The Ecological Roles of Anadara sp. in Black Tiger Shrimp Culture System

by

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ABSTRACT

The aims of this research were to characterize the effectiveness of individual filtration organisms mainly sea-weed (*Gracilaria verrucosa*) and blood cockle (*Anadara granosa*) in the black tiger shrimp (*Penaeus monodon* Fab.) polyculture system to stabilize culture water habitat. The research was performed at at the Development Centre of Marine, Brackish water and Shrimp Culture (DCMBSC) Karawang, West Java, in plastic-coated wooden tanks (0.9 x 0.6 x 1 m) with no water exchange for 53 days. The treatments were related to difference levels of weight and culture methods of *G. verrucosa* and stocking density of *A. granosa*. Culture methods included bottom method and off bottom method with the seaweed depth of about 40 cm and 0 cm from the water surface. For the bottom method, seaweed was sown at about 240 g and 640 g and for off bottom method with thallus bound of about 30 g and 80 g and hung 40 cm under water surface with interval of 25 cm. Stocking density of *A. granosa* consisted of 50, 100, and 150 inds.m^{-2}. Polyculture system shrimp and seaweed of 30 and 80 g each attachment hung 40 cm from water surface had ability to stabilize culture media and showed significantly different in shrimp growth from the other treatments except from shrimp polyculture with 240 g seaweed cultivated by bottom method. All the systems had suitable collony of micro-organism based on magnification of *Vibrio* of about 10^2 and total bacteria 10^6. Seaweed, *G. verrucosa* had ability to absorb phosphorus concentration. Polyculture system shrimp (*P. monodon*) with blood cockle (*A. granosa*) revealed that the lowest concentration of total organic matter in culture water was found in the treatment 50 individuals of blood cockle per square meter. Similar results were found for ammonia and nitrate, but not for nitrate. In term of reducing total organic matter and ammonia concentration in culture water, polyculture of tiger shrimp with *A. granosa* at 100 individuals.m^{-2} gave the best performance. Moreover, the highest growth rate of *A. granosa* was obtained by polyculture of shrimp (*P. monodon*) with *A. granosa* at a stocking density of 50 inds.m^{-2} followed by 100 and 150 inds.m^{-2}. After harvesting, shrimp and blood cockle tissue were found to have the highest concentration of carbon followed by nitrogen and phosphorus. *A. granosa* tissue showed the highest gain of phosphorus.

Key Words : *Culture water quality; cultivation method; growth rate; stocking density*.

INTRODUCTION

Brackish-water shrimp ponds and all other aquaculture environments are man-made ecosystems and represent subunits within their surrounding natural ecosystems. The major components of pond ecosystems are the cultured organisms and the aquatic environment, including all of its biological, chemical and physical characteristics and the nutrient (feed and fertiliser) inputs (Boyd, 1999). Shrimp farming can be separated into extensive, semi intensive and intensive culture systems (Midlen & Redding 2000; Direktorat Jenderal Perikanan Budidaya 2003; Pillay 2004). Extensive shrimp farming is the oldest technology and is still used by the majority of shrimp farmers in Indonesia. However, in the late 80's and early 90's, due to market forces, many black tiger shrimp farmers switched their culture practices to semi-intensive and intensive systems. Contrary to the extensive system, intensive black tiger shrimp aquaculture practices rely on high protein feed pellets to produce high rates of growth and to increase production. Intensive farming is becoming more dominant, increasing the potential impact of shrimp farming not just to the surrounding environment but also to pond system itself.

The main cause of environmental degradation in intensive culture systems is the level of input required to support high production outputs. During the culture period, a large proportion of the high protein commercial pellets are not assimilated by the shrimps (Primavera 1994) and settles down to the pond bottom as pollutant material. Approximately 10% of the feed is dissolved and 15–50% may remain uneaten (FAO 1991). The remaining 75% is ingested, but 50% is excreted as metabolic waste, producing large amounts of gaseous, dissolved and particulate waste (Lin *et al.* 1993). Subsequently, the pond effluent contains elevated concentrations of dissolved nutrients (primarily ammonia), plankton and other suspended solids (Ziemann *et al.* 1992). The dissolved nutrients and organic material in shrimp ponds stimulate rapid growth of bacteria, phytoplankton, and zooplankton (Lin *et al.* 1993). These accumulated materials may enhance eutrophication, hypernutriphication, and organic enrichment (FAO 1991; Pillay 2004) that can generate unsuitable pond water quality for black tiger shrimp, leading to disease outbreaks or even mass mortality. In addition, during the water exchange and harvesting time where the water is completely drained out, untreated wastes or accumulated organic matter are usually discharged directly into the surrounding environment. Effluent water from shrimp ponds typically contains elevated concentrations of dissolved nutrients and suspended particulates compared to influent water (Jones *et al*. 2001) mainly in the form of inorganic nitrogen and phosphorus (Pillay 2004). Therefore, in order to maintain a suitable culture medium during the rearing period for the cultured organism and alleviate negative impacts to the surrounding environment, fish farmers need to implement a sustainable and environmentally friendly culture technology, such as polyculture.

Based on the definition of polyculture as discussed in the previous chapter, more than one cultured organism can be used within a culture system, particularly where the other organisms are used to play ecological roles as filtration organisms (Lazur & Britt 1997) to support the main cultured organisms. Several previous studies noted that macro algae or seaweed – *G. verrucosa* (Troell *et al.* 1999b; Troell *et al*. 2003) and blood cockle – *A. granosa* have ability to reduce high concentration of dissolved nutrients and suspended particulates (Jones *et al*. 2001) produced by aquaculture practices (FAO 2000; Muller-Feuga 2000; Troell *et al*. 2003).

Most of the studies previously undertaken on seaweed and bivalve as filtration organisms were not carried out using a polyculture system but in separated culture units separating the main cultured organism from the filtration organism(s), so these represent co-cultivated or integrated aquaculture system rather than polyculture. Generally, filtration organisms were cultivated in several types of ponds, such as sedimentation pond (Lazur and Britt 1997), biological treatment pond, reservoir pond (Baliao & Tookwinas 2002) or drainage canal (Gunarto 2003; Shimoda *et al*. 2005; Shimoda *et al*. 2006**)**. Related to the type of water exchange, these studies were performed using flowthrough system (Jones *et al*. 1999; Jones *et al*. 2001; Baliao & Tookwinas 2002) or closed recirculation system (Gunarto 2003; Shimoda *et al*. 2005; Shimoda *et al*. 2006**)** or a combination of both (Baliao & Tookwinas 2002; Matos *et al*. 2006). Although the results of these studies showed good filtration ability in reducing concentration of aquaculture waste water, there is a need to undertake advanced research on the filtration ability of “single-filtration organism” in polyculture systems. Information on the filtration ability in more complex ecosystems would help develop more effective polyculture approaches for shrimp farmers. Therefore, this study was intended to characterize the roles of seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as single filtration organism in polyculture system with black tiger shrimp as the main cultured organism.

MATERIALS AND METHODS

The experiments were conducted at the Development Centre of Marine and Brackish water Culture (DCMBC) Karawang, West Java, in plastic-coated wooden tanks (0.9 x 0.6 x 1 m) with no water exchange throughout the 53-day experimental period (from September 10 to November 3, 2008). Samples of water quality parameters were analyzed at DCMBC Karawang, Fisheries University Jakarta (Sekolah Tinggi Perikanan Jakarta), and Tambak Pandu Karawang. The objectives of this research were to determine the ecological roles and the ability of blood cockle (*A. granosa*) as single filtration organism to stabilize environment in black tiger shrimp (*P. monodon*) polyculture system.

For this experiment black tiger shrimp were harvested from nursery ponds and blood cockle harvested from wild population were purchased from Muara Gembong, Bekasi Regency, West Java with the weight range of about 5.04 – 13.21 g and 1.50 – 2.63 g, respectively. Meanwhile, seaweed was harvested from a reservoir pond located at the DCMBSC Karawang.

Stocking density of shrimp was 4 individuals.m^{-2}. Shrimp was adapted in the concrete tanks with the size 2 x 2.5 x 1m for 3 days and examined for WSSV (White spot syndrome virus) using polymerase chain reaction (PCR) technique before stocking. Furthermore, the treatment of blood cockle consisted of 3 different levels of stocking density. In the experimental design, polyculture systems of shrimp with different levels of blood cockle stocking density produced several treatments as presented in **Table 1-1** and **Table 1-2**, respectively. Control of the experiment (abreviated by SB0) was black tiger shrimp (*P. monodon*) monoculture system (without filtrtaion organism). The placement for each treatment to the available thanks was completely randomized and all treatments had 3 replications.

Table 1-2
List of blood cockle treatment

Treatment	Description
BM50	Stocking density of blood cockle was 50 individuals.m^{-2}
BM100	Stocking density of blood cockle was 100 individuals.m^{-2}
BM150	Stocking density of blood cockle was 150 individuals.m^{-2}

There were no water exchange except the addition of water to replace loss due to seepage and evaporation. Experimental tanks were lined with 10 cm sand collected from the shore and then filled to 80 cm depth with sea water ±28 ppt and no aeration during experimental period. Shrimp were fed with commercial feed 3 times a day at 07.00, 14:00 and 20:00 h with a feeding rate of 6% of total body weight.

Measurement of water quality parameters included dissolved oxygen, temperature, salinity, pH, ammonia, nitrite, nitrate, H_2S, phosphate, total dissolved solid (TDS), total suspended solid (TSS), and total organic matter (TOM). The first four parameters were measured every 3 days at dawn – 5.00 h and noon – 11.00 h and the other parameters were measured every two weeks. The growth parameters measured were weight of shrimp and blood cockle as well as weight of seaweed; these were measured before stocking and at harvest. The measurement and taking water sample were performed at about 10 cm above bottom of experimental unit. Dissolved oxygen, temperature, salinity and pH were measured by multi-water quality parameters checker. The measurement equipmet of salinity was Atago refractosalinometer. The rest of the parameters were observed by using Sphectrophotometer Optima – SP300. The measurements all parameters followed Standard Methods of APHA (1979); Alerts & Santika (1987); Effendi (2003).

The results were analysed using one-way ANOVA and considered significant at an alpha level of 0.05 (Supranto 2004).

RESULTS

Shrimp-blood cockle polyculture system

<u>Physico-chemical aspects</u>

Some of the main water quality parameters observed includes dissolved oxygen (DO), pH, salinity, temperature, ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), and total organic matter (TOM). Results showed that concentration of dissolved oxygen was relatively lower in the early morning than in the afternoon (**Figure 1-8**). The lowest DO concentration occurred in the observation of 43-day experimental period, which is less than 2 ppm for all treatments at both of measurement time (dawn and noon). In general, the DO concentration in the BM_0 was higher than other treatments. BM_{50} showed a tendency higher DO concentration than that of BM_{100} and BM_{150}.

Temperature ranges 28 - 31.53^{O}C in the early morning and 28.53 - 32.13^{O}C in the noon. The lowest salinity was found at noon observation of about 25$^{o}/_{oo}$. All treatments showed a similar temperature change pattern and a tendency of increasing pH value. The lowest and highest pH were greater than 7 and lower than 8.5, respectively.

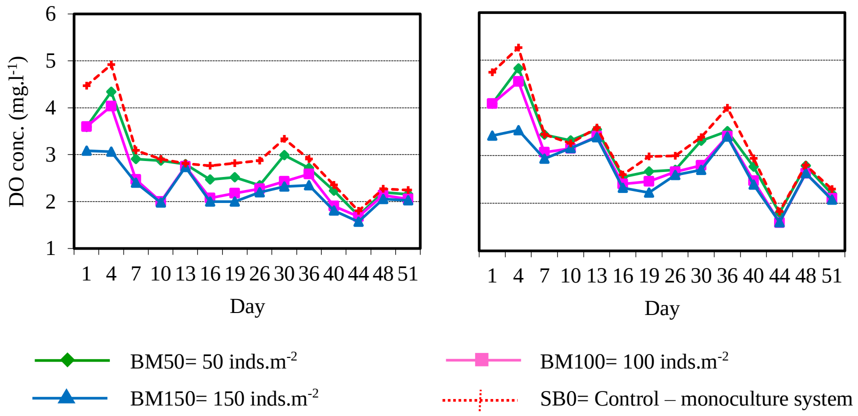


Figure 1-8

Dissolved oxygen concentration measured every 3 days in dawn (left) and noon (right) in polyculture black tiger shrimp with blood cockle

Ammonia concentration tends to decrease in all treatments during the experiment except in the SB0 as control. At harvest time or last measurement, treatment SB0 revealed highest ammonia concentration of about 0.511 ± 0.041 $mg.l^{-1}$ (**Figure 1-9a**). According to the decreasing tendency of ammonia concentration from the beginning to last measurement, BM100 and BM150 had significant higher decreasing rate than BM50 and SB0 as control. BM50 was significant different from SB0 (**Figure 1-9a** and **Appendix 1-19**).

Nitrite concentration also showed a similar pattern to the trend of ammonia and the significant highest concentration was recorded in treatment BM_{150} and SB0, of about 0.373 ± 0.107 $mg.l^{-1}$ and 0.488 ± 0.073 $mg.l^{-1}$, respectively (**Figure 1-9b)**. Treatment SB0 as control had significant lowest dropping off nitrite concentration amongst the treatments (**Figure 1-9b** and **Appendix 1-19**). Diminishing concentration of nitrite concentration of treatment BM50, BM100, and BM150 were not significant different among them but significant different from SB0 (control). The results of nitrate concentration observations did not show identical pattern (**Figure 1-9c**). Nitrate concentration of treatment BM50 and BM100 showed increasing tendency and were significant different from treatment BM150 and SB0 (**Appendix 1-21**). Treatment BM150 was significant higher rising tendency than that of treatment SB0 as control.

All treatments showed relatively increasing pattern of H_2S concentration. However, at the end of experiment, treatment BM_{50} had significantly lower H_2S (0.064 ± 0.011 $mg.l^{-1}$) concentration than treatment BM_{100} (0.082 ± 0.010 $mg.l^{-1}$) and not significant different from treatment BM_{150} (0.081 ± 0.010 $mg.l^{-1}$) and treatment BM_0 (0.078 ± 0.005 $mg.l^{-1}$) (**Figure 1-10a and Appendix 1-22**). Treatment BM50 presented best significant performance in maintaining lower hydrogen sulphide concentration and were significant different from the other treatments (**Figure 1-10a** and **Appendix 1-22**). Treatment SB0 as control had significant lower reduction of hydrogen sulphide concentration than that of BM50, BM100, and BM150.

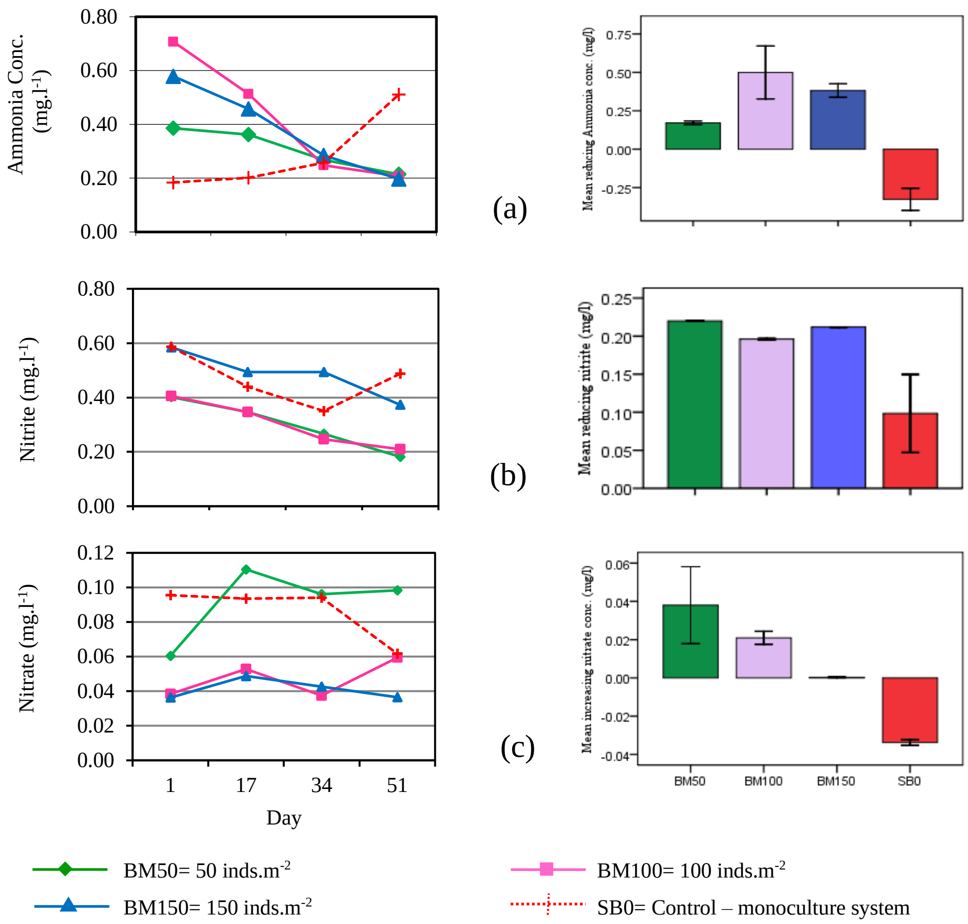


Figure 1-9

Observation results (left graphs) of and reducing concentration (right graphs) of ammonia (a), nitrite (b), and nitrate (c) in polyculture system of black tiger shrimp-blood cockle and black tiger shrimp monoculture system

During the experiment, TOM concentration presented identical decreasing tendency (**Figure 1-10b**). However, there were significant different of total organic matter concentration (TOM) among treatments (**Appendix 1-23**). Treatment BM50 had significant higher decreasing concentration than treatment BM100, BM150, and SB0 as control (**Figure 1-10b**). Meanwhile, treatment BM100 was not significant different from SB0.

At the last measurement or harvest time, the significantly lower phosphate concentration was recorded from treatment BM_{100} than SB0 ($P<0.05$) (**Figure 1-10c** and **Appendix 1-24**). However, treatment BM_{50} and BM_{150}, phosphate concentration was not significant difference from BM_{100} and BM_0. During the experiment, treatment BM50 and BM150 had significant lower diminishing PO_4 concentration ($P<0.05$) than treatment BM100 and SB0 of which were not significant different (**Figure 1-10c** and **Appendix 1-24**).

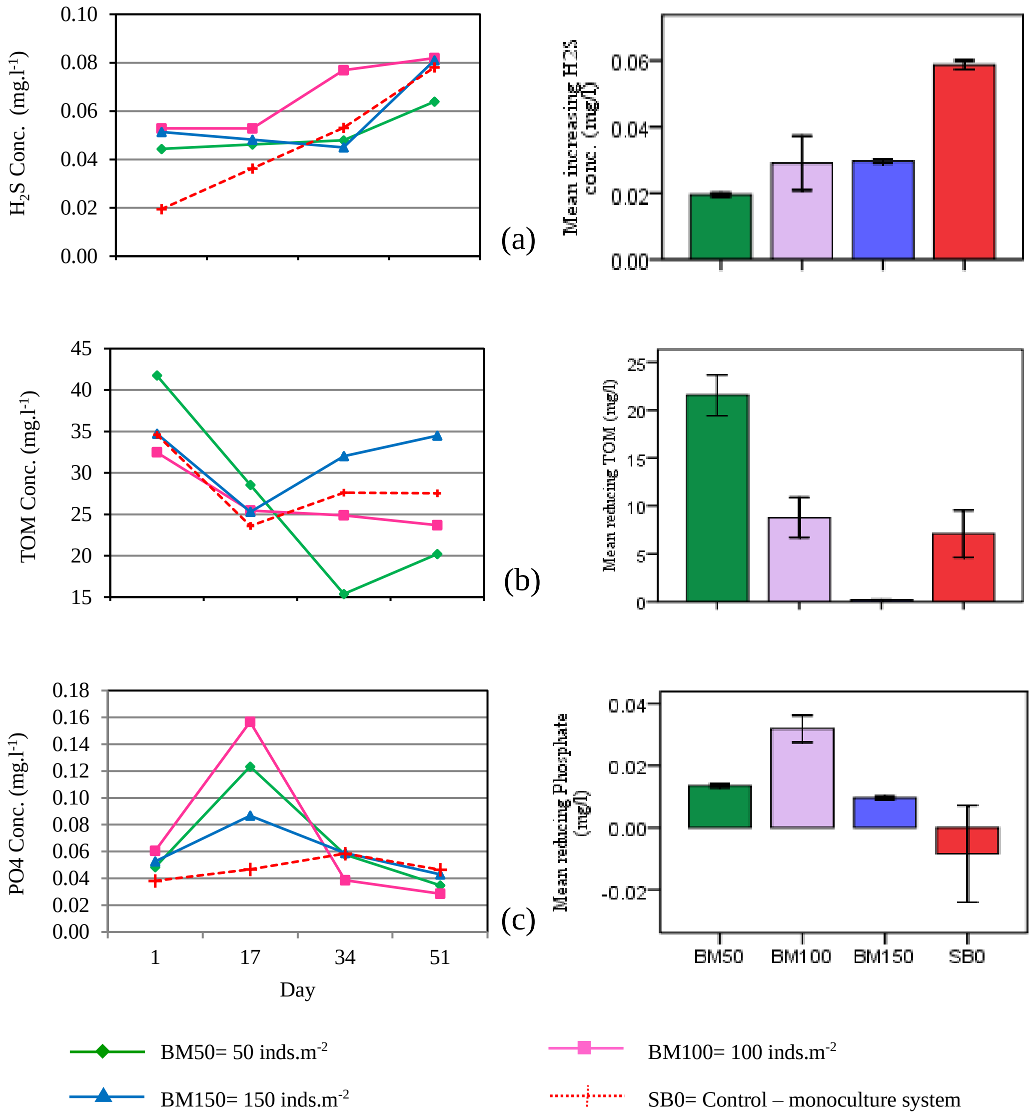


Figure 1-10

Observation results (left graphs) of and reducing concentration (right graphs) for hydrogen sulphide (a), total organic matter (b), and phosphate (c) in polyculture system of black tiger shrimp-blood cockle and black tiger shrimp monoculture system

TDS concentration tended to increase in all treatments at the end of measurements except in the treatment SB0 as control. There was significantly lower concentration of total dissolved solid of treatment SB0 than treatment BM_{100} and BM_{150} ($P<0.05$) (**Figure 1-11a** and **Appendix 1-25**). During the experiment, treatment SB0 as control showed significant higher reduction of TDS concentration than treatment BM50, BM100, and BM150 ($P<0.05$) (**Figure 1-11a** and **Appendix 1-25**).

During experiment, total suspended solid (TSS) showed a decreasing tendency and the significantly higher concentration was found in treatment SB0 (16.38 ± 1.89 mg.l^{-1}) than the other treatments (**Figure 1-11b** and **Appendix 1-26**). Treatment BM150 showed significant higher reduction of TSS concentration than treatment BM100 and SB0 ($P<0.05$) and was not significant different from treatment BM50 (**Figure 1-11b** and **Appendix 1-26**).

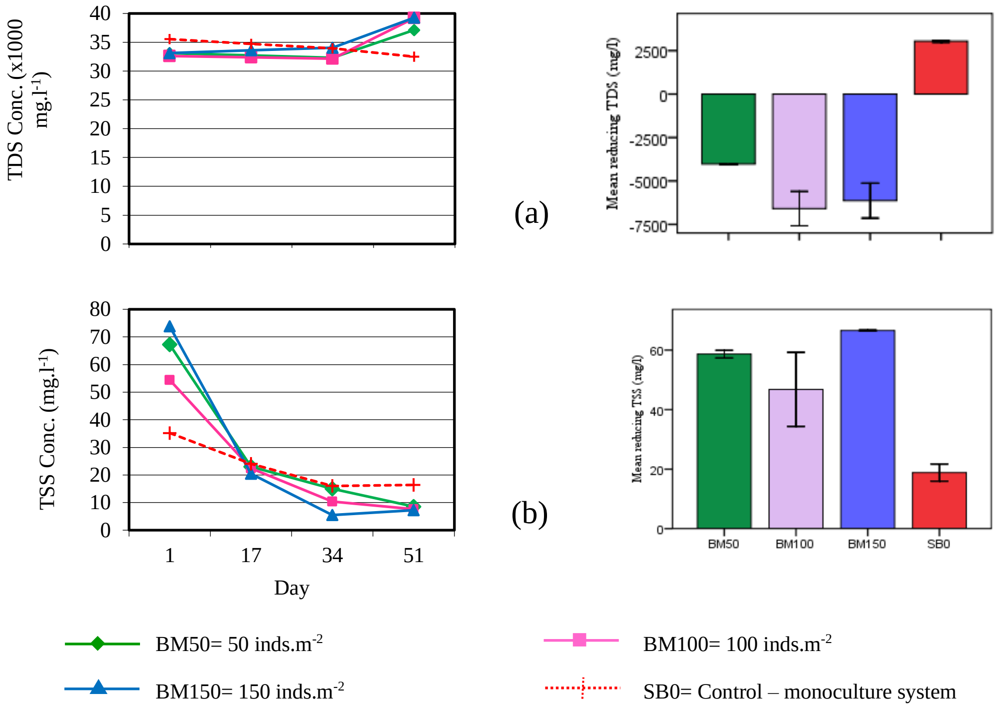


Figure 1-11

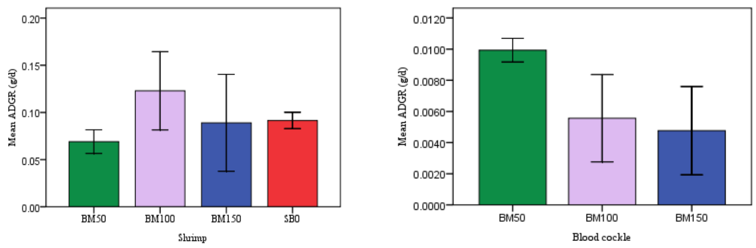
Observation results (left graphs) of and reducing concentration (right graphs) of total dissolved solid (TDS) (a) and total suspended solid (TSS) (b) in polyculture system of black tiger shrimp-blood cockle and black tiger shrimp monoculture system

Biological aspects

At the end of the experiment, half of one individual shrimp in 2 tanks or 2 replications of treatment BM100 and BM150 and all shrimps in 1 tank or replication of treatment BM50 were death. The average daily growth rate of shrimp was not significant different amongst treatments (P>0.05) (**Figure 1-12**; **Appendix 1-27**). Based on the statistical analysis, average daily growth rate of blood cokle of treatment BM_{50} was not significant different from treatment BM_{100} and significant different from treatment BM_{150} (P<0.05) (**Figure 1-12**; **Appendix 1-28**).

C content in shrimp and blood cockle tissue was higher than N and P content (**Figure 1-13**). Statistically, C, N, and P gain in shrimp tissue during experiment revealed that treatment BM_{100} and BM_{150} had significantly higher C than that of treatment BM_0 and BM_{50}; no significant difference among different stocking density for N gain; and treatment BM_{50} gained P extremely significantly higher than that of treatment BM_0, BM_{100}, and BM_{150} (**Figure1-13**). Furthermore, C gain of blood cockle tissue in the treatment BM_{100} had no significant difference than that of treatment BM_{50} but significantly higher than treatment BM_{150}. There was no significant difference of the N gain among treatments and even lost of N.

During experiment, the trend of total number of *Vibrio* of treatment SB0 as control or monoculture system showed relatively flat and bit decrease at the end of experiment. Trend of treatment BM100 and BM150 increased at the beginning and decreasing afterward that was contrary to treatment BM50 (**Figure 1-14**). There were two common trend of total bacterial. Firstly, trend of treatment BM50 and BM100 increased at the beginning and decreased afterward that was contrary to treatment BM150 and SB0 as control as second pattern (**Figure 1-14**). As presented by **Appendix 1-29,** at the end of experiment total *Vibrio* and bacteria revealed similar magnification of about 10^2 and 10^5 or 10^6, respectively. However, at the end of experiment, treatment BM50, statistically, had significant higher total number of *Vibrio* of about 6.1 x 10^2±30.41 CFU/ml than the other treatments (**Appendix 1-30).** Total number of *Vibrio* of Treatment SB0 of about 5.4 x 10^2±5.00CFU/ml was significant higher than treatment BM100 and BM150 and significant lower than treatment BM50. Meanwhile, total number of bacterial of treatment BM100 of about 4.35 x 10^6±273,008.00 CFU/ml was significant different from treatment BM50 (3.67 x 10^5±24,664.41 CFU/ml), BM150 (2.8 x 10^5±20,000.00 CFU/ml), and SB0 (2.7 x 10^5±36,055.51 CFU/ml).



BM50= 50 inds.m^{-2} BM100= 100 inds.m^{-2}

BM150= 150 inds.m^{-2} SB0= Control – monoculture system

Figure 1-12

Individual growth of shrimp and blood cockle in polyculture system

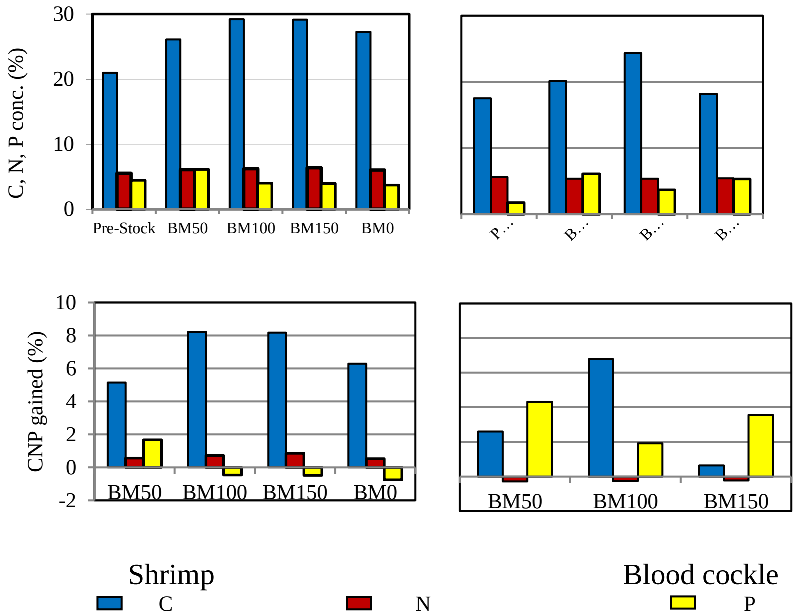


Figure 1-13

C, N, P content and gained in shrimp (left graphs) and blood cockle tissue (right graphs) in polyculture system

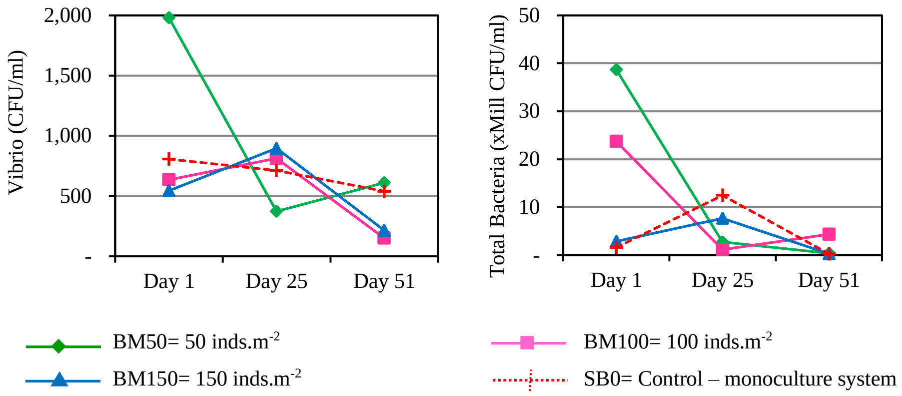


Figure 1-14

Total *Vibrio* (a) and total bacteria (b) during experiment in polyculture black tiger shrimp and blood cockle

DISCUSSION

One of the components of the shrimp pond culture ecosystem is water as the culture medium which interacts ecologically with other components. During the rearing period, water quality of shrimp pond tends to decrease due to added production inputs. Using of filtration organisms, such blood cockle (*A. granosa*), in a polyculture system seems to be able to reduce the environmental impacts of culture activities. Meanwhile, blood cockle as a filter-feeder will ingest dissolved and suspended particulates from the environment. One of the ecological consequences is to stabilize shrimp pond culture habitat.

Shrimp-Blood cockle polyculture system

In this study, blood cockle at different stocking density did not provide clear results related to several water quality parameters. Monoculture (shrimp – without blood cockle) revealed higher DO than that of polyculture shrimp-blood cockle. Among the polyculture systems, the lowest level of stocking density of blood cockle (50 inds.m^{-2}) had higher DO concentration than that of higher level of stocking density (100 and 150 inds.m^{-2}). The DO concentration decrease with increasing stocking density of blood cockles (FAO 1991). Temperature, pH, and salinity range of water were within the range of shrimp requirements (Boyd and Green 2002)**.**

The ecological impact of shrimp culture activities, generally, increases organic and inorganic concentration in the water (FAO 1991) and cause hypernutriphication and eutrophication (Pillay 2004). Some water quality parameters, such as total organic matter, ammonia, nitrite, hydrogen sulphide, total dissolved solids and total suspended solids play an important role in shrimp rearing activities. One of the advantages of implementing polyculture system using filtration organism is to stabilize these parameters.

Interesting results were observed in all polyculture treatments using shrimp and blood cockle in term of ammonia, nitrite, phosphate, and total suspended solids concentration, all of which tended to decrease throughout the culture period. Decreasing concentration of several water quality parameters were comparable to results obtained in the study reported by Jones and Preston (1999)**.** Blood cockle as a filter feeder organism stocked at the same shrimp culture unit might have ability to reduce nutrient loading resulted by shrimp rearing activities (Troell 1999) and can cope with high fluctuation physical variables of culture environment (Broom 1985). In this study, blood cockle in polyculture with shrimp might have potential for playing an important ecological role in order to maintain suitable water culture media for shrimp (Cheshuk *et al*. 2003), related to the concentration of ammonia, nitrite, and total suspended solids.

Moreover, the fluctuation of those water quality parameters during this experiment was within the range of shrimp requirement (Boyd 1990; Direktorat Jenderal Perikanan Budidaya 2003). The highest ammonia, nitrite, and total organic matter concentrations were revealed by monoculture system. This probably resulted from the lower ability of the system to oxidize and mineralize accumulated organic matter (Knud-Hansen 1998). A similar result was also obtained for total suspended solids. This result might be caused by the absence of blood cockles in the experimental monoculture system. Amongst the polyculture systems applied in these experiments, the lowest concentration of total organic matter and nitrite were recorded in the treatment with the stocking density of 50 and 100 inds. of blood cockle per square meter. However, the others water quality parameters did not appear to directly respond to the presence of blood cockle within the polyculture system.

From aquaculture and ecological points of view, related to the results of the water quality measurements, polyculture with stocking density of 100 inds.m^{-2} of blood cockle showed the best performance followed by stocking density of 50 inds.m^{-2}. However, based on the above results, blood cockle showed vary influence to the environment of shrimp culture pond. In this study, the highest weight gain of shrimp was in the polyculture system with stocking density of 100 inds. of blood cockle per square meter. Meanwhile, polyculture with stocking density 50 inds.m^{-2} of blood cockle revealed the highest growth of blood cockle followed by stocking density of 100 inds.m^{-2}. Blood cockle as filtration organism showed high ability to assimilate phosphate and carbon from the system.

CONCLUSION AND RECOMMENDATION

Conclusion

Based on the result of study concluded as follows:

1. Amongst polyculture of black tiger shrimp (*P. monodon*) with blood cockle (*A. granosa*), the lowest concentration of total organic matter and nitrite were recorded at the polyculture with the stocking density of about 50 and 100 ind. of blood cockle per square meter. Meanwhile, the rest of the observed water quality parameters revealed unclear tendency related to the present of blood cockle within the system of polyculture.
2. The highest weight gain of shrimp was in the polyculture system with blood cockle stocking density of 100 inds. per square meter. Meanwhile, polyculture with stocking density of 50 inds.m^{-2} of blood cockle revealed the highest growth rate of blood cockle followed by stocking density of 100 inds.m^{-2}.

3. Blood cockle represented by different level of stocking density did not perform clear tendency related to several water quality parameters.

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Appendix 1-19

Result of statistical analysis of mean value±SD of reducing ammonia at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	NH_3 Concentration (mg.l^{-1})		Δ NH_3
	T0	T1	
BM50	0.3857	0.2142	0.1715±0.0117a
BM100	0.7072	0.2074	0.4998±0.1725b
BM150	0.5787	0.1969	0.3818±0.0440b
SB0 (Control)	0.1841	0.5108	-0.3268±0.0717c

Note:

abc Means with different letters at the same column are significantly different at $P<0.05$

BM50 = 50 inds.m^{-2} of blood cockle　BM100 = 100 inds.m^{-2} of blood cockle

BM150 = 150 inds.m^{-2} of blood cockle　SB0 = Control – monoculture system

Appendix 1-20

Result of statistical analysis of mean value±SD of reducing nitrite at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	NO_2 Concentration (mg.l^{-1})		ΔNO_2
	T0	T1	
BM50	0.4017	0.1819	0.2199±0.00005a
BM100	0.4057	0.2097	0.1960±0.00086a
BM150	0.5849	0.3731	0.2118±0.00031a
SB0 (Control)	0.5864	0.4880	0.0984±0.05120b

Notes:

ab Means with different letters at the same column are significantly different at $P<0.05$.

BM50 = 50 inds.m^{-2} of blood cockle　BM100 = 100 inds.m^{-2} of blood cockle

BM150 = 150 inds.m^{-2} of blood cockle　SB0 = Control – monoculture system

Appendix 1-21

Result of statistical analysis of mean value±SD of reducing nitrate at first and last measurement in polyculture black tiger

TREATMENT	NO_3 Concentration (mg.l^{-1})		ΔNO_3
	T0	T1	
BM50	0.0604	0.0984	0.0380±0.0201a
BM100	0.0384	0.0594	0.0209±0.0034a
BM150	0.0363	0.0365	0.0003±0.0002b
SB0 (Control)	0.0955	0.0617	-0.0338±0.0015c

Note:

abc Means with different letters at the same column are significantly different at P<0.05

BM50 = 50 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system

Appendix 1-22

Result of statistical analysis of mean value±SD of reducing hydrogen sulphide at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	H_2S Concentration (mg.l^{-1})		ΔH_2S
	T0	T1	
BM50	0.0444	0.0639	0.0195±0.0005a
BM100	0.0528	0.0819	0.0291±0.0082b
BM150	0.0514	0.0810	0.0296±0.0006b
SB0 (Control)	0.0194	0.0781	0.0586±0.0014c

Notes:

abc Means with different letters at the same column are significantly different at P<0.05

BM50 = 50 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system

Appendix 1-23

Result of statistical analysis of mean value±SD of reducing total organic matter at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	**TOM Concentration (mg.l^{-1})**		ΔTOM
	T0	**T1**	
BM50	41.7427	20.2013	21.5413±2.130a
BM100	32.4613	23.6853	8.7760±2.102b
BM150	34.7227	34.5057	0.2170±0.021c
SB0 (Control)	34.6027	27.5127	7.0900±2.449b

Notes:

ab Means with different letters at the same column are significantly different at $P<0.05$.

BM50 = 50 inds.m^{-2} of blood cockle

BM150 = 150 inds.m^{-2} of blood cockle

BM100 = 100 inds.m^{-2} of blood cockle

SB0 = Control – monoculture system

Appendix 1-24

Result of statistical analysis of mean value±SD of reducing phosphate at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	PO_4 Concentration ($mg.l^{-1}$)		Δ PO_4
	T0	T1	
BM50	0.0483	0.0348	0.0135±0.0005a
BM100	0.0604	0.0285	0.0319±0.0043b
BM150	0.0526	0.0430	0.0096±0.0005a
SB0 (Control)	0.0381	0.0465	-0.0083±0.0156c

Notes:
abc Means with different letters at the same column are significantly different at P<0.05

BM50 = 50 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system

Appendix 1-25

Result of statistical analysis of mean value±SD of reducing total dissolved solid at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	TDS Concentration ($mg.l^{-1}$)		ΔTDS
	T0	T1	
BM50	33,110.00	37,133.33	4,023.33±18.93a
BM100	32,608.00	39,200.00	6,592.00±989.50b
BM150	33,130.33	39,266.67	6,136.33±1007.17b
SB0 (Control)	35,544.00	32,500.00	-3,044.00±55.50c

Notes:
abc Means with different letters at the same column are significantly different at P<0.05

BM50 = 50 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system

Appendix 1-26

Statistical analysis of reduction concentration of TSS in polyculture black tiger shrimp and blood cockle

TREATMENT	TSS Concentration ($mg.l^{-1}$)		ΔTSS
	T0	T1	
BM50	67.20	8.55	58.65±1.31ab
BM100	54.40	7.60	46.80±12.46a
BM150	73.80	7.22	66.58±0.28b
SB0 (Control)	35.20	16.38	18.82±2.89c

Notes:

abc Means with different letters at the same column are significantly different at P<0.05

BM50 = 50 $inds.m^{-2}$ of blood cockle
BM100 = 100 $inds.m^{-2}$ of blood cockle
BM150 = 150 $inds.m^{-2}$ of blood cockle
SB0 = Control – monoculture system

Appendix 1-27

Result of ONEWAY ANOVA Growth of black tiger shrimp by treatment in polyculture system shrimp-blood cockle

ANOVA

Growth	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.004	3	0.001	0.993	0.450
Within Groups	0.009	7	0.001		
Total	0.013	10			

Multiple Comparisons

Growth
LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BM50	BM100	-0.0538833	0.0327417	0.144	-0.131305	0.023538
	BM150	-0.0199500	0.0327417	0.562	-0.097372	0.057472
	SB0	-0.0224167	0.0327417	0.516	-0.099838	0.055005
BM100	BM50	0.0538833	0.0327417	0.144	-0.023538	0.131305
	BM150	0.0339333	0.0292850	0.285	-0.035315	0.103181
	SB0	0.0314667	0.0292850	0.318	-0.037781	0.100715
BM150	BM50	0.0199500	0.0327417	0.562	-0.057472	0.097372
	BM100	-0.0339333	0.0292850	0.285	-0.103181	0.035315
	SB0	-0.0024667	0.0292850	0.935	-0.071715	0.066781
SB0	BM50	0.0224167	0.0327417	0.516	-0.055005	0.099838
	BM100	-0.0314667	0.0292850	0.318	-0.100715	0.037781
	BM150	0.0024667	0.0292850	0.935	-0.066781	0.071715

Notes:
BM50 = 50 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system

Appendix 1-28

Result of ONEWAY ANOVA growth of blood cockle by treatment in polyculture system shrimp-blood cockle

ANOVA

Growth	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000046	2	0.000023	4.234	0.071
Within Groups	0.000033	6	0.000005		
Total	0.000079	8			

Multiple Comparisons

Growth
LSD

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
BM50	BM100	0.0043667	0.0019114	0.062	-0.000310	0.009044
	BM150	0.0051667*	0.0019114	0.035	0.000490	0.009844
BM100	BM50	-0.0043667	0.0019114	0.062	-0.009044	0.000310
	BM150	0.0008000	0.0019114	0.690	-0.003877	0.005477
BM150	BM50	-0.0051667*	0.0019114	0.035	-0.009844	-0.000490
	BM100	-0.0008000	0.0019114	0.690	-0.005477	0.003877

Note:

*. The mean difference is significant at the 0.05 level

BM50 = 50 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system

Appendix 1-29

Total colony of *Vibrio* and total bacteria in polyculture black tiger shrimp and blood cockle

Treatment	*VIBRIO*			TOTAL BACTERIA		
	Day 1	Day 25	Day 51	Day 1	Day 25	Day 51
BM50	1.98 x 10^2	3.73 x 10^3	6.1 x 10^2	3.87 x 10^7	2.73 x 10^7	3.67 x 10^5
BM100	6.34 x 10^3	8.13 x 10^2	1.5 x 10^2	2.37 x 10^7	1.13 x 10^7	4.35 x 10^6
BM150	5.43 x 10^2	8.97 x 10^2	2.15 x 10^2	2.86 x 10^6	7.66 x 10^7	2.8 x 10^5
SB0	8.07 x 10^2	7.13 x 10^2	5.4 x 10^2	1.56 x 10^6	1.25 x 10^7	2.7 x 10^5

Notes:

BM50 = 50 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system

Appendix 1-30

Result of statistical analysis of mean value±SD of *Vibrio* and total bacterial colloni at last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	*VIBRIO* (CFU/ml)	TOTAL BACTERIA (CFU/ml)
BM50	6.1 x 10^2±30.41a	3.67 x 10^5±24,664.41a
BM100	1.5 x 10^2±62.65b	4.35 x 10^6±273,008.00b
BM150	1.43 x 10^2±13.23b	2.8 x 10^5±20,000.00a
SB0	5.4 x 10^2±5.00c	2.7 x 10^5±36,055.51a

Notes:

ab Means with different letters at the same column are significantly different at $P<0.05$

BM50 = 50 inds.m^{-2} of blood cockle
BM150 = 150 inds.m^{-2} of blood cockle
BM100 = 100 inds.m^{-2} of blood cockle
SB0 = Control – monoculture system