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Effect of Different Stocking Density of Catfish on Microalgal species composition and Diversity Indices under Varying Weather Conditions

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Abstract

The threat of climate change, rising human population, and food security are some of the issues that need to be addressed urgently. Therefore the objective of this study is to document the species composition of microalgae and its diversity in different stocking densities of catfish in varying weather conditions to minimize water usage and maximize production. *Clarias gariepinus* with size 10±0.2 cm and 18.5±0.3 g were placed in a poly tank of 300L under sheltered transparent roofing. The experimental setup comprising of a control tank with 25 fishes and covered to prevent microalgal growth, 10, 15, 20, and 25 fishes for treatment 1, 2, 3, and 4, respectively. Microalgae were sampled every 2 days; different mean light intensity and temperature represent different weather conditions were recorded. The totals of 29 genera 77 species of microalgae within 5 divisions were identified. The most abundant taxa were Chlorophyta (90%). The common genus in all weather conditions were *Chlorella, Desmodesmus*, *Scenedesmu*s and *Selenastrum.* Shannon diversity index (H'), Simpson index, evenness, and species richness were diversity indices that ranged from 0.59-2.82, 0.21-0.97, to 0.52-1.04, and 2.01-5.36, respectively. The highest stocking density of catfish (25 catfish; T4) under dry weather conditions also produce the highest diversity indices.

Keywords: *Clarias gariepenus, different stocking density, different weather conditions, mixed microalgae, and species composition*

1 Introduction

The global increase in temperature is the result of a serious environmental issue like climate change which is expected to increase steadily shortly (IPCC, 2007; Vijayavenkataraman et al., 2012; IPCC, 2014). Their impacts have a profound effect on almost every aspect of life globally, especially in terms of growth rate, yield, and quality for agricultural produce (White et al., 2011; Zhang et al., 2014). This is of great significance because the agricultural sector is the main source of food to meet the increasing demand of human consumption resulting from the increasing world population, which is projected to reach nine billion by 2050. This situation would put a serious strain on global food security, energy supply, and other needs. Various countries around the world are aware of these impacts and have made efforts to solve the need for high agricultural production by improving productivity to better adapt to extreme weather tolerance and higher growth rate and yields (Wang et al., 2014; Anwar et al., 2015; Zhang et al., 2016). The aquaculture industry is a prominent industry for a food source (over 50% in terms of volume which makes it the most

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rapidly growing food-producing sector (FAO, 2017) and also a significant source of employment and revenue. As more people are becoming health conscious, the trend toward fish consumption also shows a steady increase. This is because fish is low in saturated fats, high in protein, healthy long-chain omega-3 fats and at the same time supply various essential elements including iodine, vitamin D, and calcium.

In 2015, it was estimated that the total global aquaculture production was 106 million tons live weight, valued at US$163 billion mainly consisted of farmed aquatic animals (76.6 million tons, worth US$157.9 billion), aquatic plants (29.4 million tons; valued at US$4.8 billion) and non-food products (41.000 tons; valued at US$208.2 million) (FAO, 2017). Many aquaculture methods focus on fast-growing species such as fish and shrimp but experienced some limitations such as the need to change the water regularly to remove harmful fish excrement, high ammonia that can kill fish (Okomoda et al., 2018). Besides, the discharged water eventually contaminates the aquatic environment and can cause extensive degradation to the aquatic environment and harm other aquatic organisms (Okomoda et al., 2018). These unsustainable aquaculture practices, directly and indirectly, produce negative impacts on food security and lead to pollution issues in the future.

Currently, efforts are being made to promote the development of sustainable aquaculture to supply protein sources also known as the “blue revolution” to address the challenges of climate change, water scarcity, food security, and environmental pollution (Bush et al., 2013). One approach is to incorporate the culture of microalgae in aquaculture, thereby solving many problems simultaneously without additional capital investment or economic loss (Brune et al., 2004).

Integration of microalgae in fish culture is a dynamic approach toward achieving more sustainable aquaculture to lower waste, reduce water consumption, and minimize contamination of the aquatic environment.

Moreover, allowing naturally occurring microalgae to exist in the cultural system will use nutrients like nitrogen and phosphorus (produced by uneaten feed and fish excrement in the fish tank) to prevent ammonia buildup which can kill fish.

This will allow the water to remain in the fish tank longer and reduce water replacement frequency, thus significantly decreasing the cost of water treatment by expensive technology (Renuka et al., 2014. Microalgae are promising organisms that are photosynthetic microorganisms that can fix CO_2 emissions, accumulate lipids suitable for biodiesel production, methane, hydrogen, and ethanol (Ugwu et al., 2008; Sudhakar et al., 2011; Naqqiuddin et al., 2014). Besides, microalgae are high in protein content (50% on dry weight) and a potentially excellent substitute for fishmeal (Becker, 2007). Microalgae also served as a biological indicator of water pollution because they can persist in various environments and thus be useful for nutrient sequestration and elimination of contaminated substrates from water (Renuka et al., 2014; Torres et al., 2008).

Microalgae in fish cages are known to improve the water quality as well as an aqua crop by utilizing fish wastes (Brune et al., 2004) and at the same time, fish will feed on microalgae which would lead to a decrease in the volume of fishmeal required (Becker, 2007) and enhance the survival and growth rates in other aquatic animals like prawn (Ju et al., 2012). Despite its potential, most of the productivity of microalgae is modeled based on laboratory experiments. The productivity of microalgae in an open system is greatly influenced by weather conditions such as light intensity, photoperiod, cloud covers, temperature, and rainfall. Presently there are not many studies to quantify the effect of weather conditions on microalgal productivity. Most microalgal productivities are linked to water quality in the pond (water temperature, dissolved oxygen, and ammonia concentrations), growth, survival, risks of illness, and production cost (Lauria et al., 2018). Since there are few reports on the type of microalgae found in catfish tanks under different weather conditions, this study was conducted to determine microalgae diversity in catfish tanks under different weather conditions (wet, mix, and dry weather).

2 Materials and Methods

Location of study

The experiment was set up at the compound of the Department of Biology, Faculty of Science, and Universiti Putra Malaysia (3°0'6"N 101°42'17").

Experimental facilities

African catfish *Clarias gariepinus* 10±0.2 cm and 18.5±0.3 g initial size and weight respectively were cultured in a big tank for 1 week to recondition the fish before starting the experiment. A total of 15 poly tanks, each with dimensions of (length of 0.75 m; width of 0.90 m; depth of 0.50 m) were setups for five

treatments with three replicates. Three tanks served as control and covered (no light penetration) and filled with 25 catfish. The remaining 12 tanks were exposed to ambient light: treatment tank 1 with 10 fishes, treatment tank 2 with 15 fishes, treatment tank 3 with 20 fishes, and treatment tank 4 with 25 fishes with the stipulated range for intensive culture systems. Initial water was filled at 300 L per tank. These tanks were placed under the transparent rain shelter. The aeration was supplied continuously. The growth performance and each of the diets was fed to the fish at 5% body weight, twice daily with starter feed high in protein for the first 4 weeks and then grower feed until the end of the experiment (34%, 25% crude protein content respectively, Star Feed brand) at 09.00 a.m. and 5 p.m. for 90 days.

Measurement of environmental and water quality parameters

Light intensity and environment temperature were measured using HOBO Pendant data logger (HOBO UA-002-64; Onset Computer Corporation, Bourne, MA, USA), which was placed outdoors near the area of the fish tanks and the data were retrieved at the end of the experiment. Light intensity and temperature concentration data were recorded at 30-min intervals and eventually retrieved from the logger employing HOBOware® Pro software (Onset Computer Corporation). Water quality analysis was conducted every 2 days by taking the water in sample bottles at 09.00 a.m.

Tank water samples were analyzed for pH using a pH meter (InoLab, Germany), temperature by a thermometer (LO-tox^{TM}, United Kingdom), transparency by using the Secchi disc. The concentrations of ammonia, nitrate, and phosphate were quantified by kit method (Hach DR900, USA).

Microalgae samples were collected from tanks every 2 days until the end of the experiment. Microalgae sampling was be done in triplicate for each treatment in the late afternoon around 4.00-5.00 pm. Each 100 ml sample was collected in a plastic bottle and preserved by adding two drops of 3%glutaraldehyde solution (Graham et al., 2009).

Identification of microalgae and species diversity

Let the microalgae suspension be undisturbed for at least 48 hours to settle down the phytoplankton (Graham et al., 2009). Then the upper 50ml water was removed gradually and the remaining water was used to observe algae using a compound microscope. The mixed microalgae samples were observed and identified using compound microscope Leica Model (DM750) under 100X magnification. All species of algae were identified (Prescott, 1970; Hoek et al., 1998).

The microalgae species diversity was studied according to the biodiversity index (Shannon and Wiener, 1949) which usually shows value in the range of 1.5- 3.5 (Bibi and Ali, 2013);

Shannon diversity index, $H' = -\Sigma (pi) \times \ln (pi)$

where: Σ = Summation

Pi = Number of individuals of species i/total number of samples

In = natural logarithm

Margalef's index was employed as a measure of species richness (Margalef, 1958).

Species richness index, $D = (S - 1) / \ln N$

where: S = total number of species

N = total number of individuals in the sample

In = natural logarithm

To calculate the evenness of species, Pielou's Evenness Index (e) was employed (Pielou, 1966).

Evenness, $e = H / \ln S$

where: H = Shannon – Wiener diversity index

S = total number of species in the sample

Simpson's Diversity Index is a measure of diversity that considers the number of species present, as well as the relative abundance of each species (Simpson, 1949).

Simpson's Diversity Index, $D = 1-(\sum n (n-1)/N (N-1))$

where: n = total number of organisms of a particular species

N = total number of organisms of all species

Statistical analysis

One-way ANOVA statistical analysis with Turkey multiple comparison tests using SPSS version 22 were carried out to indicate the significance of variance in species richness, evenness, and species diversity indices among different treatments. A confidence level of 95% ($P<0.05$) was selected to check the significance.

3 Results

The weather conditions and water quality throughout the study period were present in table 1, 2 and 3

Table 1. Average of environmental parameters under the different weather condition

Parameter	Dry Weather	Mix Weather	Wet Weather
Temperature (°C)	33.42±0.42[a]	28.42±0.41[b]	26.40±0.15[c]
Light intensity (µmol m^2s^{-1})	711.28±18.27[a]	301.52±47.97[b]	151.06±9.61[c]

**Each value is presented as Mean±SE. with different letters (a-d) significant different (P <0.05)*

Data From table 1, 2, and 3 suggested that temperature and mean light intensity were significantly higher ($p<0.05$) in dry weather conditions, moderate in mixed weather, and lowest in wet weather but not significant ($p>0.05$) among the treatment tanks in a particular weather conditions

Data From table 1 suggested that temperature and mean light intensity were significantly higher ($p<0.05$) in dry weather conditions (33.42oC±0.42, 711.28±18.27 µmol m^2s^{-1}), moderate in mixed weather (28.42±0.41°C, 301.52±47.97 µmol m^2s^{-1}) and lowest in wet weather (26.40±0.15°C, 151.06±9.61 µmol m^2s^{-1}).

Referring to Table 2, the mean water temperature ranged between 32.77-34.03°C in dry weather conditions, 28.13 -30.03°C in mixed weather, and 26.67-29.67°C in wet weather conditions. The water is significantly turbid ($p<0.05$) in dry weather conditions in the range of 5.56-11.32 cm. There was no significant difference ($p>0.05$) between mixed and wet weather conditions where turbidity ranged between 27.00-30.03 cm.

All in all, referring to table 2 and 3, most parameters show higher data under dry weather conditions, followed by mixed and wet weather conditions, especially, temperature, and ammonia and phosphate concentrations are higher in control under dry conditions compared to all other treatments.

Nitrate concentration (Table 3) was significantly higher ($p<0.05$) in dry weather, moderate in mixed weather, and lowest in wet weather conditions. The nitrate concentration was lowest in the control tank ($p<0.05$) than in treatment tanks with 10-25 fishes. However, among the treatment tanks, thank 1 with 10 fishes was lowest ($p<0.05$) but tanks 2, 3, and 4 did not differ significantly ($p>0.05$) in nitrate concentrations. Ammonia concentrations were highest ($p<0.05$) in the control tank than in treatment tanks (Table 3).

Among the treatment tanks, there are marginal differences in ammonium concentration among treatment tanks except for T4 where the ammonium concentration ranged between 0.62-0.84 mg/L. Phosphate levels were significantly higher in dry weather ($p<0.05$), moderate in mixed weather, and lowest in wet weather. The phosphate concentration was highest in the control tank without microalgae than in other treatment tanks.

Table 2. Physical water quality parameters between tanks and weather conditions (Similar alphabet in column and row denote no significant difference ($p<0.05$)

Parameters	Treatment	Weather conditions		
		Dry [a]	Mix [b]	Wet [c]
Temperature (°C)	Control	34.03±0.23[a]	30.03±0.28[b]	29.67±0.84[b]
	T1	32.77±0.55[a]	28.27±0.18[a]	26.67±0.67[a]
	T2	33.50±1.13[a]	28.13±0.07[a]	26.80±0.15[a]
	T3	34.03±0.58[a]	28.33±0.12[a]	27.57±0.12[ab]
	T4	33.00±0.57[a]	28.23±0.17[a]	27.00±0.18[a]
pH	Control	6.14±0.04[a]	6.94±0.02[a]	6.76±0.03[a]
	T1	7.03±0.04[a]	7.35±0.04[a]	6.76±0.07[a]
	T2	6.97±0.02[a]	6.97±0.02[a]	6.93±0.10[a]
	T3	7.03±0.04[a]	6.95±0.02[a]	7.13±0.11[a]
	T4	7.01±0.08[a]	6.98±0.08[a]	7.05±0.04[a]
Transparency (cm)	Control	7.39±0.28[b]	30.03±0.28[b]	29.67±0.84[b]
	T1	11.15±0.22[c]	28.27±0.18[a]	26.67±0.67[a]
	T2	11.32±0.26[c]	28.13±0.07[a]	26.80±0.15[a]
	T3	6.27±0.2[a]	28.33±0.12[a]	27.57±0.12[ab]
	T4	5.56±0.17[a]	28.23±0.17[a]	27.00±0.18[a]

**Control (25 catfish); T1 (10 catfish+algae); T2 (15 catfish+algae); T3 (20 catfish+algae); T4 (25 catfish+algae)*
*** Each value is presented as Mean±SE. with different letters (a-d) significant different (P <0.05)*

Table 3. Chemical water quality parameters between tanks and weather conditions (Similar alphabet in column and row denote no significant difference ($p<0.05$)

Parameters	Treatment	Weather conditions		
		Dry [a]	Mix [b]	Wet [c]
Nitrate (mg/l)	Control	1.47±0.09[a]	1.13±0.07[a]	0.05±0.06[a]
	T1	2.45±0.03[b]	1.68±0.09[a]	0.60±0.06[a]
	T2	6.38±0.09[c]	5.10±0.05[b]	4.11±0.05[b]
	T3	7.56±0.19[d]	6.53±0.14[d]	5.63±0.12[d]
	T4	6.61±0.25[c]	5.81±0.25[c]	4.92±0.15[c]
Ammonia (mg/l)	Control	3.25±0.21[b]	0.79±0.04[c]	0.61±0.02[c]
	T1	0.45±0.03[a]	0.21±0.01[a]	0.15±0.01[a]
	T2	0.39±0.02[a]	0.28±0.02[ab]	0.25±0.01[b]
	T3	0.49±0.02[a]	0.41±0.03[b]	0.33±0.03[b]
	T4	0.84±0.03[a]	0.73±0.03[c]	0.62±0.02[c]
Phosphate (mg/l)	Control	5.18±0.57[b]	3.22±0.19[bc]	2.47±0.09[c]
	T1	2.39±0.08[a]	1.13±0.03[a]	0.76±0.03[a]
	T2	3.55±0.15[ab]	2.72±0.12[bc]	2.13±0.15[bc]
	T3	4.48±0.12[bc]	3.32±0.13[d]	2.48±0.04[c]
	T4	3.35±0.16[ab]	2.54±0.23[b]	1.91±0.08[b]

**Control (25 catfish); T1 (10 catfish+algae); T2 (15 catfish+algae); T3 (20 catfish+algae); T4 (25 catfish+algae)*
*** Each value is presented as Mean±SE. with different letters (a-d) significant different (P <0.05)*

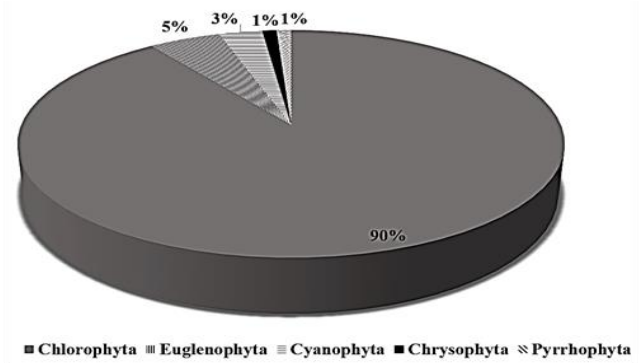

Figure 1. Percentage of total algal classification in catfish tanks under three kinds of weather

Species composition and diversity of microalgae

Concerning microalgal diversities, there are 5 microalgal divisions found in treatment tanks (1-4) in dry, mixed, and wet weather conditions comprising 29 genera and 77 species which were identified from exposed tanks (Figure 1). There were found the higher number of general and species in T4 (21 genera 42 species), T3 (18 genera 36 species), T2 (14 genera 29 species), T1 (12 genera 19 species), and control (no algae), respectively in dry weather, mixed and wet weather condition (Figure 2).

Based on weather conditions there were 21 genera and 55 species in dry weather, 16 genera and 32 species in mixed weather, and 10 genera, 23 species in wet weather. There were clear distinctions in algal composition based on weather conditions. Chlorophyta was the most common and the most dominant division in all three weather conditions and

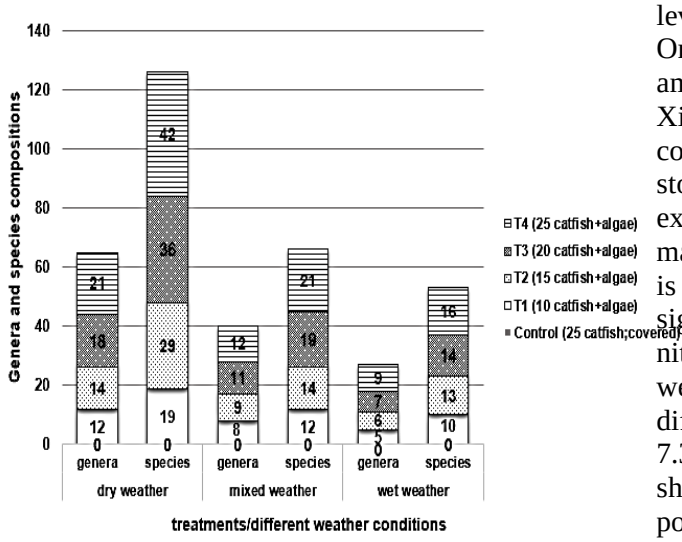

Figure 2. Algal genera and species compositions in catfish tanks under three kinds of weather

in all treatment tanks (T1-T4) which make up almost 90% of microalgae presence. Among the most dominant genera in the division, Chlorophyta is *Chlorella, Desmodesmus, Scenedesmus,* and *Selenastrum.* The next most common division is Euglenophyta comprising a distant 4% of the whole microalgal population and represented by genus *Trachelomonas* and *Euglena*. The three minor microalgal populations present in treatment tanks (T1-T4) were Cyanophyta, Chrysophyta, and Pyrrhophyta which make up 2%, 1%, and 1 % of the total microalgal population respectively. The dominant genus of Cyanophyta, Chrysophyta, and Pyrrhophyta was *Synechocystis, Chrysamoeba*, and *Peridinium*.

Diversity index (Figure 3) including Shannon index, Evenness, species richness, and Simpson index showed the highest data for Treatment 4 (25 catfish with algae) and Treatment 3 (20 catfish with algae) under dry weather condition (H'= 2.82 and 2.68, Evenness = 1.04 and 0.79, species richness= 5.36, 4.82 and Simpson index = 0.97 and 0.87). Statistical analysis showed a significant difference ($p<0.05$), between some treatments under different weather conditions for Shannon index, species richness, evenness, and Simpson index.

4 Discussion

The interesting observation in the control tank (covered tank, no algae present), the nitrogenous compound composed mainly as ammonium due to accumulation of fish excretion and particulate organic matters but in a fish tank with microalgae, the nitrate level is high and ammonium level is low (Table 3). One possible explanation is that microalgae utilize ammonium for their nitrogen source (Shi et al., 2000; Xin et al., 2010). The nitrogenous compound closely corresponds with the fish density. The higher the fish stocking density, the higher the accumulation of fish excretion, uneaten feed, and particulate organic matter. Of all the nitrogenous compounds, ammonium is a threat to fish wellbeing. Statistically, there was a significant difference in temperature, transparency, nitrate ammonia, and phosphate among different weather conditions while pH made no significant difference because it was only slightly changed (6.14-7.35) throughout the culture period. This finding showed that the presence of microalgae improved pond water quality as aquaculture wastewater treatment and also produced a useful crop that could be used as animal feed (Perschbacher, 1995; Yi and Lin, 2001; Sfez et al., 2015).

Ammonia concentrations was highest in dry weather ($p<0.05$) but no significant difference ($p>0.05$) in mixed and wet weather (Table 3) because there are no algae to uptake ammonia into a cell (Shi et al., 2000; Xin et al., 2010).

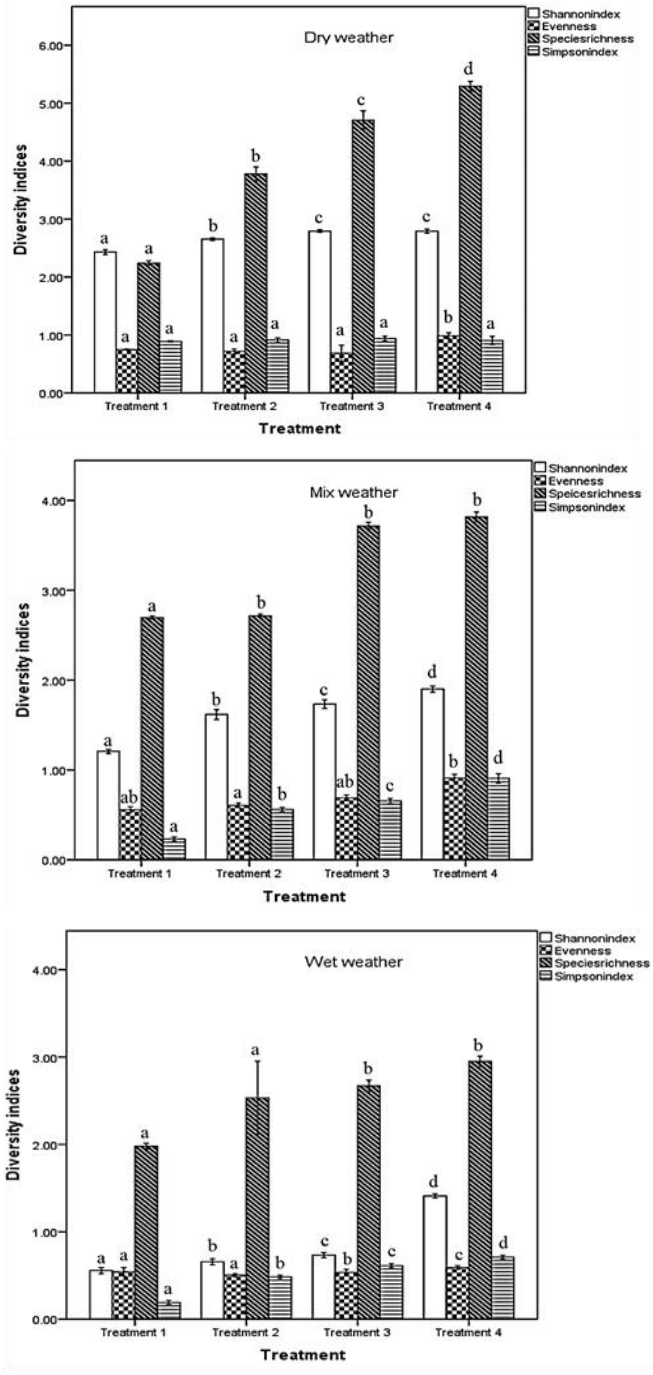

Figure 3. Variation in diversity indices in different treatment under weather conditions

** Each value is presented as Mean±SE. with different letters (a-d) significant different (P = .05) in different treatments and weather conditions*

*** T1 (10 catfish+algae); T2 (15 catfish+algae); T3 (20 catfish+algae); T4 (25 catfish+algae)*

Chlorella, *Desmodesmus*, *Scenedesmus*, and *Selenastrum* (Division Chlorophyta) were the commonly occurring genera in all weather conditions. *Chlorella* was always present in the lighted tank and all weather conditions probably due to its small size with excellent surface/volume for better nutrient uptake, fast growth, and better light and temperature tolerance (Yang et. al, 2008) thus allowing it to dominate in such environments.

Diversity index (Figure 3) including Shannon index, Evenness, species richness, and Simpson index showed the highest data for Treatment 4 (25 catfish with algae) and Treatment 3 (20 catfish with algae) under dry weather conditions. It means that the dry atmosphere was the best condition for microalgae to increase the diversity and biomass of mixed microalgae which is similar to the result reported by Renuka et al. (2014) who found the higher species diversity in the summer season because the higher temperature supported the growth and density of algae. Report to Sahab et al. (2015) the diversity of microalgae was higher in sunny weather compared to wet and mixed weather probably due to higher light intensity and longer photoperiod. Weather conditions directly or indirectly influenced water quality and diversity of natural population microalgae. Weather conditions were supported the necessary factors for microalgae growing, for example, light intensity affects water quality and microalgal diversity (Chew et al., 2018). However, the mean light intensity is also associated with the temperature where higher light intensity also comes with higher temperature. During dry weather conditions, the high average light intensity and also temperature brings about a profound effect on the fish metabolism which in turn increases nutrient like ammonia and phosphorus (Shi et al., 2000; Xin et al., 2010). When microalgal growth is optimal, it uses nutrients such as ammonium and phosphate reducing the level of that nutrient and maintaining the water quality. In control tanks without microalgae, the water quality deteriorated further. The conducive environment during high light intensity and high temperature during dry weather conditions is also reflected in the number of genera and species of microalgae presence in treatment tanks (T1-T4) (Ogbonna and Tanaka, 2000). Moreover, the Report of Richardson et al. (2005) showed that temperature is the major driving force for a seasonal session of phytoplankton. Among diverse groups, Chlorophyta has a wide range of environmental adaptability, and their abundance increases under high temperature (within the range of 20-35 ◦C) (Kagalou et al., 2006). Chlorophyta was the dominant division

in all weathers, affirming the report of Sahab et al. (2015). This could be attributed to the smaller size, higher chlorophyll content for efficient photosynthesis, and better adaptation to varying weather conditions and water quality in a fish tank. Chlorophyta was found in pH ranging from slightly acidic to neutral (6.14-7.35), which is similar to Chinnasamy et al. (2010), which showed Chlorophyta as the dominant group in pH ranging from 6.54-7.18. pH is one of the key factors that affect cell growth and diversity (Renuka et al., 2014) of microalgae. pH is affected by a metabolic process by limited the accessibility of carbon by creating CO_2, which might have led to the stop cell growth (Sharma et al., 2018; Juneja et al., 2013).

5 Conclusion

This study concluded that the diversity of microalgae is influenced by environmental factors such as mean intensity, photoperiod, temperature, pH, and concentration of nutrients. The species composition and species diversity are highest in dry weather conditions, moderate in mixed weather, and lowest in wet weather conditions.

6 Acknowledgements

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