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ARTICLE

Statistical analysis of the effects of environmental factors and fish species on class-sorted phytoplankton composition in aquaculture ponds in northern Thailand

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ABSTRACT

Understanding the phytoplankton in aquaculture ponds is critical for proper pond management. Despite the importance, the relationships between phytoplankton composition, cultured fish type, season, and nutrients were not well understood. This study statistically investigated these relationships in aquaculture ponds. Data collected at 21 tilapia and 13 catfish ponds in September 2009 (wet season), December 2009 (cold season), and March 2010 (hot season) in northern Thailand were used for the analysis. The statistical analysis showed that PO₄-P and NH₄-N concentrations in catfish ponds were significantly higher than in tilapia ponds ($p < 0.05$, Wilcoxon test). The cyanobacterial abundance in catfish ponds was significantly greater than in tilapia ponds ($p < 0.05$, Wilcoxon test). In the hot season (March), green algae were abundant ($p < 0.05$), while cyanobacteria were depleted ($p < 0.05$). Multiple linear regression model was applied to determine important factors for statistically explaining cyanobacterial abundance. The result indicated that the best model selected by AICc included season and pond type as factors influencing cyanobacterial abundance but not nutrients. However, since the effect of nutrients was included in the difference in nutrient concentration due to the difference in fish species in the ponds, it was speculated that nutrients were insignificant as explanatory variables. Furthermore, it was hypothesized that cyanobacterial abundance was reduced in March (hot season) because the predation of cyanobacteria by tilapia may be encouraged at high temperature.

1. Introduction

Phytoplankton communities significantly impact the growth and survival of the fish in aquaculture ponds (Boyd & Tucker,

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1998; Hishamunda et al., 2009). Namely, phytoplankton is considered a primary and natural food source of fish in aquaculture. Moreover, photosynthesis and respiration of phytoplankton directly control the dissolved oxygen (DO) in aquaculture ponds. Especially, geosmin and 2-MIB produced by cyanobacteria have posed a risk to the fish industry because such odorants significantly reduce the values of fish in the market (Gutierrez et al., 2013; Pimolrat et al., 2015; Schrader et al., 2011). Therefore, investigating phytoplankton, especially cyanobacteria, in aquaculture ponds is crucial in providing basic knowledge for appropriate management.

Several researchers have investigated phytoplankton in freshwater aquaculture ponds. Lukwambe et al. (2015) studied the effect of microbial agents on the phytoplankton community as well as the correlation between water quality parameters and phytoplankton density in shrimp aquaculture ponds. The abundance of 18 well-diversified phytoplankton species, including Bacillariophyta, Dinoflagellata, Cyanophyta, and Chlorophyta, was quantified. Kunlasak et al. (2013) investigated the relationships of DO with chlorophyll-a and phytoplankton composition in tilapia aquaculture ponds. The results showed that phytoplankton abundance positively correlated with the DO concentration at the daily maximum and negatively correlated with DO at the daily minimum level. In the winter, Cyanophyta decreased while Chlorophyta increased. However, the study did not analyze the effect of water quality on the phytoplankton composition. In addition, statistical elucidation of the relationships

among phytoplankton, water quality, cultured fish species, and seasonal effects has been insufficient. This study aimed to statistically clarify the effects of nutrients, seasonal information, and cultured fish types (tilapia or catfish) on phytoplankton abundance, especially cyanobacteria, in aquaculture ponds across northern Thailand.

2. Materials and Methods

2.1 Study area

The survey was conducted at 34 aquaculture ponds in Chiang Mai, Chiang Rai, Lampang, Phayao, and Sukhothai provinces in northern Thailand (Table 1). The sampling sites included 21 ponds of Nile tilapia (*Oreochromis niloticus*) and 13 ponds of hybrid catfish (*Clarias macrocephalus*, *C. gariepinus*). The water samples were collected in September 2009, December 2009, and March 2010 ($n = 39$ observations). In each pond, three locations were selected to collect water samples. At each location, two water samples were collected at around 10 cm and 30-60 cm depth (depending on the depth) from the water surface, then all collected samples (using same volume) were mixed thoroughly in a new clean bottle of 1.5 L. Since all sampling sites are privately owned ponds for commercial aquaculture, only codenames and state names are given to keep our promise not to reveal the pond names and exact locations (Table 1).

Table 1 List of sampling sites in northern Thailand

	Location	Sampling time
Catfish pond		
Pond1, Pond2, Pond3, Pond4, Pond5, Pond6, Pond7, Pond8	Chiang Mai	09/2009
Pond10, Pond11	Phayao	09/2009, 03/2010
Pond12	Phayao	09/2009
Pond31	Phayao	03/2010
Pond17	Sukhothai	12/2009
Tilapia pond		
Pond21, Pond22	Chiang Mai	12/2009
Pond23, Pond24, Pond25, Pond26, Pond27, Pond28, Pond32, Pond33, Pond34	Chiang Rai	03/2010
Pond18, Pond19, Pond20	Lampang	12/2009
Pond9	Phayao	09/2009, 03/2010
Pond29, Pond30	Phayao	03/2010
Pond13, Pond14, Pond15, Pond16	Sukhothai	12/2009

2.2 Water quality analysis

After collection, the samples were immediately transported to

the laboratory within 12 hours. Then the samples were kept in an ice box until water quality analysis the next day. Each water sample was filtrated by GF/C glass microfiber filter (Whatman, Merck Co.,

Germany) to analyze $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, and total suspended solids (TSS) based on standard analysis methods given by American Public Health Association (1998). Each analysis was performed in triplicate. In the three repeating, the coefficient of variation (CV) of analysis was controlled within 2%. These averaged values were used for statistical analysis.

2.3 Enumeration of phytoplankton

Phytoplankton was collected using a plankton net with a mesh size of 75 μm (Rigosha Co., Japan). Each sample was concentrated in a 30 mL plastic bottle and immediately preserved with 1 mL of Lugol's solution. Phytoplankton was identified under an optical microscope (BH2, Olympus Co., Japan) and enumerated using the TATAI cell counting chamber (Enosinophil counter, SLGC Ltd.). The counting was repeated three times for the same sample. It is important to note that phytoplankton cells in colonies and filaments, which have unique shapes, are difficult to count individually with a microscope. Therefore, the total number of cells in a filament or a colony was estimated from cells visible under the microscope, considering the size and shape of the colony or filament. This method was preliminary tested as follows. A researcher and a technician counted the cells in a counting chamber; then the counting results were compared to each other. In the case of the phytoplankton colony, a colony was isolated by a microcapillary pipet, then the cells in the colony were counted. After counting, the colony was transferred to a drop of water. Then the colony was dispersed to single cells by ultrasonication, and the cell number was counted again. Normally, in the case of non-colony phytoplankton, the counting error among persons was around 5-10%. However, the error was around 30% for the colony phytoplankton. Although this 30% error was not small, the effect of this error was reduced by the logarithmic transformation in statistical analysis, as described later.

2.4 Statistical analysis

The Shapiro-Wilk test was used to check the normality of the data. Because the data from both groups (tilapia ponds and catfish ponds) were non-normally distributed, the two-sample Wilcoxon test was applied to determine the differences in water quality and phytoplankton abundance between tilapia and catfish ponds. The two-sample t-test was used to compare the phytoplankton abundance among the three seasons. The hierarchical cluster analysis based on the Pearson distance measuring and the "complete" linkage method was applied to identify factors that can clearly distinguish the ponds of two fish types. Finally, the relationship between cyanobacteria and environmental factors in the aquaculture ponds was analyzed using multiple linear regression. The fish species, seasonal information (sampling time), and resulting nutrient variables from the cluster analysis were used as explanatory variables. The cyanobacterial abundance as objective variable and nutrient concentrations as explanatory variables were log-transformed to improve the normality of the data. The best-performed model was selected based on Akaike information criteria (AICc, Burnham et al., 2004). All the statistical

analyses were carried out using R version 4.2.1 (R Core Team, 2021). The significance level was chosen at 0.05.

3. Results and discussion

3.1 Water quality and phytoplankton abundance in tilapia and catfish ponds

Variations of the water quality parameters in aquaculture ponds investigated are displayed in Figure 1, where $\text{NO}_x\text{-N}$ is the summation of the nitrate and nitrite concentrations. The means of $\text{NO}_x\text{-N}$ in catfish ponds and tilapia ponds were 0.25 mg/L and 0.06 mg/L, respectively. The mean value of $\text{NO}_x\text{-N}$ in tilapia ponds was lower than in catfish ponds; however, Wilcoxon nonparametric tests showed no significant difference ($p > 0.05$). On the other hand, the mean of $\text{NH}_4\text{-N}$ concentration in catfish ponds (1.50 mg/L) was significantly greater ($p < 0.01$, Wilcoxon test) than in tilapia ponds (0.33 mg/L). $\text{PO}_4\text{-P}$ concentration in tilapia ponds was less than in catfish ponds ($p < 0.005$, Wilcoxon test). The TSS condition in the two pond types was similar ($p > 0.5$, Wilcoxon test) with a mean of 111.44 mg/L and 149.72 mg/L for tilapia ponds and catfish ponds, respectively. The difference in nutrients may depend on the culture methods where tilapia and catfish are fed differently (Boyd & Tucker, 1998). Some catfish ponds showed extremely high concentrations of ammonia. Catfish are typically cultured at high density in shallow, muddy ponds with no aeration. The ammonia in the pond is derived from fish excrement and decomposed remains of food, but oxygen is insufficient to cause active nitrification at the bottom sediment. Therefore, ammonia is likely to accumulate in the pond. The concentration distribution of $\text{NO}_x\text{-N}$, which means the sum of nitrate and nitrite, was an order of magnitude lower than that of ammonia. In tilapia ponds, a similar trend was observed. This result might be attributed to the composition of the fish feed.

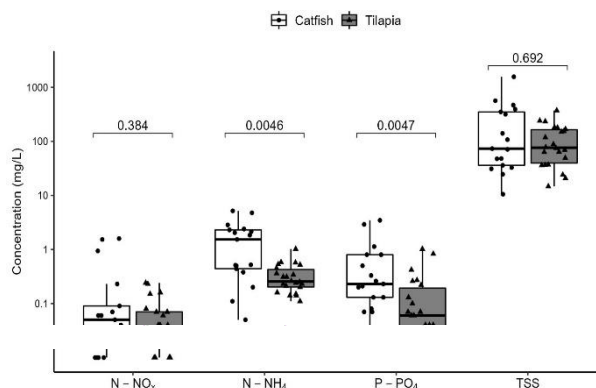


Figure 1 Comparison of water quality parameters between tilapia and catfish ponds. The p-value of Wilcoxon statistical test was shown above the boxplots. The significance level was set at $p < 0.05$.

With the difference in nutrient condition, the phytoplankton abundance in tilapia and catfish ponds was expected to be

dissimilar. The phytoplankton community in the aquaculture ponds surveyed was mainly composed of Cyanophyceae (cyanobacteria), Bacillariophyceae (diatoms), and Chlorophyceae (green algae). Therefore, these three major classes of phytoplankton cells were classified and enumerated. Cyanobacteria and green algae dominated tilapia ponds with 44.73% and 45.06% of total phytoplankton abundance, respectively. Only 10.21% of phytoplankton abundance in tilapia ponds was diatoms. In catfish ponds, cyanobacteria accounted for 93.49% of the total, followed by green algae at 4.68%, then diatoms at 1.84%. As expected, cyanobacterial abundance in catfish ponds was significantly greater than in tilapia ponds ($p < 0.001$, Wilcoxon test, Figure 2). However, there were non-significant differences between the two pond types in diatoms and green algae cell density ($p > 0.05$, Wilcoxon test, Figure 2). As nutrients have positive effects on the growth of cyanobacteria (Chorus & Welker, 2021; Huisman et al., 2005), this result was in agreement with the difference in water quality between tilapia and catfish ponds (Figure 1). Cyanobacteria have better tolerance for ammonia and uptake of N-source than green algae and diatoms (Domingues et al., 2011). As previously shown, catfish ponds had a higher concentration of ammonia than tilapia ponds. In addition, phosphate concentration in catfish ponds was higher than in tilapia ponds. These results suggest that higher N and P loading into catfish ponds by feeding promoted the growth of cyanobacteria. This could be one reason for the high density of cyanobacteria in catfish ponds. Moreover, cyanobacteria are reported as a natural food source for tilapia (Fadl et al., 2020; Hakan Turker et al., 2003; Huo et al., 2021; Turker et al., 2003). The phytoplankton abundance in tilapia ponds was significantly lower than in non-tilapia ponds (Hakan Turker et al., 2003). Therefore, the predatory effect of tilapia on cyanobacteria may be another reason for the low abundance of cyanobacteria in the tilapia ponds in this survey.

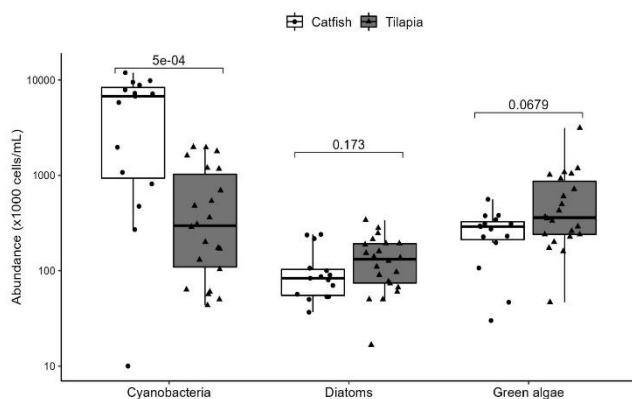


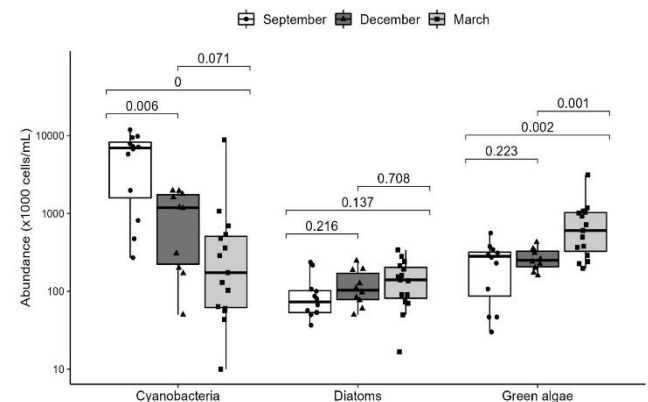
Figure 2 Comparison of phytoplankton abundance between tilapia and catfish ponds. The p -value of Wilcoxon statistical test was shown above the boxplots. The significance level was set at $p < 0.05$.

3.2 Effect of season on the phytoplankton abundance

The survey in this study was conducted during the wet season (September), cold season (December), and hot season (March) in

Thailand. As seen in Figure 3, the t -test showed that the mean abundance of cyanobacteria in September had significant differences with cyanobacteria in December ($p = 0.006$) and March ($p = 0.000$). However, at a significant level of 5%, there was no difference between cyanobacterial abundance in December and March ($p = 0.071$). The mean abundance of green algae in March was significantly greater than in September ($p = 0.001$) and December ($p = 0.002$). Nonetheless, the difference in green algae abundance between September and December was non-significant ($p > 0.05$). Figure 3 showed that all three groups of diatoms abundance (September vs. December, September vs. March, and December vs. March) had no significant differences with p -values > 0.1 . A recent systematic study compared freshwater phytoplankton growth rates for biomass production (Nalley et al., 2018). This study showed that cyanobacteria had the highest optimum temperature, followed by green algae, then diatoms. In northern Thailand, the descending order of ambient temperature is March, September, and December. Therefore, the maximum amount of cyanobacteria is expected in March. In this survey, September showed the highest abundance of cyanobacteria. However, according to Table 1, most of the ponds surveyed in September were catfish ponds, and this result may be due to the biased data collection. Namely, it is not clear from Figures 2 and Figure 3 whether pond type and season primarily affect cyanobacterial density. In addition, the filtration rate of tilapia in cyanobacteria increased with warmer water (Hakan Turker et al., 2003). Therefore, the low abundance of cyanobacteria in March may be because of predation by tilapia.

Figure 3 Comparison of phytoplankton abundance among the wet



season (September), cold season (December), and hot season (March) in Thailand. The p -value of two-sample t -test was shown above the boxplots. The significance level was set at $p < 0.05$.

3.3 Multivariate analysis for pond types, environmental factors and phytoplankton composition

Figure 1 and Figure 2 showed differences in water quality and phytoplankton composition between catfish and tilapia ponds.

Therefore, we applied cluster analysis to further clarify the factors that can characterize the ponds of tilapia and catfish. Figure 4 displayed the most accurate cluster classification which used all nutrient parameters ($\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$) and phytoplankton

abundance (cyanobacteria, diatoms, green algae). The samples were clearly clustered into two groups. Catfish ponds dominated the first group with 11/12 samples. Despite the presence of catfish ponds in the second group, this second group was dominated by tilapia ponds with 21/25 samples. This result is consistent with the results of the Wilcoxon test in Figure 1, suggesting that $\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$ might be important nutrient factors to distinguish the water quality between tilapia and catfish aquaculture ponds.

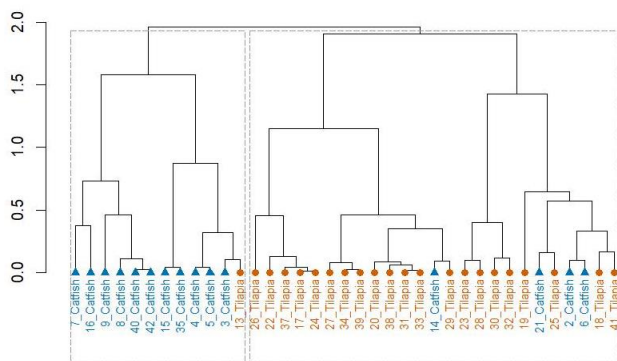


Figure 4 Hierarchical cluster dendrograms of tilapia (red round node) and catfish (blue triangle node) ponds based on $\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, cyanobacteria, diatoms, and green algae.

Table 2 Multiple linear regression models explaining cyanobacterial abundance by nutrient parameters, pond types, and seasonal information. The models were sorted based on AICc. The lower AICc indicates the better model.

Model no.	Model	AICc
1	Cyanobacteria ~ pond type + sampling time	77.39
2	Cyanobacteria ~ pond type + sampling time + NH_4	80.01
3	Cyanobacteria ~ pond type + sampling time + NO_x	80.25
4	Cyanobacteria ~ pond type + sampling time + PO_4	80.25
5	Cyanobacteria ~ pond type + sampling time + NH_4 + PO_4	83.05
6	Cyanobacteria ~ pond type + sampling time + NH_4 + NO_x	83.05
7	Cyanobacteria ~ pond type + sampling time + NO_x + PO_4	83.31
8	Cyanobacteria ~ pond type + NO_x	84.81
9	Cyanobacteria ~ pond type + PO_4	84.81
10	Cyanobacteria ~ pond type + NH_4	84.84
11	Cyanobacteria ~ pond type + sampling time + NH_4 + PO_4 + NO_x	86.32
12	Cyanobacteria ~ pond type + NH_4 +	90.33

Multiple linear regression was applied to determine the influence of environmental factors on the abundance of cyanobacteria. Table 2 compared the AICc results of models which used nutrients ($\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$), pond type (tilapia, catfish), and seasonal information (sampling time) as predictor variables. The lower AICc indicates the better model. In order to clarify the effect of cultured fish types on cyanobacterial proliferation, the pond type was included in all models as a variable of interest. Model 1 showed the lowest AICc, suggesting that pond type and season were the best-performed variables to explain the abundance of cyanobacteria, given our data set. According to the changes in AICc among models, nutrients ($\text{NO}_x\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$) in aquaculture ponds did not significantly affect the cyanobacterial abundance.

Table 3 summarizes the regression results of models 1, 2, 5, and 11. For the best model (model 1), the coefficient for tilapia was negative, indicating that the tilapia pond was associated with lower cyanobacterial abundance compared to the catfish pond. However, it is not statistically significant because the p-value is larger than 0.05. In relation to the cyanobacterial abundance in September, the abundance in December decreased but was not statistically significant. On the other hand, March showed a significant decrease ($p < 0.01$) in cyanobacterial abundance. As shown in Table 3, the second-best model (model 2), which included NH_4 as an explanatory variable, showed similar regression results, with only March showing a significant decrease. Although the positive coefficient of $\log\text{-NH}_4$ is not statistically significant ($p = 0.64698$), this result indicates that NH_4 might accelerate cyanobacterial proliferation. On the other hand, adding NH_4 to the explanatory variables increased the p-value of the tilapia pond type. The addition of PO_4 (a very large p-value = 0.8892) made both the p-value of tilapia pond type and the AICc of the model increase. When the explanatory variables included NO_x in model 11, AICc showed a large value, and p-values of explanatory variables increased. However, the March coefficient in the sampling time was statistically significant with the p-value < 0.05 . Therefore, these results clearly concluded cyanobacterial abundance significantly decreased in March (hot season). Considering the general tendency of the growth rate of cyanobacteria, this result is unexpected (Nalley et al., 2018). Moreover, although not statistically clear, it was indicated that catfish ponds tended to have more cyanobacteria than tilapia ponds. As already mentioned, it may be due to the predation of cyanobacteria by tilapia. Therefore, if we consider that hot conditions promote predation of cyanobacteria by tilapia, the decline of cyanobacteria in March, a period of high temperatures, may be explained. Furthermore, catfish ponds contained higher concentrations of nutrients ($\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$) than tilapia ponds, as shown in Figure 1. Therefore, it was speculated that nutrients were not significant factors for cyanobacterial abundance in the linear regression model because the effects of nutrients were already taken into account in the pond type. In addition, the hypertrophic state of these ponds, which

nearly saturated the nutrient level for cyanobacterial growth, might have reduced the effectiveness of nutrients as an explanatory variable. On the other hand, the data set used in this analysis is

biased, and not enough ponds were investigated. Therefore, unbiased data with a sufficient number of ponds is necessary to obtain more reliable results.

Table 3 Estimation of model parameters

	Coefficient	Standard Error	p-value	F-statistic (p-value)
<i>Cyanobacteria ~ pond type + sampling time</i>				
Intercept	3.5824	0.1809	< 2e-16 ***	9.596 (0.0001058)
December	-0.4046	0.3626	0.27261	
March	-0.9330	0.3235	0.00687 **	
Tilapia	-0.4302	0.3022	0.16395	
<i>Cyanobacteria ~ pond type + sampling time + NH₄</i>				
Intercept	3.5927	0.1844	< 2e-16 ***	7.079 (0.0003355)
December	-0.3811	0.3705	0.31138	
March	-0.9386	0.3277	0.00732 **	
Tilapia	-0.3802	0.3244	0.24987	
NH ₄	0.1219	0.2636	0.64698	
<i>Cyanobacteria ~ pond type + sampling time + NH₄ + PO₄</i>				
Intercept	3.57580	0.22246	< 2e-16 ***	5.493 (0.0009836)
December	-0.39857	0.39634	0.3224	
March	-0.94956	0.34182	0.0092 **	
Tilapia	-0.37944	0.32953	0.2584	
NH ₄	0.13476	0.28304	0.6373	
PO ₄	-0.03312	0.23586	0.8892	
<i>Cyanobacteria ~ pond type + sampling time + NH₄ + PO₄ + NO_x</i>				
Intercept	3.55410	0.33532	1e-11 ***	4.433 (0.002532)
December	-0.38798	0.42056	0.3636	
March	-0.94684	0.34881	0.0109 *	
Tilapia	-0.38095	0.33538	0.2650	
NH ₄	0.14451	0.30843	0.6428	
PO ₄	-0.02828	0.24601	0.9093	
NO _x	-0.01935	0.22070	0.9307	

Note: Significant codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 ' ' 1

4. Conclusions

The relationship between water quality and phytoplankton

community, particularly cyanobacteria, under the influences of season and cultured fish type in aquaculture ponds (pond type) was statistically investigated in this work. There were significant differences in PO₄-P and NH₄-N concentrations between tilapia and catfish ponds. The abundance of cyanobacteria in tilapia ponds was less than in catfish ponds. In addition, the cyanobacterial abundance was significantly affected by season. The multiple linear regression analysis indicated that only season and pond type

as factors influenced cyanobacteria abundance. Since the effect of nutrients was included in the difference in nutrient concentration due to the difference in fish species in the pond, it was speculated that it was not significant as an explanatory variable. This regression results showed a significant decrease of cyanobacteria in March (hot season), which can be explained by the predation of cyanobacteria by tilapia. These new findings need further confirmation by future studies. It is possible to obtain sufficient significance by expanding the target area and designing a survey with less statistical bias.

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