Abstract

This study was an evaluation of using *Spirulina platensis* for water quality control in shrimp culture tanks and the evaluation of nitrogen and phosphorus removal in the *Spirulina*-shrimp culture system under outdoor condition. The closed shrimp culture system in the this experiment consisted of three treatments *i.e.* shrimp culture without *Spirulina* (control), shrimp culture with *Spirulina* harvest (treatment 1) and shrimp culture with *Spirulina* harvest and shelter (treatment 2) and was conducted in 480 L fiberglass tanks located outdoor. The results showed that semi-continuous harvesting of *Spirulina* in treatment 1 resulted in significantly reduced ($P<0.05$) inorganic nitrogen concentrations (NH_4, NO_2 and NO_3). Without *Spirulina* (Control), considerable increment occurred with nitrogen concentrations. Change in ammonium concentration in both treatments resembled the control tank, however, treatment tanks with *Spirulina* had entirely lower nitrite and nitrate concentrations than in control. Moreover, nitrate concentrations in treatment 2 (shrimp with *Spirulina* and shelter) were lower than in treatment 1 (shrimp with *Spirulina* no shelter). In this experiment, shrimp in treatment 2 had the highest average daily growth, survival rate, biomass yield and food conversion efficiency, following by treatment 1 and control respectively. In addition, the results demonstrated that feed contributed about 51-53% nitrogen and 56-60% phosphorus of total nutrients input to the system. Major outputs of nutrients were accounted as dissolved in water fraction which ranged between 6-37% for nitrogen and 30-35% for phosphorus of the total inputs. Harvest of *Spirulina* in treatment 1 removed 4.8% of nitrogen and 8.3% of phosphorus from the culture system. Moreover, other sessile algae contaminated during the experiment also had a significant portion of nutrient removal from the tank, up to 6-11% of nitrogen and 8% of phosphorus removal. Shelter

42 in the treatment 2 significantly increased survival rate of shrimp but decreased growth
43 of *Spirulina* due to shading of the shelter.

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45 *Key words:* Water quality; Integrated culture; *Penaeus monodon*; *Spirulina platensis*;
46 Nutrients budget; Shrimp culture; Aquaculture; Microalgae

1. Introduction

Intensive culture of black tiger shrimp (*Penaeus monodon*) is widely practiced in Thailand. Traditional intense shrimp culture uses open system with high water exchange. More recently, there has been more interest in environmental friendly, closed or recirculating seawater systems with zero, or nearly zero water discharges.

Intensive shrimp culture in Thailand uses 30-57% protein feed with high feeding rates. Organic nitrogen waste from uneaten feed and shrimp excretions decomposes into toxic inorganic nitrogen compounds, including ammonia (NH_3 or NH_4) and nitrite (NO_2). With aerobic conditions, ammonia and nitrite are converted into relatively non-toxic nitrate (NO_3), but high nitrate concentrations can stress shrimp. Water exchange is therefore still recommended, especially when nitrate is ≥50 mg-N L^{-1} (Hart and O'Sullivan, 1993).

Aquaculture water treatment systems use bacteria to convert ammonium and nitrite into nitrate under aerobic condition, while nitrate removal can be accomplished using sophisticated denitrification systems under anaerobic condition (van Rijn, 1996; Abeysinghe et al., 1996). Menasveta et al. (2001) described a closed, recirculating seawater system with denitrification for shrimp broodstock culture. However, denitrification systems are complicated and impractical for large scale shrimp culture. Presently, these systems require oxygen reduction to almost zero before denitrification begins and automated controls during denitrification.

The closed recirculating system for fish and shrimp culture has seen a rapid growth in recent years. Most of the systems used in the indoor tanks consist of three main components *i.e.*, (1) sediment removal unit with sedimentation or filtration apparatus, (2) biofilter unit often with nitrofication biofilter but rarely with

denitrification, and (3) water sterilization unit by either UV or Ozone treatment. Water quality in outdoor ponds, on the other hand, depends on both physical and biological factors which mostly uncontrollable. The only water quality improvement techniques for outdoor pond are aeration with paddle wheels and water exchange.

In general, the water treatment system for indoor closed recirculating system is usually not suitable for the outdoor pond because light will interfere the system by inducing growth of phytoplankton and reducing activity of nitrifying bacteria. In the previous study (Chuntapa et al., 2003), co-culture of shrimp with *S. platensis* showed the high potential of water quality control through the microalgal harvest. Shrimp-*Spirulina* system proposed in this study requires light for *Spirulina* photosynthesis, therefore it must be used under an outdoor condition in the cement tank or plastic lined pond without soil bottom. However, to apply this concept for shrimp culture in larger scale, many factors still need to be studied. One of the most important problems is how to maintain *Spirulina* the dominant species in the tank containing other phytoplankton species. High turbidity water in the tank reduces the efficiency of light utilization by *Spirulina* and make algal harvesting more complicate.

In this experiment, The shrimp-*Spirulina* system was carried out in half-ton fiberglass tanks with very high shrimp density, 120 shrimps m^{-2} bottom area, which was three times higher than conventional intensive culture. At this high density, high concentrations of nitrogen and phosphorus waste were produced and could possibly harm shrimps if there was no attempt to treat those wastes.

Shrimp culture especially black tiger shrimp, *P. monodon*, is different from many other aquatic pelagic species because it is mostly stay at the bottom of the tank or pond. Cannibalism behavior, where the newly molted or weak shrimps were eaten by other shrimps, can be a serious problem with dense culture. An artificial shelter was

then provided as another treatment in the experiment. This shelter was a hiding area for shrimp and increased the bottom area of the tank.

Moreover, in order to evaluate the efficiency of algae in nutrient removal from the closed shrimp culture system under the ambient outdoor condition, nitrogen and phosphorus budget in the tank was studied. Mass balance of nitrogen and phosphorus in the aquaculture system, so call nitrogen and phosphorus budget, is the basic step for the quantitative study of food utilization efficiency, pond fertility, water quality and processes in the sediments (Avnimelech and Lacher (1979) cited by Thakur and Lin (2003). This can be therefore the best procedure to evaluate the efficiency of water treatment system. Since nitrogen and phosphorus is incorporated into *Spirulina* cells, determination of nitrogen and phosphorus in *Spirulina* biomass harvested from the system will illustrated the percentage of nutrients removal by the algae not by other processes such as denitrification, volatilization or water drainage. The present study aimed to investigate the efficiency of *Spirulina* for water quality control in very high shrimp culture in outdoor tanks with and without shelter using nutrients budget study.

2. Materials and methods

2.1 Spirulina platensis culture

Stock culture of *S. platensis* was obtained from the Institute of Food Research and Product Development, Kasetsart University, Thailand. *S. platensis* was maintained in 30 psu (~0.5 M NaCl) Zarrouk medium (Zarrouk, 1966) under 100 μmol photon m^{-2} s^{-1} illumination at 25-28°C. Mass culture of *S. platensis* was prepared by transferring stock algal culture into 2L Erlenmeyer flasks with air bubbling. When cultures reached mid-logarithmic growth phase, they were concentrated by filtering through 22 μm nylon

net and washed with fresh Zarrouk medium without nitrate, then added to shrimp culture tanks.

2.2 Experimental design and shrimp culture condition

In this experiment, *S. platensis* was co-cultured with shrimp in 500L fiberglass tanks (90 cm in diameter with 80 cm in height) under outdoor condition. Each tank contained 480L of 30 psu seawater with aeration through 4 air stones. Initial weight of shrimp was 4.57±1.31 g and initial density of shrimp was 72 per shrimps tank, which equal to 120 shrimps m^{-2} bottom area. This density was three times higher than that in conventional intensive shrimp culture pond. The experiment consisted of three treatments *i.e.*, shrimp culture without *S. platensis* (control), shrimp cultured with harvested *S. platensis* (treatment 1) and shrimp cultured with harvested *S. platensis* and shelter (treatment 2). Each treatment had two replicates and the experimental plan was completely randomized design. The experiment was conducted for 57 days without water exchanged except an addition of freshwater at approximately 1L day^{-1} to compensate evaporation.

The shelters used in this experiment were made of rolled into cylinder shape plastic net with 15 cm in diameter and 60 cm in length. Each set of shelter composed of totally 12 rolled net arranged in four stacks with PVC frame to maintain cubic shape. The aim of using shelters was to provide hiding area for shrimps since shrimp density in this experiment was extremely high. Diagram and photograph of the shelter is shown in Fig. 1.

Before starting experiment, water in each tank was partially sterilized by 30 minutes ozonation. Shrimps obtained from outdoor earth pond were acclimated in cement tank prior to experiment condition for 15 days before starting the experiment.

During experiment, shrimp in each tank were fed three times a day with commercial shrimp pellets (36.5% crude protein) at 4% of body weight.

2.3 Semi-continuous harvesting of S. platensis from shrimp tanks

After adding *S. platensis* into the shrimp tank, density of *S. platensis* in all tanks was regularly monitored every two days using chlorophyll-*a* (Chl-*a*) concentration and number of trichome were counted with Sedgwick-Rafter counting chamber under light microscope. Contamination of other microalgal species was also observed during trichomes counting. With semi-continuous harvesting treatments, Chl-*a* in treatment 1 and 2 tanks were maintained at 0.02-0.04 mg Chl-*a* L^{-1} by filtering appropriate water volumes from the tank through 22 μm net, then returning water back to the same tank. Concentration of Chl-*a* was determined by filtering 10 mL of water sample through Whatman GF/C filters and following by hot methanol extraction at 70°C for 2 minutes. Then optical density was measured spectrophotometrically at 630, 647, and 665 nm and Chl-*a* concentration was calculated as described in Bennet and Bogorad (1972) and Parson et al. (1989).

2.4 Water quality analysis

Throughout the experiment, water samples were collected every two days from the middle of the tank for water quality analysis. For nutrients analysis, water was filtered through GF/C glass fiber filter and kept in refrigerator (-20°C). Inorganic nutrients *i.e.*, ammonium, nitrite, nitrate and phosphate, were analyzed by colorimetric methods according to Parson et al. (1989). Total phosphorus content was analyzed by sulfuric acid - nitric acid digestion method and followed by ascorbic acid method (Takeuchi, 1988). Water temperature and pH were monitored using automatic logging pH meter (Hanna HI 98240). Salinity was measured using hand refractometer every

two days. Alkalinity was determined by titration with 0.02N H_2SO_4 until pH reach the endpoint at pH 4.5 (APHA, 1992).

2.5 Growth and survival of shrimp

Shrimp growth was determined by weighting fifteen shrimps from each tank with two decimal electronic balances every two weeks. At the end of experiment, all shrimps from each tank were harvested and weighed for production evaluation then the number of shrimp was counted for survival rate calculation.

2.6 Determination of nitrogen and phosphorus budget

2.6.1 Analysis of total nitrogen and phosphorus in shrimp feeding, shrimp carcass, molt, suspended solid and algae

Shrimp feed, body (carcass), molt, settle solid or algae were dried in the hot-air oven at 105°C for 24 hours. Dry matter was then ground and homogenized with mortar and pestle. Total nitrogen content in dry matter was determined using Kjeldahl method according to standard method for food analysis (AOAC, 1980). Total phosphorus content in dry metter was analyzed by sulfuric acid - nitric acid digestion method and followed by ascorbic acid method as described in Takeuchi (1988).

2.6.2 Nutrient budget calculation

Evaluation of nutrient budget in the present study refers to balance and dynamics of nitrogen or phosphorus in each tank. For nitrogen budget, input and output of nitrogen compounds in feed and in *S. platensis* were determined by total nitrogen analysis using Kjeldahl method (AOAC, 1980) while inorganic nitrogen (ammonium, nitrite and nitrate) in the water was analyzed by colorimetric methods as previously described (see 2.4). Shrimp, *S. platensis*, total nitrogen in the water at the beginning and entirely shrimp feed used for the whole experiment were counted as sources of nitrogen input. On the other hand, nitrogen output consisted of shrimp at the end of the

experiment, harvested *S. platensis*, total settle solid, other sessile macroalgae, shrimp molting and inorganic nitrogen in the water. Feeding rate and water exchanging data were also daily recorded. For phosphorus, total phosphorus was analyzed according to Takeuchi (1988) while phosphorus budget were evaluated with the same procedure as nitrogen budget.

The general balances of nutrient (nitrogen or phosphorus) in shrimp culture tank is described as the following equations (equation 1 to 3). The definition of each parameter is shown in Table 1.

$$\text{Nutrient input} = \text{Nutrient output} \quad (1)$$

$$^{N}\text{Shrimp}_{in} + {}^{N}\text{Feed}_{in} + {}^{N}\text{Water}_{in} + {}^{N}\textit{Spirulina}_{in} = {}^{N}\text{Shrimp}_{out} + {}^{N}\text{Water}_{out} + {}^{N}\textit{Spirulina}_{out} + {}^{N}\text{settle solid}_{out} + {}^{N}\text{Other algae}_{out} + {}^{N}\text{Molting}_{out} \quad (2)$$

$$^{N}\text{Input} - {}^{N}\text{Output} = \text{Unidentified (unaccounted) nutrient} \quad (3)$$

3. Results and Discussion

3.1 Water quality

During the first ten days of the experiment, ammonium concentration in all treatments increased up to 6-7 mg NH_4-N L^{-1} (Fig. 2-4). Thereafter, ammonium was rapidly decrease to lower than 1 mg NH_4-N L^{-1} and remained constant at this concentration untill the end of the experiment. This was possibly related to an incubation time of the nitrifying bacteria rather than phytoplankton uptake. After 10 days, nitrifying bacteria could active enough for converting all ammonium to nitrite and

finally nitrate. Increase of nitrite and nitrate after the decline of ammonium concentration during day 10-20 finally confirmed this hypothesis. Total inorganic nitrogen (TIN) in control, however, was consistently high while TIN in treatment 1 and 2 decreased after day 30. This suggested that nitrate in both treatments was eliminated by the algal activity.

It was found that phosphate concentration increased with time in all treatments (Fig. 5). The final phosphate concentration around 6-7 mg-P L^{-1} was very high compare with typical aquaculture ponds (Ray and Chien, 1992; Nelson et al., 2001). However, harvesting of *S. platensis* in treatment 1 during day 35-40 could successfully remove some phosphate out of the system.

During experimental period, pH was between from 8.5-9.1 and maximum temperature in the afternoon was between 25-29°C. Average salinity was 30 psu. Average dissolved oxygen was 5.45 ± 0.32 mg L^{-1} (Table 2). Average alkalinity was 160 (120-230 mg $CaCO_3$ L^{-1}, Fig. 6). There was no significant different (P>0.05) between water quality of control and all treatments. All mentioned parameters were within the acceptable range for growth of shrimp (Fast, 1992a, b; Chanratchakool et al., 1998) and *Spirulina* (Richmond, 1986; Vonshak, 1997).

Concentration of chlorophyll-a (Chl-*a*) which indicated the number of phytoplankton in each tank is shown in Fig. 7. For control, Chl-*a* was between 0.015 and 0.05 mg L^{-1}. This indicated the fluctuation of natural phytoplankton population found in the tank. On the other hand, number of *S. platensis* trichome in treatment 1 and 2 as showed in Fig. 8 was not related with Chl-*a* especially during the first 30 days. Cloudy weather during day 1-20 possibly one of the factor that limited growth of *S. platensis*.

After day 25, *S. platensis* became a dominant phytoplankton species in treatment 1 and semi-continuous harvesting was done in day 36, 37, 38, 41, 52, 55, 56, and 57. Unfortunately, number of *S. platensis* in treatment 2 (shrimp + *S. platensis* + shelter) was much lower than in treatment 1. This might due to nutrient limitation since TIN in treatment 2 was less than half of that in treatment 1 (Fig. 9, and 10; for control showing in Fig. 11) plus limitation of light shading under the shelter.

Phytoplankton assimilation is generally considered as the most important process that eliminates nitrogen from earthen ponds (Burford and Glibert, 1999). Moreover, Epp (2002) reported that when NH_4Cl was added directly to the tanks, the algae rapidly incorporated NH_4^+ into cellular material. According to Hargreaves (1998), estimated nitrogen uptake by phytoplankton ranged from 150 to 450 mg N m^{-2} day^{-1} in temperate aquaculture ponds.

In general, nitrogen from feed was incorporate into shrimp biomass and some was excreted as ammonia. Uneaten feed and feces were decomposed in sediment bottom which later converted into ammonia or ammonium. To prevent or reduce ammonia accumulation in the culture system, ammonia was usually transformed into nitrite and nitrate through nitrification process. Loss of ammonia from the culture system could also happy in other ways such as ammonia assimilation in plant or by volatilization directly to the atmosphere.

In Fig. 12, postulated pathway of nitrogen cycle in this study indicated that harvesting of plant is the most feasible process while volatilization and denitrification were not easy to manage. However, nitrogen elimination through settle solid harvesting could be an alternative choice. Using the data in treatment 1, after combination nitrogen in *Spirulina* (4.88%), other sessile algae (8.25%) and settle solid (10.89%), up to 24% of nitrogen could possibly be removed during culture period (see 3.3.2 Table 5).

The major source of nitrogen compounds in aquaculture pond were direct excretion of shrimp and decomposition of organic matter and uneaten shrimp feed (Avult, 1993). Kou and Chen (1991) mentioned that ammonia and nitrite in the intensive shrimp culture systems increased exponentially overtime. Chen and Tu (1991) reported that ammonia might increased to 6.5 mg NH_3-N L^{-1} even with frequent water exchange in *Penaeus monodon* grow out system. In this study, however, ammonium of all treatments including control was never exceed the acceptable concentration for aquaculture (Boyd, 1990 and Chen and Tu, 1991).

Two possible processes that reduced TIN of treatment 2 were, firstly, nitrogen uptake by other sessile macroalgae naturally found attach to the shelter and, secondly, denitrification process that occurred in microbial biofilm at the shelter surface and in the settled sediment. In fact, nitrogen content in sessile algae and sediment could be determined at the end of the experiment, however, nitrogen loss by denitrification was include in unidentified source.

Krom and Neori (1989) mentioned that nutrients, together with the high light intensity and warm temperature, support active growth of phytoplankton. Growth of *Spirulina* in treatment 1 (without shelter) was detected during the experiment but only in short period. In treatment 2 (with shelter), chlorophyll-*a* and trichome number of *Spirulina* were substantially lower than in treatment 1 and even lower than in control. Since at least 45% of tank surface area was shaded by the shelter. When combine the shading effect with high turbidity water in the tanks, sunlight could penetrate only a few centimetre below the water surface. This could be the most important limiting factor to *Spirulina* photosynthesis and growth. As recorted in the fish pond, gross productivity rate was found highest near the water surface and declined rapidly with depth because dense phytoplankton standing crop reduced light penetration (Lin, 1986). On the other

hand, this condition unlikely promoted growth of sessile macroalgae that grew attach in to the shelter top and tank surface. Therefore, to improve the efficiency of using *Spirulina* for water quality control, clear water system is one of the most important factors that need to be maintained. This can be achieved by proper aeration or alternative mixing systems that allowed some sedimentation at the bottom of the tank.

In this experiment, nitrite concentration was very high in all treatments. The highest concentration up to 30 mg NO_2-N L^{-1} was detected in control tanks. These concentrations were extremely high and could affect shrimp physiology. In normal condition, nitrite is never found accumulate in high dissolved oxygen environment since it will immediately be converted into nitrate. Accumulation of nitrite in this experiment could possibly relate with two processes, incomplete nitrification or incomplete denitrification. The pathway of nitrite accumulation found in this experiment is could be summarised as in Fig. 13. It need to be stated that nitrite concentration found in this experiment was exceptionally high. Toxicity of nitrite as reviewed by Boyd (1990) could affect shrimp around 8.5 – 15.4 mg NO_2-N L^{-1}, therefore, high nitrite concentration was one of the factor that induce stress and affect shrimp growth and survival. As this experiment was started using extreamly high density (120 shrimp m^{-2} bottom area) of moderate size shrimp (4.5 g), this density could not be compared with normal shrimp culture which range from 25 to 50 shrimp m^{-2} at post-larvae 15, (0.7 g shrimp).

In theory, incomplete nitrification happen when nitrite can not be converted to nitrate. This cause by factors that limiting activity of nitrite reducing bacteria *i.e.* *Nitrobactor*, *Nitrospira* and others. Two important factors are low oxygen concentration and low alkalinity (Bitton, 1994; Zweig *et al.*, 1999; Ruiz *et al.*, 2003). As nitrification is carried out in two steps, Ruiz *et al.* (2003) suggested that 75% of total

oxygen requirement is needed for ammonia oxidizing bacteria in the first step (equation 4). Another 25% of oxygen requirement is then needed to complete the second step by means of nitrite oxidizing bacteria (equation 5). However, Ruiz *et al.* (2003) found that DO range from 5.7 to 1.7 mg L^{-1} did not affected nitrification process in their experiment while nitrite accumulation took place when setting DO concentration at 0.7 mg L^{-1}. This low DO is possibly happen in the around the surface of the tank and sheter.

$$NH_4^+ + {}^3/_2O_2 \quad NO_2^- + H_2O + 2H^+$$

(Ammonia oxidizing bacteria require 1.5 mol of oxygen per mol of nitrogen)....(4)

$$NO_2^- + {}^1/_2O_2 \quad NO_3^-$$

(Nitrite oxidizing bacteria require 0.5 mol of oxygen per mol of nitrogen).....(5)

Incomplete denitrification (see number 3 in Fig. 14) on the other hand, is usually found when oxygen concentration in outer layer of biofilm was reduced to 1-2 mg L^{-1} by bacterial respiration (Ruiz *et al.*, 2003). This oxygen concentration might just proper for the first reducing step of denitrification process but was not low enough to complete the process since there was continuous aeration in the tank. According to Van Rijn (1996), nitrite accumulatation could be found in the recirculating water system containing high nitrate concentration. In intensive fish culture systems, nitrite was accumulated as a result of incomplete denitrification at low oxygen concentrations, or where denitrification was inhibited by limitation of organic matter (van Rijn and Rivera, 1990; van Rijn and Sich, 1992). Moreover, lack of carbon source for denitrifying bacteria might also another factor that affect denitrification process. Results from

denitrification studies published elsewhere such as Christensen and Harremoes (1978), Spotte (1979), Yang et al. (1989), Whitson et al. (1993), Bitton (1994), Aboutboul et al., 1995, Hargreaves, 1995 sited by Gross et al. (2000) and Menasveta et al. (2001), confirmed that carbon addition is essential for complete denitrification process in aquaculture system. Since there was not enough data to complete the explanation of nitrite accumulation found in this study, it has to be stated that nitrite could possibly come from either incomplete nitrification or incomplete denitrification processes.

Nitrate concentrations in both algae contained tanks (treatment 1 and 2) were lower than in control. Hence, it could be stated that ammonia was partly assimilated in the algal cells. Apart from algal assimilation, dissolved ammonia was then converted into nitrite by nitrification process under aerobic condition. On the other hand, the condition of this experiment was also a combination of both aerobic and anaerobic environment. In the water body, oxygen concentration was high due to continuous aeration but anaerobic environment could possibly happen in the biofilm layer on the tank surface and also in the sediment (including settle sediment). In treatment 1 (shrimp with *S. platensis*), surface area for bacterial biofilm was lower than in treatment 2 (shrimp with *S. platensis* and shelter). Therefore, complete denitrification process could possibly occur in biofilm layer of the shelter and this might be one of the factor that eliminated nitrogen out of the system.

3.2 Shrimp growth, survival and production yield

Summary of shrimp weight gain and food conversion is showed in Table 3. At the end of experiment, average shrimp weight in control, treatment 1 and treatment 2 were 5.63 ± 0.33, 6.45 ± 0.23 and 7.04 ± 0.04 g, respectively. Statistical analysis using ANOVA (Fig. 14) indicated that average weight of shrimp on days 45 and day 57 of the

two treatments groups were not significantly different ($P>0.05$), but both treatments had significantly greater weight than the control group on day 57 ($P<0.05$).

On the other hand, average shrimp survival at the end of experiment was only 30.6%, 46.5% and 67.4% for control, treatment 1 and treatment 2, respectively. Treatment 2 had the highest survival rate and significantly different ($P<0.05$). Treatment 1 had significantly ($P<0.05$) higher survival rate than control group.

It was found that the highest survival rate was in shrimp that cultured with *S. platensis* and shelter (treatment 2). This indicated the advantage of using shelter to reduce carnivorous behavior of shrimp and increase surface area for shrimps attachment. However, survival rate of high density shrimp culture without shelter (treatment 1) was still higher than in control. This suggested that the co-culture of *Spirulina* with shrimp could better maintain shrimp survival even at very high culture density. On the other hand, survival rate of control (without *Spirulina*) was significantly lower than other two treatments. For treatment 1 and 2, activity of algae including *Spirulina* and other sessile algae could maintain better water quality that affect shrimp survival. The average FCR content of treatment 2 in this study was 2.16, closely with other species culture in cement tank such as juvenile *Penaeus vannavei* (2.0-2.5 according to Wyban and Sweeney, 1989). Likewise, Funge-Smith and Briggs (1998) stated that FCR of shrimp culture in Earthern pond in Thailand ranged from 1.8 to over 2.0.

In the previous chapter, co-culture of shrimp with *Spirulina* had showed the excellent performance in water quality control in an experiment using clear water aquarium. The condition in this experiment was in the different manner since very high density of shrimp (120 shrimp m^{-2}) was used. This concentration was much higher than the traditional intensive culture of shrimp in Thailand that usually has around 50-100

393 shrimp m^{-2} (Briggs and Funge-Smith, 1994) but less than 50 shrimp m^{-2} was the most
394 exceptional density (Allan and Maguire, 1992). These densities were still high in
395 comparison with typical shrimp culture. With this high density, shelter in treatment 2
396 was then provided to increase attaching area for shrimp. Observation during the
397 experiment confirmed that most of shrimps were likely to stay within the shelter rather
398 than swimming around the bottom of the tank. This probably made treatment 2 the
399 highest shrimp survival rate.

400 ***3.3 Nitrogen and phosphorus budget***

401 ***3.3.1 Proximate analysis of nitrogen and phosphorus in the culture system***

402 Proximate analysis of nitrogen and phosphorus content in dry matters from the
403 last day is showed in Table 4. These tables were therefore used to calculate total
404 nitrogen and phosphorus in each tank at the beginning and at the end of the experiment.
405 Moreover, sum of dissolved inorganic nitrogen *i.e.* ammonium, nitrite and nitrate-
406 nitrogen was used to indicate total dissolved nitrogen.

407 The average nitrogen content of dry shrimp (*Penaeus monodon*) in this study
408 was 11%, closely with other studies such as in *Penaeus vannavei* (11.2% according to
409 Teichert-Coddington *et al.*, 2000) and black tiger shrimp (11.5% according to Funge-
410 Smith and Briggs, 1998). The average phosphorus content of shrimp carcass (*Penaeus*
411 *monodon*) in this study was 1.16%, also closely with previous study (1.19% according
412 to Funge-Smith and Briggs, 1998). The average phosphorus content of dry shrimp feed
413 in this study was 0.81%, closely with 0.29-0.90% as reported in Qifeng, 1991.

414 ***3.3.2 Nitrogen budget***

415 Nitrogen budget study in Table 5 revealed that the major input of nitrogen was
416 from shrimp feed and initial shrimp biomass. Nitrogen input through shrimp feed,
417 ranged from 51 to 53% of total input. These figures were lower than among the normal

418 range as previous published reports such as 82% by Muthuwan (1991) an 94% by
419 Satapornvanit (1993). The rest of input was from shrimp biomass which accounted
420 from 17 to 48% of total input. Nitrogen in the water at the beginning was very low in
421 all treatments (0.18 to 0.25%). In addition, nitrogen input as *Spirulina* biomass in
422 treatment 1 and 2 was only 2.01 and 3.52%, respectively.

423 As shown in Table 5, the major portion of the nitrogen in control tanks was
424 deposited as dissolved inorganic nitrogen. On the other hand, nitrogen of both
425 treatments was deposited in shrimp, sediment and algae while relatively smaller fraction
426 were retained by molting. Nitrogen assimilated in *Spirulina* of treatment 1 was
427 accounted by 4.88% of total nitrogen output, less than that absorbed by other sessile
428 macroalgae (8.25%). These sessile macroalgae occurred naturally around the edge of
429 the tanks during the experiment. In treatment 2, sessile macroalgae was found not only
430 at the tank wall but also attach to the shelter surface. As the result, the largest portion of
431 47.64% nitrogen output was therefore absorbed by shrimp following by sessile
432 macroalgae (14.44%), sediment (6.81%) and water (6.30%) respectively.

433 Briggs and Funge-Smith (1994) suggested that, although high protein content
434 feed was used, only 20-23% of nitrogen from feed was then retained in shrimp biomass.
435 Most of nitrogen (80%) was then excreted to surrounding environment. At the end of
436 typical shrimp culture, 27-57% of total nitrogen was discharged from the pond by water
437 exchange (Funge-Smith and Briggs, 1998; Preston *et al.* (2000) sited by Burford and
438 Williams (2000). According to Teichert-Coddington et al. (2000), at least 59% of
439 nitrogen from feed was finally waste the pond. Martin *et al.* (1998) also mentioned that
440 feeding in shrimp pond resulted in nitrogen accumulation in the sediment, so called the
441 nitrogen sink (Avnimelech and Lacher, 1979 sited by Zur, 1981; Alongi et al., 2000;
442 Jamu and Piedrahita, 2002).

Muthuwan (1991) stated that the major output of nutrients in shrimp ponds was released in discharged water. Likewise, total nitrogen in drained water of this study during harvesting were 37.97, 9.88 and 6.30 % nitrogen for control, treatment 1 and treatment 2, respectively. Teichert-Coddington et al. (2000) reported that 80% of total nitrogen loss from the pond occurred primarily from daily water exchange (72%) and pond drainage (8%). Boyd (1995) suggested that traditional pond management practices often included fertilization to promoted primary productivity. This practice is therefore confusing since most of primary nutrients for phytoplankton in the pond are readily supplied by fish excretion and feed decomposition. It was at least to be mentioned that algae in both benthic and planktonic forms could successfully reduce nitrogen waste in the discharged water from the culture system and the major sink of nitrogen in this study were sessile macroalgae (14%) in treatment 2 and suspended solid (8.25%) in treatment 1 and control.

Unidentified nitrogen was among the largest portion of nitrogen after balancing nitrogen output with the input. In this experiment, unidentified nitrogen ranged from 33% in control to 36% in both treatments. This unidentified or unaccounted source was generally found between 9 to 50% of nutrient budget in many reports elsewhere (Funge-Smith and Brigges, 1998; Lefebvre et al., 2001; and Thoman et al., 2001). Daniels and Boyd (1989) estimated that >50% of the nitrogen input via feed could be lost through the combined effects of denitrification and ammonia volatilization in polyethylene-lined, brackish water ponds. In this experiment, evaluation of denitrification process was not included in the methodology since there was not soil bottom layer. However, accumulation of nitrite found during experiment indicated a possibility of denitrification in biofilm layer of both tank wall and shelter surface. According to Thoman et al. (2001), removal of nitrogen through denitrification was the most likely explanation for

9-21% of nitrogen loss from closed recirculating mariculture systems. According to Hargreaves, 1995 sited by Gross et al. (2000), denitrification rate varies with temperature, pH, abundance of denitrifying bacteria, concentration of nitrate, organic carbon, and dissolved oxygen. In addition, Gross et al. (2000) illustrated that denitrification in aquaculture pond was approximately 38 mg N m^{-2} d^{-1}. In typical earth ponds, denitrification ranged from 17.4-57.3% of nitrogen output were recorded (Gross et al., 2000). Denitrification up to 100 kg hr^{-1} yr^{-1} was reported by Olah and Peka´r (1995) in an integrated fish culture pond. Shrimp culture in concrete tank without soil bottom at 50 shrimp m^{-2} conducted by Thakur and Lin (2003) also found that there was 36% of unaccounted nitrogen after balancing nutrient butget. Another treatment of the same report, shrimp culture in concrete tank with sediment bottom, had much lower unaccounted nitrogen (only 5.2%). This strongly suggested that bottom sediment is one of the important factor for nitrogen removal process. Ammonia volatilization, as mentioned in Daniels and Boyd (1988) and Thakur and Lin (2003), might enhanced the lost of nitrogen in the tank with vigorous aeration and high pH. Schroeder (1987) indicated that ammonia volatilization rate could be up to 50 mg N m^{-2} day^{-1},depending on the concentration of unionized ammonia, temperature, salinity as well as evaporation rate and wind speed (Hargreaves, 1998). Moreover, since dissolved organic nitrogen (DON) analysis was not included in this experiment because of technical problem, DON might be another possible source of unidentified nitrogen in the culture system.

Nitrogen budget in this study showed that 17-48% of nitrogen was incorporated into harvested shrimp, closely with other publications such as 22.8-30.7% in Thakur and Lin (2003); 22% in Brigges and Funge-Smith (1994), or even 20 to 26% of nitrogen output from fish pond (Daniels and Boyd, 1989; Krom and Neori, 1989). In this study, statistical analysis revealed that there was no significant difference ($P<0.05$) in total

feed input and also in total nitrogen input which ranged from 79.1 to 80.86 g-N per tank. Low shrimp biomass therefore related with slow growth rate and low survival rate of shrimps in all treatments as possibly the effect of high nitrite concentration as previously discussed. This was mainly the result of low survival rate in all treatments.

3.3.3 Phosphorus budget

As shown in Table. 6, phosphorus budget in all treatments related with nitrogen budget. The major input of phosphorus was also from shrimp feed (56-60%) and initial shrimp biomass (37-39%). Phosphorus input from *Spirulina* was 3.27% and 5.66% in treatment 1 and treatment 2, respectively. Phosphorus input from water, however, was very low with only 0.2-0.3% of total input.

In the present study, average phosphorus content in *Penaeus monodon* was 1.16 %, close to phosphorus in *Penaeus vannamei* that was 1.25 %. (Teichert-Coddington et al., 2000). Feed contributed 56 to 60% of total phosphorus input (see Table 5.5) which was lower than the previous reports such as 87% in Muthuwan (1991) or 91% in Satapornvanit (1993). Phosphorus content in shrimp biomass of this study ranged from 14-39% higher than 4.9-7.7 % in Brigges and Smith (1994) and 9% in Teichert-Coddington et al. (2000).

In general, unaccounted phosphorus in the earth pond is mostly the result of mud adsorption, as mud have a strong affinity to phosphorus (Boyd, 1985). Up to 84% of phosphorus input in shrimp pond was found deposite in bottom sediment (Funge-Smith and Brigges, 1998). Qifeng (1991), 34-51% of phosphorus input accumulated at the bottom sediment of fish culture tank. Even in the tanks that did not have sediment bottom such as Thakur and Lin (2003), upto 66.7% of phosphorus was found in sediment that naturally occurred during the experiment. The result of this study found that 30-35% of phosphorus output was found in water fraction of all treatments. Algae

in treatment tanks absorbed phosphorus up to 15.75% of total phosphorus in treatment 1. In detail, *Spirulina* and other sessile macroalgae in treatment 1 equally absorb phosphorus at approximately 7.48% and the rest was found in suspended solid (11%) and shrimp biomass (24%). In treatment 2, since there was low *Spirulina* biomass in both tanks, 7.29% of total phosphorus was found mainly in other sessile macroalgae. The highest number of phosphorus in shrimp biomass (39.32%) was observed in treatment 1 because it had the highest survival rate. Percentage of unidentified phosphorus output at 8-37% was lower than the percentage number that found in unidentified nitrogen output. This finding was similar to Thakur and Lin, 2003 which reported that measurable unidentified outputs were 5.2-36.0% for nitrogen, and 5.3-19.7% for phosphorus, respectively.

Muthuwan (1991) and Ritvo et al. (2002) stated that the main output of nutrients in shrimp ponds were in discharged water. Likewise, total phosphorus in drained water during harvest were 35.13, 30.31 and 33.30 % phosphorus in control, tretment 1 and treatment 2, respectively. The major sinks of phosphorus in this study were from shrimp carcass 14.80%, 24.84%, and 39.32% for control, treatment 1, and treatment 2, respectively. Another possible reason of unaccounted phosphorus might be related to practical error in settle solid collection and lost of sludge during drainage. However, more work is needed with these large culture systems. Specifically, we need to develop effective algal harvest techniques for large tanks and ponds, and we need to determine whether *S. platensis* (or another desirable alga) can be maintained as the dominant alga throughout the culture cycle.

Acknowledgement

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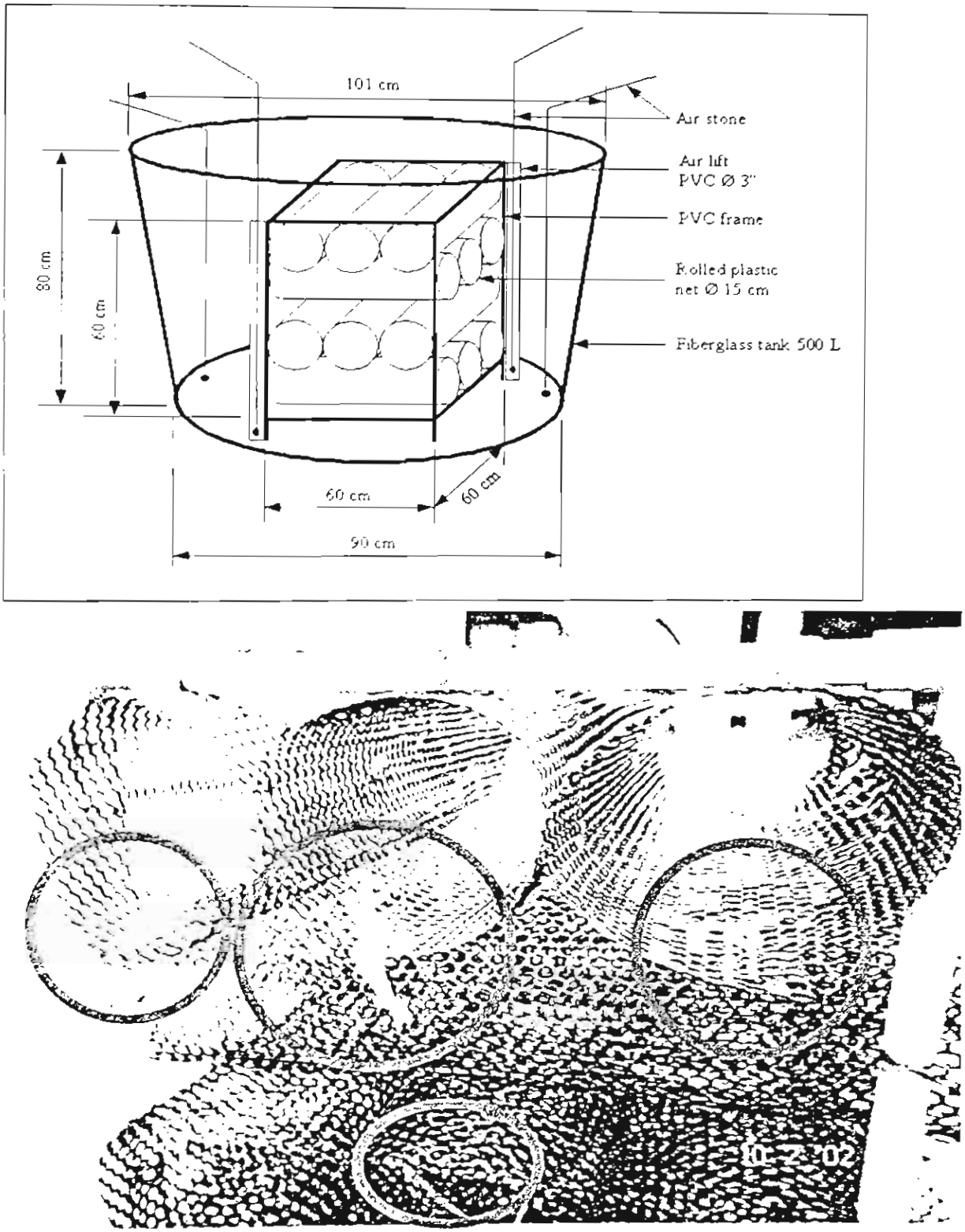

Fig. 1. Diagram (top) and photograph (bottom) of the shrimp shelter used in this experiment.

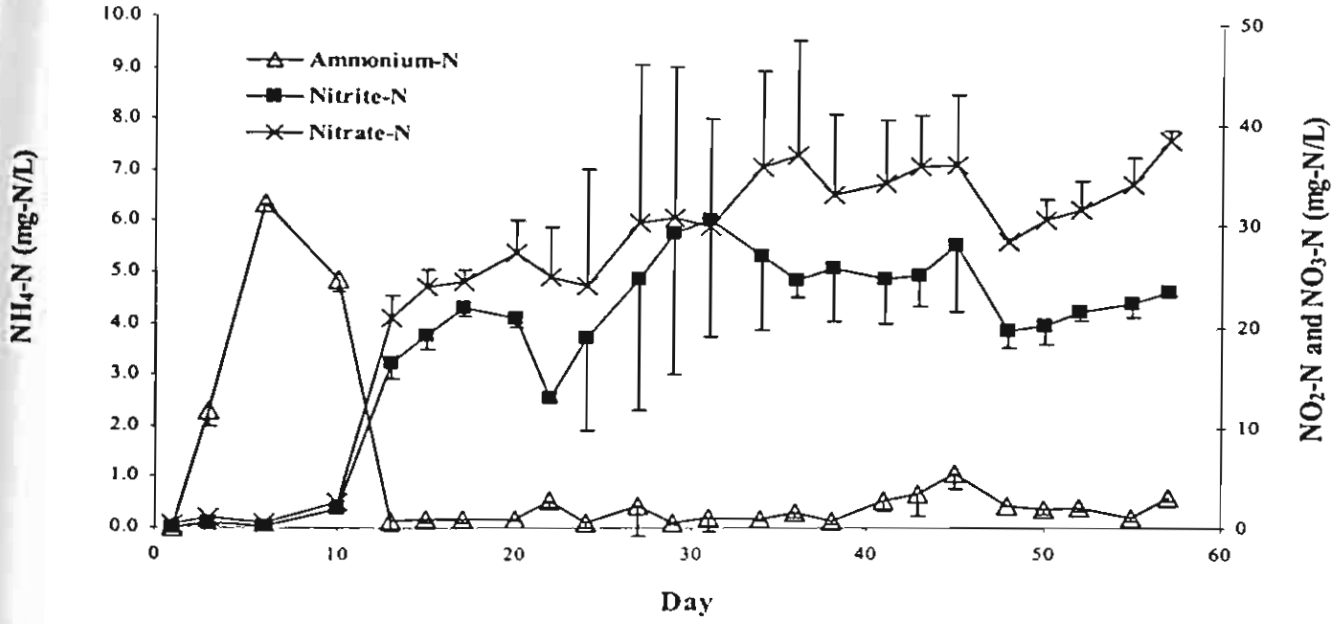

Fig. 2. Ammonium, nitrite, and nitrate concentrations during the experiment for control (high density shrimp without *S. platensis* and shelter).

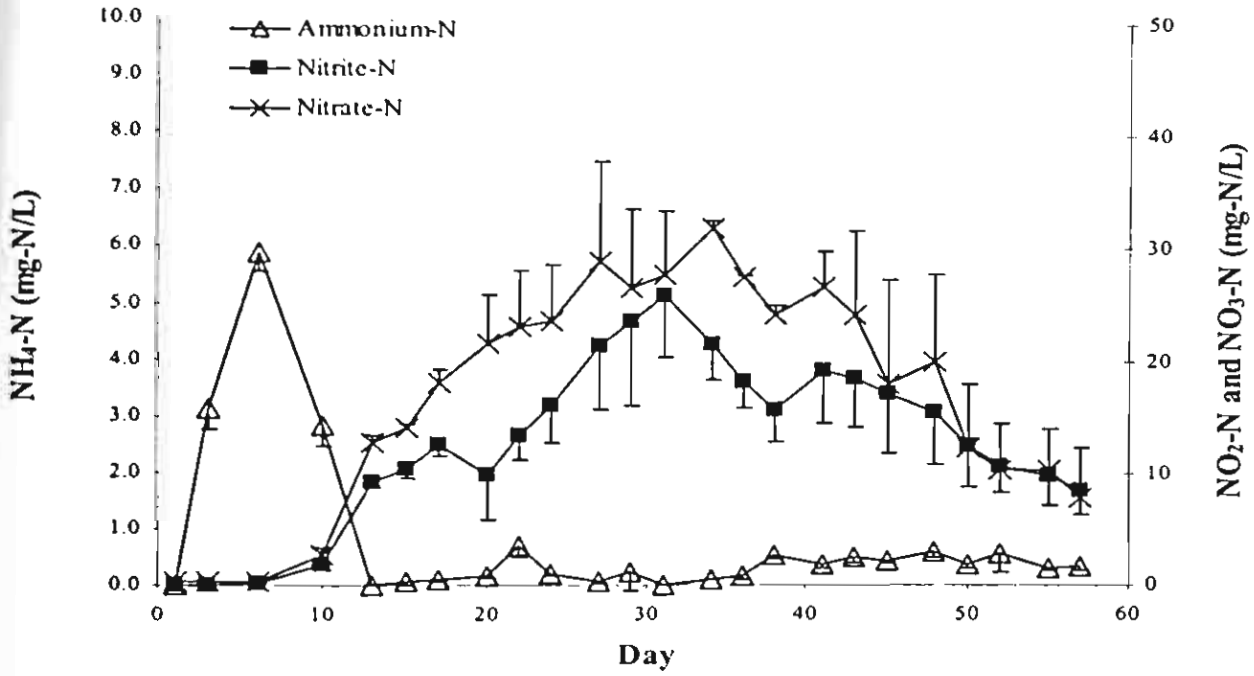

Fig. 3. Ammonium, nitrite, and nitrate concentrations during the experiment for treatment 1 (high density shrimp with *S. platensis*).

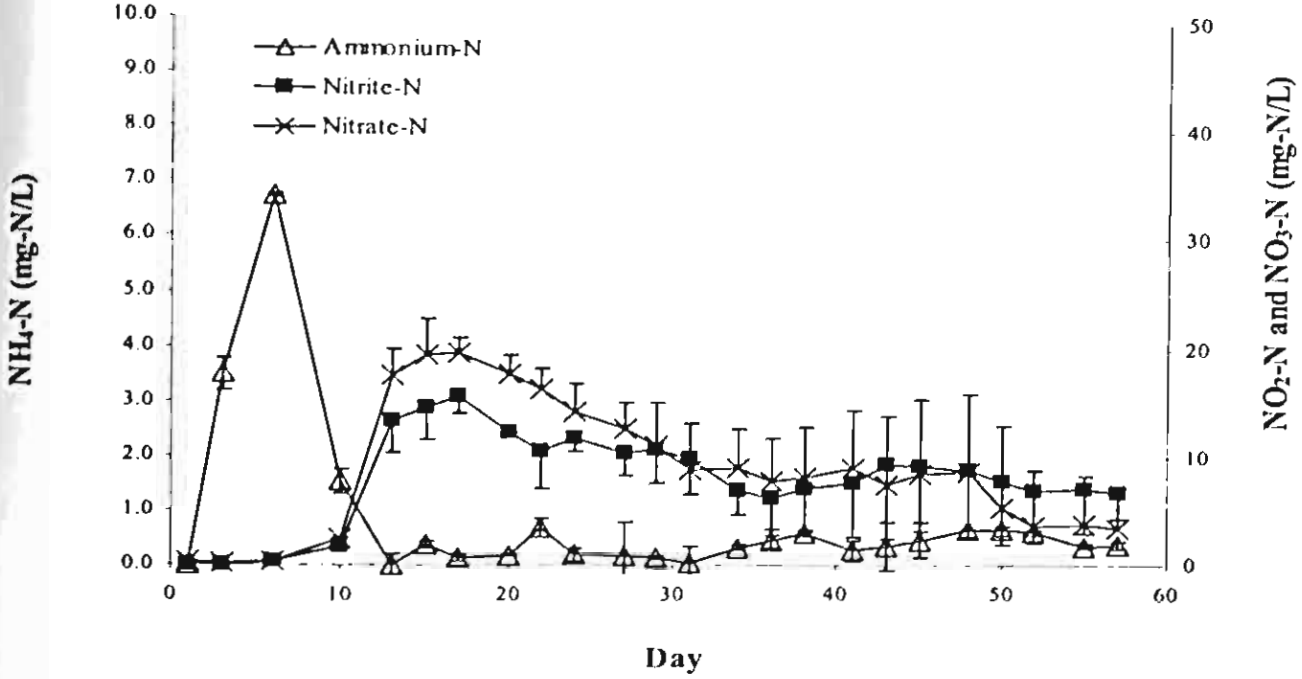

Fig. 4. Ammonium, nitrite, and nitrate concentrations during the experiment for treatment 2 (high density shrimp with *S. platensis* and shelter).

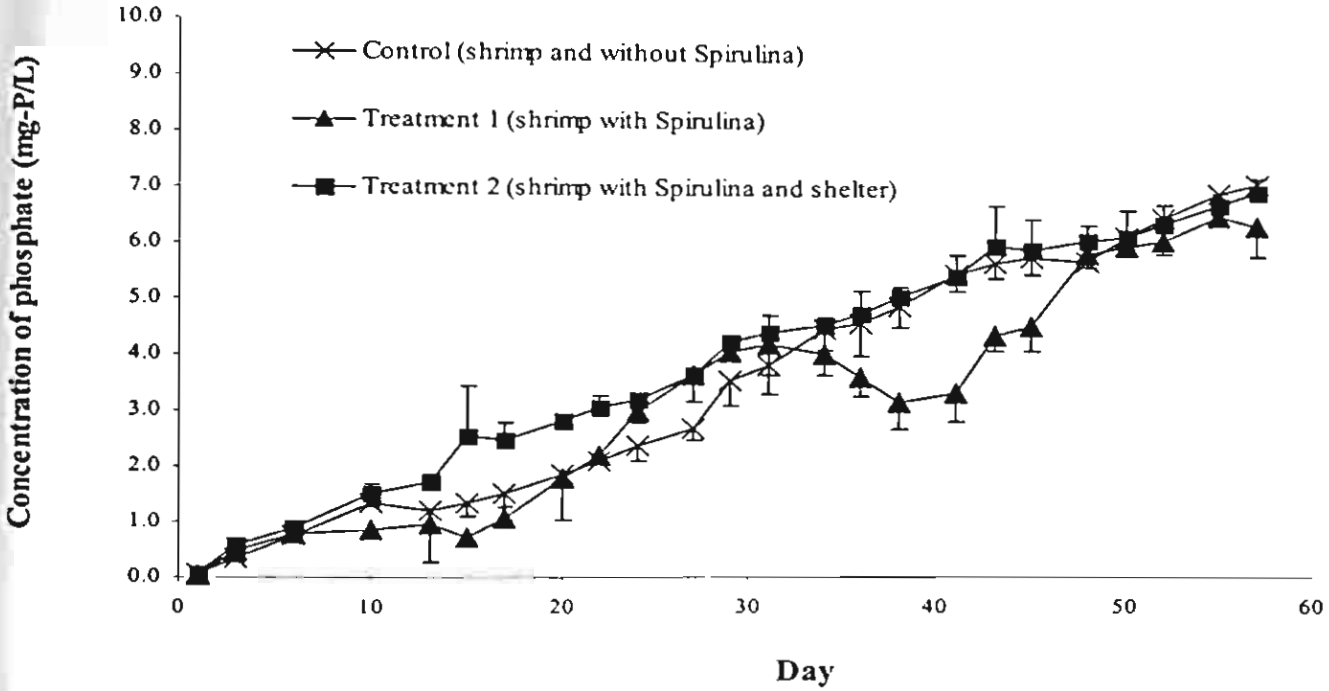

Fig. 5. Average phosphate concentrations during the experiment in control (shrimp and without *S. platensis*), treatment 1 (shrimp with *S. platensis*), and treatment 2 (shrimp with *S. platensis* and shelter) during the experiment.

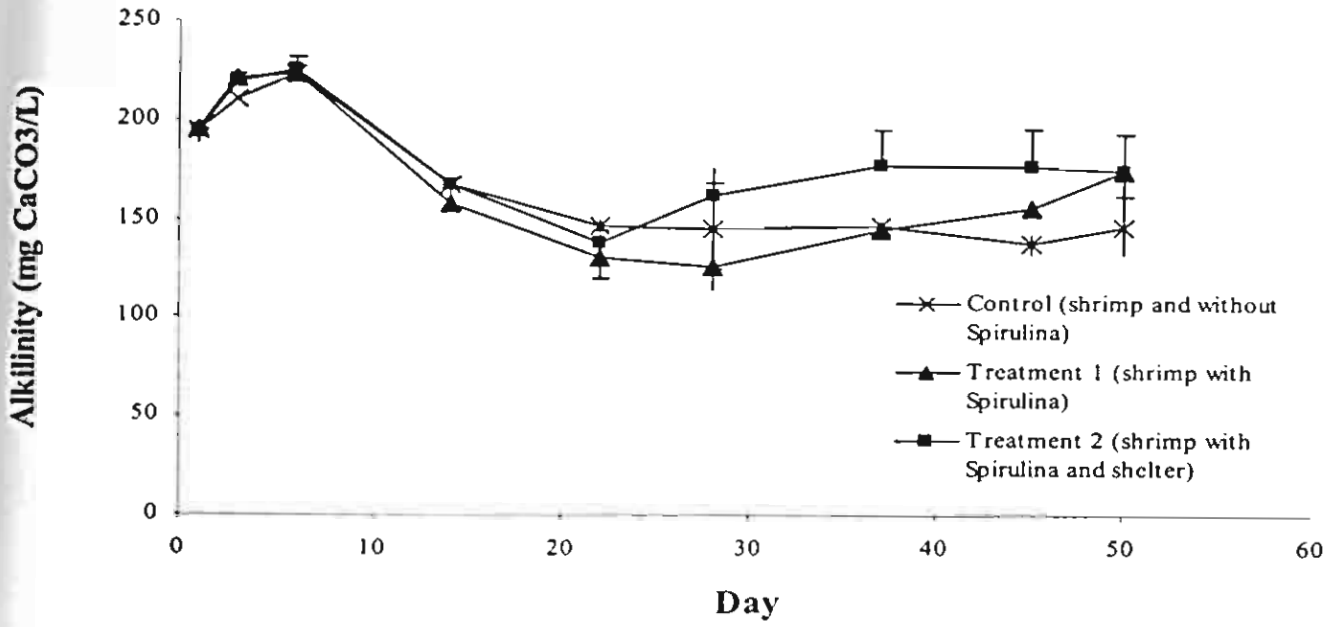

Fig. 6. Average alkalinity of control (shrimp and without *S. platensis*), treatment 1 (shrimp with *S. platensis*), and treatment 2 (shrimp with *S. platensis* and shelter).

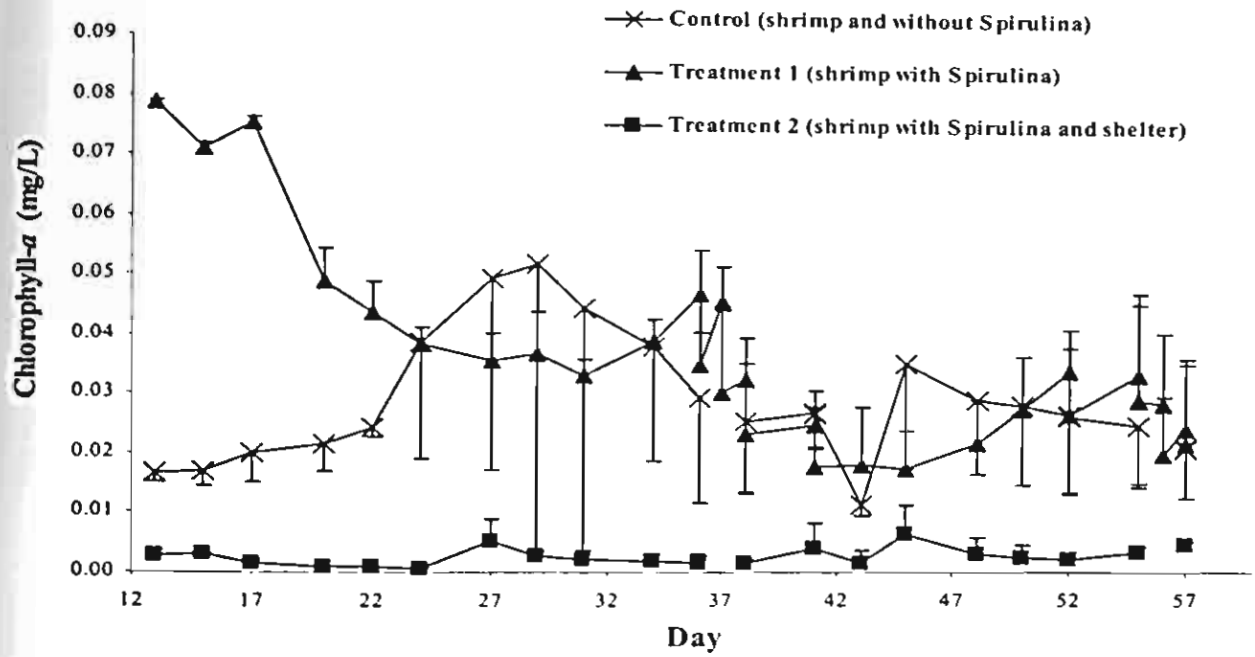

Fig. 7. Chlorophyll-*a* concentrations in shrimp culture tanks; control (shrimp and without *S. platensis*), treatment 1 (shrimp with *S. platensis*), and treatment 2 (shrimp with *S. platensis* and shelter) during the experiment. Error bars indicate standard deviation with n=2. For treatment 1, error bars were from chlorophyll-*a* data before harvesting.

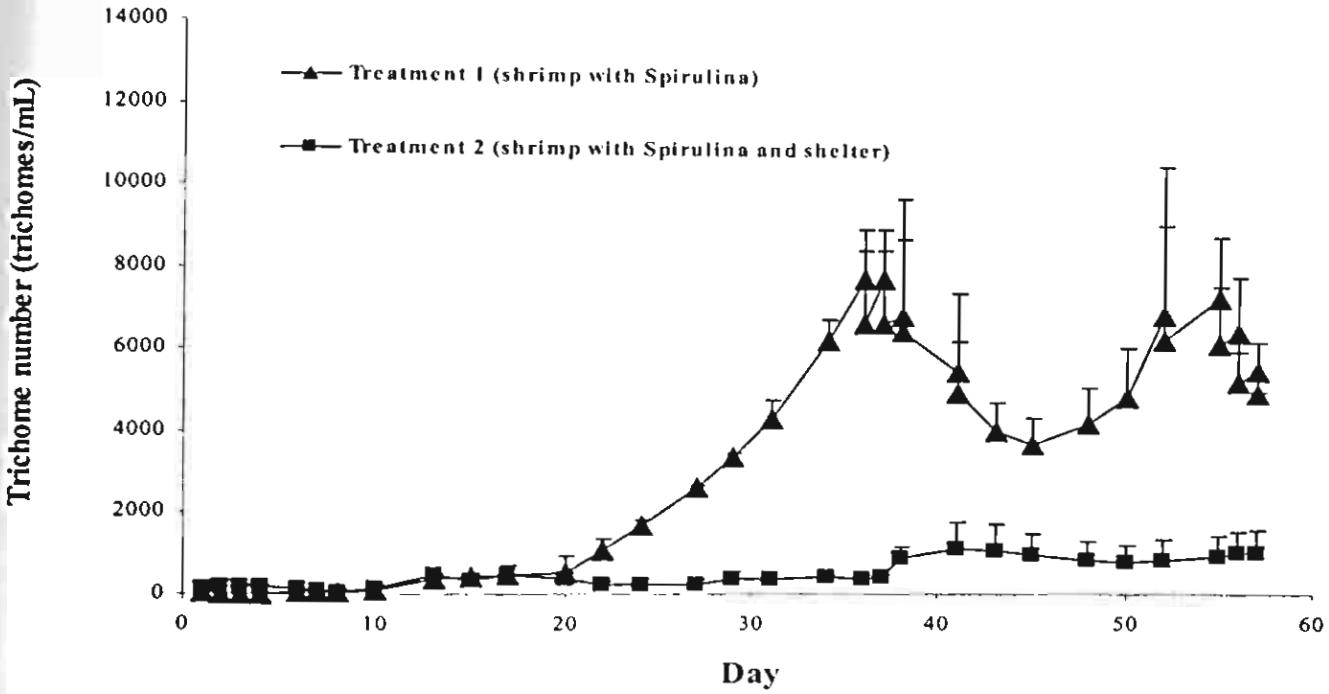

Fig. 8. Trichomes number in shrimp culture tanks; treatment 1 (shrimp with *S. platensis*), and treatment 2 (shrimp with *S. platensis* and shelter) during the experiment. Error bars indicate standard deviation with n=2. For treatment 1, error bars were from trichome data before harvesting.

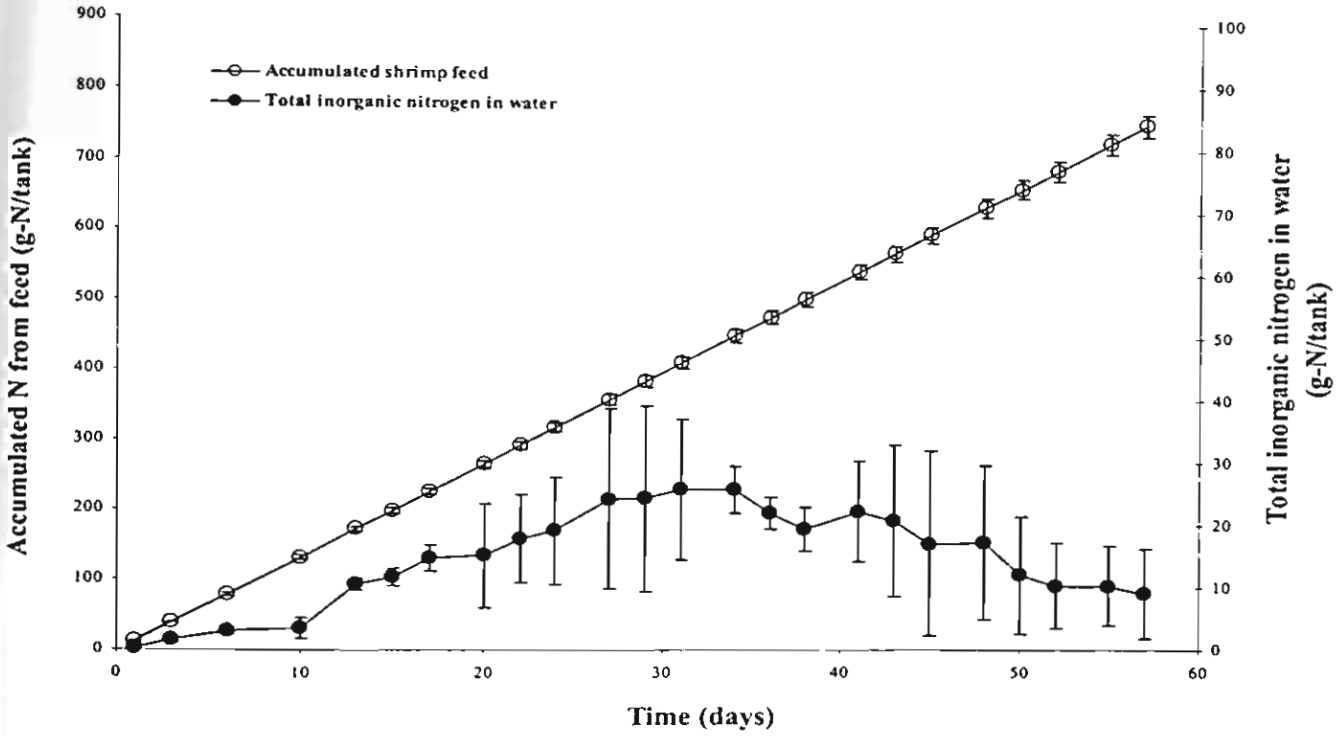

Fig. 9. Relationship between estimated nitrogen accumulation from feed and total inorganic nitrogen concentration in water of treatment 1 (shrimp with *Spirulina*).

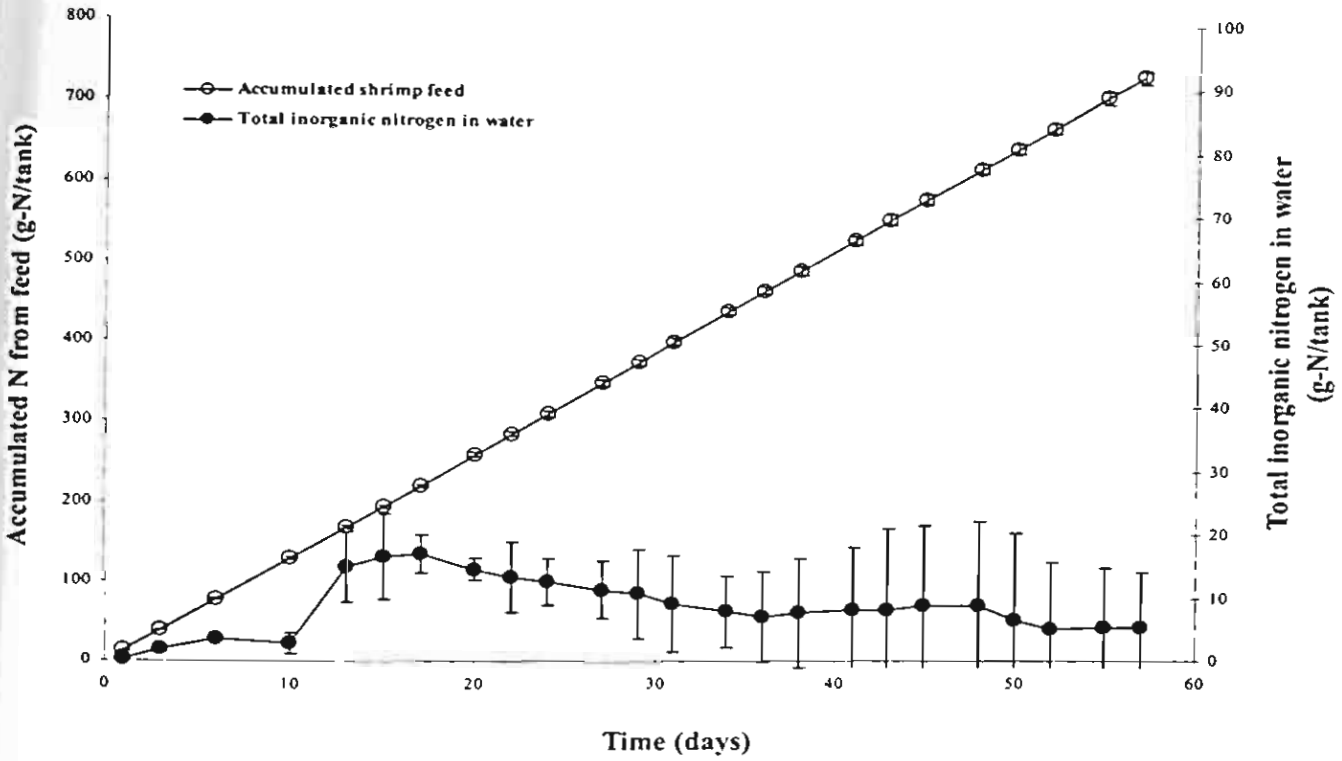

Fig. 10. Relationship between estimated nitrogen accumulation from feed and total inorganic nitrogen concentration in water of treatment 2 (shrimp with *Spirulina* and shelter).

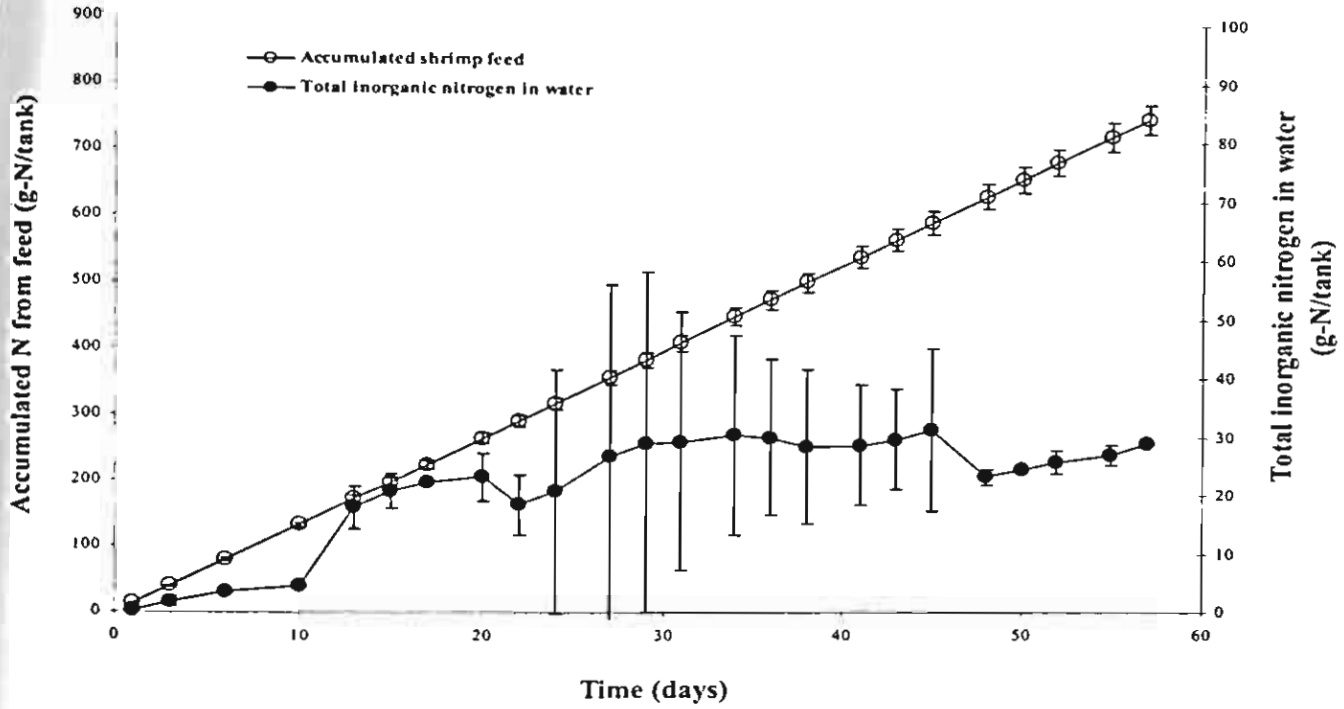

Fig. 11. Relationship between estimated nitrogen accumulation from feed and total inorganic nitrogen concentration in water of control (shrimp without *Spirulina*).

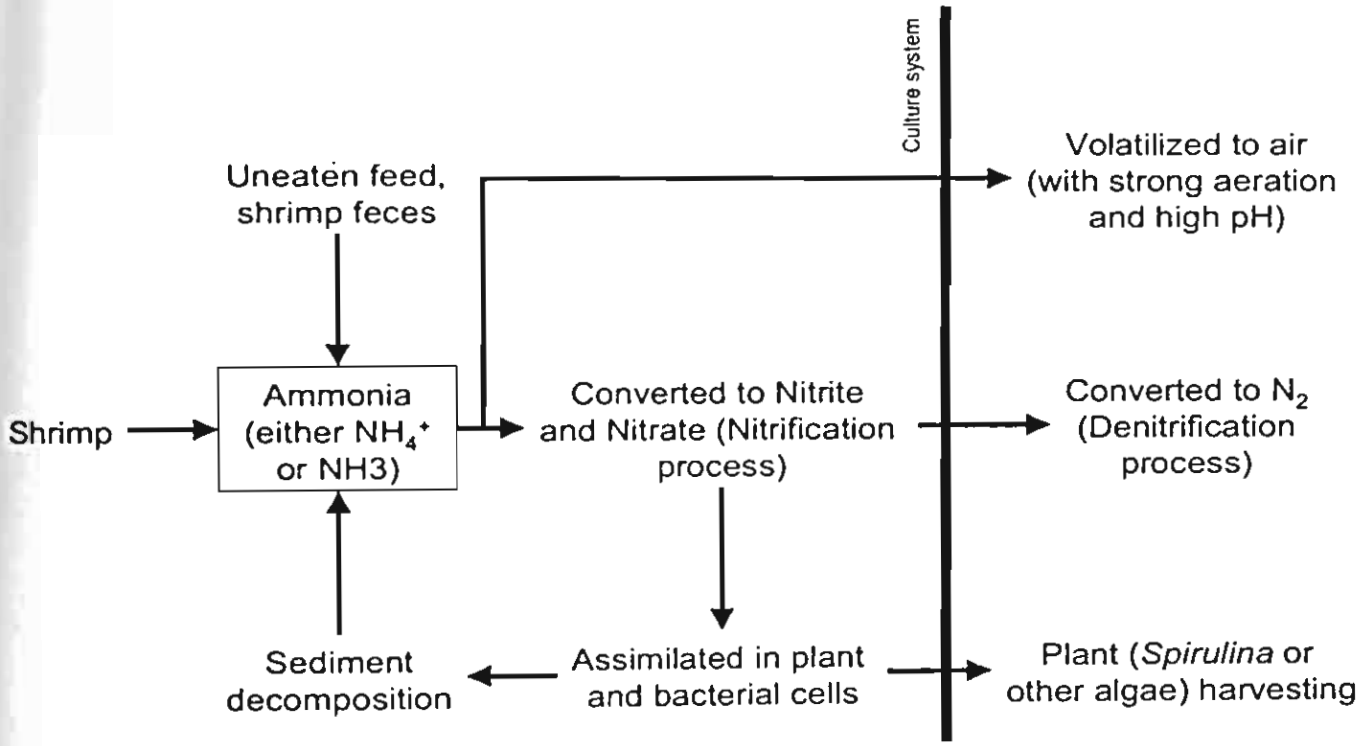

Fig. 12. Postulated pathway of nitrogen cycle in this study.

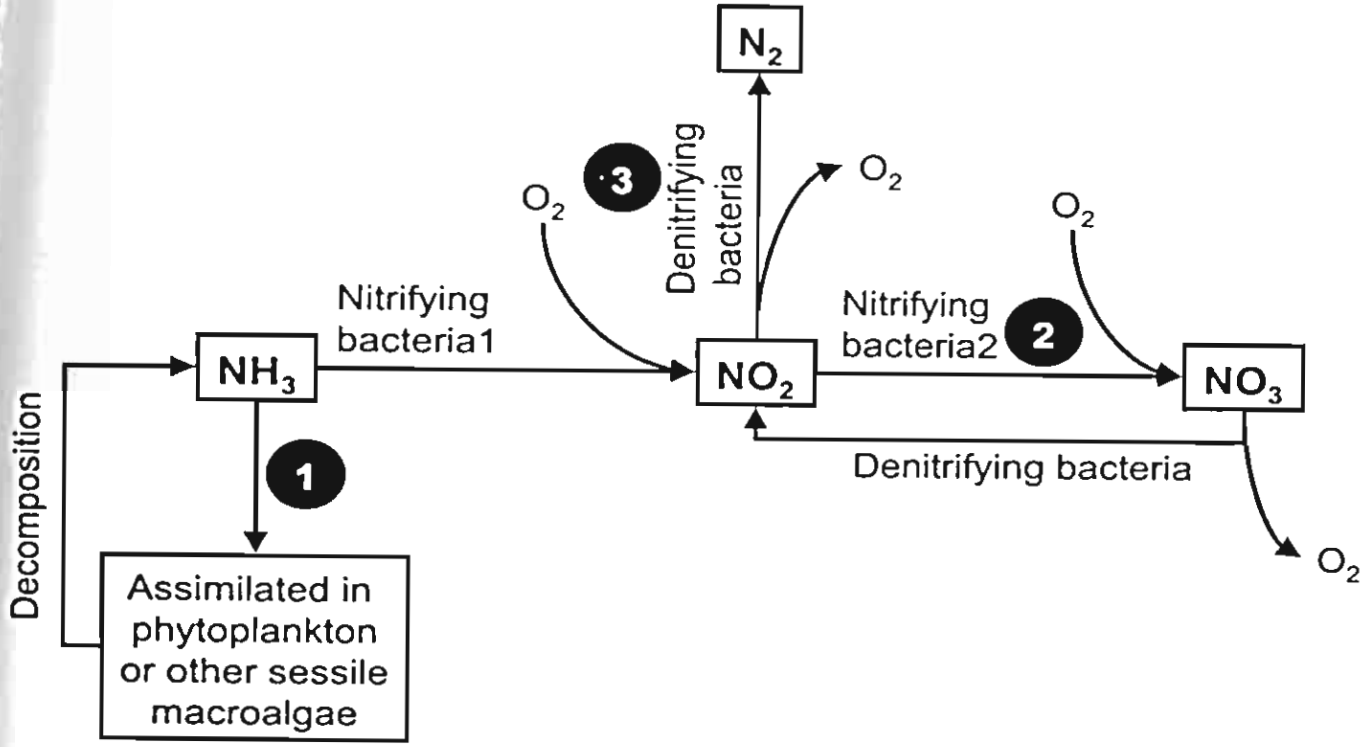

Fig. 13. Pathway of nitrite accumulation possibly occurred in this experiment. [1] Ammonia (NH_3) was partly assimilated in the algae and most of ammonia was converted to nitrite (NO_2^-). [2] Incomplete nitrification possibly caused nitrite accumulation. [3] Incomplete denitrification process also increased nitrite concentration in the water.

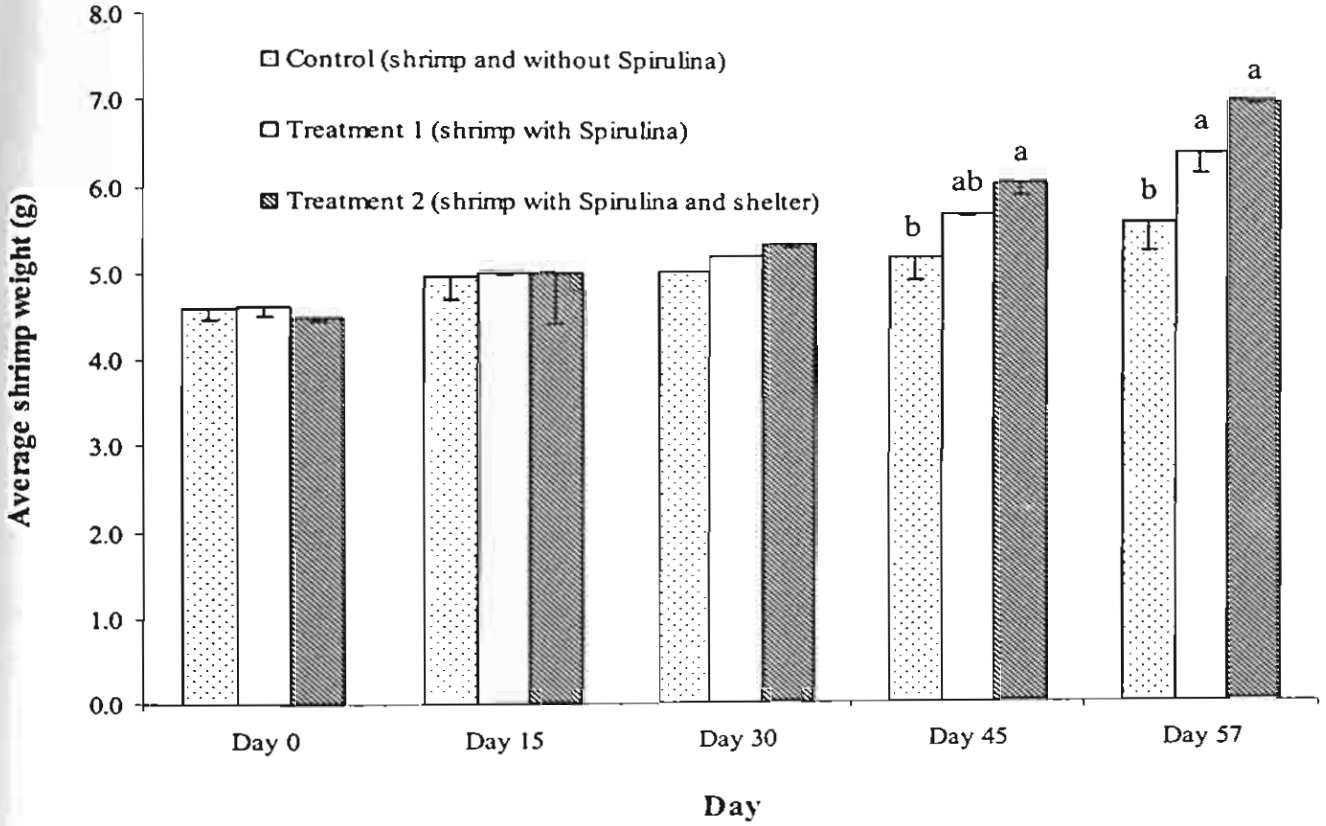

Fig. 14. Average shrimp weights of control (shrimp without *S. platensis*), treatment 1 (shrimp with *S. platensis*) and treatment 2 (shrimp with *S. platensis* and shelter). Error bars represent standard deviation (n=2) while a or b indicate significant difference (P <0.05).

Table 1. Definition of parameters used for nitrogen budget calculation in this study. The same procedure was also used for phosphorus budget calculation.

Parameters	Definition
$^{N}Shrimp_{in}$	Nitrogen in shrimp at the beginning of the experiment. = total shrimp weight × 76.4% (average percentage of dry weight per fresh weight) × 11.1% (average percentage of nitrogen in dry shrimp)
$^{N}Feed_{in}$	Nitrogen in shrimp feed. = total weight of feed used throughout the experiment × 5.85% (average percentage of nitrogen in 69.18% protein feed)
$^{N}Water_{in}$	Total inorganic nitrogen in the water at the beginning of the experiment. = concentration (g-N L^{-1}) of ammonium-N + nitrite-N + nitrate-N × 480L of water in each tank
$^{N}Spirulina_{in}$	Nitrogen in *S. platensis* cells added into shrimp tank at the beginning of the experiment. = (((1.3477*10^{-7}) *T*N)/1000) where T = amount of trichome (trichome^{-1}), N = average percentage of nitrogen content in dry *S. platensis* as 9.03%.
$^{N}Shrimp_{out}$	Nitrogen in shrimp at the end of experiment. = [total shrimp weight × 76.4% (average percentage of dry weight per fresh weight) × 11.1% (average percentage of nitrogen in dry shrimp)]

Table 1.

(Continued)

Parameters	Definition
$^{N}Water_{out}$	Total inorganic nitrogen in the water at the end of the experiment.
$^{N}Spirulina_{out}$	Nitrogen in *S. platensis* cells that were harvested out during the experiment plus Nitrogen in *S. platensis* cells at the end of the experiment.
$^{N}settle\ solid_{out}$	Nitrogen in settle solid found in the tank at the end of experiment. = volume of water in the tank (480L) × dry weight of settle solid × 4.359% of nitrogen content
$^{N}Other\ algae_{out}$	Nitrogen in other microalgae and sessile macroalgae found at the end of experiment. = total weight of dried sessile algae scratched from tank's wall and shelter × 3.89% of nitrogen content
$^{N}Molting_{out}$	Nitrogen in shrimp molt that were found in the tank at the end of experiment. = dry weight of shrimp molt × 5.35% of nitrogen content

Table 2. Chemical parameter of water quality in the experiment.

Parameters	Experiment		
	Control	Treatment 1	Treatment 2
pH	8.54-8.91	8.74-9.12	8.54-8.91
Temperature (°C)	26.80-28.95	25.30-28.05	26.80-28.95
Salinity (psu)	28-30	28-30	28-30
Alkalinity (mg as $CaCO_3$ L^{-1})	139.5-223.0	127.0-223.0	138.5-225
Dissolved oxygen (mg L^{-1})	5.14-5.91	5.43-6.24	5.78-6.58

Table 3. Summary of shrimp weight gain, survival, biomass production, total feeding and food conversion (FCR) in this experiment.

Parameters	Control (no *Spirulina*, no shelter)	Treatment 1 (with *Spirulina*)	Treatment 2 (with *Spirulina* and shelter)
Average Daily Growth (ADG g day^{-1})	0.019 ± 0.014	0.034 ± 0.019	0.047 ± 0.025
Survival Rate (%SR)	30.56 ± 1.96	46.53 ± 0.98	67.37 ± 0.98
Final Mean Body Weight (^{Final}MBW g)	5.63 ± 0.33	6.45 ± 0.23	7.04 ± 0.04
Biomass Produced (g)	124.05 ± 15.13	216.08 ± 12.27	341.55 ± 6.72
Total feed used (g)	755.30 ± 22.93	757.13 ± 15.33	736.46 ± 7.93
Food Conversion Ratio (FCR)	6.15 ± 0.93	3.51 ± 0.27	2.16 ± 0.07

Values are mean ± S.D. (n=2)

Table 4. Quantitative analysis of nitrogen and phosphorus in dry matters found in the culture system.

Source	% Protein	% Nitrogen	% Phosphorus
Shrimp feed	36.56 ± 1.71	5.85 ± 0.27	0.81 ± 0.01
Shrimp carcass	69.18 ± 1.49	11.07 ± 0.24	1.16 ± 0.17
S. platensis biomass	56.44 ± 3.46	9.03 ± 0.55	1.83 ± 0.98
Other algae (sessile form)	24.29 ± 1.01	3.89 ± 0.16	0.44 ± 0.05
Shrimp molting	33.46 ± 1.37	5.35 ± 1.37	0.31 ± 0.01
Settle solid	27.27 ± 2.98	4.36 ± 0.48	0.59 ± 0.09

Table 5. Nitrogen budget for different treatments during 57-day experiment.

Source	Control (no *Spirulina*, no shelter)	Treatment 1 (with *Spirulina*)	Treatment 2 (with *Spirulina* & shelter)
Nitrogen Input			
Shrimp (g-N per tank)	36.67 ± 1.11	36.76 ± 0.74	35.76 ± 0.39
(%)	*(46.36 ± 2.64)*	*(45.45 ± 2.15)*	*(44.74 ± 1.12)*
Shrimp feed (g-N per tank)	42.24 ± 1.28	42.34 ± 0.86	41.19 ± 0.45
(%)	*(53.40 ± 0.02)*	*(52.36 ± 0.22)*	*(51.53 ± 0.15)*
Spirulina (g-N per tank)	0	1.62 ± 0.28	2.81 ± 0.26
(%)	*(0)*	*(2.01 ± 0.38)*	*(3.52 ± 0.27)*
Water (g-N per tank)	0.19 ± 0.03	0.14 ± 0.03	0.17 ± 0.01
(%)	*(0.25 ± 0.05)*	*(0.18 ± 0.04)*	*(0.21 ± 0.01)*
Nitrogen Output			
Shrimp (g-N per tank)	13.73 ± 1.68	23.92 ± 1.36	38.07 ± 0.37
(%)	*(17.40 ± 2.64)*	*(29.59 ± 2.15)*	*(47.64 ± 1.12)*
Spirulina (g-N per tank)	0	3.96 ± 1.61	0
(%)	*(0)*	*(4.88 ± 1.91)*	*(0)*
Settle solid (g-N per tank)	8.69 ± 0.40	8.82 ± 0.96	5.44 ± 0.16
(%)	*(10.98 ± 0.18)*	*(10.89 ± 1.01)*	*(6.81 ± 0.29)*

Water (g-N per tank)	30.03 ± 0.41	7.96 ± 3.24	5.06 ± 3.04
(%)	*(37.97 ± 0.61)*	*(9.88 ± 4.16)*	*(6.30 ± 3.72)*
Other algae (g-N per tank)	0	6.68 ± 0.35	11.52 ± 1.92
(%)	*(0)*	*(8.25 ± 0.30)*	*(14.44 ± 2.60)*
Molting (g-N per tank)	0.04 ± 0.02	0.13 ± 0.01	0.20 ± 0.01
(%)	*(0.05 ± 0.03)*	*(0.16 ± 0.01)*	*(0.25 ± 0.01)*
Unidentified (g-N per tank)	26.62 ± 3.20	29.41 ± 2.98	19.63 ± 0.52
(%)	*(33.61 ± 3.04)*	*(36.35 ± 3.10)*	*(24.56 ± 0.31)*

Values are mean ± S.D. (n=2)

Table 6. Phosphorus budget for different treatments during 57-day experiment.

Source	Control (no *Spirulina*, no shelter)	Treatment 1 (with *Spirulina*)	Treatment 2 (with *Spirulina* & shelter)
Phosphorus Input			
Shrimp (g-P per tank)	3.84 ± 0.12	3.85 ± 0.08	3.75 ± 0.04
(%)	*(39.42 ± 0.02)*	*(38.15 ± 0.25)*	*(37.18 ± 0.15)*
Shrimp feed (g-P per tank)	5.88 ± 0.18	5.89 ± 0.12	5.73 ± 0.06
(%)	*(60.30 ± 0.03)*	*(58.35 ± 0.38)*	*(56.86 ± 0.23)*
Spirulina (g-P per tank)	0	0.33 ± 0.06	0.57 ± 0.05
(%)	*(0)*	*(3.27 ± 0.61)*	*(5.66 ± 0.44)*
Water (g-P per tank)	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.01
(%)	*(0.28 ± 0.05)*	*(0.23 ± 0.02)*	*(0.31 ± 0.06)*
Phosphorus Output			
Shrimp (g-P per tank)	1.44 ± 0.18	2.51 ± 0.14	3.96 ± 0.08
(%)	*(14.80 ± 2.24)*	*(24.84 ± 1.75)*	*(39.32 ± 1.36)*
Spirulina (g-P per tank)	0	0.84 ± 0.34	0
(%)	*(0)*	*(8.27 ± 3.26)*	*(0)*
Settle solid (g-P per tank)	1.31 ± 0.02	1.19 ± 0.04	0.65 ± 0.02
(%)	*(13.44 ± 0.16)*	*(11.81 ± 0.28)*	*(6.46 ± 0.27)*
Water (g-P per tank)	3.42 ± 0.12	3.06 ± 0.27	3.36 ± 0.12

%)	*(35.13 ± 0.17)*	*(30.31 ± 3.07)*	*(33.30 ± 0.70)*
Other algae (g-P per tank)	0	0.76 ± 0.04	1.30 ± 0.22
(%)	*(0)*	*(7.48 ± 0.29)*	*(7.29 ± 2.35)*
Molting (g-P per tank)	0	0.01 ± 0.00	0.01 ± 0.00
(%)	*(0.02 ± 0.01)*	*(0.07 ± 0.01)*	*(0.11 ± 0.01)*
Unidentified (g-P per tank)	3.57 ± 0.32	1.74 ± 0.13	0.79 ± 0.34
(%)	*(36.62 ± 2.22)*	*(17.23 ± 1.01)*	*(7.86 ± 3.27)*

Values are mean ± S.D. (n=2)

APPENDIX B

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Water quality control using *Spirulina platensis* in shrimp culture tanks

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Abstract

A cyanobacterium (*Spirulina platensis*) was co-cultured with black tiger shrimp (*Penaeus monodon*) for water quality control. We evaluated the effects of: (1) three *S. platensis* trial conditions on inorganic nitrogen concentrations at one shrimp density (*S. platensis* trial conditions included: absent, nonharvested and semicontinuous harvesting) and (2) two shrimp densities on inorganic nitrogen concentrations, with and without *S. platensis*. Semicontinuous harvesting of *S. platensis* at one shrimp density resulted in significantly reduced ($P<0.05$) inorganic nitrogen concentrations (NH_4, NO_2 and NO_3). With *S. platensis* absent, ammonium and nitrite concentrations ranged from 0.5 to 0.6 mg l^{-1}, while nitrate concentrations ranged from 16 to 18 mg l^{-1} by day 44. With nonharvested *S. platensis*, considerable variability occurred with nitrogen concentrations. Semicontinuous harvest of *S. platensis* reduced nitrate to 4 mg l^{-1}, while ammonium and nitrite ranged from 0.0 to 0.15 mg l^{-1}, respectively. The factorial evaluation of shrimp density versus presence and absence of *S. platensis* resulted in greatly reduced nitrogenous compounds with *S. platensis* present regardless of shrimp density, and only moderately increased nitrogen with greater shrimp density. Without *S. platensis*, all nitrogen compounds were substantially elevated and shrimp survived was significantly reduced at high shrimp density.
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Keywords: Aquaculture; Water quality; Integrated culture; Microalgae; *Spirulina platensis*; Shrimp culture; Prawn culture; *Penaeus monodon*

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1. Introduction

Intensive culture of black tiger shrimp (*Penaeus monodon*) is widely practiced in Thailand. Traditional intense shrimp culture uses open system with high water exchange. More recently, there has been more interest in environmental friendly, closed or recirculating seawater systems with zero or nearly zero water discharges.

Intensive shrimp culture in Thailand uses 30–57% protein feed with high feeding rates. Organic nitrogen waste from uneaten feed and shrimp excretions decomposes into toxic inorganic nitrogen compounds, including ammonia (NH_3 or NH_4) and nitrite (NO_2). With aerobic conditions, ammonia and nitrite are converted into relatively nontoxic nitrate (NO_3), but high nitrate concentrations can stress shrimp. Water exchange is therefore still recommended, especially when nitrate is ≥ 50 mg N l^{-1} (Hart and O'Sullivan, 1993).

Aquaculture water treatment systems use bacteria to convert ammonium and nitrite into nitrate under aerobic condition, while nitrate removal can be accomplished using sophisticated denitrification systems under anaerobic condition (van Rijn, 1996; Abeysinghe et al., 1996). Menasveta et al. (2001) described a closed, recirculating seawater system with denitrification for shrimp broodstock culture. However, denitrification systems are complicated and impractical for large-scale shrimp culture. Presently, these systems require oxygen reduction to almost zero before denitrification begins and automated controls during denitrification.

Nitrate is not easily removed by conventional water treatment. Biological nitrate removal using aerobic microalgae offers some advantage over anaerobic, microbial denitrification since both ammonia and nitrate nitrogen are readily removed and the process is less complicated (Vilchez et al., 1997). Microalgae play a dominant role in stabilizing earthen pond water quality. However, the main disadvantage of using microalgae is that algal cells are not easily removed from the culture system. If algal cells are not removed, nitrogen compounds are released back to the water. Moreover, high microalgae concentrations can cause dissolved oxygen depletions during the night due to high respiration rates. Microalgal immobilization has been used for wastewater treatment in whatever entrapment (Vilchez and Vega, 1994; Kaya et al., 1995) or attachment conditions (Garbisu et al., 1991; Gil and Serra, 1993), but these techniques are expensive and not practical on large scales with aquaculture ponds. Moreover, immobilization using carrageenan or alginate can be easily dissolved in seawater which contains salts and other ions such as phosphate and EDTA (Brodelius and Vandamme, 1987).

Ideally, microalga used with integrated aquaculture systems should have all of the following characteristics: (1) algal cells must be harvested by simple filtration; (2) easy to mass culture; (3) tolerates wide salinity range; and (4) algae are a valuable by product. With our present work, we choose the cyanobacterium *Spirulina platensis* for nitrogen removal in shrimp culture tanks since it meets all the above requirements. We previously found that *S. platensis* grew well in seawater (5–30‰ salinity) and was easily removed by filtration through 60-μm mesh net. This allowed semicontinuous harvest of *S. platensis*. Although *Spirulina* is one of the most widely studied microalgae, especially for wastewater treatment, most studies are with algal monoculture in high-rate algal pond system (Tanticharoen et al., 1993; Phang et al., 2000), not with integrated systems containing both algae and an animal crop. Our present research evaluated the efficacy of integrated algae–

shrimp culture where *S. platensis* was used for water quality control with semicontinuous algae harvest.

2. Materials and methods

2.1. S. platensis culture

Stock culture of *S. platensis* was obtained from the Institute of Food Research and Product Development, Kasetsart University, Thailand and maintained in 30 ppt (~ 0.5 M NaCl) Zarrouk medium (Zarrouk, 1966) under 200 μmol photon $m^{-2} s^{-1}$ illumination at 25–28 °C. Working algal culture was prepared by transferring stock algal culture into 2-l Erlenmeyer flasks with air injection. When cultures reached mid-logarithmic growth phase, they were concentrated by filtering through 60-μm nylon net and washed with fresh Zarrouk medium without nitrate, then added to shrimp culture tanks.

2.2. Shrimp culture condition

Black tiger shrimp were collected from earthen ponds in Pathum Thani Province and transferred to a concrete holding tank at Marine Biotechnology Research Unit, Chulalongkorn University, Bangkok for 30 days before starting the trials. All trials were performed in $0.3 \times 0.6 \times 0.3$ m^3 glass aquaria containing 30-l of seawater (30 ppt) under semitransparent roof. Aeration was provided by air stones and freshwater (0 ppt) was added daily to compensate for evaporation. Feces, uneaten feed and dead shrimp were removed daily by siphon, and water was returned to the same tank. Shrimps in each tank were fed three times daily with commercial shrimp feed (35% crude protein) at about 4% of body weight. Shrimp survival and growth were measured every 15 days. Weight was measured to 0.01 g. All treatments had three replicates, and trials were completely randomized design.

2.3. Semicontinuous harvesting of S. platensis from shrimp tanks

S. platensis densities were measured using chlorophyll-*a* (Chl-*a*) concentration and trichomes counts. Trichomes were counted using a haemacytometer with light microscope, while at the same time observing for other algal contamination. Chl-*a* analysis was measured by centrifuging (5000 rpm for 5 min) water samples. Thereafter, algal pellets were extracted in methanol and light absorption measured at 665 nm (Bennet and Bogorad, 1972). With semicontinuous harvesting treatments, Chl-*a* in all aquaria was maintained at 0.02–0.04 mg Chl-*a* l^{-1} every 2 days by filtering appropriate water volumes from the aquaria through 60-μm net, then returning water to the same aquaria.

2.4. Water quality analysis

Inorganic nitrogen compounds (NH_4–N, NO_2–N and NO_3–N) in water were measured every 2 days according to Parsons et al. (1984). pH, temperature and salinity

were also monitored. Freshwater (0 ppt) was added to compensate for evaporative water losses.

2.5. Trial I: effect of semicontinuous harvesting of Spirulina on inorganic nitrogen in shrimp culture tanks

Shrimp were cultured at 10 per aquarium (55 shrimp m^{-2}) with three treatments and three replicates per treatment: shrimp cultured without *S. platensis* (control), shrimp cultured with unharvested *S. platensis* (treatment 1) and shrimp cultured with semicontinuous harvest of *S. platensis* (treatment 2). Average initial shrimp weight was 4.6 g. This trial lasted 45 days.

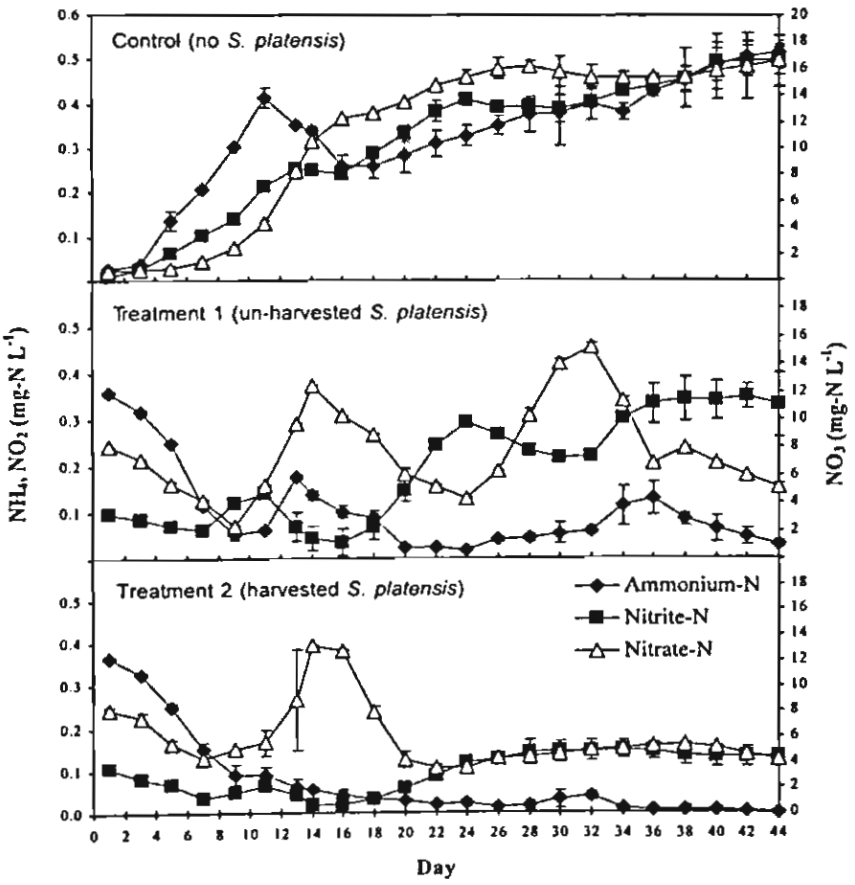


Fig. 1. Ammonium (NH_4–N), nitrite (NO_2–N) and nitrate (NO_3–N) concentrations in control tanks (shrimp without *S. platensis*), treatment 1 (shrimp without *S. platensis* harvest) and treatment 2 (shrimp with semicontinuous harvest of *S. platensis*) during trial I. Initial shrimp densities were 55 prawn m^{-2}. Error bars indicate standard deviation with $n=3$.

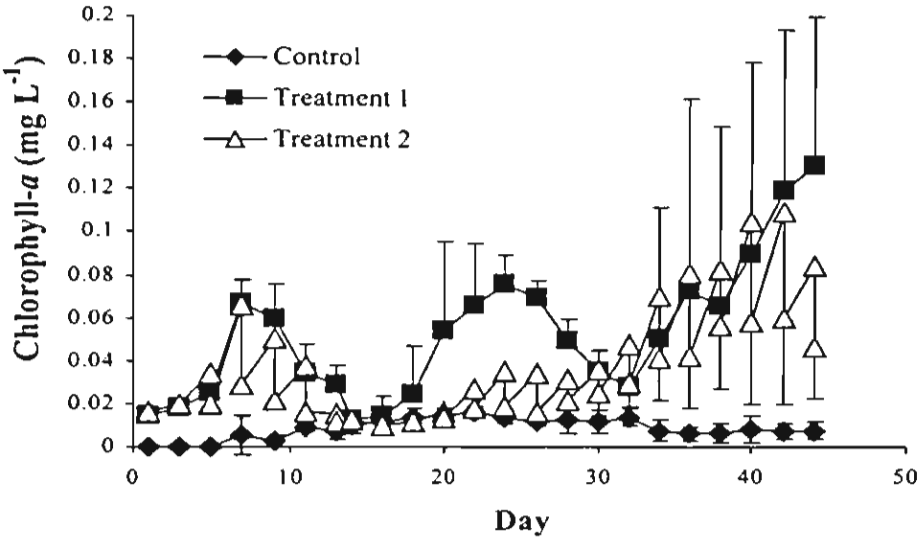


Fig. 2. Chlorophyll-*a* concentrations in shrimp culture tanks: control (shrimp without *S. platensis*), treatment 1 (shrimp with *S. platensis*, no harvesting) and treatment 2 (shrimp with semicontinuous harvest of *S. platensis*) during trial I. Error bars indicate standard deviation with $n = 3$. For treatment 2, error bars were from chlorophyll-*a* data before harvesting.

2.6. Trial II: effect of shrimp densities and semicontinuous harvest of S. platensis on inorganic nitrogen in culture tanks

Shrimp were cultured at two densities of 8 and 15 shrimp per tank (44 and 83 shrimp m^{-2}), designated low and high density, respectively. Average initial shrimp weight was

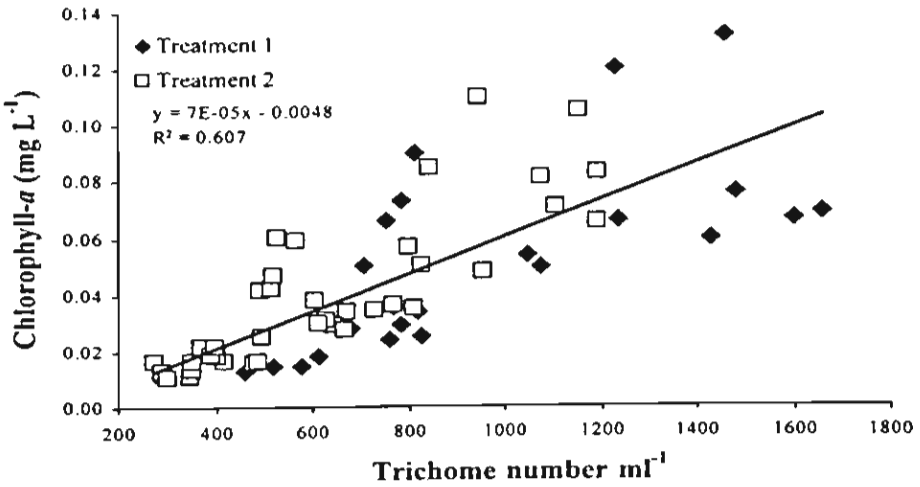


Fig. 3. Relationship between *Spirulina* trichome numbers and chlorophyll-*a* concentrations in shrimp culture tanks during trial I. Values are shown for treatment 1 (shrimp with *S. platensis*, no harvesting) and treatment 2 (shrimp with semicontinuous harvest of *S. platensis*). Regression line is for both treatments.

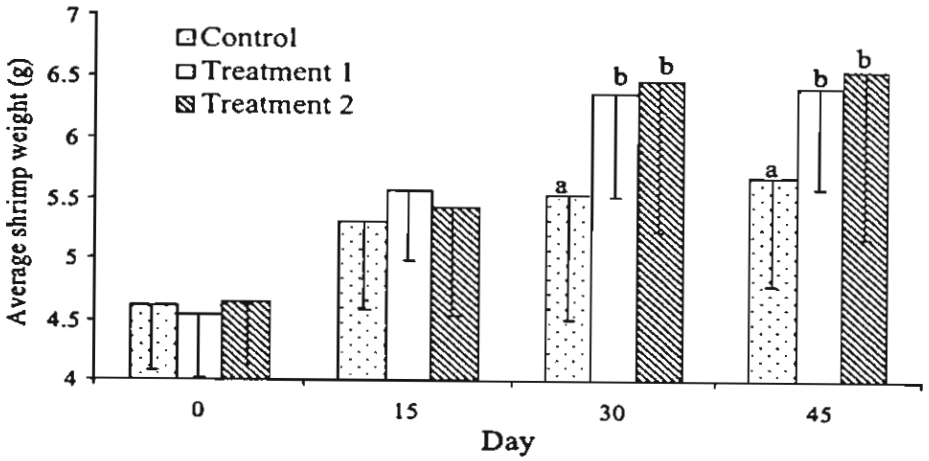


Fig. 4. Average shrimp weights in trial 1 of control (shrimp without *S. platensis*), treatment 1 (shrimp with unharvested *S. platensis*) and treatment 2 (shrimp with semicontinuous harvest *S. platensis*). Error bars represent standard deviation ($n = 30$), while a or b indicate significant differences ($P \leq 0.05$).

3.3 g. There were four treatments with three replicates per treatment: low shrimp density without *S. platensis* (control), low shrimp density with semicontinuous *S. platensis* harvest (treatment 1), high shrimp density without *S. platensis* (control 2) and high shrimp density with semicontinuous *S. platensis* harvest (treatment 2). This trial lasted 60 days.

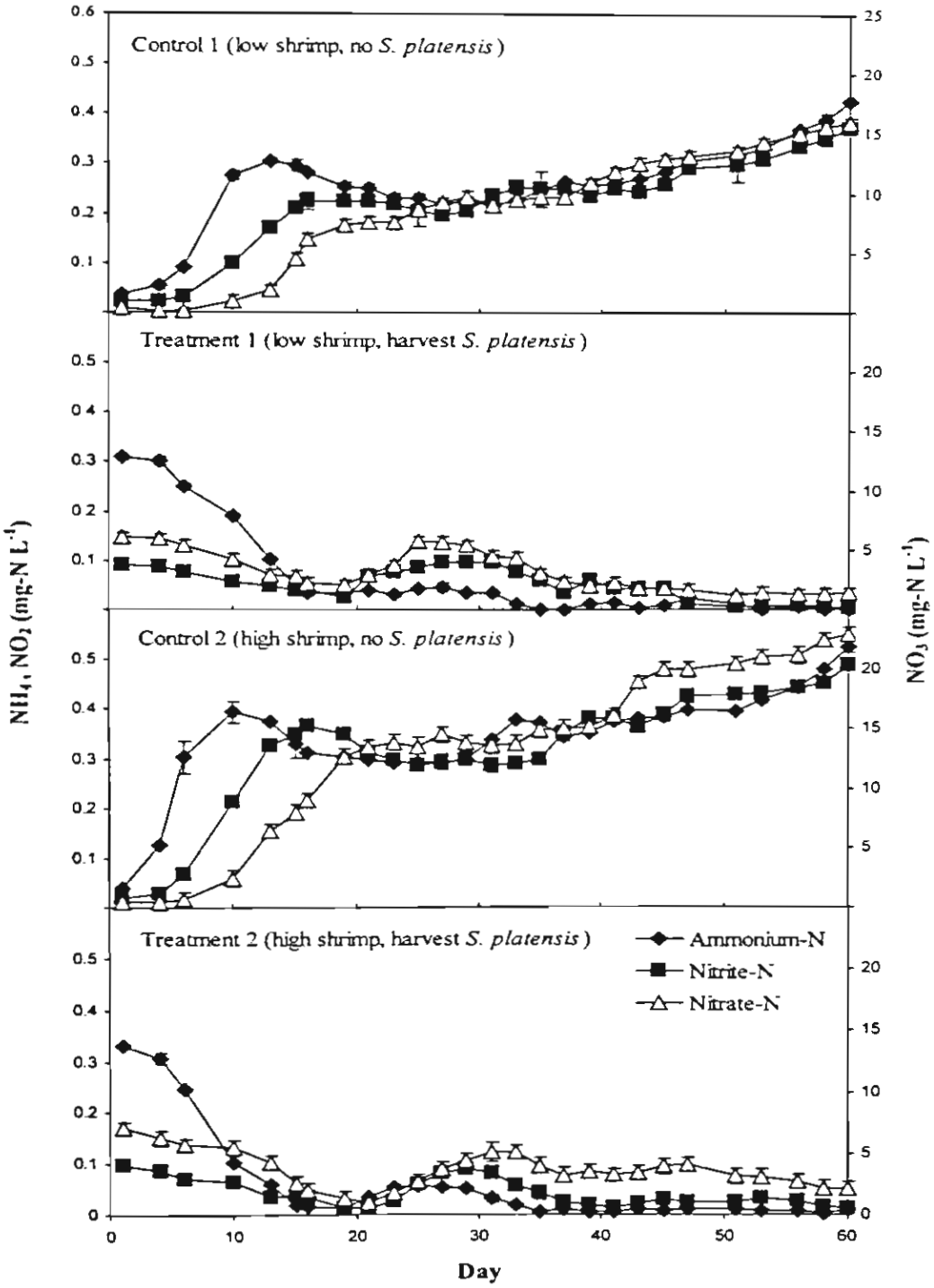
3. Results

3.1. Trial I

When concentrated *S. platensis* cells were first added to shrimp culture tanks (treatments 1 and 2), ammonium, nitrite and nitrate concentrations were greater in the treatment groups than in the control (Fig. 1). This was due to nitrogen contamination from uncompleted washed cells cultured using high nitrate Zarrouk medium. During days 1 to 7, nitrogen concentrations decreased in both treatments regulated by *S. platensis*, while nitrogen increased in the control. All nitrogen compounds in the controls increased throughout the trial with nitrate of >16 mg N l^{-1} and ammonia and nitrite of nearly 0.5 mg N l^{-1}.

Chlorophyll-*a* concentration were always less than 0.02 mg l^{-1} in trial I controls, while treatments 1 and 2, Chl-*a* averages 0.01–0.13 mg l^{-1} (Fig. 2). Microscopic

Fig. 5. Ammonium, nitrite and nitrate concentrations during trial II for control 1 (low-density shrimp without *S. platensis*), treatment 1 (low-density shrimp with semicontinuous harvesting of *S. platensis*), control 2 (high-density shrimp without *S. platensis*) and treatment 2 (high-density shrimp with semicontinuous harvesting of *S. platensis*). Shrimp density was 44 or 83 m^{-2} for low and high density, respectively. Error bars indicate standard deviation with $n = 3$.


Control 1 (low shrimp, no S. platensis)
Treatment 1 (low shrimp, harvest S. platensis)
Control 2 (high shrimp, no S. platensis)
Treatment 2 (high shrimp, harvest S. platensis)
Ammonium-N
Nitrite-N
Nitrate-N
NH4, NO2 (mg-N L-1)
NO3 (mg-N L-1)
Day

observation revealed low number of benthic and planktonic diatoms in both controls and treatments, but there was never an abundance in any tank. Cloudy weather during days 11–20 resulted in reduced *S. platensis* growth during this time in treatments 1 and 2. *S. platensis* growth increased after day 20. Trichome number and Chl-*a* were positively correlated, with $R^2 = 0.607$ ($P < 0.001$) in treatments 1 and 2. There was more variability with greater trichome number (Fig. 3). This relationship further indicates that *S. platensis* was the dominant alga in those treatments. Value for pH ranged from 7.5 to 8.5, while temperature was from 28 to 31 °C. Salinities averaged 30 ppt (29–31 ppt range), while alkalinity ranged from 120 to 150 mg l^{-1}. There were no significant differences ($P > 0.05$) between those water quality parameters for the treatments and controls.

Nitrogen compounds fluctuated the most in trial I, treatment 1 (Fig. 1). Nitrate decreased from 12 to 2 mg N l^{-1} during days 1–10, then increased to >12 by day 13 during reduced algal growth caused by cloudy weather. Nitrate increased again on day 31 with >15 mg N l^{-1} before decreasing to <6 by day 44. Nitrite concentrations ranged from <0.1 to >3 mg N l^{-1} and cycled out of synchrony with nitrate. Ammonia concentration was mostly <0.1 mg N l^{-1}.

With semicontinuous *S. platensis* harvest (trial I, treatment 2), nitrogen compounds were consistently low during most of the culture period (Fig. 1). Only nitrate increased between days 10 and 16 due to cloudy weather reduced algal growth. During days 34–44, ammonia was nearly undetectable.

Average weights of shrimp on days 30 and 45 of the two treatment groups were not significantly different, but both treatments had significantly greater weights than the

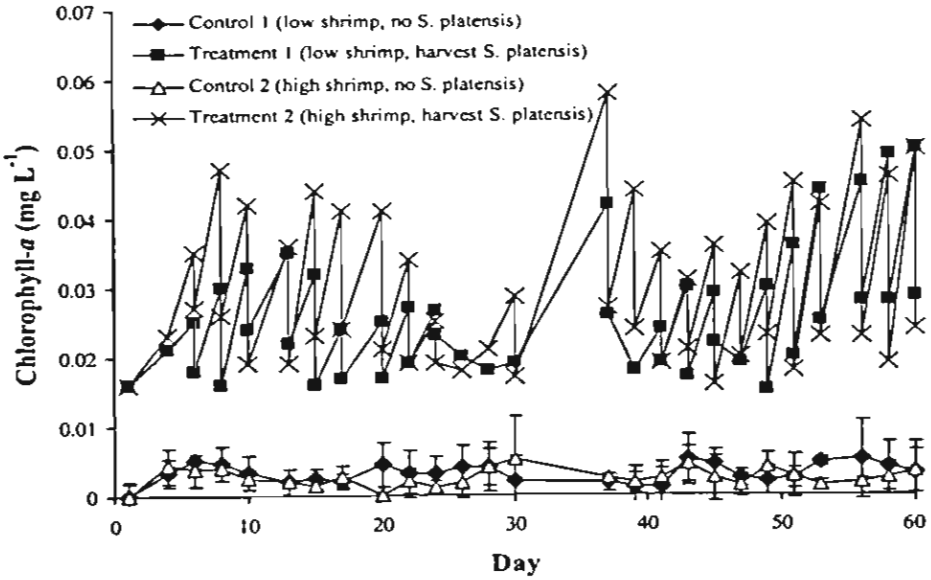


Fig. 6. Chlorophyll-*a* concentrations in trial II control 1, treatment 1, control 2 and treatment 2. Shrimp densities were 44 or 83 m^{-2} for low and high density, respectively. Error bars of control groups indicate standard deviation with $n = 3$. For treatments 1 and 2, only average data of three replicates were shown.

control group ($P<0.05$; Fig. 4). Shrimp survivals averaged 53.3 ± 11.5%, 43.3 ± 5.8% and 60 ± 10% on day 45 for control, treatment 1 and treatment 2, respectively. Average survival was not significantly different ($P>0.05$).

3.2. Trial II

Water quality in trial II can be grouped as shrimp cultured with *S. platensis* (treatments 1 and 2) and shrimp cultured without *S. platensis* (control 1 and 2). In treatments 1 and 2, semicontinuous harvesting of *S. platensis* provided excellent water quality control with both low and high shrimp densities (Fig. 5). On the other hand, ammonium, nitrite and nitrate were elevated in both control groups without *S. platensis*. Chl-*a* concentrations in both control groups were similar and much lower than treatment groups (Fig. 6). In control 2, ammonium and nitrite reached 0.5 mg N l^{-1} while nitrate increased to >20 mg N l^{-1} by day 60. Continuous exposure to these nitrogen concentrations could reduce survival.

Semicontinuous harvest of *S. platensis* maintained Chl-*a* between 0.02 and 0.05 mg l^{-1} and maintained cells in exponential growth phase throughout trial II. Decrease in inorganic nitrogen during the first 10 days of trial II was similar to the decrease in trial I. During trial II, shrimp weights were not significantly difference ($P>0.05$) between groups. However, higher density shrimp culture without *S. platensis* (control 2) had significantly ($P<0.05$) lower survival compared with other treatments (Fig. 7).

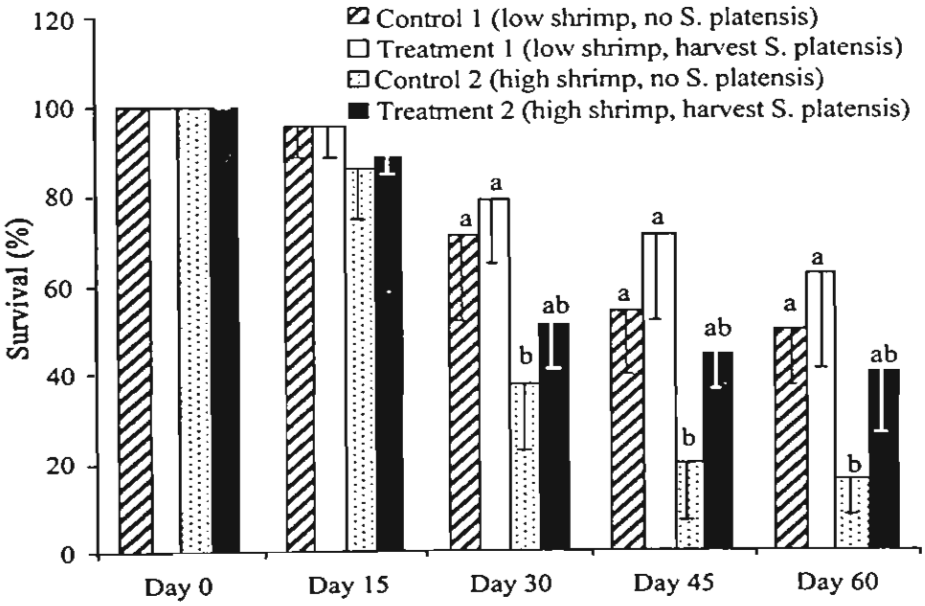


Fig. 7. Shrimp survival with and without co-culture with *S. platensis*. Shrimp densities were 44 or 83 m^{-2} with low and high densities, respectively. Error bars represent standard deviation ($n=3$), while a or b indicates statistically significant differences ($P \leq 0.05$).

4. Discussion

With intensive shrimp culture systems, low phytoplankton concentrations are an unlikely event since there is ample of nutrients from feed addition and shrimp excretion to sustain dense algal growths. In Thailand, most shrimp farmers believed that desirable "brownish-green water color" from algae will increase shrimp growth and survival. This color is usually due to diatoms and green algae. Many farmers also add fertilizer to the ponds. That sometimes causes red tides, or blooms of uncontrolled algal species such as diatom, dinoflagellates or blue-green algae in the ponds. Attempt to add bulk cultured microalgae to earthen shrimp culture ponds has been attempted during the past 10 years by both farmers and researchers in Thailand (personal communications). However, phytoplankton, which normally have a life spans of 1–2 weeks (Boyd and Musig, 1992), soon dies and decomposes releasing nitrogen into the water.

Our concept of integrating *S. platensis* with shrimp differs from other attempts of algal management since we controlled algal cells at a desired concentration. Our study was designed to maintain low cell concentration of 0.02 mg Chl-*a* l^{-1}. At this concentration, water color in our tanks was not the dark-green typical of *S. platensis* production ponds. Semicontinuous harvesting also maintained algae in exponential growth, never in stationary and death phases. This maintained healthy *S. platensis* with rapid nutrient uptake.

Typically, 70–80% of nitrogen from applied feed remains in an aquaculture pond (Boyd, 1985; Funge-Smith and Briggs, 1998). Most nitrogen loss from the pond is through denitrification from anoxic bottom sediment. However, anoxic condition or even low dissolved oxygen at the bottom should be avoided since it can negatively effect shrimp survival (Martin et al., 1998). Our study indicates a possibility for integrated culture of shrimp and *S. platensis*. Our findings clearly show that *S. platensis* reduced inorganic nitrogen and resulted in excellent water quality for shrimp, provided that some algal biomass was continuously removed. Since our trials were in glass aquaria, nitrogen cycling was more easily traced. Adaptation of this process to large earthen ponds may require a specially designed algal harvesting system. Photoinhibition, which is the inhibition of algal photosynthesis by strong sunlight, must be considered and this can be avoided by adding shade or roof. Normally, dense *S. platensis* culture could loss productivity up to 30% by photoinhibition (Vonshak et al., 1988). This should be more affectable to algal cells in our system because it is maintained at the lower density than conventional *S. platensis* culture ponds.

We found that algal contamination (mostly diatoms) in our tanks was not a problem, although there were some contaminants and ample nutrients in the tank. Low silicate concentrations could account for this.

Our integrated system of shrimp and semi-continuous harvest *S. platensis* demonstrated an excellent water quality control even at high shrimp density of 83 shrimp m^{-2}. Conventional water quality control in intensive shrimp culture systems is based on bacterial and is therefore size-limited biofiltering. We believe that our system can be more easily expanded into large tanks or ponds for various aquatic animal culture. Water quality in our shrimp tanks contain very low nitrate concentrations, and water exchange between crops was not necessary. However, more work is needed with these large culture systems. Specifically, we need to develop effective algal harvest techniques for large tanks

and ponds, and we need to determine whether *S. platensis* (or another desirable alga) can be maintained as the dominant alga throughout the culture cycle.

Acknowledgements

This work was supported by the Thailand Research Fund (TRF) through the TRF Kanchanapisek PhD scholarship for B. Chuntapa. We also acknowledge valuable help provided by Dr. Arlo W. Fast who reviewed the manuscript and made many helpful suggestions.

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บทคัดย่อในงานประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 26 (วทท. 26)

นำเสนอในรูปแบบบรรยาย

เรื่อง

การควบคุมคุณภาพน้ำในบ่อเลี้ยงกุ้งกุลาดำโดยการเลี้ยงร่วมกับสาหร่ายสไปรูลินา (CONTROL OF WATER QUALITY IN SHRIMP POND BY INTEGRATING CULTURE OF SHRIMP WITH THE MICROALGA, *Spirulina platensis*)

โดย

เบ็ญจมาศ จันทะภา
สรวิศ เผ่าทองศุข
เปี่ยมศักดิ์ เมนะเศวต

ณ ศูนย์ประชุมแห่งชาติสิริกิติ์ กรุงเทพมหานคร ระหว่างวันที่ 19 – 20 ตุลาคม 2543

การควบคุมคุณภาพน้ำในบ่อเลี้ยงกุ้งกุลาดำโดยการเลี้ยงร่วมกับสาหร่ายสไปรูลินา

CONTROL OF WATER QUALITY IN SHRIMP POND BY INTEGRATING CULTURE of SHRIMP WITH THE MICROALGA, *Spirulina platensis*

เบญจมาศ จันทะภา [a*+], สรวิศ เผ่าทองศุข[b] และ เปี่ยมศักดิ์ เมนะเศวต[ab]

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บทคัดย่อ การเลี้ยงกุ้งกุลาดำวัยรุ่นร่วมกับสาหร่ายสไปรูลินาเพื่อควบคุมคุณภาพน้ำเป็นเวลา 45 วัน โดยมีการเก็บเกี่ยวสาหร่ายแบบกึ่งต่อเนื่องซึ่งการเก็บเกี่ยวจะควบคุมสาหร่ายให้มีปริมาณคลอโรฟิลล์ประมาณ 0.02 mg Chl-*a*/ml. ผลการทดลองพบว่าคุณภาพน้ำในกลุ่มทดลอง (เลี้ยงกุ้งร่วมกับสาหร่าย) มีปริมาณความเข้มข้นของสารประกอบไนโตรเจน (NH_3-N, NO_2-N and NO_3-N) ต่ำกว่ากลุ่มควบคุม (ไม่มีสาหร่าย). การเจริญเติบโตของกุ้งโดยน้ำหนักเมื่อสิ้นสุดการทดลองพบว่า ในกลุ่มทดลองมีน้ำหนักเฉลี่ย 6.57 ± 1.15 กรัม ซึ่งสูงกว่าน้ำหนักเฉลี่ยในกลุ่มควบคุม (5.7 ± 0.91 กรัม) อย่างมีนัยสำคัญทางสถิติ ($P< 0.05$)นอกจากนี้ ผลการทดสอบการต้านทานต่อเชื้อ *Vibrio harveyi* แสดงให้เห็นว่า การเลี้ยงกุ้งร่วมกับสาหร่ายมีค่า LT_{50} เท่ากับ 97 ชั่วโมง ซึ่งสูงกว่ากลุ่มควบคุมที่มีค่าเท่ากับ 33 ชั่วโมง

Abstract: Integrated culture of juvenile shrimp and microalga *Spirulina platensis* (0.02 mg Chl-*a*/ml semi-continuous harvesting) for 45 days illustrated that water quality of the treatment group (with algae) had significantly lower nitrogen (NH_3-N, NO_2-N and NO_3-N) concentrations than control group (without algae). Growth rate of shrimp indicated by body weight was significantly higher in treatment group (6.57 ± 1.15 g) than control (5.7 ± 0.91 g) at day 45. In addition, challenge test using pathogenic bacteria *Vibrio harveyi* showed that integrated culture shrimp had higher LT_{50} (97 h.) than control (33 h.).

Experiment Procedure: Stock culture of *Spirulina platensis* was prepared using Zarrouk's medium at 30 ppt salinity. Concentrated algal cells from stock culture were then added into 0.3 x 0.6 x 0.3 m^3 glass aquariums located outdoor under semi-transparent roof. Each aquaria contained 30 L seawater and 10 *Penaeus monodon* shrimp (53 shrimp/m^2 surface area). Shrimp were fed three-times a day at 4% of body weight for both treatment (with *Spirulina*) and control (no *Spirulina* added). Concentraton of *Spirulina* cells in the treatment tanks were regulated by semi-continuous harvesting in which Chlorophyll-a (chl-*a*) concentration in the water was maintained at approximately 0.02 mg/ml. Water quality was monitored every two days. Shrimp survival and weight were recorded every 15-day. Effect of pathogenic bacterium, *Vibrio harveyi*, on shrimp survival (challenge test) was evaluated by LT_{50} at 10^6 CFU of *V. harveyi*/ml. All experimental conditions were done in three replicates.

Results, Discussion and Conclusion: In control group, concentrations of NH_3-N, NO_2-N and NO_3-N which derived from shrimp's excretion and un-eaten's feed were found accumulated in the water (Figure.1). On the other hand, integrating shrimp culture with *Spirulina* illustrated that NH_3-N, NO_2-N and NO_3-N concentrations were regulated by the algal uptake (Figure.2) and semi-continuous harvesting of algae could provided the optimum algal cells in the water. In the treatment group, average growth shrimp was significantly higher ($P<0.05$) than control group. (Figure.3), however, survival rate was not different. Preliminary study of challenge test using pathogenic bacterium *Vibrio harveyi* showed that shrimp cultured with *Spirulina* had higher 50% lethal time (LT_{50} = 97 h) than control (LT_{50} = 97 h).

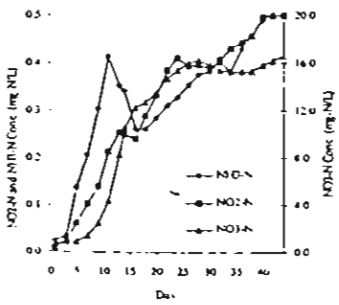

Figure 1. Average NH_3-N, NO_2-N and NO_3-N concentration (mg-N/L) in control group

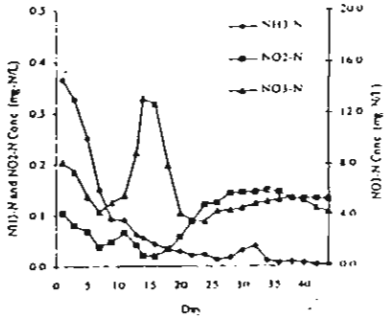

Figure 2. Average NH_3-N, NO_2-N and NO_3-N concentration (mg-N/L) in treatment group (shrimp with *Spirulina*)

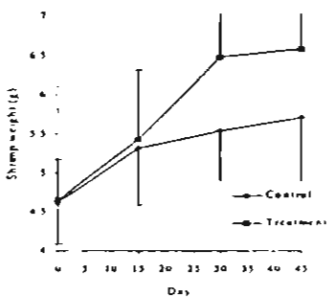

Figure 3. The average weight (g) ± SD of shrimp during 45 days of the experiment.

Keywords: water quality, semi-continuous harvesting, spirulina, intensive culture, integrating, shrimp

บทคัดย่อในงานประชุมวิชาการวิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 27 (วทท. 27)

นำเสนอในรูปแบบบรรยาย

เรื่อง

การควบคุมคุณภาพน้ำในถังเลี้ยงกุ้งกุลาดำความหนาแน่นสูงโดยการเลี้ยงร่วมกับสาหร่ายสไปรูลินา (CONTROL OF WATER QUALITY IN HIGH DENSITY PRAWN CULTURE BY INTEGRATING WITH THE MICROALGA, *Spirulina platensis*)

โดย

เบ็ญจมาศ จันทะภา
สรวิศ เผ่าทองศุข
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ณ โรงแรม ลี การ์เดนส์ พลาซ่า จังหวัดสงขลา ระหว่างวันที่ 16 – 18 ตุลาคม 2544

การควบคุมคุณภาพน้ำในฉังเลี้ยงกุ้งกุลาดำความหนาแน่นสูงโดยการเลี้ยงร่วมกับสาหร่ายสไปรูลินา

CONTROL OF WATER QUALITY IN HIGH DENSITY PRAWN CULTURE BY INTEGRATING WITH THE MICROALGA, *Spirulina platensis*

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บทคัดย่อ: ศึกษาการเลี้ยงกุ้งกุลาดำวัยรุ่นร่วมกับสาหร่ายสไปรูลินา (Division Cyanophyta) โดยมีการเก็บเกี่ยวสาหร่ายแบบกึ่งต่อเนื่อง ผลการทดลองพบว่าทำให้สามารถควบคุมคุณภาพน้ำได้อย่างน่าพอใจด้วยการเลี้ยงแบบผสมผสานในถังเดียวกัน เมื่อวิเคราะห์ผลการทดลองพบว่าปริมาณแอมโมเนียม ไนไตร์ท และไนเตรท ในบ่อที่เลี้ยงกุ้งร่วมกับสาหร่ายสไปรูลินาทั้งสองชุดทดลอง คือที่ปล่อยกุ้งที่ระดับความหนาแน่นสูงและต่ำ (83 และ 44 ตัวต่อตารางเมตร, ตามลำดับ) จะมีค่าต่ำกว่าอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับการเลี้ยงกุ้งที่ไม่เติมสาหร่ายสไปรูลินา เมื่อสิ้นสุดการทดลองพบว่าในทุกชุดทดลองให้อัตราการเติบโตที่ไม่แตกต่างกันทางสถิติ ในขณะที่อัตราการรอดพบว่า การเลี้ยงกุ้งร่วมกับสาหร่ายสไปรูลินามีค่าสูงกว่าการเลี้ยงกุ้งที่ไม่เติมสาหร่ายสไปรูลินาอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

Abstract: Cultivation of juvenile prawn (*Penaeus monodon*) with semi-continuous harvesting of microalga *Spirulina platensis* (Cyanophyta) illustrated an excellent possibility of water quality control by integrating culture system. The results indicated that ammonium, nitrite and nitrate concentration in prawn aquariums with *Spirulina* in both high or low prawn density (83 or 44 prawns/m^2 respectively) were significant lower than those culture without *Spirulina*. At the end of the experiment, no difference in growth rate of prawn was found in all treatments but prawn culture with *Spirulina* had significantly higher survival rate.

Methodology: Completely Randomize Design was used in this experiment consisted of 4 treatments *i.e.*, (1) high prawn density (83 prawn/m^2) with *Spirulina* (2) high prawn density without *Spirulina* (3) low prawn density (44 prawn/m^2) with *Spirulina* and (4) low prawn density without *Spirulina*. All treatments were in three replicates. Stock culture of *Spirulina platensis* was prepared using Zarrouk's medium at 30 ppt salinity. Concentrated algal cells from stock culture were then added into 0.3 x 0.6 x 0.3 m^3 glass aquariums located outdoor under semi-transparent roof. Each aquarium contained 30 L seawater with 15 or 8 prawns/aquarium for high and low culture densities respectively. Prawns were fed three-times a day at 4% of body weight for all treatment. Concentration of *Spirulina* cells in treatment 1 and 3 were regulated by semi-continuous harvesting in which Chlorophyll-a concentration in the water were maintained at approximately 0.02 mg/ml. Water quality was monitored every two days. Prawn survival and weight were recorded every 15-day of 60 days experiment.

Results, Discussion and Conclusion: Accumulation of inorganic nitrogen compounds derived from prawn excretion and uneaten feed decomposition in the water occur in prawn culture without *Spirulina* (treatment 2 and 4). At the end of the experiment (day 60), nitrate concentration were accumulated to 15 and 20 mg-N/L in low and high prawn density respectively. On the other hand, semi-continuous harvesting of *Spirulina* (treatment 1 and 3) significantly reduced ammonium, nitrite and nitrate concentrations in water because nitrogen waste which was taken up by algal cells were harvested out. Thus, there was no accumulation of nitrate in those treatments and the final nitrate concentration was less than 2.5 mg-N/L. Although we did not found a difference in growth rate of all treatments, survival rate in high - density prawn culture with *Spirulina* group was significantly lower ($p<0.05$) than in high-density prawn culture without *Spirulina*. This study suggested that we could increase prawn density up to 15 prawns/aquarium (83 prawns/m^2) without any effect on water quality by integrating prawn culture with semi-continuous harvesting *Spirulina*. As *Spirulina* has a large trichome and can grow in wide range of salinity from 5 - 40 ppt, it could be easily harvested by simple filtration through 70 microns mesh and can be applied to prawn culture at any salinity from nearly freshwater to seawater.

Keywords: Water Quality, High Prawn Density, Spirulina, Semi-Continuous Harvesting, Integrating Culture, Prawn

Abstract of The Fourth Asia – Pacific Marine Biotechnology Conference (APMBC'02)

Poster Presentation

Title

IMPROVEMENT OF WATER QUALITY IN SHRIMP POND BY INTEGRATING CULTURE OF SHRIMP WITH THE MICROALGA, *Spirulina platensis*

By

Benjamas Chuntapa
Sorawit Powtongsook
Piamsak Menasveta

at Hawaii Imim International Conference Center, University of Hawaii at Manoa, Honolulu, Hawaii, USA. April 22-26, 2002

Asia Pacific Marine Biotechnology Conference

Improvement of water quality in shrimp pond by integrating culture of shrimp with the microalga, *Spirulina platensis*

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The objective of this study was to evaluate possibility of water quality control in shrimp pond by integrating culture of shrimp with semi-continuous harvesting culture of the microalga, *Spirulina platensis*. In this experiment, growth rate and water quality of shrimp culture without *Spirulina* (control group) were compared with two treatment groups comprising shrimp culture with *Spirulina* (treatment 1) and shrimp culture with semi-continuous harvesting of *Spirulina* (treatment 2). Stock culture of *Spirulina platensis* was prepared using Zarrouk's medium at 30 ppt salinity. Concentrated algal cells from stock culture were added into 0.3x0.6x0.3 m^3 glass aquariums located outdoor under semi-transparent roof. Each aquarium contained 30 L seawater and 8 *Penaeus monodon* shrimp (40 shrimp m^{-2} surface area). Shrimp were fed three-times a day at 4% of body weight for all treatments. Concentration of *Spirulina* cells in the treatment 2 tank was regulated by semi-continuous harvesting in which chlorophyll-a (chl-*a*) concentration in the water was maintained at approximately 0.02 mg/ml. Water quality was monitored every two days. Shrimp survival and weight were recorded every 15-day. All experimental conditions were done in three replicates. The result indicated that concentration of NH_4^+-N, NO_2^--N and NO_3^--N of the control group which derived from shrimp excretion and uneaten feed were found accumulate in the water (Figure. 1). In treatment 1 water quality was found fluctuate as the dead algal cells released inorganic nitrogen into the system (Figure. 2). On the other hand, integrating shrimp culture with semi-continuous harvesting *Spirulina* (treatment 2) illustrated that NH_4^+-N, NO_2^--N and NO_3^--N concentrations were regulated by the algal uptake (Figure. 3) and semi-continuous harvesting of algae could provided the optimum algal cell in the water throughout the experimental period. Growth rate of shrimp in treatment 1 and 2 was significantly higher ($P<0.05$) than the control group (Figure. 4) while survival rate was not different.

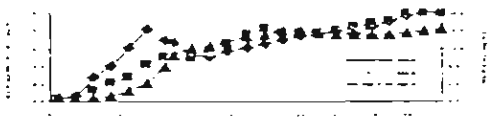

Figure. 1 Average NH_4^+-N, NO_2^--N and NO_3^--N concentration (mg-N/L) in control group (shrimp without *Spirulina*)

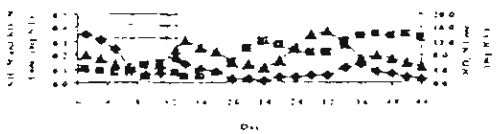

Figure. 2 Average NH_4^+-N, NO_2^--N and NO_3^--N concentration (mg-N/L) in treatment 1 (shrimp with *Spirulina*)

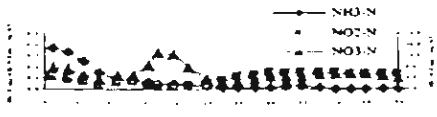

Figure. 3 Average NH_4^+-N, NO_2^--N and NO_3^--N concentration (mg-N/L) in treatment 2 (shrimp with semi-continuous harvesting *Spirulina*)

Figure. 4 The average weight (g) of shrimp during 45 days of the experiment

ภาคผนวก C

สูตรอาหาร Zarrouk medium สำหรับเพาะเลี้ยงสาหร่ายสไปรูลินา (Zarrouk medium)

To 980 ml of distilled water add:

Macronutrients;

NaCl	as needed to obtain required salinity
$CaCl_2$	0.04 g L^{-1}
$NaNO_3$	2.5 g L^{-1}
$FeSO_4.7H_2O$	0.01 g L^{-1}
EDTA (Na)	0.08 g L^{-1}
K_2SO_4	1.00 g L^{-1}
$MgSO_4.7H_2O$	0.20 g L^{-1}
$NaHCO_3$	16.8 g L^{-1}
K_2HPO_4	0.5 g L^{-1}

And 1 ml L^{-1} of A_5 and B_6 solution as listed below:

Micronutrients compose of A_5 and B_6 solution;

<u>A_5 solution</u>

$ZnSO_4.7H_2O$	0.222 g L^{-1}
$CuSO_4.5H_2O$	0.079 g L^{-1}
MoO_3	0.015 g L^{-1}
H_3BO_3	2.860 g L^{-1}

$MnCl_2.4H_2O$	1.81 g L^{-1}

B_6 solution

NH_4VO_3	229.6 x 10^{-4} g L^{-1}
$K_2Cr_2(SO_4)_4.24H_2O$	960.0 x 10^{-4} g L^{-1}
$NiSO_4.7H_2O$	478.5 x 10^{-4} g L^{-1}
$Na_2WO_4.2H_2O$	179.4 x 10^{-4} g L^{-1}
$Co(NO_3)_2.6H_2O$	439.8 x 10^{-4} g L^{-1}
$Ti_2(SO_4)_3$	400.0 x 10^{-4} g L^{-1}

Detail of preparation;

(1) Phosphorus of macronutrient should always be added last.

(2) Because of the poor solubility of NH_4VO_3, B_6 solution tends to be turbid. Thus, this solution should be well stirred before usage.

(3) Solutions A_5 and B_6 should be kept refrigerated, replacing them after 2 months.