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Diversity of Mix Microalgae in Fish Tanks under Different Weather Conditions

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Abstract

One of the biggest problems facing the world today is global warming. Many scientists believe that our production of carbon dioxide and other greenhouse gases contributed to the warming of the atmosphere and this effecting most of living organisms on earth including microalgae. Therefore, the aim of this study is to observe the changes in diversity of mixed microalgae communities in different stocking density of fish under different weather conditions within effects of environmental parameters on microalgae. Phytoplankton samples were +collected from five tanks, one tank is control tank without fish and four tanks with different densities of fish (10, 20, 40 and 80 fishes) respectively under different weather conditions for microalgal diversity study. Weather conditions and rain fall were recorded daily. Microalgae samples were collected from fish were identified, enumerated and the diversity index determined based on Shannon, Richness, Evenness and Simpson. There are 56 microalgal taxa found in the outdoor fish tanks belonging to 6 divisions represented by Chlorophyta (38) followed by Bacillariophyta (6), Cyanophyta (6), Dinophyta (3), Euglenophyta (2) and Crysophyta (1) in three weather conditions. The highest number of taxa recorded was 41 in tank 2 (20 fish), 39 taxa were observed in tank 3 (40 fish) followed by 34 taxa in tank 4 (80 fish), 33 taxa in tank 1 (10 fish) and 13 taxa in control tank without fish. In wet weather, the number of taxa was 25 followed by mix weather 24 and dry weather with 22 taxa. Favourable water quality during wet weather is probably led to highest species occurrence. Control tank without fish has 13 species represented by four microalgal division. Chlorophyta was the most common division in all tanks with 33 taxa in tank 1 (10 fishes), 41 taxa in tank 2 (20 fish), 39 taxa in tank 3 (40 fish) and 33 taxa in tank 4 (80 fish) in three weather conditions. The highest number of species occurred in wet weather and the lowest species occurrence in dry weather conditions. The most common and dominant species was *Pandorina morum* found in all tanks in different weather conditions. This study concluded that the fish stocking density and weather conditions influenced microalgal species composition and diversity.

Key words: Fish stocking density, weather conditions, Microalgae species and Diversity

Introduction

Presently the most serious and important problem humankind has ever had to face might be global warming with disastrous consequences and costly adverse effects (Shahzad, 2015). Its impact is wide ranging such as rising sea levels raise, extreme weather effects, loss of habitat and many more. The global warming and climate change occurred by natural and human causes. Most of the causes were due to release of CO_2 to the atmosphere. The best way to reduce global warming is cutting down anthropogenic emission of greenhouse gases. Numerous past and present studies have indicated that microalgae can potentially reduce greenhouse gas from coal-fired power plant and other carbon intensive industrial activities. This is possible because a microalga is small undifferentiated body with fast growth, able to live in diverse habitat, high accumulation of lipid and carbohydrate during starvation conditions and higher productivity (Bahadar et al., 2013). Microalgae can grow 20 or 30 times faster than traditional food crops, and has no need to compete for arable land (Sivasubramanian, 2016). The study on diversity of microalgae is very important to know what type of algae can be used to improve water quality. Most of the phytoplanktons can exist in open surface waters of lakes, rivers and oceans. Microalgae are also commonly found in pools, water storage tanks, and fish tanks. Many reports revealed that the microalgae abundance in ponds vary with changes in environmental factors such as light, temperature, pH and salinity (Shaari et al., 2011). Unfortunately most of the studies on microalgae are conducted in the laboratory (Ruangsomboon, 2012). Another advantage is that microalgae are capable of both carbon assimilation and O_2 productivity, thereby enhancing productivity in variety of environment (Makandar & Bhatnagar, 2010). The oldest documented use of microalgae was 2000 years ago, when the Chinese used cyanobacteria *Nostock* a food source during famine (Priyadarshani & Rath 2012). Today cultured microalgae is used as food supplement for humans, feed for land-based farm animal, and as feed for cultured aquatic species such as molluscs and the early larval stages of fish and crustaceans (Barnabé, 1994). Algae can also produce a variety of high value compounds such as fine chemicals, biofertilizer and biodiesel, without contributing to atmospheric carbon dioxide (Abdel-Raouf et al., 2012). Under favourable conditions that include adequate light availability, warm waters, and high nutrients levels, algae can rapidly grow multiply and if unchecked may cause blooms. Algal bloom can harm the aquatic environments by blocking sunlight and depleting O2 required by other aquatic organisms, restricting their growth and survival (EPA, 2013).

This diversity provides man with economic and aesthetic benefits. Climate change is predicted to change many environmental conditions that could affect the natural properties of fresh water. Biodiversity is the controlling key factor in ecology, directly linked with the functioning and regulation of the ecosystems. Measurement of environmental changes and toxicants can be achieved through using microalgae as a biological tool (Cardozo et al., 2007). Therefore, any changes in microalgae species can occur under different conditions as a response to various environmental factors. The production of algae from fish tank considered as one of the axes of economic development because the algae are a rich source of amino and fatty acids and easily absorbed by fish. This is necessary in order to complete and continue the healthy food chain. Therefore, the aim of this study is to observe the changes in diversity of mixed microalgae communities in different stocking density of fish under different weather conditions within effects of environmental parameters on microalgae.

Materials and Methods

The experiment was conducted at the Tilapia pond the fish hatchery unit of University Agriculture Park (UAP) University of Putra Malaysia. Five fibreglass tanks rectangle tanks each with dimensions (Length= 220cm, Width= 98cm, and Depth= 40cm), filled with 1000 litter dechlorinated tap water and different stocking density of red Nile tilapia (*Oreochromis niloticus*), tank 1(10 fish), tank 2 (20 fish), tank 3 (40 fish), tank 4 (80 fish) and fifth tank is control without fish. The fingerlings were approximately 1.074 g weight and 3 cm length. The commercial starter floating fish feed was calculated according to (New, 1987). Water samples were collect every two days from November 2014 to February 2015. Temperature, dissolved oxygen, PH, salinity, conductivity, and total dissolved solids were determined by Yellow Spring Instrument by multi parameter probe model (YSI-556 MPS) (Harun et al., 2015). Water transparency was measured by Secchi disk (20 cm diameter). The air temperature and light intensity was measured using light meter Licor model (L1-250). The temperature, light intensity and weather state were observed and recorded daily, three times a day at 8.00-9.00am, 12.00-1.00am, 4,00-5.00pm. The rainfall was determined using rain gauge after time of raining by using 5L size bottle with fixed funnel (Goldstein, 2002). The calculation of rain fall based on formula (Raghunath, 2006):

Rain fall gauging (mm) = (Collected water volume)/ (funnel surface area/bottle base area)

Samples of microalgae were collected every two days in different weather conditions (wet, mix, dry) by using five plastic bottles for five tanks, triplicate sampling of 500 ml water samples. All samples were preserved by Glutaraldehyde solution (Kumar, 2012), and then the samples were left constant for 48 hours to allow microalgae to settle down (Edler and Elbrächter, 2010). Then, the 400 ml of the upper water of the bottle were removed, and then remaining 100 ml were used for identification and enumeration using compound microscope at magnification of 400x. One drop of sample was placed into haemocytometer counting chamber. Each sample was counted three times, and then takes average. All species of algae were identified according to a key of freshwater algae (Bellinger and Sigee, 2010) and microalgae counted by Olympus light microscope at magnification of 400x. The diversity of microalgae was done by Shannon-Weaver index (Shannon and Weiner, 1949), Simpson index (Simpson, 1949), Evenness, and species richness (Margalef, 1958).

Statistical analysis

The data analysed by using Microsoft Excel and statistical analysis (version 20) one way ANOVA to indicate the significant of variance of physical environmental parameters, and for water quality parameters and species diversity using factorial ANOVA to determine the difference between treatments.

Results

Malaysian weather is very complex to define into distinct season. Cloudy and sunny sky and rains can occur at any time of the day. Weather conditions were classified depending on daily weather recording of the study period. The weather conditions can be classified into three weather conditions wet, mix, and dry based on scores represented in table 1 and there were 9 cycles throughout study comprising of three weather conditions each. The weather conditions scoring classified as: 1-4 (wet), 5-7 (mix), 7-10 (dry).

Table 1: The weekly weather scoring during the study period (Weather score from 1 to 10, 1-4. denote Wet weather, 5 -7 denote mixed weather and 7-10 denote Dry weather)

Days	Culture Cycles during the study period								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
1	2	6	6	3	3	6	8	8	2
2	5	7	4	6	4	1	7	8	5
3	7	5	1	5	4	1	10	9	10
4	5	1	3	5	4	2	6	9	7
5	7	4	9	6	4	6	7	8	6
6	1	7	5	2	4	4	4	7	8
7	3	6	2	6	7	5	2	7	6
8	4	6	2	3	3	6	7	8	9
9	7	5	1	5	3	1	10	10	8
10	10	4	2	1	2	7	9	8	9
Mean	5.1	5.6	3.5	4.2	6.1	3.9	7	8.2	7
Category	Mix	Mix	Wet	Wet	Mix	Wet	Dry	Dry	Dry

Based on table (2) there is a significant difference (P<0.05) between weather conditions in temperature and light intensity. The rainfall was a significant difference between weathers. As it is shown throughout table 2 there was a significant difference between weather conditions, the wet weather recorded higher rate 605.31 ml, 438.72 ml, 431.58 ml in three cycles followed by mix weather 232.77 ml, 157.28 ml, 85.76 ml, while the rate of rainfall was lower in dry weather 0, 40.8 ml, and 53.2 ml.

Table 2: Average of air temperature, light intensity and rainfall (* Temperature category: 26.79-29.87, **Light intensity category: 217.05-688.90, ***Rain category: 53.20-605.31)

Cycles Category	C1 Mix	C2 Mix	C3 Wet	C4 Wet	C5 Mix	C6 Wet	C7 Dry	C8 Dry	C9 Dry
Temperature (C°)*	29.10 ±0.42	28.20 ±0.35	28.60 ±0.40	26.79 ±0.79	28.96 ±0.37	27.09 ±0.40	28.08 ±0.54	29.87 ±0.54	29.40 ±0.46
Light intensity(μmol $m^{-2}s^{-1}$) **	505.80 ±68.93	540.26 ±60.02	466.85 ±53.45	217.05 ±21.09	522.47 ±58.66	287.28 ±43.96	562.34 ±76.44	688.90 ±63.73	588.91 ±75.67
Rainfall (ml)***	232.77 ±89.37	85.76 ±52.54	605.31 ±276.27	431.58 ±158.76	157.28 ±55.95	438.72 ±150.16	40.80 ±24.80	0.00 ±0.00	53.20 ±16.02
Category	Mix	Mix	Wet	Wet	Mix	Wet	Dry	Dry	Dry

Table 3 indicated different aspect of weather condition that exerts influence on water quality physical parameters with different stocking density of fish in three cultivation cycles. Water temperature was significantly different ($p< 0.05$) in different weather conditions but there was no significant difference ($p>0.05$) between the cycles. The highest temperature was 29.24±0.38, 27.48±0.07°C during dry and mix weather respectively in tank 4 (80 fish), whilst the lowest temperature was 26.87±°C recorded in wet weather in control (without fish) with range of 26.87 to 29.24 °C.

pH is measure of hydrogen ions, or acidity in the water which is very important for biotic and abiotic features of aquatic systems. pH value often fluctuated within the cultivation periods. The pH values fluctuates marginally 7.35±0.01, 7.25±0.02 and 7.18±0.05 during mix, wet and dry weather conditions respectively. pH of water were significantly different ($p< 0.05$) between control and other tanks but there were no significant difference ($p > 0.05$) between culture cycles and tanks with fish.

Electrical conductivity (EC) is a measure of water's capability to pass electrical flow. This ability is directly related to the concentration of ions in the water. There were no significant different ($p > 0.05$) of EC between culture cycles and tanks (with fish and control) but there were significant different ($p < 0.05$) within cultivation periods and weather conditions ranging between 0.13 to 0.17 mS/cm and the average of 0.16±0.00, 0.13±0.00 and 0.17±0.00 in mix, wet and dry weather conditions respectively.

Total dissolved solids (TDS) are the term used to describe the presence of inorganic salts and small amounts of organic matter present in water. Significant seasonal variations in total dissolved solids ($p < 0.05$) were observed. The highest value was 0.10 mg/L in mix and dry weather and the lowest value was 0.08 mg/L in wet weather its ranges from 0.08 to 0.10 mg/L. There were no significant differences ($p > 0.05$) between cultivation cycles, days, and tanks.

Mean value of dissolved oxygen recorded significant variation ($p < 0.05$) between weather conditions, tanks and days of cultivation but there was no significant difference ($p > 0.05$) between culture cycles. The highest DO value was 6.69 mg/L in control in wet weather, while the lowest value was 2.99 mg/L in tank 4 (80 fish) in dry weather conditions ranged from 2.99 to 6.69 mg/L. Salinity remained relatively high in mix and dry weather of

0.07 ppt but marginally lower in wet weather 0.06 ppt with no significant difference ($p > 0.05$) between tanks and cycles but there was significant differences ($p < 0.05$) within the cultivation period.

Secchi disk depth is measure of relative clarity of a liquid. Water transparency recorded significant differences ($p<0.05$) within weather conditions, tanks and cultivation period ($p < 0.05$) but there were no significant differences ($p > 0.05$) between cycles. The highest average secchi disk depth was in control (no fish) 0.40 m in all weather conditions and the lowest was in tank 4(80 fish) in dry weather 0.12 m with range of 0.12 to 0.40 m.

Table 3: The difference between tanks and weather conditions for physical parameters (Average of 3 cycles for every weather)

Parameters	Tanks	Weather Mix [b]	Wet [a]	Dry [c]
Temperature (°C)	Control	27.31±0.03[a]	26.87±0.11[a]	28.95±0.19[a]
	T1 (10 Fish)	27.42±0.02[a]	26.98±0.04[a]	29.09±0.12[a]
	T2 (20 Fish)	27.42±0.03[a]	26.89±0.02[a]	29.00±0.05[a]
	T3 (40 Fish)	27.31±0.04[a]	27.00±0.04[a]	29.05±0.22[a]
	T4 (80 Fish)	27.48±0.07[a]	26.98±0.05[a]	29.24±0.38[a]
		Mix [b]	Wet [a]	Dry [b]
pH	Control	7.33±0.01[b]	7.25±0.02[b]	7.18±0.05[b]
	T1 (10 Fish)	6.94±0.02[a]	6.76±0.03[a]	7.41±0.04[a]
	T2 (20 Fish)	7.35±0.04[a]	6.76±0.07[a]	7.03±0.04[a]
	T3 (40 Fish)	6.97±0.02[a]	6.93±0.10[a]	6.97±0.02[a]
	T4 (80 Fish)	6.95±0.03[a]	7.13±0.11[a]	7.03±0.04[a]
		Mix [b]	Wet [a]	Dry [c]
Conductivity ($mScm^{-1}$)	Control	0.15±0.00[a]	0.13±0.00[a]	0.17±0.00[a]
	T1 (10 Fish)	0.16±0.00[a]	0.13±0.00[a]	0.17±0.00[a]
	T2 (20 Fish)	0.16±0.00[a]	0.13±0.00[a]	0.17±0.00[a]
	T3 (40 Fish)	0.16±0.00[a]	0.14±0.00[a]	0.17±0.00[a]
	T4 (80 Fish)	0.16±0.00[a]	0.14±0.00[a]	0.17±0.00[a]
		Mix [b]	Wet [a]	Dry [c]
Total dissolved soilds (mg L¯1)	Control	0.10±0.00[a b]	0.08±0.00[a b]	0.10±0.00[a b]
	T1 (10 Fish)	0.09±0.00[a]	0.08±0.00[a]	0.10±0.00[a]
	T2 (20 Fish)	0.10±0.00[a b]	0.08±0.00[a b]	0.10±0.00[a b]
	T3 (40 Fish)	0.09±0.00[a b]	0.08±0.00[a b]	0.10±0.00[a b]
	T4 (80 Fish)	0.10±0.00[b]	0.09±0.00[b]	0.10±0.00[b]
		Mix [b]	Wet [c]	Dry [a]
Dissolved Oxygen (mg L¯1)	Control	5.16±0.04[c]	6.69±0.08[c]	4.46±0.10[c]
	T1 (10 Fish)	5.15±0.08[c]	6.42±0.19[c]	4.35±0.15[c]
	T2 (20 Fish)	4.95±0.14[b]	6.35±0.23[b]	4.00±0.03[b]
	T3 (40 Fish)	4.48±0.16[b]	5.74±0.03[b]	3.64±0.09[b]
	T4 (80 Fish)	4.10±0.22[a]	5.30±0.03[a]	2.99±0.03[a]
		Mix [b]	Wet [a]	Dry [b]
Salinity (ppt)	Control	0.07±0.00[a]	0.06±0.00[a]	0.06±0.00[a]
	T1 (10 Fish)	0.06±0.00[a]	0.06±0.00[a]	0.07±0.00[a]
	T2 (20 Fish)	0.07±0.00[a]	0.06±0.00[a]	0.06±0.00[a]
	T3 (40 Fish)	0.07±0.00[a]	0.06±0.00[a]	0.07±0.00[b]
	T4 (80 Fish)	0.07±0.00[a]	0.06±0.00[a]	0.07±0.00[b]
		Mix [b]	Wet [a]	Dry [C]
Secchi disk depth (m)	Control	0.40±0.00[d]	0.40±0.00[d]	0.40±0.00[d]
	T1 (10 Fish)	0.21±0.01[c]	0.29±0.01[c]	0.17±0.01[c]
	T2 (20 Fish)	0.18±0.01[b]	0.27±0.01[b]	0.14±0.01[b]
	T3 (40 Fish)	0.17±0.00[b]	0.25±0.01[b]	0.14±0.00[b]
	T4 (80 Fish)	0.16±0.01[a]	0.22±0.00[a]	0.12±0.00[a]

In control tank (Table 4), the total species were 13 under four divisions and division Chlorophyta was the most common. The species of microalgae distributed at 6 species in wet weather, 7 species in mix weather, and 8 species in dry weather. The most of the abundance in three weather conditions was *Navicula* sp.

Table 4: List of microalgae species in control tank (without fish) under three weather conditions (Abundance R= rare occurrence, <1000 cells/ml: F = frequent occurrence, 1000-9999 cells/ml S = subdominant, 10000-99999 cells/ml: D = dominant, >100000 cells/ml)

Division	Species	Weather conditions		
		Wet	Mix	Dry
Chlorophyta	*Coelastrum microporum*	S	-	-
	Chlorella vulgaris	F	-	F
	Dictyosphaerium pulchellum	S	-	S
	Haematococcus pluvialis	-	S	-
	Odeogonium sp.	-	F	-
	Oocystis borgei	F	-	F
	Pandorina morum	-	F	S
	Ulthrix equalise	-	D	D
Dinophyta	*Peridenium labeled*	-	-	S
Cyanophyta	*Anabena* sp.	-	S	S
	Microcystis aeruginosa	-	F	-
	Nostoc sp.	S	-	-
Bacillariophyta	*Navicula* sp.	S	F	F
Total Species	13	6	7	8

In tank 1 (10 fish), total number of 33 algal taxa were identified in three weather conditions which were distributed over 20 species in wet weather, 23 species in mix weather, and 17 species in dry weather. The 8 species was abundance in three weather conditions which include *Chlorella vulgaris*, *Coelastrum microporum*, *Dictyosphaerium pulchellum*, *Golenkinia radiate*, *Oocystis borgei*, *Pandorina morum*, *Scenedesmus quadricauda*, and *Cocconeis sp.* (Table 5).

Table 5: List of microalgae species in tank one (10 fish) in three weather conditions (Abundance R= rare occurrence, <1000 cells/ml: F = frequent occurrence, 1000-9999 cells/ml S = subdominant, 10000-99999 cells/ml: D = dominant, >100000 cells/ml)

Division	Species	Weather conditions		
		Wet	Mix	Dry
Chlorophyta	*Ankyra ankora*	-	F	F
	Botryococcus sp.	-	-	S
	Clamydomonas sp.	-	F	F
	Chlorella vulgaris	S	F	S
	Chlorogonium elongatum	S	-	-
	Coelastrum microporum	S	S	S
	Dictyosphaerium pulchellum	S	S	S
	Golenkinia radiate	S	F	S
	Haematococcus pluvialis	-	-	S
	Lagerheimia ciliate	S	F	-
	Micractinium pusillum	S	-	-
	Monoraphidium consortium	S	S	-
	Odeogonium sp.	S	S	-
	Oocystis borgei	S	D	S
	Pandorina morum	D	D	D
	Pediastrum duplex	-	F	F
	Scenedesmus acuminatus	-	S	-
	Scenedesmus acutus	S	-	F
	Scenedesmus obliqus	-	S	-
	Scenedesmus obtusus	-	S	S
	Scenedesmus quadricauda	F	S	S
	Staurodesmus dejetus	-	F	S
	Tetraedron minimum	F	F	-
	Tetraedron muticum	S	-	-
Dinophyta	*Sphaerodinium woloszynska*	R	-	-
Bacillariophyta	*Cyclotella meneghiniana*	F	-	-
	Cocconeis sp.	F	S	S
	Tabellaria sp.	F	-	-
Cyanophyta	*Gloecapsa* sp.	-	F	-
	Nostoc sp.	F	-	-
	Microcystis aeruginosa	-	F	-
Euglenophyta	*Phacus longicauda*	-	S	-
Crysophyta	*Mallomonas* sp.	-	S	F
Total species	33	20	23	17

In tank 2 (20 fish) (Table 6), total number of 40 algal taxa were identified in three weather conditions which were distributed over 25 species in wet weather, 23 species in mix weather, and 22 species in dry weather. The most species was abundance were *Ankyra ankora*, *Coelastrum microporum*, *Chlorella vulgaris*, *Haematococcus pluvialis*, *Lagerheimia ciliate*, *Pandorina morum*, *Scenedesmus obliqus*, *Scenedesmus obtusus*, *Scenedesmus quadricauda*, *Staurodesmus dejetus*, *Asterionella formosa.*

Table 6: List of microalgae species in tank two (20 fish) in three weather conditions (Abundance R= rare occurrence, <1000 cells/ml: F = frequent occurrence, 1000-9999 cells/ml S = subdominant, 10000-99999 cells/ml: D = dominant, >100000 cells/ml)

Division	Species	Weather conditions		
		Wet	Mix	Dry
Chlorophyta	*Actinastrum hantzschii*	S	-	-
	Ankyra ankora	S	F	S
	Ankistrodesmus fusiformis	-	-	S
	Botryococcus	-	-	S
	Carteria sp.	F	-	-
	Coelastrum microporum	F	S	S
	Chlorella vulgaris	S	S	F
	Chlorogonium elongatum	S	-	-
	Clamydomonas sp.		F	-
	Crucigenia quadrata	F	-	-
	Dictyosphaerium pulchellum	-	S	S
	Eustrum biverrucosum	F	-	-
	Golenkinia radiate	S	-	-
	Haematococcus pluvialis	F	F	S
	Lagerheimia ciliate	S	F	F
	Monoraphidium consortium	S	S	-
	Odeogonium sp.	-	S	-
	Oocystis borgei	-	S	D
	Pandorina morum	D	D	D
	Pediastrum duplex	-	F	-
	Pediastrum selenaea	-	-	F
	Scenedesmus acutus	-	-	F
	Scenedesmus acumitus	-	F	-
	Scenedesmus opaliensis	S	-	-
	Scenedesmus obliqus	S	F	S
	Scenedesmus obtusus	F	S	S
	Scenedesmus ornatus	-	-	F
	Scenedesmus dimorphus	S	-	-
	Scenedesmus hystrix	F	-	-
	Scenedesmus quadricauda	S	S	S
	Staurodesmus dejetus	F	S	F
	Tetraedron minimum	F	F	-
	Ulthrix sp.	-	-	D
Dinophyta	*Sphaerodinium Woloszynska*	S	-	-
Euglenophyta	*Phacus longicauda*	-	S	
Crysophyta	*Mallomonas* sp.	-	S	S
Cyanophyta	*Microcystis aeruginosa*	S	S	F
Bacillariophyta	*Asterionella formosa*	S	S	S
	Fragilaria sp.	S	-	-
	Cocconeis sp.	-	F	F
Total species	40	25	23	22

In tank 3 (40 fish) (Table 7), total number of 39 algal taxa were identified in three weather conditions which were distributed over 25 species in wet weather, 24 species in mix weather, and 15 species in dry weather. Most of the abundance was *Chlorella vulgaris*, *Coelastrum microporum*, *Golenkinia radiate*, *Oocystis borgei*, *Pandorina morum*, *Asterionella formosa*.

Table 7: List of microalgae species in tank three (40 fish) in three weather conditions (Abundance R= rare occurrence, <1000 cells/ml: F = frequent occurrence, 1000-9999 cells/ml S = subdominant, 10000-99999 cells/ml: D = dominant, >100000 cells/ml)

Division	Species	Weather conditions		
		Wet	Mix	Dry
Chlorophyta	*Actinastrum hantzschii*	S	-	-
	Ankyra ankora	-	F	-
	Chlorella vulgaris	S	S	S
	Coelastrum microporum	S	S	D
	Clamydomonas sp.		F	F
	Chlorogonium elongatum	S	-	-
	Crucigenia quadrata	-	F	-
	Dictyosphaerium pulchellum	-	S	S
	Eustrum biverrucosum	F	-	-
	Golenkinia radiate	S	S	S
	Haematococcus pluvialis	-	-	S
	Lagerheimia ciliate	S	-	-
	Monoraphidium consortium	S	S	-
	Odeogonium sp.	F	F	-
	Oocystis borgei	S	S	S
	Pandorina morum	S	D	D
	Pediastrum duplex	S	F	-
	Scenedesmus opaliensis	F	-	-
	Scenedesmus obliqus	F	-	-
	Scenedesmus obtusus	-	F	S
	Scenedesmus ornatus	-	-	F
	Scenedesmus dimorphus	S	-	-
	Scenedesmus hystrix	F	-	-
	Scenedesmus quadricauda	S	S	S
	Staurodesmus dejetus	F	-	S
	Staurodesmus leptacanthum	S	F	-
	Staurastrum cyrtocerum	F	-	-
	Staurastrum cingulum	-	F	-
	Stigeoclonium sp.	-	F	
	Tetraedron minimum	-	F	-
Dinophyta	*Sphaerodinium woloszynska*	F	-	-
	Ceratium sp.	-	F	-
Crysophyta	*Mallomonas* sp.	-	F	S
Cyanophyta	*Microcystis aeruginosa*	-	F	F
	Chroccocus sp.	F	-	-
Euglenophyta	*Phacus* sp.	F	F	-
Bacillariophyta	*Asterionella Formosa*	S	S	S
	Fragilaria sp.	S	-	-
	Cocconeis sp.	-	S	-
Total Species	39	25	24	15

In tank 4 (80 fish) (Table 8), total number of 33 algal taxa were identified in three weather conditions which were distributed over 24 species in wet weather, 21 species in mix weather, and 20 species in dry weather. The most abundance in three weather conditions were *Chlorella vulgaris,Coelastrum microporum, Dictyosphaerium pulchellum, Haematococcus pluvialis, Lagerheimia cilite, Oocystis borgei, Pandorina morum, Pediastrum duplex, Scenedesmus obtusus, Scenedesmus ornatus, Scenedesmus quadricauda, Staurodesmus dejetus.*

Table 8: List of microalgae species in tank four (80 fish) in three weather conditions (Abundance R= rare occurrence, <1000 cells/ml: F = frequent occurrence, 1000-9999 cells/ml S = subdominant, 10000-99999 cells/ml: D = dominant, >100000 cells/ml)

Division	Species	Weather conditions		
		Wet	Mix	Dry
Chlorophyta	*Ankistrodesmus fusiformis*	-	-	F
	Ankyra ankora	F	-	F
	Chlorella vulgaris	F	S	S
	Coelastrum microporum	S	S	S
	Dictyosphaerium pulchellum	F	S	S
	Eustrum sp.	F	-	-
	Golenkinia radiate	S	S	-
	Haematococcus pluvialis	S	F	S
	Lagerheimia cilite	S	F	S
	Micractinium pusillum	S	-	-
	Monoraphidium consortium	S	F	-
	Odeogonium sp.	-	S	-
	Oocystis borgei	S	S	F
	Pandorina morum	D	D	D
	Pediastrum duplex	F	F	S
	Scenedesmus acuminatus	F	-	-
	Scenedesmus obliqus	F	F	-
	Scenedesmus obtusus	F	S	S
	Scenedesmus ornatus	S	F	F
	Scenedesmus quadricauda	S	S	S
	Staurodesmus dejetus	S	S	S
	Stigeoclonium sp.	-	F	F
	Staurastrum cyrtocerum	F	-	-
	Tetraedron minimum	F	-	-
	Tetraedron muticum	F	-	-
	Ulthrix sp.	-	-	D
Bacillariophyta	*Asterionella formosa*	-	-	F
Crysophyta	*Mallomonas* sp.	-	S	F
Cyanophyta	*Microcystis aeruginosa*	S	S	-
	Snowella sp.	-	S	-
	Anabena sp.	-	-	S
Euglenophyta	*Phacus longicauda*	F	-	S
	Trachelomonas sp.	-	F	-
Total species	33	24	21	20
Total species dominance		S=12 D=1 F=6 R=1	S=9 D=2 F=10 R=0	S=12 D=1 F=5 R=0

Table 9, showed that total number of species ranged between 6 to 25 in wet weather, 9 to 24 in mix weather and 8 to 22 in dry weather, the number of species was equal in wet and mix weather which was higher than number of species in dry weather, while the total density recorded the highest rate in dry weather (73.21 to 146.50) followed by mix (61.13 to 101.36) and wet (11.71 to 46.73). For wet weather, the high density observed in tank 4, in mix weather tank 2 and in dry weather also tank 2.

Table 9 Total of Density and numbers of phytoplankton in three weather conditions

Tanks	Wet Weather		Mix Weather		Dry Weather	
	Total species	Total Density	Total species	Total Density	Total species	Total Density
control	6	11.71	9	61.13	8	73.21
(10 Fish)	20	38.29	23	70.95	17	82.62
(20 Fish)	25	30.48	23	101.36	22	146.50
(40 Fish)	25	38.66	24	82.77	15	126.46
(80 Fish)	24	46.73	21	77.74	20	138.94

Figures 1, 2, 3 presents the results of Shannon-Weiner, species richness, evenness and Simpson in control (without fish), T1 (10 fish), T2 (20 fish), T3 (40 fish), T4 (80 fish) for 3 cycles in every weather condition. For Shannon, there was a significant difference between tanks, there were ranged between 0.75 to 1.71 in wet weather, 0.42 to 1.18 in mix weather, and 0.20 to 1.09 in dry weather. There was no significant difference in species richness between four tanks but there is a significant difference between them and control there were ranged from 0.89 to 2.07 in wet weather, 1.07 to 2.18 in mix weather and 0.82 to 2.08 in dry weather. There is a significant difference in evenness between tanks as they ranged between 0.70 to 0.84 in wet weather, 0.42 to 0.59 in mix weather, and 0.25 to 0.58 in dry weather. Finally, the Simpson recorded a significant difference between tanks which ranged between 0.47 to 0.71 in wet weather, and from 0.23 to 0.54 in mix weather, and 0.11 to 0.51 in dry weather.

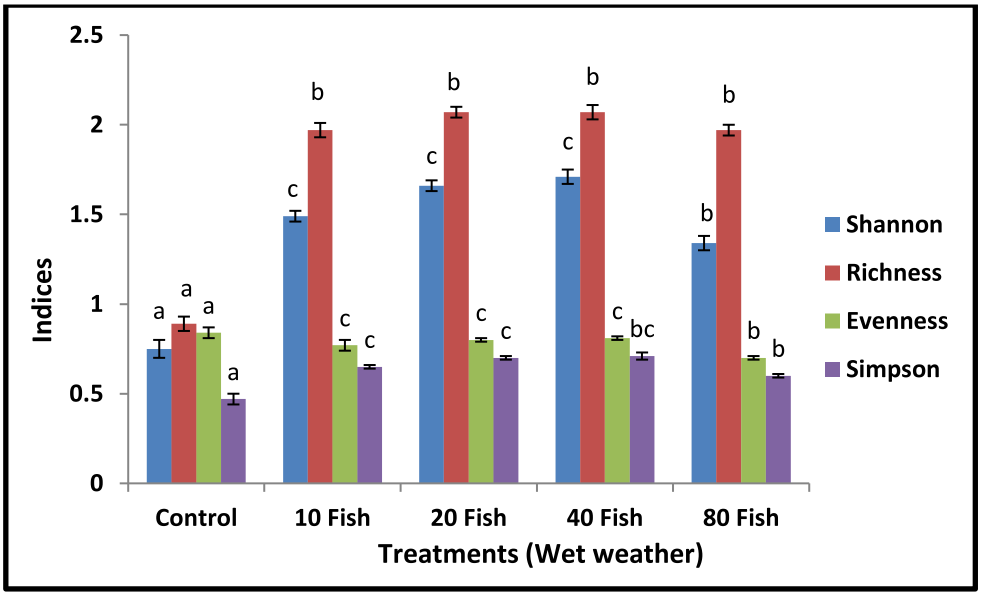

Figure 1: The average of three cycles of Shannon-Weiner, species richness, evenness and Simpson in difference density of fish from the average of three cycles. Control, T1 (10 Fish), T2 (20 Fish), T3 (40 Fish), T4 (80 Fish) in wet weather condition. Means with different letters (a-c) differ significantly (*p*< 0.05).

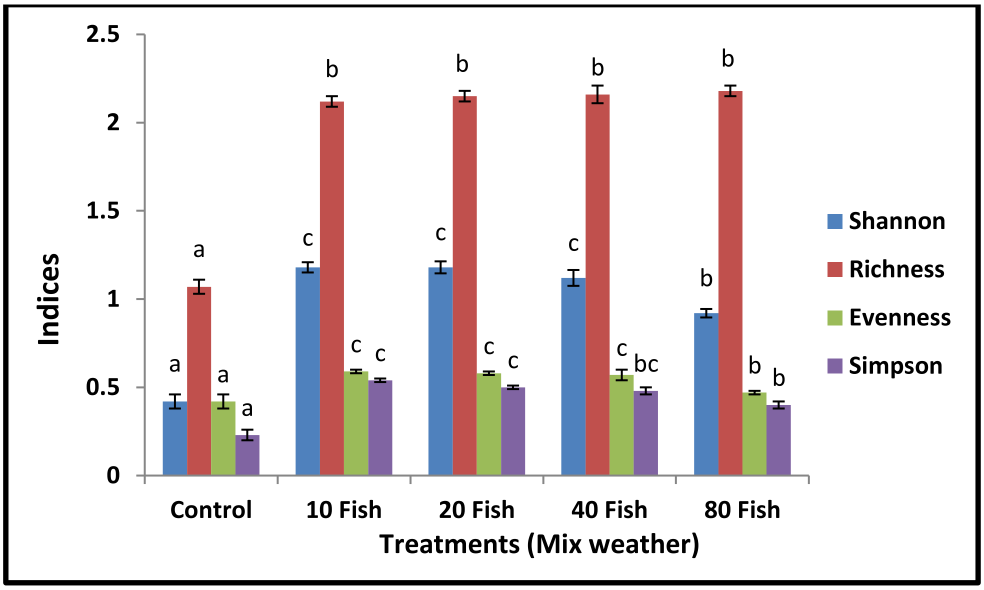

Figure 2: The average of three cycles of Shannon-Weiner, species richness, evenness and Simpson in difference density of fish from the average of three cycles Control, T1 (10 Fish), T2 (20 Fish), T3 (40 Fish), T4 (80 Fish) in mix weather condition. Means with different letters (a-c) differ significantly (*p*< 0.05)

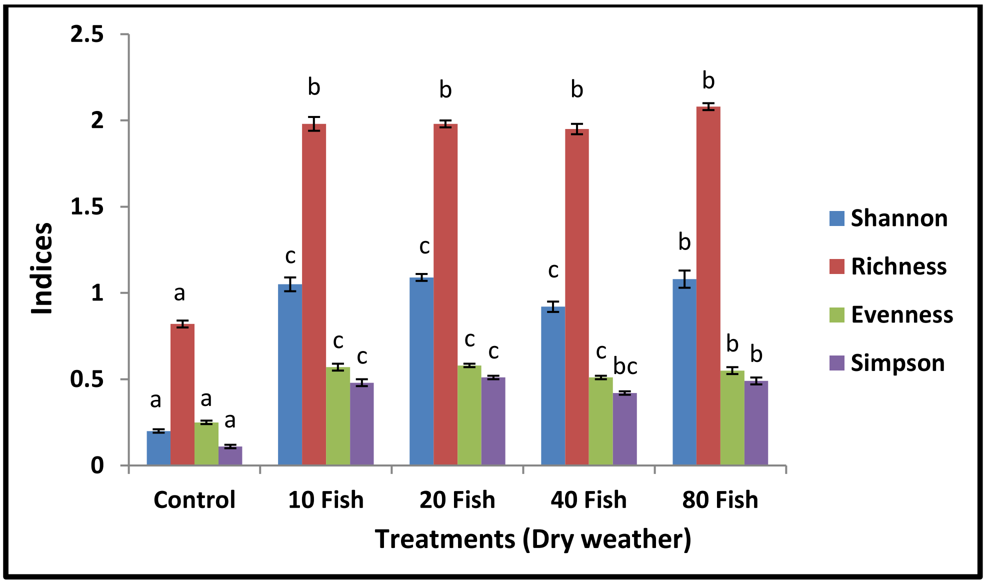

Figure 3: The average of three cycles of Shannon-Weiner, species richness, evenness and Simpson in difference density of fish from the average of three cycles. Control, T1 (10 Fish), T2 (20 Fish), T3 (40 Fish), T4 (80 Fish) in dry weather condition. Means with different letters (a-c) differ significantly (*p*< 0.05).

Discussion

The findings of this study suggest that there are two possible factors that singly or in combination that affects species composition and diversity. They are weather conditions and fish stocking density.

To suggest weather conditions have some influence on microalgal composition and diversity is not a straight forward matter because Malaysian weather has no definite pattern. Since there can be cloud cover affecting the light intensity and photoperiod, rain or clear sky and sunshine at any time of the day, week or month.

Therefore in order to quantify weather conditions, total daily rainfall, light intensity and temperature were recorded and ranked. The data indicated that there was a significantly high average rainfall ($p<0.05$) in wet weather, moderate rainfall in mixed weather and low rainfall from one week to the other. The ten days interval was chosen because normally the phytoplankton cycle was between 7-10 days (Round, 1984) which is closer to the week.

Light intensity also followed similar trend as rainfall, where the average light intensities were significantly lowest in wet conditions, moderately high in mixed weather and highest in dry seasons.

In the tropic, the light intensity is more likely influenced by the cloud covers or particulate matter from open burning but during this study there was no episode of haze or open burning that might affect the light intensity.

The mean temperature were marginally lower in wet weather, moderately high in mixed weather and highest in dry weather condition but not significantly different ($p>0.05$) within the range of 26.7-29.4 °C. The data suggests that temperature is not critical in the different weather conditions.

Weather scoring is another simple way to assess the weather conditions. By observing weather throughout the day, ranking them 1-10 with low rank 1-4 for wet weather, 5 - 7 for mixed weather and 7-10 for dry weather can adequately described the weather conditions. Mean weather score for 10 days which is equivalent to one microalgae culture cycle can revealed whether the weather conditions is wet, mixed or dry.

Another factor that might affect the species composition and diversity of mixed microalgae is fish stocking density and size of fish. Higher fish stocking density will require more food which in turn produces more waste and subsequently more nutrient for the microalgae (North et al., 2006 and Hassan, 2011).

However highest fish stocking density will not necessarily give the highest microalgal production because of too much suspended matter limit light penetration to the water body which is essential for microalgae (Parkos et al., 2003).

The results of this study showed that tank without fish did not differ much in term of temperature, pH, TDS, dissolved oxygen, salinity but the secchi disk depth were significantly higher than tanks with fish.

However among the tanks with fish, the difference between low stocking density and high fish stocking density is marginal. The differences in water quality as the results of different fish stocking density is so subtle to suggest that they contribute to different microalgae species composition and diversity

Furthermore there are not many studies regarding effect of weather conditions and fish stocking densities on phytoplankton population and diversities (Omar et al., 2016). Phytoplankton population studies suggested that weather conditions in term of amount of rainfall, weather score and mean light intensities affected the species composition, diversity and density.

In wet weather condition, possible dilution of nutrient in tanks with and without fish reduced available nutrient, thus increasing species composition and diversity (Groendahl and Fink, 2017) but decreasing species density (Cardoso et al., 2012). This is because some species cannot tolerate high nutrient levels (Morris et al., 1979). During wet weather, there are high incidence of cloud cover that reduce the light intensity (Matuszko, 2012) and duration of optimal photoperiod. There is close relationship between optimal light intensity, photoperiod and phytoplankton density (Pal et al., 2013).

In mixed weather conditions, the amount of rainfalls, mean weather score and light intensity were intermediate but the species composition and diversity were similar to wet weather conditions but microalgal density were intermediate between wet and dry weather conditions. Many past studies also reported similar observation (Khuantrairong and Traichaiyaporn, 2008; Omar et al., 2016).

In dry weather conditions, the amount of rainfall were lowest more likely to concentrate the available nutrient in the tanks, highest average light intensity and more optimal photoperiod allowing some species to grow rapidly while some species are affected. The number of species present and the diversity become low but the densities of microalgae are highest. This phenomenon is also commonly reported in other studies (Kadiri, 2000; Omar et al., 2016).

In tank without fish, there is limited nutrient available resulting very low number of microalgal species present, low species diversity and low microalgal density. Nutrient is essential for the existence of microalgae in water bodies irrespective of wet, mixed and dry weather conditions (Pate et al., 2011; Stubbs, 2016).

This finding indicated those tanks with stocking density of 10, 20, 40 and 80 species have more or less similar number of microalgal species, diversity and density in particular weather conditions suggesting that weather conditions has more profound effect on species composition, species diversity and microalgal densities.

Decrease microalgae density in rainy season could be due to reduce light intensity, decrease photoperiod, dilution of water due to rain and lower pH due to acidic rain. The light intensity another important factor it is considered important factor to photosynthesis process in order to make food then algae growth (Yoon et al., 2008). The light penetrance in water in the dry season leads to improve photosynthesis and reflect on variation in algae species. The growth rates of growing microalgae depend on the light penetration into microalgae environment (Oswald, 1988). The couler of water and amount of sediments substances and soluble elements effect on light penetration and absorbed it via microalgae.

Highest density of phytoplankton was observed in dry weather which could be as the result of high light intensities, longer period of light duration which allows utilisation of available nutrients (Silva, 2005; Badsi et al., 2012), existence of fish in tank has contributed much to the addition of some nutrients in water and help phytoplankton to grow. Decrease in microalgae density during rainy season could be due to reduce light intensity, decrease photoperiod, dilution of water due to rain and lower pH. This finding was also reported by Nweze (2006).

The phytoplankton divisions in five tanks in the three weather conditions include, Chlorophyta constituted the high rate of total taxon due to flexibility in the physiology, and activities of Chlorophyceae can allow environmental changes better than other species (Silva, 2007). Chlorophyta also has the ability to live in wide range of nutrients and physical environments due to increasing temperature in dry season could be allow the nutrients become available in the upper layer of water body. The Bacillariophyta was the second dominant division, while other divisions of phytoplankton exist in some seasons and with insignificant proportion for example Dinophyta, Crysophyta, and Euglenophyta. A Similar assemblage of algae Chlorophyta reported by Ikpi et al, (2013) in the Tropical Earthen Fish Ponds. High abundance of Chlorophyta was found in wet weather with moderate temperature (Suresh et al., 2011), this abundance might happen as a result of the cloudy weather and dilution due to rain (Paulette et al., 2011).

Chlorophyta considered as the most prevalent types of algae because the factors of water quality encouraged some species of microalgae to be dominant more than any other species because some of them especially Chlorophyta is able to tolerate some factors compare with another species. Different phytoplankton species are habitat specific in nature and their distribution reveals the features of water where they survive (Saha et al., 2000).

Among the species that belonging to Chlorophyta recorded, *Pandorina morum* can be found in all tanks with different density of fish in different weather conditions. High abundance of *Pandorina* presence in tanks was because of its ability to adapt to wide range of water physico-chemical parameter. For that reason *Pandorina* is reported to inhabit a variety of freshwater environments around the world and is common in standing bodies of water such as puddles ponds, and lakes (Van Vuuren et al., 2006).

Bacillariophyta, commonly known as diatoms are common in freshwater and marine habitats. Diatoms are sensitive to an environmental conditions changes (Reynolds, 1998). Therefore they can be used as powerful ecological tools to investigate past conditions and monitor environmental changes over time (van Vuuren et al., 2006). Dinopyta species present in high number in mix and wet weather but missing in dry weather probably due to unfavourable water quality such as high nitrogen or phosphorus. *Asterionella formosa* was subdominant in three weather conditions in tanks 20 and 40 fish but it presence in tank 4 (80 fish) was fair in dry weather conditions probably due to presence of small amounts of phosphorus. It is possible that the presence of small amount of phosphorus permits the growth of this species. *Cyclotella meneghiniana* was frequent only in wet weather conditions probably because rain water dilutes the nutrient in tank thus allowing this species to exist.

Chrysophyta represented by *Mallomonas* sp. was present in mix and dry weather but was absent in wet weather probably due to low nutrient, low light and low temperature during wet weather. Mallomonas sp. is often abundant in water containing high nutrient. Euglenophyta species often live in hard or soft water habitats of varied pH and light levels also they more abundantly in polluted water (Ersanlı & Gönülol, 2003). In this study, the presence of Euglenophyta species was subdominant in tank with high stocking densities in mix and wet weather probably due to high organic matter and moderate temperature. The presence of this division is not high because it prefers higher organic matter in the water. *Phacus longicauda* was subdominant in mix weather conditions in tanks 1, 2, 4 but the occurrence was higher in wet weather conditions.

The distribution of Cyanophyta was diverse between frequent and subdominant in different weather conditions and tanks. Many species of Cyanophyta produce populations that are toxic to humans and animals so the Cyanophyta is considered indicator for water quality. It has been observed the growth of some species of this group of algae increased with increasing temperature and organic components especially in tanks with high stocking density of fish. In this study *Microcystis aeruginosa* was found in all weather conditions due to water temperature, dissolved inorganic carbon and dissolved inorganic nitrogen is considered main factors controlling growth in various habitats. Similar studies found that water temperature is the most important factor determining the dominance *M. aeruginosa* (Imai et al., 2008).

Dinophyta were represented with 3 species and only appeared in wet weather in tanks with 10, 20, 40 fish and in control in dry weather conditions. The existence diatoms were in wet weather due to certain nutrients is present as a result of rain and this group of algae require certain amounts of nitrate to grow. Various studies have shown a similar relationship between diatoms and other nutrients, such as iron and nitrate (Takeda, 1998; Eppley & Renger, 1974). *Sphaerodinium woloszynska* was present in all tanks.

Some species of microalgae favoured low in total dissolved solids, electric conductivity, salinity and alkalinity in wet weather. Also high light intensity, alkalinity, temperature, secchi depth during dry weather conditions in tank 4 with high stocking density of fish encouraged some species of microalgae to grow such as *Botryococcus* sp., *Ankistrodesmus fusiformis*, *Scenedesmus acutus*, *Ulothrix aequalis*, *Haematococcus pluvialis*, *Anabaena* sp., *Peridenium labeled*, *Dictyosphaerium pulchellum.*

The diversity of phytoplankton is linked with several of environmental parameters such as temperature, pH, dissolved oxygen, salinity, and nutrients (Sridhar et al., 2006). The highest species diversity was observed in all tanks in mix and wet weather conditions, these results are consistent with the fact that mix conditions following wet provides an ideal condition for algae to grow, while the lowest was in dry weather conditions due to impact of high water temperature on due to the impact of high surface water on some biochemical process of microalgae species, because of dominating of microalgae species and limited number of species.

Simpson's index is a measure of diversity which takes into account the number of species present. This index was found to be high in wet and mix weather conditions and low in dry weather conditions. The value of Simpson's index ranges between 0 and 1. With this index, the result was nearer to zero (0) shows richness in diversity and result nearer to one (1) show poor in diversity. On the other hand, the values of species diversity was low in dry weather which may be due to the impact of high surface water on some biochemical process of microalgae species, because of dominating of some species of microalgae.

The total cell density of microalgae species it was diverse between five tanks, the lowest density was in control due to lack of nutrients and absence fish which considered good source for algae feeding and growth. Also total cell density was high in dry weather conditions within low species diversity. In addition, high fish stocking densities also result in large populations because of the plant nutrients that are released from fish excrement (Noga, 2011).

The physico-chemical parameter of water in tanks was influenced by the different weather conditions and different stocking density of fish. Changes in weather conditions influenced on water temperature and nutrients, resulting in decline density of some species of microalgae. The present study showed the differences between different weather conditions which are considered as the main factor impacting on phytoplankton with high number of species in mix and wet weather and high density in the dry weather. Also the seasonal succession of microalgae mentioned to Chlorophyta dominated species followed by Cyanophyta, Bacillariophyta, Dinophyta, Chrysophyta, and Euglenophyta. Findings from this study referred to that different weather conditions support Chlorophyta growth more than other divisions. Dominated species physiologically and behaviourally adapted to permanent water column mixing, high turbidity in dry weather. Also stocking density of fish was an important factor because of the effects on cell density of microalgae but at the same time was not effect on species of microalgae; species were close in all tanks.

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