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Research article

Effect of Probiotic Used on Phytoplankton Community Structure in White Shrimp (*Litopenaeus vannamei*) Pond

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Abstract: Probiotic use is one major approach to eliminating waste accumulation in shrimp farming. This study investigated the effects of probiotic use on the abundance and diversity of phytoplankton at the farm level. The results revealed 4 divisions for 12 genera of phytoplankton, consisting of Cyanophyta (*Oscillatoria* sp., *Pseudanabaena* sp., *Planktolyngbya* sp. and *Merismopedia* sp.), Chlorophyta (*Scenedesmus* sp. and *Monorapidium* sp.), Bacillariophyceae (*Nitzschia* sp., *Pleurosigma/Gyrosigma* sp. and *Navicula* sp.) and Pyrrophyta (*Protoperidinium* sp., *Ceratium* sp. and *Prorocentrum* sp.). Pyrrophyta exhibited the highest density, followed by Cyanophyta, Bacillariophyta and Chlorophyta. The cell density of 4 divisions was high at the start rearing e.g., Pyrrophyta 47,991 cells/L (85%), Cyanophyta 3,141 cells/L (5%), Bacillariophyta 3,530 cells/L (6%), and Chlorophyta 1,965 cells/L (3%). However, the density drastically changed at the end of the study in 19 days, especially in Pyrrophyta, for which cell density dropped to 4,705 cells/L (48%). The density of Cyanophyta increased towards day 7 and decreased at the end of the study (2,126 cells/L, 22%). The density of Bacillariophyceae and Chlorophyta were reduced in the initial cultivation from day 1 to day 10 but increased again before the end of the study for 1,176 cells/L (12%) and 1,698 cells/L (18%). The research indicates that adding probiotics to shrimp ponds can affect the abundance of the phytoplankton community. Probiotics can reduce an abundance of Pyrrophyta and Cyanophyta (biotoxin producing group.), while they show a trend to increase an abundance of Chlorophyta and Bacillariophyta.

Keywords: Probiotic; Shrimp; Phytoplankton; Chlorophyta; Bacillariophyta

1. Introduction

The shrimp aquaculture industry is a significant agricultural activity driving the economy in several coastal countries [1]. Economic competition results from shrimp production with the highest benefit. Huge amounts of high-protein feeds, chemicals and antibiotics are used, neglecting the maintenance of the environment both inside and outside ponds [2]. Indeed, water effluent from shrimp aquaculture should be treated before re-circulating to new crops or discarding into surrounding environments. Probiotics, which contain the most beneficial bacteria, inhibit pathogenic microorganisms, enhance and promote shrimp's immunity and growth, and improve water quality in ponds [3-5]. Water quality control by probiotics is carried out by utilizing excess nutrients in shrimp ponds. However, using probiotics by directly adding them to culturing water may influence the community structure of microbiota, such as phytoplankton. The phytoplankton plays an important role in aquatic photosynthesis, leading to oxygen production and nutrient control such as nitrate and phosphate [6]. Moreover, phytoplankton is a natural feed, providing water shade to reduce shrimp cannibalism [7]. Nutrients are necessary for both phytoplankton and probiotics. Therefore, competition between microorganisms occurred. Probiotics can use nutrients better than phytoplankton. They can control the abundance of undesire phytoplankton. Therefore, this research aimed to study the effect of probiotic use on the phytoplankton community in shrimp farms.

2. Materials and Methods

2.1 Shrimp farm

The experimental shrimp farm was in Songkhla province, southern Thailand. Farmers performed the study investigating shrimp ponds and the culture system. Shrimp ponds were prepared by clearing bottom waste and mud, followed by drying for 2–3 weeks. Lime and dolomite (1 ton each) were added to the floor of the ponds to increase pH and alkalinity. Seawater (about 48,000 m^3) was added to water depth 1.5 m in 2 rai (0.32 hectares) of the pond. Post-larva stage (PL15) of white shrimp (*Litopenaeus vannamei*) (approximately 200,000 individual/rai) was seeded in the pond. An automatic feeding machine was in operation every 5 minutes from 7 am to 6 pm. Oxygen was added by utilizing 3 mixing turbines with 50% and 100% efficiency during both day and night, respectively.

2.2 Probiotic application

Commercial probiotic product was composed of 3 significant Bacillus species, including *B. subtilis*, *B. megaterium* and *B. licheniformis*. The use of probiotics in shrimp ponds was carried on by farmers, which commenced on day 20 continuing until day 74 of culture. One hundred grams of probiotic powder were inoculated in a 200 L plastic tank containing shrimp feed of 0.5 kg, molasses of 0.5 kilograms, and tap water of 200 L. Oxygen was supplied by an air pump for 24-36 hours before use. Two hundred litres of about 10^6 CFU/ml probiotics were scattered over the pond every 3 days.

2.3 Phytoplankton collection

The collection of phytoplankton was started on day 55 of shrimp culture. Based on probiotic use, phytoplankton was collected every 3 days from day 20 until day 74 of shrimp culture. Sample collection was performed from 9–10 am before adding a new batch of probiotics to avoid the mistaken re-sampling of newly added probiotics. Phytoplankton cells from five locations in the pond were collected by passing a 20 L water sample through a 20 µm plankton net mesh. Then, cells were fixed with 10% formalin before further study in the laboratory [8].

2.4 Water parameters

Several water parameters were monitored during cultivation. Temperature, dissolved oxygen, pH and salinity parameters were measured at the time of phytoplankton collection (9–10 am) [6].

2.5 Phytoplankton study

One millilitre of phytoplankton sample was dropped into Sedgewick Rafter Cell. The morphological characteristics of phytoplankton were then studied under a light microscope (CHS, Olympus). Phytoplankton species and characteristics were identified by manual method and recorded before the cell abundance of phytoplankton was calculated with three replicates [8].

3. Results and Discussion

3.1 Phytoplankton community

The results of the phytoplankton study showed that 12 genera within 4 divisions of phytoplankton were found. The identification study exhibited 4 genera of division Cyanophyta (*Oscillatoria*, *Pseudanabaena*, *Planktolyngbya* and *Merismopedia*), 2 genera of division Chlorophyta (*Scenedesmus* and *Monorapidium*), 3 genera of division Bacillariophyceae (*Nitzschia*, *Pleurosigma* or *Gyrosigma* and *Navicula*) and 3 genera of division Pyrrophyta (*Protoperidinium*, *Ceratium* and *Prorocentrum*) (Figure 1). The total phytoplankton cells for 7 studies of 19 days were 186,967 cells/L. Highest cell abundance were the following divisions: Pyrrophyta (133,938 cells/L or 71.6%), Cyanophyta (36,991 cells/L or 19.7%), Bacillariophyta (8,984 cells/L or 4.8%) and Chlorophyta (7,053 cells/L or 3.7%), respectively (Figure 2).

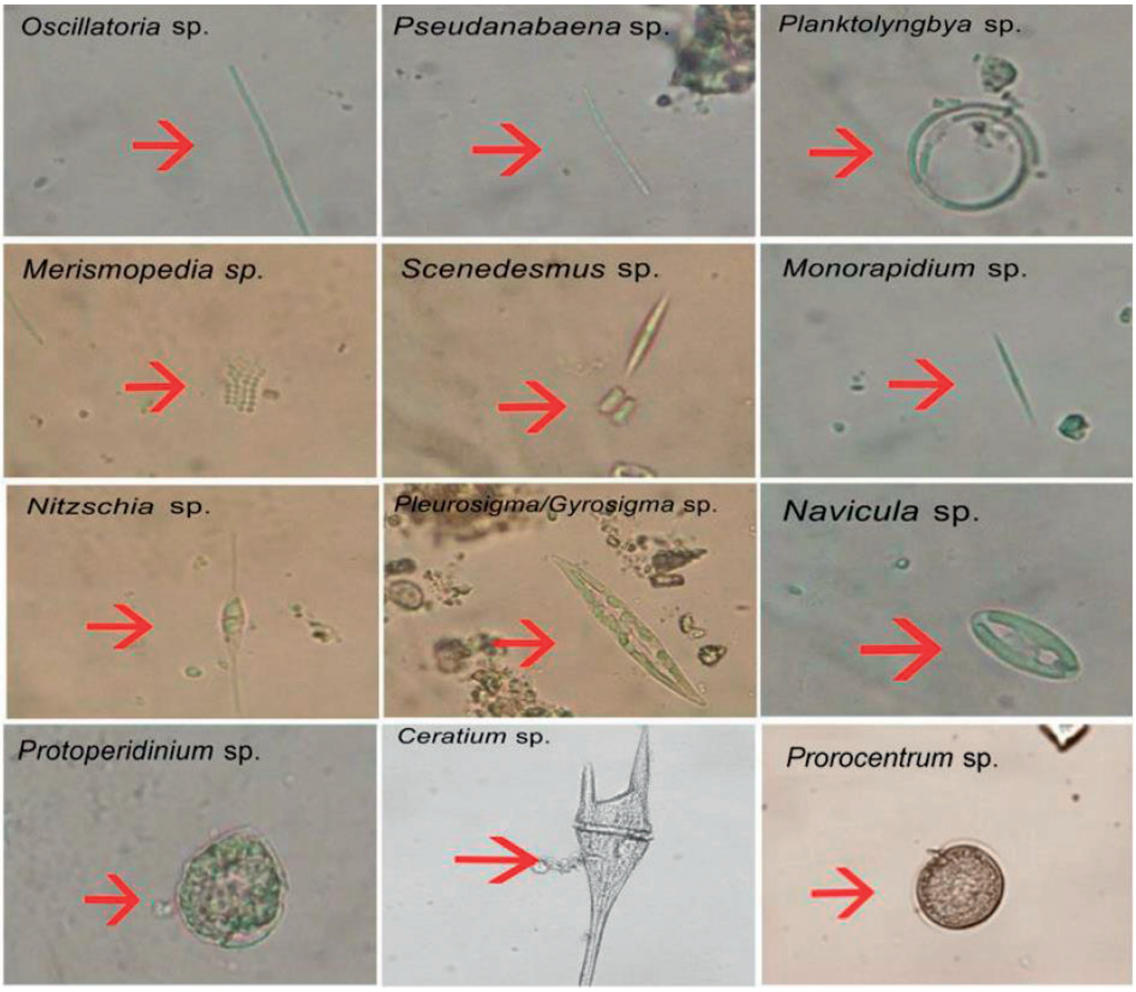

Figure 1. The morphology of phytoplankton was founded in a shrimp pond.

The most abundant genus of phytoplankton was *Protoperidinium* (133,517 cells/L), followed by *Planktolyngbya* (31,726 cells/L) and *Ceratium* sp., which were found at the lowest density (2 cells/L) (Table 1). These 4 divisions of phytoplankton commonly found in shrimp farms were previously reported. Meanwhile, this study found Pyrrophyta division had the highest concentration. In contrast, de Paiva-maia et al. [9] suggested that Cyanophyta had the highest concentration in shrimp farms (*Litopenaeus vannamei*), followed by Chlorophyta, Bacillariophyta and Pyrrophyta, respectively. [9] proposed that less concentration of Pyrrophyta resulted from the influence of probiotics.

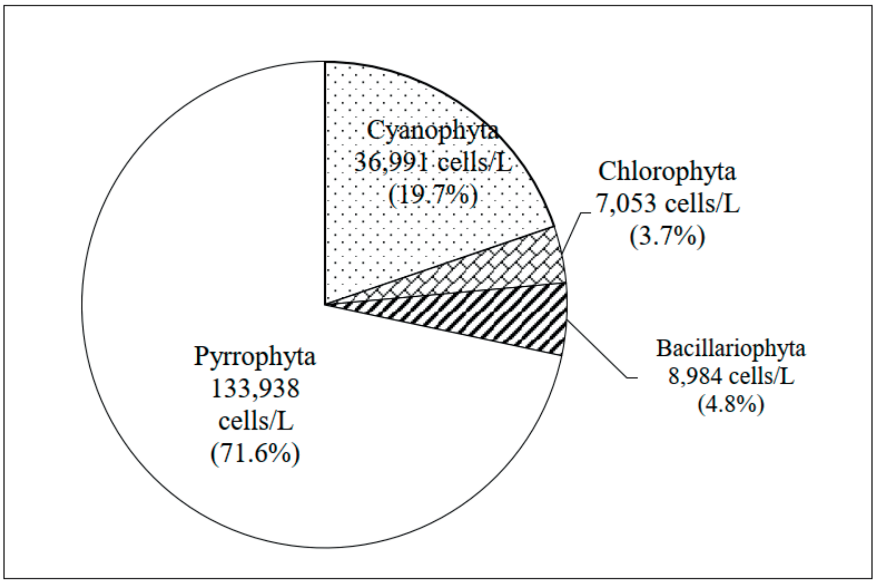

Figure 2. Phytoplankton communities in shrimp pond.

Like [9], Yusoff et al. [10] found that Cyanophyta was the highest community in treated ponds but lower than in controlled ponds as they operate with commercial bacteria. High organic substances in water are growth requirements of Pyrrophyta and Cyanophyta, which in these divisions can generally be found in eutrophic aquaculture ponds, as mentioned in previous studies [11, 12]. However, [9-10] have suggested that probiotics can be controlled to pyrrophyta and Cyanophyta. The controlling mechanisms may be nutrient use competition and antagonisms (antibiotics, enzymes, bacteriocin, etc.).

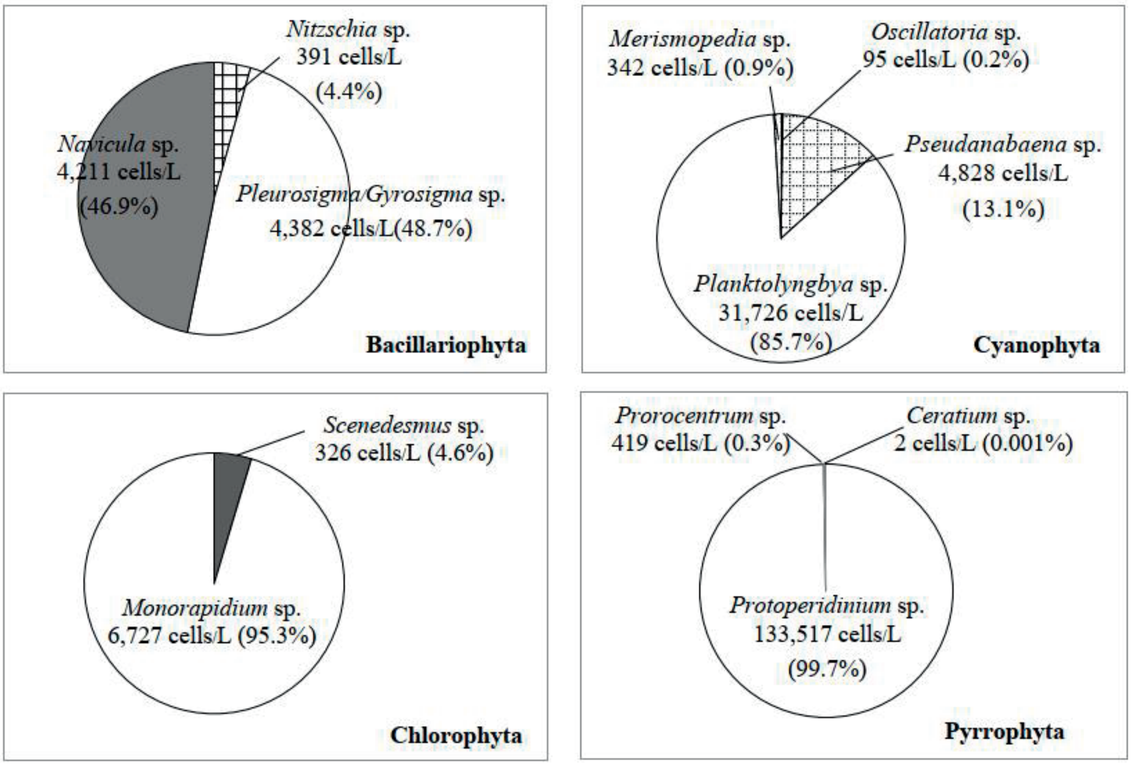

Figure 3. The abundance of phytoplankton cells is based on species.

Figure 3 showed the richness of species of pyrrophyta was *Protoperidinium* sp. (99.7%), followed by *Prorocentrum* sp. (0.3%) and *Ceratium* sp. (0.001%), respectively. The richness of species of Cyanophyta was *Planktolyngbya* sp. (85.7%), followed by *Pseudanabaena* sp. (13.1%), *Merismopedia* sp. (0.9%) and *Oscillatoria* sp. (0.2%), respectively. In contrast, the highest population species of Bacillariophyta was *Pleurosigma* sp. or *Gyrosigma* sp. (48.7%), followed by *Navicula* sp. (46.9%) and *Nitzschia* sp. (4.4%), respectively. The most abundant species of Chlorophyta were *Monorapidium* sp. (95.3%), followed by *Scenedesmus* sp. (4.6%). This study was in contrast with the report of Lukwambe et al. [13], which investigated the effects of commercial microbial agents (probiotics) on phytoplankton community structure in intensive white shrimp (*Litopenaeus vannamei*) aquaculture ponds. Their results showed that Bacillariophyta (*Navicula* sp., *Coscinodiscus* sp. and *Skeletonema* sp.) and Cyanophyta (*Oscillatoria* sp. and *Spirulina* sp.) were the highest populations. However, most *Protoperidinium* sp. occurring in shrimp ponds may negatively affect other species because it has been proposed as a biotoxin producer [14].

Table 1. The abundance of phytoplankton cells (every 3-days) from a 19-day study.

Phytoplankton	Abundance (cells/L)							
	D1	**D4**	**D7**	**D10**	**D13**	**D16**	**D19**	**Total**
Cyanophyta	3141	8600	10778	5067	5564	1715	2126	36991
- *Oscillatoria* sp.	0	7	19	15	18	15	21	95
- *Pseudanabaena* sp.	3100	95	189	363	431	249	401	4828
- *Planktolyngbya* sp.	35	8485	10531	4620	4994	1360	1701	31726
- *Merismopedia* sp.	6	13	40	68	121	91	3	342
Chlorophyta	1965	378	417	344	1553	698	1698	7053
- *Scenedesmus* sp.	50	18	18	47	103	57	33	326
- *Monorapidium* sp.	1915	360	399	297	1450	641	1665	6727
Bacillariophyta	3530	1562	323	493	1402	498	1176	8984
- *Nitzschia* sp.	176	104	97	10	4	0	0	391
- *Pleurosigma*/ *Gyrosigma* sp.	2214	992	23	120	467	221	345	4382
- *Navicula* sp.	1140	465	203	363	931	278	831	4211
Pyrrophyta	47991	9615	25976	16712	22614	6325	4705	133938
- *Protoperidinium* sp.	47829	9509	25874	16697	22607	6309	4692	133517
- *Ceratium* sp.	2	0	0	0	0	0	0	2
- *Prorocentrum* sp.	161	105	102	15	7	16	13	419
Grand total	56628	20155	37494	22616	31133	9236	9705	186967

3.2 Daily abundance of phytoplankton

The result showed that division Pyrrophyta was the most abundant in all experiments. They dominated the study period in the 48-85% (Figure. 4). Pyrrophyta had the highest cell abundance ($P<0.05$)

significantly on day 1 with 47.99± 9.41 × 10^3 cells/L (85%). While Cyanophyta, Bacillariophyta and Chlorophyta had a cell abundance on day 1 of 6%, 6% and 3%, respectively.

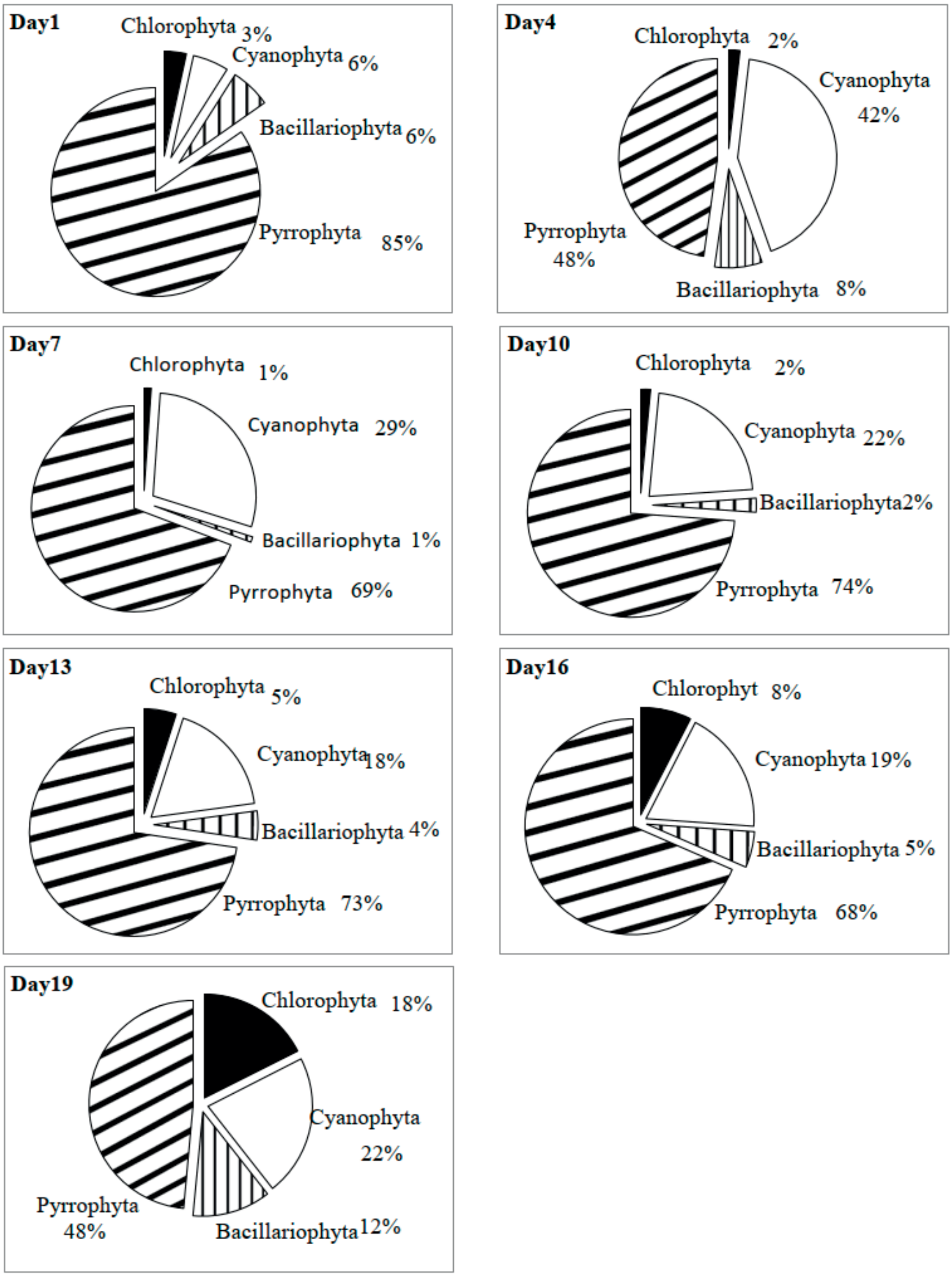

Figure 4. Abundance percentage of phytoplankton divisions every 3 days.

However, the total cell abundance of all phytoplankton on the first day of probiotic use was highest at 56.63 ± 4.51 × 10^3 cells/L (Table 2). When the probiotic was used until day 4, the Pyrrophyta had declined to 48% (9.61 ± 1.08 × 10^3 cells/L), whereas Cyanophyta had rapidly increased to 42% (8.60 ± 0.97 × 10^3 cells/L). After that, Pyrrophyta increased again with a 68-74% range on days 7, 10 and 13, then consistently decreased from day 16 to day 19. As Pyrrophyta, division Cyanophyta was the second group of abundance since days 7, 10, 13, and 16 with a range of 18-29%. On day 19, the cell abundances of division Chlorophyta and Bacillariophyta had significantly increased compared to previous times. Unfortunately, shrimps were harvested on day 19 from the first study day, and the author could not continue monitoring the phytoplankton population's dynamic. However, the result showed that using probiotics over periods (at least 19 days) will change the abundance of the phytoplankton population.

Moreover, the profile of the total abundance of all 4 divisions per day dropped from day 1 to day 19. This was revealed in each division's concentration profile, which declined since using the probiotic. The result suggested that probiotics might control the phytoplankton population. Probiotics compete with phytoplankton in shrimp ponds as they can utilize nutrients faster than phytoplankton. This is in addition to their excellent cell regeneration and faster growth [9, 15].

Moreover, probiotics may produce antagonistic and antibiotic compounds to inhibit other phytoplanktons. In our study, green algae (Chlorophyta) were found in small amounts because it is usually distributed in fresh water. There were few green algae which have resistant to saline water. According to the results, probiotics can control the abundance of harmful phytoplankton like Cyanophyta and Pyrrophyta. In contrast, probiotics have enhanced phytoplankton populations like Bacillariophyta and Chlorophyta. These two divisions are suggested as desired phytoplankton because they are important as food for higher consumers [15, 16]. Figure 4 shows the decrease of pyrrophyta and the increases of Bacillariophyceae and Chlorophyta on day 19. On the contrary, at the end of their experiment, de Paiva-maia et al. [9] reported only 2 divisions with the highest abundances in ponds, Cyanophyta and Bacillariophyta.

Table 2. The abundance of phytoplankton is based on the studied day.

Day	Abundance ($\times 10^3$ cells/L ± SD)				
	Chlorophyta	**Cyanophyta**	**Bacillariophyta**	**Pyrrophyta**	**Total**
D1	1.96 ± 0.17^{a}	3.14 ± 0.99^{a}	3.53 ± 0.13^{a}	47.99 ± 9.41^{b}	56.63 ± 4.51
D4	0.37 ± 0.08^{a}	8.60 ± 0.97^{b}	1.56 ± 0.11^{a}	9.61 ± 1.08^{b}	20.16 ± 0.54
D7	0.41 ± 0.04^{a}	10.77 ± 3.12^{b}	0.32 ± 0.07^{a}	25.97 ± 1.63^{c}	37.47 ± 1.47
D10	0.34 ± 0.06^{a}	5.06 ± 1.58^{b}	0.49 ± 0.10^{a}	16.71 ± 1.56^{c}	22.62 ± 0.86
D13	1.55 ± 0.61^{a}	5.56 ± 1.41^{b}	1.40 ± 0.16^{a}	22.61 ± 2.64^{c}	31.13 ± 1.08
D16	0.69 ± 0.06^{a}	1.71 ± 0.79^{b}	0.49 ± 0.03^{a}	6.32 ± 0.62^{b}	9.24 ± 0.39
D19	1.69 ± 0.51^{ab}	2.12 ± 0.05^{b}	1.17 ± 0.09^{a}	4.70 ± 0.32^{c}	9.71 ± 0.21
Total	7.05 ± 0.24	36.99 ± 0.95	8.98 ± 0.04	133.94 ± 3.15	186.97 ± 1.48

*Mean of the different alphabet labels within the same row indicate significant differences ($P<0.05$).
*Mean of the same alphabet label within the same row indicate no significance ($P>0.05$).

3.3 Population dynamics of phytoplankton

According to the experiment, this study found that the Cyanophyta community continuously increased from day 1 (3,141 cells/L) and showed the highest community on day 7 (10,778 cells/L), then slowly declined until day 19 (2,126 cells/L). Chlorophyta showed the most elevated community on day 1 (1,965 cells/L) and decreased to the lowest on day 10 (344 cells/L), afterwards continuously increased to 1,698 cells/L on day 19. Bacillariophyta had its highest population on day 1 (3,530 cells/L) and gradually declined to the lowest on day 7 (323 cells/L) before slowly increasing to 1,176 cells/L on day 19. The community of Pyrrophyta was at its highest on day 1 (47,991 cells/L) before fluctuating moderately until falling to a low on day 19 (4,705 cells/L). In Figure 5, all 4 divisions showed downward trends, especially Pyrrophyta. Chlorophyta, Bacillariophyceae and pyrrophyta declined on day 4, whereas Cyanophyta increased. This might be due to high nutrient loading during the initial days of the experiment, which was beneficial for their growth. Paerl and Tucker [17] mentioned that Cyanophyta prefers a low ratio of N:P as [fix N2 for their expansion and to effectively combat other phytoplankton, which probably contributed to its highest population early in the study, before slightly declining to day 19 with lower nutrient concentration. The decrease of Cyanophyta and Pyrrophyta was reported to be beneficial to shrimp culture due to their toxin and eutrophication being dangerous to shrimp [9, 10, 17].

Furthermore, at the end of the experiment, Chlorophyta and Bacillariophyceae had higher populations, which was profitable to shrimp growth as natural food, and for producing oxygen and being non-toxic [10]. However, the use of probiotics affected the population dynamic of phytoplankton. As the results showed, trends of Cyanophyta and pyrrophyta decreased, while Chlorophyta and Bacillariophyceae increased by the end of the experiment, with all divisions having smaller populations than initially. De Paiva-Maia *et al*. [9] mentioned that phytoplankton communities increased in probiotic treatments but less than in control. Nevertheless, they also confirmed that probiotics influenced dynamic phytoplankton changes.

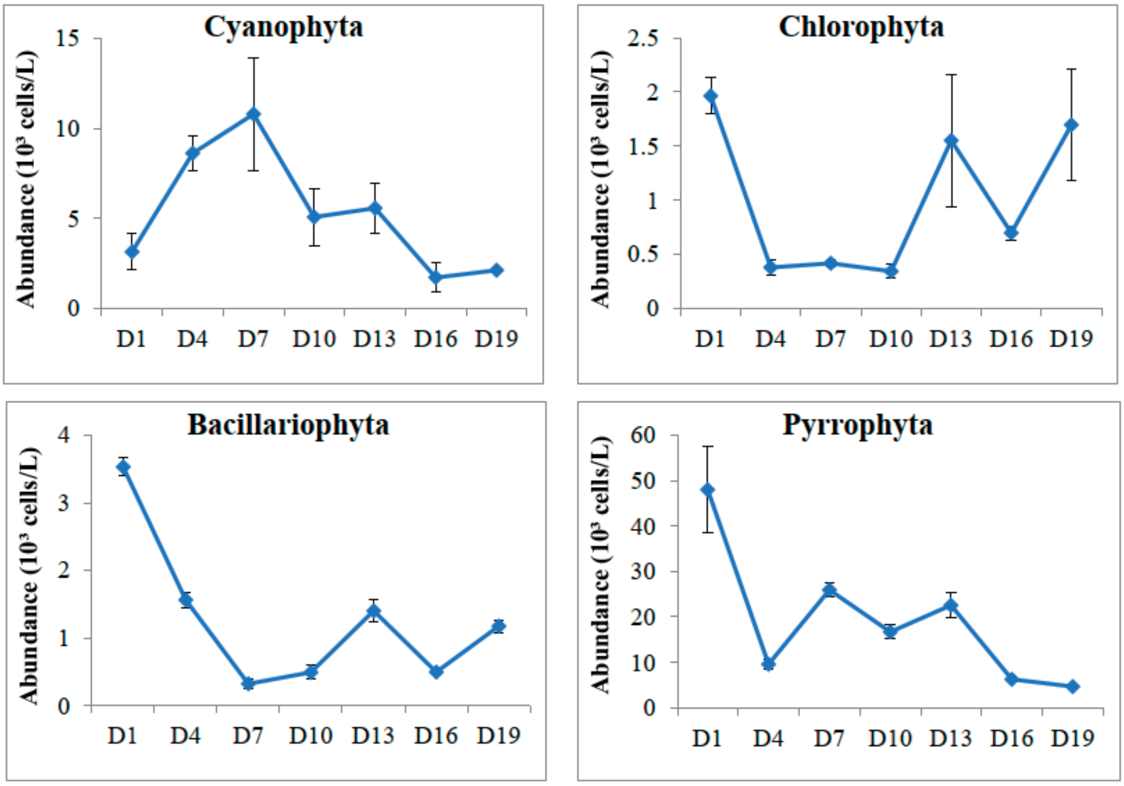

Figure 5. The abundance of phytoplankton divisions every 3 days.

3.4 Daily proportion of phytoplankton abundance

Phytoplankton abundance proportion was analyzed daily in 7 collection periods. It was found that pyrrophyta showed the highest balance and productiveness every day, followed by Cyanophyta, Bacillariophyceae and Chlorophyta, respectively (Figure 6). Bacillariophyta and Chlorophyta were useful as live feed but had low existence. In contrast, the populations of Pyrrophyta and Cyanophyta were high, as they may produce biotoxin and can be harmful to shrimp health. This study showed low populations of Pyrrophyta and Cyanophyta when probiotic was applied. Their blooming and biotoxin production can mitigate that. However, there was no control experiment without probiotics used in this study, which was the weakness of real culturing in the farm system. The study only monitored the evidence influencing probiotics on other phytoplankton in real culturing. So, it could not be assumed which phytoplankton group was the most dominant in the pond without a probiotic system. This experiment tried to compare with de Paiva-Maia *et al*.

[9], which had 2 treatments applied in shrimp ponds, with and without probiotic addition. They reported that Bacillariophyceae and Chlorophyta were the highest populations initially (~47% and 43%, respectively).

In contrast, Cyanophyta and Pyrrophyta concentrations were lower than 7%. After the probiotic was applied for 10-16 weeks, Cyanophyta and pyrrophyta increased to 32-48% and 15-39%, respectively. On the other hand, Bacillariophyceae and Chlorophyta populations fell at the study's end to 7-15% and 19-20%, respectively. Indeed, the phytoplankton abundance profile with and without probiotics was similar. However, the concentration of Cyanophyta with probiotic use was higher than the concentration without probiotic use.

In contrast, the concentration of pyrrophyta with probiotic use was lower than without probiotic use. Furthermore, Lukwambe *et al*. [13] also found higher Cyanophyta density than Bacillariophyceae after probiotic addition. From the experiment, when probiotics were applied it could be observed that Cyanophyta and pyrrophyta initially had higher populations than the other two, which was confirmed by the study of de Paiva-Maia *et al*. [9]. Despite high amounts of Cyanophyta and Pyrrophyta in this study, there was no blooming to reach the level that produces any toxicity, and the shrimp culturing cycle proceeded until harvest [10]. To develop into a bloom of Cyanophyta, typically, the high community of one or two rapid expansion species occupying for an extended period should exist [17]. Usually, Bacillariophyceae and Chlorophyta should have more population than Cyanophyta and pyrrophyta. The use of probiotics in shrimp farms can eliminate waste, improve water quality, and support shrimp health. Moreover, they also help by controlling and inhibiting the fast growth of phytoplankton belonging to a group of biotoxin producers by utilizing nutrients in the water column or producing some inhibitor agents to limit that phytoplankton. However, the intensive shrimp aquaculture system has neglected to manage desirable phytoplankton. Farmers are concerned about harmful phytoplankton because they may cause massive shrimp death. This point suggests that the decline of phytoplankton by the influence of probiotic use is not a critical problem, while probiotics' efficiency in water improvement is crucial.

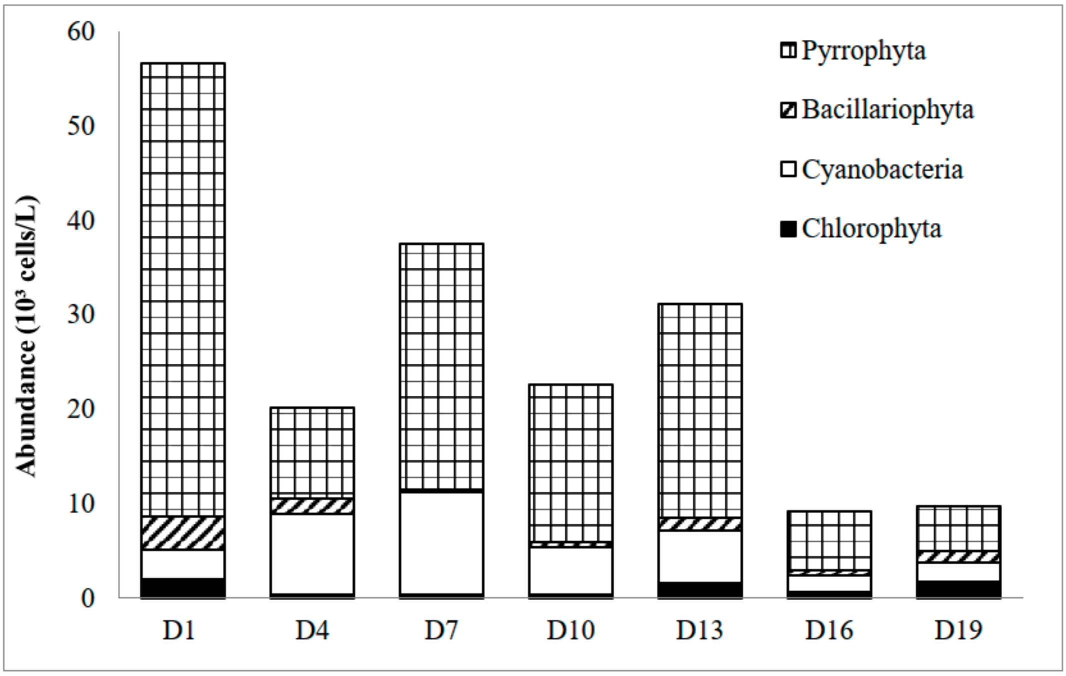

Figure 6. The daily abundance of phytoplankton.

3.5 Water Quality

Water temperature from day 1 to day 19 showed an average of 29.5 °C. In contrast, the averages of dissolved oxygen, pH and salinity of all 19 days were 6.2 mg/L, 8.0 and 11.4 ppt, respectively. The fluctuation was very low, with a minimal standard variation. None of the water quality parameters was significantly different between the sampling days ($P > 0.05$) (Table 3). The result corresponded with the study of Rajinikanth *et al*. [18] that reported all water parameters (pH, water temperature and dissolved oxygen) were stable or only slightly changed during their study.

Table 3. Water parameter.

Parameter	Day								
	D1	D4	D7	D10	D13	D16	D19	Average	SD
Temperature (°C)	29.0[a]	30.0[a]	30.5[a]	29.5[a]	29.5[a]	28.0[a]	30.0[a]	29.5	0.8165
DO (mg/L)	6.14[a]	6.50[a]	6.34[a]	6.05[a]	7.51[a]	5.15[a]	6.00[a]	6.2	0.7050
pH	8.00[a]	8.02[a]	8.04[a]	8.00[a]	8.18[a]	8.01[a]	8.04[a]	8.0	0.0634
Salinity (ppt)	13.0[a]	10.5[a]	10.0[a]	12.0[a]	13.0[a]	10.0[a]	11.0[a]	11.4	1.3138

*Mean of the same alphabet label within the same row indicate no significance ($P>0.05$).

4. Conclusions

Using probiotics can control an abundance of phytoplankton in shrimp ponds. Phytoplankton found in ponds of white shrimp (*Litopenaeus vannamei*) in the 19 days of the experiment (every 3 days sampling) was 12 genera from 4 divisions. This study found species from Cyanophyta (blue-green algae) were *Oscillatoria* sp., *Pseudanabaena* sp., *Planktolyngbya* sp., and *Merismopedia* sp. *Chlorophyta* (green algae) species found were *Scenedesmus* sp. and *Monorapidium* sp., while *Nitzschia* sp., *Pleurosigma/Gyrosigma* sp., and *Navicula* sp. represented Bacillariophyceae (diatom). Members of Pyrrophyta (dinoflagellate) were *Protoperidinium* sp., *Ceratium* sp., and *Prorocentrum* sp., where the highest cell abundance was also found for this division. The abundance profile of all divisions reflected the effects of probiotics on the phytoplankton community. Probiotics can control an abundance of division Pyrrophyta and Cyanophyta. In contrast, probiotics can enhance an abundance of division Bacillariophyceae and Chlorophyta at the end of the study. Water parameters in this study, e.g., temperature, dissolved oxygen, pH, and salinity, were highly stable and showed no significant difference. Probiotics solely influenced changes in the phytoplankton population for improving water quality and phytoplankton density control.

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