Filter Feeding by Blood Cockle, *Anadara granosa*, for Water Quality Improvement in Closed Culture System of Pacific White Shrimp (*Litopenaeus vanamei*)

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ABSTRACT

The possibility of using blood cockle as a means to improve water quality in Pacific white shrimp culture system through the removal of particulate matter from water was investigated. Juvenile Pacific white shrimp (*Litopenaeus vanamei*) was cultured with and without blood cockle, *Anadara granosa*, in an outdoor closed culture system (without water exchange) for 2 months. Results of this experiment showed thatblood cockle is very effective in removing particulate matter, particulate nitrogen, phytoplankton and particulate phosphorus from Pacific white shrimp culture water. Co-culture of blood cockle with Pacific white shrimp resulted in decreasing turbidity by 59.0%, total particulate matter by 60.0%, particulate organic matter by 66.0%, chlorophyll *a* by 85.2%, particulate nitrogen by 75.0%, and particulate phosphorus by 55.4%. Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, particulate phosphorus and total ammonia nitrogen were 5.0 NTU, 49.6 µg/L, 12.4 mg/L, 8.1 mg/L, 0.595 mg/L, 0.100 mg/L, and 0.354 mg/L, respectively, in Pacific white shrimp and blood cockle treatment compared to average values of 12.2 NTU, 335.2 µg/L, 31.0 mg/L, 23.8 mg/L, 2.377 mg/L, 0.224 mg/L, and 0.277 mg/L, respectively, in Pacific white shrimp treatment. Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus in Pacific white shrimp and blood cockle treatment were significantly lower ($P<0.05$) than those of Pacific white shrimp treatment but there was no significant difference ($P>0.05$) between average value of total ammonia nitrogen, production rate, survival rate and growth rate of Pacific white shrimp of the two treatments.

Keywords: Pacific white shrimp, *Litopenaeus vanamei*, blood cockle, *Anadara granosa*, closed culture system

INTRODUCTION

Shrimp pond water quality problems can be traced to two basic sources: (1) the excess feed that enriches the pond water and produces unstable algal population and

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increases the suspended organic detritus density in the water column, and, (2) the excessive settlement of suspended solids to the bottom (Wang, 1990). According to Funge-Smith and Briggs (1998), feed was the principal source of nitrogen and phosphorus in intensive shrimp culture systems. Approximately 80% of nitrogen added to ponds as shrimp feed was not retained as shrimp biomass (Briggs and Funge-Smith, 1994; Jackson *et al*., 2003). Remaining nitrogen fuels plankton and microbial production within ponds, often resulting in negative effects on pond water and sediment quality, such as anoxia, nutrient toxicity, and blooms of undesirable algal species (Moriarty, 1997; Burford and Glibert, 1999). Subsequently, pond operators periodically flush ponds to minimise these effects, discharging untreated pond water into nearby rivers, creeks and estuaries (Paez-Osuna *et al*., 1997; Funge-Smith and Briggs, 1998; Jackson *et al*., 2003), leading to deleterious effects in receiving waters such as eutrophication (Sansanyuth *et al*., 1996; Naylor *et al*., 1998 cited by Costanzo *et al*., 2004).

Bivalve filter feeders play a key role in many coastal ecosystems due to their high filtration capacity and culture density (Nunes *et al*., 2003). As filter feeders, bivalves feed mostly on phytoplankton and suspended organic matter, which are normally found in high concentrations in shrimp ponds as a result of fertilization and unconsumed feed (Chamberlain, 1988 cited by Costanzo *et al*., 2004). Thus, filter feeders are able to utilize natural productivity and organic matter in ponds and convert them into biomass. At the same time, these filter feeders contribute to the control of phytoplankton blooms which could cause problems in the culture system (Smith, 1985; Smith and Piedrahita, 1988; Shpigel and Blaylock, 1991). Mollusks also help to reduce organic matter content in the water column and sediments. This organic matter mostly came from unconsumed feed and, at high levels, may cause a serious problem in the ponds.

Blood cockle (*Anadara granosa*) is a marine bivalve with an economic value, and cultured in several coastal mud-flats in Thailand. Natural population of blood cockle is also found in several coastal mud-flats in the Gulf of Thailand. In this study we investigated the possibility of using blood cockle to improve water quality in Pacific white shrimp intensive closed culture systems by studying the effect of blood cockle-Pacific white shrimp co-culture on particulate matter, particulate organic matter, particulate nitrogen, particulate phosphorus, chlorophyll *a* concentration and other related water quality parameters, and on the production and growth of Pacific white shrimp.

MATERIALS AND METHODS

The experiment was conducted in 75x155x60 cm (WxLxH) fiberglass tanks with water recirculation system but without water exchange. A cement board was used to partition the fiberglass tanks into two compartments for Pacific white shrimp and blood cockle. The bivalve compartment was 75x40x60 cm (WxLxH) and shrimp compartment was 75x115x60 cm height

(WxLxH). In the shrimp compartment, 90 cm long baffle was placed perpendicularly to the tank partition to direct water flow. Water depth in the tanks was 50 cm. Water from the lower part of the bivalve section was airlifted into the shrimp section by two 0.5 inch PVC tubes, flowed around center baffle back to the bivalve section through a plastic screen on a rectangular hole (15x30 cm) on another side of the partition. The hole was cut at 10 cm above tank bottom level. Recirculation rate of water through airlift system was 4 L/min. Two plastic trays (35 x55x15 cm height) were placed on a PVC stand in the bivalve section. The first tray was placed 10 cm above tank bottom and the second tray was 10 cm above the first tray. Two air stones were placed on the tank bottom under the trays. Stocking rate of the bivalve was 1.0 kg/tank (0.5 kg/tray). Two air stones were placed in shrimp compartment on both sides of the center baffle (Figure 1).

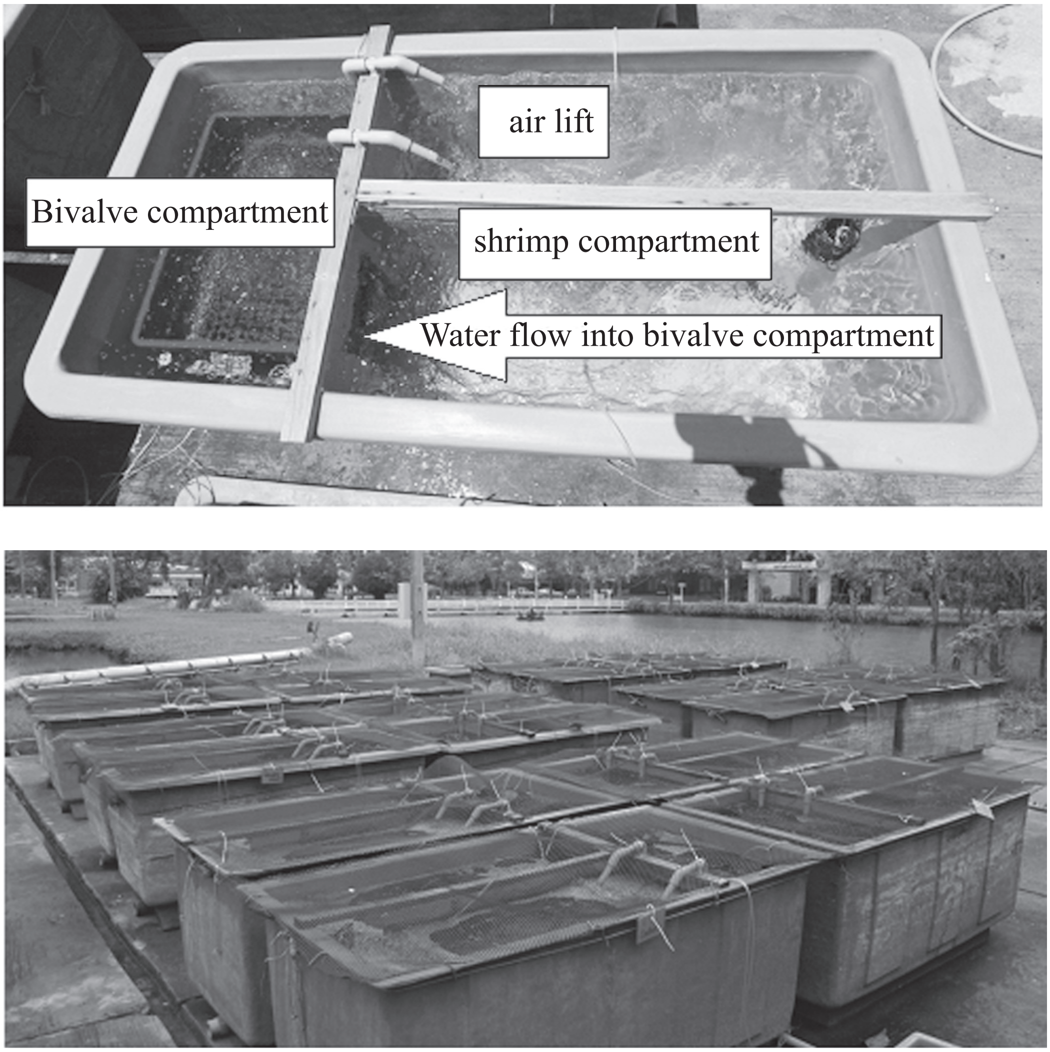


Figure 1. Experimental tanks.

Water used in this experiment was diluted salt water mixed with water from a shrimp pond. Salinity was 17.5 ppt. The volume of water in the shrimp compartment was 0.43 m^3 while that in the bivalve compartment was 0.15 m^3., for a total volume in the experimental tank of 0.58 m^3. Shrimp were stocked at 50 shrimp/tank for a stocking density of for shrimp was 50/0.58 m^3 or 86/0.58 m^3 of total water volume. Average initial weight of shrimp were 10.3 in shrimp treatment and 9.9 g in shrimp and blood cockle treatment. Blood cockle was were stocked at the rate of 1.0 kg/ 0.58 m^3 or 1.72 kg/ $m^3$of total water volume. Average initial weight of blood cockle was 12.6 g.

The experiment consisted of three replications. Three similar tanks were also set up as control with the same stocking rate of shrimp, but no bivalves were stocked in the plastic baskets in the bivalve compartment. Shrimp were fed twice daily at the recommended feeding rate of the commercial feed pellets used. Experimental period was 8 weeks. Water samples were collected once a week from the shrimp compartment to analyze for dissolved oxygen, pH, turbidity, total particulate matter, particulate organic matter, particulate nitrogen, particulate phosphorus, chlorophyll *a*, total ammonia nitrogen, pH, salinity and dissolved oxygen. Experimental animals were counted and weighted at the beginning and at the end of the experiment to calculate survival and growth rates.

Total particulate matter was analyzed by filtering the samples through GF/C glass fiber filter, dried in an oven at 103-105^{o}C overnight, then weighed (APHA *et al*., 2005). The samples were then transferred to a muffle furnace and ignited at 450^{o}C for 4 h, cooled in a dessicator and weighed to obtain particulate organic matter fraction which is equal to the weight lost after 4 h ignition in the muffle furnace. Particulate nitrogen was analyzed using Kjeltec 1035 analyzer unit, Tecator: Digestion block, Foss, Model 2520. Particulate phosphorus was analyzed spectrophotometrically after percholic acid digestion. Chlorophyll *a* content was analyzed using spectrophotometric determination method after extracting with acetone (APHA *et al*., 2005). Turbidity was measured by Turbidimeter HACH 2100Q. Dissolved oxygen was measured by YSI Dissolved oxygen meter. pH of water was measured by YSI pH meter. Total ammonia nitrogen was measured by Phenate Method (APHA *et al*., 2005). Data were analyzed for mean and standard deviation and compared by t-test.

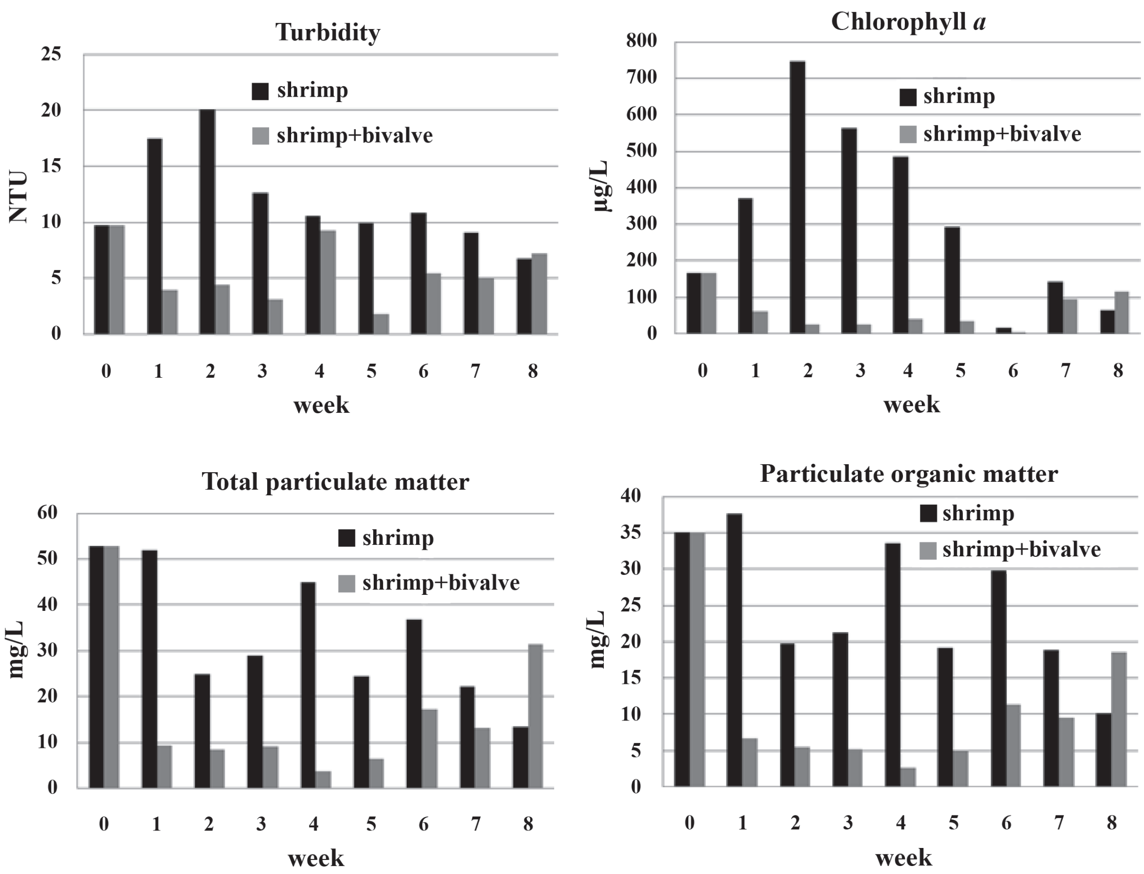
RESULTS AND DISCUSSION

Results from this experiment indicated that introduction of blood cockle into closed culture system of Pacific white shrimpcan improve water quality in the culture system by reducing water turbidity, phytoplankton, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus (Table 1, Figure 2). Statistical analysis indicated that average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus in tanks with Pacific white shrimp and blood cockle were significantly lower ($P \leq 0.05$) than those in tanks with Pacific white shrimp only.

Table 1. Comparison of average values of turbidity, total particulate matter (TPM), particulate organic matter (POM), chlorophyll *a*, total ammonia nitrogen (TAN), particulate nitrogen (PN), particulate phosphorus (PP), dissolved oxygen (DO) and pH of water in tanks culturing shrimp and shrimp + blood cockle (mean±S.D.)

water quality parameter	shrimp	shrimp + bivalve	% decrease
Turbidity (NTU)	12.2±4.5[a]	5.0±2.4[b]	59.0
TPM (mg/L)	31.0±12.8[a]	12.4±8.7[b]	60.0
POM (mg/L)	23.8±9.0[a]	8.1±5.1[b]	66.0
Chlorophyll *a* (µg/L)	335.2±256.6[a]	49.6±38.3[b]	85.2
TAN (mg/L)	0.277±0.217[a]	0.354±0.310[a]	-
PN (mg/L)	2.377±0.973[a]	0.595±0.204[b]	75.0
PP (mg/L)	0.224±0.086[a]	0.100±0.046[b]	55.4
DO (mg/L)	6.9±0.23[a]	6.7±0.21[a]	-
pH	8.1±0.1[a]	7.9±0.09[a]	-
Salinity (ppt)	15.3±3.1[a]	15.3±3.0[a]	-

Average values denoted by different superscript in each parameter are statistically significant different (P≤0.05)



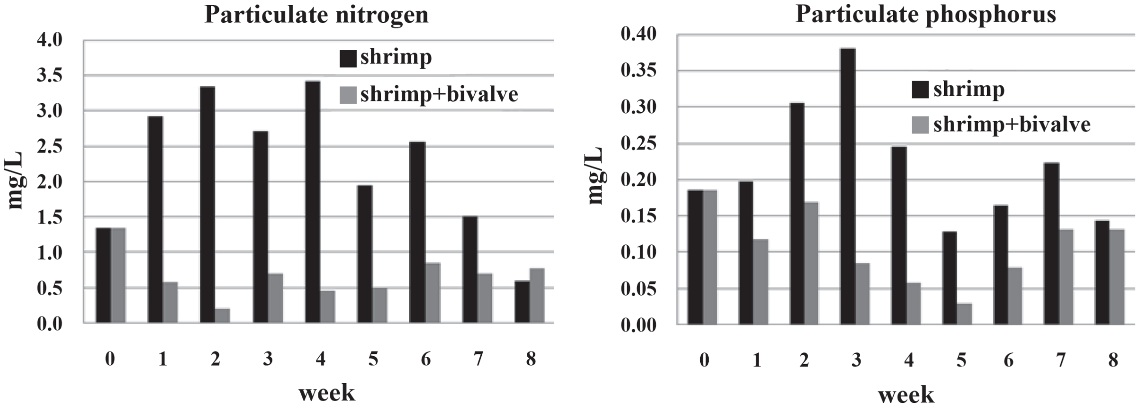


Figure 2. Average values of total particulate matter, chlorophyll *a*, particulate organic matter, particulate nitrogen, particulate phosphorus and turbidity of water in tanks culturing shrimp and shrimp + blood cockle.

Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, and particulate phosphorus in treatment with Pacific white shrimp and blood cockle were 5.0±2.4 NTU, 49.6±38.3 µg/L, 12.4±8.7 mg/L, 8.1±5.1 mg/L, 0.595±0.204 mg/L and 0.100±0.046 mg/L, respectively, compared to average values of 12.2±4.5 NTU, 335.2±256.6 µg/L, 31.0 ±12.8 mg/L, 23.8±9.0 mg/L, 2.377±0.973 mg/L and 0.224±0.086 mg/L, respectively in treatment with Pacific white shrimp (Table 1).

Initial water contained 52.9 mg/L of total particulate matter, 35.0 mg/L of particulate organic matter,1.344 mg/L of particulate nitrogen, 0.186 mg/L of particulate phosphorus, 166.8 µg/L of chlorophyll *a*, 0.320 mg/L of total ammonia nitrogen and 6.4 mg/L of dissolved oxygen. pH of water was 8.1, salinity was17.5 ppt, and water turbidity was 9.7 NTU.

In the first two weeks, weekly average values of turbidity, chlorophyll *a*, particulate nitrogen, and particulate phosphorus steadily increased in the treatment with Pacific white shrimp only. In the third week, the average values of all of these parameters except particulate phosphorus started to decrease. The decreasing trend werecontinued until week 5 for turbidity, week 6 for chlorophyll *a*, and week 3 for particulate nitrogen, then increased again and fluctuated until the end of the experiment. Average values of particulate phosphorus continuously increased from weeks 1 to 3 then began to decrease from weeks 4 to 5. Very small changes in the average values of total particulate matter and particulate organic matter were observed in week 1. Average values of both parameters decreased sharply in week 2 then fluctuated until the end of the experiment (Figure 1). Weekly average values from weeks 1 to 8 in Pacific white shrimp tanks were 17.5, 20.1, 12.6, 10.6, 9.9, 10.8, 9.1 and 6.7 NTU for turbidity, 371.3, 745.8, 561.7, 484.9, 293.6, 16.0, 143.9 and 64.1 µg/L for chlorophyll *a*, 52.0, 24.8, 29.0, 44.9, 24.5, 36.9, 22.1 and 13.4 mg/L for total particulate matter, 37.6, 19.8, 21.2, 33.5, 19.2, 29.8, 18.8 and 10.2 mg/L for particulate organic matter, 2.927, 3.346, 2.711, 3.421, 1.941, 2.567, 1.514 and 0.585 mg/L for particulate nitrogen and 0.198, 0.306, 0.380, 0.245, 0.128, 0.165, 0.223 and 0.143 mg/L for particulate phosphorus (Figure 2).

In the treatment with Pacific white shrimp and blood cockle,weekly average values of all the water quality parameters decreased significantly in week 1 and then fluctuated until the end of the experiment. Weekly average values from weeks 1 to 8 in these Pacific white shrimp tanks were 3.9, 4.4, 3.1, 9.3, 1.8, 5.4, 5.1 and 7.2 NTU for turbidity, 60.4, 25.8, 25.1, 39.0, 33.3, 2.7, 95.4, and 115.1 µg/L for chlorophyll *a*, 9.4, 8.4, 9.2, 3.6, 6.5, 17.2, 13.1 and 31.4 mg/L for total particulate matter, 6.7, 5.5, 5.2, 2.6, 5.0, 11.3, 9.6 and 18.6 mg/L for particulate organic matter, 0.581, 0.209, 0.691, 0.452, 0.509, 0.844, 0.695 and 0.778 mg/L for particulate nitrogen and 0.118, 0.169, 0.085, 0.058, 0.029, 0.078, 0.132 and 0.132 mg/L for particulate phosphorus (Figure 2).

The decrease in total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus in the Pacific white shrimp and blood cockle treatment indicated the possibility of using blood cockle to improve water quality in intensive culture system of Pacific white shrimp. According to the results of this study, blood cockle could reduce turbidity, particulate matter, particulate organic matter, chlorophyll *a*, particulate nitrogen and particulate phosphorus in water in intensive culture system of Pacific white shrimp by 59.0, 60.0, 66.0, 85.2, 75.0 and 55.4%, respectively. Sterling and Okumus (1995) and Neori, *et al*. (2004) reported that culturing oyster or mussel with salmon could reduce nutrient concentration in the effluent. Freshwater mussel (*Diplodon chilensis*) was reported to be able to reduce chlorophyll *a* and total phosphorus in water in closed culture system of salmon in outdoor experimental tanks (Soto and Mena, 1999). Jones *et al*. (2001) reported that Sydney rock oyster (*Saccostrea commercialis*) could removed large concentrations of phytoplankton and other suspended solids from shrimp pond effluent resulting in the reduction of total Kjeldahl nitrogen and total phosphorus. Shpigel *et al*. (1997) also reported that turbidity and chlorophyll *a* concentration in gilt-head seabream (*Sparus aurata*) farm effluent were reduced significantly by filtration of bivalves, *Crassostrea gigas* and *Tapes philippinarum*.

There was no statistically significant difference ($P \geq 0.05$) in the average values of total ammonia nitrogen and dissolved oxygen between the treatments of Pacific white shrimp only and Pacific white shrimp with blood cockle. Average values of total ammonia and dissolved oxygen were 0.277 mg/L and 6.9 mg/L in Pacific white shrimp only treatment, and 0.354 and 6.7 mg/L in Pacific white shrimp with blood cockle treatment (Table 1). Weekly average values from weeks 1 to 8 were between 0.118 and 0.785 mg/L for total ammonia nitrogen in Pacific white shrimp only treatment, and between 0.089 and 1.248 mg/L in Pacific white shrimp with blood cockle treatment. Weekly average values from weeks 1 to 8 of dissolved oxygen were between 6.5 and

7.2 mg/L for Pacific white shrimp only treatment, and between 6.3 and 7.0 mg/L in Pacific white shrimp with blood cockle treatment (Figure 3).

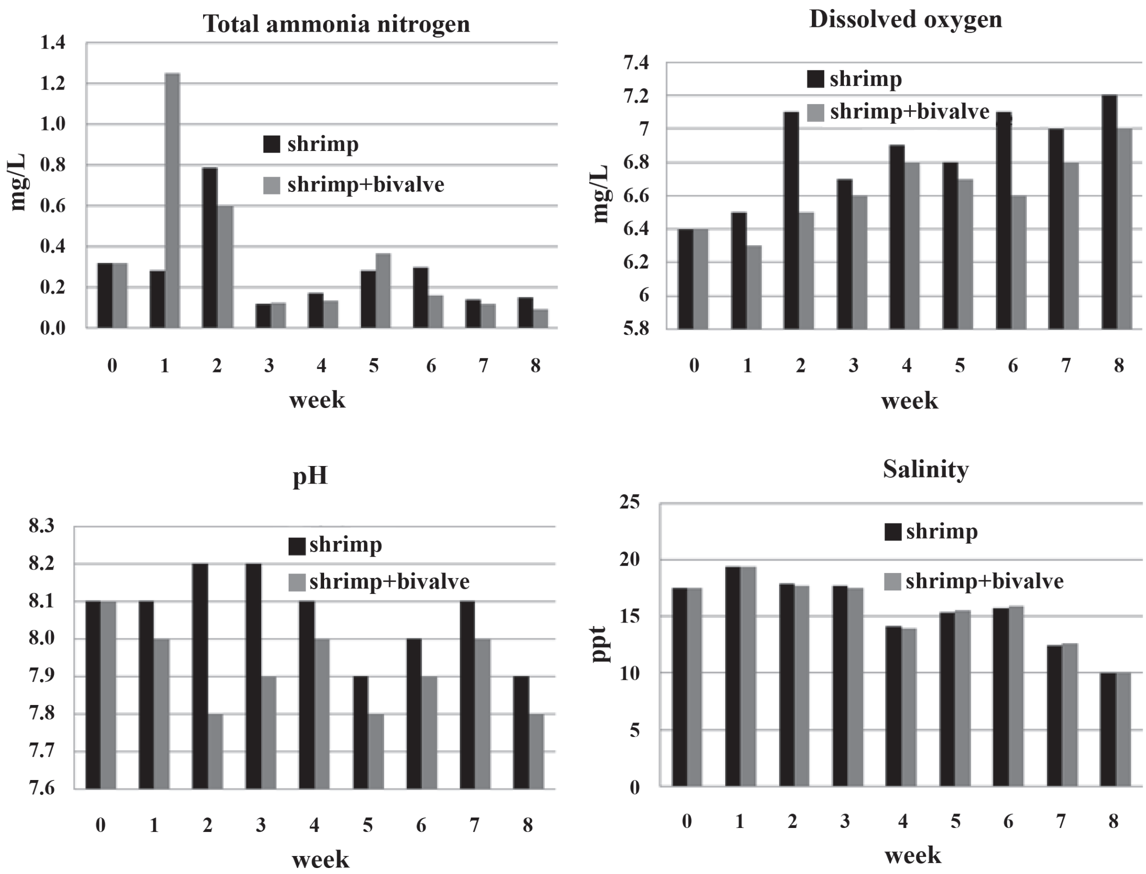


Figure 3. Average values of total ammonia nitrogen, dissolved oxygen, pH and salinity of water in tanks culturing shrimp and shrimp + blood cockle.

Average pH value throughout experimental period in the Pacific white shrimp only treatment was 8.1, which was not significantly different ($P \geq 0.05$) from the average value of pH 7.9 in the Pacific white shrimp with blood cockle treatment (Table 1). Weekly average values from weeks 1 to 8 of water pH were between 7.9 and 8.2 in Pacific white shrimp only treatment, and between 7.8 and 8.0 in Pacific white shrimp with blood cockle treatment (Figure 2). Weekly average values from weeks 1 to 8 of water salinity were between 10.0 and 19.4 ppt in Pacific white shrimp only treatment, and between 10.1 and 19.4 ppt in Pacific white shrimp with blood cockle treatment (Figure 3).

According to the result of this study, introduction of blood cockle into closed culture system of Pacific white shrimp did not reduce total ammonia nitrogen concentration in water. This was different from the findings of Soto and Mena (1999) which reported that freshwater mussel, *D. chilensis*, reduced the concentration of ammonia by one order of magnitude after 18 days until 39 days in closed culture system of salmon in an outdoor tank experiment.

In the shrimp with bivalve treatment, blood cockle removed large portions of phytoplankton and other particulate matter (uneaten feed, shrimp feces and dead algal cells) from the water column resulting in the reduction of chlorophyll *a*, particulate organic matter, particulate nitrogen, particulate phosphorus and turbidity of water. Part of the filtered particulate matter was ingested and assimilated by the bivalves while another part including feces and pseudofeces settled down to the bottom of the bivalve compartment. Less sediment was found in the shrimp compartment in the shrimp with bivalve treatment. In the Pacific white shrimp without blood cockle treatment, most settleable particulate matter also settled down in the empty bivalve compartment. Bottom sediment disturbance by shrimp generated resuspension of settleable particulate matter which was carried out from the shrimp compartment with water which flowed into the bivalve compartment.

There were no significant differences ($P \geq 0.05$) in average total weight at harvest, size at harvest, the increase in bodyweight, survival rate and feed conversion ratio of shrimp between the two treatments. In the Pacific white shrimp only treatment, average total weight of shrimp at harvest was 1333.3 g per tank which was equivalent to a production rate of 2.3 kg/m^3. The average body weight of shrimp at harvest was 27.9 g. Average increase in of body weight was 17.4 g/shrimp/8 weeks and average feed conversion ratio was 1.8. In the Pacific white shrimp with blood cockle treatment, the average total weight of shrimp at harvest was 1365.0 g per tank which was equivalent to a production rate of 2.4 kg/m^3. The average size of shrimp at harvest was 27.9 g. Average increase in body weight was 17.7 g/shrimp/8 weeks and average feed conversion ratio was 1.7. The average survival rate of shrimp in the Pacific white shrimp only treatment was 95.0%, while that in the Pacific white shrimp with blood cockle treatment was 96.7% (Table 2).

Average survival rate of blood cockle was 94.3% and average growth rate was 0.2 g/individual/8weeks (Table 3). In this experiment, there was neither water exchange nor sediment removal. A thick layer of sediment was found at the bottom of the bivalve compartment after harvest. Toxic metabolites generated from the decomposition of organic sediment were likely to be the cause of the slow growth rate of the cockle. Periodically removal of sediment from the bivalve compartment should be an effective means to improve environmental conditions. In addition, the high stocking density of the bivalves could have negatively aeffected the bivalve themselves.

Even though higher production of shrimp was not obtained from co-culture of blood cockle with Pacific white shrimp, the result of this experiment indicated that co-culture of blood cockle and -Pacific white shrimp can be used as a means to reduce particulate nutrients and particulate organic matter including phytoplankton in shrimp intensive culture systems. This set-up will result in better environmental conditions in the culture tanks and less pollutant (organic matter, phytoplankton and nutrients) loading in the effluents.

Table 2. Survival rate, growth rate, final body weight and feed conversion ratio of Pacific white shrimp cultured with and without blood cockle (mean±S.D.)

Treatment	shrimp	shrimp + bivalve
Initial number	50	50
Initial total weight (g)	517 ± 37.9^{a}	497 ± 32.1^{a}
Average initial weight (g/shrimp)	10.3 ± 0.8^{a}	9.9 ± 0.6^{a}
Survival rate (%)	95.0 ± 6.4^{a}	96.7 ± 3.1^{a}
Final total weight (g)	1333.3 ± 115.5^{a}	1365.0 ± 37.7^{a}
Final average weight (g)	27.9 ± 0.7^{a}	27.9 ± 0.9^{a}
Average growth rate (g/shrimp/8 week)	17.4 ± 1.3^{a}	17.7 ± 0.3^{a}
Average growth rate (%)	174.3 ± 13.3^{a}	177.3 ± 3.5^{a}
Feed conversion ratio	1.8 ± 0.3^{a}	1.7 ± 0.1^{a}

Average values denoted by different superscript in each parameter are statistically significant different (<0.05)

Table 3. Survival rate, growth rate and final body weight of blood cockle cultured with Pacific white shrimp (mean±S.D.)

Initial number	79-82
Initial total weight (g)	1000
Average initial weight (g/individual)	12.6±0.3
Survival rate (%)	94.3±5.6
Final total weight (g)	940.0±52.9
Final average weight (g)	12.8±0.3
Average growth rate (g/individual/8weeks)	0.2±0.0
Average growth rate (%)	1.7±0.4

CONCLUSION

Results from this investigation indicated that blood cockle, *A. granosa*, can be used to improve water quality in intensive culture system of shrimp by reducing total particulate matter, particulate organic matter, phytoplankton, particulate nitrogen and particulate phosphorus. This will directly result in the reduction of pollutant loading in shrimp farm effluents and better environmental conditions in shrimp culture system. However, more studies are needed to obtain proper stocking densitiesy of bivalves and shrimp to obtain optimum production.

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