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PRESENCE AND ABUNDANCE OF CYANOBACTERIA IN SELECTED AQUACULTURE PONDS IN PERAK, MALAYSIA AND THE RELATIONSHIPS WITH SELECTED PHYSICOCHEMICAL PARAMETERS OF WATER

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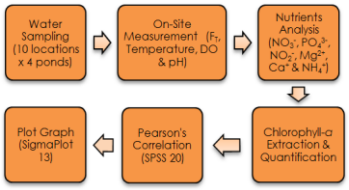
Graphical abstract



Abstract

The presence of cyanobacteria in aquaculture ponds can disrupt the water quality. This study was conducted to determine the abundance of cyanobacteria in selected aquaculture systems in Perak, Malaysia. This study also aimed to identify the relationships between cyanobacterial biomass with physicochemical properties of water. In this study, a total of 40 aquaculture fish ponds were sampled between December 2013 to March 2014 from 10 locations in Perak, Malaysia. The results revealed that cyanobacteria were present in all of the sampled aquaculture ponds. Among all, 75% of the sampled ponds contained chlorophyll-*a* exceeded 50 µg chl-a L^{-1} and the highest chlorophyll-*a* concentration was recorded at 436 µg chl-*a* L-1. This indicates that cyanobacteria are present in abundance in aquaculture water and could pose health risk to human through aquaculture fish consumption. Pearson's correlation analysis suggests that cyanobacterial biomass was significantly correlated to temperature, DO and pH. However, there was no correlation detected among nutrients with cyanobacterial concentration in water column. Regular monitoring of temperature, DO and pH are crucial for an early detection of cyanobacterial occurrence in aquaculture industry in order to avoid economic losses, as well as adverse health impact to consumer.

Keywords: Cyanobacteria, chlorophyll-*a*, instantaneous chlorophyll fluorescence, fish, aquaculture, physicochemical parameters

Abstrak

Kehadiran bakteria-siano dalam kolam akuakultur boleh mengganggu kualiti air. Kajian ini dijalankan untuk menentukan kepesatan bakteria-siano dalam sistem akuakultur terpilih di Perak, Malaysia. Kajian ini juga bertujuan untuk mengenalpasti hubungan antara biomas bakteria-siano dengan ciri fisikokimia air. Dalam kajian ini , sebanyak 40 buah kolam ikan akuakultur telah disampel di antara Disember 2013 hingga Mac 2014 daripada 10 lokasi di Perak, Malaysia. Hasil penelitian menunjukkan bahawa bakteria-siano hadir dalam semua kolam akuakultur yang disampel. Di antara semua kolam yang terpilih dalam kajian ini, 75% daripadanya mengandungi klorofil-*a* melebihi 50 µg chl-a L^{-1} dan kepekatan tertinggi dicatatkan pada 436 µg chl-*a* L^{-1}. Ini menunjukkan bahawa bakteria-siano hadir dalam kuantiti yang besar dalam air akuakultur dan boleh menimbulkan risiko kesihatan kepada manusia melalui pemakanan ikan ternakan. Analisis korelasi Pearson menunjukkan bahawa biomas backteria-siano adalah sangat signifikan terhadap suhu, DO dan pH. Walau bagaimanapun, tidak ada sebarang hubungan diperhatikan di antara nutrien dengan kepekatan bakteria-siano dalam ruang air. Pemantauan secara berkala terhadap suhu, DO dan pH air adalah penting untuk pengesanan awal kejadian bakteria-siano dalam industri akuakultur bagi mengelakkan sebarang kerugian ekonomi serta kesan sampingan terhadap kesihatan pengguna

Kata kunci: Bakteria-siano, klorofil-*a*, floresens klorofil instan, ikan, akuakultur, parameter fizikokimia

1.0 INTRODUCTION

Excessive growth of cyanobacteria is a common concern in aquaculture ponds [1]. This phenomenon causes depletion of oxygen level in water column of aquaculture ponds and subsequently leads to mortality of aquatic species [1-2]. Harmful algal blooms (HABs) in aquaculture industry can cause serious economic losses. Preliminary study on the effect of the bloom to United States economy reported that the country lost more than USD 40 millions per year and at least USD 1 billion per decade [1, 3]. In order to minimise the unnecessary losses in aquaculture sector, an effective approach for aquaculture monitoring must be developed. To tackle this issue, it is very important to draw attention to the root cause which is the environment where the aquatic organisms thrive.

Some cyanobacteria species are capable of producing secondary metabolites called cyanotoxin. Microcystin is the most commonly found cyanobacterial toxin in freshwater system [4-5] and its contamination in aquaculture industry is not a new issue [6-7]. Microcystin enters fish body through gills, diet and food chain [4-5], destroys the liver tissues and causes fish death [5, 8]. Besides, this cyanotoxin also can accumulate in fish tissues and pose health risk to human when consumed [4, 7]. In addition, some cyanobacteria are also capable to synthesise two highly odorous compounds called geosmin and 2-methylisoborneal (MIB) that can cause earthy-musty taste on fish [9-10].

Environmental factors such as total nitrogen, total phosphorus, water temperature and pH were found to be closely related to cyanobacterial occurrence in water column [11]. pH and DO in particular were reported to have strong correlations with algal concentration in water column [12-13] thus, regular monitoring of these two parameters is essential as an alert to the occurrence of severe cyanobacterial bloom [13]. Apart from that, cyanobacteria capability to out-compete for nutrients and its tolerance to warm water temperature [1] causes problem when this particular algae take the advantage of eutrophic aquaculture situations especially in Malaysia climate. Magnesium and calcium, two types of nutrients responsible for water hardness as well as alkalinity on the other hand, were reported to have an inverse relationship with algal growth [14].

Many studies have been conducted since the last two decades to investigate the correlation between environmental variables with cyanobacterial concentration in water bodies. However, these studies were mainly focused on the oceans and lakes [11]. Moreover, there are still limited number of published literatures found on research conducted in Malaysia to assess the relationship between physicochemical parameters of water with cyanobacterial biomass in aquaculture ponds. Available studies [15-16] carried out in a single location is inadequate to represent Malaysia aquaculture system as a whole. Furthermore, research on HABs in Malaysia was reported to be still insufficient to guarantee public health and food safety [17].

Since there are lacks of cyanobacteria research in Malaysia especially in aquaculture industry, this study was conducted to determine the abundance of cyanobacterial biomass in aquaculture system in Perak, Malaysia. On top of that, this study also aims to identify the relationship between environmental factors (e.g. temperature, DO, pH and nutrients) with relative abundance of cyanobacteria in fish aquaculture ponds by assessing the relationships in ten different locations. This research is crucial for the purpose of aquaculture monitoring particularly in Malaysia aquaculture system. Besides, this study is also important for public health risk protection to ensure safe fish supply to be delivered to consumers.

2.0 EXPERIMENTAL

2.1 Location and Description of Study Ponds

Forty aquaculture ponds from a total of ten locations in Perak, Malaysia were selected in this study. In more specific, the ponds are located in Behrang, Tapah, Temoh, Chenderiang and Air Kuning (Figure 1). The Global Positioning System (GPS) coordinates of sampling locations are as shown in Table 1. All of the chosen aquaculture ponds comprised of earth ponds in use for fish production business. The fish farms ranged from small, medium and up to big farms with about 100 ponds and red tilapia was found to be the most common fish selected for the business. Water source was obtained from the nearby natural water bodies.

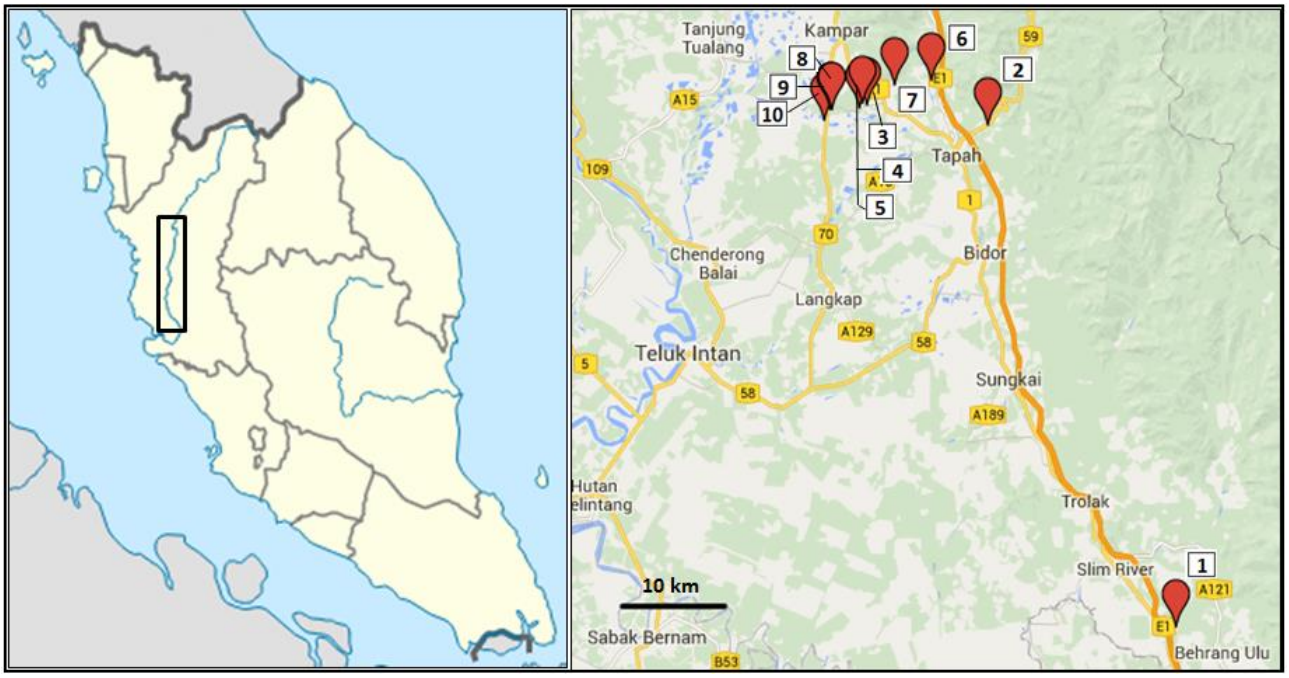


Figure 1 Map of ten study locations around Perak, Malaysia. (1) Behrang; (2) Tapah ; (3) Temoh I; (4) Temoh II; (5) Temoh III; (6) Chenderiang I; (7) Chenderiang II; (8) Air Kuning I; (9) Air Kuning II; (10) Air Kuning III. (Bar = 10 km)

Table 1 Global Positioning System (GPS) coordinates of sampling location

Location	GPS Coordinate
1	3.783455, 101.458448
2	4.225602, 101.290220
3	4.244946, 101.177267
4	4.244775, 101.173662
5	4.245974, 101.182073
6	4.267876, 101.238398
7	4.263939, 101.205611
8	4.238325, 101.148491
9	4.238753, 101.149800
10	4.229358, 101.142354

2.2 Water Sampling

Sampling was carried out in each aquaculture system between December 2013 to March 2014. For each farm, a total of four ponds were sampled randomly. Water temperature, dissolved oxygen (DO) and pH were measured on-site with portable probe (YSI 550A) at a depth of 0.5m from the water surface. Water sample was then grabbed from 0.15m below the water surface. Approximately 2 mL of the grabbed water sample was placed in a cuvette to measure the instantaneous chlorophyll fluorescence (F_T) of cyanobacteria with AquaPen-C (AP 100) while the remaining water was stored immediately into High-Density Polyethylene (HDPE) bottle. The sample bottles were placed in a cooler container containing ice in order to maintain the freshness of sample as well as to protect the samples from sunlight. Water samples were brought back to laboratory for subsequent analysis.

2.3 Nutrients Analysis

Nutrients were analysed with ion chromatography (DIONEX ICS-1100 RFIC, ASDV) within two days after the sample collection [18]. Six types of nutrients which can be classified into two groups were quantified in this study: (i) Anions: nitrate, phosphate, nitrite; and (ii) Cations: magnesium, calcium and ammonium. Water samples were pre-filtered through 0.45 µm membrane filter prior to analysis. Ultra-pure water (Sartorius Stedim Biotech) was used throughout this study and ion analysis was carried out according to Jackson (2000) [18]. The results were compared against ion standards. Mobile phases used were (i) Anions: 0.5M sodium carbonate concentrate and 0.5M sodium bicarbonate concentrate; and (ii) Cations: Methanesulfonic acid.

2.4 Chlorophyll-*a* Extraction and Quantification

Chlorophyll-*a* is a photosynthetic pigment commonly found in both eukaryotic (algae) as well as prokaryotic algae (cyanobacteria). This pigment has been widely used as a proxy for total phytoplankton biomass [19-20] and normally associated with the presence of cyanobacteria [21].

Chlorophyll-*a* was extracted and quantified according to Standard Methods [22]. Water samples ranged between 60 mL to 650 mL, were filtered through glass fiber filter paper (GF/C 47mm Whatman Glass Microfibre filters paper) to collect algal cells as much as possible. Filter papers containing algal cells were then freeze-thawed three times prior to extraction with 10 mL 90% v/v acetone (Bendosan, AR

grade) in centrifuge tubes. Sonicator was used to break the algal biomass in cold water bath for 10 minutes. After that, the sample extracts were subjected to centrifugation for 5 minutes at 3800 rpm to separate particulate materials from chlorophyll suspension. The absorbance of chlorophyll-*a* extracts were measured with a spectrophotometer (PRIM 1835 by SECOMAM CE) at 750nm and 665nm against 90% v/v acetone blank before and after acidification with 0.2 mL 1% v/v hydrochloric acid (HCl). Total chlorophyll-*a* was calculated with revised Lorenzen's 1967 [23] equation [24].

2.5 Statistical Analysis

Data collected were analysed with Statistical Package for Science Social (SPSS) program version 20. Pearson's correlation was used to determine the strength of correlation between temperature, DO, pH and nutrients with cyanobacterial biomass. Data were log-transformed to reduce the heteroscedasticity and to standardise the variance, when necessary. The graphs were plotted with SigmaPlot version 13.

3.0 RESULTS AND DISCUSSION

3.1 Validation of Total Chlorophyll-*a* for Cyanobacterial Biomass Estimation

The validity of total chlorophyll-*a* to be used to estimate cyanobacterial biomass was confirmed through the correlation established between total chlorophyll-*a* with instantaneous chlorophyll fluorescence (F_T) of cyanobacteria. Instantaneous chlorophyll fluorescence (F_T) has been used for decades to measure chlorophyll fluorescence in the living algal cells [25] as well as to detect the presence of cyanobacteria in water column as it provides rapid and real-time status of cyanobacteria [26]. AquaPen-C (AP 100) quantifies F_T at red-orange excitation light (620nm) for excitation through phycobilins.

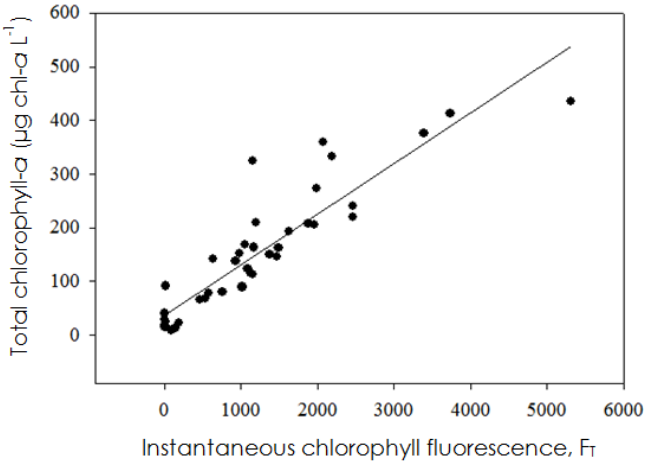


Figure 2 Regression analysis for relationship between total chlorophyll-*a* and instantaneous chlorophyll fluorescence at 620nm (y = 0.09x + 38.08)

Cyanobacteria were found to be present in all selected aquaculture ponds during the sampling period and a strong positive correlation was obtained between F_T with total chlorophyll-*a* of phytoplanktons (P <0.001, r =0.91) (Figure 2). Highly correlated result indicated that the variability of chlorophyll-*a* quantified in this study can be used as a proxy to estimate the variability of cyanobacterial biomass.

3.2 Occurrence and Abundance of Cyanobacterial Biomass in Aquaculture Systems

Most of the ponds (75%) were experiencing cyanobacterial bloom as the chlorophyll-*a* concentrations were above the level of bloom definition of 50 µg chl-*a* L^{-1} [27]. The highest concentration of cyanobacteria was detected in a pond in location 6 which was 436 µg chl-*a* L^{-1}, followed by location 9 (414 µg chl-*a* L^{-1}) and location 5 (377 µg chl-*a* L^{-1}). The lowest biomass was observed in location 10 as all of the sampled ponds were having cyanobacterial concentrations below 50 µg chl-*a* L^{-1} (Figure 3).

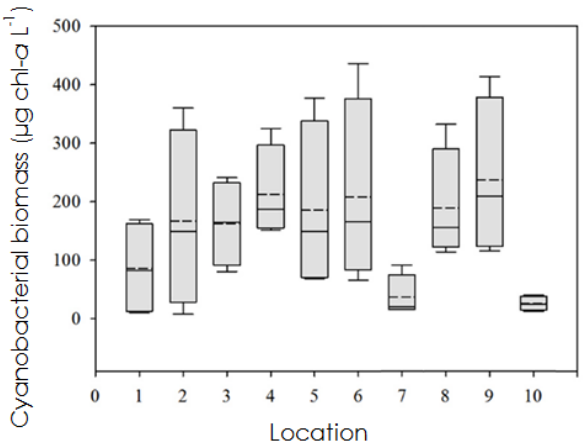


Figure 3 Boxplots of cyanobacterial biomass in ten selected study ponds around Perak, Malaysia during sampling period. (1) Behrang; (2) Tapah; (3) Temoh I; (4) Temoh II; (5) Temoh III; (6) Chenderiang I; (7) Chenderiang II; (8) Air Kuning I; (9) Air Kuning II; (10) Air Kuning III

A productive aquaculture pond normally has about 50 to 200 µg L^{-1} chlorophyll-*a* [2]. In this study, 45% of the sampled ponds were having biomass within the productive range while 35% of them had above 200 µg chl-*a* L^{-1}. In comparison to other studies conducted in aquaculture ponds in Malaysia, these results can be considered alarming. A research conducted in Indigenous Fisheries Research and Production Centre (IFRPC) reported that 83% of the quantified chlorophyll-*a* were below 20 µg chl-*a* L^{-1} and the highest recorded biomass was 172.12 µg chl-*a* L^{-1} [15].

3.3 Physicochemical Characteristics of the Study Aquaculture Ponds

Physicochemical characteristics of water sample varied between locations and ponds (Table 2). The temperature ranged between 28.9°C to 34.4°C, and most ponds were having temperature below 32°C. Water temperature between 25°C to 32°C was reported to be optimum for the growth of freshwater species [2]. DO, the most crucial water parameter in aquaculture water [2] ranged between 5.57 mg L^{-1} to 11.54 mg L^{-1} in this study. This range of DO concentration is classified as best condition for aquatic growth [2]. The water pH during sampling ranged between 7.19 to 9.74. Aquaculture water with pH 7 to 9 is categorised as an ideal pH for the growth of fish as well as crustaceans while pH 9 to 11 can cause slow development to aquatic species [2]. Among all of the analysed dissolved inorganic nutrients in water sample, only nitrate fell in the range of desired concentration (0.2 – 10 mg L^{-1}) [2]. Magnesium concentration was much lower than the preferred concentration of 100 mg L^{-1}. Some of the collected water samples had ammonium and calcium below and within the acceptable ranges. No data recorded for phosphate and nitrite as the concentrations were below the detection limit.

Table 2 Physicochemical characteristics of the selected aquaculture ponds

Loc	Temp (°C)	DO (mg L^{-1})	pH	Nutrients (mg L^{-1})			
				NO_3^-	Mg^{2+}	Ca^+	NH_4^+
1	29.7±0.4	5.57±0.93	7.19±0.34	0.34±0.02	0.84±0.22	8.86±4.68	0.0079±0.0010
2	29.4±0.8	9.44±4.38	8.05±1.56	0.58±0.56	0.64±0.22	5.70±1.62	0.0497±0.0905
3	29.1±0.7	5.63±1.51	7.75±0.76	0.28±0.02	1.03±0.58	4.02±1.78	0.0006±0.0000
4	28.9±0.6	7.59±0.71	7.25±0.12	0.34±0.05	0.23±0.09	2.26±1.39	0.0031±0.0024
5	32.1±1.3	9.25±3.16	8.89±0.98	0.28±0.03	0.93±0.90	3.54±2.66	0.0478±0.0083
6	29.2±0.8	9.71±1.56	8.27±1.25	0.36±0.16	0.59±0.46	3.70±3.57	0.0106±0.0103
7	31.8±0.2	5.69±1.48	7.97±0.76	0.35±0.07	1.37±0.89	5.64±3.91	0.4103±0.8166
8	30.8±0.5	11.54±1.73	9.74±0.37	0.34±0.05	2.87±0.73	13.69±3.48	0.0091±0.0069
9	31.4±0.5	10.98±4.57	9.53±0.98	0.29±0.05	3.37±0.26	17.88±4.75	0.2204±0.3781
10	34.4±0.3	7.61±1.95	8.20±0.74	0.31±0.09	1.63±0.18	16.79±4.40	0.5533±1.0721

Note: Data above are expressed in Mean ± Standard deviation.

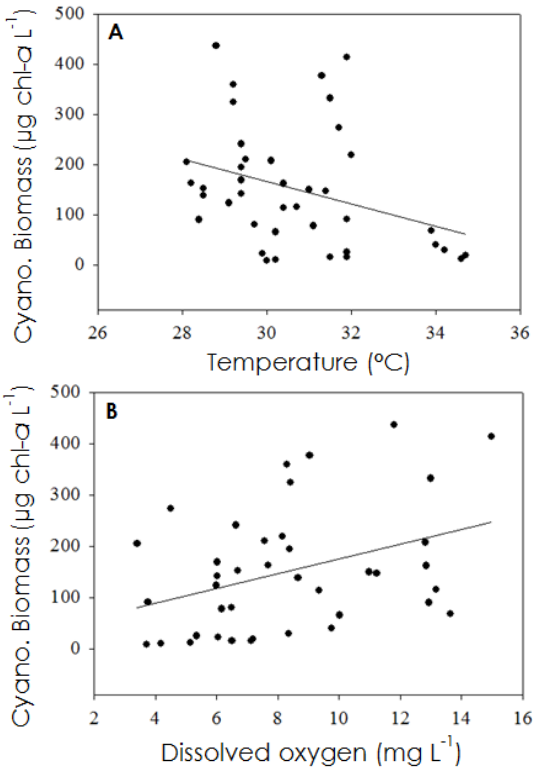
3.4 Relationship Between Physicochemical Parameters of Water with Cyanobacteria

A significant correlation (P<0.05) was found between temperature, DO and pH with cyanobacterial biomass, however, no relationship was detected between any of the nutrients with cyanobacterial intensity in water column (Table 3). Besides, there were no phosphate and nitrite analysed in the water sample.

Table 3 Relationship between physicochemical parameters of water with cyanobacterial biomass in selected aquaculture ponds

Parameter	Chlorophyll-*a*
(a) Temperature (°C)	-0.434**
(b) Dissolved oxygen (mg L^{-1})	0.424**
(c) pH	0.321*
(d) Nutrients (mg L^{-1})	
- Nitrate	-0.145
- Phosphate	-
- Nitrite	-
- Magnesium	-0.113
- Calcium	-0.239
- Ammonium	-0.138

Note: *p < 0.05; **p < 0.01; " - " = no correlation



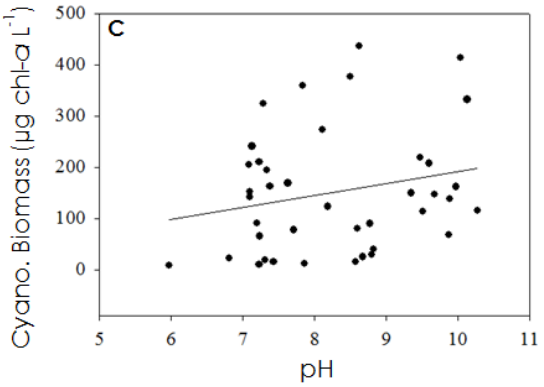


Figure 4 Regression analysis for relationships between temperature, DO and pH with cyanobacterial biomass in selected aquaculture ponds. (A) Temperature (y = -22.47x + 840.18), (B) DO (y = 14.43x + 31.19) and (C) pH (y = 23.27x – 41.81)

The water temperature in all sampled ponds was quite high, and most of them were around 30°C during sampling (Table 2). Most species of cyanobacteria attained optimum growth rate within the temperature range of 25°C [28] to 35°C [29-30]. Other studies reported that some species of cyanobacteria such as *Microcystis sp*. can out-compete other phytoplanktons at 30°C and above [31]. As we can see from the result (Figure 4), temperature has negative correlation with cyanobacterial biomass whereby the chlorophyll-*a* concentration dropped significantly at temperature above 32°C. This suggests that cyanobacteria may be able to thrive to a certain extent of high temperature in freshwater system. Similar finding was also reported by a research conducted in Sarawak whereby a significant negative correlation was observed between cyanobacterial cell density with temperature when the range of temperature was between 27.10°C to 32.30°C, however, no correlation observed at higher temperature (26.98°C - 34.41°C) [15].

A significant correlation was also observed between DO with cyanobacterial biomass (Figure 4). DO was reported to have a link with chlorophyll-*a* concentration [32] especially in a reservoir with low water exchange or closed fish farms [33]. This was because of the direct effect of algal photosynthesis on DO concentration [33]. Apart from algal photosynthesis, aquatic respiration also contributing to this effect and they were reported to show diurnal variations in eutrophic water [11]. Table 2 shows that DO measured in the selected aquaculture ponds in Perak varied significantly from 3.41 mg L^{-1} to 14.98 mg L^{-1}. Similar result was also reported by a research conducted on *Tor tambroides* ponds in Serian, Sarawak [15].

Water pH was detected to have a positive correlation with cyanobacterial biomass in this study (Figure 4). Cyanobacteria activity and intensity in water column were reported to be influenced by the water pH [34]. During intense bloom, photosynthetic activities of phytoplanktons increase and cause depletion of free carbon dioxide. This subsequently leads to increase in pH value which favours the dominance of cyanobacteria [35]. At high pH, not all types of phytoplanktons are capable to utilise carbon as efficient as cyanobacteria. Dominance of cyanobacteria was due to its lower half-saturation constants (Ks) for CO_2 and its competitive advantage over other phytoplanktons as cyanobacteria can use both free CO_2 as well as bicarbonate (HCO_3^-) as source of carbon during photosynthesis [35-36]. In addition, buoyancy characteristic in some cyanobacteria also allow this species to move up and down for the carbon and further reduce the carbon dioxide to a level below the utilisation limit of other phytoplanktons [36-37].

Many scientific literatures stated that cyanobacterial proliferation is closely related to nutrient concentration in water bodies [27]. This phenomenon was also reported in aquaculture ponds [38]. On the contrary, there are also literatures stated that cyanobacterial abundance in aquaculture ponds was affected by a combination of multiple environmental factors and not solely due to nutrients [15-16]. This was also proven in this study which found no correlation between any of the six examined nutrients with cyanobacteria proliferation in aquaculture ponds. This suggests that temporal and spatial variations may exist between nutrients and cyanobacterial biomass likewise their compositions as well as toxicity [27].

Phosphate was below detection level in the water sample (Table 2). The main reason for this could probably due to minimal input of phosphorus into the aquaculture water body since the concentration of phosphorus in most fish feeds are relatively quite low. Unlike plant fertilizer, fish manure normally contains insignificant amount of phosphorus. Phosphorus is one of the 20 inorganic minerals which composed about 1.0% - 2.5% of fish diet [39]. Undetected phosphate concentration could also be because of the ion chromatography analysis that only quantifies soluble reactive phosphate (SRP) in the form of orthophosphate instead of the total phosphorus itself [18]. Good control system in terms of isolated location and aquaculture pond management plays an important role as well in minimizing external eutrophication from disrupting the water quality. This finding indicated that orthophosphate may or may not give impact on cyanobacteria proliferation in aquaculture ponds. Although it was assumed that cyanobacteria favours high concentration of phosphorus and nitrogen, there was also report stated that cyanobacterial bloom often taking place when the concentration of SRP is the lowest [27]. Insignificant relationship between orthophosphate with cyanobacteria cell density and chlorophyll-*a* concentration was also observed in both studied aquaculture ponds in Serian, Sarawak [15-16].

Similar to phosphate, nitrite was also not detected in this study (Table 2). Nitrite present in aquaculture water is commonly the intermediate product of nitrification process [40]. In aquaculture pond, large amount of nitrogen is introduced into the water body through fish feeds [2] as most of the fish fertiliser for growing fish

contains about 32% - 45% of protein [39]. Despite so, nitrite normally did not accumulate in water column as it will be quickly converted into nitrate [41]. Besides, ammonia assimilation by phytoplanktons and other aquatic plants also limits the concentration of ammonia available for nitrification, hence further reduce the production of nitrite [41]. Nitrite concentration in aquaculture water was also reported to be very low, and the acceptable range is below 0.1 mg L^{-1} [2]. Water analysis conducted in freshwater fish (*Tor tombroides*) ponds in Sarawak detected between 0.001 mg L^{-1} to 0.007 mg L^{-1} nitrite in the water sample throughout the study period and reported that this parameter has no significant relationship with both cyanobacteria cell density as well as chlorophyll-*a* concentration in aquaculture water body [15].

4.0 CONCLUSION

The presence of cyanobacteria was detected in all selected aquaculture ponds in Perak, Malaysia and the concentrations varied between location and pond. Seventy-five percent (75%) of the sampled ponds were suffering from cyanobacterial bloom as the biomass were above 50 µg chl-*a* L^{-1}. Cyanobacteria intensity of up to 436 µg chl-*a* L^{-1} could pose health risk to human particularly to Malaysian through freshwater fish consumption. This study showed that temperature, DO and pH have significant relationships with cyanobacterial biomass. Temperature, a variable found to have a negative correlation with chlorophyll-*a* concentration in this study showed significant decline in biomass at temperature above 32°C. There were no correlations obtained between nutrients with cyanobacteria intensity in water column. This confirmed that nutrients are not the only factors affecting cyanobacterial abundance in aquaculture ponds, but rather combination of multiple environmental factors. The findings of this study highlighted the needs to monitor temperature, DO and pH in order to provide an alert to the occurrence of cyanobacteria in aquaculture industry. This is critical to prevent significant economic losses in aquaculture business, as well as negative impact on public health. Occasional nutrient monitoring is advisable, however, it might not be necessary if the aquaculture ponds are not exposed to external eutrophication sources such as agriculture and residential areas.

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