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DYNAMICS OF NITROGEN IN INTENSIVE CULTURE OF WHITELEG SHRIMP IN (*Litopenaeus vannamei*) TANK INTEGRATED WITH HYBRID CONSTRUCTED WETLANDS

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ABSTRACT

The objective of this study was to evaluate dynamics of nitrogen in intensive whiteleg shrimp (Litopenaeus vannamei) culture in tanks and integration with hybrid constructed wetlands (CWs). The CWs planted with Typha orientalis (C. Presl) and unplanted CWs were arranged in three replications, while control (without CWs) was duplicates. Water from the shrimp tanks was distributed to the hybrid CWs and their outlets were recycled back to the shrimp tanks that were studied at 5-10‰ of salinity. The hybrid CWs consists of a vertical subsurface flow (VF), horizontal subsurface flow (HF) and surface flow (SF). The entire systems were operated at a recirculation rate of 11.4 L/min (equivalent to 100% volume shrimp tank per day). Water quality in the shrimp tanks was monitored every two weeks. The results showed that while the concentrations of NO_2-N and NH_3-N were increased overtime but they were still in the suitable range for whiteleg shrimp growth. Taken together the results indicated that the wetland system helped to maintain water quality in intensive recirculating shrimp culture tanks without water exchange.

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1 INTRODUCTION

In recent years, brackish-water shrimp farming in the Mekong Delta of Vietnam is fast growth and brings high economic efficiency. The shrimp farming area accounted for 638,422.7 hectares by the end of 2014 (fulfilling 95.4% of the year's plan and 109.6% as compared to the previous year) (Directorate of Fisheries, 2014). In particular, farming area of whiteleg shrimp (*Litopenaeus vannamei*) was 79,630 hectares, achieving 113.8% of the 2013 year's plan (Directorate of Fisheries, 2014). However, shrimp farming is encountering many difficulties and challenges due to the substantial diminishing in water quality of cultivation areas anh thus caused a major loss (Tan, 2006). In addition, aquaculture activities caused locally environmental pollution of water bodies in the Mekong Delta (Vietnam Environment Administration, 2012). Nowadays treatment of wastewater from intensive culture ponds of whiteleg shrimp using biological products (EMs – Effective Microorganisms) and adsorption materials (lime powder, Zeolite, Diatomic,…) is applying in many places. Although these methods help to secure pond water quality, they are costly and decline economic efficiency from farmers.

Integration of constructed wetlands (CWs) in recirculating aquaculture system (RAS) to treat pond wastewater was highly feasibility, increased water use efficiency by recycling and recirculating and minimized water discharge to environment (minimal-or zero-water exchange systems) which was studied in recent years (Nguyen *et al.*, 2012; Nhien *et al.*, 2013; Trang and Brix, 2014). Lin *et al.* (2003) confirmed that surface flow (SF) and horizontal subsurface flow (HSSF) CWs helped to improve water quality in RAS of whiteleg shrimp culture. To our knowledge, application of *Typha orientalis* C. Presl planted in CWs to purify wastewater from RAS of whiteleg shrimp has not studied yet in Vietnam. For this reason, this research was carried out to study dynamics of nitrogen in recirculating whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) system integrated into hybrid CWs planted *Typha orientalis* C. Presl.

2 MATERIALS AND METHODS

2.1 Experimental setup

Six composite tanks (1 m^3) were used for rearing whiteleg shrimp. There were six hybrid constructed wetlands (CWs) systems consisted of a vertical subsurface flow (VF, cylindrical in 49 x 49 cm), a horizontal subsurface flow (HF, rectangular in 60 x 40 x 30 cm), and a surface flow (SF, rectangular in 94 x 84 x 43 cm) wetlands in series (Fig. 1). The VF systems were set up in the plastic containers with a system of perforated Ø 21 mm pipes that was installed to facilitate distribution of the influent equally over the surface of the VF. Water level was controlled below the HF systems' media at 5 cm while water level was higher than media surface at 10 cm in the SF systems. Each of CWs was filled with a-60 L of mixed recycling pottery, beehive coal and oyster shell at a ratio of 1:2:3 ($2.0<d\leq5.0$ mm). The CWs media had porosity of 62.1 ± 1.2%.

The hybrid CWs were used as biofilters for the shrimp tanks, in which three systems were planted with *Typha orientalis* C. Presl at a density of 3 plants/VF tank; 4 plants/HF tank and 12 plants/SF tank to acheive a density of 15 plants/m^2 (Nguyen *et al.*, 2012), and the other three were unplanted. Two plastic shrimp tanks were conducted at the same time to evaluate water quality in the shrimp tanks without treatment by CWs (as control treatment). The experiment was completely randomized design with three replications, except for the control shimp tanks were arranged in two replications.

2.2 System operation and monitoring

The entire systems were operated at an recirculation rate of 11.4 L/min (equivalent to 100% volume shrimp tank per day). This flow rate (11.4 L/min) was controlled by a timer which was set at 100 seconds pumping and 28 minutes stop. Water from the shrimp tanks was distributed to the hybrid CWs and their outlets were collected at the sumps before pumped back to the shrimp tanks (Fig. 1).

Postlarvae (PL) of whiteleg shrimp (*Litopenaeus vannamei* Boone, 1931) were introduced into the culture tank of each system, with a stocking density of 150 PL/m^3. The shrimps were fed 4 times/day with commercial floating pellets containing 40% crude protein (Frystart Flake, Thaland) during the first two weeks of culture. The feeding rate was based on 8% of body weight and adjusted according to the intake rate of the shrimp. The study was monitored for 12 weeks.

In the first month, the system was operated with tap water that supplemented with NPK (20-20-15) at a dosage of 80 g/a shrimp-tank in order to let the plants and microorganisms in wetlands to adapt to environment beside to make the culture water a light green color with plankton growth. Then, water in shrimp tanks was mixed together well in 24 hours before tranfering shrimp to the culture tanks. After which, water in the shrimp tanks was increased salinity at 5‰ for two weeks. In the following two weeks, salinity was increased up to 10‰ and maintained to the end of the experiment to ensure that plants and shrimps could grow.

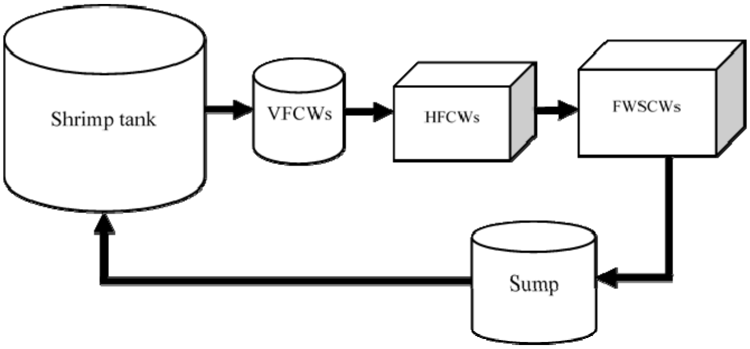

Fig. 1: Schematic diagram of the integration of intensive shrimp culture tank and hybrid constructed wetlands (CWs)

2.3 Water sampling and analysis

Water samples were taken every two weeks at 6:00 - 7:00 AM. Temperature, dissolved oxygen (DO), pH and salinity of the water in the culture tanks were measured at the experimental site using respective portable meters. Water samples were collected and transferred immediately to the laboratory for analysis of Ammonium nitrogen (NH_4-N), Nitrite nitrogen (NO_2-N) and Nitrate nitrogen (NO_3-N). All the analytical measurements were carried out according to Standard Methods (APHA *et al.*, 1998). Ammonia nitrogen (NH_3-N) concentration was calculated regarding to Albert (1973).

2.4 Statistical analysis

Data were tested for normal distribution, variance homogeneity (Levene's test) and logarithmically transformed if necessary. Differences in water quality were identified by Multifactor ANOVA (3 treatments and 7 sampling times) using Type III sum of squares. Tukey Honestly Significant Differences (HSD) was used to compare significant differences among treatments at the 5% probability level. The software Statgraphics Centurion XV (StatPoint, Inc., USA) was used for all statistical analyses.

3 RESULTS AND DISCUSSION

3.1 Temperature, pH, salinity and DO values in the shrimp tanks

There were no significant differences for temperature among treatments but it changed among sampling times ($p<0,001$) due to weather changing during study period (from April to July). However, temperature in the shrimp tanks was in the range of 25 - 29.8 °C (Fig. 2a). It was in the optimum level for requirements on rearing water quality for whiteleg shrimp growth (Ministry of Agriculture and Rural Development [MARD], 2010).

The pH values can affect to the physical, chemical and biological elements of the environment and shrimp health. In particular, an increase of pH and temperature values is the cause of increase of NH_3-N concentration that can toxic to the aquatic organisms. Similar pH values were measured among three treatments and sampling times ($p>0.05$). During the experiment, the pH in the shrimp tanks ranged between 7.45 and 8.23 (Fig. 2b) that was in the optimum level for whiteleg shrimp growth (MARD, 2010).

Salinity in this study was controlled during study to fulfill requirements for whiteleg shrimp and *Typha orientalis* growth. Salinity was intended to increase from 5‰ (for every 2 weeks) until 10‰ at the end of the study. However, the measured salinity in the shrimp tanks was in the range of 6.2-11.2‰ (Fig. 2c). Compared to the control treatment (without CWs) the salinity in the CWs with and without *Typha orientalis* was lower ($p<0.001$). It might be due to higher evapotranspiration occuring in the constructed wetlands. The salinity values in this study was in the permitted limit for whiteleg shrimp growth (MARD, 2010).

Dissolved oxygen (DO) is the most critical water quality in shrimp tank. Similar to pH, no difference of DO concentrations were detected among planted, unplanted and control treatments ($p>0.05$). The average DO values were 4.79±0.6 mg/L and 4.68±0.5 mg/L in the planted and unplanted treaments, respectively. Additionally, DO values in the control shrimp tanks was 3.51±0.3 mg/L. The former DO values were in the optimum level while the latter DO value was in the permitted limit for whiteleg shrimp growth (MARD, 2010).

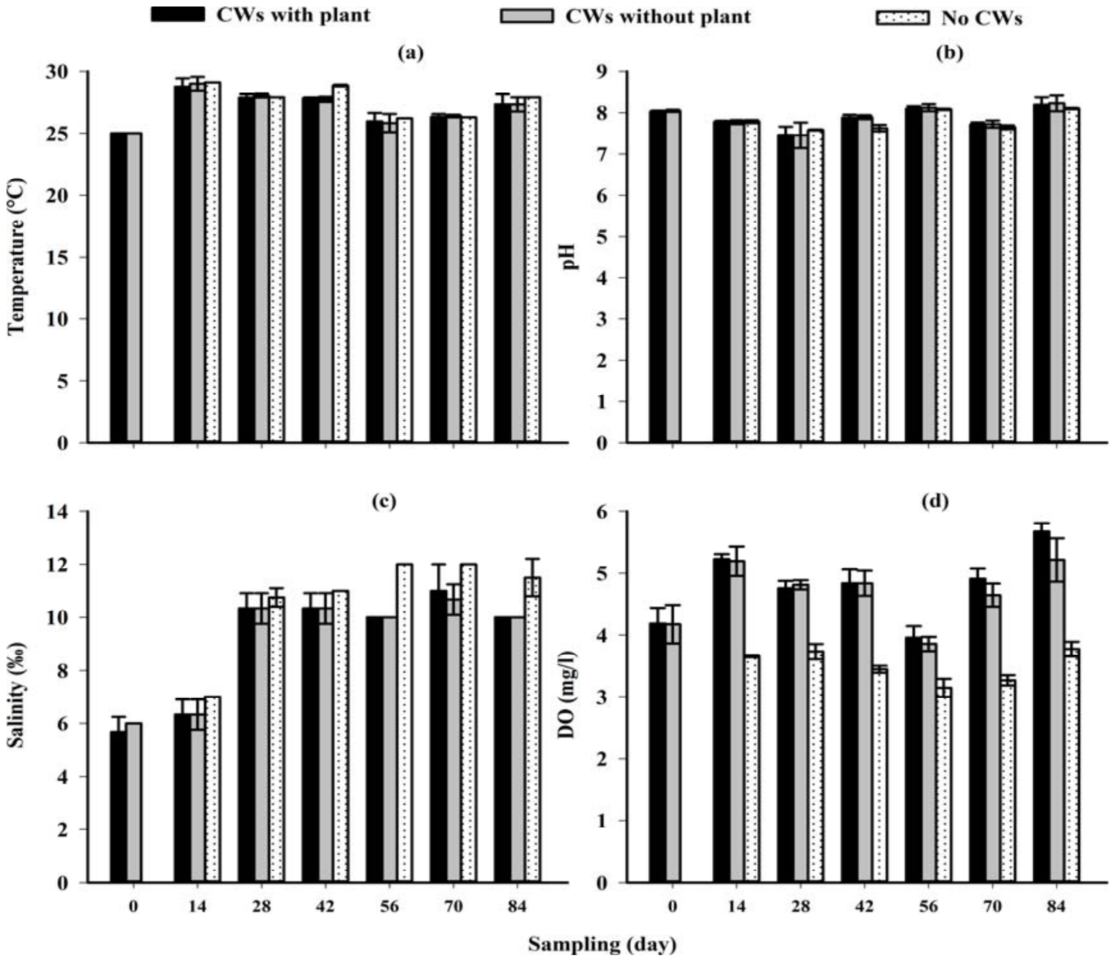

Fig. 2: Temperature (a), pH (b), salinity (c) values and DO concentrations (d) in the shrimp tanks of the three treatemnts CWs with and without plant and control (no CWs)

3.2 Nitrogen concentrations in the shrimp tanks

3.2.1 Ammonium and ammonia nitrogen

Ammonium nitrogen (NH_4-N) concentrations in the shrimp tanks were similar between planted and unplanted treatments ($p>0.05$) but they were higher than those in the control treatment ($p<0.05$). A significant difference was found among sampling times ($p<0.001$) for NH_4-N concentrations (Fig. 3a). There was a significant accumulation of NH_4-N concentration in the control treatment (system without constructed wetlands), but not so much in the systems integrated with CWs. The later CWs systems had similar NH_4-N concentration to the other study, in which a low shrimp density of 110 shrimp/m^3 was cultured (Luan *et al.*, 2015). Regarding to Lin and Chen (2001), the safe NH_4-N level for whiteleg shrimp growth should be lower than 2.44 mg/L.

Ammonia nitrogen (NH_3-N) in water was calculated according to Albert's formula (1973). The NH_3-N concentrations in the shrimp tanks were similar between planted and unplanted treatments ($p>0.05$) but a significant difference was detected among sampling times ($p<0.001$) (Fig. 3b). NH_3-N ranged from 0.02-0.05 mg/L in the planted and unplanted treatments while it varied from 0.22 to 0.38 mg/L in the control treatment. The NH_3-N concentrations were accumulated overtime. The former NH3-N values were in the optimum level while the latter NH_3-N value was in the permitted limit for whiteleg shrimp growth (MARD, 2010). Taken together, NH_3-N concentrations in this experiment had not a significant effect on the shrimp growth.

3.2.2 Nitrite and nitrate nitrogen

Nitrite is toxic to shrimp and exposure to high concentrations may cause retarded growth and mortalities (Gross *et al.*, 2004). The NO_2-N concentrations in the shrimp tanks in this study did not significantly differ between planted and unplanted treatments (*p>0.05*). In general, NO_2-N concentrations in shrimp tanks were prone to accumulate overtime (Fig. 3c). The concentrations of NO_2-N in the planted CWs were in the range of 0.25 - 0.31 mg/L which was in permitted level for whiteleg shrimp growth (MARD, 2010; <0.35 mg/L). However, NO_2-N concentration in the unplanted CWs was slightly higher (0.26-0.41 mg/L). In contrast, NO_2-N concentrations in the control treatment (no CWs) were significantly much higher than both unplanted and planted CWs. It ranged from 1.9 to 6.1 mg/L which considered as toxic to shrimp and led to high shrimp mortality (65%). Gross *et al.* (2004) suggested a safe concentration for whiteleg shrimp production in ponds to be less than 0.45 mg/L NO_2-N.

The NO_3-N concentration is not harmful for shrimp growth, however, higher accumulation of NO_3-N might be toxic to shrimp (Kuhn *et al.*, 2011). In this study, NO_3-N concentration likely accumulated overtime (*p<0.001*). The NO_3-N concentration was significantly higher in unplanted system than in *Typha orientalis* (C. Presl) planted system (*p<0.001*). Importantly, NO_3-N concentration in the control treatment was the highest. In particular, the NO_3-N concentrations in the planted, unplanted and control treaments were in the range of 2.74-6.15, 2.59-7.44 and 4.59-8.58 mg/L, respectively. The possible explaination for the nitrate accumulation was due to without water exchange in the study and shrimp feeding daily.

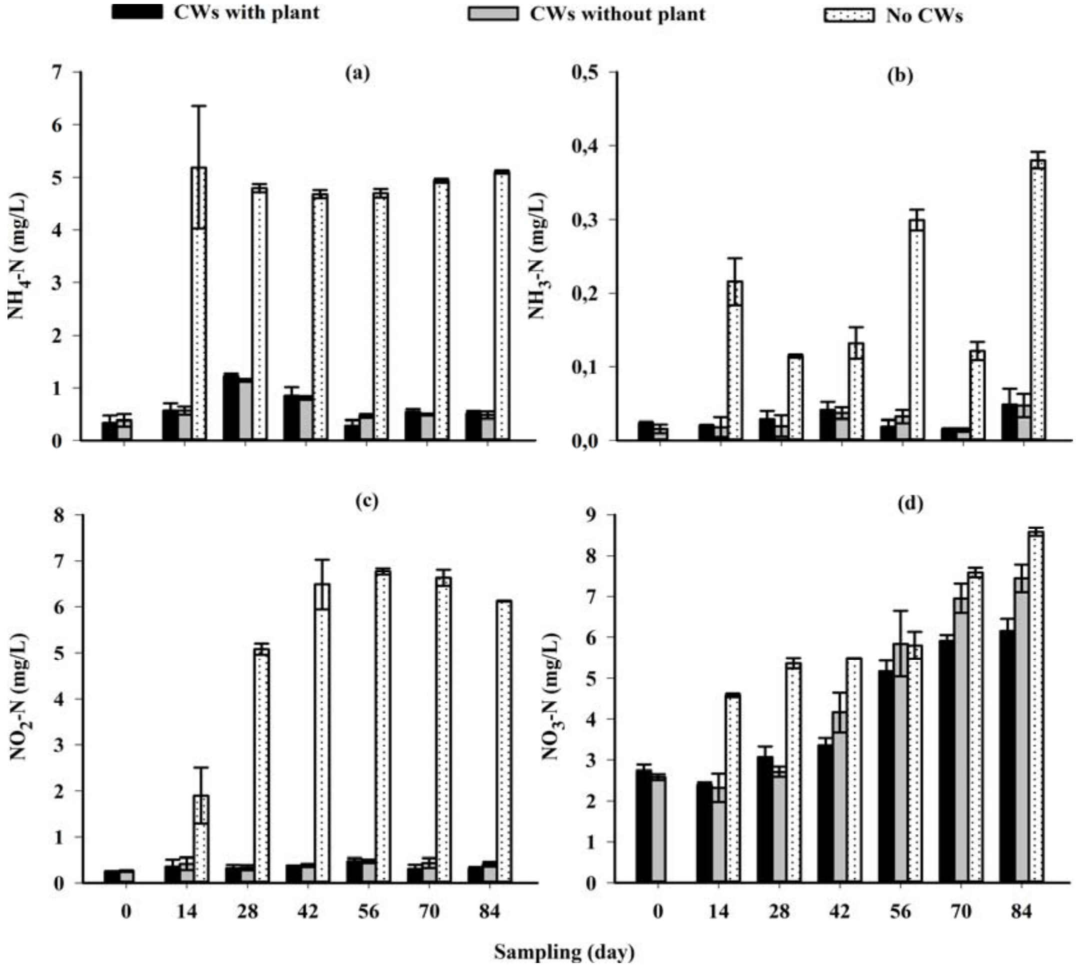

Fig. 3: NH_4-N (a), NH_3-N (b), NO_2-N (c) and NO_3-N concentrations (d) in the shrimp tanks of the three treatemnts CWs with and without plant and control treatment (no CWs)

3.3 Shrimp growth

There were significant differences among the three treatment systems for shrimp growth, except for survival rate (Table 1). Similar amount of feeding to the shrimp in the three systems was approx. 646 g per tank (Table 1). However, the harvested biomass after 89 culture days was the highest in the CWs system with plants leading to the highest specific growth rate was also recorded in this system ($p<0.05$). A low survival rate was achieved similarly in all three systems ($p>0.05$), but a better feed conversion ratio was achieved in the CWs with plants. It concludes that the constructed wetlands with the presence of *Typha orientalis* C. Presl helped maintaining water quality for better shrimp growth compared to the other studied systems.

The study was conducted for 12 weeks without water exchange. There was only 716.7, 733 and 90 L of tap water added to the CWs with, without plants and no CWs, respectively, in order to replace water loss due to sampling and evapotranspiration.

Table 1: Shrimp performance in the CWs with and without plants and control treatment (No CWs)

	Treatments			***F-values***
	CWs with plants	**CWs without plants**	**No CWs**	
Mean initial weight (g/m^3)	7.5	7.5	7.5	-
Total feeding (g/m^3)	646.14	646.14	646.14	-
Total biomass (g/m^3)	234.25±13.9^a	163.67±3.0^b	169.32±9.25^b	11.8*
Specific growth rate (%/day)	3.86±0.07^a	3.46±0.02^b	3.50±0.06^b	14.38*
Feed conversion ratio	2.87±0.17^b	4.14±0.08^a	4.01±0.23^a	16.91*
Survival (%)	32.67±0.38	28.0±0.67	28.0±4.0	2.21^{ns}

Mean with different superscript in a row are significantly defferent (P<0,05, n=3)

**p<0.05; ns: not significant difference*

4 CONCLUSION

This study demonstrated that the hybrid constructed wetlands integrated into indoor recirculating systems for culturing intensive whiteleg shrimp could effectively reduce an accumulation of NH_4-N, NH_3-N, NO_2-N and NO_3-N from the culture tanks. The CWs with the presence of *Typha orientalis* C. Presl provided high water quality and good culture environment, consequently increasing the growth of shrimp compared to the unplanted CWs and without CWs systems.

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