Ammonia, Phosphate, Total Suspended Solid and Chlorophyll *a* Removal in Mangrove Habitat Receiving Shrimp Pond Effluents

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Abstract

Diseases continue to devastate the shrimp industry. One culture system that has the potential to abate disease occurrence, improve shrimp survival and environment-friendly is aquasilviculture. Aquasilviculture is the culture of aquatic organism with mangroves inside the pond (mixed system) or in the receiving environment (separate). A previous study reported that the presence of mangroves in the receiving environment enhances shrimp survival via an improved incoming water quality. The present study determined the time required for a mangrove habitat to remove nutrients from shrimp (*Penaeus monodon*) farm effluents and the factors affecting mangrove efficiency to remove nutrients. Results showed that ammonia, phosphate, chlorophyll a and total suspended solids (TSS) were fluctuating but statistically lower in water drained into mangrove habitat (Mangrove to Pond Area Ratio, (MPR)=2:1 and MPR=4:1) compared to area without mangroves (MPR=0). At MPR=4:1, ammonia is removed from the water after 3 days; TSS after 2 days; phosphate and chlorophyll a after 7 days. At MPR=2:1, only ammonia can be efficiently removed after 3 days. These results further showed that the type of nutrient and MPR affect the efficiency of mangroves to remove nutrients from shrimp farm effluents.

The growth of plants in areas receiving and not receiving shrimp farm effluents were compared by measuring the monthly increase in the seedling height and the increase in the stem length between two nodes in saplings and trees. After 3 months, increase in growth was greater in plants in area receiving shrimp farm effluents compared to those not receiving, except for the seedlings. This indicates that mangroves purify the water by nutrient uptake as supported by the data showing greater increase in stem length in saplings and trees.

Introduction

Diseases continue to devastate the shrimp industry. One culture system that has the potential to abate disease occurrence and improve shrimp survival is aquasilviculture. Aquasilviculture is the culture of aquatic organism with mangroves. There are 2 types of aquasilviculture system: mixed, wherein mangroves are inside the culture pond; and separate, wherein the mangroves are in the receiving environment (Primavera *et al*., 2000). Less disease incidence is reported in areas with mangroves (Belak *et al*., 1999). No Whitespot Syndrome Virus (WSSV) outbreak was observed in shrimp cultured in pens inside mangrove habitat despite WSSV outbreak in adjacent ponds (Lebata *et al*., 2012). High mangrove-to-pond area ratio (MPR) is a WSSV protective factors (Tendencia *et al*., 2011). One-way mangrove influences disease occurrence could be via an improved water quality (Tendencia *et al*., 2012). Higher phosphorus (P) and nitrogen (N) removal percentage in ponds with mangrove seedlings than in ponds without seedlings were observed in the Pacific whiteleg shrimp *Litopenaeus vannamei* experimental ponds (Moroyoqui-Rojo *et al*., 2012). Several studies have reported the capability of mangroves to remove nutrients from pond effluents and this depends on the ratio of mangrove forest: shrimp pond area (MPR) (Robertson and Philipps, 1995; Shimoda *et al*., 2005, 2007; Primavera *et al*., 2007). Reported MPR varied depending on the type of nutrient to be removed and the type of system. An MPR of 4:1 was reported to establish a healthy ecosystem (Saenger *et al*., 1983). In a separate system, an MPR of 2 to 22:1 is required to filter the N and P loads (Robertson and Philipps, 1995); while an MPR of 0.04 to 0.12: 1 is required to completely remove the dissolved inorganic nitrogen (Rivera-Monroy *et al*.,1999). In mixed mangrove and pond systems, MPR of 2.1 to 5.2: 1 is required to remove the N remaining in the aquaculture pond (Shimoda *et al*., 2007), while 6.2 to 8.9:1 is required to fully process the P (Shimoda *et al*., 2005). Impounded mangroves of 1.8-5.4 ha are required to remove the nitrate wastes from 1 ha of shrimp pond (Primavera *et al*., 2007).

Other importance of mangroves, aside from being a biofilter is that some parts of the mangrove tree, particularly the leaves, have antimicrobial properties (Sudheer *et al*., 2011). The leaves may also provide a suitable substrate for the growth of favourable periphytic biofilm (Tran & Yakupitiyage, 2005; Gatune *et al*., 2012). Furthermore, leaf litter enriches the environment during decomposition. The presence of mangrove can also mitigate the effects of climate change through carbon sequestration (Donato *et al*., 2011) and lowers air and water temperature (Le, 2006).

This paper determined the time required for a mangrove habitat to remove nutrients (i.e. N, P) from shrimp farm effluents; and, the factors affecting mangrove efficiency to remove nutrients.

Materials and method

Two experiments were done using earthen ponds. The number, size and purpose of the ponds used in the two experiments are presented in **Table 1.** Aeration was provided using a paddle wheel. No water change was implemented in the entire duration of the experiments. Water loss due to evaporation was replenished as needed using water from a reservoir to maintain 1.0 m water depth. Shrimp were fed commercial pellet (please see **Table 2** for the proximate analysis) ad libitum until termination.

In the first experiment, MPR=2:1 vs MPR=0, *P. monodon* postlarvae (ABW= 0.006) were stocked at 20 ind/m^2 in a 700 m^2 earthen pond. Pond effluents were drained into 2 types of receiving environment: with mangroves (area=1400 m^2; MPR=2:1) and without mangroves (4 m^2; MPR=0) after 50 days of shrimp culture. MPR used was lower than the reported MPR needed for a healthy ecosystem, thus, this experiment was not replicated.

In the second experiment, MPR=4:1 vs MPR=0, *P. monodon* postlarvae

Table 1. Number, size and purpose of the ponds used in the two experiments

Pond No	# of units	Description	Purpose	Size	
				Experiment 1	Experiment 2
1	1	With mangrove	Settling pond*	1.4 ha	1.4 ha
2	1	Without mangrove	Culture pond	0.7 ha	0.35ha
3	1	With few mangrove	Reservoir	0.6 ha	0.6 ha
4	1	Without mangrove	Settling Pond*	0.1 ha	0.1 ha

*Received pond effluents

Table 2. Proximate analysis of the commercial pellet used to feed the experimental shrimp

Composition	Amount (dry weight basis)
Crude protein (% min)	47
Crude fat (% min)	8
Crude fiber (% max)	3
Crude ash (% max)	16
Moisture (% max)	12

(ABW=0.005) were stocked at 25 ind/m^2 in a 350 m^2 earthen pond. Pond effluents were drained into 2 types of receiving environment: with mangroves (area=1400 m2; MPR=4) and without mangroves (4 m2; MPR=0) after 120 days of shrimp culture. The experiment was replicated three times using the same set of ponds.

In both experiments, water samples for ammonia and phosphate analyses were taken from the receiving environments daily on the first week after water drain and weekly thereafter until the 4th week. Water samples for chlorophyll a and total suspended solids (TSS) analyses were taken from the receiving environment on days 0, 1, 6, 7, 14, 21, 28 and days 0, 1, 2, 3, 5, 7, 14, 21, 28, respectively. Water parameters were measured following procedures described in the 23rd edition of the Standard Methods for the Examination of Water and Wastewater (2017). Ammonia-nitrogen content was measured using the phenate method (APHA AWWA WEF 4500-NH_3); phosphate, using the ascorbic acid method (APHA AWWA WEF 4500-P); TSS, filtration-oven drying method (APHA AWWA WEF 2540); and chlorophyll a using spectrophotometry (APHA AWWA WEF 10200).

Mangrove community structure (MCS) was done before the start of the first trial of Experiment 2 and before the start of every trial of the experiment and after the third trial. MCS was done approximately 1 month after draining shrimp farm effluent. Density was calculated using a formula of Krebs (1999):

Density = Number of individuals per plant type / the area of the sample (ha)

In a separate investigation, the effect of shrimp farm effluent on mangrove plant was done by measuring plant growth in areas receiving and not receiving shrimp farm effluents. Plant growth in the two environments were compared by measuring the monthly increase in seedling height and stem length between two nodes in saplings and trees. The measurement was done monthly for 3 months on 10 trees, 10 saplings, and 10 seedlings, each site, that were tagged and labelled, so that the same plant is measured during samplings.

Statistical analysis

Water nutrient levels were analysed using ANOVA and repeated measures using SPSS V 23.

Results

In Experiment 1, ammonia, phosphate, chlorophyll a and total suspended solids were fluctuating but generally lower, especially the ammonia level, in water drained into habitat with mangroves compared to those without mangrove (**Table 3**). Ammonia was not detected in habitat with mangrove 3 days after draining; phosphate after 5 days; while levels of these nutrients in ponds without mangrove remained high until after 14 days. Using repeated measures, ammonia and chlorophyll a were significantly reduced in the receiving environment with mangroves (MPR=2:1) (**Table 4**).

In Experiment 2, ammonia, phosphate, chlorophyll a and total suspended solids (TSS) were fluctuating (**Table 5**). Phosphate was not detected in habitat with mangrove 5 days after draining; TSS after 28 days. Chlorophyll a was reduced three times after 28 days. TSS in the receiving environment without mangrove increased after 2, 5, and 28 days of incubation. Phosphate was removed from both environments. Using repeated measures, significantly lower levels of ammonia, total suspended solid and chlorophyll a were observed in the habitat with mangroves that received shrimp farm effluents compared to that without mangrove (**Table 6**).

Results of the mangrove community structure is presented in **Table 7**. The number of trees, saplings and seedlings increased after receiving shrimp pond effluents after each experimental trial. In the investigation of the effect of pond effluent on mangrove, after 3 months, increase in plant growth was greater in area receiving shrimp farm effluents compared to those not receiving, except for the seedlings (**Table 8**).

Discussion

This study confirms previous reports that mangroves are capable of removing nutrients from pond effluent. Suspended solids, total phosphorus content, ammonia-nitrogen, and nitrate-nitrogen are efficiently removed in microcosms planted with Avicennia marina, Rhizophora stylosa, and Lumnitzera racemose (Su *et al*., 2019). Nutrient removal in ponds with mangrove seedlings is higher than in ponds without seedlings, thus improving water quality and reducing nutrients in the effluent (Moroyoqui-Rojo *et al*., 2012). In this study, only ammonia was removed from shrimp pond effluent drained into an area with mangrove at MPR=2:1. At higher MPR of 4:1, other parameters such as phosphate, TSS and chlorophyll a were also removed. This observation is in consonance with previous reports that MPR affects the efficiency of mangroves to remove nutrients from pond effluents. Reported MPR required to remove

Table 3. Levels of ammonia, phosphate, total suspended solids (TSS) and chlorophyll a observed in the receiving environment with, MPR=2:1, and without mangroves (Experiment 1). Values in the same column with the same superscript are not significantly different (P>0.05)

Day	Ammonia (ppm)		Phosphate (ppm)		TSS (ppm)		Chlorophyll a (ppm)	
	With mangroves	Without Mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves
0	.09^{c}	.09^{b}	.035^{c}	.01^{a}	355^{bc}	355	nm	nm
1	0.17^{d}	.17^{e}	.035^{c}	.025^{b}	455^{d}	340	nm	nm
2	nm	nm	Nm	nm	nm	nm	nm	nm
3	.00^{a}	.05^{a}	.01^{ab}	.02^{b}	375^{cd}	344	nm	nm
4	.00^{a}	.12^{cd}	.02^{bc}	.01^{a}	nm	nm	nm	nm
5	.00^{a}	.095^{bc}	.00^{a}	.01^{a}	246^{a}	304	nm	nm
6	.045^{ab}	.19^{e}	.055^{c}	.06^{ac}	nm	nm	39	39
7	.00^{a}	.08^{b}	.02^{bc}	.01^{a}	282^{ab}	348	38	44
14	.01^{ab}	.14^{d}	.02^{bc}	.02^{b}	340^{bc}	359	37	38
21	nm	nm	Nm	nm	nm	nm	nm	nm
28	nm	nm	Nm	nm	nm	nm	nm	nm

nm= not measured

Table 4. Mean ammonia, phosphate, total suspended solids (TSS) and chlorophyll a level observed in the receiving environment with (MPR=2:1) and without mangroves (MPR=0) (Experiment 1)

Parameter	Unit	With mangrove	Without mangrove	P-value
Ammonia-N	ppm	0.035	0.043	0.002
Phosphate-P	ppm	0.019	0.018	0.423
TSS	ppm	255.38	256.25	0.925
Chlorophyll a	ppm	37.71	40.85	<0.000

Table 5. Levels of ammonia, phosphate, total suspended solids (TSS) and chlorophyll a observed in the receiving environment with, MPR=4:1, and without mangroves (Experiment 2). Values in the same column with the same superscript are not significantly different (P>0.05)

Day	Ammonia (ppm)		Phosphate (ppm)		TSS (ppm)		Chlorophyll a (ppm)	
	With mangroves	Without mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves	With mangroves	Without mangroves
0	$.08^{d}$	$.08^{b}$	$.085^{cd}$	$.085^{e}$	118^{e}	118^{cd}	118^{e}	118^{e}
1	$.035^{bcd}$	$.89^{i}$	$.085^{cd}$	$.055^{abcd}$	34^{ed}	46^{b}	90^{d}	101^{d}
2	$.06^{cde}$	$.36^{g}$	$.095^{d}$	$.055^{cde}$	160^{b}	147^{f}	nm	nm
3	$.045^{bc}$	$.25^{e}$	$.075^{cd}$	$.055^{cde}$	31^{c}	43^{g}	nm	nm
4	$.07^{de}$	$.23^{e}$	$.115^{d}$	$.015^{abc}$	nm	nm	nm	nm
5	$.225^{f}$	$.165^{d}$	$.00^{a}$	$.006^{a}$	110^{a}	165^{c}	nm	nm
6	$.195^{f}$	$.13^{c}$	$.045^{bc}$	$.011^{ab}$	nm	nm	nm	nm
7	$.15^{e}$	$.405^{g}$	$.00^{a}$	$.05^{bcde}$	14^{a}	111^{c}	33^{a}	68^{b}
14	$.045^{bc}$	$.53^{h}$	$.03^{ab}$	$.07^{de}$	82^{cd}	123^{d}	74^{c}	76^{c}
21	$.01^{a}$	$.055^{b}$	$.015^{ab}$	$.00^{a}$	16^{a}	17^{a}	49^{b}	61^{ab}
28	$.03^{ab}$	$.015^{a}$	$.00^{a}$	$.00^{a}$	12^{a}	138^{e}	43^{b}	58^{a}

nm= not measured

Table 6. Mean ammonia, phosphate, total suspended solids (TSS) and chlorophyll a level observed in the receiving environment with (MPR=4:1) and without mangroves (MPR=0) (Experiment 2)

Parameter	Unit	With mangroves	Without mangroves	P-value
Ammonia-N	ppm	0.087	0.283	<0.000
Phosphate-P	ppm	0.045	0.036	0.176
TSS	ppm	64	100	<0.000
Chlorophyll a	ppm	67.94	80.31	<0.000

Table 7. Mangrove community structure of the mangrove area receiving shrimp pond effluent

Sampling Period	Trees (number/ha)	Saplings (number/ha)	Seedlings (number/ha)
A	980	1040	1850
B	1185	1333	2444
C	1466	1570	2963
D	1833	1800	3585

A= before the start of Experiment 2 Trial 1

B= before the start of Experiment 2 Trial 2

C= before the start of Experiment 2 Trial 3

D= one month after Experiment 2 Trial 3

Table 8. Average monthly increase in mangrove growth observed in mangrove habitat receiving and not receiving shrimp farm effluent over a 3-month period

	Average monthly increase in growth (mm)		
	Trees	Saplings	Seedlings
Receiving	5	12	52
Not Receiving	4	9	66

nutrients from pond effluent varies and has a wide range. An MPR of 2 to 89.9:1 is required to filter the phosphorus load (Robertson and Philipps, 1995; Shimoda *et al*., 2005); while an MPR of 1.8 to 5.4: 1 is required to remove nitrogen (Shimoda *et al*., 2007; Primavera *et al*., 2007) from farm effluents. The variability in the MPR required to remove nutrients from pond effluent could be attributed to the mangrove hydrodynamics (Robertson and Philipps, 1995). However, MPR of 4:1 reported to establish a healthy ecosystem (Saenger *et al*., 1983) can be implemented or used as rule of thumb. This is supported by the result of this study wherein NH4+, PO43-, TSS, and chlorophyll a in shrimp farm effluent were efficiently reduced in MPR=4:1.

As long as there is mangrove in the receiving environment or MPR is not zero, MPR does not seem to affect nitrogen removal. Nitrogen is removed from the pond effluent through nitrification and denitrification processes during which nitrogen is released into the environment making it available for plant growth (Dai *et al*., 2021; Wang *et al*., 2019). Bacteria responsible for the two processes, nitrification and denitrification, are abundant in mangrove habitats (Dai *et al*., 2021).

Phosphate is removed from the pond effluent by plant uptake and by the incorporation of phosphate into TSS (Ouyang and Guo, 2016; Ruzhitskaya and Gogina, 2017). The latter explains for the increase in the TSS level in receiving environments without mangrove; and the non-significant difference in the phosphate level in pond effluent drained in both types of receiving environments and in both MPR. Phosphate in the pond effluent bonded with the TSS in all types of environments.

Mangroves strip pond effluent of nutrients through plant assimilation. This is evident in this study by the increasing number of trees, saplings and seedlings after each experiment. Furthermore, this is also shown in the greater increase in plant growth in environment receiving shrimp farm effluents compared to those not receiving, except for the seedlings. The increase in the number and growth of mangroves in receiving environment with mangrove is consistent with the findings of Capdeville *et al*., (2019). Some of the seedlings being monitored for growth in the environment with mangroves also died. The lesser increase in growth and mortality observed in the seedlings in receiving environment with mangroves could be due to eutrophication. The increase in nutrient resulted in the increase in the number of seedlings that resulted in an overcrowding and eventually some of the older seedlings died and the others did not grow well.

In summary, the study demonstrated the efficiency of mangrove forest to remove nutrients, TSS and chlorophyll a from shrimp pond effluents. Although the levels of ammonia, phosphate, chlorophyll a and TSS were fluctuating, they are statistically lower in effluents drained into the mangrove habitat (MPR=2:1 and MPR=4:1) compared to area without mangroves. At MPR=4:1, ammonia is removed from the water after 3 days; TSS after 2 days; phosphate and chlorophyll after 7 days. At MPR=2:1, only ammonia can be efficiently removed and after 3 days. Mangroves purify the shrimp farm effluent by nutrient uptake as shown in the greater increase in stem length in saplings and trees in environments receiving the effluent.

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