

# Relationships of Dissolved Oxygen with Chlorophyll-a and Phytoplankton Composition in Tilapia Ponds

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Received July 2013

## ABSTRACT

This study investigated the relationships among the parameters of dissolved oxygen, chlorophyll-a and phytoplankton composition in tilapia ponds. Each pond (a total of 18 ponds) was sampled once in the dry, winter season between January and March and again early in the rainy season between May and June. The data were analyzed by examining correlations among parameters as affected by season, altitude and culture system. Observations were made at sites located in 5 selected provinces of northern Thailand: Chiangrai, Chiangmai, Phayao, Lampang and Nakornsawan. Mean elevation of these areas range from 25 to 582 meters above sea level (masl) and were categorized into low (<400 masl) and high (>400 masl) elevation sites. Ponds were 0.8 - 2.0 m deep, 0.16 - 0.64 ha in area and could be further categorized into high and low input systems. Mean air temperature in winter ranged between 16.5°C - 35.8°C while mean water temperature ranged between 25.5°C - 27.1°C. In rainy season, air temperature ranged between 22.0°C - 37.3°C and water temperature ranged between 29.4°C - 31.8°C. The amount of chlorophyll-a in both seasons were comparable ( $p > 0.05$ ), but chlorophyll-a in high input system was significantly higher ( $p < 0.05$ ) than in low input ponds. Only weak correlation was found between chlorophyll-a,  $DO_{max}$  and  $DO_{min}$ . Multifactor-ANOVA was used to analyze the difference of total bacteria and filamentous cyanobacteria in ponds based upon elevation, culture systems and season. Result shows that there is a significant interaction observed between elevation, culture system and season ( $p < 0.05$ ). Species diversity and composition of phytoplankton in fish ponds in 2 seasons revealed the presence of 90 genera of phytoplankton under all 7 divisions. Divisions Chlorophyta and Cyanophyta had the most number of genera identified in both seasons with *Pediastrum* spp., and *Scendesmus* spp., and *Anabaena* spp. as dominant genera/genus, respectively.

**Keywords:** Dissolved Oxygen; Chlorophyll-a; Phytoplankton Composition; Tilapia Ponds; Elevation, Season

## 1. Introduction

Increased demand for high protein food and apparent declines in capture fisheries together have helped drive rapid expansion of the aquaculture industry in the past two decades. The aquaculture industry however is facing challenges such as high cost of inputs and climatic changes. Climate effect such as increase in temperature is observed to increase disease transmission, deplete oxygen, increase incidence of harmful algal blooms in ponds to mention a few [1].

Tilapia culture is one of the major aquaculture industries in Thailand with river cage and earthen pond cultures were both practiced. However, some tilapia cage farmers have switched from river to earthen pond due to difficulties with extreme water flows and poor water quality. There are substantial differences in tilapia culture systems among places depending on various constraints and opportunities such as topography (lowland

and upland) and availability of water and alternative nutrient inputs [2]. Elevation above sea level, for instance, influences air and water temperature in pond culture [3].

Physical, chemical and biological water quality in fish pond ecosystem influences growth and survival rates as well as reproduction and likelihood of disease infection [4]. Dissolved oxygen (DO), in particular, is an important factor for fish respiration and phytoplankton dynamics. DO content typically correlates with phytoplankton density in fish ponds. Maintenance of phytoplankton populations at desired levels is an important but difficult aspect of fish pond management. Many fish culture manuals stress that an algal bloom must be maintained to improve oxygen levels, to prevent macrophyte growth, and to provide natural foods, either directly or indirectly, for fish in the pond [5-7]. At the same time, however, uncontrolled algal growth causes many serious problems for aquaculturists [8]. Thus, proper management of phytop-

lankton growth is a major goal of modern pond aquaculture [7-10].

While phytoplankton affects water quality in several ways [7,8], the difficulty and importance of managing phytoplankton stem primarily from the complex relationship between algal dynamics and dissolved oxygen levels. Phytoplankton are the major source of dissolved oxygen in fish ponds as well as—directly as consumers and indirectly as the source of detritus upon which most bacterial respiration is based—the major sink for oxygen [11,12]. Dissolved oxygen levels depend primarily on the relative magnitudes of photosynthetic oxygen generation and total plankton respiration [13]. Given the complexity of this relationship and the importance of dissolved oxygen to aquacultural production [14], the interaction between dissolved oxygen and phytoplankton biomass should be examined in detail.

Water quality in aquaculture ponds is influenced by management and other external factors such as culture system, stocking densities, water exchange practices and sources, and fertilizer application. The main objective of this research was to determine the association between chlorophyll-a, phytoplankton composition, and dissolved oxygen in tilapia ponds from different culture systems in winter and rainy seasons. This information will be then used to develop techniques to manage phytoplankton and dissolved oxygen dynamics in fish ponds.

## 2. Materials and Methods

### 2.1. Study Sites

In this study, data was collected in the dry, winter season, between January and March and in the early rainy season between May and June 2013. Observations were made in 18 ponds at sites located in 5 selected provinces of northern Thailand: Chiangrai, Chiangmai, Phayao, Lampang and Nakornsawan. Mean elevation of these areas range from 25 to 582 meters above sea level (masl). Ponds were 08 - 2.0 m deep and 0.16 - 0.64 ha in size at different elevations above sea level (low: <400 masl and high: >400 masl) and employed different culture systems: high input (high load of nutrient resulting either from intensive feeding or from manure fertilization) and low input (low load of nutrient where feeding was sporadic).

### 2.2. Water Sampling and Analysis

Pond water samples were assessed to determine levels of algal biomass, total bacteria and other water quality parameters. Dissolved oxygen was monitored in situ along with pH, temperature, turbidity and conductivity using the multi-meter TOA DKK WQC-22A (Japan). Water samples were collected for nutrient analyses (nitrate-N, nitrite-N, total ammonia-N, and orthophosphate-P) and

chlorophyll-a determination in the laboratory following standard methods [15]. For hydro-biological analysis, phytoplankton was sampled by filtration of 5-L pond water with a net of 10- $\mu$ m mesh. Samples were concentrated in a 30-mL plastic bottle and immediately preserved with 1 mL Lugol's solution. Species and count of phytoplankton were determined using an Olympus BH2 microscope with the aid of a 1000 oil immersion objective. Total bacterial analysis was done using the pour plate technique as described in ISO, 1999 and SCA, 2002 [16,17]

### 2.3. Statistical Analysis

Analysis of Variance (ANOVA) was used to compare water pond parameters across the two seasons and elevation groups. Paired sample T-test was used to compare the differences of water quality variables between the two seasons, elevation groups and culture systems.

## 3. Results and Discussion

### 3.1. Effect of Season on the Relationship between Chlorophyll-a and Dissolved Oxygen

Monitoring of tilapia ponds was carried out during winter and early rainy season. Mean chlorophyll-a concentrations, DO<sub>max</sub> and DO<sub>min</sub> were comparable between the two seasons (Table 1). Mean DO (max and min) concentrations were not significantly different between the two seasons. A significant negative correlation was found between chlorophyll-a and DO<sub>min</sub> in both seasons (Figure 1). There was no association with DO<sub>max</sub>. The frequency of DO<sub>min</sub> levels below 1-ppm threshold value (red line in figures) were comparable between the two seasons.

As a general rule of thumb, DO level in ponds correlates with the amount of chlorophyll-a which results from phytoplankton biomass. In water bodies when the amount of phytoplankton increases, the amount of chlorophyll-a increases as well [18] and so does DO due to algal photosynthesis during daylight (positive relationship with DO<sub>max</sub>). However, as phytoplankton biomass

**Table 1. Means ( $\pm$ SD) of chlorophyll-a, DO<sub>max</sub> and DO<sub>min</sub> in winter and rainy season.**

Parameter	Season	
	Winter	Rainy
Mean Chlorophyll-a ( $\mu$ g·L <sup>-1</sup> )	177.8 $\pm$ 174.0 <sup>ns</sup>	204.8 $\pm$ 220.1 <sup>ns</sup>
Mean DO max (mg·L <sup>-1</sup> )	7.78 $\pm$ 1.94 <sup>ns</sup>	7.59 $\pm$ 4.60 <sup>ns</sup>
Mean DO min (mg·L <sup>-1</sup> )	1.96 $\pm$ 2.19 <sup>ns</sup>	1.12 $\pm$ 1.68 <sup>ns</sup>

Values with the same letter superscripts in rows are not significantly different ( $P > 0.05$ ).

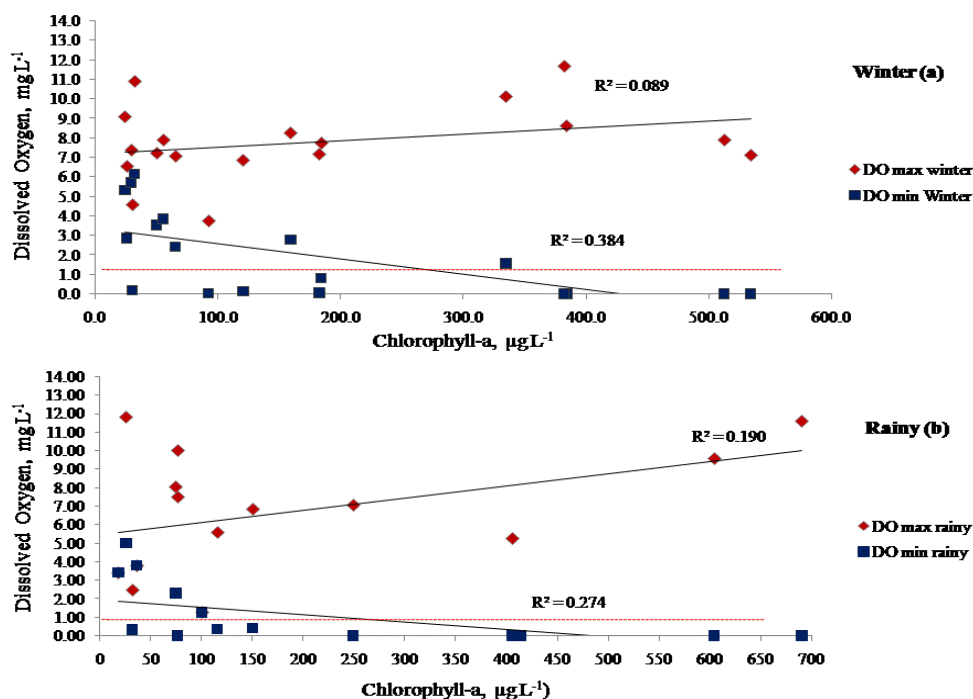


Figure 1. Correlation of  $DO_{max}/DO_{min}$  versus chlorophyll-a for winter (a) and rainy (b) season.

increases, respiration during nighttime can deplete DO concentrations to critical values (negative relationship with  $DO_{min}$ ). The lack of relationship of chlorophyll-a with  $DO_{max}$  and significant negative association with  $DO_{min}$  in both seasons could be explained that DO was affected not only by algal photosynthesis but also by aquatic respiration, oxidative decomposition of organic compounds (from fish and animal wastes), water exchange rate and artificial pond aeration (in the case of commercial ponds). Differences in culture practices among ponds surveyed in the study may have also influenced relationships and will now be considered in more details.

### 3.2. Effect of Culture System on the Relationship between Chlorophyll-a and Dissolved Oxygen

Mean chlorophyll-a concentration was significantly lower in tilapia ponds which adopt low input culture system compared to those that receive high inputs (Table 2). Mean DO (max and min) concentrations of ponds receiving low and high inputs were not significantly different. A significant positive correlation between chlorophyll-a and  $DO_{max}$  was observed in high input ponds but not low-input ponds (Figure 2). A significant negative correlation of chlorophyll-a with  $DO_{min}$  was found in low-input ponds, but not high input ponds (Figure 2).

High input ponds, which comprise both surveyed commercial and integrated fish farming ponds, are expected

Table 2. Means ( $\pm$ SD) of chlorophyll-a,  $DO_{max}$  and  $DO_{min}$  in high and low input-based culture systems.

Parameter	Culture System	
	High input	Low input
Chlorophyll-a ( $\mu\text{g}\cdot\text{L}^{-1}$ )	273.0 $\pm$ 199.3 <sup>a</sup>	45.1 $\pm$ 26.8 <sup>b</sup>
DO max ( $\text{mg}\cdot\text{L}^{-1}$ )	8.20 $\pm$ 3.46 <sup>ns</sup>	6.78 $\pm$ 3.10 <sup>ns</sup>
DO min ( $\text{mg}\cdot\text{L}^{-1}$ )	0.65 $\pm$ 1.22 <sup>ns</sup>	3.20 $\pm$ 2.08 <sup>ns</sup>

Values with the same letter superscripts in rows are not significantly different ( $P > 0.05$ ).

to have higher chlorophyll-a concentrations compared to low input (subsistence) ponds due to their higher nutrient loads. Commercial tilapia farms usually adopt intensive culture system of production with high stocking rates which rely heavily on the use of commercial feeds rather than on natural food. As fish waste and uneaten feed enter the pond system, nutrients accumulate in the bottom, released into the water and taken up by phytoplankton bloom. Similarly, integrated ponds (with pig and chicken) produce high loads of nutrients from manure fertilization for the purpose of producing natural food for the fish. The utilization of organic manure as the principal nutrient input to the pond is a traditional management practice in freshwater fish farming in Thailand.

In high input culture system, changes in  $DO_{max}$  slightly follow adjustments in chlorophyll-a concentration showing a weak positive relationship. However, the negative correlation observed in low input culture system between

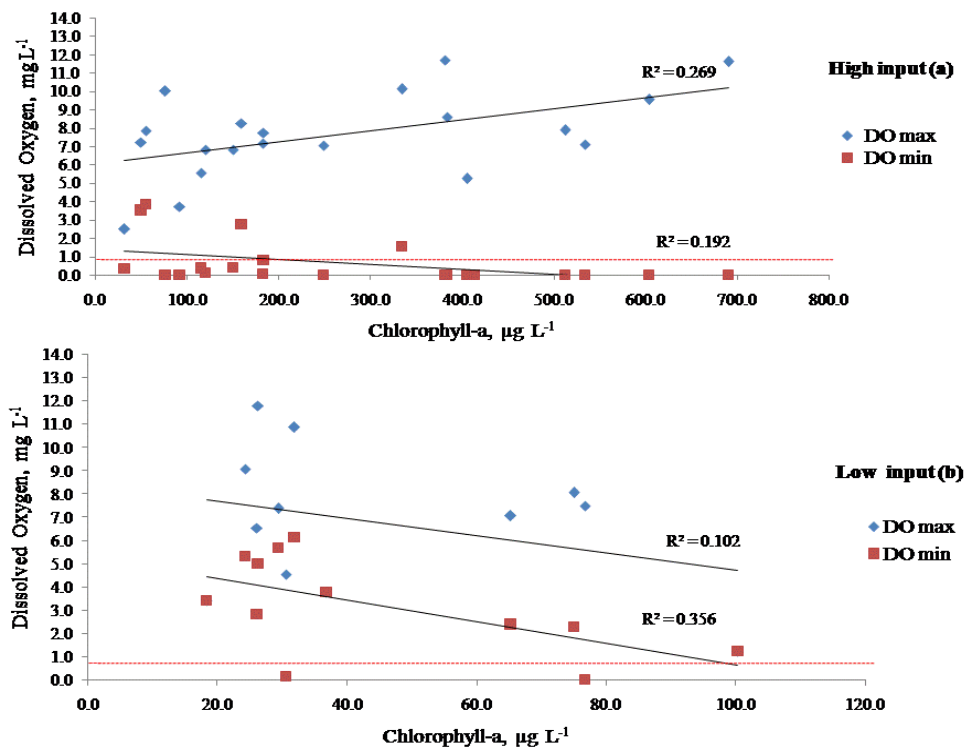


Figure 2. Correlation of  $DO_{max}/DO_{min}$  versus chlorophyll-a for high input (a) and low input (b) culture system.

chlorophyll-a and  $DO_{max}$  contradicts the general rule and no explanation can currently be found with regards to this result.

### 3.3. Effect of Elevation on the Relationship between Chlorophyll-a and Dissolved Oxygen

Mean chlorophyll-a, mean  $DO_{max}$  and  $DO_{min}$  in ponds surveyed from low elevation sites were not significantly different from ponds in high elevation as shown (Table 3). In low elevation sites, chlorophyll-a and  $DO_{max}$  was positively correlated with each other, whilst chlorophyll-a and  $DO_{min}$  were correlated negatively (Figure 3). In high elevation sites chlorophyll-a was significantly negatively correlated with  $DO_{min}$  (Figure 3).

The present study covered a wide range of tilapia production systems (commercial, integrated and subsistence) at low elevation sites. Water quantity is not a major constraint, but these areas may acquire other impacts making changes to culture systems e.g. floods, capital cost or market access. These impacts may cause farmers to switch from commercial to integrated system (fish and pig or chicken/multiple fish species) to reduce production cost but the quality of tilapia maybe affected in terms of off-flavor due to uncontrolled cyanobacterial growth in high-nutrient waters of an integrated system. The integrated system of culture could blend well with the resource-poor small-scale aquaculture farmers especially

Table 3. Means ( $\pm$ SD) of chlorophyll-a,  $DO_{max}$  and  $DO_{min}$  at <400 and >400 masl.

Parameter	Elevation, masl	
	<400	>400
Chlorophyll-a ( $\mu\text{g}\cdot\text{L}^{-1}$ )	219.0 $\pm$ 222.0 <sup>ns</sup>	155.6 $\pm$ 153.1 <sup>ns</sup>
$DO_{max}$ ( $\text{mg}\cdot\text{L}^{-1}$ )	7.19 $\pm$ 4.28 <sup>ns</sup>	8.27 $\pm$ 1.71 <sup>ns</sup>
$DO_{min}$ ( $\text{mg}\cdot\text{L}^{-1}$ )	1.00 $\pm$ 1.65 <sup>ns</sup>	2.27 $\pm$ 2.19 <sup>ns</sup>

Values with the same letter superscripts in rows are not significantly different ( $P > 0.05$ ).

those dependent on aquatic resource for their livelihoods since this strategy would make them more resilient to climate variability. On the other hand, to resist the impact of flooding in low elevation sites, the farmer can strengthen and increase the height of the perimeter dikes. The farmer can likewise deploy nets on the top of the dykes so that when a flood occurs, the fish remain in the ponds. At high elevation sites, it could be the proper area for subsistence system since water scarcity, fingerlings quality and lower temperature are major conditions.

Higher elevation sites and high input systems were more likely to have  $DO_{min}$  values below the threshold level of 1 ppm than at lower elevation sites and low input systems, respectively (Table 4). Chlorophyll-a in high input system were significantly higher than in low input system. Seasonal and elevation differences were not significant (Table 4).

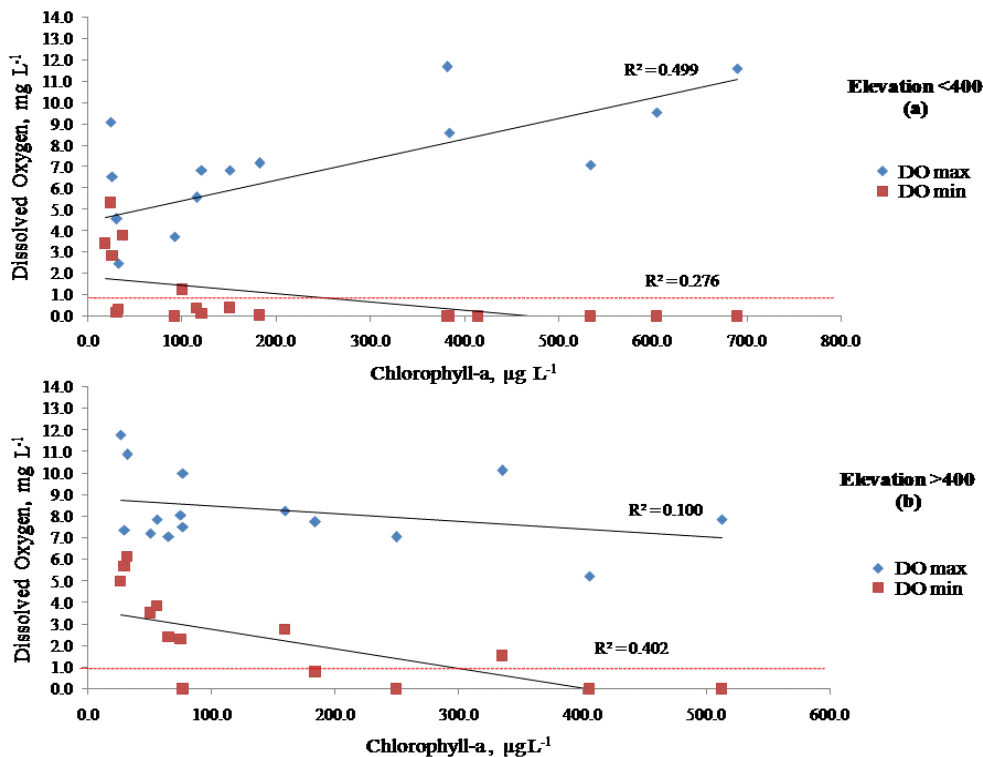


Figure 3. Correlation of DO<sub>max</sub>/DO<sub>min</sub> versus chlorophyll-a for <400 masl (a) and >400 masl (b) sites.

Table 4. Percentage (%) of tilapia ponds with DO<sub>min</sub> below the threshold value (1 ppm) and effect of season, culture system and elevation on chlorophyll-a.

Factor	Mean Chlorophyll-a (µg·L <sup>-1</sup> )	Number of ponds		(%)
		Total (n)	No. with <1 ppm DO <sub>min</sub> .	
<b>Season</b>				
Winter	177.8 ± 174.0 <sup>ns</sup>	18	9	50.00
Rainy	204.9 ± 220.1 <sup>ns</sup>	15	10	66.67
<b>Culture system</b>				
high input	273.0 ± 199.3 <sup>a</sup>	22	17	77.27
low input	45.1 ± 26.9 <sup>b</sup>	12	2	16.67
<b>Elevation</b>				
<400	218.9 ± 222.0 <sup>ns</sup>	18	13	72.22
>400	155.6 ± 153.1 <sup>ns</sup>	15	6	40.00

Values with different letter superscripts in the column are significantly different (P < 0.05).

### 3.4. Effect of Elevation, Culture System and Season on Total Bacterial Load

Mean total bacteria concentration in pond water at high input system in low elevation sites (<400 masl) was significantly higher (P < 0.05) than in high elevation sites (>400 masl) whilst in low input systems at high elevation sites mean total bacteria concentration was significantly higher (P < 0.05) than at low elevation sites (Table 5).

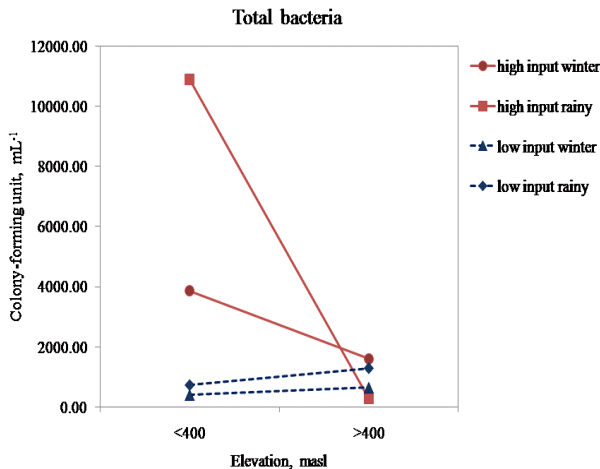
Multifactor-ANOVA was used to evaluate differences in total bacterial concentration in ponds based upon elevation, culture system and season. Significant interaction among elevation, culture system and season for total bacteria concentration were observed (Figure 4).

In general, temperature varies inversely with elevation. This is one of the reasons why lower bacteria activity was seen in higher altitude ponds (>400 masl). Mean

**Table 5. Total bacteria ( $\times 10^3$ ) ( $\pm$ SD) in ponds at different elevation in both culture system.**

Culture System	Elevation, masl	
	<400	>400
High input	7.37 $\pm$ 5.30 <sup>a</sup>	1.17 $\pm$ 1.11 <sup>b</sup>
Low input	0.29 $\pm$ 0.16 <sup>b</sup>	0.97 $\pm$ 0.59 <sup>a</sup>

Values with different letter superscripts in rows are significantly different ( $P < 0.05$ ).



**Figure 4. Total bacteria in ponds at different elevation and culture system in both seasons.**

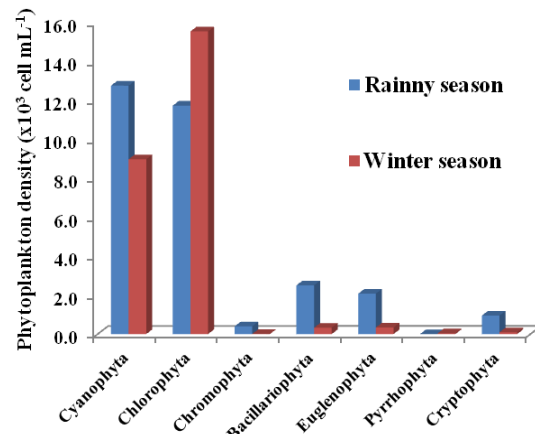
temperatures at low elevation sites are higher by 2 degrees than at high elevation sites. High organic loading also increased nutrient and bacterial levels in high input pond systems at <400 masl. Commercial and integrated culture systems (high input systems) have higher fish stocking density and feeding rate and therefore the amount of organic matter and waste (including excess uneaten feeds) are expectedly high for these systems which increase bacterial load in water. This could deplete DO from water during the decomposition of these materials at the bottom. Farms, especially those which grow tilapia in commercial ponds like the one in Phayao (>400 masl), use aerators to counteract DO depletion during nighttime.

However, for low input pond system at high elevation sites, bacterial level is higher than the hotter low elevation sites. This observation contradicts the result obtained for high input pond system. Temperature has been shown to be one of the limiting factors for bacterial growth particularly in relatively low temperatures (<12°C - 15°C) although many of bacteria are adapted to colder environment, hence, the result. It is also possible that subsistence ponds in higher altitudes had higher organic matter content than in lower altitude ponds which could have significantly contribute to the bacterial load in the water.

### 3.5. Phytoplankton Density and Effect of Elevation, Culture System and Season on Filamentous Cyanobacteria Biomass

For the assessment of diversity and composition of phytoplankton in the surveyed ponds, a total of 90 genera of phytoplankton from 7 divisions were identified covering the 2 sampling seasons. Division Chlorophyta (49 genera, 54.4%) was the most abundant followed by Bacillariophyta (13 genera, 14.4%), Cyanophyta (10 genera, 11.1%), Cryptophyta (7 genera, 7.8%), Chromophyta (5 genera, 5.6%), Euglenophyta (5 genera, 5.6%), and Pyrrophyta (1 genus, 1.1%) which was found only in winter. In terms of phytoplankton density, Cyanophyta (cyanobacteria) dominated the pond waters in winter whilst Chlorophyta were the prevalent group during rainy season. There were no significant differences in total biomass of both Cyanophyta and Chlorophyta between seasons (Figure 5). Among the phytoplankton identified in the study, *Pediastrum* spp. and *Scendesmus* spp., both chlorophytes, and *Anabaena* spp., a cyanobacteria, dominated the pond waters.

Mean density of filamentous cyanobacteria was significantly higher ( $P < 0.05$ ) in high input system at low elevation sites (<400 masl) than in high elevation sites (>400 masl). However, result for low input system shows otherwise, where density of filamentous cyanobacteria was observed to be significantly higher ( $P < 0.05$ ) at high elevation sites as opposed to low elevation sites (Table 6).



**Figure 5. Phytoplankton densities in ponds in 2 seasons.**

**Table 6. Cell density ( $\times 10^3$ ) ( $\pm$ SD) of filamentous cyanobacteria in ponds at different elevation in both culture system.**

Culture System	Elevation, masl	
	<400	>400
High input	7.96 $\pm$ 5.37 <sup>a</sup>	1.43 $\pm$ 0.1 <sup>b</sup>
Low input	0.34 $\pm$ 0.16 <sup>b</sup>	1.78 $\pm$ 0.6 <sup>a</sup>

Values with different letter superscripts in rows are significantly different ( $P < 0.05$ ).

Multifactor-ANOVA was used to analyze the difference of filamentous cyanobacterial biomass in ponds based upon elevation, culture system and season (Figure 6). Results show significant interactions among elevation, culture system and season for filamentous cyanobacteria.

Water temperature plays a significant role in the dissemination of phytoplankton [19]. Seasonal variability changes algal density and diversity. The study shows that the amount of phytoplankton in rainy season is generally higher than in winter, except for Chlorophyta, which conforms to the amount of chlorophyll-a in the ponds. Furthermore, the quantity of filamentous cyanobacteria in high input system was higher than in low input system owing to the huge amount of nutrients present in the former. The water temperature during the study ranged between 28°C - 32°C. High fish stocking densities (>10,000 fish ha<sup>-1</sup>) and feeding rates (exceeding 70 kg ha<sup>-1</sup>.d<sup>-1</sup>) resulted in high waste loading rates that often caused excessive eutrophication in fish ponds, leading to the proliferation of cyanobacteria [20] in high input system, especially during the rainy season. Generally, cyanobacteria require higher temperature than any other algae in order to increase its growth rate [21,22] hence, the high temperature during rainy season supports highly visible blooms of cyanobacteria, in particular, *Anabaena* spp. One major implication with filamentous cyanobacteria, such as *Anabaena* spp., dominating the pond waters is the production of off-flavors, which could be acquired by the fish and adversely affect market demand.

### 3.6. Managing Chlorophyll-a and Dissolved Oxygen in Aquaculture Ponds

Chlorophyll-a and dissolved oxygen are relevant parameters to be managed in an aquaculture pond system. Phytoplankton, which is indexed by chlorophyll-a, can develop conspicuously in pond waters, leading to eutrophic conditions, especially during climate-driven low-water periods. Similarly, dissolved oxygen is important to the health of aquatic ecosystems and a key indicator in determining water quality. Uncontrolled phytop-

lankton growth (high chlorophyll-a) can be a serious problem for the farmers as it takes more oxygen out of the water during the night than what remains in solution from daytime photosynthesis. Moreover, phytoplankton die offs can increase bacterial decomposition and the reduction in normal oxygen production can lead to oxygen depletions, high ammonia levels, and stressed or dead fish. To alleviate this problem mechanical aeration must be applied to meet the increased demand for oxygen and prevent oxygen depletion and subsequent fish losses or stress [22]. In high input systems nutrient reduction is arguably the best strategy to reduce the incidence of algae, especially harmful cyanobacterial blooms [23]. Thus, maintaining the suitable amount of phytoplankton in fish pond is important.

An important limitation of this study was the restriction of sampling to just two time periods. Observations over more months and multiple years are needed to fully the effects of seasons and other climate-driven factors on chlorophyll-a and dissolved oxygen relationships.

## 5. Conclusion

The relationships between chlorophyll-a, and DO max or DO min were complex, being effected by season, nutrient inputs and elevation. Taken together findings suggest that other factors other than algal photosynthesis were involved. As would be expected chlorophyll-a was much higher in high input than in low input ponds. Significant interactions were observed for total bacteria and filamentous cyanobacteria between elevation, culture system and season. Divisions Chlorophyta and Cyanophyta had the most number of genera identified in both seasons with *Pediastrum* spp., and *Scendesmus* spp., and *Anabaena* spp. as dominant genera/genus, respectively.

## 6. Acknowledgements

The work was carried out with the aid of a grant from the International Development Research Centre, Ottawa, Canada, as a contribution to the AQUADAPT project.

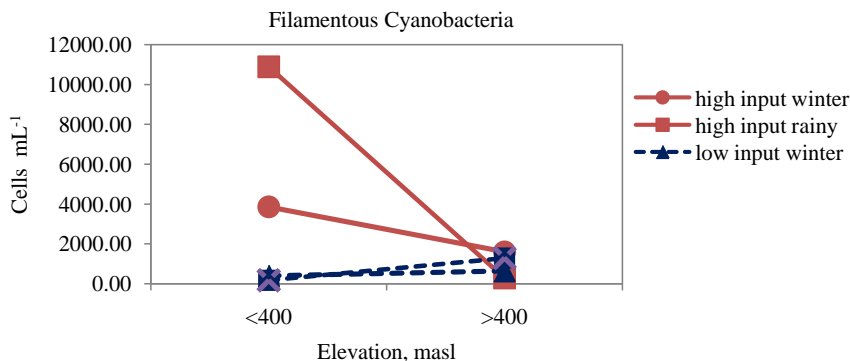


Figure 6. Density of filamentous cyanobacteria in ponds at different elevation and culture system in 2 seasons.

Special thanks to Redel Gutierrez of Maejo University for editing this paper.

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