Assessment of the bivalves stocking density for maximizing nutrients removal from shrimp farm effluents

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Abstract. Intensive shrimp farming effluent contains pollutants that include high levels of organic matter, nutrients and bacteria. This research investigated the effects of bivalve different stocking densities on nutrient removal from a shrimp farm effluent. Oyster *(Crassostrea rivularis)* was tested with three densities of 30, 40 and 50 oysters m^{-2}. They were placed in three plastic trays (350x200x65 mm) and hung in a 1 m^3 tank at three depths of the water column (20, 50 and 80 cm from the surface). Clam *(Corbicula fluminea)* was tested at three densities of 50, 75 and 100 clams m$^{-2.}$ Clams were distributed evenly on the bottom of the 1 m^3 tank. The experiment was conducted over eight days and replicated three times at shrimp farming in Dien Mon commune, Phong Dien district, Thua Thien Hue province, Vietnam. The removal rate of nutrients (NO_2-N, NO_3-N, TN, PO_4-P, TP) and total coliforms increased with increasing oyster stocking density from 30 to 50 oysters m^{-2} and decreasing clam density from 100 to 50 clams m^{-2}. The removal of total nitrogen (TN), total phosphorus (TP), total suspended solid (TSS) and total coliform occurred in the range of 45-56, 35-45, 51-80 and 94-98%, respectively. The removal efficiency of NO_2-N, NO_3-N, TN and total coliform by oysters was higher than by clams in all treatments, while the removal of PO_4-P, TP by clams was slightly higher than by oysters. The study also indicated that the highest removal of NO_2-N, NO_3-N, TN, TSS and total coliforms was achieved being 75.1%, 96.5, 56.2, 80.1, and 98.8%, respectively, at the highest stocking density of oysters (50 oyster m^{-2}), while the removal of TP by clams was highest (72.7%) at the lowest stocking density (50 clams m-^{2}).

Key Words: white-leg shrimp, shrimp wastewater, oyster, clam.

Introduction. The nutrient concentration of intensive shrimp faming wastewater has been estimated as the difference between the nutrients contained in the feed and nutrients retained by the biomass. The loss of nitrogen (N) and phosphorus (P) from shrimp farms into the wastewater may reach 89% and 102% respectively (Verdegem 2013). The study of Buhmann & Papenbrock (2012) indicated that high N and P concentrations can cause algal blooms and eutrophication in ecosystems. The impact of organic matter from shrimp effluent on the coastal environment and its influence on N and P loads has also been studied (Martínez-Durazo et al 2019). The N and P discharged may come from intensive or semi-intensive culture systems and often in the form of ammonium (NH_4^+), nitrite (NO_2-N), nitrate (NO_3-N) and phosphate (PO_4^+) (Iber & Kasan 2021; Lich et al 2022). In the early stage of shrimp production, large amounts of feed containing N, P, potassium (K) and some trace elements are put into the water to improve the growth of algae that provide shrimp with sufficient feed. In the middle and late stages of shrimp cultivation, most of the feed remains in the water, with some becoming suspended solids and some releasing much of its N and P upon dissolution. Hao (1996) found that feed and shrimp meal remaining in the water can significantly increase the amount of the soluble organic N and P above that produced by shrimp excrement. Studies were carried out to understand the relationship between pond age and total N in the bottom of the pond by investigating 120 shrimp ponds in the Mekong Delta and the central area of Vietnam (Le 2008; Anh et al 2010). The results showed that the total N increased with pond age, and had a positive linear relationship with pond age with a correlation coefficient between 0.4703–0.8874. Traditional shrimp production mainly relies on the exchanging of pond water and discharging N and P rich effluents into the surrounding waters, causing degradation of water quality, alteration of phytoplankton community structure and eutrophication (Lich et al 2016).

In the last decade, several researchers focused on the removal of nutrients from shrimp farm effluents using bivalves, seaweeds or a polyculture system (Ertl et al 2016; Brito et al 2018). These studies indicated that oysters and clams have the potential to improve the water quality from shrimp farm effluent. The Sydney rock oyster (*Saccostrea glomerata*) filters nutrients, total suspended solids (TSS) and phytoplankton from the water column, lowering turbidity and increasing water quality (Ertl et al 2016). The use of *Anomalocardia brasiliana* to treat effluents from shrimp faming reduced waste solids and increased the nitrification process from 72 to 96 h, indicating the clam potential for bioremediation of shrimp wastewater (Brito et al 2018). However, information available on the stocking density of oysters and clams for the removal of nutrients and comparison of bivalvess are lacking. This study investigated the stocking density of two local bivalve species, the oyster (*Crassostrea rivularis*) and the Asian clam (*Corbicula fluminea*) for maximizing the nutrient removal from intensive white-leg shrimp (*Litopenaeus vannamei*) farming effluent.

Material and Method. Two bivalves species: oyster (*C. rivularis*) (Figure 1a) and Asian clam (*C. fluminea*) (Figure 1b) were collected at Tam Giang lagoon. After collection, the bivalves were transported to Phu Thuan Center for Training and Practice of Aquaculture, Hue University of Agriculture and Forestry, where they were washed with seawater to remove sand, mud, epiphytes and sediments. After these procedures, the bivalves were kept in plastic trays and hung in tanks containing 500 L of seawater collected from the intensive shrimp farming in Dien Mon commune, Phong Dien district, Thua Thien Hue province and maintained at the following conditions: temperature of 24-28^{o}C, salinity of 18‰, dissolved oxygen (DO) >4 mg L^{-1}, and pH between 7.5-8. The bivalves were kept in separate tanks and maintained for the acclimatization process for one week. During the acclimatization period, a combination of powdered rice, fish and maize was fed at the rate of 3-5% of the total bivalve biomass in the treatment tank daily at 8:00 AM.

Oysters (50.2±3.4 g wet weight) were tested at three densities including 30 (OY-30), 40 (OY-40) and 50 (OY-50) oysters m^{-2} (approximately 1500, 2000 and 2500 g m^{-3}) and a control (0 oyster m^{-2}). Clams (20.1±1.7 g wet weight) were tested at three densities of 50 (CL-50), 75 (CL-75) and 100 (CL-100) clams m^{-2} (approximately 1000, 1500 and 2000 g m^{-3}) and control (CT) (0 clams m^{-2}). Oysters were placed in three plastic trays (350x200x65 mm) and hung in a 1 m^3 tank at three depths of the water column (20, 50 and 80 cm from the surface). Each tray contained 10-15 oysters. Clams were distributed evenly on the bottom of the tank. The experiment was conducted over 8 days with three parallel replications, in the farm area.

Wastewater from intensive shrimp farms was collected from a wastewater canal at the last month of the shrimp production cycle. The DO of the wastewater was enhanced and NH_3 was removed by a splash board treatment over three hours. The treated wastewater was pumped into tanks stocked with 500 g of finfish (*Mugil cephalus*) and 200 g of mangrove snail (*Cerithidea obtuse*) for biodegradation of organic matter during an 84 h period before the experiment and finally moved into the batch exchange before being used for this experiment. One m^3 of shrimp wastewater was supplied into each treatment tank. The oxygen level in the treatment tanks was maintained at >4 mg L^{-1} during the experimental process. The effects of bivalve density on nutrient removal were investigated during the 8 days of treatment process.

Water samples were collected from a water depth of 25-30 cm at 5 positions inside the tank, including the four corners and the center, at 7:00 AM every day during the experiment. The samples from the 5 points were mixed together and a 1 L sample collected from the homogenously mixed composite was stored in 1 L polyethylene amber container. The value of total ammonia (TAN) and NO_2-N were analysed daily in the experment site by a test kit. Other parameters including NO_3-N, PO_4-P, total nitrogen (TN), total phosphorous (TP), total suspended solids (TSS) and total coliform were measured every two days in the laboratory of the Faculty of Environment, Hue University of Science, according to the standard method (APHA et al 2012).

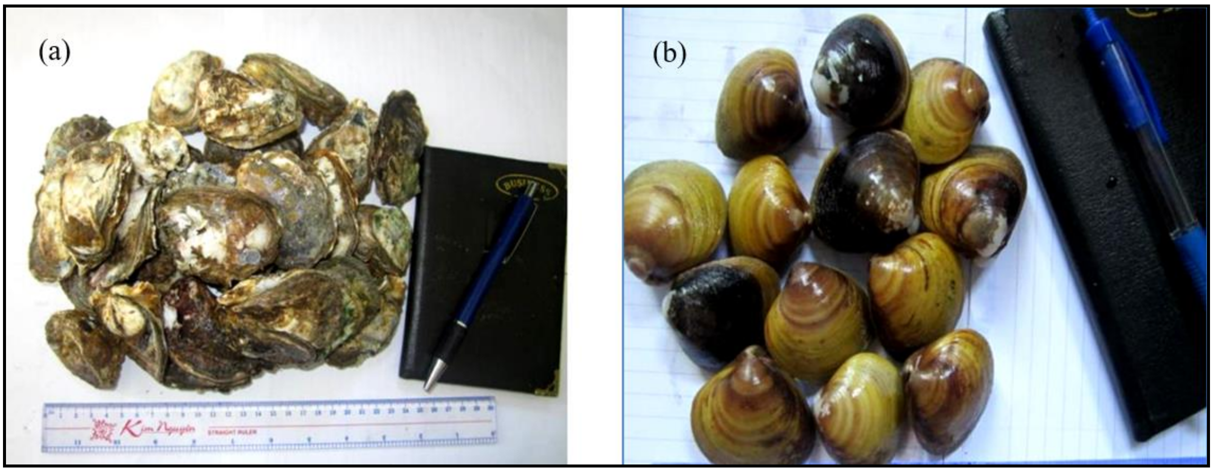

Figure 1. Two local bivalves species were used in the experiments; (a) oyster (*Crassostrea rivularis*); (b) Asian clam (*Corbicula fluminea*).

The mean and the standard deviation (SD) values of the effluent water quality indicators of the treatments were calculated. The treatment efficiency was calculated by using the mean difference between initial and final values. The experiments were analyzed by using one-way ANOVA followed by the Tukey post-hoc test and Tamhane's test (when equal variances were not assumed). SPSS® software Version 21 was used for the mentioned analyses. The differences were considered significant at $p<0.05$. The time series was interpreted by using a straightforward analysis of the plotted graphs prepared in MS-Excel®.

Results and Discussion

Effects of different C. rivularis stocking densities on water quality. The initial value of TAN was 2.41 mg L^{-1} and it was significantly removed during the experimental process. In the first four days of the treatment, TAN significantly decreased with increasing oyster stocking densities. At the lowest density (OY-30), the concentration of TAN was reduced from 2.41 mg L^{-1} to 1.39 mg L^{-1}, while in the medium (OY-40) and high (OY-50) oyster stocking densities, it was reduced to 1.13 mg L^{-1} and 0.91 mg L^{-1}, respectively. However, after this period, TAN slightly increased in all treatments and achieved its highest level at the end of the experiment in treatment OY-50. In the control, TAN slightly decreased during the first two days of treatment, but afterwards, TAN significantly increased until the end of the experiment. TAN was reduced by 35.7%, 34.2% and 25.2% in treatments OY-30, OY-40 and OY-50, respectively, after 8 days of experimentation and there were significant differences between treatments OY-30 and OY-50 ($p<0.05$) (Table 1).

Table 1 showed that in treatments OY-30 and OY-40, the concentration of NO_2-N decreased during the experiment, and at the end of experiment, NO_2-N was reduced by 58.3% and 48.5%, respectively. In treatment OY-50, NO_2-N decreased significantly in the first three days of the experiment, but after this period, it fluctuated, ranging from 0.88 to 1.19 mg L^{-1}. The removal of NO_2-N in the treatment of OY-50 (75.1%) was higher than that in treatments OY-30 (58.3%) and OY-40 (48.5%). The concentration of NO_3-N fluctuated during the sampling periods.

Table 1

Removal of TAN, NO_2-N NO_3-N, TN, PO_4-P, TP, TSS and total coliforms (%) at three different *Crassostrea rivularis* densities (30, 40 and 50 oysters m^{-2}) and the control after 8 days treatment

Parameters	*Treatments*								
		Control (CT) (no oysters)		*OY-30 (30 oysters m^{-2})*		*OY-40 (40 oysters m^{-2})*		*OY-50 (50 oysters m^{-2})*	
	Initial	*Final*	*Reduction (%)*	*Final*	*Reduction (%)*	*Final*	*Reduction (%)*	*Final*	*Reduction (%)*
TAN (mg L^{-1})	2.41± 0	2.82± 0.05	-17± 2.07^{c}	1.55± 0.03	35.8± 1.27^{a}	1.58± 0.03	34.3± 1.26^{ab}	1.8± 0.01	25.2± 0.48^{b}
NO_2-N (mg L^{-1})	4.37± 0	5.05± 0.13	-15.6± 2.86^{c}	1.82± 0.04	58.3± 0.92^{a}	1.37± 0.03	48.5± 0.7^{a}	1.09± 0.08	75.1± 1.87^{b}
NO_3-N (mg L^{-1})	4.11± 0	3.13± 0.09	23.8± 2.26^{c}	1.18± 0.02	71.2± 0.61^{a}	0.48± 0.17	88.3± 4.22^{a}	0.14± 0.02^{b}	96.5± 0.37^{b}
TN (mg L^{-1})	17.57± 0.03	15.37± 0.24	12.6± 1.39^{c}	9.7± 0.12	44.7± 0.6^{a}	8.9± 0.18	49.5± 1.13^{ab}	7.7± 0.16	56.2± 1.02^{b}
PO_4-P (mg L^{-1})	0.86± 0.01	1.31± 0.08	-49.8± 9.91^{b}	0.48± 0.03	43.6± 3^{a}	0.44± 0.03	49± 2.41^{a}	0.48± 0.03	44± 2.35^{a}
TP (mg L^{-1})	3.89± 0	4.56± 0.06	-17.1± 1.57^{b}	2.52± 0.07	35± 1.71^{a}	2.39± 0.07	38.5± 1.71^{a}	2.13± 0.07	45.2± 1.85^{a}
TSS (mg L^{-1})	216.6± 1.15	160.3± 7.03	25.8± 3.25^{c}	106.6± 5.51	50.7± 1.85^{a}	88± 3	59.3± 1.48^{a}	43± 7.81	80.1± 3.62^{b}
Total coliforms (10^3 MPN 100 mL^{-1})	57± 0	48.34± 2.31	15.2± 4.05^{b}	3.37± 0.65	94.1± 1.11^{a}	2.67± 0.58	95.3± 1.02^{a}	0.67± 0.29	98.8± 0.5^{a}

Note: TAN - total ammonia; TN - total nitrogen; TP - total phosphorus; TSS - total suspended solids; different superscripts show significant differences ($p<0.05$); the minus sign shows that the concentrations at the end of experimentation were higher than the initial value.

The concentration of NO_3-N increased by 12.1, 14.7 and 16.4% compared to the initial value in the first two days in treatments OY-30, OY-40 and OY-50, respectively, while it did not change in the control. However, after this period, there was a slight decrease in concentration until the end of the experiment with an overall reduction of approximately 71.2, 88.3 and 96.5% compared to the initial value in treatments OY-30, OY-40 and OY-50, respectively and 23.8% in control.

The effects of different oyster stocking densities on the removal of TN and TP are presented in Figure 2. Figure 2(a) shows that TN significantly decreased during the experiment in all treatments and in the control. The concentration of TN decreased from 17.54 to 9.7 mg L^{-1} for treatment OY-30, from 17.61 to 8.9 mg L^{-1} for treatment OY-40 and from 17.59 to 7.7 mg L^{-1} for treatment OY-50. Figure 2(a) shows that in the first four days of treatment, TN was significantly reduced at the same rate in all treatments. After this period, at the highest stocking density of oysters, the removal of TN was significantly higher than that in the low and medium stocking densities. Within 8 days of treatment, TN was reduced by 44.7, 49.5 and 56.2% in treatments OY-30, OY-40 and OY-50, respectively. Treatment OY-50 achieved a higher removal of TN, although no significant differences were found ($p>0.05$). TN was also reduced from 17.54 to 15.37 mg L^{-1} in the control, but the decrease was significantly lower compared to the treatment with oysters at densities from 30 to 50 oyster m^{-2} ($p<0.05$).

The concentration of PO_4-P decreased to almost half the initial value from 0.86 to 0.48, 0.44 and 0.48 mg L^{-1} in treatments OY-30, OY-40 and OY-50, respectively, while it significantly increased in the control to 1.31 mg L^{-1} (49.8%). Statistical analysis indicated no significant differences for the removal of PO_4-P among the three oyster treatments. Figure 2b showed that TP was reduced from 3.89 to 2.52, 2.39 and 2.13 mg L^{-1} in treatments OY-30, OY-40 and OY-50, respectively, but it increased to 17.1% in the control after 8 days of treatment. The experimental results indicated that, as oyster stocking density increased, the concentration of TP decreased and was lowest at the highest (OY-50) stocking density. This result may suggest that the concentration of nutrients in the treatment pond decreased with increasing oyster stocking density. The comparison of the three oyster stocking densities showed that, at the highest stocking density treatment, there was a significantly higher bioconversion of nutrients than in treatments with low and medium oyster stocking densities ($p<0.05$).

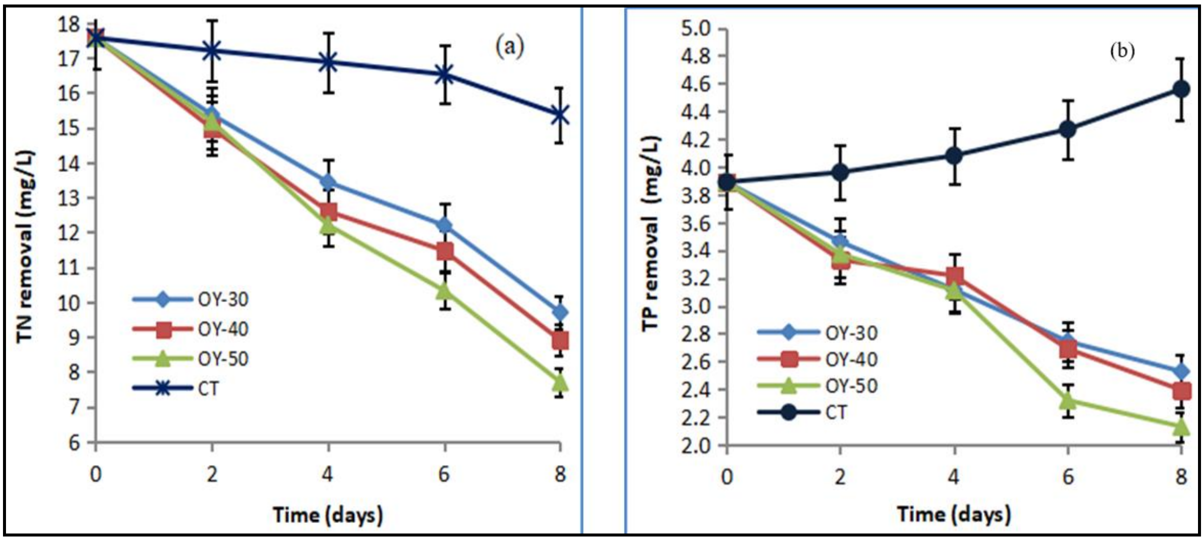

Figure 2. Removal of total nitrogen (TN) (a) and total phosphorous (TP) (b) at three *Crassostrea rivularis* densities (30, 40 and 50 oysters m^{-2}) and the control during 8 days.

Table 1 showed that *C. rivularis* were effective in removing TSS from shrimp wastewater with a significant reduction at all densities. TSS concentration in treatment OY-50 was higher than that in the other treatments. At the end of the experiment, TSS was reduced by 50.76, 59.32 and 80.09% in low, medium and high *C. rivularis* stocking densities, respectively. TSS also decreased in the control by 25.8% and was significantly lower compared to the treatments. This result indicated that the removal of TSS increased with increasing oyster stocking density. Table 1 also shows that total coliforms concentration decreased in all treatments. Total coliforms concentration in treatment OY-30 was higher than that in treatments OY-40 and OY-50. After 8 days of experimentation, the total coliforms were significantly reduced by 94.1, 95.3 and 98.83% in low, medium and high stocking densities, respectively, while it was reduced by only 15.2% in the control. The results suggest that the reduction of total coliforms slightly increased with increasing oyster stocking density, but there were no statistically significant differences among the three stocking densities.

Effects of different C. fluminea stocking densities on water quality. The concentrations of TAN and NO_2-N fluctuated during the sampling periods in all three treatments. TAN significantly decreased from 2.58 to 1.68 mg L^{-1} in treatment C-50, while it decreased only slightly to 2.05 and 2.11 mg L^{-1} in treatments CL-75 and CL-100, respectively, in the first three days of treatment. After this period, TAN still decreased until the end of treatment to 1.57 mg L^{-1} in treatment C-50, while it fluctuated around 1.8-2 mg L^{-1} in treatments C-75 and C-100. In the control, TAN slightly increased during the treatment duration. After 8 days of treatment, TAN was reduced by 39.3, 29.3 and 20.9% in treatments CL-50, CL-75 and CL-100, respectively, while it increased by 22.2% in the control compared to the initial value. NO_2-N was significantly reduced from 4.74 to 1.16, 1.61 and 2.01 mg L^{-1} in treatments CL-50, CL-75 and CL-100, respectively, within 8 days of treatment. At the end of treatment, NO_2-N was reduced by 75.5, 66 and 57.6% in treatments CL-50, CL-75 and CL-100, respectively, while it increased by 11.4% in the control compared to the initial value (Table 2).

NO_3-N significantly increased in the first two days of the experiment, but after this period, it slightly decreased in all treatments. At the end of the experiment, the lowest level of NO_3-N was observed in the treatment with lowest clam stocking density. NO_3-N decreased from 4.19 to 2.19, 2.89 and 2.55 mg L^{-1} (47.8, 31 and 39.1% in treatments C-50, CL-75 and CL-100), respectively, while it was reduced only with 15.2% in the control. A significant difference between the treatments and control ($p<0.05$) was observed. The concentration of PO_4-P was significantly reduced from 0.87 to less than 0.5 mg L^{-1} in all treatments, while it significantly increased in the control to 1.34 mg L^{-1} within 8 days of experimentation. The removal of PO_4-P was slightly increased with increasing clam stocking density. At the end of the experiment, the highest removal of PO_4-P was achieved in treatment CL-100, but no significant differences were found between results in all clam stocking densities.

Table 2

Removal of TAN, NO_2-N NO_3-N, TN, PO_4-P, TP, TSS and total coliforms at three different *Corbicula fluminea* densities (50, 75 and 100 clams m^{-2}) and the control after 8 days treatment

Parameters	*Treatments*								
		Control (No clams)		*C-50 (50 clams m^{-2})*		*C-75 (75 clams m^{-2})*		*C-100 (100 clams m^{-2})*	
	Initial	*Final*	*Reduction (%)*	*Final*	*Reduction (%)*	*Final*	*Reduction (%)*	*Final*	*Reduction (%)*
TAN (mg L^{-1})	2.58 ± 0.01	3.15 ± 0.03	-22.2 ± 1.12^{a}	1.57 ± 0.05	39.27 ± 1.98^{b}	1.82 ± 0.08	29.32 ± 0.06^{bc}	2.04 ± 0.06	20.93 ± 2.54^{c}
NO_2-N (mg L^{-1})	4.74 ± 0	5.28 ± 0.04	-11.39 ± 0.84^{a}	1.16 ± 0.03	75.52 ± 0.76^{b}	1.61 ± 0.04	66.03 ± 0.84^{b}	2.01 ± 0.03	57.59 ± 0.55^{c}
NO_3-N (mg L^{-1})	4.19 ± 0	3.55 ± 0.09	15.15 ± 1.55^{a}	2.19 ± 0.03	47.81 ± 0.6^{b}	2.89 ± 0.08	31.01 ± 1.04^{bc}	2.55 ± 0.09	39.05 ± 1.69^{b}
TN (mg L^{-1})	14.34 ± 0.02	12.85 ± 0.1	10.48 ± 0.68^{a}	5.38 ± 0.33	62.45 ± 2.32^{b}	7.17 ± 0.06	50.03 ± 0.37^{c}	11.88 ± 0.08	17.21 ± 0.54^{a}
PO_4-P (mg L^{-1})	0.87 ± 0	1.34 ± 0.02	-54.02 ± 2.3^{a}	0.49 ± 0.03	44.06 ± 3.51^{b}	0.44 ± 0.03	49.03 ± 3.04^{b}	0.37 ± 0.05	57.47 ± 5.27^{c}
TP (mg L^{-1})	5.17 ± 0.02	6.16 ± 0.05	-19.08 ± 0.91^{a}	1.41 ± 0.07	72.74 ± 1.34^{b}	1.79 ± 0.07	65.38 ± 1.35^{bc}	2.17 ± 0.09	58.09 ± 1.69^{c}
TSS (mg L^{-1})	212 ± 1	161.7 ± 4.04	23.74 ± 1.7^{a}	93.67 ± 7.02	56.03 ± 3.22^{b}	97.67 ± 5.5	53.92 ± 2.93^{b}	117 ± 5.29	44.82 ± 2.26^{b}
Total coliforms (10^3 MPN 100 mL^{-1})	62 ± 0	50.7 ± 0.58	17.37 ± 2.2^{a}	12 ± 1	80.65 ± 1.61^{b}	11.67 ± 0.15	81.18 ± 2.46^{b}	10 ± 1	83.71 ± 1.38^{b}

Note: TAN - total ammonia; TN - total nitrogen; TP - total phosphorus; TSS - total suspended solids; different superscripts show significant differences ($p<0.05$); the minus sign shows that the concentrations at the end of experimentation were higher than the initial value.

The effect of different clam stocking densities on the removal of TN and TP is shown in Figure 3. The data indicated that the different clam stocking densities significantly affected the reduction of TN. The concentrations of TN decreased during the experiment in all treatments and the control. TN was reduced from 14.34 to 5.38, 7.17 and 11.88 mg L^{-1} in treatments CL-50, CL-75 and CL-100, respectively. The highest removal was achieved at the low stocking density (CL-50) and the lowest removal at the high stocking density (CL-100). TN was reduced in low and medium stocking densities (60.45 and 50.03%, respectively), while it was reduced by only 17.21% at the highest stocking density (100 clams m^{-2}). There were significant differences in TN removal among the three stocking densities ($p<0.05$). In the control, TN was also reduced from 14.34 to 12.85 mg L^{-1}, but it was much lower than that in treatments with clam stocking at densities from 50 to 100 clams m^{-2}.

Figure 3b indicates that, in the first two days of treatment, TP in treatment CL-50 was initially reduced less than that observed in the treatments CL-75 and CL-100. However, after two days of treatment until the end of the experiment, the reduction of TP in the treatment with low stocking density was significantly higher than that observed in the medium and high stocking densities. Within 8 days of treatment, TP was reduced from 5.17 to 1.41, 1.79 and 2.17 mg L^{-1} in treatments CL-50, CL-75 and CL-100, respectively. The experimental results indicated that as the clam stocking density increased, the removal of TP decreased and was lowest at the highest (CL-100) stocking density.

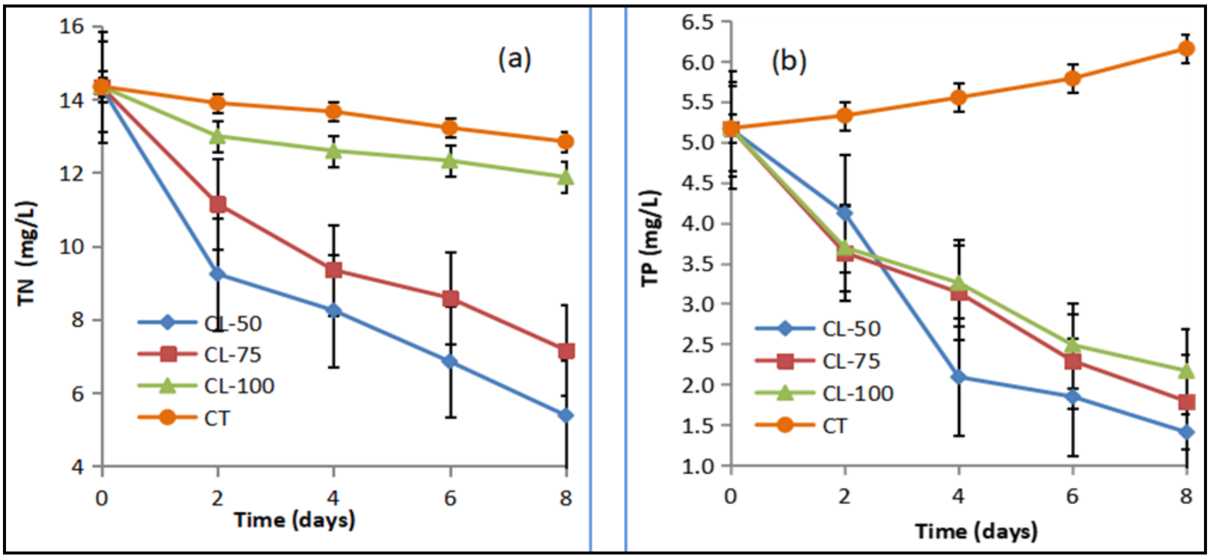

Figure 3. The removal of total nitrogen (TN) (a) and total phosphorous (TP) (b) in three different clam densities of 50 (CL-50), 75 (CL-75) and 100 (CL-100) clams m^{-2}.

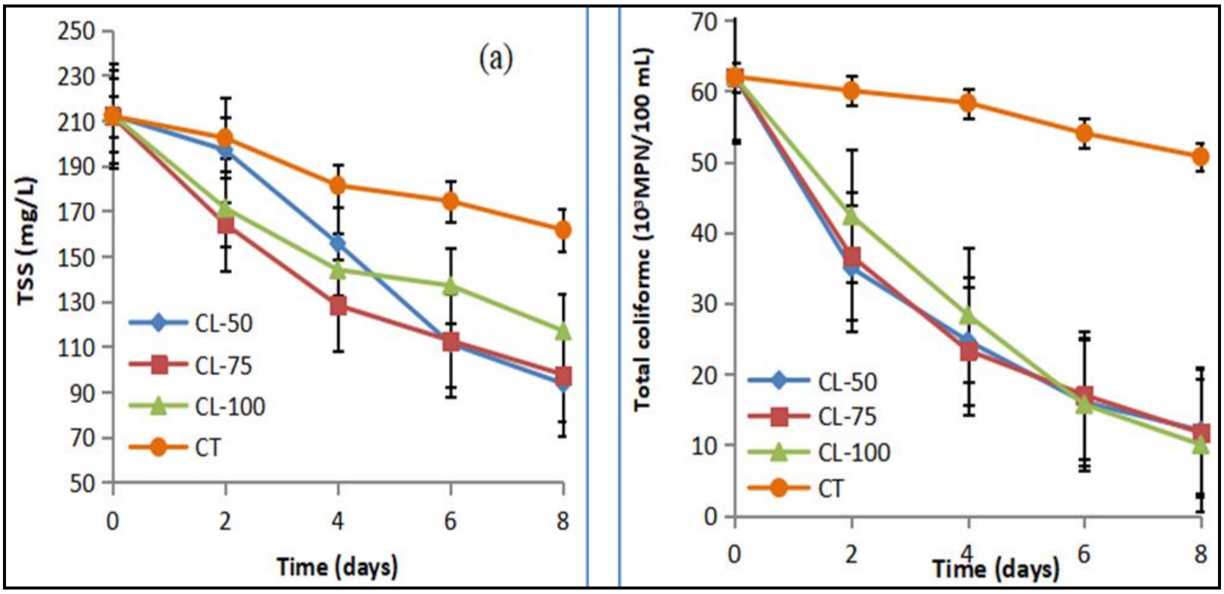

Figure 4. The removal of total suspended solids (TSS) (a) and total coliforms (b) in three different clam densities of 50 (CL-50), 75 (CL-75) and 100 (CL-100) clams m^{-2}.

Figure 4a indicates that the TSS concentration decreased in all treatments. In the first four days of treatment, TSS removal in treatment C-50 was slightly lower than in treatments CL-75 and CL-100. However, after this period, the removal of TSS in treatment CL-100 was slower than that in treatments CL-50 and CL-75. At low stocking density, TSS was reduced from 212 to 93.67 mg L^{-1} within 8 days, representing approximately 56.03% of the initial effluent value. In high and medium stocking densities, the concentration of TSS was reduced from 212 to 97.67 and 117 mg L^{-1} (53.92% and 44.82%), respectively, but there were no statistically significant differences among the three treatments. TSS was also reduced in the control from 212 to 161.7 mg L^{-1}, being approximately 23.74%.

Figure 4b shows that total coliforms concentration significantly decreased in all three treatments. The final level of total coliforms concentration in treatment CL-50 was higher than in treatments CL-75 and CL-100. After 8 days, the total coliforms concentration was significantly reduced by 94.09, 95.32 and 98.83% in low, medium and high stocking densities, respectively. Total coliforms concentration was also slightly reduced in the control (17.4%), but the removal was significantly lower compared to treatments with stocking clam densities from 50 to 100 clams m^{-2} ($p<0.05$).

Previous research has shown that filtration by bivalves such as oysters or clams can significantly reduce the concentration of TN, TP, TSS as well as bacteria in shrimp farm effluent, but may increase TAN and inorganic matter concentrations through bivalves excretion (Jones et al 2002; Khoi & Fotedar 2012). In the present study, the high density of *C. rivularis* and low density of *C. fluminea* effectively removed TN, TP, NO_3-N, PO_4-P, total coliforms, as well as TSS. Similarly, Jones & Preston (1999) and Jones et al (2001) reported that Sydney rock oysters (*Crassostrea virginica*) could remove phytoplankton, suspended solids and bacteria from shrimp pond effluent. Khoi & Fotedar (2012) used blue mussels at different densities (32, 64, 128 and 256 mussels m^{-2}) and showed that high mussel densities effectively removed TN, TSS and total bacteria, while PO_4-P and TP increased with increasing mussel stocking densities. This finding is similar to the finding in the present study for *C. fluminea*, but contradicts that of *C. rivularis* and the findings of Jones et al (2001, 2002) for *C. virginica*. In the present study, TP was reduced by 45.3% at the highest *C. rivularis* density and by 72.7%, at the lowest *C. fluminea* density, while the study of Jones & Preston (1999) reported that *C. virginica* can remove up to 67% TP from shrimp pond effluents. *C. fluminea* showed higher performance in the removal of TP concentration than oysters due to *C. fluminea* filtering the effluent in the tank bottom, while oysters filtered it in the water column. Other clam species (*Anomalocardia brasiliana*) showed potential for the reduction of NO_2-N, NO_3-N, alkalinity, TSS and orthophosphate in 96 h (Brito et al 2018). The use of the clam *A. brasiliana* at 2.5 kg wet weight per m^3 of shrimp effluent, for the treatment of effluents in a biofloc system with a salinity of 25‰ for 72–96 h, provides good services to the culture environment (Brito et al 2018). Although shrimp effluent contains a high proportion of small particles rich in P, which may be filtered by oysters and mussels, in the tank bottom the nutrient concentration equilibrium may be higher than in the water column. Moreover, oyster and clam excretion will probably settle in the tank bottom, resulting in an increased TP, especially at high stocking densities, if bivalves are used alone.

Conclusions. The removal rate of NO_2-N, NO_3-N, TN, TP, TSS, as well as total coliforms increased with increasing oyster stocking density from 30 to 50 oysters m^{-2}, while TAN removal efficiency achieved was highest at the lowest oyster stocking density. However, the final levels of TAN at these three oyster densities were lower than the threshold (2 mg L^{-1}) of the wastewater standard. The nutrients (N and P) removal of the treatment with stocking oyster densities from 30 to 50 oyster m^{-2} (equivalent biomass 1500 g to 2500 g in 1 m^3 wastewater) were significantly higher compared to the control. PO_4-P removal was not significantly different in the three oyster stocking densities, but was significantly higher compared to the control. Thus, the optimal oyster density for maximizing nutrient removal efficiency was 50 oysters m^{-2}, approximately 2500 g m^{-3}. The study also indicated that the removal of nutrients including N and P in shrimp wastewater in the treatment with low clam stocking density was significantly higher than that in treatments with medium and high stocking densities (50 to 100 clams m^{-2}, approximate biomass from 1000–2000 g m^{-2}). The concentrations of TSS and total coliforms were significantly reduced in all three treatments, but there were no significant differences between the three clams stocking densities. Thus, the removal efficiency of TP decreased with increasing clam stocking density. The optimal clam density for maximizing nutrient removal efficiency was 50 clams m^{-2} (approximately 1000 g m^{-3}).

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Conflict of Interest. The author declares that there is no conflict of interest.

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