School of Science
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Nutrient Cycle in an Integrated Recirculating Aquaculture System

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DECLARATION

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PREAMBLE

The purpose of this research is to assess the nutrient flow in an integrated closed recirculating aquaculture system (IRAS) and evaluate the nutrient uptake capacities of green seaweed (*Ulva lactuca*) and blue mussel (*Mytilus edulis*) when cultivated with western king prawn (*Penaeus latisulcatus*) effluents. The effectiveness of seaweed and mussel in nutrient removal was accomplished by evaluating the water quality, nutrient conversion rates and nutrient budgets, growth and survival of prawns when they were integrated with seaweed and mussel in IRAS.

This thesis consists of nine chapters. Chapter 1 is in the form of an introduction which briefly highlights the current issue of aquaculture industry, negative impacts of prawn intensive farming and the current published knowledge on the nutrient uptake capacities of mussels and seaweed; and status of integrated aquaculture system. This chapter also justifies and underlines the need to undertake the current research.

Chapter 2 reviews the research into prawn aquaculture industry; and biological and aquaculture aspects of western king prawn, blue mussel and green seaweed. The relevant information on the negative impacts of prawn farming, prawn effluent characteristic and integrated aquaculture models are described in this chapter. Nutrient uptake capacities of mussels and seaweeds are also main part of this chapter. Nutrient retention and benefit of integrated aquaculture system are mentioned.

Chapters 3 described the general materials and methodology applied in the project. Description and operation of the IRAS, collections of animals and plants, management of tanks during the trials, data collection and analysis are presented.

Chapter 4, 5, 6, 7 and 8 detail the main research of this thesis and attempt to evaluate the relationship between nutrient discharge and intensification levels, nutrient removal rate and nutrient retention of green seaweed and blue mussels when

integrated with western king prawns in IRAS by conducting series of laboratory based experiments. All these chapters form an essential component of this research and can be viewed as independent experiments bound by a common theme. Moreover, information about western king appears to be briefly repetitive, it was essential to maintain the flow of information for the chapters which were already published or submitted.

Chapter 4 includes an experiment that investigate the effects of different stocking densities of western king prawn on water quality, survival and growth performances of prawns, nutrient conversion rate and nutrient budget in the IRAS.

Chapter 5 presents an experiment with aiming to find out the optimum level of feeding rate for western king prawn culture in the IRAS. Growth, survival, water quality parameters and nutrient budget of prawns fed different feed rates are described and discussed in this chapter.

Chapter 6 details the experiment to investigate the ability of green seaweed inclusion into western king prawn culture in the IRAS. The optimum stocking density and feeding rate of western king prawn in Chapter 4 and 5 are applied. Water quality, nutrient removal rate of seaweed, nutrient utilization efficiency and nutrient budget when prawns integrated with seaweed are examined.

In Chapter 7, the inclusion of blue mussels into western king prawn culture in IRAS is investigated. The optimum stocking density and feeding rate of western king prawn in Chapter 4 and 5 are applied. Water quality parameters, growth and survival of prawn and mussel; and nutrient conversion rate and nutrient budget are investigated.

Chapter 8 presents an experiment to investigate the relationship between different blue mussel stocking densities and biomass of western king prawn on nutrient removal capacities of various components of IRAS. The optimum feeding rate results from Chapter 5 is used for this experiment. Water quality in culture media, prawn and mussel growth and survival rates, nutrient contents in animals and sediments are examined.

Chapter 9 summarizes the nutrient retention in different integrated culture models. The chapter also synthesises the main findings of this project. The data collected from this research are also compared to obtain a more complete picture in the application of integrated closed recirculating aquaculture system. Effects of feeding rate and stocking density of western king prawns reared in the monoculture are combined and presented. An assessment of the role of integrated culture is also discussed. The main conclusions are highlighted which are then followed by the recommendations for future research.

ABSTRACT

Intensive marine aquaculture may cause negative impacts on the aquatic environment by causing water pollution, algae bloom and reduction in biodiversity of the surrounding aquatic environment. The nutrient-rich effluents from aquaculture activities contain nitrogen and phosphate which serve as a nutrient source for bivalve, algae and invertebrates. Seaweed and mussels have been integrated with marine species in culture systems to remove the waste from aquaculture farms. The research has explored the nutrient uptake capacities of green seaweed (*Ulva lactuca*) and blue mussel (*Mytilus edulis*) integrated with western king prawn (*Penaeus latisulcatus*) in the integrated closed recirculating aquaculture systems (IRAS) and has evaluated the nutrient retention of seaweed and animals in the system.

One experimental unit of an IRAS consisted of three tanks: a mussel tank, a prawn tank and a waste-collection tank. The mussel tank and the prawn tank were set on the top and lower tiers of a metal frame; the waste-collection tank was on the floor. Water was pumped from the waste-collection tank to the reservoir tank by a submersible pump, circulated to the prawn tank and then returned to the waste collection tank through gravity.

A series of experiments were conducted under laboratory conditions to investigate the water quality; survival, growth of western king prawn and nutrient budget in the IRAS. The research results proved that the recirculating culture system could maintain acceptable water quality for western king prawn at stocking densities till 16 prawn m^{-2} and at feeding rate of 3.0% of wet weight biomass. Increasing the stocking densities results in decreasing the water quality in the culture media, the growth and survival of western king prawn reared in the recirculating aquaculture system. In contrast, increasing feeding rate did not improved growth and survival rate of western king prawn but feed utilization efficiency decreased significantly with increasing the feeding rates. Total phosphorus (TP) and orthophosphate (PO_4^{3-}) concentrations increased linearly with increasing the feeding rates of western king prawn. High percentage of nutrient inputs accumulated into tank bottom at higher

stocking densities whereas over 50% of nutrient inputs were in discharged water at harvest in lower stocking densities.

Inclusion green seaweed into western king prawn culture system improved the water quality in the IRAS. Concentrations of total ammonia nitrogen (TAN), nitrate (NO_3^-) and PO_4^{3-} in the integrated culture system were lower than those in the monoculture system. Green seaweed effectively removed 24.02-99.05% TAN and 13.80-96.40% PO_4^{3-} in the culture media. Feed utilization efficiency in integrated culture was significantly enhanced by 24.90 % nitrogen (N) and 19.41% phosphorus (P). Nutrient budget revealed that western king prawn and green seaweed retained 28.00-31.90% and 6.53-29.71% N of total nitrogen (TN) inputs at harvest, respectively while P retention was 13.46-14.63% and 1.62-13.50% of TP inputs, respectively. The rest of total nutrient input was in discharged water and tank sediments.

Integrated culture of blue mussel and western king prawn could improve the water quality in the IRAS though effectively removing of the total bacteria (TB), total suspended solids (TSS) and total nitrogen (TN) in the cultured media. However, concentrations of NO_2^-, NO_3^- and PO_4^{3-} were increased due to the mussel excretion. Growth and survival of western king prawn were not affected by adding blue mussels into western king prawn culture in IRAS at stocking rate of 250 mussel m^{-2} and 16.07 prawn m^{-2}. When stocking rates exceed density of 312.5 mussel m^{-2} and =21.36 prawn m^{-2}, growth and survival of both western king prawn and blue mussel in the IRAS were declined significantly due to the low water quality and crowded effects in the culture media. Inclusion of blue mussels into western king prawn culture was enhanced the feed utilization efficiency further up to 10.63 % N and 4.89 % P and reduced the nutrient discharged through draining at harvest. N and P contents accumulated in mussel tanks increased linearly with the increasing mussel stocking densities in the IRAS. The results indicate that the use of integrated aquaculture system is a step forward to achieve sustainability in aquaculture.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
AusAID	Australian Agency for International Development
BOD	Biological Oxygen Demand
C	Carbon
CARL	Curtin Aquatic Research Laboratories
CFU mL^{-1}	Colony forming unit per millilitre
d	Day
DIN	Dissolved inorganic nitrogen
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
FCR	Feed conversion ratio
g	Gram
h	Hour
IRAS	Integrated Recirculating Aquaculture System
kg	Kilogram
L	Litre
LSD	Least Significant Difference
m^2	Square meter
mg L^{-1}	Milligram per litre
mL	Millilitre
N	Nitrogen
P	Phosphorus
ppt	Parts per thousand, ‰
RAS	Recirculation Aquaculture System
s	Second
SE	Standard Error
SGR	Specific Growth Rate
SPSS	Statistical Package for the Social Science

TAN	Total Ammonia Nitrogen
TB	Total bacteria
TN	Total Nitrogen
TP	Total phosphorus
TSS	Total Suspended Solids
WA	Western Australia

LIST OF SOME COMMON AND SCIENTIFIC NAMES USED IN THIS THESIS

Banana prawn	*Penaeus merguiensis* (De Man, 1888)
Blue mussel	*Mytilus edulis* (Linnaeus, 1758)
Blue prawn	*Penaeus stylirostris* (Stimpson, 1874)
Brown mussel	*Perna indica* (Kuriakose & Nair, 1976)
Brown tiger prawn	*Penaeus esculentus* (Haswell, 1879)
Chinese prawn	*Penaeus chinensis* (Osbeck, 1765)
Constricted tagelus	*Sinonovascula constricta* (Lamarck, 1818)
Gray prawn	*Penaeus setiferus* (Linnaeus, 1767)
Green mussel	*Perna viridis* (Linnaeus, 1758)
Green ormer	*Haliotis tuberculata* (Linnaeus, 1758)
Green seaweed	*Ulva lactuca* (Linnaeus, 1753)
Hairy cockle	*Scapharca inaequivalvis* (Bruguiere, 1789)
Hard clam	*Meretrix meretrix* (Linnaeus, 1758)
Japanese abalone	*Haliotis discus hannai* (Ino, 1953)
Kuruma prawn	*Penaeus japonicus* (Bate, 1888)
Manila clam	*Tapes philippinarum* (Adams & Reeve, 1850)
Nile tilapia	*Oreochromis niloticus* (Linnaeus, 1758)
Pearl oyster	*Pinctada martensii* (Dunker, 1872)
Pocketbook mussel	*Lampsilis cardium* (Rafinesque, 1820)
Scallop	*Placopecten magellanicus* (Gmelin, 1791)
School prawn	*Metapenaeus macleayi* (Haswell, 1879)
Seabream	*Sparus aurata* (Linnaeus, 1758)
Sydney rock oyster	*Saccostrea commercialis* (Iredale and Roughley, 1933)
Tiger prawn	*Penaeus monodon* (Fabricius, 1798)
White prawn	*Litopenaeus vannamei* (Boone, 1931)
Witch prawn	*Penaeus canaliculatus* (Olivier, 1811)
Yabby	*Cherax destructor* (Clark, 1936)
Pacific oyster	*Crassostrea gigas* (Thunberg, 1793)

LIST OF PUBLICATIONS

1. Khoi, L.V., and R. Fotedar, 2010. Effects of stocking density on nutrient budget and growth of the western king prawn (*Penaeus latisulcatus* Kishinouye) in a recirculating aquaculture system. *Aquaculture Research*, 41, e624-e633.

2. Khoi, L.V., and R. Fotedar, 2011. Integration of western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) and green seaweed (*Ulva lactuca* Linnaeus, 1753) in a closed recirculating aquaculture system. *Aquaculture*,322-323: 201-209,

3. Khoi, L.V., R. Fotedar, and M. Kumar, 2012. Effects of feeding rates on the growth, water quality and nutrient budget of western king prawn (*Penaeus latisulcatus* Kishinouye) reared in recirculating aquaculture systems. *Aquaculture Research*, DOI: 10.1111/j.1365-2109.2012.03120.x

4. Khoi, L.V., and R. Fotedar, 2012.. Integration of blue mussel (*Mytilus edulis* Linnaeus, 1758) with western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) in a closed recirculating aquaculture system under laboratory conditions. *Aquaculture,* 354-355:84-90.

5. Khoi, L.V., and R. Fotedar. Submitted. Effects of blue mussel (*Mytilus edulis*) densities and western king prawn (*Penaeus latisulcatus*) biomass on growth, survival and water quality in a closed recirculating aquaculture system under laboratory conditions.

CHAPTER 1 INTRODUCTION

1.1 BACKGROUND INFORMATION

1.1.1 Environmental impacts of aquaculture

Aquaculture is the fastest growing food producing sector in the world and remains a growing, vibrant and important production sector for high protein food. The global aquaculture production reached 52.5 million tonnes, with an estimated value of US$98.4 billion (excluding aquatic plant) in 2008 (FAO 2010b). While the fish supply from marine capture fisheries appears to have reached a plateau, the aquaculture sector maintained an average annual growth rate of 5.3% in volume terms between 2006 and 2008; and 8.3% worldwide between 1970 and 2008 (FAO 2010b). Aquaculture continues to grow more rapidly than all other animal food-producing sector. World aquaculture output has increased substantially, from less than 1.0 million tonnes of annual production in 1950 to the 52.5 million tonnes reported for 2008, increasing at three times the rate of world meat production (2.7% from poultry and livestock together) in the same period (FAO 2010b). Production from aquaculture is mostly destined for human consumption. Globally, aquaculture accounted for 45.7% of the world's fish food, production for human consumption in 2008, up from 42.6% in 2006. Aquaculture production supplied the rest of the world with 26.7% of its food fish, up from 4.8% in 1970 (FAO 2010b).

Intensive aquaculture activity provides large quantities of high-protein feed to culture species, however, nutrient retention capacity for N and P is usually low and variable among the species, resulting in significant releases of both dissolved and particulate wastes to the surrounding environment. Studies of intensive prawn farms in Thailand found that only 21% of the prawn feed N was recovered as harvested prawn while 35% was discharged to the environment (Briggs and Funge-Smith 1994). The wastewater composition of intensive aquaculture systems mainly comprise of solid wastes, dissolved metabolic waste, dissolved nutrients from feeds and faeces, and biocide and pharmaceutical residues (Midlen and Redding 1998). Troell et al. (1999) reported that supplied N discharged into surrounding waters in the form of ammonium (NH_4^+) is about 50-60%. Only about 10% of dissolved

nutrient is released from prawn pond into environment (Briggs and Funge-Smith 1994). Although the dissolved nutrients are an important nutrient source for plant and other species in the aquatic environment, their increased availability may have a significant impact to the balance of natural ecosystems. The negative impacts of high rich-nutrient flux may result in increased algae growth and biomass, reduction of biodiversity, toxic and non-toxic algae blooms and stimulating dinoflagellate growth (Troell et al. 1997).

To reduce the organic matters and nutrients from the culturing system, the reduction of feed waste by improvement of feed quality and feeding management (Smith et al. 2002; Pan et al. 2005) and waste water treatment systems have been studied. For water treatment system, sedimentation ponds effectively reduced suspended particles (Teichert-Coddington et al. 1999; JacksonPrestonBurford et al. 2003). However, sedimentation is less effective in reducing TN and TP concentrations, especially if sedimentation ponds are operated for a long period with continuous water flow (Muangkeow et al. 2007). Several studies (Kaewsuralikhit 1994; Khidprasert 1995; Jones and Preston 1999; Li and Dong 2000; Jones et al. 2001; Tian et al. 2001) have been reported on integrated culture of prawn with bivalves and/or seaweed. Previous studies showed that oysters remove suspended particulates and phytoplankton, while seaweed absorbs dissolved nutrients in the prawn effluents (Wang 1990; Jones et al. 2001; Jones et al. 2002).

The western king prawn is widely distributed throughout the Indo-West Pacific region and are considered to be important potential aquaculture species. Due to its established markets in Asia and Australia (Andrews and Bowen 1992), and its tolerance to a wide range of salinity and temperature (Penn 1980; Ramasamy and Pandian 1984) including tropical, tropical and temperate areas, the species is becoming the major candidate for farming in Australia as it suits Australia's wide range of climates. They have been cultured in China (Wang et al. 2004), Japan (Shokita 1970, 1984), Thailand (Ling 1973) and India (Kathirvel et al. 1986; Kathirvel and Selvaraj 1987; Kathirvel et al. 1987) as well as in Australia with many recent attempts to culture this species in Western Australia (Sang and Fotedar 2004a; Sang and Fotedar 2004b; Prangnell and Fotedar 2005, 2006b, 2006a; Hai et al. 2007; Prangnell 2007; Hai et al. 2009b, 2009a; Hai and Fotedar 2009; Prangnell and

Fotedar 2009; Hai et al. 2010a; Hai et al. 2010b; Khoi and Fotedar 2010, 2011). Western king prawn was integrated culture with seaweeds, *Sagrassum* spp. in the static water tank culture (Mai, 2010).

Green seaweed is probably the most suitable candidate for integrated aquaculture because its fast growth rate might be of importance for ease of culture and to out compete potential epiphytes or other species (Winberg et al. 2009). Green seaweed has been grown in many parts of the world in pilot commercial systems (DeBusk et al. 1986; Neori et al. 1991; Neori et al. 2003) including integrated multi-trophic systems where green seaweed culture is combined with aquaculture of marine animals (Cohen and Neori 1991; Jiménez del Río et al. 1996; Neori et al. 1996; Neori et al. 1998; Neori et al. 2000; Schuenhoff et al. 2003). It is efficient removes ammonium (Bracken and Stachowicz 2006) and has a morphology well suited to tumble culture (tank cultivation of seaweeds using air agitation.

Blue mussels are distributed widely widely distributed in the boreal regions of the northern hemisphere (Seed 1976) and are considered ideal candidates for aquaculture (Hickman 1992). They are one of the most important aquaculture species in Western Australia with the production of 505.6 tonnes in 2009/2010 and the value of $1.8 millions (Department of Fisheries 2011). Integrated culture of mussels with Atlantic salmon (*Salmo salar*) Cheshuk et al. 2003) and other salmon species (Wallace 1980; Taylor et al. 1992; Stirling and Okumus 1995) were carried out in open-waters.

1.1.2 Nutrient uptake capacity of seaweed and mussel

Owing to high capacity for absorption and metabolism of dissolved N and P, seaweed species have been served as trappers to remove the dissolve nutrients in the waste water. The approach was initiated in the middle of the 1970's by Ryther et al.(1975) and recently gained more interests (Neori et al. 1991; Buschmann et al. 1994; Neori et al. 1996; Chopin et al. 1999; Chow et al. 2001; George et al. 2004; Hernández et al. 2006). Vandermeulen and Gordin (1990) reported that *Ulva* efficiently removed up to 85% of TAN from fishpond effluent water. Shpigel et al. (1993) reported removal efficiencies by green seaweed of over 90% from TAN concentrations of 2 g m^{-2} day^{-1} and stocking densities of 1 kg m^{-2}. Jiménez del Río

et al. (1996) obtained dissolved inorganic nitrogen (DIN) removal rate of 2.2 g DIN m^{-2} day^{-1} during the summer and 1.1 g DIN m^{-2} day^{-1} during winter by seaweed, *Ulva rigida*. Working with a 3.3 m^3 system of seabream (*Sparus aurata*), Japanese abalone (*Haliotis discus hannai*) and seaweeds, Neori et al. (2000) found that green seaweed removed 67% of its TAN input. In a three-stage seaweed system connected to an intensive fishpond, Schuenhoff et al. (2003) obtained TAN removal efficiencies of 70% by green seaweed and Neori et al. (2003) obtained a real removal rates of up to 2.9 g m^{-2} day^{-1} with removal efficiencies of 85–90% in a novel three-stage system of green seaweed. Most of the studies concluded that the high rich-nutrient effluents from aquaculture systems may be suitable for *Ulva* spp. growth and development (Cohen and Neori 1991; Neori et al. 1996; Neori et al. 1998).

Mussels feed on the smaller, non-settleable particles such as phytoplankton, bacteria and other organic materials (Jones and Preston 1999). They can significantly reduce the concentrations of TB, phytoplankton, TN and TP, and other suspended particles in prawn pond effluent (Jones and Preston 1999; Jones et al. 2001; Ramos et al. 2008; Ramos et al. 2009). Jones and Preston (1999) reported that filtration by the high density of Sydney rock oysters (*Saccostrea commercialis*) reduced the effluent TSS to 49% of the initial level, the TB numbers to 58%, TN to 80% and TP to 67%. The combined effects of settlement and Sydney rock oyster filtration reduced the concentration of chlorophyll *a to* 8% of the initial effluent value. However, bivalves can increase the concentration of dissolved nutrients such as PO_4^{3-} and NH_4^+ in the water column through excretion (Prins and Smaal 1994; Jones et al. 2001).

1.1.3 Integrated recirculating aquaculture system

Integrated recirculating aquaculture system is an integrated system where the main and subordinate species are separated by different units and the aquaculture effluents flow toward the culture units of the subordinate species; in some of these systems the water is discharged to receiving ecosystems, whereas in others it is re-circulated (Chien and Tsai 1985). The secondary species thrive in the effluents from the ponds (or tanks) of the main species, feeding on non-consumed food, organic matter and other nutrients. This process improves the quality of the discharged water so that it

can be reused and decreases the environmental impact of the aquaculture (Martínez-Porchas et al. 2010). Integrations of *Ulva* with fish (Cohen and Neori 1991; Neori et al. 1996; Neori et al. 2000; Schuenhoff et al. 2003), abalone (Neori et al. 1998; Shpigel et al. 1999; Bolton et al. 2009) and bivalves (Neori et al. 1998) were carried out in semi-recirculating integrated culture systems (Schuenhoff et al. 2003; Troell et al. 2003). There is no available information on integration of western king prawn with green seaweed in recirculating aquaculture system. In the semi-recirculating systems, farming effluents from fish ponds flow through seaweed tanks, which serve as biofilters for removing dissolved nutrient and then part of effluents circulated back to the fish pond. This process reduces nutrient concentration in the effluent but fails to remove nutrients completely in wastewater (Buschmann et al. 1994; Buschmann et al. 1996; Schuenhoff et al. 2003). Due to increase concerns about pollution and disease infection through water intake, IRAS have been developed recently (Muangkeow et al. 2007).

Integrated and intensive culture of white prawn (*Litopenaeus vannamei*) with herbivorous fish (mullet) and oysters in closed recirculation system has been proposed by Sandifer and Hopkins (1996). Integration of white prawn and Nile tilapia (*Oreochromis niloticus*) in closed recirculation system has been reported by Muangkeow et al. (2007; 2011) in tank culture. No information on integrated of western king prawn with green seaweed or blue mussel in the IRAS is available.

Seaweeds and bivalves have a significant capacity to convert a large quantity of waste nutrients into valuable products (Troell and Norberg 1998; Troell et al. 2003; Wang 2003). Overall, at least 60% of the nutrient input can reach commercial products when fish or prawns are cultured with seaweed (ShpigelNeori et al. 1993). This rate is nearly three times higher than modern monoculture fish net/pen farms (Neori et al. 2004). Approximately 24.6% N inputs were retained in harvested products in Chinese prawn (*Penaeus chinensis*), tilapia hybrids (*Oreochromis niloticus* X O. *mossambics*) and constricted tagelus (*Sinonovascula constricta*) in closed pond poly-culture and higher than monoculture (prawn only) (Zhen-xiong et al. 2001). Feed utilization efficiency was higher in integrated pond culture (prawn-tilapia and bivalves) than in monoculture (Li and Dong 2000; Zhen-xiong et al.

2001). However, there are no published data on the growth performance, survival rate and nutrient retention of prawns and mussels in an IRAS.

1.2 AIM OF THE STUDY

The research will assess the nutrient flow in IRAS and evaluate the nutrient uptake capacity of green seaweed or blue mussel when cultivated with western king prawn effluents. The research will also evaluate the nutrient conversion rate of seaweed and mussels in the IRAS.

1.3 OBJECTIVES

The aim of the research will be achieved by meeting the following objectives:

1. To assess the nutrient composition of effluents from western king prawn cultured in the integrated recirculating system.

2. To determine the relationship between nutrient retention and level of intensification (feed levels and stocking densities) of western king prawn culture.

3. To investigate the relationship between the biomass of green seaweed and its nutrient-uptake capacity when green seaweed is integrated with western king prawn in the integrated closed recirculating aquaculture system.

4. To investigate the relationship between the stocking density of blue mussel and its nutrient-uptake capacity when blue mussel is integrated with western king prawn in the integrated closed recirculating aquaculture system.

5. To assess the growth performance and survival of western king prawn; growth and yield of green seaweed; and nutrient budget in the integrated closed integrated aquaculture.

6. To investigate the growth performance, survival rate and nutrient retention of western king prawns and blue mussels in the integrated closed recirculating aquaculture system.

7. To investigate the effects of stocking rates of blue mussels and western king prawns on water quality, growth and survival of animals reared in integrated closed recirculating aquaculture system.

1.4 SIGNIFICANCE

The research aims to make contributions in prawn effluent management for sustainable prawn industry by contributing in understanding of nutrient removal capacities of blue mussels and seaweed. The significances of the current research are as follows:

1. The research will contribute in sustaining the production of western king prawn aquaculture via an application of suitable density and feeding rate.

2. .

3. The study will assist in understanding the mechanisms and principles of using seaweed and mussels as bio-filters in the integrated closed recirculating aquaculture system.

4. The current study will contribute in understanding the nutrient budget and nutrient retention in integrated closed recirculating aquaculture system.

5. The research will assist in understanding the nutrient uptake capacities of green seaweed and blue mussels in indoor culture conditions.

6. The research will add value to the recirculating aquaculture systems.

7. The research will contribute in sustaining the prawn aquaculture industry and protect the environment through managing the prawn effluents from the intensive prawn farming.

8. The findings of this study may be used as references for the other related studies in integrated aquaculture system.

CHAPTER 2 LITERATURE REVIEW

2.1 BRIEF OVERVIEW OF PRAWN FARMING

2.1.1 Prawn farming and culture systems

Penaeid prawn farming began hundreds of years ago in countries of South-East Asia and has been one of the most economically successful of all intensive aquaculture industries. Crustacean production contributed 9.5% (5.00 million tonnes) to world aquaculture production by quantity and 23.1% (US$22.7 billion) in 2008 (FAO 2010b). In 2009, more than 3,495 thousand tonnes of commercial shrimp/prawns were farmed worldwide and valued at over $14.6 billion (FAO 2011). Prawn was the most important product economically, being 15.4% of the global values of fishing products in international trade in 2008 (FAO 2010a). The major species currently farmed are the tiger prawn *(Penaeus monodon*), the banana prawn (*Penaeus merguiensis*) and the Chinese prawn (FAO 2010a). In Australia, the prawn farming industry produces more than 5,381 tonnes of prawns in 2009-2010 (ABARE 2011), valued at over $77 millions, and is based on approximately 900 hectares of ponds and hatcheries (Department of Primary Industries and Fisheries 2008). The industry is based primarily in Queensland and New South Wales. Prawn farming is also conducted in the Northern Territory near Darwin and is under development on the northern coastline of Western Australia (Owens 2006).

Traditional prawn farming (e.g. extensive pond farming) dominates many regions and is characterised by ponds of irregular shape and size (Tookwinas 1999). Seedstock normally come from the wild and supply is seasonal dependent (FAO 1986a). Extensive farming employs very low stocking densities, supplement feed is not given and water management is by tidal exchange (FAO 1986a). Thus, extensive prawn ponds produce limited wastes (Pa´ez-Osuna 2001b).

Semi-intensive or intensive systems obtain a higher production per unit area than extensive systems (New and Wijkstrom 1990; ShpigelNeori et al. 1993). These systems often involve high rearing densities (e.g. above 15-20 prawns m^{-2}) to enhance production (Bratvold et al. 2004). High quality feed inputs are also used to

ensure satisfactory survival, growth rates and feed conversion efficiency of the cultured species (Brzeski and Newkirk 1997) but only a minor part of these nutrients is taken up by the cultured species. The remainder is released as wastes where they can have negative impacts on water quality and for the environment (Naylor et al. 2000). From an economical and ecological point of view, the management of aquaculture wastes is of particular concern (Shpigel et al. 1993b; Bratvold et al. 2004) for a sustainable development of the prawn farming.

2.1.2 Effect of stocking density and feeding rate to prawn culture

Stocking density is a major factor affecting production parameters, such as growth, survival yield of prawns and water quality in culture system. The past research has shown an inverse relationship between the stocking density and growth of prawn (Sandifer et al. 1987; Ray and Chien 1992; Williams et al. 1996; Tseng et al. 1998; Arnold et al. 2006) and water quality (Martin et al. 1998;). For example, high growth rate was found at stocking densities of 40 and 80 prawn m^{-2} compared to160 prawn m^{-2} in the recirculating system (Tseng et al. 1998). Reduced growth and survival of prawn reared at high density can be due to the competition for the space, poor water quality (Forster and Beard 1974) and cannibalism (Abdussamad and Thampy 1994). In addition, higher stocking density can result in an increased waste load (Martin et al. 1998; Pa´ez-Osuna 2001b). Increasing prawn stocking density resulted in an increasing input of organic matter into the pond, through feed pellets. As a result, more wastes were produced (Pa´ez-Osuna 2001b). Studies have clearly shown that Biochemical Oxygen Demand (BOD), ammonia (NH_3), chlorophyll *a*, and TSS increase with stocking density (Robertson and Phillips 1995; Patnaik and Lawrence 2011). For example, dissolved oxygen (DO) and TAN were significantly higher at density 1602 prawn m^{-3} and feed rate 2.5 g prawn $^{-1}$ $week^{-1}$ compared to lower densities (658 and 111 prawn m^{-3}) and feed rates (1.0, 1.5 and 2.0 g $prawn^{-1}week^{-1}$) (Patnaik and Lawrence 2011). When organic wastes accumulated in the sediment (unconsummated feed pellets, faeces, etc.) are degraded, NH_3, NO_2^- and NO_3^- are also formed (Blackburn et al. 1988).

Feed rate is one of the most important variable components and has a major influence on the productivity. Optimal feeding rate is essential in maximizing conversion rate from feed to prawn. The accuracy of determine the feeding rate is mainly based on the estimate of the density and size of the stock (FAO 1986a). The amount of feed input to culture pond is related to the variable cost and associated with the prawn productivity. At the same time the wastes produced by feed and then released into natural waters can cause negative impact on the environment (Cho and Bureau 2001). The amount of wastes is increased when inappropriate feed management strategies and overestimated feed inputs are employed. The excess feed through deterioration of water quality can lead to poor growth and survival with a consequent reduction in production and economic returns (Wyban et al. 1989). Therefore, optimum feeding rate can increase the economic returns of prawn farming through better feed utilisation and reduced environmental impacts.

Feeding rate depends on the species, development stages, culture system and the nutrient composition of the feed. Robertson (1988) recommended the optimum feeding rate for maximum growth of 5 to 6 g tiger prawn was at 10% prawn biomass per day as dry pellets whilst feed input rate for 0.1-1.8 g banana prawn was estimated at 11-12% prawn biomass in aquaria (Sedgwick 1979b).

2.2 OVERVIEW OF RESEARCHES ON WESTERN KING PRAWN BIOLOGY AND AQUACULTURE

2.2.1 Taxonomy, distribution, biology and environmental requirements

Western king prawn also called bamboo prawn, is a popular species in Australia and Japan and is widely distributed throughout the Indo-West Pacific region (Dore and Frimodt 1987). A taxonomic classification of western king prawn is as follows (Holthuis 1980):

Kingdom: *Animalia*
Phylum: *Arthropoda*
Subphylum: *Crustacea*
Class: *Malacostraca*
Subclass: *Eumalacostraca*

Superorder: *Eucarida*
Order: *Decapoda*
Suborder: *Dendrobranchiata*
Superfamily: *Penaeoidae*
Family: *Penaeidae*
Genus: *Penaeus (Melicertus)*
Species: *latisulcatus*

Authority: Kishnouye 1896

Juveniles of western king prawn occur predominantly on intertidal sand- and mud-flats, generally located between shallow subtidal/intertidal seagrass beds and mangroves higher on the shoreline (Penn et al. 1989; Potter et al. 1991), and are often found in estuaries (Dore and Frimodt 1987). This species prefers to live on barren sand- and mud-flats (Tanner and Deakin 2001), gravel substrates or in shallow marine water down to a 90 m depth (Dore and Frimodt 1987; Andrews and Bowen 1992). They are nocturnal and bury in the sediment during the daytime (Rasheed and Bull 1992) to avoid predators (Tanner and Deakin 2001) and light (Wassenberg and Hill 1994). Their behaviour and physiology is similar to kuruma prawn (*Penaeus japonicus*) and with witch prawn (*Penaeus canaliculatus*) (Dore and Frimodt 1987).

Western king prawns mature at the age of 1 to 2 years (Penn 1980). At the first year of life, females can reach maturity when they are as small as 10.8cm in length (Abdel Razek et al. 1994). The mature prawns reach a carapace length of 23mm for males and 25mm for females (Penn 1980). Their reproduction times and frequencies varied by location. For instance, during summer in temperate water of Cockburn South, Western Australia (Penn 1980), and year-round reproduction with two peaks (spring and autumn), at Shark Bay and Exmouth Gulf, Western Australia (Rothlisberg et al. 1987). Spawning takes place off-shores and planktonic stages migrate to inshore towards the end of larval development (Kangass 1999). Larvae move at random and are carried into the nursery areas by currents but in some cases, tides cycles influence the distribution pathway of western king prawn (Penn 1975). They remain in estuaries for a year before moving offshore (Potter et al. 1991;

Kangass 1999). Western king prawns, like all penaeid prawns, feed on benthic fauna and detritus (Rasheed and Bull 1992; Wassenberg and Hill 1994).

Western king prawns are eurythermic species and can tolerate a wide range of temperatures between 10-32^{o}C (Tseng 1987). The optimum temperature of this species is 23-26^{o}C for culture and 22-26^{o}C for optimum spawning (Tseng 1987). There is no maturation or spawning when temperatures are less than 17^{o}C (Penn 1980). Larvae growth at a temperature ranges of 21-30^{o}C (Rothlisberg and Jackson 1987) and maximum growth of the early stage of this species is at temperature range of 25-28^{o}C (Prangnell 2007).

Salinity is an important influence on the western king prawn distribution within temperate estuaries with larger prawns more susceptible to declining salinity (Potter et al. 1991). Western king prawns can tolerate a wide range of salinities of 20-50 ppt (Ramasamy and Pandian 1984). The maximum survival of this species is achieved at salinities between 25-45 (Ramasamy and Pandian 1984), and 22-34 ppt (Sang and Fotedar 2004a). Prawns can tolerate sudden salinity decrease from 32 to 25 ppt and from 27 to 20 ppt, and even tolerate sudden increases in medium potassium concentration from 78 to 284 mg L^{-1} and 78 to 365 mg L^{-1} in inland saline water (Prangnell 2007).

Photo: Ngo Van Hai

Plate 2.1 Western king prawn

2.2.2 Cultivation of western king prawn

Western king prawn have been considered to be a prospective species for culture (Kathirvel and Selvaraj 1987). The eggs and larvae of this species have been successfully reared under controlled conditions in Japan (Shokita 1970) while a semi-commercial hatchery for seed production has been set up in South Australia, Australia (Pawnall 1974). However, the western king prawn hatchery in South Australia is no longer in operation. Attempts were made to culture this species in India (Kathirvel et al. 1986; Kathirvel and Selvaraj 1987; Kathirvel et al. 1987), Japan (Shokita 1984) and Australia (Sang and Fotedar 2004a; Sang and Fotedar 2004b; Prangnell and Fotedar 2005, 2006b; Hai et al. 2007; Hai et al. 2009b, 2009a; Khoi and Fotedar 2010). Prawns have been reared in saline water in Western Australia (Prangnell and Fotedar 2005, 2006b; Prangnell 2007), in application of customised probiotics (Hai et al. 2009b, 2009a) and in integrated cultures with the seaweeds, *Sargassum* spp. (Mai et al. 2010).

Western king prawns have been reared at different stocking densities, depending on the prawn sizes, culture systems and the water types. Western king prawns were stocked at densities of 18.29 prawn m^{-2} in integrated culture of the prawn and seaweed, *Sagrassum* sp. (Mai et al. 2010). . They were also reared at density of 23.0 prawn m^{-2} or fourteen prawns (PL40) per tank (250L plastic tank and 88 cm diameter) (Prangnell and Fotedar 2006b) and 32.91 prawn m^{-2} or nine prawns (5.32 ± 0.12 g) per tank (125 L tank and 59 cm diameter) (Prangnell and Fotedar 2009) in inland saline water. The highest prawn stocking density with a prawn mean weight of 2.95g was 40.23 prawn m^{-2} in the study conducted by Sang and Fotedar (2004) in different salinity ranges (10-46 ppt).

Various feed types and feeding rates have been provided for western king prawn in indoor culture conditions. Prawns were fed commercial diets in studies by Mai et al. (2010), Hai and Fotedar (2009) and Hai et al. (2009b, 2009a); flesh blue mussel (Sang and Fotedar 2004a; Prangnell and Fotedar 2005); and combination of commercial diets and flesh mussels (Prangnell and Fotedar 2006b). Feed was given twice a day at rates of 3-5% body weight (Hai et al. 2009b, 2009a; Hai and Fotedar 2009) or 2.5% body weight per day (Mai et al. 2010). Prangnell and Fotedar (2006b)

fed western king prawn three times a day with the following schedule: frozen *Artemia* on days 0–60 of the experiment; crumble (Skretting Nutra TP) on days 0–39 (20.4% body weight d^{-1}); live adult *Artemia* on days 23–194; 2 mm pellets (Skretting Nutra TP) on days 15–201 (3.3% bw d^{-1}); 4 mm pellets (Skretting Classic SS) on days 115–201 (1.3% bw d^{-1}); thawed mussel (*Perna canaliculus*) on days 184–197 (1.2% bw d^{-1}); and fresh blue mussel on days 197–201 (3.0% bw d^{-1}).

2.3 OVERVIEW OF RESEARCH ON GREEN SEAWEED AND BLUE MUSSEL

2.3.1 Green seaweed

Green seaweed (*Ulva lactuca*) is a common macroalgae occurring worldwide and native to Australia. It inhabits both intertidal and subtidal zones (Taylor 1957) and has a high temperature and irradiance tolerance range (Winberg et al. 2009). The optimum temperature range for growth of green seaweed in North Sea is 15-20^{o}C with a slight decrease at 20^{o}C (Fortes and Lüning 1980) but Enright (1979) found the temperature optimum for growth of green seaweed at 20^{o}C. Green seaweed survives a wide range of salinity (2-35 ppt), and lives on a variety of substrata (Niesenbaum 1988).

Green seaweed is able to photosynthesize and grows under very low photon flux densities (Vermaat and Sand-Jensen 1987). Light compensation point for growth is at 2.5 µmol $m^{-2}s^{-1}$ and is similar to the minimum light compensation point for photosynthesis of green seaweed (Sand-Jensen 1988a). Photosynthetic light harvesting efficiency is highest in green seaweed acclimated to intermediate light levels of 8.8 and 25.0 µmol $m^{-2}s^{-1}$ and is associated with maximum chlorophyll density and light absorptance (Sand-Jensen 1988b). Adaptation to low light in green seaweed is achieved by increasing chlorophyll content, light absorption, photosynthesize efficiencies and by decreasing relative growth rate and respiration (Vermaat and Sand-Jensen 1987). However, photosynthetic processes in green seaweed are independent of water flow and even stressed by high currents when light levels are below saturation (Koch 1993).

Ulva spp. can utilize both dissolved free carbon dioxide (CO_2) and bicarbonate ions (HCO_3^-) as the exogenous carbon source for photosynthesis (Beer and Eshel 1983; Drechsler and Beer 1991). The uptake of NO_3^- and NH_4^+ is inhibited by 5 K Pa CO_2 (Magnusson et al. 1996). Several authors showed that seaweeds have a high infinity for dissolve inorganic carbon which is only weakly affected by DO levels, whereas pH level approaching 10 is highly inhibitory (Beer and Eshel 1983; Colman 1984; Sand-Jensen and Gordon 1984). *Ulva* sp. photosynthesis decreases at pH 8.0–9.0 while it reaches maximum values of production between pH 6.0 and 8.0; and its optimum is between pH 6.0 and 7.5. The decrease in photosynthetic rate below pH 6.0 is lower (12.30%) than above pH 8.0 (81.03%)(Menéndez et al. 2001).

Ulva spp. are good colonizers and tolerate nutrient pollution better than most macroalgae (Sze 1998). Anderson (1942) showed that NO_3^- is the preferred N source for green seaweed but NH_4^+ is preferred when both NO_3^- and NH_4^+ are at low concentration. *Ulva* efficiently removes up to 85 % of the NH_4^+ from fish pond wastewater in darkness or light independently of temperature fluctuations (Vandermeulen and Gordin 1990). In addition green seaweed maintains the ability to take up NH_4^+ and NO_3^- under conditions of rapidly changing salinity within the range of 20-30 ppt (Lartigue et al. 2003). Other NH_4^+ salts, esters, amides, and amino acids can also be utilized by green seaweed (Kylin 1945). Green seaweed is able to absorb PO_4^{3-} (Steffensen 1976) and may remove 50% PO_4^{3-} content in sewage (Tsagkamilis et al. 2010).

Plate 2.2 Green seaweed (*Ulva lactuca*)

Growth rate of *Ulva* spp. shows a seasonal trend and is highest in summer (Robertson-Andersson et al. 2008). Growth rates for seaweed, *Ulva fenestrata* under experimental conditions were 16% wet wt day^{-1} (Björnsäter and Wheeler 1990). This was lower than those obtained for seaweed, *U. curvata*, 52% wet wt day^{-1} in short-term laboratory studies (Duke et al. 1989); green seaweed, 18.6% wet wt day^{-1} (Neori et al. 1991) and seaweed, *U. fasciata*, 36% wet wt day^{-1} (Lapointe and Tenore 1981) . The low growth rate for seaweed, *U. fenestrata* may be attributed to the lower water temperature (13°C) (Björnsäter and Wheeler 1990). The SGR obtained by Neori et al. (1991) were low and can be due to the scaling up of the tank sizes as the other studies were experimental, small scale setups.

Species of the genus *Ulva* are grown for human food in eastern countries (Ohno 1993) and since 1990 have been authorised for human food in France. Seaweeds are low-calorie foods, with a high concentration of minerals (Mg, Ca, P, K and Na), vitamin, protein, and indigestible carbonhydrates and a low lipid contents (Norziah and Ching 2000; Wong and Cheung 2000). Thus, they are extensively used as food, food ingredients, as ingredients in cosmetics and fertilizers (Winberg et al. 2009);

and feed sources for other high-valued aquaculture animals in integrated aquaculture systems (Neori et al. 2000; Chopin et al. 2001; Neori et al. 2004). Green seaweeds are used as feed for abalone or sea urchin (Neori et al. 2000; Neori et al. 2004); especially as abalone grow out has been demonstrated to produce faster growth rates when fresh rather than dry or pelleted feed is used (Troell et al. 2006).

Ulva species have also been grown in many parts of the world in pilot commercial systems (DeBusk et al. 1986; Neori et al. 1991; Neori et al. 2003) including integrated multi-trophic systems where *Ulva* culture is combined with aquaculture of marine animals (Cohen and Neori 1991; Jiménez del Río et al. 1996; Neori et al. 1996; Neori et al. 1998; Neori et al. 2000; Schuenhoff et al. 2003). A major reason for the widespread use of *Ulva* is that many species of this genus can thrive unattached in sheltered marine waters and estuaries, and have particular affinities for growth in high N concentrations, thereby removing large amounts of dissolved nutrients. Therefore, unattached *Ulva* species are perfect aquaculture candidates, and much research has been carried out on their growth in various systems (Henley 1992).

Ulva spp. vary greatly in nutrient contents due to environmental factors such as temperature, salinity, light and nutrients (Marinho-Soriano et al. 2006). Authors reported the seasonal changes in tissue N and P of *Ulva* (Duke et al. 1987; Robertson-Andersson et al. 2008). The seaweeds experienced lower tissue N but higher specific growth rate (SGR) in summer (Robertson-Andersson et al. 2008) whereas higher phosphate content was observed in autumn and winter months (Robertson-Andersson 2003). Therefore they can be used as bio-indicator of nutrients in the water column because of their ability to assimilate rapidly surrounding nutrients and their tissue content can reflect the local nutrient regime within relatively a short time period (Ryther 1981).

2.3.2 Blue mussel

Blue mussel (*Mytilus edulis*) is widely distributed in the boreal regions of the northern hemisphere, from the western border of the Kara Sea, south to the Mediterranean, North Carolina, California and Japan; it is absent from the high

Arctic waters of Siberia, Franz Josefland and Spitzbergen (Seed 1976). It also occurs in European waters, extending from the White Sea, Russia as far as south as the Atlantic coast of Southern France (Gosling 1992). Blue mussel has a wide distributional pattern, expanding its range from the high intertidal to subtidal regions and its salinity range from estuarine areas to fully oceanic seawaters. It lives on a variety of substrata, such as rock, stones, shingle, dead shells and even compacted of mud and sand (Seed 1976)

Blue mussel is euryhaline and tolerant of a wide range of salinity compared to other biogenic reefs species. It occurs in marine as well as in brackish waters (Baltic) down to 4 ppt (Kautsky 1982a). Almada-Villela (1984) reported greatly reduced shell growth for a period of up to a month or so upon exposure to 16 ppt compared to 26 or 32 ppt, while exposure to 22 ppt caused only a small drop in growth rate.

Plate 2.3 Blue mussels (*Mytilus edulis*)

Temperature is an important factor affecting the distribution of mussels. Stubbings (1954) showed that limited mussel distribution to the southern hamisphere coinsides more or less with the maximum surface isotherm of 27 ^{o}C. Blue mussels can survive even when tissue temperatures fall below -10 °C (Williams 1970), with large adults

surviving laboratory conditions of -16 °C for 24 hours (Bourget 1983).The species is well acclimated for a 10-20 °C temperature range (Thompson and Newell 1985), with an upper sustained thermal tolerance limit of about 29 °C for adults (Almada-Villela et al. 1982). High water temperatures coupled with low food rations have been demonstrated to reduce growth in blue mussel (Bayne and Widdows 1978).

Mussels feed on the smaller, non-settleable particles such as phytoplankton, bacteria and other organic material. Small inorganic particles are also filtered, coagulated into larger, more settleable particles and egested as pseudofaeces (Tenore and Dunstan 1973). During filtration, filter-feeder bivalves sort particles by size, weight (Yonge 1926) and chemical composition (Loosanoff 1949). Oysters preferentially ingest organic material, reject inorganic material, and preferentially ingest N rich over C (carbon) rich particles (Newell and Jordan 1983). Rejected material is expelled as pseudofaeces, and when food concentration exceeds the digestive capabilities of the gut, pseudofaeces may contain some digestible food. However, bivalves filtration can be reduced or cease completely when sediment loads are too high, (Loosanoff and Tommers 1948). Therefore, it is essential to reduce the concentration of suspended particles by sedimentation prior to filtration by bivalves. The organic particles ingested by the oysters are incorporated into tissue, thereby capturing wasted nutrients and converting them into a secondary cash crop (Jones et al. 2002).

Suspension-feeding bivalves increase the quantity and quality of sediment organic matter through the production of faeces and pseudofaeces (Giles and Pilditch 2006). They feed on small particles from the water column which are either bound in mucus and rejected as pseudofaeces (Jones and Preston 1999). Once faeces and pseudofaeces of bivalves reach the bottom seabed, they are called biodeposits. Biodeposits have high nutritional value (Kautsky and Evans 1987; Giles and Pilditch 2004) and show high bacterial activities (Kaspar et al. 1985). Biodeposition increases the flux of organic matter to the sediment and the remineralisation of biodeposits increases sediment oxygen demand and supplies regenerated nutrients to the overlying water. Furthermore, biodeposition can alter denitrification (Newell et al. 2002; Christensen et al. 2003) and burial rates (Hatcher et al. 1994; Newell 2004) leading to the removal of nutrients from the water column.

Marine mussels are widely cultivated for their proteinaceous meat and are considered ideal candidates for aquaculture (Hickman 1992). They now occupy a premier position in the world statistics for aquaculture production (FAO 1990) and mussel cultivation is a growing industry in developing countries (Silas 1980). The global mussel aquaculture production in 2009 was 1,764,630 tonnes; and Australia produced 3,362 tonnes blue mussel in 2009 (FAO 2011). Mussel culture methods, as practiced in many countries, are carried out by using a variety of culture methods based on the prevailing hydrographical, social and economic conditions. They include bottom culture, intertidal and shallow water culture methods as rack culture, hanging method, tray culture, Wig-wam culture or Rope-web culture (Aypa 1990). In the deep-water culture, long-line culture and raft culture methods are popular (Aypa 1990). There are two popular culture methods in Australia namely longlines and raft cultures (Department of Primary Industries 2012).

2.4 ENVIRONMENTAL IMPACTS OF PRAWN FARMING

2.4.1 Environmental impacts of prawn farming

Prawn farming can generate diverse environmental impacts on the environment (Troell et al. 1999; Neori et al. 2000; Matos et al. 2006; Zhou et al. 2006). Negative effects of the prawn farming include physical habitat destruction, introduction of diseases and alien species (Troell et al. 2003), pond sediment disposal, chemical and biological products residues, capture of wild postlarvae and wild prawn stocks, abandoned prawn ponds and the release of large amount of wastes into the environment (Pa´ez-Osuna 2001b). These impacts depend on several factors: location of farms, management and use of technology during pond operation, culture surface and scales of production, and depurative capacity of receiving water bodies (Pa´ez-Osuna 2001b; Alonso-Rodríguez and Páez-Osuna 2003).

Intensive prawn farming result in the discharge of significant amounts of nutrients into adjacent waterways with a high proportion of these nutrients originating from the commercial feed (Briggs and Funge-Smith 1994). Thakur and Lin (2003) reported that N inputs in the form of feed ranged from 76.4 to 92.4%, and P input through feed ranged from 69.8 to 90.6% of the total inputs in the closed prawn

culture system of tiger prawn. However, nutrient retention capacity for N and P, being provided through feeds, is usually low and variable in different prawn species and farming systems (Table 2.1). Studies of intensive prawn farms in Thailand found that only 21% of the prawn feed N was recovered as harvested prawn while 35% was discharged to the environment (Briggs and Funge-Smith 1994). A major portion of the N (31%) and most of the P (84%) was retained in the sediments (Briggs and Funge-Smith 1994). Likewise, studies of semi-intensive farms in Honduras showed that 72% of the N entering the ponds was discharged to the environment as a result of water exchange (Teichert-Coddington et al. 2000). The direct discharge of waste nutrients from prawn farms into adjacent environments has raised concerns about the sustainability of prawn farming (Phillips et al. 1993; Primavera 1994; Naylor et al. 2000). In addition, the discharge of untreated pond effluent represents an economic loss of costly nutrients, thereby reducing farm profitability (JacksonPrestonThompson et al. 2003)

Table 2.1 Retention of feed nitrogen and phosphorus in penaeid prawn biomass for different prawn species and culture systems

Species	Culture model	Feeding rate (% BW^{-1})	FCR	N retention (%)	P retention (%)	References
Tiger prawn	Extensive culture	4-20	1.6-2.2	19.8-45.3	-	(Hari et al. 2005)
White prawn	ICRS[a]	3-8	1.13-1.27	38.5-47.6	18.9-23.4	(Muangkeow et al. 2007)
White prawn	Tank culture	8-10	1.22-1.43	34.5-42.3	15.1-18.6	(Pan et al. 2005)
White prawn	Polyculture tank[c]	Ad lid.	1.46-2.08	19.9-29.2	6.5-9.9	(Yuan et al. 2010)
Chinese prawn	Polyculture pond[b]	2-8	-	12.6-17.8	5.4-7.4	(Tian et al. 2001)

BW: body weight; ad lib.: ad libitum feeding; FCR: Feed conversion ratio
[a] Integrated closed recirculation system
[b] Nutrient inputs were feed and fertilizer; Polyculture pond: integrated culture of Chinese prawn (*Penaeus chinensis*), hybrid tilapia (*Oreochromis mossabicus x O. niloticus*) and constricted tagelus (*Sinonovacula constricta*)
[c] Polyculture culture of red tilapia (*Oreochromis* spp.) and white prawn (*Litopenaeus vannamei*)

The release of wastes mainly depends on species, culture system, feeding level, feed composition, prawn size, and temperature (Schneider et al. 2005). The impacts of these releases ultimately depend on local/regional hydrodynamic conditions, the physical, chemical and biological characteristics of the receiving ecosystem (Troell 2009). Generally, N and P are two main nutrient components of prawn ponds effluents (Troell et al. 1997; Tovar et al. 2000; Burford et al. 2003; Matos et al. 2006; Zhou et al. 2006). Effluents from prawn pond are typically enriched in suspended solid, nutrient and BOD with concentration largely depending on whether the management is intensive or semi-intensive (Sandifer and Hopkins 1996). Amount and components of suspended solids and dissolved nutrients released to surrounding environment depend on number of factors, for example, species, culture system, pond management (Pa´ez-Osuna 2001a), level of intensification (Alonso-Rodríguez and Páez-Osuna 2003). Ratios of N and P in the prawn effluents range from 1.1 to 61.0 in semi-intensive systems and from 5.5 to 67.0 in the intensive systems (Alonso-Rodríguez and Páez-Osuna 2003). Troell et al. (1999) reported that 50-60% of N discharged into surrounding waters is in the form of NH_4^+. The dissolved nutrients may stimulate the rapid growth of virus, bacteria and toxic and/or non-toxic algal blooms (Lin et al. 1993; Troell et al. 1997; Troell et al. 1999; Neori et al. 2000). Consequently, the outbreak of infectious disease such as pathogenic bacteria and virus can occur (Primavera 1997).The major crash of the shrimp industry in Taiwan, then in the Upper Gulf of Thailand, and the losses from shrimp diseases in the Philippines, Indonesia, and China have all been linked to waste production exceeding the assimilative capacity of the local water bodies (Lin 1989; FAO-NACA 1994). Chen and Sheng (1992) reported deterioration of water quality due to pond effluents in Shandong and Hebei in China. On the west coast of Sri Lanka, major shrimp mortalities were blamed on pollution of the main water supply canal (the 'Dutch Canal') by pond effluents (Jayasinghe 1994). (Flegel 1997). These wastes also cause increased BOD and solids accumulation (Tovar et al. 2000) which could induce toxic conditions for aquatic animals. As a consequence, nutrient loading can lead to a decline in farm productivity or even the collapse of an aquaculture industry as it already has in some countries, for example, the prawn industry in China (Msuya et al. 2006) and fish culture in the Philippines (Rodrigueza and Montaño 2007). Therefore, there is a risk of eutrophication in receiving water

bodies of farm effluents and more efforts should be made to manage effluents form prawn farms in order to maintain sustainable prawn farming development.

2.4.2 Management of prawn effluents

The prawn aquaculture industry has adopted a number of strategies to reduce nutrient wastes and its impacts on the local environment. Teichert-Coddington et al. (1999) proposed two general approaches to modify the environmental impacts of effluents. One strategy is simply to reduce the quantity of effluents and the other is to improve the quality of effluents before discharge. Reducing the nutrient loading from aquaculture has been applying, including the improvement of feed utilization by the animals and the treatment of the effluent with biological or chemical filters (Matos et al. 2006). Since feed loss is also an economic problem for the aquaculture (Subasinghe et al. 2003), much research has been completed on improving the feed efficiency (Houlihan et al. 2001). Filtration methods that reduce NH_4^+ in recirculation aquaculture systems have also been applied (Neori et al. 2004). However, some of these methodologies only transform the nutrients into less toxic forms and do not really reduce the "output" of nutrients to the environment. In addition, they are often expensive and involve a high degree of technology (Matos et al. 2006). Consequently, the treatment of the effluents is still more the exception than the rule in aquaculture (Troell et al. 1999; Troell et al. 2003).

Various methods to minimize the environmental impacts of prawn farming include sedimentation tanks (Teichert-Coddington et al. 1999; Nunes et al. 2006), elimination of water exchange rates (Hopkins et al. 1995), use of wetlands (Tilley et al. 2002), and using filter-feeder mollusks (Shpigel and Neori 1996; Jara-Jara et al. 1997; Shpigel et al. 1997; Lefebvre et al. 2000), seaweed (Nelson et al. 2001; Msuya et al. 2006). Popular methods of treated aquaculture effluent are shown in Table 2.2. These methods have proven to reduce nutrients loading in aquaculture, for example, constructed wetlands can remove 82-90% TP, 92-93% PO_4^{3-} and 86-89% TN from a fish farm (Summerfelt et al. 1999), and 30% TP in prawn culture (Tilley et al. 2002). The denitrification process control NH_4^+ and NO_2^- within acceptable ranges (below 0.5 and <0.2 mg L^{-1}, respectively) for culture of tiger prawn broodstock (Menasveta et al. 2001).

Table 2.2 Common methods for the treating aquaculture effluents

Methods	References
Water exchange	Hopkins et al.(1993)
Sedimentation	Wang (1990), Teichert-Coddington et al. (1999), Boyd (2000), Jones et al. (2001)
Constructed wetlands	Summerfelt et al. (1999), Negroni (2000), Gautier et al. (2001), Tilley et al. (2002)
De-nitrification	Aboutboul et al. (1995), Menasveta et al. (2001), Erler et al. (2004)
Microbial agents (bacteria)	van Rijn (1996), Chuntapa et al. (2003), Liu and Han (2004)
Filter-feeder bivalves	Jones and Preston (1999), Ramos et al. (2008), Jones et al. (2002)
Using microalgae	Chuntapa et al. (2001), Sreesai and Pakpain (2007), Brune et al. (2003)
Using seaweeds as bio-filter	Ryther et al. (1975), Neori et al., (1996), Neori et al. (2003), Matos et al.(2006), Mai et al. (2010), Mao et al. (2009), Rodrigueza and Montaño (2007)

Troell and Norberg (1998) reported that waste materials (faeces and waste feed) from aquaculture effluents are being broken down into finer particles and dissolved nutrients, suggesting that filter feeders may be suitable for absorbing the particulate wastes while seaweeds could be suitable for absorbing dissolved nutrients. Therefore, integrated effluent treatments which combine of sedimentation process and bivalves and/or seaweed can be applied in order to gain more economical efficiency and ecological balance in the aquaculture industry (Chopin et al. 2001).

2.5 INTEGRATED AQUACULTURE SYSTEMS

2.5.1 Integrated aquaculture systems

Integrated aquaculture system is defined as 'an output from one subsystem in an integrated farming system which otherwise may have been wasted becomes an input to another subsystem resulting in a greater efficiency of output of desired products from the land/water area under a farmer's control' (Edwards et al. 1988). Integrated pond systems are applied traditionally in Asia (Schneider et al. 2005) and principally

originated in China, Japan and South Korea (Goldman et al. 1974; Ryther et al. 1975). Wastes in the integrated system serve as nutrient for phototrophic and detrivorous/heterotrophic conversion into plants, bacteria, and invertebrates (Riise and Roose 1997; Liu and Cai 1998), therefore higher nutrient retention is a result of nutrient re-use by primary and secondary producers (Liu and Cai 1998). Consequently, integrated aquaculture system can limit the environmental impact of waste from fish/prawn cultivation and enhance the total productivity (Troell et al. 2003).

Aquatic polyculture is traditionally practiced in the Pacific and Indian Ocean-bordering nations, particularly China. Rice/fish culture, popular in Europe in the 19th–early 20th centuries, has been practiced in China for millennia (Fernando 2002). Earthen marine ponds, associated with natural or agriculture plants (such as mangroves and rice) are used on a wide scale for extensive prawn farming in China, Indonesia, Ecuador, India, the Philippines, Taiwan, Thailand, Japan and more recently in Vietnam (Binh et al. 1997; Alongi et al. 2000). In Northern Europe, ducks, fish and crayfish have been raised together in freshwater ponds (Maki 1982). The ducks eat the algae and small fish, and deposit manure, which promotes further growth of algae and other aquatic plants. Crayfish eat these plants, as do other herbivorous fish. People then harvest the fish, ducks and crayfish (Maki 1982). This type of polyculture is a managed imitation of a natural ecosystem. The culture of microalgae in wastewater from animal feedlots has also been researched and practiced in several countries for years, but is not discussed in this chapter.

Integrated aquaculture can be land-based or open-water systems and may include several combinations of species (Neori et al. 2004). Both systems have mainly built on technical approaches involving mechanical separation, sedimentation and filtration (Cripps 1994). The land-based systems have been successfully implemented (Littlewood 1990; Neori et al. 1991; HopkinsHamiltonSandifer et al. 1993; Lin et al. 1993; ShpigelLee et al. 1993; Buschmann et al. 1994; Buschmann et al. 1996; Neori et al. 2000; Muangkeow et al. 2007) in small-scale (indoor/outdoor tank culture) systems (Buschmann et al. 1994; Buschmann et al. 1996; Neori et al. 2003; Muangkeow et al. 2007) and large-scale (pond culture) systems (Maguire et al. 1981; Wang et al. 1998). Polyculture in earthen brackish water ponds has been

practised for a long time, with extensive polyculture systems of prawn, fish, agriculture plants (including also mangroves and rice) found mainly in China, Indonesia, Ecuador, India, the Philippines, Taiwan Province of China, Thailand, Japan and more recently in Viet Nam (Binh et al. 1997; Brzeski and Newkirk 1997; Neori et al. 2004).

The concept of open-water integrated culture was proposed by Kautsky et al. (1997). Traditional integrated open water mariculture systems, located principally in China, Japan, and South Korea, have a long history. These operations have consisted of fish net pens, shellfish and seaweed placed next to each other in bays and lagoons (Neori et al. 2004). Filter-feeder bivalves and/or seaweeds are cultured adjacent to meshed fish cages, reducing nutrient loadings by filtering and absorbing nutrient particles and/or dissolved inorganic nutrients produced from within the fish nets/pens (Troell et al. 1999; Lombardi et al. 2006) as well as any phytoplankton production stimulated by introduced dissolved nutrient wastes (Cheshuk et al. 2003). Factors such as sea tides, currents and waves make dispersion of wastes difficult to predict (Troell and Norberg 1998). These factors also make it difficult to design any experiments and to collect data in coastal areas (Zhou et al. 2006). Therefore, a few studies have investigated the possibilities of integrating seaweed or bivalves as bio-filters into open-water farming (Troell et al. 1997; Troell and Norberg 1998; Sarà et al. 2009) but there are conflicting conclusions regarding the potential for open-water integrated culture to enhance bivalve production and, by implication, to significantly reduce fish farm wastes (Cheshuk et al. 2003). Studies have shown that bivalves are capable of utilising fish farm wastes as an additional food supply (Lefebvre et al. 2000), likely explaining the increased growth displayed by mussels (Wallace 1980) and oysters (Jones and Iwama 1991) grown adjacent to fish cages. However, other studies have reported no, or minimal, growth enhancement of bivalves cultured in an integrated bivalve–fish system (Taylor et al. 1992; Stirling and Okumus 1995).

The appropriate organisms used in integrated aquaculture systems are chosen based on the roles and functions they play in the ecosystem, their economic value and/or their acceptance by consumers. Martínez-Porchas et al.(2010) indicated that the conditions in the culture units in the integrated systems have to meet the biological requirements of the subordinate species that is being co-cultures, otherwise serious

risks of losing all of part of the economic and human effort invested may occur. Another consideration is the stocking rate in integrated culture management. Yi and Fitzsimmons (2004) showed that overstocking complicates the management and is not suitable in the long culture period. An optimum stocking rate of Chinese prawn and tilapia hybrids was 6 prawn m^{-2} and 0.32 tilapia m^{-2}, respectively (Wang et al. 1998).

Integrated aquaculture models can be classified as fish-macroalgae (Cohen and Neori 1991; Neori et al. 1991; Neori et al. 2000), fish-macroalge-abalone (Neori et al. 2000), prawn-microalgae-oyster (Wang 2003), fish-bivalve-macroalgae (ShpigelNeori et al. 1993), prawn-fish (Tian et al. 2001; Muangkeow et al. 2007). An extensive review on analysing nutrient conversions in integrated intensive aquaculture systems is provided by Schneider et al (2005).

2.5.2 Integrated recirculating aquaculture systems

Integrated recirculation aquaculture system implies a polyculture system where the main and subordinate species are separated by different units and the effluents of main species circulate through the culture units of the subordinate species (Chien and Tsai 1985). The subordinate species feed on non-consumed food, organic matter and other nutrients from the main species effluents. This process improves the quality of the discharged water so that it can be reused and decreases the environmental impact of the aquaculture (Martínez-Porchas et al. 2010). Several authors have been worked with semi-recirculating integrated aquaculture systems (Shpigel and Neori 1996; Neori et al. 1998; Neori et al. 2003; Schuenhoff et al. 2003) where parts of the treated effluents recirculate back to the culture system. Shpigel and Neori (1996) have proposed three designs for the semi-recirculating integrated culture of mollusc with seaweed and fish in land-based facilities and showed that these designed systems significantly reduce the operational and environmental risks involved with mariculture. However, this process reduces nutrient concentration in the effluent but fails to remove nutrients completely in wastewater (Buschmann et al. 1994; Buschmann et al. 1996; Schuenhoff et al. 2003).

Integrated closed recirculation systems have been developed because of growing concern of water pollution and avoidance of disease infection through water intake. In this system, wastewater with high organic particles and nutrients from intensive prawn pond flows to the treatment ponds and then recycles back to the prawn pond (Muangkeow et al. 2007). Lin (1995) proposed an integrated closed recirculation system composed of prawn, sedimentation, biological treatment (fish, mussel, etc.) and aeration ponds. Integrated and intensive culture of white prawn with herbivorous fish (mullet) and oysters in closed recirculation system has been proposed by Sandifer and Hopkins (1996). Recently, Muangkeow et al. (2007; 2011) have reported the integration of white prawn and Nile tilapia in an integrated closed aquaculture system.

2.5.3 Integrated aquaculture systems of prawn and other species

Integration of prawns and seaweed has been studied in several countries. Cultivation of seaweed, *Gracilaria parvispora* in prawn-farm effluent ditches and floating cages has been trialled in Hawaii, USA by Nelson et al. (2001). Results showed that the mean relative growth rates (RGRs) of effluent-enriched thalli in the cage system ranged from 8.8% to 10.4% day^{-1}, a significant increase over the growth (4.6% day^{-1}) of thalli fertilized with inorganic fertilizer. Thalli were also grown directly in the effluentditch, where mean growth rates of 4.7% day^{-1} were obtained, less than in cage-culture (Nelson et al. 2001).In Australia, Jones et al. (2002) reported that seaweed, *G. edulis* has effectively improved water quality of the effluents from a commercial farm of kuruma prawn. Co-culture of seaweeds, *Sargassum* spp. and western king prawn was conducted by Mai et al. (2010) and results that by integrating seaweed into prawn culture, the concentrations of TAN, NO_2^-, NO_3^-, DIN, TN, PO_4^{3-} and TP were significantly lower in the integrated culture system than in the prawn monoculture. The integration of *Gracilaria* with prawn farming have been done in Malaysia (Phang et al. 1996), Brazil (Marinho-Soriano et al. 2002) and China (Chang and Wang 1985; Yin 1987; Wei 1990; Liu et al. 1997). *Ulva* sp. has been used in laboratory-scale integrated culture with prawns in Japan (Ali et al. 1994).

Prawns have also been reported in integrated cultures with bivalves (Maguire et al. 1981; Jones et al. 2001), fish (Tian et al. 2001; Erler et al. 2004; Muangkeow et al. 2007) and seaweed (Kaewsuralikhit 1994; Khidprasert 1995; Bunting 2006). Adding a secondary or subordinate species improves the performance of the main cultured organism (Wang et al. 1998; Tian et al. 2001). For instance, Pacific oyster (*Crassostrea gigas*) and black clam (*Chione fructifraga*) and Wami tilapia (*Oreochromis urolepis hornorum*) have been co-cultured with penaeid prawns, improving the production parameters of the prawns (Martinez-Cordova and Martinez-Porchas 2006; Tendencia et al. 2006).

2.6 METHODS FOR TREATING AQUACULTURE EFFLUENTS

Several integrated aquaculture systems have been designed with different approaches to illustrate performances of nutrient retention or nutrient recycling more effectively and efficiently than other methods (Troell et al. 2003). Sedimentation (Wang 1990; Teichert-Coddington et al. 1999; Boyd 2000; Jones et al. 2001) and constructed wetlands (Gautier et al. 2001; Konnerup et al. 2011) and filter bivalve culture (Wang 1990; HopkinsHamiltonSandifer et al. 1993; Jones and Preston 1999; Jones et al. 2001; Jones et al. 2002) have been used to eliminate suspended solids while seaweed (Buschmann et al. 1994; Buschmann et al. 1996; Neori et al. 1996; Neori and Shpigel 1999; Neori et al. 2000; Chopin et al. 2001; Neori et al. 2003) effectively absorb dissolved nutrients in the prawn effluents.

2.6.1 Sedimentation

Sedimentation is the most feasible means to treat aquaculture pond effluents (Pillay 1992; Midlen and Redding 1998), mainly because of the huge volume of water involved. The ability of sedimentation ponds to improve water quality have been assessed in laboratory scale by Boyd et al. (1998) with emphasis on the removal of solids. Prawn effluents contain suspended solids, predominantly phytoplankton, protists and bacteria (Jones et al. 2002). The major source of TSS in pond effluent are suspended soil particles from erosion, and particulate organic matter resulting from feed and fertilizer-driver wastes and plankton production (Pillay 1992; Boyd and Tucker 1998). Gautier, Amador, and Newmark (2001) concluded that suspended

soil particles are mainly responsible for the increase in TSS in pond drainage. Sedimentation ponds remove effectively TSS concentration in water discharged from prawn farms. Teichert-Coddington et al. (1999) reported that 88% of the TSS in effluents from an intensive prawn farm at the time of harvest are removed after 1/4 day of sedimentation. 18-26% of N and P can be removed from prawn ponds by using sedimentation (Teichert-Coddington et al. 1999). Factors may influence the effectiveness of sedimentation ponds in reducing TSS, including effluent composition, residence time, pond design, pond management, and biological processes such as presence of filter-feeding organisms (Preston et al. 2000). Sedimentation ponds are less effective in reducing the concentration of TP and TN, due to decomposition of organic matters that have settled in the sedimentation ponds if sedimentation ponds are operated for a long periods with continuous water flow (JacksonPrestonBurford et al. 2003). In addition, several authors reported that sedimentation alone is inefficient in removing particulate matters (Henderson and Bromage 1988; Hennessy 1991). Therefore, it is necessary to develop nutrient removal systems that are more actively managed than passive sedimentation ponds. A possible approach is to combine passive sedimentation with active recapture of nutrients through the harvest of a second cultured species.

2.6.2 Using seaweeds as biofilters in integrated aquaculture systems

Most of the N and P in an aquaculture system come from animal excretion and is available in the form of NH_4^+ and PO_4^{3-} (Matos et al. 2006). In intensive aquaculture systems, where water is re-utilized, biological filters perform nitrification, oxidizing NH_4^+ to form NO_3^- (Troell et al. 2003). Therefore, in these systems, N becomes also available in the form of NO_3^-. These forms of N and P are the preferred sources of nutrients for many seaweed species (Lobban and Harrison 1994; Troell et al. 2003; Carmona et al. 2006).

Seaweed has been shown to remove nutrients from the effluents of aquaculture farms (Nelson et al. 2001; Marinho-Soriano et al. 2002) (Table 2.3). The first seaweed usage to absorb dissolved inorganic nutrients from aquaculture effluents was suggested by Ryther et al. (1975). Seaweeds used as biofilters in integrated systems has been successfully demonstrated at laboratory scales (Troell et al. 2003) to large

field scales (Neori et al. 2004); tank cultures (Buschmann et al. 1994; Jiménez del Río et al. 1996; Neori et al. 2000; Cruz-Suárez et al. 2010) to pond cultures (Marinho-Soriano et al. 2002; Msuya et al. 2006). Seaweeds have been used to remove dissolved inorganic nutrients from wastewater in fish (Ryther et al. 1975; Vandermeulen and Gordin 1990; Buschmann et al. 1994; Buschmann et al. 1996; Neori et al. 1996; Martínez-Aragón et al. 2002), prawn (Nelson et al. 2001; Msuya et al. 2006) and mollusc (Mao et al. 2009) aquaculture prior to disposal; and proven to effectively reduce the nutrient load in effluents and maintain water quality at acceptable levels (Marinho-Soriano 2007; Neori et al. 2007) (Table 2.3). Bunting (2006) constructed an integrated prawn–shellfish–seaweed polyculture system and observed that hairy cockle (*Scapharca inaequivalvis*) and seaweeds, *Gracilaria* spp. reduced the TAN, TN and TP concentrations from prawn effluents by 61, 72 and 71%, respectively. Kaewsuralikhit (1994) and Khidprasert (Khidprasert 1995) found that seaweeds, *Gracilaria salicornia*, *G. Fisheri*, *Caulerpa macrophysa* and *Sargassum polycystum* were capable of effectively assimilating TAN, NO_2^- and NO_3^- (>40%). All these reports clearly indicate that a potential alternative to diminish the pollution associated with aquaculture effluent is bioremediation throughout seaweed species that are capable of assimilating and using the wastes (Kang et al. 2008).

The genus *Ulva* have been demonstrated to work effectively in integrated systems with main species (Neori et al. 2000; Neori et al. 2004). *Ulva* spp. are efficient removes ammonium (Bracken and Stachowicz 2006) (Table 2.5) and has a morphology well suited to tumble culture (tank cultivation of seaweeds using air agitation). Fast growth rate of the species might be of importance for ease of culture and to out compete potential epiphytes or other species (Winberg et al. 2009). *Ulva* sp. significantly reduced the concentration of DIN from seabream farm ponds effluents in Eilat (Aqaba), Israel (Neori et al. 1996; Neori et al. 2003). Neori et al. (1998) reported that green seaweed removed less than 25% of the PO_4^{3-} added in a complex integrated system, however they found seawed removal rate was up to 84.8% of phosphorus over 24 h. Neori et al. (2000) and Schneider et al. (2005) also reported a high growth rate of *Ulva* when co-cultured with seabream and Japanese abalone, but harvested biomass was limited to low-profit agar extraction (Neori et al. 2000; Carmona et al. 2006).

Biofiltering efficiency of seaweed rely on the different species, stocking densities (Matos et al. 2006; Schuenhoff et al. 2006), nutrient concentration and types, water exchange rates (Matos et al. 2006; Schuenhoff et al. 2006). Seaweed, *Gracilaria chilensis* removed 32% of the PO_4^{3-} in an integrated salmon-seaweed tank culture system (Buschmann et al. 1996) and 27% in effluents from salmon cages (Troell et al. 1997). The highest phosphate uptake was found for seaweed, *Ulva rotundata* (2.86 μmol PO_4^{3-} g^{-1} dry wt h^{-1}) at the highest water flow (2 volume per day) among three seaweeds, *U. rotundata*, *U. intestinalis* and *G. gracilis* (Martínez-Aragón et al. 2002). PO_4^{3-} uptake rate of seaweeds is reduced during the dark period (Tsagkamilis et al. 2010). Lavery and Comb (1991) studied kinetic parameters in a eutrophic estuary and found that *Ulva* and *Chaetomorpha* species showed higher PO_4^{3-} uptake rates during the light period than in the dark. The lower PO_4^{3-} uptake during the night observed in green seaweed may be related to the photosynthetic activity of the plant (Tsagkamilis et al. 2010).

Table 2.3 Efficiency of some seaweed in removing dissolved inorganic nutrients from aquaculture effluents

Species	Culture methods	Nutrients	Removal rate (%)	References
Asparagopsis armata	Integrated culture of seaweed-fish	TAN	18.0-86.0	Schuenhoff et al. (2006)
Chondrus crispus	Integrated culture of seaweed-fish	TN	41.40	Matos et al. (2006)
Enteromorpha sp.	Integrated culture of prawn-fish-seaweed	TN	43.0-46.0	Erler et al. (2004)
Enteromorpha intestinalis	Integrated culture of fish-seaweed	PO_4^{3-}	85.3-99.6	Martínez-Aragón et al. (2002)
Gracilaria sp.	Integrated culture of fish-seaweed	TAN	50.0-95.0	Shpigel et al. (1993)
		PO_4^{3-}	27.0	Buschman et al. (1994), Buschman et al. (1996), Troell et al. (1999)
		TN	41.0	
		TP	52.0	
G. bursa pastoris	Integrated culture of fish-seaweed	TN	76.7	Matos et al. (2006)
G. conferta	Integrated culture of seaweed, abalone, fish and	TN	3.0-88.0	Neori et al. (1998)

	clams			
G. chilensis	Integrated culture of fish-seaweed	DIN	5.0	Troell et al. (1997)
		PO_4^{3-}	27.0	
G. caudate	Integrated culture of prawn-seaweed	TAN	60.0	Marinho-Soriano (2007)
		NO_3^-	50.0	
		PO_4^{3-}	12.0	
G. gracilis	Integrated culture of fish-seaweed	PO_4^{3-}	71.4-98.0	Martínez-Aragón et al. (2002)
G. edulis	Integrated treatment of prawn-oyster-seaweed	TAN	76.0	Jones et al. (2001)
		NO_3^-	30.0	Jones et al. (2002))
		PO_4^{3-}	35.0	
		TN	66.0	
		TP	56.0	
G. longissima	Integrated culture of fish-seaweed	TAN	19.1	Hernández et al. (2005)
		DIN	17.0	
		PO_4^{3-}	3.2	
Kappaphycus alvarezii	Integrated culture of oyster-seaweed	TAN	41.0-66.0	Qian et al. (1996)
K. striatum				Rodrigueza and Montaño (2007)
Laminaria saccharina	Integrated culture of fish-seaweed	DIN	26.0-40.0	Subandar et al. (1993)
Palmaria palmate	Integrated culture of fish-seaweed	NUF	41.0	Matos et al. (2006)
Paracentrotus lividus	Integrated culture of fish-seaweed	TAN	66.0-70.0	Schuenhoff et al. (2003)
		PO_4^{3-}	20.0	
Porphyra amplissima	Integrated culture of fish-seaweed	TP	70.0-100.0	Carmona et al. (2006)
		PO_4^{3-}	35.0-91.0	
Ulva sp.	Integrated culture of fish-seaweed	TAN	85.0	Vandermeulen and Gordin (1990)
Ulva lactuca	Integrated culture of fish-seaweed	TAN	67.0-90.0	Krom et al. (1995), Schuenhoff et al. (2003)
		PO_4^{3-}	9.0-21.0	
		TN	73.0-80.0	Neori et al. (1998; 2000; 2003),
		TN	42.0-66.0	Msuya and Neori (2008)
		NUF	16.0-50.0	
U. reticulate	Integrated culture of fish-seaweed	TAN (outflow)	63.0 – 65.0	Mwandya et al. (2001)
			44.0	Msuya et al. (2006))
		TAN	33 – 58	

		(inflow)		
		PO_4^{3-}		
U. rigida	Integrated culture of fish-seaweed	TAN	76.0	Jiménez del Río et al. (1994)
U. rotundata	Integrated culture of fish-seaweed	TAN	24.4	Hernández et al. (2005)
		DIN	54.0	Martínez-Aragón et al. (2002)
		PO_4^{3-}	8.9	
		PO_4^{3-}	60.7-96.2	

Buschmann et al. (1996) reported that seaweed, *G. chilensis* is able to remove 50% of NH_4^+ in winter, increasing up to 95% in spring in a tank salmon-seaweed cultivation system. Troell et al. (1997) estimated that, extrapolating to a large scale, seaweed, *G. chilensis* co-cultivated with salmon had the potential to remove at least 5% DIN released from the fish farm. Neori et al. (1998) reported that green seaweed and seaweed, *G. conferta* remove about 34% TAN supplied in a complex integrated culture.

Green seaweeds may have two or three times more protein content than brown seaweeds (Burtin 2003), especially *Ulva* grown in the integrated aquaculture systems have generally higher protein content than in wild harvest. The protein contents of wild harvest *Ulva* range between 3.7-24.0% (Nisizawa et al. 1987; Simpson and Cook 1998; Wong and Cheung 2001). Average tissue protein values of green seaweed is 34% when integrated with sea urchin (*Paracentrotus lividus*), Japanese abalone and seabream (Schuenhoff et al. 2003), and 28% in integrated aquaculture system of Japanese abalone, seabream and green seaweed (Neori et al. 2000). Thus, they are extensively used as food, food ingredients, as ingredients in cosmetics and fertilizers (Winberg et al. 2009); and feed sources for other high-valued aquaculture animals in integrated aquaculture systems (Neori et al. 2000; Chopin et al. 2001; Neori et al. 2004). *Ulva* spp. are used as feed for abalone or sea urchin (Neori et al. 2000; Neori et al. 2004); especially as abalone grow out has been demonstrated to produce faster growth rates when fresh rather than dry or pelleted feed is used (Troell et al. 2006). In addition, *Ulva* spp. are low-calorie foods, with a high concentration of minerals (Mg, Ca, P, K and Na), vitamin, protein, and indigestible

carbohydrates and a low lipid contents (Norziah and Ching 2000; Wong and Cheung 2000).

Generally, increasing seaweed stocking density may result in higher nutrient removal rates however, seaweed yield and growth rate will decrease when stocking densities exceed the optimum (under not N-limiting conditions) (Jiménez del Río et al. 1996) due to light limitation (Duke et al. 1989; Neori et al. 1991). The optimum density for maximum yield and growth rate in tank culture differs from species to species; and depends on the culture system, nutrient and environmental factors. Green seaweed optimal stocking density is obtained at 1-2 kg m^{-2} and at TAN fluxes of about 0.5 moles m^{-2} $d^{-}1$ (Neori et al. 1991), that is higher than 0.8 kg m^{-2} (Neori et al. 1991) in a rapid seawater exchange (12 tank volume per day) with the green seaweed kept in suspension by continuous aeration. As might be expected, the biofiltration rates were higher under higher nutrient loading levels, while the biofiltration efficiencies were higher under lower nutrient loading levels (Chopin et al. 2001).

Aeration plays an important role for the seaweed tank culture since it accelerates nutrient diffusion under nutrient limitation, most likely by thinning the diffusive boundary layer around the fronds (Gonen et al. 1993). In addition, aeration may increase supply of inorganic carbon, expulsion of excess photo-synthetically generated oxygen from the fronds, and moving the fronds vertically through the exponentially decaying light field in the tanks (Vandermeulen and Gordin 1990; Neori et al. 2004). Rosenberg and Ramus (1982) and Duke et al. (1989) found light irradiances for *Ulva* photosynthesis range between 35 and 80 μmol photons m^{-2} s^{-1}.

Water velocity affected biomass yields and biofiltration performance of green seaweed under low nutrient concentration in the laboratory experiments (Msuya and Neori 2008). Parker (1981) showed that the application of simulated current consistently enhanced growth rates in green seaweed under laboratory conditions. Under the controlled conditions of the indoor experiments, water velocity influenced photosynthetic performance of the seaweed by increasing photosynthetic quantum yields (Msuya and Neori 2008). Madsen et al. (Madsen et al. 2001) showed that photosynthetic rates of the seaweeds were positively correlated with water velocity up to 10 cm s^{-1}. Gonen et al. (1993) found up to 50% increase in photosynthetic

rates in seaweed, *Gracilaria conferta* when water velocity was increased from 0.0 to about 1.5 cm s^{-1}. Dodds (1991) found a 50% photosynthetic increase in seaweed, *Cladophora glomerata* for a water velocity change from 0 to 8 cm s^{-1}. An extensive review on general aspects of seaweed biofiltration in mariculture is provided by Neori et al. (2004).

2.6.3 Using bivalves as biofilters in integrated aquaculture systems

Various filter-feeding bivalves have been used as biofilters in integrated aquaculture systems (Table 2.4). They usually belong to three main groups: Oysters (Maguire et al. 1981; Jones and Iwama 1991; Shpigel et al. 1993a; Shpigel et al. 1997; Lefebvre et al. 2000), mussels and clams (Hopkins et al. 1993a; Shpigel and Neori 1996; Shpigel et al. 1997). Bivalves have been integrated with fish (Shpigel and Neori 1996; Shpigel et al. 1997; Lin et al. 2001; Cheshuk et al. 2003), prawns (Hopkins et al. 1993a; Martínez-Córdova et al. 2010; Tendencia 2007). Integrated culture of bivalves with other marine species may be carried out in open-waters or on land-based systems (Table 2.4).

Table 2.4 Filter-feeding bivalves used as biofilters in treating aquaculture effluents

Species	Culture type	Effluent source	References
Crassostrea gigas	Open-water system	Salmon farm *(Oncorhynchus tshawytscha*	(Jones and Iwama 1991; ShpigelLee et al. 1993; Shpigel et al. 1997; Lefebvre et al. 2000)
C. commercialis	Land-based system	Pond-effluent *Metapenaeus macleayi*	(Maguire et al. 1981; Jones and Preston 1999; Jones et al. 2001; Jones et al. 2002)
Mercenaria mercenaria/ Crassostrea virginica	Pond culture	*Litopeneaus vannamei*	(HopkinsHamiltonSandifer et al. 1993)
Tapes philippinarum	Land-based system	*Sparus aurata*	(Shpigel and Neori 1996; Shpigel et al. 1997)
Tridacna derasa, T. gigas, T. maxima, T. squamosa	Land-based system	*Lutjanus analis*	(Lin et al. 2001)
Crassostrea rhizophorae	Land-based system	*Litopenaeus vannamei*	(Ramos et al. 2009)

Ruditapes decussates	Land-based system	Turbot *Psetta maxima*	(Jara-Jara et al. 1997)
Chione fluctifraga	Land-based system	*Litopenaeus vannamei*	(Martínez-Córdova et al. 2011)
Mytilus planulatus	Open-water	Atlantic salmon (*Salmo salar*)	(Cheshuk et al. 2003)
M. edulis	Open-water	Salmon farm	(Wallace 1980; Taylor et al. 1992; Stirling and Okumus 1995)
Perna indica/ P. viridis	Pond Polyculture	*Penaeus monodon*	(Tendencia 2007)
Crassostrea gigas/*Chione fluctifraga*	Pond polyculture	*Litopenaeus vannamei*	(Martinez-Cordova and Martinez-Porchas 2006)

Filter-feeder bivalves can significantly reduce the concentrations of bacteria, phytoplankton, TN, TP and other suspended particles in prawn pond effluent (Jones and Preston 1999; Jones et al. 2001; Ramos et al. 2008; Ramos et al. 2009) (Table 2.5). Removal efficiency of the oyster, *Crassostrea rhizophorae* was highest at 6 h, with 62.1% removal by turbidity, 69.4% by TSS, 35.4% by total volatile solids, 100% by chlorophyll *a*, and 17.2% by BOD_5, when compared with the control tank (Ramos et al. 2008). However, nutrients removal efficiency of bivalves in integrated effluent treatments relies on filter-feeding species (Ramos et al. 2009), stocking density (Jones and Preston 1999), culture system (Jones et al. 2002) and initial nutrient effluent concentration (Jones et al. 2001) (Table 2.5). Filtration of oysters can be reduced or ceased completely when sediment loads are too high (Loosanoff and Tommers 1948). Other studies have observed the problems associated with a high concentration of suspended solids on the health of oysters (HopkinsHamiltonSandifer et al. 1993). In addition, high biofouling has significantly impacted on growth and survival rates of bivalves in integrated aquaculture systems (Jones et al. 2001; Jones et al. 2002). Thus, Jones et al. (2001) suggested that integrated aquaculture systems should have sedimentation ponds in order to reduce the level of suspended solid before prawn effluents can go through oyster ponds and/or seaweed ponds.

Table 2.5 Removal efficiency of some filter-feeding bivalves in removing dissolved inorganic nutrients from aquaculture effluents

Species	Culture method/system	Suspended solid/nutrient	Removal rate (%)	References
Crassostrea rhizophorae	Integrated treatment of prawn effluents	Turbidity	62.10	(Ramos et al. 2008)
		TSS	69.40	
		TVS	35.40	
		Chlorophyll a	100.00	
		BOD_5	17.20	
C. rhizophorae	Integrated treatment of prawn effluents	Turbidity,	62.10	(Ramos et al. 2009)
		TSS	70.60	
		TVS	36.10	
		Chlorophyll a	100.00	
		BOD_5	17.20	
C. gigas	Integrated treatment of prawn effluents	Turbidity,	56.30	(Ramos et al. 2009)
		TSS	41.20	
		TVS	27.80	
		Chlorophyll a	51.40	
		BOD_5	8.00	
Saccostrea commercialis	Integrated treatment of prawn effluents	TSS	88.23	(Jones et al. 2001)
		Chlorophyl a	91.54	
		TKN	32.68	
		TP	37.11	
		Bacteria	68.42	
S. commercialis	Integrated treatment of prawn effluents	TSS	49.00	(Jones and Preston 1999)
		TN	80.00	
		TP	67.00	
		Bacteria	58.00	
S. commercialis	Flow-through system	Total particulate	29.00	(Jones et al. 2002)
		Chlorophyl a	39.00	
		TN	66.00	
		TP	56.00	
		Bacteria	35.00	
S. commercialis	Recirculating flow regime	TSS	16.00	(Jones et al. 2002)
		Chlorophyl a	4.00	
		Bacteria	12.00	

Bivalves can increase the concentration of dissolved nutrients, such as PO_4^{3-} and NH_4^+ in the water column through excretion (Prins and Smaal 1994; Jones et al. 2001). Oysters excrete NH_4^+, amino acids, urea, uric acid (Hammen et al. 1966) and PO_4^{3-} (Pomeroy et al. 1965; Dame 1996). Nutrient release rates rely on the species, culture system and dissolved nutrients. Qian et al. (1996) reported that the N release rates of pearl oyster (*Pinctada martensii*) was 0.52 μmol h^{-1} of NH_4^+ and 0.44 μmol h^{-1} of NO_3^- per oyster, while for Sydney rock oyster, these values were 0.52 and 0.28 μmol h^{-1}, respectively (Jones et al. 2001).

Several authors have found significantly enhanced growth of shellfish (*e.g.* oysters and mussels) when grown with salmon (Jones and Iwama 1991; Stirling and Okumuş 1995; Sarà et al. 2009). Stirling and Okumuş (1995) reported that growth rate of blue mussels, suspended in salmon cages is dependent of seasonal changes with maximum tissue growth occurred during April-May and shell length during June-August. Blue mussel annual length increments is 25.1-25.9 mm at sites in Loch Etive and 20.1-22.8 mm in Loch Leven, Scotland (Stirling and Okumuş 1995). Generally, growth rates decline with increasing stocking densities of juvenile Sydney rock oysters (Holliday et al. 1991; Holliday et al. 1993), larvae hard clam (*Meretrix meretrix)* (Liu et al. 2006) and scallop (*Placopecten magellanicus)* (Parsons and Dadswell 1992) while survival rates of these bivalves are independent of stocking densities (Holliday et al. 1991; Parsons and Dadswell 1992; Liu et al. 2006).

2.6.4 Nutrient retention efficiencies in integrated aquaculture systems

Seaweeds and bivalves have a significant capacity to convert a large quantity of waste nutrients into valuable products (Troell and Norberg 1998; Troell et al. 2003; Wang 2003). The efficiency of nutrient retention of different integrated system modules differs and depends on the nutritional values of the feed which in turn depends on the specific demands of the cultured species (Schneider et al. 2005). The nutrient retention efficiencies of some integrated systems are shown in Table 2.6. Overall, at least 60% of the nutrient input can reach commercial products when fish or prawns are cultured with seaweed (ShpigelNeori et al. 1993). This rate is nearly three times higher than modern monoculture fish net/pen farms (Neori et al. 2004).

Schneider et al. (2005) also reported that when either macroalgae, microalgae and/or macrophytes are integrated with fish culture, the total nutrient retention of feed increases by 20-50% in N and up to 53% in P. Shpigel et al.(1993) and Neori et al. (2000) also reported that *Ulva* species may retain between 20-30% feed N in an integrated aquaculture system.

Table 2.6 Comparison of some integrated aquaculture systems for intensive production and waste/nutrient conversion into a harvestable product(s)

Integrated systems	**Species**	**Harvestable product (g N retention kg^{-1} feed**	**Harvestable product (% N retention)**	**References**
Fish	*Sparus aurata*	14.0	20.0	(Neori et al. 2000)
Seaweed	*Ulva lactuca*	23.0	32.0	
Fish	*Sparus aurata*	14.0	20.0	(Neori et al. 2000)
Seaweed	*Ulva lactuca*	23.0	32.0	
Abalone	*Haliotis discus hannai*	9.0	12.0	
Prawn	*Penaeus vannamei*	17.0	21.0	(Wang 2003)
Microalgae	*Chaetoceros* sp.	40.0	50.0	
Oyster	*Crassostrea virginica*	5.8	7.0	
Fish	*Spaurus aurata*	16.6	26.0	(ShpigelNeori et al. 1993)
Bilvalves	*Crassostrea gigas/Tapes semidecussatus*	9.3	4.5	
Macroalgae	*Ulva lactuca*	14.2	22.4	
Prawn	*Penaeus vannamei*	-	38.5-45.0	(Muangkeow et al. 2007)
Fish	*Oreochromis niloticus*	-	6.08-6.95	

Nutrient retention in filter-feeder bivalves also rely on the culture system, culture species and the nature of integrated model (ShpigelNeori et al. 1993; Wang 2003) and are likely lower than in seaweed (see Table 2.6). A higher efficiency of nitrogen utilization has been observed in integrated/polyculture systems of bivalves with other marines species compared with monoculture systems (Zhen-xiong et al. 2001). Li and Dong (2000) worked with four closed-polyculture systems: prawn-tagelus, prawn-scallop, prawn-tilapia and prawn-tilapia-tagelus and found the highest economic and efficiency were obtained in prawn-tilapia-tagelus system which raised the production by 28% and the utilization efficiency of input N by 85%. Similarly,

Zhen-xiong et al. (2001) reported the highest N efficiency (20.1%) was observed in prawn-fish-tegalus polyculture system and the lowest value of 12.2% was in monoculture system (prawn only). Moreover, the closed-polyculture systems reduced the N discharge ratio to 6-8% instead of 40-90% in the usual open culture systems (Li and Dong 2000).

2.7 BENEFITS OF INTEGRATED AQUACULTURE SYSTEMS

The economic value of integrated/polyculture systems has been investigated since 1985 (Neori et al. 2004). Integrated aquaculture systems can diversify the production- mix in changing market conditions and add another source of income (Buschmann et al. 1994; Neori et al. 1996; Troell et al. 1997; Neori et al. 2007). The production of species in integrated aquaculture systems is at least equal to, or greater than, those attained from monocultures systems (Neori et al. 2000; Newell 2004). For example, by integrating seaweed, *Gracilaria* with salmon, approximately 48.9 kg m^{-2} $year^{-1}$ of seaweed, *Gracilaria* was harvested annually in a pond with salmon, equivalent to US$ 34,000 (Troell et al. 1999). Eventually, a farm model of land-based integrated seabream-shellfish-seaweed can harvest 25 tons of fish, 50 tons of bivalves and 30 tons of seaweed annually (Neori et al. 2004). Integrated mussel-salmon aquaculture system in cage culture would enhance productivity by 20%, compared to monoculture of mussel or salmon; investment in an integrated salmon–mussel aquaculture system is shown to produce a positive net present value (NPV) (£1.425 million) which exceeds the combined NPV of salmon monoculture (£0.922 million) and mussel monoculture (£0.353 million)(Whitmarsh et al. 2006). Hence, using bivalves/seaweeds for biofiltration in aquaculture systems has both ecological and economic benefits.

Integrated aquaculture systems are practical and functional in most cases and usually lead to reduced effluent being discharged into open water bodies (Neori et al. 2004; Martínez-Porchas et al. 2010). The subordinate species in the integrated systems can fed on and assimilate most of the wastes generate from fish/prawn farming. Thus, integrated culture systems improve the water quality in the culture media and reduce the negative ecological impacts (Martínez-Porchas et al. 2010). Moreover, it the

quality of the wastewater in improved enough, it could be re-used in closed or recirculating systems.

Several reports have indicated that prawn performance is enhanced by the presence of a secondary species. Martínez-Córdova and Martínez-Porchas (2006) concluded that the presence of Pacific oysters and black clam polyculture with white prawn in ponds have a beneficial effect on the productive performance of white prawn and pacific oyster, black clam was a good candidate to be produced in prawn ponds. In addition, integration of tilapia with prawn has shown that tilapia feeding on excess organic matter, improves the water quality and thus increases the prawn production (Akiyama and Anggawati 1998). Similar conclusion was drawn by Wang et al.(1998) in an experimental polyculture of Chinese prawn–hybrid tilapia.

The optimisation of an integrated aquaculture system depends on the objectives to achieve, i.e. biofilteration versus biomass production (Neori et al. 2003; Schuenhoff et al. 2003). Maximising bivalves and seaweeds production and nutrient removal efficiency in an integrated aquaculture system could be difficult to achieve. Schuenhoff et al. (2006) pointed out that an integrated aquaculture system has a high biomass yield of seaweed, the drawback is that nutrient removal efficiency decreases. To achieve elevated biomass production of seaweed, water exchange rates should not limit the availability of both carbon and nitrogen to seaweed growth (Schuenhoff et al. 2006). However, Msuya et al. (2006) reported that the higher water exchange rates may result in the higher nutrient removal rates. In addition, the growth rates of cultured species can reduce due to the fouling and mortality of the seaweed in an integrated aquaculture system (Msuya et al. 2006). Thus, the biomass production per unit area of each species in an integrated aquaculture system may be lower than monoculture system. In addition, efficient treatment of waste water from aquaculture ponds usually involves a high level of technology which requires high investment in terms of the set up and running costs (Neori et al. 2004; Matos et al. 2006). Finally, adding a subordinate species risks introducing pathogens into the culture system. Naylor et al. (2001) highlighted that a common problem in the aquaculture industry is the transport and transmission of pathogens as a result of the use of exotic species.

In conclusion, intensive aquaculture industry faces several problems including contamination caused by farm effluent. Integrated culture appears to be an adequate approach to minimize or eventual solve these problems. Owning to high capacity for absorption and metabolism of nutrients, seaweeds and filter-feeding bivalves have been shown to assimilate most of the wastes generated from farm effluents, with a consequent decrease in nutrients excess, improvement in the water quality and diminution of the environmental impact resulting from the effluent discharges. In addition, integrated culture may improve yield of culture species and allow famers produce different by-products with a commercial value. However, there is no published information on the effects of stocking densities and feeding rates on the performance of western king prawn and nutrient budget in IRAS under laboratory condition. Further, limited published information is available on the integrated culture of decapods with seaweed and filter-feeder bivalves in the IRAS. This research, therefore, focuses on the nutrient flow and integrated cultures of western king prawn with seaweed or mussels in the IRAS.

CHAPTER 3 GENERAL MATERIALS AND METHODS

3.1 EXPERIMENT UNITS

All experiments (Chapters 4-8) were conducted in a purpose-build research room at the Curtin Aquatic Research laboratories (CARL) located at Technology Park, Bentley (31.98^{o}S, 115.88^{o}E), Perth, Western Australia. This room (9.7m length, 4.4 m width and 2.5 m height) was specifically designed for aquatic sciences research and thus contains no windows, allowing researchers to control light conditions, air flow and temperature.

IRAS (Plate 3.1) with tank dimensions described in Table 3.1 were used in the trials.

Table 3.1 Prawn tank specifications in the closed recirculation aquaculture system

Reservoir tank	Internal diameter	0.60 m
	Average tank depth	0.46 m
	Surface area	0.28 m^2
	Effective water volume	130 L
Prawn tank	Average tank depth	0.42 m
	Surface area	0.75 m^2
	Bottom area	0.56 m^2
	Effective water volume	300 L
Waste collection tank	Internal diameter	0.58 m
	Average tank depth	0.63 m
	Surface area	0.26 m^2
	Effective water volume	160 L

Each IRAS consisted of three tanks namely: a reservoir tank, a prawn tank and a waste-collection tank and were located on parallel stands (Plate 3.1). Each stand held three IRAS in which the reservoir tank and the prawn tanks were set on the top and lower shelves of the stand, respectively; the waste-collection tank was on the floor.

During the trials, the reservoir, prawn and waste-collection tanks were filled with 80, 120 and 100 L, respectively, of seawater.

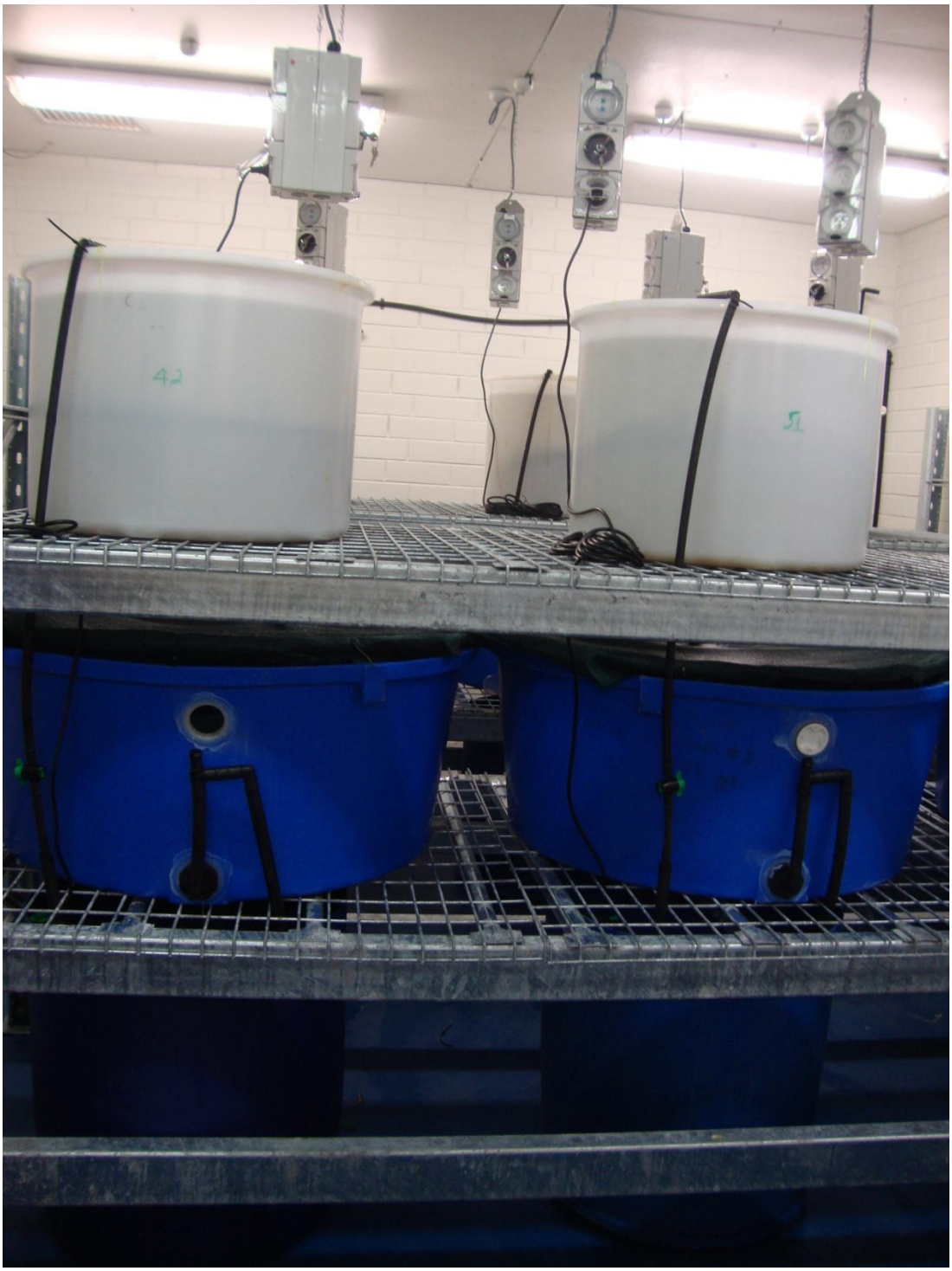

Plate 3.1 Integrated recirculating aquaculture system used in all trials

A submersible pump (Weipro, Model: WH-1000) took water from 10 cm below the water surface of the waste-collection tank to the reservoir tank, circulated it through the prawn tank and then went back to the waste-collection tank by gravity. Flow rate into each tank was 9-10 mL s^{-1} and recirculation ran constantly, giving a retention time in each reservoir tank of approximately 2.3 hours. Prawn tanks were constantly aerated using two air stones connected to a 19 mm polyethylene pipe that was plumbed into the 50 mm CARL mainline. The prawn tanks were covered with black polyethylene (mesh size of 1.0-1.5 cm diameter) put in brackets over the top of each tank to prevent prawns from escaping out.

3.2 COLLECTION OF SPECIES

Western king prawn were collected from the Canning river, Walter Point, Bicton, Western Australia, Australia (32^{o}01 S, 115^{o}45 E) using a hand drag net with the mesh size of 6.0 mm. They were transported in 50L containers with continuous aeration to the CARL. Western king prawns were held at seawater salinity (35 ppt) in 300L tanks for 15 days at CARL and fed a commercially formulated feed ST#1 (43% protein, 6% fat and 2% fibre) (Ridley Aqua-feed, Ridley AgriProducts Pty, Victoria, Australia) until the commencement of the experiments.

Green seaweed were collected from shallow waters of Canning river in Walter point, Bicton, Western Australia (32^{o}00'S, 115^{o}47'E). Plants were picked up by hands and immediately transported in containers filled with ocean water to CARL. Sand, mud and epiphytes were removed manually by rinsing with seawater.

Blue mussel were collected by hands from the Walter Point jetty, Canning river, Bicton, Western Australia, Australia (32^{o}00'39.86"S, 115^{o}47'21.18E). They were transported while wet and cooled with ice packs to the CARL. Sand, mud and epiphytes were removed manually by rinsing mussels with seawater and then mussels were kept in 300 L tanks with moderate aeration at salinity of 35 ppt for 24 hours before experiments commenced.

3.3 PRAWN FEEDING AND TANK MANAGEMENT

Western king prawns were fed a commercially formulated feed ST#1 (43% protein, 6% fat and 2% fibre) (Ridley Aqua-feed, Victoria, Australia) during rearing periods. Mortalities and exuviae were removed as soon as they were noticed. The condition of dead prawns was also recorded.

Uneaten food and prawn wastes were siphoned out after 2-hour of feeding and were filtered through a filter net with mesh size of 100 μm. The solid residues in the filter net were released to the waste collection tank of the IRAS while the filtrate was returned to the prawn tank. At harvest, reservoir and waste collection tanks were drained and the deposited sediment was collected to determine the total mass of sediment.

3.4. DATA COLLECTION

3.4.1. Water quality parameters and bacteria culture

DO and pH were measured daily with a dissolved oxygen meter (YSI model 58, USA) and a pH meter (Cyberscan, pH300, EUtech Instrument, Singapore), respectively. The salinity was measured by a portable refractometer (RHS-10ATC, Huake instrument, Zhejiang, China) every two days and maintained between 34-35 ppt throughout the experiment by adding the freshwater to make up the loss due to the evaporation. The water temperature of prawn tank was maintained at about 25^{o}C by using one automatic submersible heater (Sonpar®, Model: HA-200, Zhongshan, Guangdong, China).

Samples of NH_3, NO_2^-, NO_3^- and PO_4^{3-} were collected weekly and filtered through GF/C glass fibre filters before analysis.

NH_3 concentration was determined by Phenate method (4500-NH_3 F) (APHA 1998).

NO_2^- concentration was measured by Colorimetric method (4500-NO_2^- B) (APHA 1998).

PO_4^{3-} concentration was determined by Ascorbic acid method (4500-P E) (APHA 1998).

NO_3^- concentration was measured by the method 8191 using a DR/890 Colorimeter (Hach Company, Loveland, Colorado, USA). DIN was sum of TAN, NO_2^-, NO_3^-

TN and TP concentrations were measured fortnightly by digesting unfiltered samples using persulfate oxidation method. TP was then determined by the ascorbic acid method (APHA, 1998). TN was measured through determination of NO_3^- in the digested samples using the method 8191 (Hach Company, Loveland, Colorado, USA).

TSS samples (Chapter 7 and Chapter 8) were collected fortnight and were filtered on Whatman GF/C filters and dried at 60^{o}C until constant weight.

The bacterial (Chapter 7 and Chapter 8) load in the rearing medium was determined at the commencement of the experiment and monthly thereafter. Water samples were processed for TB count by serially diluting the sample 10-fold using 0.85% autoclaved seawater. Portions from each dilution were plated onto marine agar (1.5% sodium chlorine w/v). Bacteria that grew on the marine agar plates were counted after 24 hours incubation at 30^{o}C.

3.4.2 Prawns and mussels growth and survival rates

Western king prawn weights were measured using a potable balance (ACB 600H, Adam Equipment Inc., Danbury CT 06810, USA) with a precision of 0.01 while blue mussels shell length (shell length: the maximum distance between the anterior and posterior margins of the shell) were measured to the nearest 0.05 mm with a calliper at the termination of the experiment (Chapter 7 and Chapter 8).

Survival rates were calculated by the formulae: Survival rate (%) = 100 x (n_t/n_0), where n_t and n_0 are number of animal(s) at time (t) and experiment start (0), respectively.

3.4.3 Determination of N and P contents of animals and plant

Animals were frozen to -20 ^{o}C at the conclusion of the experiment and later defrosted to obtain the N and P contents in their dry muscles. Whole western king prawns were dried in ceramic crucibles at 105 ^{o}C to a constant weight to obtain dry weight in an electric oven (Thermotec 200 Oven, Contherm Scientific Ltd, Hutt city, New Zealand). The dried prawns were pooled for each tank and ground to a powder with a mortar and pestle.

Total protein nitrogen content was measured by the standard method as described in Association of Official Analytical Chemists (AOAC) (1990) with a Kjeltec Auto 1030 Analyser (Tecator, Höganäs, Sweden). For total phosphorus, 1.0 g of dry prawn powder per pooled tank was ignited at 550 ^{o}C for 24 h and digested following the method by Cheng et al. (2005) and then the total phosphorus content was measured by the molybdovanadate methods (AOAC 1990). The mussels, wastes and feed were also dried at 105 ^{o}C to a constant weight while dried green seaweed samples were obtained by drying at 60 ^{o}C to a constant weight and had protein and P contents measured by the above described methods.

CHAPTER 4 EFFECTS OF STOCKING DENSITIES ON WESTERN KING PRAWN CULTURE

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4.1 INTRODUCTION

Stocking density is a major factor affecting production parameters, such as growth, survival yield of prawns (Arnold et al. 2006) and water quality (Thakur and Lin 2003) in culture system. Generally, an increase in stocking density results in a decrease in growth and survival (Williams et al. 1996) and water quality (Thakur and Lin 2003). Reduced growth and survival of prawn reared at high density can result from competition for the space, poor water quality (Forster and Beard 1974) and cannibalism (Abdussamad and Thampy 1994).

An important requirement of intensive prawn aquaculture is to provide large quantities of high-nutritious feed as the quantity of feed provided is a direct function of stocking densities used. Approximately 30% of the nutrients added through the feed are incorporated into the fish biomass at harvest in an intensive aquaculture system (Hall et al. 1992). For the prawn culture in a pond environment, it ranges from 6 – 21% of the total nutrient inputs (Briggs and Funge-Smith 1994; Primavera 1994). As a result, a large amount of nutrients, in the form of uneaten feed and excretory products are directly discharged into the surrounding environment and may result in the deterioration of water quality. This in turn can make the environment unsuitable for prawn culture when exceeding the assimilating capacity of the receiving waters (Thakur and Lin 2003). Therefore, environmental manipulation to boost aquaculture production requires an understanding of the basic physical, chemical and biological processes occurring in the system. To understand the chemical processes, information on the fate of the added nutrient, particularly nitrogen and phosphorus, is essential. The chemical budget can be formulated to account all sources of nutrient gains and losses (Islam et al. 2004) and the basic step to estimate food utilization efficiency, water quality and processed in the sediments of prawn ponds.

Western king prawn is distributed widely throughout the Indo-West Pacific region and around most Australian coastal waters (Dore and Frimodt 1987). It is an economically important species with the estimated annual catch of 1317 tonnes, which makes up approximately 50% of prawn catch in the Western Australia (Fletcher and Santoro 2008). On the other hand, attempts have been made to culture this species in Japan (Shokita 1984), India (Kathirvel et al. 1986) and Australia (Sang and Fotedar 2004a; Sang and Fotedar 2004b; Hai et al. 2007; Mai et al. 2008; Hai et al. 2009b, 2009a; Hai and Fotedar 2009; Hai et al. 2010a; Hai et al. 2010b; Mai et al. 2010). The suitable salinity for aquaculture of western king prawn is reported to be at 34 mg L^{-1} (Sang and Fotedar 2004a). However, there is no information available on the impact of stocking density on the western king prawn growth performance and nutrient budget. The purpose of the present study was to investigate the water quality, nutrient distribution and the growth performances of western king prawn reared at different stocking density in a recirculating aquaculture system.

4.2 MATERIALS AND METHODS

4.2.1 Materials

Western king prawn juveniles (6.02±0.03 g weight) were collected in Bicton in Canning river, Western Australia (32°01′ S, 115°45′ E) in January 2008. Collection and acclimation of western king prawn were presented in the Section 3.2.

The trial was conducted using 12 IRAS at the CARL, located at Bentley, Western Australia. The description and operation of IRAS were presented in the Section 3.1.

4.2.3 Experimental design

Western king prawns were stocked at densities of 4, 8, 16 and 32 prawn m^{-2} and reared for 70 days; this is approximately equated to a biomass of 12, 24, 48 and 96 prawns $IRAS^{-1}$. Three replicate tanks were randomly assigned to each treatment.

The feed was provided once a day and at a rate of 3% of the wet body weight per day. The amount of food was adjusted monthly in relation to the survival and body weight of the western king prawns in each recirculating unit after growth rate sampling. Tank and waste management during the trial period were described in the Section 3.3.

4.2.4 Sample collection and analysis

Western king prawn growth rate was calculated monthly to a 2-decimal place electronic scale, after being blotted dry with a paper towel to remove excess moisture. At the termination of the trial, prawns were counted, weighed individually and then frozen at -20 ^{o}C for further analysis.

Dry weights of formulated feed, whole prawn and waste samples were obtained by drying in ceramic crucibles at 105 ^{o}C to a constant weight. Determination of protein and P contents of whole western king prawn; feed and wastes were described in the Section 3.4.3.

DO, water temperature, salinity and pH were measured in all of the prawn tanks. Determinations of these parameters were presented in the Section 3.4.1.

TAN, NO_2^-, NO_3^- and PO_4^{3-} in the prawn tanks were measured weekly whereas TP and TN samples were collected fortnight. Determinations of these parameters were presented in the Section 3.4.1.

The nutrient budget of N and P was determined based on the inputs such as water, stocked prawns and the feed; and outputs from the harvested prawn, drained water and sediments. The nutrient inputs and outputs in the form of water represented the nutrient contained in the water on the day of prawn stocking and harvesting respectively. Sediment samples were collected by draining the sediment-tanks and then the deposited sediment samples were collected to determine the total mass of sediment deposited. Three sediment samples from each recirculation system were used to determine the total phosphorus and nitrogen concentration and the values were averaged.

4.2.5 Data analysis

Prawn biomass, SGR, survival rate and nutrient conversion rate were calculated by the formulae:

Prawn biomass (g) = sum of individual weight (g)

SGR (% day^{-1}) = 100 x ($lnW_t - lnW_0$)/t (Hopkins 1992):

Nutrient (P/N) conversion rate: 100 x (A-B)/C; Where W_t and W_0 are total weight at time t and the start, respectively.. A is the total nitrogen/phosphorus at harvest; B is the total nitrogen/phosphorus of the prawns when starting the experiment; C is the total nitrogen/phosphorus in feed input.

Nutrient inputs in the form of feed and nutrient inputs and outputs in the form of prawn were calculated as follows:

Nutrients (N/P) in feed = nutrients concentration in feed x total amount of feed supplied.

Nutrients (N/P) in prawn = nutrients concentration in prawn x total prawn biomass.
Unaccounted nutrients were calculated by subtracting out the total nutrient output in prawns, sediments and water at harvest from the total input.

All statistical analysis was performed using the Statistical Package for the Social Science (SPSS) version 15.0 (SPSS, Chicago, IL, USA) for Windows package and results were presented as means ±SE (standard error). To assess normality of distribution a Shapiro–Wilk test was used and homogeneity of variance was tested using Lavene's F test. When necessary, data were transformed using square root or arcsine transformation in order to fit normal distribution curve. If the data still did not have normal distribution and homogeneous variance, the Kruskal–Wallis was conducted to test the overall difference of all treatments. When overall differences

were significant, Mann–Whitney test was used to means between individual treatments. One-way analysis of variance (ANOVA) was performed to examine differences parameters among treatments. Significant ANOVA's were followed by Least significant Difference (LSD) testing to identify differences among treatments. Differences were considered significant at the 5% level of probability.

4.3 RESULTS

4.3.1 Water quality parameters

Increase in the stocking densities of western king prawn juveniles resulted in a decrease in mean DO concentration and pH value. The lowest mean DO and pH was observed in a culture system stocked at a density of 32 prawn m^{-2} (5.37 ± 0.14 mg L^{-1} and 7.72 ± 0.02, respectively) which was significantly ($P<0.05$) lower than mean DO and pH of tanks stocked with other prawn densities (Table 4.1). There was no significant difference ($P>0.05$) in mean DO between two culture systems stocked at 4 and 8 prawn m^{-2} (Table 4.1).

Table 4.1 Effects of four stocking densities of western king prawn on the water quality in a recirculating system

	Stocking rates (prawn m^{-2})			
	4	**8**	**16**	**32**
DO (mg L^{-1})	5.94±0.01^{a}	5.88±0.01^{a}	5.78±0.09^{b}	5.37±0.14^{c}
pH	8.10±0.01^{a}	8.08±0.01^{a}	7.99±0.01^{b}	7.72±0.02^{c}
[1]TAN (mg L^{-1})	0.17±0.03^{a}	0.21±0.04^{a}	0.25±0.04^{a}	0.39±0.07^{b}
NO_3^- (mg L^{-1})	4.07±0.34^{ab}	3.82±0.36^{b}	5.46±0.59^{ac}	6.31±0.69^{c}
NO_2^- (mg L^{-1})	0.30±0.06^{a}	0.31±0.05^{a}	0.31±0.05^{a}	0.33±0.06^{a}
TN (mg L^{-1})	4.99±0.58^{a}	4.75±0.61^{a}	5.88±0.78^{a}	6.46±0.87^{a}
PO_4^{3-} (mg L^{-1})	0.34±0.03^{a}	0.54±0.05^{b}	0.77±0.07^{c}	1.02±0.08^{d}
TP (mg L^{-1})	0.54±0.08^{a}	0.81±0.10^{b}	0.93±0.11^{cb}	1.13±0.12^{c}

[1] Data were transformed (square root) prior to statistical analysis. Values (mean ± SE) within a row sharing a common superscript are not significant different (LSD test; $P<0.05$; n=3).

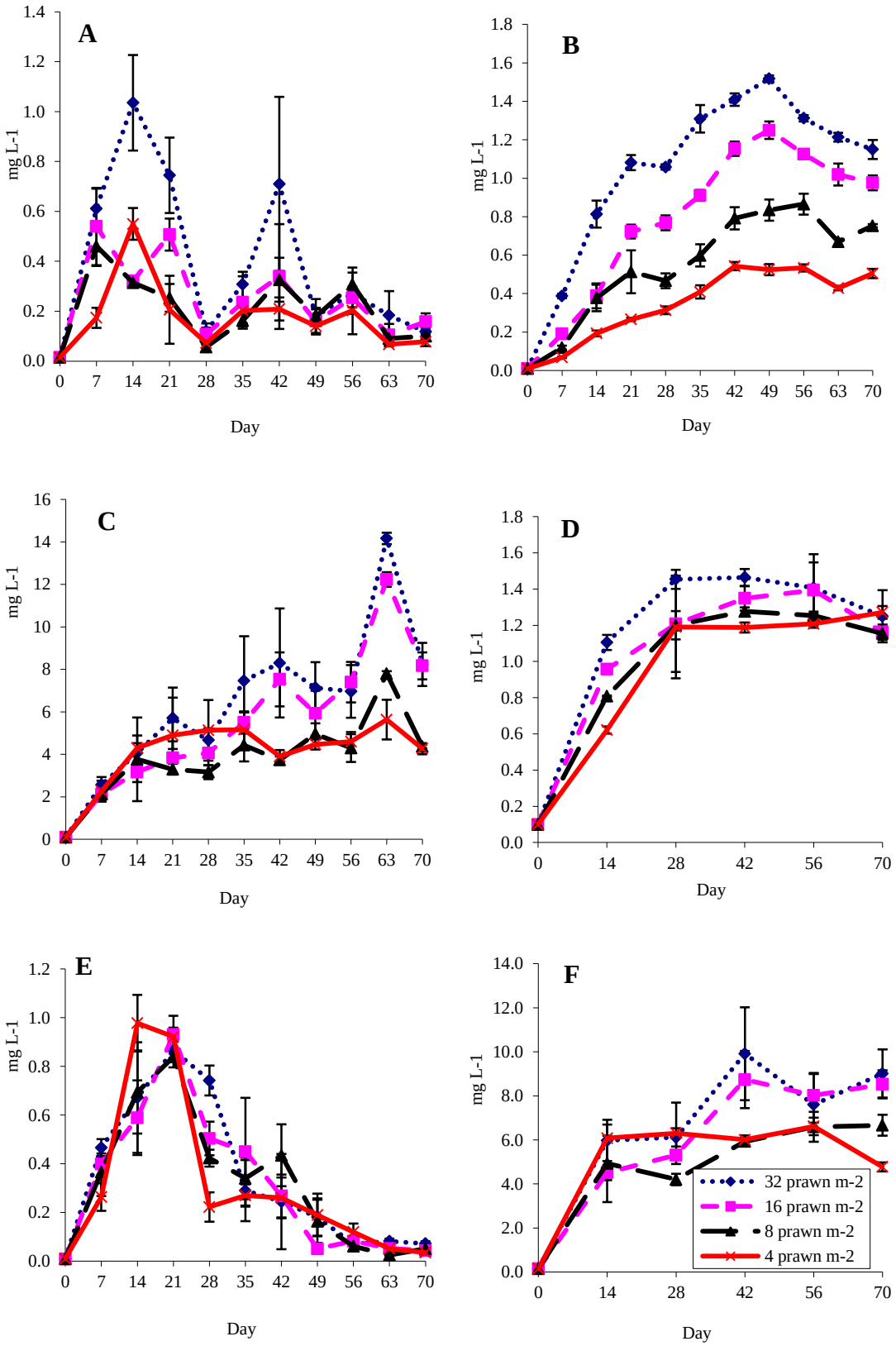

Figure 4.1 Mean concentrations of TAN (A), PO_4^{3-} (B), NO_3^- (C), TP (D), NO_2^- (E), TN (F) in prawn tanks at four stocking densities in a recirculating system during the 70-day trial. Error bars indicate standard error (SE).

TAN fluctuated greatly over the trial period (Figure 4.1A) ranging from 0.02 to 1.41 mg L^{-1} and was affected by the stocking densities. The highest TAN concentration was observed in day 14 of culture period with value of 1.03±0.19 mg L^{-1} at 32 prawn m^{-2}. Mean TAN was 0.39±0.07 mg L^{-1} at 32 prawn m^{-2} which was significantly higher ($P<0.05$) than TAN for the other stocking densities (Table 4.1). The mean total TAN was independent of stocking densities of 4, 8 and 16 prawn m^{-2}.

There was no significant difference ($P>0.05$) in mean water NO_2^- and TN concentrations among any stocking densities (Table 4.1). The NO_3^- of water tanks reared at 32 and 16 prawn m^{-2} were significantly higher ($P<0.05$) than 8 and 4 prawn m^{-2}; and the lowest (3.83 ± 0.36 mg L^{-1}) was recorded in density of 8 prawn m^{-2}. The mean TP of water tanks was highest at prawns reared at 32 and 16 prawn m^{-2}, which was significantly higher ($P<0.05$) than in prawns reared at 4 prawn m^{-2}. The mean PO_4^{3-} concentrations varied from 0.34 ± 0.03 mg L^{-1} to 1.02 ± 0.08 mg L^{-1} (Table 4.1) and was the highest at density of 32 prawn m^{-2}; the lowest was at the density of 4 prawn m^{-2}.

4.3.2 Growth and survival

The western king prawn growth and survival rate decreased with increasing the stocking density. After 70 days of culture, the mean prawn weight was significantly higher ($P<0.05$) at stocking densities of 4 (7.89±0.0.17 g) and 8 m^{-2} (7.82±0.11 g) than 16 (7.46±0.08 g) and 32 m^{-2} (7.26±0.04 g). SGR ranged from 0.24-0.34 % d^{-1} and the highest SGR of the prawns was found at densities with 4 and 8 prawn m^{-2} while the lowest was at a density of 32 prawn m^{-2} (Table 4.2). After 70-day culture, prawn survival was 35.42±2.08% at a density of 32 m^{-2}, which was significantly lower than for prawns stocked at the other densities. There was no significant difference ($P>0.05$) in survival between stocking densities of 8 (75.00±14.43%) and 16 m^{-2} (58.33±8.83%) at the conclusion of the trial. All prawn mortalities showed signs of cannibalism.

Table 4.2 Effects of four stocking densities of western king prawn reared in a recirculating system on the growth performance, survival rate and nutrient conversison rates

	Stocking rates (prawn m^{-2})			
	4	**8**	**16**	**32**
Prawn stock				
Mean weight (g)	6.09±0.08[a]	5.93±0.05[a]	5.99±0.07[a]	6.06±0.03[a]
Biomass (g)	12.19±0.18[a]	23.74±0.22[a]	47.94±0.63[a]	97.02±0.50[a]
Prawn harvest				
Mean weight (g)	7.89±0.0.17[a]	7.82±0.11[a]	7.46±0.08[b]	7.26±0.04[b]
[2]Survival rate (%)				
Day 28	100.00 ±0.00[a]	100.00±0.00[a]	75.00±7.22[b].	68.55±3.61[b]
Day 56	100.00± 0.00[a]	83.33±3.33[ba]	75.00±7.22[b]	43.75±9.55[c]
Day 70	100.00±0.00[a]	75.00±14.43[ba]	58.33±8.83[b]	35.42±2.08[c]
[1]SGR (% d^{-1})	0.34±0.01[a]	0.36±0.01[a]	0.29±0.01[b]	0.24±0.00[c]
[2]N conversion (%)	4.35±2.36[a]	4.11±2.77[a]	0.94±1.89[a]	-11.89±2.14[b]
[2]P conversion (%)	1.86±2.06[a]	1.07±2.05[a]	-0.90±4.89[a]	-7.96±0.38[a]

[1] Data were transformed (square root) prior to statistical analysis. [2] Percentage data were transformed (arcsine) prior to statistical analysis. Values (means ± SE) within a row sharing a common superscript are not significant different (LSD test; $P<0.05$; n=3).

4.3.3 Nutrient conversion rate

The mean N and P contents of the commercial formulated pellet ST #1 were 7.24% and 1.18% wet weight, respectively. Mean N and P contents in western king prawns at harvest ranged from 2.26 to 3.02% and 0.20 to 0.24% of the wet weight, respectively. No significant difference in either the N or P concentrations in the western king prawns was found among any culture systems. N conversion at density of 32 prawn m^{-2} was significantly lower than at other densities. The N conversion rates at densities of 4, 8 and 16 prawn m^{-2} were not significant ($P>0.05$) different (Table 4.2). P conversion into western king prawn biomass at harvest ranged from -7.96 to 1.86% of total P inputs and was not influenced by any stocking densities.

4.3.4 Nutrient budget

The lowest mean N retention (9.34±1.60%) in the prawn biomass at harvest was observed at a density of 32 prawn m^{-2} which was significantly lower ($P<0.05$) than the stocking density of 16 prawn m^{-2}. Mean N retained in western king prawns was higher a densities of 16 (20.13±2.36%) than 8 (17.80±3.92%) and 4 m^{-2} (16.15±1.86%) but was not significant ($P>0.05$) different (Table 4.3). The percentage N accumulated into tank bottom was significantly higher ($P<0.05$) at higher stocking densities. The N in the discharged water at harvest increased with decreasing the stocking densities and was significantly lowest (23.34 ± 2.60%) at 32 prawn m^{-2}. The highest unaccounted N input was obtained in the treatment with 32 prawn m^{-2} and the lowest was in 4 prawn m^{-2} ($P<0.05$) (Table 4.3).

Table 4.3 Nitrogen budget of western king prawn reared at four stocking densities in a recirculating aquaculture system during a 70- day trial

	Stocking rates (prawn m^{-2})			
	4	**8**	**16**	**32**
Input-N				
Water	0.05(2.03±0.04^{a})*	0.05(1.33±0.06^{b})	0.05(0.81±0.05^{c})	0.05(0.39±0.00^{d})
Prawn	0.27(12.42±0.18^{a})	0.49(14.43±1.64^{a})	1.08(19.40±1.29^{b})	2.19(18.93±0.14^{b})
Feed	1.89(85.55±0.21^{a})	2.86(84.24±1.68^{a})	4.49(79.80±1.34^{b})	9.33(80.68±0.14^{b})
Total	2.21(100.00±0.00)	3.39(100.00±0.00)	5.61(100.00±0.00)	11.56(100.00±0.00)
Output-N				
Water	1.43(64.82±3.52^{a})	2.00(58.92±2.58^{a})	2.56(45.59±2.49^{b})	2.70(23.34±2.60^{c})
Prawn	0.36(16.15±1.86^{ab})	0.60(17.80±3.92^{ab})	1.12(20.13±2.36^{b})	1.08(9.34±1.60^{a})
Waste	0.28(12.79±1.53^{a})	0.41(12.14±2.60^{a})	1.31(23.17±1.90^{b})	5.68(49.23±2.91^{c})
Unaccounted	0.14(6.23±1.65^{a})	0.38(11.14±3.58^{ab})	0.63(11.12±2.87^{ab})	2.09(18.09±1.79^{b})
Total	2.21(100.00±0.00)	3.39(100.00±0.00)	5.61(100.00±0.00)	11.56(100.00±0.00)

*Percentage of the compartment (% $IRAS^{-1}$) versus total input-N (g $IRAS^{-1}$) is given in parentheses. Values (mean±SE) within a row sharing a common superscript are not significant different (LSD test; $P<0.05$; n=3).

The P inputs in the form of feed ranged from 84.50 ± 1.07 to 86.45 ± 0.11% of total P input and no significant differences (P>0.05) were found among any stocking densities. The P input sunk into the tank bottoms and P from the discharged water showed a similar trend as was found for the N budget. The P accumulated in tank bottom was highest at density of 32 prawn m^{-2} (49.11 ± 1.82%). The P in discharged water was significantly higher at densities of 4 and 8 prawn m^{-2}.

Table 4.4 Phosphorus budget of western king prawn reared at four stocking densities in a recirculating system during a 70-day trial

	Stocking rates (prawn m^{-2})			
	4	**8**	**16**	**32**
Input-P				
Water	0.03(8.23±0.17^{a})*	0.03(5.56±0.26^{b})	0.03(3.50±0.22^{c})	0.03(1.71±0.02^{d})
Prawn	0.03(7.17±0.10^{a})	0.05(8.60±1.02^{a})	0.10(12.01±0.85^{b})	0.21(11.85±0.09^{b})
Feed	0.31(84.60±0.25^{a})	0.47(85.84±1.22^{a})	0.73(84.50±1.07^{a})	1.52(86.45±0.11^{a})
Total	0.36 (100.00±0.00)	0.54 (100.00±0.00)	0.86(100.00±0.00)	1.76(100.00±0.00)
Output-P				
Water	0.24(65.62±2.45^{a})	0.35(65.20±2.32^{a})	0.38(44.28±1.27^{b})	0.37(21.36±2.60^{c})
Prawn	0.03(8.75±0.82^{a})	0.05(9.47±2.74^{a})	0.10(11.25±4.24^{a})	0.09(4.97±0.29^{a})
Waste	0.07(19.01±2.33^{a})	0.10(17.82±3.45^{a})	0.29(33.17±2.89^{b})	0.86(49.11±1.82^{c})
Unaccounted	0.02(6.61±0.82^{a})	0.04(7.51±1.30b^{a})	0.10(11.30±1.85^{b})	0.43(24.56±1.37^{c})
Total	0.36(100.00±0.00)	0.54(100.00±0.00)	0.86(100.00±0.00)	1.76(100.00±0.00)

*Percentage of the compartment (% $IRAS^{-1}$) versus total input-P (g $IRAS^{-1}$) is given in parentheses. Values (mean ± SE)within a row sharing a common superscript are not significant different (LSD test; P<0.05; n=3).

4.4 DISCUSSION

The present study demonstrated that the recirculating culture system could maintain acceptable water quality for western king prawn culture. However, the increase in TAN concentrations with higher densities of prawn is a direct consequence of TAN being the major excretory product of prawns (Burford and Williams 2001). Although there are no published studies on lethal TAN levels for culturing juvenile western

king prawn, the TAN concentration in our studied are below 1.8 mg L^{-1} recommended for culture of green tiger prawn (*Penaeus semisulcatus*) (Wajsbrot et al. 1990). NO_2^- concentrations in the present study remained below 1.0 mg L^{-1} in all stocking densities, which is below the concentration of 6.0 mg L^{-1} reported to affect the growth in penaeid prawns (Wickins 1976).

Concentrations of DO and pH in the current study were lower at higher prawn stocking densities. However, the prawn growth was unlikely to be limited by DO and pH concentrations as they fell in the favourable limit for some penaeid production. Tsai (1990) considered pH values of below 4.8 or above 10.6 lethal to penaeids with an optimum range of 6.6 to 8.5; prawn growth and food conversion efficiency were reduced outside of optimum range. Apud et al. (1985) observed that a pH of 5 or below in ponds caused mortality of penaeids while lethal values of low pH for the crayfishes, *Procambarus clarki* and *Orconectes rusticus* in acidified water were 2.5 to 2.8 (Morgan and McMahon 1982). In acid waters, crustaceans and fish may experience impaired ionic regulation (Morgan and McMahon 1982; Havas and Hutchinson 1983). Crustaceans can, however, regulate internal pH to some extent through Cl-/HCOr and Na+/H+ exchanges via the gills (Wickins 1984b). The dissolution of exoskeletal calcium carbonate (Defur et al. 1980; Morgan and McMahon 1982) and the release of ammonia and amino acids (Brehm and Meijering 1986) can also buffer pH changes to some extent.

The PO_4^{3-} and TP concentrations in the present study increased with the intensification of stocking densities; the PO_4^{3-} remained lower than the TP concentration, indicating a large portion of the phosphorus in the water was contained in the suspended solids (Midlen and Redding 1998). The soluble phosphorus may react with Fe^{+3}, Al^{+3}, Ca^{+2} or soil colloids and get bound (Boyd 1995) to be later absorbed in the soil sediment. The uneaten food and prawn faeces in the prawn-tank were removed; a large portion of the soluble phosphorus and suspended particles remained in the water column and resulted in an increase in TP and PO_4^{3-} concentrations.

The SGR of western king prawn in the present study (0.24±0.00 to 0.36±0.01 % d^{-1}) are lower (0.45±0.04 % d^{-1} in 42 days at a stocking density of 20 prawn m^{-2}) than in the study conducted by Prangnell and Fotedar (2005). This could be the prawns in Prangnell and Fotedar (2005)'s study were fed to satiation chopped, thawed green mussel, *Perna canaliculatus* or other factors not yet identified. However, the inverse relationship between stocking density and prawn growth are similar to the tiger prawn (Allan and Maguire 1992; Ray and Chien 1992). The lower growth rate at higher stocking densities is direct result of the crowding effect. Rasheed and Bull (1992) observed the behaviours of western king prawn in tanks and found that prawns at high density spent more time active, searching and in contact and less time feeding and remained stationary than did prawns at lower density. Forster and Bread (1974) suggested that crowded condition may result in additional stressors such as poor water quality and insufficient space. Poor water quality may in turn, influence the physiological state of the prawns whilst violating behaviour requirement for space may affect growth though hormonal response or disruption of feeding efficiency (Al-Ameeri and Cruz 2006). In the present study, water quality parameters were all within the safe range for prawn culture (ChenLiu et al. 1990; Tsai and Chen 2002), therefore stress due to space availability may have resulted the reduced feed intake and could be the primary factor for growth inhibition of the prawn stocked at higher densities.

Feeding rate and feeding frequency are not likely to be responsible for the lower growth. Feeding frequency in our study was quite low, compared to previous study for western king prawn (2 feedings d^{-1})(Hai et al. 2009a). Velasco et al. (1999) found that growth of white prawn and water quality in culture tanks were significantly unaffected when prawns were fed more than once per day. Feeding rate in the present study was in range of 3.0-5.0% wet body weight d^{-1} for the western king prawn as reported by Hai et al. (2009).

Western king prawn survival observed in the present study ranged from 35.42±2.08 to 100.00±0.00% which is considerably higher than the 13.64 ± 5.87% of survival at stocking density of 40 prawn m^{-2} after 60 days of culture (Sang and Fotedar 2004a). However, PL_{40} western king prawn at a stocking density of 28 prawn m^{-2} showed

higher survival (67.92 ± 5.75%) when cultured under similar culture system and conditions (Prangnell and Fotedar 2006b). The crowding effect and cannibalism are responsible for high percentage of mortality in freshwater prawns (*Macrobrachium rosenbergii)* (Sandifer and Smith 1975). Arnol et al. (2005) suggested that reduced survival rate at higher densities was generated from an increase in negative behavioural interaction such as cannibalism. The dead western king prawns in the present study were freshly moulted and showed the signs of cannibalism; suggesting that stress caused due to a high density resulted in moult deaths and subsequent cannibalism.

The N and P conversion rates in the present study were lower than observed in white prawn (38.5–47.6% and 18.9–23.4%, respectively) (Muangkeow et al. 2007) and for Chinese prawn (23.4% and 14.7%, respectively) (Tian et al. 2001). The lowest N conversion rate was observed at the 32 prawn m^{-2}, which is probably due to lower prawn survival rate and mean weight at harvest. Thus, efficiency of feed utilization was not obtained at the highest stocking density. The results also suggested that increasing stocking density up to 16 prawn m^{-2} in the recirculating system had not affected the prawn N conversion rate.

The nutrient budget showed that 9.34 ± 1.60–20.13±2.36% of N input were incorporated into western king prawn biomass (Table 4.3). The percentages of total N recovered as harvested prawn are lower compared with other reports for tiger prawn culture of nearly 22% (JacksonPrestonThompson et al. 2003) in intensive prawn farming and 23-31% (Thakur and Lin 2003) in a closed culture system. However, these values are comparable to those reported by Islam et al. (2004) with 12.1% of the N incorporated into prawns biomass when reared in earthen ponds. The results in the present study suggest that N utilization efficiency was not improved in the IRAS, compared to other culture systems. Feed is one of the highest variable costs of prawn farming (Lawrence and Lee 1997) and feed wastage both affects farm profitability and has the potential to contribute to eutrophication of surrounding waterways and (Naylor et al. 1998). P retained by western king prawns was slightly lower (10-13%) than reported by Thakur and Lin (2003) and not significantly

different among treatments; implying that efficiency of the proportional recovery of P was not affected by the stocking densities.

Unaccounted nutrient losses in our study was lower than 32.5 - 39.3% reported by Perez-Velazquez et al. (2008) in a zero-water exchange culture system of white prawn and appeared to be comparable to 5.2-36.0% (Thakur and Lin 2003) for tiger prawn in a closed culture system. N may have been lost through denitrification, NH_3 volatilization and/or diffusion at a higher pH levels (Briggs and Funge-Smith 1994). In the present study, water was circulated from one tank to the others; therefore NH_3 may have been lost though the water movement. Other source of the nutrient loss in the present study may be at the harvest stage. The recirculating system has no sediment in the tank bottoms therefore, some of the wastes deposited in the bottom-tank could have washed out of the tanks with the drained water during harvest.

In the study, higher western king prawn stocking densities of 16 and 32 m^{-2} were recorded to have the highest percentage of N and P inputs trapped in the bottom of waste-collection tanks while the remaining lower densities achieved the highest nutrient loss through water effluents (Table 4.3 and 4.4). Major outputs of nutrients are in the discharge water in the exchanged-water prawn ponds (Teichert-Coddington et al. 2000). However, loss of nutrient through sediments is higher than the water borne loss in a closed prawn culture system due to rapid accumulation of sediments in prawn ponds (Briggs and Funge-Smith 1994; Thakur and Lin 2003). Thakur and Lin (2003) reported 14-53% and 39-67% of N and P inputs, respectively trapped in sediments, and Jackson et al. (2003) up to 57% of N discharged in effluents in an intensive prawn farm. In both cases, a significant proportion could be attributed to organic nutrient.

The study revealed that the recirculating culture system could maintain acceptable water quality for the western king prawn within the tested stocking density. Western king prawn can be reared at stocking densities till 16 prawn m^{-2} in term of N conversion rate, TAN and PO_4. The lower growth of prawn also demonstrates that other limiting factors could be present in the current recirculating system. Further research should focus on the effects of varying feeding rates at a constant stocking

density on the water quality and the growth performances of the western king prawns.

CHAPTER 5 EFFECTS OF FEEDING RATES ON WESTERN KING PRAWN CULTURE

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5.1 INTRODUCTION

Feed rate depends on the species, development stages, culture system and the nutrient composition of the feed. Growth of tiger prawn was 4% higher in the higher feeding rate (5% wet prawn biomass day^{-1}) than in the lower feeding rate (2.5% wet prawn biomass day^{-1}) in fibreglass pools with sediment (Allan et al. 1995). The optimum feeding rate for maximum growth of 5 to 6 g tiger prawn was at 10% prawn biomass per day as dry pellets in prawn farming (Robertson 1988) whilst feed input rate for 0.1-1.8 g banana prawn was estimated at 11-12% prawn biomass in aquaria (Sedgwick 1979b). The accuracy of determine the feeding rate is based mainly on the estimate of the density and size of the stock (FAO 1986b) For western king prawn, a commercially important species in Australia, no data are available on the optimum feeding rate of formulated diet when reared in recirculating aquaculture systems. The only studies available on western king prawns are on their growth and survival rates in inland saline water (Prangnell and Fotedar 2005, 2006b), probiotic usage (Hai et al. 2009a; Hai and Fotedar 2009) and physiological conditions in different environment conditions (Sang and Fotedar 2004b; Hai and Fotedar 2009).

In previous study, Khoi and Fotedar (2010) investigated the effects of stocking density on the nutrient budget and growth of the western king prawn in a recirculating aquaculture system. Limited information on the impact of feeding rates on the water quality and growth particularly related to western king prawn juveniles was found. Feeding rate significantly influenced production (biomass) but did not affected survival of juvenile crayfish, *Cherax destructor* fed at 5, 10 and 15% BW d^{-1} (Mills and McCloud 1983a). Maguire and Leedow (1983) fed school prawns, *Metapenaeus macleayi* (Haswell) at six feeding rates of 0, 2.5, 5.0, 7.5,10.0 and 12.5% of wet prawn biomass d^{-1} in brackish water farming ponds in Australian and found that feeding rate increased in mean male and female prawn weight, biomass and food quotient and these parameters increased as feed rate rose in the range 0-5% but above this level there was little growth response. Western king prawn is a

commercially important prawn and the contribution to environmentally sustainable farming development such as culture in re-circulating system will potentially benefit the growth of culture industry. The hypothesis of this study was that more feeding rates would result in higher nutrient intake and hence higher growth rates of the prawns. The aim of this study was to investigate the water quality, nutrient distribution and the growth performances of western king prawn by applying different feeding rates in a recirculating aquaculture system.

5.2 MATERIALS AND METHODS

Over 150 western king prawns with an initial mean weight of 4.60±0.13 g were caught by using a drag net in Canning river, Western Australia (32°01′ S, 115°45′ E). Collection of prawns and acclimation protocol were described in the Section 3.2.

5.2.1 Experiment setup

The experiment was conducted using 12 IRAS at the CARL, located at Bentley, Western Australia. The description and operation of IRAS were presented in the Section 3.1.

Four feeding levels of 3.0; 4.5; 6.0 and 7.5% of prawn wet weight day^{-1} were set up in triplicate. The western king prawns were stocked at a density of 16 prawn m^{-2} and cultured for 98 days. Feed (ST#1) was given twice a day, and western king prawns were measured monthly for the total weight and immediately returned to the tank after measuring. Tank and waste management during the trial period were described in the Section 3.3.

5.2.2 Data collection

Western king prawn growth rate was measured monthly for the total weight .The weight gain (%) and (SGRs) (% d^{-1}) of the western king prawns were calculated by using the equations Weight gain = 100 x $(Wt-W_0)/W_0$ and SGRs = 100 x $(\ln W_t - \ln W_0)/t$; W_t and W_0 are the weight of the prawns at current time (t) and at the commencement of the experiment (0), respectively.

DO, pH and salinity were measured as described in the Section 3.4.1. TAN, NO_2^-, NO_3^- and PO_4^{3-} samples were collected weekly and determined as described in Section 3.4.1. DIN is the sum of TAN, NO_2^- and NO_3^- concentrations in each prawn tank.

TP and TN were collected fortnightly and measured as described in the Section 3.4.1.

The whole western king prawns, feed pellets and waste deposits were dried in an oven at 105 °C for a constant weight. Total protein and phosphorus contents of these samples were determined as described in the Section 3.4.3.

Nutrient inputs were measured initially in the water, in the stocked prawns and feed. Nutrient outputs were also determined from harvested prawns, drained water and waste deposits. Waste deposited samples were collected by draining the waste-collection tanks; and then the waste deposit was left to dry for two days to determine the total quantity of the deposited waste.

Food conversion ratio (FCR) was calculated based on the quantity of food input and the increase in biomass as follow: $FCR = F_i/\Delta_w$; Where, F_i and Δ_w are the total food input and the total weight increase respectively.

Nutrient (P/N) conversion rate was calculated using the formula (Muangkeow et al. 2007): N/P conversion rate (%) = 100 x (A-B)/C; Where, A and B are the total nitrogen/phosphorus of prawns when finishing and starting the experiment, C is the total nitrogen/phosphorus in feed input.

5.2.3 Statistical analysis

The SPSS statistical program for Windows package (version 15.0) was used to analyse data and results were presented as means ±SE (standard error). ANOVA and LSD *post hoc* tests were used to determine the significant differences at P = 0.05 between mean water parameters, prawn survival and growth rates, and nutrient

budget of the prawns reared at different feeding rates. Where the data did not have normal distribution and homogeneous variance, the Kruskal–Wallis was used to test the overall difference of all treatments. In the case of significant treatment effects, Mann–Whitney's test was applied to analyse the significant difference between the means of each treatment.

5.3 RESULTS

5.3.1 Growth, FCR and survival

Feeding rates influenced significantly ($P<0.05$) the SGRs and weight gain of western king prawns (Table 5.1). The highest SGRs (0.76 ± 0.02 % day^{-1}) was observed in the western king prawns fed 3.0%, and SGRs and weight gain of western king prawns were independent of the feeding rates from 4.5 to 7.5% (Table 5.1). Western king prawn survival rate for all feed rates were high (83.33-85.55%) and there was not significant effect of feed rate on survival rate ($P>0.05$) (Table 5.1). Western king prawns fed the feeding rates of 3.0 % and 4.0% had significantly ($P<0.05$) lower FCR than those fed feeding rates of 6.0 and 7.5% (Table 5.1).

Table 5.1 Weight gain, SGRs, survival rate, FCR and nutrient conversion rates of the western king prawn reared 98 days in a recirculating system at different feeding rates.

	Feeding rates (% wt d^{-1})			
	3.0	**4.5**	**6.0**	**7.5**
Weight gain (%)	88.81 ± 2.85^{a}	79.87 ± 8.88^{b}	74.89 ± 4.87^{b}	74.71 ± 5.99^{b}
SGR (% d^{-1})	0.76 ± 0.02^{a}	$0.68\pm0.0.06^{b}$	0.66 ± 0.03^{b}	$0.66\pm0.0.04^{b}$
*Survival rate (%)	88.55 ± 5.55^{a}	83.33 ± 9.62^{a}	83.33 ± 0.00^{a}	88.55 ± 5.55^{a}
FCR	3.42 ± 0.39^{a}	4.25 ± 0.38^{a}	6.23 ± 0.37^{b}	8.80 ± 0.94^{c}
N conversion (%)	10.91 ± 1.56^{a}	5.32 ± 3.19^{ab}	4.13 ± 0.02^{b}	3.80 ± 0.61^{b}
P conversion (%)	8.11 ± 1.18^{a}	3.90 ± 1.13^{b}	3.51 ± 0.17^{b}	3.74 ± 0.41^{b}

Values in the same row sharing a common superscript letters (a, b, c) are not significant different (LSD test; $P<0.05$; n=3). *Data transformed (arcsine) prior to statistical analysis.

5.3.2 Nutrient conversion

Nutrient conversion rates increased significantly with decreasing the feeding rates. The conversion rate in the feeding rates of 3.0% (10.91±1.56%) was significantly higher ($P<0.05$) than those in the feeding rates of 6.0 and 7.5% (Table 5.1). There was no significant ($P>0.05$) difference in N conversion rates between the feeding rates of 6.0% and 7.5% or feeding rates of 3.0% and 4.5% (Table 5.1). The highest P conversion rate was also recorded at the feeding rate of 3.0% (8.11±1.18%), this was significantly higher ($P<0.05$) than for western king prawns reared at other feeding rates. The P conversion rate of western king prawns did not change significantly ($P>0.05$) between feeding rates of 4.5 and 7.5%(Table 5.1).

5.3.3 Water quality variables

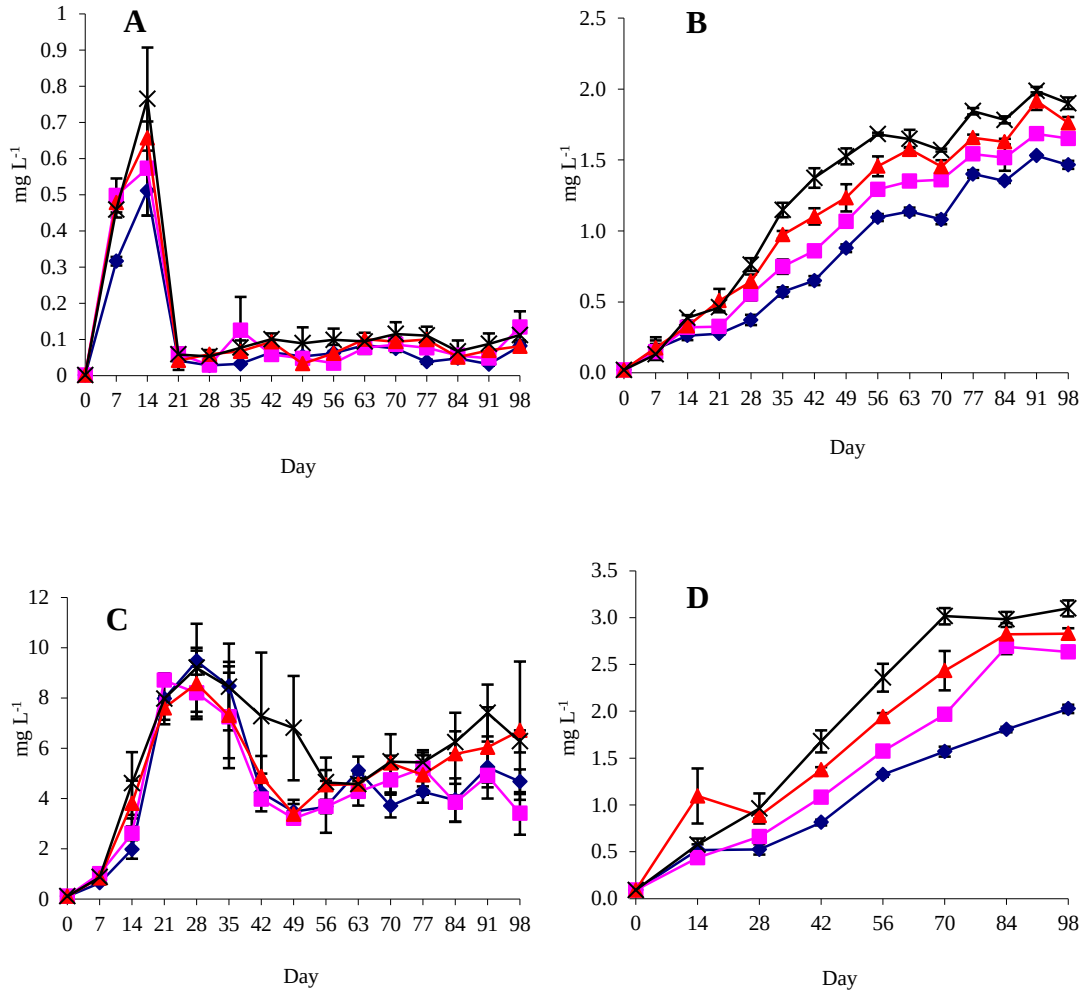
Increasing the feeding rate from 3.0% to 7.5% of body weight had effects on DIN and NO_3^- concentrations of water tanks. Mean DIN were 5.20±0.24 and 5.11±0.24 g mL^{-1} in water tanks fed at 3.0 and 4.5% respectively, which were significantly lower ($P<0.05$) than in those fed at 6.0 and 7.5% (Table 5.2). NO_3^- was significantly higher in the prawn tanks receiving 7.5% (5.68±0.45 mg L^{-1}) than those receiving the feeding rates of 3.0% (4.46±0.41 mg L^{-1}) and 4.5% (4.33±0.38 mg L^{-1}) (Table 5.2). TAN of water tanks ranged from 0.01 to 0.94 mg L^{-1} and mean TAN was not significantly different among treatments. Similarly, the NO_2^- and TN concentrations were not significantly ($P>0.05$) affected by the feeding rates.

Table 5.2 Summary of water quality variables obtained during 98 days trial in prawn tanks where western king prawns were fed at feeding rates of 3.0, 4.5, 6.0 and 7.5% of wet body weight

	Feeding rates (% wt d^{-1})			
	3.0	**4.5**	**6.0**	**7.5**
TAN (mg L^{-1})	0.18 ±0.02^{a}	0.20 ± 0.04^{a}	0.21±0.05^{a}	0.22±0.03^{a}
NO_3^- (mg L^{-1})	4.46±0.41^{a}	4.33±0.38^{a}	4.96±0.42^{ab}	5.68±0.45^{b}
DIN (mg L^{-1})	5.20±0.24^{a}	5.11±0.24^{a}	5.79±0.34^{b}	6.69±0.61^{b}
PO_4^{3-} (mg L^{-1})	0.82±0.07^{a}	0.96±0.08^{ab}	1.05±0.09^{bc}	1.21±0.10^{c}

TP (mg L^{-1})	1.08±0.14[a]	1.39±0.19[ab]	1.68±0.20[b]	1.84±0.23[b]
NO_2^-(μg L^{-1})	338.47±6.37[a]	3.55.63±21.98[a]	281.03±20.79[a]	396.81±32.80[a]
TN (mg L^{-1})	5.98±0.08[a]	6.75±0.16[a]	6.64±0.22[a]	7.25±0.48[a]

Values within a row sharing a common superscript are not significant different (LSD test; $P<0.05$; n=3). The linear relationship between TP (Y) and feeding rate (X) ($Y=0.0947X+0.5971$; $R^2=0.9981$); and PO_4^{3-} (Y) and feeding rate (X) ($Y=0.1717X+0.5996$; $R^2=0.9823$).



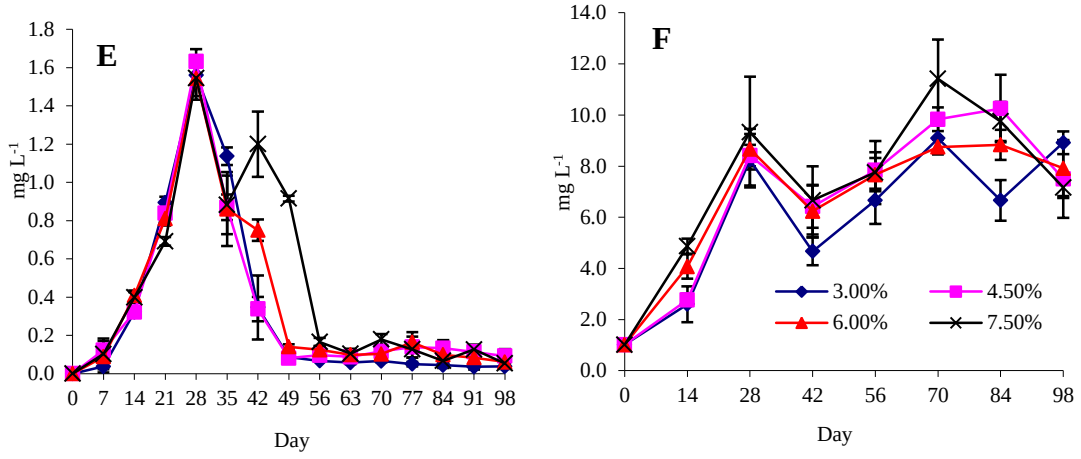


Figure 5.1 Mean concentrations of TAN (A), PO_4^{3-} (B), NO_3^- (C) TP (D), NO_2^- (E) and TN (F) at different feeding rates over 98-day trial of the western king prawn reared in a recirculating aquaculture system

The PO_4^{3-} and TP concentrations in water tanks increased linearly with the increase of feeding rates (R^2=0.9823; R^2=0.9981, respectively) (Figure 5.1B and 5.1D). Mean $PO_4^{3-}$and TP of water tanks receiving the feeding rates of 3% was significantly lower than those receiving the feeding rates of 6.0 and 7.5% (Table 5.2). Feeding rates of 4.5, 6.0 and 7.5% did not significantly (P>0.05) affect the TP concentrations in the prawn tanks. DO throughout the trial was between 4.93±0.35 and 7.27±0.15 mg L^{-1} and mean DO concentrations were significantly (P<0.05) lower in the prawn tanks given at feeding rates of 7.5 % (4.93±0.35 mg L^{-1}) and 6.0 % (5.27 ± 0.28 mg L^{-1}) than in those given at feeding rates of 4.50 % (5.51±0.45 mg L^{-1}) and 3.0 % (6.27±0.15 mg L^{-1}). Mean pH was 7.91± 0.56 and varied from 7.78 ± 0.34 to 8.21± 0.23. The lowest value was recorded at prawn tanks given at feeding rate of 7.5% (7.78±0.34), which was significantly (P<0.05) lower than those at other feeding rates.

Table 5.3 Nitrogen budget of western king prawn reared in a recirculating system during a 98-day trial

	Feeding rate (%)			
	3.0	**4.50**	**6.0**	**7.50**
Input-N				
Water	$0.31\pm0.00_1$ (5.86 ± 0.01^a)	$0.31\pm0.00_1$ (4.22 ± 0.08^b)	$0.31\pm0.00_1$ (3.23 ± 0.00^c)	$0.31\pm0.00_1$ (2.88 ± 0.11^d)
Prawn	$0.55\pm0.01_1$ (10.48 ± 0.13^a)	$0.58\pm0.01_1$ (7.99 ± 0.19^b)	$0.59\pm0.01_1$ ($6.25\pm0.10_c$)	$0.57\pm0.02_1$ ($5.37\pm0.37_d$)
Feed	$4.36\pm0.00_1$ (83.65 ± 0.12^a)	$6.37\pm0.13_2$ (87.79 ± 0.25^b)	$8.58\pm0.00_3$ (90.52 ± 0.09^c)	$9.78\pm0.43_4$ (91.75 ± 0.48^d)
Total	$5.22\pm0.01_1$ (100.00 ± 0.00^a)	$7.26\pm0.13_2$ (100.00 ± 0.00^a)	$9.48\pm0.01_3$ (100.00 ± 0.00^a)	$10.66\pm0.42_4$ (100.00 ± 0.00^a)
Output-N				
Water	$2.68\pm0.13_1$ (51.28 ± 2.61^a)	$2.25\pm0.46_1$ (30.80 ± 5.69^b)	$2.38\pm0.33_1$ (25.07 ± 3.45^b)	$2.15\pm0.13_1$ (20.32 ± 1.96^b)
Prawn	$0.83\pm0.13_1$ (15.99 ± 2.41^a)	$0.65\pm0.32_1$ (8.82 ± 4.17^{ab})	$0.60\pm0.00_1$ (6.37 ± 0.04^b)	$0.63\pm0.09_1$ (6.01 ± 0.99^b)
Waste	$0.97\pm0.14_1$ (18.51 ± 2.61^a)	$2.65\pm0.62_2$ (36.85 ± 9.10^b)	$4.78\pm0.05_3$ (50.47 ± 0.55^b)	$5.90\pm0.74_3$ (45.54 ± 5.12^b)
Uncounted	$0.74\pm0.08_1$ (14.20 ± 1.55^a)	$1.70\pm0.20_2$ (23.53 ± 2.85^{bc})	$1.72\pm0.36_2$ (18.10 ± 3.80^{ab})	$1.98\pm0.15_3$ (28.13 ± 2.46^c)

Data of gram per recirculating system column within a row sharing a common superscript (1, 2, 3, 4) are not significant different (LSD test; $P<0.05$; n=3). Data of percentage column within a row sharing a common superscript letters (a, b, c and d) are not significantly different (LSD test; $P<0.05$; n=3)
Percentage data were transformed (arcsine) prior to statistical analysis.

Table 5.4 Phosphorus budget of western king prawn reared in a recirculating system during a 98-day trial

	Feeding rates (%)			
	3.0	**4.5**	**6.0**	**7.5**
Input-P				
Water	$0.027\pm0.000_{1}(2.83\pm0.00^{a})$	$0.027\pm0.000_{1}(1.99\pm0.04^{b})$	$0.027\pm0.000_{1}(1.50\pm0.00^{c})$	$0.027\pm0.000_{1}(1.33\pm0.06^{d})$
Prawn	$0.062\pm0.001_{1}(6.45\pm0.08^{a})$	$0.065\pm0.001_{1}(4.81\pm0.12^{b})$	$0.067\pm0.001_{1}(3.71\pm0.06^{c})$	$0.064\pm0.002_{1}(3.17\pm0.22^{d})$
Feed	$0.865\pm0.000_{1}(90.72\pm0.08^{a})$	$1.263\pm0.026_{2}(93.19\pm0.15^{b})$	$1.700\pm0.000_{3}(94.78\pm0.06^{c})$	$1.939\pm0.086_{4}(95.50\pm0.28^{d})$
Total	$0.954\pm0.001_{1}(100.00\pm0.00)$	$1.355\pm0.026_{2}(100.00\pm0.00)$	$1.794\pm0.001_{3}(100.00\pm0.00)$	$2.030\pm0.084_{3}(100.00\pm0.00)$
Output-P				
Water	$0.542\pm0.008_{1}(56.79\pm0.89^{a})$	$0.807\pm0.023_{2}(59.61\pm2.38^{a})$	$0.847\pm0.036_{2,3}(47.19\pm1.96^{b})$	$0.894\pm0.024_{3}(44.23\pm2.38^{b})$
Prawn	$0.089\pm0.006_{1}(9.29\pm0.68^{a})$	$0.077\pm0.011_{1}(5.67\pm0.71^{b})$	$0.085\pm0.002_{1}(4.72\pm0.09^{b})$	$0.091\pm0.006_{1}(4.53\pm0.39^{b})$
Waste	$0.189\pm0.007_{1}(19.83\pm0.73^{a})$	$0.327\pm0.030_{2}(24.08\pm1.80^{a})$	$0.618\pm0.043_{3}(34.45\pm2.39^{b})$	$0.748\pm0.038_{4}(32.18\pm3.19^{b})$
Unaccounted	$0.134\pm0.005_{1}(14.10\pm0.54^{a})$	$0.144\pm0.006_{1}(10.65\pm0.53^{a})$	$0.245\pm0.042_{1,2}(13.63\pm2.34^{a})$	$0.296\pm0.128_{2}(19.05\pm5.50^{a})$

Data of gram per recirculating system column within a row sharing a common superscript (1, 2, 3, 4) are not significant different (LSD test; $P<0.05$; n=3). Data of percentage column within a row sharing a common superscript letters (a, b, c and d) are not significantly different (LSD test; $P<0.05$; n=3)
Percentage data were transformed (arcsine) prior to statistical analysis.

5.3.4 Nutrient budget

The majority of nutrient inputs were from feed which accounted for 83- 91% of nitrogen and 90-95% of phosphorus of the total inputs. On average, 6.01% to 15.99% of the N-input and 4.53 to 9.29% of the P-input were retained as the harvested prawns (Table 5.3 and Table 5.4). N retention at the feeding rate of 3% was significantly greater ($P<0.05$) than those at the feeding rates of 6.0% and 7.5%, whilst no significant difference between N retention subject to 4.5, 6.0 and 7.5% of feeding rates was found. The highest P retention was recorded in the recirculating system receiving the feeding rate of 3.0%. The P percentage retained in the prawn biomass at harvest was not significantly affected by feeding levels of 4.5, 6.0 and 7.5% (Table 5.4).

The highest amount of N was accumulated in the waste collection tank receiving feeding rates of 6.0 (4.78±0.50 g) and 7.5% (5.90±0.78 g) which was significantly higher ($P<0.05$) than western king prawns fed feeding rates of 3.0 and 4.5% (Table 5.3). Feeding rates however did not influence the amount of N loss through effluent water at the harvest. On the contrary, the percentage of P loss at harvest in the drained water ranged between 44.23% and 56.79% of total P-input and the amount of P discharged in the drained water was highest in the feeding rates of 7.5 % and 6.0% (Table 5.4). This was significantly higher ($P<0.05$) than for recirculating units receiving feeding rates of 3.0 and 4.5%.

5.4 DISCUSSION

These results are contrary to the hypothesis that western king prawns would grow faster if they were provided with more commercial prawn diet. The growth results obtained for each feeding rate indicated that there was little value in supplying feed at rates in excess of 3.00% of biomass d^{-1}. Results were contrary to the study of Maguire and Leedow (1983) whom reported the increase of feeding rates (0-12.5 % BW d^{-1}) resulted in an increase of mean male and female prawn weight, biomass and food quotient and these parameters increased as feed rate rose in the range 0-5% but above this level there was little growth response. In the present study, as feeding rate increased from 3.0 to 7.5% of body weight d^{-1}, there was a decline on SGRs, weight

and biomass gain of western king prawn. , indicating that adding more feed to the culture system does not necessarily increase the western king prawn growth but can cause deleterious effects on the water and sediment quality. Maguire and Leedow (1983) estimated that in brackish water farming pond the optimum feeding rates for small juvenile school prawns, *Metapenaeus macleayi* in terms of growth was 4.7% of wet biomass d^{-1}, with dry artificial diets of 39.1% protein. Western king prawn in the study were fed a commercial diet with higher protein content (43%) than those in the study of Maguire and Leedow (1983), suggesting that protein content of given diet may affect the feed intake of the prawns. Feed intake may vary with temperature (Maguire 1980), dietary food attractant (Deshimaru and Yone 1978a), protein (Deshimaru and Yone 1978b) and energy content (Sedgwick 1979a). SGRs values in the present study estimates are comparable to those reported by Hai et al. (2009a) with SGRs of 0.74-0.85% d^{-1} when rearing western king prawn using the same formulated diet as in the present trial and feeding rates of 3-5%. However, the SGR are lower compared with 1.2 % d^{-1} (Sang and Fotedar 2004a) when western king prawn were fed flesh blue mussels or 0.83-0.88 % d^{-1} (Hai and Fotedar 2009) when western king prawn were given supplemented prebiotics (Bio-Mos® and β-1,3-D-glucan) commercial diets. Many attempts have been made to culture western king prawn in India (Kathirvel et al. 1986; Kathirvel and Selvaraj 1987; Kathirvel et al. 1987), China (Wang et al. 2004) and Western Australia (Sang and Fotedar 2004a; Sang and Fotedar 2004b; Prangnell and Fotedar 2005, 2006b, 2006a; Prangnell 2007; Mai et al. 2008; Prangnell and Fotedar 2009; Mai et al. 2010). These studies focused on the physiology conditions (Prangnell and Fotedar 2006a; Prangnell and Fotedar 2009), integrated culture (Mai et al. 2010) and artificial breeding(Kathirvel et al. 1987) of western king prawns. There are lacks of researches on the nutritional requirements of juvenile western king prawns. Moreover, growth of prawns was affected by feed types. Crear et al. (2000) evaluated six commercial prawn pelleted feeds and fresh blue mussel, *Mytilus edulis* as feeds for juvenile rock lobster, *Jasus edwardsii*. Three of prawn feeds were formulated for the kuruma prawn and the other three for tiger prawn. Lobster grew significantly better on the mussel than on the prawn pelleted feeds. Therefore, it is recommended to have studies on the nutrient requirements and the optimum feed formulation for western king prawn. Survival rate of the western king prawn in this study was unaffected by feeding rates. Similar results were obtained by Mill and McCloud (1983b) for the yabby (*Cherax*

destructor) reared in ponds, and Allan et al. (1995) for tiger prawn juveniles reared in fibreglass pools and Maguire and Leedow (1983) for school prawn (*Metapenaeus macleayi*) cultured in brackish water ponds.

Lower FCR values and higher nutrient conservation rates were observed at feeding rates of 3.0% and 4.5% biomass d^{-1}, suggesting that there was little feed efficient utilization in supplying feedat rates in excess of 4.5% of biomass d^{-1}. Boyd et al. (1979) reported lower feed conversion value was sign of poor feed quality and low utilization. The P conversion rate in the present study was close to Chinese prawn reported by Tian et al. (2001) whilst the N and P conversion rates were lower than reported by Muangkeow et al. (2007) for white prawn (38.5–47.6% and 18.9–23.4% respectively). This was due to higher FCR (3.42-8.80) in the study than 1.13-1.27 in the work conducted by Muangkeow et al (2007).

Growth of western king prawn in the present study was not limited by the water quality as all water quality parameters were within the acceptable limits for penaeid production (ChenLiu et al. 1990; ChenTing et al. 1990). Although there are no known studies on lethal DO and pH levels for culturing juveniles western king prawn, DO concentrations and pH values in the current study are above concentrations of 4.0 mg L^{-1} and within the range of 7.5-8.5, respectively, that are recommended for culture of tiger prawn (Chien 1992). Sub-lethal low pH can affect maturation and reproduction in crustaceans (Walton et al. 1982; Zimmer and Starr 1984). Low pH can also influence the impact of potential toxins, e.g. ammonia (Alabaster and Lloyd 1980; Colt and Armstrong 1981) and heavy metals (Boyd 1989). Growth and moulting frequency of prawn, *Penaeus occidentalis* and tiger prawn were reduced and carapace dry weight increased when prawns were exposed for 36 to 56 days to seawater with a pH reduced from 7.9 to 6.4 by the addition of carbon dioxide (Wickins 1984a). TAN values were low (0.01-0.94 mg L^{-1}) in the present study, compared to earlier reports by (Chen and Tu 1991) for tiger prawn post larvae but it was comparable to those reported by Khoi and Fotedar (2010) (0.02-1.41 mg L^{-1}) for western king prawn. These authors claimed that high TAN levels in aquaculture system are directly dependant on the feeding rates (Boyd 1990; Briggs and Funge-Smith 1994). It is in contrast to the current results that TAN is not affected by feeding rates indicates that feed input was below the carrying capacity of the recirculating systems. TAN levels peaked on 14^{th} day of the culture followed by an increase in NO_2^- concentration on 28^{th} day, showing that it took 4 weeks to establish the nitrification process to initiate. This duration was slightly shorter than the 8 weeks needed to establish the nitrification process in tiger prawn concrete culture tanks by Thakur and Lin (2003). Nitrification is affected by dissolved oxygen concentration, temperature, substrate concentration, pH, numbers of nitrifying bacteria, and availability of surfaces. Many of these factors are interrelated and their effect on nitrification is complex (Hargreaves 1998).

The NO_3^- concentrations and DIN in this study increased with increasing feeding rates. However, NO_3^- concentrations are below the tolerance levels of NO_3^- for seawater culture (less than 20 mg L^{-1} NO_3^-) (Spotte 1979), indicating that NO_3^- concentrations in the recirculating system in this study were at a safe level for the

culture of western king prawns. NO_3^- concentration in the recirculating water system can reach 500 mg L^{-1} (Pierce et al. 1993), however, it may vary from 2.26 to 4.52 mg L^{-1} in commercial ponds rearing penaeid prawns (Muir et al. 1991).

In the present study, higher feeding levels resulted in a higher PO_4^{3-} and TP concentrations which are in agreement with Boyd (1990) who reported the increase in TP when feeding rates increased in channel catfish ponds. The PO_4^{3-} and TP in the study (Table 5.1) are higher than reported by Thakur and Lin (2003) for tiger prawn (311-384 μg L^{-1} and 517-864 μg L^{-1}, respectively) reared in the concrete tanks without water exchange. Boyd (1990) mentioned that dissolved orthophosphate concentrations in highly eutrophic water are usually not greater than 5- 20 μg L^{-1} and seldom exceed 100 μg L^{-1}.

The difference can be explained by the fact that there are no sediments in the tanks, therefore, the phosphorus concentration equilibrium in the recirculating system may skewed in favour of the water. In addition, PO_4^{3-} concentration remained much lower than TP concentration, suggesting that a large portion of water phosphorus was contained in suspended solids. Boyd (1990) stated that anaerobic pond mud strongly absorbs phosphate and is eventual recipient of the most of the P added to the aquaculture ponds. Midlen and Redding (1998) estimated over half of the P inputs are bound in the soils of the pond bottom in a relative insoluble form.

The results of the present study confirm previous observations that in intensive prawn farms, most of the N and P originate from added feeds (Briggs and Funge-Smith 1994; Funge-Smith and Briggs 1998; Thakur and Lin 2003). However, the percentage of total N recovered as harvested prawn in the present study was approximately 6.01-15.99% and close to the 21% obtained in a previous study of intensive prawn farms in Thailand (Briggs and Funge-Smith 1994). These results are lower than those estimated for tiger prawn (Funge-Smith and Briggs 1998; Thakur and Lin 2003; Hari et al. 2005) and white prawn (Muangkeow et al. 2007). The highest nutrient retained by the western king prawn was in the feeding rate of 3.0%, implying the highest feed efficiency. The nitrogen and phosphorus nutrient losses in the current study increased with the feeding levels and are higher compared to other works (Teichert-Coddington et al. 2000; Thakur and Lin 2003; Islam et al. 2004). Nitrogen nutrient budget revealed that a large amount of N-inputs in feeding rates of 6.0% and 7.5% was deposited into tank bottoms as the wastes in the recirculating system, indicating that some signs of overfeeding were occurring at these feeding

rates. That concurred with Burford and Wiliams (2001) who claimed that the higher amount of nutrient waste generated could be due to overfeeding, quality of ingredients or suboptimal feed formulation.

The study reveals that feeding level of 3.0% wet weight is suitable for western king prawn growth in term of water quality and nutrient conversion rate in the recirculating aquaculture system. However, the lower growth rate and higher FCR of the western king prawns in the present study indicate that there may be other limiting factors for their growth. Future research should focus on the optimum feed formulation for western king prawn culture.

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CHAPTER 6 INTEGRATING PRAWN CULTURE WITH SEAWEED

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6.1 INTRODUCTION

An important principle of intensive aquaculture is to provide large quantities of high-nutrient feed to cultured animals. However, only 30% of N added through feed is removed through fish harvest in an intensive fish farming (Hall et al. 1992). For western king prawn culture, only 9.34-20.13% N and 4.97-11.25% P inputs are retained in prawn biomass and the rest are wasted in water and sediment (Khoi and Fotedar 2010). The remaining amount of the dissolved nitrogen which is released to the surrounding environment depends on the species, culture systems, feed quality and feeding management (Smith et al. 2002). Troell et al. (1999) reported that NH_4^+ accounts for about 50-60% of supplied N discharged into the surrounding waters. Therefore, it is necessary to develop water treatments methods for discharged water in order to minimise the negative impacts of intensive farming activities to the surrounding environment.

The dissolved nitrogen compounds can be removed by several methods. Jiménez del Río et al. (1996) proposed three approaches for waste water treatments (i) microbial oxidation by means of the active sludge technique or biological beds, (ii) two step removal process, where waste waters are used to grow bivalves and the residual nutrients dissolved into the water are removed by seaweeds and (iii) removed by direct absorption of dissolved N by seaweeds. Each technique has advantages and disadvantages and the simplest and cheapest technique is to use the seaweeds as a direct biofilter.

Recently, integrated closed recirculation systems have been considered as an effective strategy to minimize wastes from culturing system and avoid disease infections through water intake (Muangkeow et al. 2007). In these systems, water with high organic particles and nutrients from intensive prawn ponds flow to the treatment ponds and then recycle back to the prawn ponds. Dissolved nutrients in prawn effluent are predominantly ammonium nitrogen (Jones et al. 2001) which

makes *Ulva* sp. suitable for integrating with prawn systems as Ulvales have high tolerance and affinity for NH_4^+ uptake (Lehnberg and Schramm 1984). Previous studies have focussed on the integration of *Ulva* sp. with finfish (Jiménez del Río et al. 1996) and abalone (Neori et al. 1998; Neori et al. 2000). Little information is available on integrated seaweeds and prawns, except for Ulva co-cultured with white prawns (Copertino et al. 2009; Cruz-Suárez et al. 2010). Yamasaki et al. (1997) showed that integration of kuruma prawn larvae and seaweed, *Ulva pertusa* resulted in higher survival and better growth of prawn larvae, and lower bacterial density in seaweed treatments.

Western king prawns are widely distributed throughout the Indo-west pacific region (Dore and Frimodt 1987), and throughout warm and temperate waters of the Australian coast. Studies on the culture of the species have been documented by Sang and Fotedar (2004a; 2004b), Prangnell and Fotedar (2005, 2006b), Hai et al. (2009b, 2009a;) and Khoi and Fotedar (2010). There is a little information available on integrating seaweeds, *Sargassum* spp. culture with western king prawns except research by Mai et al. (2008; 2010). This study aims to investigate the water quality, nutrient conversion and nutrient budget when western king prawns are integrated with green seaweed in a closed recirculating aquaculture system.

6.2 MATERIALS AND METHODS

6.2.1 Collection of western king prawns and seaweeds

Western king prawn with an initial weight of 4.80±0.01g were caught and acclimated as described in the Section 3.2.

Green seaweed were collected from shallow waters of Canning river in Walter Point, Bicton Western Australia (32^{o}00'S, 115^{o}47'E), as described in the Section 3.2.

6.2.2 Design of IRAS

The experiment was carried out using fifteen experimental units as described in Section 3.1 in indoor tanks of CARL under laboratory conditions. The reservoir tanks of IRAS in this trial were referred as seaweed tanks.

The seaweed tanks were aerated at a moderate rate by pipes embedded in the round bottom. Holes of 2.0 mm were spaced at 8.0-10.0 cm intervals along the pipes, so that the large bubbles were produced which stirred the seaweed gently. Light was provided by banks of blue/white (2 white tubes x 2 blue tubes) T5 HO aquarium tubes (Horizon 2ft, Octopus company, Lansvale, NSW, Australia) suspended 30 cm above the seaweed tanks, which delivered irradiance of 80 microeinsteins m^{-2} s^{-1} with a 16:8 h light: dark cycle. Light spectra were approximately 420 nm.

6.2.3 Experimental set up

A preliminary trial of NH_4^+ uptake by green seaweed was conducted to determine appropriate range of ratios of seaweed to prawn biomass for the experiment. During the preliminary trial, thirty-six 1-L beakers were filled with sea water (34-35 ppt) and NH_4Cl to give four initial NH_4^+ concentrations from 0.25, 0.55, 0.88 and 1.24 mg L^{-1} and then green seaweed were added to beakers at three stocking densities of 1, 2 and 4 g of fresh weight L^{-1} or 0.24, 0.48 and 0.97 g of dry weight L^{-1}. Three replicates were set up for each treatment and arranged in a completely randomized design. Aeration was supplied during the trial and the trial lasted for 3 hours. Based on the rates of NH_4^+ uptake by green seaweed and published NH_4^+ excretion rates for western king prawn (Khoi and Fotedar 2010), ratios of green seaweed to western king prawn biomass were selected that fell within or immediately outside the theoretical ratio required to control NH_3.

Prawn stocking density in the main experiment was based on the Chapter 4 results. Nine western king prawns were stocked per tank which equals to16 prawn m^{-2} and biomass of approximately 40 g $IRAS^{-1}$. In order to test the effects of different ratios of western king prawn to green seaweed stocking densities on growth, water quality and nutrient budget, five treatments up in triplicate were set. Treatment 1 had no green seaweed (monoculture) and was set as a control treatment. In treatments 2 to 5, green seaweed was stocked into seaweed tanks at densities of 0.25, 0.50, 1.00 and

2.00 kg of fresh weight m^{-2} (equal to 1.00, 2.00, 4.00 and 8.00 g L^{-1} or biomass of 80, 160, 320 and 640g per tank), respectively. Therefore, ratios of the green seaweed to western king prawn biomass were 2, 4, 8 and 16.

Feeding rate was based on the Chapter 5 results. Western king prawns were fed twice a day at a rate of 3.0% body weight per day using a commercially formulated feed ST#1 (43% protein, 6% fat and 2% fibre) (Ridley Aqua-feed, Ridley AgriProducts Pty, Victoria, Australia) and cultured for 42 days. Prawn wastes and tank management are described in Section 3.3.

6.2.4 Data collection

6.2.4.1 Water quality

TAN, NO_2^-, NO_3^- and PO_4^{3-} samples in prawn and seaweed tanks were determined weekly as described in Section 3.4.1. Nutrient removal (NR) of the seaweed in the closed integrated systems was calculated following the equation $NR=100x(N_t-N_p)$; N_t and N_p are nutrient concentrations in the integrated treatments and the prawn monoculture, respectively.

Temperature, pH, DO and salinity were measured as described in the Section 3.4.1. Salinity was maintained between 34-35ppt by adding freshwater.

6.2.4.2 Prawns and seaweed growth

Green seaweed were weighted after every 7days by removing all thalli from the seaweed tank with a net and spinning them in a washing machine (Whirlpool, model 6LBR7255BQ2, Benton Harbour, Michigan, USA) for 20s to remove water retained in the surface of the seaweed. The thalli were immediately transferred by covered bucket to a mechanical weighing scale (Model GX-4000, A&D Company Limited, Tokyo, Japan). Yields(Y) were calculated according to the equation $Y= (B_t-B_0)/(t*A)$; where B_t and B_0 are the weight of the total seaweed biomass at a current time (t) and at the commencement of the experiment (0), A is the culture area in m^2. Western king prawns were also measured for total weight at the conclusion of the

experiment (42 days). The SGRs (% d^{-1}) of the prawns and seaweed were calculated by using the equations SGR = 100 x ($\ln W_t - \ln W_0$)/t; W_t and W_0 are the weight of the prawns or seaweed at current time (t) and at the commencement of the experiment (0), respectively and t is the number of rearing days (d).

6.2.4.3 Nitrogen and phosphorus determinations

The whole western king prawns, pelleted feed and waste deposits samples were dried as described in Section 3.4.3 while dried weight and ash content of green seaweed samples were obtained by drying at 60 ^{o}C to a constant weight and burned at 550^{o}C for 24hrs in an electric furnace, respectively. Determinations of protein and phosphorus contents of the dried samples were described in Section 3.4.3. Total C of seaweed samples was determined with a CS 2000 analyser (Eltra, Neuss, Germany). Nutrient conversion rates (%) of feed N and P by western king prawns and green seaweed is defined as conversion rate= 100 x (A-B)/C (Muangkeow et al. 2007), where: A, total nitrogen/phosphorus at harvest; B, total nitrogen/phosphorus at stocking; C, total nitrogen/phosphorus in the input feed.

Nutrient budget was calculated based on the initial nutrient inputs from the water, stocked green seaweed, stocked western king prawns and feed; and nutrient outputs from harvested prawns and seaweed, drained water and waste deposits. At the termination of experiment, submerged pumps were switched off and water samples were collected from all tanks in the recirculating systems to determine the total nutrient contents in the drained water. Waste deposited samples were collected by draining the waste-collection tanks; and dried and analysed by the above methods.

6.2.5 Data analysis

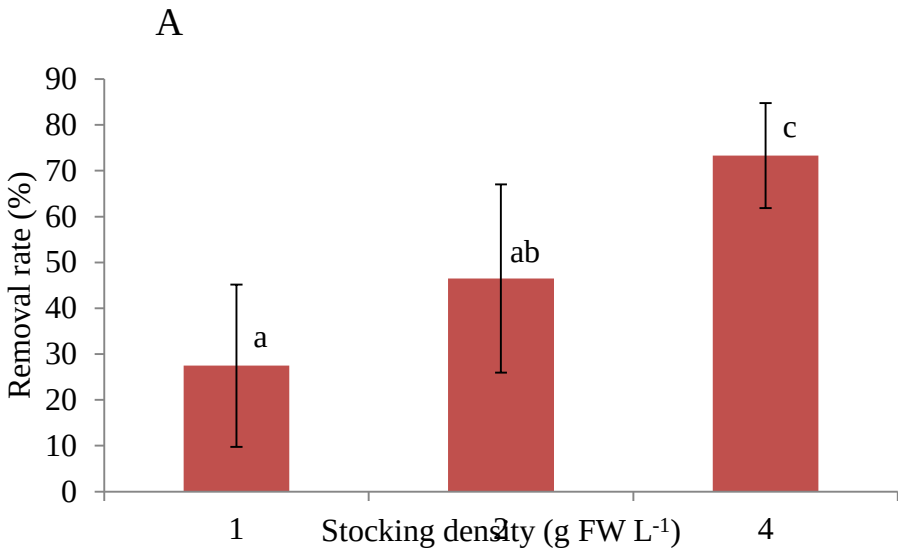
All data were analysed using SPSS for Windows version 17.0. Data were tested for normality and homoscedasticity before applying parametric and non-parametric tests as appropriate. ANOVA and LSD *post hoc* tests were used to determine the significant differences at P = 0.05 between mean water parameters, prawn survival and growth rates, nutrient conversion rate and nutrient budget reared at different

seaweed stocking densities. Where the data did not have normal distribution and homogeneous variance, the Kruskal–Wallis was used to test the overall difference of all treatments. In the case of significant treatment effects, Mann–Whitney's test was applied to analyse the significant difference between the means of each treatment.

6.3 RESULTS

6.3.1 Water quality

NH_4^+ removal rates in the preliminary experiment were significantly ($P<0.05$) proportional to green seaweed stocking densities but inversely proportional to NH_4^+ concentrations (Figure 6.1). No significance was found ($P>0.05$) between green seaweed removal rates at 0.56 and 0.88 NH_4^+ concentrations.



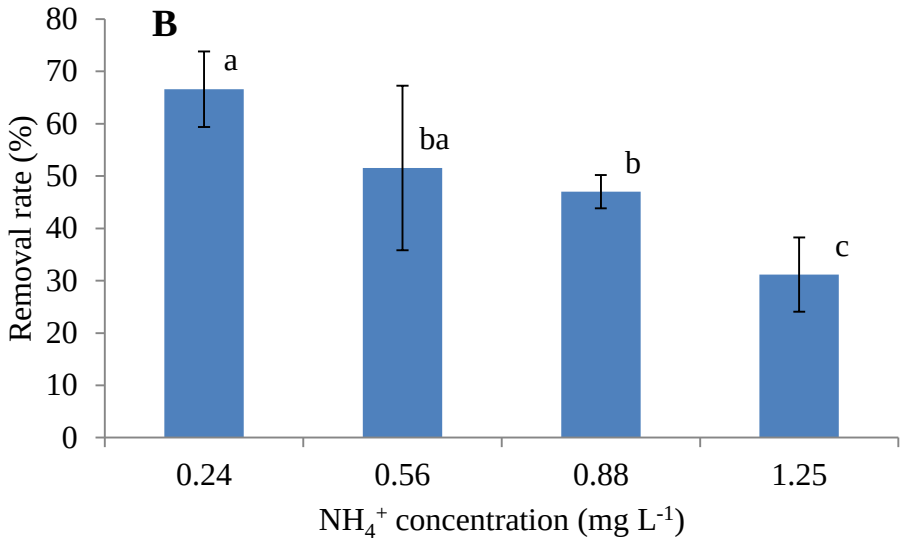


Figure 6.1 Mean NH_4^+ removal rate (%) of green seaweed at different stocking densities (A) and NH_4^+ concentrations (B) after 3-hour experiment (Means±SE) in the preliminary experiment. Different letters indicate a statistical difference (P<0.05).

In the main experiment, recirculating aquaculture system that included green seaweed had significantly lower concentrations of inorganic nitrogen and phosphorus during the course of the experiment. Concentrations of TAN and NO_3^- in integrated prawn tanks were significantly lower (P<0.05) than those in the control tanks (Table 6.1). The lowest concentrations of TAN and NO_3^- were recorded at the highest green seaweed stocking density which were significantly (P<0.05) lower than other seaweed densities. Mean PO_4^{3-} concentrations in prawn tanks increased as the trial progressed (Figure 6.2D) and were not significantly affected by any green seaweed stocking densities (Table 6.1). pH values were significantly (P<0.05) lower in monoculture tanks than in integrated culture tanks (Table 6.1) but were not significantly (P>0.05) affected by adding green seaweed into western king prawn culture system. Water quality parameters in seaweed tanks of the integrated culture systems showed a similar trend to those in the prawn tanks (Table 6.2).

Table 6.1 Overall means of some water quality parameters of prawn tanks in the IRAS reared western king prawn integration with green seaweed during 42-day trial

		Green seaweed stocking densities (kg m^{-2})			
	Prawn Monoculture	**0.25**	**0.50**	**1.00**	**2.00**
TAN (mg L^{-1})	0.49 ± 0.05^{a}	0.34 ± 0.11^{b}	0.33 ± 0.03^{b}	0.38 ± 0.08^{b}	0.29 ± 0.08^{c}
NO_3^- (mg L^{-1})	6.73 ± 0.13^{a}	3.09 ± 0.60^{bc}	3.39 ± 0.26^{b}	$2.77\pm0.51b^{c}$	1.85 ± 0.28^{d}

NO_2^- (µg L^{-1})	222.97±4.93[a]	217.09±6.84[a]	217.51±7.10[a]	177.19±2.18[b]	174.87±2.92[b]
PO_4^{3-} (mg L^{-1})	0.45 ±0.02[a]	0.24±0.01[b]	0.24 ± 0.01[b]	0.23 ± 0.01[b]	0.24 ± 0.02[b]
pH	8.03±0.02[a]	8.40±0.01[b]	8.39±0.00[b]	8.37±0.02[b]	8.37±0.03[b]

Values in the same row sharing a common superscript letters (a, b, c, d) are not significant different (LSD test; P<0.05; n=3).

Mean TAN and PO_4^{3-} removal rates of green seaweed over 42-day trial ranged from 24.02-99.05% and 13.80-96.40% (Figure 6.3), respectively. The removal rates of TAN by green seaweed at 2.00 kg m^{-2} and 1.00 kg m^{-2} seem to remain constant over the study period except for the drop at day 28 of the culture period (Figure 6.3A). This coincided with the highest mortalities of seaweed while TAN removal of 0.50 kg m^{-2} and 0.25 kg m^{-2} increased over the experimental period (Figure 6A). The overall mean TAN removal rates ranged from 59.90-81.14% of TAN excreted by the prawns and the highest value was recorded at green seaweed density of 1.00 kg m^{-2} (81.14±5.83%) and 2.00 kg m^{-2} (75.12±9.95%) which are significantly higher than those at other stocking densities (Table 6.2). Overall mean PO_4^{3-} removal rate was from 50.27 to 55.33% (Table 6.2) and decreased as the trial progressed (Figure 6.3B).

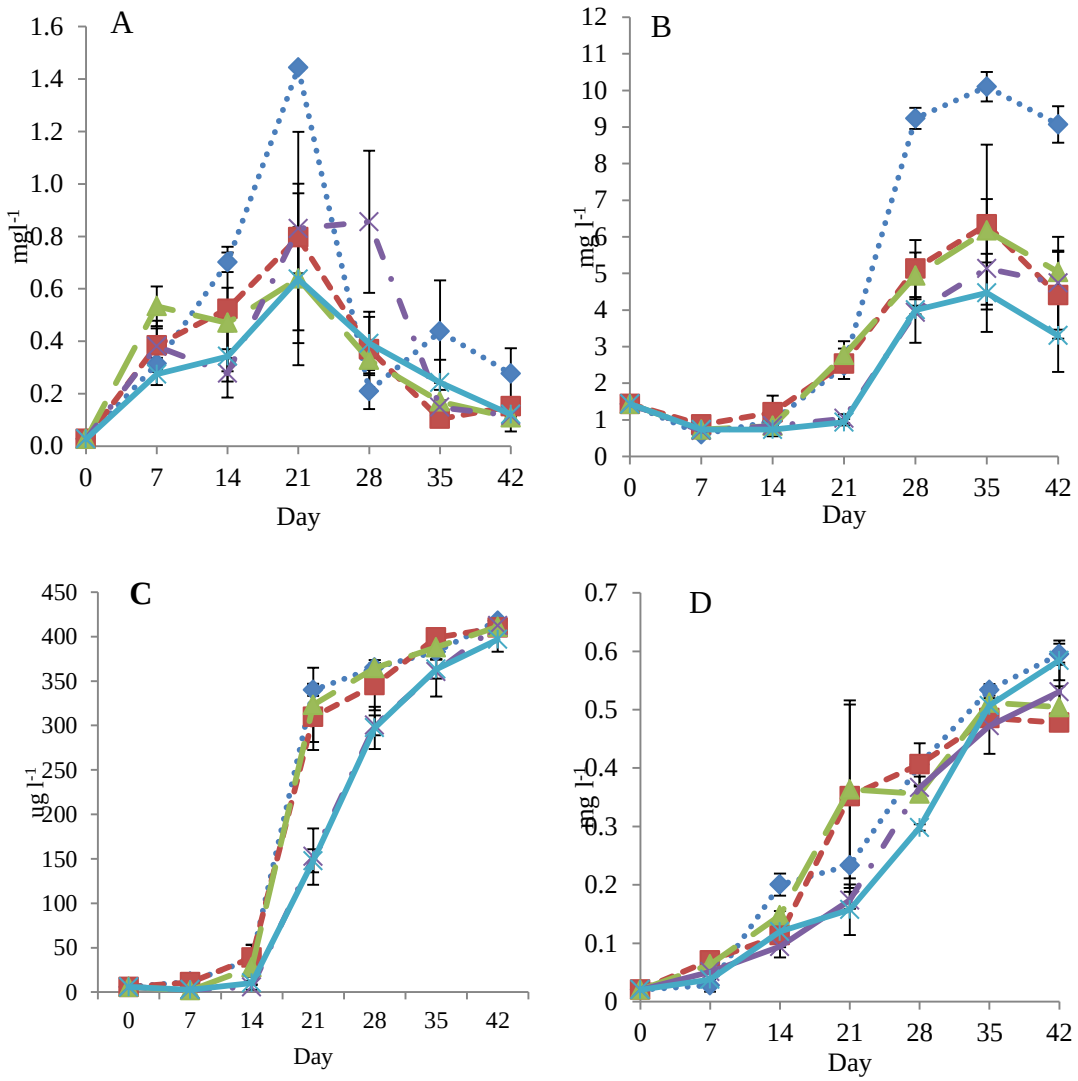
Table 6.2 Overall means of some water quality parameters of seaweed tanks and TAN and PO_4^{3-} removal rates in the IRAS reared western king prawn integration with green seaweed during 42-day trial

	Prawn	**Green seaweed stocking densities (kg m^{-2})**			
	Monoculture	**0.25**	**0.50**	**1.00**	**2.00**
TAN (mg L^{-1})	0.44±0.04[a]	0.22±0.08[b]	0.21±0.04[b]	0.12±0.03[c]	0.11±0.05[c]
NO_3^- (mg L^{-1})	4.72±0.07[a]	3.05±0.36[b]	3.07±0.23[b]	2.580.22[b]	1.84±0.20[c]
NO_2^- (µg L^{-1})	218.99±3.48[a]	216.07±9.15[b]	209.65±6.56[b]	176.80±4.33[b]	159.93±3.54[b]
PO_4^{3-} (mg L^{-1})	0.43±0.01[a]	0.24±0.01[b]	0.24±0.01[b]	0.23±0.01[b]	0.24±0.02[b]
pH	7.85±0.01[a]	8.44±0.04[b]	8.44±0.01[b]	8.40±0.03[bc]	8.36±0.04[c]
TAN removal (%)	-	59.90±4.04[a]	60.31±5.24[a]	81.14±5.83[b]	75.12±9.94[b]
PO_4^{3-} removal (%)	-	50.27±2.24[a]	51.46±0.33[a]	55.33±1.81[a]	53.38±3.15[a]

Values in the same row sharing a common superscript letters (a, b, c) are not significant different (LSD test; P<0.05; n=3).

6.3.2 Growth and survival rates of western king prawn and green seaweed

Increasing green seaweed stocking density did not significantly affect the growth and survival rate of the western king prawns (Table 6.3). Overall specific growth rates of western king prawns ranged from 0.70 to 0.76 (% d^{-1}) and were not significant ($P>0.05$) difference among treatments.



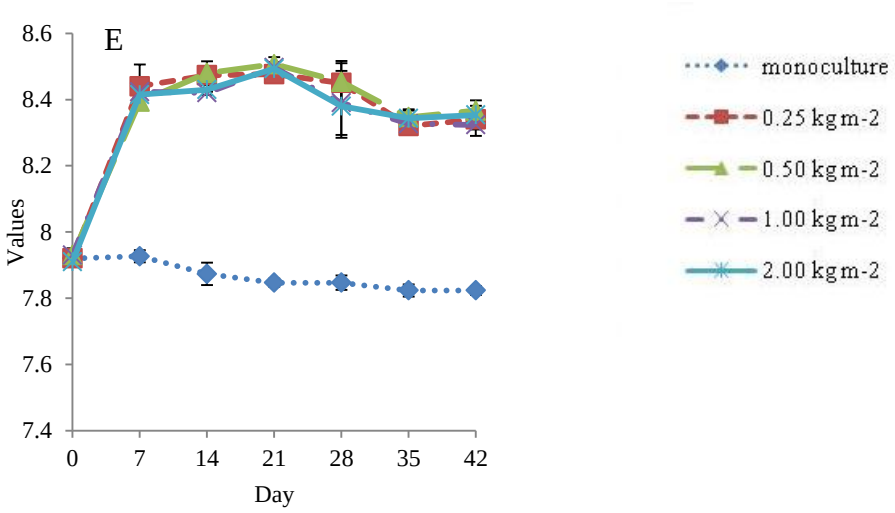


Figure 6.2 Concentrations of TAN (A), NO_3^- (B), NO_2^- (C), PO_4^{3-} (D) and pH (E) in the prawn tanks of five treatments in the IRAS during 42- day experiment. Error bars indicate SE.

The growth and yield of green seaweed increased in the beginning of the experiment with first week showing maximum growth. Maximum growth (SGR)(7.4% d^{-1}) were recorded at the lowest density (0.25 kg m^{-2}) (Figure 6.4A). Seaweed yield increased with the increasing stocking densities and reached a maximum (56.7 g fresh weight m^{-2}) at a density of 1.00 kg m^{-2} (Figure 6.4B). The lowest yield was observed at the highest density (2 kg m^{-2}) which was significantly lower than those of other densities. After day 7 of the culture period, the seaweed growth rates were reduced between 0.28-0.64 % d^{-1} and a reduced yield was observed after 14 days, with appearance of white and empty filaments (ghost tissue). This biomass reduction was higher at 2.00 kg m^{-2} and the lowest value was recorded at 0.25 kg m^{-2} (Figure 4B).

Table 6.3 Prawn growth and survival rates, C:P and C:N ratios in dried green seaweed tissues and protein contents (dry weight) of western king prawn and green seaweed reared in IRAS during 42 days of the trial

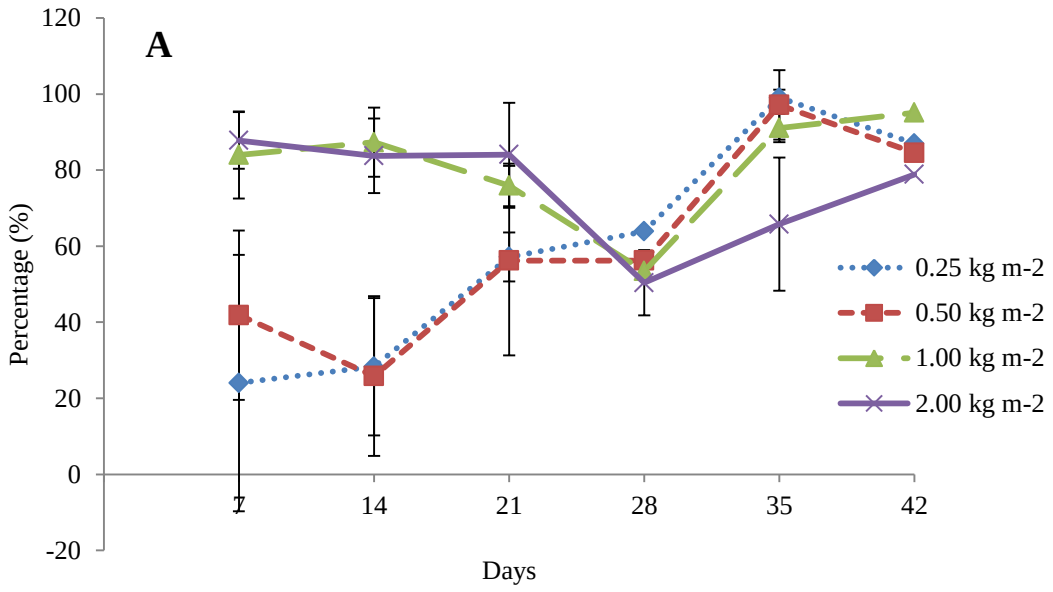
	Prawn Monoculture	**Green seaweed stocking densities (kg m^{-2})**			
		0.25	**0.50**	**1.00**	**2.00**
Initial weight (g)	4.82±0.16[a]	4.77±0.18[a]	4.87±0.17[a]	4.72±0.19[a]	4.80±0.22[a]
Final weight (g)	6.01±0.18[a]	5.91±0.17[a]	6.11±0.35[a]	5.95±0.42[a]	6.07±0.27[a]
Biomass gain (g)	8.19±2.68[a]	9.11±0.65a	8.91±1.12[a]	10.54±1.45[a]	10.10±1.28[a]
Prawn SGR (% d^{-1})	0.75±0.06[a]	0.70±0.08[a]	0.72±0.09[a]	0.75±0.08[a]	0.76±0.09[a]

*Prawn survival (%)	100.00±0.00[a]	95.83±4.17[a]	100.00±0.00[a]	91.67±4.17[a]	100.00±0.00[a]
C:P ratio	**194:1	55:1	47:1	44:1	41:1
C:N ratio	**24:1	8:1	8:1	9:1	10:1
Prawn protein (%)	55.97±1.47[a]	60.50±2.02[a]	59.87±2.64[a]	58.99±3.32[a]	59.83±1.87[a]
Seaweed Protein (%)	**5.41±0.22[a]	25.15±1.27[b]	23.89±0.52[b]	22.78±1.10[bc]	20.27±0.83[c]
Carbon content (%)	**21.83±0.5[a]	31.30±.60[b]	30.60±0.50[b]	30.93±0.60[b]	31.33±1.03[b]

*Data transformed (arcsine) prior to statistic analysis.
** N and P values of the initial seaweed stock. Values in the same row sharing a common superscript letters (a, b, c) are not significant different (LSD test; P<0.05; n=3).

6.3.3 Ratios of C:P and C:N in seaweed tissues, seaweed protein content, total carbon in seaweed tissues and nutrient conversion rates

Ratio of C:P in dried green seaweed tissues in the IRAS ranged from 41:1 to 55:1 and decreased with increasing green seaweed stocking densities while C:N showed a inverse trend (Table 6.3).



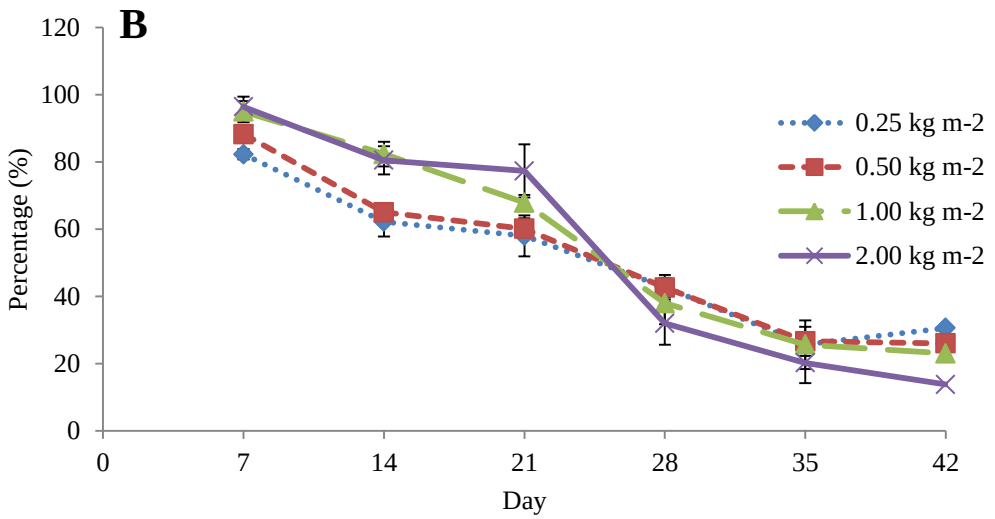


Figure 6.3 Mean removal rates of green seaweed for TAN (A) and PO_4^{3-} (B) over 42-day experiment. Error bars indicate SE.

Over 42-day trial, the protein tissue content of green seaweed showed an increasing trend with decreasing green seaweed density. Protein content of green seaweed in the IRAS was significantly different (P<0.05%) among treatments and was higher than the protein content of the initial green seaweed stock (Table 6.3). The lowest protein value (20.27±0.83%) of green seaweed tissue was recorded at 2.00 kg m^{-2} and was significantly lower than at density of 0.50 kg m^{-2} (23.89±0.52%) and 0.25 kg m^{-2} (25.150±1.27%). The mean green seaweed protein content was independent of stocking densities of 0.25, 0.50 and 1.00 kg m^{-2} (Table 6.3). C contents of green seaweed tissues in the integrated culture were from 30.60 to 31.33% and were significantly higher than that in the initial seaweed stock (Table 6.3). Inclusion green seaweed into western king prawn culture was not significantly (P>0.05) affected the C contents in the seaweed tissues.

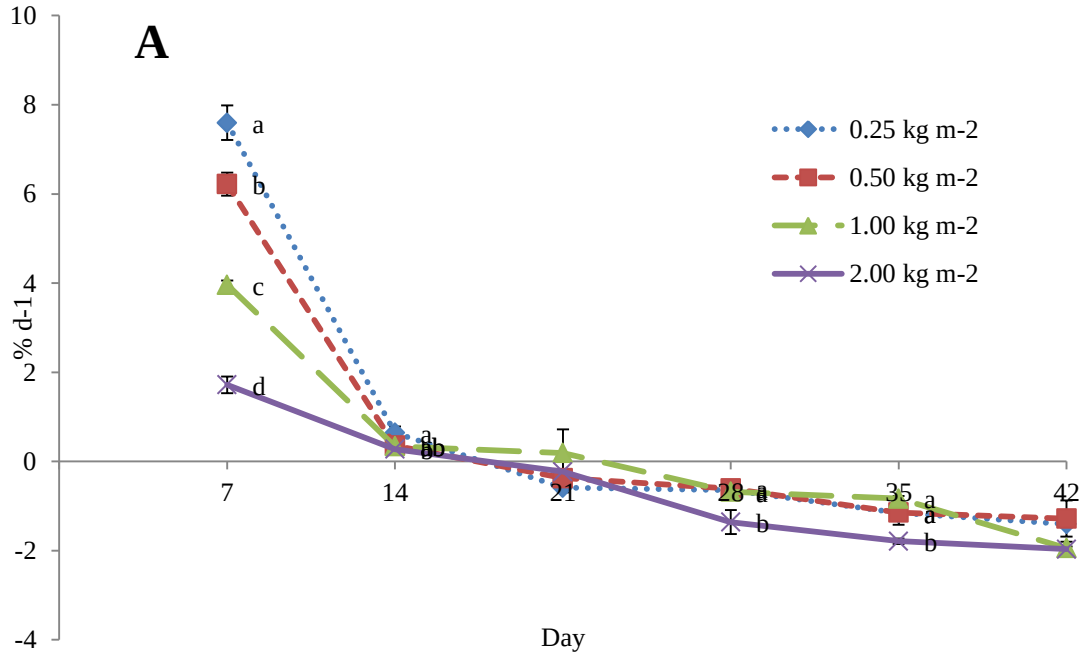
Table 6.4 The conversion rate of feed N and P (in percentage) into western king prawn and green seaweed biomass in the IRAS

		Green seaweed stocking densities (kg m^{-2})			
	Prawn Monoculture	**0.25**	**0.50**	**1.00**	**2.00**
Nitrogen (%)					
Prawn	7.56±3.05[a]	7.92±2.53[a]	6.28±1.35[a]	9.39±3.87[b]	10.22±1.43[b]
Seaweed	-	6.03±0.57[a]	13.13±2.64[b]	20.07±1.40[c]	22.25±3.32[c]

Prawn+seaweed	7.56±3.05[a]	13.95±2.80[a]	19.42±2.11[b]	29.46±5.15[bc]	32.46±2.27[c]
Phosphorus (%)					
Prawn	2.81±0.31[a]	2.83±0.61[a]	2.66±0.33[a]	3.01±0.30[a]	2.80±0.43a
Seaweed	-	2.15±0.17[a]	6.07±1.28[b]	10.92±0.66[c]	19.42±0.90[d]
Prawn+seaweed	2.81±0.31[a]	4.98±0.77[a]	8.73±1.59[b]	13.93±0.95[c]	22.22±1.30[d]

Values in the same row sharing a common superscript letters (a, b, c, d) are not significant different (LSD test; P<0.05; n=3).

The conversion of feed N into western king prawn biomass showed an increasing trend with increasing green seaweed density in the integrated systems. There was a significant difference (P<0.05) among green seaweed stocking densities where the highest values were recorded at densities 2.00 kg m^{-2} (10.22±1.43%) and 1.00 kg m^{-2} (9.39±3.87%) (Table 6.4). Conversion rate of N to seaweed biomass was also significantly different (P<0.05) and the highest values were observed at green seaweed densities of 2.00 and 1.00 kg m^{-2}; the lowest value was at density of 0.25 kg m^{-2} (Table 6.4). Feed N utilization efficiency showed significant difference (P<0.05) between treatments (Table 6.4) with low rate in the prawn monoculture system (7.56±3.05%) and high rate in the integrated systems.



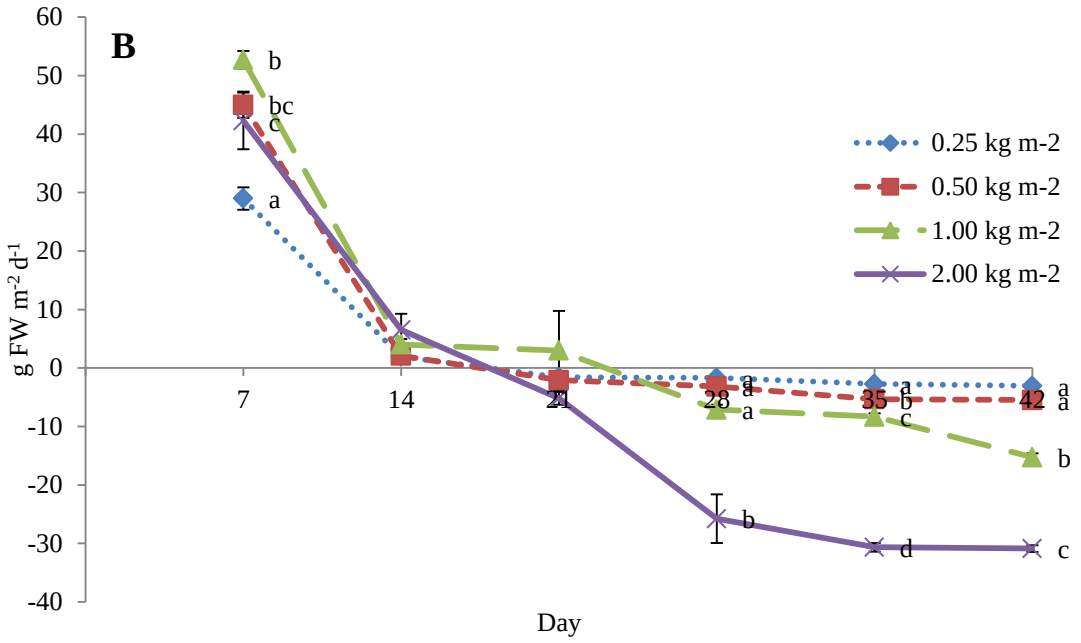


Figure 6.4 SGR (A) and yield (B) of green seaweed integration with the western king prawn in an IRAS during 42-day trial. Different letters indicate a statistic difference. Error bars indicate SE.

The conversion of feed P into the green seaweed biomass showed a similar trend as N conversion. The P conversion rate to western king prawn ranged from 2.81 to 3.01%, without significant differences due to different green seaweed densities. Feed P utilization efficiency showed significant ($P<0.05$) differences among stocking green seaweed densities (Table 6.4). The values increased with increasing green seaweed densities and the lowest value was observed in the prawn monoculture system and an integrated culture system with green seaweed density of 0.25 kg m^{-2}.

6.3.4 Nutrient budget

Approximately 28.00-31.90% of N input was retained in western king prawn biomass at harvest while 6.53-29.71% of N input was retained by green seaweed biomass. No significant differences ($P>0.05$) in N retention in western king prawns between treatments was found. The retained N in green seaweed tissue was significantly different ($P<0.05$) between densities and the highest value was recorded at 2.00 kg m^{-2} and 1.00 kg m^{-2} which were higher than other stocking densities. The lowest N retention was observed at a density of 0.25 kg m^{-2} (Table 6.5).

Table 6.5 Nitrogen budget in percentage of western king prawn integration with green seaweed in an IRAS during a 42-day culture

	Prawn Monoculture	Green seaweed stocking densities (kg m^{-2})			
		0.25	**0.50**	**1.00**	**2.00**
Input-N (%)					
Water	6.40±0.01[a]	6.25±0.00[b]	6.12±0.01[c]	5.82±0.01[d]	5.26±0.02[e]
Prawn	26.85±0.17[a]	25.95±0.05[b]	24.92±0.14[c]	23.85±0.14[d]	22.40±0.29[e]
Seaweed	-	2.60±0.00[a]	5.10±0.01[b]	9.68±0.02[c]	17.50±0.07[d]
Feed	66.75±0.27[a]	65.20±0.04[b]	63.86±0.12[c]	60.66±0.11[d]	54.84±0.20[e]
Output-N (%)					
Water	11.09±0.38[a]	8.30±1.18[b]	7.77±0.76[b]	6.54±0.97[bc]	4.61±0.63[c]
Prawn	31.90±2.05[a]	31.12±1.62[a]	28.94±0.87[a]	29.54±2.41[a]	28.00±0.92[a]
Seaweed	-	6.53±0.37[a]	13.48±1.68[b]	21.85±0.84[c]	29.71±1.84[d]
Waste	11.84±2.31[a]	19.47±0.90[a]	19.33±0.45[a]	17.22±1.91[a]	26.40±1.45[b]
Unaccounted	45.17±4.63[a]	30.58±2.81[b]	30.49±0.75[b]	24.85±378[bc]	11.28±2.25[c]

Values in the same row sharing a common superscript letters (a, b, c, d, e) are not significant different (LSD test; $P<0.05$; n=3).

The highest N input loss through discharged water at harvest was observed in prawn monoculture system and the lowest was recorded at 2.00 and 1.00 kg m^{-2} (Table 6.5). Waste N% output was significantly ($P<0.05$) higher at stocking density of 2.00 kg m^{-2} (26.40±1.45%) than other seaweed densities. Unaccounted N loss at 2.00 kg m^{-2} was significantly lower ($P<0.05$) than those at other green seaweed stocking densities in the integrated systems.

The retained P content at harvest was 13.46-14.63% in western king prawn biomass and 1.62-13.50% in the green seaweed of the total P-input. P contents in western king prawn tissue was not significantly affected ($P>0.05$) by integrating green seaweed with western king prawns (Table 6.6). However, P content removed by the green seaweed increased with the increase in green seaweed biomass input. The P loss through drained water was highest in the prawn monoculture and the lowest was observed at densities of 2.00 and 1.00 kg m^{-2} of the integrated systems (Table 6.6).

Table 6.6 Phosphorus budget in percentage of western king prawn integration with green seaweed in an IRAS during a 42-day culture

	Prawn Monoculture	Green seaweed stocking densities (kg m^{-2})			
		0.25	**0.50**	**1.00**	**2.00**
Input-P (%)					
Water	11.04±0.01[a]	10.92±0.00[b]	10.81±0.01[c]	10.55±0.01[d]	10.03±0.01[e]
Prawn	12.48±0.09[a]	12.21±0.02[b]	11.86±0.04[c]	11.66±0.05[d]	11.52±0.10[e]
Seaweed	-	1.22±0.00[a]	2.41±0.00[b]	4.70±0.00[c]	8.95±0.01[d]
Feed	76.48±0.08[a]	75.65±0.01[b]	74.928±0.04[c]	73.09±0.04[d]	69.51±0.08[e]
Output-P (%)					
Water	19.13±0.66[a]	14.50±2.06[ab]	13.72±1.34[b]	11.87±1.76[bc]	8.80±1.19[c]
Prawn	14.63±0.24[a]	14.36±0.45[a]	13.85±0.29[a]	13.85±0.26[a]	13.46±0.24[a]
Seaweed	-	1.62±0.13[a]	4.39±1.08[b]	7.34±1.12[c]	13.50±0.64[d]
Waste	32.36±2.42[a]	41.47±1.93[b]	40.03±1.83[b]	31.63±1.80[a]	36.07±0.56[ab]
Unaccounted	33.88±2.69[a]	28.05±3.99[a]	27.99±1.68[a]	35.31±1.77[a]	28.17±2.83[b]

Values in the same row sharing a common superscript letters (a, b, c, d, e) are not significant different (LSD test; $P<0.05$; n=3).

6.4 DISCUSSION

In the present study, water recirculation through the seaweed acting as a biofilter was found to be beneficial in terms of water quality and levels of nutrient. Maximum levels of TAN in seaweed and prawn tanks were observed in prawn monoculture culture systems (Figure 6.2) and TAN concentrations never exceeded 1.5 mg L^{-1} and always were well below the lower limit of NH_3 toxicity to penaeid species (ChenTing et al. 1990). TAN levels peaked on 21th day of the culture followed by an increase in NO_2^- concentration, showing that it took 3 weeks to establish the nitrification process. This duration was shorter than the 8 weeks needed to establish the nitrification process in tiger prawn concrete culture tanks (Thakur and Lin 2003). pH has strong effect on nitrification rates and the reactions occur fastest when pH is from 8 - 9 (Henze et al. 1997). Inclusion green seaweed into prawn culture enhanced pH values which promote the nitrification process.

Concentrations of TAN, NO_3^-, PO_4^{3-}-P in the integrated culture system were lower than those in prawn monoculture system, suggesting that green seaweed could effectively remove N and P nutrient. NH_4^+ uptake rate of green seaweed in the preliminary experiment increased with increased stocking densities (1 to 4 g fresh weight L^{-1}) and NH_4^+ concentrations (0.25 to 1.25 mg NH_4^+ L^{-1}) (Figure 6.1). This was again confirmed in the main experiment in which N removal rates achieved the maximum value at 1.00 kg m^{-2} (equal to 4g L^{-1}) (Table 6.2). However, there was a decrease of N uptake of green seaweed at the highest density of 2.00 kg m^{-2}. This may be associated with the poor light penetrate (Neori et al. 1998) and CO_2 depletion (Lapointe and Tenore 1981).

Dissolved phosphorus in the present study increased over the course of the trial though part of the phosphate was removed by the seaweed. The fact that the tank bottoms in the had little sediments, indicated that the nutrient concentration equilibrium in the recirculating system skewed in favour of the water which resulted in an increase in inorganic N and P concentration over the course of the trial. Chen et al. (1989) mentioned that sediments play an important role in the balance of an aquaculture system and can act as buffers. Anaerobic pond mud strongly absorbs phosphate and is eventual recipient of the most phosphorus added to the aquaculture ponds (Boyd 1990). Therefore, it is estimated that over half of the P from the inputs are bound in the soil in a relative insoluble form (Midlen and Redding 1998).

pH value in the present study was lower in monoculture system than in the closed integrated culture systems. This can be explained by the fact that *Ulva* sp. can utilize both dissolved free CO_2 and HCO_3^- as the exogenous C source for photosynthesis (Beer and Eshel 1983). As CO_2 is removed, carbonate accumulates and hydrolyzes, and the pH increases (Boyd 1990). HCO_3^- concentration in seawater is higher than dissolved free CO_2 (Beer and Eshel 1983) and HCO_3^- absorption in turn by seaweed may also contribute to the increase of pH in media to 8.3 (Boyd 1990). CO_2 released from the animal and bacteria respiratory process, organic matter decomposition and direct diffusion from the atmosphere could react with H_2O to librate hydrogen ion. Therefore, in the absence of seaweed in monoculture, pH was dropped.

Maximum TAN removal rates waere recorded at green seaweed stocking density of 1.00 and 2.00 kg m^{-2}. The result was in accordance with the data reported by Debusk et al (1990) and Neori et al (1991) for green seaweed in intensive fishpond systems. However, TAN removal efficiency in the present study was higher than 49-56% (Neori et al. 1991) from the marine fishpond effluents and 55% (Neori et al. 1998) from an integrated culture system of Japanese abalone and macroalgae but was similar to that reported by Schuenhoff et al. (2003), (64% TAN removal) for an integrated culture of seabream and green seaweed.

Inclusion of *Ulva* into western king prawn culture has no effect on the survival and growth performance of the western king prawns. These results were similar to the findings of Mai et al (2010) when western king prawn was integrated with seaweed, *Sargassum* sp. in static culture systems. Survival rate in the current study ranged from 91.67-100.00% which is higher than 60-80% survival (Hai et al. 2009a) when the western king prawns were reared in different probiotics. The SGR of western king prawns were comparable to those reported by Hai et al. (2009a) and Mai et al (2010) and higher than 0.24-0.34 % d^{-1} obtained by Khoi and Fotedar (2010) at different western king prawn stocking densities. However, the SGR in the current trial was lower than 1.2% d^{-1} as published by Sang and Fotedar (2004a) at 34 ppt, probably because of different feed types used. In the current study, the western king prawns were fed by a commercially formulate diet, while western king prawns in Sang and Fotedar (2004a) research were provided with fresh blue mussel.

The growth rate and yield of *Ulva* increased with increasing stocking densities but decreased at the highest stocking (2.00 kg m^{-2}). Neori et al. (1991) found that under N-sufficient conditions, increasing stocking densities can decrease the yield of green seaweed. This relationship is due to light limitation, even though each thallus was cycled from the top to the bottom of the tanks by bubble induced water circulation (Vandermeulen 1989). Lapointe and Tenore (1981) suggested that insufficient carbon supply may decrease seaweed growth at higher stocking densities. Beer and Eshel (1983) stated that *Ulva* utilizes both dissolve free CO_2 and HCO_3^- as the carbon sources for photosynthesis. Thus, with a fixed supply of C during the trial period, increasing stocking density is likely to lead to a carbon limitation in the integrated systems. In the present study, total carbon contents were not affected by

seaweed stocking densities (Table 6.3), suggesting that carbon is unlikely to a limiting factor for seaweed growth. Msuya and Neori (2008) showed that biomass yield of the seaweed depends only on nutrient load level. Thus at lower stocking densities of green seaweed, high nutrients concentration in the cultured environment may be responsible for the decline of yield and growth of green seaweed.

Sze (1998) stated that *Ulva* spp. are good colonizer and tolerate pollution better than most macroalgae and can be used as bio-indicators of nutrients in the water column since their ability to assimilate surrounding nutrients is rapid which is clearly reflected by their tissue nutrient content within a relatively short period of time (Ryther 1981). Fenchel and Jørgensen (1977) stated that C:N ratio of benthic plant ranges from 10:1 to 70:1 and that the C:P ratio is about 200:1. Both C:N and C:P ratios in the present study (Table 6.3) were well below the estimates of Fenchel and Jørgensen (1977). Neori at el. (1991) reported that C: N ratio in green seaweed cultivated in marine fishpond effluents showed a negative hyperbolic correlation with N-lux, dropping from over 15 to approximately 7 when grown at low and high N-fluxes, respectively.. Several authors have reported critical nitrogen for different species of Ulva to be around 2.3% (Villares and Carballeira 2004). In the present study, tissue N values ranger from 3.0 to 4.3% (dry weight) which suggested that green seaweed was possibly N-limited. The results confirmed the findings of various authors (Hansiak 1979; Lapointe and Tenore 1981) who claimed that C:N ratio of benthic plants decreases with increasing DIN concentration.

In general, green seaweeds may have two or three times more protein content than brown seaweeds (Burtin 2003). In the present study, seaweed protein values in the closed integrated systems ranged from 20.27 to 25.15%, which were markedly higher than in initial thalli (mean of 5.41%), and were higher than the seaweed, *Padina pavonia* (12%) obtained by Fouad et al. (1977) and the seaweeds, *Entermorpha* spp. (9-14%) obtained by Aguilera-Morales et al. (2005). *Ulva* protein content increased with increasing rates of ammonium supply per unit area (Chopin et al. 2001) and nutrient loading (Msuya and Neori 2008). Prawn effluents from IRAS usually contain higher N and P contents than nutrients in seawater, that is led to increase protein content of seaweeds in integrated culture systems. The high N contents fell well within the range found in *Ulva* spp. grown in eutrophic fish-farm

effluents (Neori et al. 1998; Neori et al. 2000) but were lower than 34-40% protein of green seaweed integration with sea urchin, Japanese abalone and seabream (Schuenhoff et al. 2003). The chemical composition of seaweed may vary according to the species, habitat, environmental conditions and other factors (Marinho-Soriano et al. 2006).

In the current study, approximately 6.28-10.22% and 6.03-22.25% of feed N was converted into western king prawn and green seaweed biomass at harvest, respectively. Results in the present study are comparable to 13-21% (Schuenhoff et al. 2003) but lower than 24.6% of N input (Neori et al. 2000) which was assimilated by green seaweed in a semi-recirculating integrated system with abalone and seabream. Similarly, only 2.15-19.42% of feed P was locked up in new green seaweed biomass, which fell within the range of 5.65-6.38% of P feed retained in seaweed biomass in a semi-integrated system (Schuenhoff et al. 2003). The rest of the feed inputs were in the form of unassimilated nutrients, released from the prawn tank as inorganic nutrient, faeces and uneaten food. In this experiment, the green seaweed conversion rate was higher for N than P (6.03-22.25% and 2.15-19.42% for N and P, respectively) (Table 6.4), suggesting green seaweed efficiently removes more excess nitrogen in a prawn culture system than phosphorus. The feed N utilization efficiency was greater in higher green seaweed stocking densities (1.00 and 2 kg m^{-2}) than in lower densities (0.25 and 0.50 kg m^{-2}) (Table 6.4). Moreover, the highest growth and yield of green seaweed were achieved at stocking density of 1.00 kg m^{-2}. These suggest that stocking density of 1.00 kg m^{-2} is suitable in term of growth rate, yield and nutrient conversion rate in the IRAS.

The main source of N and P inputs in aquaculture ponds is feed. In the present study, percentage contribution of feed to the total N and P inputs ranged from 54.84-66.75% and 69.51-76.58%, respectively, and were lower than 82-95% N and 38-91% P as reported by Briggs and Funge-Smith (1994) and Thakur and Lin (2003). Nutrient budget showed that approximately 28.00-31.12% N and 13.46-14.63% P of the total nutrient inputs were retained in western king prawn biomass at the end of the 42 days. This result is in agreement with the findings of Thakur and Lin (2003) in a closed prawn culture system who reported that about 23-31% N and 10-13% P

were assimilated in tiger prawn tissue. These results are also comparable to those estimated for tiger prawns (Funge-Smith and Briggs 1998; ; Hari et al. 2005) and white prawns (Teichert-Coddington et al. 2000). However, nutrients retained by western king prawns were not significantly different among treatments; implying that in terms of the proportional recovery of the nutrient from closed prawn culture system, efficiency was not affected by integration of green seaweed and western king prawn. Results showed that seaweed biomass were decline over time and the highest biomass loss was at stocking density of 2 kg m^{-2} thus the highest N loss through sediment at this density in the present study was due to the accumulation of the N which was decayed from dead seaweed.

Unaccounted loss of nutrients in our study ranged between 11.28-45.17% N and 27.99 – 33.88% P of the total inputs; and were higher than 5.2-36.0% N and 5.3-19.7% P in closed culture systems of tiger prawn (Thakur and Lin 2003) but lower than 66% in prawn farms in North-West coast of Mexico (Paez-Osuna et al. 1999). Nitrogen might have lost from the prawn culture system either by NH_3 gas volatilization and/or denitrification (Boyd 1990) which are often not measured directly (JacksonPrestonThompson et al. 2003). Therefore, in most studies, including the present one, these factors are estimated indirectly as the difference between the nitrogen inputs and outputs. Ammonia volatilization is promoted by two sets of factors: those favoring the NH_3 side of the water column NH_4^+/NH_3 equilibrium (most importantly, overall TAN concentration and high pH, but also higher temperature and salinity); and those promoting the effective transfer across the water–air phase boundary (wind, mechanical aeration, temperature and mixing) (JacksonPrestonThompson et al. 2003). Daniels and Boyd (1988) stated that NH_3 gas volatilization is probably the main cause of nitrogen loss and is further enhanced by vigorous aeration and high pH in the tanks. However, higher N loss in the present study was observed in prawn monoculture tanks where pH values were relatively low, compared to the integrated culture treatments. Moreover, Chiayvareesajja and Boyd (1993) found that heavy aeration can marginally reduce TAN concentrations under the laboratory setup but would not reduce the TAN concentrations appreciably even aeration rates were tripled in aquaculture ponds. Therefore, there were other factors e.g mixing that contributed for the unaccounted N loss in the culture systems. P was lost from water exchange (56%) and during pond harvest

(9%) (Teichert-Coddington et al. 2000) in semi-intensive prawn farms in Honduras. In the study, P loss was likely that some of the sediment deposited in the bottom tanks can wash out from the tanks with the drained water during harvest .

The integrated culture system described here can improve the feasibility of land-based mariculture as it can reduce the risks of the nutrient release into the environment. However, the high mortality and lower growth rate of seaweed in each stocking density demonstrates that other limiting factors can be incorporated when green seaweed is integrated with western king prawns. Future research should focus on the underlying mechanisms which are responsible for N and P removal from the culture media as high nutrients could prove toxic to green seaweed cultivation and thus in turn can increase the nutrient loading.

Future research could also investigate incorporating different seaweed species into the IRAS.

CHAPTER 7 INTEGRATING PRAWN CULTURE WITH MUSSELS

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7.1 INTRODUCTION

Intensive marine prawn aquaculture can introduce significant quantities of nutrient wastes from uneaten feed, faeces and excretory products into the local environment (Midlen and Redding 1998). Prawn feeds typically contain 33-45% protein and 1.2-1.3% phosphorus (P) (Muangkeow et al. 2011). Only 24-37% of N and 13-28% of P in such feeds are converted into prawn biomass at harvest (Briggs and Funge-Smith 1994; Thakur and Lin 2003; Muangkeow et al. 2007). Unincorporated N and P are discharged to waste or are retained in culture water and sediment (Briggs and Funge-Smith 1994; Burford et al. 2002). There are many concerns regarding the environmental impacts from the aquaculture wastes along with the decline in prawn growth and survival rate (Khoi and Fotedar 2010). Therefore, one of the major challenges for the sustainable prawn culture, and the aquaculture industry in general is to minimise the concurrent environmental degradation with the intensification of the industry.

Integrating the culture of filter-feeding bivalve molluscs with prawns has long been advocated as another potential strategy to alleviate waste loadings and environmental impacts associated with intensive prawn culture (Jones et al. 2001). Previous studies have shown that filtration by bivalves can significantly reduce the concentrations of bacteria, TN and TP and other suspended particles from prawn effluent (Maguire et al. 1981; Jones et al. 2001; Jones et al. 2002; Tendencia 2007). However, they can increase the concentration of $PO_4^{3-}$and NH_4^+ through excretion (Prins and Smaal 1994; Jones et al. 2001). There is little information on the integrated culture of bivalves and prawns in the IRAS. Martinez-Cordova and Martinez-Porchas (2006) reported the survival of white prawn, Pacific oyster and black clam were 48.3-63.1% and 61.9 - 67.3%, 10.7-16.2% and 45.5- 50.2%, respectively, in a polyculture in earthen ponds. Presently, the impact of blue mussel stocking densities on western king prawn growth and survival rates in an integrated aquaculture system is unknown.

The western king prawn is a popular species in Australia and Japan and is widely distributed throughout the Indo-West Pacific region (Dore and Frimodt 1987). Attempts have been made to culture this species in India, Japan (Shokita 1984) and Australia (Sang and Fotedar 2004a; Sang and Fotedar 2004b; Prangnell and Fotedar 2005, 2006b; Hai et al. 2007; Hai et al. 2009b, 2009a; Khoi and Fotedar 2010). Effects of stocking density on the nutrient budget and growth of the western king prawn in a recirculating aquaculture system have been reported by Khoi and Fotedar (2010). Polyculture of western king prawn and seaweeds, *Sargassum* spp. has been examined by Mai et al. (2008; 2010) in indoor tanks. The aim of this study was to examine the effects of different blue mussel stocking densities when integrated with western king prawn on water quality, growth, survival and nutrient contents in mussels and prawn muscles and tank sediments.

7.2 MATERIALS AND METHODS

7.2.1 Integrated recirculating culture systems

The experiment was carried out in CARL, Perth, Western Australia, Australia under laboratory conditions. Description and operation of the IRAS were presented in Section 3.1. The reservoir tanks (Chapter 4 and Chapter 5) in the IRAS are referred as mussel tanks in this trial.

7.2.2 Animals and experimental setup

Western prawn prawns were collected from the Canning river, Walter Point, Bicton, Western Australia, Australia (32^{o}01′ S, 115^{o}45′ E) and were acclimated as described in Section 3.2.

Blue mussels were collected by hand from the Walter Point jetty, Canning river, Bicton, Western Australia, Australia (32^{o}00’39.86”S, 115^{o}47’21.18E). They were transported and acclimated as described in Section 3.2.

Blue mussels were placed on trays with diameter of 50 cm, which were hanged 20 cm below the water surface of the mussel tanks. Trays were constructed using extruded polypropylene, UV-stabilised, oyster mesh (mesh size of 6mmx6mm) (Nylex® Corporation Pty Ltd, Australia) panel attached to 10 mm PVC pipe frame. That allowed mussel faeces and wastes can settle to the bottom of the mussel tanks.

A preliminary experiment was conducted to test the removal rate of mussels. During the preliminary trial, eighteen 1-L beakers were filled with sea water (35ppt) and microalgae, *Tetraselmis* sp. were added to beakers at three stocking densities of 5 x 10^3, 10 x 10^3 and 20 x 10^3 cell mL^{-1}. The blue mussel (initial mean length of 48.1±0.6 mm) were stocked at two density of 1 and 3 mussel per beaker. Three replicates were set up for each treatment and arranged in a completely randomized design. Aeration was supplied during the trial and the trial lasted for 30 minutes. Filtration rate was calculated as follows: $F= V. (Ln(C_0)-Ln(C_t))/Nt$; V= water volume, Ln: natural logarithm, C_0 and C_t: concentration at time 0 and time t, N: number of mussels.

Table 7.1 Western king prawn biomass and blue mussel stocking density in the experiment

Treatments	**Prawn**		**Mussel**		**Ratio of prawn biomass and mussel number**	**Ratio of mussel number and prawn biomass**
	Biomass (g)	**Density (prawn m^{-2})**	**Number**	**Density (mussel m^{-2})**		
1	40	16.07	0	0	-	-
2	40	16.07	10	31.25	4.00	0.25
3	40	16.07	20	62.50	2.00	0.50
4	40	16.07	40	125.00	1.00	1.00
5	40	16.07	80	250.00	0.50	2.00

In the main experiment, nine western king prawns with initial mean weight of 4.69±0.02 g were stocked into prawn tanks at a density of approximately 16 prawn m^{-2}, which equal approximately 40 g $tank^{-1}$. In order to test the effects of different

ratios of western king prawn to blue mussel stocking densities on growth and survival, water quality and nutrient compositions of prawns, mussels and wastes, five treatments were set up (Table 7.1). Treatment 1 had no mussel and was set as a control treatment. From treatment 2 to 5, blue mussels (initial mean length of 50.46±0.03 mm) were stocked into mussel tanks at densities of 10, 20, 40 and 80 mussels per tank or 31.25, 62.50, 125.00 and 250 mussel m^{-2} and hence ratios of blue mussel numbers and western king prawn biomass in the IRAS were 0.25, 0.50, 1.00 and 2.00, respectively. Three replicates were set for each treatment and arranged in a completely randomized experiment design.

Western king prawns were fed twice a day at a rate of 3% wet body weight per day using a commercially formulated feed ST#1 (43% protein, 6% fat and 2% fibre) (Ridley Aqua-feed, Victoria, Australia) and prawns were cultured for a period of 98 days. Tanks and wastes management were presented in Section 3.3. At harvest, mussel and waste collection tanks were drained and the deposited sediment was left to dry before collected to determine the total mass of sediment.

7.2.3. Data collection

7.2.3.1 Water quality parameters and bacteria culture

TAN, NO_2^-, NO_3^- and PO_4^{3-} samples were collected weekly and measured as described in Section 3.4.1. DIN was sum of the TAN, NO_2^-, NO_3^- concentrations. TN and TP were determined fortnightly as described in Section 3.4.1.

TB and TSS were determined as described in Section 3.4.1. TSS concentration was calculated as follows: TSS (mg L^{-1})=(W_d-W_f)/V. Where W_d is the dry weight (in mg), W_f is the weight of the filter (in mg), V is the volume of the sample (in L).

Water temperature, salinity and pH were measured as described in Section 3.4.1. water temperature was maintained between 24 and 25^oC whereas salinity was maintained between 34-35 ppt throughout the trial by adding freshwater water.

7.2.3.2 Prawns and mussels growth and survival rates

Western king prawn weights were measured using a portable balance (ACB 600H, Adam Equipment Inc., Danbury CT 06810, USA) with a precision of 0.01 g at day 0; 42 and 98 (harvest day) of the trial period while blue mussel shell lengths (shell length: the maximum distance between the anterior and posterior margins of the shell) were measured to the nearest 0.05 mm with a calliper at the beginning and termination of the main experiment.

SGR of the western king prawns and blue mussels were calculated by using the equation: SGR (% d^{-1}) = 100 x ($\ln W_t - \ln W_0$)/t; Where W_t and W_0 are the weight/length of the prawns or mussels at current time (t) and at the commencement of the experiment (0), respectively and t is the number of rearing days (d).

Survival rates were calculated by the formulae: Survival rate (%) = 100 x (n_t/n_0), where n_t and n_0 are number of prawns or mussels at time (t) and experiment start (0), respectively.

7.2.3.3 N and P contents of animals and wastes

Thirty post-acclimated mussels were frozen to −20 °C for further analysis of nitrogen and phosphorus contents and referred as stocked mussels. At the conclusion of the experiment, remaining prawns and mussels were also frozen and then defrosted to obtain the nitrogen and phosphorous contents in their dry muscles. The shell and flesh meat were dissected out of each mussel weighed and dried individually in ceramic crucibles at 105^{o}C for 24 hours to obtain dry weight in an electric oven (Thermotec 200 Oven, Contherm Scientific Ltd, Hutt city, New Zealand). The dried mussels were pooled for each tank and ground to a powder with a mortar and pestle. Determination of N and P contents of the whole prawn, wastes, mussel meat and prawn feed were described in Section 3.4.3.

Nutrient (P/N) conversion rate: 100 x (A-B)/C; Where A is the total nitrogen/phosphorus at harvest; B is the total nitrogen/phosphorus of the prawns and

mussels when starting the experiment; C is the total nitrogen/phosphorus in feed input. P and N conversion rates may refer as efficiencies of N and P feed utilization. Nutrient budget was calculated based on the initial nutrient inputs from the water, stocked mussel, stocked prawns and feed; and nutrient outputs from harvested prawns and mussels, drained water and waste deposits (waste-collection and mussel tanks). At the termination of experiment, submerged pumps were switched off and water samples with volume of 200 ml were collected from all tanks in the recirculating systems to determine the total nutrient contents in the drained water. The samples then were analysed soon after the collection. Waste deposited samples were collected by draining the waste-collection and mussel tanks; and dried and analysed by the above methods.

7.2.4 Data analysis

The SPSS statistical program (version 17.0) was used to analyse data. ANOVA and LSD *post hoc* tests were used to determine significant differences between mean water quality parameters, nutrient conversion rate, survival and growth rates of western king prawns and blue mussels reared in the IRAS. All significant test are at the $P<0.05$ level. All data were tested for normal distribution by Kolmogorov-Smirnov tests and for homogeneity of variance by Levene's test. Where the data did not have normal distribution and homogeneous variance, the Kruskal–Wallis test was used to test the overall difference of all treatments. In the case of significant treatment effects, Mann–Whitney's test was applied to analyse the significant difference between the means of each treatment. Relationship between total phosphorus, orthophosphorus concentrations, nitrogen contents and mussel–prawn ratios was examined by Spearman rank correlation.

7.3 RESULTS

In the preliminary trial, filtration rate was significantly ($P<0.05$) higher at density of three mussel L^{-1} than at one mussel L^{-1}. Removal rate was significantly ($P<0.05$) affected by microalgae densities. The highest removal rate (1.89 ± 0.15 l h^{-1}) was observed at lower *Tetraselmis* sp. density ($5x10^3$ cell mL^{-1}) which was significantly

higher than 1.33±0.12 l h^{-1} and 1.26±0.16 l h^{-1} at algae densities of 10x10^3 and 20 x10^3 cell mL^{-1}, respectively.

7.3.1 Water quality parameters

In the main experiment, TSS decreased with increasing mussel stocking densities and was significantly (P<0.05) affected by adding blue mussels into the IRAS (Table 7.2). TN concentrations were significantly (P<0.05) lower in tanks stocked at 62.50, 125.00 and 250.00 mussel m^{-2} than in those stocked at 32.25 mussel m^{-2} and prawn monoculture.. TSS removal rates ranged between 1.39 and 85.23% and mean values were significantly (P<0.05) higher at stocking density of 250.00 mussel m^{-2} (51.52%) than at stocking densities of 31.25 mussel m^{-2} (13.83%), 62.50 mussel m^{-2} (21.42%) and 125 mussel m^{-2} (33.50%). There were signs of fine solids and biofouling such as macroalgaes and banacles in the shells of mussels at the lower mussel stcoking densities of 32.25 and 62.50 mussel m^{-2} at the end of the trial. The TB load was significantly (P<0.05) lower in all mussel and prawn integrated culture treatmentsthan in prawn monoculture treatment (Table 7.2) and decreased with increasing mussel densities.

Table 7.2 Mean concentrations of TAN, DIN, TN, TP, TB and TSS in the integrated culture of blue mussel and western king prawn during 98-days trial

	Prawn Monoculture	**Mussel stocking densities (mussel m^{-2})**			
		32.25	**62.50**	**125.00**	**250.00**
TN (mg L^{-1})	7.83±0.50^{a}	7.66±0.46^{a}	7.38±0.20^{b}	7.51±0.13^{b}	7.41±0.14^{b}
TSS (mg L^{-1})	165.95±2.83^{a}	144.05±6.41^{b}	131.81±1.33^{b}	112.59±6.71^{c}	81.76±2.22^{d}
TB (10^5 CPU mL^{-1})	11.31±0.08^{a}	7.56±0.30^{b}	5.06±0.72^{bc}	5.48±0.67^{bc}	4.44±0.30^{c}
DIN (mg L^{-1})	6.05±0.36^{a}	6.11±0.51^{a}	6.20±0.54^{a}	6.61±0.71^{a}	7.20±0.55^{b}
PO_4^{3-} (mg L^{-1})	0.90±0.02^{a}	0.98±0.05^{ab}	1.04±0.02^{bc}	1.10±0.02^{c}	1.20±0.03^{d}
TP (mg L^{-1})	1.19±0.04^{a}	1.33±0.14^{ab}	1.42±0.03^{bc}	1.56±0.05^{c}	1.75±0.07^{d}
TAN (mg L^{-1})	0.18±0.00^{a}	0.16±0.02^{a}	0.17±0.01^{a}	0.22±0.03^{a}	0.21±0.03^{a}

Values are means±SE. Values followed by different letters of the same row are significantly difference at α=0.05.

TP and PO_4^{3-} concentrations significantly ($P<0.05$) increased with increasing blue mussel stocking densities in the IRAS. The highest values of TP and PO_4^{3-} were observed at tanks stocked 250.00 mussel m^{-2} (1.20 and 1.75 mg L^{-1}, respectively), which were significantly ($P<0.05$) higher than those stocked other blue mussel stocking densities (Table 7.2). The mussel stocking density showed a strong positive correlation with the mean TP ($R^2=0.9567$) and PO_4^{3-} ($R^2=0.9252$) concentrations. TP concentrations in the IRAS were 11.89 (32.25 mussel m^{-2}), 17.72 (62.50 mussel m^{-2}), 24.70 (125.00 mussel m^{-2}) and 35.81 % (250.00 mussel m^{-2}) higher than that in monoculture. The DIN concentration was significantly higher ($P<0.05$) in 250.00 mussel m^{-2} than in other mussel stocking densities (Table 7.2). The inclusion of blue mussels into the IRAS did not affect the TAN in the culture media at all treatments

7.3.2 Survival and growth rates of western king prawn and blue mussel

7.3.2.1 Survival and growth rates of western king prawn

Survival rates of western king prawns at the conclusion of the experiment ranged from 81.48-85.19% (Table 7.3) and were not significantly ($P>0.05$) affected by inclusion of blue mussels into the IRAS. Similarly, SGR of western king prawns were not significantly ($P>0.05$) different among the treatments over the experiment period (Table 7.3).

Table 7.3 The survival rates and growth performances of western king prawn and blue mussel in the integrated culture of blue mussel and western king prawn during 98-day trial

	Prawn -Monoculture	**Mussel stocking densities (mussel m^{-2})**			
		32.25	**62.50**	**125.00**	**250.00**
Prawn (g)					
Initial weight	4.65±0.02[a]	4.65±0.03[a]	4.67±0.02[a]	4.74±0.04[a]	4.71±0.05[a]
Harvest weight	8.06±0.18[a]	8.44±0.35[a]	8.66±0.70[a]	8.47±0.13[a]	8.29±0.35[a]
SGR (% d^{-1})					
Day 0-day 42	0.67±0.12[a]	0.80±0.04[a]	0.82±0.02[a]	0.76±0.03[a]	0.73±0.04[a]
Day42- day98	0.58±0.03[a]	0.66±0.05[a]	0.72±0.07[a]	0.67±0.01[a]	0.59±0.06[a]
Day 0-day98	0.61±0.03[a]	0.74±0.04[a]	0.77±0.03[a]	0.69±0.01[a]	0.65±0.02[a]

Survival rate (%)	85.19±9.80[a]	85.19±9.80[a]	85.19±3.70[a]	81.48±7.41[a]	81.48±3.70[a]
Mussel (mm)					
Initial length	-	50.50±0.10[a]	50.48±0.14[a]	50.52±0.18[a]	50.37±0.23[a]
Harvest length	-	53.03±0.04[a]	53.42±0.20[ab]	53.80±0.15[b]	54.52±0.18[c]
SGR (% d^{-1})	-	0.058±0.002[a]	0.068±0.002[ab]	0.075±0.006[b]	0.094±0.007[c]
Survival rate (%)	-	90.00±5.77[a]	90.00±2.89[a]	72.50±11.27[a]	80.00±11.46[a]

Values are means±SE. Values followed by different letters of the same row are significantly difference at α=0.05.

7.3.2.2 Growth and survival rates of blue mussel

Increasing the blue mussel stocking density did not significantly (P>0.05) alter the survival rates of blue mussels in the IRAS (Table 7.3). SGR were significantly (P<0.05) affected by adding the different blue mussel stocking densities. The highest SGR was recorded at mussel stocking density of 250.00 mussel m^{-2} which was significantly (P<0.05) higher than that at other blue mussel densities (Table 7.3). There was a strong positive correlation (R^2=0.998) between blue mussel stocking densities and SGRs in the IRAS.

7.3.3 Nitrogen and phosphorus content in animals and wastes; and nutrient conversion rates

7.3.3.1 Nutrient content in animals and wastes

N contents in western king prawns biomass ranged from 2.31 to 2.46% in wet weight and were not significantly (P>0.05) different among the treatments over the experimental period (Table 7.4). Increasing blue mussel stocking density in the IRAS resulted in an increase N content trend in mussel tissues. The highest N value (9.64±0.67%) was recorded at blue mussels reared at 250.00 mussel m^{-2} which was higher significantly (P<0.05) higher than those at stocking densities of 31.25 and 62.50 mussel m^{-2}. N contents in mussels tissues reared in the IRAS were significantly (P<0.05) lowered than N values in the initial mussels stock (Table 7.4).

Table 7.4 Nitrogen and Phosphorus contents (%) in western king prawns (in wet weight) and mussels (in dry weight), wastes (dry weight) and N: P ratio in the IRAS during 98-day trial

	Prawn Monoculture	Stocked mussel	Mussel socking densities (mussel m^{-2})			
			31.25	**62.50**	**125.00**	**250.00**
Nitrogen						
Prawn	2.40±0.08[a]	-	2.40±0.14[a]	2.31±0.05[a]	2.46±0.10[a]	2.43±0.08[a]
Mussel meat		9.99±0.30[a]	8.49±0.48[b]	8.92±0.73[b]	9.12±0.28[bc]	9.64±0.67[c]
Waste collection tank	2.15±0.36[a]	-	2.32±0.14[a]	2.06±0.23[a]	2.16±0.14[a]	2.24±0.22[a]
Mussel tank	1.47±0.04[a]	-	1.72±0.22[b]	1.77±0.04[b]	1.83±0.04[b]	2.07±0.17[c]
Phosphorus						
Prawn	0.31±0.04[a]	-	0.29±0.04[a]	0.32±0.03[a]	0.31±0.11[a]	0.36±0.02[a]
Mussel meat		1.37±0.09[a]	1.13±0.10[b]	1.18±0.04[b]	1.14±0.04[b]	1.20±0.03[b]
Waste collection tank	2.98±0.20[a]	-	3.15±0.46[a]	3.40±0.25[a]	3.21±0.02[a]	3.20±0.09[a]
Mussel tank	0.75±0.06[a]	-	0.81±0.20[a]	0.68±0.20[a]	0.64±0.13[a]	0.70±0.12[a]
N:P ratio	1.76		1.94	2.40	2.66	2.77

Values are means±SE. Values followed by different letters of the same row are significantly difference at α=0.05.

Inclusion of blue mussels into western king prawn culture in the IRAS significantly increased ($P<0.05$) N contents in sedimentation wastes at mussel tanks. N contents were significantly ($P<0.05$) lower in sediment wastes at mussel control tanks than in those at integrated culture tanks (Table 7.4). There were a strong positive correlation (R^2=0.9932) between mussel stocking densities and nitrogen contents in deposited wastes.

P contents in whole western king prawns and sediment wastes at mussel tanks and waste-collection tanks in the IRAS were not significantly ($P>0.05$) affected by inclusion blue mussels into western king prawns culture system (Table 7.4). P contents in dry meat of mussels were significantly ($P<0.05$) higher in the stocked mussels than in the harvested mussels in the integrated mussel-prawn treatments.

However, different mussel stocking densities did not significantly (P>0.05) affect the P contents of mussels tissue at the termination of the experiment. N and P ratios in the sedimentation wastes at the mussel tanks ranged from 1.76 to 2.77 and increased with the increasing of blue mussel stocking densities. There was a positive correlation (R^2=7875) between blue mussel stocking densities and N:P ratios in the deposited wastes from the mussel tanks.

7.3.3.2 Conversion rate of feed N

The conversion rate of feed N into western king prawn biomass was not significantly (*P*>0.05) affected by including different blue mussel stocking densities into western king prawn culture, whereas N conversion into total mussel biomass differed significantly (P<0.05) between blue mussel stocking densities (Table 7.5). The highest conversion rate of feed N (12.24±2.86%) was observed at the highest blue mussel stocking density (250.00 mussel m^{-2}) which was significantly (P<0.05) higher than those at other mussel stocking densities. Feed utilization efficiency showed a significant (P<0.05) lower at blue mussel densities of 31.25 and 62.50 mussel m^{-2} than those at higher densities of 125.00 and 250.00 mussel m^{-2} (Table 7.5). Utilization efficiencies of feed N were 0.46 (31.25 mussel m^{-2}), 0.86 (62.50 mussel m^{-2}), 2.38 (125.00 mussel m^{-2}) and 10.63% (250.00 mussel m^{-2}) higher than those at monoculture. Inclusion blue mussels into the IRAS increased significantly (P<0.05) feed N deposited in bottom of the mussel tanks in the IRAS.

Table 7.5 The conversion rate of feed N and P into western king prawn and blue mussel biomass in the IRAS.

	Prawn Monoculture	**Mussel stocking densities (mussel m^{-2})**			
		31.25	**62.50**	**125.00**	**250.00**
Nitrogen (%)					
Prawn	10.98±0.84^{a}	10.87±1.52^{a}	8.37±2.54^{a}	8.74±0.86^{a}	9.37±1.47^{a}
Mussel	-	0.57±0.16^{a}	3.46±0.11^{b}	4.61±0.59^{b}	12.24±2.86^{c}
Prawn+mussel	10.98±0.84^{a}	11.44±1.17^{a}	11.84±0.93^{a}	13.36±1.56^{ba}	21.61±2.35^{b}
Mussel tank	1.15±0.12^{a}	1.70±0.47^{a}	1.99±0.15^{ab}	2.29±0.14^{bc}	3.11±0.33^{c}

Phosphorus(%)					
Prawn	4.66±1.71[a]	4.27±3.25[a]	4.32±1.82[a]	3.32±0.22[a]	5.72±0.24[a]
Mussel	-	0.71±0.06[a]	2.13±0.69[b]	2.07±0.64[b]	3.83±0.66[c]
Prawn+mussel	4.66±1.71[a]	4.98±2.39[a]	6.45±1.13[a]	5.39±0.39[a]	9.55±0.80[b]
Mussel tank	1.99±0.35[a]	3.30±0.28[a]	2.59±0.86[a]	2.71±0.61[a]	3.57±0.84[a]

Values in the same row sharing a common superscript letters (a, b, c, d) are not significant different (LSD test; P<0.05; n=3).

7.3.3.3 Conversion rate of feed P

Approximately 3.32-5.72% feed P was retained in western king prawn biomass at harvest. The effect of blue mussel stocking densities on conversion of feed P followed the same pattern as that for conversion of feed N; adding different stocking densities of blue mussels into western king prawn culture had no significant effect on conversion of P into western king prawn biomass (P>0.05), but there was a significant effect of blue mussel stocking densities on the conversion into total biomass of culture animals (P<0.05) (Table 7.5). Tanks with highest blue mussel densities had a higher rate of P conversion into total biomass of animals than did those without mussel (monoculture) and other blue mussel stocking densities (Table 7.5). Utilization efficiencies of feed P in the integrated culture were 0.32 (31.25 mussel m^{-2}), 1.79 (62.50 mussel m^{-2}), 0.73 (125.00 mussel m^{-2}) and 4.89% (250.00 mussel m^{-2}) higher than those in the monoculture.

7.3.4 Nutrient budget

On average, 13.95-23.50% and 12.78-57.72% of total N inputs were retained in western king prawns and blue mussels biomass at harvest, respectively. Higher N retention was found in monoculture and the treatment stocked 31.25 mussel m^{-2}, which were significantly (P<0.05) higher than those stocked 125.00 and 250.00 mussel m^{-2} (Table 7.6). N retention in blue mussel biomass showed a significant increase with increasing blue mussel stocking densities (Table 7.6). N loss through water discharge at harvest and unaccounted N loss were 15.39-38.21% and 12.17-31.49%, respectively and showed a significant decline with increasing blue mussel stocking densities.

Table 7.6 Nitrogen budget in percentage of western king prawn integration with blue mussel in an IRAS during a 98-day culture

	Prawn Monoculture	Mussel stocking densities (mussel m^{-2})			
		31.25	62.50	125.00	250.00
Input-N (%)					
Water	3.42±0.00^{a}	3.00±0.00^{b}	2.67±0.00^{c}	2.18±0.00^{d}	1.61±0.00^{e}
Prawn	14.48±0.03^{a}	12.69±0.05^{b}	11.31±0.02^{c}	9.36±0.05^{d}	6.86±0.05^{e}
Mussel	-	12.37±0.01^{a}	22.01±0.01^{b}	36.04±0.02^{c}	53.00±0.03^{d}
Feed	82.09±0.03^{a}	71.94±0.04^{b}	64.01±0.02^{c}	52.41±0.03^{d}	38.53±0.02^{e}
Output-N (%)					
Water	38.21±1.73^{a}	34.23±1.25^{b}	27.35±3.09^{c}	22.94±0.30^{c}	15.39±0.30^{d}
Prawn	23.50±0.68^{a}	20.51±3.87^{ab}	16.67±1.64^{bc}	13.95±0.47^{cd}	10.47±0.53^{d}
Mussel	-	12.78±0.12^{a}	24.23±1.36^{b}	38.46±0.82^{c}	57.72±1.10^{d}
*Collection tank	5.86±0.94^{a}	6.02±1.53^{a}	3.95±0.32^{ab}	3.33±0.53^{ab}	3.06±0.25^{b}
**Mussel tank	0.95±0.10^{a}	1.22±0.34^{a}	1.27±0.09^{a}	1.20±0.07^{a}	1.20±0.13^{a}
Unaccounted	31.49±2.05^{a}	25.23±2.34^{ab}	26.52±3.09^{ac}	20.13±1.38^{bc}	12.17±1.05^{d}

Values in the same row sharing a common superscript letters (a, b, c, d, e) are not significant different (LSD test; $P<0.05$; n=3). *Percentage of nitrogen input was trapped in the collection tanks; ** percentage of nitrogen input was sunk in mussel tanks.

P content retained in western king prawn biomass at harvest ranged from 7.99 to 11.24% of total P inputs and was not significantly ($P<0.05$) affected by inclusion blue mussels into the IRAS (Table 7.7). P retention in mussel tissues showed a similar trend to N retention. Approximately 22.45-31.17% P inputs were settled in collection tanks in the IRAS whereas P discharged through draining at harvest ranged between 29.87-40.12% of total P inputs.

Table 7.7 Phosphorus budget in percentage of western king prawn integration with blue mussel in an IRAS during a 98-day culture

	Prawn Monoculture	Mussel stocking densities (mussel m^{-2})			
		31.25	62.50	125.00	250.00
Input-P (%)					

Water	1.58±0.00^{a}	1.49±0.00^{b}	1.40±0.00^{c}	1.25±0.00^{d}	1.03±0.00^{e}
Prawn	7.02±0.02^{a}	6.58±0.03^{b}	6.21±0.02^{c}	5.60±0.05^{d}	4.62±0.04^{e}
Mussel	-	6.22±0.07^{a}	11.74±0.18^{b}	21.18±0.33^{c}	42.03±0.08^{d}
Feed	91.40±0.02^{a}	85.71±0.06^{b}	80.66±0.17^{c}	71.98±0.28^{d}	59.58±0.02^{e}
Output-P (%)					
Water	39.74±0.79^{a}	40.12±3.15^{a}	38.86±0.35^{a}	36.50±0.14^{a}	29.87±0.16^{b}
Prawn	11.24±1.57^{a}	10.24±2.80^{a}	9.70±1.50^{a}	7.99±0.10^{a}	8.02±0.17^{a}
Mussel	-	6.83±0.91^{a}	13.45±0.73^{b}	22.67±0.53^{c}	37.06±0.34^{d}
*Collection tank	30.17±1.99^{a}	31.15±5.59^{a}	28.71±5.62^{a}	22.45±2.33^{b}	22.56±0.77^{b}
**Mussel tank	1.82±0.32^{a}	2.83±0.24^{a}	2.09±0.70^{a}	1.95±0.43^{a}	2.13±0.50^{a}
Unaccounted	18.85±2.46^{a}	11.65±3.19^{ab}	9.29±4.62^{bc}	10.40±1.88^{ac}	2.49±0.54^{c}

Values in the same row sharing a common superscript letters (a, b, c, d, e) are not significant different (LSD test; $P<0.05$; $n=3$). *Percentage of nitrogen input was trapped in the collection tanks; ** percentage of nitrogen input was sunk in mussel tanks.

7.4 DISCUSSION

Previous studies have shown that filtration by bivalves can significantly reduce the concentrations of bacteria, TN and TP and other suspended particles in prawn effluents but may increase NH_4^+ and PO_4^{3-} concentrations; and other inorganic matters through bivalve excretion (Prins and Smaal 1994; Jones and Preston 1999; Jones et al. 2001). In the present study, higher mussels effectively removed the TN, TB and TSS. Similarly, oysters (*Crassostrea virginica*) can remove bacteria, phytoplankton and other suspended solids from the prawn pond effluent (Jones and Preston 1999; Jones et al. 2001). Loosanoff (1949) and Jones et al. (2002) reported that filter-feeder bivalves feed on the small rich organic particles, such as bacteria and organic matter debris. They sort particles by size, weight (Yonge 1926) and chemical composition (Loosanoff 1949). Newell and Jordan (1983) reported that oyster preferentially ingest organic material and reject inorganic material (Newell and Jordan 1983). Therefore, bivalves can effectively reduce suspended particles in the water colume. The present study showed that presence of higher blue mussel stocking density (up to 250.00 mussel m^{-2}) can effectively removed suspended solids and controls the growth of bacteria in the closed integrated system. Higher mussel density would translate to more organisms to filter or remove the bacteria and

suspended solids from the water. That was in comfirmation with the findings of the preliminary trial. However, higher mussel stocking densities (250.00 mussel m^{-2}) resulted in higher DIN concentrations while low mussel densities (31.25, 62.50 and 125.00 mussel m^{-2}) were not significantly affect the DIN concentrations in the IRAS. Several authors reported that mussels can increase DIN concentrations in culture media due to the excretion (Dame et al. 1991; Prins and Smaal 1994; Jones et al. 2001). Jones et al.(2001) reported that mean NO_3^-/NO_2^-concentration was reached 13.0 µM in Sydney rock oyster tanks when oysters were used as the biofilter to treat prawn effluents, compared to 1.4 µM in control tank (without oysters).

PO_4^{3-} and TP concentrations in the current study increased with increasing blue mussel stocking densities. The increase in PO_4^{3-} was probably due to the mussel excretion (Asmus et al. 1995) and was similar to several other studies (Dame et al. 1991; Jones et al. 2001). Jones et al.(2001) found that PO_4^{3-} concentrations increased from 0.5 to 2.0 µM in the control treatment (tanks without Sydney rock oysters) and from 0.5 to 3.3 µM in the oyster treatment (tanks with oysters) in an integrated treatment of prawn effluents. In the present trial, TP concentrations increased with increasing mussel stocking densities.. The result is in contradiction to the findings of Jones et al (2001; 2002) for Sydney rock oyster. Jones and Preston (1999) reported that oyster may remove 67% TP in the prawn farm effluents. The fact that prawn pond effluent often contains a high proportion of small clay particles (Hopkins et al. 1995) rich in phosphate (Pomeroy et al. 1965) which may be filtered by bivalves while the tank bottoms in the closed recirculating system had little sediments and non-clay suspended solids, thus the nutrient concentration equilibrium can skew in favour of the water and suspended solids which resulted in an increase in inorganic P and TP concentrations over the course of the experiment.

TAN concentrations in the present study were not affected by the inclusion of blue mussels into the IRAS. This finding is in line with the findings of Pietros and Rice (2003) who reported that NH_3^--N concentrations in tanks with eastern oysters (*Crassostrea virginica*) were similar to control tanks without oysters. In contrast, the lowest NH_3^--N concentrations was found at the highest combined density of Pacific oyster (16 pcs m^{-2}) and black clam (10 pcs m^{-2}) with the white prawn in the polyculture ponds (Martinez-Cordova and Martinez-Porchas 2006). NH_3^--N

concentrations in the current study were well below the lower limit of NH_3 toxicity to penaeid species (ChenLiu et al. 1990), suggesting that during the experimental period, total biomass of blue mussels and western king prawns were below the carrying capacity of the system, and the deleterious nitrogenous waste was effectively removed by phytoplankton, microbial activities (Shilo and Rimon 1982) and bivalves (Jones et al. 1996; Jones and Preston 1999; Jones et al. 2001; Jones et al. 2002).

The SGRs of western king prawns in the study (0.61-0.77 % d^{-1}) are higher than in the study conducted by Prangnell and Fotedar (2005). However, the higher SGRs were not a consequence of the blue mussel inclusion as the control treatment also resulted in the same SGR. The highest blue mussel growth rate was obtained at 250.00 mussel m^{-2}, suggesting that blue mussel grew well at the stocking rate of 250.00 mussel m^{-2} and 40 g of prawn biomass (16.07 prawn m^{-2}) in the IRAS. Several authors (Kautsky 1982b; Boromthanarat and Deslou-Paoli 1988) reported that high mussel stocking density has a negative effect on the mussel growth. On the contrary, Lauzon-Guay et al. (2005) found that mussels at low (146-335 pcs per 30cm of sock) and high (357-787 pcs per 30 cm of sock) initial densities were of the same length by the end of the experiment. In the present study, mussel SGR's increased significantly with increasing mussel stocking densities. Mussels feed on particles suspended in the surrounding water by filtration. Mussel filtration rate is mainly controlled by food concentration (Widdows et al. 1979), temperature and particles size (Thompson and Bayne 1974); and quality and quantity of seston (Bayne et al. 1993; Wong and Cheung 1999). Studies on physiological response of bivalves to increasing suspended sediment show a decrease in clearance rate (Allan et al. 1995; Aguilera-Morales et al. 2005) and growth (Fernando 2002; Watanabe 2002). Shin et al (2002) reported that mussel, *Perna viridis* can survive in a high level of suspended solids (from 0 to 1200 mg/L) over a period of 96h but there were morphological damages of the ctenidia when mussels were under 14-d exposure of suspended solids from 0 to 600 mg/l, followed by 14-d recovery in natural seawater, that exert sub-lethal effects resulting in reduced activities in feeding, respiration and even growth in the longer term. Loosanoff and Tommers (1948) and Winter (1978) reported that when sediment loads are too high, mussel filtration can be reduced or ceased completely. Water flow rate can have a significant impact on the ability of

mussels to filter particulates. High-water flow rates can enhance mussels filtration (Loosanoff and Tommers 1948), but low water flow rates enable more effective settling of particulates (Henderson and Bromage 1988). Water flow rate in the present study, was set at 10 mL s^{-1} and there were signs of particulated solids and fouling of mussels shells in the lower blue mussel stocking densities (31.25 and 62.50 mussel m^{-2}) at the end of the experiment, suggesting that low growth rates of mussels reared at lower mussel-prawn ratios are probably related to the high suspended solids and low water flow rates in these IRAS culture medias.

N content in blue mussel tissues increased with increasing mussel stocking densities and was higher in blue mussels of the initial stocks than those reared in the IRAS. Higher suspended solids in the culture media impacted on filtration rate of mussels (Loosanoff and Tommers 1948) and may cause the low nutrient contents in mussels meat in lower mussel stocking densities. In addition, suspension feeding bivalves have phytoplankton as the main component of their diet, in the shallow coastal environment (Shumway et al. 1987; Mac Donald and Ward 1994), and this component includes a variety of species differing in cell size, shape and other structural features. Food availability is main factor controlling the growth rate of suspension-feeding (Winter 1978) and referred as the phytoplankton dynamic (Rosenberg and Loo 1983). Strohmeier et al.(2008) reported the reduction of mussel meat ratio (%), chemical compositions and biomass in the centre of a mussel long-line culture farm where the phytoplankton concentration was 20 to 91% less in the centre of the farm compared to the reference sites. Therefore, the lower nutrient contents in mussels reared in the IRAS was probably due to high suspended solids and the absence of phytoplankton in culture media.

N contents in the deposited wastes at mussel tanks ranged 1.47-2.07% dry weight and correlated positively with the mussel stocking density. Inclusion blue mussels into western king prawn culture in the IRAS significantly affected the N contents in the wastes at mussel tanks. Previous studies have shown that mussel increases the quantity and quality of deposited organic matter through the production of faeces and pseudo-faeces (Kaspar et al. 1985; Giles and Pilditch 2006). More than 48 % of both the carbon and nitrogen consumed were expelled as faeces (Hawkins and Bayne 1985). Wong and Cheung (1999) reported that pseudo-faeces production of the green

mussel (*Perna viridis*) was a positive function of the rate of particle filtration; and when sestons had a lower organic content, more pseudo-faeces was released. Faeces and pseudo-faeces have higher sinking velocities than their constituent particles and therefore increase nutrient sedimentation (Giles and Pilditch 2006).

The N and P conversion rates by western king prawn in the present study ranged from 8.37–10.98% and 3.32–5.72%, respectively (Table 7.5). N conversion rate was close to that reported by Khoi and Fotedar (2011) for western king prawn (5.76-10.22%) in the same IRAS. However, the value was lower than N conversion rate (12.6–17.8%) of Chinese prawn reported by Tian et al (2001). The rates of conversion of feed N and P by blue mussels in the present study (Table 7.5) were closed to those reported by Tian et al. (2001) for constricted tagelus (N, 1.42-7.31%; P, 0.58-3.21%) in a closed polyculture of Chinese prawn, tilapia hybrids and constricted tagelus. Feed utilization efficiencies of the integrated culture of western king prawns and blue mussels in the present study were 11.44-21.61% N and 4.98-9.55% P. Feed N efficiency was lower than 28.0-38.7% (Xiongfei et al. 2005) in a closed-recirculating polyculture of white prawn and constricted tagelus in ponds but were comparable to 12.2-20.1% N (Zhen-xiong et al. 2001) in closed prawn culture systems in ponds. Jordan and Valiela (1982) reported that ribbed mussel (*Guekensia demissa*) absorb approximately 50% of N which they were filtered. About 4% of the N absorbed by the mussels is secreted in byssal threads, 20% invested for growth, and 21% released in gametes (Jordan and Valiela 1982). Feed N and P conversion rates of blue mussels in this study in different treatments showed similar trends; they were low in the lower mussel stocking densities of 31.25 and 62.50 mussel m^{-2} and obtained the highest value at the 250.00 mussel m^{-2}. A similar trend was noticed for the prawn growth rate. As discussed above, higher suspended solid had negative impacts on the growth of the mussels at lower stocking densities (31.25 and 62.50 mussel m^{-2}) in the IRAS. This results in declining the growth rate and then biomass gain of mussels integrated with western king prawn in IRAS.

Feed are the major nutrient inputs in water exchange prawn ponds (Briggs and Funge-Smith 1994) and closed tank culture system (Thakur and Lin 2003) and account for approximately 82-95% N and 38-91% P of total nutrient inputs (Briggs and Funge-Smith 1994). In the present study, percentage contribution of feed to the

total nutrient inputs in the monoculture and lower mussel stocking densities (31.25 and 62.50 mussel m^{-2}) was in the same range as reported previously (Briggs and Funge-Smith 1994; Thakur and Lin 2003), however feed contributes only 38-52% N and 60-72% P in the total nutrient inputs in higher mussel stocking densities (125.00 and 250.00 mussel m^{-2}). Nutrient budget showed that western king prawns could assimilate only 10.47-23.50% N and 7.99-11.24 P whereas blue mussels retained 12.78-57.72% N and 6.83-37.06% P of the total nutrients inputs. Nutrient budget values of prawns are comparable to those reported by Khoi and Fotedar (2010, 2011) for western king prawn in the same culture systems and Islam et al. (2004) with 12.1% of the N incorporated into tiger prawn biomass when reared in earthen ponds. However, the estimates in the present study are lower compared with other reports for tiger prawn culture of nearly 22% (JacksonPrestonThompson et al. 2003) and 23-31% N and 10-13% P (Thakur and Lin 2003) in a closed tank culture system.

The major outputs of nutrients in the closed culture system were in sediment (Briggs and Funge-Smith 1994; Thakur and Lin 2003). In contrast, the present study showed that loss of N in water discharge (15.39-38.21%) at harvest was higher than loss N through sediment (3.06-6.02%). The sink of P in the sediment accounted for 22.56-31.15% P of the total inputs whereas the drained water at harvest contained 29.87-40.12% P. Enell and Ackefors (1991) mentioned that approximately 50% of the N and P that settle on the bottom is translocated back into water column.

The study showed that the blue mussels can grow in the IRAS and effectively remove the TB, TSS and TN in the cultured water. The stocking densities of blue mussels had no effects on the growth and survival rates of western king prawns reared in IRAS. The suitable stocking rate of western king prawns and blue mussels in the IRAS were 250.00 mussel m^{-2} and 16.05 prawn m^{-2} in term of mussel growth, TN and TB removal rates. Low blue mussel growth rate is related to the depletion of food availability and water flow rate. Inclusion of blue mussels into western king prawn culture was enhanced the feed utilization efficiency and reduced the nutrient discharged through draining at harvest. Future research should focus on the optimum blue mussel stocking and western king prawn biomass reared in the closed integrated recirculation system in order to enhance the nutrient removal efficiency of mussel and the mussel growth rate.

CHAPTER 8 EFFECTS OF DIFFERENT MUSSEL STOCKING DENSITIES AND PRAWN BIOMASS

8.1 INTRODUCTION

Intensive prawn farming invariably results in the discharge of significant amounts of nutrients into adjacent waterways with a high proportion of these nutrients originating from the commercial feed (Briggs and Funge-Smith 1994). Nutrient retention capacity for N and P, being provided through feeds, is usually low and variable in fish and prawn farming. Studies of intensive prawn farms in Thailand found that only 21% of the prawn feed N was recovered as harvested prawn while 35% was discharged to the environment (Briggs and Funge-Smith 1994). The direct discharge of waste nutrients from prawn farms into adjacent environments has raised concerns about the sustainability of prawn farming (Phillips et al. 1993; Primavera 1994; Naylor et al. 2000).

Integrated aquaculture of filter-feeding bivalves with cultured animals can minimise the environmental impacts of farm effluents, the main reason for this is that bivalves species can fed on and assimilate most of the wastes generated from prawn effluents (Martínez-Porchas et al. 2010). Jones et al. (2001) reported that presence of Sydney rock oyster diminished the concentration of suspended particles, phytoplankton and bacteria in the prawn effluent. Higher efficiency of nitrogen utilization has been observed in polyculture/integrated aquaculture systems compared with monoculture systems (Li and Dong 2000; Xiongfei et al. 2005). Tendencia (2007) reported that molluscs and Chinese prawn integrated cultures have been widely practiced in China.

In intensive prawn farming, increasing prawn stocking density resulted in an increasing input of organic matter into the culture environment, through feed pellets. Several reports have indicated that NH_3 and TSS increase with stocking densities (Robertson and Phillips 1995; Patnaik and Lawrence 2011). In the integrated culture system, higher bivalves density would translate to more organisms to filter or remove the bacteria and suspended solids from the waste water (Jones and Preston 1999) but presence of bivalves in the culture media may increase concentrations of

PO_4^{3-} and NH_4^+ in culture media through the excretion of bivalves (Prins and Smaal 1994; Jones et al. 2001). Therefore, stocking rate of culture species is an important concern in integrated culture management (Martínez-Porchas et al. 2010). Presently, an optimum stocking rate of bivalves and prawns in the integrated closed recirculating culture system is unknown. In previous chapter (Chapter 7), results showed that blue mussels can grow in the IRAS and effectively remove the TB, TSS and TN in the cultured water of western king prawns. The highest blue mussel growth was observed at the stocking density of 250 mussel m^{-2} and western king prawn biomass of 40 g (16.07 prawn m^{-2}). Therefore, the aim of this study was to examine the effects of integration of different blue mussel stocking densities and different western king prawn biomass on water quality; growth and survival of western king prawns and blue mussels, nutrient contents in mussels, prawns meat and tank sediments.

8.2 MATERIALS AND METHODS

8.2.1 Test animals

Western king prawns (initial average weight of 4.18±0.23 g) were collected from the Canning river, Walter Point, Bicton, WA, Australia ($32^o01'$ S, $115^o45'$ E) and were acclimated as described in the Section 3.2.

Blue mussels with an initial mean length of 48.21±0.10 mm were collected and acclimated as described in the Section 3.2.3. Mussels were placed on trays with diameter of 50 cm, which were hanged 20 cm below the water surface of the mussel tanks. Trays were constructed using extruded polypropylene, UV-stabilised, oyster mesh (mesh size of 6mm x 6mm) (Nylex® Corporation Pty Ltd, Australia) panel attached to 10 mm PVC pipe frame, allowing blue mussel faeces and wastes can settle to the bottom of the mussel tanks.

8.2.2 Recirculating aquaculture system

The experiment was carried out using fifteen IRAS, in CARL, Perth, Western Australia under laboratory conditions. Description and operation of the IRAS were

presented in Section 3.1. Aeration was supplied to prawn and mussel tanks by two air stones suspended at mid-column of water.

8.2.3 Experimental set up and feeding of prawns

To test the effects of different stocking rates of western king prawn and blue mussel on growth, survival and water quality, five integrated culture treatments were set up (Table 8.1). From treatment 1 to treatment 3, prawns were stocked at densities of 21.36, 27.77 and 34.18 prawn m^{-2} (biomass of 50, 65 and 80 g $IRAS^{-1}$), respectively; and mussels were stocked at densities of 312.50, 406.25 and 562.50 mussel m^{-2} (number of 100, 130 and 180 mussel $IRAS^{-1}$), respectively (Table 8.1). Stocking rates of the blue mussel number and western king prawn biomass in the IRAS were 2.0 in the first three treatments. For treatment 4 and 5, prawn was stocked at densities of 27.77 and 34.18 prawn m^{-2} (biomass of 65 and 80 g $IRAS^{-1}$), respectively, while mussel densities were kept at a density of 250.00 mussel m^{-2} (number of 80 mussel $IRAS^{-1}$). Stocking rates of mussel numbers and prawn biomass in the treatment 4 and 5 were 1.2 and 1.0, respectively (Table 8.1). Three replicates in a completely randomized experimental design were used for each treatment. .

Table 8.1 Western king prawn biomass and blue mussel stocking densities in the experiment

Treatments	Prawn		Mussel		Ratio of prawn biomass and mussel number	Ratio of mussel number and prawn biomass
	Biomass (g)	Density (prawn m^{-2})	Number	Density (mussel m^{-2})		
1	50	21.36	100	312.50	0.5	2.0
2	65	27.77	130	406.25	0.5	2.0
3	80	34.18	160	562.50	0.5	2.0
4	65	27.77	80	250.00	0.8	1.2
5	80	34.18	80	250.00	1.0	1.0

Western king prawns were fed twice a day at a rate of 3% wet body weight using a commercially formulated feed ST#1 (43 protein, 6% fat and 2% fibre) and were

cultured for a period of 91 days. Waste and tank management were described in Section 3.3. At the termination of the experiment, mussel and waste-collection tanks drained and the deposited sediment was collected for further analysis.

8.2.4 Data collection

8.2.4.1. Growth and survival rates of animals

Western king prawns were measured for total weight after 42 days and at the conclusion of the experiment (91 days). Prawns and mussel measurements were described in the Section 3.4.2. Specific growth rate (SGR) of the western king prawns and blue mussels were calculated by using the equation: SGR (% d^{-1}) = 100 x ($\ln W_t - \ln W_0$)/t; Where W_t and W_0 are the weight/length of the prawns or mussels at current time (t) and at the commencement of the experiment (0), respectively and t is the number of rearing days (d).

Survival rates were calculated by the formulae: Survival rate (%) = 100 x (n_t/n_0), where n_t and n_0 are number of prawns or mussels at time (t) and experiment start (0), respectively

8.2.4.2 Water quality parameters and bacterial load

Water temperature, pH and salinity were determined as described in the Section 3.4.1. Salinity was adjusted between 34-35 ppt throughout the trial by adding freshwater to compensate for salinity rise caused by evaporation.

TAN, NO_2^-, NO_3^- and PO_4^{3-} were collected weekly and measured as described in the Section 3.4.1. TN, TP and TSS were collected fortnightly and determined as described in the Section 3.4.1.

The bacterial load in the rearing medium was determined at the commencement of the experiment and monthly thereafter. Determination of the TB was presented in the Section 3.4.1.

8.2.3.3 Nitrogen and Phosphorus contents of prawns, mussels and wastes

Western king prawns and blue mussels were frozen to -20^{o}C at the conclusion of the experiment and later defrosted to obtain the nitrogen and phosphorous contents. The shell and flesh meat were dissected out of each mussel from the IRAS, weighed and dried individually in ceramic crucibles at 105^{o}C to a constant weight to obtain dry weight in an electric oven (Thermotec 200 Oven, Contherm Scientific Ltd, Hutt city, New Zealand). The dried mussel was pooled for each tank and ground to a powder with a mortar and pestle. Total protein nitrogen and phosphorus contents were measured as described in the Section 3.4.3.

8.2.4 Data analysis

The SPSS statistical program (version 17.0) was used to analyse data. ANOVA and LSD *post hoc* tests were used to determine significant differences between mean water quality parameters, survival and growth rates of prawns and mussels reared in the closed integrated culture systems. All significant test are at the $P<0.05$ level. All data were tested for normal distribution by Kolmogorov-Smirnov tests and for homogeneity of variance by Levene's test. Where the data did not have normal distribution and homogeneous variance, the Kruskal–Wallis was used to test the overall difference of all treatments. In the case of significant treatment effects, Mann–Whitney's test was applied to analyse the significant difference between the means of each treatment.

8.3 RESULTS

8.3.1 Survival and growth rates of western king prawns and blue mussels

8.3.1.1 Prawn survival and growth rates

Increasing stocking densities of blue mussels and western king prawns in the IRAS significantly ($P<0.05$) reduced prawn survival. At the conclusion of the experiment, prawn survival rates was the highest in IRAS stocked 312.50 mussel m^{-2} and 21.36 prawn m^{-2}; and 250.00 mussel m^{-2} and 27.77 prawn m^{-2}, which were significantly

(P<0.05) higher than other stocking rates (Table 8.2). Survival rates of prawns were significantly (P<0.05) higher at lower prawn density (27.77 prawn m^{-2}) than at higher (34.18 prawn m^{-2}) when blue mussels were stocked at the same initial densities of 250.00 mussel m^{-2}.

Western king prawn survival was significantly (P<0.05) affected by adding blue mussels into the western king prawn culture in IRAS. Higher western king prawn survival was found at lower mussel density (250.00 mussel m^{-2}) when prawns were reared at the same densities of 27.77 prawn m^{-2}. However, when western king prawn stocking density was 34.18 prawn m^{-2} (Table 8.2), survival of prawns in the IRAS was significantly (P>0.05) independent of blue mussel inclusion s into the prawn culture.

Table 8.2 SGR and survival rates of western king prawn and blue mussel at different stocking rates in the IRAS during 91-day trial

	Stocking rates of mussel (mussel m^{-2}) and prawn (prawn m^{-2})				
	312.50	**406.25**	**562.50**	**250.00**	**250.00**
	21.36	**27. 77**	**34.18**	**27. 77**	**34.18**
Prawn Survival (%)					
Day 42	77.78±5.56^{a}	41.67±4.17^{bc}	38.60±6.33^{b}	66.67±8.33^{ac}	49.12±12.65^{bc}
Day 91	63.89±7.35^{a}	22.92±2.08^{b}	19.30±1.75^{b}	45.83±7.51^{a}	24.56±8.77^{b}
SGR (% d^{-1})					
Day0-Day42	$_{1}$0.59±0.04^{a}	$_{1}$0.51±0.05^{a}	$_{1}$0.33±0.03^{b}	$_{1}$0.54±0.03^{a}	$_{1}$0.54±0.06^{a}
Day 42-Day91	$_{1}$0.48±0.06^{a}	$_{2}$0.34±0.02^{b}	$_{1}$0.30±0.04^{b}	$_{2}$0.35±0.03^{b}	$_{1}$0.40±0.05^{ab}
Day0-Day91)	$_{1}$0.57±0.03^{a}	$_{1}$0.40±0.02^{b}	$_{1}$0.31±0.01^{c}	$_{1}$0.52±0.02^{ε}	$_{1}$0.46±0.05^{b}
Mussel					
Survival (%)	51.67±3.53^{a}	12.31±2.04^{b}	3.13±0.36^{c}	44.58±1.50^{ε}	21.25±2.60^{d}
SGR (% d^{-1})	0.070±0.002^{a}	0.058±0.004^{b}	0.045±0.003^{c}	0.065±0.007^{ab}	0.064±0.007^{ab}

Values are means±SE. Values followed by different letters of the same row are significantly difference at α=0.05.

The SGR of western king prawns decreased significantly ($P<0.05$) as prawn biomass and/or mussel density increased. The highest values were recorded at the stocking rates of 312.50 mussel m^{-2} and 21.36 prawn m^{-2} (0.57±0.03 % d^{-1}); and 250 mussel m^{-2} and 27.77 prawn m^{-2} (0.52±0.02 % d^{-1}), and were significantly ($P<0.05$) higher than other stocking rates in the IRAS. When prawns were stocked at the same initial density of 27.77 or 34.18 prawn m^{-2}, growth of prawns were significantly ($P<0.05$) higher in lower initial mussel densities (Table 8.2).

8.3.1.2 Mussel survival and growth rates

Survival and growth rates of blue mussels showed similar trends as of western king prawns (Table 8.2). The lowest survival (3.13±0.36%) was observed at the highest mussel stocking density (stocking rate of 562.50 mussel m^{-2} and 34.18 prawn m^{-2}). Survival of blue mussels was significantly ($P<0.05$) higher at western king prawn density of 27.77 prawn m^{-2} than at the density of 34.18 prawn m^{-2} when blue mussels were stocked at the same initial density (250 mussel m^{-2}). The lowest SGR (0.045±0.003 % day^{-1}) was found in the highest blue mussel density (562.50 mussel m^{-2}) and western king prawn density (34.18 prawn m^{-2}) and was significantly ($P<0.05$) lower than other stocking rates (Table 8.2). There was no significant difference ($P>0.05$) in the SGR of blue mussels in IRAS stocked at the same initial densities of 250.00 mussel m^{-2} or 27.77 prawn m^{-2}.

Table 8.3 Overall concentrations of TAN, NO_3^-, NO_2^-, TP , TSS and TB in the IRAS of blue mussel and western king prawn during 91-day trial

	Stocking rates of mussel (mussel m^{-2}) and prawn (prawn m^{-2})				
	312.50 21.36	**406.25 27. 77**	**562.50 34.18**	**250.00 27. 77**	**250.00 34.18**
TAN (mg L^{-1})	0.30±0.02^{a}	0.32±0.01^{a}	0.46±0.03^{b}	0.30±0.01^{a}	0.35±0.02^{a}
NO_3^- (mg L^{-1})	13.65±0.65^{a}	15.22±0.42^{ac}	18.04±0.73^{b}	14.10±1.38^{ac}	16.12±2.02^{bc}
NO_2^- (mg L^{-1})	0.28±0.01^{a}	0.26±0.02^{a}	0.28±0.01^{a}	0.25±0.01^{a}	0.26±0.01^{a}
PO_4^{3-} (mg L^{-1})	1.23±0.04^{a}	1.43±0.02^{bd}	1.54±0.03^{b}	1.29±0.05^{ac}	1.38±0.04^{dc}
TP (mg L^{-1})	1.69±0.08^{a}	2.09±0.09^{b}	2.69±0.09^{c}	1.82±0.03^{ad}	1.94±0.02^{bd}

TB (x10^5 CPU mL^{-1})	16.35±0.60^{a}	20.52±1.53^{b}	22.19±1.59^{b}	22.02±1.26^{b}	20.25±0.73^{b}
TSS (mg L^{-1})	148.21±3.67^{ac}	154.35±4.86^{ab}	164.64±2.62^{b}	137.23±4.30^{c}	125.50±1.58^{d}

Values are means ± SE. Values followed by different letters of the same row are significantly difference at α=0.05.

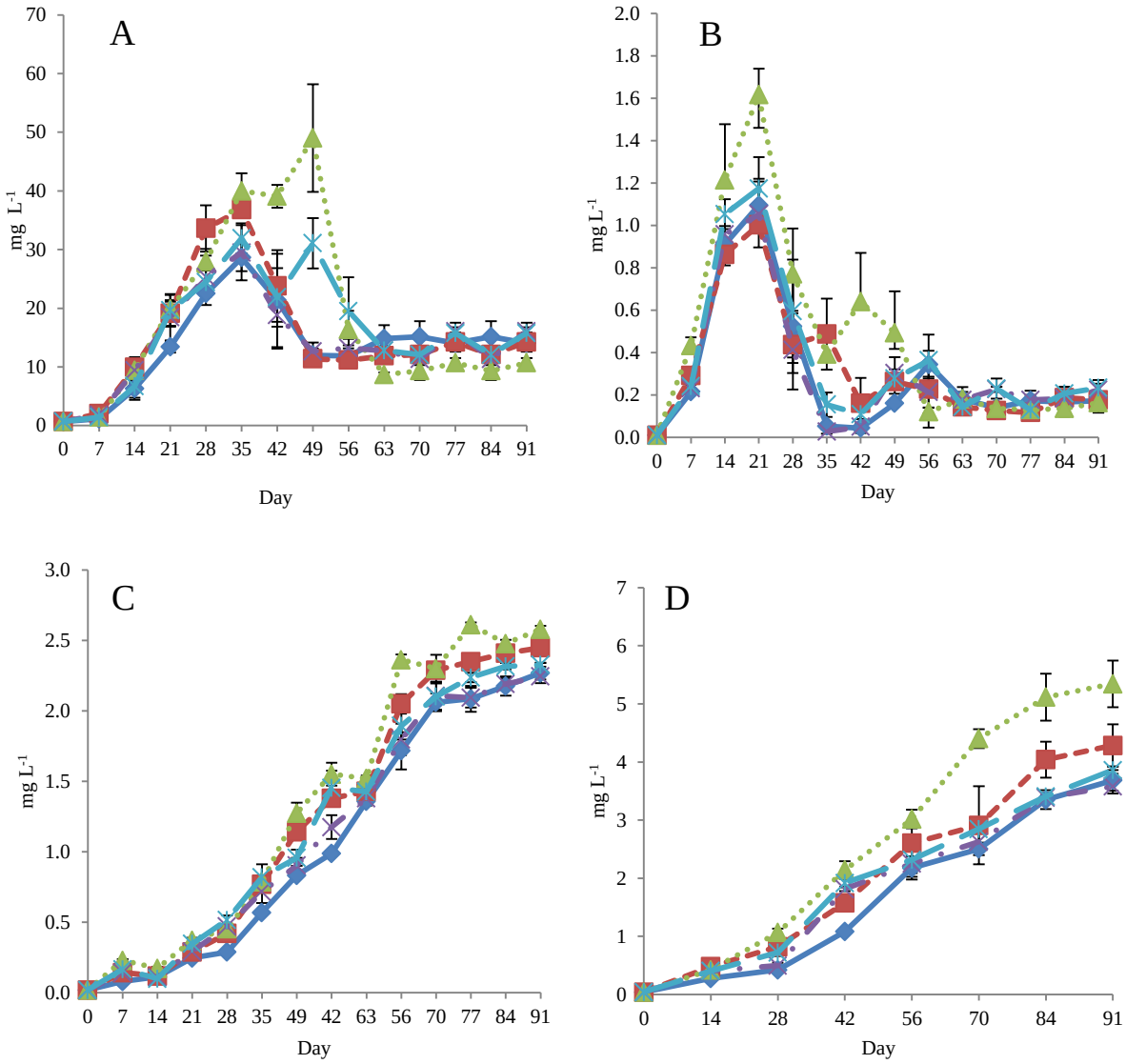
8.3.2 Water quality parameters

Overall concentrations of TAN, NO_3^-, PO_4^{3-} and TP showed an increasing trend when both blue mussel and western king prawn densities increased (Table 8.3). TAN concentrations ranged from 0.01 to 1.62 mg L^{-1} and peaked at day 21 of the trial (Figure 8.1B). The highest TAN overall concentration was found in the stocking rate of 562.50 mussel m^{-2} and 34.18 prawn m^{-2} (Table 8.3) and was significantly ($P<0.05$) higher than those in other treatments. NO_2^--N concentrations were independent ($P>0.05$) to the increase of blue mussel and western king prawn densities.

Concentrations of TP, PO_4^{3-} and TB showed an increasing trend (Table 8.2) over the course of the experiment (Figure 8.1C and 8.1D) and were significantly ($P<0.05$) dependant on the increases of either blue mussel or western king prawn stocking densities and both (Table 8.3). Highest mean TP concentrations were observed in the highest blue mussel and western king prawn stocking densities and was significantly ($P<0.05$) higher than those in other treatments. TB was significantly lower in stocking rate of 21.36 prawn m^{-2} and 312.50 mussel m^{-2} than other stocking rates. No significant difference in TB in the culture media among treatments with blue mussel stocking densities exceeding 312.50 mussel m^{-2} and western king prawn densities being excess of 21.36 prawn m^{-2} was found.

TSS increased significantly and peaked at day 28 and 42 and then decreased over the remainder of the experiment (Figure 8.1E). Mean TSS values, on day 14 of the culture period, were significantly ($P<0.05$) different among stocking rates. The lowest value 64.83 mg L^{-1} was observed at the highest mussel density (562.50 mussel m^{-2}) which was significantly lower than other mussel densities. The highest TSS on the day 28 was recorded at mussel density of 562.50 mussel m^{-2} (367.00 mg L^{-1}). However, overall mean TSS were in the range between 125.50 and 164.64 mg

L^{-1} and the lowest value was found in the stock rate of 250 mussel m^{-2} and 34.18 prawn m^{-2}.



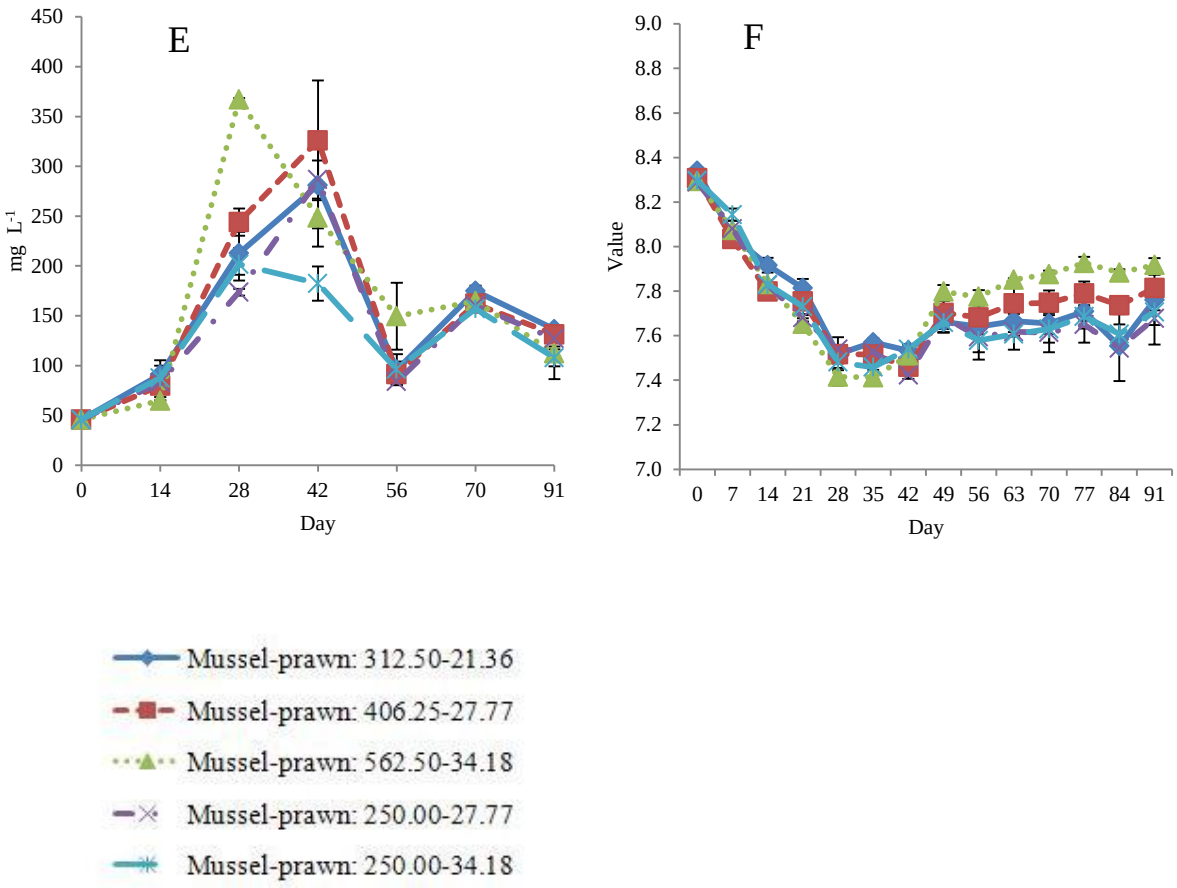


Figure 8.1 Concentrations of NO_3^- (A), TAN (B), PO_4^{3-} (C), TP (D), TSS (E) and pH (F) in the prawn tanks of five treatments in the IRAS during 91- day experiment. Error bars indicate SE.

Mean pH reduced from the beginning to the first month and then increased slightly through the rest of the experiment (Figure 8.1F). pH values were in the range of 7.35-8.41 and were below 7.8 during most of the experimental period. The lowest value (7.35) was observed at the stocking rate of 562 mussel m^{-2} and 34.18 prawn m^{-2} at day 28 of the trial period.

8.3.3. Nitrogen and phosphorus content in animals and wastes

8.3.3.1 Nitrogen content

N content in whole western king prawns carcass and wastes deposited in waste collection tanks ranged from 2.31 to 2.60% and 2.12 to 2.49%, respectively, and

were not significantly (P>0.05) different among any treatments at the termination of the experiment (Table 8.4). N contents in mussels' meat and wastes at mussel tanks in the IRAS displayed a decreasing trend when blue mussels stocking densities increased from 312.50 to 562.50 m^{-2} (Table 8.4). The lowest N values were observed at blue mussel stocking density of 562.50 m^{-2} which were significantly (P<0.05) lower than in the other treatments. No significant difference (P>0.05) in N contents of mussels' meat and wastes among IRAS stocked with the same initial mussel density of 250.00 mussel m^{-2} was found.

Table 8.4 Nitrogen contents in western king prawns (wet weight); and blue mussels and wastes (dried weight) in the integrated culture of blue mussel and western king prawn during 91-day trial

	Stocking rates of mussel (mussel m^{-2}) and prawn (prawn m^{-2})				
	312.50	**406.25**	**562.50**	**250.00**	**250.00**
	21.36	**27. 77**	**34.18**	**27. 77**	**34.18**
Whole prawn (%)	2.42±0.09^{a}	2.51±0.13^{a}	2.43±0.07^{a}	2.31±0.08^{a}	2.60±0.02^{a}
Mussel meat (%)	8.31±0.21^{a}	7.81±0.25^{b}	7.54±0.18^{c}	7.98±0.28^{ab}	7.82±0.08^{b}
Wastes (%)					
Mussel tank	2.30±0.16^{a}	2.16±0.10^{a}	1.67±0.17^{b}	1.97±0.20^{ab}	1.98±0.08^{ab}
Waste tank	2.40±0.20^{a}	2.49±0.26^{a}	2.12±0.06^{a}	2.22±0.21^{a}	2.25±0.25^{a}

Values are means ± SE. Values followed by different letters of the same row are significantly difference at α=0.05.

8.3.3.2 Phosphorus content

Increasing both blue mussel and western king prawn stocking densities did not significantly (P>0.05) affect P contents in whole western king prawns and wastes deposited in waste-collection tanks in the IRAS (Table 8.5).

Table 8.5 Phosphorus contents in western king prawn (wet weight) ;blue mussels and wastes (dried weight) in integrated culture system of blue mussel and western king prawn during 91-day trial

	Stocking rates of mussel (mussel m^{-2}) and prawn (prawn m^{-2})				
	312.50	**406.25**	**562.50**	**250.00**	**250.00**
	21.36	**27. 77**	**34.18**	**27. 77**	**34.18**

Prawn (%)	0.28±0.03^{a}	0.26±0.00^{a}	0.25±0.01^{a}	0.27±0.04^{a}	0.27±0.01^{a}
Mussel meat (%)	1.13±0.06^{a}	0.92±0.06^{b}	0.81±0.03^{b}	0.98±0.03^{a}	0.84±0.06^{bc}
Wasters (%)					
Mussel tank	0.85±0.10^{abc}	0.73±0.03^{a}	0.75±0.05^{ab}	1.13±0.17^{c}	1.07±0.12^{bc}
Waste tank	4.16±0.15^{a}	3.47±0.62^{a}	4.48±0.33^{a}	3.85±0.47^{a}	3.95±0.09^{a}

Values are means ± SE. Values followed by different letters of the same row are significantly difference at α=0.05.

The highest values of P contents in mussel meat were observed at stocking rates of 312.50 mussel m^{-2} and 21.36 prawn m^{-2}, which were significantly ($P<0.05$) higher than other stocking rates (Table 8.5). Among the treatments stocked with the same blue mussel density (250.00 mussel m^{-2}), P content in mussel meat was higher in tanks stocked with lower prawn density (27.77 prawn m^{-2}) (Table 8.5).

8.4 DISCUSSION

One of the important factors affecting growth, survival of prawns and water quality in culture system is stocking density. Several authors (Sandifer et al. 1987; Ray and Chien 1992; Williams et al. 1996; Tseng et al. 1998; Arnold et al. 2006) (Martin et al. 1998;) reported an inverse relationship between the stocking density and growth of prawn. In the present study also, increase in blue mussel and western king prawn stocking densities in the IRAS resulted in reducing the animal growth, survival and water quality. The SGRs of western king prawns in the current study (0.31-0.57 % d^{-1}) are lower than 0.74-0.86 % d^{-1} in the study conducted by Hai et al. (2009a) and comparable to 0.24-0.36 % d^{-1} obtained by Khoi and Fotedar (2010). Survival rate of western king prawn in the present study is considerably lower than the 60-80 % of survival rate recorded after the application of different probiotics (Hai et al. 2009a) and 55-60% of survival when integrated with seaweeds, *Sargassum* spp. (Mai et al. 2010). The dead prawns in the present study were freshly moulted and showed the signs of cannibalism; suggesting that stress caused due to a high density resulted in moult deaths and subsequent cannibalism. Reductions of prawn growth and survival reared at high density are likely to result from competition for the space and/or poor water quality (Forster and Beard 1974) and increase in cannibalism (Abdussamad

and Thampy 1994). NH_3^- concentrations in all treatments and tanks in the present study, are higher than lethal levels of 0.18 mg L^{-1} NH_3^- at 22 ^oC for green tiger prawn juveniles (Kir et al. 2004) and 0.14 mg L^{-1} for Chinese prawn juveniles (ChenTing et al. 1990), suggesting that poor water quality may be the primary factor for the growth inhibition and reduced survival rates of western king prawns.

Factors implicated in regulating the growth rate of mussels include density (Kautsky 1982a; Boromthanarat and Deslou-Paoli 1988), competition (Harger 1970) and food quality and quantity (Widdows et al. 1979). In the present study, mussel growth decreased significantly as the mussel densities increased and the lowest values were observed at the highest mussel stocking densities of 562.50 mussel m^{-2}. This is in line with the finding of Liu et al. (2006) who showed that hard clam larvae reared at highest density have the smallest mean size, whereas larvae reared at the lowest density have the largest mean size. Similar observation was observed in ropes culture of mussels,*Mytilus galloprovincialis*, at different denstities (220, 370, 500, 570, 700, 800 and 1150 ind m^{-1}) (Cubillo et al. 2012). *M. galloprovincialis* cultured at lower densities presented higher growth rate and consequently reached greater weight and length values at the end of the experimental period than thosed cultured at higher densities.

Growth rates of blue mussels were significantly correlated by the abundance of dinoflagellates (Coe 1974). As mussels in the current study were not supplemented with any phytoplankton, the low growth rates of mussels probably can be contributed to the lack of phytoplankton in the mussel diet. The highest TSS values in the present study were observed between 28^{th} and 42^{nd} day of the trial. This may be due to the decomposing dead mussels in the IRAS. Widdows et al. (1981) reported that growth of the blue mussels are severely reduced in polluted environments. Working on adult mussels (*Dreissena polymorpha*) under laboratory conditions at 20 ± 1°C, Madon et al.(1998) also demonstrated that mussels in turbid rivers exhibited lower growth potential and do not stabilize at the high population densities, compared to mussels reared in the lakes.

Mass mortality of blue mussels in the present study was observed in the first month of the trial and was dependant on the increased mussel and prawn densities. Water quality and food availability are likely to be responsible for the lower survival rates of mussels in the IRAS. NH_3 concentrations were reached the highest values

between day 21 and 28 of the culture period whereas pH values were reduced below 7.5 (Figure 8.1B and Figure 8.1F). Arthur et al. (1987) reported that molluscs are sensitive to certain contaminants, especially NH_3, relative to other invertebrates and fishes. NH_3 concentrations in the current study are higher than lethal level of 0.093 mg L^{-1} for pocketbook mussel (*Lampsilis cardium*) (Newton et al. 2003). Michaelidis et al. (2005) also found that a pH reduction in sea-water below 7.5 is harmful for shelled molluscs and a reduction in sea-water pH to 7.3 may be fatal for the mussels. Prawns excrete two principal toxic metabolites to the water: NH_3 and CO_2 . In the IRAS where water is not changing, the accumulation of metabolic CO_2 results in pH reduction (Eshchar et al. 2006). Thus, water quality was probably a major cause of the high mortality and low growth rates of mussels in the IRAS.

The present study showed that increase in mussel stocking densities did not enhance the efficiency of mussels in removing suspended solids and bacteria in the IRAS. Results were in contrast with several authors who reported that mussels effectively removed the TB and TSS from the culture media (Jones and Preston 1999; Jones et al. 2001; Tendencia 2007). Tendencia (2007) showed that the presence of brown mussels (*Perna indica*), green mussels and oysters, *Crassostrea* sp. in a simulated prawn culture system could efficiently control the growth of luminous bacteria and their densities affected the removal efficiency. In the present study, blue mussels effectively removed the TSS at day 14 of the trial (Figure 8.1E) and the lowest TSS was found at the highest mussel stocking density (562.50 mussel m^{-2}). However, mass mussel mortality in the first month resulted in reducing the number of mussels in treatments while the TSS values in IRAS increased considerably with the progress of the trial (Muangkeow et al. 2007) and decomposition of dead mussels.

In the present study, N and P contents in mussel meat and wastes in the mussel tanks showed higher values in the tanks stocked with lower mussel densities. N content in mussel meat at the harvest was comparable to 8. % N in dry meat of Manila clam (*Tapes philippinarum*) (Mann 1979) and 6.5-8.0% N in the dry meat of freshwater mussels (*Parreysia corrugate*) (Nagabhushanam and Lomte 1971). The low nutrient contents in mussel meat in the IRAS may be explained by reduction of filtration rate of mussels caused by high TSS in the IRAS.

Mass mortalities in higher mussel densities were observed at early culture period and were probably responsible for the low nutrient contents into the deposited wastes of the mussel tanks as nutrient contents in the deposited wastes in mussel tanks were lower at higher mussel stocking densities. Filter-feeder bivalves feed on the small rich organic particles, such as bacteria and organic matter debris (Loosanoff 1949; Jones et al. 2002). However, only 50% of nitrogen filtered is absorbed by the ribbed mussels and approximately 50% N deposited as faeces and pseudo-faeces (biodeposition) (Jordan and Valiela 1982). Therefore, mussels increase the quantity and quality of deposited organic matter through the production of faeces and pseudo-faeces (Kaspar et al. 1985; Giles and Pilditch 2006). Higher number of mussels in the IRAS may catch up more nutrients suspended in water column and then deposit them into bottom of mussel tanks.

Stocking rate of culture species is one of important aspects in integrated culture management (Martínez-Porchas et al. 2010). In the present study, increasing either mussel or prawn densities resulted in reduced growth and survival rates of western king prawns and blue mussels; plus it led to deterioration in water quality. With the stocking densities of mussel ranging from 250.00 to 562.50 mussel m^{-2} and prawn ranging 21.36-34.18 prawn m^{-2}, the suitable stocking rates in the IRAS to optimise growth and survival of blue mussels and western king prawns were 312.50 mussel m^{-2} and 21.36 prawn m^{-2}. Yi and Fitzsimmons (2004) reported overstocking complicated the management and was not sustainable in the long run. Tian et al (2001) showed that the optimum stocking ratio of 2-cm long Chinese prawns, 150-g of tilapia hybrid and 3-cm long constricted tagelus in a closed-integrated pond culture was 7.2 prawn m^{-2}, 0.08 tilapia m^{-2} and 14 tagelus m^{-2}. Muangkeow et al (2007) reported that the optimum stocking rates of white prawn and Nile tilapia to optimise the nutrient conversion rate to culture animals without lowering prawn growth were 40 prawn m^{-2} and 0.4 tilapia m^{-2}; and 40 prawn m^{-2} and 1.0 tilapia m^{-2}.

The study reveals that increase in blue mussel density and western king prawn biomass leads to decreased growth, survival, water quality and nutrient content in waste deposits of the mussel tanks. The low mussel and prawn growth and survival rates can be connected to the high suspended solid and NH_3 concentrations; and low pH values in the culture media. The suitable mussel density and prawn biomass in

the recirculation system should not exceed the mussel density of 312.50 mussel m^{-2} and prawn of 21.36 prawn m^{-2}.

CHAPTER 9 GENERAL DISCUSSION

9.1 INTRODUCTION

Penaeid prawn culture is one of the most profitable aquaculture activity due to the higher commercial value of the generated product, representing an annual global economic profit of US$14 647 million in 2010 (FAO 2011). Despite the benefits and progress of penaeid prawn aquaculture, the activity faces several problems, including introduction of non-native species into the wild, and modification of coastal habitats (Naylor et al. 2000), disease outbreaks (Morales-Covarrubias 2004), and decreasing prices (FAO 2007). Other problem related to the prawn industry include eutrophication and nitrification of discharged or receiving ecosystems, which can generate explosive growth of phytoplankton and the proliferation of pathogenic microorganisms (Martínez-Porchas et al. 2010). This potential adverse environmental impacts from untreated effluent have raised concerns about the sustainability of prawn farming (Phillips et al. 1993) and (Primavera 1994). Thus, it has prompted the search for cost-effective methods for improving effluent water quality prior to its discharge into receiving waters.

In order to reduce the organic load and nutrients from the culturing system, the reduction of feed waste by improvement of feed quality, better feed management (Smith et al. 2002; Pan et al. 2005) and waste water treatment systems have been studied. Sedimentation ponds can effectively reduce suspended particles (Teichert-Coddington et al. 1999; JacksonPrestonBurford et al. 2003) but sedimentation is less effective in reducing TN and TP concentrations, especially if sedimentation ponds are operated for a long periods with continuous water flow (JacksonPrestonBurford et al. 2003). Purpose-built microbial mat systems (Paniagua-Michel and Garcia 2003) and wetlands (Tilley et al. 2002; Lin et al. 2003; Lin et al. 2005) show higher efficiency in improving prawn effluent quality. However, nutrient and organic compounds are accumulated in sediments or retained as organisms such as bacteria, benthic microalgae and plant, which are necessary to be removed from time to time

A potentially viable alternative of biological treatment of the effluent is to use filter-feeder bivalves and macroalgae to remove suspended particulates and nutrients

(ShpigelNeori et al. 1993). Integrated culture of prawns with bivalves or seaweed has improved the water quality, minimized the ecological impacts of prawn farming (Jones et al. 2001; Nelson et al. 2001; Bunting 2008) and enhanced the efficiency of feed utilization (Zhen-xiong et al. 2001) but this practice faces some problems and disadvantages. Factors such as the species involved, the size of the organisms, the stocking density, stressful conditions and the food quantity and quality need to be considered (Wang 2007; Copertino et al. 2009) for avoiding any disaster. It has been documented that high densities of subordinate species and stressful conditions diminish prawn growth (Muangkeow et al. 2007; Wei et al. 2008).

Stocking rate and feed input need to be considered for sustainable production in the integrated culture. Optimum stocking density and feeding rate can enhance the growth and survival of organism; and maximise the production of culture system (Maguire and Leedow 1983; Tian et al. 2001; Liu et al. 2006; Muangkeow et al. 2007). Overstocking can complicate the management and is not sustainable in the long run (Yi and Fitzsimmons 2004) whereas overfeeding may result in heavy nutrient loads and deteriorate water quality of the culture system. High biofouling and concentration of particles can have the negative effects on growth of oysters (Jones et al. 2001; Jones et al. 2002) while seaweed growth can be limited by epiphyte and high water turbidity (Phang et al. 1996; Nelson et al. 2001). This chapter reviews and combines the discussion of previous trials of this project which involved monoculture of prawns and integrated culture of prawns with seaweed and bivalves.

9.1 INTERACTIVE EFFECTS OF STOCKING DENSITY AND FEEDING RATES IN MONOCULTURE OF PRAWNS

The current research has revalidated that increasing stocking densities and feeding rates of cultured species can result in the decrease of growth and deterioration of water quality in the cultural environment (Khoi and Fotedar 2010). The negative effects of an increase in stocking density on growth of western king prawn in the present study is consistent with other intensive production studies on juvenile brown tiger prawn (*Penaeus esculentus*) (Arnold et al. 2006), grey prawn (*Penaeus*

setiferus) and white prawn (Williams et al. 1996) in a semi-closed recirculating system; and tiger prawn (Tseng et al. 1998) in a closed recirculating system. Maguire and Leedow (1983) found that growth of school prawn in ponds declined while survival rate was unaffected when stocking density increased from 6.1 to 21.2 prawn m^{-2}. Likewise, Araneda et al. (2008) reported a decrease in growth rates and survival of white prawn, cultured in freshwater (0 ppt[1]) at three densities (90, 130 and 180 prawn m^{-2}), as stocking density increased. Nonetheless, no differences in final weight, weight gain (%), and survival were found among prawn stocked at 28.4, 56.8, and 85.2 prawn m^{-2}. Reduced growth of juvenile prawn cultured at higher densities is thought to result from a combination of factors, which include: excretory products, faeces and unconsumed food items accumulate in the culture water causing toxic effects on prawns (Ray and Chien 1992), a decrease in the availability of space and natural food sources (Maguire and Leedow 1983) and the degradation of water quality (Nga et al. 2005). Concentrations of DO, pH, TAN, NO_2, NO_3 in current project where prawn stocking density was below 16 prawn m^{-2} were maintained within levels recommended for optimal growth and survival of post-larvae and juvenile penaeid species (ChenLiu et al. 1990; Chien 1992). Therefore, it is reasonable to assume that reduced growth at higher densities of western king prawn was likely to be influenced by a decrease in the availability of space and natural food sources.

The quantity of wastes generated was proportional to levels of intensification in the recirculating aquaculture system. The accumulation of waste-N in the sediments increased with stocking density and feeding rates. An increase of stocking densities of western king prawn from 4 prawn m^{-2} to 32 prawn m^{-2} resulted in increasing percentage of waste-nitrogen in tanks bottom from 12.79 to 49.23% of total nitrogen input (Khoi and Fotedar 2010). Waste-N values increased from 18.51 to 50.47% when feeding rates was increased from 3.0 to 7.5% (Chapter 5). Similar trends have been observed for tiger prawn (Thakur and Lin 2003) in a RAS and for blue prawn (*Penaeus stylirostris*) (Martin et al. 1998) in pond culture. Moreover, intensified levels (stocking density and feed rate) of western king prawn culture affected the water quality in culture media. Concentrations of DIN, PO_4^{3-} and TP in the IRAS increased with the increasing stocking densities of western king prawn (Khoi and Fotedar 2010) and feeding rates (Chapter 5) while TAN concentration was only

affected by stocking densities but not by feeding rates. Several reports have indicated that TAN increase with stocking densities (Robertson and Phillips 1995; Patnaik and Lawrence 2011). In contrast, TAN was independent to stocking densities reported by Wyban et al. (1987) for white prawn in manure-fertilizer ponds, Thakur and Lin (2003) for tiger prawn in closed tanks. DO levels in the recirculating culture system were dependent to stocking densities of western king prawn and feeding rates. Increase in both stocking density and feeding rate resulted in reducing the DO levels in culture media (Khoi and Fotedar 2010)(Chapter 5). The results are in line with Patnaik and Lawrence (Patnaik and Lawrence 2011) who found that DO and TAN were higher at density 1602 prawn m^{-3} and feed rate 2.5 g prawn $^{-1}$ $week^{-1}$ than at densities of 658 and 111 prawn m^{-3}; and feed rates of 1.0, 1.5 and 2.0 g $prawn^{-1}week^{-1}$; and have confirmed that degree of intensification, i.e., higher stocking density, feeds and fertilizers, produces an increased waste load (Pa´ez-Osuna, 2011b).

Table 9.1 Interactive effects of feeding rates and stocking densities on TAN, DO, DIN, PO_4^{3-} and TP in recirculating aquaculture system reared western king prawn

Treatments		TAN (mg L^{-1})	DIN (mg L^{-1})	DO(mg L^{-1})	PO_4^{3-} (mg L^{-1})	TP (mg L^{-1})
4.0*	3.0**	0.17±0.03	4.5±0.05	5.94±0.01	0.34±0.03	0.54±0.08
8.0	3.0	0.21±0.04	4.34±0.26	5.88±0.01	0.54±0.05	0.81±0.10
16.0	3.0	0.25±0.04	5.32±0.14	5.78±0.09	0.77±0.07	0.93±0.11
32.0	3.0	0.39±0.07	7.03±0.07	5.37±0.14	1.02±0.08	1.13±0.12
16.0	3.0	0.18±0.02	5.20±0.24	6.27±0.15	0.82±0.07	1.08±0.14
16.0	4.5	0.20±0.04	5.11±0.24	5.51±0.45	0.96±0.08	1.39±0.19
16.0	6.0	0.21±0.05	5.79±0.34	5.27 ± 0.28	1.05±0.09	1.68±0.20
16.0	7.5	0.22±0.03	6.69±0.79	4.93±0.35	1.21±0.10	1.84±0.23

*Values in this column present feed rates (% wet weight) used in the experiments.
** Values in this column present different western king prawn stocking densities (prawn m^{-2}).
Values are means ± SE.

Feeding rates did not improve growth and survival of western king prawn cultured in the RAS. SGR of western king prawns had an inverse relationship with the increase of the feeding rates (Chapter 5). Similar finding has been observed by Mill and McCloud (1983b) for the yabby reared in ponds Allan et al. (1995) for tiger prawn

juveniles reared in fibreglass pools and Maguire and Leedow (1983) for school prawn cultured in brackish water ponds. SGR are lower compared to 1.2 % d^{-1} (Sang and Fotedar 2004a) when western king prawn were fed fresh blue mussels at the rate of 0.83-0.88 % d^{-1} (Hai and Fotedar 2009) when western king prawn were given supplemented prebiotics (Bio-Mos® and β-1,3-D-glucan) commercial diets. In addition, FCR values showed the same trend as the growth rate indicating low utilization of feed (Boyd et al. 1979) as adding more feed to the culture system does not necessarily increase the prawn growth but can cause detrimental effects on the water and sediment quality. Since water quality parameters were within the favourable limits for penaeid production (ChenLiu et al. 1990; ChenTing et al. 1990), feed may be a limiting factors for the growth of western king prawn in the recirculating aquaculture system. Future research should focus on the optimum feed formulation for western king prawn culture.

Nutrient analysis showed that 9.34-20.13% N and 4.97-11.25% P input were retained in prawn biomass at harvest and 23.34-64-82% N and 21-36-65.62% P were in the discharged water (Khoi and Fotedar 2010) in the recirculating aquaculture system. These N values are lower than other reports for tiger prawn culture of approximately 22% (JacksonPrestonThompson et al. 2003) and 23-31% (Thakur and Lin 2003) in a closed tank culture system. Nutrients trapped in sediment were lower than 14-53% N and 39-67% P (Thakur and Lin 2003) in the closed culture system. This is due to the fact that there was no additional or artificial and/or natural substrate in the bottom of the tanks. Boyd (1995) reported that sediments act as receptors to absorb solid and dissolved nutrients from the water column.

In semi-intensive aquaculture a greater proportion of input N is from non-feed sources—either entrained in intake water (e.g. up to 63% of total N intake, (Teichert-Coddington et al. 2000) or added through fertilization (e.g. 20%, (Paez-Osuna et al. 1999)) while in intensive prawn farms, most of the N (>90%) originates from added feeds (Briggs and Funge-Smith 1994). In the IRAS, percentage contribution of feed to the total nutrient inputs was in the same range as reported previously (Khoi and Fotedar 2010; Khoi et al. 2012). In addition, percentage nitrogen and phosphorus inputs in the form of feed were significantly higher in the treatment with higher stocking density (Khoi and Fotedar 2010) and feeding rate (Khoi and Fotedar 2012);

the result is in agreement with the previous reports (Briggs and Funge-Smith 1994; Martin et al. 1998; Thakur and Lin 2003) in intensive prawn farming systems. Nutrient percentage removed from the system via prawn harvest in ranges from 22.8-30.7% N and 10.5-/12.8% P (Thakur and Lin 2003) in a closed intensive tank culture of tiger prawn and 22%N in intensive prawn farms in Australia(JacksonPrestonThompson et al. 2003); these values are higher than the percentage of total N and P recovered as harvested western king prawn in the IRAS (Khoi and Fotedar 2010; Khoi et al. 2012). The difference may be due to the lower growth rate of western king prawn and high FCR in the IRAS (Khoi and Fotedar 2010). Feed is one of the highest variable costs of shrimp farming (Lawrence and Lee 1997) and feed wastage has the potential to contribute to eutrophication of surrounding waterways (Naylor et al. 1998) . Higher western king prawn stocking densities (16 and 32 m^{-2}) (Khoi and Fotedar 2010) or feeding rates (6.0 and 7.5 % d^{-1}) have the highest percentage of N and P inputs trapped in the bottom tanks in the IRAS. The results are similar to major outputs of nutrients in intensive prawn culture systems reported by Briggs and Funge-Smith (1994); and Thakur and Lin (2003). In contrast, at lower feeding rates (3.0 and 4.5% d^{-1}) and densities (4 and 8 prawn m^{-2}), major loss of nutrient through water born are higher than sediment loss (Khoi and Fotedar 2010; Khoi et al. 2012). The fact that there are no sediments in the tanks; therefore, the nutrients in settled wastes in bottom tanks of the IRAS are possibly translocated back to the water column

9.2 INTEGRATED CULTURE OF PRAWNS AND SEAWEED

Green seaweed effectively removed TAN, NO_2^-, NO_3^- and PO_4^{3-} in prawn tanks in the integrated culture of green seaweed and western king prawn in the IRAS (Khoi and Fotedar 2011). Similar observations in nutrients (TAN, NO_2 and NO_3) removal capacity of seaweeds, *Gracilaria salicornia*, *Gracilaria fisheries*, *Caulerpa macrophysa*, *Sargassum polycystum* from pond based prawn effluents were reported by Kaewsuralikhit (1994) and Khidprasert (1995). Mai et al. (2010) also reported that the nutrient uptake of seaweeds, *Sargassum* spp. integrated with western king prawn in indoor tank culture. TAN removal rate of green seaweed in the IRAS was obtained at the maximum value of 81.14% (Khoi and Fotedar 2011) (Table 9.2) and

was in accordance with 65% reported by Msuya et al. (2006) in an integrated pond culture of fish and seaweed, *Ulva reticulate*. TAN removal efficiency of green seaweed in the present study was lower than 85-90% (Neori et al. 2003) in a novel three-stage system of green seaweed but similar to that reported by Schuenhoff et al. (2003), (64% TAN removal) for semi-closed integrated culture of sea bream and green seaweed.

Table 9.2 TAN removal, feed utilization efficiency of green seaweed; and nutrients retention in seaweed biomass at harvest during 42-day culture in the IRAS

	TAN removal (%)	**Nitrogen Utilization efficiency (%)**	**Phosphorus Utilization Efficiency (%)**	**Nitrogen retention in seaweed biomass (%)**	**Phosphorus retention in seaweed biomass (%)**
Monoculture	-	**7.56±3.05[a]**	**2.81±0.31[a]**	-	-
[*]0.25 kg m^{-2}	59.90±4.04[a]	13.95±2.80[a]	4.98±0.77[a]	6.53±0.37[a]	1.62±0.13[a]
0.50 kg m^{-2}	60.31±5.24[a]	19.42±2.11[b]	8.73±1.59[b]	13.48±1.68[b]	4.39±1.08[b]
1.00 kg m^{-2}	81.14±5.83[b]	29.46±5.15[bc]	13.93±0.95[c]	21.85±0.84[c]	7.34±1.12[c]
2.00 kg m^{-2}	75.12±9.94[a]	32.46±2.27[c]	22.22±1.30[d]	29.71±1.84[d]	13.50±0.64[d]

Values within a colume sharing a common superscript are not significant different (LSD test; $P<0.05$; n=3). [*] Values in the column present different green seaweed stocking densities (kg m^{-2}).

Inclusion of seaweed into prawn culture in the IRAS has improved the efficiency utilization of feed in the integrated culture, compared to the monoculture. Khoi and Fotedar (2011) reported that efficiency utilizations of feed nitrogen in integrated culture were 2-4 times higher than that in monoculture (Table 9.2) while values of feed phosphorus were 2-10 times higher in integrated culture than in prawn monoculture. Conversion rate of feed nitrogen into seaweed biomass in the IRAS was 6.03-22.25% (Khoi and Fotedar 2011) and is in agreement with that reported by Schuenhoff et al. (2003) (13-21%) but is lower than 24.6% (Neori et al. 2000) in a semi-recirculating integrated system of Japanese abalone and seabream. Approximately 2.15-19.42% of feed phosphorus was locked up in new seaweed biomass at harvest in the present project and is fell within the range of 5.65-6.38% of P feed retained in seaweed biomass in a semi-integrated aquaculture system (Schuenhoff et al. 2003). Therefore, conversion rate of feed nutrients into green

seaweed biomass was higher for nitrogen than phosphorus (Khoi and Fotedar 2011), suggesting green seaweed efficiently removes more excess nitrogen in IRAS than phosphorus. Consequently, integrated culture of prawn and seaweed decrease nutrient excess, improve the water quality and diminish the environmental impact resulting from effluent discharges.

Inclusion seaweed into prawn culture in the IRAS had no effect on the survival and growth performance of the western king prawns. That is in contract with several previous reports which have indicated that prawn performance is enhanced by the presence of a secondary species (Martínez-Porchas et al. 2010). Akiyama and Anggawati (1998) reported that tilapia in the integrated culture with prawn in ponds feed on excess organic matter, improving water quality and thus increasing prawn production. Seaweeds absorb dissolved nutrient produced by main species (Wang 1990; Jones et al. 2001) and improve the water quality in the integrated culture system (Nelson et al. 2001; Bunting 2006). Concentrations of environmental factors such as TAN, NO_2^-, NO_3^- and PO_4^{3-} in the IRAS were in the save levels for western king prawn cultivation (Khoi and Fotedar 2011). Moreover, the main and subordinate species in the integrated culture system are separated by different units, thus there are no competition for space and food; crowding effects and cannibalism among them. Therefore, green seaweed in the IRAS is unlikely to improve the growth performance of western king prawn.

The growth, yield and N uptake of *Ulva* increased with increasing stocking densities and the optimum stocking density was obtained at 1.00 kg wt m^{-2} in the IRAS (Khoi and Fotedar 2011). The results are in agreement with those reported by Jiménez del Río et al. (1996) for seaweed, *Ulva rigida* (1.07 kg wt m^{-2} or 2.5 g wt L^{-1}) in a tank culture of seaweed as biofilters for dissolved inorganic nitrogen from seabream effluents and are higher than 0.8 kg wt m^{-2} of green seaweed in seawater exchange culture (DeBusk et al. 1986). However, there was a decrease of N uptake of seaweed at the higher density of 2.00 kg wt m^{-2}. This may be associated with the poor light penetrate (Neori et al. 1998) and CO_2 depletion (Lapointe and Tenore 1981). Neori et al. (1991) found that under N-sufficient conditions, increasing stocking densities up to 2 kg wt m^{-2} can decrease the yield of green seaweed due to light limitation,

even though each thallus was cycled from the top to the bottom of the tanks by bubble induced water circulation (Vandermeulen 1989).

Growth and yield of seaweed were affected by heavy nutrients loads in the IRAS. Khoi and Fotedar (2011) observed high mortality of green seaweed in the IRAS after 15 days of culture. Mai et al. (2010) also reported high mortality of seaweeds, *Sargassum* spp. in an integrated culture of seaweed and prawn in indoor tank culture after 7 days of the culture period. Chemical analysis showed that both C:N and C:P ratios in green seaweed tissues were lower than 10:1 and 200:1 ratios, recommended by Fenchel and Jørgensen (1977), respectively, suggesting that high N and P are limited factors for growth and survival of seaweed in the IRAS.

Nutrient budget revealed integrating culture o in IRAS may improve the nutrients utilization efficiency. The percentage of total nutrient recovered as harvested seaweed was 6.53-29.71% N and 1.62 – 13.50% P in an integrating green seaweed and western king prawn in the IRAS (2011). That confirmed the work conducted by Zhen-xiong et al. (2001) whom stated that higher efficiency of nitrogen utilization has been observed in polyculture systems compared with monoculture systems. Thus, integrated cultures allow farmers to produce other species with commercial value and to increase the economical profitability of their farms with little or no financial investment because most of the costs have already been met (Purcell et al. 2006). In the IRAS, N unaccounted loss are 11.28-45.17% N and 27.99 – 33.88% P of the total inputs (Khoi and Fotedar 2011). Martin et al. (1998) estimated 15% of nitrogen unaccounted for ponds stocked at 4 prawn m^{-2}, however, higher losses, up to 66% have also been reported (Paez-Osuna et al. 1999). Denitrification and ammonia volatilization are two potential losses of nitrogen that are often not measured directly. Therefore in most studies, including the present one, these factors are estimated indirectly as the difference between the nitrogen inputs and outputs (JacksonPrestonThompson et al. 2003). Ammonia volatilization is promoted by two sets of factors: those favoring the NH_3 side of the water column NH4/NH3 equilibrium (most importantly, overall TAN concentration and high pH, but also higher temperature and salinity); and those promoting the effective transfer across the water–air phase boundary (wind, mechanical aeration, temperature and mixing). Inclusion of seaweed into prawn culture enhances the pH in the culture media (Khoi and Fotedar 2011). Water mixing is also generated by circulation of water among

tanks in the IRAS. Thus, the N unaccounted loss may be ammonia volatilization in the IRAS.

9.3 PRAWN AND BIVALVES IN THE IRAS

Adding mussels into prawn culture enhanced the efficiency of the nitrogen from the feed and phosphorus utilization in the IRAS compared to the monoculture systems. In the present project, conversion rate of N from feed and P were significantly higher in integrated culture of western king prawn and blue mussel than in monoculture and feed utilization efficiency in integrated culture was 10.63% N and 4.89% P higher than in monoculture (Chapter 7). Similar positive observations have been reported for white prawn and constrict tagelus (Zhen-xiong et al. 2001) in closed recirculating pond culture system; and Chinese prawn, tilapia and constricted tagelus (Tian et al. 2001) in closed-polyculture system in ponds. Li and Dong (2000) showed that integrated culture Chinese prawn, red tilapia, contricted tagelus and scallop raised the production by 28% and the utilization efficiency of input nitrogen by 85%; and reduced the nitrogen discharged ratio to 6-8% instead of 40-90% in the usual open culture systems. Nutrients discharged through draining were significantly lower in integrated culture than in monoculture (Chapter 7) in the current project and were lower than 3.0-6.0% of total nitrogen input reported by Zhen-xiong et al. (2001). Thus, integrated culture of prawn and mussel in the IRAS can contribute to minimizing the environmental impact of any farm effluents (Martínez-Porchas et al. 2010).

Table 9.3 Growth rate (SGR), survival and nutrients retention of blue mussel and western king prawn in the integrated recirculating aquaculture system

Treatments		SGR (% d^{-1})		Survival (%)		N retention (%)
*Prawn	Mussel**	Prawn	Mussel	Prawn	Mussel	
16.07	0.00	0.61±0.03	-	85.19±9.80	-	10.98±0.84
16.07	31.25	0.74±0.04	0.058±0.002	85.19±9.80	90.00±5.77	11.44±1.17
16.07	62.50	0.77±0.03	0.068±0.002	85.19±3.70	90.00±2.89	11.84±0.93
16.07	125.00	0.69±0.01	0.075±0.006	81.48±7.41	72.50±11.27	13.36±1.56
16.07	250.00	0.65±0.02	0.094±0.007	81.48±3.70	80.00±11.46	21.61±2.35

21.36	312.50	0.57±0.03	0.070±0.002	63.89±7.3	51.67±3.53	-
27.77	406.25	0.40±0.02	0.058±0.004	22.92±2.08	12.31±2.04	-
34.18	562.50	0.31±0.01	0.045±0.003	19.30±1.75	3.13±0.36	-
27.77	250.00	0.52±0.0	0.065±0.007	45.83±7.51	44.58±1.50	-
34.18	250.00	0.46±0.05	0.064±0.007	24.56±8.77	21.25±2.60	-

* Prawn stocking density in prawn m^{-2}; ** Mussel stocking density in mussel m^{-2}

Blue mussels effectively removed the TB, TSS and TN in the cultured media in the IRAS (Chapter 7). TSS removal rates ranged between 13.83 and 51.52% when blue mussel stocking densities were below 250.00 m^{-2} and western king prawn stocking densities did not exceed 16.07 prawn m^{-2}. Similar observation has been reported for Sydney rock oyster (Jones and Preston 1999; Jones et al. 2001), brown mussel and green mussel (Tendencia 2007), Manila clam (Shpigel and Neori 1996). TSS removal efficiency of blue mussel are in line with giant oyster (41.2%) (Ramos et al. 2009) and Sydney rock oyster (49%) (Jones and Preston 1999) in integrated treatment of prawn effluents. Bunting (2006) observed that hairy cockle and seaweed, *Gracilaria* spp. reduced the TAN, TN and TP concentrations from prawn effluents by 61, 72 and 71%, respectively. However, water quality in the IRAS did not improve when blue mussel stocking densities exceeding 250 mussel m^{-2} (Chapter 8). Results showed both dissolved and solid nutrients in culture tanks increased with the increasing stocking densities of blue mussels and western king prawn. That is in contrast to the findings of Tendencia (2007); and Jones and Preston (1999) whose reported that the bivalves significantly reduced the concentration of TN, TP, TSS and TB in prawn effluents with the highest oyster density having the greatest effect. Increase in blue mussels and western king prawn stocking densities resulted in increased feed inputs and consequently suspended particles (Thakur and Lin 2003; Muangkeow et al. 2007) in the IRAS. However, nutrient removal of bivalves was limited by high concentration of particles (Jones et al. 2001; Jones et al. 2002). Loosanoff and Tommers (1948) reported that oyster filtration can be reduced or ceased completely if sediment loads are too high. It is assumed that high concentration of suspended solid is a limited factor for nutrient removal of blue mussels in the IRAS.

Growth and survival of blue mussels is dependent on mussel stocking density and prawn biomass in the IRAS. When western king prawns were stocked at 16.07 prawn m^{-2} and mussel densities increased from 31.25 to 250 mussel m^{-2}, SGR of mussels increased from 0.058 to 0.094 % d^{-1}, respectively (Chapter 7). When both prawn and mussels densities exceeded 16.07 prawn m^{-2} and 250 mussels m^{-2}, the growth and survival rates of mussels were reduced (Chapter 8). The results are in agreement with Liu et al. (2006) for hard clam larvae, Taylor et al. (1997) for pearl oysters and (MacDonald 1988) for scallop (*Patinopecten yessoensis)* larvae. Factors implicated in regulating the growth rate of mussels may include density ??(Kautsky 1982a; Boromthanarat and Deslou-Paoli 1988), competition (Harger 1970) and food quality and quantity (Widdows et al. 1979) and food sources (Seed 1976). Blue mussels in current project were not given microalgae while growth rates of blue mussels were significantly correlated by the abundance of dinoflagellates (Coe 1974). Water quality was not suitable for growth and survival of blue mussels in IRAS as pH levels (below 7.5) and NH_3 concentrations (over 0.30 mg L^{-1}) were outside the safe ranges for pocketbook mussel. Furthermore, Jones et al (2001; 2002) reported that high concentrations of particles have a negative effects on growth of filter-feeder bivalves and health of oysters (HopkinsHamiltonSandifer et al. 1993). Hence, food source, water quality and stocking densities can influence the growth and survival of blue mussels in the IRAS.

9.4 CONCLUSIONS AND RECOMMENDATIONS

9.4.1 Conclusions

Based on the results obtained from the current experiments, the following points highlight the conclusions and confirm the objectives of the research have been achieved:

1. Concentrations of TAN, NO_2^--N, NO_3^--N in the western king prawn effluent in the IRAS are below the lethal levels for the cultivation of penaeid prawn species. TP and PO_4^{3-}concentrations in IRAS increase linearly with

increasing the western king prawn feeding rates and stocking densities (Objective 1).

2. The recirculating aquaculture system could maintain acceptable water quality for the western king prawn culture within the density range of 4-32 prawn m^{-2}. Increasing the stocking densities and feeding rates reduce growth and survival rate of western king prawns in the recirculating aquaculture system and decrease the water quality in the culture media (Objective 2).

3. Higher percentage of input nutrients accumulate into tank bottom at a higher stocking densities whereas at lower socking densities more than 50% of input nutrients are discharged *via* effluents waters at harvest (Objective 2).

4. Feeding rate of 3.0% of body weight is the optimum within the range of 3.0 to 7.5% and suitable for western king prawn growth, water quality and nutrient conversion rate in the recirculating aquaculture system (Objective 2).

5. Feed utilization efficiency and nutrient retention in prawn biomass increase with decreasing feeding rates in the IRAS (Objective 2).

6. Including green seaweed into western king prawn culture improves the water quality and feed utilization efficiency in the IRAS (Objective 3).

7. At harvest retention of nutrient inputs in western king prawn and green seaweed biomass is approximately 6.28-10.22% and 6.03-22.25%, respectively (Objective 3).

8. Feed utilization efficiency of integrated culture of green seaweed and western king prawn culture is enhanced by 24.90 % nitrogen and 19.41% phosphorus, compared to monoculture in IRAS (Objective 3).

9. Western king prawn growth and survival rates are not affected by the presence of green seaweed in the IRAS (Objective 5).

10. Mean TAN and PO_4^{3-} removal rates of green seaweed over 42-day trial range from 24.02-99.05% and 13.80-96.40%, respectively (Objective 3).

11. Blue mussels effectively remove a proportion of the TSS, TB and TN from the western king prawn effluent in the IRAS (Objective 4).

12. Blue mussels stocked at densities below 250.00 mussel m^{-2} do not affect the growth and survival of western king prawn in the IRAS (Objective 7).

13. Integrated culture of blue mussels and western king prawns in the IRAS improves the feed utilization efficiency further up to 10.63 % nitrogen and 4.89 % phosphorus and reduce the nutrient discharged at harvest in the IRAS (Objective 6).

14. Optimum stocking rate of blue mussel and western king prawn in terms of feed conversion rate and nutrient removal rate are 250.00 mussel m^{-2} and 16.07 prawn m^{-2}, respectively (Objective 6) .

15. The suitable blue mussel density and western king prawn biomass in the recirculation system should not exceed the density of 312.50 mussel m^{-2} and prawn of 21.36 prawn m^{-2} (Objective 7).

16. Nitrogen and phosphorus contents accumulated in mussel tanks increase linearly with the increasing blue mussel stocking densities in the IRAS (Objective 6).

9.4.2 Recommendations

Based on the outcomes from the current research, the following recommendations are made for future research:

1. Future researches should focus on the nutrient requirements and the optimum feed formulation for western king prawn culture in the IRAS as the lower growth and higher FCR of the western king prawn than previous research indicated the presence of other limiting factors other than studied in this research.

2. Green seaweed showed lower survival and growth rates in integrated culture with western king prawn in the IRAS. High nutrients were likely to be responsible for toxication to seaweed culture. Future research should focus on the underlying mechanisms which are responsible for optimisation of seaweed cultures; and different seaweed species cultivated in IRAS.

3. Higher scale (commercial) studies should be carried out in order to evaluate the value of integrating seaweed or blue mussel with western king prawn in IRAS.

4. The study which could investigate the combined effects of integrating blue mussels and green seaweed with western king prawn in the IRAS is recommended.

5. Studies on the effects of stocking density and feeding rates on western king prawn culture at different life cycle stages at commercial and large scale are needed.

6. Separate studies on the optimum as well as lethal levels of TAN, NO_2^-, NO_3^- and pH under laboratory conditions are warranted to understanding the optimum environment to culture western king prawns.

7. The study which investigates the effects of light and water flow rates on the growth, yield and nutritional compositions of green seaweed integrated culture with western king prawn in the IRAS is also recommended.

8. Studies on the nutritional values of blue mussels and green seaweed reared in integrated culture in the IRAS are needed to evaluate the quality of by-product in the IRAS.

Future research should focus on economic analysis of the IRAS system.

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