Engng.J.CMU.(2020) 27 (2), 16-27

วารสารวิศวกรรมศาสตร์
มหาวิทยาลัยเชียงใหม่
Engineering Journal
Chiang Mai University

# Seasonal Variation of Water Quality and Microcystin Concentration in Kandawgyi Lake, Yangon City, Myanmar

**Khine Lwin Aye[1], Tomoaki Itayama[1], Tan Duc Nguyen[1], Kyaing[2], Kyi Pyar Shwe[2], Niwooti Whangchai[3], Reunkaew Praphrute[4], Akihide Tada[1]***

[1]Graduate School of Engineering, Nagasaki University, Nagasaki, Japan
[2]Department of Civil Engineering, Yangon Technological University, Yangon, Myanmar
[3]Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chaing Mai, Thailand
[4]Institute of Product Quality and Standardization, Maejo University, Chaing Mai, Thailand
*Corresponding Author: E-mail address: atada @ nagasaki-u.ac.jp

## ABSTRACT

This study is the first report on seasonal variations in water quality parameters and cyanotoxin microcystin (MC) concentration during one year in Kandawgyi Lake, Yangon City, Myanmar. Water quality parameters were measured and water samples were collected once a month from August 2018 to June 2019 at four observation sites (St-1~4). The seasonal changes of DO, pH and water temperature and turbidity were similar among the four observation sites. The observed DO values were always over the saturation levels due to the high photosynthetic activity in the hypereutrophic lake. However, chlorophyll-a as a surrogate of phytoplankton biomass was correlated negatively with DO. The multivariate linear regression model (linear model) analysis suggested that Kandawgyi lake was in the N-limitation state. Also, the lower N:P ratio (< 10) of lake water supported this fact. The linear model analysis elucidated the dependency on the water temperature for the three MC variants. The highest concentration of MC-YR and MC-LR at a lower temperature of less than 29°C was observed. The higher value of MC-RR in concentration was found at the temperature ranged was 29°C to 31°C than other temperature ranges. In terms of seasonal variation, MC-LR, which is more toxic than MC-RR and MC-YR, tended to show higher concentration in cool-season of Yangon city. It is known that the production ratio of MC variants is depending on the species or genetic variants of toxic cyanobacteria producing MCs. Therefore the seasonal variations of MC variants suggested the seasonal succession of toxic cyanobacteria. Moreover, it was thought that the variations of MC variants among the sites could be involved by the low mixing condition of the lake water. Therefore, this result has shown that lake monitoring with sufficient frequency and observation points in lakes with low water mixing is essential for monitoring water safety.

**Keywords:** hypereutrophication, toxic cyanobacteria, microcystin, temperature, nutrients

## 1. INTRODUCTION

Lakes or reservoirs in the urban area of developing countries frequently face severe deterioration of the water quality due to the discharging of untreated wastewater into water bodies [1]. Kandawgyi Lake's view and sampling details were presented in Figure 1 and Table 1. It is located in Yangon city of Myanmar. The lake and the parking surroundings are the most famous recreation places in Yangon. The lake has seven major inlets and one outlet. The domestic wastewater discharged to each channel through inlet. As a result, every time look the algae bloom. Hence it is apparent that the lake is in the eutrophic or hypertrophic state.

The blooms ruin the lake view for tourists, and it frequently generates a strong odor for the surroundings [2]. It is easily recognized from such a situation of Kandawgyi Lake that the bloom is consisting of cyanobacteria. The lake may have suitable conditions that the toxic cyanobacteria bloom occurs in terms of rich nutrients (N and P), light condition and temperature condition [3]. However, no



Received 2 October 2019
Revised 28 November 2019
Accepted 28 November 2019

investigation has been conducted to elucidate the actual eutrophication state and the character of the algal bloom of Kandawgyi Lake yet though it is a noticeable lake in the largest city of Myanmar. Hence, we need to survey the lake intensively. In particular, we have to focus on cyanotoxin microcystins (MCs) with the substantial hepatotoxicity, because many articles already reported that abundant MCs were detected in algal blooms in eutrophic or hypereutrophic lakes and reservoirs [4, 5, 6]. MCs have been the hazards for water safeness in the world [7, 8, 9].

In addition, some researchers reported that the lower N:P ratio in water bodies is a favorable condition for cyanobacterial bloom consisting of *Microcystis sp.* producing MCs [10,11]. Therefore, total nitrogen (TN) and total phosphorus (TP) in the lake water have to be measured. Furthermore, we have to consider the variants of MCs in the lake, because there were 90 kinds of analogous compounds of MCs, which have different toxicity levels [11]. In many studies, MC-LR (leucine-arginine), MC-YR (tyrosine-arginine) and MC-RR (arginine-arginine) in the variants of MCs were mainly measured [6, 8, 10]. In particular, MC-LR has been used as the guideline value of MC in drinking water by WHO [9]. Moreover, it is necessary to compare the water quality parameters and MCs in considering the differences of precipitation and temperature during three seasons that rainy season, summer season and cool season in Myanmar. In particular, the nutrient loading from the surroundings could be increased in the rainy season. Moreover, the temperature is one of the most significant factors for the growth of cyanobacteria and toxin production [3, 7]. Therefore, in this study, we aimed to elucidate the relation between microcystins and influencing factors N, P and temperature in Kandawgyi lake by the monthly survey of lake. It was expected that the result was useful for baseline data for water quality management.

## 2. MATERIALS AND METHODS

### 2.1 Field Survey and Sample Collection

The map of the study area shows in Figure 1, and the general features of the study area (Kandawgyi Lake) describe as Table-1[2]. The field observation conducted from August 2018 to June 2019. Four observation sites were selected far from inlet points to avoid the direct discharge of domestic wastewater effect from a point source. Moreover, observation sites were selected regarding the flow direction. The water temperature, turbidity, pH and DO were measured by using a multi-item water quality measurement sensors (Model– AAQ1183, JFE ALEC Co. Ltd ;) at four sites St.1, St.2, St.3, and St.4 (see Figure 1). For the entire observation period, the collection time of each site conducted the same time and procedures. For St-1 (start 13:21 ~ end 13:31), St-2 (start 13:34 ~ end 13:44, St-3 (start 13:47 ~ end 13:58) and St-4 (start 14:03 ~ end 14:13). Surface water samples were collected from about 20 ~ 30 cm depth from the surface to avoid the bloom. The water samples were kept in a cool box with enough ice packs for transporting to the laboratory. The samples for TN, TP, and MCs were frozen until the analysis.

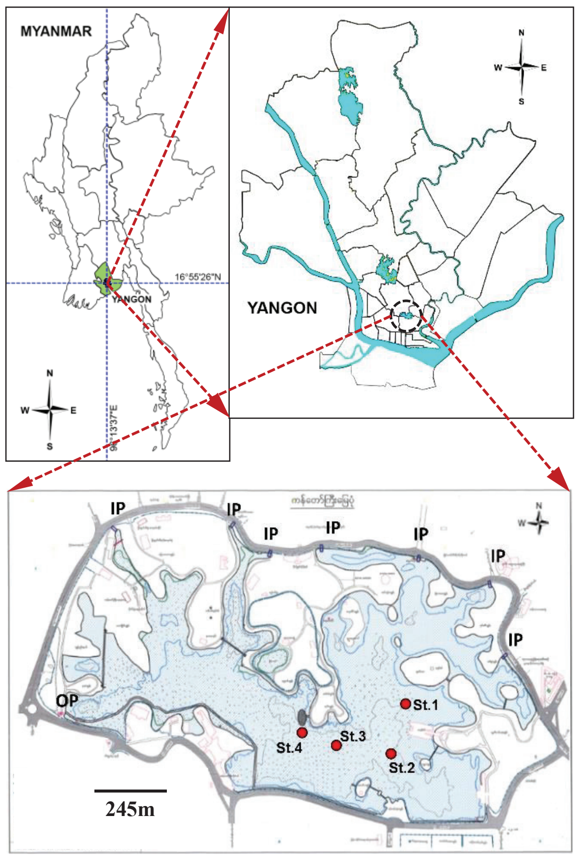


**Figure 1** Maps of study area

**Table 1** Limnological features of Kandawgyi Lake

| | |
|---|---|
| Constructed Year | 1879 |
| Latitude | 16°47′43.75″N |
| Longitude | 96°9′59″ E |
| Average Depth of Lake | 1.83 m |
| Maximum Depth | 3.66 m |
| Water Surface Area | 0.6475 $km^2$ |
| Catchment Area | 1.421 $km^2$ |

### 2.2 Water Quality Analysis

The concentration of TN and TP in the unfiltered samples was measured with three repeats using the potassium persulfate method [12]. Chlorophyll-a (Chl-a) was extracted from the cell residue on a GF/C filter using 90% hot methanol and quantified spectrometrically [13]. The Chl-a measurement for samples from August ~ February was repeated twice, and that for samples from April ~ June was repeated three times.

### 2.3 Microcystin Analysis

The MCs analysis was carried out based on acetic acid extraction and HPLC [14, 15, 16]. 100% acetic acid of a particular grade (Merck, USA) of 1 ml was added to 20 ml samples and shook it for 30min. Then, the ultra-sonication of each sample for 5 min was repeated until the complete cell disruption. The cell debris was removed from the sample water after the cell disruption by 47 mm GF/C filter (Whatman Co., USA) before they pass through the solid phase extraction (SPE) column. The cartridge of SPE of 60mg/3ml capacity (Strata-X, Phenomenex Inc., USA) was washed by 100% methanol of 5ml (HPLC grade, Merck.) and Milli-Q water of 20 ml twice with 5 ml/min.

The sample water was passed through the washed SPE cartridge with 5 ml/min, and then the column was washed by 20% methanol of 5ml and Milli-Q water of 15 ml (twice). Finally, MCs were eluted from the SPE cartridge to a test tube by 100% methanol of 3 ml. It was dried 30 minutes with $N_2$ gas flow (100 ml/min) with heating (60~70°C). The dried samples were dissolved into 75% methanol of 0.5 ml, and then it was filtrated by membrane filter with 0.22 µm pore size (Nylon, Starlab Scientific, Germany) to transfer each HPLC vial. Standard solutions of MC-LR, -YR and -RR (Enzo Life Science Inc., USA) were also prepared for the quantification.

HPLC (Agilent 1200 series, USA) was used for the quantification of MCs with C-18 column (Agilent, Zorbax, Eclipse, 5 µm, 3 mm × 150 mm). The column temperature was set at 40 °C and a diode-array detector at 238 nm. Solvent A is distilled water containing 0.05% (*v*/*v*) trifluoroacetic acid (TFA, HPLC grade, Sigma Aldrich, Co. LTD, USA) and Solvent B is acetonitrile (HPLC grade, Merck Co., Germany). The ratio of mobile phase 'A' and 'B' was changed from 70 %: 30% to 100% of 'A' solution (acetonitrile) for 44 min in the linear gradient method. The injection volume is 10 µL. Three kinds of microcystin variants (RR, YR, and LR) were detected at 238nm. Each peak was identified by the retention time of each standard.

### 2.4 Statistical Analysis

All statistical computations were performed by using R (version 3. 4. 3), which is a language and environment for statistical computing [17]. In particular, to evaluate the influencing factors on Chla and MCs, a multivariate linear regression model was used. We wrote the multivariate linear regression model as a linear model hereafter. In the analysis, the z-scores of explanatory valuables were applied to compare the magnitude of the effect in the explanatory variables, which have different average and standard deviation [18, 19]. The z-score calculation method was shown in Appendix 2. Moreover, the Akaike Information Criteria (AIC) was used for model selection [19]. A linear model with a smaller AIC is generally identified as a better model. The statistically significance level was classified based on the null hypothesis rejection probability p-value as follows:

'***' < 0.001 < '**' <0.01<'*' <0.05

Then, $p < 0.05$ was adopted as a statistically significant level in this study.

## 3. RESULTS AND DISCUSSION

### 3.1 Seasonal variations of Water Quality Parameters

Figure 2 presents the variation of water temperature, DO, pH, and turbidity, which were measured from 13:20 to 14:10. All numeric data are presented in Table A2 in Appendix 3. The water temperature of each site presented similar seasonal fluctuation with the temperature range between 28°C and 34°C. The observed dissolved oxygen (DO) between 10.2 mg/L and 19.9 mg/L was apparently over the saturation level in consideration with the water temperature. The high DO value means high photosynthetic activity [20, 21]. In this survey, we observed a high pH value between 8.7 and 10.8, and it also indicated the high activity of the phytoplankton photosynthesis [20, 21]. Chl-a showed monthly fluctuation between 97 µg/L and 417 µg/L in concentration at four observation sites as illustrated in Figure 3 (and see Table A3 in Appendix 3). The lowest value of Chl-a was observed in September and the highest value was recorded on the 1st of June. A positive correlation between DO and Chl-a as a surrogate of phytoplankton biomass was expected, but Pearson's correlation was a negative value ($r = -0.593$, $p < 0.01$). This result suggested that much organic matter, produced by phytoplankton, could be degraded by active bacteria which consumed a lot of DO. Especially, Chl-a in June 2019 showed the noticeable raising, as illustrated in Figure 3. However, the DO showed the decrement. The climate condition was rain at this time (see Table A1 of Appendix 1), then the turbidity value was the highest value of 95 F.T.U, as shown in Figure 2(d). Thus, the DO was dropped because the low light condition limits the photosynthetic activity. On the other hand, as the turbidity has a clear positive correlation with Chl-a ($r = 0.686$, $p < 0.001$), it was suggested that the significant part of the changes of the turbidity depended on the phytoplankton growth.

The marker IP shown in the map of Figure 1 indicates the inlet points. In this survey, we frequently saw that the grey or black colored water flowed into the lake at these inlet points during the rainy season. The northern part of the basin of the lake is a commercial area where many restaurants and hotels have been operated. The wastewater from these buildings has flowed into the lake without any treatment, according to the information of the Yangon City Development Committee (YCDC). The untreated wastewater flowing from the drainage channels to the inlet points in the lake during the rainy season was involved in the nutrient loading. On the other hand, as the rain was very few in cool-season and summer season, we never saw the flowing water in the drainage channels. Nevertheless, the increments of TN and TP of the lake water in the rainy season were not so clear as shown in Figure 4(a-b). It is difficult to discuss further the sources of nutrients N and P to the lake until hydrological and hydraulic studies will be conducted. Thus, we used TN and TP as the surrogates of comprehensive nutrients N and P loads according to a common technique in limnology [21]. TN was varied between 1.6 mg-N/L and 3.2 mg-N/L as illustrated in Figure 4 (see Table A2 in Appendix 3). The average TN: TP ratio of 3.9 was calculated from Table A2. The variation pattern of TN was similar to the Chl-a. However, the temporal change of TP ranged from 0.20 mg-P/L to 0.87 mg-P/L and did not show a clear correspondence with changes in Chl-a .

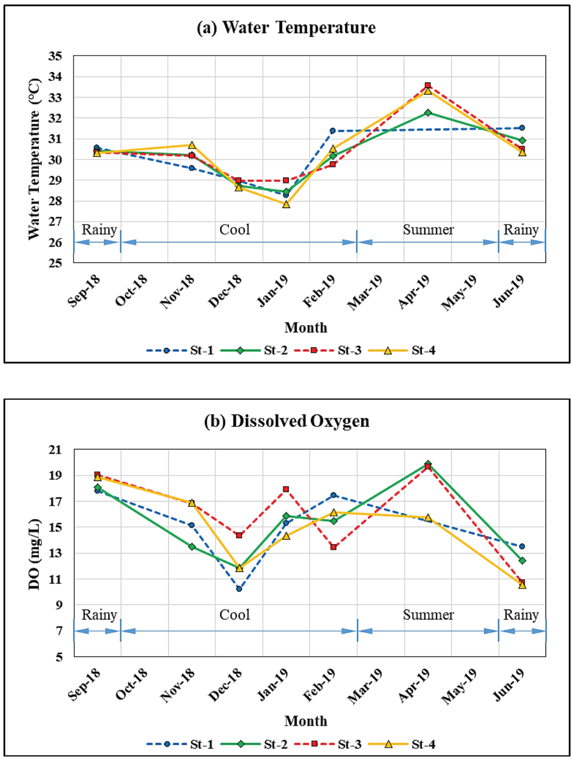


**Figure 2** (a-d) Seasonal variations of water quality parameters

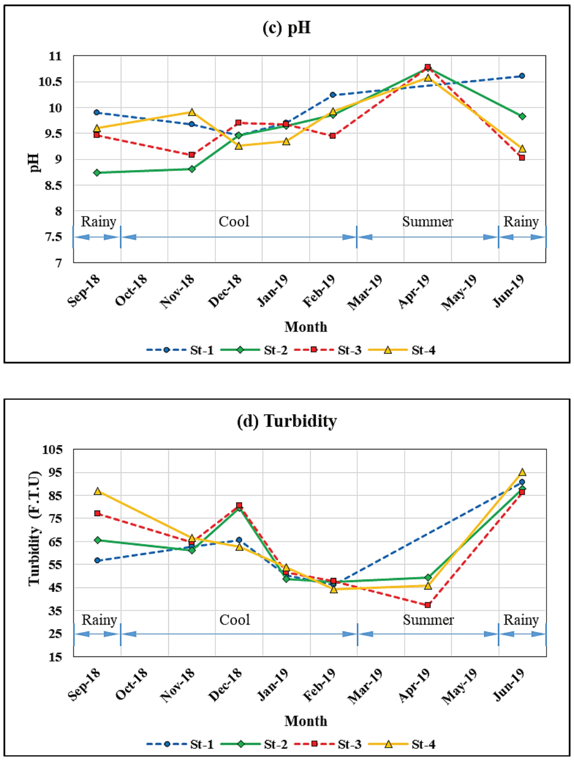


**Figure 2** (cont) (a-d) Seasonal variations of water quality parameters

Linear model analysis was used to clarify the effect of temperature and nutrients N and P for the change of Chl-a. Here, TN, TP, temperature, and Chl-a were logarithmically converted. Besides, the respective z-score was calculated to compare the effect of TN, TP and temperature from the magnitude of each regression coefficient (see Appendix 2) [18]. The summary result of the linear model is presented in Table 2. In model 1, the coefficient of z-log (TN) was more significant than the others, and the p-value less than 0.1 was calculated. Therefore, it was thought that TN relatively affected the Chl-a despite statistically no significant of the linear model ($p = 0.169$).

On the other hand, the effects of z-log (TP) and water temperature were not completely significant. Selecting only z-log (TN) as explanatory variables, the AIC value for the regression decreased as shown in model 2 of Table 2 [19]. It means that TN mainly affected to the increment of phytoplankton biomass (Chl-a). Thus, it was suggested that the limiting factor for the growth of the phytoplankton was nitrogen (N) in Kandawgyi Lake.

Moreover, the fact that TN: TP ratio of the lake water was 3.9 confirms that the lake was the nitrogen limitation state [22, 23]. In such cases, it is preferable to calculate the trophic state index (TSI) from the average value of not TP but Chl-a shown in Table A2. As a result, a value of 83 was obtained as TSI of Kandawgyi Lake. This value completely indicated that the lake was a hypereutrophic state [24].

**3.2 Seasonal variations of microcystin variants**

Three MC variants, MC-RR, MC-YR and MC-LR were detected in each site during the observation period. The order of the averaged concentration of MC variants was MC-RR (24.5 µg/L), MC-LR (7.3 µg/L) and MC-YR (4.3 µg/L) (see Table A2 in Appendix 3).Figure 5 illustrated the fluctuations of the total of the three MCs at each site, respectively. Whereas the changes of Chl-a at the four observation sites almost synchronized, MCs fluctuated with the different patterns among the sites. Furthermore, the ratios among MC-RR, MC-YR and MC-LR showed the natural seasonal fluctuations at the four observation sites as illustrated in Figure 6(a-d). In particular, MC-RR was always the highest percentage in the three MCs except for in December 2018 at St-4 and April 2019 at St-1.

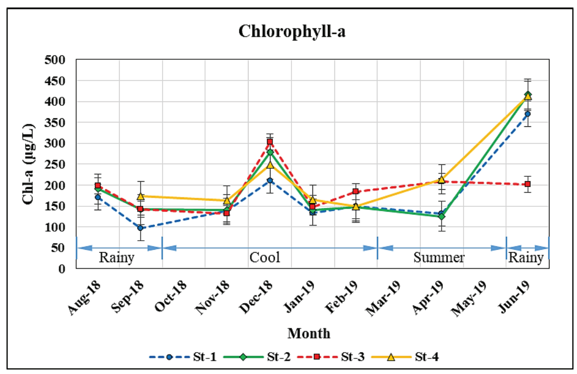


**Figure 3** Seasonal variations of Chl-a

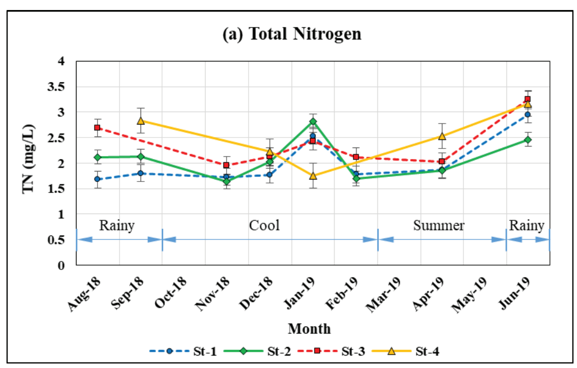


**Figure 4** (a-b) Seasonal variations of TN and TP

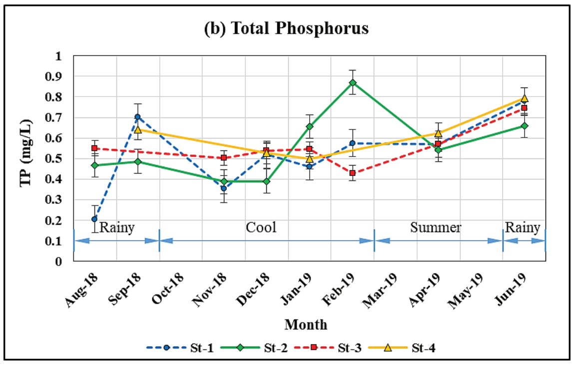


**Figure 4** (cont) (a-b) Seasonal variations of TN and TP

**Table 2** Linear models for Chl-a

Model 1. logChl-a~ z- logTP +z-logTN+z-Temp

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| z- log(TP) | 0.007 | 0.046 | 0.158 | 0.876 |
| z- log(TN) | 0.082 | 0.045 | 1.831 | 0.084 |
| z-Temp | 0.009 | 0.027 | 0.315 | 0.757 |

F statisics: 1.88, p-value: 0.169, AIC: -10.862

Model 2. logChl-a~ z- logTN

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| z- log(TN) | 0.085 | 0.035 | 2.454 | 0.023* |

F-statistic: 6.02, p-value: 0.023, AIC: -14.657

It was illuminated that MC-LR, which is more toxic than MC-RR and MC-YR [8], tended to show higher concentration in cool season and summer season as shown in Figure 6 (a-d). In order to clearly present the effect of temperature, Figure 7(a-c) illustrated each bar plot of the temperature effect on the concentration of MC-RR, MC-YR and MC-LR, respectively. The concentration of MC-RR was the highest in the temperature range of M (29°C~31°C). MC-YR and MC-LR showed the highest concentration in the temperature range of L (<29°C). However, all three MC variants showed the lowest concentration in the temperature range of H (>31°C). It is thought that nutrients N and P affect MC production cyanobacteria [9, 10].

Thus, three explanatory variables TN, TP and water temperature were selected for the linear model analysis in order to elucidate the influencing factors on the change of the concentration of each MC variant in the lake. Here, z-log(TN) and z-log(TP) were used as well as the linear model analysis for Chl-a. The objective variables, MC-RR, MC-YR, and MC-LR were also logarithmically converted. In addition, the water temperature was converted to a category variable using the same categorization in Figure 7. Then, the model selection method based on AIC was again applied to find a better model [19]. In Table 3(a-c), the results of the linear model analysis on MC-RR, MC-YR, and MC-LR were presented. Table 3(a) showed that water temperature within the range of M (29~31°C) has a significant positive effect on MC-RR concentration though both TN and TP were no contribution factors.

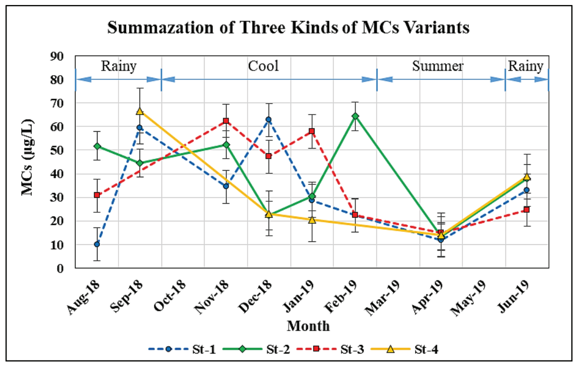


**Figure 5** Seasonal variations of microcystin concentration at four observation sites

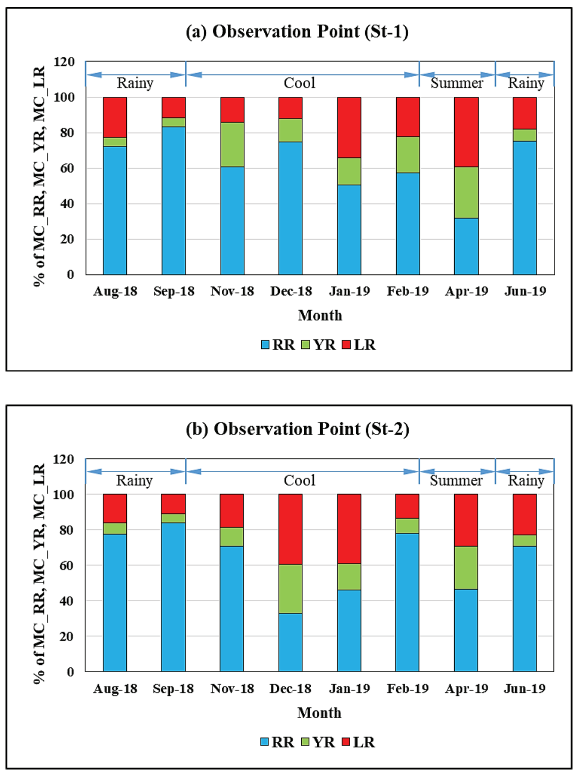


**Figure 6** (a-d) Spatial and Temporal Changes of RR, YR&LR (%)

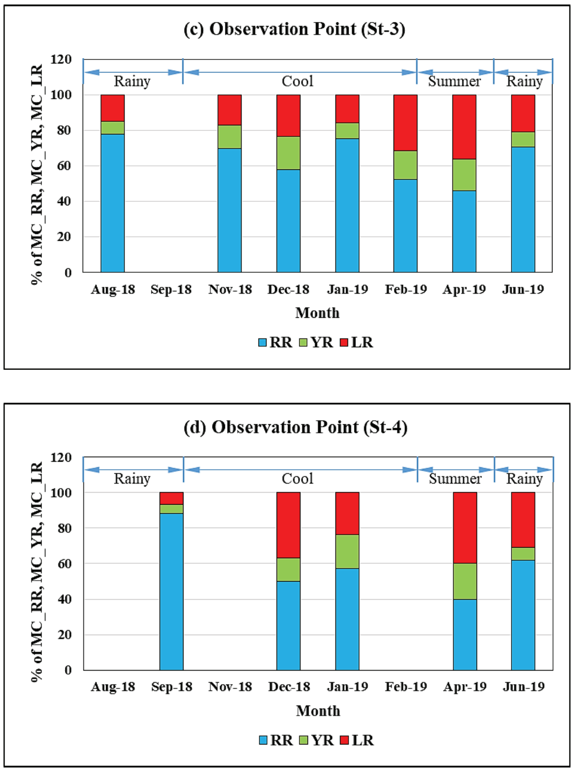


**Figure 6** (cont) (a-d) Spatial and Temporal Changes of RR, YR&LR (%)

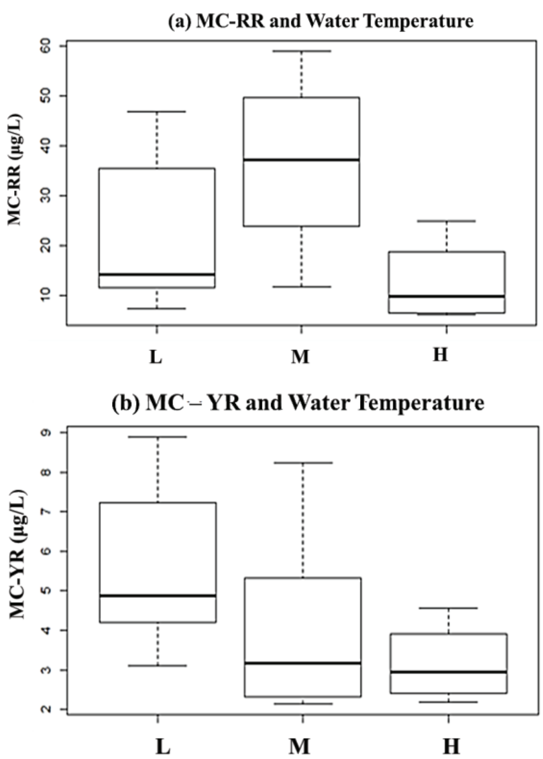


**Figure 7** (a-c) Effect of water temperature on MC variants, L: water temperature <29°C, M: 29°C <water temperature <31°C, H: water temperature >31°C

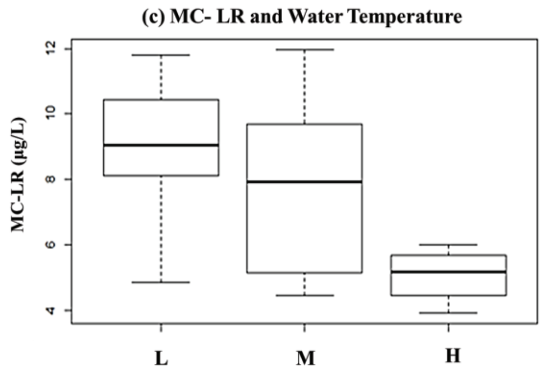


**Figure 7** (cont) (a-c) Effect of water temperature on MC variants, L: water temperature <29°C, M: 29°C <water temperature <31°C, H: water temperature >31°C

As presented in Table 3(b-c), the water temperature of the range of L significantly affected MC-YR and MC-LR in concentration. According to recent researches, it was found that MC production ability per cell decreased as the temperature increased [25, 26]. The result was consistent with our results. However, in another research, MCs production increased with the high temperature (32°C) in the non-axenic culture of *Microcystsis aergunosa* [26]. In contrast, the opposite result was obtained about the axenic culture [27]. Since the non-axenic condition is a natural condition for the lake water, it seemed that this result didn't support our survey results. In addition, we need to consider the seasonal succession of several toxic cyanobacteria species in a natural lake. Each strain in toxin-producing *Microcystis sp.* is known to produce different amounts of MC variants [28, 29].

Furthermore, a result from survey research in England elucidated that the ratios among MC variants produced from four toxic cyanobacteria species (*Microcystis sp. Oscillatoria sp., Anabaena sp.,* and *Aphanizomenon sp.*) were different each other [30]. Therefore, it was reasonably thought that the observed variation of the ration among MC variants could be due to the seasonal succession of toxic cyanobacteria species. Changes of the nutrients N and P generally relate to the species succession in the lake ecosystem [29]. However, no significant contributions ($p>0.05$) of both TN and TP for MCs were clearly found in Table 3(a-c). It is well known that the low N:P ratio is a favorable condition for the growth of *Microcystis sp.* [23].

Nevertheless, the effect of P can be saturated because Kandawgyi Lake is in the hypereutrophic state with a very low N:P ratio of 3.9.

On the other hand, we already elucidated that N loading promoted the increment of the biomass of phytoplankton in this lake, and the temperature effect on the growth of phytoplankton was not significant. Besides, the result of the linear model analysis suggested that the composition of toxic cyanobacteria species, which related the composition of MC variants, was determined by not nutrients N and P but temperature. This finding is not only interesting from the viewpoints of limnology but also important from the viewpoints of lake water quality management because the changes of toxic cyanobacteria and MC variants composition directly linked to the safe of the lake water as already mentioned [9, 10, 11]. In a further study, we need to directly clarify the seasonal variation of species composition of toxic cyanobacteria [30].

**Table 3** Results of the linear model analysis for MC variants.

(a) Linear models for MC-RR

Model 1: logMC-RR ~ z-logTN+z-logTP+Temp

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| z-logTN | 0.012 | 0.074 | 0.161 | 0.874 |
| z-logTP | -0.049 | 0.077 | -0.644 | 0.528 |
| Temp-L | 0.191 | 0.175 | 1.091 | 0.291 |
| Temp-M | 0.474 | 0.161 | 2.941 | 0.009** |

F-statistic: 2.53, p-value: 0.078 AIC = 11. 303

Model 2. logMC-RR~ Temp

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| Temp-L | 0.223 | 0.159 | 1.402 | 0.177 |
| Temp-M | 0.474 | 0.154 | 3.079 | 0.006** |

F-statistic: 5.24, p-value:0.015 AIC= 7.927

(b) Linear models for MC-YR

Model 1. logMC-YR~ z- logTN+z-logTP+Temp

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| z- logTN | -0.059 | 0.036 | -1.655 | 0.116 |
| z- logTP | -0.070 | 0.038 | -1.866 | 0.079· |
| Temp-L | 0.211 | 0.086 | 2.464 | 0.025* |
| Temp-M | 0.083 | 0.079 | 1.059 | 0.304 |

F-statistic: 6.65, p-value: 0.002 AIC = -20.294

**Table 3** (cont) Results of the linear model analysis for MC variants.

Model 2. logMC-YR~ Temp

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| Temp-L | 0.238 | 0.105 | 2.263 | 0.036* |
| Temp-M | 0.065 | 0.102 | 0.637 | 0.532 |

F-statistic: 3.38, p-value: 0.056 AIC= -10.264

(c) Linear models for MC-LR

Model 1. logMC-LR~ z- logTN+z-logTP+Temp

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| z- logTN | 0.029 | 0.036 | 0.805 | 0.432 |
| z- logTP | -0.043 | 0.038 | -1.139 | 0.270 |
| Temp-L | 0.209 | 0.085 | 2.445 | 0.026* |
| Temp-M | 0.167 | 0.078 | 2.132 | 0.048* |

F-statistic: 2.56, p-value: 0.077 AIC = -20.323

Model 2. logMC-LR~ Temp

| Variable | Estimate | SE | t-value | p |
|---|---|---|---|---|
| Temp-L | 0.241 | 0.079 | 3.030 | 0.007** |
| Temp-M | 0.172 | 0.077 | 2.238 | 0.037* |

F-statistic: 4.61, p-value: 0.023 AIC= -22.673

Estimate of categorical variable Temp = L (M) shows an additive effect for each objective variable measured from the effect at Temp=H [25].

Moreover, it was thought that the variations of MC variants among the four sites could be involved by the low mixing condition of the lake water. The differences of MC variants among the four sites, of course, indicated the differences of the community of toxic cyanobacteria among the sites as already discussed. The four observation sites in Figure 1 were expected to show the averaged and representative dynamic character about the water quality parameters because the sites were apart from each inlet point (IP) of the drainage channels as nutrients sources (see Figure 2 and Figure 3). However, the seasonal variation of MC variants resulted from the seasonal succession of toxic cyanobacteria, was almost independently caused at each site despite the continuous water body among the sites as shown in Figure 5. This is another significant finding in this study. Hence, it is important to monitor the safeness of lake water with sufficient frequency and observation points, in case of lakes with a low degree of water mixing such as Kandawgyi Lake. Of course, preliminary hydrological and hydrological studies are a prerequisite for the optimal determination of location and frequency.

## 4. CONCLUSIONS

The seasonal variation of water quality parameters and microcystin (MC) concentration in the Kandawgyi Lake were studied. The lake was identified in the hypereutrophic state due to the very high Chl-a of 196μg/L on average, and in the N-limitation state with the lower N:P ratio of 3.9. MC concentration was quantified for the lake water samples at each site, and three MC variants MC-RR, MC-LR and MC-YR were detected. The order of the averaged concentration of MC variants was MC-RR (24.5 μg/L), MC-LR (7.3 μg/L) and MC-YR (4.3 μg/L). Each concentration of MC variants was depended on not TN and TP but temperature, though Chl-a, as a surrogate of phytoplankton biomass, was affected by not temperature but TN. In particular, the lower concentration of three MC variants was measured in higher temperature conditions (>31°C). Moreover, the variations of MC variants among the sites could be involved by the low mixing condition of the lake water, because it was thought that the seasonal changes of MC variants were due to the succession of toxic cyanobacteria independent in each site.

## 5. ACKNOWLEDGMENT

The authors sincerely appreciate to Yangon City Development Committee (YCDC), because they permitted our survey and access for several unpublished information on Kandawgyi Lake and the basin. Moreover, we sincerely acknowledge international research team members who supported to collect the water samples and experimental works in this study. Also, we would like to thank our friend Dr. Rameshprabu Ramaraj, for valuable comments and English grammatically collections.

This work is as part of the Ph.D. study, and the authors gratefully acknowledge the Japanese Government (Monbukagakusho: MEXT) scholarship organization.

## REFERENCES

[1] Yunlonga, S., Jinsonga, Z., Jiab, Z., Lic, W., Aiminc, C., and Yid, T. Spatial and Temporal Variations of Water Quality and Trophic Status in Xili Reservoir: a Subtropics Drinking Water Reservoir of Southeast China. IOP Conference Series: Earth and Environmental Science, 2017; 100(1).

[2] Soe, P. E. Study on the Eutrophication Status of Kandawgyi Lake, Yangon Technological University, Master Thesis, 2013.

[3] Cuvin-Aralar, M. L., Fastner, J., Focken, U., Becker, K. and Aralar, E. V. Microcystins in Natural Blooms and Laboratory Cultured *Microcystis Aeruginosa* from Laguna de Bay, Philippines. *Systematic and Applied Microbiology*, 2002; 25(2): 179-182.

[4] Jacoby, J. M., Collier, D. C., Welch, E. B., Harby, F. J. and Caryton, M. Environmental Factors Associated with a Toxic Bloom of *Microcystis Aeruginosa*. *Canadian Journal of Fisheries and Aquatic Science*, 2000; 57: 231-240.

[5] Joung, S. H., Oh, H. M., Ko, S. R. and Ahn, C. Y. Correlations Between Environmental Factors and Toxic and Non-Toxic Microcystis Dynamics During Bloom in Daechung Reservoir, Korea. *Harmful Algae*, 2011; 10: 188–193.

[6] Lee, T. A., Rollwagen-Bollens, G., Bollens, S. M. and Faber-Hammond, J. J. Environmental Influence on Cyanobacteria Abundance and Microcystin Toxin Production in a Shallow Temperate Lake. *Ecotoxicology and Environmental Safety*, 2015; 114: 318–325.

[7] De Figueiredo, D. R., Azeiteiro, U. M., Esteves, S. M., Goncalves, F. J. M. and Pereira, M. J. Microcystin-Producing Blooms—A Serious Global Public Health Issue. *Ecotoxicology and Environmental Safety*, 2004; 59: 151-163.

[8] Gupta, N., Pant, S. C., Vijayaraghavan, R. and Rao, P. V. L. Comparative Toxicity Evaluation of Cyanobacterial Cyclic Peptide Toxin Microcystin Variants (LR, RR, YR) in Mice. *Toxicology*, 2003; 188: 285–296.

[9] Xie, L. Q., Xie, P. and Tang, H. J. Enhancement of Dissolved Phosphorus Release from Sediment to Lake Water by Microcystis Blooms—An Enclosure Experiment in a Hyper-Eutrophic, Subtropical Chinese Lake. *Environmental Pollution*, 2003; 122: 391–399.

[10] Xie, L. Q., Xie, P., Li, S. X., Tang, H. J. and Liu, H. The Low TN: TP Ratio, A Cause, or a Result of Microcystis Blooms?. *Water Research*. 2003; 37: 2073–2080.
[11] Prommana, R., Peerapornpisal, Y., Whangchai, N., Morrison, L. F., Metcalf, J. S., Ruangyuttikarn, W., Towproma, A. and Codd, G. A. Microcystins in Cyanobacterial Blooms from Two Freshwater Prawn (Macrobrachium Rosenbergii) Ponds in Northern Thailand. *Science Asia*, 2006; 32: 365-370.
[12] American Public Health Association. In Standard Methods for the Examination of Water and Waste Water. 20th Eds, APHA: Washington, DC, 1998.
[13] Saijo, Y. Methods for the Quantitative Determination of Chlorophyll. *Journal of Limnology*, 1975; 36: 103–109.
[14] Fastner, J., Codd, G. A., Metcalf, J. S., Woitke, P., Wiedner, C., Utkilen, H. An International Comparison Exercise for the Determination of Purified Microcystin-LR and Microcystins in Cyanobacterial Field Material. *Analytical and Bioanalytical Chemistry*, 2002; 374: 437–444.
[15] Lawton, L., Edwards, C. and Codd, G. A. Extraction and High-Performance Liquid Chromatographic Method for the Determination of Microcystins in Raw and Treated Waters. *The Analyst*, 1994; 119: 1525–1530.
[16] Meriluoto, J. and Codd, G. A. Toxic Cyanobacterial Monitoring and Cyanotoxin Analysis. Abo Akademi University Press, Abo, Finland, 2005; 149.
[17] R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2017. [online] available: https://www.R-project.org/.
[18] Kleinbaum, D. G., Kupper, L. L., Muller, K. E. and Nizam, A. Applied Regression Analysis and Other Multivariable Methods. 3rd Edition, Duxbury Press, Toronto, 1998.
[19] Parzen, E., Tanabe, K. and Kitagawa, G. Information Theory and an Extension of the Maximum Likelihood Principle. Springer, New York, 1998; 199-215.
[20] Stumm, W. and Morgan, J. J. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters. 3rdEds, John Wiley & Sons, New York, 1996.
[21] Wetzel, R. G. Limnology: Lake and River Ecosystems, 3rdEds, Academic Press, 2001.
[22] Sakamoto, M. Primary Production by Phytoplankton Community in Some Japanese Lakes and Its Dependence on Lake Depth. *Journal of Arch Hydrobiology*, 1966; 62: 1–28.
[23] Simith, V. H. The Nitrogen and Phosphorus Dependence of Algal Biomass in Lakes: An Empirical and Theoretical Analysis. *Limnology and Oceanography*, 1982; 27(6): 1101-1112.
[24] Carlson R E. A Trophic State Index for Lakes. *Limnology and Oceanography*, vol. 1977; 22(2): 361–369.
[25] Bui, T., Dao, T. S., Vo, T. G. and Lurling, M. Warming Affects Growth Rates and Microcystin Production in Tropical Bloom-Forming Microcystis Strains. *Toxins*, 2018; 10(3): 123.
[26] Peng, G., Martin, R. M., Dearth, S. P., Sun, X., Boyer, G. L., Campagna, S. R., Lin, S. and Wilhelm, S. W. Seasonally Relevant Cool Temperatures Interact with N Chemistry to Increase Microcystins Produced in Lab Cultures of *Microcystis Aeruginosa* NIES-843. *Environmental Science & Technology*, 2018; 52 (7): 4127–4136.
[27] Dziallas, C., Grossart, H. P. Increasing Oxygen Radicals and Water Temperature Select for Toxic *Microcystis* sp. *Plos One*, 2011; 6(9).
[28] Su, X., Xue, Q., Steinman, A. D., Zhao, Y. and Liqiang Xie, L. Spatiotemporal Dynamics of Microcystin Variants and Relationships with Environmental Parameters in Lake Taihu. China, *Toxins*, 2015; 7 (8): 3224-3244.
[29] Watanabe, M. F., Oishi, S., Harda, K., Matsuura, K., Kawai, H. and Suzuki, M. Toxins Contained in *Microcystis* Species of Cyanobacteria (Blue-Green Algae). *Toxicon*, 1988; 26(11): 1017-1025.
[30] Andrew, D. T., Monika, D.-R., Alison, O. N., Coates, L., Lewis, A., and Katy, K. Analysis of Microcystins in Cyanobacterial Blooms from Freshwater Bodies in England. *Toxins*, 2018; 10(39).

# APPENDIX

**Appendix 1**

**Table A1** Weather Conditions at Observation Date

| Date | Maximum Air Temp(°C) | Minimum Air Temp(°C) | Rainfall (mm) | Remarks |
|---|---|---|---|---|
| 18/08/2018 | 31 | 22 | 3 | Rain |
| 21/09/2018 | 32.2 | 19.5 | 12 | Rain |
| 11/11/2018 | 32.5 | 20 | 11 | Rain |
| 15/12/2018 | 31.6 | 18.5 | 0 | Cloudy |
| 13/01/2019 | 33 | 19.2 | 0 | Fair |
| 25/02/2019 | 35.8 | 19.5 | 0 | Fair |
| 06/04/2019 | 39.2 | 20.5 | 0 | Fair |
| 01/06/2019 | 33.3 | 26 | 1 | Rain |

**Appendix 2**

z-score of valuable X

$$z-X=\frac{X-mean(X)}{SD(X)}$$

z-score of logarithmic transformed valuable X

$$z-\log(X)=\frac{\log(X)-mean(\log(X))}{SD(\log(X))}$$

where mean(X) shows the average and SD(X) shows the standard deviation.

**Appendix 3**

**Table A2** Overall mean and standard deviation of water temperature, Chl-a, TN, TP and MCs

| Variables | Mean±SD |
|---|---|
| Water Temperature (°C) | 30.2±1.41 |
| Chl-a (µg/L) | 196.3±82.9 |
| Turbidity (F.T.U) | 64.39±17.4 |
| TN (mg-N/L) | 2.21±0.48 |
| TP (mg-P/L) | 0.56±0.14 |
| MC-RR (µg/L) | 24.5±16.3 |
| MC-YR (µg/L) | 4.3±2.3 |
| MC-LR (µg/L) | 7.3±2.6 |

**Table A3** Measurement Data of Water Temperature, TN, TP, Chl-a, MC-RR, MC-YR and MC-LR

Notice: N.A (data not available)

| Month | Sites | Water Temp. (°C) | TN (mg-N/L) Mean±SD | TP (mg-P/L) Mean±SD | Chl-a (µg/L) Mean±SD | Turb. (F.T.U.) | MC_RR (µg/L) | MC_YR (µg/L) | MC_LR (µg/L) |
|---|---|---|---|---|---|---|---|---|---|
| Aug | St-1 | N.A | 1.68±0.05 | 0.20±0.07 | 171.0±3.1 | N.A | 7.28 | 0.54 | 2.28 |
| | St-2 | N.A | 2.12±0.09 | 0.47±0.03 | 191.1±6.2 | N.A | 40.13 | 3.31 | 8.33 |
| | St-3 | N.A | 2.69±0.04 | 0.55±0.01 | 198.6±9.1 | N.A | 24.00 | 2.18 | 4.62 |
| | St-4 | N.A | N.A | N.A | N.A | N.A | N.A | N.A | N.A |
| Sep | St-1 | 30.56 | 1.80±0.06 | 0.70±0.32 | 97.3±9.1 | 56.76 | 49.56 | 2.97 | 6.89 |
| | St-2 | 30.43 | 2.13±0.08 | 0.49±0.02 | 141.7±6.1 | 65.61 | 37.29 | 2.33 | 4.86 |
| | St-3 | 30.35 | N.A | N.A | 141.6±1.2 | 77.04 | N.A | N.A | N.A |
| | St-4 | 30.31 | 2.83±0.16 | 0.64±0.02 | 173.7±4.2 | 87.05 | 58.87 | 3.36 | 4.45 |

**Table A3** (cont) Measurement Data of Water Temperature, TN, TP, Chl-a, MC-RR, MC-YR and MC-LR

Notice: N.A (data not available)

| Month | Sites | Water Temp. (°C) | TN (mg-N/L) Mean±SD | TP (mg-P/L) Mean±SD | Chl-a (µg/L) Mean±SD | Turb. (F.T. U.) | MC_ RR (µg/L) | MC_ YR (µg/L) | MC_ LR (µg/L) |
|---|---|---|---|---|---|---|---|---|---|
| Nov | St-1 | 29.57 | 1.73±0.14 | 0.35±0.01 | 138.7±1.3 | 62.63 | 20.91 | 8.67 | 4.89 |
| | St-2 | 30.22 | 1.64±0.11 | 0.39±0.00 | 141.1±2.1 | 61.15 | 37.05 | 5.60 | 9.69 |
| | St-3 | 30.16 | 1.77±0.07 | 0.50±0.02 | 132.1±2.1 | 64.54 | 43.37 | 8.24 | 10.70 |
| | St-4 | 30.70 | N.A | N.A | 163.3±0.7 | 66.40 | N.A | N.A | N.A |
| Dec | St-1 | 28.98 | 2.03±0.12 | 0.52±0.01 | 210.7±67.2 | 65.73 | 46.86 | 8.24 | 7.71 |
| | St-2 | 28.72 | 2.13±0.31 | 0.39±0.01 | 277.7±1.2 | 79.50 | 7.32 | 6.22 | 8.76 |
| | St-3 | 28.99 | 1.95±0.14 | 0.54±0.06 | 302.2±22.5 | 80.61 | 27.23 | 8.88 | 11.17 |
| | St-4 | 28.65 | 2.23±0.03 | 0.53±0.01 | 248.0±7.9 | 62.68 | 11.59 | 3.10 | 8.52 |
| Jan | St-1 | 28.27 | 2.53±0.17 | 0.46±0.02 | 134.1±0.0 | 50.38 | 14.42 | 4.40 | 9.71 |
| | St-2 | 28.44 | 2.82±0.08 | 0.66±0.00 | 140.3±1.1 | 48.74 | 13.96 | 4.62 | 11.81 |
| | St-3 | 28.98 | 2.43±0.39 | 0.55±0.02 | 147.1±4.2 | 51.63 | 43.48 | 5.12 | 9.30 |
| | St-4 | 27.86 | 1.76±0.14 | 0.50±0.00 | 165. 6±5.0 | 53.96 | 11.73 | 4.00 | 4.84 |
| Feb | St-1 | 31.38 | 1.78±0.12 | 0.57±0.01 | 149.7±21.6 | 46.33 | 12.76 | 4.56 | 4.99 |
| | St-2 | 30.17 | 1.69±0.42 | 0.87±0.01 | 147.1±1.3 | 47.33 | 50.32 | 5.34 | 8.73 |
| | St-3 | 29.74 | 2.12±0.18 | 0.43±0.02 | 184.1±7.4 | 47.72 | 11.70 | 3.63 | 7.13 |
| | St-4 | 30.54 | N.A | N.A | 149.5±4.0 | 44.37 | N.A | N.A | N.A |
| April | St-1 | N.A | 1.86±0.07 | 0.57±0.01 | 132.1±54.7 | N.A | 3.76 | 3.46 | 4.68 |
| | St-2 | 32.26 | 1.86±0.05 | 0.54±0.01 | 125.5±14.8 | 49.28 | 6.25 | 3.25 | 3.92 |
| | St-3 | 33.57 | 2.03±0.29 | 0.57±0.02 | 209.3±38.7 | 37.28 | 6.80 | 2.64 | 5.37 |
| | St-4 | 33.32 | 2.53±0.60 | 0.62±0.17 | 213.9±4.66 | 45.82 | 5.56 | 2.90 | 5.55 |
| June | St-1 | 31.50 | 2.95±0.51 | 0.78±0.02 | 370.3±231.4 | 90.78 | 24.89 | 2.18 | 5.98 |
| | St-2 | 30.93 | 2.47±0.40 | 0.66±0.02 | 417.1±92.5 | 87.84 | 26.84 | 2.31 | 8.72 |
| | St-3 | 30.51 | 3.24±0.35 | 0.75±0.02 | 202.0±128.1 | 86.41 | 17.39 | 2.15 | 5.14 |
| | St-4 | 30.35 | 3.17±0.48 | 0.79±0.02 | 412.9±58.3 | 95.33 | 23.94 | 2.90 | 11.95 |