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Changes in phytoplankton populations within integrated culture systems of caged Nile tilapia with open-pond Pacific white shrimp and Giant freshwater prawn

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Abstract The phytoplankton population in cage culture systems in earthen ponds was reported. The results can be used as a guideline for effective management planning of such system. The changes in phytoplankton numbers were studied at three tilapia farms in Ratchaburi Province, Western Thailand. The two ponds were sampled every month for two crops in each farm in a study period of three months. A total of 36 species of phytoplankton were recorded. Green algae were dominant in terms of the number of species (21 species), and blue-green algae were dominant in terms of quantity (78.5% of average total density). The density of phytoplankton in the ponds increased as the culture period progressed. The average phytoplankton density was between 66,003.2 cells/L and 188,187.2 cells/L. The ecological indices showed that the diversity of the phytoplankton decreased and some species of phytoplankton were more prominent than others duration study period. It can be concluded that the relationship between phytoplankton and environmental factors was increased in phytoplankton volume in the pond and related to water-quality factors. The accumulation of waste in ponds is related to change in phytoplankton population, namely: total suspended solids (TSS) in water (r = 0.563), total ammonia (r = 0.514), nitrite (r = 0.521), total nitrogen (r = 0.722), phosphate-phosphorus (r = 0.558) and biological oxygen demand (BOD) (r = 0.530).

Keywords: Nile tilapia, Pacific white shrimp, Giant freshwater prawn, cage culture, pond culture

Introduction

In the tilapia culture pond, two main negative impacts, such as the changes in water quality and the off-flavor problem in fish, are affected by the phytoplankton group (Tucker, 2000; Smith *et al*., 2008; Wangwibulkit, 2008; Suriyaphan, 2008; Gutierrez *et al.*, 2013). With respect to the natural food of

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aquatic animals, phytoplankton is the primary source and the starting point in the food web within ponds. Nile tilapia can feed on both living and dead phytoplankton in suspension in the water. The preparation of natural food at the beginning of a crop is necessary (Suriyaphan, 2008). Regarding water-quality changes in ponds, photosynthesis and respiration of phytoplankton result in high oxygen concentrations in the daytime and low at night. Such day-night disparity in O_2 also has effects on pH levels, ammonia toxicity, hydrogen sulfide levels, and the growth of aquatic animals. Water-quality problems in most ponds are caused by the inability to control the volume of phytoplankton in ponds. Sudden changes in water quality cause animal stress and yield reduction (Bhatnagar and Devi, 2013; Kunlasak *et al*., 2013). With regard to off-flavor problems in fish and shrimp, blue-green algae, including *Anabeana*, *Oscilatoria*, *Nostoc*, and *Microcystis*, are phytoplankton that release odorous compounds into fresh water. Such microcystins (toxins produced by the algae) cause many of the off-flavor problems, characterized by unpleasant odors present in the body of aquatic animals. The most prevalent off-flavors in aquaculture products are caused by the organic compounds, geosmin and 2-methylisoborneol (MIB) (Tucker, 2000; Gutierrez *et al.,* 2013). These chemicals will flourish alongside both blue-green algae blooms and mass deaths. Geosmin and MIB are released in high volume and will accumulate in the tissues of aquatic animals (Pimolrat *et al*., 2015), especially tissues that have a high-lipid (fat and oil) composition. When accumulated in the tissues, such compounds are difficult to disperse. Other off-flavor problems can be caused by aquatic animals eating such chemicals directly or via absorption through various parts of their skin or tissue (Wangwibulkit, 2008). In other reports, the current tilapia culture in Thailand is rife with high-stock densities. Such populations require high volumes of feed. Thus wastewater in ponds, caused by the animals' excreta and the high volumes of uneaten feed remaining, leads to high growth in phytoplankton. This in turn leads to greater incidence of compounds that cause the off-flavor problems in pond product (Rahman *et al*., 2008; Dhawan and Laur, 2002; Tavares *et al*., 2010).

Nile tilapia farming is one of the most important aquaculture businesses in Thailand. Both the domestic and foreign markets have significant demand for the Kingdom's tilapia production. The Department of Fisheries found in 2016 that Thailand tilapia production had reached was about 176,400 tons (Nurit, 2016). In 2017, Thailand tilapia production had risen to 185,000 tons (Nurit, 2017; 2018) and was likely to expand in 2018, to around 189,000 tons (Nurit, 2018). The majority of farmers prefer high-density tilapia farming to increase productivity. The tilapia culture system in Thailand can be separated into three types according to the culture characteristics: cage culture in rivers, pond culture, and cage culture in earthen ponds.

At present, tilapia cage culture in rivers has been found to be more problematic due to the degradation of natural water sources and the inability to control environmental factors. Such problems have resulted in serious damage to the livelihoods of many tilapia farmers. However, in standard pond culture, there are problems with feed remaining in ponds, feed failing to be evenly distributed across ponds resulting in the fish of unequal sizes, harvesting problems, and the off-flavor problems from phytoplankton proliferation discussed above. All have resulted in damages to farmers' output (Surajit *et al*., 2009). As a result of these problems, many farmers have become increasingly more interested in cage culture systems in earthen ponds (Gutierrez *et al.*, 2013), which is a combination of both systems, and aims for good yields and better control of environmental factors.

In recent times, government agencies, large farms and many private companies have begun to support cage culture systems in earthen ponds. This is because such systems: enable better control of environmental factors, reduce the damage caused by disease in contrast to open systems, and make feeding and harvesting product easier. As a result, the newer system is attracting greater attention from farmers than it had in the past. However, knowledge of the management of environmental factors in cage culture systems in earthen ponds is still relatively poorly disseminated – notably, data on changes in phytoplankton populations in this system's ponds. Therefore, it was necessary to study the related environmental factors and their relationships to farming efficacy. It was the goal to use as a guideline for effective management planning ahead of the implementation of such system.

Materials and methods

Study area and culture conditions

Changes in phytoplankton populations of tilapia (*Oreochromis niloticus*) raised via cage culture in earthen ponds, polycultured with Pacific white shrimp (*Litopenaeus vannamei*) and freshwater prawn (*Macrobrachium rosenbergii*), were studied at tilapia farms in Bang Phae District, Ratchaburi Province, Western Thailand. Samples were taken at three farms (two ponds per farm) every month for two crops. Most of the ponds are small, covering areas of around 0.48-0.96 hectare. At each farm, there are 6-8 ponds. Each cage for raising tilapia are enclosed with 5-6-centimeter mesh, and are 7 meters wide, 15 meters long, and 2 meters high. Cages are installed numbering 4-10 per pond, with an aerator in each cage. The culture period covered by this study was three months per crop. The culture patterns and relevant information were gathered via in-depth interviews of farmers.

Water-quality sampling and analysis

Sixteen water-quality parameters that affected tilapia cage culture in earthen ponds were monthly investigated. Water-quality samples were collected at three farms from two ponds per farm, and sampling was replicated three times per pond and two crops per farm (one crop period was three months). The sampling period was separated into four periods – one week before fish stocking, during the first month, during the second month, and during the third month (during the harvest period).

The study period took place from September 2015to May 2016, and all water-quality measurements were conducted according to APHA, AWWA and WEF (2012) standard methods for examination of water and wastewater. Four indexes of the water-quality factors were analyzed in the field, including temperature by thermometer, transparency by Secchi Disk, pH by pH meter (YSI PRO20) and dissolved oxygen by DO meter (YSI PRO20). However, other indexes were analyzed in the laboratory, including total suspended solids using the glass-fiber-filter method, total alkalinity by the titrimetric method, total hardness by the EDTA titrimetric method, nitrite-nitrogen by the colorimetric method, nitrate-nitrogen by the cadmium-reduction method, total ammonia-nitrogen via the phenol-hypochlorite reaction method, total nitrogen by persulfate digestion, Cd reduction/diazotization method, orthophosphate by the ascorbic acid method, phosphate-phosphorus and total phosphorus by persulfate digestion, ascorbic acid method, BOD by the five-day BOD test and chlorophyll-a by the spectrophotometric method.

Total phytoplankton estimation

Phytoplankton specimens were collected by filtration of 50 Ls of the water samples using a 20-micrometer plankton net. The samples were fixed with 4% formalin for further analysis in the laboratory. Phytoplankton samples were collected in the same location as the sampling sites for water specimens. The samples were collected in triplicate in the pond and sampling was conducted every month for the two crops. Species lists and the density of phytoplankton specimens were proved under a microscope.

Statistical analysis

Pond-water-quality data were analyzed using descriptive statistics including maximum-minimum, mean and standard deviation, to explain the overall water-quality changes in the ponds. Ecological structure data of phytoplankton including density, diversity index, evenness index, and dominant index were based on Krebs (2001). Analysis of the phytoplankton data was also carried out using descriptive statistics to describe the overall changes in the composition of species in the ponds. The data from water-quality and phytoplankton analysis were averaged from all ponds and two crop cycles.

Correlation analysis of phytoplankton populations and the water-quality factors were tested under parametric statistical conditions beforehand. If the data were normally distributed, Pearson correlations were not used in the analysis of the relationship. However, if the data were not normally distributed, the Spearman correlation statistics were performed in the analysis of the relationship, at a 95-percent-confidence level. Linear regression was analyzed for essential factors that had a high correlation coefficient ($r = 0.7-0.9$).

Results

Culture pattern, pond characteristics, and production

Characteristics of tilapia cage culture in earthen ponds with open-pond Pacific white shrimp and freshwater prawn in the study areas are displayed in Table 1. On pond preparation, the ponds were dredged up at about 1-3 year intervals. The duration of the pond drying was generally not more than five days, and the average range was 1-3 days. During the drying period, farmers applied lime into pond basins. The main water supply was from irrigation canals in the area. Pond water was generally circulated among ponds inside the farm without releasing effluent. However, water shortages occurred at times due to: low rainfall and closure of the floodgate from the dam at the upper reaches of the irrigation canal. Moreover, most farms did not have water storage ponds.

Pond water depth was maintained at 1.5 meters. The salinity of the water during crop growth was in the range of 1-5 ppt. At the beginning of the crops, the farmers adjusted pond water salinity at the level 2-3 ppt by using salt from salt fields before stocking shrimp to the amount of 2-4 sacks/pond (50 kg/sack). The average stocking density of 200-gram tilapia was 1,000 fish/cage, with 4-10 cages per pond, while shrimp and prawn were stocked in the open water in ponds. The stocking rate of post-larva-15 Pacific white shrimps and two-month-old freshwater prawn were about 80,000-150,000 shrimps/pond, and

10,000-23,000 prawns/pond respectively. During the culture period, if the salinity was lower than 2 ppt, commercial minerals were added to the ponds. Additional commercial microorganisms were applied to improve water and pond soil every 15 days.

Based on interviews with farmers in the field, it was found that the average yield of the tilapia was 971.88 - 1,094.10 kg/cage, the production of shrimp averaged 581.75 - 906.50 kg/pond, and freshwater prawn production averaged 392.50 - 487.50 kg/pond. According to the growth preferences of Nile tilapia, it was found that the feed conversion ratio (FCR) in this study was in the range of 1.20 - 1.34. Weight gain per day was 6.43 - 7.45 grams /body/day, the average percentage of weight gain was 385.94 - 447.05 and the average specific growth rate was 1.32-1.42 percent/day.

Table 1. Average culture pattern and growth performance of tilapia polycultured with Pacific white shrimp and freshwater prawn in two crops

Culture pattern and growth performance	Farm name		
	JD	JN	AP
Tilapia (average per crop)			
- Stocking density (fish/cage)	1,000	1,000	1,000
- Fish weight (g)	200	200	200
- Total feed (kg/cage/crop)	1,055.00±38.13	1,031.25±42.65	1,070.35±69.07
- Total fish product (kg/cage)	1,016.50±76.50	971.88±58.07	1,094.10±62.57
- Feed conversion ratio	1.33±0.14	1.34±0.07	1.20±0.08
- Weight gain per day (g/fish/day)	6.80±0.64	6.43±0.48	7.45±0.52
- Weight gain (%)	408.25±38.25	385.94±29.04	447.05±31.28
- Specific growth rate (%)	1.35±0.06	1.32±0.05	1.42±0.05
Pacific white shrimp and Freshwater prawn (average per crop)			
- Pacific white shrimp stocking density (shrimp/ m^2)	13 - 16	16	13 - 17
- Pacific White shrimp size	post-larvae 15	post-larvae 15	post-larvae 15
- Freshwater prawn stocking density (prawn/ m^2)	2 - 3	3	2 - 3
- Freshwater prawn (age)	2 month	2 month	2 month
- Total feed (kg/pond/crop)	1,546.00±137.59	1,364.75±107.54	1,419.25±23.06
- Total shrimp product (kg/pond)	581.75±201.41	762.50±62.92	906.50±109.68
- Total prawn product (kg/pond)	392.50±102.43	487.50±25.00	431.25±90.32

Water-quality factors in the ponds

In this study, water samples were collected to a total of 144samples from all 6 ponds and 2 crop cycles. The results are reported in Table 2. Most water-quality indexes were in line with the appropriate criteria for freshwater aquaculture, and the indexes, namely BOD, transparency and total suspended solids in water showed high accumulations in the ponds during the culture period.

The average BOD value in the ponds was higher than the standard criteria at the end of the crops. The average transparency was lower than the standard criteria during the culture period after the first month. These may have come as a result of the accumulation of waste feed and excreta from the aquatic animals in the pond throughout the culture period. It was noticed that the average TSS was higher than the standard criteria at the beginning of crops until the end of crops, and the high TSS before culture was probably due to the effect of the high accumulation of nutrient rich in organic matter in the pond-bottom soil from previous crops. There is a risk of hypoxia in the ponds, especially at night, which can directly affect fish production in the ponds.

Farmers have to add more water to ponds in the case of water- level decreases only because of the limited water within the irrigation systems in the study areas. The installation of aerators in each cage and the switching on of aerators almost all the time (devices are only turned off at feeding times) has become the solution for dissolved oxygen depletion. Results for dissolved oxygen levels in the culture period were in the appropriate range. However, the levels of dissolved oxygen was beyond saturation point due to photosynthesis of phytoplankton, especially at the end of the crop during daylight hours. Farmers should be aware of the hypoxia problem at night. Farmers should regularly check aerators to make sure they are in good working condition at all times. Alternatively, farmers should allocate areas of farms to build storage ponds for water exchange during times of insufficient supply from irrigation channels.

Species composition of phytoplankton in ponds

The composition and quantity of phytoplankton species in the ponds were studied. Four groups of phytoplankton were found, namely: blue-green algae, green algae, diatoms, and dinoflagellate. A total of 36species of phytoplankton were recorded. The data were presented in Table 3.

Green algae was the dominant phytoplankton in that it had the highest number for species diversity. There were 21species of green algae. Blue-green algae (Cyanophyta) was the dominant phytoplankton by quantity, and this

Table 2. Average water-quality parameters in two crops

Parameter	Beginning crop	1st month	2nd month	3rd month	Standard
Temperature (^oC)	(28.6-35.4) 31.73±2.30	(29.2-33.5) 31.78±1.26	(29.7-33.5) 31.74±1.30	(25.5-34.6) 30.43±3.01	25-32 ACFS (2016)
Transparency (cm)	(20-70) 36.39±15.04	(20-50) 30.00±9.05	(20-40) 25.42±7.22	(15-35) 21.25±6.08	30 – 60 ACFS (2016)
Total suspended Solids (mg/L)	(7.3-222.3) 107.75±55.64	(116.7-324.0) 235.56±55.39	(223.3-466.7) 325.81±72.07	(266.7-666.7) 401.57±110.38	≤ 25 ACFS (2016)
Dissolved oxygen (mg/L)	(4.62-16.77) 8.18±3.68	(6.61-17.36) 11.62±3.85	(8.46-19.87) 12.18±3.01	(7.44-15.58) 10.78±2.81	≥ 4 ACFS (2016)
pH	(7.42-9.14) 7.86±0.50	(7.35-9.26) 8.29±0.56	(7.94-9.21) 8.44±0.37	(7.51-8.76) 8.23±0.35	6.5 – 8.5 ACFS (2016)
Alkalinity (mg/L as $CaCO_3$)	(105.0-211.7) 143.08±36.32	(111.3-247.7) 172.44±50.54	(110.7-274.3) 188.42±58.80	(82.3-465) 203.75±122.15	50 – 200 ACFS (2016)
Hardness (mg/L as $CaCO_3$)	(310-1,060.0) 603.2±228.46	(246.7-1,043.3) 583.25±236.23	(326.7-1,316.7) 646.11±228.75	(253-973.3) 631.31±248.01	80 – 200 ACFS (2016)
Total Ammonia (mg/L as N)	(0.005-0.031) 0.015±0.008	(0.007-0.064) 0.033±0.018	(0.023-0.184) 0.086±0.050	(0.054-0.436) 0.183±0.108	≤ 0.5 ACFS (2016)
Nitrite (mg/L as N)	(0.002-0.052) 0.013±0.016	(0.003-0.075) 0.022±0.024	(0.005-0.131) 0.055±0.050	(0.008-0.925) 0.168±0.251	<0.4 Bhatnagar and Devi (2013)
Nitrate (mg/L as N)	(0.007-0.165) 0.052±0.048	(0.010-0.191) 0.089±0.064	(0.055-0.239) 0.141±0.061	(0.074-0.542) 0.246±0.161	< 4.0 Bhatnagar and Devi (2013)
Total nitrogen (mg/L as N)	(0.070-0.745) 0.435±0.283	(0.269-1.291) 0.717±0.379	(0.331-1.983) 1.216±0.425	(0.967-2.682) 1.578±0.460	< 4.0 ACFS (2016)
Orthophosphate (mg/L as P)	(0.002-0.008) 0.004±0.0017	(0.002-0.010) 0.006±0.0022	(0.004-0.015) 0.008±0.0034	(0.005-0.078) 0.017±0.002	≤ 0.2 Bhatnagar and Devi (2013)
Total filterable dissolved Phosphate (mg/L as P)	(0.005-0.016) 0.010±0.004	(0.010-0.021) 0.014±0.003	(0.014-0.068) 0.028±0.017	(0.014-0.163) 0.047±0.041	<0.2 Bhatnagar and Devi ((2013
Total phosphorus (mg/L as P)	(0.011-0.043) 0.023±0.012	(0.012-0.107) 0.040±0.024	(0.026-0.121) 0.053±0.027	(0.032-0.275) 0.090±0.070	< 0.5 ACFS (2016)
BOD (mg/L)	(1.55-12.55) 9.39±3.08	(10.13-24.60) 14.90±4.04	(13.81-26.37) 19.59±4.26	(15.57-33.70) 23.85±5.6	≤ 20 ACFS (2016)
Chlorophyll a (mg/m^3)	(63.48-74.10) 66.75±3.34	(71.22-130.77) 83.18±17.14	(67.84-161.45) 101.25±37.36	(69.20-171.00) 119.67±52.73	100-300 Bhujel (2014)

could be found throughout the culture period. It had an average density of from 2, 603.9to 174, 983.1cells/L, and green algae (Chlorophyta) showed an average density of from 10, 736.8to 45, 410.3cells/L. Green algae showed dominance at the beginning of the crop, while density was relatively stable at the beginning and at the final harvest. In contrast, the blue-green algae group presented a minor population at the beginning of the crop. After that, an increase in its population over time was recorded toward the end of crop. Phytoplankton in other groups in the ponds of the study area were found in small amounts. If considered at the genus level, it is found that *Oscillatoria* is the dominant genus of phytoplankton by quantity (62.52% of all phytoplankton), followed by the genus *Scenedesmus* (14.63% of all phytoplankton) and the genus *Spirulina* (12.57% of all phytoplankton), respectively. The data of each genus are shown in Table 3.

Table 3. List of phytoplankton composition and density in beginning crop and harvesting period

Group	Genus	Plankton density (unit/L)			
		Beginning crop	Harvesting period	Total	%*
Blue-green algae	*Merismopedia*	89.7 ±105.9	2,651.0 ± 2,524.6	5,950.4 ±1,063.8	1.49
	Microcystis	208.4 ±294.7	5,106.9 ±339.7	7,314.3 ±2,321.5	1.84
	Oscillatoria	1,383.5 ± 839.2	145,641.7± 84,408.0	248,899.2±68,505.3	62.52
	Spirulina	891.5 ± 1,160.2	21,580.4 ± 25,023.7	50,037.4 ±11,095.8	12.57
	Anabaena	0.8 ±1.2	3.1 ±2.0	9.4 ±2.5	0.00
	Cylindrospermopsis	29.4 ±38.5	1.1 ±1.6	195.2 ±41.2	0.05
	Raphidiopsis	0.6 ±0.8	0.0 ±0.0	4.2 ±1.7	0.00
Total		2,603.9 ± 2,436.4	174,983.1 ± 57,201.4	312,408.7±81,917.7	78.47
Green algae	*Actinastrum*	0.6 ±0.8	87.7 ±124.0	5,41.3 ±137.8	0.14
	Pediastrum sp.1	340.7 ±408.2	435.1 ±114.3	2,293.4 ±489.7	0.58
	Pediastrum sp.2	83.0 ±38.1	1,778.5 ±322.2	3,623.2 ±735.2	0.91
	Pediastrum sp.3	0.8 ±1.2	34.1 ±48.3	79.5 ±22.7	0.02
	Tetraedron	24.2 ±4.7	3.1 ±4.3	105.9 ±33.0	0.03
	Coelastrum	596.1 ±270.1	2,341.8 ± 2,072.1	8,340.1 ±1,385.3	2.09
	Crucigenia	12.1 ±14.3	126.5 ±97.3	1,095.6 ±242.0	0.28
	Scenedesmus sp.1	9,553.6 ± 9238.6	3,338.1 ±457.8	46,696.3 ±12,903.6	11.73
	Scenedesmus sp.2	1,574.7 ± 2002.8	1,315.9 ±268.1	11,557.8 ±2,963.4	2.90
	Scenedesmus sp.3	0.0 ±0.0	42.9 ±60.7	174.7 ±62.1	0.04
	Staurastrum	27.9 ±37.1	5.8 ±5.9	89.0 ±25.1	0.02
	Euglena sp.1	100.9 ±142.7	270.5 ±278.9	710.9 ±79.8	0.18

Table 3. (Con.)

Group	Genus	Plankton density (unit/L)			
		Beginning crop	Harvesting period	Total	%*
	Euglena sp.2	131.0 ± 180.6	225.3 ± 287.2	1,397.9 ± 244.1	0.35
	Lepocinclis	86.6 ± 122.5	508.7 ± 719.4	1,624.7 ± 382.1	0.41
	Phacus sp.1	220.3 ± 306.9	15.7 ± 20.7	472.8 ± 83.6	0.12
	Phacus sp.2	0.3 ± 0.4	9.6 ± 13.6	83.5 ± 18.9	0.02
	Phacus sp.3	56.1 ± 78.6	45.7 ± 14.0	530.7 ± 96.5	0.13
	Strombomonas	0.0 ± 0.0	0.0 ± 0.0	37.3 ± 11.4	0.01
	Trachelomonas	15.0 ± 21.2	55.0 ± 74.6	86.4 ± 22.6	0.02
	Unidentified green algae	0.0 ± 0.0	362.8 ± 513.0	465.2 ± 171.3	0.12
Total		12,823.8 ± 11,946.9	11,002.7 ± 4,353.9	79,973.6 ± 16,970.0	20.09
Diatom	*Cyclotella*	129.1 ± 160.6	2,048.3 ± 2,581.5	5,205.3 ± 967.5	1.31
	Gyrosigma	1.7 ± 2.4	0.0 ± 0.0	3.4 ± 1.0	0.00
	Navicula	4.7 ± 2.8	12.2 ± 17.3	54.6 ± 8.9	0.01
	Campylodiscus	1.4 ± 1.9	0.3 ± 0.4	14.7 ± 5.5	0.00
	Surirella	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 1.1	0.00
	Unidentified diatom	9.1 ± 9.7	24.7 ± 35.0	38.3 ± 10.6	0.01
Total		145.9 ± 168.8	2,085.5 ± 2,633.4	5,318.7 ± 975.1	1.34
Dinoflagellate	*Ceratium*	0.0 ± 0.0	89.2 ± 116.7	103.0 ± 42.6	0.03
	Unidentified dinoflagellate	62.1 ± 87.8	26.2 ± 2.1	317.1 ± 49.0	0.08
Total		62.1 ± 87.8	115.9 ± 115.3	420.4 ± 40.0	0.11
Total phytoplankton		15,635.6 ± 14,639.9	188,187.2 ± 55,596.1	398,121.3±74,944.1	100

Remark: *% = Percentage of average total phytoplankton.

Changes in phytoplankton composition in the ponds

The composition and density of phytoplankton in the ponds of this study can be classified into four groups (Tables 3 and 4), namely: (1) Blue-green algae group, with *Oscillatoria* as the dominant plankton. Overall, the average number of blue-green algae at the beginning of the crop was 2,603.9 ± 2,436.4 cells/L, and at the end of the crop, it was 174,983.1 ± 57,201.4 cells/L. (2) Green algae group, with *Scenedesmus* as the dominant plankton. Overall, green algae had averaged at the beginning of the crop 12,823.8 ± 11,946.9 cells/L, and at the end of the crop, 11,002.7 ± 4,353.9 cells/L. (3) Diatom group, with *Cyclotella* as the dominant plankton. Overall, the average amount at the beginning of the crop was 145.9 ± 168.8, and at the end of the crop, was 2,085.5 ± 2,633.4 cells/L. (4) Dinoflagellate, only two species were found: *Ceratium* sp. and an unidentified specimen. Overall, the average amount at the beginning crop was 62.1 ± 87.8 cells/L, and at the end of the crop was 115.9 ± 115.3 cells/L.

Table 4. Average phytoplankton density in two crops

Phytoplankton group	Phytoplankton density in culture period (unit/L)			
	Beginning crop	1st month	2nd month	3rd month
Blue-green algae	2,603.9±2,436.4 (16.65 %)	18,334.2±789.4 (27.78 %)	116,487.5±29,966.2 (90.8 %)	174,983.1±57,201.4 (92.98 %)
Green algae	12,823.8±11,946.9 (82.02 %)	45,410.3±13,777.2 (68.80 %)	10,736.8±3,356.6 (8.37 %)	11,002.7±4,353.9 (5.85 %)
Diatom	145.9±168.8 (0.93 %)	2,171.5±1,972.8 (3.29 %)	915.8±533.1 (0.71 %)	2,085.5±2,633.4 (1.11 %)
Dino-flagellate	62.1 ±87.8 (0.40 %)	87.2 ±73.4 (0.13 %)	155.2±186.0 (0.12 %)	115.9±115.3 (0.06 %)
Total	15,635.6±14,639.9	66,003.2±14,887.2	128,295.3±26,956.8	188,187.2±55,596.1

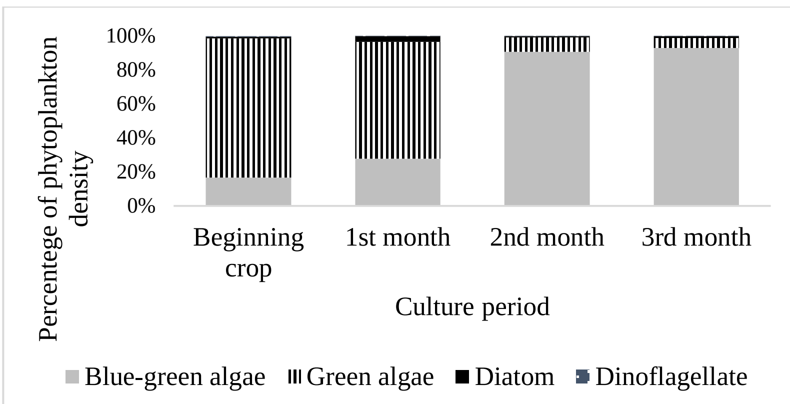

Figure 1. Percentege of phytoplankton density in culture period

When considering the average total density of all phytoplankton in the pond, it was found that the amount of phytoplankton in the pond increased over time. In the pre-stocking period, the average quantity was 15, 635.6 ± 14, 639.9 cells/L. At the beginning of the crop (first month), the average volume was 66, 003.2 ± 14, 887.2cells /L. During the second month, the average volume was 128, 295.3 ± 26, 956.8cells/L, and at the end of the crop (third month), the average volume was 188, 187.2 ± 55, 596.1cells/L. Changes in the composition of phytoplankton (Table 4) in the ponds under study focus on explaining the two dominant phytoplankton groups, blue-green algae and green algae, because both groups were more than 95% of the total amount of phytoplankton in the ponds. In the pre-stocking period, green algae were the dominant group (82.02% of all phytoplankton), and blue-green algae were about 16.65%. The volume of green algae in the ponds under study decreased over the length of the culture period, but the volume of blue-green algae increased during the culture period. At the end of the crop (third month), blue-green algae reached the highest quantity to 174,983.1 $\pm$57,201.4 cells/L (92.98%), and the green algae decreased for only 5.85% (Figure 1).

Ecological index of phytoplankton in the ponds

From the average data of two crops, four groups of phytoplankton were found. In the pre-stocking period, the average diversity index of phytoplankton was 1.18 ± 0.39, the evenness index was 0.13 ± 0.05, and the dominant index was $0.87 \pm$ .0.05This could explain why, at that time, there were some phytoplankton that were more distinguished than the phytoplankton of other groups; it was a group of green algae with an average volume to 82.01% of all phytoplankton. During the culture period, it was found that the phytoplankton had a diversity index in the first month (1.22 $\pm$0.48) that was higher than it was in the pre-stocking period. This could explain why, during the first month of the crop, the amount of phytoplankton in other groups began to increase, especially in the blue-green algae group. In the first month, the amount of phytoplankton in the pond was slightly different and this period showed the highest diversity index in the culture period. The culture period during the second and third months showed that the diversity index of phytoplankton had decreased. At that time, the evenness index decreased over time, but the dominant index increased. Moreover, the dominant index had the highest value during the third month, which included the end of the crop and the harvesting period. The results showed that after the first month of the culture period, blue-green algae began to increase in quantity and replaced the green algae that had previously been high in the pre-stocking period. At the end of the crop, blue-green algae were the group with the highest density in the sampling area. The average density of blue-green algae was 174,983.1 $\pm$177,562.9 cells/L (92.98% of all phytoplankton). The dominant genus of blue-green algae group was *Oscillatoria*, with an average volume of between 1,383.5 and 145,641.7 cells/L, followed by *Spirulina* with an average volume between 891.5 and 21,580.4 cells/L (Table 5).

An overview of the ecological indices data can conclude that the diversity index of phytoplankton in the ponds under study decreased as the culture period progressed. But, the dominance index increased as the culture period progressed and the evenness index was at a very low level. All ecological indices displayed that the diversity of the phytoplankton decreased and some species of phytoplankton were more prominent than others as time progressed.

Table 5. Ecological indices of phytoplankton in culture period

Culture period	Ecological indices		
	Diversity index	**Evenness index**	**Dominance index**
Beginning crop	1.18 ± 0.39	0.13 ± 0.05	0.87 ± 0.05
1st month	1.22 ± 0.48	0.11 ± 0.05	0.89 ± 0.05
2nd month	0.92 ± 0.54	0.08 ± 0.05	0.92 ± 0.05
3rd month	0.91 ± 0.63	0.08 ± 0.06	0.92 ± 0.06

Correlation of phytoplankton and environmental factors in the ponds

Based on the analysis of the correlation coefficient between the ecological structure of the phytoplankton populations in the ponds and environmental factors (Table 6), there was a moderate correlation, with total suspended solids ($r = 0.563$), total ammonia ($r = 0.514$), nitrite ($r = 0.521$), total nitrogen ($r = 0.622$), phosphate-phosphorus ($r = 0.558$), and BOD ($r = 0.530$), statistically ($p < 0.05$).

If considered as groups of phytoplankton, this study is focused on explaining the two main phytoplankton groups: green algae and blue-green algae. Both groups presented a quantity of more than 95% of the total phytoplankton community in the ponds. The average volume of green algae when performing the correlation coefficient with water-quality factors revealed that there was no statistical correlation ($p > 0.05$). However, the average volume of blue-green algae had a high correlation level statistically ($p < 0.05$) with total nitrogen ($r = 0.722$) (Fig 2), and a statistically moderate positive relationship ($p < 0.05$) with total suspended solids in water ($r = 0.670$), total ammonia ($r = 0.658$), nitrite ($r = 0.515$), nitrate ($r = 0.541$), orthophosphate ($r = 0.524$), phosphate-phosphorus ($r = 0.684$) and BOD ($r = 0.628$) was discovered.

If considering the relationship of the ecological index of phytoplankton with water-quality factors, it was found that the diversity index was not statistically correlated with other water-quality factors ($p > 0.05$), excepting total ammonia with low negative correlate ($r = -0.336$, $p < 0.05$). These results correspond with the evenness index, which had a low negative relationship with total ammonia ($r = -0.427$), phosphate-phosphorus ($r = -0.326$) and chlorophyll a ($r = -0.43$). In terms of the dominant index, there was a low level of correlation with the total ammonia ($r = 0.427$), phosphate-phosphorus ($r = 0.326$) and chlorophyll a ($r = 0.439$).

Concerning the linear regression between the volume of blue-green algae and total nitrogen in sediment, it was presented that both variables linearly related with statistically significance ($p < 0.05$) and the total nitrogen in the sediment could predict the volume of blue-green algae. The equation can be written as follows.

Table 6. Correlation coefficient of phytoplankton and water quality in tilapia cage culture in earthen ponds with open-pond Pacific white shrimp and Freshwater prawn

Water-quality parameters	Correlation coefficient (r)					
	Density of phytoplanktons			Ecological indices		
	Blue-green algae	Green algae	Total phytoplankton	Diversity index	Evenness index	Dominance index
Temperature	-0.107	-0.059	-0.050	0.046	0.067	-0.067
Transparency	-0.447*	0.071	-0.381*	-0.078	-0.010	0.010
Total suspended Solids	0.670*	-0.062	0.563*	-0.069	-0.209	0.209
Dissolved oxygen	0.259	0.178	0.261	0.059	-0.034	0.034
pH	0.247	0.224	0.303*	0.026	-0.044	0.044
Alkalinity	0.147	-0.143	0.084	-0.052	-0.107	0.107
Hardness	0.126	-0.181	0.040	-0.129	-0.163	0.163
Total Ammonia	0.658*	-0.280	0.514*	-0.336*	-0.427*	0.427*
Nitrite	0.515*	0.101	0.521*	-0.008	-0.114	0.114
Nitrate	0.541*	0.021	0.482*	0.095	-0.004	0.004
Total nitrogen	0.722*	-0.113	0.622*	0.053	-0.093	0.093
Orthophosphate	0.524*	-0.079	0.416*	0.021	-0.067	0.067
Total filterable dissolved phosphate	0.684*	-0.191	0.558*	-0.202	-0.326*	0.326*
Total phosphorus	0.466*	-0.082	0.368*	0.085	-0.028	0.028
Chlorophyll a	0.972*	0.179	0.994*	-0.224	-0.439*	0.439*
BOD	0.628*	-0.132	0.530*	-0.161	-0.293*	0.293*

Remark: * = The relationship between phytoplankton and water quality was related with a statistical level of 0.05.

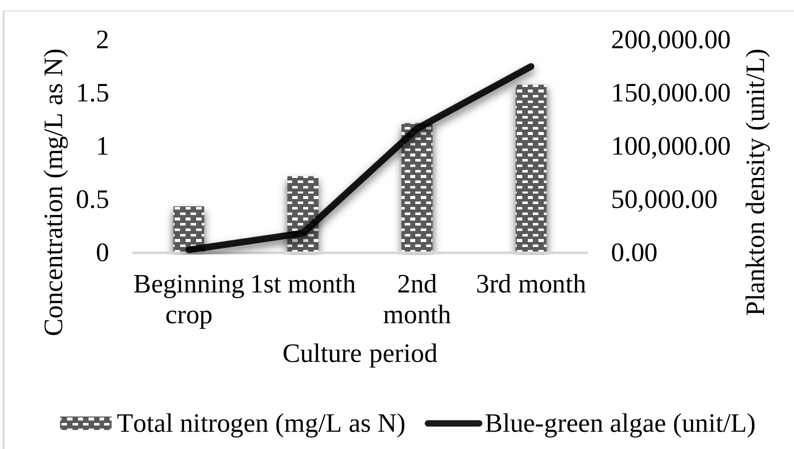

Figure 2. Concentration of total nitrogen and blue-green algae density in tilapia cage culture in earthen ponds with open-pond Pacific white shrimp and freshwater prawn

$$Y = 121{,}368.7(X) - 41{,}635.7;\ (R^2 = 0.32)$$

Y = volume of blue-green algae (cells/L), X = total nitrogen in the sediment (percentage).

In an overall examination of the phytoplankton relationship in the pond, it could be concluded that the increase in the average total phytoplankton volume is associated with the change in water-quality factors. It indicated that the accumulation of waste increased toward the end of the culture period, especially the total suspended solids in water (r = 0.563), total ammonia (r = 0.514), nitrite (r = 0.521), total nitrogen (r = 0.722), phosphate - phosphorus (r = 0.558) and BOD (r = 0.530) related with phytoplankton.

Discussion

In the culture systems observed in this study, there were specific characteristics that different from other system. In the system, there was the tilapia raised in cages in earthen ponds and shrimps raised in open ponds. The system incorporates the commercial feeding of both shrimp and tilapia in large quantities because farmers emphasize the production of both tilapia and shrimp at commercial levels. Farmers from the area provided information that the primary objective of raising product in this system is to reduce damage from epidemics among aquatic animals because fish diseases do not infect to shrimp and likewise shrimp diseases do not infect fish. Also, the seasons for prevalence of epidemics in both animal types are different.

In this system, there were 36 species of phytoplankton discovered in the ponds. Green algae was dominant in terms of species number, and blue-green algae was dominant in terms of the quantity. Following the reports of phytoplankton composition in tilapia ponds of Kunlasak *et al*. (2013) and carp ponds of Ciric *et al*. (2015). Phytoplankton composition in the tilapia cage culture system in earthen ponds, and tilapia pond culture system in Thailand, following Pimolrat *et al*. (2015) are compared in Table 7. The comparison shows that the results of this study of phytoplankton composition were consistent those of the pond culture system. Both systems found dinoflagellate phytoplankton. Generally, this kind of phytoplankton is used as an indicator of water with high accumulations of organic matter. But in this study, dinoflagellate was found in low volumes (0.06 - 0.40% of the total phytoplankton volume) in the ponds (Tables 3 and 4, Fig. 1).

Table 7. Species composition of phytoplankton in tilapia culture systems in Thailand

Species composition of phytoplankton	Cage culture in earthen ponds [1] (percent)	Earthen ponds[2] (percent)
Blue-green algae	19.4	26.0
Green algae	58.3	53.0
Diatom	16.7	20.0
Dinoflagellate	5.6	1.0
Total species number	36	No data

Source: 1 = this study ,2 = Pimolrat *et al*., 2015.

The volume of phytoplankton in the ponds under study increased during the culture period. The density of phytoplankton in this study and two tilapia culture systems: pond culture (Srisapoome *et al*., 2016) and cage culture in the river (Insumran *et al*., 2017), were also compared. The results showed that the average phytoplankton density of the cage culture in the river was between 204 and 1,800 cells/L. However, in this study, the average was between 66,003.2 and 188,187.2 cells/L, indicating that in the open-environment systems, such as rivers, where water is continually circulating, there was a relatively low accumulation of waste in the system. Also, in open systems, the volume of phytoplankton varies according to the season and soil erosion into the water source (Insumran *et al*., 2017). However, in the pond system, the composition of phytoplankton was liable to change according to the volume of organic matter added to the system (Tavares *et al*., 2010). Furthermore, the increase in waste in the culture period effected an increase in phytoplankton (Pimolrat *et al*., 2015). The data corresponds to the water-quality index, which indicates an accumulation of organic matter in the system, namely BOD or TOC. In the cage culture in the river system, the BOD data were low. With an average of 2.24 - 3.83 mg /L, during this study, the BOD data in the culture period were high. With an average of 9.39 - 23.85 mg/L (Table 2). In pond culture systems, Srisapoome *et al*. (2016) reported that the average data of TOC were between 7.78 and 23.06 mg/L. This indicated that the cage culture in the river system had lower organic matter accumulation than a cage culture in the pond or the standard pond culture.

Regarding the ecological indices of phytoplankton in Table 5, this study indicated that the diversity index (H’) of the phytoplankton decreased as the study period progressed. Comparing with the criteria of Krebs (2001), it was found that during the pre-stocking period and the beginning of the crop (first month), the diversity index had a moderate level ($1 < H' < 3$), but after that until the harvesting period, the diversity index had a low level ($H' < 1$). A report from

Indonesia (Yanuhar *et al*., 2016) has explained that the moderate level of the diversity index would indicate that the ecology in the ponds was rich in phytoplankton. However, the low diversity index level showed that some species of phytoplankton were more prominent than others. The evenness index was shown to decrease as the culture period advanced, but the dominant index increased. Such data showed that during the pre-stocking period, the green algae group had a more prominent volume (82.01%) than other groups. At the beginning of the crop, other groups began to climb in volume until plankton in the ponds were not very different. Later, the diversity and evenness indexes fell as the culture period progressed. However, the rise in the dominant index indicated that blue-green algae had climbed in volume. It was dominant in the pond, especially during the end of the crops (92.98%). *Oscillatoria* was the dominant genus in the period. Increases in the volume of organic matter in the pond directly affected the quantity of phytoplankton (Tavares *et al*., 2010).

Overall, on the relationship between phytoplankton and environmental factors, it can be concluded that the increase of the phytoplankton volume in the ponds during this study was related to water-quality factors. The results (Table 6) indicated that the accumulation of waste in the ponds was related to the change of phytoplankton, namely: total suspended solids in water were (r = 0.563), total ammonia (r = 0.514), nitrite (r = 0.521), total nitrogen (r = 0.722), phosphate-phosphorus (r = 0.558) and BOD (r = 0.530). The results were consistent with the report by Pimolrat *et al*. (2015), which concluded that with tilapia culture in earthen ponds, nitrogen compounds, especially total ammonia, were an important factor effecting the phytoplankton volume. This was because ammonia is an important nutrient for the growth of phytoplankton in the blue-green algae group. Moreover, the report of Kanokrung *et al*. (2017) concluded that phosphorus was an essential mineral for the growth of phytoplankton in ponds. Therefore, if water-quality controls at the farms were inefficient or failed to meet suitable standard criteria, as reported by ACFS (2016) and Bhatnagar and Devi (2013), especially factors that effected organic matter accumulation in ponds, such conditions may affect pond production (Shoko *et al*., 2014). Mainly, phosphorus and nitrogen groups were the primary nutrients to effect blue-green algae increasing in aquaculture ponds (Dhawan and Laur, 2002; Tavares *et al*., 2010). In this study, it was found that total nitrogen had a high level of correlation (r = 0.722) (Table 6) with the density of the blue-green algae group. Pimolrat *et al*. (2015) reported that blue-green algae has a high growth rate when the water temperature exceeds 25^{o}C and high nutrient content in the form of nitrogen, because nitrogen is the main nutrient factor for the growth of blue-green algae. As far as the blue-green algae group was concerned, it was the most common group in the tilapia culture ponds under study (92.98%) at the end of a crop. *Oscillatoria* was a dominant species of blue-green algae group, an average volume between 1, 383.5and 145, 641.7 cells/L. It was phytoplankton essential to the creation of geosmin or MIB. Both organic compounds are the main source of the off-flavor problem in fish products, which is a significant impact in the commercial tilapia farming industry (Tucker, 2000; Smith *et al*., 2008). If fish absorb these algal products into their bodies, the compounds' unpleasant odors will emanate from the fatty tissue of the fish (Pimolrat *et al*., 2015). The quality of fish meat is one of the most important factors affecting the price of fish products. The odor problem has resulted in a 30% reduction in the price of catfish in the United States of America (Engle *et al*., 1995).

From the correlation coefficient analysis of the average volume of blue-green algae in this study, the increase of the total nitrogen content in the pond could result in significantly increased volumes blue-green algae. This finding aligns with those of Schrader and Dennis (2005). Controlling the volume of blue-green algae at an appropriate level is beneficial to aquaculture in terms of water-quality control, reducing the off-flavor problem in fish products. Farmers should focus on controlling waste in ponds, which is a crucial factor that results in the increase of total nitrogen content in ponds, such as feeding in appropriate volume and removal of residual feed particles, which is a major organic matter source (Rahman *et al*., 2008). Reportedly, it is easy to remove feed waste from cage culture systems in earthen ponds. Installation of aerators is another way to reduce the impact of phytoplankton proliferation. Notably, dissolved oxygen in the ponds at night is reduced by the respiration of phytoplankton. Also, during the day, the operation of aerators was also beneficial for the process of organic matter decomposition in ponds (Kunlasak *et al*., 2013).

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