosphorus (TP) and phosphate (Nash *et al.* 2009; Zhao *et al.* 2015).

The accurate monitoring of TP in water is an important link in the control of eutrophication (Du *et al.* 2018). TP monitoring can be divided into in-situ methods, in which water samples are analyzed to determine the relationship between the concentrations of TP and optically active substances (chlorophyll-a [CA] and total suspended solids [SS]), and ex-situ methods, in which the TP concentration is calculated from the inversion of optically active substances (Song *et al.* 2014).

It has been discovered that eutrophication is responsible for the massive spread of aquatic macrophytes in water (Selman *et al.* 2008). Monitoring of aquatic macrophytes in freshwater ecosystems provides essential evidence for the development of control measures to conserve both water quality and quantity (Dube *et al.* 2017). The phycocyanin (PC) concentration is an indicator of cyanobacterial blooms in a given water body (Varunan & Shanmugam 2017). An accumulating body of evidence indicates that the intensity of the response to the increasing PC concentration differs considerably across taxa and that these changes may cause a shift in the phytoplankton community (Xiao *et al.* 2018). Toxic cyanobacterial blooms are increasingly prevalent in freshwater systems (Thomson-Laing *et al.* 2020) and threaten the drinking water supply (Garnier *et al.* 2005). The World Health Organization (WHO) recommends a drinking water maximum of 1 μg/L for microcystins, a class of cyanotoxins (Chorus & Bartram 1999). Traditionally, non-native aquatic species like hyacinth (*Eichhornia crassipes*) and other land cover types have been monitored by field surveys, chemical spraying, or biological and physical removal (Dube *et al.* 2017). These techniques are time-consuming, and the analysis is largely laboratory-based; there remains a need for an easy-to-use, rapid, in-situ method to assess aquatic macrophytes and allow water managers to make decisions quickly (Thomson-Laing *et al.* 2020).

Chlorophyll fluorescence is a sensitive tool to detect the photosynthetic activity of aquatic macrophytes (Iriel *et al.* 2015). The light energy absorbed in plants can undergo three deactivation pathways: electron transfer (which leads to photosynthesis), energy dissipation as heat, or energy emission as fluorescence (Iriel *et al.* 2015). Therefore, detecting chlorophyll fluorescence emitted from aquatic macrophytes may be useful for evaluation of eutrophic water. Durrieu *et al.* (2006) and Védrine *et al.* (2003) used biosensors based on chlorophyll fluorescence inhibition for real-time detection and on-line monitoring of heavy metals and pesticides. Iriel *et al.* (2015) found that fluorescence properties were sensitive to the presence of arsenic in aquatic media. Chlorophyll fluorescence from *Spirodela oligorrhiza* plants was used to evaluate water quality (Romanowska-Duda *et al.* 2005). The level of chlorophyll fluorescence has been proposed as an indicator of chromium contamination in waters (Chen *et al.* 2018). Measurement of chlorophyll fluorescence has even been suggested to counter biological threats to water bodies (Kalaji *et al.* 2014). Limitations remain to the application of fluorescence probes due to various interfering factors, such as temperature and turbidity, that vary among environmental samples (Bertone *et al.* 2018; Choo *et al.* 2019).

Lakes in urban areas are especially sensitive to daily human activities. The Bi Lake is the main lake in Taipei and serves the general public, offering a children's playground, tennis court, and swimming area. Therefore, the Bi Lake is a suitable case study to investigate effects of climate warming and anthropogenic activities on eutrophic water. In this study of Bi Lake, chlorophyll fluorescence was used in an innovative manner to detect PC, and the results were compared with the Carlson's Trophic State Index (CTSI)-derived level of eutrophication in the lake. The in-situ detection of PC was expected to be a more effective method for classification of eutrophication than laboratory-based measurement of CTSI indicators. In addition, effects of environmental factors on chlorophyll fluorescence were also determined by the strength of the correlation, which has not been discussed in previous studies.

2. MATERIALS AND METHODS

2.1. Study area and water sampling

As shown in Figure 1, Bi Lake, with 13 ha water surface area and 1.33–1.43 m depth, is located to the northeast of Taipei (Taipei City Government 2019). It is an old lake that dates back to the era of Japanese occupation, at which time it was used for irrigation. The input to the lake is rain water, and no water flows out. A water gate is used to adjust the water level of the lake in case of extreme weather. The flow rate of Bi Lake is 15–25 m^3/s (Taipei City Government 2019), and hydraulic retention time of the water is 2.07 h as calculated in this study. To evaluate the effects of anthropogenic activities on water quality, three water samples were collected at each of five sites – the bridge (B), management room (M), swimming area (S), wooden plank walkway (P), and water gate (W)–every month for a period of 12 months (October 2019–September 2020). The wastewater generated from the swimming pool is discharged into local sewage systems without affecting the water quality of Bi Lake. Water quality indicators including transparency, dissolved oxygen (DO) concentration, and temperature

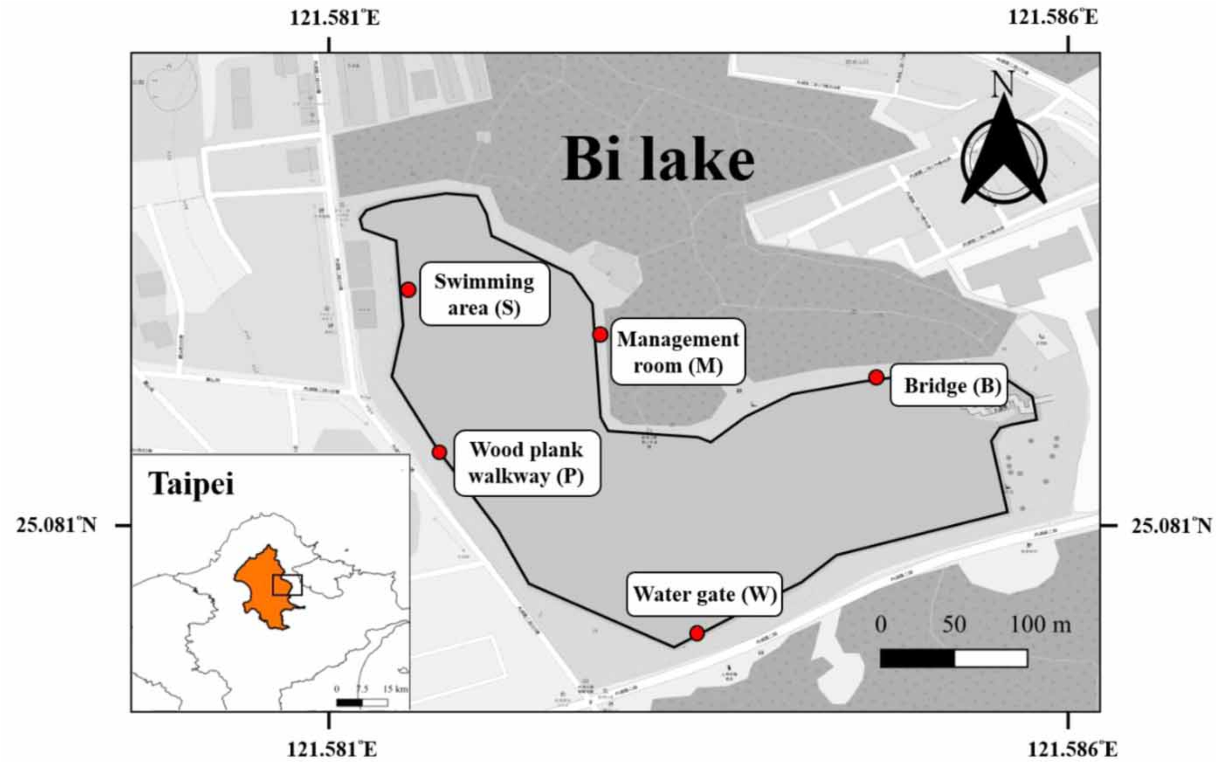

Figure 1 | Location of Bi lake in Taipei city.

were measured with a Pro 2030 YSI detector (Xylem, USA). In addition, 3-L water samples were extracted and placed in a wide-mouth plastic bottle to in-situ measure turbidity, chromaticity by the Smart 3 Colorimeter manufactured by Lamotte, Washington, D.C. (Figure 2(a)).

2.2. Analysis of CTSI and PC concentration

The CTSI requires the determination of CA concentration, TP concentration, and transparency. The transparency of the water was measured using a 20-cm-diameter Secchi disc. The maximum depth at which the disc could be seen when lowered into the water was marked and measured. The CA and TP concentrations of the collected water samples were analyzed as described below. In the laboratory, the TP concentration was analyzed by the method prescribed in NIEA W427.53B, which has been certified by the Taiwan EPA. The CA concentration was in-situ measured using an hVI fluorescent detection system (High View Innovation Co., Ltd, Taiwan) integrated into a handheld fluorometer platform (Figure 2(b)).

The above-mentioned hVI fluorescent detection system was in-situ measured to estimate the concentrations in cyanobacterial cultures. The two classes of photosynthetic pigments in cyanobacteria are PC with a large molecular weight, and the carotenoids and chlorophylls with a smaller molecular weight (Davaeifar *et al.* 2019). To calibrate the hVI system, a 0.1-mL water sample was placed in a glass cuvette (provided by the manufacturer), and the fluorescence readout was generated within 30 s. The whole process was performed in triplicate, and the average results are presented.

The CTSI was calculated using the following formulae,

$$\mathrm{TSI(CA)} = 9.81\ln \mathrm{CA} + 30.6 \tag{1}$$

$$\mathrm{TSI(SD)} = 60 - 14.41\ln \text{Secchi depth} \tag{2}$$

$$\mathrm{TSI(TP)} = 14.42\ln \mathrm{TP} + 4.15 \tag{3}$$

$$CTSI = \frac{TSI(CA) + TSI(SD) + TSI(TP)}{3} \tag{4}$$

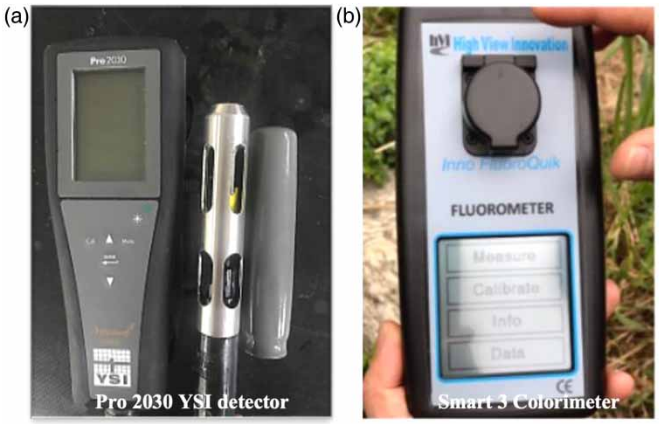

Figure 2 | The instruments used for in-situ measurement.

where TSI is the CTSI value, SD is the Secchi depth, and ln is natural logarithm. The CA and TP concentrations were measured in units of μg/L. The Secchi depth was measured in meters (m). As shown in Equation (4), the mean value of TSI(TP), TSI(CA), and TSI(SD) was taken as the final CTSI value. Based on their CTSI values, lakes are classified as oligotrophic (CTSI < 40), mesotrophic (40≤CTSI≤50), or eutrophic (50 < CTSI). The simple correlation is used to explore the relationship between the water quality factors, and the Pearson correlation coefficient is used to determine the strength of the correlation.

2.3. Calculation of CA and PC by spectrophotometer

To calibrate the results of in-situ measurements, the PC concentration (μg/L) was determined by spectrophotometry (UNICO S-2150, USA) at different wavelengths in the laboratory. Two-point calibration was established each time before measuring. The wavelength at 665 and 750 nm were applied to calculated CA concentration (μg/L); the wavelength at 615 and 652 nm were used to evaluated PC concentration (μg/L), respectively. Finally, the measured readings are brought into the equation formula as follows (Bennett & Bgorad 1973; Wasmund *et al.* 2006; Lan *et al.* 2011),

$$CA\left(\frac{\mu g}{L}\right) = \frac{(\lambda_{665} - \lambda_{750}) \times 15 \times 1000}{83.4 \times V} \quad (5)$$

$$PC\left(\frac{\mu g}{L}\right) = \frac{\lambda_{615} - 0.474 \times \lambda_{652}}{5.34} \quad (6)$$

where λ represents the absorb at different wavelength, and V is the volume of the water sample (L).

3. RESULTS AND DISCUSSION

3.1. Results of climate warming on water quality

In general, temperature plays an important role in the composition and succession of the aquatic macrophytes (Ye *et al.* 2011; O'Neil *et al.* 2012; Paerl & Paul 2012). Figure S1 shows the relationships between DO concentration and the water/outdoor room temperature. Water temperature was measured during in-situ sampling, and monthly average room temperature within recent 30 years was acquired from Central Weather bureau (2021). The DO concentration decreased as the water/outdoor air temperatures rose in summer. The highest water and outdoor air temperatures were 33.2 °C and 30.1 °C, resulting in the minimum DO concentration of 5.14 mg/L in July. Deoxygenation, which can kill fish on still summer nights, becomes worse as temperature increases (Moss *et al.* 2011). In contrast, the highest DO concentration was 8.74 mg/L in February, which is close to the saturated DO concentration (9.2 mg/L) in water and provides a favorable environment for aquatic creatures. In Taipei city, the daily average outdoor air temperature can exceed 29 °C in July and August; therefore, it is particularly important to investigate eutrophication of urban lakes in these 2 months.

The monthly average water temperature was slightly higher than the outdoor air temperature in summer but lower than it in winter. Water systems act as a buffer solution for aquatic creatures. Both chlorophytes and cyanobacteria grow in excessive quantities when the daily average outdoor air temperature reaches 30 °C (Fu *et al.* 2015; Ma *et al.* 2015; Harris *et al.* 2016). Higher temperature favors an increased density of phytoplankton that is responsible for algal blooms in lakes (Kosten *et al.* 2012; Tian *et al.* 2012; Harris *et al.* 2016). Aquatic macrophytes tend to increase in abundance and expand their geographic range in summer (Jones & Brett 2014). Deterioration of water quality is especially frequent at summer time of year (Lu *et al.* 2019).

In Taiwan, surface water is classified into five grades, and urban lakes are required to satisfy the fifth grade, which makes them subject to environmental protection. The water in Bi Lake meets the fifth (worst) grade requires a DO concentration above 2 mg/L and no floating dirt on the surface. Changing temperature affects the structure of biological organization in freshwater and interacts with other stressors (Woodard *et al.* 2013).

3.2. Anthropogenic activities on water quality

In summer, the higher water temperature results in higher turbidity and chromaticity. The turbidity and chromaticity showed a strong linear relationship ($R^2 = 0.85$). In detail, the median values of the turbidity were highest in the swimming area (S) (44.00 NTU), followed by the bridge (B) (35.80 NTU), water gate (W) (34.00 NTU), management room (M) (33.43 NTU), and wooden plank walkway (P) (32.00 NTU) (Figure 3(a)). The sampling locations with higher turbidity showed higher upper extreme values and outliers. On average, the lower quartile and upper quartile of turbidity were between 19 NTU and 56 NTU in the case of Bi Lake. For comparison, in the Huangpu River system, the turbidity values were over 100 NTU (Zhang *et al.* 2017). According to the Tap Water Quality Standard in Taiwan, the turbidity of tap water is required to be below 4 NTU, whereas that of raw water should be below 500 NTU.

The median values of the chromaticity were highest at the bridge (B) (552.27 Pt), followed by the swimming area (S) (495.37 Pt), management room (M) (426.73 Pt), wooden plank walkway (P) (393.37 Pt), and water gate (W) (346.35 Pt) (Figure 3(b)). The highest upper extreme value was 791.39 Pt found in the swimming area (S). The data range between upper quartile and lower quartile was wider than the turbidity shown in Figure 3(a). Obviously, the swimming area (S) and the bridge (B) are both popular with general public and had high turbidity and chromaticity. Deterioration of water quality was due to anthropogenic activities. Nutrients from anthropogenic activities flowing into the lake eventually become an internal source of eutrophication. The lake begins to turn visibly green as the water fertility increases from moderate to high, such as approximately 10 parts per billion (ppb) of the photosynthetic pigment chlorophyll (Jones & Brett 2014). Chlorophyll degradation products may at times constitute a significant fraction of the total green pigments present in water. The turbidity of the lake water met the standard for a backup source of tap water in Taipei but the chromaticity exceeded the limit. The chromaticity must be below 15 Pt for both tap water and raw water.

3.3. Results of CTSI

Table 1 shows the CTSI values of the Bi Lake. Overall, the CTSI values were lower in winter and higher in summer. The worst (i.e., highest) CTSI was 50, measured in April, showing that the water quality was worst during this transitional season. At this time of year, the bottom sediments are stirred up with higher turbidity permeated the entire lake (Fig. S2). On average, the level of eutrophication was higher at the swimming area (S) (CTSI≈47) and wooden plank walkway (P) (CTSI≈46). The results were consistent with the above finding that the swimming area (S) had higher turbidity and chromaticity. Fish farming has been found to be the most significant contributor to eutrophication globally (Silvenius *et al.* 2017). Fishing activities were quite popular at the bridge (B) and its sampling water was at mesotrophic level (CTSI≈45) in this study. Analyses have confirmed the role of local human activity as the main environmental driver of eutrophication and degradation of water quality (Jenny *et al.* 2016).

The CTSI is determined by the CA concentration, TP concentration, and transparency. However, their linear relationships with the measured CTSI were weaker than that of the PC concentration, as shown in Figure 4(a)–4(d). The linear relationship between CTSI and PC had an R^2 value of 0.605 (Figure 4(d)); and the R^2 value was increased to 0.861 when comparing TP and PC concentrations. The correlation between the TP and CA concentrations is stronger in more phytoplankton-dominated water (Wu *et al.* 2010; Chen *et al.* 2015; Hui & Yao 2016). The TP pollution may cause an increase in the PC concentration which represents an accessory pigment to chlorophyll in this study. Du *et al.* (2018) found that some TP present in granular form may be adsorbed on algae particles. Studies found that when eutrophication increases and the nitrogen-to-phosphorus ratio is low (TP concentration high), cyanobacteria become dominant (Isles *et al.* 2017; Sondergaard *et al.* 2017). The turbidity and CTSI had a minor correlation with an R^2 value of 0.454 in the case of Bi Lake (Figure 4(e)).

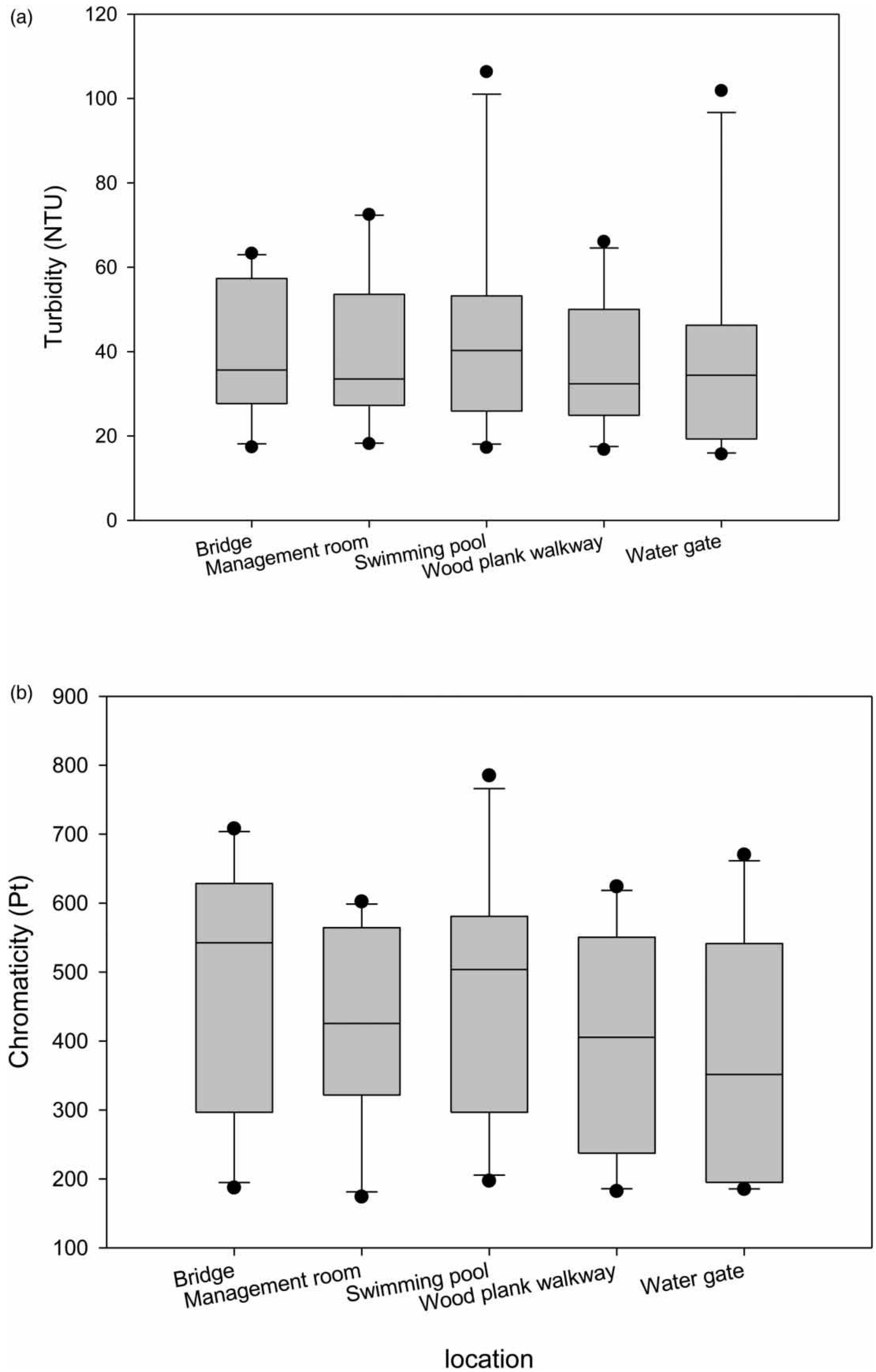

Figure 3 | Median values of turbidity (a) and chromaticity (b) at the five sampling locations of Bi lake.

3.4. Calculation of PC on eutrophic lake

The most common fluorescent proteins produced by cyanobacteria are PC in freshwater. Changes in the PC concentration may affect the photosynthesis of algae (Chi *et al.* 2020). The median PC concentration in Bi Lake ranged from 75.55 to

Table 1 | CTSI values of the five sampling locations of Bi lake

Sampling location Time (Year/Month)	Bridge (B)	Management room (M)	Swimming area (S)	Wooden plank walkway (P)	Water gate (W)	Monthly average
2019/Oct.	51	47	50	49	45	48
2019/Nov.	39	42	40	41	43	41
2019/Dec.	38	40	42	40	42	40
2020/Jan.	44	40	44	42	42	42
2020/Feb.	41	45	41	46	43	43
2020/Mar.	48	47	47	47	52	48
2020/Apr.	50	49	53	51	50	51
2020/May	50	45	54	48	44	48
2020/Jun.	46	45	51	47	44	47
2020/Jul.	44	45	47	46	47	46
2020/Aug.	42	45	52	45	43	45
2020/Sep.	45	41	44	45	44	44
Average value	45	44	47	46	45	

80.24 μg/L, as shown in Figure 5. The highest median PC concentration was that of the water sampled from the swimming area (S) (80.24 μg/L), followed by the water gate (W) (79.88 μg/L) and the bridge (B) (76.34 μg/L). The upper extreme values >100 μg/L were detected in the Bi Lake. Among them, the data range between upper quartile and lower quartile was relative wider in the water gate (113.16–38.88 μg/L). The adjustment of water level may dilute the PC concentration which is affected by human activities, such as in the swimming area (S) in this study. These results are higher than the WHO alert level 1 (<30 μg/L) of the PC concentration in lake water (Shin *et al.* 2018).

It was found that the PC concentration depended not only on the water quality but also on the water temperature. Paerl & Paul (2012) suggested that chlorophytes normally exhibit optimal growth rates at temperatures above 20 °C. Therefore, the wavelength of the water samples in Figure 6 shows higher PC's peaks in winter than summer with high temperature. As shown in Figure 6, the wavelength of the water samples from Bi Lake had sharp peaks at 615 nm which represents the environment is PC (cyanobacteria) dominant in winter. In contrast, the CA peaks were dominant at 663 nm to 665.6 nm (Qin et al., 2013) in summer found in this study. Calculation of CA and PC from their emitted wavelength were ranged from 17.25 to 30.54 μg/L (for CA) and 37.62 to 164.68 μg/L (for PC) shown in Table S1 and they were verified by highly correlated to in-situ measurement of chlorophyll fluorescence (R^2>0.91). However, the linear relationship of PC and CA was not positive correlated. Researchers found that PC is a water-soluble protein, whereas CA is insoluble (Davaeifar *et al.* 2019). However, Thomson-Laing *et al.* (2020) identified linear relationships ($R^2 = 0.4$–0.88) between the PC concentration and cyanobacterial biovolumes in individual lake analyses. In this study, the PC concentration was evidently not the main factor affecting the chromaticity of the lake ($R^2 = 0.03$). Researchers have attempted to purify PC-containing water using zero-valent iron (Liu *et al.* 2019) and chitosan (Lee *et al.* 2016).

4. CONCLUSIONS

This study took the 13-ha Bi Lake in northern Taipei as a case study to evaluate the effects of climate and anthropogenic activities on eutrophication in lake waters. In addition to CTSI, chlorophyll fluorescence was used in an innovative manner to quantify the PC concentration as a rapid alert for eutrophic water. Here, the DO concentration had the opposite trend to the outdoor air/water temperature. The highest water temperature and the outdoor air temperature were 33.2 °C and 30.1 °C, respectively, coinciding with the lowest DO concentration of 5.14 mg/L in July. The turbidity and chromaticity showed a strong linear relationship ($R^2 = 0.85$). Both the swimming area (S) and the bridge (B) are popular with the general public and had the worst turbidity (35.80–44.00 NTU) and chromaticity (495.37–552.27 Pt). The chromaticity was too high for the lake to be a suitable backup source of tap water in Taipei.

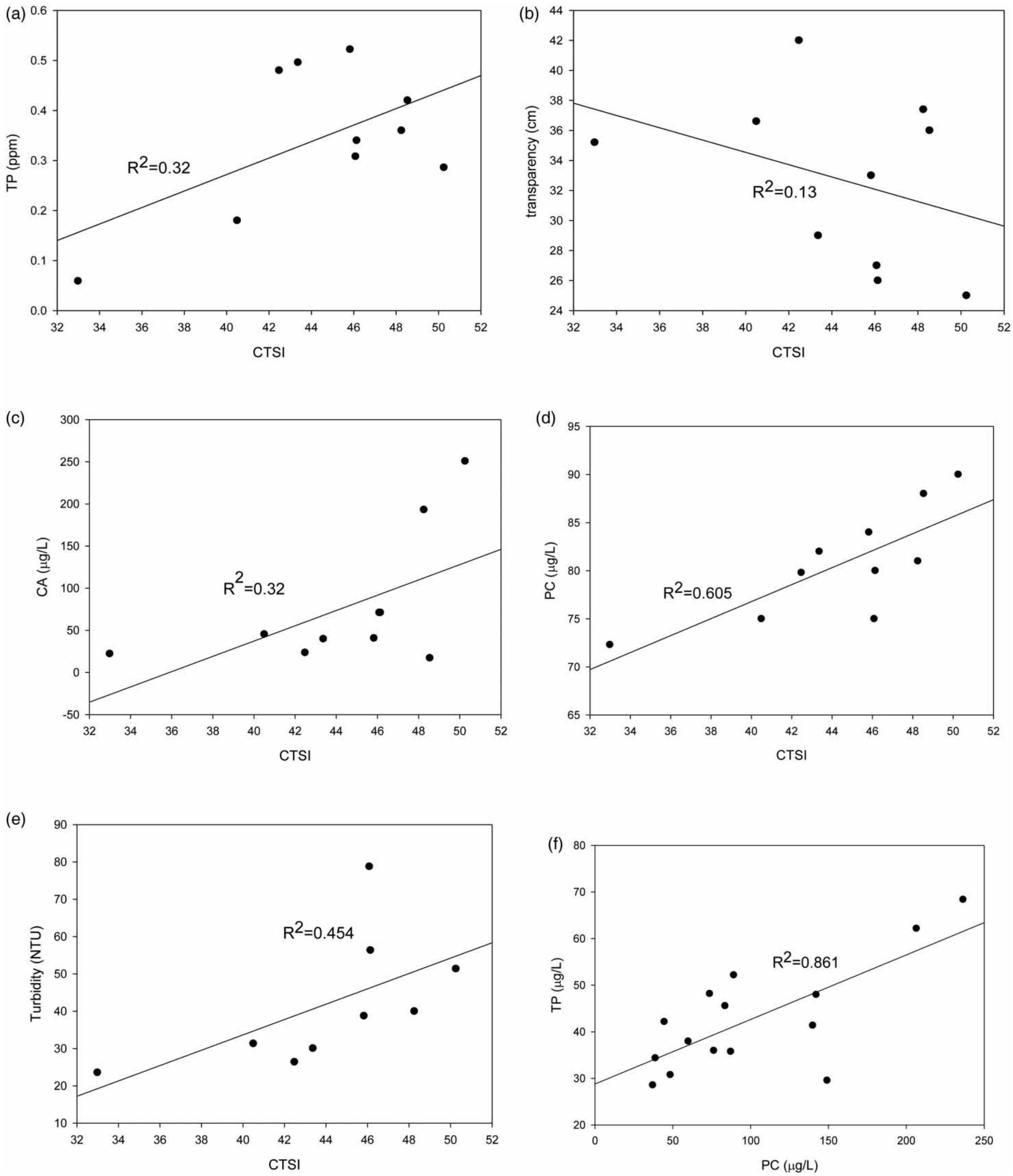

Figure 4 | Linear correlations between pairs of water quality indicators.

Overall, the CTSI values were lower in winter and higher in summer. The water sampled from the swimming area (S) had the highest CTSI value (~47), followed by the wooden plank walkway (P) and the bridge (B). When the chlorophyll fluorescence is used to quantify the PC concentration, the CTSI had a stronger linear relationship with the PC concentration than with any other indicator ($R^2 = 0.605$). This suggests that the PC concentration could be a rapid screening indicator for CTSI. The median PC concentration in Bi Lake ranged from 73.11 to 81.57 μg/L and was affected by the water quality,

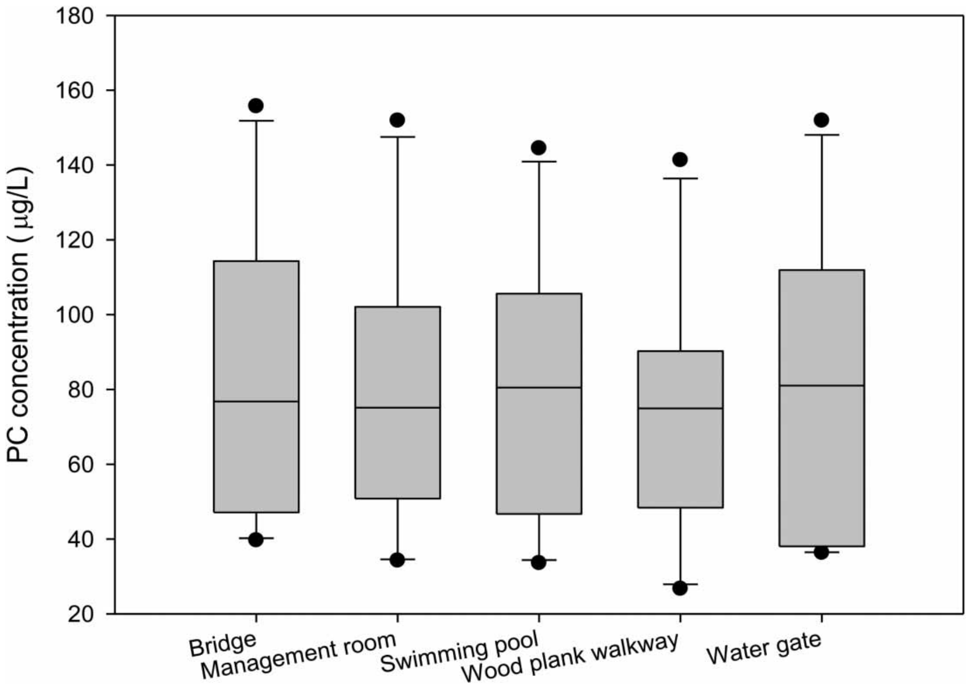

Figure 5 | Mean values of PC at the five sampling locations of Bi lake.

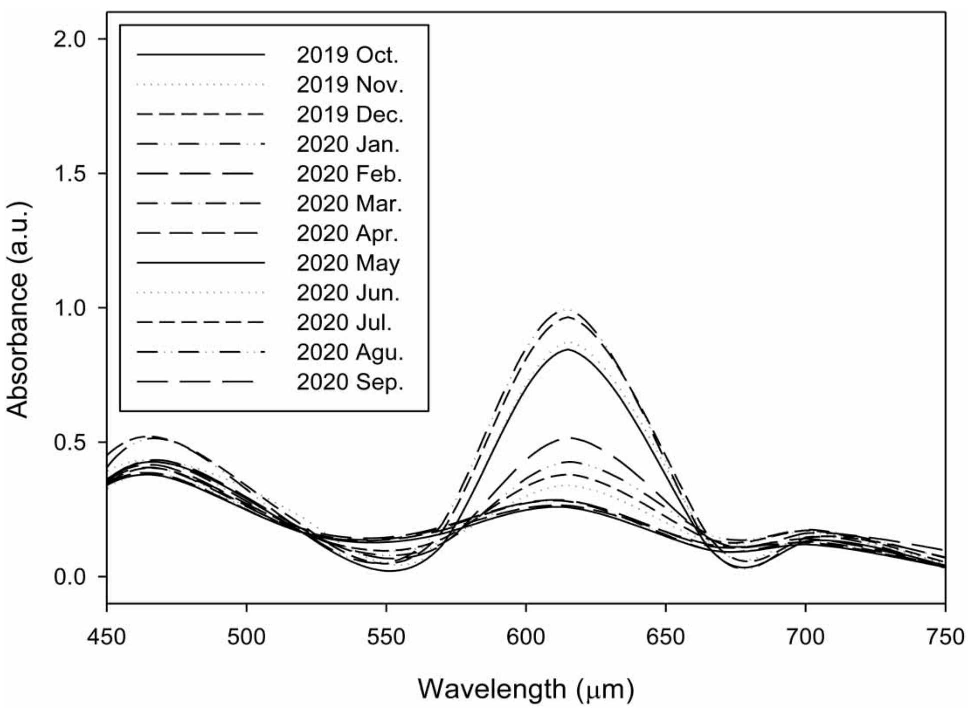

Figure 6 | The wavelength of the water samples in Bi lake.

water temperature, and human activities. Calculation of CA and PC from their emitted wavelength were highly correlated to in-situ measurement of chlorophyll fluorescence (R^2>0.91). The wavelength of the water samples had sharp peaks at 615 nm, which represent the environment is PC (cyanobacteria) dominant in winter of the Bi Lake. The linear relationship between the TP and PC concentrations had an R^2 value of 0.861, a higher value than found in other studies. In future studies, the relationship between TP and PC concentrations may be detailed studies.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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