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DIVERSITY AND SIMILARITY AMONG CYANOBACTERIA ASSEMBLAGES FROM SELECTED AQUATIC ECOSYSTEMS IN SARAWAK USING β –INDICES

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ABSTRACT

A study was carried out to evaluate the diversity and similarity of cyanobacterial populations in selected Sarawak aquatic ecosystem using β-indices. Eight stations including aquaculture ponds, cage cultures, waterfall and artificial lake located in Serian, Bau and Batang Ai areas were selected. A total of 43 species belonging to 30 genera of cyanobacteria were recorded. The most distributed pattern among all sampling stations belongs to the genera *Chroococcus, Lyngbya, Nostoc* and *Oscillatoria.* The highest β diversity values were found among non-contiguous stations. Besides, no identical or totally different cyanobacteria diversity values were obtained among those non-contiguous stations. The highest β diversity value (0.84) was found among stations with contrasting environmental characteristics. The wide range of β-diversity and similarity suggested that different locations and types of aquatic ecosystems may have variations in physico-chemical properties of the water and eventually lead to the different composition of cyanobacteria.

Keywords: cyanobacteria, composition, diversity, β-indices, Sarawak

INTRODUCTION

Cyanobacteria, which are also known as blue green algae, belong to the class Cyanophyceae. These microalgae have the characteristics of both bacteria, which have prokaryotic cell organization and algae, which has the ability to carry out photosynthesis as in plants. They have the ability to grow in most diverse ecological conditions and are very beneficial to mankind. For example, *Spirulina* is a source of food for the native people near Lake Chad, Africa (Sze 1998). In other circumstances, however, they may produce toxins that can cause deleterious effects on human and animal health (Skulberg *et al.* 1993).

Presently, there is still insufficient regional information on diversity and species numbers for the microalgae (Norton *et al.* 1996) particularly cyanobacteria. There is optimism that future research in uninhabited areas such as Antarctica, Asia and Australia for microalgae particularly cyanobacteria will be established to collect more data (Norton *et al.* 1996). A survey of the algae inhibiting waterholes in an area of New South Wales in Australia revealed no fewer than 530 taxa (except diatoms) and this case will possibly contribute to the future science fields (Ling & Tyler 1996). Sarawak, which is the largest state in Malaysia, is rich in diversity and natural resources. However, there are still inadequate number of research regarding microalgae diversity particularly cyanobacteria.

Abang (2003) has carried out survey in various natural water bodies in Sarawak and identified eleven genera namely *Anabaena, Aphanizomenon, Aphanocapsa, Aphanotheca, Borzia, Chrooccoccus, Merismopedia, Microcystis, Oscillatoria, Planktolyngbya* and *Spirulina.* Ramlah (2005) has identified nine genera in selected aquaculture ponds namely *Anabaena, Anacystis, Calothrix, Chamaesiphonales, Gloeotrichia, Lyngbya, Microcystis, Oscillatoria*, and *Spirulina*. Based on Ressom *et al*. (1994) and Skulberg *et al*. (1993), out of 15 genera identified by Abang (2003) and Ramlah (2005), there were seven potential toxin-producer cyanobacteria, namely *Anabaena, Aphanizomenon, Gloeotrichia, Lyngbya, Microcystis, Oscillatoria* and *Spirulina.*

During dry season of 2005, 38 genera of cyanobacteria were recorded to have inhibited Sungai Semadang and Sungai Bengoh. Ten of these

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genera were listed under potential toxin-producing genera (Ruhana *et al*. 2006). They also reported that the four common genera found in almost all sampling stations along the rivers namely, *Lyngbya, Oscillatoria, Scytonema* and *Spirulina* were also categorized as potential toxin producers as suggested by Ressom *et al*. (1994) and Skulberg *et al*. (1993). These works had shed some lights on diversity of cyanobacteria in selected Sarawak waters. However, if comparison is made between number of studies and data collected with the large number of aquatic environments in Sarawak, one may say that the knowledge about cyanobacteria with respect to their diversity is very limited.

The component of diversity that measures the differences among communities in terms of species composition is β-diversity (Whittaker 1972; Magurran 1988). Several factors that have been recognized to influence the species turnover, are mainly related to the environment and heterogeneity (Goettsch & Hernandez 2006). In contrast, β-similarity is the mean proportion habitat or communities occupied by a single species. It allows comparison of habitat similarity of two different study systems and provides information about the degree of partitioning of habitat by species Routledge (1977).

The objective of this study was to evaluate the diversity and similarity of cyanobacterial populations in selected Sarawak aquatic ecosystems with using β- indices. In this study, the formula provided by Wilson & Shmida (1984), was selected on the basis that it can measure the continuity of species between communities (Koleff *et al*. 2003). Furthermore, there are also different indices that allowed evaluating the biotic similarities among different communities or regions such as Jaccard's similarity index (Magurran 1988). Thus, to complement the β diversity analysis, the similarity among the different assemblages was measured using Jaccard's similarity index.

MATERIALS & METHODS

Cyanobacterial Collection

Eight stations including aquaculture ponds, cage cultures, waterfall and artificial lake located in Serian, Bau, and Batang Ai area were selected in this study. The details of the locations are stated in Table 1. Sampling was conducted in November 2007.

Cyanobacterial samples were collected using Van Dorn Bottle and sieved through 20 µm mesh size sieve. Specimen retained in the sieves were kept in separate bottles, preserved with Lugol's solution and transported back to labs for identification. Due to limited budget and time constraint, sampling exercises were carried out once for every location. For each sampling, samples were collected in three replicates.

Table 1. Global Positioning System (GPS) coordinates and brief description of sampling stations

Station	GPS Coordinate	Location Description
1	N 01° 0.8641', E 110° 34.829'	Ranchan Pool, located near Serian town. Popular recreational waterfall area among the locals. Approximately 80 km from Kuching city.
2	N 01° 24.889', E 110° 08.904'	Tasik Biru, located in Bau district. Formed from an open cast gold mining pit. The water contains high concentration of arsenic as declared by NREB* Sarawak.
3	N 01° 11.701', E 110° 31.216'	Located at IFRPC**, Tarat, Serian. Earth pond layered with black HDPE^{+}, has been stocked with 120 individuals of F1 *Tor tambroides*$^{++}$ fries.
4	N 01° 12.139', E 110° 31.380'	Located at IFRPC, Tarat, Serian. Earth pond that has been stocked with 70 individuals of F1 *T. tambroides* juveniles.
5	N 01° 11.247', E 111° 51.732'	Cage culture situated at Batang Ai dam area, stocked with 45 individuals of F1 *T. tambroides.* The depth of the cage net was approximately 2.5 m
6	N 01° 10.883', E 111° 51.958'	Cage culture at Batang Ai dam, stocked with 52 individuals of F1 *T. tambroides*. Near to Station 5, about the same description as Station 5.
7	N 01° 10.551', E 111° 42.776'	Outside the cages, depth was approximately 30 m.

8	N 01° 08.369’, E 111° 52.218’	Earth pond at Batang Ai Inland Fisheries Station that has been stocked with 50 individuals of *T. tambroides* juveniles. Very shallow pond (Depth: ±0.2 m)

Stations 1, 3 and 4 were located in Serian whereby stations 5-8 were located in Batang Ai, Sibu. Station 2 located in Bau.

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$^{+}$ High Density Polyethylene

$^{++}$ Species of Mahseers (locally known as “empurau” or “kelah”)

Cyanobacterial identification

Observation of cyanobacteria was carried out using the Inverted Light Microscope Olympus M1025 – Microscope Research Fluorescence Model 1X51RFLCCD. Species identification was based on keys according to Anagnostidis & Komarek (1985, 1986, 1988, 1989, 1991), Bold & Wayne (1985), Graham & Wilcox (2000), Hoek *et al.* (1995), Prescott (1978), Aishah (1996) and Sze (1998). List of cyanobacteria found was compiled.

β-Indices Analysis

The relative frequency (*f*) of each species in the sampling stations was estimated according to the formula described by Goettsch & Hernandez (2006):

$$f = ss / ts$$

where,

ss = number of stations/sites in which the species occurs

ts = total number of stations/sites

The β-diversity values were estimated using the formula described by Wilson & Shmida (1984), which was as follows:

$$\beta = (b + c) / 2a + b + c$$

where,

β = beta diversity

a = total number of cyanobacteria species that occur in both habitats

b = total number of cyanobacteria species that occur in the neighboring habitat but not in the focal one

c = total number of cyanobacteria species that occur in the focal habitat but not in the neighboring one

In order to complement the β-diversity analysis, Jaccard’s index of similarity (Mueller-Dombois & Ellenberg 1974) was calculated. The similarity values were obtained by the formula:

$$C_J = a / a + b + c$$

where,

C_J = index of similarity

a = total number of cyanobacteria species that occur in both habitats

b = total number of cyanobacteria species that occur in the neighboring habitatbut not in the focal one

c = total number of cyanobacteria species that occur in the focal habitat but not in the neighboring one.

RESULTS AND DISCUSSION

Species Composition

A total of 43 species belonging to 30 genera were recorded in all sampling sites (Table 2 & Table 3).

Oscillatoria was the best represented genus, as they comprised 9% of the species composition in the samples. The results obtained during this study have a slight difference with the previous study done by Abang (2003) and Ramlah (2005) with the absence of four genera namely *Anacystis*, *Aphanizomenon*, *Calothrix* and *Planktolyngbya*. However, this study is in agreement with those two studies with respect to the presence of *Anabaena*, *Microcystis*, *Oscillatoria* and *Spirulina* (Table 2). The number of sampling sites involved, sampling time, weather and types of sampling sites were the possible contributors to the variation in cyanobacterial composition found in the above mentioned studies.

This study showed variation in terms of species number among different localities. The highest number of species was found in station 4 (earth pond, Tarat), where 18 species were recorded (Figure 1). In contrast station 2 which recorded 5 species was the lowest. These differences were perhaps related to the types of the sampling sites *i.e.* HDPE (High Density Polyethylene) pond, earth pond, cage culture ponds, dam water, lake and waterfall.

Table 2. Comparisons of cyanobacteria genera between two previous studies and this study

Cyanobacteria Genera	Abang (2003)	Ramlah (2005)	This study
Anabaena	+	+	+
Anabaenopsis	-	-	+
Anacystis	-	+	-
Aphanizomenon	+	-	-
Aphanocapsa	+	-	+
Aphanothece	+	-	+
Arthrospira	-	-	+
Borzia	+	-	+
Calothrix	-	+	-
Chamaesiphon	-	+	+
Chroococcus	+	-	+
Cylindrospermopsis	-	-	+
Dactyloccopsis	-	-	+
Gloeocapsa	-	-	+
Gloeotrichia	-	+	-
Gloeothece	-	-	+
Lyngbya	-	+	+
Marssoniella	-	-	+
Merismopedia	+	-	+
Microcystis	+	+	+
Myxobactron	-	-	+
Nostoc	-	-	+
Oscillatoria	+	+	+
Phormidium	-	-	+
Planktolyngbya	+	-	-
Pleurocapsa	-	-	+
Pseudoanabaena	-	-	+
Pseudocapsa	-	-	+
Rhabdoderma	-	-	+
Scynechocystis	-	-	+
Synechococcus	-	-	+
Scytonema	-	-	+
Spirulina	+	+	+
Tolypothrix	-	-	+
Tychonema	-	-	+
Number of Genera	11	9	30

The symbol '+' represents present while symbol '-' represents the absence of the genera.

Table 3 shows the individual species distribution pattern in all sampling stations. The most distributed pattern was observed for four species, which were *Chroococcus* sp., *Lyngbya* sp., *Nostoc* sp. and *Oscillatoria* sp. These species are widespread and have an almost continuous distribution among the sampling stations. Genus *Chroococcus* was found in almost all aquatic ecosystems and some terrestrial habitats (Graham & Wilcox 2000). Based on Sze (1998), genera *Lyngbya* sp., *Nostoc* sp. and *Oscillatoria* sp. could be found in almost all stations and they could act as indicator for eutrophic and polluted water.

Microcystis spp. was more abundant in earth aquaculture ponds, which were station 4 and 8 compared to other locations (Table 3). This was expected since *Microcystis* is one of the dominant cyanobacteria that is associated with almost permanent blooms in tropical freshwaters that are exposed to constant sunshine, warmth and nutrients such as phosphate, silicate, nitrate, carbon dioxide and lime (Frankelin 1972). Formation of the bloom near the surface of the pond was essentially due to the buoyant nature of the cyanobacteria (Chorus & Cavalieri 2000).

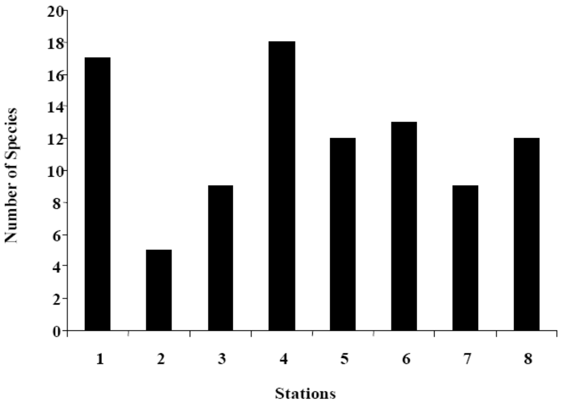

Figure 1. Variation of cyanobacteria species number in all sampling stations.

Table 3. Presence (●) of cyanobacteria species and total number of species per sampling station

	Species	Stations studied							
		1	2	3	4	5	6	7	8
1	*Anabaena circinalis*				●				
2	*Anabaena planctonica*				●				
3	*Anabaena* sp.				●	●			
4	*Anabaenopsis* sp.				●				
5	*Aphanocapsa endophytica*	●							
6	*Aphanocapsa* sp.	●				●	●		●
7	*Aphanothece microspora*	●							
8	*Aphanothece* sp.	●				●	●		●
9	*Arthrospira* sp.	●							
10	*Borzia* sp.	●							
11	*Chamaesiphon* sp.							●	
12	*Chroococcus limneticus*				●				
13	*Chroococcus minor*	●							
14	*Chroococcus* sp.	●	●	●	●	●		●	●
15	*Cylindrospermopsis* sp.	●			●				
16	*Dactyloccopsis* sp.	●	●				●	●	●
17	*Gloeocapsa* sp.			●		●	●	●	●
18	*Gloeothece* sp.						●		
19	*Lyngbya birgei*				●				
20	*Lyngbya* sp.	●			●	●	●	●	●
21	*Marssoniella* sp.						●		
22	*Merismopedia* sp.	●							
2	*Microcystis aeruginosa*				●				
24	*Microcystis flos-aquae*				●				
25	*Microcystis* sp.				●				●
26	*Myxobactron* sp.					●		●	
27	*Nostoc* sp.	●	●	●	●	●	●		●
28	*Oscillatoria quasiperforata*			●					
29	*Oscillatoria nigra*			●					
30	*Oscillatoria princeps*				●				
31	*Oscillatoria* sp.	●	●	●	●	●			●
32	*Phormidium* sp.		●			●	●		●
33	*Pleurocapsa* sp.			●	●				
34	*Pseudoanabaena* sp.	●							●
35	*Pseudocapsa* sp.							●	
36	*Rhabdoderma* sp.						●		
37	*Scynechocystis aquatilis*			●					
38	*Synechococcus* sp.	●					●		
39	*Synechocystis* sp.			●	●		●		●
40	*Scytonema* sp.	●				●	●	●	
41	*Spirulina* sp.				●				
42	*Tolypothrix* sp.					●			
43	*Tychonema* sp.							●	
	Number of species	17	5	9	18	12	13	9	12

Relative Frequency

Figure 2 shows the number of species grouped into different values of relative frequency (see also Table 4). In total, there were six different values of relative frequency for the 43 species. The result showed that only six species (14%) have frequencies above the mean value (f = 52.1%). This was clear indicator of the high discontinuous or the markedly restricted distribution of cyanobacteria among sampling sites. Thus, it is suggested that cyanobacteria are common microorganism in aquatic ecosystems but several species require specific conditions to grow and flourish.

The lowest frequency value (f = 12.5%) in all samplings sites correspond to 26 species found which notably constitute 60.5% of the species. It is important to mention that some genera especially *Anabaena*, *Microcystis*, *Oscillatoria* and *Spirulina* that were classified as potentially toxin producer by Ressom *et al.* (1994) and Skulberg *et al.* (1993) is extremely narrow in distribution (Table 4). However, not all of the low frequency species have narrow distribution, for example *Anabaena* are widespread among the filamentous, heterocyst forming genera (Stewart 1973) and *Microcystis* which are usually found in eutrophic ecosystems (Wetzel 1983) have faster growth rates (Mardhiah 2006).

It was likely that low frequency of some species can be associated with the specific environment characteristics that limit the spread. For instance, *Microcystis* require suitable light conditions, less resource competition, suitable acidity or alkalinity, cellular nutrient storage and competition for trace elements (Yoshinaga *et al.* 2006).

The highest occurrence frequency value of f = 87.5 was recorded for *Chroococcus* sp. and *Nostoc* sp., indicating their very large distribution range. This data was supported by Graham & Wilcox (2000) and (Stewart 1973), who documented that these two genera were commonly found in almost all tropical freshwater ecosystem. However, the frequency of the occurrence of cyanobacteria may vary depending on the type of environment, light intensity, temperature, nutrients dynamics, population stability, growth rate and temperature (Mur *et al.* 1999).

Table 4. The frequency of all cyanobacteria species in this study

	Species	Frequency (%)
1	*Anabaena circinalis*	12.5
2	*Anabaena planctonica*	12.5
3	*Anabaena* sp.	12.5
4	*Anabaenopsis* sp.	12.5
5	*Aphanocapsa endophytica*	12.5
6	*Aphanocapsa* sp.	50.0
7	*Aphanothece microspora*	12.5
8	*Aphanothece* sp.	50.0
9	*Arthrospira* sp.	12.5
10	*Borzia* sp.	12.5
11	*Chamaesiphon* sp.	12.5
12	*Chroococcus limneticus*	12.5
13	*Chroococcus minor*	12.5
14	*Chroococcus* sp.	87.5
15	*Cylindrospermopsis* sp.	25.0
16	*Dactyloccopsis* sp.	62.5
17	*Gloeocapsa* sp.	62.5
18	*Gloeothece* sp.	12.5
19	*Lyngbya birgei*	12.5
20	*Lyngbya* sp.	75.0
21	*Marssoniella* sp.	12.5
22	*Merismopedia* sp.	12.5
23	*Microcystis aeruginosa*	12.5
24	*Microcystis flos-aquae*	12.5
25	*Microcystis* sp.	25.0
26	*Myxobactron* sp.	25.0
27	*Nostoc* sp.	87.5
28	*Oscillatoria quasiperforata*	12.5
29	*Oscillatoria nigra*	12.5
30	*Oscillatoria princeps*	12.5
31	*Oscillatoria* sp.	75.0
32	*Phormidium* sp.	50.0
33	*Pleurocapsa* sp.	25.0
34	*Pseudoanabaena* sp.	25.0
35	*Pseudocapsa* sp.	12.5
36	*Rhabdoderma* sp.	12.5
37	*Scynechocystis aquatilis*	12.5
38	*Synechococcus* sp.	25.0
39	*Synechocystis* sp.	50.0
40	*Scytonema* sp.	50.0
41	*Spirulina* sp.	12.5
42	*Tolypothrix* sp.	12.5
43	*Tychonema* sp.	12.5

β-diversity Estimation

The β-diversity values for all pairs of contiguous and non-contiguous stations were higher than zero (Table 5) indicating that all stations were different in terms of their cyanobacteria species composition. Similarly, no total species turnover was recorded, even among the most distance station, as none of the values reached β = 1. The highest recorded value was β = 0.87 for station 4 (earth pond, Serian) and 7 (outside cage, Batang Ai) which only shared two cyanobacteria species (*Chroococcus* sp. and *Lyngbya* sp.). Station 4 and 7 were very different habitats and their distance was very far (approximately 300 km). This situation suggested that different types of water bodies and distance of the stations will have variations in physico-chemicals of the water and contributed to the increase of the cyanobacterial diversity.

As far as the analysis of contiguous station was concerned (Table 5), station pairs 3-4 and 6-7 have the highest β diversity value (β = 0.73). This can be clearly explained by the fact that station 3 and station 4 belong to different types of aquaculture ponds. Station 3 was an earth pond, layered with black HDPE whereas station 4 was an ordinary earth pond. In addition, brownish bloom on the surface of station 4 was observed during the sampling visits. Station 6 and 7 were also different types of aquatic ecosystems. station 6 comprises cage cultures whereas station 7 is the Batang Ai dam area. Therefore, there is frequent input of nutrients from fish pellet in station 6 compared to station 7.

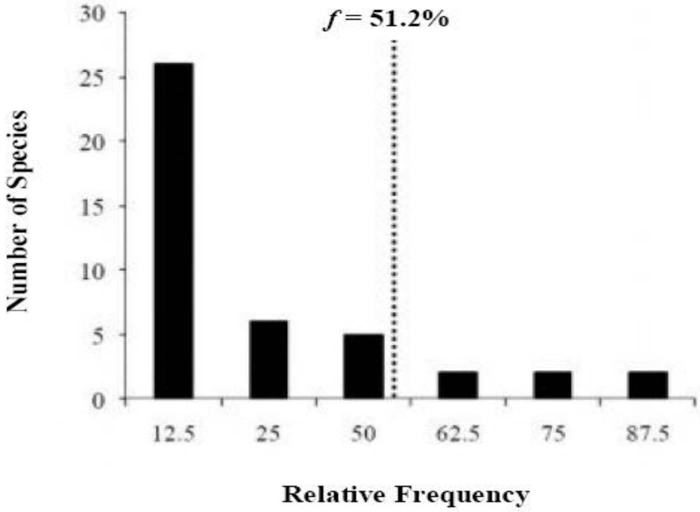

Figure 2. Frequency distribution on the species in the sampling stations. The dotted line indicates the mean value of the frequencies.

Table 5. β-diversity (above diagonal) and Jaccard's similarity values (below diagonal) among sampling stations

C_J / β	1	2	3	4	5	6	7	8
1	■	0.73	0.81	0.78	0.67	0.68	0.76	0.64
2	0.15	■	0.70	0.79	0.68	0.75	0.78	0.63
3	0.10	0.18	■	†0.73	0.72	0.79	0.82	0.68
4	0.13	0.1	†0.16	■	0.75	**0.84	0.87	0.71
5	0.19	0.19	0.16	0.14	■	†0.64	†0.68	*0.60
6	0.19	0.14	0.12	0.09	†0.22	■	†0.73	0.61
7	0.13	0.13	0.10	*0.07	†0.19	†0.15	■	0.72
8	0.22	0.23	0.19	0.17	**0.25	0.24	0.16	■

Some species of cyanobacteria prefer specific water environment. For instance, *Microcystis aeruginosa* and *Anabaena* sp. were only found in station 4 where the water was turbid and potentially eutrophic. These species were usually observed in undesirable water quality and related to toxic effect conditions (Sze 1998). Likewise, most species of *Anabaena* can be found in the water bloom with *Microcystis aeruginosa* (Bold & Wynne 1985), which was also observed only in station 4. This phenomenon suggested that contrasting types of ponds tend to have variation in physico-chemical properties of the water which eventually lead to the different composition of cyanobacteria.

An additional factor determining species turnover is tolerance of the different species cyanobacteria towards water quality. Thus, *Chroococcus* and *Nostoc*, which are common to the locality, are known to tolerate diverse environmental parameters, *Chroococcus* can be found in almost all aquatic ecosystems. Graham & Wilcox (2000), supported this contention. Meanwhile, the widespread distribution of nitrogen-fixing *Nostoc* (Stewart 1973), can be attributed to the presence of akinetes, which are formed after a period of active growth. This thick- walled cell survives in a dormant state when conditions are unfavourable (Sze 1998).

In contrast, the narrow distribution range of some species may be explained at least in part, by their low establishment ability and require specific aquatic conditions. For example, the presence of *Microcystis aeruginosa* and *Anabaena* spp., which are potentially toxic species, usually related to eutrophic and undesirable water quality.

The β-diversity values were higher among non-contiguous pairs of stations as compared to the contiguous ones. The highest value correspond to the station pairs 4-7 ($\beta = 0.87$). This pair of stations was differed in type of ecosystems, environmental characteristics and the distance (approximately 300km).

Similarity Analysis

β-diversity and similarity are opposite concepts. Hence, their corresponding numerical values are expected to be inversely proportional to each other (Table 5). Overall, the similarities among all sampling stations were between 0.07 and 0.20 indicating low numbers recorded in all sampling stations. The highest similarity value ($C_J = 0.25$) was observed between station 5 and station 8 pair. Cyanobacteria species and the lowest β-diversity correspond to these stations.

On the other hand, station pair 4-7, which share only two species, had the lowest similarity ($C_J = 0.07$) and the highest β diversity. In agreement with the above statements, no similarity values equal to one were observed, as a reflection of the fact that none of the station pairs were identical in terms of cyanobacteria species composition.

The different frequency of the cyanobacteria assemblages may be due to combinations of several factors. The most important factor is probably the great differences of the ecosystem being studied (Goettsch & Hernandez 2006). Since variations in ecosystem also mean variation in physico-chemical properties of the water, then there will also be variations in the composition of cyanobacteria. In this connection, the fidelity of several cyanobacteria species in type and physico-chemical composition was probably responsible, at least partially, for the highly discontinuous distribution (Goettsch & Hernandez 2006).

The observation clearly showed gradual spatial changes in cyanobacteria species composition, which is indicated by relatively low similarity and high β-diversity values. However, it must be emphasized that species turnover is attributable to the replacement of different species in spatial aspects, which was not the case in this study. The β-diversity reported here was strongly determined by the intermittent pattern of distribution of many cyanobacteria species.

CONCLUSION

The diversity and similarity of cyanobacterial population in selected Sarawak freshwater ecosystem had been documented. A total of 43 species belonging to 30 genera were recorded. The most distributed genera were *Chroococcus, Lyngbya, Nostoc* and *Oscillatoria*. The highest β-diversity values were found among non-contiguous stations. No identical or totally different cyanobacteria diversity values were obtained among those non-contiguous stations. Moreover, the highest β-diversity values were found among sites with contrasting environmental characteristics. The wide range of β-diversity and similarity suggested that different locations and types of aquatic ecosystems may have variations in physico-chemical properties of the water and eventually lead to the different composition of cyanobacteria.

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