The ability of sea-weed to absorb organic and inorganic substances in tiger prawn culture system

by Cek Turnitin

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The ability of sea-weed to absorb organic and inorganic substances in tiger prawn culture system

By

Moch. Nurhudah[1], Jatna Supariatna[2], Patria Mufti[2], Michael Rimmer[3]

[1] Sekolah Tinggi Perikanan, Jakarta,
[2] University of Indonesia – UI, Department of Biology
[3] Senior Research Fellow – ACIAR Expert

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ABSTRACT

The aims of this research were to characterize the ecological roles and the ability of *Gracilaria verrucosa* to stabilize the environment in commercial scale of polyculture system of black tiger shrimp (*Penaeus monodon*) culture. The research was performed from August 2009 to January 2010 at Sungai Buntu Village, Cilebar District, Karawang Region. There were 6 experimental pond units consisted of three ponds with area of 1,8[illegible] m^2 and the other three ponds were 2,624 m^2. The embankments height of all ponds was 1.2 m. The experiment followed a completely randomized design with treatment of polyculture black tiger shrimp – seaweed (off bottom method with 80 g thallus.bound^{-1} submerged 40 cm under surface water with hung interval of 1.5 m between 2 ropes) and monoculture system as control and each treatment had three replications. The stocking density of PL_{12} of black tiger shrimp (*P. monodon*) as experimental cultured organism was 4 $PLs.m^{-2}$. Shrimp polyculture system with seaweed (*G. verrucosa*) cultivated by off method had ability to stabilize culture media. The maintaining ability of several water and soil quality parameters of shrimp polyculture was significant difference from monoculture system. The shrimp growth, productivity, and survival rate in polyculture system was significantly higher than monoculture system ($P<0.005$). Shrimp polyculture system had suitable collony of micro-organism based on magnification of *Vibrio* of about 10^2 and total bacteria 10^5. Seaweed, *G. verrucosa* had ability to absorb carbon, nitrogen, and phosphorous substance from culture water habitat. Total number and species of plankton in culture habitat and total haenocyte count (THC) were also significantly different between shrimp polyculture and monoculture system ($P<0.05$).

Key Words : *Culture habitat; growth rate; productivity; survival rate; water quality* .

INTRODUCTION

In the beginning of 2010, the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia, has decleared a newly increased target of aquaculture production to 353% in 2014. It means that the annual aquaculture production growth rate must be more than 80%. Meanwhile, at the beginning of 2000, aquaculture production growth rate was anually about 10%. Therefore, serious effort and comprehensive approach should be employed in order to lift up the production. The most common technology to increase production is by employing intensive culture system. On the other hand, intensive technology has high probability to generate negative impacts to the culture and the surrounding environment (FAO 1991; Lin *et al*., 1993). Therefore, imploying sustainable and environmentally friendly technology, polyculture system by employing filtration organism, for instance, is one of the promising technologies (Midlen & Redding 2000; Pillay 2004). Employing additional organism in the culture unit of polyculture system is traditionally most intended to get considerable economic as well as ecological benefits of resources and a higher resilience against environmental fluctuation (Chien & Liao, 1995). Moreover, based on the physiological nature of second cultured organism, *G. verrucosa*, for instance, could play important ecological role as filtration organism. *G. verrucosa* has the ability to absorb most soluble nutrients (Troell 1999; Chow *et al*. 2001) resulted from shrimp culture practices and and have mechanisms for storing large reserved nutrients (Vergara *et al*. 1993).

Majority documentation and information on *G. verrucosa* as filtration organism were mostly employed in the different culture unit either by flowthrough (Jones 1999) or recirculation system (Shimoda *et al*. 2005; Shimoda *et al*. 2006). The purpose of employing filtration organism by flowthrough system is mainly to reduce concentration of organic and inorganic matter resulted from culture practices as waste water before draining out to surrounding environment. Meanwhile, in the recirculation system, it is intended to enhance waste water quality before reusing it as suitable culture habitat. Moreover, the research scale was generally laboratory scale. In consequence, most of the ecological parameters were totally under-control. Therefore, field scale research is crucial for providing scientific judgment & culture practice guidance to shrimp farmers or even become a Best Management Practice.

Study on polyculture system of black tiger shrimp with *G.verrucosa* as single filtration organism especially by commercial or field scale is a strategic research and nowdays needed by artisanal aquaculture in Indonesia in order to overcome ecological problems. Previous researches in laboratory scale had been proved that seaweed as filtration organism is able to reduce ecological impact of aquaculture as described by Chow *et al*. (2001); Jones *et al*. (2001); Baliao & Tookwinas (2002); Shimoda *et al*. (2005); Shimoda *et al*. (2006; Matos *et al*. (2006). In contrast, commercial or field scale studies are very rare. Field conditions are usually characterized by a more complex environmental variables and their reversal interaction which lead to unpredictable effects. Ecologically, aquaculture practices tend to manipulate aquaculture ecosystems in order to achieve higher production than in nature by employing culture technology and management and change the equilibrium of the natural ecosystem and even generate serious ecological pressure. Manipulation of culture unit has consequences of changing of ecosystem components and followed by changing of ecological processes. Simultaneously, structure and ecological function of culture unit as an ecosystem will be influenced. Therefore, study on commercial or field scale of polyculture system is needed in order to analyse the sustainability of polyculture techniques at a commercial scale.

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MATERIALS AND METHODS

Pond management and experimental design

The field experiment was carried out from August 2009 to January 2010 at Sungai Buntu Village, Cilebar District, Karawang Region. At the experimental site, there are 27 earthen ponds with variety of surface area from 1,881 to 2,624 m^2 which consist of 16 rearing ponds and the rest as sedimentation and reservoirir pond. There were 6 experimental pond units consisted of three ponds with area of 1,881 m^2 and the other three ponds were 2,624 m^2. The embankments height of all ponds was 1.2 m (**Table 3-1**). The experiment were started by draining out pond compeletely to eradicate all unexpected organisms (flora & fauna) and dried for 20 days and repairing the embankments and slopes and pond construction in whole as well. The fertilizer dose of 1,000 kg ha^{-1} organic manure was applied to all rearing ponds a week before pond watering or 2 weeks before stocking. The
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ponds were filled with seawater gradually to a depth up to about 90 cm. Seawater was passed through a serial sedimentation ponds prior to be kept for few days in the reservoir tanks. Each pond was equiped by a paddle wheel as emergency aeration to anticipate dissolved oxygen deficiency.

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The experiment followed a completely randomized design with treatment of polyculture black tiger shrimp – seaweed (off bottom method with 80 g

thallus.bound^{-1} submerged 40 cm under surface water with hung interval of 1.5 m between 2 ropes) and monoculture as control and each treatment had three replications. All ponds were stocked with 4 PLs shrimp.m^{-2}. PL_{12} of black tiger shrimp (*P. monodon*) as experimental organism was purchased from private hatchery in Indramayu, West Java. Shrimps were fed commercial pelleted shrimp feed containing approximately 40% crude protein, 9% fat, 12% moisture, and 4% ash. Daily feed rations were divided into three equal portions and given at 06.00, 12.00, and 18.00 h. Feeding rates were adjusted about weekly based on the remaining amount of uneaten feed on the control point- set net. Seaweed (*Gracilaria verrucosa*) as filtration organism was purchased from Muara Gembong, Bekasi Regency, West Java. Seaweed was tied on the rope with interval of about 2 m of about 80 g mass at each attachment and hung at intervals of 25 cm and merged about 40 cm under the water surface. The total amount of cultivated seaweed was about 800 kg.ha^{-1}.

Table 3-1.

Experimental pond area and stocking density of black tiger shrimp

Culture Technology	Rep.	Area (m^2)	Shrimp Density	
			Stocking (PLs.m^{-2})	Total (PLs)
Polyculture	1	2,624	4	10,496
	2	1,881	4	7,524
	3	1,881	4	7,524
Monoculture	1	2,624	4	10,496
	2	2,624	4	10,496
	3	1,881	4	7,524

Note: Rep.= Replication; PL = Post Larvae

Water quality observation

Measurement of physico-chemical of water quality parameters included dissolved oxygen, temperature, salinity, pH, water transparancy, total alkalinity, total ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), H_2S, phosphate (PO_4), total dissolved solid, total suspended solid, and total organic matter. The first five parameters were measured weekly at 05.00 h and 11.00 h and the rest parameters were measured just before stocking of shrimp and triweekly during experimental period. Water sampling was performed at about 15 cm above pond bottom. Dissolved oxygen, temperature, salinity and pH were measured by multi-water quality parameters checker. The measurement equipment of salinity and water transparancy were Atago refractosalinometer and Secchii disk with the diameter of 25 cm, respectively. The rest of the parameters were observed by using Sphectrophometer Optima – SP300. The measurements all parameters followed Standard Methods – APHA (1979); Alerts & Santika (1987); Effendi (2003).

A series of physico-chemical parameters of pond bottom soil viz. texture (3 fractions – sand, silt, and clay), pH, carbon, organic matter, pontential and availability of phosphrus, nitrogen, and cation exchange capacity (CEC) were also investigated. Soil sampling was carried out just after draining, before watering, and at/after harvesting time at in-let water and out-let water. In addition, biological factors included shrimp and seaweed growth, plankton community, bacterial collony, white spot virus of shrimp, total haemocyte count, and tissue content (C, N, P) of shrimp and seaweed. The weight of black tiger shrimp as growth parameters were measured once every two weeks after 50 days of stocking and at harvesting time. Shrimp samples were collected by using feeding control point-set net during rearing period and lift net just before harvesting time. The seaweed growth parameters were observed twice during rearing period, at day 75 and 100 after cultivation. Observation of seaweed weight was carried out at 40 seaweed bonds originated from separated four ropes in a experimental pond. Plankton water samples of about 30 – 50 litre were passed through a 30-μm mesh plankton net. Qualitative & quantitative investigation of plankton were based on Davis (1955); Sournia (1978); Sahlan (1982).

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Data Analysis

The data were statistically analysed using SPSS version 16.0 to perform an analysis of differences between treatments (polyculture of filtration organism) by t-test and considering significant at an alpha level of 0.05 (Prasisto 2002; Supranto 2004). Resulted analysis were directed to explain the condition of water culture media and the ability of filtration organism to maintain the quality of culture water habitat of black tiger shrimp.

RESULTS

Physico-chemical parameters

Pond bottom soil quality

Ecologically, shrimp pond (*tambak*) soil is one of the main important ecosystem aspect in determining favourable culture habitat. Several parameters of soil quality included soil texture, carbon, nitrogen, phosphorus, total organic matter, and cation exchange capacity were investigated. Soil texture of all research unit (pond) were dominated by sand fraction (**Appendix 3-1**). All soil physico-chemical parameters except nitrogen and carbon of both culture techniques at harvesting time (**Figure 3-1; Figure 3-2** and **Table 3-2**) were significant different ($P<0.05$). Concentration of carbon (**Figure 3-1**), CN ratio (**Figure 3-1**), total organic matter (**Figure 3-2**), and phosphate (**Figure 3-2**) in polyculture system were significant different from monoculture system ($P<0.05$). Furthermore, CN ratio of polyculture system at harvest time was significant different from monoculture system ($P<0.05$) and revealed lower ratio. CN ratio in monoculture system was significantly different ($P<0.05$) between stocking and harvest time but contrary result was showed by polyculture system.

Table 3-2.

Mean value±SD of pond bottom soil quality parameters of polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Parameter	Stocking		Harvesting	
	Polyculture	Monoculture	Polyculture	Monoculture
C	0.778±0.010a	0.785±0.034a	0.688±0.070a	0.858±0.041b
N	0.047±0.021a	0.045±0.009a	0.0267±0.006a	0.023±0.003a
C/N	18.890±7.622a	17.885±3.542a	26.339±3.860a	37.023±3.148b
TOM	9.374±0.098a	9.573±0.667a	8.469±0.440a	10.301±0.482b
PO4	0.378±0.018a	0.489±0.078a	0.332±0.053a	0.526±0.059b
CEC	196.474±7.402a	193.899±15.483a	199.830±17.398a	215.970±29.943a

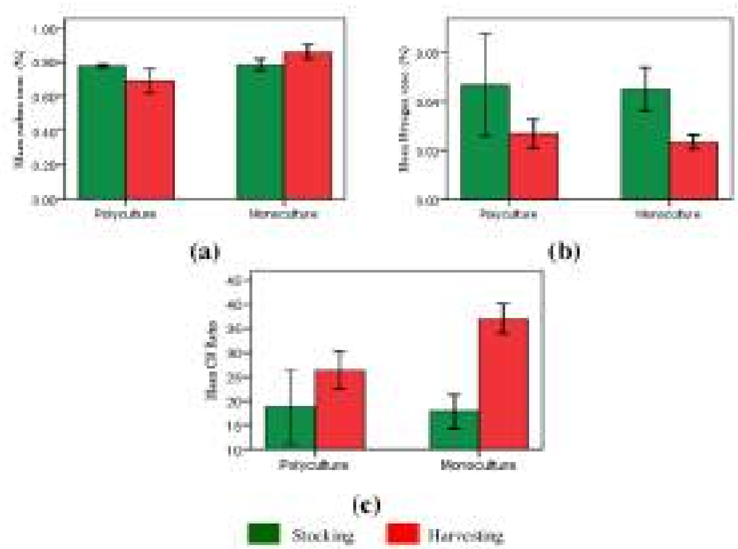

Figure 3-1

Mean value±SD of carbon (a), nitrogen (b), and C/N ratio (c) of pond bottom soil in polyculture system of black tiger shrimp-seaweed and monoculture system

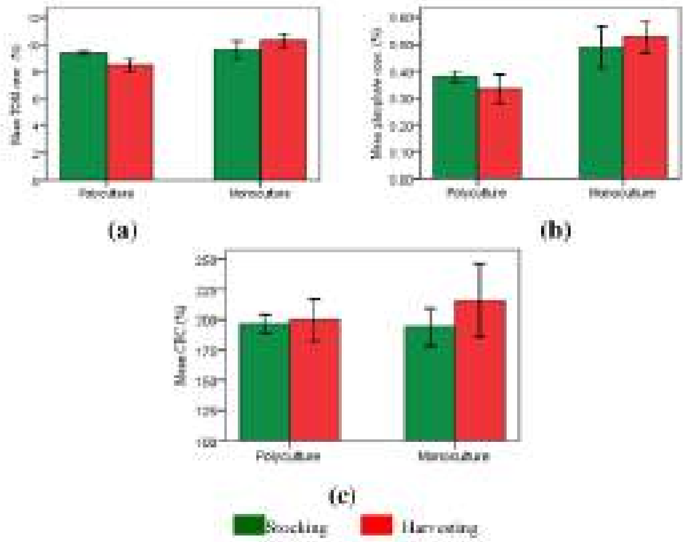

Figure 3-2

Mean value±SD of total organic matter (a), phosphate (b), and cation exchange capacity (c) of pond bottom soil in polyculture system of black tiger shrimp-seaweed and monoculture system

Water quality parameters

Some of the main water quality parameters observed included dissolved oxygen (DO), pH, salinity, temperature, ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), total organic matter (TOM), hydrogen sulphide (H_2S), phosphate (PO_4), total alkalinity, water trancparancy, total disolved solid (TDS), and total suspended solid (TSS). Results showed that concentration of dissolved oxygen were relatively lower in the early morning than in the afternoon (**Figure 3-3a**). In general, all treatments showed similar pattern of DO concentration tendency and start to decrease at day 43th and increase at day 71th then decrease again at day 92th till end of experimental period. DO in polyculture system showed higher concentration than monoculture system (**Figure 3-3a** and **Appendix 3-2**). The lowest and highest pH were found between 8.28 and 8.88 (**Figure 3-3c** and **Appendix 3-3**) of which recorded at day-85th and day 125th dawn measurement in monoculture system, respectively. Temperature ranges in polyculture and monoculture system were about 29.00 – 30.75^{O}C and 29.17 – 30.77^{O}C in the early morning and 30.58 - 34.30^{O}C and 30.52 – 34.23^{O}C in the afternoon, respectively (**Table 3-3b** and **Appendix 3-4**). It means that diurnal fluctuation of temperature in both culture technology, polyculture and monoculture, was less than 2^{O}C in early morning and less than 4^{O}C in the afternoon. During experiment period, the fluctuation of water salinity was about 8.67 ppt. The highest and lowest average salinity of about 46.67 ppt and 38 ppt were recorded in polyculture system at dawn and noon, respectively (**Table 3-3d** and **Appendix 3-5**).

At the first month of experiment, water colour of all pond cultures were clear green (**Table 3-3**) and even one of replications of polyculture system had clear green colour for whole experiment period. On the other hand, the colour of one replication of monoculture system changed already to yellowish green at day 29. Water transparancy seems to increase at first two months of experimental period and then decrease after ward. Decreasing trend of transparancy was started after day 50th of experiment (**Figure 3-4a** and **Appendix 3-6**). Moreover, polyculture system revealed significantly better capacity in maintaining total suspended solid (TSS) (**Figure 3-4b** and **Appendix 3-7**) and total dissolved solid (TDS) (**Figure 3-4c** and **Appendix 3-7**) concentration during experimental period. Based on that results, polyculture systems were able to reduce or stabilize TDS and TSS concentration.

During experiment, concentration of ammonium, ammonia, and nitrite except nitrate in polyculture system revealed generally similar tendency pattern as and lower than in monoculture system (**Figure 3-5**). Ammonium, ammonia and nitrite concentration tends to increase in all culture systems at first month of experiment period and then level off. After first measurement, polyculture system revealed significant lower concentration of ammonium, ammonia and nitrite than monoculture system (**Figure 3-5**).

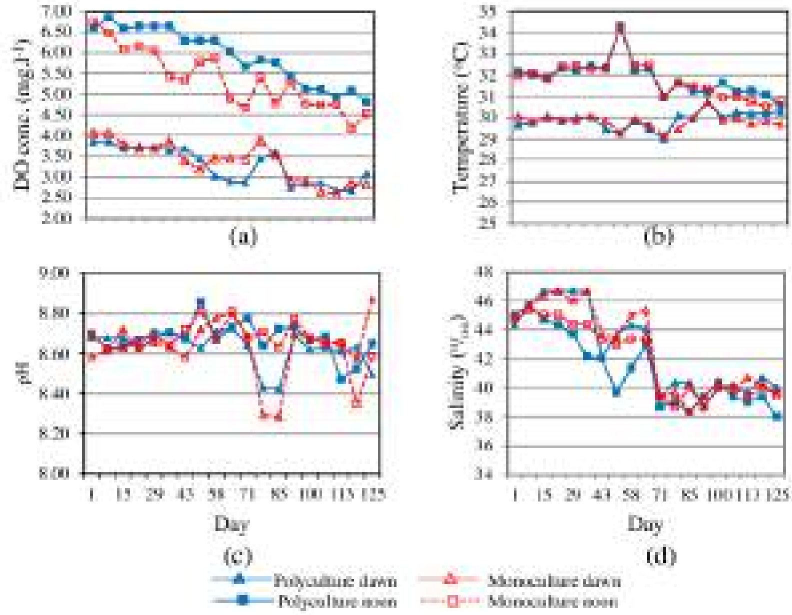

Figure 3-3

Observation results in dawn and noon of dissolved oxygen concentration (a), temperature (b), pH (c), and salinity (d) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Table 3-3

Results of water colour observation of polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system during experimental period

CULTURE SYSTEM		Day																		
		1	7	15	22	29	36	43	51	58	64	71	78	85	92	100	106	113	118	125
Polyculture	1	CG	CG	CG	CG	CG	CG	CG	CG	G	G	G	G	G	G	CG	CG	CG	CG	CG
	2	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	G	G	G	YG	YG
	3	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG
Monoculture	1	CG	CG	CG	CG	CG	YG	YG	G	G	G	G	G	G	G	G	G	G	G	G
	2	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	G	G	G	YG	YG
	3	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	G	G	G	G	G

Notes:
R = Replicate G = Green CG = Clear green YG = Yellowish green

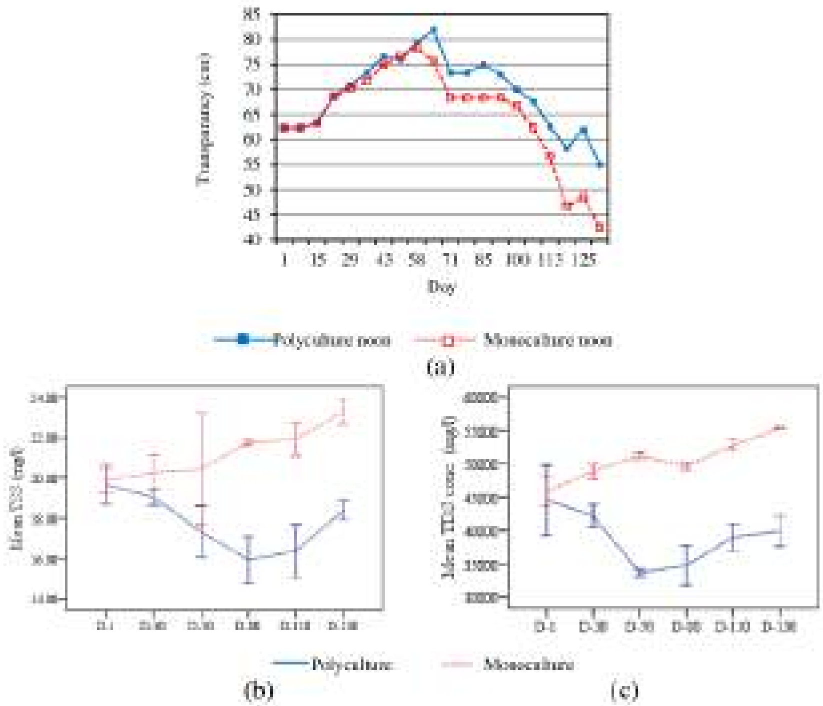

Figure 3-4

Mean value±SD of Secchi disk visibility (a), total suspended solid (b), and total dissolved solid (c) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Polyculture system started to reveal significantly lower concentration of ammonium than monoculture system at third measurement ($P<0.05$) (**Figure 3-5a**). Monoculture system had increase tendency for whole experiment period. Meanwhile, polyculture tended to decrease after second measurement at day 30^{th}. Similar to ammonium, ammonia concentration in polyculture system showed significant different from monoculture system ($P<0.05$) at second measurement (**Figure 3-5b** and **Appendix 3-8**). Nitrite concentration presented a similar pattern of the ammonia concentration (**Figure 3-5c**). Significantly higher ability of decreasing nitrite concentration was showed by polyculture system than monoculture system. Nitrate concentration presented an opposite pattern to the trend of another nitrogen specieses concentration. After second measurement, nitrate concentration of polyculture system of about 0.3412±0.057 mg.l-1 was significant lower than monoculture system (0.0854±0.009 mg.l-1) **Figure 3-5d** and **Appendix 3-8**).

During experiment, concentration of total organic matter, phosphate, and hydrogen sulphyde except total alkalanity in polyculture system were generally significant lower than in monoculture system (**Figure 3-6** and **Appendix 3-9**). At the beginning, concentration of total organic matter of all experimental culture unit were higher than that of following measurement (**Figure 3-6a**). Total organic matter concentration at last measurements in polyculture and monoculture system were 35.4833± 2.1597 mg.l-1 and 46.9067± 1.1912 mg.l-1, respectively. In term of phosphate concentration, monoculture system had increase tendency for whole experiment period and after day 30 showed significant higher concentration than polyculture (**Figure 3-6b**). Meanwhile, total alkalinity of both culture systems were not significant different (**Figure 3-6c**). Similar to phosphate concentration, polyculture system found significantly lower concentration of hydrogen sulphyde than monoculture system at third measurement (**Figure 3-6d**).

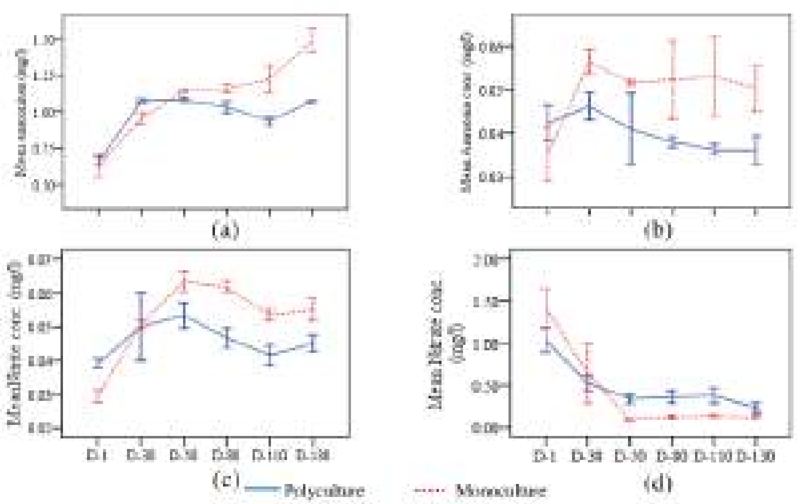

Figure 3-5

Mean value±SD of ammonium (a), ammonia (b), nitrite (c), and nitrate (d) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

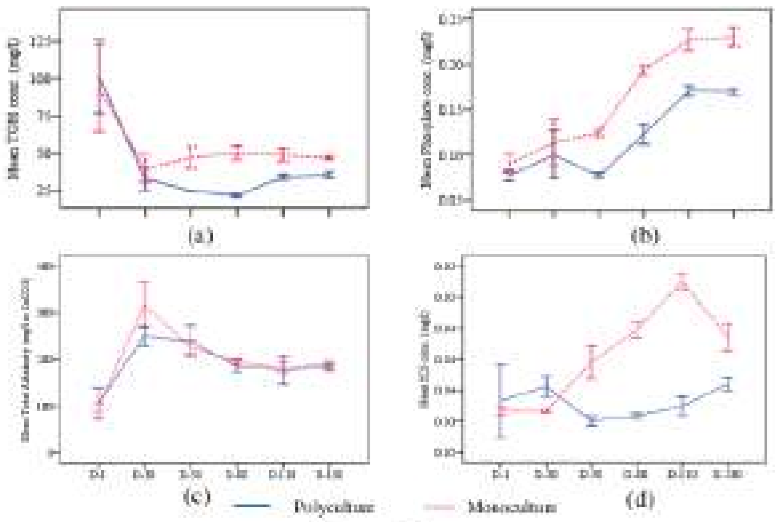

Figure 3-6

Mean value±SD total organic matter (a), phosphate (b), total alkalinity (c), and hydrogen sulphide (d) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Biological parameters

The experiment was performed by applying 2 different culture systems, those were black tiger shrimp polyculture with seaweed and black tiger shrimp monoculture system. **Table 3-4** presents results of statistical analysis of experimental variables. At the end of culture practices for about 140 days as experimental period, mean shrimp production (±SD) of polyculture and monoculture system were about 159±44.1928 kg and 150±22.7156 kg, respectively (**Appendix 3-10** and **3-11**). Statistically, polyculture system shtowed significant higher survival rate and productivity than that of monoculture system (**Figure 3-7c and 3-7d** and **Appendix 3-11**). From aquaculture's goal point of view, polyculture system of black tiger shrimp and seaweed revealed better performance than that of monoculture system. The pattern of shrimp growth showed gradually rise and then tremendously increased at last observation (**Figure 3-7a** and **Figure 3-7b**). It was probably caused by difference sampling technique. First technique, shrimp samples were collected from set net as feeding check point. The other sampling technique was by using lift net. Comparably, the shrimp growth rate in polyculture system was significant different from monoculture system ($P<0.05$) (**Figure 3-7b**). Furthermore, during experimental period, total haemocyte count was found not significant different at first sampling (day 75) and was significant different at harvest time (**Figure 3-8**).

Seaweed weight sampling was done at day 75 and 100 of experimental period. Just after first sampling, the seaweed weigth of each attachment was reduced until of about 80 – 100 g. First sampling recorded that mean weight±SD of seaweed bonds was 506.50±20.27 g. Related to the first cultivation weight, it had weight gain of about 426.50±20.27g or 533.13±25.34% with Average Daily Growth (ADG) Rate of 5.69±0.27 $g.d^{-1}$ (**Appendix 3-12**). Results of second sampling were not comparable to previous sampling. All seaweed bonds exhibited reduction weight and had negative weight gain and ADG.

Table 3-4.

Result of statistical analysis of mean value±SD of several variables of polyculture of black tiger shrimp-seaweed and black tiger shrimp monoculture system

No.	Variable	Culture Technology	
		Polyculture	**Monoculture**
A.	**Black tiger shrimp**		
1.	Absolute growth rate (g)	6.1719±0.4504a	5.3217±0.2318b
2.	Survival rate (%)	85.41±1.27a	68.84±1.47b
3.	Productivity ($kg.m^{-2}$)	0.0758±0.0037a	0.0646±0.004b
4.	THC – day 70	920.67±54.78a	885.3333±93.09a
5.	THC – day 135	1100.83±36.26a	985.83333±31.26b
6.	Carbon (%)	24.32±1.2314a	24.47±0.5498a

7.	Nitrogen (%)	10.13±0.3937a	10.36±0.2066a
8.	Phosphat (%)	4.30±0.2650a	4.59±0.4580a
B.	**Seaweed**	**(Stocking)**	**(Harvesting)**
	Carbon (%)	17.60±1.45a	24.18±1.77b
	Nitrogen (%)	1.52±0.02a	1.95±0.11b
	Phosphat (%)	3.51±0.87a	7.90±0.22b
D.	**Plankton**		
	Density (D120) (cell/l)	1173±33a	1965±55b
	Dominancy index (D120)	0.1279±0.0047a	0.1540±0.0026b
E.	**Microbial (CFU/ml)**		
	Total *Vibrio* (D120)	165±33a	1254±551b
	Total bacterial (D120)	1.4 x 10^5±26,457a	1.01 x 10^6±162,506b

Notes:

- ab means with different letters at the same row are significantly different at P<0.05.

Furthermore, C, N, and P content in shrimp and seaweed tissue were also measured. Statistical analysis of collected data on C, N, P content of shrimp tissue were intended to figure out the different between both culture systems. However, in case of seaweed, the analysis were intended to compare between C, N, P content at stocking and harvesting time. Carbon content in shrimp (**Figure 3-9c**) and seaweed tissue (**Figure 3-9a**) was higher than N and P content. Statistically, however, C, N, and P content in shrimp tissue between polyculture and monoculture systems were not found significant different (**Figure 3-9c**). C, N, P content in seaweed tissue at harvest time of about 24.18±1.77 %, 1.95±0.11 %, and 7.99±0.22 %, respectively, were significant different from stocking time (**Figure 3-9a**). Seaweed tissue gained significant higher carbon than nitrogen and phosphorus (**Figure 3-9b**).

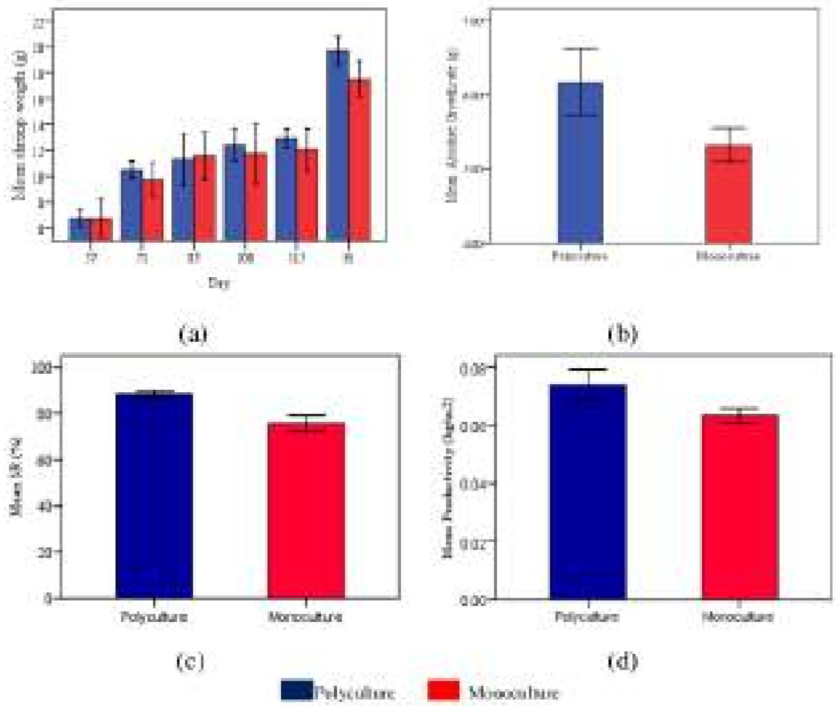

Figure 3-7

Mean value±SD of black tiger shrimp weigth (a) and absolute growth rate (b), survival rate (c), and productivity (d) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

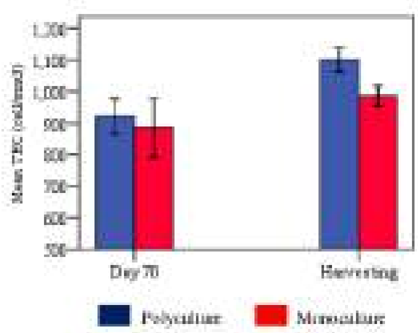

Figure 3-8

Mean value±SD of total haemocyte count (THC) in polyculture and monoculture system

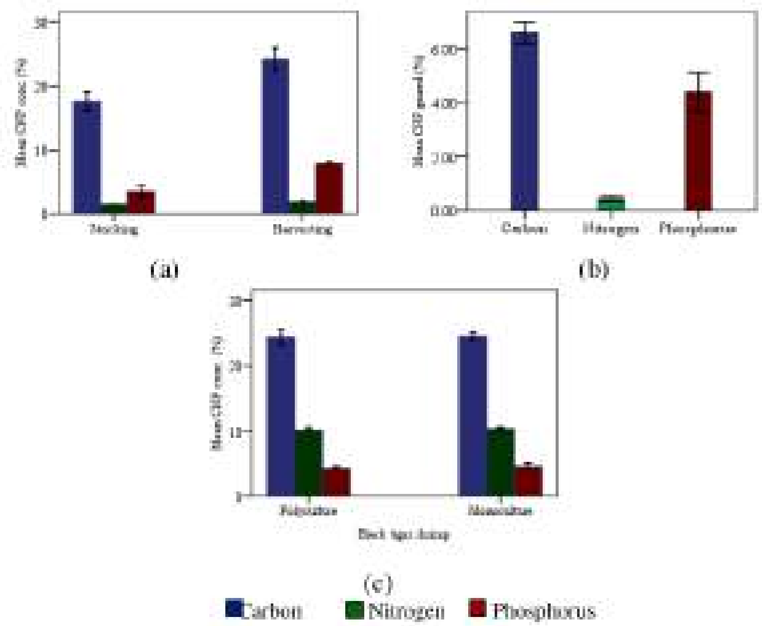

Figure 3-9

Mean value±SD of C, N, P concentration (a) and C, N, P gained (b) in seaweed tissue (upper) and C, N, P concentration in shrimp tissue (c) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

One of the important biological indicators is plankton community. Two plankton variables were analyzed included plankton density and dominancy index. The graph of plankton density has peak at the midle part and lower of both side of graph (**Figure 3-10a**). Statistically, results of two first observation demonstrated that plankton densities were not significant different between poluculture and monoculture system (**Figure 3-10a**) and then revealed significant different. At day 60th, plankton density of monoculture system was significant higher than polyculture system. At the end of experimental period, plankton density of polyculture system decreased. The other plankton variable also showed similar results. First and third sampling results revealed that dominancy indexes were not significant different between polyculture and monoculture system. The rests revealed significant different (**Figure 3-10b**). However, dominancy index of all sampling results of both culture system were found less than 0.5 of which indicated that there were no dominancy of certain plankton species.

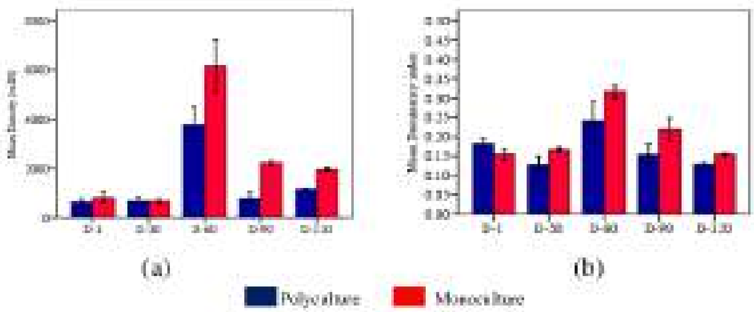

Figure 3-10

Mean value±SD of plankton density (a) and dominancy index (b) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Another investigated biological factor as research variables were total *Vibrio* and total bacterial. Similar trend of total *Vibrio* were observed between polyculture and monoculture system (**Figure 3-11a**) but not for the total bacterial count. Polyculture system had high total bacterial (570,000±105,000 CFU.ml^{-1}) at first observation and tend to decrease afterward (**Figure 3-11b**). The highest total bacterial of about 3,510,000±684,324 CFU.ml^{-1} was revealed by monoculture system at day 60th of culture period and tend to decrease afterward. Statistically, there were significant different of total *Vibrio* and total bacteria between polyculture and monoculture system at all sampling series except first observation for total *Vibrio* and two first observation for total bacteria (**Appendix 3-13**). However, at all observation series of experiment, total *Vibrio* and bacteria of both culture system revealed similar magnification of about 10^2 or 10^3 and 10^5 or 10^6, respectively (**Appendix 3-13**).

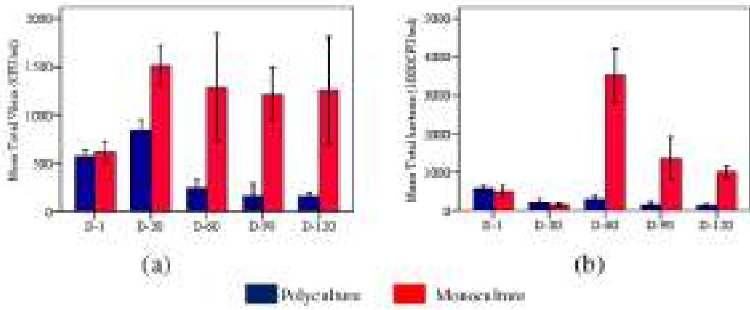

Figure 3-11

Mean value±SD of total *Vibrio* (a) and total bacterial count (b) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

DISCUSSION

Physico-chemical parameters

Black tiger shrimp (*P. monodon*) polyculture system with *Gracilaria verrucosa* as single-filtration organism was able to maintain a suitable culture habitat and lead to high growth, survival rate, and shrimp produtivity. The performance of *G. verrucosa* as filtration organism showed comparable results to the previous study as single filtration in the polyculture system (Nurhudah *et al.* 2009) and in the integrated system (Shpigel *et al*. 1993; Troell et al. 1999; Chow *et al*. 2001; Jones *et al*. 2001; Baliao & Tookwinas 2002; Neori *et al*. 2004). Suitable culture water quality during culture period brought about better performance of cultured and filtration organism.

There are two main important component of culture unit system mainly soil as culture container and water as culture habitat. Reciprocal interaction between those components form an ecosystem of culture environments that may influence aquaculture production. Texture and physico-chemical characteristic of of pond bottom soil were relatively similar amongst culture unit. However, at the end of experiment or at harvesting time, several soil quality parameters included carbon, CN ratio, total organic matter, and phosphate were significant different between shrimp polyculture system and monoculture system. Those parameters concentration indicated that shrimp polyculture system had faster decomposition process than monoculture system (Boyd 1990). Carbon organic is utilized by microbes as energy to degrade accumulated organic matter on the pond bottom soil. Narrow CN ratio also present the high rate of decomposition. Therefore, shrimp polyculture system could maintain stable decomposition process and establish suitable culture water habitat for black tiger shrimp (Direktorat Jenderal Perikanan Budidaya 2003).

Dissolved oxygen, temperature, salinity, transparancy, and pH were more frequent observed. Dissolved oxygen concentration at noon observation in the shrimp polyculture system showed higher concentration than in the monoculture system. It was caused by the occurence of *Gracilaria verrucosa* that performe photosynthesis during day time and supply most dissolved oxygen in the culture water habitat (Wetzel 1983; Boyd 1990; Midlen & Redding 2000; Pillay 2004). Temperatur and salinity of all culture system showed similar figures and probably due to similar effect of local weather and seawater source. The last mentioned parameter of all culture unit was generaly similar and within the range of shrimp culture requirement. The stable pH was supposed related to the high buffer capacity of seawater (Boyd 1990). It was supported by results of salinity observation during experiment that commonly more than $40^{o}/_{oo}$.

Secchi disk visibility expressed transparancy of culture water. Shrimp polyculture system started showing better transparancy just after measurement at day-58. It was comparable to the TSS, TDS concentration, and water colour. Generally, all culture systems had water colour of clear green at the beginning and then change to green or even yellowish green at second month of rearing or experiment period. The ability of *Gracilaria verrucosa* as filtration organism to maintain level of water transparancy, TSS, and TDS concentration were similar results to previous studies (Shpigel *et al*. 1993; Troell *et al*. 1999; Jones *et al*. 2001). From aquaculture point of view, those water quality parameters were within the range of shrimp culture requirement (Boyd 1990; Direktorat Jenderal Perikanan Budidaya 2003).

Nitrogen is one of water quality parameters as main limiting factors for shrimp culture. Polyculture system had better ability to maintain concentration of some nitrogen concentration that is combarable to the former studies (Shpigel *et al*. 1993; Troell *et al*. 1999; Chow *et al*. 2001; Jones *et al*. 2001; Baliao & Tookwinas 2002; Neori *et al*. 2004). Identical results were discovered in term of total organic matter, phosphate, and hydrogen sulfide. However, total alkalinity was much likely not tobe influenced by the different culture system but supposed by buffering capacity of seawater (Boyd 1990) and within range of shrimp culture requirement (Direktorat Jenderal Perikanan Budidaya 2003; Effendi 2003).

Biological parameters

Based on the aquaculture definition, achievement of aquaculture practices is mostly related to production level of main cultured species, black tiger shrimp (*P. monodon*). Polyculture system had better growth, survival rate, and productivity of shrimp as main cultured species than that of monoculture system. Those figures are identical to the former studies in laboratory scale with applying polyculture system (Nurhudah *et al*. 2009) and integrated system with common goals to reduce dissolved and suspended matter in waste water of aquaculture practices (Shpigel *et al*. 1993; Troell *et al*. 1999; Chow *et al*. 2001; Jones *et al*. 2001; Baliao & Tookwinas 2002; Neori *et al*. 2004). Similar result was recorded for total haemocyte count (THC) at day 135 and comparable to the laboratory scale of study carried out by Maftuch (2009) that *Gracilaria verrucosa* containing β-glucan of which could increase the number of haemocyte. Disimilarity was found for CNP content in shrimp tissue that not significant different between poluculture and monoculture. It was probably caused by the availability of such nutrients were quit enough for supplying all aquatic organisms requirement. Seaweed as photo-autotrophic organism showed ability in absorbing carbon, nitrogen, and phosphorous from the environment.

Several micro-organism indicator indicated that shrimp polyculture system revealed stable population. Density and dominancy index of plankton in shrimp polyculture system expresed more stable ecosystem (Krebs 1994) than monoculture system. Total *Vibrio* and bacterial data showed comparable results to previous study (Jones *et al*. 2001). It's probably due to the suitable habitat established of bacterial in monoculture system (Maier *et al*. 1999).

CONCLUSION AND RECOMMENDATION

Conclusion

Based on the result of study was concluded as follows:

1. Polyculture system of shrimp (*P. monodon*) with seaweed (*G. verrucosa*) cultured by off bottom method had ability to stabilize culture media. The tendency of all water and soil quality parameters in polyculture system showed significantly better concentration than that of monoculture system.
2. Growth rate, survival rate, and productivty of shrimp of polyculture system were better than that of monoculture system.
3. Shrimp polyculture system had suitable collony of micro-organism based on magnification of *Vibrio* of about 10^2 and total bacteria 10^5 and high total haemocyt count as well as showed lower dominancy of plankton.
4. Seaweed, *G. verrucosa* had ability to absorb carbon, nitrogen, and phosphorous substance from culture water habitat.

Recommendation

Based on the conclusion aboved, some recommendations are as follows:

1. Shrimp polyculture system with *G. verrucosa* cultivated by off bottom method with 80 g thallus.bound^{-1} submerged 40 cm under surface water with interval of 25 cm and 1.5 m between 2 ropes might be practiced by shrimp farmers in order to ensure high productivity and avoid environment degradation.

2. Further research on the shrimp polyculture system with higher stocking density of shrimp in order to figure out optimum shrimp productivity and seaweed (*G. verrucosa*) ability in maintaining culture water habitat.

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Appendix 3-1

Mean value±SD of pond soil texture of polyculture system of black tiger shrimp-seaweed and black tiger shrim monoculture system

Culture Technology		Soil Texture (%)		
		Sand	Silt	Clay
Polyculture	1	92.00	3.50	4.50
	2	84.00	8.00	8.00
	3	81.00	8.50	10.50
Mean value±SD		**85.67±5.69**	**6.67±2.75**	**7.67±3.01**
Monoculture	1	90.50	5.00	4.50
	2	88.00	6.00	6.00
	3	83.50	8.50	8.00
Mean value±SD		**87.33**±3.55	**6.50**±1.80	**6.17**±1.76

Appendix 3-2

Average results of dissolved oxygen ($mg.l^{-1}$) in polyculture and monoculture system in dawn and noon measurements during experiment

Culture Technology	Time	Day																			
		1	**7**	**15**	**22**	**29**	**36**	**43**	**51**	**58**	**64**	**71**	**78**	**85**	**92**	**100**	**106**	**113**	**118**	**125**	**135**
Polyculture	Dawn	3.86	3.86	3.69	3.73	3.73	3.65	3.69	3.44	3.02	2.90	2.87	3.45	3.60	2.75	2.83	2.86	2.67	2.67	3.07	2.55
	Noon	6.59	6.85	6.59	6.65	6.65	6.65	6.30	6.30	6.30	6.03	5.67	5.83	5.77	5.43	5.13	5.12	4.94	5.08	4.82	4.86
Monoculture	Dawn	4.07	4.07	3.77	3.71	3.71	3.86	3.40	3.18	3.45	3.45	3.43	3.89	3.51	2.92	2.92	2.66	2.61	2.84	2.83	2.34
	Noon	6.75	6.49	6.08	6.17	6.06	5.43	5.33	5.78	5.86	4.90	4.67	5.41	4.78	5.29	4.76	4.74	4.74	4.17	4.53	4.96

Appendix 3-3

Average results of pH in polyculture and monoculture system in dawn and noon measurements during experiment

Culture Technology	Time	Day																			
		1	**7**	**15**	**22**	**29**	**36**	**43**	**51**	**58**	**64**	**71**	**78**	**85**	**92**	**100**	**106**	**113**	**118**	**125**	**135**
Polyculture	Dawn	8.68	8.68	8.68	8.63	8.68	8.70	8.67	8.63	8.71	8.73	8.65	8.43	8.42	8.69	8.62	8.63	8.61	8.63	8.50	8.49
	Noon	8.69	8.63	8.64	8.67	8.70	8.71	8.68	8.86	8.67	8.74	8.78	8.64	8.72	8.73	8.67	8.69	8.47	8.53	8.65	8.69
Monoculture	Dawn	8.58	8.62	8.72	8.63	8.66	8.63	8.58	8.72	8.78	8.80	8.69	8.29	8.28	8.71	8.68	8.65	8.66	8.57	8.88	8.43
	Noon	8.70	8.62	8.63	8.66	8.69	8.64	8.72	8.81	8.68	8.81	8.68	8.71	8.63	8.77	8.68	8.66	8.65	8.35	8.59	8.56

Appendix 3-4

Average results of water temperature (^{O}C) in polyculture and monoculture system in dawn and noon measurements during experiment

Culture Technology	Time	Day																			
		1	**7**	**15**	**22**	**29**	**36**	**43**	**51**	**58**	**64**	**71**	**78**	**85**	**92**	**100**	**106**	**113**	**118**	**125**	**135**
Polyculture	Dawn	29.67	29.77	30.05	29.85	29.98	30.03	29.47	29.27	29.83	29.47	29.00	30.10	29.98	30.75	30.00	30.23	30.17	30.20	30.28	28.13
	Noon	32.18	32.00	31.83	32.32	32.22	32.43	32.33	34.30	32.20	32.28	30.97	31.63	31.27	31.20	31.65	31.22	31.27	31.08	30.58	30.08
Monoculture	Dawn	30.05	29.78	29.98	29.90	29.87	30.07	29.83	29.28	29.93	29.63	29.17	29.48	29.95	30.77	29.87	29.92	29.70	29.82	29.68	28.48
	Noon	31.97	32.12	31.88	32.42	32.45	32.28	32.28	34.23	32.45	32.48	30.98	31.63	31.48	31.33	30.95	31.00	30.75	30.52	30.78	30.53

Appendix 3-5

Average results of salinity ($^{O}/_{OO}$) in polyculture and monoculture system in dawn and noon measurements during experiment

Culture Technology	Time	Day																			
		1	**7**	**15**	**22**	**29**	**36**	**43**	**51**	**58**	**64**	**71**	**78**	**85**	**92**	**100**	**106**	**113**	**118**	**125**	**135**
Polyculture	Dawn	44.33	45.67	46.67	46.67	46.67	46.67	42.00	43.67	44.33	44.00	39.33	40.33	40.33	38.67	40.00	40.17	39.67	40.67	40.00	34.75
	Noon	45.00	45.67	44.67	44.33	43.67	42.17	42.00	39.67	41.33	43.00	38.67	39.00	38.33	39.33	40.33	39.33	39.00	39.33	38.00	34.75
Monoculture	Dawn	44.83	45.67	46.33	46.67	46.00	46.67	43.67	43.50	45.00	45.33	39.33	39.67	38.33	39.33	40.33	39.83	40.67	40.17	39.50	34.25
	Noon	44.67	45.33	45.00	45.00	44.33	44.33	43.33	43.00	43.33	43.33	39.33	38.67	40.00	38.67	40.00	40.00	39.33	40.00	39.50	34.25

Appendix 3-6

Average results of transparancy (Sechii disk visibility) (cm) in polyculture and monoculture system in dawn and noon measurements during experiment

Culture Technology	Day																			
	1	**7**	**15**	**22**	**29**	**36**	**43**	**51**	**58**	**64**	**71**	**78**	**85**	**92**	**100**	**106**	**113**	**118**	**125**	**135**
Polyculture	62	62	63	69	71	73	77	76	79	82	73	73	75	73	70	68	63	58	62	55
Monoculture	62	62	63	69	70	72	75	77	78	76	68	68	68	68	67	62	57	47	49	43

Appendix 3-7

Average results of total suspended solid (TSS) and total dissolved solid (TDS) ($mg.l^{-1}$) in polyculture and monoculture system in dawn and noon measurements during experiment

Parameter	Culture Technology											
	Polyculture						Monoculture					
	D-1	**D-30**	**D-50**	**D-80**	**D-110**	**D-130**	**D-1**	**D-30**	**D-50**	**D-80**	**D-110**	**D-130**
TSS	19.67	19.37	17.33	15.93	16.37	18.38	19.87	20.27	20.43	21.77	21.92	23.28
TDS	44,559.00	42,258.00	33,673.33	34,771.67	39,006.67	39,918.33	45,893.33	48,890.67	51,208.67	49,673.00	52,763.00	55,390.00

Appendix 3-8

Average results of measurement of ammonium, ammonia, nitrite, and nitrate in polyculture and monoculture system in dawn and noon measurements during experiment

Parameter	Culture Technology											
	Polyculture						Monoculture					
	D-1	D-30	D-50	D-80	D-110	D-130	D-1	D-30	D-50	D-80	D-110	D-130
NH_4	0.6605	1.0744	1.0813	1.0335	0.9378	1.0710	0.6325	0.9555	1.1460	1.1573	1.2235	1.4850
NH_3	0.0423	0.0462	0.0408	0.0380	0.0362	0.0361	0.0352	0.0564	0.0516	0.0523	0.0530	0.0503
NO_2	0.0392	0.0500	0.0533	0.0467	0.0417	0.0450	0.0295	0.0507	0.0633	0.0617	0.0533	0.0550
NO_3	1.0371	0.5269	0.3412	0.3640	0.3805	0.2377	1.4190	0.6510	0.0854	0.1207	0.1353	0.1167

Appendix 3-9

Average results of measurement of total organic matter (TOM), phosphate (PO_4), total alkalinity, and hydrogen sulphide (H_2S) in polyculture and monoculture system in dawn and noon measurements during experiment

Parameter	Culture Technology											
	Polyculture						Monoculture					
	D-1	D-30	D-50	D-80	D-110	D-130	D-1	D-30	D-50	D-80	D-110	D-130
TOM	101.38	32.76	24.77	21.54	33.91	35.48	93.58	39.58	47.06	50.25	48.81	46.90
PO_4	0.08	0.10	0.08	0.12	0.17	0.17	0.09	0.11	0.12	0.19	0.22	0.23
Alkalinitas	105.33	250.00	238.33	185.81	176.72	182.81	97.00	316.67	225.87	194.28	182.77	190.56
H_2S	0.03	0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.04	0.04	0.05	0.04

Appendix 3-10

Total harvested biomass with shrimp size of polyculture of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Culture Technology		Biomass (kg) with size (Inds.kg^{-1})							Total (kg)
		30	35	40	45	50	60	70	
Polyculture	1	42.00	31.50	31.50	31.50	31.50	21.00	21.00	210
	2	20.25	13.50	13.50	20.25	13.50	27.00	27.00	135
	3	19.80	6.60	19.80	19.80	19.80	19.80	26.40	132
Monoculture	1	16.60	16.60	33.20	33.20	24.90	24.90	16.60	166
	2	8.00	16.00	16.00	24.00	24.00	40.00	32.00	160
	3	18.60	18.60	31.00	18.60	12.40	12.40	12.40	124

Appendix 3-11

Total area, shrimp density and survival rate, and total biomass of polyculture of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Culture Technology		Area (m^2)	Total Density (PLs)	Biomass		SR (%)
				Total (kg)	Productivity (kg.m^{-2})	
Polyculture	1	2,624	10496	210	0.0800	89.03
	2	1,881	7524	135	0.0718	89.26
	3	1,881	7524	132	0.0702	86.84
Monoculture	1	2,624	10496	166	0.0633	74.33
	2	2,624	10496	160	0.0610	79.65
	3	1,881	7524	124	0.0659	73.34

Notes:
SR = Survival rate

Appendix 3-12

Mean value±SD of of seaweed weigth sampling of polyculture system of black tiger shrimp-seaweed

Variable	Replication			
	1	2	3	Mean
Stocking (g)	80	80	80	80
1st Sampling (Day 75)				
Sample weight (g)	512.63±99.68	483.88±95.69	523.00±104.80	506.50±20.27
Weight gain (g)	432.63	403.88	443.00	426.50±20.27
Weight gain (%)	540.78	504.84	553.75	533.13±25.34
ADG ($g.d^{-1}$)	5.77	5.39	5.91	5.69±0.27
2nd Sampling (Day 100)				
Sample weigth (g)	66.13±17.38	58.00±19.99	58.50±19.42	60.88±4.55
Weight gain (g)	-13.88	-22.00	-21.50	-19.1250±4.55
Weight gain (%)	-17.34	-27.50	-26.88	-23.9063±5.70
ADG ($g.d^{-1}$)	-0.14	-0.22	0.22	-0.19±0.05

Appendix 3-13

Mean value±SD of microbial observation of polyculture system of black tiger shrimp-seaweed and black tiger shrim monoculture system

Culture Technology		Day 1	30	60	90	120
Total *Vibrio* (CFU/ml)						
Polyculture	1	6.3×10^2	7.8×10^2	2.49×10^2	1×10^2	1.65×10^2
	2	5.95×10^2	7.8×10^2	3.3×10^2	80	1.98×10^2
	3	5×10^2	9.7×10^2	1.5×10^2	3.2×10^2	1.32×10^2
Mean value±SD		575±67a	843±109a	243±90a	166±133a	165±33a
Monoculture	1	5.8×10^2	1.34×10^3	7.2×10^2	1.13×10^3	6.36×10^2
	2	5.25×10^2	1.44×10^3	1.28×10^3	1.53×10^3	1.43×10^3
	3	7.4×10^2	1.75×10^3	1.84×10^3	9.9×10^2	1.7×10^3
Mean value±SD		615±111a	1510±213b	1280±560b	1215±281b	1254±551b
Total Bacteria (CFU/ml)						
Polyculture	1	4.95×10^5	2.8×10^5	4.3×10^5	2.3×10^5	1.5×10^5
	2	6.9×10^5	1.1×10^5	2.2×10^5	1.4×10^5	1.6×10^5
	3	5.25×10^5	2.4×10^5	2.4×10^5	9×10^4	1.1×10^5
Mean value±SD		570,000± 105,000a	210,000± 88,881a	296,666± 115,902a	153,333± 70,945a	140,000± 26,457a
Monoculture	1	7.05×10^5	2×10^5	3.15×10^6	7.5×10^5	8.5×10^5
	2	4.15×10^5	1.3×10^5	3.1×10^6	1.53×10^6	1.01×10^6
	3	3.8×10^5	1.5×10^5	4.3×10^6	1.78×10^6	1.18×10^6
Mean value±SD		500000± 178395a	160000± 36055a	3510000± 684324b	1353333± 537246b	1011666± 162506b

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