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Design of Integrated Aquaculture of the Pacific White Shrimp, Tilapia and Green Seaweed

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Abstract: In this study, an integrated aquaculture system, based on shrimp-fish-seaweed, was designed and tested for its ability to treat shrimp pond effluent. Marine shrimp (*Penaeus vannamei*), herbivorous fish (*Oreochromis* sp.) and seaweed (*Entermorpha* sp.) were co-cultured in a recirculation system. The experimental results indicated that the integrated aquaculture, that included water-recirculation, could reduce nitrogenous-waste accumulation in shrimp ponds by retaining some nitrogen content in fish and seaweed biomass. 88% of the nitrogen introduced by feeding was collected through this system. Most nitrogen content (45%) was found in the form of pond sediment. 24% of the original nitrogen was retained in the form of aquaculture biomass, i.e. 15%, 6% and 3% for shrimp, fish and seaweed respectively.

Keywords: shrimp pond effluent; integrated aquaculture system

1. Introduction

Since Thailand became an international leader in marine shrimp production in the1990s, intensive shrimp farming areas have rapidly expanded along almost every coastal province in the country. Intensive shrimp farming requires high protein diets to maintain productivity. Most food eaten by shrimps is eventually excreted as metabolic waste which adds inorganic nutrients and organic matter to the shrimp pond water and sediment. Thus, the ponds become eutrophic, due to the active decay of uneaten food and assimilation of metabolic wastes carried out by microorganisms, e.g. bacteria, fungi, protozoa and phytoplankton. The higher abundance of these microorganisms requires more oxygen for the decomposition of the organic compounds. The final products of this degradation process are inorganic compounds, such as ammonia (NH_3), nitrites (NO_2^-), nitrates (NO_3^-), and phosphates (PO_4^{2-}).

The traditional method for maintaining good quality pond water has been frequent water exchange, but this results in high pollution of the receiving water body. The environmental impacts of untreated effluent have raised concerns about the sustainability of shrimp farming. The treatment of this wastewater must be considered as an integral part of the whole culture system. However, this may be difficult in practice since traditional methods of wastewater treatment are ineffective and prohibitively expensive for application in treating shrimp pond effluent [1]. One major problem is the diluted nature and high volume of aquaculture effluent in comparison with traditional forms of wastewater [2]. Hence, this has prompted the search for cost-effective methods of improving the effluent quality prior to discharge into the environment. Biological treatment is an attractive option for the treatment of wastewater because of its advantages, which include an environment-friendly process, low operating cost and the gain of value-added products,.

Many aquatic species were studied in the treatment of aquaculture wastewater. Bivalves, such as oysters and green mussels, can be used as primary filter feeders to remove the fine organic/inorganic matter from suspension as they coagulate this material into larger, more settle-able particles and egest them as pseudo-feces [3-4]. Herbivorous fish, such as tilapia or mullet, can also be used as filter feeders on plankton, detritus and other bottom deposits [5-6]. However, although bivalves and fish can reduce particulate concentration to a certain extent, the concentration of ammonia could be increased through their excretion [7]. To overcome this kind of problem, various species of micro/macro algae can be used to assimilate large quantities of dissolved nutrients. For example, the rhodophyte *Gracilaria edulis* rapidly assimilates ammonia [8] and another rhodophyte, *G. fisheri*, has been effectively used to assimilate waste nitrogen from shrimp pond effluent [4,9]. In fresh-water systems, macrophytes such as duckweed (*Lemnaceae* sp.) are commonly used aquatic plants for wastewater treatment, and can also be used to treat shrimp pond effluent [10].

In Thailand, the Department of Fisheries (DOF) proposed a conceptual design for the biological treatment of shrimp-farming effluent [11]. The proposed process used a combination of physical treatments, e.g. aeration and sedimentation, and biological treatment in an integrated aquaculture system. The shrimp pond effluent was drained through sequences of treatment ponds in the same order as the natural food chain, e.g. sedimentation pond, aquatic animal pond, aquatic plant pond, and chemical treatment pond, in which organic and inorganic matter in shrimp pond effluent could serve as food for other aquatic organisms. The treated effluent could be recycled back into the shrimp pond. Through this method, not only could the environment problems caused by shrimp-farming effluent be reduced, but the farmers would also gain additional profits from the co-cultured aquatic species.

This work aimed to obtain an insight into the performance of the integrated aquaculture system, based on the use of shrimp, fish, and seaweed, in terms of nitrogen budgets for the treatment of shrimp pond effluent.

2. Experimental

2.1 Design of Integrated Aquaculture System

Nitrogen budget in the integrated aquaculture system

In order to design the integrated system for the treatment of shrimp pond effluent, the estimated nitrogen budget of shrimp pond effluent and those of each treatment pond should be determined. The nitrogen budget and the nitrogen compounds of the shrimp pond effluent could be expressed as a fraction of the total N introduced in the shrimp feed. Shrimps were expected to assimilate 22% of the N, and a further 21% of the N would be removed by de-nitrification, volatilization and settle in the shrimp pond as sediment. The remaining 57% N would dissolve and be suspended in the water. From the 57% carried in the water, 26% would be in the form of phytoplankton and the remaining 31% would be dissolved N [12].

For the treatment ponds, fish was assumed to filter the 80% of particulate nitrogen (PN) from the shrimp pond effluent. Assuming the fish N assimilation efficiency was 24%, then 5% of original feed N would convert to fish biomass, while 16% of the original N would be excreted in the form of settling particles (8%) and dissolved nutrients (8%). At this rate, the fish pond effluent would contain 44% of the original N introduced by feeding, which was 4% of PN and 39% of dissolved nitrogen (DN). The seaweed production unit was estimated to remove the 90% of DN in fish pond effluent, thus taking up 36% of the original feed N. The final effluent would contain only 9% of the original N (5% PN and 4% DN). The flow diagram of nitrogen in the proposed integrated system is shown in Fig. 1.

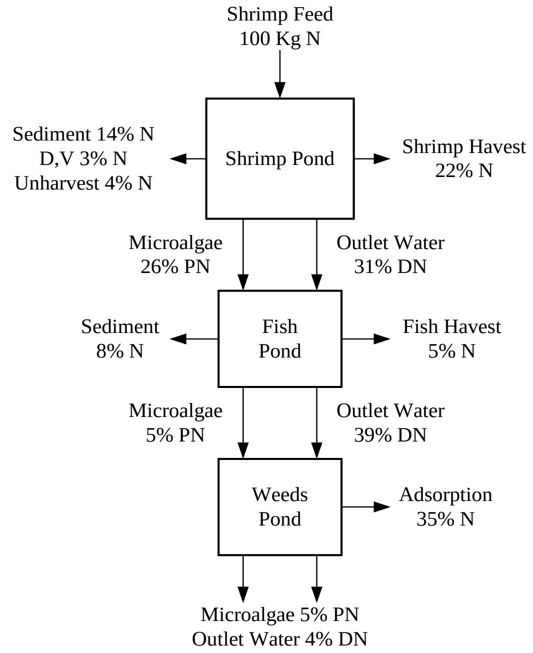


Figure 1. Flow diagram of nitrogen budgets in the proposed integrated system (PN; particulate nitrogen, DN; dissolved nitrogen, D; de-nitrification, V; volatilization).

System dimensions

The information about nitrogen budgets requires the estimation of the nitrogen discharge from the shrimp ponds, in order to design the treatment ponds. For example, a 1-ha intensive shrimp pond (10,000-m^2 with 1-m depth), with an annual production of 10,000-kg shrimp biomass and a feed conversion ratio (FCR) of 2.0, will be fed with 20,000-kg feed. This produces a nitrogen loading of 1,400-kg N (7.0% N content diet feed) per year or 4-kg N daily. If 57% of this amount (2.3-kg N) accumulated in the water column, 4,600 m^3 will be required for the water exchange (50% daily exchange rate) to maintain the total nitrogen (TN) concentration under 0.5-mg N L^{-1}.

The amount of co-culture species required for nitrogen removal in the effluent as previously discussed was determined based on their nitrogen removal rates. The area requirement for treatment ponds could be estimated based on knowledge of the proper stocking density. Using the tilapia filtration rate on green algae of 250-µg C fish (size of 1.4 g) $^{-1}$ h^{-1} [13] together with a 5.7 C:N mass fraction ratio in algae composition [14], the filtration rate in Table 1 could be estimated as 0.8-g PN kg^{-1} (wet weight) day^{-1}. The DN removal rate in Table 1 was calculated by assuming that the DN in the effluent from fish ponds was mainly NO_3-N, and by using the maximum nitrate uptake rate for *Enteromorpha* (V_{max}) and the half-saturation constant for nitrate (K_s) from the literature [15], namely, 2.4 µmol.NO_3 g dw^{-1} d^{-1} and 0.17 µmol.NO_3 L^{-1}. Based on this information, only 9% of N content introduced to a shrimp pond would remain in the seaweed pond effluent. This suggests the possibility of either discharge or recycling of the effluent.

Expected yield

An annual production of 10,000 kg shrimp requires approximately 1,400 kg N. 5% of this original quantity (70 kg N) would yield 2,700-kg of tilapia, with 2.6% N content of fresh meat [16]. The seaweed bio-filter was expected to assimilate 490 kg N or 35% of the total N input which yielded 223,000 kg (or stocking density at 2.23 kg m^{-3}) of seaweed biomass based on 0.22%of the nitrogen content of the fresh seaweed [17].

Integrated aquaculture system

The performance of the integrated aquaculture system, based on shrimp-fish-seaweed culture, was tested in laboratory-scaled experiments. The pacific white shrimp (*P. vannamei*), hybrid red tilapia (*Oreochromis sp.*) and green macro-algae (*Enteromorpha sp.*) were chosen for this study. The pond volume of shrimp pond to fishpond and seaweed pond was set at a ratio of 400:40:80 (liter). All ponds were placed outdoors. Details of each component in the integrated system are showed below.

The shrimp pond: Shrimp, with in average size of 10 g, were stocked in a 400-L circular plastic pond (0.9-m diameter and 0.6-m depth) at a total weight of 150-160 g per pond. A commercial diet, with about 5.6% of N content (35% of crude protein), was fed at a rate of 3% of shrimp body-weight per day. Aeration was supplied via a submersible pump to ensure a dissolved oxygen (DO) level above 5.0 mg L^{-1}. The salinity was adjusted to 10 ppt.

The fishpond: Total biomass of 40 g wet weight of red tilapias was stocked in a 40-L plastic pond. Neither aeration nor artificial feed was supplied to this pond. These fish were supposed to feed themselves with uneaten shrimp-feed and phytoplankton that possibly developed in the system.

The seaweed pond: Seaweed, 175 g wet weight, was stocked in a plastic basket floating in a 80-L plastic pond, without aeration. The salinity was adjusted to the same value as those in shrimp and fish ponds, i.e. 10 ppt.

Shrimp pond effluent sequentially over-flowed through the fishpond and seaweed pond and was then recycled back to the shrimp pond using a centrifugal pump. The schematic diagram of the integrated shrimp-tilapia-seaweed system is shown in Fig. 2.

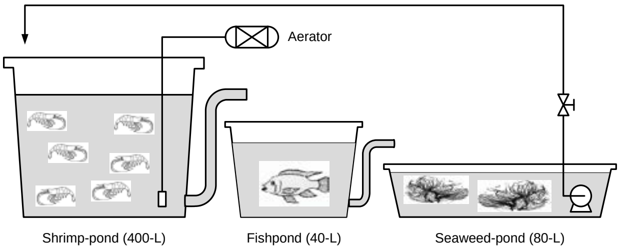


Figure 2. Schematic diagram of integrated shrimp-tilapia-seaweed system

Table 1. Design parameters for an integrated system to treat the effluent from a 1-ha intensive shrimp pond.

	Removal rate	% Removal	Biomass required	Stocking density	Pond volume required
Tilapia	0.8-g PN kg^{-1} d^{-1}	80	1,000 kg	1-kg m^{-3}	1,000 m^3
Seaweed	0.4-g DN kg^{-1} d^{-1}	90	3,500 kg	2-kg m^{-3}	1,800 m^3

2.2 Analytical procedures

Water quality

DO, temperature and pH in all experimental ponds were measured using an OAKTON model DO300 oxygen meter and a Sartorius model PB-11 pH meter, respectively. Salinity was measured with a hand refractometer. Dissolved nutrients, i.e. total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and ortho-phosphate (PO_4-P), were determined according to the standard methods for examination of water and wastewater [18]. Total nitrogen (TN) was analyzed using the persulfate digestion method described by Valderrama [19] and followed by nitrate analysis. Filtered samples were analyzed for DN using the same persulfate digestion method. PN was calculated as the deference between TN and DN. Chlorophyll a (CHLa), the most abundant form of chlorophyll, was used as an index for phytoplankton biomass and measured using the standard method for water and wastewater analysis [20]. Sediment samples were collected at the end of each experiment and tested for nitrogen content as the total Kjeldahl Nitrogen (TKN) [20].

Growth performance of aquaculture

The total weights of shrimp, fish and seaweed were determined at the beginning of the experiments. At harvest, the number of shrimp and fish were counted. The total weights of each aquaculture species were determined. Nitrogen conversions by growth of aquaculture were determined from nitrogen content in feed pellet and shrimp, fish and seaweed samples at the beginning and end of the experiments. The total nitrogen in dried shrimp, fish, and seaweed samples and total nitrogen in feed pellet were oven-dried at 60 °C to a constant weight and determined as the total Kjeldahl nitrogen (TKN) [20]. The growth performance of shrimp and tilapia were measured based on total weight gain, growth rate and survival rate. The efficiency of shrimp in converting feed mass into increased body mass was measured as a feed conversion ratio (FCR); the ratio between shrimp weight gained per unit weight of feed consumed.

3. Results and Discussion

The performance of integrated aquaculture systems in treatment of shrimp pond effluent was assessed in terms of total nitrogen budgets in each aquaculture species pond.

Water quality

Water temperature in all ponds varied from 26.0 to 30.2°C. Levels of pH, DO and salinity ranged from 6.4-8.3, 5.5-8.3 mg L^{-1} and 7-10 ppt, respectively. Changes in water quality parameters in each aquaculture pond are showed in Fig. 3. TN concentrations in all ponds tended to gradually increase with the time. TAN in all ponds increased sharply in the first week and began to decline thereafter. Accumulation of NO_2-N and NO_3-N followed TAN within a couple weeks, respectively. These patterns corresponded to the development of nitrifying bacteria, i.e. ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), in aquaculture systems [21]. PN concentrations in the shrimp pond increased during the first 4-weeks and then

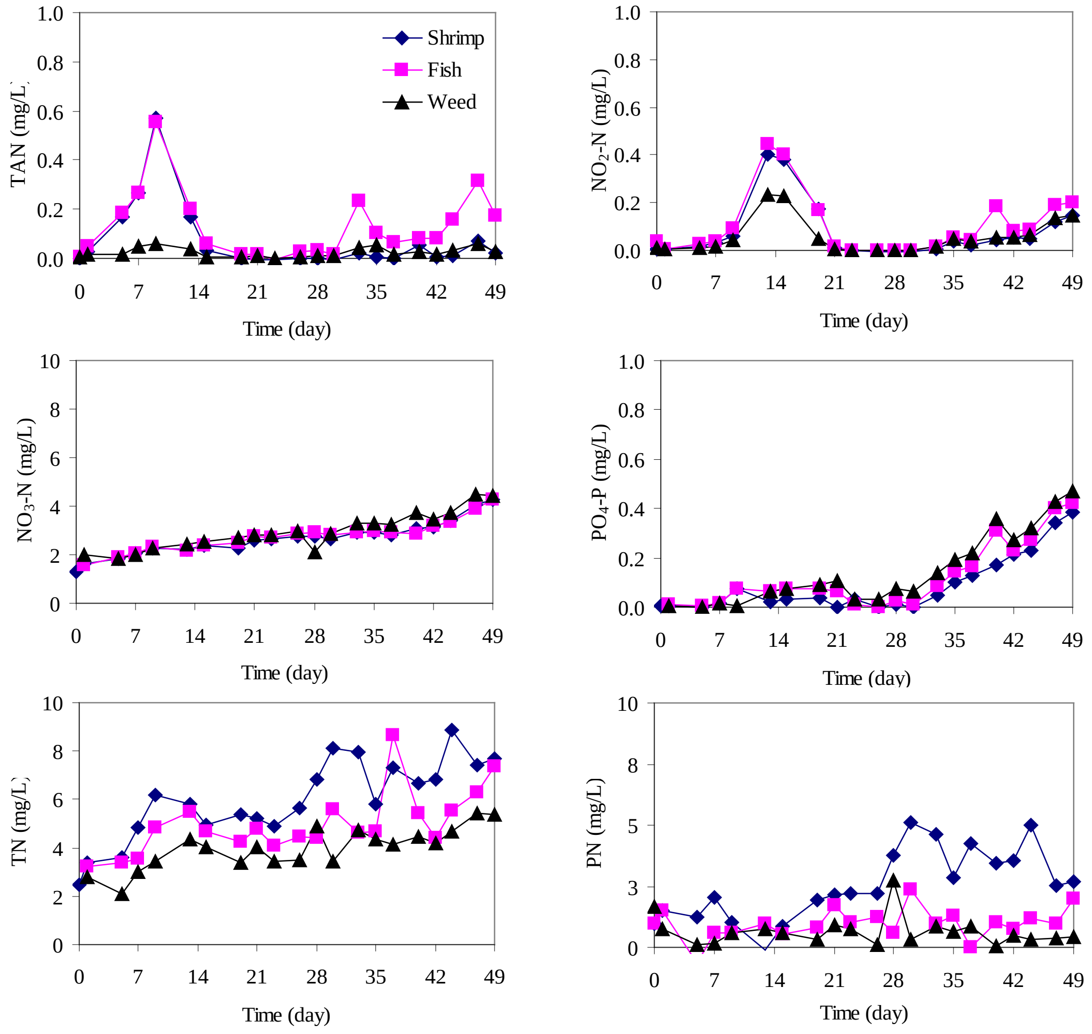
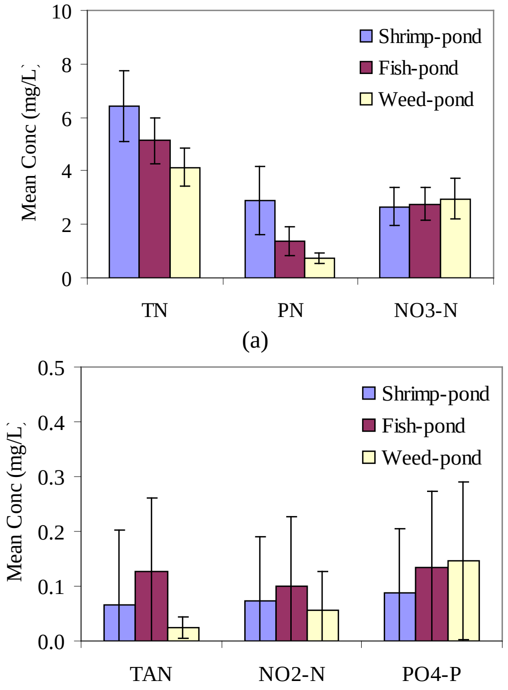


Figure 3. The water quality trends in the integrated aquaculture system at a recirculation rate of 50% of shrimp pond volume per day.

fluctuated between 3-5 mg L^{-1}. The fluctuation might be caused by the limitation of light penetration into the water due to a shading effect from high phytoplankton density. Consequently, the growth of plankton was also limited. However, PN concentration in the fish pond and seaweed pond were maintained at a lower level. PO_4-P concentrations built up slowly during the first 4-weeks and begun to rapidly increase thereafter, which might have limited plankton growth due to the shading effect. However, PO_4-P levels were usually low, i.e. below 0.5 mg L^{-1}, and showed slightly differences between each pond.

Fig. 4 shows the mean concentrations of nutrients in each pond. TN and PN tended to gradually decrease as the effluent flowed to the fish pond and seaweed pond. However, the concentration of NO_3-N, which was the main fraction of DN in the effluent. The concentration of TAN, NO_2-N and PO_4-P in all ponds were found at very low levels and showed no significant differences between each pond. These low ambient nutrient concentrations indicated that dissolved nutrients were efficiently assimilated by bacteria, phytoplankton and seaweed developed in this system. However, significant differences in TN and PN were found between the shrimp pond and the seaweed pond.



(a)

(b)

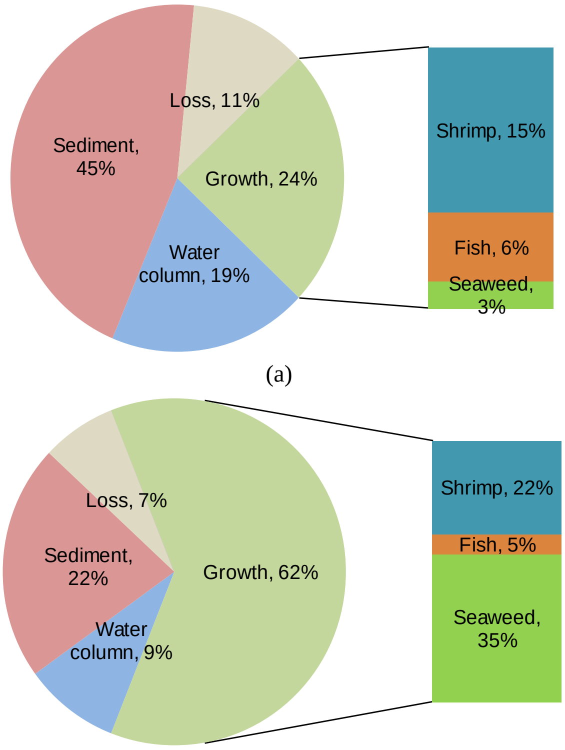
Figure 4. Mean concentration of nitrogen contents in integrated aquaculture system at a recirculation rate of 200-L d^{-1}.

Growth performance of aquacultures

The total shrimp yield was 53.4 g with the mean N content in shrimp being 2.9% wet weight. Daily growth averaged only 0.11 g per day individual. The survival rate was high (100%). However, the feed conversion ratio (FCR) was high (3.4) since feed was not adjusted according to shrimp consumption. The feeding rate was fixed in the range of 3% of shrimp body weight per day. No fish mortality was observed in this study with an average daily growth of 0.41 g per day. The total fish yield was 20.1 g with the mean N content in tilapia being 3.2% wet weight. Seaweed grew at a mean daily growth rate of 0.4%, yielding on average 130 g fresh weight at the harvesting period. By average, the seaweed biomass had 90% of moisture content with 12% of protein (% dry weight).

Conversion of feed nitrogen

Since the experiment was done in a closed system, only some tap water was added to compensate for evaporation loss. For the nitrogen, the main input to this integrated aquaculture system was diet feed protein. Nitrogen content of commercial feed pellet was 5.6% wet weight. The N conversions in the aquaculture system were assumed as accumulation in water column, assimilation by aquaculture and sedimentation as pond sediments. The nitrogen conversions in the integrated system are shown in Fig. 5.



(a)

(b)

Figure 5. N conversion (% of N feed) in the integrated aquaculture systems; (a) Experimental results, (b) Expected results

N conversions by shrimp were only 15%, which was slightly lower than the designed value, but comparable to those reported in literature, i.e. 18-22% of total feed [12,22]. The N conversion by tilapia (6%) was comparable to the designed value. However, N conversion by seaweed (3%) was far lower. That made the overall N conversion by growth of aquaculture in the integrated system (24%) only one-third of the design value (62%). The specific growth rate of seaweed in this study was only 0.005 d^{-1}, which was lower than the 0.02 d^{-1} growth rate recorded by seaweed in estuaries [15]. The reason for the low N uptake by seaweed probably resulted from non-optimum growth conditions, which consequently lowered its growth rate. In general, the key factors that control macro-algae in natural conditions were defined as a function of light, temperature, nutrients (N and P) and salinity. In this study, average light intensity (95 µmol m^{-2} s^{-1}) and temperature (28.2°C) ranged in the optimum growth condition for *Enteromorpha*, i.e. 100-500 µmol m^{-2} s^{-1} and 15-30°C respectively [15]. The concentration of NH_4-N, which was a preferable N-source for algae, in this study was low 0.07 mg N L^{-1}, however, the average concentration of NO_3-N, which is a less energetically efficient N-source, in this study (2.8 mg N L^{-1}) was still higher than the half-saturation constant for nitrate of *Enteromorpha*, i.e. 0.18 mg N L^{-1} or 12.72 µmol NO_3 L^{-1}. It indicated that N-limitation was not observed in this study. Hence, the only factor that could have possibly affected seaweed growth was salinity. Under non-limiting nutrient conditions, *Enteromorpha* showed higher growth rates within the salinity range of 10 to 22 ppt. Its growth varied along a normal distribution curve with salinity and the optimum salinity range for growth was 18-22, which was far from the range recorded in this study (7-10 ppt). Nevertheless, *Enteromorpha* was more affected by lower than by higher salinity [23]. Below 3 ppt, seaweed ceased to grow and death occurred within a few hours at 0 ppt.

Only small amounts of sediment could be collected from shrimp ponds due to the continuous aeration that was applied to create a well-mixed condition. However, the amount of sediment N collected from fish and seaweed ponds in the integrated system was large (45%). The C/N ratio of the sediment (5.6) was similar to a Redfield's ratio (5.7). The CHLa content in the sediment (0.6 % dry weight) fell within a range of 0.5 and 2%, the general range of phytoplankton [24]. All this information pointed to the distinct possibility that the pond sediment was phytoplankton. Microscopic observation also indicated that these phytoplankton were in the form of the aggregate of various algal species, including green algae, e.g. *Chlorella*, *Scenedesmus* and filamentous blue-green algae, e.g., *Oscillatoria*. In this study, the N accumulation in sediment was two times higher than the designed value, but comparable to values reported by Lorenzen *et al*. [25], which predicted that 48 to 66% of feed N would settle to the pond bottom in the form of phytoplankton. During this sedimentation, excessive organic matter accumulation probably occurred and lead to problems associated with the pond bottom, e.g. oxygen consumption and release of nutrients from re-mineralization [26]. Therefore, addition of physical process, such as filtration or sedimentation, to remove excessive phytoplankton would be recommended to reduce the accumulation of nitrogen in form of organic matter at the pond bottom. Though phytoplankton and the organic particulate fraction were normally not easily settled due to natural buoyancy, or having a specific gravity close to that of seawater [27]. However, the aggregated microalgae found in this study exhibited a high settling velocity (2.54 m d^{-1}) compared to that of mixed phytoplankton communities (0.02-1.1 m d^{-1}) [28]. Hence, the microalgae in our process could be easily settled and removed by sedimentation, especially, in the non-aerated pond, i.e., fish and seaweed ponds.

The N content collected from this integrated system accounted for 88-95% of feed N. The rest was assumed to be lost to the atmosphere via volatilization of ammonia and de-nitrification. Volatilization was the transmission of NH_3 from the water column to the atmosphere. However, due to ammonia, volatilization was promoted by favoring the NH_3 side of the water column NH_4^+-NH_3 equilibrium, which was mostly governed by overall TAN concentration and high pH. Hence, volatilization was not expected to account for a significant loss of N in typical marine aquaculture ponds with a TAN less than 1.0 mg L^{-1} and pH in the range of 7.2-8.3 [25,29]. Since average TAN levels in this study were very low (0.07 mg L^{-1}), the loss of N by volatilization was negligible. The major loss of N presumably resulted from de-nitrification.

4. Conclusions

The integrated aquaculture recirculation system could control the water quality in a shrimp pond within the preferred range. The average TAN and NO_2-N concentrations were found at very low levels. The aquaculture also reduced nitrogenous waste accumulation in the shrimp pond by retaining some N content in the form of fish and seaweed biomass. 88% of the nitrogen introduced as feed was collected from the system. Most of the nitrogen content (45%) was found in the form of sediment, following the development of the phytoplankton in shrimp pond. The 24% of N introduced as feed was retained in the form of aquaculture biomass, i.e. 15%, 6% and 3% for shrimp, fish, and seaweed, respectively. The aquaculture production obtained in this study was lower than expected, especially for the seaweed biomass. This was probably due to the non-optimum growth conditions, such as salinity. However, based on the nitrogen budgets obtained in this integrated aquaculture, a better co-culture system should be comprised of only shrimp and fish. In this case, the fishpond area could be increased for better sedimentation efficiency and tilapia could be stocked at a higher density, in order to sufficiently remove the excess settled phytoplankton.

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