



UNIVERSITAS INDONESIA

**ENVIRONMENTALLY FRIENDLY CULTURE
TECHNOLOGY OF BLACK TIGER SHRIMP
(*Penaeus monodon* Fabricius, 1798) USING BIOFILTRATION
Gracilaria verrucosa (Hudson) Papenfuss, 1950 AND
Anadara granosa Linnaeus, 1758**

DISSERTATION

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**FACULTY OF MATHEMATICS AND NATURAL SCIENCES
GRADUATE STUDY PROGRAM BIOLOGY
INDONESIA UNIVERSITY
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**in fulfilment of the requirement for the Doctor degree
in Conservation Biology**

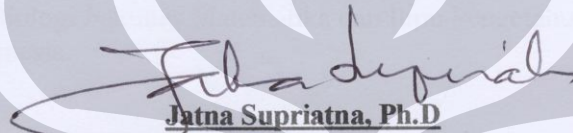
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BIOFILTRATION *Gracilaria verrucosa* (Hudson)
Papenfuss, 1950 AND *Anadara granosa* Linnaeus,
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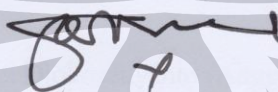

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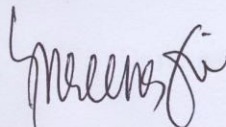
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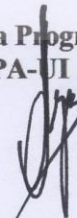

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RINGKASAN

Penelitian ini dilakukan untuk memberikan informasi ilmiah tentang keuntungan secara ekologi dari sistem budidaya udang polikultur dengan menggunakan filter biologi. Tujuan penelitian secara garis besar adalah mengembangkan kegiatan budidaya udang yang bertanggung jawab dan ramah lingkungan serta menjamin keberlanjutannya. Tiga jenis organisme yang digunakan dalam penelitian yaitu udang windu (*Penaeus monodon*), rumput laut (*Gracilaria verrucosa*), dan kerang darah (*Anadara granosa*). Penelitian dilakukan melalui 3 tahap, yaitu:

- 1) Tahap 1, karakterisasi efektivitas rumput laut (*G. verrucosa*) dan kerang darah (*A. granosa*) sebagai filter biologi tunggal dalam polikultur udang windu (*P. monodon*). Hasil penelitian tahap ini disajikan dalam “**Chapter 1. Characterization of filtration organisms – seaweed *Gracilaria verrucosa* and blood cockle – *Anadara granosa* in black tiger shrimp *Penaeus monodon* Fab. polyculture system**”.
- 2) Tahap 2, karakterisasi efektivitas rumput laut (*G. verrucosa*) dan kerang darah (*A. granosa*) sebagai filter biologi gabungan dalam polikultur udang windu (*P. monodon*). Perlakuan polikultur udang windu didasarkan pada hasil penelitian Tahap 1. Hasil penelitian tahap ini disajikan dalam “**Chapter 2. Characterization of dual-filtration organisms of seaweed – *Gracilaria verrucosa* and blood cockle – *Anadara granosa* in black tiger shrimp (*Penaeus monodon* Fab.) polyculture system**”.
- 3) Tahap 3, karakterisasi efektivitas rumput laut (*G. verrucosa*) sebagai filter biologi tunggal dalam polikultur udang windu dalam percobaan skala komersial atau lapangan. Percobaan tahap ini merupakan tindak lanjut dari percobaan tahap sebelumnya tetapi dilaksanakan dalam skala lapangan. Hasil penelitian tahap ini disajikan dalam “**Chapter 3. Study on effectiveness of seaweed–*Gracilaria verrucosa* as filtration organism in**

**commercial scale of black tiger shrimp (*Penaeus monodon* Fab.)
polyculture system”.**

Penelitian Tahap 1 dan 2 merupakan percobaan skala laboratorium menggunakan wadah kayu (ukuran 0,9 x 0,6 x 1 m) dilapisi plastik tanpa pergantian air selama percobaan dan dilakukan di Balai Pengembangan Budidaya Air Payau dan Laut (BPBAPL), Desa Sungai Buntu, Kecamatan Cilebar, Kabupaten Karawang, Jawa Barat. Penelitian Tahap 3 dilakukan di tambak milik pembudidaya yang terletak di desa yang sama dengan penelitian tahap sebelumnya. Parameter fisika-kimia kualitas air yang diamati meliputi oksigen terlarut, suhu, salinitas, pH, ammonia, nitrit, nitrat, asam sulfat, fosfat, total padatan terlarut, total padatan tersuspensi, dan total bahan organik. Aspek biologi yang diamati meliputi pertumbuhan (berat) udang, rumput laut, kerang, total bakteri dan *Vibrio*, kandungan karbon, nitrogen, dan fosfor dalam daging udang, kerang, dan thalus rumput laut. Disamping itu, di percobaan Tahap 3 dilakukan juga pengamatan terhadap beberapa parameter fisika kimia kualitas tanah, kecerahan, warna air, komunitas plankton, dan total jumlah haemosit. Analisa laboratorium untuk beberapa parameter ekologi dilakukan di BPBAPL, Sekolah Tinggi Perikanan Jakarta, dan Tambak Pandu Karawang. Padat tebar udang sebagai biota utama yang dibudidayakan adalah 4 ind.m⁻² untuk seluruh perlakuan.

Penelitian Tahap 1 terdiri dari beberapa perlakuan yaitu perbedaan cara penanaman (di dasar, di kedalaman sekitar 40 cm dari permukaan, dan di permukaan) dan jumlah penebaran (240 gram dengan 30 g per ikatan dan 640 g dengan 80 g per ikatan) rumput laut. Adapun percobaan kerang darah dengan perlakuan perbedaan padat tebar yaitu 50 individu.m⁻², 100 individu.m⁻², dan 150 individu.m⁻². Tujuan penelitian ini adalah menentukan peran ekologi dan kemampuan mempertahankan kualitas lingkungan dari rumput laut (*G. verrucosa*) dan kerang darah (*A. granosa*) sebagai filter biologi tunggal dalam lingkungan polikultur udang windu (*P. monodon*). Hasil penelitian menunjukkan bahwa konsentrasi oksigen terlarut meningkat sesuai dengan metode penanaman rumput

laut. Polikultur (udang windu – *G. verrucosa*) dengan metode lepas dasar digantung 40 cm di bawah permukaan air menunjukkan kandungan oksigen terlarut yang lebih tinggi dibanding dengan sistem polikultur lainnya dan monokultur. Parameter ammonia, nitrit, nitrat, asam sulfat, total bahan organik dan total padatan terlarut di semua polikultur udang dengan *G. verrucosa* cenderung menurun sesuai dengan waktu pembudidayaan. *G. verrucosa* dalam sistem polikultur memiliki potensi untuk memainkan peran secara ekologi untuk mempertahankan media air pembudidayaan yang sesuai untuk kehidupan udang terkait dengan kandungan ammonia, nitrit, nitrat, total bahan organik dan total padatan terlarut. Ditinjau dari tingkat produksi budidaya dan ekologi maka polikultur udang dengan rumput laut jumlah tebar 240 dan 640g yang ditanam dengan metode lepas dasar dengan digantung 40 cm dari permukaan memiliki kemampuan yang baik dalam mempertahankan kualitas air budidaya sebagaimana yang dibutuhkan udang dan pertumbuhan udangnya relatif lebih tinggi dibanding polikultur lainnya. Ditinjau dari pertumbuhan udang, polikultur udang dengan rumput laut metode tanam disebar di dasar sebanyak 240 g dan metode lepas dasar digantung 40 cm di bawah permukaan air baik per ikatan 30 g (total 240 g) maupun 80 g (total 640 g) menunjukkan pertumbuhan yang berbeda nyata ($P < 0,05$) dengan polikultur udang dengan rumput laut perlakuan lainnya.

Sementara itu, polikultur udang dengan kerang darah 50 individual.m⁻² memiliki kandungan oksigen terlarut lebih tinggi dari pada perlakuan dengan padat tebar yang lebih tinggi (100 maupun 150 individual.m⁻²). Namun demikian, seluruh perlakuan termasuk monokultur sebagai kontrol menunjukkan pola kecenderungan konsentrasi oksigen terlarut yang serupa dan selalu lebih rendah dari 3 mg.l⁻¹ kecuali pada hari 30 pengamatan dalam wadah kontrol memiliki nilai lebih dari 3 mg.l⁻¹. Konsentrasi ammonia, nitrit, nitrat, asam sulfat, total bahan organik dan total padatan terlarut di semua polikultur udang dengan kerang darah cenderung menurun sesuai dengan waktu pembudidayaan. Kerang darah sebagai organisme pemakan penyaring yang dibudidayakan secara polikultur memiliki kemampuan mengurangi nutrisi yang berlebihan hasil dari kegiatan budidaya. Dari sudut pandang produksi budidaya dan ekologi, polikultur dengan kerang

darah kepadatan 100 individual.m⁻² memiliki kualitas air yang cukup baik diikuti dengan kepadatan 50 individual.m⁻². Namun demikian, ditinjau dari pertumbuhan udang windu, maka tidak ada perbedaan yang nyata antar polikultur dengan kepadatan kerang yang berbeda.

Percobaan Tahap 2 bermaksud membandingkan antara teknologi udang polikultur dengan filter biologi gabungan dan monokultur. Polikultur udang dengan empat jenis filter biologi gabungan, yaitu 1) 30 g rumput laut per ikatan (total 240 g) + 50 individu.m⁻² kerang darah, 2) 30 g rumput laut per ikatan (total 240 g) + 100 individu.m⁻² kerang darah, 3) 80 g rumput laut per ikatan (total 640 g) + 50 individu.m⁻² kerang darah, dan 4) 80 g rumput laut per ikatan (total 640 g) + 100 individu.m⁻² kerang darah. Ikatan rumput laut digantung sekitar 40 cm di bawah permukaan air. Tujuan penelitian ini adalah menentukan peran ekologi dan kemampuan mempertahankan kualitas lingkungan dari rumput laut (*G. verrucosa*) dan kerang darah (*A. granosa*) sebagai filter biologi gabungan dalam lingkungan pembudidayaan polikultur udang windu (*P. monodon*).

Parameter kualitas air yang meliputi ammonium, ammonia, nitrit, asam sulfat, fosfat dan total bahan organik di sistem polikultur udang dengan filter biologi gabungan relatif lebih tinggi dibanding sistem monokultur dan gambaran sebaliknya untuk konsentrasi oksigen terlarut. Pada saat siang hari, polikultur udang dengan 80 g rumput laut per ikatan (total 640 g) + 50 individu.m⁻² kerang darah masih dapat meningkatkan konsentrasi oksigen terlarut hingga 3 mg.l⁻¹. Namun demikian, secara umum polikultur udang dengan filter gabungan rumput laut (*G. verrucosa*) dan kerang darah (*A. granosa*) tidak mampu mempertahankan kualitas air lingkungan pembudidayaan. Udang sebagai organisme yang dibudidayakan mengalami kematian hampir di setiap ulangan dari setiap perlakuan kecuali untuk polikultur udang dengan dengan 80 g rumput laut per ikatan (total 640 g) + 50 individu.m⁻² kerang darah hanya satu ulangan yang mengalami kematian massal.

Berasarkan hasil penelitian Tahap 1 dan 2, maka penelitian lapangan (Tahap 3) dengan menerapkan perlakuan polikultur udang dengan rumput laut (*G.*

verrucosa) sistem lepas dasar dengan kedalaman 40 cm dari permukaan dan berat per ikatan adalah 80 g dan monokultur sebagai kontrol dengan masing-masing 3 kali ulangan. Jumlah rumput laut sekitar 800 kg.ha⁻¹. Petak tambak penelitian dikelompokkan menjadi 2 berdasarkan luasnya, yaitu 1.881 m² dan 2.624m² masing-masing tiga petak penelitian.

Polikultur udang windu (*P. monodon*) dengan rumput laut *G. verrucosa* mampu untuk mempertahankan habitat pembudidayaan yang selanjutnya memberikan pengaruh positif berupa pertumbuhan, kelangsungan hidup, dan produktivitas udang yang tinggi. *G. verrucosa* sebagai filter biologi menunjukkan hasil yang sesuai dengan penelitian sebelumnya dalam sistem polikultur (Nurhudah et al. 2009) dan dalam budidaya terpadu (Shpigel et al. 1993; Troell et al. 1999; Chow et al. 2001; Jones et al. 2001; Baliao & Tookwinas 2002; Neori et al. 2004).

Hasil penelitian menunjukkan bahwa beberapa parameter kualitas tanah petak tambak saat panen menunjukkan perbedaan nyata ($P < 0,05$) antara polikultur dan monokultur, yaitu jumlah dari kandungan karbon (C), perbandingan karbon dan nitrogen (C/N), total bahan organik, dan fosfat. Sementara itu, jumlah nitrogen dan kapasitas tukar kation (KTK) antara polikultur dan monokultur tidak berbeda nyata. Secara umum, budidaya sistem polikultur menunjukkan kemampuan untuk mempertahankan beberapa parameter kualitas air sesuai dengan kebutuhan udang yang dibudidayakan. Pada siang hari, polikultur udang windu – *G. verrucosa* menunjukkan kandungan oksigen terlarut yang lebih tinggi dibanding dengan monokultur. Walaupun demikian, pada pagi hari setelah hari 90 menunjukkan kecenderungan konsentrasi oksigen terlarut yang hampir sama yaitu kurang dari 3 mg.l⁻¹ tetapi masih lebih besar dari 2,5 mg.l⁻¹. Parameter kualitas air lainnya yang meliputi ammonium, ammonia, nitrit, nitrat, asam sulfat, fosfat, total bahan organik, total padatan tersuspensi, dan total padatan terlarut dalam polikultur udang dengan *G. verrucosa* adalah berbeda nyata ($P < 0,05$) dibanding monokultur khususnya setelah bulan kedua periode pemeliharaan kecuali untuk total alkalinitas.

Beberapa parameter biologi yang diamati dan digunakan sebagai tolak ukur keberhasilan sistem pembudidayaan yang terkait dengan udang windu dan rumput laut adalah pertumbuhan, tingkat kelangsungan hidup, tingkat produksi (produktivitas), total haemosit, kandungan karbon-nitrogen-fosfor dalam daging, plankton, dan bakteri (total *Vibrio* dan total bakteri). Pada saat panen, pertumbuhan, produktivitas, dan tingkat kelangsungan hidup udang, total jumlah haemosit, kepadatan dan indeks dominansi plankton serta total *Vibrio* dan bakteri dalam polikultur adalah berbeda nyata ($P < 0,05$) dibanding dengan monokultur. Sementara itu, kandungan karbon, nitrogen, dan fosfor dalam daging udang tidak berbeda nyata antara sistem polikultur dan monokultur, tetapi berbeda nyata untuk hasil analisa dari thalus *G. verrucosa* ($P < 0,05$).

Ditinjau dari hasil analisis parameter kualitas tanah dasar tambak dan kualitas air habitat pembudidayaan maka tambak dengan sistem polikultur udang windu (*P. monodon*) dengan rumput laut (*G. verrucosa*) mampu menyerap beberapa bahan anorganik dalam habitat pembudidayaan. Polikultur udang windu (*P. monodon*) dengan rumput laut (*G. verrucosa*) merupakan teknologi budidaya yang ramah lingkungan karena tidak hanya mempertahankan kualitas air habitat pembudidayaan tetapi juga memperbaiki kualitas air hasil pembudidayaan yang akan di buang ke lingkungan sekitar saat pergantian air dan pemanenan. Disamping itu, ditinjau dari hasil panen dibandingkan dengan monokultur maka teknologi polikultur menunjukkan pertumbuhan dan tingkat kelulusan hidup serta produksi udang yang lebih tinggi.

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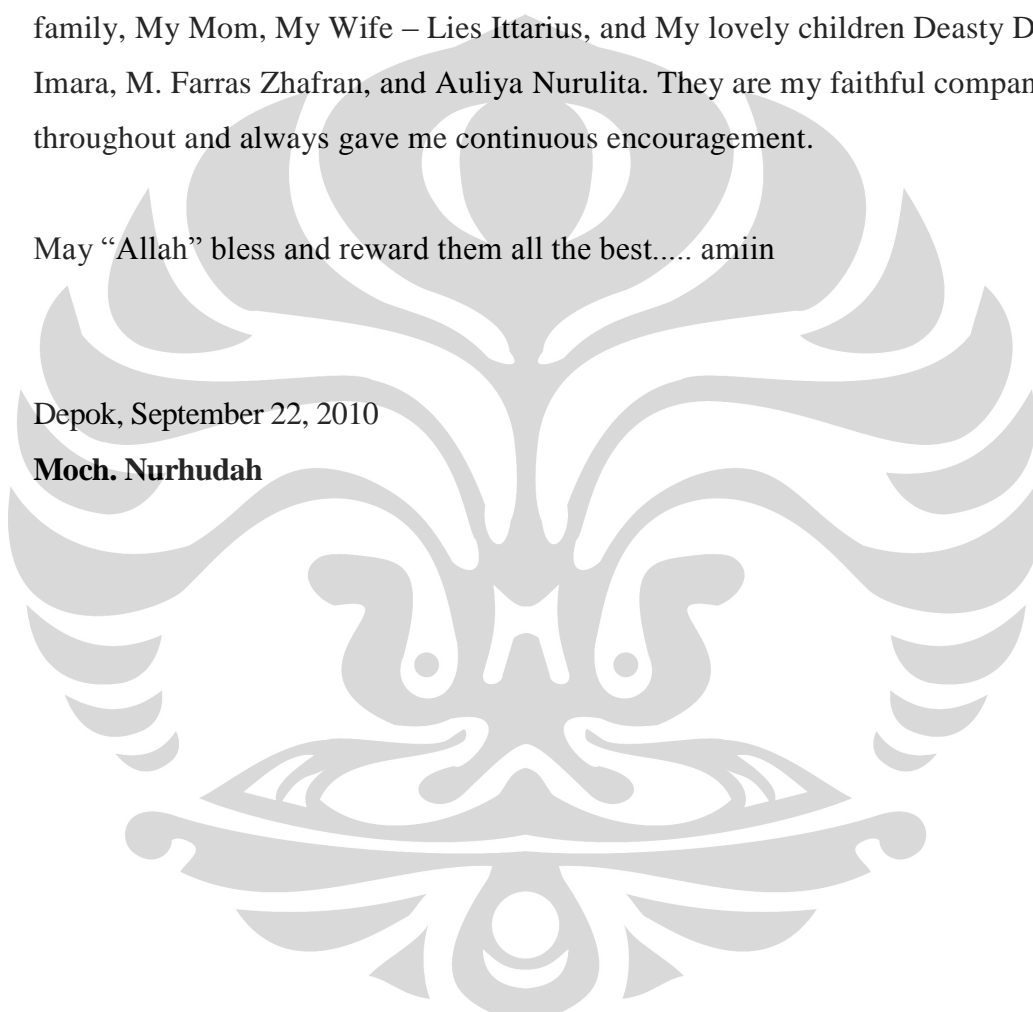
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Depok, September 22, 2010

Moch. Nurhudah



GENERAL INTRODUCTION

In Indonesia, shrimp culture has made significantly contribution to total fishery production. From 2002 to 2007, the contribution of shrimp culture to total fisheries production is about 31% per year for production volume and about 65% for production value (Direktorat Jenderal Perikanan Budidaya 2007, 2008). In addition, the Directorate General for Culture Fisheries estimated that the potential area for future development is about 700,000 ha (Direktorat Jenderal Perikanan Budidaya 2003, 2007).

Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants (Barnabe 1990; FAO 1991). Aquaculture practices tend to manipulate ecosystems in order to achieve higher production than natural one by employing culture technology and management. In Indonesia, aquaculture practices are generally divided into three categories those are intensive, semi-intensive, and traditional or extensive system. Generally, the density of black tiger shrimp in intensive, semi-intensive and traditional culture technology are more than 15 ind.m⁻², 7–14 ind.m⁻², and less than 7 ind.m⁻², respectively (Direktorat Jenderal Perikanan Budidaya 2003).

In general, aquaculture is undertaken in man-made ecosystems 'culture units' which are generally made up of biotic and abiotic components. The biotic component includes cultured shrimp/fish, filtration organisms, plankton, exotic organisms entering along with in-flowing water, and microbes. The abiotic component comprises of physical and chemical aspects of both soil and water as the culture medium. All culture ecosystem components, those are biotic and abiotic, perform ecological functions and interact with each other. Therefore, a change in one of these ecosystem components may be followed by changes in the other components, the cultured organisms or even the whole culture system (Mukherjee 1997). The three major ecological processes are production,

consumption, and decomposition (Zonneveld *et al.* 1991), all of which influence structure and ecological function of the system (Folke *et al.* 2002).

Ecologically, aquaculture practices tend to change the equilibrium of the natural ecosystem and may even generate serious ecological pressure. Practices which generate ecological pressure include: high stocking density (Surawidjaja 2006), feeding, and utilization of bioactive compounds (including pesticides and antibiotics) (FAO 1991). Moreover, at harvesting time waste water from the culture system may be drained into natural ecosystem. Consequently, the natural surrounding environment may be considerably disturbed (Donovan 2001; FAO 2006; FAO 2007; Tucker *et al.* 2008). More intensive production of shrimp culture requires more inputs and is more likely to cause environmental degradation either in the pond itself or in the surrounding environment (Flaherty & Karnjanakorn 1995; Jenkins 1995; Neiland *et al.* 2001; Szuster 2003). The consequences of these characteristics are a decrease in the shrimp's immune system which makes the shrimp vulnerable to disease, reduced growth, increases mortality, which in turn resulting in lower harvesting volume and even mass mortality of shrimp (Departemen Kelautan dan Perikanan 2005). According to Troell *et al.* (1999b), the natural self purification rate of waste water of intensive culture, termed *the footprint for waste assimilation*, would need an area of 7.4 – 21.6 times the area of its culture system itself to assimilate aquaculture wastes. The impacts of aquaculture activities may include: reduction of carrying capacity of aquaculture area, water quality deterioration, and disease out breaks. Therefore, proper procedure of aquaculture activities should be done in order to cope with brackish water aquaculture collapse. Implementation of aquaculture technology and management is intended to stabilize those processes and eliminate environmental changes in order to improve production.

In the last decade, international attentions to the environment problems and food security have strongly influenced aquaculture practices. Some importer countries declared a strick regulation related to the responsible aquaculture such as utilisation of certain antibiotic and conversion of mangrove forest to brackish

water pond. Therefore, aquaculture practices must apply environmentally friendly and responsible technology. There are several aquaculture technologies that can improve environmental sustainability, including polyculture (Pillay 2004), cultivation rotation system between shrimp and other organisms (Jonnes 1999), and integrated aquaculture systems (Jones *et al.* 2000; Ryder *et al.* 2004). The organisms commonly cultivated in brackish water polyculture systems are shrimp, milk-fish, seaweed, and bivalves (Midlen & Redding 2000; Pillay 2004). The advantages of having a polyculture system are that by culturing several species that belong to the different trophic levels, resources are used more efficient and the system is more resilient against environmental fluctuations (Troell *et al.* 1999a). Furthermore, the polyculture system can more readily cope with self-pollution by reducing the concentration of organic and inorganic substances, and economically earn additional income resulted from secondary or tertiary cultured organisms, e.g. seaweed and bivalve (Jonnes 1999).

This research was undertaken in order to provide a scientific assessment of the ecological benefits of polyculture system by employing bio-filtration organisms. The overall goal of this research was to develop responsible and environmentally friendly shrimp culture practices in order to conserve aquaculture resources as well as ensure the sustainability of shrimp culture. There are three specific goals, namely:

1. to evaluate the effectiveness of seaweed and blood cockle either as individual or as dual-filtration organisms in reducing organic and inorganic matter concentration during the culture period;
2. to analyse the effectiveness of polyculture techniques to maintain the culture medium from the perspectives of ecological stability and provision of an environment suitable for shrimp survival and growth; and
3. To analyse the sustainability of polyculture techniques at a commercial scale.

Those goals are more focused on the ecological aspects of polyculture, given that the criteria for sustainable aquaculture are based largely on the

ecological and economical viability of farming technology and business practices (Boyd and Schmittou 1999).

Three organisms were employed in these research experiments: black tiger shrimp (*Penaeus monodon*) as the main culture organism, seaweed (*Gracilaria verrucosa*) and blood cockle (*Anadara granosa*) as filtration organisms (Shimoda *et al.* 2006). The culture procedure for all experimental activities was based on those of the Direktorat Jenderal Perikanan Budidaya (2003) and Baliao & Tookwinas (2002). The selection of ecological parameters was based on Rand (1979), Alerts & Santika (1987), Zonneveld *et al.* (1991) and Effendi (2003). These activities consisted of 3 (three) stages as follows:

1. characterization of the effectiveness of individual filtration organisms (seaweed and blood cockle) in the black tiger shrimp polyculture system;
2. characterization of the effectiveness dual-filtration organisms (seaweed and blood cockle) in the black tiger shrimp polyculture system, and
3. The effectiveness of filtration organisms in commercial scale.

Two ecological parameters were chosen on these experiments, biotic and abiotic. The biotic aspects include black tiger shrimp, filtration organisms, plankton, and bacteria. Parameters of those two first organisms comprised of growth, population, and carbon-nitrogen-phosphorus (CNP) content, while other organisms, bacteria and plankton were measured on population density. The abiotic aspects include physico-chemical parameters of water: dissolved oxygen, temperature, salinity, water transparency, ammonia, NO₂, NO₃, pH, H₂S, PO₄, total organic matter (TOM), total dissolved solids (TDS) and total suspended solids (TSS), and soil quality (i.e texture, pH, organic matter, carbon, nitrogen, phosphorous, cation exchange capacity).

Literatures on environmentally friendly and sustainable black tiger shrimp culture using filtration organisms such as seaweed and filter-feeding organisms are very limited. Lazur & Britt (1997); Chow *et al.* (2001); Jones *et al.* (2001); Baliao & Tookwinas (2002); Neori *et al.* (2004); Shimoda *et al.* (2005); Matos *et al.* (2006); Shimoda *et al.* (2006) had studied biofilter system for integrated aquaculture but

not polyculture system either using single, dual or multi-filtration organisms. According to the definition of polyculture suggested by Midlen & Redding (2000), Hawerton (2001), and Pillay (2004), the research outlined above was not in true polyculture systems, but was mostly using a monoculture system complemented by employing filtration organisms.

PROBLEM STATEMENT

Aquaculture practices try to manipulate natural ecosystem to establish appropriate culture unit in order to increase yield. In aquaculture, the density of the target (production) organism is usually much higher than in wild populations. For example, the stocking density in extensive black tiger shrimp culture is about 40 times higher than that found in nature or in wild population (Surawidjaja 2006). In addition, during the rearing period, some of the supplied food is not consumed and will settle down to the pond bottom (Hargreaves and Tucker 2004). This excessive feed may cause environmental problems related to increase suspended and dissolved solids or even process of hypernutrification and eutrophication. The possible impacts of shrimp farm effluent on water quality include increased fluctuation of diurnal dissolved oxygen levels (Donovan 2001), organic matter rich sediment accumulation (Funge-Smith and Briggs 1998) and degraded water quality (FAO 2006; FAO 2007; Tucker *et al.* 2008). Therefore, aquaculture technology needs to be improved to make production more efficient and to minimize negative ecological impacts. This can only be achieved by application of scientific principles to improve production methods and environmental management (Boyd and Schmittou 1999).

Due to the limited scientific information on black tiger shrimp polyculture related to environmentally friendly technology by employing filtration organisms, this research was undertaken to address the following issues:

1. Does the seaweed, *G. verrucosa* as a single filtration organism has the ability to absorb inorganic matter in the culture medium? Can they also stabilize the culture ecosystem?

2. Does the blood cockle, *A. granosa* as a single filtration organism has the ability to absorb organic matters in the culture medium and stabilize the culture ecosystem?
3. Do the seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as dual-filtration organism have the ability to absorb inorganic and organic matters in the culture medium and stabilize the culture ecosystem?
4. How effective are these polyculture systems in maintaining the pond culture ecosystem?

BENEFITS OF STUDY

The benefits of the study on black tiger shrimp (*P. monodon*) polyculture systems with seaweed (*G. verrucosa*)-blood cockle (*A. granosa*) are as follows:

1. To increase pond productivity by optimizing utilization of each individual niche within the pond culture ecosystem.
2. To reduce the ecological impacts of black tiger shrimp aquaculture practices.
3. To reduce the risk of shrimp crop failure due to the high mortality rate or even mass mortality related to environment factors.
4. To develop an environmentally friendly and sustainable black tiger shrimp culture that can be adopted by shrimp farmers.
5. To support the revitalization and rehabilitation of brackish water shrimp culture.

HYPOTHESES

1. Seaweed (*G. verrucosa*) as photoautotrophic organism is able to absorb inorganic substances in shrimp culture system.
2. Blood cockle (*A. granosa*) as filter feeder organism is able to absorb organic substances in shrimp culture system.
3. Seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) in combination as filtration organism are able to absorb inorganic and organic substances and stabilize ecosystem of black tiger shrimp pond culture.

4. Polyculture of black tiger shrimp (*P. monodon*) with seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) in certain percentage will be on ideal system to mitigate ecological impacts and an environmentally friendly and ecologically sustainable as well as productive aquaculture technology.

As written in all previous paragraphs of this chapter, the **Figure 0-1** presents the framework approach of research direction which includes ecological impacts of aquaculture practices, scope of research and common goals. Upper part of the picture presents the ecological impacts of aquaculture. Lower part is main idea of environmentally friendly aquaculture in order to cope with aquaculture impacts.

Regarding the specific goals of the study, the role of seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as single filtration organism and as dual-filtration organism in the black tiger shrimp (*P. monodon*) polyculture system was presented as Chapter 1 and 2, respectively. Two articles from Chapter 1 were presented at the World Ocean Conference held in Manado, North Sulawesi from 12 – 14 May 2009; the title of those articles were 1) **The ecological roles of *Anadara* sp. in black tiger shrimp culture system**, 2) **The ability of seaweed to absorb organic and inorganic substances in black tiger shrimp polyculture system**, as oral and poster presentation, respectively. Subsequently in Chapter 3, the commercial or field scale experiment of seaweed (*G. verrucosa*) as filtration organism in the black tiger shrimp (*P. monodon*) polyculture system is elucidated. One article from Chapter 3 was presented at the International symposium on small islands and coral reef in Sail Banda event held in Ambon, Maluku from August 4 – 5, 2010 with the title of “**Environmentally friendly shrimp culture technology: the effectiveness of seaweed–*Gracilaria verrucosa* as filtration organism in maintaining physico-chemical culture water quality in commercial scale of black tiger shrimp (*Penaeus monodon* Fab.) polyculture system**”. The last chapter “General Discussion”, it synthesizes all results of the study as presented in Chapter 1, 2, and 3 in order to portray an environmentally friendly aquaculture technologies.

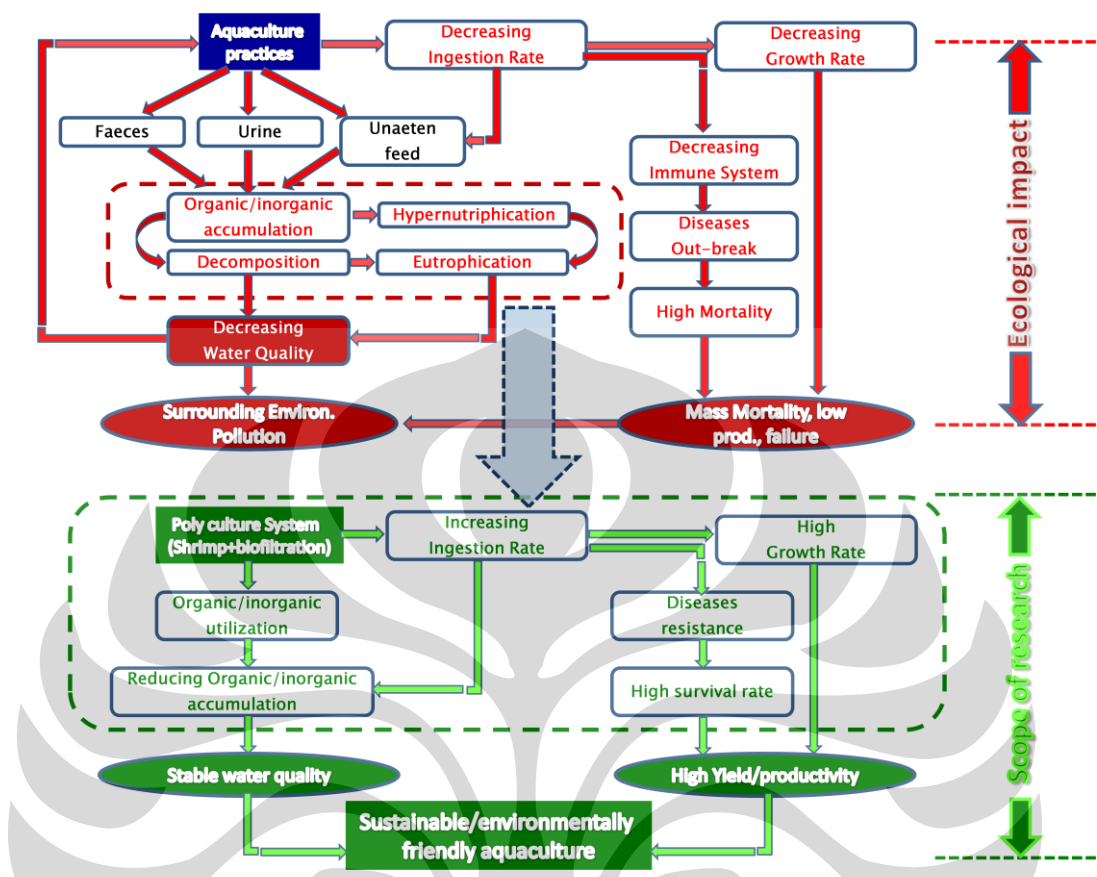


Figure 0-1
 Relationship between problem statement and scope of research

CHAPTER 1

Characterization of filtration organisms – seaweed *Gracilaria verrucosa* and blood cockle – *Anadara granosa* in black tiger shrimp *Penaeus monodon* Fab. polyculture system

ABSTRAK

Tujuan dari penelitian ini adalah menentukan efektivitas rumput laut (*Gracilaria verrucosa*) dan kerang darah (*Anadara granosa*) sebagai filter biologi dalam budidaya udang windu (*Penaeus monodon* Fab.) sistem polikultur. Penelitian dilakukan di Balai Pengembangan Budidaya Perikanan Laut, Air Payau dan Udang, Sungai Buntu, Karawang, Jawa Barat dalam wadah kayu yang dilapisi plastik (ukuran 0,9 x 0,6 x 1 m) tanpa pergantian air selama 53 hari. Perlakuan penelitian ditentukan berdasarkan perbedaan berat dan metode penanaman rumput laut (*G. verrucosa*) serta padat tebar kerang darah (*A. granosa*). Metode penanaman meliputi metode dasar dan lepas dasar dengan kedalaman rumput laut sekitar 40 cm dan 0 cm dari permukaan air. Untuk metode dasar, rumput laut disebar merata di dasar wadah penelitian sebanyak 240 g dan 640 g dan untuk metode lepas dasar berat rumput laut per ikatan adalah 30 g (total berat 240 g) dan 80 g (total berat 640 g) yang digantung dengan interval jarak sekitar 25 cm. Padat tebar kerang darah (*A. granosa*) adalah 50, 100, dan 150 individu.m⁻². Sistem polikultur udang dengan rumput laut ditanam dengan metode lepas dasar kedalaman 40 cm dari permukaan air dengan berat rumput laut 30 dan 80 gram per ikatan mampu mempertahankan keseimbangan lingkungan pembudidayaan dan memiliki tingkat pertumbuhan udang yang berbeda nyata ($P < 0.05$) dengan perlakuan lain tetapi tidak berbeda nyata dengan perlakuan rumput laut yang ditanam di dasar dengan jumlah penebaran 240 g. Seluruh sistem polikultur memiliki jumlah koloni mikroorganisme yang sesuai berdasarkan besaran pangkat dari total *Vibrio* sekitar 10² dan total bakteri sekitar 10⁶. Rumput laut memiliki kemampuan untuk menyerap fosfor dari media kultur. Sistem polikultur udang dengan kerang darah padat tebar 50 individu.m⁻² menunjukkan konsentrasi terendah untuk total bahan organik dalam air media budidayanya. Hasil serupa diperoleh untuk kandungan ammonia dan nitrit, kecuali untuk nitrat. Berkenaan dengan pengurangan konsentrasi total bahan organik dan ammonia dalam air media budidaya, polikultur udang dengan kerang darah kepadatan 100 individu.m⁻² memiliki kemampuan yang tinggi. Lebih lanjut, tidak ditemukan perbedaan yang nyata untuk tingkat pertumbuhan udang antar semua perlakuan. Namun demikian, pertumbuhan kerang darah tertinggi ditunjukkan oleh polikultur udang – kerang darah dengan kepadatan 50 individu.m⁻² diikuti oleh kepadatan 100 dan 150 individu.m⁻². Setelah panen, hasil analisa daging udang dan kerang darah memiliki kandungan karbon paling tinggi diikuti oleh nitrogen dan fosfor. Tingkat penyerapan yang paling tinggi oleh kerang darah adalah terhadap fosfor.

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 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.m². 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 10² and total bacteria 10⁶. 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.m² 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.m² followed by 100 and 150 inds.m². 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, hypernutrification, 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 sea-weed (*G. verrucosa*) and 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. In term of seaweed, the treatments were related to differences of cultivation methods and the weight of seaweed. Seaweed cultivation methods included “bottom method” and “off bottom method” with two culture depth treatments i.e. 40 cm and 0 cm from the water surface. For the bottom method, seaweed treatments were sown and for the off bottom method, seaweed was tied with about 30 g and 80 g mass at each attachment and hung at intervals of 25 cm. Furthermore, the treatment of blood cockle consisted of 3 different levels of stocking density. In the experimental design, polyculture systems of shrimp with

combinations of weight and cultured methods of seaweed and 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 (abbreviated by SB0) was black tiger shrimp (*P. monodon*) monoculture system (without filtration organism). The placement for each treatment to the available tanks was completely randomized and all treatments had 3 replications.

Table 1-1
List of seaweed treatment

Treatment	Description	Total Weight (g)
SS30	seaweed was evenly sown	240
SS80	seaweed was evenly sown	640
SL430	Seaweed was tied with about 30 g at each attachment and hung with depth of 40 cm at intervals of 25 cm	240
SL480	Seaweed was tied with about 80 g at each attachment and hung with depth of 40 cm at intervals of 25 cm	640
SL030	Seaweed was tied with about 30 g at each attachment and hung on the water surface at intervals of 25 cm	240
SL080	Seaweed was tied with about 80 g at each attachment and hung on the water surface at intervals of 25 cm	640

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 equipment of salinity was Atago refractosalinometer. The rest of the parameters were observed by using Spectrophotometer 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-seaweed polyculture system

Physico-chemical aspects

Some of the main water quality parameters observed included 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 were relatively lower and more flat trend in the early morning

than in the afternoon (**Figure 1-1**). One week after stocking, the highest DO concentration in the morning and at noon were observed in treatment SL430 of about 3.36 mg.l^{-1} and 4.86 mg.l^{-1} (**Appendix 1-1**), respectively. In general, all treatments showed similar pattern of stable tendency of DO concentration. However, just after day 30 of dawn observation and day 10 of noon observation, treatment SL430 and 480 revealed higher DO concentration than the other treatments (**Figure 1-1**).

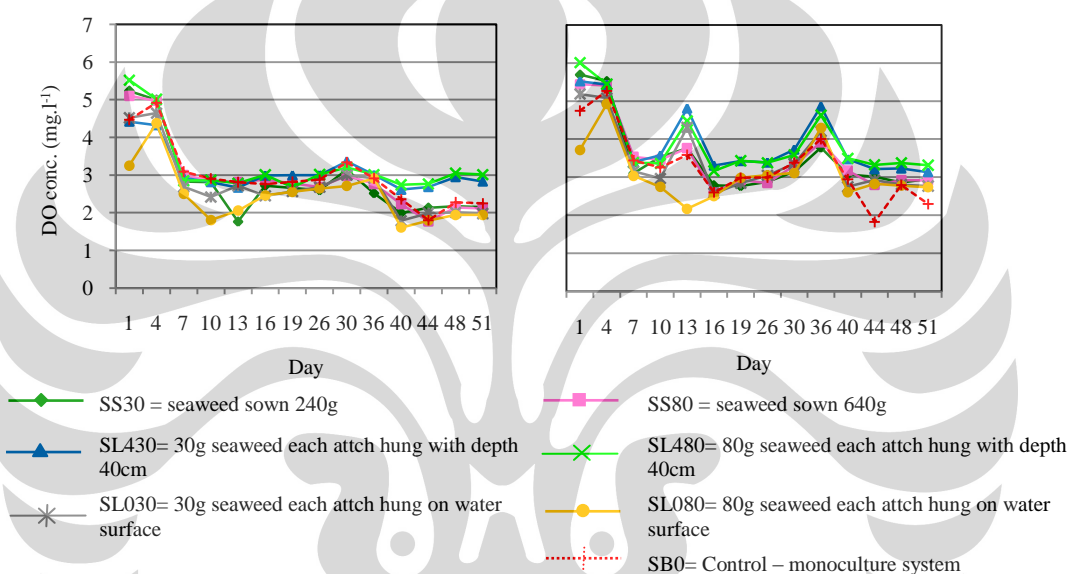


Figure 1-1

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

All treatments showed a similar pattern of tendency of temperature, pH, and salinity. Temperature ranges were about $28 - 31.30^{\circ}\text{C}$ in the early morning and $28.57 - 32.13^{\circ}\text{C}$ in the afternoon (**Appendix 1-3**). Started from measurement at day 10, water temperature showed rising tendency. pH values did not exceed 8.5 and remained above 7.0 throughout the experimental period (**Appendix 1-2**). The lowest (7.07) & highest (8.44) pH values were recorded on day 4 at dawn and on day 36 at noon, respectively. During the experimental period, the fluctuation of water salinity was about 4 ppt (**Appendix 1-4**). However, the lowest and the highest salinity at dawn measurement was $2^{\circ}/_{00}$ higher than that of at noon.

Ammonia concentration tended to be low in all treatments just after day 17 of the experimental period and increase afterward, except treatment SL430 and SL480 (**Figure 1-2a**). However, according to the decreasing ammonia concentration, treatment SS30, SL430, SL480, and SL030 were significant different decreasing rate ($P < 0.05$) than treatment SS80, SL080, and SB0 (**Figure 1-2a** and **Appendix 1-5**). Nitrite concentration presented a similar pattern to the trend of ammonia (**Figure 1-2b**). The treatment SL430 and SL480 showed contrary trend and had significant higher ability ($P < 0.05$) of decreasing nitrite concentration than the other treatments (**Figure 1-2b** and **Appendix 1-6**). The lowest concentration of last measurement and highest ability decreasing concentration were recorded at treatment SL430 of about 0.1005 mg.l^{-1} and $0.5289 \pm 0.194 \text{ mg.l}^{-1}$ and SL480 of about 0.1589 mg.l^{-1} and $0.3889 \pm 0.070 \text{ mg.l}^{-1}$, respectively (**Figure 1-2b** and **Appendix 1-6**). The rest parameters had significant higher concentration at last measurement and lower decreasing concentration of nitrite. Nitrate concentration of treatment SL030 & control (SBO) showed decreasing tendency and the other treatments presented opposite tendency (**Figure 1-2c**). Treatment SS80 and SL480 revealed higher decreasing concentration of nitrate than the others (**Figure 1-2c** and **Appendix 1-7**).

Contrary to the previous parameters, hydrogen sulphide revealed increasing at all treatments (**Figure 1-3a**). Reducing hydrogen sulphide concentration of treatment SS30 and SL430 was not significant different from SS80 but significant different from the other treatments ($P < 0.05$) (**Figure 1-3a** and **Appendix 1-8**). Treatment SB0 revealed significant increase in hydrogen sulphide concentration (**Appendix 1-8**). During the experiment, TOM concentration presented decreasing tendency (**Figure 1-3b**). At the end of the experiment, treatment SL480 revealed significant different from the other treatments (**Appendix 1-9**). Treatment SL480 showed significantly higher ($20.0470 \pm 0.359 \text{ mg.l}^{-1}$) ability in reducing TOM concentration than that of the other treatments (**Figure 1-3** and **Appendix 1-9**).

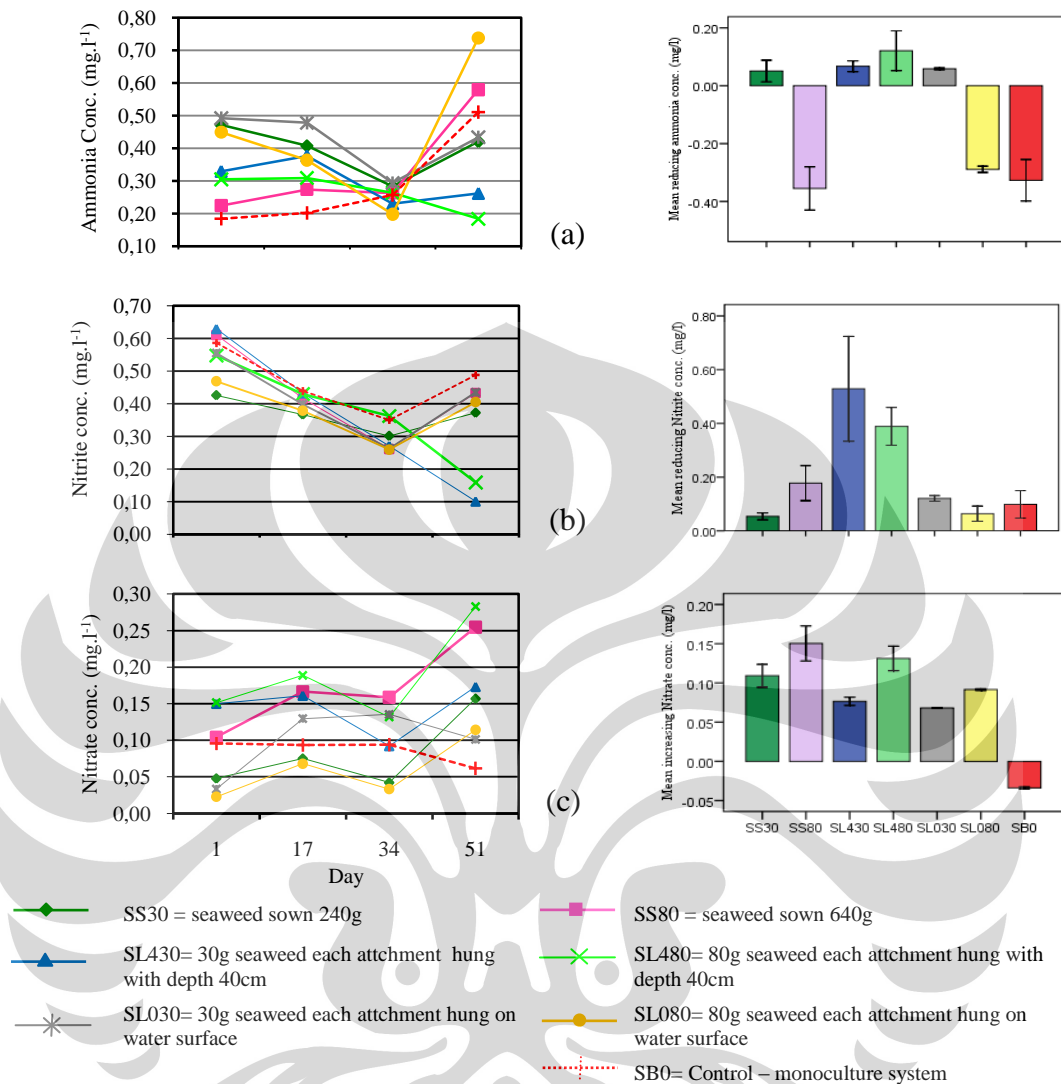


Figure 1-2

Measurement results (left graphs) of and reucing concentration (right graphs) for ammonia (a), nitrite (b), and nitrate concentration (c) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Generally, PO_4 concentration of most treatments tended to increase at the beginning of experiment period and decrease afterward except treatment SL480. Treatment SL480 showed relatively flat trend of PO_4 concentration from first till last measurement (**Figure 1-3c**). Treatment SL030 was not significant different from treatments SS30 and SB0 as control but significantly sound lower than that of the rest parameters (**Figure 1-3c** and **Appendix 1-10**).

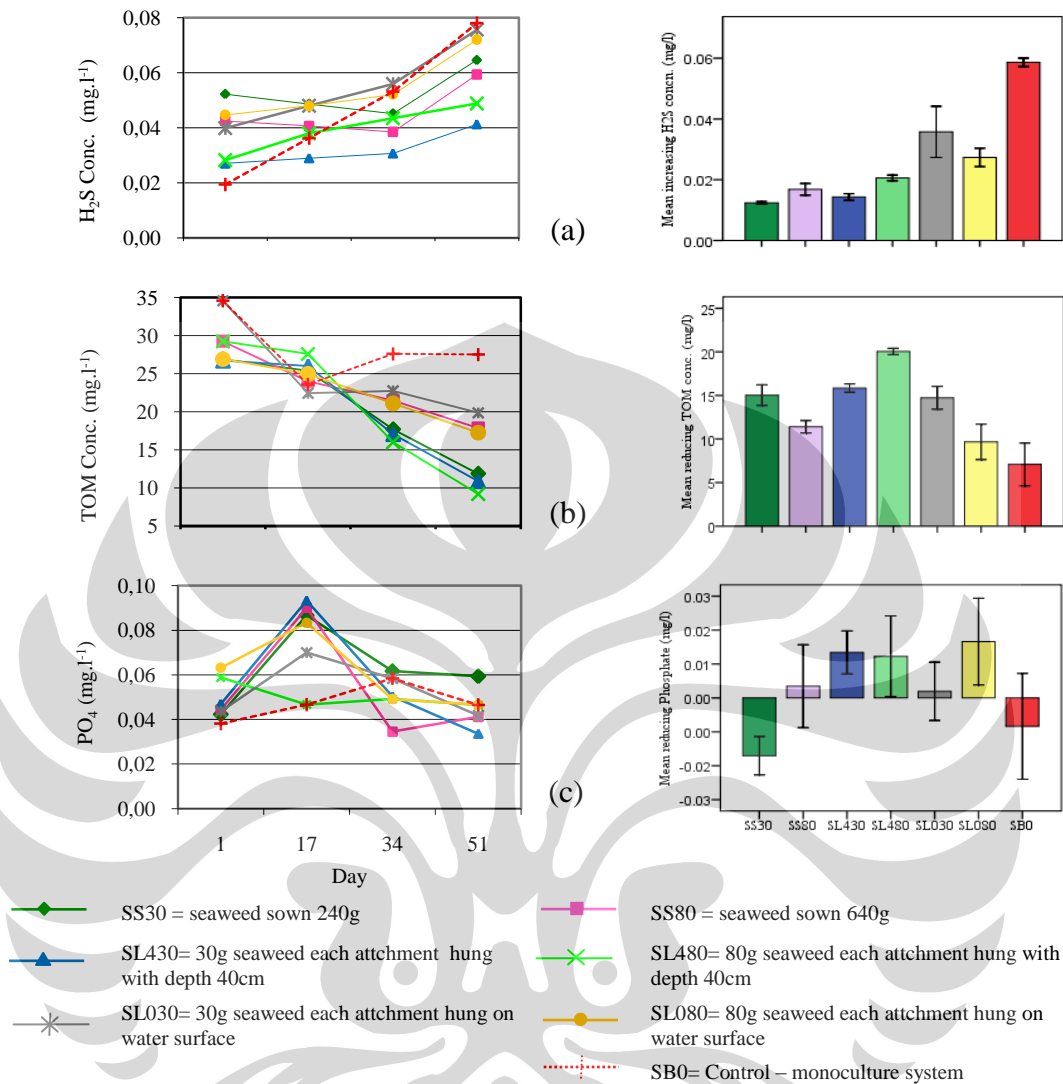


Figure 1-3

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

During the experiment, TDS concentration of treatment SL480 tended to decrease significantly (**Figure 1-4 - upper**). At the end of the experiment, treatment SL480 and SB0 as control demonstrated significantly higher ability in reducing TDS concentration ($P < 0.05$) than the other treatments (**Figure 1-4 - upper** and **Appendix 1-11**). Meanwhile, treatment SS30 and SS80 had significantly lower ability in maintaining concentration of TDS than the other

treatments. All treatments except SB0 as control presented increase TSS concentration at the end of experiments (**Figure 1-4** – lower). Treatment SB0 as control by employing monoculture system showed significantly higher reducing TSS concentration ($P<0.05$) than the other treatments (**Appendix 1-12**).

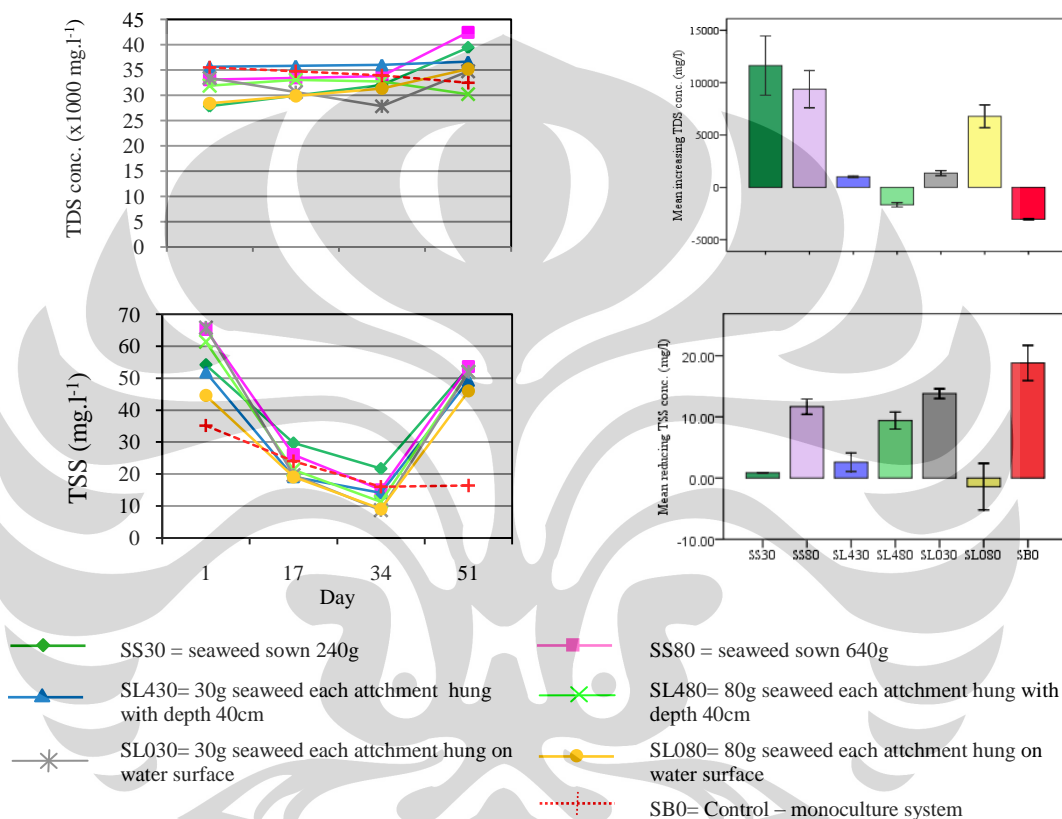


Figure 1-4

Measurement results of (left graphs) and maintaining capacity (right graphs) for Total Dissolved Solid (TDS) (upper) and Total Suspended Solid (lower) in polyculture system of black tiger shrimp-seaweed and black tiger shrimp monoculture system

Biological aspects

The main indicators of success in aquaculture practices are the mortality and growth rate of shrimp as the main cultured organism. During this experiment, all individuals of shrimp in one replication of treatment SS30, SS80, SL080 and 1 individual shrimp in one replicate each of treatment SL480 and SL30 died (**Appendix 1-14**).

The average harvested weight in all treatments varied from 11.85 g to 19.98 g per individual shrimp. Related to the shrimp growth, the average daily growth rate was not significant different amongst treatment S30, SL430 and SL480 and significant different from treatments SS80, SL30, SL80, and SB0 ($P < 0.05$) (**Figure 1-5; Appendix 1-13; Appendix 1-14; Appendix 1-15; Appendix 1-16**).

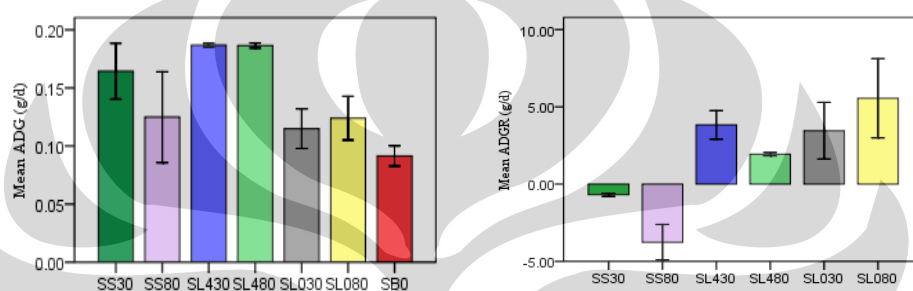


Figure 1-5

Average daily growth rate of shrimp (left) and seaweed (right) in polyculture system

The seaweed used in this experiment had a wide range of biomass at harvest. The polyculture black tiger shrimp with bottom method of both 240 g (treatment SS30) and 640 g (treatment SS80) had lower average harvested biomass of about 203.55 g and 440.80 g than that of stocking, respectively (**Appendix 1-16**). The heaviest weight gain was performed by treatment SL080 (polyculture with 80 g seaweed of each attachment) with the average daily growth rate of about 5.5527 ± 2.561 g. However, its average daily growth rate had not significant different compare to treatment SL430 and SL030S.

Furthermore, C, N, and P content in shrimp and seaweed tissue were also measured. C content in shrimp and seaweed tissue was higher than N and P content (**Figure 1-6**). Statistically, concentration of C in shrimp tissue of treatment SL480 was not significant different from treatment SL030 but significant different concentration from the other treatments ($P < 0.05$). Nitrogen concentration of treatments SS30 was not significant different from treatment

SL480 and SL030. However, the treatment SL480 and SL030 were found no significant different from the rest treatments. The Phosphorous concentration of treatment SL080 was significant different from the other treatments ($P < 0.05$).

In seaweed tissue (**Figure 1-6**), SL430 and SL480 had significant highest concentration of C but there was no significant difference between treatments SL480, SS30, SS80 and SL080. The lowest concentration was found in treatment SS80 and SL030. Concentration of N was not significantly different amongst the treatments. Concentration of P of treatment SS30 were not significant different from treatment SL480 but were significantly different from the other treatments. The lowest concentration of P was attributed by treatment SS80 and SL030.

During experiment, total number of *Vibrio* in treatments SS80, SL430 and SB0 showed a relatively flat trend and bit decrease at the end of experiment. However, *Vibrio* numbers in the other treatments showed a tendency to increase at the beginning at the experiment and decrease later (**Figure 1-7**). A different trend was observed for total bacterial. There were two common trend of total bacterial. Firstly, the trend of treatments SL080, SL430, SL480, and SB0 was similar to that of total *Vibrio* (**Figure 1-7**). Secondly, a tendency in reduction in numbers through the course of the experiment was seen in treatments SS30, SS80, and SL030. As presented in **Appendix 1-17**, at the end of experiment total *Vibrio* and bacteria had similar levels of about 10^2 (total *Vibrio*) and $10^5 - 10^6$ (bacteria), respectively. However, at the end of the experiment treatments SS80 and SL480 had higher total number of *Vibrio* than the other treatments of about $8.13 \times 10^2 \pm 90.19$ CFU/ml and $8.80 \times 10^2 \pm 131.15$ CFU/ml, respectively (**Appendix 1-18**). Number of total *Vibrio* in treatments SS30, SL030, and SB0 were significantly higher than in SL430, SL080 and significantly lower than SS80 and SL480. The number of total bacteria in treatment SS80 was significantly higher than SS30, SL430, SL030, SL080, and SB0. SL030 showed the highest total bacteria count amongst the treatments. SL080 was not significant different from SB0 (control) and showed the lowest number of total bacteria amongst the others.

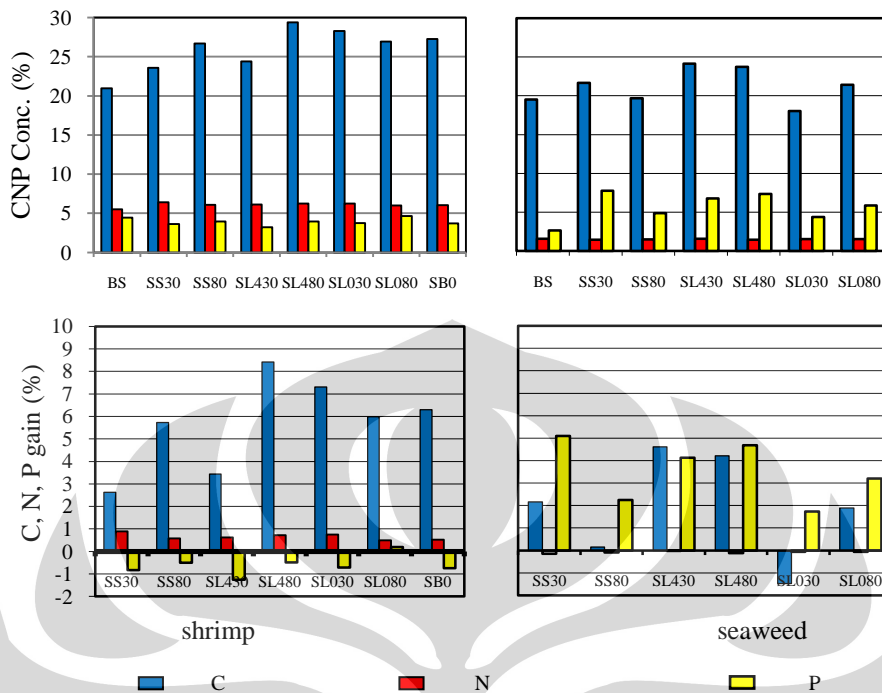


Figure 1-6
C, N, P content and gained in shrimp (left graphs) and seaweed tissue (right graphs) in polyculture system

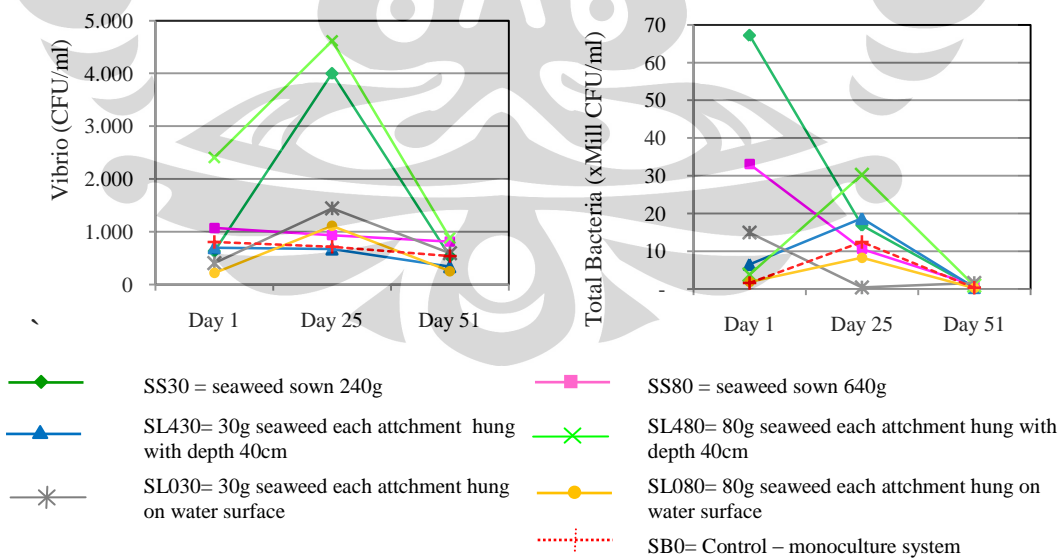


Figure 1-7
Total *Vibrio* (left) and bacteria (right) during experiment in polyculture black tiger shrimp with seaweed

Shrimp-blood cockle polyculture system

Physico-chemical aspects

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 $^{\circ}\text{C}$ in the early morning and 28.53 - 32.13 $^{\circ}\text{C}$ in the noon. The lowest salinity was found at noon observation of about 25‰. 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.

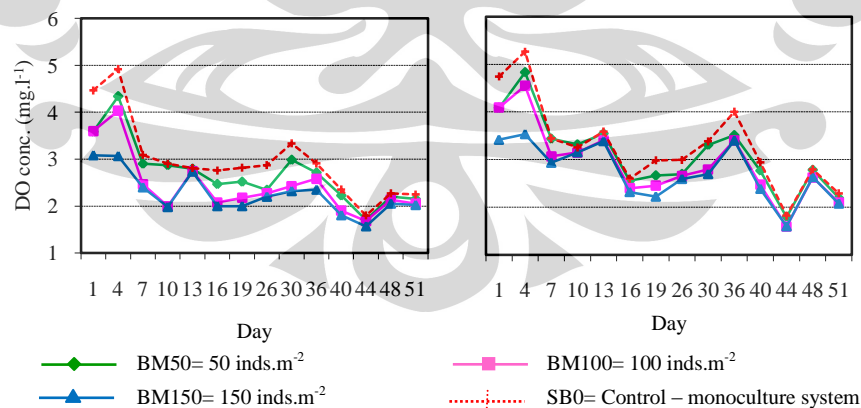


Figure 1-8

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 $0.511 \pm 0.041 \text{ mg.l}^{-1}$ (**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 BM₁₅₀ and SB0, of about $0.373 \pm 0.107 \text{ mg.l}^{-1}$ and $0.488 \pm 0.073 \text{ mg.l}^{-1}$, 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 H₂S concentration. However, at the end of experiment, treatment BM₅₀ had significantly lower H₂S ($0.064 \pm 0.011 \text{ mg.l}^{-1}$) concentration than treatment BM₁₀₀ ($0.082 \pm 0.010 \text{ mg.l}^{-1}$) and not significant different from treatment BM₁₅₀ ($0.081 \pm 0.010 \text{ mg.l}^{-1}$) and treatment BM₀ ($0.078 \pm 0.005 \text{ mg.l}^{-1}$) (**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.

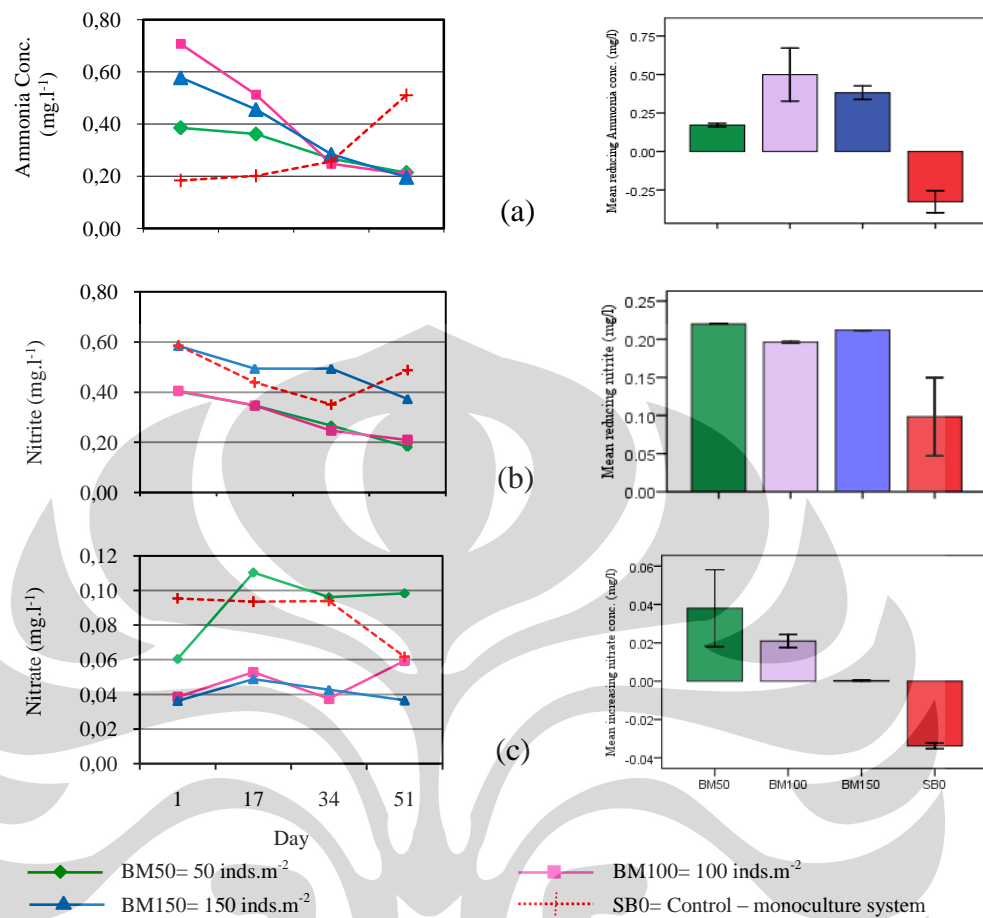


Figure 1-9

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 BM₁₀₀ than SB0 ($P < 0.05$) (**Figure 1-10c** and **Appendix 1-24**). However, treatment BM₅₀ and BM₁₅₀, phosphate

concentration was not significant difference from BM_{100} and BM_0 . During the experiment, treatment BM_{50} and BM_{150} had significant lower diminishing PO_4 concentration ($P < 0.05$) than treatment BM_{100} and SB_0 of which were not significant different (**Figure 1-10c and Appendix 1-24**).

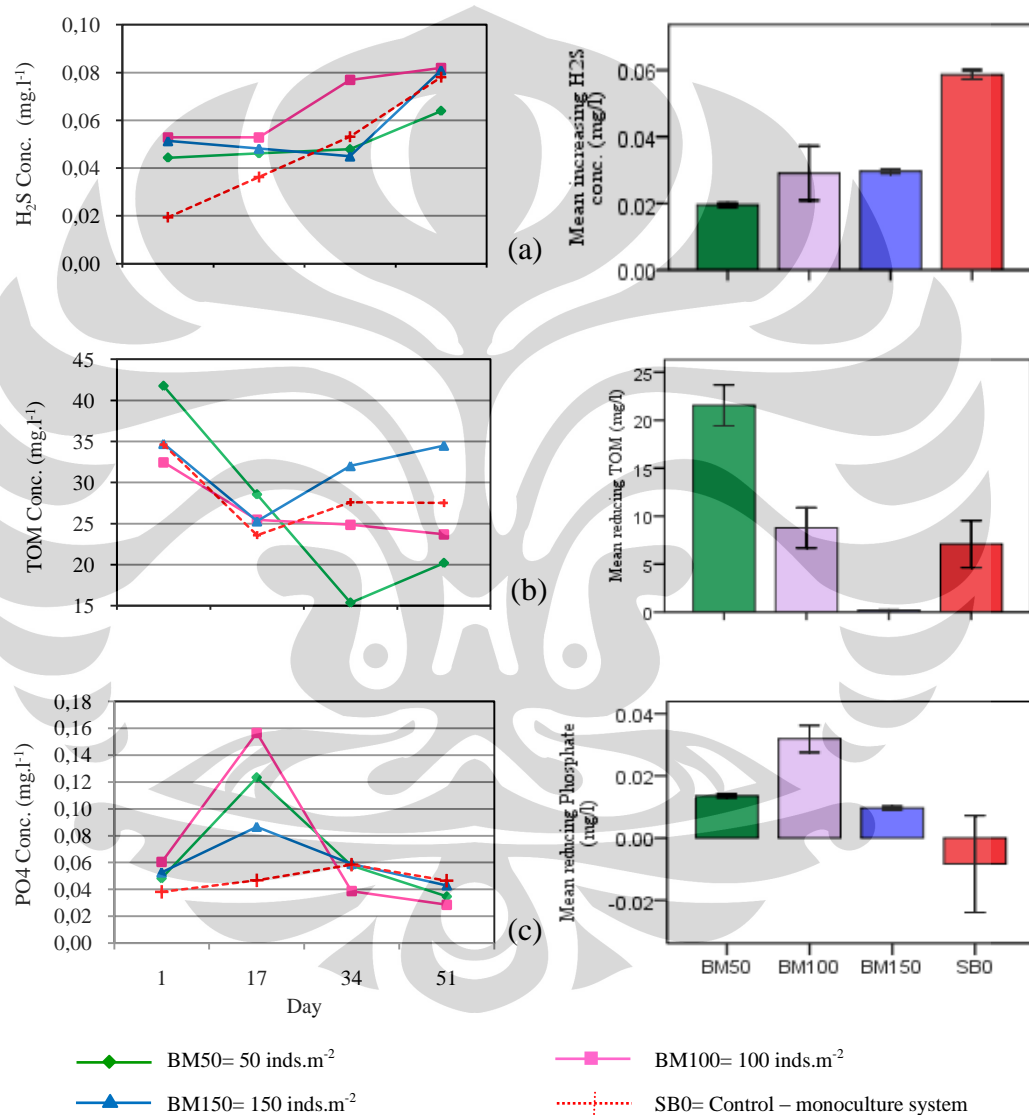


Figure 1-10

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 BM₁₀₀ and BM₁₅₀ ($P < 0.05$) (**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 ($16.38 \pm 1.89 \text{ mg.l}^{-1}$) 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**).

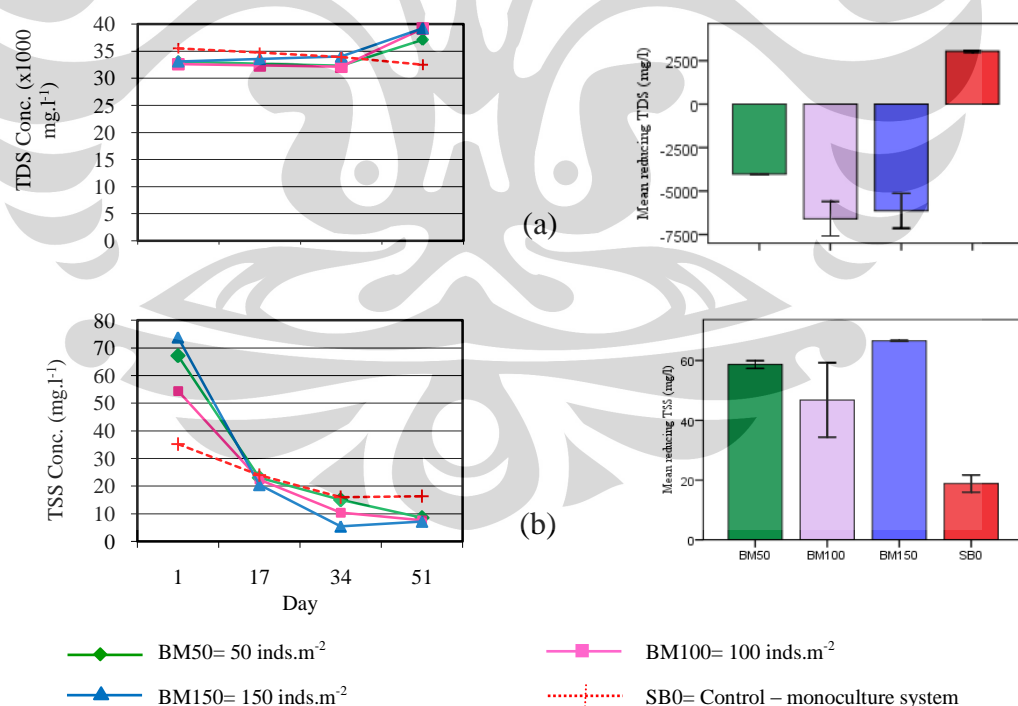


Figure 1-11

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 cockle of treatment BM₅₀ was not significant different from treatment BM₁₀₀ and significant different from treatment BM₁₅₀ ($P<0.05$) (**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 BM₁₀₀ and BM₁₅₀ had significantly higher C than that of treatment BM₀ and BM₅₀; no significant difference among different stocking density for N gain; and treatment BM₅₀ gained P extremely significantly higher than that of treatment BM₀, BM₁₀₀, and BM₁₅₀ (**Figure 1-13**). Furthermore, C gain of blood cockle tissue in the treatment BM₁₀₀ had no significant difference than that of treatment BM₅₀ but significantly higher than treatment BM₁₅₀. 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 $6.1 \times 10^2 \pm 30.41$ CFU/ml than the other treatments (**Appendix 1-30**). Total number of *Vibrio* of Treatment SB0 of about

$5.4 \times 10^2 \pm 5.00$ CFU/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 $4.35 \times 10^6 \pm 273,008.00$ CFU/ml was significant different from treatment BM50 ($3.67 \times 10^5 \pm 24,664.41$ CFU/ml), BM150 ($2.8 \times 10^5 \pm 20,000.00$ CFU/ml), and SB0 ($2.7 \times 10^5 \pm 36,055.51$ CFU/ml).

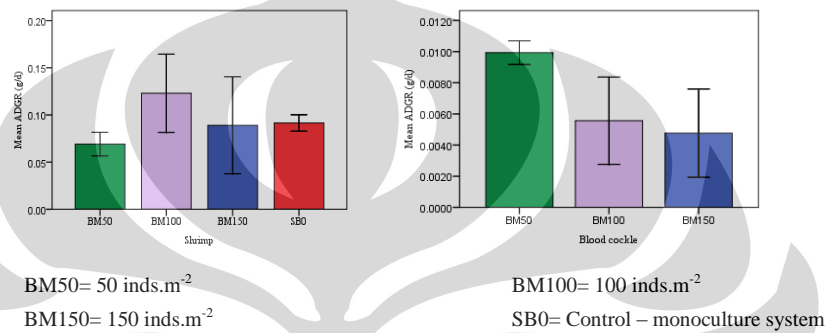


Figure 1-12
 Individual growth of shrimp and blood cockle in polyculture system

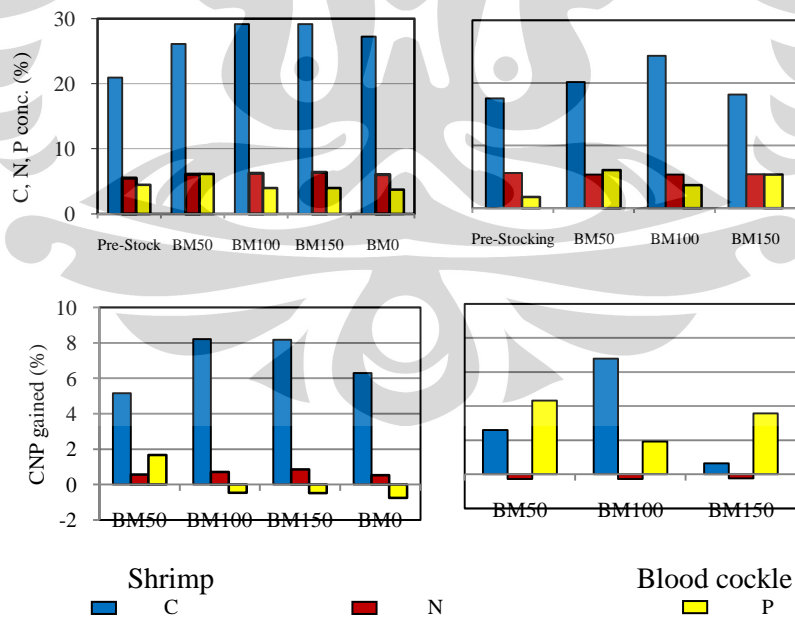


Figure 1-13

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

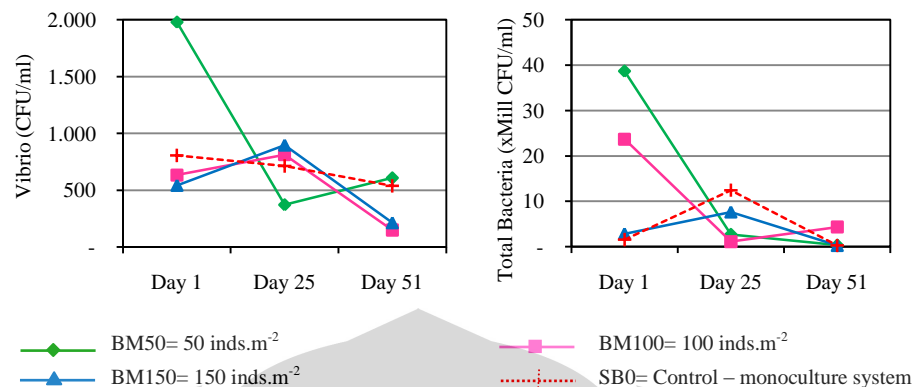


Figure 1-14

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 as seaweed (*G. verrucosa*) and blood cockle (*A. granosa*), in a polyculture system seems to be able to reduce the environmental impacts of culture activities. Seaweed as a phototrophic organism will absorb micro- and macro- dissolved nutrients from the environment. Meanwhile, blood cockle as a filter-feeder will ingest dissolved and suspended particulates from the environment. Therefore, both those filtration organisms naturally seem to be able to reduce organic and inorganic substances in the culture medium. One of the ecological consequences is to stabilize shrimp pond culture habitat.

Shrimp-seaweed polyculture system

In this study, employing *G. verrucosa* with different levels of stocking weight and cultivation methods improved water quality. The concentration of dissolved oxygen concentration increased with the method of seaweed cultivation. Black tiger shrimp Polyculture with *G. verrucosa* hung 40 cm under water surface

showed higher dissolved oxygen concentration than the other treatments. It probably due to diffusion and solubility rate of oxygen in water resulted from photosynthesis (Boyd 1992), the concentration in dawn was lower than in the afternoon and the position of seaweed in water column. After day 30 of dawn observation of experimental period, DO concentration in the shrimp polyculture with seaweed cultivated by bottom and on the surface water long-line methods decreased much sharper than in shrimp polyculture with seaweed cultivated by hung 40 cm from water surface. The position of seaweed cultivation to the water surface influence sun light penetration, photosynthesis rate and subsequently related to the adding up of dissolved oxygen to the culture water. Bottom method culture will cause lower intensity of sun light and photosynthesis rate of seaweed. On the other hand, on the water surface position, seaweed will exposure to the highest sun light intensity but oxygen resulted from photosynthesis process might easily be released out to the air due to the low water solubility rate. Therefore, during the experiment, polyculture (shrimp – *G. verrucosa*) with seaweed hung 40 cm under water surface provided more than the minimum requirement of DO concentration according to the requirement for black tiger shrimp culture of more than 3 mg.l⁻¹ (Boyd 1992; Boyd and Green 2002; Darmono 1993). Suitable dissolved oxygen concentration of shrimp culture water is one of main important water quality parameters to support growth and survival of shrimp and the other ecological processes within culture ecosystem (Boyd 1990; Hawerton 2001).

Interesting results were observed in all polyculture system black tiger shrimp (*P. monodon*) with *G. verrucosa* in term of ammonia, nitrite, nitrate, hydrogen sulphide, total organic matter and total dissolved solid concentration of which tended to decrease with culture period. Aquaculture practices lead to have organic matter rich sediment accumulated on the pond bottom and degraded water quality from which may cause eutrophication, hypernutrification, and low DO concentration (Donovan 2001; Pillay 2004; FAO 2006). Decreasing concentration of several water quality parameters were comparable to the study reported by Jones and Preston (1999). Seaweed as a phototropic organism cultivated along

with shrimp in the same culture unit might have the ability to lift up DO concentration of culture water habitat and ensure ecological process included production, consumption, and decomposition (Zonneveld *et al.* 1991; Wetzel 1993). It might reduce nutrient loading resulted by shrimp rearing activities (Troell 1999). Dissolved oxygen concentration trend appeared to have relationship with the concentration trend of ammonia, nitrite, nitrate, total organic matter, and phosphate. The decomposition process of organic substances was supported by availability of dissolved oxygen concentration (Boyd 1990; Knud-Hansen 1998; Effendi 2003). Since, ammonia concentration in aquaculture ecosystem tends to increase with culture period resulted from fish excretion (Hargreaves & Tucker 2004). In this study, *G. verrucosa* in polyculture with shrimp might have potential for playing an important ecological role in order to maintain suitable water culture media for shrimp, related to the DO, ammonia, nitrite, nitrate, hydrogen sulphide, total organic matter and total dissolved solids concentration.

Moreover, the range of those water quality parameters was within the range of black tiger shrimp requirement (Boyd 1990). The highest ammonia, nitrite, and total organic matter concentration was found at the monoculture system. This may have resulted from the lower ability of the system to oxidize and mineralize accumulated organic matter (Knud-Hansen 1998). Contrary to results mentioned previously, total suspended solid was not affected by the inclusion of *G. verrucosa*.

Black tiger shrimp (*Penaeus monodon*) as the main culture organism can be used as a biological indicator of achievement of aquaculture practices. The best growth rate in these experiments was observed from the shrimp polyculture system with 30 and 80 g seaweed hung 40 cm under the water surface and seaweed cultivated by bottom method with total weight of 240 g. However, these growth rates were lower than those found by Lumare (1993). Another result of biological factors investigations were bacterial colony and C, N, P content in the shrimp and seaweed tissue. All different culture systems did not affect the colony of *Vibrio* and total bacteria. It is contrary to the study performed by Jones and

Petterson (1991) and Troll *et al.* (1999). In general, shrimp absorbed nitrogen from the environment and released phosphorus to the system. In contrast, seaweed could absorb phosphorus from the system. It seems to be useful in maintaining P concentration within the system. Because, in aquaculture practices, phosphorus input through feed range from 68.8 to 90.6% of the total (Thakur & Lin 2003). Besides that, phosphorus has tendency to be demobilized to the system (Boyd 1990).

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 hypereutrophication 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. Black tiger shrimp (*P. monodon*) polyculture with seaweed (*G. verrucosa*) was tied with about 30 g at each attachment and hung with depth of 40 cm from water surface had ability to stabilize culture media by maintaining several water quality parameters.
2. The best growth rate was achieved by polyculture system of black tiger shrimp (*P. monodon*) with 30 and 80 g seaweed (*G. verrucosa*) at each attachment and hung with depth of 40 cm from water surface and 240 g seaweed cultivated by bottom method.
3. All the systems had suitable collony of micro-organism based on magnification of *Vibrio* of about 10^2 and total bacteria of 10^6 .
4. 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.
5. 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.
6. Blood cockle represented by different level of stocking density did not perform clear tendency related to several water quality parameters.

Recommendation

Based on the conclusion aboved, some recommendations for the next stage of study are the polyculture of black tiger shrimp (*P. monodon*) and dual-filtration

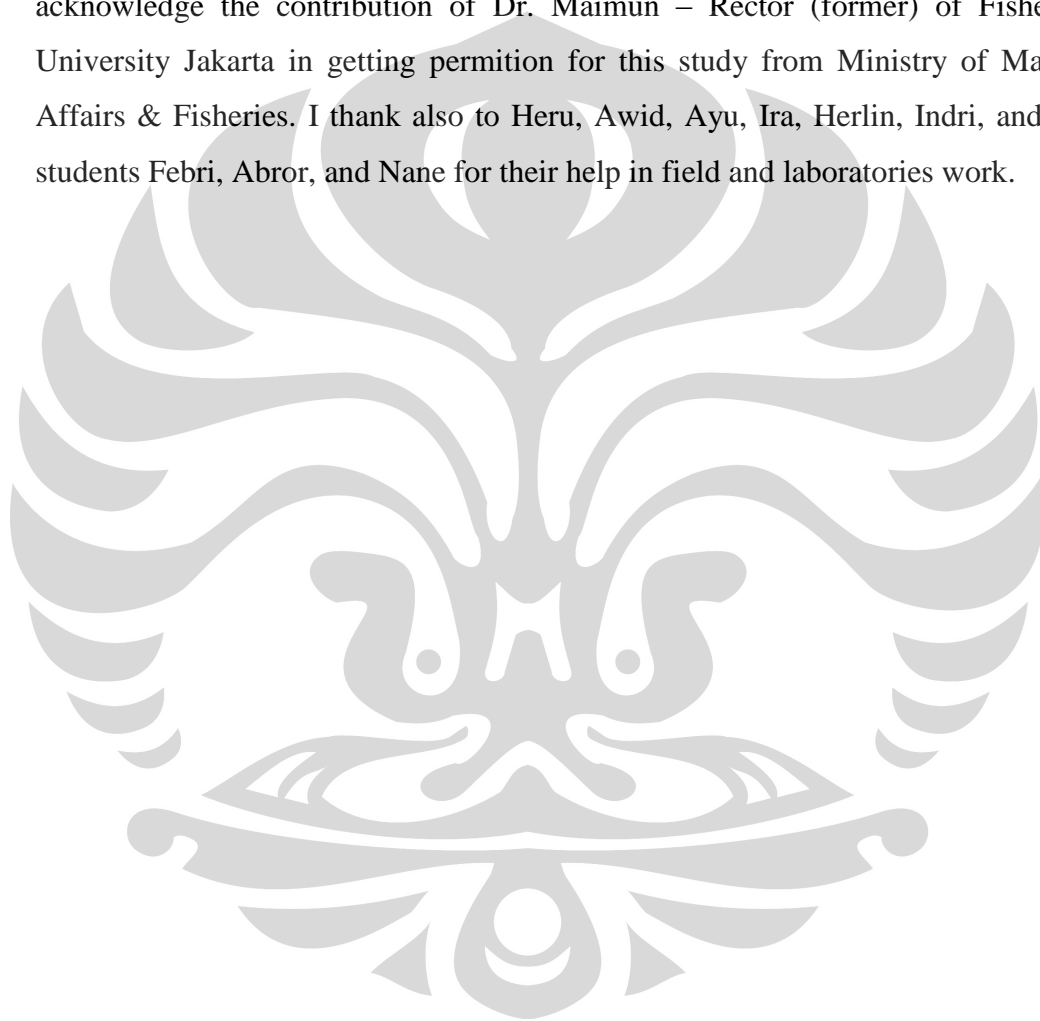
organism i.e. seaweed and blood cockle with difference level stocking density.

There will be four different kinds of polyculture system, as follows:

1. Polyculture – black tiger shrimp (*P. monodon*) (4 inds.m⁻²) and Seaweed (*G. verrucosa*) (30 g at each attachment or total weight 240 g and hung with depth of 40 cm at intervals of 25 cm) and blood cockle (*A. granosa*) (50 inds.m⁻²).
2. Polyculture – black tiger shrimp (*P. monodon*) (4 inds.m⁻²) and Seaweed (*G. verrucosa*) (30 g at each attachment or total weight 240 g and hung with depth of 40 cm at intervals of 25 cm) and blood cockle (*A. granosa*) (100 inds.m⁻²).
3. Polyculture – black tiger shrimp (*P. monodon*) (4 inds.m⁻²) and Seaweed (*G. verrucosa*) (80 g at each attachment or total weight 640 g and hung with depth of 40 cm at intervals of 25 cm) and blood cockle (*A. granosa*) (50 inds.m⁻²).
4. Polyculture – black tiger shrimp (*P. monodon*) (4 inds.m⁻²) and Seaweed (*G. verrucosa*) (80 g at each attachment or total weight 640 g and hung with depth of 40 cm at intervals of 25 cm) and blood cockle (*A. granosa*) (100 inds.m⁻²).

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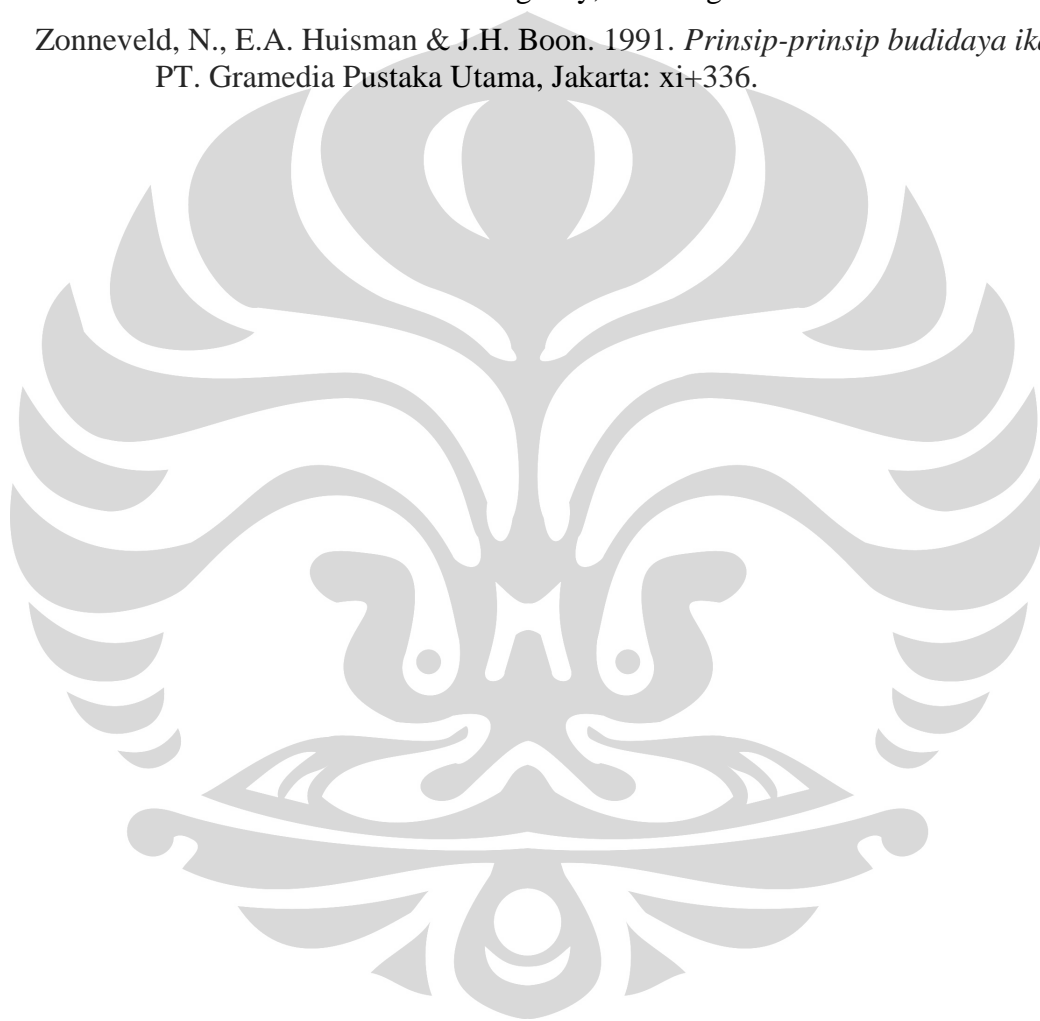


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Appendix 1-1

Results of dissolved oxygen (mg.l^{-1}) measurement in dawn in polyculture black tiger shrimp seaweed

Treatment	Day													
	1	4	7	10	13	16	19	26	30	36	40	44	48	51
Dawn														
SS30	5.23	5.00	2.82	2.81	1.76	2.72	2.66	2.59	3.14	2.51	1.99	2.13	2.17	2.15
SS80	5.10	4.97	3.00	2.85	2.81	2.87	2.78	2.68	2.98	2.77	2.22	1.77	2.14	2.11
SL430	4.42	4.32	2.93	2.81	2.67	2.99	2.99	3.00	3.36	3.01	2.61	2.68	2.95	2.83
SL480	5.52	5.01	2.85	2.86	2.78	3.00	2.68	3.01	3.20	3.01	2.74	2.76	3.05	3.01
SL030	4.52	4.64	2.69	2.42	2.72	2.45	2.56	2.68	3.00	2.91	1.79	1.99	2.01	1.98
SL080	3.25	4.37	2.50	1.80	2.06	2.46	2.55	2.64	2.71	2.91	1.61	1.79	1.94	1.94
SBO	4.47	4.92	3.09	2.90	2.81	2.76	2.82	2.87	3.34	2.91	2.36	1.80	2.27	2.25
Noon														
SS30	5.69	5.52	3.11	3.53	3.76	2.79	2.77	2.87	3.15	3.78	3.07	3.01	2.86	2.95
SS80	5.44	5.41	3.53	3.46	3.76	2.60	2.92	2.85	3.31	3.92	3.17	2.80	2.92	2.92
SL430	5.52	5.43	3.40	3.57	4.81	3.31	3.43	3.39	3.73	4.86	3.47	3.21	3.23	3.13
SL480	6.01	5.44	3.38	3.36	4.48	3.16	3.43	3.38	3.59	4.63	3.50	3.33	3.38	3.31
SL030	5.18	5.08	3.16	2.97	4.31	2.58	2.84	3.05	3.36	4.20	2.75	2.92	2.80	2.77
SL080	3.72	4.92	3.04	2.74	2.17	2.50	2.99	3.05	3.11	4.29	2.61	2.82	2.78	2.74
SBO	4.74	5.26	3.44	3.26	3.59	2.60	2.98	2.99	3.38	4.00	2.94	1.83	2.80	2.29

Note:

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SBO = Control – monoculture system

Appendix 1-2

Results of pH measurement in dawn in polyculture black tiger shrimp seaweed

Treatment	Day													
	1	4	7	10	13	16	19	26	30	36	40	44	48	51
Dawn														
SS30	7.92	7.13	7.67	7.60	7.91	8.07	8.00	8.06	8.21	8.15	8.08	8.02	8.13	8.13
SS80	7.91	7.07	7.40	7.53	7.95	8.04	8.00	8.38	8.30	8.12	8.23	8.20	7.99	8.08
SL430	7.91	7.13	7.60	7.73	8.28	8.09	7.99	8.35	8.26	8.12	8.14	8.16	8.26	8.33
SL480	7.91	7.07	7.47	7.47	7.86	7.88	8.00	8.41	8.35	7.98	8.01	8.01	8.30	8.21
SL030	7.91	7.13	7.53	7.60	7.96	7.97	7.97	8.29	8.26	8.20	8.22	8.19	8.18	8.15
SL080	7.84	7.13	7.73	7.60	7.81	7.88	8.01	8.43	8.27	8.24	8.16	8.15	8.17	8.24
SBO	7.88	7.07	7.67	7.53	8.15	8.20	8.00	8.10	8.08	8.32	8.20	8.15	8.12	8.14
Noon														
SS30	7.68	7.66	7.73	8.01	7.98	8.01	8.05	8.38	8.15	8.11	8.14	8.17	8.21	8.23
SS80	7.75	7.67	7.73	7.93	8.00	8.16	8.06	8.41	8.17	8.17	8.10	7.93	8.09	8.22
SL430	7.88	7.68	7.93	8.07	8.06	7.91	8.04	8.41	8.20	8.36	8.04	8.22	8.33	8.43
SL480	7.64	7.51	7.53	7.83	7.92	8.11	8.04	8.41	8.18	8.44	8.11	8.15	8.33	8.31
SL030	8.21	7.64	7.80	7.98	8.11	8.02	8.01	8.33	8.16	8.28	8.08	8.12	8.28	8.38
SL080	7.59	7.58	8.07	7.87	7.82	7.88	8.06	8.43	8.23	8.30	8.16	7.99	8.27	8.34
SBO	7.97	7.81	7.67	8.08	8.14	7.96	8.04	8.20	8.01	8.15	8.20	8.26	8.22	8.28

Note:

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SBO = Control – monoculture system

Appendix 1-3

Results of temperature ($^{\circ}\text{C}$) measurement in dawn in polyculture black tiger shrimp seaweed

Treatment	Day														
	1	4	7	10	13	16	19	26	30	36	40	44	48	51	
DAWN															
SS30	28.97	28.73	28.00	29.67	29.97	30.77	30.67	29.93	28.93	30.37	30.47	29.92	29.47	29.43	
SS80	28.90	28.27	28.00	29.67	30.00	30.17	30.25	30.33	29.33	31.07	31.08	30.73	30.10	29.83	
SL430	29.07	28.83	28.00	30.33	29.83	30.00	30.17	30.33	29.30	30.90	31.10	30.47	30.00	29.73	
SL480	29.07	29.17	28.00	29.67	30.07	30.50	30.40	30.33	29.37	30.73	30.83	30.43	29.77	29.63	
SL030	29.00	28.73	28.00	30.33	30.03	30.57	30.60	30.20	29.43	30.63	30.93	30.20	29.97	29.87	
SL080	29.40	28.63	28.00	30.33	30.00	30.03	30.23	30.43	29.40	31.03	31.23	30.47	30.10	29.97	
SB0	29.07	28.00	28.00	29.67	29.90	30.37	30.32	30.07	29.37	31.10	31.30	30.13	30.13	30.13	
NOON															
SS30	29.40	28.53	30.00	28.93	30.37	30.70	30.88	31.37	30.53	32.13	32.00	30.23	31.10	31.23	
SS80	29.17	28.57	30.00	29.10	30.17	30.37	30.75	31.40	30.47	31.80	31.88	30.33	31.07	31.07	
SL430	29.40	28.53	30.00	28.83	30.13	30.67	30.47	31.37	30.33	31.90	31.97	30.30	30.87	30.87	
SL480	29.37	28.70	30.00	28.93	30.40	30.50	30.73	31.43	30.53	31.90	31.77	30.30	30.80	30.87	
SL030	29.33	28.57	30.00	29.13	30.13	30.37	30.97	31.30	30.53	31.83	31.87	30.37	30.97	31.07	
SL080	29.77	28.80	30.00	28.90	30.23	30.20	30.63	31.40	30.43	31.77	32.10	30.33	31.03	31.13	
SB0	29.43	28.70	30.00	29.13	30.53	30.47	30.68	31.10	30.47	31.60	32.23	30.43	31.10	31.40	

Note:

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-4

Results of Salinity (‰) measurement in dawn in polyculture black tiger shrimp seaweed

Treatment	Day													
	1	4	7	10	13	16	19	26	30	36	40	44	48	51
DAWN														
SS30	31.33	33.00	31.33	29.67	29.67	30.00	30.00	32.00	27.67	34.33	33.00	33.00	29.33	30.00
SS80	31.33	33.33	32.33	29.67	31.00	30.33	30.00	34.00	28.67	36.33	34.50	32.67	30.00	30.00
SL430	31.33	33.00	34.00	30.33	31.00	30.00	30.00	34.67	29.00	35.33	32.67	32.00	30.00	30.33
SL480	32.00	33.33	32.00	29.67	30.67	30.33	30.00	31.67	28.00	35.00	33.83	33.00	29.67	30.33
SL030	31.00	33.00	33.00	30.33	30.33	30.00	30.00	33.67	28.67	35.33	33.00	32.67	30.00	30.00
SL080	30.67	33.67	35.00	30.67	30.00	30.00	30.00	34.33	29.00	35.33	32.67	32.67	30.00	30.00
SB0	32.00	33.67	33.00	29.67	31.00	31.00	30.33	34.00	28.00	35.33	32.67	32.67	29.67	30.00
NOON														
SS30	26.00	25.33	28.00	30.33	27.67	31.33	30.00	29.67	28.33	33.33	33.50	30.00	29.33	30.00
SS80	26.00	25.33	29.00	30.33	29.33	32.00	29.33	29.33	29.00	32.67	33.83	31.00	29.33	29.33
SL430	26.00	25.67	29.00	30.67	29.00	33.00	29.67	30.33	29.33	33.33	32.33	30.33	29.00	29.67
SL480	26.00	25.00	28.67	30.33	30.00	32.00	30.00	29.33	28.00	32.00	34.17	29.00	28.67	30.00
SL030	25.67	25.33	28.67	30.67	27.00	32.33	30.00	30.00	29.33	32.00	33.33	31.00	29.00	29.00
SL080	25.33	26.00	29.67	31.00	28.33	33.33	29.33	29.00	28.67	33.00	32.33	29.00	29.00	29.67
SB0	25.67	24.67	28.67	30.33	29.67	32.00	30.00	29.67	28.67	32.33	33.00	29.00	28.67	29.33

Note:

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-5

Result of statistical analysis of mean value \pm SD of reducing ammonia at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	NH ₃ Concentration (mg.l ⁻¹)		Δ NH ₃
	T0	T1	
SS30	0.4715	0.4208	0.0507 \pm 0.0375a
SS80	0.2245	0.5795	-0.3551 \pm 0.0748b
SL430	0.3293	0.2620	0.0673 \pm 0.188a
SL480	0.3048	0.1837	0.1211 \pm 0.0686a
SL030	0.4925	0.4338	0.0588 \pm 0.0326a
SL080	0.4495	0.7383	-0.2888 \pm 0.0108b
SB0 (Control)	0.1841	0.5108	-0.3268 \pm 0.0717b

Notes:

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

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-6

Result of statistical analysis of mean value \pm SD of reducing nitrite at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	NO ₂ Concentration (mg.l ⁻¹)		Δ NO ₂
	T0	T1	
SS30	0.4259	0.3723	0.0535 \pm 0.013a
SS80	0.6106	0.4331	0.1774 \pm 0.065a
SL430	0.6293	0.1005	0.5289 \pm 0.194b
SL480	0.5479	0.1589	0.3889 \pm 0.070b
SL030	0.5554	0.4350	0.1205 \pm 0.010a
SL080	0.4691	0.4054	0.0637 \pm 0.028a
SB0 (Control)	0.5864	0.4880	0.0984 \pm 0.051a

Notes:

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

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-7

Result of statistical analysis of mean value \pm SD of reducing nitrate at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	NO ₃ Concentration (mg.l ⁻¹)		Δ NO ₃
	T0	T1	
SS30	0.0478	0.1569	0.1091 \pm 0.146a
SS80	0.1043	0.2547	0.1504 \pm 0.022b
SL430	0.1500	0.1732	0.0232 \pm 0.005cd
SL480	0.1514	0.2827	0.1313 \pm 0.016b
SL030	0.0334	0.1014	0.0680 \pm 0.0005c
SL080	0.0227	0.1142	0.0915 \pm 0.001ad
SB0 (Control)	0.0955	0.0617	-0.0338 \pm 0.0015e

Notes:

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

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-8

Result of statistical analysis of mean value \pm SD of reducing hydrogen sulphide at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	H ₂ S Concentration (mg.l ⁻¹)		Δ H ₂ S
	T0	T1	
SS30	0.0523	0.0647	0.0124 \pm 0.0004a
SS80	0.0425	0.0593	0.0168 \pm 0.0020ab
SL430	0.0270	0.0413	0.0143 \pm 0.0011a
SL480	0.0282	0.0488	0.0206 \pm 0.0010b
SL030	0.0399	0.0757	0.0358 \pm 0.0084c
SL080	0.0447	0.0720	0.0273 \pm 0.0030d
SB0 (Control)	0.0194	0.0781	0.0586 \pm 0.0014e

Notes:

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

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-9

Result of statistical analysis of mean value \pm SD of reducing total organic matter at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	TOM Concentration (mg.l ⁻¹)		Δ TOM
	T0	T1	
SS30	26.8987	11.8630	15.0357 \pm 1.184a
SS80	29.2657	17.8573	11.4083 \pm 0.717b
SL430	26.7127	10.8643	15.8483 \pm 0.481a
SL480	29.2460	9.1990	20.0470 \pm 0.359c
SL030	34.5847	19.8553	14.7293 \pm 1.310a
SL080	26.9260	17.2503	9.6757 \pm 2.027b
SB0 (Control)	34.6027	27.5127	7.0900 \pm 2.449d

Notes:

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

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-10

Result of statistical analysis of mean value \pm SD of reducing phosphate at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	PO ₄ Concentration (mg.l ⁻¹)		Δ PO ₄
	T0	T1	
SS30	0.0423	0.0593	-0.0170 \pm 0.0057a
SS80	0.0445	0.0411	0.0034 \pm 0.0122b
SL430	0.0470	0.0336	0.0134 \pm 0.0064b
SL480	0.0588	0.0465	0.0123 \pm 0.0119b
SL030	0.0437	0.0417	0.0019 \pm 0.0086ab
SL080	0.0632	0.0466	0.0166 \pm 0.0128b
SB0 (Control)	0.0381	0.0465	-0.0083 \pm 0.0156ab

Notes:

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

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-11

Result of statistical analysis of mean value \pm SD of reducing total dissolved solid at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	TDS Concentration (mg.l ⁻¹)		Δ TDS
	T0	T1	
SS30	27,838.67	39,466.67	11,628.00 \pm 2,825.10a
SS80	33,094.00	42,466.67	9,372.67 \pm 1,770.84a
SL430	35,623.33	36,633.33	1,010.00 \pm 92.71b
SL480	31,898.00	30,233.33	-1,664.67 \pm 213.85c
SL030	33,402.33	34,766.67	1,364.33 \pm 244.36b
SL080	28,414.33	35,200.00	6,785.67 \pm 1085.28d
SB0 (Control)	35,544.00	32,500.00	-3,044.00 \pm 55.51c

Notes:

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

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-12

Result of statistical analysis of mean value \pm SD of reducing total suspended solid at first and last measurement in polyculture black tiger shrimp and seaweed

Treatment	TSS Concentration (mg.l ⁻¹)		Δ TSS
	T0	T1	
SS30	54.20	53.33	0.87 \pm 0.06a
SS80	65.34	53.67	11.67 \pm 1.25bc
SL430	51.93	49.33	2.60 \pm 1.51a
SL480	61.40	52.00	9.40 \pm 1.39c
SL030	65.80	52.00	13.80 \pm 0.79b
SL080	44.60	46.00	-1.40 \pm 3.81a
SB0 (Control)	35.20	16.38	18.82 \pm 2.89d

Notes:

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

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-13

Average weight at stocking-harvesting time and absolute and daily growth rate of black tiger shrimp

Treatment	R	Average Weight (g)		Growth rate	
		Stocking	Harvesting	ABS. (g)	Daily (g.d ⁻¹)
SS30	1	8.8700	0.0000	0.0000	0.0000
	2	6.8650	14.1750	7.3100	0.1379
	3	8.6100	18.7250	10.1150	0.1908
	Mean	8.1150	10.9667	5.8083	0.1096
SS80	1	9.0600	15.9100	6.8500	0.1292
	2	8.3650	14.7450	6.3800	0.1204
	3	5.8750	0.0000	0.0000	0.0000
	Mean	7.7667	10.2183	4.4100	0.0832
SL430	1	5.5900	15.5700	9.9800	0.1883
	2	9.0200	18.8450	9.8250	0.1854
	3	6.9350	16.8300	9.8950	0.1867
	Mean	7.1817	17.0817	9.9000	0.1868
SL480	1	11.3400	21.2400	9.9000	0.1868
	2	11.2250	20.9800	9.7550	0.1841
	3	7.7300	17.7100	9.9800	0.1883
	Mean	10.0983	19.9767	9.8783	0.1864
SL030	1	8.1500	14.2100	6.0600	0.1143
	2	8.3250	14.4800	6.1550	0.1161
	3	11.4550	17.5000	6.0450	0.1141
	Mean	9.3100	15.3967	6.0867	0.1148
SL080	1	9.5400	15.9000	6.3600	0.1200
	2	10.1650	0.0000	0.0000	0.0000
	3	7.5450	14.3200	6.7750	0.1278
	Mean	9.0833	10.0733	4.3783	0.0826
SB0	1	6.0050	10.1350	4.1300	0.0779
	2	9.9600	0.0000	0.0000	0.0000
	3	10.3800	13.5700	3.1900	0.0602
	Mean	8.7817	7.9017	2.4400	0.0460

Note:

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-14

Shrimp mortality rate in polyculture black tiger shrimp and seaweed

Treatment	Replication		
	1	2	3
SS30	100	0	0
SS80	0	0	100
SL430	0	0	0
SL480	0	0	0
SL030	0	0	0
SL080	0	100	0
SB0 (Control)	0	100	0

Note:

SS30 = seaweed sown 240g

SL430= 30g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SS80 = seaweed sown 640g

SL480= 80g seaweed each attachment hung with depth 40cm

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-15

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

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.024	6	0.004007	13.7398	0.000153
Within Groups	0.003	11	0.000292		
Total	0.027	17			

Multiple Comparisons

Treatment		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
(I)	(J)				Lower Bound	Upper Bound
SS30	SS80	.0395500*	.0170779	.041	.001962	.077138
	SL430	-.0224000	.0155899	.179	-.056713	.011913
	SL480	-.0220000	.0155899	.186	-.056313	.012313
	SL030	.0495333*	.0155899	.009	.015220	.083846
	SL080	.0404500*	.0170779	.037	.002862	.078038
	SB0	.0729333*	.0155899	.001	.038620	.107246
SS80	SS30	-.0395500*	.0170779	.041	-.077138	-.001962
	SL430	-.0619500*	.0155899	.002	-.096263	-.027637
	SL480	-.0615500*	.0155899	.002	-.095863	-.027237
	SL030	.0099833	.0155899	.535	-.024330	.044296
	SL080	.0009000	.0170779	.959	-.036688	.038488
	SB0	.0333833	.0155899	.055	-.000930	.067696
SL430	SS30	.0224000	.0155899	.179	-.011913	.056713
	SS80	.0619500*	.0155899	.002	.027637	.096263
	SL480	.0004000	.0139440	.978	-.030291	.031091
	SL030	.0719333*	.0139440	.000	.041243	.102624
	SL080	.0628500*	.0155899	.002	.028537	.097163
	SB0	.0953333*	.0139440	.000	.064643	.126024
SL480	SS30	.0220000	.0155899	.186	-.012313	.056313
	SS80	.0615500*	.0155899	.002	.027237	.095863
	SL430	-.0004000	.0139440	.978	-.031091	.030291
	SL030	.0715333*	.0139440	.000	.040843	.102224
	SL080	.0624500*	.0155899	.002	.028137	.096763
	SB0	.0949333*	.0139440	.000	.064243	.125624
SL030	SS30	-.0495333*	.0155899	.009	-.083846	-.015220
	SS80	-.0099833	.0155899	.535	-.044296	.024330
	SL430	-.0719333*	.0139440	.000	-.102624	-.041243
	SL480	-.0715333*	.0139440	.000	-.102224	-.040843
	SL080	-.0090833	.0155899	.572	-.043396	.025230
	SB0	.0234000	.0139440	.121	-.007291	.054091
SL080	SS30	-.0404500*	.0170779	.037	-.078038	-.002862
	SS80	-.0009000	.0170779	.959	-.036688	.036688
	SL430	-.0628500*	.0155899	.002	-.097163	-.028537
	SL480	-.0624500*	.0155899	.002	-.096763	-.028137
	SL030	.0090833	.0155899	.572	-.025230	.043396
	SB0	.0324833	.0155899	.061	-.001830	.066796
SB0	SS30	-.0729333*	.0155899	.001	-.107246	-.038620
	SS80	-.0333833	.0155899	.055	-.067696	.000930
	SL430	-.0953333*	.0139440	.000	-.126024	-.064643
	SL480	-.0949333*	.0139440	.000	-.125624	-.064243
	SL030	-.0234000	.0139440	.121	-.054091	.007291
	SL080	-.0324833	.0155899	.061	-.066796	.001830

*. The mean difference is significant at the 0.05 level.

Appendix 1-16

Result of statistical analysis of mean value \pm SD of daily growth rate of 53 rearing period of black tiger shrimp and seaweed in polyculture system

Treatment	Shrimp			Seaweed		
	W0 (g)	W1 (g)	DGR (g.d ⁻¹)	W0 (g)	W1 (g)	DGR (g.d ⁻¹)
SS30	8.12	16.45	0.1644 \pm 0.0240a	240	203.55	-0.6877 \pm 0.104a
SS80	7.77	15.33	0.1248 \pm 0.0391b	640	440.80	-3.7584 \pm 1.153b
SL430	7.18	17.08	0.1868 \pm 0.0015a	240	443.16	3.8331 \pm 0.923c
SL480	10.10	19.98	0.1864 \pm 0.0021a	640	742.56	1.9352 \pm 0.107cd
SL030	9.31	15.40	0.1148 \pm 0.0170b	240	423.19	3.4565 \pm 1.832cd
SL080	9.08	15.11	0.1239 \pm 0.0189b	640	934.29	5.5527 \pm 2.561c
SB0 (Control)	8.78	11.85	0.0691 \pm 0.0086b			

Note:

abcd Means with different letters at the same column are significantly different at $p < 0.05$

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-17

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

Treatment	<i>Vibrio</i> (CFU/ml)			Total Bacteria (CFU/ml)		
	Day 1	Day 25	Day 51	Day 1	Day 25	Day 51
SS30	6.30 x 10 ²	4 x 10 ³	5.33 x 10 ²	6.72 x 10 ⁷	1.69 x 10 ⁷	4 x 10 ⁵
SS80	1.07 x 10 ³	9.33 x 10 ²	8.13 x 10 ²	3.31 x 10 ⁷	1.06 x 10 ⁷	1.003 x 10 ⁶
SL430	6.95 x 10 ²	6.7 x 10 ²	3.36 x 10 ²	6.49 x 10 ⁶	1.87 x 10 ⁷	3.7 x 10 ⁵
SL480	2.41 x 10 ³	4.62 x 10 ³	8.80 x 10 ²	3.8 x 10 ⁶	3.03 x 10 ⁷	9.15 x 10 ⁵
SL030	4.03 x 10 ²	1.44 x 10 ³	5.93 x 10 ²	1.49 x 10 ⁷	3.7 x 10 ⁵	1.6 x 10 ⁶
SL080	2.17 x 10 ²	1.11 x 10 ³	2.5 x 10 ²	1.84 x 10 ⁶	8.26 x 10 ⁶	2.33 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

Note:

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-18

Result of statistical analysis of mean value \pm SD of *Vibrio* and total bacterial colloni at last measurement in polyculture black tiger shrimp and seaweed

Treatment	<i>Vibrio</i> (CFU/ml)	Total Bacteria (CFU/ml)
SS30	$5.33 \times 10^2 \pm 20.82a$	$4 \times 10^5 \pm 48,000.00a$
SS80	$8.13 \times 10^2 \pm 90.19b$	$1.003 \times 10^6 \pm 37,527.77b$
SL430	$3.36 \times 10^2 \pm 15.28c$	$3.7 \times 10^5 \pm 75,498.34a$
SL480	$8.80 \times 10^2 \pm 131.15b$	$9.15 \times 10^5 \pm 80,000.00b$
SL030	$5.93 \times 10^2 \pm 60.28a$	$1.6 \times 10^6 \pm 50,000.00c$
SL080	$2.5 \times 10^2 \pm 45.00c$	$2.33 \times 10^5 \pm 15,275.25d$
SB0	$5.4 \times 10^2 \pm 5.00a$	$2.7 \times 10^5 \pm 36,055.51d$

Notes:

abcd Means with different letters at the same column are significantly different at $p < 0.05$

SS30 = seaweed sown 240g

SS80 = seaweed sown 640g

SL430= 30g seaweed each attachment hung with depth 40cm

SL480= 80g seaweed each attachment hung with depth 40cm

SL030= 30g seaweed each attachment hung on water surface

SL080= 80g seaweed each attachment hung on water surface

SB0 = Control – monoculture system

Appendix 1-19

Result of statistical analysis of mean value \pm 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	$0.1715 \pm 0.0117a$
BM100	0.7072	0.2074	$0.4998 \pm 0.1725b$
BM150	0.5787	0.1969	$0.3818 \pm 0.0440b$
SB0 (Control)	0.1841	0.5108	$-0.3268 \pm 0.0717c$

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 \pm SD of reducing nitrite at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	NO ₂ Concentration (mg.l ⁻¹)		Δ NO ₂
	T0	T1	
BM50	0.4017	0.1819	0.2199 \pm 0.00005a
BM100	0.4057	0.2097	0.1960 \pm 0.00086a
BM150	0.5849	0.3731	0.2118 \pm 0.00031a
SB0 (Control)	0.5864	0.4880	0.0984 \pm 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 \pm SD of reducing nitrate at first and last measurement in polyculture black tiger

TREATMENT	NO ₃ Concentration (mg.l ⁻¹)		Δ NO ₃
	T0	T1	
BM50	0.0604	0.0984	0.0380 \pm 0.0201a
BM100	0.0384	0.0594	0.0209 \pm 0.0034a
BM150	0.0363	0.0365	0.0003 \pm 0.0002b
SB0 (Control)	0.0955	0.0617	-0.0338 \pm 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 \pm SD of reducing hydrogen sulphide at first and last measurement in polyculture black tiger shrimp and blood cockle

TREATMENT	H ₂ S Concentration (mg.l ⁻¹)		Δ H ₂ S
	T0	T1	
BM50	0.0444	0.0639	0.0195 \pm 0.0005a
BM100	0.0528	0.0819	0.0291 \pm 0.0082b
BM150	0.0514	0.0810	0.0296 \pm 0.0006b
SB0 (Control)	0.0194	0.0781	0.0586 \pm 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 \pm 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)		Δ TOM
	T0	T1	
BM50	41.7427	20.2013	21.5413 \pm 2.130a
BM100	32.4613	23.6853	8.7760 \pm 2.102b
BM150	34.7227	34.5057	0.2170 \pm 0.021c
SB0 (Control)	34.6027	27.5127	7.0900 \pm 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

BM100 = 100 inds.m⁻² of blood cockle

BM150 = 150 inds.m⁻² of blood cockle

SB0 = Control – monoculture system

Appendix 1-24

Result of statistical analysis of mean value \pm 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 \pm 0.0005a
BM100	0.0604	0.0285	0.0319 \pm 0.0043b
BM150	0.0526	0.0430	0.0096 \pm 0.0005a
SB0 (Control)	0.0381	0.0465	-0.0083 \pm 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

BM100 = 100 inds.m⁻² of blood cockle

BM150 = 150 inds.m⁻² of blood cockle

SB0 = Control – monoculture system

Appendix 1-25

Result of statistical analysis of mean value \pm 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)		Δ TDS
	T0	T1	
BM50	33,110.00	37,133.33	4,023.33 \pm 18.93a
BM100	32,608.00	39,200.00	6,592.00 \pm 989.50b
BM150	33,130.33	39,266.67	6,136.33 \pm 1007.17b
SB0 (Control)	35,544.00	32,500.00	-3,044.00 \pm 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

BM100 = 100 inds.m⁻² of blood cockle

BM150 = 150 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
BM150 = 150 inds.m⁻² of blood cockle

BM100 = 100 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

BM150 = 150 inds.m⁻² of blood cockle

BM100 = 100 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	1.98×10^2	3.73×10^3	6.1×10^2	3.87×10^7	2.73×10^7	3.67×10^5
BM100	6.34×10^3	8.13×10^2	1.5×10^2	2.37×10^7	1.13×10^7	4.35×10^6
BM150	5.43×10^2	8.97×10^2	2.15×10^2	2.86×10^6	7.66×10^7	2.8×10^5
SB0	8.07×10^2	7.13×10^2	5.4×10^2	1.56×10^6	1.25×10^7	2.7×10^5

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 1-30

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	$6.1 \times 10^2 \pm 30.41a$	$3.67 \times 10^5 \pm 24,664.41a$
BM100	$1.5 \times 10^2 \pm 62.65b$	$4.35 \times 10^6 \pm 273,008.00b$
BM150	$1.43 \times 10^2 \pm 13.23b$	$2.8 \times 10^5 \pm 20,000.00a$
SB0	$5.4 \times 10^2 \pm 5.00c$	$2.7 \times 10^5 \pm 36,055.51a$

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

BM100 = 100 inds.m⁻² of blood cockle

BM150 = 150 inds.m⁻² of blood cockle

SB0 = Control – monoculture system

CHAPTER 2

Characterization of dual-filtration organisms of seaweed – *Gracilaria verrucosa* and blood cockle – *Anadara granosa* in black tiger shrimp (*Penaeus monodon* Fab.) polyculture system

ABSTRAK

Tujuan dari penelitian ini adalah menentukan efektivitas dari rumput laut (*Gracilaria verrucosa*) dan kerang darah (*Anadara granosa*) sebagai filter biologi gabungan dalam budidaya udang windu (*Penaeus monodon*) sistem polikultur. Penelitian dilakukan di Balai Pengembangan Budidaya Perikanan Laut, Air Payau dan Udang, Sungai Buntu, Karawang, Jawa Barat dalam wadah kayu yang dilapisi plastik (ukuran 0,9 x 0,6 x 1 m) tanpa pergantian air selama 57 hari. Padat tebar udang adalah 4 individu.m⁻² dan diuji menggunakan “PCR” untuk mengetahui infeksi virus bercak putih (WSSV- White spot syndrome virus) sebelum ditebar. Percobaan menggunakan rancangan acak lengkap dengan perlakuan penelitian adalah polikultur udang menggunakan filter biologi gabungan rumput laut (*G. verrucosa*) dan kerang darah (*A. granosa*). Metode penanaman rumput laut adalah lepas dasar dengan berat rumput laut per ikatan adalah 30 (total berat rumput laut 240g) dan 80 g (total berat rumput laut 640g) digantung pada kedalaman sekitar 40 cm dari permukaan air dengan interval jarak sekitar 25 cm. Padat tebar kerang darah adalah 50 dan 100 individu.m⁻². Perlakuan penelitian dikelompokkan menjadi 4 jenis polikultur, yaitu polikultur udang (4 individu.m⁻²) dengan 1) 30 g rumput laut per ikatan dengan kerang darah 50 individu.m⁻² 2) 30 g rumput laut per ikatan dengan kerang darah 100 individu.m⁻² 3) 80 g rumput laut per ikatan dengan kerang darah 50 individu.m⁻² 4) 80 g rumput laut per ikatan dengan kerang darah 100 individu.m⁻². Adapun sebagai kontrol adalah monokultur udang (tanpa filter biologi). Setiap perlakuan diterapkan 3 ulangan. Berdasarkan hasil yang diperoleh disimpulkan bahwa polikultur udang dengan filter biologi gabungan tidak bekerja dengan sempurna dalam kaitannya dengan menstabilkan air habitat pembudidayaan. Konsentrasi oksigen terlarut, ammonium, ammonia, nitrit, nitrat, hidrogen sulfid, fosfat, dan total bahan organik tidak sesuai dengan persyaratan hidup udang yang dibudidayakan. Disamping itu, hasil pengamatan total bakteri dan *Vibrio* dari sistem polikultur jumlah koloninya melebihi yang seharusnya.

ABSTRACT

The aim of this research was to characterize the effectiveness of dual filtration organisms of sea-weed *Gracilaria verrucosa* and blood cockle – *Anadara granosa* in the black tiger shrimp (*Penaeus monodon*) polyculture system to stabilize culture water habitat. The research was performed at 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 57 days. Stocking density of shrimp was 4 individual.m⁻² and examined for WSSV (White spot syndrome virus)-free using PCR technique before stocking. The experiment followed completely

randomized design with the treatments of polyculture system using dual-filtration organism seaweed (*G. verrucosa*) and blood cockle (*A. granosa*). Seaweed cultivation was off bottom method and tied with about 30 g (total weight 240 g) and 80 g (total weight 640 g) mass at each attachment and hung at a depth of 40 cm under surface water with interval of 25 cm. Blood cockle densities were 50 and 100 ind.m⁻². There were four kinds of polyculture system, those were polyculture system of 4 ind.m⁻² of black tiger shrimp (*P. monodon*) with 1) 30 g seaweed per attachment and 50 ind.m⁻² 2) 30 g seaweed per attachment and 100 ind.m⁻² 3) 80 g seaweed per attachment and 50 ind.m⁻² 4) 80 g seaweed per attachment and 100 ind.m⁻². Furthermore, shrimp monoculture system was set up as control. The placement for each treatment grouping to the available tanks was completely randomized and all treatments had 3 replications. Based on the results of study, it is concluded that shrimp polyculture systems with dual filtration organisms did not work properly in order to stabilize culture water habitat. The concentration of dissolved oxygen, ammonium, ammonia, nitrite, hydrogen sulphide, phosphate and total organic matter were not in the range of shrimp culture requirement. Shrimp polyculture system had also exceeded total number of *Vibrio* and bacterial.

Key Words : *Culture habitat; dual filtration organism; growth rate; water quality.*

INTRODUCTION

Aquaculture is the farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants (Barnabe 1990; FAO 1991). Aquaculture practices tend to manipulate aquaculture ecosystems in order to achieve higher production than in nature by employing culture technology and management. High production input is supplemented into the culture system especially for intensive culture technology and described as “through put-based systems” (Folke and Kautsky 1992). It means that only a few part of production input is taken up by cultured species and the rest is accumulated to the pond bottom ecosystem and released as wastes to the surrounding environment during water exchange and harvesting time. Month by month of culture period of black tiger shrimp culture, accumulation of particulate matter and dissolved nutrients concentration on the pond bottom tend to increase and even exceed the threshold concentration of cultured organism requirement (FAO 1991; Midlen and Redding 2000).

Accumulated substances consist of particulate matter and dissolved nutrients or organic and inorganic matter (Jones *et al.* 2001). Most of accumulated materials may naturally be decomposed (decomposition process) into simple form

and then be utilized by photo-autotrophic organism, so called production process, to form more complex organic compounds such as protein and carbohydrate. The third ecological process, so called consumption, is performed by heterotrophic organisms such as omnivorous, herbivorous, and carnivorous organisms. Those ecological processes have close relationship with physico-chemical parameters of pond water and pond bottom soil. Excessive accumulation of organic and inorganic matters on the bottom could cause hypernutrification and eutrophication conditions. Those conditions are attributed by plankton bloom and water quality degradation and will probably generate adverse impact to cultured organism (Pillay 2004). In addition, during water exchange and harvesting time, waste water of culture practices are flushed out as high organic content pollutant to the surrounding environment. Several aquaculture technologies such as polyculture, integrated, closed recirculation, etc. are intended to maintain water culture habitat quality and to reduce impact to surrounding environment. In polyculture system, there are two or more organisms cultured in one culture unit. One of the organisms is main cultured species and the rest are additional species and to be filtration organisms in order to stabilize culture habitat.

Based on the former study, there were principally two kinds of filtration organism related to their ecological roles. First, a group of autotrophic organisms that have the ability to absorb dissolved nutrients, such as seaweed (Jones *et al.* 2001; Baliao & Tookwinas 2002; Neori *et al.* 2004) and mangrove (Robertson and Phillips. 1995; Shimoda *et al.* 2005; Shimoda *et al.* 2006). Secondly, filter feeder organisms such as oyster (Chow *et al.* 2001; Jones *et al.* 2001) and mussel (Baliao & Tookwinas 2002) that are able to reduce particulate materials. In term of culture technologies, those studies were mostly performed in the integrated culture system of which filtration organisms were stocked in separated culture unit and to be single filtration organism (Shpigel *et al.* 1993). So, we are lacking on scientific information either on combination of several filtration organisms or employing filtration organism in polyculture system (Pillay 2004).

MATERIALS AND METHODS

The experiment was conducted 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 throughout the 57-day experimental period (from May 28 to July 23, 2009). Several water quality parameters were analyzed at DCMBSC Karawang and Fisheries University Jakarta. The objectives of this research were to determine the ecological roles and the synergy ability of dual-filtration organism of seaweed (*Gracilaria verrucosa*) and blood cockle (*Anadara granosa*) to stabilize environment of black tiger shrimp (*Penaeus monodon*) polyculture system.

Black tiger shrimp (*P. monodon*) as main cultured organism and seaweed (*G. verrucosa*) as filtration organism were provided by DCMBSC. The mean shrimp size was 5.58 ± 1.2328 g in weight and 9.15 ± 0.6840 cm in length. Blood cockle (*A. granosa*) as filtration organism was purchased from Muara Gembong, Bekasi Regency, West Java with the mean weight of about 6.22 ± 0.4341 g. The shrimp and seaweed were harvested from rearing ponds and blood cockle was harvested from wild population.

The experiment was carried out by preparing two culture technologies, those were shrimp polyculture system with combined filtration organism (*G. verrucosa* and *A. granosa*) and shrimp monoculture system (without filtration organism) as control. Stocking density of shrimp was 4 individuals.m⁻². Shrimp was acclimated in the fiber tanks with the size of 2 x 1.5 x 0.5 m for 3 days and examined for WSSV (White spot syndrome virus)-free using PCR technique before stocking. Seaweed was cultivated by off bottom method and tied with about 30 g and 80 g mass at each attachment and hung at a depth of 40 cm under surface water with interval of 25 cm. Blood cockle densities were 50 and 100 inds.m⁻². There were four kinds of polyculture system, those were polyculture system of 4 inds.m⁻² of black tiger shrimp (*P. monodon*) with:

- 1) Treatment 1 – 30 g seaweed per attachment and 50 ind.m⁻² (abbreviated as SB35).

- 2) Treatment 2 – 30 g seaweed per attachment and 100 ind.m⁻² (abbreviated as SB310).
- 3) Treatment 3 – 80 g seaweed per attachment and 50 ind.m⁻² (abbreviated as SB85).
- 4) Treatment 4 – 80 g seaweed per attachment and 100 ind.m⁻² (abbreviated as SB810).

The experiment was using completely randomized design and the placement of each treatment was arranged to the available tanks and all treatments had 3 replications. There were no water exchange except the addition of water to replace the lost 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 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 physico-chemical of water quality parameters included dissolved oxygen, temperature, salinity, pH, ammonia, nitrite, nitrate, H₂S, phosphate, total dissolved solid, total suspended solid, and total organic matter. The biological parameters included growth of shrimp, blood cockle and seaweed, and total *Vibrio* and bacteria. The first four parameters were measured every 3 days at 5.00 and 11.00 h. and the rest parameters except growth parameters were measured once every three week. The growth parameter consisted of weight of shrimp, blood cockle and seaweed were measured before stocking and at harvest time. The measurement and water sampling were performed at about 20 cm above pond bottom. Dissolved oxygen, temperature, and pH were measured by multi-water quality parameters checker. The measurement equipment of salinity was Atago refractosalinometer. The rest of the parameters were observed by using Spectrophotometer Optima – SP 300. The measurements all parameters followed Standard Methods – APHA (1979); Alerts & Santika (1987); Effendi (2003).

Collected Data were used to explain the ability of filtration organisms to avoid a decrease in water quality of shrimp media. Therefore, the analysis of the

differences between the treatment of their bio-filtration was used one-way ANOVA and considered significant at an alpha level of 0.05 (Prasisto 2002; Supranto 2004). However, due to the total mortality of most replications of all treatments except shrimp monoculture system (without filtration organism) as control, collected data were not analysed statistically.

RESULTS

One indicator of achievement of aquaculture practices is the mortality rate of black tiger shrimp as main cultured organism. **Table 2-1** and **Appendix 2-1** indicate, in terms of mortality rate of shrimp, the best results were found at the treatment SB0 as control which was shrimp monoculture system without employing seaweed and blood cockle as filtration organisms. The worst one with 100% mortality of all replications was treatment SB810 – polyculture black tiger shrimp with 80 g seaweed per attachment and 100 inds.blood cockle.m⁻². The treatment SB35 (30 g seaweed per attachment & 50 ind.m⁻² of blood cockle) and SB310 (30 g seaweed per attachment & 100 inds.m⁻² of blood cockle) had shrimp mortality of 100% of 2 replications and 50% of one replication. Treatment SB85 – black tiger shrimp polyculture system with 80 g seaweed per attachment and 50 inds.m⁻² of blood cockle revealed better mortality rate than the other treatments. Moreover, blood cockle as filtration organism had similar mortality rate to the shrimp (**Table 2-1**). In addition, data on mortality rate of black tiger shrimp and blood cockle were collected at day 20th of experiment period. The routine observation of all research variables (physico-chemical and biological) of replications that showed 100% mortality rate of black tiger shrimp was terminated at day 20th, i.e. treatment SB35 replication 1 and 2, treatment SB310 replication 2 and 3, treatment SB85 replication 3, and all replications of treatment SB810. Therefore, Collected data on growth rate of shrimp was not analysed statistically due to the mass mortality of the majority replications of each treatment.

Table 2-1
Mortality rate of shrimp and blood cockle (%)

Treatment	Shrimp			Blood Cockle		
	Replication					
	1	2	3	1	2	3
SB35	100	100	50	62	100	38
SB310	50	100	100	40	100	100
SB85	0	0	100	26	26	8
SB810	100	100	100	100	100	100
SB0	0	0	0	-	-	-

Note:

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²
- SB310: 30 g seaweed per attachment and 100 ind.m⁻²
- SB85: 80 g seaweed per attachment and 50 ind.m⁻²
- SB810: 80 g seaweed per attachment and 100 ind.m⁻²
- SB0: shrimp monoculture system

During the experiment, recorded DO concentration tended to decrease. The higher dissolved oxygen concentration was attributed by shrimp monoculture system (SB0) followed by shrimp polyculture with 80 g seaweed per attachment and 50 ind.m⁻² of blood cockle (treatment SB85) (**Figure 2-1a**). The minimum & maximum of those concentrations of SB0 varied from 2.47 to 3.10 mg.l⁻¹ in the early morning and from 2.76 to 3.56 mg.l⁻¹ in the afternoon. The variation of DO concentration of SB85 were from 0.99 to 3.10 mg.l⁻¹ in the early morning (**Appendix 2-2**) and from 1.89 to 4.21 mg.l⁻¹ in the afternoon (**Appendix 2-3**). The other treatments showed lower concentration than that of two previous treatments. Simultaneous observation of another water quality parameters were conducted which included pH, salinity and temperature. Higher pH was attributed by shrimp monoculture system (SB0) followed by shrimp polyculture with 80 g seaweed per attachment and 50 ind.m⁻² of blood cockle (treatment SB85) (**Figure 2-3** and **Appendix 2-4**). Salinity and temperature of all treatments showed relatively similar pattern. The variation of salinity and temperature were 27.67 – 34.00 ppt (**Appendix 2-5**), and 25.10 – 31.00°C (**Appendix 2-6**), respectively.

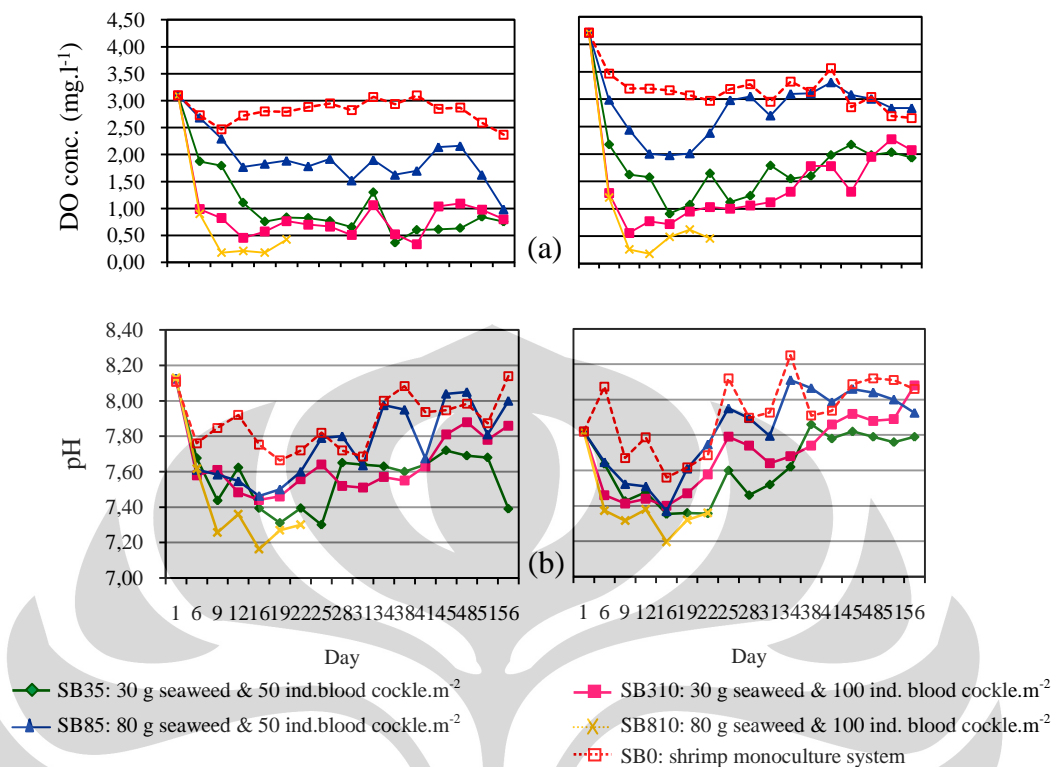


Figure 2-1

Water quality parameters included DO (a) and pH (b) measured every 3 days in dawn (left graphs) and noon (right graphs) in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Ammonium concentration tended to increase in all treatments during the experiment. Sharp increase were observed from first to second measurements (**Figure 2-2a** and **Appendix 2-7**). After day-20th of experiment period, monoculture system (SB0) as control showed relatively stable ammonia concentration of about 0.415 ± 0.024 mg.l⁻¹ and the highest concentration was recorded in experiment with treatment SB310-30 g seaweed with 100 ind. of blood cockles (**Figure 2-2b** and **Appendix 2-7**). Most of treatments except for SB810-polyculture shrimp with 80 g of seaweed.bound⁻¹ and 100 inds. of blood cockles revealed relatively level off trends of ammonia concentration from first to second measurement and then increase afterward. The lowest and the highest concentration were recorded in monoculture system (SB0) as control and shrimp polyculture with 30 g of seaweed.bound⁻¹ and 100 ind. of blood cockles (SB310).

Similar to ammonium, nitrite concentration also tended to increase in all treatments during the experiment (**Figure 2-2c** and **Appendix 2-7**). After day-20th of experiment period, monoculture system (SB0) as control showed relatively lower concentration of about $0.0550 \pm 0.0044 \text{ mg.l}^{-1}$ and the highest concentration was recorded in experiment treatment of 80 g seaweed with 100 inds. of blood cockles. Amongst nitrogen parameters, nitrate concentration of all treatments revealed different trend, mostly decrease in all treatments from first to second measurements and then increase tenderly until end of experiment (**Figure 2-2d**). More detail results of the observation were presented at **Appendix 2-7**.

Hydrogen sulphide and phosphate concentration dynamic showed similar trend. Its concentration of all experiment treatments revealed increase trend and then level off at the end observation (**Figure 2-3a** and **Appendix 2-8**). Furthermore, total organic matter (TOM) had similar trend at the beginning but then increase at the end measurement (**Figure 2-3b** and **Appendix 2-8**). Phosphate and TOM of monoculture system had lower concentration than other treatments. Similar pattern of TDS and TSS was observed of all treatments. Its tended to increase after second measurements (**Figure 2-3d** and **Figure 2-3e**).

The remaining harvested experimental unit showed that treatment SB310 had highest shrimp growth rate amongst them followed by treatments SB85, SB0 and SB35 (**Figure 2-4** and **Appendix 2-9**). Low growth rate was also recorded for seaweed (**Appendix 2-10**) and blood cockle (**Appendix 2-11**) as filtration organism. The treatment SB85 revealed highest weight gained of seaweed and blood cockle followed by treatment SB35 and SB310. The worst gain was showed by treatment SB810.

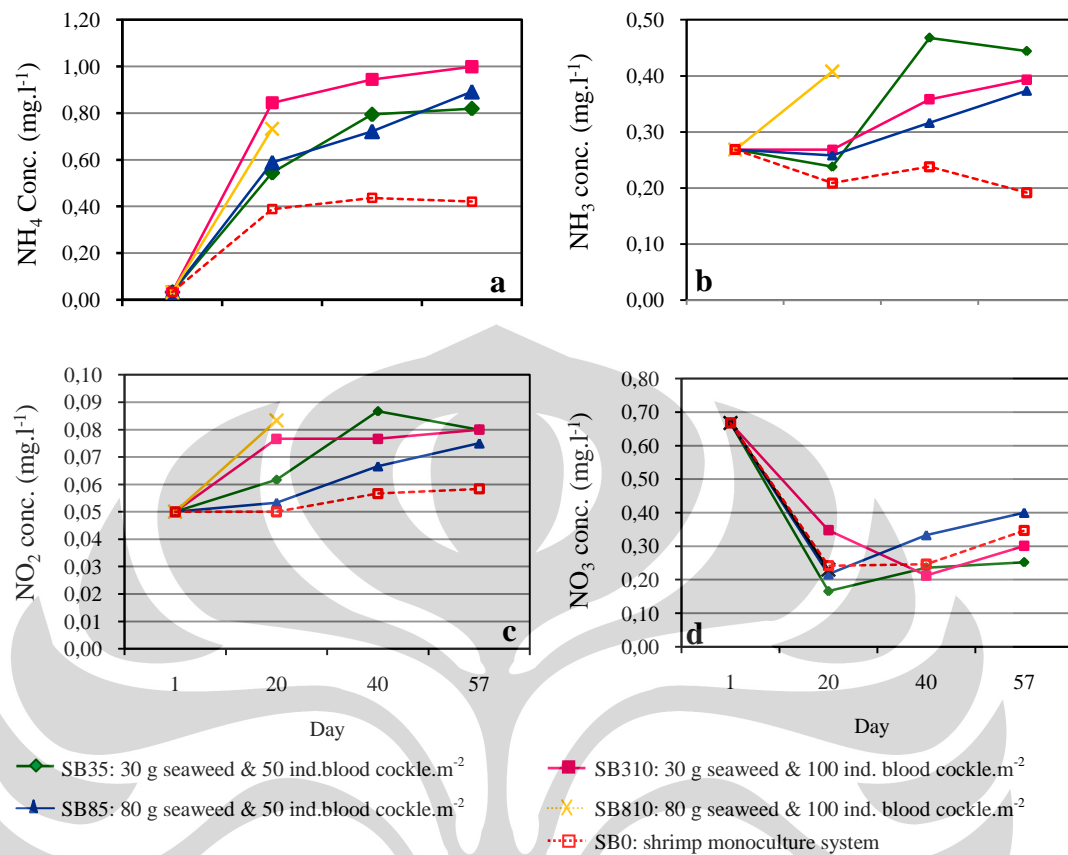


Figure 2-2

Ammonium (a), ammonia (b), nitrite (c), and nitrate concentration (d) in black tiger shrimp polyculture system with seaweed and blood cockle as dual-filtration organism

Total number of *Vibrio* of all treatments increased relatively from the first until third observation and then level off or bit decrease at the end of experiment (Figure 2-5). Similar trends were observed for total bacteria but tend to increase at last observation (Figure 2-5). The total number of *Vibrio* and bacteria of SB0 as control and SB85 (shrimp polyculture system with 80 g seaweed and 50 ind. of blood cockle.m⁻²) were lower than the other treatments. As presented by Appendix 2-12, at the end of experiment total *Vibrio* revealed similar magnification of about 10³. Treatment of SB0 as control showed stable trend of total bacteria with magnification range from 10⁴ to 10⁵ CFU.ml⁻¹.

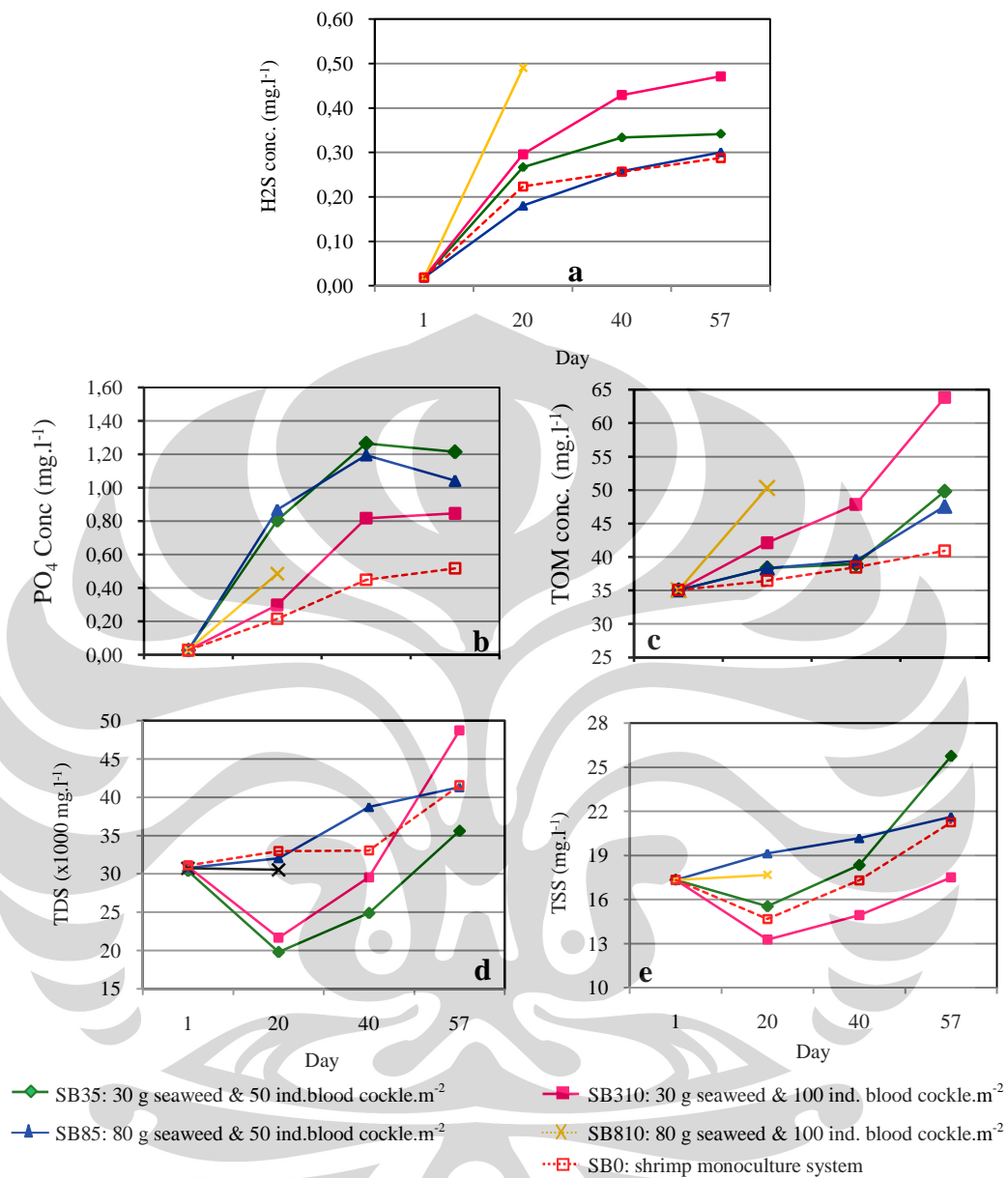


Figure 2-3

Hydrogen sulphide (a), phosphate (b), total organic matter (c), total dissolved solid (d), and total suspended solid (e) concentration in black tiger shrimp polyculture system with seaweed and blood cockle as dual-filtration organism

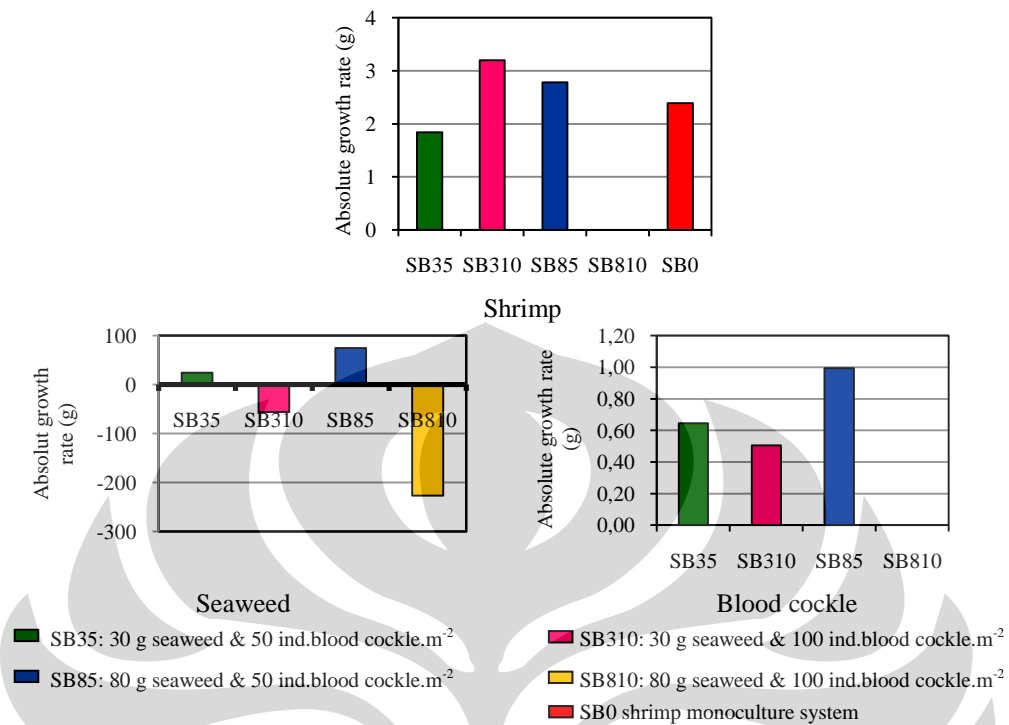


Figure 2-4.

Absolute growth rate (g) of black tiger shrimp, seaweed, and blood cockle in polyculture system during experiment

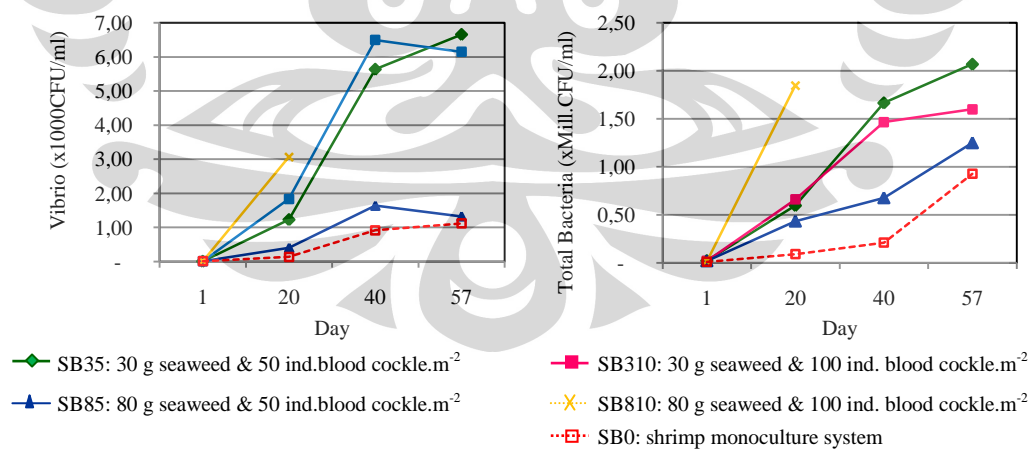


Figure 2-5.

Total *Vibrio* and total bacteria in black tiger shrimp polyculture system with seaweed and blood cockle as dual-filtration organism during experiment

DISCUSSION

In general, aquaculture is undertaken in man-made ecosystems 'culture units' which are generally made up of biotic and abiotic components. In this study, the main biotic component includes cultured shrimp and combined filtration organisms (seaweed – *G. verrucosa* and blood cockle – *A. granosa*). The abiotic component comprises physical and chemical aspects of water as the culture medium. Biotic and abiotic perform ecological functions and interact with each other. Therefore, a change in one of these ecosystem components may be followed by changing in the other components, the cultured organisms or even the whole culture system. One of the main water quality parameters and limiting factors in aquaculture practices is dissolved oxygen (DO). Aquatic organisms need oxygen for 24 hours for their respiration. Oxygen sources are mainly coming from photosynthesis mechanism and secondly diffusion from the air (Wetzel 1993; Howerton 2001). As a main source of oxygen in the culture water, photosynthesis rate is controlled by several factors such as light intensity and plant abundant (Boyd 1990). In this study, *G. verrucosa* is the expected main oxygen source of culture unit and as filtration organism. On the other hand, during night time (no photosynthesis process), dissolved oxygen tends to decrease due to respiration of all aquatic organisms included shrimp, seaweed, blood cockle, and microorganism. Oxygen deficiency will also influence the other ecological process i.e. decomposition, consumption, and production rate (Zonneveld *et al.* 1991). At day 12th, all treatments except treatment SB0 as control revealed sharp decrease of dissolved oxygen concentration. Diurnal fluctuation pattern between pH and dissolved oxygen of all treatments were also similar. During day time, pH will increase with photosynthesis process due to the plenty of dissolved oxygen concentration (Boyd 1990). Other parameters such as ammonium, ammonia, nitrite, nitrate, hydrogen sulphide, total organic matter, and phosphate expressed also similar pattern to the dissolved oxygen.

Seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as dual-filtration organism in the polyculture system of black tiger shrimp were not able to

maintain a suitable culture habitat and lead to low growth, survival rate, and shrimp productivity. Shrimp mortality also increased except in the control and in Treatment of SB85 (80 g seaweed.bound⁻¹ and 50 inds.m⁻² of blood cockle). Most of main water quality parameters such as dissolved oxygen, ammonium, ammonia, nitrite, hydrogen sulphide, phosphate and total organic matter in the shrimp polyculture system with dual filtration organisms were relatively higher concentration than in shrimp monoculture system. It indicated that the combination of both filtration organisms in shrimp polyculture system was not properly working. The performance of seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as dual-filtration organism showed opposite results to the previous study as single filtration in the polyculture system (Nurhudah *et al.* 2009a; Nurhudah *et al.* 2009b) and in the integrated system (Troell *et al.* 1999; Shpigel *et al.* 1993; Chow *et al.* 2001; Jones *et al.* 2001; Baliao & Tookwinas 2002; Neori *et al.* 2004). Those were probably due to the lack of dissolved oxygen supply (**Figure 2-3a**) that was needed for decomposing of accumulated organic matter in the culture habitat (Boyd 1990). In polyculture system, there were more introduced species organisms within the culture unit than monoculture system, meaning that more oxygen will be needed for the system. Therefore, the worst dissolved oxygen level was found just before daybreak due to the respiration process of all organisms. Furthermore, TDS and TSS concentration had no clear patterns. Seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as filtration organism were not able to reduce concentration of TDS and TSS as had been mentioned by previous studies (Jones *et al.* 2001).

Ecologically, decreasing culture water habitat quality is much likely be having influences to the biotic components mainly shrimp as cultured species, seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as filtration organism, and bacterial population. As presented by **Table 2-1**, most of the shrimp polyculture system had high mortality rate especially with total mortality rate of the shrimp polyculture system with 80 g of seaweed per bound and 100 ind. of blood cockles.m⁻². The worst performances of this polyculture systems were also found for seaweed (*G. verrucosa*) and blood cockle (*A. granosa*). On the other hand,

shrimp monoculture system had survival rate of 100%. The worst performance of aquatic organisms in culture system were probably caused by poor water quality of culture habitat of which had reciprocal relationship with organisms density in the culture system. At day 20th of experimental period, some water quality parameters showed inappropriate concentration related to the shrimp culture requirements. All shrimp polyculture system had lower dissolved oxygen concentration than shrimp culture requirement (Boyd 1990; Direktorat Jenderal Perikanan Budidaya 2003; Effendi 2003). The other water quality parameters such as ammonium, ammonia, nitrite, hydrogen sulphide, phosphate and total organic matter had possibility influenced the culture organisms growth and survival rate. In addition, it could generate excessive dissolved nutrients concentration and plankton bloom (increasing growth and productivity), so-called hypernutrification and eutrophication (Pillay 2004). Moreover, during the experiment, total *Vibrio* and total bacterial in shrimp monoculture system were lower than that of polyculture system.

CONCLUSION AND RECOMMENDATION

Conclusion

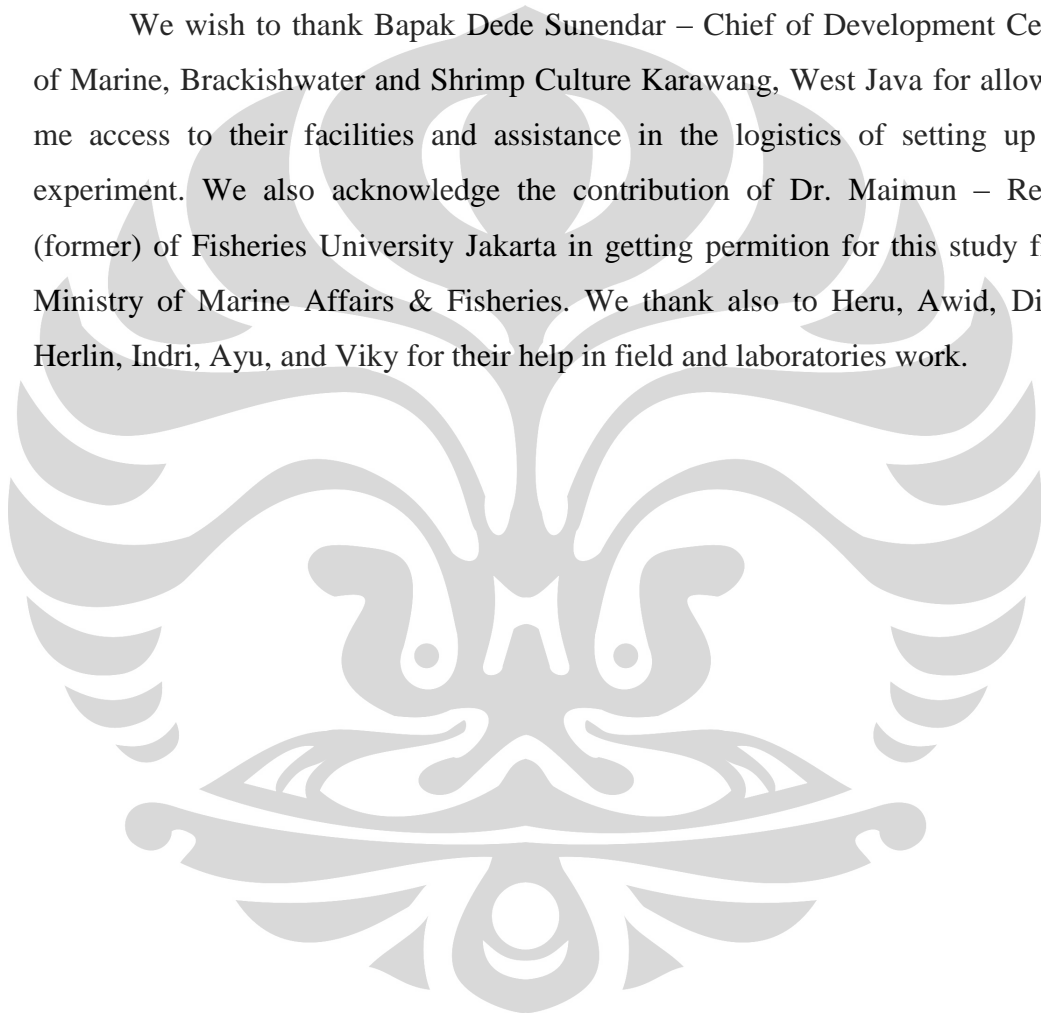
Shrimp polyculture systems with dual filtration organisms (seaweed-*G. verrucosa* and blood cockle-*A. granosa*) did not work properly in order to stabilize culture water habitat. The concentration of dissolved oxygen, ammonium, ammonia, nitrite, nitrate, hydrogen sulphide, phosphate and total organic matter were not in the range of shrimp culture requirements. DO concentration tended to decrease sharply from first to day 10th of experimental period. The lowest DO concentration was 2.47 mg.l⁻¹. Ammonium, ammonia, nitrite, and total organic matter concentration raised significantly from first to second observation. The low ability of polyculture system to maintain a suitable culture habitat had lead to low growth, survival rate, and shrimp productivity.

Recommendation

It is recommended that further study should focus on the study of shrimp polyculture system with *G. verrucosa* as single filtration organism.

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Appendix 2-1

Mortality rate of shrimp (%) by day of observation during experiment

Treatment	R	Day											
		1	5	10	15	20	25	30	35	40	45	50	55
SB35	1	0	0	0	0	0	100	100	100	100	100	100	100
	2	0	0	0	0	0	0	100	100	100	100	100	100
	3	0	0	0	0	0	0	50	50	50	50	50	50
SB310	1	0	0	0	0	0	50	50	50	50	50	50	50
	2	0	0	0	0	0	100	100	100	100	100	100	100
	3	0	0	0	0	0	100	100	100	100	100	100	100
SB85	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	100	100	100	100	100	100
SB810	1	0	0	0	0	100	100	100	100	100	100	100	100
	2	0	0	0	0	100	100	100	100	100	100	100	100
	3	0	0	0	0	0	100	100	100	100	100	100	100
SB0	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0

Note: - R = Replication- SB35: 30 g seaweed per attachment and 50 ind.m⁻²- SB85: 80 g seaweed per attachment and 50 ind.m⁻²

- SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²- SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-2

Results of dissolved oxygen (mg.l^{-1}) measurement in dawn in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Treatment	R	Day																
		1	6	9	12	16	19	22	25	28	31	34	38	41	45	48	51	56
SB35	1	3.10	0	1.81	1.53	1.03	0.5	0.54	0	0	0	0	0	0	0	0	0	0
	2	3.10	0	1.62	1.79	1.01	0.49	0.73	0.00	0.00	0	0	0	0	0	0	0	0
	3	3.10	0	2.19	2.06	1.28	1.28	1.23	0.82	0.77	0.65	1.30	0.36	0.60	0.61	0.63	0.84	0.75
	Mean		3.10	0	1.87	1.79	1.11	0.76	0.83	0.82	0.77	0.65	1.30	0.36	0.60	0.61	0.63	0.84
SB310	1	3.10	0	1.92	1.75	0.39	1.24	1.44	0.70	0.66	0.51	1.06	0.52	0.34	1.04	1.09	0.98	0.80
	2	3.10	0	0.82	0.6	0.81	0.22	0.8	0.00	0.00	0	0	0	0	0	0	0	0
	3	3.10	0	0.22	0.12	0.16	0.26	0.06	0.00	0.00	0	0	0	0	0	0	0	0
	Mean		3.10	0	0.99	0.82	0.45	0.57	0.77	0.70	0.66	0.51	1.06	0.52	0.34	1.04	1.09	0.98
SB85	1	3.10	0	2.14	2.35	1.64	2.51	1.74	1.98	2.06	1.82	2.08	1.46	1.54	2.3	2.28	1.41	1.02
	2	3.10	0	2.91	2.74	2.35	1.67	2.15	1.59	1.78	1.22	1.72	1.79	1.86	1.98	2.04	1.84	0.95
	3	3.10	0	3.01	1.80	1.32	1.31	1.77	0.00	0.00	0	0	0	0	0	0	0	0
	Mean		3.10	0	2.69	2.30	1.77	1.83	1.89	1.79	1.92	1.52	1.90	1.63	1.70	2.14	2.16	1.63
SB810	1	3.10	0	0.18	0.05	0.47	0.24	0.08	0.00	0.00	0	0	0	0	0	0	0	0
	2	3.10	0	0.42	0.25	0.08	0.16	0.02	0.00	0.00	0	0	0	0	0	0	0	0
	3	3.10	0	2.11	0.24	0.09	0.14	1.17	0.00	0.00	0	0	0	0	0	0	0	0
	Mean		3.10	0	0.90	0.18	0.21	0.18	0.42	0.00	0.00	0	0	0	0	0	0	0
SB0	1	3.10	0	2.81	2.62	3.40	3.34	2.98	2.97	3.15	2.92	3.22	2.97	3.10	2.75	2.78	2.39	2.63
	2	3.10	0	2.56	2.04	2.65	2.45	2.98	2.77	3.05	2.92	3.31	2.97	3.10	2.85	2.94	2.39	2.03
	3	3.10	0	2.82	2.74	2.12	2.62	2.42	2.91	2.64	2.63	2.68	2.87	3.09	2.95	2.89	2.99	2.45
	Mean		3.10	0	2.73	2.47	2.72	2.80	2.79	2.88	2.95	2.82	3.07	2.94	3.10	2.85	2.87	2.59

Note: - R = Replication

- SB35: 30 g seaweed per attachment and 50 ind.m^{-2}

- SB85: 80 g seaweed per attachment and 50 ind.m^{-2}

- SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m^{-2}

- SB810: 80 g seaweed per attachment and 100 ind.m^{-2}

Appendix 2-3

Results of dissolved oxygen measurement in noon in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Treatment	R	Day																
		1	6	9	12	16	19	22	25	28	31	34	38	41	45	48	51	56
SB35	1	4.21	2.11	1.42	1.18	1.12	0.60	1.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	4.21	1.92	1.21	1.34	0.49	1.43	1.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	4.21	2.49	2.24	2.21	1.12	1.20	2.12	1.12	1.24	1.79	1.55	1.60	1.98	2.17	1.98	2.03	1.93
	Mean		4.21	2.17	1.62	1.58	0.91	1.08	1.65	1.12	1.24	1.79	1.55	1.60	1.98	2.17	1.98	2.03
SB310	1	4.21	2.22	0.89	0.92	1.25	0.93	1.70	1.00	1.06	1.12	1.31	1.78	1.78	1.31	1.95	2.27	2.07
	2	4.21	1.12	0.19	0.50	0.50	0.80	1.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	4.21	0.52	0.60	0.90	0.41	1.12	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean		4.21	1.29	0.56	0.77	0.72	0.95	1.03	1.00	1.06	1.12	1.31	1.78	1.78	1.31	1.95	2.27
SB85	1	4.21	2.44	2.84	1.96	2.25	2.01	2.25	3.28	3.46	3.19	3.20	3.20	3.47	3.23	3.20	3.12	3.12
	2	4.21	3.21	2.14	2.18	1.43	2.16	2.71	2.68	2.64	2.21	2.99	3.01	3.14	2.93	2.81	2.55	2.55
	3	4.21	3.31	2.34	1.87	2.25	1.86	2.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean		4.21	2.99	2.44	2.00	1.98	2.01	2.38	2.98	3.05	2.70	3.10	3.11	3.31	3.08	3.01	2.84
SB810	1	4.21	0.48	0.51	0.34	0.16	0.38	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	4.21	0.72	0.08	0.06	0.80	0.08	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	3	4.21	2.41	0.19	0.14	0.50	1.40	0.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Mean		4.21	1.20	0.26	0.18	0.49	0.62	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SB0	1	4.21	3.11	3.31	3.36	3.06	3.02	3.22	3.27	3.20	2.61	3.47	3.08	4.01	3.21	3.39	3.05	3.05
	2	4.21	3.26	3.15	3.15	3.20	3.11	2.98	3.07	3.20	2.61	3.56	3.08	4.01	3.21	3.39	3.05	3.05
	3	4.21	4.02	3.12	3.07	3.22	3.08	2.71	3.21	3.41	3.64	2.93	3.24	2.67	2.14	2.34	1.97	1.87
	Mean		4.21	3.46	3.19	3.19	3.16	3.07	2.97	3.18	3.27	2.95	3.32	3.13	3.56	2.85	3.04	2.69

Note: - R = Replication

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²
 - SB85: 80 g seaweed per attachment and 50 ind.m⁻²
 - SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²
 - SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-4

Results of pH measurement in dawn and noon in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Treatment	Day																
	1	6	9	12	16	19	22	25	28	31	34	38	41	45	48	51	56
Dawn																	
SB35	8.12	7.68	7.44	7.62	7.39	7.31	7.39	7.30	7.65	7.64	7.63	7.60	7.64	7.72	7.69	7.68	7.39
SB310	8.12	7.58	7.61	7.48	7.44	7.46	7.56	7.64	7.52	7.51	7.57	7.55	7.63	7.81	7.88	7.78	7.86
SB85	8.13	7.61	7.58	7.55	7.46	7.50	7.60	7.79	7.80	7.64	7.98	7.95	7.68	8.04	8.05	7.81	8.00
SB810	8.13	7.62	7.26	7.36	7.16	7.27	7.30										
SB0	8.11	7.76	7.85	7.92	7.75	7.66	7.72	7.82	7.72	7.69	8.00	8.08	7.94	7.95	7.98	7.87	8.14
Noon																	
SB35	7.82	7.64	7.43	7.48	7.35	7.36	7.36	7.60	7.46	7.52	7.62	7.86	7.78	7.82	7.79	7.76	7.79
SB310	7.82	7.46	7.41	7.44	7.40	7.47	7.58	7.79	7.74	7.64	7.68	7.74	7.86	7.92	7.88	7.89	8.08
SB85	7.82	7.65	7.52	7.51	7.37	7.61	7.75	7.95	7.90	7.80	8.11	8.07	7.99	8.06	8.04	8.00	7.93
SB810	7.82	7.37	7.32	7.38	7.20	7.32	7.36										
SB0	7.82	8.07	7.67	7.79	7.56	7.62	7.69	8.12	7.90	7.93	8.25	7.91	7.94	8.09	8.12	8.11	8.06

Note: - R = Replication

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²
- SB85: 80 g seaweed per attachment and 50 ind.m⁻²
- SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²
- SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-5

Results of salinity (‰) measurement in dawn and noon in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Treatment	Day																
	1	6	9	12	16	19	22	25	28	31	34	38	41	45	48	51	56
Dawn																	
SB35	30.00	28.33	31.33	30.33	29.00	28.00	28.33	30.00	30.00	31.00	30.00	32.00	32.00	32.00	33.00	32.00	33.00
SB310	30.00	28.67	31.33	31.33	29.00	28.00	30.67	31.00	32.00	30.00	31.00	31.00	31.00	30.00	31.00	30.00	31.00
SB85	30.00	28.00	32.33	31.67	29.00	28.33	31.33	31.50	33.50	30.50	33.50	33.50	33.00	33.50	34.00	33.50	34.00
SB810	30.00	27.67	30.67	30.67	28.00	28.33											
SB0	30.00	28.33	31.00	30.33	29.00	28.67	31.00	31.33	30.33	30.33	30.33	31.00	30.67	30.67	31.67	30.00	32.33
Noon																	
SB35	31.00	29.67	31.33	31.00	28.67	28.33	30.00	31.00	30.00	30.00	31.00	32.00	32.00	31.00	31.00	32.00	34.00
SB310	31.00	30.67	30.67	31.33	28.67	28.67	31.67	31.00	32.00	31.00	31.00	31.00	32.00	32.00	32.00	33.00	34.00
SB85	31.00	30.00	31.33	32.00	28.67	28.33	32.33	32.00	31.50	30.50	32.50	33.00	33.50	34.00	34.00	33.50	35.50
SB810	31.00	29.33	30.67	30.67	29.33	28.33											
SB0	31.00	29.00	30.67	30.67	29.00	29.00	31.67	32.00	31.33	31.00	31.33	31.67	31.67	31.67	32.33	30.67	31.33

Note: - R = Replication

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²
 - SB85: 80 g seaweed per attachment and 50 ind.m⁻²
 - SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²
 - SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-6

Results of temperature ($^{\circ}\text{C}$) measurement in dawn and noon in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Treatment	Day																
	1	6	9	12	16	19	22	25	28	31	34	38	41	45	48	51	56
Dawn																	
SB35	28.90	28.70	28.07	27.90	28.23	28.80	28.20	28.40	27.60	28.20	27.10	27.60	26.20	25.30	25.10	26.40	27.30
SB310	28.77	28.70	28.17	27.93	28.33	28.73	28.23	27.50	27.70	28.40	27.20	28.10	26.50	25.40	25.50	26.90	27.80
SB85	28.70	28.67	28.13	28.07	28.13	28.27	28.13	27.20	27.55	28.80	26.85	27.65	26.25	25.70	25.25	26.55	27.75
SB810	28.70	28.60	28.43	28.10	27.93	28.27											
SB0	28.83	28.73	27.91	27.90	27.77	28.30	28.47	27.47	27.73	28.30	27.10	27.90	26.20	25.63	25.37	26.70	27.57
Noon																	
SB35	28.50	28.60	28.23	28.20	29.27	28.47	28.57	28.70	28.00	28.60	27.40	28.60	28.60	31.00	25.10	27.00	27.50
SB310	28.50	28.60	28.40	28.27	30.17	28.67	28.40	27.80	28.40	27.70	27.50	30.30	29.50	28.00	25.30	27.40	28.00
SB85	28.50	28.40	28.20	28.07	29.10	28.53	28.13	27.50	28.25	27.45	27.15	30.00	30.15	29.00	25.10	27.20	28.00
SB810	28.50	28.33	28.43	28.23	28.97	28.50	28.50										
SB0	28.50	28.60	28.27	28.27	29.80	28.47	28.23	27.77	29.83	28.73	27.40	29.60	29.70	29.57	25.40	27.10	27.93

Note: - R = Replication

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²

- SB85: 80 g seaweed per attachment and 50 ind.m⁻²

- SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²

- SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-7

Results of ammonium, ammonia, nitrite, and nitrate measurement in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Treatment	Day			
	1	20	40	57
Ammonium				
SB35	0.0332	0.5443	0.7943	0.8197
SB310	0.0332	0.8443	0.9443	0.9990
SB85	0.0332	0.5883	0.7217	0.8915
SB810	0.0332	0.7327		
SB0	0.0332	0.3883	0.4363	0.4208
Ammonia				
SB35	0.2687	0.2380	0.4678	0.4440
SB310	0.2687	0.2683	0.3581	0.3934
SB85	0.2687	0.2583	0.3164	0.3738
SB810	0.2687	0.4079		
SB0	0.2687	0.2089	0.2381	0.1920
Nitrite				
SB35	0.0500	0.0617	0.0867	0.0800
SB310	0.0500	0.0767	0.0767	0.0800
SB85	0.0500	0.0533	0.0667	0.0750
SB810	0.0500	0.0833		
SB0	0.0500	0.0500	0.0567	0.0583
Nitrate				
SB35	0.6676	0.1657	0.2362	0.2520
SB310	0.6676	0.3478	0.2125	0.3011
SB85	0.6676	0.2164	0.3333	0.4000
SB810	0.6676	0.2303		
SB0	0.6676	0.2419	0.2466	0.3467

Note:

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²
 - SB85: 80 g seaweed per attachment and 50 ind.m⁻²
 - SB0: shrimp monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²
 - SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-8

Results of water quality parameters measurement of Hydrogen sulphide, phosphate, total organic matter, total dissolved solid, and total suspended solid in polyculture black tiger shrimp and dual filtration organisms (seaweed and blood cockle)

Treatment	Day			
	1	20	40	57
H₂S				
SB35	0.0190	0.2667	0.3333	0.3416
SB310	0.0190	0.2958	0.4292	0.4713
SB85	0.0190	0.1806	0.2579	0.3004
SB810	0.0190	0.4903		
SB0	0.0190	0.2233	0.2567	0.2875
PO₄				
SB35	0.0282	0.8048	1.2651	1.2144
SB310	0.0282	0.2980	0.8175	0.8460
SB85	0.0282	0.8687	1.1951	1.0425
SB810	0.0282	0.4854		
SB0	0.0282	0.2157	0.4489	0.5175
TOM				
SB35	35.08	38.34	38.93	49.82
SB310	35.08	42.13	47.87	63.83
SB85	35.08	38.34	39.40	47.60
SB810	35.08	50.32		
SB0	35.08	36.46	38.48	40.93
TDS				
SB35	30,390.00	19,800.67	24,893.33	35,622.00
SB310	30,946.00	21,671.00	29,561.67	48,730.00
SB85	30,768.67	32,059.67	38,739.33	41,311.50
SB810	30,733.33	30,531.33		
SB0	31,112.00	32,976.33	33,060.33	41,575.00
TSS				
SB35	17.33	15.53	18.33	25.75
SB310	17.33	13.27	14.93	17.50
SB85	17.33	19.13	20.17	21.63
SB810	17.33	17.67		
SB0	17.33	14.67	17.30	21.25

Note:

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²
 - SB85: 80 g seaweed per attachment and 50 ind.m⁻²
 - SB0: shrimp monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²
 - SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-9

Weight at stocking-harvesting time and absolute and daily growth rate of black tiger shrimp

Treatment	Rep.	Average Weight (g)		Growth	
		Stocking	Harvesting	ABS (g)	ADG (g.d ⁻¹)
SB35	1	5.93	0.00	0.00	0.00
	2	6.39	0.00	0.00	0.00
	3	4.80	5.16	1.84	0.03
	Mean	5.70	5.16	1.84	0.03
SB310	1	5.19	10.18	3.20	0.06
	2	4.52	0.00	0.00	0.00
	3	6.02	0.00	0.00	0.00
	Mean	5.24	10.18	3.20	0.06
SB85	1	5.27	7.40	2.13	0.04
	2	5.42	8.85	3.43	0.06
	3	4.80	0.00	0.00	0.00
	Mean	5.16	8.12	2.78	0.05
SB810	1	6.48	0.00	0.00	0.00
	2	6.73	0.00	0.00	0.00
	3	4.93	0.00	0.00	0.00
	Mean	6.04	0.00	0.00	0.00
SB0	1	5.17	8.54	3.37	0.06
	2	6.59	8.15	1.56	0.03
	3	5.53	7.77	2.25	0.04
	Mean	5.76	8.15	2.39	0.04

Note: - R = Replication

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²

- SB85: 80 g seaweed per attachment and 50 ind.m⁻²

- SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²

- SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-10

Weight at stocking-harvesting time and absolute growth rate of seaweed

Treatment	Rep.	Average Weight (g)		ABS Growth (g)
		Stocking	Harvesting	
SB35	1	240	220.52	-19.48
	2	240	293.29	53.29
	3	240	278.96	38.96
	Mean	240	264.26	24.26
SB310	1	240	280.53	40.53
	2	240	209.69	-30.31
	3	240	60.85	-179.15
	Mean	240	183.69	-56.31
SB85	1	640	701.30	61.30
	2	640	714.56	74.56
	3	640	729.14	89.14
	Mean	640	715.00	75.00
SB810	1	640	384.25	-255.75
	2	640	349.80	-290.20
	3	640	505.35	-134.65
	Mean	640	413.13	-226.87

Note: - Rep = Replication

- ABS: Absolute

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²

- SB85: 80 g seaweed per attachment and 50 ind.m⁻²

- SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-11

Weight at stocking-harvesting time and absolute growth rate of blood cockle

Treatment	R	Stocking			Harvesting			ABS Growth (g)
		Ind.	Weight (g)		Ind.	Weight (g)		
			Total	Average		Total	Average	
SB35	1	50	301.05	6.02	19	157.00	8.26	2.24
	2	50	294.96	5.90	31	181.14	5.84	-0.06
	3	50	337.18	6.74	31	201.25	6.49	-0.25
	Mean	50	311.06	6.22	27	179.80	6.87	0.64
SB310	1	100	632.99	6.33	60	470.71	7.85	1.52
	2	100	659.20	6.59	0	0.00	0.00	0.00
	3	100	600.77	6.01	0	0.00	0.00	0.00
	Mean	100	630.99	6.31	60	470.71	7.85	0.51
SB85	1	50	268.49	5.37	37	230.79	6.24	0.87
	2	50	287.85	5.76	46	343.63	7.47	1.71
	3	50	305.07	6.10	37	240.53	6.50	0.40
	Mean	50	287.13	5.74	40	271.65	6.74	0.99
SB810	1	100	652.97	6.53	0.00	0.00	0.00	0.00
	2	100	678.83	6.79	0.00	0.00	0.00	0.00
	3	100	652.15	6.52	0.00	0.00	0.00	0.00
	Mean	100	661.32	6.61	0.00	0.00	0.00	0.00

Note: - R = Replication

- Ind.: Individuals

- ABS: Absolute

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²

- SB85: 80 g seaweed per attachment and 50 ind.m⁻²

- SB810: 80 g seaweed per attachment and 100 ind.m⁻²

Appendix 2-12

Total colony of *Vibrio* and total bacteria in polyculture black tiger shrimp with seaweed by day of observation during experiment

Treatment	<i>Vibrio</i> (CFU/ml)				Total Bacteria (CFU/ml)			
	D1	D20	D40	D57	D1	D20	D40	D57
SB35	7	1.2×10^3	5.64×10^3	6.67×10^3	2.17×10^4	5.97×10^5	1.77×10^6	2.07×10^6
SB310	7	1.8×10^3	6.5×10^3	6.15×10^3	2×10^4	6.6×10^5	1.47×10^6	1.6×10^6
SB85	7	4×10^2	1.64×10^3	1.33×10^3	2×10^4	4.33×10^5	6.77×10^5	1.25×10^6
SB810	7	3.07×10^3	7.47×10^3	-	1.67×10^4	1.85×10^6	2.07×10^6	-
SB0	7	1.3×10^2	9.13×10^2	1.12×10^3	1×10^4	9×10^4	2.09×10^5	9.27×10^5

Note: - R = Replication

- SB35: 30 g seaweed per attachment and 50 ind.m⁻²
 - SB85: 80 g seaweed per attachment and 50 ind.m⁻²
 - SB0: monoculture system

- SB310: 30 g seaweed per attachment and 100 ind.m⁻²
 - SB810: 80 g seaweed per attachment and 100 ind.m⁻²

CHAPTER 3

Study on effectiveness of seaweed–*Gracilaria verrucosa* as filtration organism in commercial scale of black tiger shrimp (*Penaeus monodon* Fab.) polyculture system

ABSTRAK

Tujuan dari penelitian ini adalah menentukan peran ekologi dan kemampuan rumput laut (*Gracilaria verrucosa*) menstabilkan lingkungan budidaya udang windu (*Penaeus monodon*) sistem polikultur skala komersial. Penelitian dilakukan dari bulan Agustus 2009 sampai Januari 2010 di Desa Sungai Buntu, Kecamatan Cilebar, Karawang, Jawa Barat. Penelitian menggunakan 6 petak tambak dengan 3 petak luasnya 1.881 m² dan 3 petak lagi 2.624 m² dengan tinggi pematang sekitar 1,2 meter. Perlakuan penelitian adalah polikultur udang dengan filter biologi rumput laut (*G. verrucosa*) dengan metode tanam lepas dasar dan jumlah rumput laut per ikatan 80 g digantung 40 cm di bawah permukaan air dan dengan jarak antar tali 1,5 meter. Sebagai kontrol adalah monokultur udang. Jumlah ulangan adalah tiga untuk masing-masing perlakuan. Padat tebar udang adalah 4 PL₁₂.m⁻². Polikultur udang dengan rumput laut yang ditanam secara lepas dasar mampu menstabilkan lingkungan pembudidayaan. Kemampuan mempertahankan beberapa parameter kualitas air dan tanah adalah berbeda nyata antara polikultur dan monokultur (P<0.05). Pertumbuhan udang, produktivitas, dan tingkat kelulusan hidup udang antara sistem polikultur dan monokultur adalah berbeda nyata (P<0.05). Tambak polikultur memiliki jumlah koloni mikro-organisme yang sesuai dengan persyaratan dikaitkan nilai pangkatnya, yaitu 10² untuk *Vibrio* dan 10⁵ untuk total bakteri. Rumput laut (*G. verrucosa*) mampu menyerap karbon, nitrogen, dan fosfor dari air media budidaya. Jumlah dan jenis plankton, serta jumlah haemosit udang dari sistem polikultur juga berbeda nyata dari sistem monokultur (P<0.05).

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 1,881 m² and the other three ponds were 2,624 m². 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 PL₁₂ 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 10² and total bacteria 10⁵. 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 declared 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 annually 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, employing 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 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 nowadays 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.

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 m² which consist of 16 rearing ponds and the rest as sedimentation and reservoir pond. There were 6 experimental pond units consisted of three ponds with area of 1,881 m² and the other three ponds were 2,624 m². The embankments height of all ponds was 1.2 m (**Table 3-1**). The experiment were started by draining out pond completely 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 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 equipped by a paddle wheel as emergency aeration to anticipate dissolved oxygen deficiency.

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. PL₁₂ 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 transparency, total alkalinity, total ammonia (NH₃), nitrite (NO₂), nitrate (NO₃), H₂S, phosphate (PO₄), 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

transparency were Atago refractosalinometer and Secchii disk with the diameter of 25 cm, respectively. The rest of the parameters were observed by using Spectrophotometer 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, potential and availability of phosphorus, 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- μ m mesh plankton net. Qualitative & quantitative investigation of plankton were based on Davis (1955); Sournia (1978); Sahlan (1982).

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

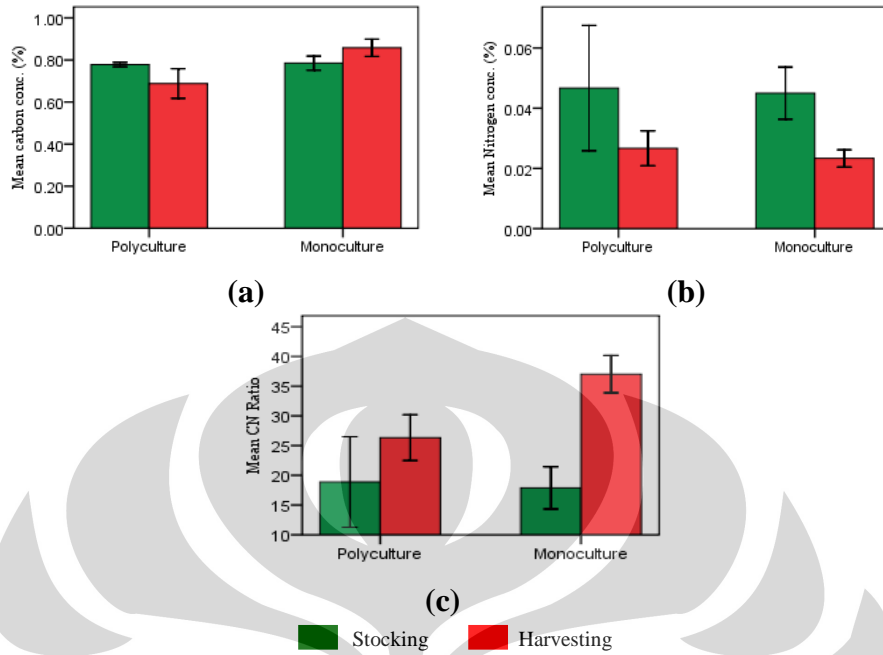


Figure 3-1

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

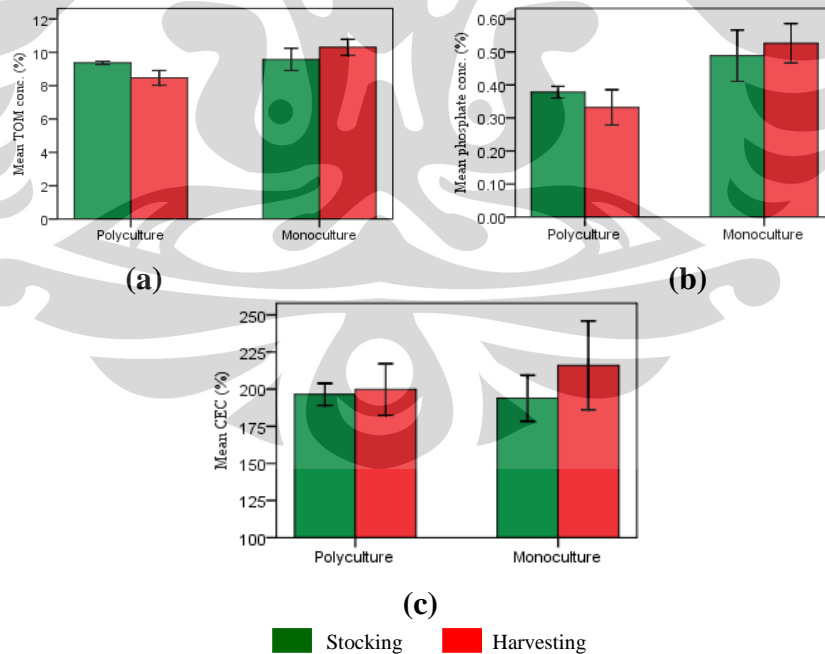


Figure 3-2

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 (NH₃), nitrite (NO₂), nitrate (NO₃), total organic matter (TOM), hydrogen sulphide (H₂S), phosphate (PO₄), total alkalinity, water transparency, total dissolved 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^oC and 29.17 – 30.77^oC in the early morning and 30.58 - 34.30^oC and 30.52 – 34.23^oC 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^oC in early morning and less than 4^oC 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 transparency seems to increase at first two months of experimental period and then decrease after ward. Decreasing trend of transparency 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**).

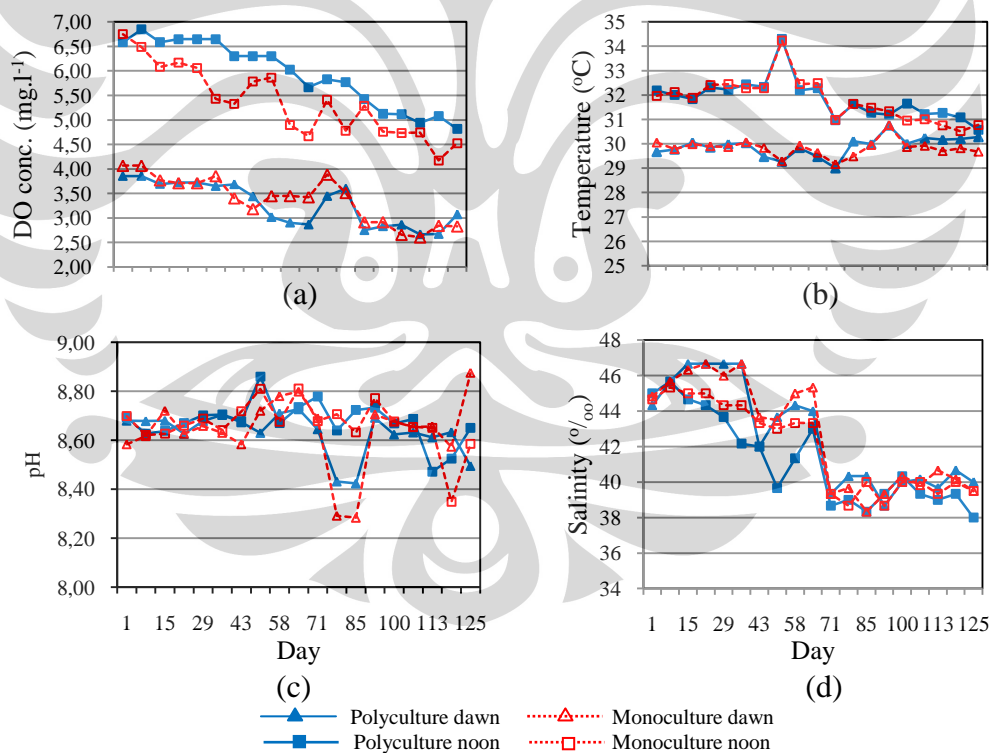


Figure 3-3

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	CG	CG	CG	CG	CG	CG
	2	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	CG	G	G	G	YG	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

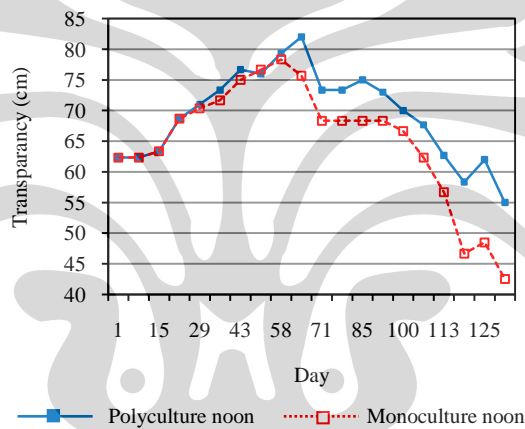
Notes:

R = Replicate

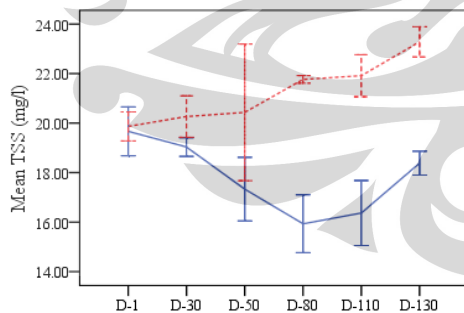
G = Green

CG = Clear green

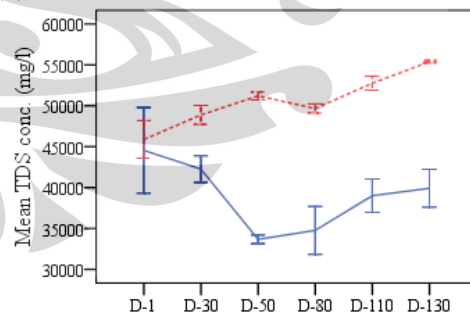
YG = Yellowish green



(a)



(b)



(c)

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 30th. 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 $0.3412 \pm 0.057 \text{ mg.l}^{-1}$ was significant lower than monoculture system ($0.0854 \pm 0.009 \text{ mg.l}^{-1}$) **Figure 3-5d** and **Appendix 3-8**).

During experiment, concentration of total organic matter, phosphate, and hydrogen sulphide except total alkalinity 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 $35.4833 \pm 2.1597 \text{ mg.l}^{-1}$ and $46.9067 \pm 1.1912 \text{ mg.l}^{-1}$, 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 sulphide than monoculture system at third measurement (**Figure 3-6d**).

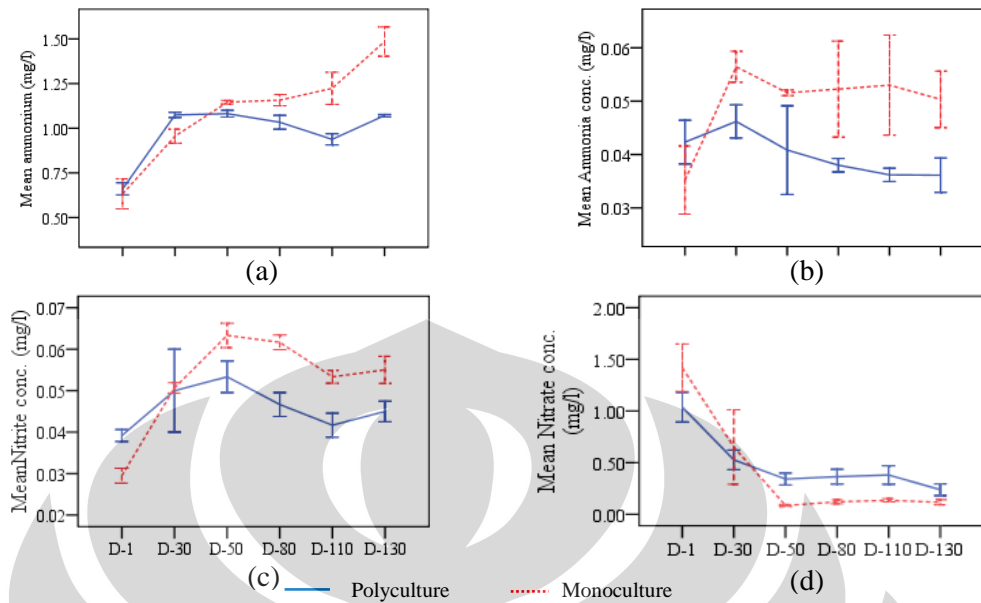


Figure 3-5

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

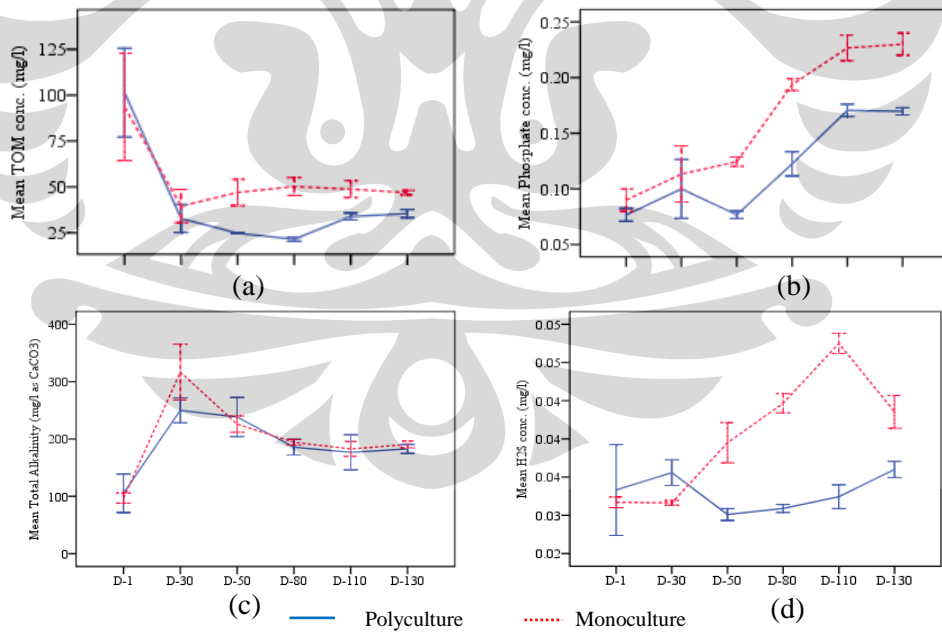


Figure 3-6

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 (\pm 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 showed 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 weight of each attachment was reduced until of about 80 – 100 g. First sampling recorded that mean weight \pm SD of seaweed bonds was 506.50 ± 20.27 g. Related to the first cultivation weight, it had weight gain of about 426.50 ± 20.27 g or $533.13\pm 25.34\%$ 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 \pm 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 \pm 0.4504a	5.3217 \pm 0.2318b
2.	Survival rate (%)	85.41 \pm 1.27a	68.84 \pm 1.47b
3.	Productivity (kg.m ⁻²)	0.0758 \pm 0.0037a	0.0646 \pm 0.004b
4.	THC – day 70	920.67 \pm 54.78a	885.3333 \pm 93.09a
5.	THC – day 135	1100.83 \pm 36.26a	985.83333 \pm 31.26b
6.	Carbon (%)	24.32 \pm 1.2314a	24.47 \pm 0.5498a
7.	Nitrogen (%)	10.13 \pm 0.3937a	10.36 \pm 0.2066a
8.	Phosphat (%)	4.30 \pm 0.2650a	4.59 \pm 0.4580a
B.	Seaweed	(Stocking)	(Harvesting)
	Carbon (%)	17.60 \pm 1.45a	24.18 \pm 1.77b
	Nitrogen (%)	1.52 \pm 0.02a	1.95 \pm 0.11b
	Phosphat (%)	3.51 \pm 0.87a	7.90 \pm 0.22b
D.	Plankton		
	Density (D120) (cell/l)	1173 \pm 33a	1965 \pm 55b
	Dominancy index (D120)	0.1279 \pm 0.0047a	0.1540 \pm 0.0026b
E.	Microbial (CFU/ml)		
	Total <i>Vibrio</i> (D120)	165 \pm 33a	1254 \pm 551b
	Total bacterial (D120)	1.4 x 10 ⁵ \pm 26,457a	1.01 x 10 ⁶ \pm 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 $24.18 \pm 1.77\%$, $1.95 \pm 0.11\%$, and $7.99 \pm 0.22\%$, respectively, were significant different from stocking time (**Figure 3-9a**). Seaweed tissue gained significant higher carbon than nitrogen and phosphorus (**Figure 3-9b**).

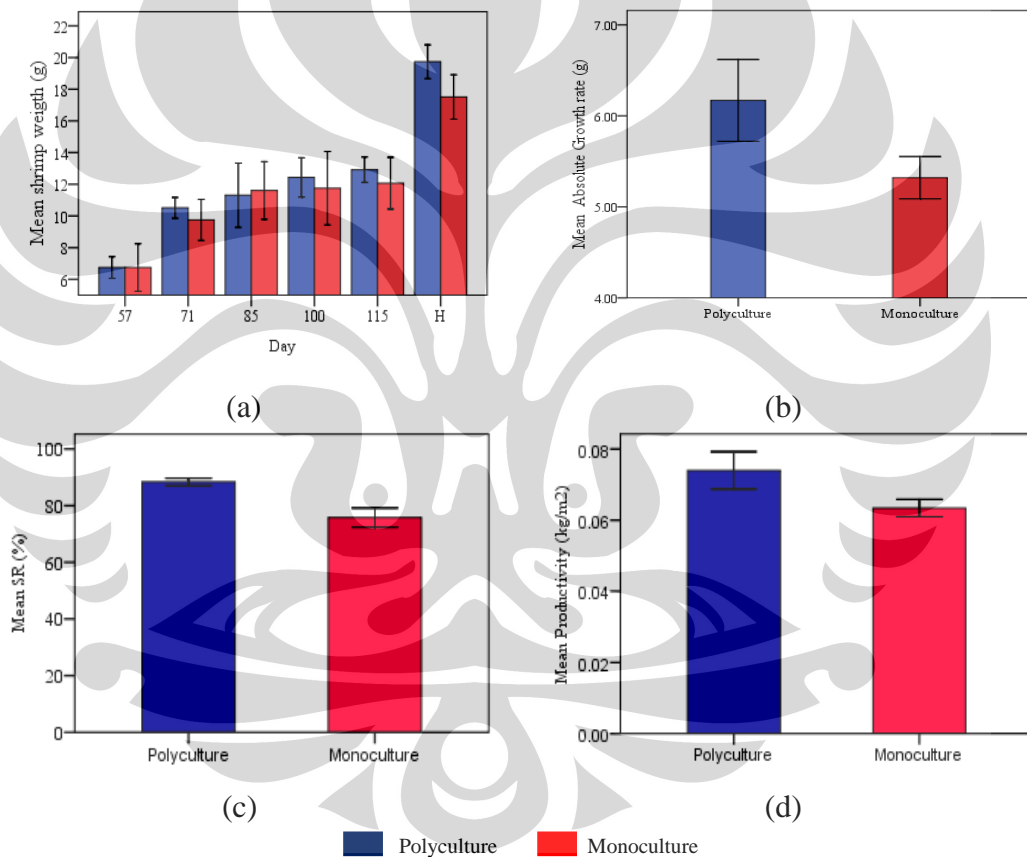


Figure 3-7

Mean value \pm SD of black tiger shrimp weight (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

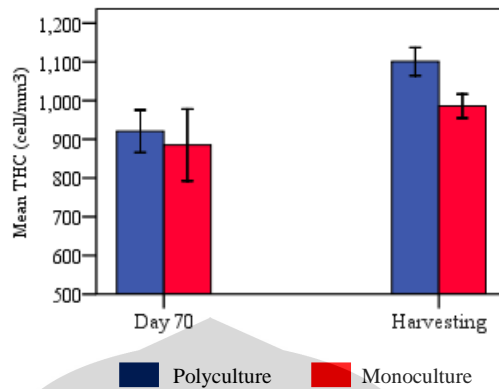


Figure 3-8

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

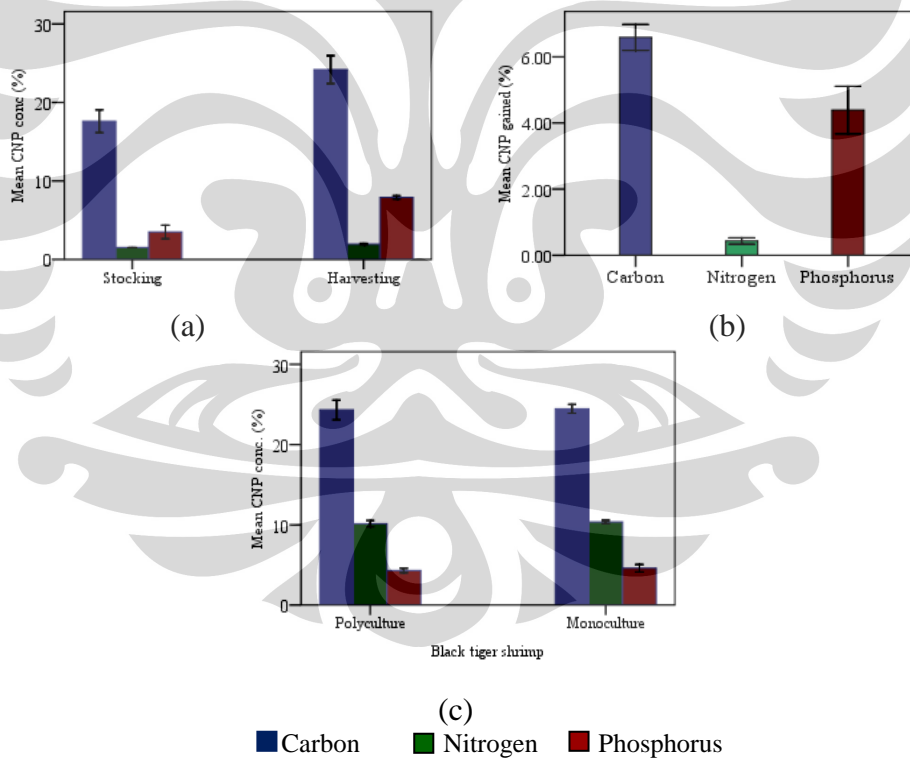


Figure 3-9

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 dominance index. The graph of plankton density has peak at the middle 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 polyculture 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 dominance indexes were not significant different between polyculture and monoculture system. The rests revealed significant different (**Figure 3-10b**). However, dominance index of all sampling results of both culture system were found less than 0.5 of which indicated that there were no dominance of certain plankton species.

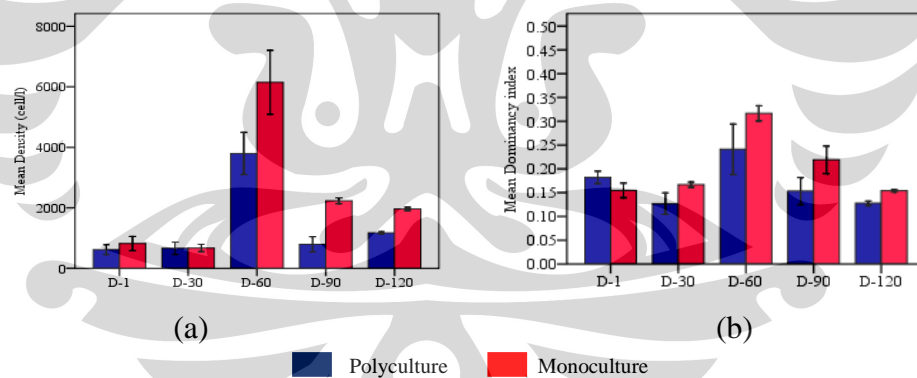


Figure 3-10

Mean value \pm SD of plankton density (a) and dominance 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 ($570,000\pm 105,000$ CFU.ml⁻¹) at

first observation and tend to decrease afterward (**Figure 3-11b**). The highest total bacterial of about $3,510,000 \pm 684,324$ 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 10² or 10³ and 10⁵ or 10⁶, respectively (**Appendix 3-13**).

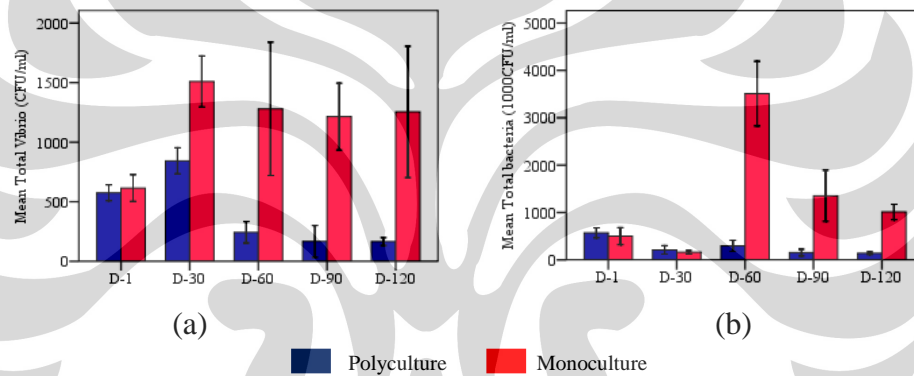


Figure 3-11

Mean value \pm 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 productivity. 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 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, transparency, 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 occurrence 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 generally 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‰.

Secchi disk visibility expressed transparency of culture water. Shrimp polyculture system started showing better transparency 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 transparency, 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 comparable 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 to be 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.

ACKNOWLEDGEMENTS

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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 shrimp 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 ($^{\circ}\text{C}$) 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 (‰) 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 transparency (Secchi 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 (PO₄), total alkalinity, and hydrogen sulphide (H₂S) 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 ₂ S	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 \pm SD of of seaweed weigh 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 \pm 99.68	483.88 \pm 95.69	523.00 \pm 104.80	506.50 \pm 20.27
Weight gain (g)	432.63	403.88	443.00	426.50 \pm 20.27
Weight gain (%)	540.78	504.84	553.75	533.13 \pm 25.34
ADG (g.d ⁻¹)	5.77	5.39	5.91	5.69 \pm 0.27
2nd Sampling (Day 100)				
Sample weight (g)	66.13 \pm 17.38	58.00 \pm 19.99	58.50 \pm 19.42	60.88 \pm 4.55
Weight gain (g)	-13.88	-22.00	-21.50	-19.1250 \pm 4.55
Weight gain (%)	-17.34	-27.50	-26.88	-23.9063 \pm 5.70
ADG (g.d ⁻¹)	-0.14	-0.22	0.22	-0.19 \pm 0.05

Appendix 3-13

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

Culture Technology	Day					
	1	30	60	90	120	
Total <i>Vibrio</i> (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	3.13×10^6	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

GENERAL DISCUSSION

Aquaculture practices tend to manipulate aquaculture ecosystems in order to achieve higher production than in nature by employing culture technology and management (FAO 1991). Ecologically, this practice tends to change the equilibrium of the natural ecosystem and may even generate serious ecological pressure. Many literatures revealed, increasing production through intensified aquaculture systems has been affecting the carrying capacity of the environment on which the farming activity takes place (Flaherty & Karnjanakorn 1995; Jenkins 1995; Szuster 2003), and therefore threatens further developments. More intensive production for shrimp culture requires more inputs and is more likely to cause environmental degradation either in the pond itself or in the surrounding environment as receiving water (Donovan 2001).

The shrimp's habit of slowly nibbling feed particles causes substantial nutrient losses even if the pellets are of good quality (Antony and Philip 2006). Therefore, the negative impact of aquaculture derives mainly from particulate and dissolved nutrients from animal excretion and uneaten food. In order to reduce or even eliminate those negative environmental impacts, sustainable and environmentally friendly aquaculture technology must be implemented not just in the individual level of culture unit but in much wider region as a group of ecological unity.

One of appropriate aquaculture technologies is polyculture system (Pillay 2004) of different trophic level of organisms. Its advantages are more efficient resource utilization and built resilient system against environmental fluctuations (Troell *et al.* 1999a). Polyculture system can more readily cope with self-pollution by reducing the concentration of organic and inorganic substances, and economically earn additional income resulted from secondary or tertiary cultured organisms, e.g. seaweed and bivalve (Jonnes 1999). Seaweed as a photo-autotrophic organism and mussel as filter feeder have ability to reduce dissolved

nutrient (Troell 1999) and particulate matter (Shpigel 1993) loading resulted by shrimp rearing activities.

In this dissertation, the results of the first stage experiment are presented in the first chapter that figured out the ability of *G. verrucosa* and *A. granosa* as single filtration organism in black tiger shrimp (*P. monodon*) polyculture system. The best performance of each single filtration organism of the first stage experiment was employed as dual-filtration organisms in black tiger shrimp (*P. monodon*) polyculture system as the second stage experiment. This dissertation was also to discover the optimum role of filtration organism in maintaining suitable habitat of *P. monodon*. Those studies were carried out in laboratory scale. Results of previous experiment stages were adopted for the commercial or field scale experiment as third stage experiment or last research stage. The field scale study was intended to explain synergism between main cultured species *P. monodon* and additional cultured species function as filtration organism.

Single- versus dual-filtration organism

G. verrucosa as single filtration organism in black tiger shrimp (*P. monodon*) polyculture system has two function possibilities of culture methods namely bottom and off bottom method. The first method, seaweed was sawn evenly on the experimental unit bottom. The last one consists of the surface water and sub-merge method. Each method has ecological consequences in relation to photosynthesis process, light penetration, water transparency, particulate and dissolved substances. In this experiment, *A. granosa* as single filtration organism in black tiger shrimp (*P. monodon*) polyculture system was cultured in different level of stocking density consisted of 50, 100, and 150 inds.m⁻². Stocking density of *A. granosa* influences filtration capacity in order to make up water quality of the culture habitat.

Polyculture system *P. monodon* and *G. verrucosa* as single filtration organism showed interesting figure in terms of dissolved oxygen concentration. The off bottom culture methods of using *G. verrucosa* sub merge of about 40 cm

from the surface water revealed better dissolved oxygen concentration during the experimental period. Oxygen is a limiting factor for cultured organism performance and has closed relationship with all ecological process in the culture system. Therefore, positive ecological impacts of using filtration organism *G. verrucosa* in shrimp (*P. monodon*) polyculture system are not just stabilizing dissolved oxygen concentration but also maintaining suitable concentration of organic and inorganic nutrients i.e. total organic matter, ammonia, and nitrite. The weight and the position of seaweed in the water column of culture unit influence their ability in playing ecological role. The off bottom method submerged 40 cm from the surface water with the seaweed weight of about 80 gram per attachment (total weight 640 g) showed better performance in maintaining culture water habitat than that of bottom method and hung on surface water. It is probably related to the amount of and solubility rate of the oxygen released by photosynthesis process. Through photosynthesis, the lowest concentration of dissolved oxygen was produced by bottom cultured seaweeds, followed by 40cm sub-merged seaweeds, however seaweeds cultured on the water surface produced the highest concentration of dissolved oxygen. Seaweeds cultured on the surface acquired higher light intensity as inhabiting light and lower oxygen melting level due to an increase in surface water temperature. Nevertheless, the highest oxygen melting level, dissolved oxygen concentration, and oxygen distribution to the whole water column was generated by seaweeds planted 40 cm under the water surface. The availability of dissolved oxygen would play essential role in organic matters decomposition in the bottom area, which then provide nutrients for photo-autotrophic organisms. This has been scientifically proved in the laboratory-scale of the first stage research.

Polyculture system of *P. monodon* with *A. granosa* as single filtration organism also showed high ability to assimilate phosphate and carbon from the system. The lowest concentration of total organic matter and nitrite were recorded at the polyculture with the stocking density of about 50 and 100 individuals of blood cockle per square meter. In term of dissolved oxygen parameter, all culture system using blood cockle (*A. granosa*) as filtration organism demonstrated lower

concentration (less than 0.2 mg.l^{-1}) than shrimp monoculture of more than 2 mg.l^{-1} (without *A. granosa*).

G. verrucosa and *A. granosa* have different position in the trophic level and have different ecological roles. Based on the previous paragraph, those filtration organisms showed ability in maintaining or stabilizing water quality of culture habitat of polyculture system *P. monodon*. Seaweed (*G. verrucosa*) as photo-autotrophic organism and blood cockle (*A. granosa*) as filter feeding organism showed comparable ability to some previous studies as reported by Baliao & Tookwinas (2002), Chow *et al.* (2001), Jones *et al.* (2001), Shimoda *et al.* (2005), Shimoda *et al.* (2006), and Neori *et al.* (2004). However, those previous studies were performed in integrated aquaculture, while this research was exploring polyculture system of shrimp with filtration organism (seaweed and blood cockle). Thus, culturing second organism in polyculture is intended not only to increase production but also to enhance ecological roles.

Unfortunately, unexpected results were discovered for the dual-filtration organisms (*G. verrucosa* and *A. granosa*) in the shrimp polyculture system. Most of treatments were not able to maintain suitable water quality parameters and revealed high mortality. Seaweed (*G. verrucosa*) and blood cockle (*A. granosa*) as dual-filtration organism in the polyculture system of black tiger shrimp were not able to maintain a suitable culture habitat, and lead to low growth, low survival rate or even mass mortality, and reduced shrimp productivity. Most of main water quality parameters such as ammonium, ammonia, nitrite, hydrogen sulphide, phosphate and total organic matter in the shrimp polyculture system with dual filtration organisms were relatively higher than those in shrimp monoculture system. Dissolved oxygen concentration tended to decrease, lower than the minimum requirement of *P. monodon* of about 3 mg.l^{-1} (Alabaster and Loyd 1980; Boyd 1990; Direktorat Jenderal Perikanan Budidaya 2003). Other water quality parameters include all nitrogen parameters, total organic matter and biological aspects were found similar results. Consequently, shrimp growth and survival rate were very low and even mass mortality occurred for all replication of shrimp

polyculture with 80 g.per attachment of *G. verrucosa* culture on surface water stocking density of *A. granosa* of 150 ind.m⁻¹. Presumably, the system was not able to support total cultured organism. It indicated that combination of both filtration organisms in shrimp polyculture system was not properly working.

Commercial scale of shrimp polyculture system with seaweed

Black tiger shrimp polyculture system with *G. verrucosa* as single-filtration organism cultivated by off bottom method with 80 g.bound⁻¹ submerged 40 cm from water surface and hung interval on 25 cm was able to maintain a suitable culture habitat, producing high growth, survival rate, and shrimp productivity. Suitable culture water quality during the culture period resulted in better performance of cultured and filtration organism, and other biological aspects such as plankton and bacterial population. Dissolved oxygen concentration at noon observation in the shrimp polyculture system revealed higher concentration than in the monoculture system. It was due to the occurrence of *G. verrucosa* that performs photosynthesis during day time and supplied most dissolved oxygen in the culture water habitat (Wetzel 1983; Boyd 1990; Midlen & Redding 2000; Pillay 2004).

Oxygen availability in aquaculture habitat will support the effectiveness of the ecological processes in aquaculture system, namely production, consumption, and decomposition processes. Production process of photo-autotrophic organisms will be best supported by the availability of the required organic matters. Consumption process of heterotrophic organisms is highly influenced by the supply of natural food and water quality required by the shrimp to grow well. Higher consumption by shrimp will consequently boost the immuno-competence of the shrimps towards disease resistance. Higher availability of dissolved oxygen in aquaculture medium will also increase the oxidation process and organics matters mineralization accumulated in the pond bottom. Thus, this can avoid the increase in concentration of certain hazardous matters such as ammonia, nitrite, and hydrogen-sulphid, which will eventually create suitable habitat for the shrimp in the pond bottom.

Moreover, *G. verrucosa*, as suggested by Maftuch (2009), released a certain substance that could improve immune system for *P. monodon*. It indicates that *G. verrucosa* as single filtration organisms in shrimp polyculture system is properly working by supporting main cultured species *P. monodon*. As suggested by Anderson (1992), the system is much likely to be able to establish well reciprocal interaction amongst main components of culture system mainly environment (culture habitat), pathogen, and host or cultured species. Furthermore, the system will improve shrimp immune system, without using antibiotics or any kinds of chemical or bioactive compounds to control or prevent diseases outbreak, and enhance production. Thus, shrimp (*P. monodon*) polyculture system using seaweed (*G. verrucosa*) filtration organism is an environmentally friendly technology. Detail technical description of that technology is as follows:

- 1) Shrimp (*P. monodon*) stocking density is 4 PLs.m⁻².
- 2) Seaweed (*G. verrucosa*) cultivation method is off bottom or long-line method. Seaweed weight is 80 g.bound⁻¹ with the interval 25 cm between attachment and 1.5 m between ropes, submerged 40 cm from water surface.

This study investigated two of three variables of sustainable aquaculture system suggested by Boyd & Schmittou (1999). They were production technology and environmental aspect, excluding economical aspects. In general, seaweeds play important roles in maintaining proper culture habitat for shrimp by absorbing inorganic substances. Polyculture system between *P. monodon* and *G. verrucosa* fulfilled the requirement as sustainable technology (Clem 1999; Martinez-Cordero and Leung, 2004; Pillay 2004). In terms of production volume as described in Chapter 3, polyculture system revealed higher survival rate and production of shrimp of about 7.68% and 7.71%, respectively than that of monoculture system. Increasing production means increasing income. Additional income will also come from seaweed production. In this study, at day 75 of the experimental period, the seaweed average production was 2,435.51±430.09 kg.ha⁻¹. Besides that, applying environmentally friendly aquaculture will have possibility to have wide

international market access due to the strict regulation from importer countries related to the environmental consideration. However, additional information on socio-economic aspects and more deep research on production technology as well as environmental aspects need to be discovered. Several important topics for future research are related to the seaweed culture method and water depth, stocking density of shrimp and blood cockle and seaweed weight, seaweed weight and shrimp immune system.

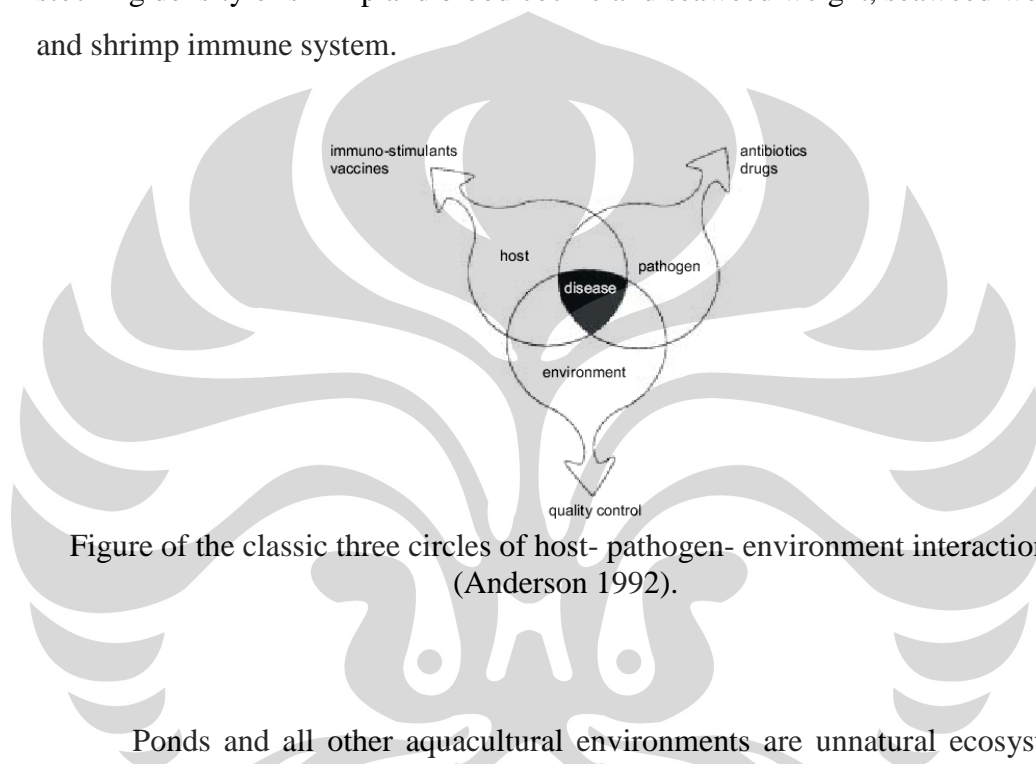
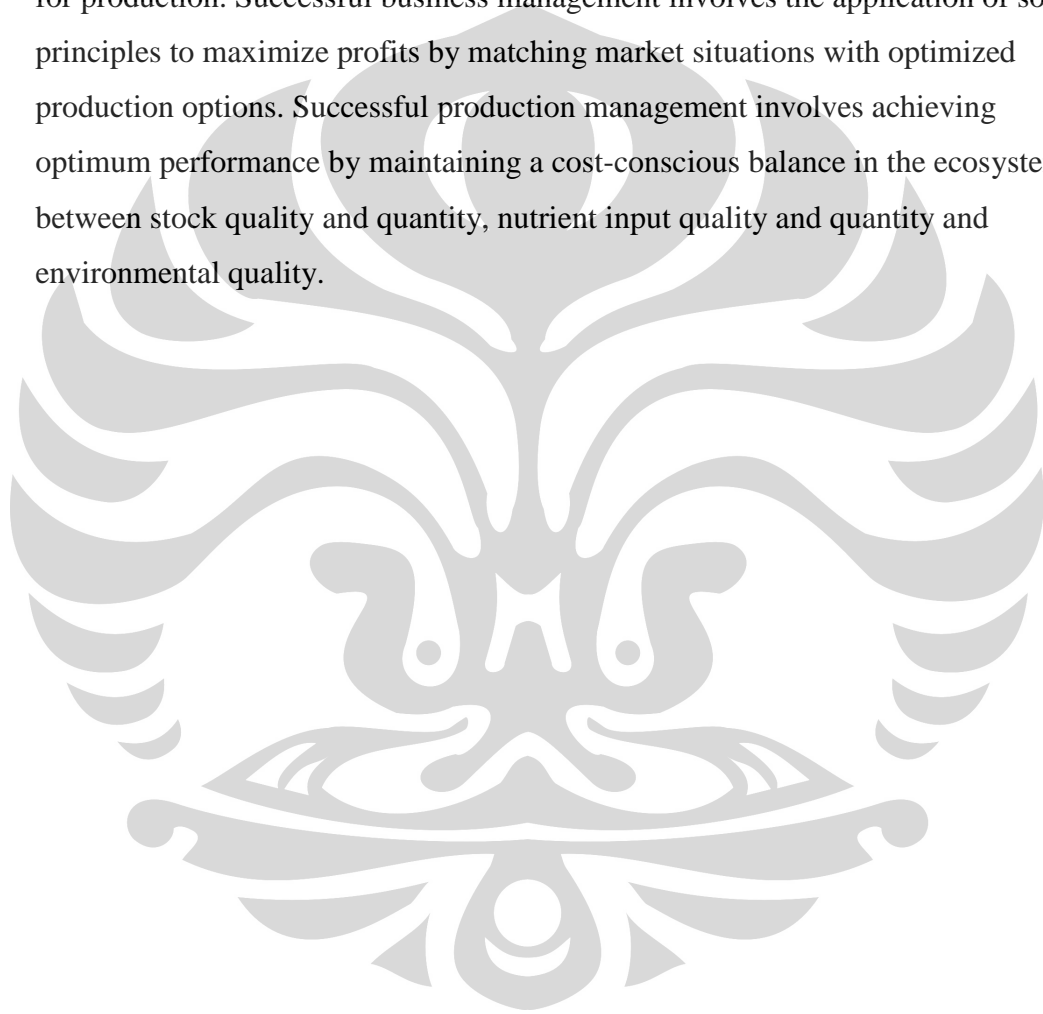


Figure of the classic three circles of host- pathogen- environment interactions (Anderson 1992).

Ponds and all other aquacultural environments are unnatural ecosystems and actually subunits within their surrounding natural ecosystems. The major components of pond ecosystems are the cultured organisms, the water environment, including all of its biological, chemical and physical characteristics and the nutrient (feed) inputs. Management of the ecosystem components, by applying ecological principles, allows the aquaculturist to maintain stability in the culture system to achieve an aquacultural yield. The greater the aquaculturist's understanding and application of ecological principles, the higher the effectiveness and efficiency and the lower the risks of producing the yield. Herein lies many problems. Lack of understanding and misconceptions of principles by all sectors, including farmers and scientists, are directly or indirectly responsible

for many, perhaps most, of the problems associated with destabilizing culture systems, especially those causing pollution within and outside the culture system.

Markets determine aquaculture opportunity, and ecological and economic principles determine choices of aquaculture practices for packaging sustainable aquaculture technologies. Markets are the driving force and the only justification for production. Successful business management involves the application of sound principles to maximize profits by matching market situations with optimized production options. Successful production management involves achieving optimum performance by maintaining a cost-conscious balance in the ecosystem between stock quality and quantity, nutrient input quality and quantity and environmental quality.



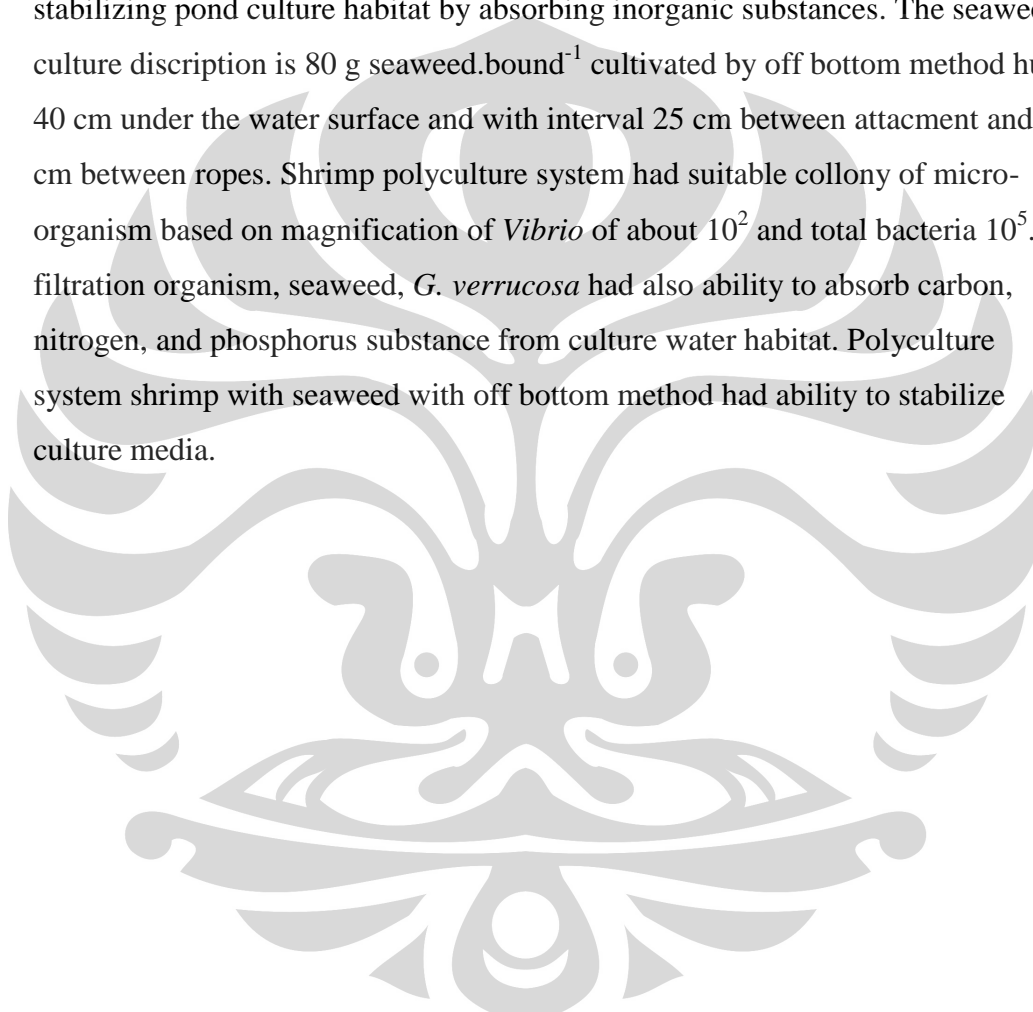
GENERAL CONCLUSION

Naturally, seaweed (*G. verrucosa*) as photo-autotrophic organism and blood cockle (*A. granosa*) as filter feeding organism have ability to absorb inorganic and organic matter in the culture water habitat and stabilize the culture ecosystem, respectively. Furthermore, based on the natural and biological characteristic, seaweed (*G. verrucosa*) has potency to be cultured as filtration organism in polyculture system with black tiger shrimp (*P. monodon*). Amongst culture technologies, shrimp polyculture system with 30 and 80 g seaweed per bound cultivated by off bottom method hung 40 cm under the water surface with the interval 25 cm between attachments has ability to improve water quality of culture habitat. Therefore, seaweed (*G. verrucosa*) as single filtration organism in shrimp polyculture system can play an important role in reducing aquaculture ecological impacts. In addition, that technology revealed better survival, productivity, and growth rate of shrimp than shrimp monoculture system. Moreover, blood cockle (*A. granosa*) as filter feeding organism in shrimp polyculture system revealed also ability in stabilizing water culture habit and improving shrimp production. The highest weight gain of shrimp was in the polyculture system with stocking density 100 inds. of blood cockle per square meter. Polyculture with stocking density 50 inds. of blood cockle revealed the highest growth of blood cockle followed by stocking density of 100 inds.m⁻². As single filtration organism *G. verrucosa* and *A. granosa* has better performance in maintaining water quality either related to the biological or physico-chemical aspects.

Based on definition suggested by Pillay (2004), the number of cultured organism in polyculture system can be more than two organisms (one main cultured organism and more than one additional cultured organism). However, contrasting results were found in the black tiger shrimp polyculture system using dual filtration organism *G. verrucosa* and *A. granosa*. After day 10 of observation, water quality was reduced and after day 20 the growth and survival rate of shrimp

were very low or even mass mortality was found. In general, polyculture system of black tiger shrimp (*P. monodon*) with dual-filtration organism of *G. verrucosa* and *A. granosa* was not able to stabilize culture habitat.

In the commercial or field scale shrimp polyculture system using seaweed (*G. verrucosa*) as single filtration organism revealed better performance in stabilizing pond culture habitat by absorbing inorganic substances. The seaweed culture description is 80 g seaweed.bound⁻¹ cultivated by off bottom method hung 40 cm under the water surface and with interval 25 cm between attachment and 150 cm between ropes. Shrimp polyculture system had suitable colony of micro-organism based on magnification of *Vibrio* of about 10² and total bacteria 10⁵. As filtration organism, seaweed, *G. verrucosa* had also ability to absorb carbon, nitrogen, and phosphorus substance from culture water habitat. Polyculture system shrimp with seaweed with off bottom method had ability to stabilize culture media.



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